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Interakcja pomiędzy zaangażowanymi w odpowiedź presyjną receptorami kannabinoidowymi CB_1 i angiotensynowymi w jądrze przykomorowym podwzgórza czuwających szczurów z nadciśnieniem pierwotnym i normotensyjnych.

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Rozdział 2. Wykaz stosowanych skrótów

2-AG, 2-arachidonyloglicerol

ACE, enzym konwertujący angiotensynę

ACE2, enzym konwertujący angiotensynę typu 2

ACEA, arachidonylo-2'-chloroetyloamid

AEA, anandamid

Ang 1-7, angiotensyna 1-7

Ang I, angiotensyna I

Ang II, angiotensyna II

AT_1R, receptor angiotensyny II typu 1

AT_2R, receptor angiotensyny II typu 2

BP, ciśnienie krwi

CB_1R, receptor kannabinoidowy typu 1

CB_2R, receptor kannabinoidowy typu 2

CBD, kannabidiol

CBG, kannabigerol

CBN, kannabinol

CHO, jajnik chomika chińskiego

COX-1, cyklooksygenaza konstytutywna

COX-2, cyklooksygenaza indukowana

COVID-19, choroba spowodowana przez koronawirus 2019

DAGL, lipaza diacyloglicerolowa

DAG, diacyloglicerol

DBP, rozkurczowe ciśnienie krwi

eCB, endokannabinoidy

ECS, układ endokannabinoidowy

EPSP, postsynaptyczny potencjał pobudzający

FAAH, hydrolaza amidowa kwasów tłuszczowych

GABA, kwas γ-aminomasłowy

Glu, kwas glutaminowy

GPCR, receptory sprzężone z białkami G

HR, częstość akcji serca

i.p., dootrzewnowo

i.v., dożylnie

MAGL, lipaza monoacyloglicerolowa

MasR, receptor Mas

MBP, średnie ciśnienie krwi

MetAEA, methanandamid

NA, noradrenalina

NTS, jądro pasma samotnego

OUN, ośrodkowy układ nerwowy

PPAR, receptor aktywowany przez proliferatory peroksysomów

PVN, jądro przykomorowe podwzgórza

RAS, układ renina-angiotensyna

RSNA, aktywność nerwów współczulnych nerek

RVLM, dogłowowo brzuszno-boczny obszar rdzenia przedłużonego

SARS-CoV-2, koronawirus ciężkiego ostrego zespołu oddechowego

SBP, skurczowe ciśnienie krwi

SD, szczury Sprague Dawley

SHR, szczury ze spontanicznym nadciśnieniem

THC, Δ^9-tetrahydrokannabinol

THCV, tetrahydrokannabiwaryna

TRPV1, receptor waniloidowy przejściowego potencjału typu 1

WKY, szczury Wistar Kyoto

Rozdział 3. Wprowadzenie

W ostatnich latach pojawia się coraz więcej doniesień dotyczących wzajemnych powiązań między układem endokannabinoidowym (ECS), a układem renina-angiotensyna (RAS). Powyższa tematyka stała się jeszcze bardziej aktualna w dobie pandemii COVID-19 wywoływanej przez koronawirus zespołu ostrej niewydolności oddechowej 2 (SARS-CoV-2), ponieważ udowodniono, że wnika on do wnętrza komórek gospodarza poprzez przyłączenie się do ACE2, jednego z kluczowych składowych RAS [1]. Wpływ kannabinoidów na ACE2 oraz na inne mechanizmy istotne przy zwalczaniu następstw COVID-19 omówiłem w rozdz. 3.4. pracy przeglądowej.

Zarówno układ endokannabinoidowy jak i RAS zaangażowane są w patofizjologię układu krążenia, a coraz więcej leków na nadciśnienie wykorzystuje poszczególne punkty uchwytu układu angiotensynowego [Tab. 1. w pracy przeglądowej]. Dokładniejsze zbadanie interakcji pomiędzy tymi układami może mieć zatem potencjalne znaczenie terapeutyczne.

W tekście mojej rozprawy doktorskiej odwołuję się do odpowiednich tabel i rycin pracy oryginalnej (Mińczuk i wsp., 2022, *Cells*) i przeglądowej (Mińczuk i wsp., 2022, *Int. J. Med. Sci*.).

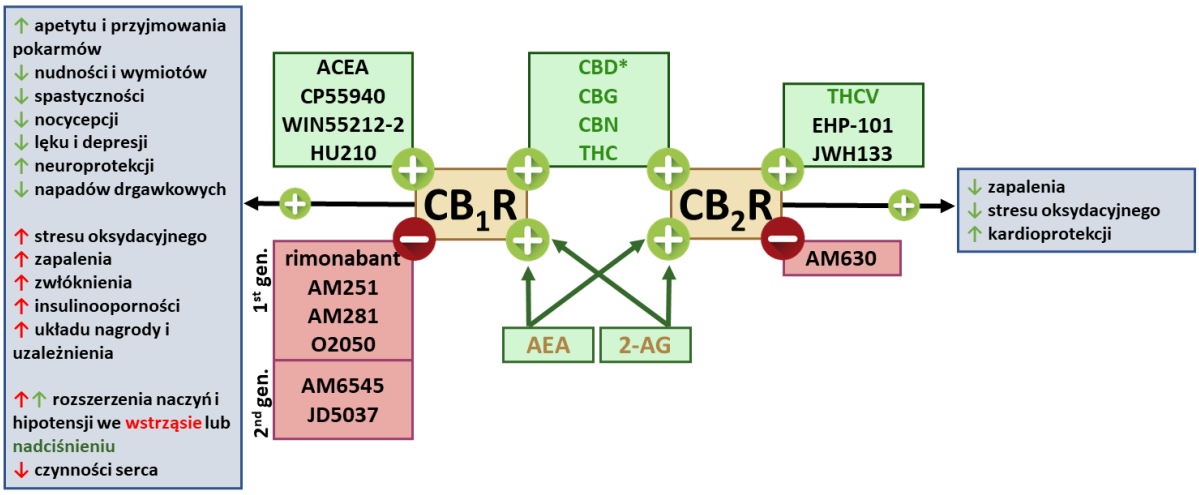
Kannabinoidy i układ endokannabinoidowy

Kannabinoidy są związkami lipofilnymi oddziałującymi na receptory kannabinoidowe. Kojarzone są głównie z pozyskiwaną z konopi siewnych (*Cannabis sativa*) marihuaną, która stanowi jedną z najpopularniejszych używek na świecie ze względu na swoje działanie prowadzące do złudnego odprężenia i krótkotrwałej euforii [2]. Według danych Biura Organizacji Narodów Zjednoczonych ds. Narkotyków i Przestępczości (UNODC) na rok 2019 około 4% całej populacji, a więc ok. 200 milionów osób w wieku 15 - 64 lat przynajmniej raz zażyło marihuanę. Chińska i hinduska medycyna już od około 4 tysięcy lat wykorzystuje lecznicze właściwości marihuany w tym jej działanie przeciwbiegunkowe, przeciwbólowe, psychotropowe, tłumiące nudności i wymioty, pobudzające apetyt, zmniejszające poczucie lęku, niepokoju, a także zapobiegające śmierci uszkodzonych neuronów, co ma istotne znaczenie dla osób chorujących na nowotwory, cierpiących na brak apetytu i spadek masy ciała [3].

Pierwszą substancją wyizolowaną z *Cannabis sativa* był Δ^9-tetrahydrokannabinol (THC). Obecnie wśród kannabinoidów wyróżniamy trzy zasadnicze grupy [4-6]:

- **kannabinoidy roślinne** (fitokannabinoidy), do których należy wspomniany wyżej THC, a także niepsychoaktywne: kannabidiol (CBD), kannabigerol (CBG) i kannabinol (CBN);
- **endokannabinoidy** (eCB; wytwarzane między innymi przez organizmy człowieka i zwierząt), wśród których do najlepiej zbadanych zaliczamy anandamid (AEA) i 2- arachidonyloglicerol (2-AG);
- **kannabinoidy syntetyczne**, otrzymywane na drodze modyfikacji naturalnych fitokannabioidów lub syntezy laboratoryjnej (np. CP55940, JWH133, czy WIN- 55212- 2).

Kannabinoidy działają głównie za pośrednictwem dwóch metabotropowych receptorów kannabinoidowych sprzężonych z białkami G (GPCR): CB_1 (CB_1R) i CB_2 (CB_2R), a także za pośrednictwem innych receptorów, takich jak receptory metabotropowe GPR55 oraz GPR18, jonotropowe waniloidowe przejściowego potencjału 1 (transient receptor potential vanilloid 1; TRPV1), czy steroidowe aktywowane przez proliferatory peroksysomów (peroxisome proliferator-activated receptor; PPAR). Najliczniej występującymi receptorami kannabinoidowymi są CB_1R, które ulegają ekspresji głównie w ośrodkowym układzie nerwowym (OUN) oraz w niewielkim stopniu w tkankach obwodowych, w tym na neuronach obwodowych, w układzie krążeniowo-oddechowym (naczynia systemowe i płucne), nerkach, wątrobie i innych tkankach. Natomiast CB_2R, zlokalizowane są głównie obwodowo, zwłaszcza na komórkach układu odpornościowego. Na rycinie 1 przedstawiłem przykładowych agonistów i antagonistów CB_1R i CB_2R oraz ich główne efekty pobudzenia. [4, 6-10].



Rycina 1. Przykładowe związki pobudzające receptory kannabinoidowe (CB_1R, CB_2R) oraz efekty ich pobudzenia. Zielone kółka ze znakiem plus oznaczają (częściowy) agonizm danego receptora; czerwone kółka ze znakiem minus oznaczają antagonizm lub odwrotny agonizm; związki syntetyczne, roślinne i endokannabinoidy zapisane są odpowiednio czarną, zieloną i brązową czcionką; strzałki w górę - wzrost; strzałki w dół - spadek; zielone strzałki - działanie pożądane; czerwone strzałki - działanie niepożądane; 1. gen. - antagoniści pierwszej generacji; 2. gen. - antagoniści drugiej generacji; *słabe powinowactwo; 2-arachidonyloglicerol (2-AG); anandamid (AEA); ACEA - arachidonylo-2'-chloroetyloamid; CBD - kannabidiol; CBG - kannabigerol; CBN - kannabinol; THC - Δ^9-tetrahydrokannabinol; THCV - tetrahydrokannabiwaryna.

Do układu endokannabinoidowego oprócz receptorów kannabinoidowych i eCB należą także enzymy zaangażowane w syntezę i degradację eCB. Co ważne, kannabinoidy wywierają wielokierunkowe działanie na organizm nie tylko poprzez interakcję z odpowiednimi receptorami, ale także pośrednio poprzez metabolity powstające w wyniku ich degradacji. Za rozkład AEA i 2-AG odpowiedzialne są odpowiednio hydrolaza amidowa kwasów tłuszczowych (FAAH) oraz lipaza monoacyloglicerolowa (MAGL). W wyniku tych procesów może powstać kwas arachidonowy, który następnie, w szlaku zależnym od cyklooksygenazy 1/2 (COX-1/COX-2), przekształcany jest w pochodne eikozanoidów, o szerokim spektrum działania w organizmie.

Składowym układu endokannabinoidowego, od początku jego odkrycia, przypisywano potencjalne znaczenie terapeutyczne. W swojej rozprawie doktorskiej najwięcej uwagi poświęcam CB_1R ze względu na fakt, że przegląd literatury dotyczący interakcji tego układu z RAS dotyczy głównie tych receptorów. Jak zaznaczyłem na Rycinie 1, efekty pobudzenia CB_1R mogą być zarówno korzystne, jak i szkodliwe, a ponadto ich funkcja może ulec zmianie w różnych stanach patologicznych. Niestety, pomimo intensywnych badań wciąż jedynie nieliczne leki (zestawione w Tabeli 1 pracy

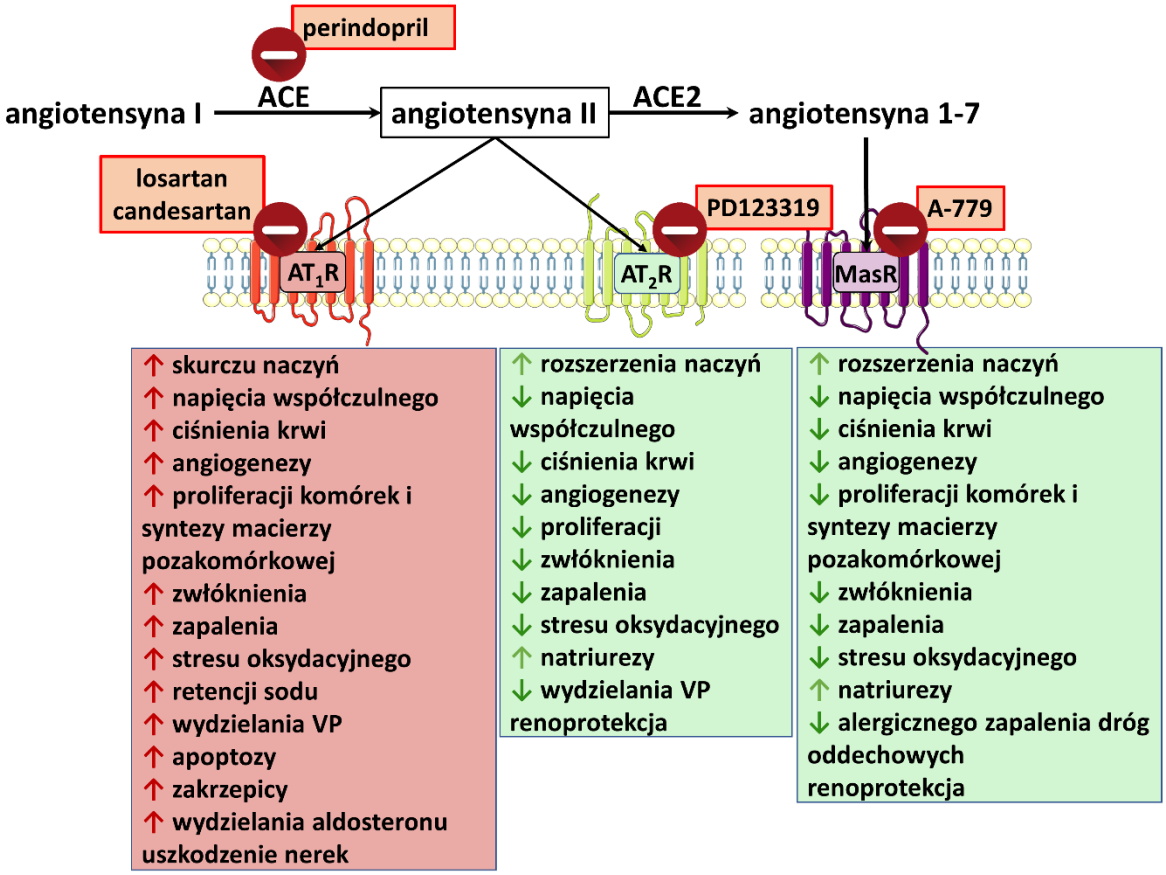
przeglądowej) zostały oficjalnie zatwierdzone do użycia. Wskazaniem do stosowania syntetycznych analogów THC, dronabinolu i nabilonu (zatwierdzonych m.in. przez amerykańską Agencję ds. Żywności i Leków), jest leczenie utraty apetytu i masy ciała oraz nudności, a kannabidiolu lekoopornej postaci padaczki [9, 11-13]. Na podstawie badań przedklinicznych sugeruje się, że antagoniści CB_1R mogą mieć potencjalne znaczenie terapeutyczne w leczeniu cukrzycy [14, 15], przewlekłej choroby nerek [16], stłuszczenia wątroby [17], zaburzeń wynikających z nadużywaniem alkoholu [14] czy COVID-19 [18]. Niestety, jedyny do tej pory oficjalnie zatwierdzony do użycia antagonista CB_1R pierwszej generacji, działający ogólnoustrojowo rimonabant, jako lek przeciw otyłości i uzależnienia od nikotyny, został w 2008 r. wycofany po około 2 latach stosowania, ze względu na poważne działania niepożądane ze strony OUN [14]. W chwili obecnej dużo nadziei przypisuje się antagonistom CB_1R drugiej generacji, czyli związkom działającym jedynie obwodowo (np. AM6545, JD5037).

Układ renina-angiotensyna

Układ renina-angiotensyna odgrywa istotną rolę jako układ hormonalny biorący udział w regulacji ciśnienia tętniczego krwi (BP) oraz utrzymania prawidłowego stężenia sodu i wody (głównie przy udziale aldosteronu), zarówno na poziomie narządów, jak i komórek [19, 20]. Powstawanie biologicznie czynnych składników RAS, w skrócie można przedstawić następująco: renina katalizuje przemianę angiotensynogenu do angiotensyny I (Ang I), która przy udziale enzymu konwertującego angiotensynę (ACE) jest przekształcana do angiotensyny II (Ang II) (Ryc. 2). Ang II jest głównym efektorem RAS, charakteryzującym się bardzo szerokim działaniem zachodzącym za pośrednictwem aktywacji kilku szlaków transdukcji sygnału, przy czym receptory Ang II typu 1 (AT_1R) są uznawane za dominujące. Ang II działa również za pośrednictwem receptorów Ang II typu 2 (AT_2R) lub jest degradowana przez enzym konwertujący angiotensynę typu 2 (ACE2) do działającej za pośrednictwem receptorów Mas (MasR) angiotensyny 1-7 (Ang 1-7) [21, 22]. Wszystkie powyższe receptory należą do GPCRs.

Jak zaznaczono na Rycinie 2, RAS składa się z dwóch działających przeciwstawnie osi: tzw. klasycznej, stymulującej szereg niepożądanych efektów, w skład której wchodzą ACE/Ang II/AT_1R oraz tzw. alternatywnej, indukującej pożądane działania, do której zaliczamy AT_2R oraz ACE2/Ang-(1-7) i MasR. Efekty

pobudzenia poszczególnych receptorów przedstawiono szczegółowo na Rycinie 2. Wzrost aktywności jednej z osi powoduje spadek aktywności drugiej i odwrotnie [20, 23, 24]. Co ważne, zaburzenie równowagi pomiędzy nimi, przy przewadze osi ACE/Ang II/AT_1R prowadzi do rozwoju wielu stanów patologicznych, w tym w układzie sercowo-naczyniowym (niewydolność serca, zawał serca, nadciśnienie tętnicze), zaburzeń metabolicznych (cukrzyca), funkcji nerek, wątroby, przewodu pokarmowego, endokrynologicznych, neurodegeneracyjnych, hematologicznych, rozrodczych, mięśniowych, a także tych w układzie oddechowym [20, 24-27]. Nie należy jednak zapominać o korzystnym wpływie pobudzenia AT_1R np. przy wstrząsie septycznym (Tabela 1, praca przeglądowa).



Rycina 2. Uproszczony schemat układu renina-angiotensyna (RAS) i niektórych związków modyfikujących jego działanie. RAS składa się z dwóch działających przeciwstawnie osi: stymulującej szereg niepożądanych efektów (tzw. klasycznej; czerwony prostokąt), w skład której wchodzą konwertaza angiotensyny (ACE), angiotensyna II (Ang II) i receptory angiotensyny II typu 1 (AT_1R) oraz osi ochronnej (tzw. alternatywnej; zielone prostokąty) utworzonej przez receptory Ang II typu 2 (AT_2R) oraz konwertazę angiotensyny typu 2 (ACE2) oraz angiotensynę-(1-7) (Ang-(1-7) i jej receptory Mas (MasR); VP, wazopresyna; czerwone kółka ze znakiem minus oznaczają antagonizm, odwrotny agonizm lub hamowanie danego mechanizmu; strzałki w górę - zwiększenie; strzałki w dół - zmniejszenie; zielone strzałki - działanie pożądane; czerwone strzałki - działanie niepożądane.

Co ciekawe, Ang 1-7, pomimo że jest znana głównie z działania rozszerzającego naczynia krwionośne i obniżania ciśnienia [23, 28], po podaniu ośrodkowym (w tym iniekcji do jądra przykomorowego podwzgórza; PVN) zwiększa ciśnienie tętnicze [29, 30]. „Odwrotne" efekty pobudzenia receptora Mas są zatem niezwykle ciekawym tematem zachęcającym do dalszych badań.

Biorąc pod uwagę szereg niekorzystnych działań Ang II za pośrednictwem AT_1R na układ krążenia (Rycina 2), składowe RAS (w tym ACE i AT_1R) stanowią niezwykle ważne punkty uchwytu działania szeregu leków w leczeniu nadciśnienia tętniczego czy niewydolności serca. (Tabela 1, praca przeglądowa).

Jądro przykomorowe podwzgórza

Jądro przykomorowe podwzgórza jest złożoną strukturą zaangażowaną m.in. w ośrodkową regulację układu sercowo-naczyniowego. PVN modyfikuje m.in. aktywność układu współczulnego, która zależy od układów: pobudzającego glutaminianergicznego i hamującego GABA-ergicznego (kwas γ-aminomasłowy; GABA). Zaburzenie równowagi pomiędzy neuronami glutaminianergicznymi a GABA- ergicznymi prowadzi do nadciśnienia [31, 32].

W PVN zlokalizowane są zarówno składowe ECS jak i RAS [28, 29, 33]. Ang II działająca za pośrednictwem AT_1R należy przy tym do czynników pobudzających układ sympatyczny [31], a wspomniane poprzednio leki stosowane w nadciśnieniu tętniczym (głównie inhibitory ACE i antagoniści AT_1R) działają także na mechanizmy ośrodkowe [28, 33, 34].

Zaangażowanie Ang II w PVN w patofizjologii nadciśnienia tętniczego potwierdzają następujące fakty: (1) w PVN człowieka stwierdzono wyższą ekspresję proreniny w nadciśnieniu tętniczym, która wykazywała dodatnią korelację z ciśnieniem tętniczym krwi [35]; (2) mikroiniekcja Ang II do PVN stymulowała silniejsze odpowiedzi presyjne i/lub wzrost aktywności współczulnej u szczurów z nadciśnieniem naczyniowo-nerkowym [36, 37]; (3) u szczurów z nadciśnieniem naczyniowo-nerkowym i ze spontanicznym nadciśnieniem pierwotnym (SHR) występowała wyższa ekspresja

AT_1R w PVN [37-39] niż u ich normotensyjnych odpowiedników oraz (4) delecja genu receptora AT_{1a} obniżała BP u SHR, ale nie u normotensyjnych szczurów Wistar [40].

Wspomniane wcześniej dwa układy w PVN regulujące napięcie układu współczulnego, pobudzający glutaminianergiczny i hamujący GABA-ergiczny, modulowane są nie tylko przez Ang II, ale także inne związki, w tym między innymi działające na CB_1R [41]. Receptory kannabinoidowe CB_1 są zlokalizowane głównie presynaptycznie, a ich pobudzenie hamuje uwalnianie różnych neuroprzekaźników, w tym kwasu glutaminowego i GABA [42]. Wcześniejsze badania w naszym Zakładzie wykazały bowiem, że mikroiniekcja do PVN stabilnego analogu AEA, methanandamidu (MetAEA) oraz agonisty CB_1R CP55940 indukuje dwukierunkowe zmiany ze strony układ sercowo-naczyniowego. Odpowiedź presyjna obserwowana dopiero po uprzednim dożylnym podaniu antagonisty CB_1R AM251 była przy tym hamowana przez dodatkowe podanie AM251 do PVN, ale nie przez antagonistę CB_2R SR144528 [41, 43].

Rozdział 4. Cel pracy z uzasadnieniem podjętej tematyki badawczej

Jądro przykomorowe podwzgórza jest jedną ze struktur OUN zaangażowaną w regulację układu krążenia, którego funkcja ulega zaburzeniu w nadciśnieniu tętniczym, stanowiącym istotny czynnik ryzyka zgonów z przyczyn sercowo-naczyniowych. W PVN zlokalizowane są składowe układu endokannabinoidowego oraz RAS, które modyfikują ciśnienie krwi i częstość akcji serca.

Wpływ kannabinoidów na układ krążenia zależy od użytego modelu doświadczalnego. Wykazano przy tym, że dożylne podanie THC, AEA czy CP55940 obniża BP u szczurów uśpionych, a podwyższa je u zwierząt czuwających. Odpowiedź presyjna na podanie kannabinoidów przeważa też po ich podaniu ośrodkowym. Jest także wyższa u zwierząt z nadciśnieniem w porównaniu do ich normotensyjnych kontroli (prace przeglądowe, [44, 45]).

Dotychczasowe badania wykonane w naszym Zakładzie wykazały, że podany dożylnie antagonista receptora AT_1 losartan nie tylko hamuje, ale nawet odwraca presyjny efekt uzyskany w wyniku mikroiniekcji agonisty CB_1R CP55940 do PVN uśpionych szczurów normotensyjnych [41, 43]. Stosując ten sam model doświadczalny, Gyombolai i wsp. (2012) [46] zaobserwowali z kolei, że antagonista CB_1R AM251 redukował presyjne działanie Ang II przy czym obydwa związki podawano do PVN.

Jak do tej pory istnieje zaledwie jedna publikacja, której autorzy sugerują istnienie interakcji Ang 1–7 z układem kannabinoidowym [47]. Wykazano w niej, że infuzja Ang 1–7 do macicy szczurów powoduje wzrost ekspresji receptorów CB_1 i CB_2.

Biorąc powyższe pod uwagę, **<u>celem mojej rozprawy doktorskiej było</u>**:

1. zbadanie potencjalnej interakcji w PVN szczurów czuwających pomiędzy CB_1R a receptorami AT_1 i AT_2 dla Ang II i receptorami Mas dla Ang 1–7,
2. zbadanie wpływu nadciśnienia pierwotnego na efekt presyjny stymulowany pobudzeniem receptorów w PVN AT_1 i AT_2 dla Ang II, Mas dla Ang 1–7 i kannabinoidowych CB_1,
3. określenie mechanizmów interakcji pomiędzy ECS i RAS oraz ich potencjalnego znaczenia terapeutycznego na podstawie dostępnej literatury.

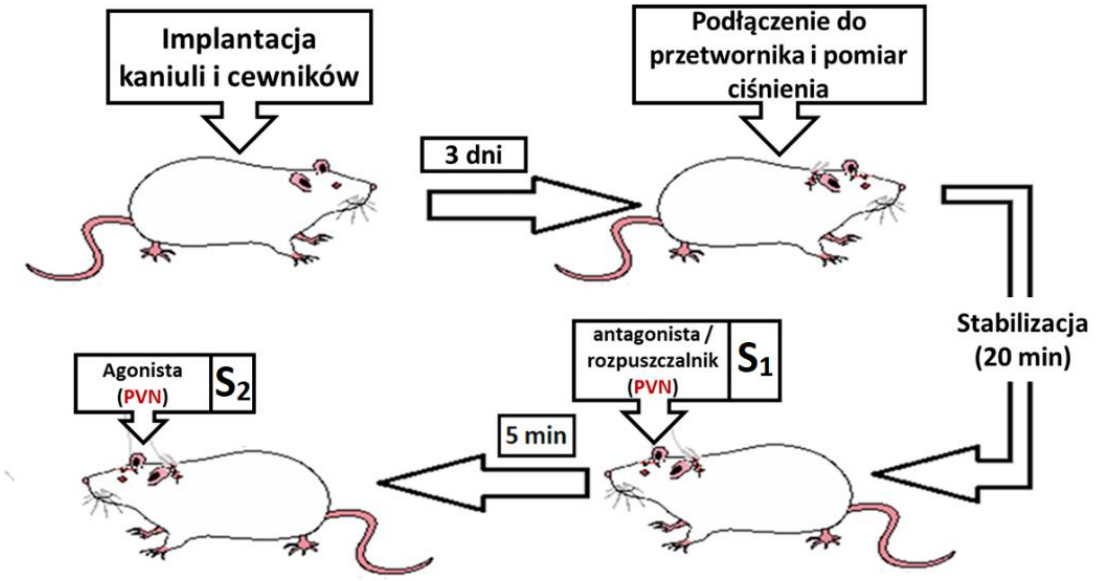
Rozdział 5. Realizacja celów naukowych, materiały i metody badawcze, podsumowanie wyników badań i dyskusja

Materiały i metody badawcze

W doświadczeniach wykorzystałem samce szczurów ze spontanicznym nadciśnieniem pierwotnym (SHR) i ich normotensyjnej kontroli, szczurów Wistar Kyoto (WKY). Wszystkie procedury chirurgiczne i protokoły eksperymentalne zostały zatwierdzone przez Lokalną Komisję Etyczną ds. Zwierząt w Olsztynie (numer pozwolenia: 77/2019; data zatwierdzenia: 29 października 2019 r.).

Wykaz zasadniczych czynności w trakcie przeprowadzanych doświadczeń:

1. wstępny pomiar ciśnienia tętniczego krwi u szczurów czuwających - metoda bezkrwawa, mankietowa, na ogonie szczura w celu weryfikacji BP u SHR i WKY - przydział zwierząt do odpowiednich grup badawczych;
2. szczury znieczulone dootrzewnowo (i.p.) pentobarbitonem sodowym w dawce 300 μmol/kg:
    a. implantacja kaniuli do PVN,
    b. implantacja cewników do tętnicy szyjnej i żyły udowej;
3. **zasadnicze doświadczenia** - mikroiniekcja badanych związków do PVN i pomiar BP oraz częstości akcji serca (HR) u szczurów czuwających; wyniki dotyczące HR nie zostały uwzględnione w publikacji ze względu na ich niewielkie wzrosty oraz duże zróżnicowanie;
4. potwierdzenie prawidłowego umiejscowienia kaniuli (przy użyciu błękitu Evansa, Rycina 1., praca oryginalna);
5. izolacja tkanek i analiza biochemiczna poszczególnych części mózgowia (metoda Western blot);
6. przegląd literatury dotyczącej interakcji pomiędzy ECS i RAS



Rycina 3. Ogólny schemat doświadczeń. Po implantacji kaniuli i cewników następował 3-dniowy okres rekonwalescencji. Po podłączeniu cewników do systemu pomiaru ciśnienia, po 20 minutach stabilizacji, do jądra przykomorowego podwzgórza (PVN) podawany był antagonista/rozpuszczalnik (S_1), a po kolejnych 5 minutach, odpowiedni agonista (S_2).

Protokół doświadczeń

Ogólny schemat moich doświadczeń przedstawiłem na Rycinie 3. U każdego szczura wykonałem dwie jednostronne mikroiniekcje do PVN (S_1 i S_2). Ang II, Ang 1-7 lub CP55940 były podawane podczas S_2 odpowiednio w dawkach: 0.3 nmol/szczura 0.3 nmol/szczura i 0.1 nmol/szczura. Antagoniści receptorów AT_1 (losartan, 20 nmol/szczura), AT_2 (PD123319, 10 nmol/szczura), Mas (A-779, 3 nmol/szczura) i CB_1 (AM251, 30 nmol/szczura) byli wstrzykiwani do PVN w trakcie S_1, 5 min przed S_2 (lub po powrocie BP do wartości sprzed wstrzyknięcia). Grupy kontrolne otrzymywały odpowiedni rozpuszczalnik dla poszczególnych ligandów. Wszystkie doświadczenia z CP55940 wykonano w obecności podanego dożylnie AM251 (3 μmol/kg).

Dokładny opis procedur i analizy statystycznej wyników znajduje się w rozdziale 2. dołączonej do rozprawy pracy oryginalnej.

Podsumowanie wyników i dyskusja

Celem mojej pracy było określenie potencjalnej interakcji w PVN pomiędzy receptorami kannabinoidowymi CB_1 oraz AT_1 i AT_2 dla Ang II a także Mas dla Ang 1-7,

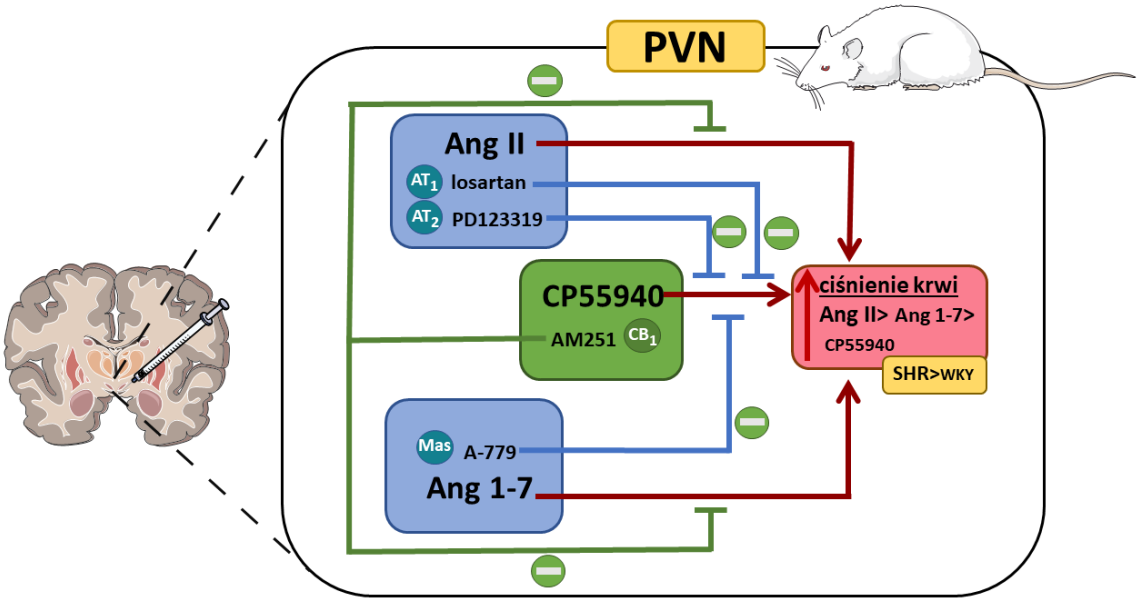
biorącymi udział w regulacji ciśnienia tętniczego u czuwających szczurów z nadciśnieniem tętniczym pierwotnym i ich normotensyjnej kontroli Wistar Kyoto (WKY). Dodatkowo dokonałem przeglądu całej dostępnej literatury dotyczącej interakcji pomiędzy ECS i RAS co pozwoliło mi na porównanie mechanizmów odpowiedzialnych za tę interakcję oraz jej potencjalnego znaczenia terapeutycznego.

Jak wspomniałem powyżej, wszystkie doświadczenia wykonałem na czuwających szczurach ze spontanicznym nadciśnieniem i ich normotensyjnych kontrolach, szczurach Wistar Kyoto. W badaniach zastosowałem SHR ponieważ: (1) są modelem najczęściej występującego u ludzi nadciśnienia pierwotnego; (2) występujące u nich podwyższone BP wynika częściowo ze zwiększonej aktywności układu współczulnego zależnego od PVN i RAS oraz (3) niemal wszystkie klasy dopuszczonych do obrotu leków przeciwnadciśnieniowych działają u nich hipotensyjnie [48, 49]. Rzeczywiście, jak wykazałem, SBP, DBP i MBP były wyższe o około 60% u SHR w porównaniu z WKY. Przeprowadzenie doświadczeń na zwierzętach czuwających wynika z faktu, że kannabinoidy (np. THC, AEA lub CP55940) podwyższają BP u szczurów czuwających, a obniżają je u zwierząt uśpionych [44, 45]. Jako agonistę receptorów kannabinoidowych zastosowałem CP55940, charakteryzującego się wysokim powinowactwem zarówno w stosunku do receptorów CB_1 jak i CB_2 [50]. Jednak w poprzednich badaniach naszej grupy wykazaliśmy, że odpowiedź presyjna powstała w wyniku podania CP55940 do PVN była zmniejszona jedynie przez mikroiniekcję do PVN antagonisty receptorów CB_1 AM251, ale nie antagonisty receptorów CB_2 SR144528 [43]. CP55940 podawany do PVN stymulował dwukierunkowe zmiany BP i HR u szczurów uśpionych uretanem [41, 43]. W niniejszej pracy skoncentrowałem się jednak wyłącznie na badaniu odpowiedzi presyjnej CP55940, ponieważ antagonista AT_1R losartan podany dożylnie (i.v.) nie modyfikował spadku BP indukowanej mikroiniekcją agonisty receptorów CB, natomiast odwracał wzrost BP indukowany podaniem CP55940 do odpowiedzi hipotensyjnej [41].

Jak wspomniałem we wstępie, mikroiniekcja CP55940 do PVN podwyższała BP w obecności podanego uprzednio antagonisty receptorów CB_1 AM251 [41, 43]. W związku z powyższym, rutynowo wykonywałem mikroiniekcje CP55940 do PVN po wcześniejszej dożylnej iniekcji AM251. Co ciekawe, AM251 podawany zarówno dożylnie jak i do PVN zwiększał BP odpowiednio o około 15-20 mmHg i 5-10 mmHg, zarówno u WKY, jak i u SHR (Rycina 2, praca oryginalna). Lekkie działanie presyjne

AM251 wynika prawdopodobnie z antagonizowania wpływu endokannabinoidów pobudzających odpowiednio hamujące receptory presynaptyczne CB_1 zlokalizowane obwodowo na zakończeniach nerwów współczulnych unerwiających naczynia oporowe [51] lub receptorów CB_1 w PVN odpowiedzialnych za spadek BP (tak zwana pierwsza faza odpowiedzi na CP55940, [41, 43]). Umiarkowany, natychmiastowy i krótkotrwały wzrost BP wywołany przez mikroiniekcję AM251 do PVN był również obserwowany wcześniej u szczurów znieczulonych uretanem przez Gyombolai i wsp. (2012), lecz nie przez naszą grupę [43]. W naszych poprzednich doświadczeniach dożylna iniekcja AM251 także nie modyfikowała BP u szczurów znieczulonych uretanem, co wynikało zapewne z niższego napięcia układu współczulnego i prawdopodobnie także niższej aktywności układu endokannabinoidowego u zwierząt uśpionych niż u czuwających [41, 43].

Szczegółowe wyniki doświadczeń stanowiących podstawę mojej rozprawy doktorskiej opublikowałem w mojej pracy oryginalnej (Mińczuk i wsp., 2022, Cells) . W skrócie przedstawia je Rycina 4.



Rycina 4. Podsumowanie najważniejszych wyników pracy oryginalnej Mińczuk i wsp. (2022). Angiotensyna II (Ang II), angiotensyna 1-7 (Ang 1-7) oraz agonista receptorów kannabinoidowych CP55940 podawane do jądra przykomorowego podwzgórza (PVN) czuwających szczurów ze spontanicznym nadciśnieniem (SHR) i ich normotensyjnej kontroli, zwierząt Wistar Kyoto (WKY), stymulowały wzrost ciśnienia krwi (BP) wyższy u SHR niż WKY. Najsilniejsze działanie wykazywała Ang II, a najsłabsze Ang 1-7. Antagonista receptorów AT_1 losartan oraz AT_2 PD123319 hamowały odpowiedź presyjną indukowaną przez Ang II i CP55940. Antagonista receptorów Mas A-779 zmniejszały wzrost BP stymulowany Ang 1-7 i CP55940. Antagonista receptorów CB_1 AM251 osłabiał wzrost BP w odpowiedzi na Ang II i Ang 1-7.

W swoich doświadczeniach stwierdziłem, że mikroiniekcja Ang II, Ang 1-7 i CP55940 (w obecności AM251 i.v.) do PVN stymulowały wzrost ciśnienie skurczowego (SBP), rozkurczowego (DBP) i średniego (MBP) u czuwających WKY i SHR. Najsilniejszą odpowiedź presyjną wywoływała Ang II, a najsłabszą Ang 1-7 (odpowiednio o około 20 i 6-8 mmHg u WKY). Sun i wsp. (2012) wykazali, że te same dawki Ang II i Ang 1-7, które stosowałem w niniejszej pracy, podane do PVN wywołały porównywalny wzrost MBP u czuwających szczurów Sprague-Dawley.

Na uwagę zasługuje fakt, że mikroiniekcja CP55940 do PVN (w obecności AM251 i.v.) podwyższała BP zarówno u szczurów czuwających (Rycina 6., praca oryginalna) jak i uśpionych uretanem [41, 43]. Natomiast, jak wspomniałem powyżej, podanie dożylne kannabinoidów głównie zwiększa BP u zwierząt czuwających, ale obniża je u uśpionych [44]. Powyższe porównanie sugeruje, że za działanie presyjne kannabinoidów odpowiedzialne są mechanizmy ośrodkowe.

W przeprowadzonych doświadczeniach wyraźnie wykazałem, że odpowiedzi presyjne na Ang II wynikały odpowiednio z pobudzenia receptorów AT_1 i AT_2. Były one bowiem całkowicie lub silnie hamowane przez wcześniejsze podawanie do PVN ich antagonistów, odpowiednio losartanu i PD123319, przy czym losartan działał znacznie silniej (Rycina 4., praca oryginalna). Jak wspomniałem we wstępie, receptory AT_1 w PVN pośredniczą w odpowiedzi presyjnej na Ang II. Natomiast ośrodkowe receptory AT_2 znane są głównie z ich wpływu obniżającego BP [52]. Jednak podobnie jak w wykonanych przeze mnie doświadczeniach, odpowiedź presyjna na podanie Ang II do PVN u uśpionych szczurów normotensyjnych była silnie hamowana nie tylko przez losartan, ale także przez PD123319 [53, 54], przy czym Camargo i wsp. (2002) stwierdzili, że podobnie jak w przypadku moich wyników, hamujący wpływ PD123319 był słabszy niż losartanu. Ponadto, Sun i wsp. (2012) wykazali, że losartan hamował efekt presyjny wywołany przez podaną do PVN Ang II, ale nie przez Ang 1-7, co potwierdza swoiste działanie losartanu w stosunku do receptorów AT_1. Wzrost BP stymulowany przez Ang 1-7 był natomiast zmniejszany przez A-779, co wskazuje na udział receptorów Mas (Rycina 4., praca oryginalna). Jak wspomniałem powyżej, udział receptorów CB_1 w działaniu presyjnym CP55940 po jego podaniu do PVN został przez nas udowodniony wcześniej [43], dlatego nie powtarzałem tych doświadczeń.

Jak do tej pory nikt nie badał efektów presyjnych Ang II, Ang 1-7 oraz CP55940 po ich podaniu do PVN u szczurów z nadciśnieniem spontanicznym. Moje wyniki są więc pierwszymi, które udowadniają, że wszystkie powyższe odpowiedzi były istotnie wyższe (lub miały tendencję do bycia wyższymi) u SHR w porównaniu z WKY. Jak do tej pory, wyższe wzrosty ciśnienia tętniczego wywołane mikroiniekcją do PVN Ang II [36, 37] oraz Ang 1-7 [37, 55] były wykazywane tylko u szczurów z nadciśnieniem naczyniowo-nerkowym.

Wzrost/spadek ciśnienia tętniczego w odpowiedzi na dany ligand zależy od wartości podstawowego ciśnienia tętniczego. Nie można zatem wykluczyć, że silniejsze odpowiedzi obserwowane u SHR wynikały z wyższego bazalnego BP występującego u tych zwierząt. Jednak wyniki moich badań Western blot oceniających gęstość poszczególnych receptorów wskazują także na inną możliwość. Samodzielnie nie oceniałem jedynie gęstości receptorów AT_1, gdyż była już zbadana wcześniej. Okazało się, że w PVN SHR występuje wyższy, w porównaniu z WKY, poziom receptorów AT_1 [38, 39] i CB_1 [30] zlokalizowanych presynaptycznie głównie na neuronach glutaminianergicznych [39, 56, 57] oraz GABA-ergicznych [41]. Ich pobudzenie odpowiednio zwiększa aktywność glutaminianergiczną i zmniejsza aktywność GABA- ergiczną. Jak wspomniałem we wstępie aktywność układu współczulnego zależy między innymi od równowagi pomiędzy neuronami glutaminianergicznymi i GABA- ergicznymi w PVN (Rycina 8., praca oryginalna). Zarówno nasilenie wpływu pobudzającego, jak i zmniejszenie wpływu hamującego układ sympatyczny może stymulować wzrost ciśnienia tętniczego. Czyli wzrost gęstości receptorów AT_1 i CB_1 w PVN szczurów SHR w porównaniu do WKY może być przynajmniej częściowo odpowiedzialny za wyższe BP u tych zwierząt. Co ważne, ekspresja receptorów AT_1 i CB_1 jest również wyższa u SHR w porównaniu do WKY w innych kluczowych dla regulacji układu krążenia regionach mózgu, takich jak dogłowowo brzuszno-boczny obszar rdzenia przedłużonego (RVLM) i jądro pasma samotnego (NTS), w których podobnie jak w PVN ostateczny wpływ na BP jest zależny od interakcji neuronów glutaminianergicznych i GABA- ergicznych (dla AT_1R: [38, 58]; dla CB_1R: [30]). Na wyższy poziom CB_1R, ale nie endokannabinoidów w RVLM SHR zwrócili uwagę wcześniej Wang i wsp. (2017) [59].

Co ciekawe, wykonana przeze mnie analiza Western blot wykazała, odwrotnie niż w przypadku receptorów AT_1 i CB_1, wyższą ekspresję receptorów AT_2 i Mas

u normotensyjnych WKY niż u SHR zarówno w PVN, RVLM jak i NTS. Powszechnie przyjmuje się, że receptory AT_2 i Mas przeciwdziałają efektowi presyjnemu powstałemu w wyniku stymulacji AT_1R poprzez wzmocnienie działania GABA-ergicznego, bezpośrednio w wyniku nasilenia neuroprzekaźnictwa GABA-ergicznego (pobudzające AT_2R są zlokalizowane głównie na neuronach GABA-ergicznych; [52]) lub pośrednio poprzez stymulację uwalniania GABA za pośrednictwem tlenku azotu, jak wykazano w PVN zarówno w przypadku receptorów AT_2 [60], jak i Mas [61]. Zmniejszenie ekspresji receptorów AT_2 i Mas, a tym samym hamującego wpływu na układ sympatyczny może więc przyczyniać się do wzrostu ciśnienia tętniczego u SHR. Co ciekawe, gęstość receptorów Mas w PVN była wyższa u szczurów z nadciśnieniem naczyniowo-nerkowym niż u szczurów normotensyjnych [55].

W kontekście uzyskanych przeze mnie wyników, pojawia się istotne pytanie, jak można wyjaśnić działanie presyjne Ang 1-7 i udział receptorów AT_2 we wzroście BP indukowanym przez Ang II? Niestety w dostępnej literaturze można znaleźć jedynie pojedyncze publikacje na ten temat. Yu i wsp., (2019) [62] sugerują, że Ang 1-7 podawana do PVN podwyższa BP w wyniku nasilenia stresu oksydacyjnego i stanu zapalnego, gdyż antagonista Mas A-779 podany do PVN nie tylko obniżał BP czy poziom noradrenaliny (NA) w osoczu, ale także zmniejszał poziom reaktywnych form tlenu i cytokin prozapalnych w PVN. Z kolei Nasimi i wsp., (2021) [63] tłumaczy zależny od pobudzeniem receptorów AT_2 wzrost BP ich wpływem na transmisję wazopresynergiczną w PVN, ponieważ wzrost BP i HR stymulowany mikroiniekcją wazopresyny do PVN był bardzo silnie hamowany przez PD123319, a jedynie częściowo przez losartan.

Dowody na istnienie interakcji pomiędzy układami (endo)kannabinoidowym a RAS w PVN

Następujące fakty potwierdzają istnienie interakcji pomiędzy modyfikującymi funkcje układu krążenia, zlokalizowanymi w PVN receptorami CB_1 dla kannabinoidów, receptorami AT_1 i AT_2 dla Ang II oraz receptorami Mas dla Ang 1-7:

Po pierwsze, antagonista receptorów CB_1 AM251 podany do PVN zmniejszał lub miał tendencję do zmniejszania odpowiedzi presyjnej na Ang II zarówno u WKY, jak i SHR, a nawet odwracał wzrost BP wywołany przez Ang 1-7 do odpowiedzi

hipotensyjnej. Hamujący wpływ AM251 na odpowiedź presyjną Ang II (obydwa związki podawano do PVN) wykazali wcześniej Gyombolai i wsp. (2012).

Po drugie, odpowiedź presyjną na CP55940 silnie zmniejszało wcześniejsze podanie do PVN antagonistów receptorów AT_1, AT_2 i Mas, odpowiednio losartanu, PD123319 i A-779. W naszych poprzednich pracach losartan i.v. nie modyfikował odpowiedzi sercowo-naczyniowej na AEA i.v. (w tym jej fazy presyjnej; [64]), a wręcz odwracał wzrost BP i HR indukowany przez CP55940 podany do PVN do spadku BP i HR [41]. Tak drastycznej odwrotnej zmiany w odpowiedzi na CP55940 w obecności losartanu nie uzyskałem w doświadczeniach realizowanych przeze mnie. Jednak w tym przypadku losartan podawałem lokalnie do PVN, w przeciwieństwie do iniekcji i.v. w poprzedniej pracy [41].

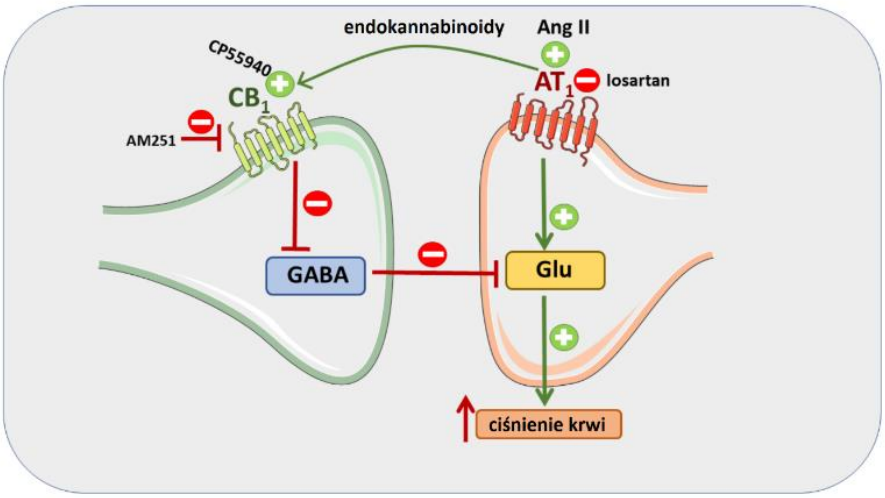
Po trzecie, antagonista receptorów Mas A-779 obniżał BP w porównywalnym stopniu zarówno u WKY, jak i u SHR, ale tylko w obecności AM251 i.v. Natomiast losartan i PD123319 nie modyfikowały BP zarówno przy braku jak i w obecności AM251 i.v. Na podstawie powyższych wyników można przypuszczać, że endokannabinoidy działające na receptory CB_1 w PVN współdziałają z endogennym tonem presyjnym wywoływanym przez Ang 1-7, ale nie przez Ang II. Udział endogennej Ang 1-7 w PVN w regulacji ciśnienia krwi wykazano wcześniej u uśpionych szczurów z nadciśnieniem tętniczym wywołanym dietą wysokosodową lub nadciśnieniem nerkowym, ale nie u ich odpowiednich kontroli normotensyjnych, u których A-779 (3 nmol/szczura) podany do PVN obniżał ciśnienie tętnicze, aktywność nerwów współczulnych nerek (RSNA) i poziom noradrenaliny w osoczu [52, 62]. Uzyskane przeze mnie wyniki są natomiast pierwszymi, wskazującymi na potencjalną interakcję pomiędzy Ang 1-7 i jej receptorami Mas z receptorami kannabinoidowymi CB_1 w ośrodkowym układzie nerwowym. Dotychczas istnienie takiej interakcji sugerowano w macicy szczura, w której miejscowa infuzja Ang 1-7 zwiększała ekspresję receptorów CB_1 i CB_2 [47].

Powstaje pytanie, jak można wyjaśnić interakcję między receptorami CB_1 a receptorami AT_1, AT_2 i Mas?

W 2007 r. Turu i wsp. wykazali po raz pierwszy istnienie interakcji pomiędzy receptorami CB_1 i AT_1 na komórkach CHO (Chinese hamster ovary; jajnika chomika chińskiego), udowadniając, że pobudzenie receptorów AT_1 prowadzi do uwolnienia 2-AG, który działając na CB_1R może modyfikować odpowiedź stymulowaną

przez AT_1R. Pionierskie obserwacje Turu i wsp. (2007) [65] zostały potwierdzone w kolejnych publikacjach omówionych szczegółowo w mojej pracy przeglądowej oraz w skrócie w dalszej części mojej rozprawy.

Przestawiony powyżej mechanizm może mieć miejsce także w PVN. Jak opisałem wcześniej, napięcie układu współczulnego zależy między innymi od wzajemnej równowagi pomiędzy pobudzającymi neuronami glutaminianergicznymi i hamującymi GABA-ergicznymi [31, 32, 52]. Presynaptyczne receptory AT_1 i CB_1, odpowiednio nasilające i hamujące uwalnianie odpowiedniego neuroprzekaźnika, są zlokalizowane na obydwu typach neuronów [66, 67], przy czym pobudzające AT_1R przeważają na neuronach glutaminianergicznych [39], a CP55940 zwiększa BP, działając głównie na hamujące CB_1R na neuronach GABA-ergicznych [41]. Przypuszczalne mechanizmy zaangażowane w odpowiedź presyjną wywołaną przez Ang II i CP55940 oraz interakcję pomiędzy receptorami AT_1 i CB_1 zostały schematycznie przedstawione na Rycinie 5 i omówione w jej legendzie. Jak na razie istnieje zbyt mało danych na temat interakcji receptorów CB_1 z receptorami AT_2 i Mas, żeby można było zaproponować jakiś mechanizm.

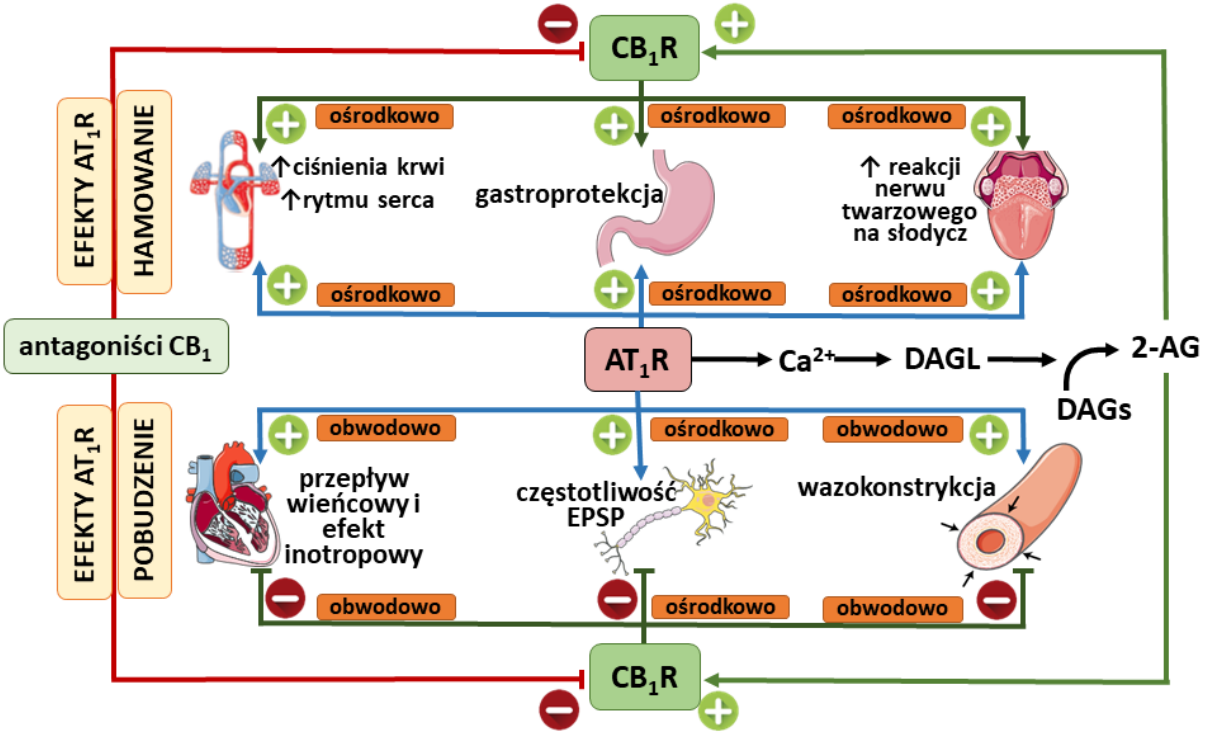


Rycina 5. Przypuszczalne mechanizmy zaangażowane w odpowiedź presyjną wywołaną przez Ang II i CP55940 oraz interakcję pomiędzy receptorami AT_1 i CB_1 w jądrze przykomorowym podwzgórza (PVN). Stymulowanie presynaptycznych pobudzających receptorów AT_1 przez Ang II z jednej strony nasila uwalnianie kwasu glutaminowego (Glu), a z drugiej prawdopodobnie prowadzi do uwalniania endokannabinoidów. Endokannabinoidy lub egzogennie podany CP55940 hamują uwalnianie GABA (kwas γ-aminomasłowy) w wyniku pobudzenia presynaptycznych hamujących receptorów CB_1. Prowadzi to do większego uwalniania Glu ze względu na zmniejszenie hamującego działania GABA. Odpowiedź presyjna wywołana przez Ang II może być osłabiona przez antagonistę receptora CB_1, AM251, hamującego presynaptyczne hamujące CB_1R na neuronach GABA-ergicznych. Z kolei odpowiedź presyjną wywołaną przez CP55940 można zahamować antagonistą receptora AT_1 losartanem, który prawdopodobnie zmniejsza uwalnianie endokannabinoidów.

W pracy przeglądowej omówiłem jeszcze inne potencjalne mechanizmy interakcji ECS z RAS zaproponowane na podstawie wyników uzyskanych w doświadczeniach in vivo i in vitro, takie jak heterodimeryzacja AT_1R-CB_1R, czy zmiana gęstości poszczególnych receptorów w wyniku pobudzenia lub blokowania receptorów drugiego układu.

Znaczenie interakcji pomiędzy receptorami AT_1 i CB_1.

Systematyczny przegląd literatury w bazie PubMed wykazał jedynie 43 publikacje dotyczące interakcji pomiędzy układem (endo)kannabinoidowych a RAS (w tym także jego powiązania z SARS-CoV-2, COVID czy ACE2, których nie omawiałem w swojej rozprawie). Najwięcej z nich dotyczy uwalnianych pod wpływem pobudzenia AT_1R endokannabinoidów, które działając na CB_1R modyfikowały odpowiedź stymulowaną AT_1R. Niestety, tylko w paru przypadkach mierzono poziom 2- AG lub AEA. Najczęściej zastosowano ocenę pośrednią badając wpływ antagonistów CB_1R na efekt pobudzenia AT_1. Analiza wszystkich powyższych publikacji pozwoliła na wyciągnięcie dodatkowego wniosku w mojej rozprawie. Okazało się bowiem, że wpływ antagonistów CB_1R na efekt pobudzenia receptorów AT_1 zależy od tego czy pobudzenie obydwu receptorów indukuje odpowiedzi skierowane w tę samą stronę czy przeciwną. Wszystkie publikacje zostały schematycznie przedstawione i omówione na rycinie 6 i w jej legendzie. Przykładowo, najwięcej z powyższych publikacji dotyczy izolowanych naczyń krwionośnych, kurczonych i rozkurczanych w wyniku pobudzenia odpowiednio AT_1R i CB_1R, a antagoniści CB_1R nasilali efekt pobudzenia AT_1R. Z kolei inhibitory rozkładu eCBs, w tym głównie 2-AG, osłabiali działanie Ang II. Biorąc pod uwagę potencjalne znaczenie terapeutyczne antagonistów CB_1R, takich jak AM6545, czy JD5037 oraz inhibitorów rozkładu eCB, które w przyszłości mogą być znaczenie przy leczeniu bólu, stwardnienia rozsianego, choroby Parkinsona czy depresji [6], przy ich stosowaniu należy brać pod uwagę ich ewentualną interakcję z AT_1R.



Rycina 6. Wpływ antagonistów receptora CB_1 (CB_1R) na efekty wywoływane pobudzeniem receptorów AT_1 (AT_1R). Stymulacja AT_1R prowadzi do generowania sygnału Ca^{2+} i pośrednio, w wyniku pobudzenia lipazy diacyloglicerolowej (DAGL), pośredniczy w biosyntezie 2- arachidonyloglicerolu (2-AG) z diacylogliceroli (DAGs). **Antagoniści CB_1R mogą osłabiać efekty pobudzenia AT_1R** w przypadku takiego samego kierunku końcowego efektu zachodzącego w wyniku pobudzenia CB_1R i AT_1R, co wykazano w przypadku wzrostu ciśnienia tętniczego krwi i częstości akcji serca [30], gastroprotekcji [68] po ich ośrodkowym podaniu lub wzmocnieniu odpowiedzi nerwu twarzowego na słodkie związki [69]. **Antagoniści CB_1R mogą wzmocnić efekty pobudzenia AT_1R** w przypadku przeciwstawnych efektów pobudzenia CB_1R i AT_1R, co wykazano w przypadku ich ujemnego [70] i dodatniego efektu inotropowego w sercu [71], zmniejszenia lub zwiększenia postsynaptycznych potencjałów pobudzających (EPSP) komórek jądra nadwzrokowego [72] lub odpowiednio rozszerzenia i zwężenia naczyń krwionośnych [73].

W pracy przeglądowej omówiłem jeszcze przykłady interakcji farmakokinetycznej. Chciałbym w tym miejscu podkreślić potencjalne prawdopodobieństwo występowania takiej interakcji w przypadku szeregu szeroko dostępnych bez recepty niewystandaryzowanych produktów ze składników otrzymywanych z konopi z lekami działającymi na RAS.

Podsumowując, wyniki moich badań jednoznacznie wskazują na istnienie interakcji pomiędzy układem angiotensynowym i (endo)kannabinoidowym w jądrze przykomorowym podwzgórza zaangażowanymi w regulację układu krążenia, ponieważ antagoniści AT_1R, AT_2R oraz MasR osłabiali odpowiedź presyjną wynikającą z pobudzenia CB_1R. Z drugiej antagonista CB_1R zmniejszał wzrost BP zachodzący

za pośrednictwem receptorów dla Ang II i Ang 1-7. Hamujący wpływ antagonisty CB_1R jest zgodny z wnioskiem wynikającym z dokładnego przeglądu literatury dotyczącej interakcji obydwu układów. Wynika z niej bowiem jednoznacznie, że antagoniści CB_1R modyfikują działanie Ang II, osłabiając je, bądź wzmacniając, w zależności od końcowego kierunku końcowego efektu. Niewątpliwie cenne byłyby dodatkowe doświadczenia potwierdzające powyższą obserwację, szczególnie w układzie, w którym by jednocześnie badano wpływ antagonistów CB_1R na odpowiedzi zachodzące za pośrednictwem AT_1R o tym samym i przeciwnym kierunku co pobudzenie CB_1R. Niewątpliwie istnienie takiej interakcji należy brać pod uwagę przy stosowaniu leków działających za pośrednictwem AT_1R i będących wciąż na etapie badań przedklinicznych antagonistów CB_1R (w tym drugiej generacji) lub inhibitorów rozkładu endokannabinoidów. Dalszego wyjaśnienia i potwierdzenia wymaga też wzrost ciśnienia krwi zachodzący w wyniku pobudzenia powszechnie znanych ze swojego działania hipotensyjnego AT_2R i MasR oraz ich wzajemne oddziaływanie z CB_1R.

W swojej pracy wykazałem także, że u zwierząt z nadciśnieniem spontanicznym odpowiedź presyjną indukowana pobudzeniem AT_1R, AT_2R oraz MasR jest silniejsza niż w normotensji, co wynika prawdopodobnie z odmiennego wzrostu gęstości badanych receptorów angiotensynowych i kannabinoidowych w jądrach mózgu kluczowych do regulacji układu krążenia. Istotne byłoby powtórzenie moich badań na innych modelach nadciśnienia, w tym nadciśnieniu indukowanym otyłością, które w dobie pandemii staje się coraz większym problemem.

Biorąc pod uwagę wzrastające użycie dostępnych bez recepty niewystandaryzowanych produktów ze składników otrzymywanych z konopi należy pamiętać o ich potencjalnej interakcji farmakodynamicznej i farmakokinetycznej z lekami działającymi na RAS.

Rozdział 6. Wnioski

1. Mikroiniekcje Ang II, Ang 1-7 i CP55940 (po wcześniejszym dożylnym podaniu antagonisty receptora CB_1 AM251) do jądra przykomorowego podwzgórza (PVN) podnoszą ciśnienie krwi u czuwających szczurów, odpowiednio przez receptory $AT_{1/2}$, Mas i CB_1.
2. Efekty presyjne Ang II, Ang 1-7 i CP55940 po ich podaniu do PVN są silniejsze u szczurów ze spontanicznym nadciśnieniem (SHR) niż u ich normotensyjnej kontroli (WKY), co częściowo może wynikać z wyższej ekspresji receptorów dla AT_1 i CB_1 i niższej dla AT_2 i Mas receptorów w PVN, dogłowowo brzuszno-bocznym obszarze rdzenia przedłużonego (RVLM) i jądrze pasma samotnego (NTS) u SHR niż u WKY.
3. W PVN istnieje interakcja pomiędzy receptorami kannabinoidowymi CB_1 a receptorami dla Ang II i Ang 1-7, odpowiedzialnymi za stymulację odpowiedzi presyjnej.
4. Antagoniści receptorów kannabinoidowych CB_1 modyfikują odpowiedzi stymulowane pobudzeniem receptorów AT_1 dla Ang II w zależności od tego czy pobudzenie obydwu receptorów indukuje efekty skierowane w tę samą czy przeciwną stronę:
   - osłabiają odpowiedź na pobudzenie receptorów AT_1 w przypadku porównywalnych efektów pobudzenia receptorów AT_1 dla Ang II,
   - wzmacniają odpowiedź na pobudzenie receptorów AT_1 w przypadku przeciwstawnych efektów pobudzenia receptorów AT_1 dla Ang II.
5. Przy terapeutycznym stosowaniu związków oddziaływujących na układ (endo)kannabinoidowy i układ renina-angiotensyna należy brać pod uwagę wystąpienie potencjalnej interakcji farmakodynamicznej lub farmakokinetycznej pomiędzy nimi.

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Rozdział 8. Streszczenie w języku polskim

W ostatnich latach pojawia się coraz więcej doniesień dotyczących wzajemnych powiązań między układem endokannabinoidowym (ECS), a układem renina- angiotensyna (RAS), które są zaangażowane między innymi w patofizjologię układu krążenia. Składowe obydwu układów są obecne w jądrze przykomorowym podwzgórza (PVN), które zaangażowane jest m.in. w ośrodkową regulację ciśnienia krwi i częstości akcji serca poprzez modyfikację aktywności układu współczulnego.

Celem mojej pracy było zbadanie potencjalnej interakcji pomiędzy zaangażowanymi w regulację układu krążenia receptorami kannabinoidowymi CB_1 a receptorami AT_1 i AT_2 dla angiotensyny II i receptorami Mas dla angiotensyny 1–7 w PVN szczurów czuwających, a także określenie wpływu nadciśnienia pierwotnego na efekt presyjny stymulowany pobudzeniem powyższych receptorów.

Doświadczenia przeprowadziłem na czuwających samcach szczurów ze spontanicznym nadciśnieniem pierwotnym (SHR) i ich normotensyjnych kontrolach szczurach Wistar Kyoto (WKY). Mikroiniekcja do PVN agonistów receptorów AT_1/AT_2, Mas i CB_1, odpowiednio angiotensyny II (Ang II), angiotensyny 1-7 (Ang 1-7) i CP55940, stymulowały wzrost ciśnienia krwi (BP) wyższy u SHR niż WKY. Najsilniejsze działanie wykazywała Ang II, a najsłabsze Ang 1-7. Antagonista receptorów AT_1 losartan oraz AT_2 PD123319 hamowały odpowiedź presyjną indukowaną przez Ang II i CP55940. Antagonista receptorów Mas A-779 zmniejszały wzrost BP stymulowany Ang 1-7 i CP55940. Antagonista receptorów CB_1 AM251 osłabiał wzrost BP w odpowiedzi na Ang II i Ang 1-7. Efekty presyjne Ang II, Ang 1-7 i CP55940 po ich podaniu do PVN były silniejsze u SHR niż u WKY, co częściowo może wynikać z wyższej ekspresji receptorów dla AT_1 i CB_1 i niższej dla AT_2 i Mas receptorów w PVN, dogłowowo brzuszno-bocznym obszarze rdzenia przedłużonego (RVLM) i jądrze pasma samotnego (NTS) u SHR niż u WKY.

Podsumowując, uzyskane przeze mnie wyniki wykazały istnienie w PVN wyraźniej interakcji pomiędzy zaangażowanymi w indukowanie odpowiedzi presyjnej receptorami AT_1/AT_2 dla Ang II, Mas dla Ang 1-7 oraz CB_1 dla (endo)kannabinoidów. Natomiast, szczegółowy przegląd dostępnej literatury na temat interakcji pomiędzy układami ECB i RAS, pozwolił na wyciągnięcie dwóch dodatkowych wniosków wskazujących na potencjalne znaczenie tej interakcji: (1) antagoniści receptorów kannabinoidowych CB_1 modyfikują efekty pobudzenia receptorów AT_1 dla Ang II

w zależności od tego czy pobudzenie obydwu receptorów indukuje efekty skierowane w tę samą czy przeciwną stronę oraz (2) przy terapeutycznym stosowaniu związków oddziaływujących na układ (endo)kannabinoidowy i układ renina-angiotensyna należy brać pod uwagę wystąpienie potencjalnej interakcji farmakodynamicznej lub farmakokinetycznej pomiędzy nimi.

Streszczenie w języku angielskim

In recent years, there have been more and more reports on the interaction between the endocannabinoid system (ECS) and the renin-angiotensin system (RAS), which are involved, among other things, in the pathophysiology of the cardiovascular system. Components of both are present in the paraventricular nucleus of the hypothalamus (PVN), which takes part in the central regulation of blood pressure and heart rate by modifying the activity of the sympathetic nervous system.

The aim of my study was to investigate the potential interaction between involved in cardiovascular regulation cannabinoid CB_1 receptors and AT_1 and AT_2 receptors for angiotensin II and Mas receptors for angiotensin 1-7 in the PVN of conscious rats, and to determine the effect of primary hypertension on the pressure effect stimulated by activation of the above receptors.

I performed the experiments on conscious male rats with spontaneous primary hypertension (SHR) and their normotensive controls Wistar Kyoto (WKY) rats. Microinjection of the AT_1/AT_2, Mas, and CB_1 receptor agonists angiotensin II (Ang II), angiotensin 1-7 (Ang 1-7), and CP55940 into the PVN, respectively, stimulated an increase in blood pressure (BP) higher in SHR than in WKY. Ang II had the strongest effect while Ang 1-7 had the weakest effect. AT_1 receptor antagonist losartan and AT_2 receptor antagonist PD123319 inhibited the pressure response induced by Ang II and CP55940. The Mas receptor antagonist A-779 decreased the BP elevation stimulated by Ang 1-7 and CP55940. The CB_1 receptor antagonist AM251 attenuated the increase in BP in response to Ang II and Ang 1-7. The pressor effects of Ang II, Ang 1-7, and CP55940 after their administration into the PVN were stronger in SHR than in WKY, which may in part be due to the higher expression of AT_1 and CB_1 receptors and lower expression for AT_2 and Mas receptors in the PVN, rostral ventrolateral medulla (RVLM) and the nucleus tractus solitarii (NTS) in SHR than in WKY.

In summary, my results demonstrated the existence of a more pronounced interaction in the PVN between the AT_1/AT_2 receptors for Ang II, Mas for Ang 1-7, and CB_1 for (endo)cannabinoids involved in inducing the pressure response. In contrast, a detailed review of the available literature on the interaction between the ECB and RAS systems allowed us to draw two additional conclusions indicating the potential importance of this interaction: (1) cannabinoid CB_1 receptor antagonists modify the effects of AT_1 receptor

stimulation for Ang II depending on whether stimulation of both receptors induces effects directed in the same or opposite direction, and (2) the occurrence of a potential pharmacodynamic or pharmacokinetic interaction between them should be considered when compounds affecting the (endo)cannabinoid system and the renin-angiotensin system are used therapeutically.

Rozdział 9.

Article

Cross-Talk between CB_1, AT_1, AT_2 and Mas Receptors Responsible for Blood Pressure Control in the Paraventricular Nucleus of Hypothalamus in Conscious Spontaneously Hypertensive Rats and Their Normotensive Controls

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Abstract: We have previously shown that in urethane-anaesthetized rats, intravenous injection of the angiotensin II (Ang II) AT_1 receptor antagonist losartan reversed the pressor effect of the cannabinoid CB_1 receptor agonist CP55940 given into the paraventricular nucleus of hypothalamus (PVN). The aim of our study was to determine the potential interactions in the PVN between CB_1 receptors and AT_1 and AT_2 receptors for Ang II and Mas receptors for Ang 1-7 in blood pressure regulation in conscious spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY) rats. The pressor effects of Ang II, Ang 1-7 and CP55940 microinjected into the PVN were stronger in SHRs than in WKYs. Increases in blood pressure in response to Ang II were strongly inhibited by antagonists of AT_1 (losartan), AT_2 (PD123319) and CB_1 (AM251) receptors, to Ang 1-7 by a Mas antagonist (A-779) and AM251 and to CP55940 by losartan, PD123319 and A-779. Higher (AT_1 and CB_1) and lower (AT_2 and Mas) receptor expression in the PVN of SHR compared to WKY may partially explain the above differences. In conclusion, blood pressure control in the PVN depends on mutual interaction of CB_1, AT_1, AT_2 and Mas receptors in conscious spontaneously hypertensive rats and their normotensive controls.

Keywords: angiotensin II; angiotensin 1-7; AT_1 receptor; AT_2 receptor; blood pressure; cannabinoid CB_1 receptor; CP55940; Mas receptor; paraventricular nucleus of hypothalamus; spontaneously hypertensive rats

1. Introduction

Hypertension is one of the main risk factors for cardiovascular diseases but in ~90-95% of the patients with so-called primary, essential, or idiopathic hypertension its specific etiology is unclear [1] Genetic and environmental factors and alterations of peripheral and central sites involved in blood pressure (BP) regulation are being considered [2,3].

For central regulation of cardiovascular functions, brain stem areas like the rostral ventrolateral medulla (RVLM) and the nucleus tractus solitarii (NTS) and the hypothalamic paraventricular nucleus (PVN) play an important role [1,4]. The PVN is a main source of stimulation for sympathetic outflow that is finely tuned by both sympathoexcitatory (glutamate) and sympathoinhibitory (γ-aminobutyric acid; GABA) transmitters. The hypothalamic balance of inhibitory and excitatory synaptic inputs is impaired in primary hypertension [1,4].

The PVN contains all components of the renin-angiotensin system (RAS), including angiotensin II (Ang II), angiotensin 1-7 (Ang 1-7) and the receptors of Ang II (AT_1, AT_2) and Ang 1-7 (Mas), which are key modulators in the cardiovascular function [5–7]. Ang II is regarded as one of the sympathoexcitatory transmitters in the PVN [1]. Two classes of RAS blockers, i.e. angiotensin-converting enzyme (ACE) inhibitors (e.g. enalapril) and AT_1 receptor antagonists (e.g. losartan), serve as first-line drugs for the treatment of essential hypertension according to the guidelines of American [3] and European [8] heart societies.

The following facts confirm the involvement of Ang II in the PVN in the pathophysiology of hypertension: (1) in the human PVN, expression of prorenin in hypertension positively correlated with the level of blood pressure [9]; (2) Ang II microinjected into the PVN induced stronger pressor responses and/or increases in sympathetic activity in renovascular hypertensive rats than in their normotensive controls [10,11]; (3) a higher expression of AT_1 receptors (AT_1Rs) was found in the PVN of renovascular and spontaneously hypertensive rats (SHR) [11–13] than in normotensive animals and (4) BP decreased in SHR but not in Wistar rats in response to AT_{1a} receptor gene silencing [14].

Ang 1-7 is mainly known for its vasodilatory/hypotensive responses (for review, see [5,7]), however, its central administration (including intra-PVN injections) increases BP [5,11]. Pressor responses were enhanced and concomitantly a higher density of Mas receptors (MasRs) in the PVN was observed in renovascular hypertensive rats compared to their normotensive controls [11,15]. The MasR antagonist A-779 microinjected into the PVN reduced BP and sympathetic activity in high salt hypertensive rats but not in their controls [16].

Cannabinoids like the phytocannabinoid Δ^9-tetrahydrocannabinol (THC), the synthetic cannabinoid CP55940 or the endocannabinoid anandamide (AEA) exert complex effects on the cardiovascular system that are influenced by hypertension. Intravenous (i.v.) injection mainly decreases BP in anaesthetized rats but increases it in conscious animals (for review, see [17,18]). We have shown previously that microinjection of methanandamide (MethAEA; the stable analogue of AEA) and CP55940 into the PVN led to a cannabinoid CB_1 receptor (CB_1R)-mediated hypotension in urethane-anaesthetized rats; this hypotension

was, however, reversed into a pressor effect by the CB_1R antagonist AM251 when administered via the i.v. route [19].

Two pieces of evidence confirm the existence of an interaction between Ang II and CB_1Rs in the PVN of urethane anaesthetized normotensive rats. Firstly, in our previous work [20], the AT_1R antagonist losartan i.v. not only inhibited the pressor effect of CP55940 (plus AM251 i.v.) administered into the PVN but even reversed it into a hypotensive one. Secondly, Gyombolai et al. [21] showed, that AM251 reduced the increase in BP elicited by microinjection of Ang II (both compounds were given to the PVN). Moreover, the enhancement of CB_1 and CB_2 receptor expression in the rat uterus in response to local Ang 1-7 infusion [22] indicates a potential interaction between CB receptors and Ang 1-7.

The aim of our study on conscious rats was to determine the interactions in the PVN between cannabinoid CB_1Rs with AT_1 and AT_2 receptors for Ang II and Mas receptors for Ang 1-7 involved in blood pressure regulation. SHR, which represent a model of essential hypertension in humans and in which most antihypertensive drugs are active [23], were compared to their normotensive controls, Wistar Kyoto rats (WKY).

2. Materials and Methods

2.1. Animals

Male spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats, weighing 250–300 g, were purchased from the Center for Experimental Medicine of the Medical University of Białystok (Poland). After an acclimatization period of three days, each SHR and WKY rat was assigned randomly to one of the experimental groups. All surgical procedures and experimental protocols were executed in accordance with European Directive (2010/63/EU) and Polish legislation and were approved by the Local Animal Ethics Committee in Olsztyn (Poland; approval code: 77/2019; approval date: 29 October 2019). The study was performed following the principles of replacement, refinement or reduction (the 3Rs). Rats were housed at constant humidity (55 ± 5%) and temperature (22 ± 1 °C) and were kept under a 12/12 h light/dark cycle. Animals had free access to standard pelleted rat chow and tap water.

2.2. Placement of Cannula for PVN Microinjection

Rats were anaesthetized intraperitoneally (i.p.) with pentobarbitone sodium (300 μmol/kg). To access the PVN, each rat was placed in a stereotaxic frame (World Precision Instruments; Sarasota, FL, USA). The stereotaxic coordinates for the PVN, according to the Paxinos and Watson rat brain atlas [24] were as follows: 1.8 mm caudal from bregma, 0.5 mm lateral (right) to the midline, and 8.0 mm below the skull surface. Two 1 mm holes were drilled into the skull, one using the above-mentioned coordinates, for guide cannula implantation. The cannula had an outer (OD) and inner (ID) diameter of 0.5 and 0.3 mm, respectively, and a length of 7.5 mm (therefore, its lower end was 0.5 mm above the injection site, thus preventing potential damage of the PVN). The second hole was drilled few millimeters besides, for placing a 1 mm metal screw for better fixing the cannula in place with dental cement. The cannula was then plugged with a 30 G dummy wire cannula.

2.3. Placement of Catheters for BP Measurements and Injections

Right after cannulation, under the same anaesthesia, animals were placed on a heated table. One cm incisions were made between the scapulae, and on the neck and groin area. Carotid artery, and femoral vein were exposed and separated from adjacent tissues. Next, polyurethane catheters (1 mm OD, 0.63 mm ID), filled with heparinized saline, were inserted into carotid artery and femoral vein, and their extravascular parts were tunneled under the skin and exteriorized on the neck. Immediately after insertion, the catheter was flushed with heparinized saline (100 units/ml) in order to prevent formation of a blood clot. Rats were then returned to their individual cages and allowed to recover for 3 days. During that time, catheters were flushed daily with heparinized saline to ensure their patency. During surgery animals received buprenorphine (0.05 mg/kg subcutaneously) and all surgical sites were disinfected with betadine (100 mg/ml) before making any incisions. During the recovery period, rats were given paracetamol (100-300 mg/kg/day) in the drinking water.

2.4. Microinjections

All PVN microinjections were performed on freely moving, conscious rats in a volume of 200 nl and completed in ten seconds. The injection cannula was longer by 0.5 mm than the guide cannula to make sure that the compounds were delivered to the correct site. When multiple injections were administered, the next injection was only given when the BP had returned to the baseline level. At the end of the experiment, 100 nl of 2% Evans Blue was injected into the microinjection site for histological identification of the PVN with an optical microscope. Only animals with the microinjection site within the confines of the PVN were included in the data analysis. A representative photo of the microinjection site evaluated by Evans Blue is shown in Figure 1.

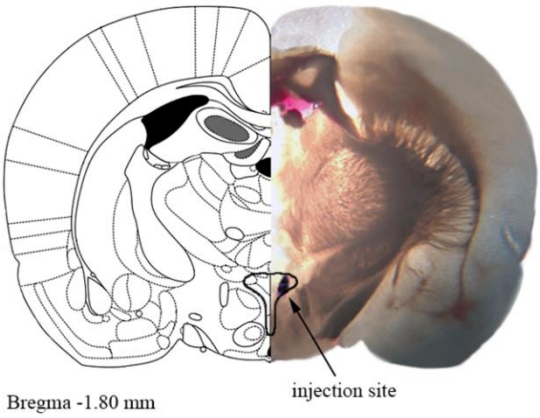

Figure 1. A representative photograph of the microinjection site in the paraventricular nucleus of hypothalamus (PVN), coupled with the matching slide from the Paxinos and Watson rat brain atlas [24]. One mm thick brain slice with the injection site shown by Evans blue dye. The drawn outline represents the confines of the PVN.

2.5. Experimental Protocol

Each rat received two unilateral microinjections into the PVN (S1 and S2). The agonists Ang II, Ang 1-7 or CP55940 were administered into the PVN once (S2) at the following doses: 0.3 nmol/rat [10,11], 0.3

nmol/rat [11] and 0.1 nmol/rat [20], respectively. Antagonists of AT_1 (losartan, 20 nmol/rat; [25]), AT_2 (PD123319, 10 nmol/rat; [25]), Mas (A-779, 3 nmol/rat; [11]) and CB_1 (AM251, 30 nmol/rat, [19]) receptors were injected into the PVN 5 min before the respective agonists (or after BP had returned to preinjection values; S1). Control groups received the respective vehicle for the particular receptor ligands. All experiments with CP55940 were performed in the presence of AM251 (3 μmol/kg) given i.v. (into the femoral vein in a volume of 0.5 mL/kg) in order to prevent the CP55940-induced hypotension (according to [20]). Intravenous injection of AM251 induced a long-lasting pressor effect (for details, see Results) and the microinjection of the respective receptor antagonists into the PVN took place after return of basal BP to preinjection values.

Cardiac parameters were recorded from the right carotid artery of unrestrained conscious rats by a pressure transducer system (model MLT844, ADInstruments, Sydney, Australia) with computer data acquisition (Bridge Amp/PowerLab 4/35, ADInstruments, Sydney, Australia). We did not analyze heart rate since the changes induced by the particular ligands were too small and showed a big scatter of variation.

2.6. PVN tissue Microdissection

At the end of the experiment, rat brains were sectioned into 1 mm slices using a rat brain matrix (Zivic Instruments, Pittsburgh, PA, USA) at the appropriate coordinates taken from the Paxinos and Watson rat brain atlas [24] for PVN, RVLM, and NTS. Tissues were then isolated by the use of a punch-out technique, flash-frozen in liquid nitrogen, and stored at −80 °C until analyzed by Western blotting.

2.7. Western blot Analysis

Routine Western blotting procedures were used. Briefly, samples of PVN, RVLM, and NTS from each rat were homogenized in ice-cold M-PER lysis buffer (Thermo Scientific, Rockford, IL, USA) containing a cocktail of protease inhibitors (Roche Diagnostics GmbH, Mannheim, Germany). Total protein concentration was determined using the bicinchoninic acid method (BCA) (Pierce Rapid Gold BCA Protein Assay Kit, Thermo Fisher Scientific, Waltham, MA, USA) with bovine serum albumin as a standard. Next, homogenates were reconstituted in Laemmli buffer with β-mercaptoethanol, separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were incubated with the primary antibodies of interest (anti-CB_1R, ab259323; anti-AT_2R, ab92445; anti-MasR, ab200685; Abcam, Cambridge, UK) followed by incubation with appropriate secondary antibodies conjugated with horseradish peroxidase (ab6721, Abcam, Cambridge, UK). Protein bands were visualized using an enhanced chemiluminescence substrate (Thermo Scientific, Rockford, IL, USA) and quantified using ImageJ software (v1.53f51). Protein expression was standardized to β-actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH), depending on the molecular weight of the specific antibodies.

2.8. Data Analysis

Results are given as means ± SEM; n refers to the number of rats. In order to quantify the effects of antagonists on the cardiovascular

effects of Ang II, Ang 1-7 or CP55940, the agonist-induced maximal change in BP was calculated from the respective basal systolic, diastolic and mean BP (SBP, DBP and MBP) averaged over the 5 minutes before injection of the particular agonist. This procedure was chosen to minimize the influence of inter-subject variability on final data. For comparison of the mean values between the WKY and SHR group, the t-test for unpaired data was used. When two groups were compared with the same control, one-way analysis of variance (ANOVA) followed by the Dunnett post hoc test was used. Differences were considered as significant when $p < 0.05$. Statistical analysis was performed using Graph Pad Prism 5 (GraphPad Software, La Jolla, CA, USA).

2.9. Drugs

A-779 (5-L-isoleucine-7-D-alanine-1-7-angiotensin II, trifluoroacetate salt); AM251 [(N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide)]; angiotensin 1-7; angiotensin II (Tocris Bioscience, Bristol, UK); betadine (Egis Pharmaceuticals PLC, Budapest, Hungary); buprenorphine (Richter Pharma AG, Wels, Austria); CP55940 [(−)-cis-3-[2-hydroxy4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol] (Sigma Aldrich, St. Louis, MO, USA); losartan potassium (Tocris Bioscience, Bristol, UK); paracetamol (Sequoia, Warsaw, Poland); PD123319 ((6S)-1-[[4-(dimethylamino)-3-methylphenyl]methyl]5-(2,2-diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid, di(2,2,2-trifluoroacetate)) (Sigma Aldrich, St. Louis, MO, USA); pentobarbitone sodium (Biowet, Puławy, Poland).

Drugs were dissolved in saline with the following exceptions. AM251 was dissolved in a mixture of ethanol, Cremophor El, DMSO and saline (1:1:1:9.5) for i.v. injections and in DMSO and saline (1:9) for PVN injections. CP55940 was dissolved in a 19% solution of cyclodextrin. None of the used vehicles significantly altered any of the cardiovascular parameters by itself (data not shown).

3. Results

3.1. General

Basal systolic (SBP), diastolic (DBP) and mean (MBP) blood pressure, measured immediately before the administration of the antagonists (S_1) into the PVN, was significantly higher in spontaneously hypertensive rats (200 ± 4, 168 ± 3 and 184 ± 3 mmHg, respectively; $n = 80$; $p < 0.001$) than in normotensive Wistar Kyoto rats (129 ± 2, 105 ± 3, 117 ± 2 mmHg, respectively; $n = 80$). The CB_1R antagonist AM251 given i.v. (3 μmol/kg) and into the PVN (30 nmol/rat) increased BP by about 15-20 mmHg and 5-10 mmHg, respectively (Figures 2a, b). The AM251-induced pressor effects lasted for ~8-15 and ~5-7 min after its application i.v. and into the PVN, respectively. The increases in BP elicited by i.v. AM251 injection were slightly higher in SHR in comparison to WKY although a significant difference was obtained for MBP only. The AT_1 receptor antagonist losartan 20 nmol/rat and the AT_2 receptor antagonist PD123319 10 nmol/rat, respectively, microinjected into the PVN did not modify basal BP by themselves (results not shown). Interestingly, as shown in Figures 2c, d, the MasR antagonist A-779 (3 nmol/rat) given into the PVN did not have any effect on BP in the WKY or SHR group by itself.

However, after blockade of the CB_1Rs with AM251 i.v. it decreased SBP, DBP and MBP by about 5-10 mmHg both in WKY and in SHR.

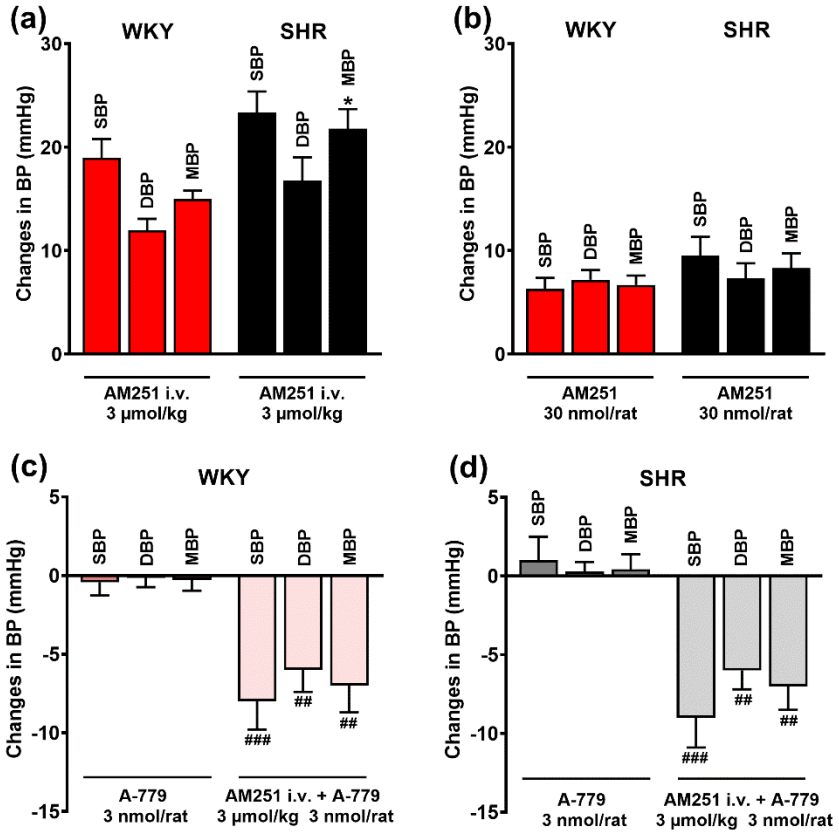

Figure 2. Effects of AM251 and A-779 on basal systolic (SBP), diastolic (DBP) and mean (MBP) blood pressure in conscious Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). The CB_1 receptor antagonist AM251 was injected intravenously (**a**) or into the paraventricular nucleus of hypothalamus (PVN) (**b**). The Mas receptor antagonist A-779 was injected into the PVN. Results are presented as mean ± SEM (n = 21-27 for A; n = 10 for B; n = 6-7 for (**c**) and (**d**). *p < 0.05 compared to WKY; ##p < 0.01, ###p <0.001 compared to A-779 in the absence of AM251.

3.2. Involvement of AT_1, AT_2 and Mas Receptors in the Pressor Responses of Ang II and Ang 1-7 Microinjected into the PVN

The AT_1 and AT_2 receptor agonist Ang II 0.3 nmol/rat, the MasR agonist Ang 1-7 0.3 nmol/rat and the CB_1R agonist CP55940 0.1 nmol/rat (given in the presence of AM251 3 µmol/kg, i.v.) microinjected into the PVN enhanced SBP, DBP and MBP both in WKY and in SHR (for original tracings, see Figure 3). In WKY Ang II induced comparable increases in SBP, DBP and MBP by about 20 mmHg (Figure 4a). They were significantly higher in SHR by about 60%, 40% and 50%, respectively (Figure 4b). All pressor effects of Ang II both in WKY (Figure 4a) and in SHR (Figure 4b) were almost completely blocked by the previous injection of the AT_1R antagonist losartan (20 nmol/rat) into the PVN and inhibited by ~75% by the AT_2R antagonist PD123319 (10 nmol/rat).

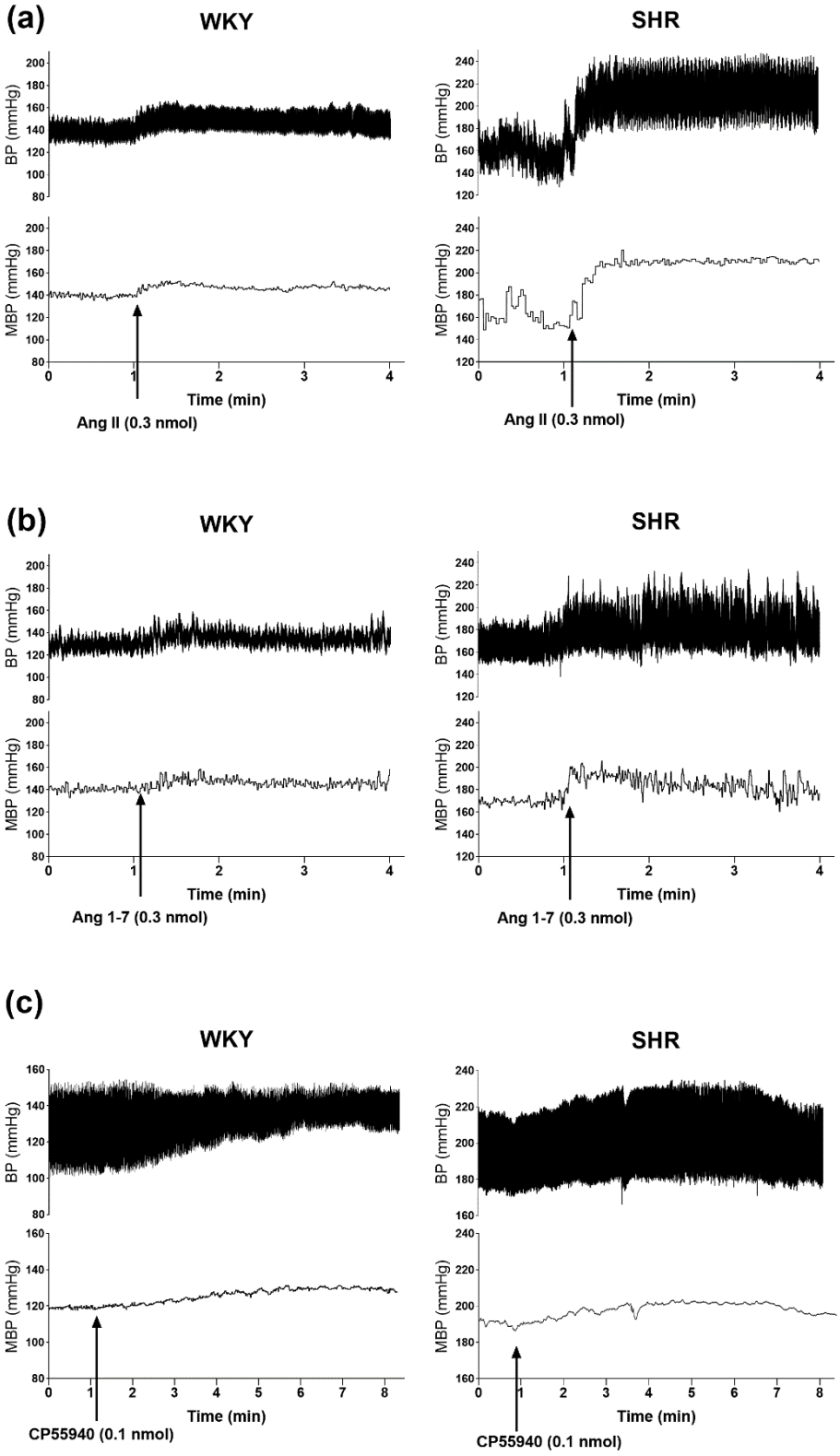

Figure 3. Representative original traces of the pressor effects of angiotensin II (**a**), angiotensin 1-7 (**b**) and CP55940 (after i.v. administration of AM251) (**c**) injected into the paraventricular nucleus of hypothalamus (PVN) on blood pressure (BP) and mean blood pressure (MBP) in conscious Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). Arrows show the moment of application of the particular agonist.

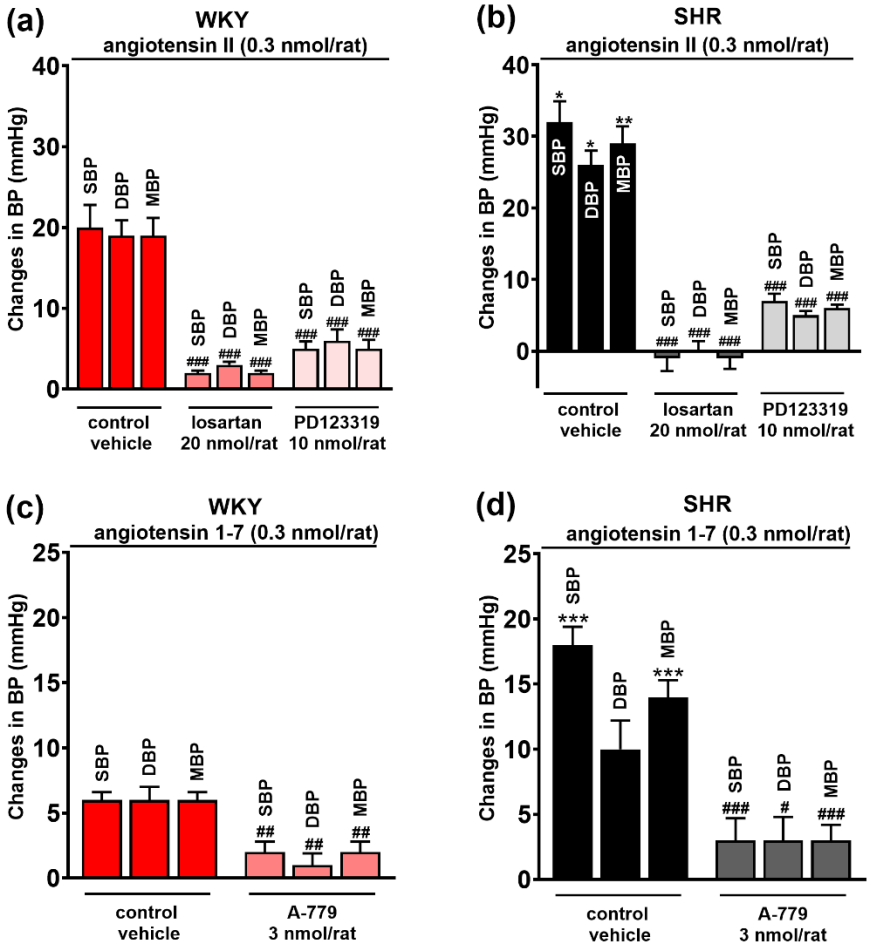

Figure 4. Effect of angiotensin II ($AT_{1/2}$ receptor agonist) (**a**,**b**) and angiotensin 1-7 (Mas receptor agonist) (**c**,**d**) on systolic (SBP), diastolic (DBP) and mean (MBP) blood pressure, and their interaction with AT_1, AT_2 and Mas receptor antagonists (losartan, PD123319 and A-779, respectively) injected into the paraventricular nucleus of hypothalamus (PVN) in conscious Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). Results are presented as mean ± SEM (n = 7-8) and calculated as change in basal values determined before agonist injection. #p < 0.05, ##p < 0.01, ###p < 0.001 compared to the corresponding control group; *p < 0.05, **p < 0.01, ***p < 0.001 compared to WKY.

Compared to Ang II, Ang 1-7 increased BP to a lesser extent both in WKY and SHR (Figures 4c, d). In WKY, SBP, DBP and MBP increased by ~6 mmHg, while SHR showed significantly higher values in SBP (by ~200%) and MBP (by ~100%) compared to WKY; the increase in DBP (by ~50%) did not reach statistical significance. The pressor effects of Ang 1-7 both in WKY and in SHR were diminished by the previous injection of the MasR antagonist A-779 (20 nmol/rat) by ~75% (Figures 4c, d).

3.3. Involvement of CB_1 Receptors in the Pressor Effects of Ang II and Ang 1-7 Microinjected into the PVN

As shown in Figure 5, the CB_1R antagonist AM251 injected into the PVN before Ang II, and Ang 1-7 given also into PVN, modulated their pressor responses. AM251 significantly lowered the rise in MBP induced by Ang II and tended to cause lower Ang II-induced increases

in DBP and MBP in WKY (Figure 5a). In SHR, AM251 reduced the Ang II-induced increase in SBP, DBP and MBP by 55%, 85% and 65%, respectively (Figure 5b). Interestingly enough, the pressor effects of Ang 1-7 (SBP, DBP and MBP) were not only reduced by AM251, but even reversed into hypotensive responses both in WKY (Figure 5c) and SHR (Figure 5d).

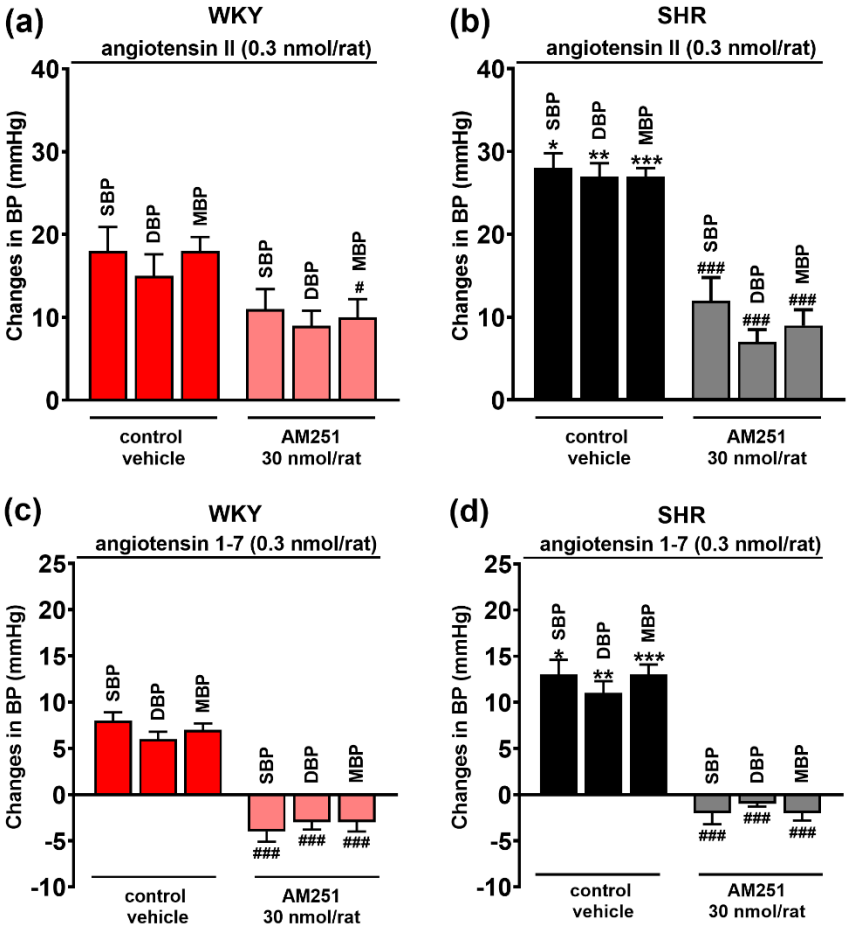

Figure 5. Influence of the CB_1 receptor antagonist AM251 on the pressor effect of angiotensin II ($AT_{1/2}$ receptor agonist) (**a**, **b**) and angiotensin 1-7 (Mas receptor agonist) (**c**, **d**) injected into the paraventricular nucleus of hypothalamus (PVN) in conscious Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). Results, presented as mean ± SEM (n = 7–8), are calculated as change in basal values determined before agonist/antagonist injection. #p < 0.05, ###p < 0.001 compared to the corresponding control group. *p < 0.05, **p < 0.01, ***p < 0.001 compared to WKY.

3.4. Involvement of AT_1, AT_2 and Mas Receptors in the Pressor Effect of the CB_1 Receptor Agonist CP55940 Microinjected into the PVN

Like in our previous study [20], CP55940 increased BP subsequent to i.v. injection of the CB_1R antagonist AM251. In the present experiments CP55940 increased SBP, DBP and MBP by ~8-15 mmHg in WKY (Figure 6a) and by ~15-18 mmHg in SHR (Figure 6b); the difference was statistically significant for SBP. All antagonists under study, losartan (AT_1R), PD123319 (AT_2R) and A-779 (MasR) significantly reduced the pressor responses to CP55940 both in

normotensive (Figure 6a,c) and hypertensive (Figure 6b,d) rats; in SHR, the reduction ranged from ~50% (PD123319 for SHR) to ~85% (A-779 for SHR).

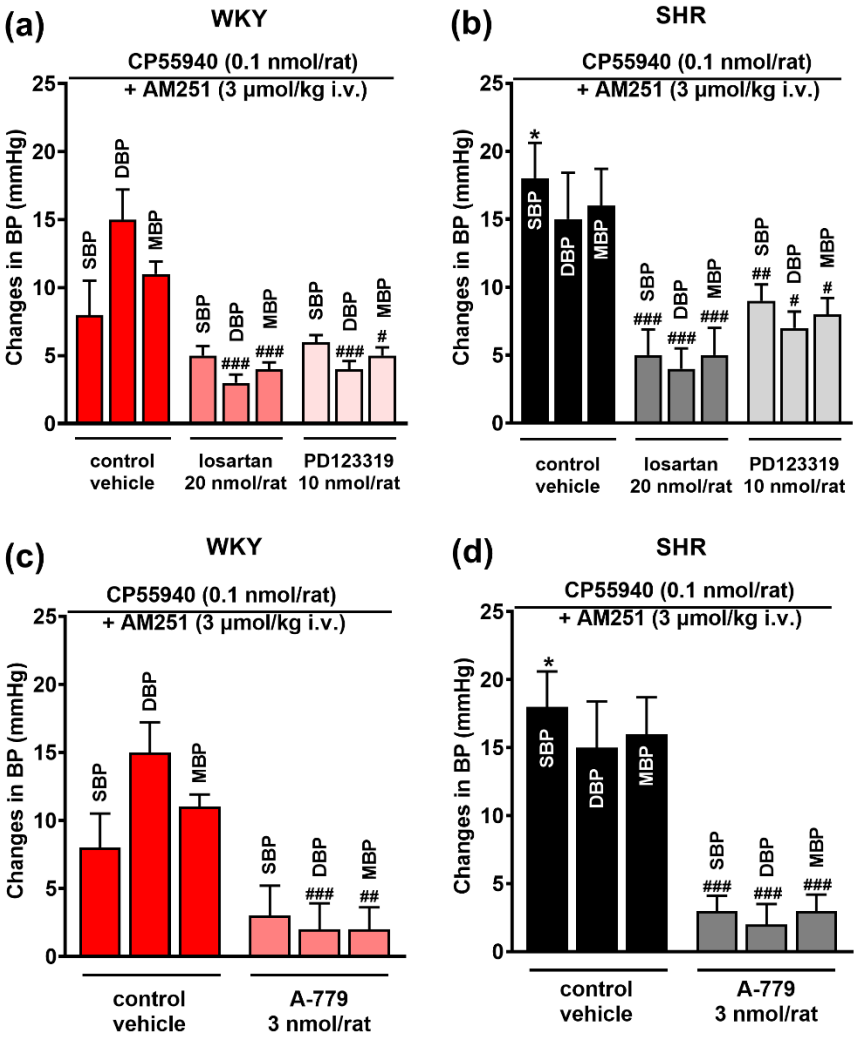

Figure 6. Influence of AT_1 and AT_2 (losartan and PD123319; (**a**,**b**), and Mas (A-779; (**c**,**d**) receptor antagonists on the pressor effect of the CB_1 receptor agonist CP55940 injected into the paraventricular nucleus of hypothalamus (PVN) in conscious Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). All experiments were performed after i.v. injection of the CB_1 receptor antagonist AM251. Results, presented as mean ± SEM (n = 5-7), are calculated as the change in basal values determined before agonist injection. #p < 0.05, ##p < 0.01, ###p < 0.001 compared to the corresponding control group; *p < 0.05 compared to WKY.

3.5. Comparison of Cannabinoid CB_1, Mas, and AT_2 Receptor Expression in the PVN, RVLM and NTS of WKY and SHR rats

As shown in Figure 7a, Western blot analysis revealed an about twofold higher expression of CB_1Rs in the PVN and RVLM of SHR, compared to their normotensive controls (WKY); there were no differences in the NTS. By contrast, the density of AT_2 and Mas receptors was altered in an opposite direction; this held also true for the NTS (Figures 7b, c). AT_2R expression was about 2-4-fold and Mas receptor expression about 1.5-2-fold higher for WKY than for SHR.

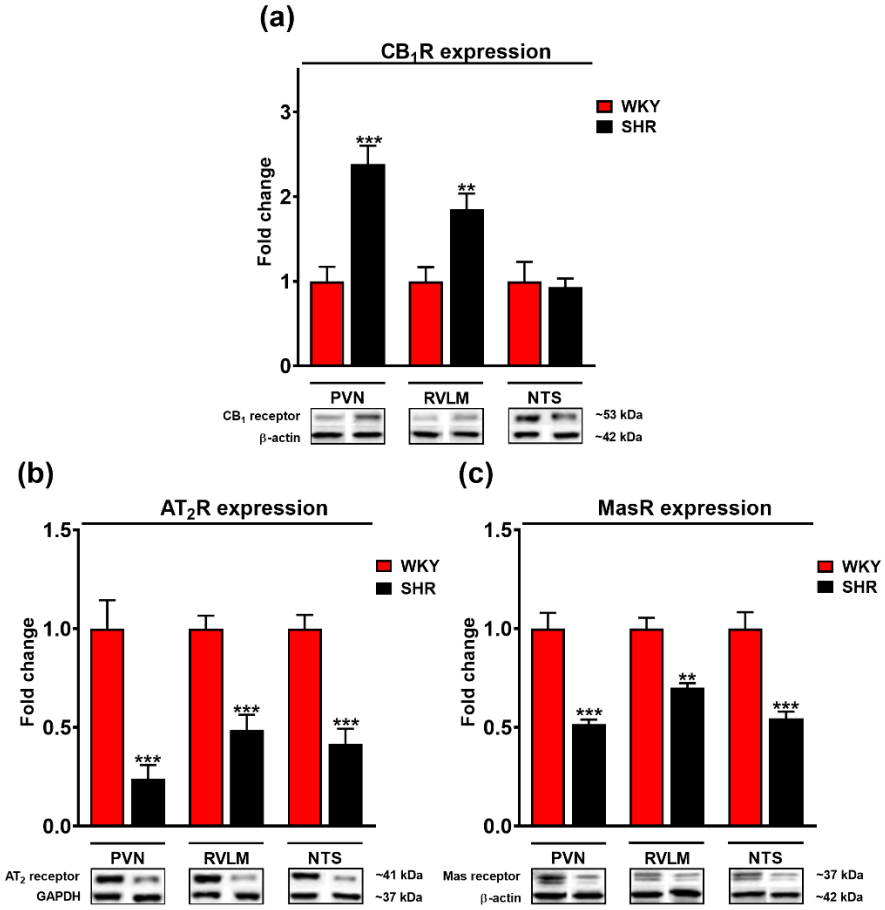

Figure 7. Fold change of cannabinoid CB_1 (**a**), AT_2 (**b**) and Mas (**c**) receptors and representative Western blots of the paraventricular nucleus of hypothalamus (PVN), rostral ventrolateral medulla (RVLM) and nucleus tractus solitarii (NTS) in Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). β-actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as loading control. Results presented as mean ± SEM (n = 6). **p < 0.01, ***p < 0.001 compared to WKY.

4. Discussion

In the present study, we identified interactions between cannabinoid CB_1Rs and $AT_{1/2}$ receptors for Ang II and MasRs for Ang 1-7 in the PVN involved in blood pressure regulation. The experiments were performed on conscious rats and revealed quantitative differences between SHR and WKY. Conscious rats were used since anaesthesia reverses the BP response to cannabinoids (e.g. THC, AEA or CP55940) to prolonged hypotension (for review, see [17,18]). We used the $CB_{1/2}$ receptor agonist CP55940 [26] since the effect of this drug microinjected into the PVN was reduced by the microinjection of the CB_1R antagonist AM251 but not the CB_2R antagonist SR144528 into the PVN [19]. We restricted ourselves to pressor responses since in our previous experiments the fall in BP elicited by microinjection of CP55940 into the PVN was only reduced by AM251 but not by antagonists of other receptors including AT_1Rs [20]. CP55940 was

routinely examined after the previous i.v. administration of AM251 that prevents the hypotensive response to CP55940 [19,20].

Ang II (0.3 nmol/rat), Ang 1-7 (0.3 nmol/rat) and CP55940 (0.1 nmol/rat; after i.v. administration of AM251) microinjected into the PVN increased SBP, DBP and MBP in conscious WKY and SHR. The strongest pressor response was elicited by Ang II and the weakest one by Ang 1-7 (by ~20 and ~6 mmHg in WKY, respectively). Sun et al. [11] showed that the same doses of Ang II and Ang 1-7, given into the PVN, induced comparable increases in MBP in conscious male Sprague-Dawley rats. CP55940 given into the PVN (after i.v. administration of AM251) increased BP both in conscious (current study) and urethane-anaesthetized rats [19,20]. By contrast, as mentioned above, i.v. administration of cannabinoids mainly increased BP in conscious but decreased it in anaesthetized animals [17] suggesting that central mechanisms are responsible for the pressor effects of cannabinoids.

The pressor responses to Ang II and Ang 1-7 result from the activation of AT_1/AT_2 receptors and Mas receptors, since they were almost prevented or strongly inhibited by the respective antagonists losartan/PD123319 and A-779 (given into the PVN). AT_1Rs in the PVN are well known to mediate the pressor response to Ang II. By contrast, central AT_2Rs are rather known to counteract the effects of Ang II (for review, see [27]). As in our study, not only losartan but also PD123319 strongly reduced the Ang II-induced increases in BP in the PVN of anaesthetized rats [28,29]. Sun et al. [11] showed that losartan inhibited the pressor effects induced by Ang II but not by Ang 1-7, which were reduced by A-779, confirming the involvement of AT_1- and MasRs in the increases in BP elicited by Ang II and Ang 1-7, respectively. As mentioned above, the involvement of CB_1Rs in the pressor effect of CP55940 after its administration into the PVN has been proven in our previous study [19].

To the best of our knowledge, we are the first to demonstrate that the pressor responses to Ang II, Ang 1-7 and CP55940 were significantly higher (or tended to be higher) in SHR than in WKY. Higher increases in BP induced by Ang II [10,11] and Ang 1-7 [11,15] have so far been shown in renovascular hypertensive rats only. Increases/decreases in blood pressure in response to a given ligand depend on the value of the basal BP. Thus, we cannot exclude the possibility that the higher responses observed in SHR were related to higher basal BP. However, we would like to underline that the higher pressor effects in SHR, when compared to WKY, may result from different densities of the particular receptors in the brain regions responsible for cardiovascular system regulation. The balance of excitatory and inhibitory synaptic inputs depending on glutamatergic and GABAergic transmission is responsible for the final integration of the sympathetic outflow by the PVN [1,4,27]. In the PVN, facilitatory AT_1Rs and AT_2Rs are expressed primarily on glutamatergic and GABAergic neurons, respectively. In addition, AT_1Rs diminish GABAergic input [13,27,30,31]. Presynaptic inhibitory CB_1Rs are localized both on glutamatergic and GABAergic synapses [32]. However, we have provided evidence previously that CP55940 increased BP acting predominantly at inhibitory presynaptic CB_1Rs on GABAergic neurons [20]. Both an enhancement of sympathoexcitatory and a reduction of sympathoinhibitory inputs might lead to increases in BP.

Compared to WKY, the PVN of SHR has higher levels of AT_1 [12,13] and CB_1 (current study) receptors. AT_1 and CB_1 receptor expression are also higher in SHR than WKY in other brain regions, crucial for cardiovascular regulation, like RVLM and NTS in which similarly to the PVN glutamate and GABA interact to modulate BP (for AT_1Rs - [12,33]; for CB_1Rs - current study). A higher level of CB_1Rs but not of endocannabinoids in the RVLM of SHR has been described previously by Wang et al. [34]. To summarize, enhanced AT_1R (stimulation of glutamatergic neurons) and CB_1R (inhibition of GABAergic neurons) densities in the PVN of SHR might explain the higher BP response to Ang II and CP55940 in SHR than in WKY (Figure 8).

With respect to AT_2 and Mas receptors, the Western blotting analysis showed a significantly higher expression in WKY than SHR in each of the three brain regions, PVN, RVLM and NTS. AT_2 and Mas receptors are known to counteract the pressor effect of AT_1R stimulation by enhancement of GABAergic input, directly by a facilitatory effect on GABAergic neurotransmission (AT_2Rs are located predominantly on GABAergic neurons; [27]) or indirectly via nitric oxide stimulating GABA release determined in the PVN both for AT_2 [35] and Mas [36] receptors. Thus, the decrease of AT_2 and Mas receptor expression and of the sympathoinhibitory input observed in our study might contribute to the higher level of BP in SHR. Of course one should keep in mind that a change in receptor expression does not always correlate to functionality and our results require confirmation by studies dedicated to the downstream pathways regarding specific receptor activation. Interestingly, the density of MasRs in the PVN was higher in renal hypertensive rats (2K1C) compared to their normotensive controls [15]. However, the question arises how can we explain the pressor effects of Ang 1-7 and the involvement of AT_2Rs in the increase in BP induced by Ang II? The enhancement of BP elicited by Ang 1-7 has so far been explained by the production of reactive oxygen species (ROS; [16]) and the pressor response induced by AT_2Rs is probably mediated via vasopressinergic neurons in the PVN [37], since the increases in BP and HR induced by vasopressin microinjected into the PVN of anaesthetized rats were partially attenuated by the AT_1R antagonist losartan and strongly reduced by the AT_2R antagonist PD123319 [37].

The following facts confirm the existence of an interaction between cannabinoid CB_1Rs, AT_1 and AT_2 receptors for Ang II and MasRs for Ang 1-7 in the PVN. Firstly, the CB_1R antagonist AM251 given into the PVN reduced or tended to diminish the pressor response to Ang II both in WKY and SHR and even reversed the increases in BP elicited by Ang 1-7 to hypotensive responses. An inhibitory effect of AM251 on the pressor response to Ang II (both given into the PVN) was previously shown by Gyombolai et al. [21]. Secondly, the pressor response to CP55940 was strongly reduced by previous topical application of the AT_1, AT_2 and Mas receptor antagonists losartan, PD123319 and A-779, respectively. In our previous papers, losartan i.v. did not modify the cardiovascular response to AEA i.v. (including its pressor phase; [38]) and the hypotensive and bradycardic responses to CP55940 microinjected into the PVN. On the other hand, it even reversed the increase in BP and HR induced by this latter compound given into the PVN to a fall in BP and HR [20]. In the current study, losartan did not

reverse the pressor response to CP55940 into a fall in BP, probably since it was given locally into the PVN in contrast to i.v. injection in the previous paper [20]. Thirdly, topically administered A-779 decreased BP by itself to a comparable degree both in WKY and SHR but only after the previous i.v. administration of AM251. By contrast, losartan and PD123319 failed to modify BP both in the absence or presence of AM251 i.v. Thus, it seems, that endocannabinoids acting on CB_1 receptors in the PVN interact with the endogenous pressor tone elicited by Ang 1-7 but not Ang II. Similarly, it has been shown previously, that A-779 (3 nmol/rat) given into the PVN reduced BP, renal sympathetic nerve activity and plasma noradrenaline level in anaesthetized high salt-induced or renal hypertensive rats but not in the respective control animals [11,16]. We are the first to demonstrate an interaction of Ang 1-7 and its MasRs with cannabinoid CB_1Rs in the central nervous system. So far, the existence of such an interaction was suggested only in the rat uterus in which local Ang 1-7 infusion enhanced CB_1 and CB_2 receptor expression [22].

How can we explain the interaction between CB_1 receptors with AT_1, AT_2 and Mas receptors (Figure 8)? Paracrine transactivation of CB_1 receptors by endocannabinoids released after activation of AT_1Rs by Ang II in cells expressing both AT_1 and CB_1 receptors diminished the response to Ang II in various cultured cells [39] and vessels (for review, see [40]) and in the heart [41]. On the basis of the latter data, one might assume that AT_1R activation in the PVN leads to the release of endocannabinoids which in turn activate presynaptic inhibitory CB_1Rs mainly on GABAergic neurons (for detailed explanation, see above). Thus, the CB_1R antagonist AM251 inhibits the pressor response to Ang II since it antagonizes presynaptic CB_1Rs responsible for the reduction of GABA release. Consequently, the enhanced inhibitory influence on the glutamatergic input reduces the pressor response to Ang II. On the other hand, in our hands, losartan inhibited the pressor response to CP55940 which mainly results from the reduction of the inhibitory GABAergic tone via activating of presynaptic CB_1Rs by their agonist (see above). Since the final BP response in the PVN is determined by the balance between GABAergic and glutamatergic inputs, we suppose that losartan blocking AT_1Rs on glutamatergic neurons might reduce the pressor response to CP55940. We are not able to comment on the mechanisms related to AT_2 and Mas receptors because only very limited data regarding their pressor effects are available so far. One should keep in mind that impaired GABAergic and/or enhanced glutamatergic inputs result in elevated sympathetic outflow and hypertension (including SHR) [4]. In our hands, AM251 reduced the pressor response to Ang II in SHR more strongly than in WKY confirming the above suggestion that it mainly inhibits presynaptic inhibitory CB_1 receptors located mainly on GABAergic neurons.

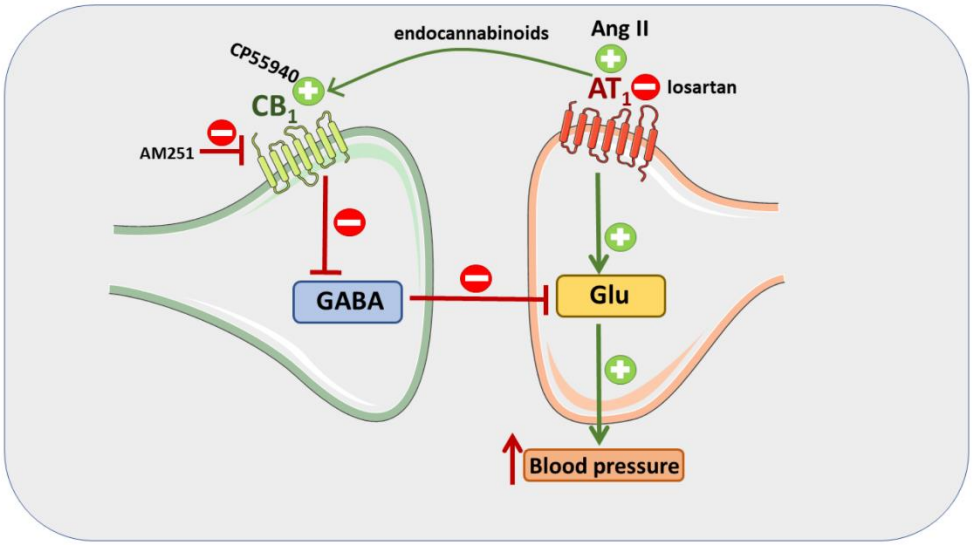

Figure 8. Mechanisms involved in the pressor response induced by Ang II and CP55940. Ang II, via AT_1 receptors, leads to endocannabinoid formation in the paraventricular nucleus of hypothalamus (PVN). Endocannabinoids in turn inhibit GABA release by activation of CB_1 receptors. Since little GABA is released, much glutamate (Glu) can be released, also since facilitatory AT_1 receptors on the glutamatergic neuron further increase Glu release. The extents of Glu release and of the pressor response are correlated. The pressor response in SHR is increased since AT_1 and CB_1 receptor density in their PVN is increased. On the other hand, the pressor response induced by Ang II can be attenuated by the CB_1 receptor antagonist AM251. In addition, the pressor response induced by the CB_1 receptor agonist CP55940 can be antagonized by the AT_1 receptor antagonist losartan.

Interestingly, CB_1R antagonists enhanced the vasoconstrictor responses to Ang II in various isolated arteries (for review, see [40]) but decreased the increase in BP induced by microinjection of Ang II into the PVN (current paper), confirming that CB_1R activation in the peripheral vascular bed and in the PVN is mainly responsible for hypotensive and hypertensive effects of endocannabinoids, respectively (for review, see [18]).

In this context, it is also of interest to comment on the effect of the CB_1R antagonist AM251 when given alone. AM251 administered i.v. and into the PVN increased BP by about 15-20 mmHg and 6-8 mmHg, respectively, both in WKY and SHR. Its effect following i.v. injection probably results from the activation of peripheral presynaptic inhibitory CB_1Rs located on sympathetic nerve endings innervating resistance vessels [42]. Accordingly, the fall in BP in response to CP55940 in anaesthetized rats was reversed to hypertension by the peripheral CB_1R antagonist AM6545 [19]. On the other hand, the weaker enhancement of BP after its application into the PVN might be caused by the blockade of CB_1Rs responsible for the fall in BP by endocannabinoids released in the PVN (first phase of the response to CP55940; [19,20]. An increase in BP by AM251 given into the PVN was also observed in urethane-anaesthetized [21] (0.3 nmol/rat) but not by our group [19]. In our previous study, AM251 given i.v. also failed to affect BP in urethane-anaesthetized rats; they have a lower sympathetic

tone (lower basal BP than in the present paper; [19,20]) and probably also a lower activity of the endocannabinoid system.

5. Conclusions

We show that microinjection of Ang II, Ang 1-7 and CP55940 (after i.v. administration of the CB_1 receptor antagonist AM251) into the PVN induced pressor responses in conscious rats via $AT_{1/2}$, Mas and CB_1 receptors, respectively, and that the pressor responses were stronger in SHR than in WKY. The more pronounced pressor effect in SHR may partially result from its higher (AT_1 and CB_1) and lower (AT_2 and Mas) receptor expression in PVN, RVLM and NTS compared to WKY. With respect to the PVN, we also showed a mutual interaction between cannabinoid CB_1 receptors and receptors for Ang II and Ang 1-7 responsible for stimulation of the pressor response. These interactions, the mechanisms of which have to be clarified in future studies, have to be considered when compounds acting at CB_1 and AT_1 receptors are used for therapeutic purposes.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S7abc: Uncropped Western blot images.

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Rozdział 10.

Review

Cross-Talk between the (Endo)Cannabinoid and Renin-Angiotensin Systems: Basic Evidence and Potential Therapeutic Significance

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Abstract: This review is dedicated to the cross-talk between the (endo)cannabinoid and renin angiotensin systems (RAS). Activation of AT_1 receptors (AT_1Rs) by angiotensin II (Ang II) can release endocannabinoids that, by acting at cannabinoid CB_1 receptors (CB_1Rs), modify the response to AT_1R stimulation. CB_1R blockade may enhance AT_1R-mediated responses (mainly vasoconstrictor effects) or reduce them (mainly central nervous system-mediated effects). The final effects depend on whether stimulation of CB_1Rs and AT_1Rs induces opposite or the same effects. Second, CB_1R blockade may diminish AT_1R levels. Third, phytocannabinoids modulate angiotensin-converting enzyme-2. Additional studies are required to clarify (1) the existence of a cross-talk between the protective axis of the RAS (Ang II—AT_2 receptor system or angiotensin 1-7—Mas receptor system) with components of the endocannabinoid system, (2) the influence of Ang II on constituents of the endocannabinoid system and (3) the (patho)physiological significance of AT_1R-CB_1R heteromerization. As a therapeutic consequence, CB_1R antagonists may influence effects elicited by the activation or blockade of the RAS; phytocannabinoids may be useful as adjuvant therapy against COVID-19; single drugs acting on the (endo)cannabinoid system (cannabidiol) and the RAS (telmisartan) may show pharmacokinetic interactions since they are substrates of the same metabolizing enzyme of the transport mechanism.

Keywords: cannabinoids; endocannabinoids; angiotensin II; angiotensin 1-7; RAS; COVID-19

1. Introduction

In recent years more and more publications have appeared regarding the cross-talk between the (endo)cannabinoid and renin angiotensin (RAS) systems. This issue has become even more important in the era of the coronavirus disease 2019 (COVID-19) pandemic since angiotensin-converting enzyme type 2 (ACE2), one of the key RAS components, was described as a receptor required for the entry of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into host cells [1–4]. The primary aim of our review is to compare all publications regarding the interaction between (endo)cannabinoids and RAS and to find as many common effects and mechanisms as possible. The secondary aim is dedicated to the determination of its potential therapeutic significance.

2. Materials and Methods

To find the most relevant articles dealing with the interaction between angiotensin and cannabinoid systems, we performed a comprehensive search in the PubMed database (closed on 20 May 2022). The following key phrases were used in the search engine: "angiotensin cannabinoid" (which yielded 94 results), "angiotensin 1-7 cannabinoid" (4 results), "ACE cannabinoid" (15 results), "ACE2 cannabinoid" (13 results), "ACE2 cannabinoid COVID" (11 results) and "cannabinoid SARS-CoV-2" (68 results). Taking approved therapeutic usage into consideration, search phrases also consisted of: "sativex", "nabilone", "dronabinol", and "epidiolex" coupled with the word "angiotensin" (a total of 13 results).

First, the titles and then the abstracts and full texts of the identified papers were analyzed, and duplicated articles or those with non-relevant content were removed from the list. Only articles in English were considered. Non-relevant studies included, for example, no described interaction between the two systems of interest or "ACE" being an acronym for unrelated definitions such as "central amygdala". Finally, 43 publications were included in this review, which can be seen systematically summarized in Tables 1–4. Additional papers listed in the References were used to provide more background on angiotensin and cannabinoid systems, as well as SARS-CoV-2. The screening process is summarized in Figure 1, illustrating the PRISMA flowchart [5].

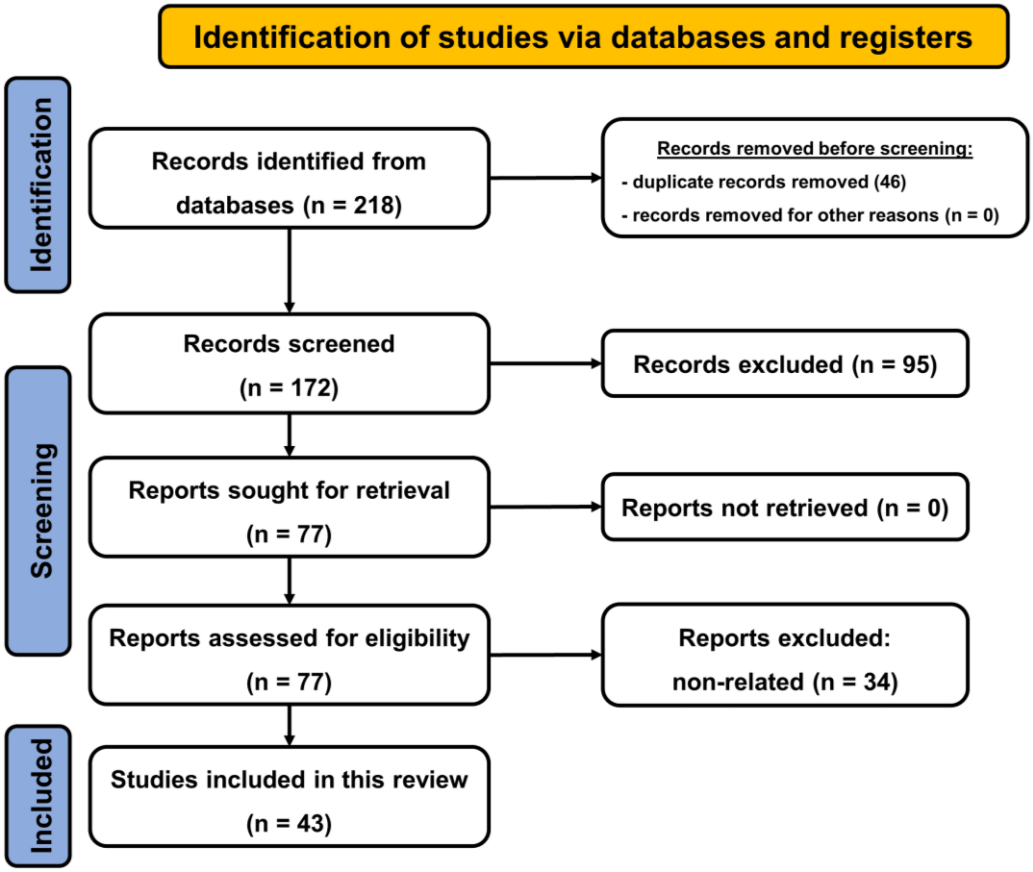

Figure 1. The PRISMA Flow Diagram.

To make reading fluent, the current article is structured into paragraphs, first describing the cannabinoid and angiotensin systems (Sections 3.1.1 and 3.1.2) and then the interaction between both systems (Sections 3.2–3.5). Interactions are being described in the following order: overall cross-talk between RAS and eCBS (Section 3.2 and Table 2), the interaction between angiotensin 1-7 and eCB-mediated effects (Section 3.3 and Table 3), and interaction relevant to COVID-19 (Section 3.4 and Table 4).

3. Results and Discussion

3.1. Components and Main Effects of (Endo)Cannabinoid and Renin-Angiotensin Systems

3.1.1. (Endo) Cannabinoid System

Cannabinoids are lipophilic compounds that belong to three groups: (1) phytocannabinoids, i.e., compounds naturally occurring in the hemp plant Cannabis sativa var. indica, including the psychoactive Δ^9-tetrahydrocannabinol (THC) and non-intoxicating compounds such as cannabidiol (CBD), cannabigerol (CBG) or cannabinol (CBN); (2) synthetic compounds, e.g., CP55940, WIN55212-2 and JWH133 and (3) endocannabinoids (eCBs), e.g., anandamide (AEA) or 2-

arachidonoylglycerol (2-AG), or endocannabinoid-like compounds, e.g., palmitoylethanolamide (PEA). eCBs and endocannabinoid-like compounds are produced by the body; eCBs have an affinity to cannabinoid receptors (CBRs), whereas endocannabinoid-like compounds, despite their similar chemical structure to eCBs, do not have an affinity for CBRs [6–8].

Cannabinoids exert their effects mainly via two G protein-coupled receptors (GPCRs): cannabinoid CB_1 (CB_1Rs) and CB_2 (CB_2Rs) receptors. Both CB_1 and CB_2 receptors act via $G_{i/o}$-protein-dependent pathways and inhibition of adenylyl cyclase. Moreover, their activation may lead to stimulation of mitogen-activated protein kinases (MAPK) and, in the case of CB_1Rs, modulation of calcium and potassium channels [6,9,10]. The most abundant cannabinoid receptors are CB_1Rs, which are expressed mainly in the central nervous system (CNS) and also in low but functional levels in most peripheral tissues, including peripheral neurons, the cardiopulmonary system (systemic and pulmonary vessels), kidney, liver, and other tissues. CB_2Rs are localized mainly peripherally, especially on immune cells. They inhibit inflammation and oxidative stress [6,7,9,11–13]. Cannabinoids also act via other types of G protein-coupled receptors (GPR55 and GPR18) and other receptor families (ionotropic vanilloid TRPV1 and peroxisome proliferator-activated receptor (PPAR) [6,9,10].

Cannabinoid receptors and eCBs belong to the endocannabinoid system (Figure 2), including enzymes involved in the synthesis and degradation of eCBs described in detail in our previous review [8]. Because of the complexity of the synthesis and metabolism of eCBs, only those receptors and enzymes that are important for the understanding of the current review are described below and presented in Figure 2. Briefly, 2-AG is formed almost exclusively by hydrolysis of diacylglycerols (DAGs) by diacylglycerol lipase (DAGL). Cannabinoids exert a multidirectional effect on the body not only through interaction with appropriate receptors but also indirectly through metabolites resulting from their degradation. Fatty acid amide hydrolase (FAAH) is responsible for the degradation of AEA, while monoacylglycerol lipase (MAGL) is particularly involved in the degradation of 2-AG. As a result of their degradation, arachidonic acid (AA) may be formed and converted into eicosanoid metabolites by cyclooxygenase 1/2 (COX-1/COX-2) with a broad spectrum of activity in the body. The eCB system, with its widespread distribution in the body, represents an important signaling pathway involved in many biological processes. Moreover, the eCB system seems to be a promising point for new therapeutic strategies, in many cases, including FAAH and MAGL inhibitors such as URB597 and JZL184, respectively (for review, see [8]).

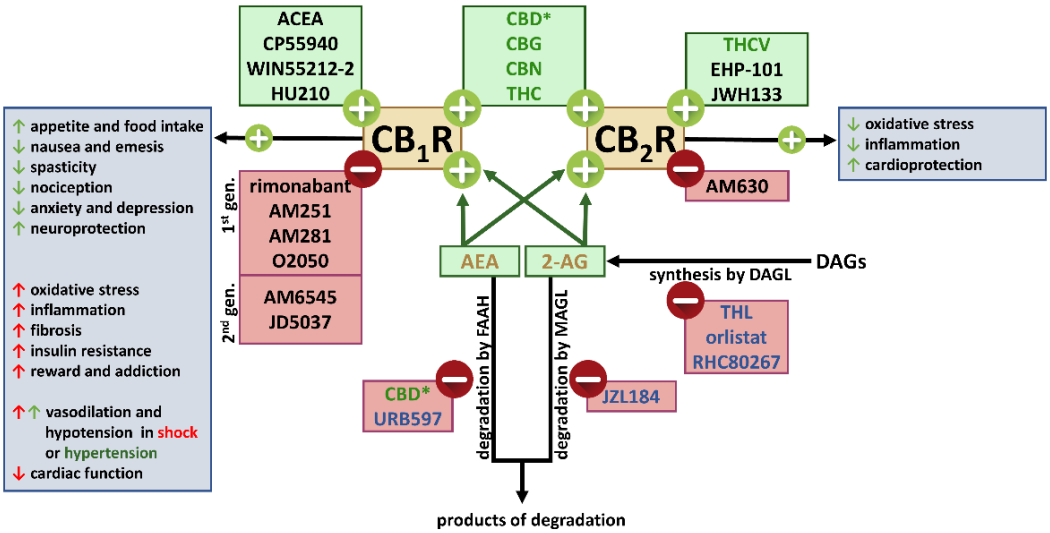

Figure 2. Simplified diagram of compounds modifying the (endo)cannabinoid system as far as they are considered in this review. The ECS comprises endocannabinoids such as anandamide (AEA), 2-arachidonoylglycerol (2-AG), enzymes for their biosynthesis [diacylglycerol lipase (DAGL)] and degradation [fatty acid amide hydrolase (FAAH), and monoacylglycerol lipase (MAGL)] and cannabinoid receptors (CB_1R, CB_2R). Green circles with a plus sign describe (partial) agonism at the respective receptor; red circles with a minus sign describe antagonism, inverse agonism, or inhibition at the respective mechanism. Synthetic, plant-derived compounds and endocannabinoids are written in black, green, and brown font, respectively; the blue font is for enzyme inhibitors. Up arrows, increase; down arrows, decrease; green arrows, desired effects; red arrows, undesired effects. 1st gen., first-generation antagonists; 2nd gen., second-generation antagonists; * weak affinity. ACEA, Arachidonyl-2′-chloroethylamide; CBD, cannabidiol; CBG, cannabigerol; CBN, cannabinol; DAGs, diacylglycerols; THC, Δ^9-tetrahydrocannabinol; THCV, tetrahydrocannabivarin; THL, tetrahydrolipstatin.

CB_1R function undergoes modification under many pathological circumstances. Intriguingly, as shown in Figure 2, both positive and negative changes in their effects may occur under the same pathological condition (described in detail previously by [6,9,11–14]). Thus, CB_1R activation may have beneficial effects against loss of appetite and body weight, nausea, spasticity in multiple sclerosis, pain (especially peripherally-restricted CB_1R agonists), anxiety- and depressive-like behavior, posttraumatic stress disorder, neuroprotection, and epilepsy [9,15–18] or may lead to vasodilatation in arteries of hypertensive animals [19,20]. THC itself (international non-proprietary name, dronabinol), its synthetic analog nabilone, and the mixture of THC and cannabidiol (nabiximols) are approved by the US Food and Drug Administration and other national or supranational drug agencies for some of the above indications (Table 1 [21]). However, CB_1R activation can also lead to cognitive impairment, agitation and acute psychosis [22,23], vasodilatation and/or hypotension in various forms of shock, decreased cardiac function (cardiomyopathies, and heart failure), diet-

induced obesity, development of non-alcoholic fatty liver disease and peripheral insulin resistance [6,7,9,15]. Deleterious consequences of the activation CB_1Rs result from their ability to increase reactive oxygen species (ROS) generation and pro-inflammatory responses leading to endothelial and cardiomyocyte cell remodeling/fibrosis, death, and cardiovascular, metabolic, renal, respiratory, and hepatic dysfunction, detected in both preclinical and clinical studies [6,9,11,13,15,24]. In addition to the agonists mentioned above, one CB_1R antagonist, rimonabant, was available until 2008 as an anti-obesity drug (Table 1).

Table 1. Approved drugs targeting the (endo)cannabinoid and renin-angiotensin systems [1].

System	Class	Mechanism	Approved Drug	Indications
(endo)cannabinoid system	agonists	unselective cannabinoid receptor agonist	dronabinol (Δ^9-tetrahydrocannabinol, THC)	anorexia and weight loss in HIV patients, nausea and vomiting in cancer chemotherapy
		unselective cannabinoid receptor agonist	nabilone	like dronabinol
	antagonists	CB_1 receptor antagonist	rimonabant [2]	obesity
	other	weak activity towards the cannabinoid system, antioxidant drug	cannabidiol	neuropathic pain; Lennox-Gastaut and Dravet syndrome
		(see dronabinol and cannabidiol)	nabiximols (1:1 formulation of dronabinol and cannabidiol)	neuropathic pain in multiple sclerosis, intractable cancer pain
renin-angiotensin system	agonists	unselective AT receptor agonist	angiotensin II	increase in blood pressure in adults with septic or other distributive shock
	antagonists	renin inhibitor	e.g., aliskiren	essential hypertension
		AT_1 receptor antagonist	e.g., candesartan, valsartan	essential hypertension, congestive heart failure
		aldosterone receptor antagonist	e.g., eplerenone	congestive heart failure
	other	angiotensin converting enzyme inhibitor	e.g., perindopril	essential hypertension, congestive heart failure

[1] Based essentially on references from subsections 3.1.1, 3.1.2., and [21]. [2] Withdrawn from the market in 2008.

3.1.2. Renin-Angiotensin System

The RAS plays a significant role in the regulation of blood pressure and sodium and water homeostasis and occurs as (1) circulating hormonal system, (2) local or paracrine RAS expressed in many organs (both in the CNS and in the periphery), and (3) intracellular or intracrine RAS in several cell types [25,26]. The RAS comprises different peptides with opposing biological effects (Figure 3). Briefly, angiotensinogen is converted into angiotensin I (Ang I) by renin and subsequently into angiotensin II (Ang II) by the angiotensin-converting enzyme (ACE). Ubiquitous actions of the main effector of the RAS, namely Ang II, are related to the activation of several signal transduction pathways, which are predominantly attributed to the Ang II type 1 receptor (AT_1R). In addition, Ang II also acts through the Ang II type 2 receptor (AT_2R), or is degraded by ACE2 to Ang 1-7 acting via the Mas receptor (MasR) [27,28]. All receptors are GPCRs; for details of the most important signaling pathways of AT_1Rs, see Section 3.2.1.

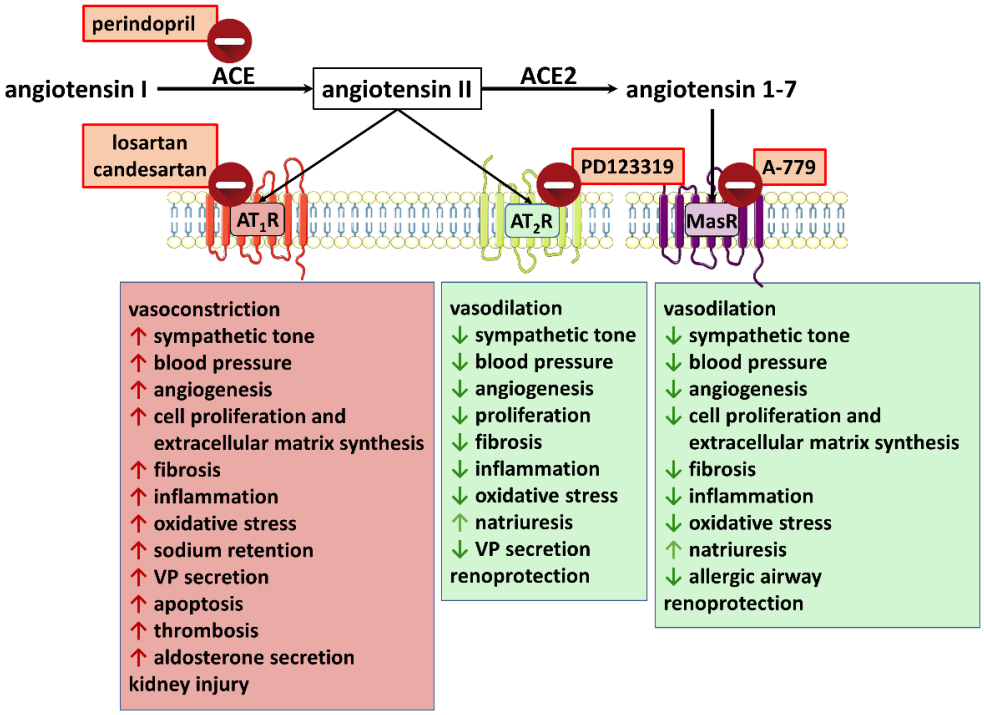

Figure 3. Simplified diagram of the renin-angiotensin system (RAS) and modifying drugs mentioned in this review. The RAS consists of two axes that counteract each other: a deleterious one (so-called classic; red rectangle) containing angiotensin-converting enzyme (ACE)/angiotensin II (Ang II)/Ang II type 1 receptors (AT_1Rs) and a protective one (so-called alternative; green rectangles) constituted by (i) Ang II type 2 receptors (AT_2Rs) and (ii) angiotensin-converting enzyme type 2 (ACE2)/angiotensin 1-7) and its Mas receptors (MasR). VP, vasopressin. Red circles with minus signs describe antagonism, inverse agonism, or inhibition at the respective mechanism. Up arrows, increase; down arrows, decrease; green arrows, desired effects; red arrows, undesired effects.

As shown in Figure 3, the RAS consists of two counter-regulatory arms: the classic one (red rectangle; deleterious axis) containing ACE/Ang II/AT_1Rs and the alternative one (green rectangles; beneficial axis) constituted by (i) AT_2Rs and (ii) ACE2/angiotensin 1-7 and its MasRs. The effects following activation of the particular receptors are shown in Figure 3. The increase in the activity of one axis results in the decrease of the other [26,29,30]. Importantly, disturbance of the balance between the deleterious and the protective axis with the predominance of the ACE/Ang II/AT_1R axis leads to many pathologies, including metabolic (diabetes mellitus), renal, cardiovascular (heart failure, myocardial infarction, hypertension), lung, hepatic, digestive, endocrine, neurodegenerative, hematological, reproductive, and muscular disease [4,26,30–32]. Even if the classic arm plays an important and unpleasant role in many disease states of humans, it is not per se deleterious. One should consider that well-being and even survival would not be possible if this arm were missing in the presence of severe loss of fluid and sodium and/or marked hypotension.

The RAS is the target of several (classes of) drugs (Figure 3, Table 1). Angiotensin II itself is used for the treatment of septic shock. Antagonists at the different levels of the RAS system, i.e., renin, angiotensin II and aldosterone, are used to treat essential hypertension and/or congestive heart failure. For the latter two indications, inhibitors of the degradation of angiotensin I to angiotensin II (ACE inhibitors) are important drugs.

3.2. Examples of Cross-Talk between the (Endo) Cannabinoid and Renin-Angiotensin Systems

Details of particular publications regarding the cross-talk between the (endo)cannabinoid and renin-angiotensin systems are shown in Table 2. We have concentrated mainly on changes in components of both systems but not on physiological or pathophysiological effects induced by activation of the individual receptors. For a description of all compounds mentioned in our review, see Figures 2 and 3.

Table 2. Examples of the cross-talk between the (endo)cannabinoid and renin-angiotensin systems.

Species	Model	Agonist Concentration (μM) or Dose	Effect	(Functional) Antagonist; Concentration In Vitro (μM) or Dose	Influence on the Agonist Effect	Final Conclusion of the Authors	References
cells: Chinese hamster; human; African green monkey	CHO; HEK293; COS7 cells (co-expressing AT_1Rs and CB_1Rs) from ovaries, kidneys, and fibroblasts, respectively	Ang II (0.1)	↑2-AG ↔AEA ↑G_o protein activation	AM251 (10) THL (1)	↓Ang II-induced G_o protein activation	**AT_1R stimulation leads to DAGL**-mediated transactivation of **CB_1Rs** in an autocrine and paracrine manner	[33,34]
cells: mouse	neuro2A cells, a neuroblastoma cell line co-expressing CB_1Rs and AT_1Rs	Ang II (0.01–10)	↑pERK levels via $G_{\alpha i}$ instead of $G_{\alpha q}$ the expression of AT_1R shifts CB_1Rs from an intracellular compartment to the plasma membrane	losartan CB_1R-targeting siRNA RIM (1) THL (1) HU210 (0.0001)	Ang II-induced ↑pERK ↓ by losartan, CB_1R-targeting siRNA, RIM, and THL; ↑ by HU210 (occurring in the presence of a very low non-signaling concentration of Ang II only)	**AT_1Rs and CB_1Rs form receptor heteromers**; blocking CB_1R activity prevented the Ang II-mediated pathologic effect	[35]
cells: rats	hepatic stellate cells from control rats (cHSCs) and rats treated with ethanol for 8 months (eHSCs)	Ang II (1)	CB_1R, AT_1R and AT_1R-CB_1 heteromer levels in eHSCs > cHSCs; ↑pERK levels, ↑mitogenic and ↑profibrogenic markers in eHSCs > cHSCs	RIM (1)	↓Ang II-induced changes		
			Blood Vessels				
rats **Wistar**	aortic VSMCs	Ang II (0.1)	↑2-AG level ↑Ca^{2+} signal	THL (1) JZL184 (1)	↓ and ↑ of Ang II-induced 2-AG formation and Ca^{2+} signal by THL and JZL184, respectively	Ang II stimulates **eCB (2-AG) release** from the vascular wall that reduces the vasoconstrictor effects of Ang II via **CB_1R activation** **(eCBs act as protective negative feedback in response to Ang II)**	[36]
rats and/or mice	aortic rings from rats aortic rings	Ang II (0.001–0.1)	concentration-dependent contraction	WIN-2 (10) O2050 (1)	vasodilation to WIN-2; not detected in $CB_1^{-/-}$		

	from $CB_1^{-/-}$ and WT mice			THL (1) JZL184 (1)	O2050, THL↑ , and JZL184↓ vasoconstrictor effect of Ang II; amplificatory effect of O2050 in WT only		
rats and/or mice	skeletal muscle arterioles, saphenous arteries	Ang II (0.001–0.1)	concentration-dependent contraction	WIN-2 (1) O2050 (1) RIM (1) AM251 (1) THL (1)	vasodilation to WIN-2; not detected in $CB_1^{-/-}$ ↑vasoconstrictor effect of Ang II in WT but not in $CB_1^{-/-}$	Ang II stimulates **eCB release** from the vascular wall that reduces the vasoconstrictor effects of Ang II via **CB_1R activation** **(eCBs act as protective negative feedback in response to Ang II)**	[37]
rats Wistar	intramural coronary resistance arterioles	Ang II (0.0001–10)	concentration-dependent contraction	WIN-2 (0.0001–1) O2050 (1) THL (1)	vasodilatation to WIN-2 reduced by O2050 and AM251 ↑vasoconstrictor effect of Ang II	Ang II stimulates **eCB release** from the vascular wall that reduces the vasoconstrictor effects of Ang II via **CB_1R activation** **(eCBs act as protective negative feedback in response to Ang II)**	[38]
rats Wistar	pulmonary arteries	Ang II (0.0001–0.03)	concentration-dependent contraction	AM251 (1) RHC80267 (40) JZL184 (1) URB597 (1)	AM251 and RHC80267 ↑ but JZL184 ↓ vasoconstrictor effect of Ang II; URB597 ↔	Ang II stimulates **eCB (2-AG) release** from the vascular wall that reduces the vasoconstrictor effects of Ang II via **CB_1R activation** **(eCBs act as protective negative feedback in response to Ang II)**	[39]
rats	uterine artery from hypertensive TgA and normotensive SD rats	Ang II (0.00001–0.01)	concentration-dependent contraction, stronger in TgA	URB597 (1) JZL184 (1) RIM (1)	↓responses to Ang II in SD and TgA ↓responses to Ang II in TgA ↔responses to Ang II in SD and TgA	**eCBs** reduce the Ang II-induced contraction **in a CB_1R-independent manner** in the early stages of hypertensive pregnancy **(eCBs act as protective negative feedback in response to Ang II)**	[40]
rats SD mice	VSMCs from rat and mouse thoracic aortas with CB_1R expression	Ang II (1)	↑ROS production ↑NADPH oxidase activity	RIM (0.1–1) or AM251 (1) CP55940 (1)	↓AT_1Rs and decrease in the Ang II-induced ↑ROS production and ↑NADPH oxidase activity ↑AT_1Rs	CB_1R inhibition (*in vitro* and *in vivo*) has atheroprotective effects by **down-regulation of AT_1Rs,** decreased vascular ROS, and thus improved endothelial function in hypercholesterolemic $ApoE^{-/-}$ mice	[41]

mice	ApoE$^{-/-}$ treated with a cholesterol-rich diet		development of atherosclerotic plaques, ↓aorta relaxation, ↔aortic AT_1R level	RIM (10 mg/kg/day; p.o.) for 7 weeks	↓aortic AT_1Rs and improvement of endothelial function, no effect on atherosclerotic plaques		
				Heart			
rats SD	isolated Langendorff-perfused hearts	Ang II (0.001–0.1) 2-AG (1) WIN-2 (1)	↓CF and moderate negative inotropic effect 2-AG and WIN-2: ↑CF WIN-2: negative inotropic effect	O2050 (1) + Ang II orlistat (10) + Ang II	↓cardiac effects of Ang II	besides direct cardiac responses, Ang II induces indirect ones via **<u>eCBs</u>** (probably 2-AG) activating **<u>CB_1Rs</u>**: - direct positive inotropy reversed into a negative one, ↓oxygen demand - direct coronary constriction attenuated, (↑)oxygen supply **(eCBs act as <u>protective negative feedback</u> in response to Ang II)**	[42]
mice	streptozotocin-induced diabetes	diabetic cardiomyopathy	↑myocardial CB_1 and AT_1R expression and AEA level connected with cardiac dysfunction, inflammation, oxidative/nitrative stress	RIM or AM281 (10 mg/kg; i.p. daily for 11 weeks) or CB_1R deletion ($CB_1^{-/-}$ mice)	pharmacological inhibition or genetic deletion of CB_1Rs—improvement of diabetic cardiac dysfunction connected with ↓AT_1Rs and CB_1Rs in LV	overactivation of the eCB system and CB_1Rs may play an important role in the pathogenesis of diabetic cardiomyopathy by **<u>facilitating AT_1R</u>** expression and signaling	[43]
rats Wistar	isolated Langendorff-perfused hearts underwent ischemia + reperfusion	ischemia and reperfusion	↑stroke size, ↓ventricular function; ↑ cardiac AT_1R level and ↔ cardiac AT_2R level	CBD (5 mg/kg; i.p. daily for 10 days)	↓stroke size and ↑ventricular function; ↓ cardiac AT_1R level and ↑ cardiac AT_2R level	cardioprotective effect of CBD might result from an **<u>increase in cardioprotective AT_2Rs</u>** stimulating counter-regulatory effects on the AT_1Rs	[44]
mice	Ang II-induced fibrosis and inflammation	Ang II infusion (1 µg/kg/min *[preventive]* or 500 *[therapeutic]* for 4 weeks)	fibrosis and inflammation in the heart, aorta, lung, kidney, and skin	EHP-101 (2, 5 or 20 mg/kg for 4 or 2 weeks)	↓cardiac, aortic, lung, kidney, and skin fibrosis and inflammation in the preventive or therapeutic model	EHP-101 (dual agonist of **CB_2Rs** and PPARγ) can alleviate cardiac, aortic, lung, kidney, and skin inflammation induced by Ang II	[45]

				Blood Pressure			
mice **$CB_1^{-/-}$** **$CB_1^{+/+}$**	anesthetized	Ang II (1 µg/kg/min)	↑BP in WT and $CB_1^{-/-}$	O2050 (10 mg/kg; p.o.)	↑ pressor effect of Ang II in WT, not in $CB_1^{-/-}$	confirmation of in vitro experiments on isolated arteries that Ang II stimulates **release of eCBs (2-AG)** from the vascular wall that reduce vasoconstrictor effects of Ang II via **CB_1R activation**	[36]
rats SD	conscious	Ang II-induced hypertension (60 ng/min; s.c. for 10–12 days)	↑BP	AM251 (3 mg/kg; i.v.) URB597 (10 mg/kg; i.v.) in pentobarbital-anaesthetized rats	AM251 ↑BP and URB597 ↓BP in Ang II-induced hypertension but not in normotension	the Ang II-induced hypertension is diminished by **eCBs acting at CB_1Rs**; effect of URB597 reduced by AM251	[19]
rats **Wistar**	conscious	Ang II (500 ng/kg/h) + VP (50 ng/kg/h for 4 days)- induced hypertension	↑BP	AEA (3 mg/kg) URB597 (3 mg/kg) WIN-2 (150 µg/kg) AM251 (3 mg/kg)	AEA, WIN-2 ↓BP in Ang II-VP-induced hypertension; URB597 enhanced the effect of AEA; AM251 blocked the effect of WIN-2	the Ang II-VP induced hypertension might be diminished by **eCBs acting at CB_1Rs**	[46]
rats **SHR** **WKY**	conscious		BP was higher in SHR than in WKY	RIM 3 mg/kg i.v. URB597 1.7 mg/kg i.v.	RIM ↑BP and URB597 ↓BP in SHR but not in normotensive WKY	in SHR in which RAS is overactivated **eCBs acting at CB_1Rs** reduce BP	[19]
rats	conscious (mRen2)27 hypertensive rats or normotensive SD	(mRen2)27: higher RAS activity		RIM (10 mg/kg; p.o. acutely or daily for 28 days)	acutely: ↓BP and ↓HR in hypertensive but not in SD chronically: ↓BP and ↓HR; ↔plasma Ang II, ↔Ang 1-7;	**upregulated ECS** contributes to hypertension and impaired autonomic function in this Ang II-dependent model; systemic **CB_1R** blockade may be an effective therapy for **Ang II-dependent**	[47]

					↔ACE; improvement of sympathetic and parasympathetic BRS	**hypertension** and the associated metabolic syndrome	
rats	anaesthetized (mRen2)27 hypertensive, ASrAOGEN and SD rats	(mRen2)27: higher RAS activity; AsrAO-GEN: low glial angiotensinogen	levels in NTS: *2-AG*: (mRen2)27 > SD > ASrAOGEN; *AEA*: (mRen2)27 ≈ SD ≈ ASrAOGEN dorsal medulla: CB_1: ASrAOGEN < (mRen2)27 ≈ SD; CB_2: no differences	RIM (0.36 and 36 pmol/rat; NTS)	↑BRS in (mRen2)27; ↓BRS in ASrAOGEN; ↔BRS in SD	upregulated **brain ECS in Ang II**-dependent hypertension may contribute to the impaired baroreceptor sensitivity in this model of hypertension	[48]
rats	obese fa/fa Zucker rats and control lean fa/+ Zucker rats; isoflurane-anaesthetized	acute Ang II (30 and 100 ng/kg, i.v.)	stronger pressor response in obese than in lean rats	RIM (3 or 10 mg/kg, p.o.) for 12 months	normalized the acute pressor response to Ang II in obese rats to the level of lean rats	authors suggest that **chronic CB_1R blockade** by RIM might **reduce** vascular **AT_1R** expression; an indirect mechanism related to the decrease in the cholesterol level should also be taken under consideration	[49]
rats **SHR** **WKY**	conscious		SHR in comparison to WKY: higher BP, carotid, mesenteric artery: ↑AT_1Rs, ↑ACE kidney: ↔AT_1Rs, ↔AT_2Rs, ↑ACE	PEA (30 mg/kg; s.c. for 5 weeks)	BP in SHR↓ SHR arteries: ↓AT_1Rs, ↓ACE level SHR kidney: ↓AT_1Rs, ↑AT_2Rs, and ↓ACE level associated with ↓oxidative and nitrosative stress	PEA lowers BP and protects against hypertensive renal injury partially via **reduction** in vascular **AT_1Rs** and Ang II-mediated effects and via **modulation of the RAS, leading the AT_1/AT_2 balance** towards an anti-hypertensive status	[50,51]
rats **WKY**	cultured lymphocytes from WKY	Ang II (0.01–1)	concentration-dependent ↓AEA transporter activity and ↑ROS level	losartan (10 and 100)	↓Ang II effects on AEA transporter activity and ROS level	Ang II plays a critical role in mediating the decrease in **AEA transporter activity** in SHR; probably via AT_1Rs	[52]
rats **SHR** **WKY**	conscious		SHR: ↑plasma Ang II and ↑AEA level; ↓AEA transporter activity in comparison to WKY	losartan (15 or 30 mg/kg; p.o. for 2 weeks)	restoration of reduced AEA transporter activity; ↓plasma AEA level		

			Nervous System				
rats **Wistar**	urethane- anesthetized	Ang II (0.14 nmol/rat; PVN)	↑BP	AM251 (0.48 nmol/rat; PVN)	AM251 reduced the Ang II-mediated BP increase and slightly increased BP by itself	Ang II-induced hypertension involves CB_1Rs in the PVN	[53]
rats **Wistar**	urethane- anaesthetised (microinjection into the PVN, doses in nmol/rat)	CP55940 (0.1) CP55940 (0.1) + AM251 (3 µmol/kg; i.v.)	↓BP, ↓HR ↑BP, ↑HR	losartan (10 µmol/kg; i.v.) losartan (10 µmol/kg; i.v.)	no effect reversed ↑BP, ↑HR to ↓BP, ↓HR	presynaptic inhibitory CB_1Rs on GABAergic neurons in the PVN activated by **eCBs released in response to Ang II** modify the glutamatergic neurotransmission enhanced by presynaptic AT_1R activation	[54]
rats **SHR** **WKY**	conscious (all compounds microinjected into the PVN, nmol/rat)	Ang II (0.03) or CP55940 (0.1) + AM251 (3 µmol/kg; i.v.)	↑BP stronger in SHR than in WKY	losartan (20) PD123319 (10) AM251 (30)	↓pressor effect of Ang II and CP55940 ↓pressor effect of Ang II and CP55940 ↓pressor effect of Ang II	mutual interaction in the PVN between CB_1Rs and receptors for Ang II responsible for stimulation of the pressor response (probably via stimulation of CB_1R by eCBs **released in response to Ang II)**	[55]
mice	magnocellular neurosecretory cells from the supraoptic nucleus	Ang II (0.1)	↑frequency of mEPSCs	AM251 (2)	↑effect of Ang II	**eCBs released in response to Ang II** modulate the excitatory synaptic inputs **via negative feedback**	[56]
rats Wistar **mice** **$CB_1^{+/+}$** **$CB_1^{-/-}$**	conscious	Ang II (191 pmol/rat; i.c.v.) Ang II (191 pmol/mouse i.c.v.)	↓ethanol-induced gastric lesions (reduced by candesartan 5.2 and 31.7 nmol/rat; i.c.v.) gastroprotection in $CB_1^{+/+}$ as opposed to $CB_1^{-/-}$	AM251 (1.8 nmol/rat; i.c.v.) THL (0.2 nmol/rat; i.c.v.)	inhibition of the gastroprotective effect of Ang II	**Ang II stimulates eCB release via activation of central AT_1R receptors,** and activation of **CB_1Rs** induces gastroprotection in a vagus-mediated mechanism (inhibition by vagotomy and atropine)	[57]
mice **$CB_1^{+/+}$** **$CB_1^{-/}$**	response of the chorda tympani (CT) nerve in anesthetized mice	$CB_1^{+/+}$: Ang II (100–5000 ng/kg; i.p.)	gustatory nerve responses ↓ to NaCl and ↑ to sweeteners, blocked by candesartan	$CB_1^{-/-}$: Ang II (100–5000 ng/kg; i.p.)	gustatory nerve responses ↓ to NaCl and ↔ to sweeteners	enhancing effect of Ang II on sweet taste responses mediated by AT_1 and CB_1Rs; authors suggest that **Ang II, via AT_1Rs, stimulates the release of 2-AG** that may act as an autocrine enhancer for **CB_1Rs** on sweet taste cells	[58]

rats **SHR** **WKY**	<u>astrocytes</u> basal CB_1R densities: brainstem: SHR<WKY cerebellum: SHR>WKY	Ang II (0.1)	SHR: ↓CB_1R and ↑CB_1R densities and phosphorylation in brainstem and cerebellar astrocytes, respectively; opposite effects in WKY	losartan (10) PD123319 (10) ACEA (0.01)	- effects of Ang II were inhibited by losartan (brainstem) and by losartan and PD123319 (cerebellum) - ACEA reduced the AT_1R-mediated MAPK activation in brainstem and cerebellar astrocytes	Ang II, mostly via the AT_1R, is capable of **<u>altering CB_1R expression</u>** and phosphorylation in astrocytes isolated from the brainstem and cerebellum under hyper- and normotensive conditions; possible role in neuroinflammatory and attention-deficit hyperactivity disorders, respectively	[59,60]
rats **SHR** **WKY**	astrocytes isolated from the brainstem and from cerebellum	Ang II (0.1)	↓IL-10 and ↑IL-1β gene expression in astrocytes from both brain regions of SHR and WKY	ACEA (0.01)	co-treatment of Ang II and ACEA resulted in the neutralization of Ang II-mediated effect in WKY but not SHR	Ang II and ACEA have opposing roles in the regulation of inflammatory gene signature in astrocytes isolated from SHR and Wistar rats (possible **<u>functional antagonism</u>**)	[61]
mice **$CB_2^{-/-}$** **$CB_2^{+/+}$**	hippocampus slices		$CB_2^{-/-}$: ↓ACE level, and ↑aβP in comparison to WT		CB_2R deletion:↑aβ neurotoxicity associated with ↓level of ACE (that degrades aβ)	**<u>activation of CB_2Rs increases ACE level</u>** that degrades aβ; possible significance in Alzheimer's disease	[62]
	N2a cells overexpressing aβP	JWH133	↑ACE level, ↓aβP	AM630	all JWH133 effects were attenuated		
				Kidney			
humans	podocytes	Ang II (0.1)	↑AEA, ↑2-AG ↑AT_1Rs and CB_1Rs	JD5037 (100) or losartan (10)	↓ all changes induced by Ang II	**<u>peripheral CB_1R</u>** blockade might possess therapeutic potential in disease(s) connected with **enhanced RAS**	[63]
rats	Zucker diabetic fatty rats with nephropathy; control lean rats	diabetic compared to lean rats	↑plasma Ang II and aldosterone levels; ↓AT_1Rs in renal cortex	JD5037 (3 mg/kg p.o. for 3 months) losartan (20 mg/kg p.o. for 28 days)	↓plasma Ang II and aldosterone levels; ↓AT_1Rs in renal cortex ↔plasma Ang II and ↓aldosterone levels; ↓CB_1Rs in renal cortex		
mice	streptozotocin-induced diabetic		↑glomerular CB_1 and ↑AT_1Rs; ↔CB_2Rs	AM6545 (10 mg/kg; i.p.) alone or together with	<u>Single treatments</u> ↔glomerular CB_1-, CB_2Rs, and ↓AT_1Rs; ↓progression of	The superior effect of **<u>dual therapy (peripheral CB_1R antagonist + ACE inhibitor</u>**) on albuminuria, nephrin loss, and inflammation suggest that CB_1R blockade may be a valuable option as an additional	[64]

nephropathy	perindopril (2 mg/kg; p.o.) for 14 weeks	albuminuria, down-regulation of nephrin and podocin, ↓inflammation, and ↓expression of markers of fibrosis Combined treatment ↔glomerular CB_1-, CB_2Rs and ↓AT_1Rs; also reversal of albuminuria	therapy, although single and combined treatment only reduce glomerular AT_1Rs without affecting CB_1Rs and CB_2Rs.

↓—decrease; ↑—increase; ↔—no change. **2-AG**, 2-arachidonoyl glycerol; **A549**, alveolar epithelial cell line; **AβP**, amyloid-β protein; **ACE**, angiotensin-converting enzyme; **ACE2**, angiotensin-converting enzyme 2; **ACEA**, arachidonyl-2′-chloroethylamide; **AEA**, anandamide; **Ang II**, angiotensin II; **Ang 1-7**, angiotensin 1-7; **ApoE**, apolipoprotein E; **ASrAOGEN**, transgenic rats characterized by a transgene producing antisense RNA against angiotensinogen in the brain; **AT_1R**, Ang II receptor type 1; **AT_2R**, Ang II receptor type 2; **BRS**, baroreceptor sensitivity; **CB_1R**, cannabinoid receptor type 1; **CB_2R**, cannabinoid receptor type 2; **CBD**, cannabidiol; **CBG**, cannabigerol; **CBN**, cannabinol; **CF**, coronary flow; **CHO**, Chinese hamster ovary cells; **DAGL**, diacylglycerol lipase; **eCBs**, endocannabinoids; **ECS**, endocannabinoid system; **EHP-101 (VCE-004.8)**, oral lipidic formulation of the novel non-psychotropic cannabidiol aminoquinone; **ERK**, extracellular signal-regulated kinases; **FAAH**, fatty acid amide hydrolase; **hACE2**, human ACE2; **hiPSC-CMs**, human iPSC-derived cardiomyocytes; **HSC,** hepatic stellate cells; **IFN-γ**, interferon γ; **i.c.v.**, intracerebroventricular; **IL-1β**, interleukin-1β; **IL-10**, interleukin-10; **i.p.**, intraperitoneal; **i.v.**, intravenous; **HR**, heart rate; **LDH**, lactate dehydrogenase; **LV**, left ventricle; **MAGL**, monoacylglycerol lipase; **MAPK**, mitogen-activated protein kinase; **mEPSCs,** miniature excitatory postsynaptic currents; **(mRen2)27**, Ang II-dependent hypertension model; **NA,** noradrenaline; **NTS**, solitary tract nucleus; **PEA**, *N*-palmitoylethanolamide; **pERK**, phospho-ERK; **p.o.**, per os; **PVN**, paraventricular nucleus of hypothalamus; **RIM,** rimonabant; **RAS**, renin angiotensin system; **ROS**, reactive oxygen species; **s.c.**, subcutaneous; **SD**, Sprague-Dawley rats; **SHR**, spontaneously hypertensive rat; **TgA**, transgenic rat, model of preeclampsia; **THC**, Δ^9-tetrahydrocannabinol; **THCV**, tetrahydrocannabivarin; **THL**, tetrahydrolipstatin; **TMPRSS2**, transmembrane serine protease 2; **TNF-α**, tumor necrosis factor α; **URB597**, an inhibitor of FAAH (fatty acid amide hydrolase); **WIN-2**, WIN55212-2; **WKY**, Wistar Kyoto rats; **WT**, wild type; **VSMCs**, vascular smooth muscle cells; **VP**, vasopressin.

3.2.1. Angiotensin II Causes Transactivation of Cannabinoid-Mediated Effects

The AT_1Rs for Ang II interact with various G proteins, including $G_{q/11}$ [53,65,66]. Activation of $G_{q/11}$ protein-coupled receptors and the subsequent stimulation of phospholipase C leads to Ca^{2+} signal generation and indirectly causes rapid biosynthesis of eCBs (mainly 2-AG) by DAGL activation (Figure 4). As mentioned above, 2-AG exerts its effects mainly via CB_1Rs and CB_2Rs [8]. This reasoning suggests that Ang II might cause transactivation of cannabinoid-mediated effects.

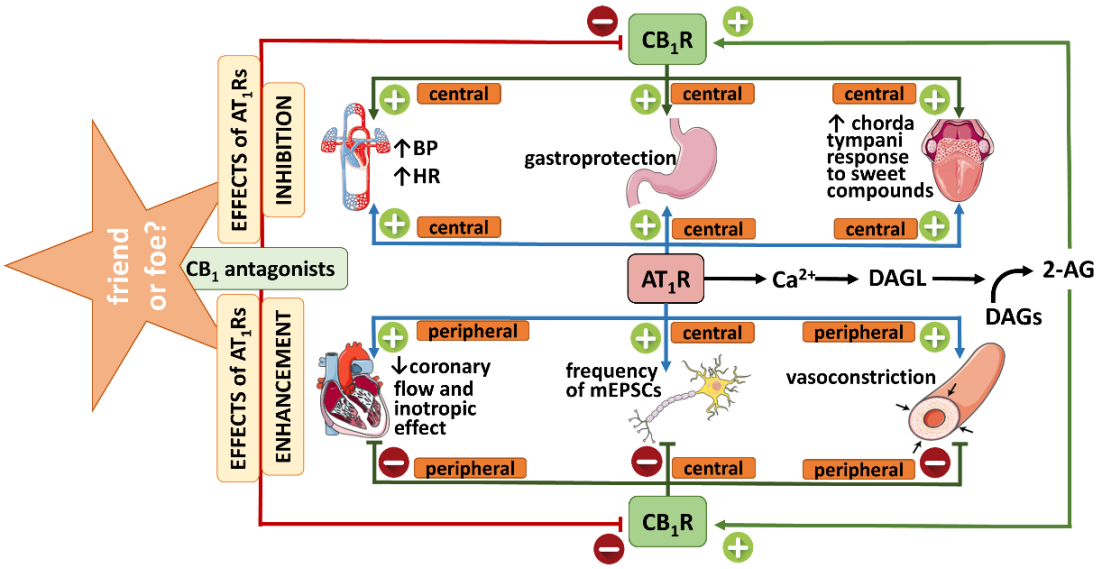

Figure 4. Influence of CB_1 receptor (CB_1R) antagonists on the effects elicited by activation of AT_1 receptors (AT_1R). Stimulation of AT_1Rs leads to Ca^{2+}-signal generation and rapid biosynthesis of 2-arachidonoylglycerol (2-AG) from diacylglycerols (DAGs) by diacylglycerol lipase (DAGL) activation. If CB_1R and AT_1R activation lead to the same effect, CB_1R antagonism will inhibit the AT_1R-mediated effect, i.e., will decrease blood pressure (BP) and heart rate (HR), gastro-protection, or the chorda tympani response to sweet compounds (upper part of the figure). In the case of opposite effects of CB_1R and AT_1R activation, CB_1R blockade will enhance the AT_1R-mediated effects, i.e., its positive inotropic action, its facilitatory effect on miniature excitatory postsynaptic currents (mEPSCs) or its vasoconstrictor effect (lower part of the figure). For the respective literature, see Table 2 and Section 3.2.1.

The following facts determined by Turu et al. [33] on Chinese hamster ovary (CHO) cells co-expressing CB_1Rs and AT_1Rs clearly confirm the above working hypothesis. Firstly, Ang II increased levels of 2-AG (measured by mass spectrometry) but not of AEA. Secondly, Ang II elicited G_o protein activation (determined by detecting the dissociation of G protein subunits with bioluminescence resonance energy transfer; BRET) that was blocked both by the CB_1R antagonist AM251 and the DAGL inhibitor tetrahydrolipstatin (THL); AM251 and THL by themselves inhibited basal CB_1R activity. The pioneering observations by Turu et al. [33] have been confirmed and extended by the same group on HEK293 and COS7 cells from kidney and fibroblasts, respectively [34]. Thus, Ang II caused CB_1R-dependent

activation not only in an autocrine but also in a paracrine manner [34], i.e., when AT_1Rs and CB_1Rs are localized on the same or separate cells, respectively.

The latter interaction obtained on cell culture has been verified in blood vessels and other body systems. First of all, Szekeres et al. [36] showed in vitro that Ang II enhanced the calcium signal and the 2-AG level in rat aortic vascular smooth muscle cells (VSMCs). Both responses to Ang II were reduced by the DAGL inhibitor THL and enhanced by the MAGL inhibitor JZL184 leading to inhibition of 2-AG synthesis and degradation and finally to a decrease and increase in 2-AG levels, respectively. The results obtained on cell culture were further confirmed on isolated vessels examined in the organ bath, including the wire myograph for studies on small resistance arteries. As shown in Table 2, Ang II caused a concentration-dependent vasoconstriction of rat and/or mice aortic rings [36], skeletal muscle arterioles and saphenous arteries [37], and intramural coronary resistance arterioles [38]. All vasoconstrictory responses to Ang II were enhanced by antagonists of CB_1Rs (the inverse agonists rimonabant and AM251 or the neutral antagonist O2050) and by the DAGL inhibitor THL via reduction of the CB_1R-mediated vasodilatation or 2-AG levels, respectively. On the other hand, the MAGL inhibitor JZL184, increasing 2-AG levels, reduced the above effects of Ang II. Moreover, the CB_1R antagonists had an amplificatory influence only in wild-type but not in CB_1R knockout mice [36,37]. The presence of CB_1Rs in the particular vessels or aortic VSMC has been confirmed with immunohistochemistry or using the Western blot technique [36–38] or functionally by studying the vasodilatory effect of the CB_1R agonist WIN55212-2 with or without CB_1R blockade [36–38]. Moreover, WIN55212-2-induced vasodilatation was not observed in $CB_1^{-/-}$ mice [36,37].

An interaction between Ang II and CB_1Rs resembling that in systemic arteries (see above) was also observed in rat pulmonary arteries, in which CB_1Rs are present in smooth muscle, as determined using immunohistochemical staining [39]. Thus, the Ang II-induced concentration-dependent contraction of pulmonary arteries was amplified by AM251 and the DAGL inhibitor RHC8026 and diminished by inhibition of 2-AG but not AEA degradation by JZL184 and URB597, respectively. Unfortunately, the Ang II-evoked response in human pulmonary arteries was very weak and did not allow for examining its potential interaction with CB_1-Rs [39].

In uterine arteries isolated from control and TgA rats (model that mimics many features of preeclampsia), the vasoconstrictor responses to Ang II (which were stronger in TgA than in control animals) were decreased by inhibitors of FAAH (URB597) and/or MAGL (JZL184) but not by the CB_1R antagonist rimonabant [40]. Authors suggest that eCBs released in response to Ang II reduced the Ang II-induced contraction in a CB_1R- independent manner. Indeed, the non-selective COX inhibitor indomethacin reduced the maximal contraction and sensitivity to Ang II in arteries from TgA rats, suggesting that eCBs (both AEA and 2-AG) released by Ang II were quickly converted to

vasoactive eicosanoids via COX, yielding vasodilator prostaglandins and/or prostacyclin.

In summary, the above in vitro experiments on isolated arteries clearly show the existence of protective negative feedback in response to the vasoconstrictory effect of Ang II. Thus, Ang II acting via AT_1Rs not only contracts particular arteries but also stimulates a DAGL-dependent release of eCBs (mainly 2-AG) from vascular endothelium or smooth muscles that cause vasodilatation in a CB_1R-dependent (Figure 4) or independent manner, thereby diminishing the initial response to Ang II.

An interaction between AT_1Rs and eCBs is also suggested to be present in the heart ([42]; Table 1, Figure 4). In rat isolated Langendorff-perfused hearts Ang II reduced coronary flow (CF) and caused a moderate negative inotropic effect, although it is known to exert a positive inotropic effect in other heart preparations (e.g., on rat cardiomyocytes in the study by Rajagopal et al. [67]). In Langendorff-perfused hearts, the activation of CB_1Rs (the presence of which was confirmed by immunohistochemistry) by WIN55212-2 and 2-AG increased CF and decreased cardiac contractility in a manner sensitive to the CB_1R antagonist O2050. However, in contrast to previous experiments on rat coronary arteries [38], O2050 and the DAGL inhibitor orlistat did not amplify but reduced both cardiac effects of Ang II. Miklós et al. [42] argue that the final response to Ang II in the heart is determined by an interplay between coronary arteries and cardiomyocyte contractility, and the degree of CF results primarily from the cardiac oxygen demand and not only from the direct vascular effects of vasoactive agents. Authors suggest that CB_1R blockade by O2050 prevented the CB_1R-induced negative inotropic effect elicited by 2-AG released under Ang II stimulation. Ang II then increases cardiac contractility (otherwise masked by flow deprivation) and, subsequently, the overall oxygen demand and the CF.

The question arises whether the protective negative feedback of Ang II—eCBs—CB_1Rs observed in vitro is relevant also under in vivo conditions. The answer is "yes", as shown in Table 1. First of all, Szekeres et al. [36] showed that, as in experiments on isolated arteries, the increase in blood pressure (BP) elicited by intravenous (i.v.) infusion of Ang II was enhanced by the CB_1R antagonist O2050 in anesthetized $CB_1^{+/+}$ R wild type as opposed to $CB_1^{-/-}$ R knockout mice. A similar mechanism may occur in experiments performed on conscious rats with hypertension induced by infusion of Ang II [19] or Ang II plus vasopressin [46]. Thus, the CB_1R antagonist AM251 enhanced and the FAAH inhibitor URB597 reduced BP [19], and URB597 enhanced the fall in BP elicited by AEA [46]. Similarly, in spontaneously hypertensive (SHR) rats (in which the RAS is overactivated [68]) but not in their normotensive Wistar Kyoto (WKY) controls, the CB_1R antagonist rimonabant and the FAAH inhibitor URB597 enhanced and reduced BP, respectively [19]. The blockade of the hypotensive effect of AEA and WIN55212-2 by AM251 confirms the involvement of CB_1Rs in the hypotensive action of both compounds [46].

Interestingly, an opposite effect of CB_1R blockade was obtained in transgenic (mRen2)27 rats, i.e., a monogenetic model of Ang II-dependent hypertension in which the mouse Ren2 renin gene was transfected into the genome of the Sprague-Dawley (SD) rat. (mRen2)27 rats have the phenotype of chronic hypertension with markedly impaired baroreflex control over heart rate (HR) and increased body weight. Unlike in SHR or hypertension models induced by Ang II [19] or Ang II with vasopressin infusion [46], acute or chronic administration of rimonabant decreased BP and body weight in (mRen2)27 rats but not in their normotensive SD controls [47]. One can exclude that the reduction of body weight is responsible for the fall in BP since it resulted not only from chronic but also from acute administration of the CB_1R antagonist. Moreover, chronic rimonabant administration improved sympathetic and parasympathetic baroreflex sensitivity for HR control but failed to change plasma Ang II and Ang 1-7 levels or ACE activity. The authors concluded that an upregulated endocannabinoid system contributes to hypertension and impaired autonomic function in this Ang II-dependent model and that systemic CB_1R blockade may be an effective therapy in this type of hypertension [47]. In a subsequent study, Schaich et al. [48] confirmed their conclusion showing enhanced levels of 2-AG (but not AEA) in the solitary tract nucleus (NTS) of (mRen2)27 rats in comparison to SD or ASrAOGEN rats (the latter have a low level of glial angiotensinogen). Microinjection of rimonabant into the NTS dose-dependently enhanced baroreceptor sensitivity in (mRen2)27 but decreased it in ASrAOGEN animals. Thus, upregulation of the brain endocannabinoid system may be responsible for the impairment of baroreceptor sensitivity in this Ang II-dependent model of hypertension.

In this context, one should keep in mind that Ang II administered centrally and peripherally increases BP [69], whereas cannabinoids can elicit different cardiovascular responses (including hypotension and hypertension) [70,71]. Interactions between the (endo)cannabinoid and RAS systems may not only occur in vessels but also in the CNS. The paraventricular nucleus of the hypothalamus (PVN) is one of the crucial brain regions involved in the regulation of cardiovascular functions, in which the balance between sympathoexcitatory glutamatergic and sympathoinhibitory GABAergic transmission is responsible for the final integration of the sympathetic outflow [72]. Gyombolai et al. [53] were the first to show that microinjection of the CB_1R antagonist AM251 into the PVN of anesthetized rats reduced the increase in BP elicited by application of Ang II to the same nucleus, although the underlying mechanism has not been clarified. We found that the cannabinoid receptor agonist CP55940 given into the PVN of rats anesthetized with urethane decreased BP but in the presence of AM251 given i.v. produced a clear pressor response, sensitive to AM251 given into the PVN [54]. The AT_1R antagonist losartan given i.v. inhibited the depressor response to CP55940 only but reversed its pressor response to a fall in BP. In our most recent study, performed on conscious SHR and their normotensive (WKY) controls in which all compounds were microinjected into the PVN, the pressor responses to

Ang II and to CP55940 (both of which were stronger in SHR than in WKY) were inhibited by antagonists of AT_1 and AT_2 receptors, losartan and PD123319, respectively. On the other hand, AM251 diminished the increase in BP induced by Ang II [55]. The pressor effects of Ang II and CP55940 given into the PVN result mainly from the activation of facilitatory AT_1Rs on glutamatergic [73,74] and inhibitory CB_1Rs on GABAergic [54,55] neurons, respectively (Figure 5). In addition, Ang II may lead to the release of eCBs that, via CB_1Rs, reduce the GABAergic tone and eventually lead to increased activity of the glutamatergic neurons associated with a pressor effect. On the other hand, AM251 blocking presynaptic CB_1Rs on GABAergic neurons may increase the GABAergic inhibitory influence on the sympathoexcitatory glutamatergic tone stimulated by Ang II and, in this way, reduces its pressor response (Figure 5). The potential mechanism responsible for the interaction of AT_2Rs (which, unlike the AT_1Rs, are not coupled to $G_{q/11}$ proteins; [75]) with CB_1Rs remains to be established. AT_2Rs are known mainly to counteract the pressor effect of AT_1R stimulation by enhancing GABAergic neurotransmission (AT_2Rs are located predominantly on GABAergic neurons; [76]).

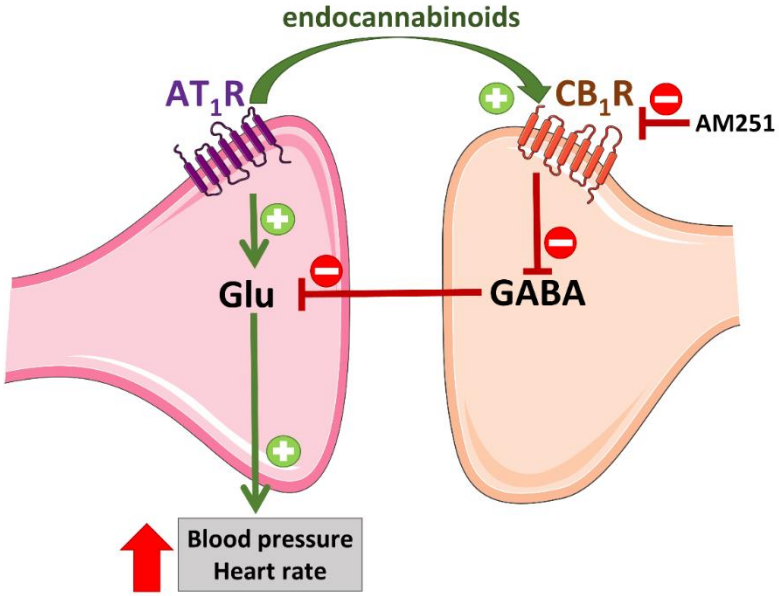

Figure 5. Potential mechanisms of the cross-talk between AT_1 receptors (AT_1Rs) and cannabinoid type 1 receptors (CB_1Rs) in the paraventricular nucleus of the hypothalamus. AT_1R activation increases blood pressure and heart rate due to a direct and indirectly mediated increase in glutamate (Glu) release. The indirect effect involves an inhibitory γ-aminobutyric acid (GABA) interneuron. In detail, AT_1R activation increases the release of endocannabinoids (mainly 2-arachidonoyl-glycerol) acting at presynaptic cannabinoid CB_1 receptors (CB_1Rs), activation of which decreases the inhibitory influence of GABA on sympathoexcitatory glutamatergic neurons. On the other hand, the CB_1R antagonist AM251 blocking presynaptic CB_1Rs increases the inhibitory influence of GABA on glutamatergic neurons excited by AT_1R activation, resulting in a decreased Ang II-induced pressor response. Facilitatory influences are shown by green arrows and plus signs, whereas inhibitory effects are represented by red bars and minus signs.

Interestingly, the mechanism related to the Ang II-induced transactivation of the cannabinoid-mediated effects is not restricted to the cardiovascular system. Thus, AM251 enhanced the Ang II-induced miniature excitatory postsynaptic currents (mEPSCs) in supraoptic magnocellular neurons from the supraoptic nucleus (SON), which is crucial for body fluid homeostasis (Table 2, Figure 4). The results suggest that eCBs released by Ang II modulate the excitatory synaptic inputs via presynaptic inhibitory CB_1Rs [56]. The authors based their conclusion on the results of their previous publication, in which they proved that CP55940 reduced the frequency of spontaneous mEPSCs in magnocellular neurosecretory neurones of the SON in a manner sensitive to AM251 [77]. On the other hand, there may be an alternative explanation for the interaction. Thus, CB_1Rs are pre-coupled receptors, and an inverse agonist like AM251 is expected to lead to an increase in mEPSCs even in the absence of eCBs. Thus, Ang II and AM251 may increase mEPSCs via two independent mechanisms, i.e., via agonism at facilitatory AT_1Rs and inverse agonism at inhibitory CB_1Rs, respectively.

Gyires et al. [57] showed that the gastroprotective effect of Ang II given intracerebroventricularly (i.c.v.) against ethanol-induced gastric lesions in conscious rats was reduced not only by the AT_1R antagonist candesartan but also by AM251 and the 2-AG synthesis inhibitor THL (all compounds were injected i.c.v.). Moreover, the beneficial influence of Ang II was absent in $CB_1^{-/-}$ knockout mice. The authors suggest that Ang II caused gastroprotection via stimulation of central AT_1Rs that led to the release of eCBs in a DAGL-dependent manner and the subsequent activation of CB_1Rs (Figure 4). CB_1Rs, like AT_1Rs, are known to exert beneficial effects on gastrointestinal function [57].

Shigemura et al. [58] proved that intraperitoneal (i.p.) administration of Ang II decreased the response of the gustatory nerve to NaCl both in the wild type and in $CB_1^{-/-}$ knockout mice. By contrast, the genetic deletion of CB_1Rs prevented the enhancement of the response to sweeteners induced by Ang II. Authors suggested that activation of AT_1Rs by Ang II stimulates 2-AG release, which may act as an autocrine enhancer for CB_1Rs on sweet taste cells (Table 2, Figure 4). The CB_1R-dependent enhancement of gustatory nerve responses to sweeteners induced by AEA or 2-AG had previously been demonstrated in mice by Yoshida et al. [78].

The authors of all the above publications argue that Ang II causes the release of eCBs (mainly 2-AG). Unfortunately, as shown in Table 2, this interesting hypothesis has been confirmed directly by determining eCB levels in cell culture and vessels only [33,36]. In the other studies it is based mainly on indirect proof using inhibitors of eCB synthesis and/or degradation and CB_1R antagonists.

3.2.2. Other Forms of Interaction between Angiotensin II and Cannabinoid-Mediated Effects

Numerous authors [43,47,48,53,54,63,64] argue that the interaction between CB_1 and AT_1 receptors they found is based on AT_1R-CB_1R heteromerization. However, this has so far been proven only in the

excellent study by Rozenfeld et al. [35], in which coimmunoprecipitation, resonance energy transfer assays, and receptor- and heteromer-selective antibodies were used. The authors demonstrated that $G_{\alpha q}$-coupled AT_1Rs and $G_{\alpha i}$-coupled-CB_1Rs form receptor heteromers in cells in which both receptors occur naturally (hepatic stellate cells [HSCs] isolated from ethanol-administered rats), and naturally (CB_1) and recombinantly (AT_1) (cultured Neuro 2A cells) (Table 2). The following results on cultured Neuro 2A cells co-expressing CB_1 and AT_1 receptors clearly confirm the existence of AT_1R-CB_1R heteromerization: (1) Ang II-induced an increase in ERK1/2 phosphorylation via $G_{\alpha i}$ (for CB_1Rs) instead of $G_{\alpha q}$ (for AT_1Rs), which was reduced by decreasing CB_1R levels by siRNA, and by rimonabant or THL; (2) the CB_1R agonist HU210 stimulated ERK1/2 phosphorylation only in the presence of a very low non-signaling concentration of Ang II; (3) an increase in intracellular Ca^{2+} levels is induced by activation of AT_1 but not CB_1 receptors, yet requires the presence of CB_1Rs since it was attenuated by siRNA targeting CB_1Rs; (4) the expression of AT_1Rs shifted the localization of CB_1Rs from an intracellular compartment to the plasma membrane. The physiological relevance of AT_1R-CB_1R heteromerization has been demonstrated in HSCs isolated from ethanol-treated rats and their controls. Firstly, the levels of CB_1Rs, AT_1Rs, and AT_1R-CB_1 heteromers were upregulated in ethanol-treated HSCs compared to controls. Secondly, the CB_1R antagonist rimonabant prevented Ang II-mediated mitogenic signaling and profibrogenic gene expression. The authors concluded that enhanced CB_1R expression in activated HSCs might affect AT_1R properties and contribute to the profibrogenic effect of Ang II.

Attenuation of the AT_1R-mediated effect by CB_1R blockade is likely for the postganglionic sympathetic neurons, and AT_1R-CB_1R heteromerization might explain this phenomenon. In general, sympathetic neurons are equipped with presynaptic inhibitory $G_{i/o}$ protein- and facilitatory G_q and G_s protein-coupled receptors. Although $G_{i/o}$ and G_s protein-coupled receptors in the mouse atrium act independently [79], this does not hold for the interplay between the $G_{i/o}$ and G_q protein-coupled receptors [79,80]. Thus, if the presynaptic inhibitory α_2-adrenoceptor (an example of a $G_{i/o}$ protein-coupled receptor) is blocked by an α-adrenoceptor antagonist, the AT_1R-mediated facilitation of noradrenaline release from the sympathetic neurons is impaired [79]. Facilitation can be restored if an agonist of another $G_{i/o}$ protein-coupled receptor is added, e.g., a δ-opioid receptor agonist or neuropeptide Y (acting via presynaptic Y_2 receptors). The same occurs if the presynaptic bradykinin (B_2) receptor is considered instead of the AT_1R [79]. In harmony with these findings, AT_1R (and B_2 receptor)-mediated facilitation of noradrenaline release is impaired in α_2-adrenoceptor knockout mice [80]. Unfortunately, the interaction between the presynaptic AT_1R and CB_1R has not been examined so far.

In a few studies, antagonists of CB_1Rs caused down-regulation of AT_1Rs and subsequently also the pathological effects elicited by Ang II. Such changes have been observed in vitro and in vivo following acute and chronic administration of CB_1R antagonists. Thus, in aortic VSMCs,

rimonabant and AM251 (but not the CB_2R antagonist SR144528) reduced the oxidative stress induced by Ang II. Moreover, blockade by CB_1Rs or their stimulation by CP55940 led to down- and up-regulation of AT_1Rs, respectively. The level of CB_1Rs was not affected by rimonabant in VSMCs ([41]; Table 1). Chronic administration of rimonabant for 7 weeks to apolipoprotein E-deficient ($ApoE^{-/-}$) mice on a cholesterol-rich diet did not prevent atherosclerotic plaque development, collagen content, and macrophage infiltration but, like in the above in vitro experiments, reduced oxidative stress and improved aortic endothelium-dependent vasodilation associated with the down-regulation of AT_1Rs [41].

Similarly, application of the CB_1R antagonist rimonabant or AM281 for 11 weeks or genetic deletion of CB_1Rs in mice reduced AT_1 and CB_1 receptor levels in the left cardiac ventricle and improved cardiac dysfunction in streptozotocin-induced diabetes ([43] Table 2). Moreover, treating obese Zucker rats with rimonabant for 12 months reduced the pressor response to acute i.v. injection of Ang II in comparison to vehicle-treated control animals. The CB_1R blockade also improved renal function and metabolic profile and increased the survival of obese Zucker rats ([49], Table 2). The authors suggested that rimonabant might reduce the vascular AT_1R expression. However, an indirect mechanism related to the decrease in cholesterol level should also be considered.

The paper by Jourdan et al. ([63]; Table 2) refers to the interaction between AT_1 and CB_1 receptors in kidneys. In an in vitro study on human podocytes, the peripheral CB_1R antagonist JD5037 and the AT_1R antagonist losartan diminished the Ang II-induced enhancement of AEA and 2-AG levels and the increased expression of both CB_1 and AT_1 receptors. These results are in line with in vivo observations on Zucker diabetic fatty rats with nephropathy, which had higher plasma levels of Ang II and aldosterone as well as up-regulated AT_1 and CB_1 receptors in the renal cortex in comparison with control lean rats [63]. Chronic administration of JD5037 for 3 months or losartan for 4 weeks prevented/attenuated the development of nephropathy and decreased the expression of AT_1 and CB_1 receptors in the renal cortex. Authors suggest that activation of renal CB_1Rs aggravates the deleterious effects of RAS activity. Indeed, in rats with streptozotocin-induced diabetes, dual 14-week therapy with the peripheral CB_1R antagonist AM6545 and the ACE inhibitor perindopril was more effective than monotherapies. Thus, single antagonists produced various beneficial effects but only combined treatment very markedly reduced albuminuria ([64]; for details, see Table 2). Importantly, diabetic nephropathy was associated with higher glomerular levels of CB_1 and AT_1 receptors. Both mono- and combined therapy failed to modify glomerular CB_1R expression but normalized AT_1R levels. Altogether, the studies by Jourdan et al. [63] and Barutta et al. [64] suggest that both RAS inhibition and CB_1 receptor blockade have a beneficial influence on the locally increased responsiveness to Ang II.

The studies by Mattace Raso et al. [50,51] and Franco-Vadillo et al. [44] document a beneficial and CB_1R-independent influence of two

cannabinoids on the harmful effects of Ang II (for details, see Table 1). A decrease in AT_1R densities was found in the heart [44], carotid and mesenteric arteries [51], and kidney [50] in response to chronic administration of CBD for 10 days to rats [44] or PEA for 5 weeks to hypertensive SHR and their normotensive WKY counterparts [50,51]. CBD or PEA treatment also reduced detrimental changes in cardiac [44], vascular and renal [50,51] tissues, and BP in SHR [50,51]. CBD and PEA show an overlap in their biochemical effects [81]. CBD is the major nonpsychoactive cannabinoid constituent of marijuana and has a low affinity for CB_1 and CB_2 receptors (Figure 2; [82]). PEA belongs to so-called endocannabinoid-like compounds with similar chemical structures to eCBs but is devoid of affinity for any CB receptor [8]. Apart from regulating the endocannabinoid system, CBD has a complex pharmacodynamic profile, including PPARγ [82]. The primary mechanism of action of PEA is its direct activation of PPARα receptors [81]. PPARγ agonists are known to decrease BP in humans, possibly through the suppression of the RAS, including the inhibition of AT_1R expression [83]. Similarly, activation of PPARα decreases the Ang II-mediated rise in BP [84]. Thus, PPARs may represent the intermediate link in the interaction between CBD, PEA, and RAS, although this hypothesis needs to be proven by further studies. Of course, other explanations of the influence of CBD and PEA on AT_1R densities are possible, especially when considering the pleiotropic action of CBD.

Beneficial effects against Ang II-induced fibrosis and inflammation in mouse heart, aorta, lung, kidney, and skin were obtained after chronic administration of EHP-101, an oral lipidic formulation of the novel non-psychotropic cannabidiol aminoquinone VCE-004.8, a dual agonist of CB_2R and PPARγ receptors ([45]; Table 2). Both properties may contribute to a functional antagonism against the detrimental consequence of AT_1R stimulation.

An opposite cross-talk, i.e., the ability of Ang II to reduce CB receptor densities, has been identified in brainstem and cerebellar astrocytes that play critical roles in hypertension and attention-deficit hyperactivity disorder, respectively, and in which activation of astroglial AT_1Rs and CB_1Rs has been determined to increase and to neutralize the inflammatory state, respectively ([59,85]; for details, see Table 2). Authors compared the effects of Ang II on CB_1Rs in astrocytes isolated from hypertensive SHR and normotensive WKY rats [59]; they found that basal expression of CB_1Rs in brainstem astrocytes was lower and that in cerebellar astrocytes was higher in SHR than in WKY rats. Ang II infusion further decreased (brainstem) and increased (cerebellum) CB_1R expression via AT_1Rs (brainstem) and both AT_1Rs and AT_2Rs (cerebellum). In further studies, Haspula and Clark [60] showed that stimulation of CB_1Rs by their potent agonist ACEA led to inhibition of AT_1R-mediated MAPK activation. On the other hand, Ang II caused phosphorylation and, probably, inactivation of CB_1Rs. Moreover, ACEA caused the neutralization of Ang II-mediated changes in IL-10 and IL-1β gene expression in astrocytes from both brain regions of SHR and WKY [61].

Interesting results were obtained by Shi et al. [52], who found that Ang II decreased the activity of the AEA transporter in cultured lymphocytes from WKY in a manner sensitive to the AT_1R antagonist losartan; this in vitro observation was confirmed under in vivo conditions in SHR and WKY (Table 2). Plasma Ang II and AEA levels were higher in SHR than in WKY. The higher AEA level probably resulted from the lower activity of the AEA transporter in SHR. Chronic administration of losartan not only dose-dependently reduced BP but also increased AEA transporter activity and diminished plasma AEA levels. The authors concluded that Ang II plays a critical role in mediating the decrease in AEA transporter activity in SHRs, probably via AT_1Rs [52].

Importantly, there are more and more examples that cannabinoids modulate ACE levels or activity. Wang et al. [62] were probably the first to show that the CB_2R agonist JWH133 increased ACE levels in Neuro-2a cells in a manner sensitive to the CB_2R antagonist AM630. ACE levels were also lower in hippocampus slices isolated from knockout $CB_2^{-/-}$ mice in comparison to the wild-type animals (Table 2). Further examples of the influence of cannabinoids on ACE and, in particular, on ACE2 are given in part 3.4.

3.2.3. Typology and Potential Therapeutic Significance of the Cross-Talk between (Endo) Cannabinoids and the Renin-Angiotensin System

A detailed analysis of the results shown in Table 2, based on 33 papers, reveals that the cross-talk between the (endo) cannabinoid and renin-angiotensin systems mainly occurs in the following two ways (a. and b.).

a. Stimulation of AT_1Rs by Ang II induces the release of eCBs (mainly 2-AG), which acts at CB_1Rs, thereby modifying the final effects of Ang II. eCB release by Ang II was either determined directly in various cultured cells [33,34], aortic VSMCs [36], and human podocytes [63], or indirectly in isolated blood vessels [36–41], isolated heart [42], or conscious rats [19,46] by studying the effects of inhibitors of eCB synthesis or degradation. CB_1R antagonists modified the effects induced by AT_1R activation both under in vitro conditions in cell culture [33,34], human podocytes [63], isolated blood vessels [36–39,41] isolated heart [42], magnocellular neurosecretory cells from the supraoptic nucleus [56], and under in vivo conditions in conscious and/or anaesthetized rats or mice [19,46,54,55,57,58].

Depending on the model (Table 2 and Figure 4), CB_1R blockade or genetic deletion of CB_1Rs may enhance or reduce the effects elicited by AT_1R activation. The final result of the cross-talk between AT_1 and CB_1 receptors depends on whether CB_1R activation induces an effect in the same or the opposite direction compared to AT_1R stimulation. In the case of opposite effects mediated via CB_1 and AT_1 receptors, CB_1R antagonists will increase the AT_1R-mediated effects. This holds for some blood vessels of rodents [36–39], the rat heart [42], and mEPSCs in magnocellular neurosecretory cells from the supraoptic nucleus of mice [56]. On the other hand, CB_1R antagonists will reduce the AT_1R-

mediated effects if CB_1 and AT_1 receptor activation elicit responses in the same direction (Table 2; Figure 4). This holds for the pressor effect of Ang II after its application into the PVN of the rat [53–55], the gastroprotective influence of Ang II given i.c.v. to rodents [57], and the responses to sweeteners in mice [58] (Table 2; Figure 4).

b. A decrease in AT_1R expression due to CB_1R antagonism has been shown less frequently. Thus, acute application of CB_1R antagonists reduced AT_1R levels in rodent VSMCs [41] and human podocytes [63], and their chronic administration diminished the AT_1R expression in the aorta of $ApoE^{-/-}$ mice [41], mouse heart [43], and kidney [63,64] isolated from diabetic mice and/or rats (Table 2; Figure 4). A decrease in AT_1Rs was also observed in response to chronic treatment of rats with cannabidiol (heart [44]) and PEA (kidney, arteries [44,51]).

So far, the practical relevance of the cross-talk between ECS and RAS for humans cannot be appreciated. In this respect, CB_1R antagonists are interesting and are discussed as future drug strategies for the treatment of diabetes [86,87], chronic kidney disease [88], fatty liver disease [89], alcohol use disorder [86], and COVID-19 [90]. The CB_1R antagonist rimonabant was used as an anti-obesity drug [15,86,91] but withdrawn from the market in 2008 because of severe central side effects [86]. Second-generation CB_1R antagonists (e.g., AM6545, JD5037), peripherally restricted, may offer advantages over rimonabant. In this context, detailed studies are necessary to clarify whether they increase vascular and cardiac harmful AT_1R-mediated responses or, by contrast, reduce the detrimental responses to AT_1R stimulation or expression of AT_1Rs. One should also keep in mind that FAAH and MAGL inhibitors, which might become future drugs for the treatment of pain, traumatic brain injury, multiple sclerosis, Parkinson's disease, anxiety-related disorders, and depression [8], may also modify the responses induced by AT_1R activation (Table 2).

3.3. Interaction between Angiotensin 1-7 and (Endo) Cannabinoid-Mediated Effects

So far, only two publications show that not only Ang II but also Ang 1-7 seems to modulate cannabinoid-related effects (Table 3). In the first, Brosnihan et al. [92] showed that the infusion of Ang 1-7 altered the endocannabinoid system in the decidualized uterus of pseudopregnant rats. A Low AEA level is preferred for fertilization, implantation, decidualization, and placentation, whereas a high AEA level causes embryotoxicity. Cannabinoid CB_1R activation impaired decidualization. The role of 2-AG and CB_2Rs is still not established in early pregnancy [92,93]. Chronic infusion of Ang 1-7 for 5 days caused an up-regulation of CB_1R, CB_2R, and MAGL mRNA in the decidualized uterine horn. However, as decidualization seems to be inhibited by activation of CB_1Rs, a higher expression level may result in implantation failure, spontaneous miscarriage, or preeclampsia [93].

In the second publication, we identified the cross-talk between MasRs for Ang 1-7 and CB_1Rs in the PVN of conscious hypertensive

SHR and their normotensive controls, WKY ([55]; Table 3). Microinjection of Ang 1-7 or the CB_1R agonist CP55940 into the PVN produced pressor responses, which were stronger in SHR than in WKY. (Note that CP55940 was microinjected after i.v. administration of the CB_1R antagonist AM251 to unmask the pressor effect of CB_1R activation in the PVN; [94]). The increase in BP induced by Ang 1-7 was not only prevented by the MasR antagonist A-779, but surprisingly reversed into a hypotensive response with AM251. On the other hand, the pressor response to CP55940 was reduced not only by AM251 injected into the PVN [94] but also by A-779. Additionally, A-779 decreased BP by itself, but only after the previous i.v. administration of AM251, suggesting a CNS-based interaction between eCBs acting on CB_1Rs with the endogenous pressor tone elicited by Ang 1-7.

In summary, two in vivo studies on rats suggest that a cross-talk between Ang 1-7 (and its MasRs) and the ECS appears to exist. So far, there are no results/suggestions regarding the mechanism (s) underlying this cross-talk and its potential therapeutic consequence (s).

Table 3. Examples of the cross-talk between the (endo)cannabinoid and angiotensin 1-7.

Species	Model	Basal Treatment (Concentration (μM) or Dose)	Effect	Intervention (Concentration (μM) or Dose)	Influence on the Agonist Effect	Final Conclusion of the Authors	References
rats	ovariectomized pseudopregnant	steroid treatment and bolus of oil leading to decidualization	uterus: ↓CB_1Rs, ↑CB_2Rs, ↔MAGL, ↓FAAH	Ang 1-7 (24 μg/kg/h; i.u. for 5 days)	uterus: ↑CB_1Rs, ↑CB_2Rs, ↑MAGL, ↔FAAH	Ang 1-7 augments the expression of CB_1Rs, CB_2Rs, and MAGL in the decidualized uterus and thus may interfere with early events of decidualization	[92]
rats **SHR** **WKY**	conscious (compounds microinjected into the PVN, doses in nmol/rat; if not stated otherwise)	Ang 1-7 (0.03) or CP55940 (0.1) + AM251 3 μmol/kg i.v.	both treatments: ↑BP stronger in SHR than in WKY	A-779 (3) AM251 (30)	↓pressor effect of Ang 1-7 and CP55940 ↓pressor effect of Ang 1-7	mutual interaction in the PVN between the CB_1Rs and the receptors for Ang 1-7 responsible for stimulation of the pressor response	[55]

↓—decrease; ↑—increase; ↔—no change; **Ang 1-7**, angiotensin 1-7; **BP**, blood pressure; **CB_1R**, cannabinoid receptor type 1; **CB_2R**, cannabinoid receptor type 2; **FAAH**, fatty acid amide hydrolase; **i.u.**, intrauterine; **i.v.**, intravenous; **MAGL**, monoacylglycerol lipase; **PVN**, paraventricular nucleus of the hypothalamus; **SHR**, spontaneously hypertensive rats; **WKY**, Wistar Kyoto rats.

3.4. Influence of Cannabinoids on ACE2 Activity and Other Parameters Relevant in the Fight against COVID-19

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection was rapidly spreading worldwide and led to over a hundred million detected infections and more than 6,200,000 deaths by 26 May 2022 (https://covid19.who.int/). Problems with vaccination of part of the population and mutations of the virus mean that SARS-CoV-2 infections may continue for many more years [26]; new promising therapies against COVID-19 are constantly being proposed. Recently, many studies have been published about the relationship between the RAS and the pathophysiology and severity of COVID-19. A key role is played by ACE2, which, however, represents a "double-edged sword" [26]. On the one hand, ACE2 is a SARS-CoV-2 entry receptor, and increased ACE2 expression may facilitate virus entry into cells [1–3]. On the other hand, viral binding reduces ACE2 levels at the cell surface, and the shift of the RAS balance towards the deleterious over the protective axis (increase in the ACE/ACE2 ratio) may lead to a progression of disease severity (Figure 3) [95,96]. High ACE/ACE2 ratios occur in males, geriatric, and smoking patients, and many pathologies (especially cardiovascular, pulmonary, and renal diseases and obesity) are recognized as comorbidities that may aggravate the COVID-19 infection. Low ACE/ACE2 ratios are found in women, physically trained individuals, and patients well-treated with ACE inhibitors. The beneficial properties of ACE2 can be achieved either by inhibiting the ACE/Ang II/AT_1R axis or by promoting the ACE2/Ang 1-7/MasR axis as a result of reducing the ACE/ACE2 ratio and/or by directly stimulating RAS anti-inflammatory components such as soluble ACE2, Ang 1-7 analogs, MasR, or AT_2R agonists (Figure 3).

Cannabinoids have been proposed as potential therapies or adjuvant drugs against the SARS-CoV-2 infection from the beginning of the COVID-19 pandemic because of their anti-inflammatory and immunomodulatory activities [4,90,97,98].

Two previous publications, which appeared before the onset of the COVID-19 pandemic, showed that modifying the (endo)cannabinoid system may modulate ACE activity/level (Table 2). Thus, chronic administration of the endocannabinoid-like compound PEA reduced ACE levels in the aorta and kidney of SHR, which were enhanced compared to the normotensive WKY [50]. Furthermore, the CB_2R agonist JWH133 increased the ACE level in N2a cells in a manner sensitive to the CB_2R antagonist AM630; the ACE level in hippocampus slices from $CB_2^{-/-}$ mice was lower than in their wild-type littermates [62]. Unfortunately, the promising observations have not been confirmed in conscious (mRen2)27 hypertensive rats (Table 2) and in human iPSC-cardiomyocytes infected with SARS-CoV-2 (Table 4). Thus, although chronic administration of rimonabant reduced BP and HR in hypertensive rats, the level of ACE was not affected [47]. Moreover, although the potent CB_1/CB_2R agonist WIN55212-2 inhibited cytotoxicity and the release of proinflammatory cytokines, it failed to affect ACE2 levels [99]. Concerning the latter study (and other studies

listed in Table 4), it is of interest that tocilizumab, an antibody acting against the receptor of the proinflammatory cytokine interleukin-6, represents an important therapeutic strategy for the treatment of severe forms of SARS-CoV-2 [100].

Table 4. Influence of cannabinoids on ACE2 activity and other relevant effects in the fight against COVID-19.

Model	Agonists	Effects	Final Conclusion of Authors	References
human iPSC-cardiomyocytes infected with SARS-CoV-2	WIN55212-2	↔ **ACE2 levels**; ↔viral infection and replication; ↓release of proinflammatory cytokines and cytotoxic damage	therapeutic potential of cannabinoids in protecting the heart against SARS-CoV-2 infection is not related to modification of ACE2 levels	[99]
in silico docking studies	CBD THC CBN	CBD: **hACE2** (↓), main virus protease activity ↓ THC: hACE2 and main virus protease (↓) CBN: inactive	THC and CBD might inhibit the SARS-CoV-2 infection via their influence on hACE2 and viral proteases	[101]
in silico docking studies	8 phyto-compounds derived from cannabis, including CBD, THC, and CVN	CBD and CVN showed the strongest potency in docking to **ACE2**, TMPRSS2, NRP1, IL-6, and TNF-α	CBD and CVN may be beneficial for the treatment of COVID-19 and post-COVID-19 neuronal symptoms	[102]
artificial 3D human models of oral, airway, and intestinal tissues treated with TNF-α and IFN-γ	13 high-CBD *Cannabis sativa* extracts	**↓ACE2** and TMPRSS2 in oral, lung, and intestinal epithelia constituting important routes of SARS-CoV-2 invasion	*Cannabis sativa* extracts may become a useful and safe addition to the prevention/treatment of COVID-19 as an adjunct therapy; the modulation of ACE2 levels may be an effective strategy for decreasing disease susceptibility	[103]

alveolar epithelial A549 cell line **macrophage cell line KG1**	extract from *Cannabis sativa* strain Arbel (CBD, CBG, THVC, and terpenes)	A549: ↓**ACE2** expression together with ↓IL-6, IL-8, CCL2 macrophage: ↑IL-6 and ↑IL-8 levels	further studies are needed to determine the therapeutic significance of cannabis in COVID-19 treatment due to its positive (A549 cells) and negative effects (macrophages)	[104]
human colon Caco-2 cell line	CBD	↓**ACE2** (concentration-dependent) ↑cell viability, ↓all proinflammatory markers	further studies are needed to clarify the consequences of ACE2 down-regulation and its impact on susceptibility to SARS-CoV-2	[105]
human lung fibroblast WI-38	high-CBD/low-THC cannabis extracts CBD	↓**ACE2**, TMPRSS, COX2, IL-6, and IL-8 ↓**ACE2**, TMPRSS	further studies are needed to identify the proper ratios of a combination of single ingredients to find an ideal formulation for future potential clinical studies/use	[31]
human H1299 lung adenocarcinoma cells	industrial hemp essential oil: E-caryophyllene and α-pinene were the prominent terpenes and CBDA was the main terpenophenol	↓gene expression of **ACE2** and TMPRSS2	hemp essential oils are promising agents to be further investigated with the final goal of optimizing their use in protective devices for counteracting the SARS-CoV-2 virus entry into the human host	[106]
Caco-2 **293T-ACE2** **Vero E6 cell lines**	extracts of hemp and isolates of specific cannabinoids: CBDA and CBGA	cannabinoid acids (CBDA and CBGA) lower SARS-CoV-2 entry into Vero E6 cells through spike binding	CBDA and CBGA (allosterically) block cellular entry of pseudovirus and live SARS-CoV-2 alpha variant B.1.1.7 and beta variant B.1.351	[107]

↓—decrease; (↓)—moderate decrease; ↑—increase; ↔—no change; **ACE2,** angiotensin-converting enzyme 2; **Caco-2**, human colorectal adenocarcinoma cell line; **CBD,** cannabidiol**; CBDA**, cannabidiolic acid; **CBG**, cannabigerol; **CBGA**; cannabigerolic acid; **CBN**, cannabinol; **CCL2,** C–C Motif Chemokine Ligands (CCLs) 2; **COX2,** cyclooxygenase 2; **COVID-19**; coronavirus disease 2019; **CVN,** cannabivarin**; hACE2,** human angiotensin-converting enzyme; **hiPSC-CMs**, human iPSC-derived cardiomyocytes; **IL-6**, interleukin-6; **IL-8**, interleukin-8; **NRP1**, neuropilin-1; **SARS-CoV-2**, severe acute respiratory syndrome coronavirus 2; **TMPRSS2**, transmembrane serine protease 2; **THC**, Δ^9-tetrahydrocannabinol; **TNF-α**, tumor necrosis factor α.

Research dedicated to a potential therapeutic role of cannabinoids in the fight against COVID-19 significantly increased from the beginning of the pandemic onward. As shown in Table 4, the results are related mainly to changes in ACE2 activity/level under treatment of various phytocannabinoids. Thus, in an in silico docking study, both CBD and THC revealed a moderate inhibitory effect against human ACE2 and a strong (CBD) and moderate (THC) inhibitory effect on the main virus protease [101]. In another in silico study, CBD and cannabivarin were the most potent among 8 compounds derived from the cannabis plant to inhibit ACE2 [102]. Moreover, CBD decreased ACE2 expression in human 3D tissue models of oral, airway, and intestinal tissues (that serve as routes for the invasion of SARS-CoV-2) [103] and in human cell lines ([31,105]; Table 4).

Cannabis contains cannabinoids, flavonoids, diterpenes, triterpenes, and lignans [107], which may show a synergistic, so-called "entourage" effect with CBD, i.e., the whole plant extract can have a higher potency and can be more beneficial than the individual compounds [31,108,109]. This putative entourage effect suggested previously for the therapeutic activity of CBD in COVID-19 infection (reviewed in [4]) does not appear to be mediated by direct CB_1R or CB_2R activation [110]. As shown in Table 3, high-CBD cannabis extracts downregulated ACE2 expression in 3D models of the oral cavity, intestinal, and lung epithelia [103]. Likewise, high-CBD/low-THC cannabis extracts [31], CBD-enriched Cannabis sativa Arbel strain extract [104], or industrial hemp oil containing terpenes and cannabidiolic acid (CBDA) and cannabigerolic acid (CBGA) [106,107] reduced ACE expression in cell lines [31,104,106]. Moreover, CBD-enriched extracts prevented infection of human epithelial cells by a pseudovirus expressing the SARS-CoV-2 spike protein and the entry of the SARS-CoV-2 alpha variant B.1.1.7 and the beta variant B.1.351 into cells at concentrations that are clinically achievable [107].

It should be highlighted that, as shown in Table 4, cannabis extracts, including various phytocannabinoids and terpenes, not only down-regulate ACE2 but can also suppress transmembrane serine protease 2 (TMPRSS2; another virus entry site into the cytoplasm of host cells) [31,103,106], main viral protease [101], neuropilin 1 (NRP1) [102], or inflammation-promoting agents such as IL-6 or IL-8 [31,102,104], CCL2 [104] or cyclooxygenase-2 [31]. However, one should keep in mind that CBD itself is characterized by a bell-shaped dose-response curve associated with a narrow therapeutic window, which makes its effective clinical use difficult. Accordingly, the phytocannabinoid formulations (e.g., CBD with CBG and THCV) may show better and safer activity because such treatment can prevent the bell-shaped dose-response typical for CBD [104].

To summarize the 9 papers in Table 4, cannabinoids in plant extracts may inhibit SARS-CoV-2 via different mechanisms, potentially leading to enhanced effectiveness compared to the individual compounds [4,31,107]. Unfortunately, proinflammatory effects may occur as well [31,103,104]. Thus, their potential therapeutic use in the fight against COVID-19 (e.g., as adjunct therapy) must be approached with caution and requires further research.

3.5. Pharmacokinetic Interactions between Drugs Acting on the (Endo)Cannabinoid and Renin-Angiotensin Systems

This review is dedicated to interactions between the (endo)cannabinoid and renin-angiotensin systems at the cellular level. However, if one considers the combined administration of drugs acting on either system, possible pharmacokinetic interactions should also be considered, i.e., one drug may interfere with the metabolism or the transport of another. Unexpectedly, this type of interaction escaped our PubMed-based search. In detail, THC, and, in particular, CBD, is known to interact with drug-metabolizing enzymes like CYP2C8/9 in the liver [111–115]. Moreover, they may affect transport proteins like the bile salt export pump important for losartan and telmisartan excretion. Third, they may, like ACE inhibitors, increase transaminase activity, thereby enhancing their side effects [114]. It is of interest that a wide range of over-the-counter marijuana and CBD-based products is now available, frequently characterized by an unknown CBD content (e.g., [4]). Thus, one should consider the possibility of unexpected pharmacokinetic interactions with RAS-based drugs against hypertension or heart failure.

In summary, strictly speaking, the interactions described in this section do not represent crosstalk between the ECS and the RAS but rather an interplay between individual drugs acting on either system. This interplay can be explained by the fact that the involved drugs, accidentally, share chemical properties, with the consequence that they are common substrates of metabolizing enzymes or transport mechanisms. The practical relevance is high. The concentration or half-time of one drug may be increased by the other, or side effects, e.g., on the liver, may be exaggerated.

4. Conclusions

During the past 15 years, there has been a noteworthy progression in the number of publications regarding the cross-talk between the (endo)cannabinoid and renin-angiotensin systems. One important mechanism is that AT_1R activation by Ang II leads to the release of eCBs (mainly 2-AG), which, by acting at CB_1Rs, modify the response to AT_1R stimulation. The interplay, which has been precisely determined in the case of various arteries, should also be examined in detail in other organs, especially in the heart (considered in a single paper only). Another major mechanism is that CB_1R antagonists diminish AT_1R levels. A third mechanism is that phytocannabinoids influence ACE2. Moreover, the following interesting observations shown so far only in very few publications require further studies, i.e., (1) the existence of a potential cross-talk between the protective axis of the RAS (Ang 1-7 or AT_2Rs) with components of the (endo)cannabinoid system, (2) the influence of Ang II on components of the endocannabinoid system like the levels of CB_1Rs, CB_2Rs or the AEA transporter and (3) the (patho)physiological significance of AT_1R-CB_1R heteromerization. Beyond the specific cross-talk between the RAS and the ECS, pharmacokinetic interactions have to be considered. They occur because single drugs acting on the RAS (telmisartan) or ECS (cannabidiol) accidentally share metabolizing enzymes or transport mechanisms.

The cross-talk may have consequences for drug therapy. (1) Thus, CB_1R antagonists, the therapeutic significance of which is suggested for

various diseases, may enhance (mainly vasoconstriction) or reduce (primarily effects mediated by the CNS) responses elicited by AT_1Rs. The final reaction depends on whether stimulation of AT_1Rs and CB_1Rs induces opposite or the same effects, and potentially beneficial though also detrimental consequences may occur. (2) Despite initially promising results indicating beneficial effects of phytocannabinoids against COVID-19 (especially as adjuvant therapy), additional studies are required to determine their potential therapeutic role and the underlying mechanism (s) in this disease. (3) Pharmacokinetic interactions of drugs acting on the RAS and ECS may lead to an increase in the concentration and half-time of one of the drugs, and to exaggerated side effects, e.g., in the liver.

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Rozdział 11. Oświadczenie autora rozprawy doktorskiej

Białystok, 07.06.2022

Krzysztof Mińczuk

Uniwersytet Medyczny w Białymstoku
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Oświadczenie autora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Mińczuk, K.; Schlicker, E.; Malinowska, B. Cross-Talk between CB_1, AT_1, AT_2 and Mas Receptors Responsible for Blood Pressure Control in the Paraventricular Nucleus of Hypothalamus in Conscious Spontaneously Hypertensive Rats and Their Normotensive Controls. Cells 2022, 11, 1542. https://doi.org/10.3390/cells11091542*

wchodzącej w skład mojej rozprawy doktorskiej polegał na opracowaniu koncepcji i planu badań oraz metod badawczych, prowadzeniu części eksperymentalnej badań, analizie statystycznej wyników i ich interpretacji oraz przygotowaniu manuskryptu artykułu, co określam jako 75% udziału w przygotowaniu wyżej wymienionej publikacji.

2. Mińczuk, K.; Baranowska-Kuczko, M.; Krzyżewska, A.; Schlicker, E.; Malinowska, B. Cross-Talk between the (Endo)Cannabinoid and Renin-Angiotensin Systems: Basic Evidence and Potential Therapeutic Significance. Int. J. Mol. Sci. 2022, 23, 6350. https://doi.org/10.3390/ijms23116350

wchodzącej w skład mojej rozprawy doktorskiej polegał na przeglądzie literatury, analizie i interpretacji danych oraz przygotowaniu manuskryptu artykułu, co określam jako 75% udziału w przygotowaniu wyżej wymienionej publikacji.

Mińczuk Krzysztof
Podpis autora rozprawy doktorskiej (czytelny)

B. Malinowska
Podpis promotora (czytelny)

**W przypadku każdej z włączonych do cyklu prac zaleca się złożenie oświadczenia przez autora wskazujące na jego merytoryczny oraz procentowy wkład w powstanie pracy [np. twórca hipotezy badawczej, pomysłodawca badań, wykonanie specyficznych badań (np. przeprowadzenie konkretnych doświadczeń, opracowanie i zebranie danych, wykonanie zestawień statystycznych itp.), wykonanie analizy wyników, przygotowanie manuskryptu artykułu, i inne]. Określenie wkładu autora powinno być na tyle precyzyjne, aby umożliwić dokładną ocenę jego udziału i roli w powstaniu każdej z prac.*

Rozdział 12. Oświadczenia współautorów rozprawy doktorskiej

Białystok, 07.06.2022

Barbara Malinowska

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Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

1. *Mińczuk, K.; Schlicker, E.; Malinowska, B. Cross-Talk between CB_1, AT_1, AT_2 and Mas Receptors Responsible for Blood Pressure Control in the Paraventricular Nucleus of Hypothalamus in Conscious Spontaneously Hypertensive Rats and Their Normotensive Controls. Cells 2022, 11, 1542. https://doi.org/10.3390/cells11091542*

wchodzącej w skład rozprawy doktorskiej Pana mgr inż. Krzysztofa Mińczuka polegał na współuczestniczeniu w opracowaniu koncepcji i planu badań oraz metod badawczych i interpretacji wyników badań oraz edytowaniu manuskryptu artykułu.*

2. Mińczuk, K.; Baranowska-Kuczko, M.; Krzyżewska, A.; Schlicker, E.; Malinowska, B. Cross-Talk between the (Endo)Cannabinoid and Renin-Angiotensin Systems: Basic Evidence and Potential Therapeutic Significance. Int. J. Mol. Sci. 2022, 23, 6350. https://doi.org/10.3390/ijms23116350

wchodzącej w skład rozprawy doktorskiej Pana mgr inż. Krzysztofa Mińczuka polegał na współuczestniczeniu w opracowaniu koncepcji manuskryptu oraz jego edytowaniu.*

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionych prac przez Pana mgr inż. Krzysztofa Mińczuka jako części rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

B. Malinowska

.....
Podpis (czytelny)

W przypadku prac dwu- lub wieloautorskich zaleca się złożenie oświadczenia **przez współautora wskazujące na jego merytoryczny (a NIE procentowy) wkład w powstanie pracy [np. twórca hipotezy badawczej, pomysłodawca badań, wykonanie specyficznych badań (np. przeprowadzenie konkretnych doświadczeń, opracowanie i zebranie danych, wykonanie zestawień statystycznych itp.), wykonanie analizy wyników, przygotowanie manuskryptu artykułu, i inne]. Określenie wkładu danego współautora powinno być na tyle precyzyjne, aby umożliwić dokładną ocenę jego udziału i roli w powstaniu każdej pracy.*

Rheinische Friedrich-Wilhelms-Universität

Institut für Pharmakologie und Toxikologie
Direktor: Prof. Dr. Alexander Pfeifer

Universitätsklinikum Bonn

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Venusberg-Campus 1

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To whom it may concern

Statement of the co-author

I declare that my contribution in the preparation of the publications:

1. *Mińczuk, K.; Schlicker, E.; Malinowska, B. Cross-Talk between CB_1, AT_1, AT_2 and Mas Receptors Responsible for Blood Pressure Control in the Paraventricular Nucleus of Hypothalamus in Conscious Spontaneously Hypertensive Rats and Their Normotensive Controls. Cells 2022, 11, 1542. https://doi.org/10.3390/cells11091542*

which forms part of the doctoral dissertation of Mr. Krzysztof Mińczuk is restricted to interpreting the results and revising the text prepared for submission;

2. *Mińczuk, K.; Baranowska-Kuczko, M.; Krzyżewska, A.; Schlicker, E.; Malinowska, B. Cross-Talk between the (Endo)Cannabinoid and Renin-Angiotensin Systems: Basic Evidence and Potential Therapeutic Significance. Int. J. Mol. Sci. 2022, 23, 6350. https://doi.org/10.3390/ijms23116350*

which forms part of the doctoral dissertation of Mr. Krzysztof Mińczuk consists of contributing to the conception of the manuscript and revising the text prepared for submission.

At the same time, I agree to submit the above-mentioned papers by Mr. Krzysztof Mińczuk as a part of his doctoral dissertation in the form of a thematically coherent series of papers published in scientific journals.

Eberhard Schlicker

Prof. Dr. Eberhard Schlicker

Białystok, 07.06.2022

Marta Baranowska-Kuczko

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Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

2. *Mińczuk, K.; Baranowska-Kuczko, M.; Krzyżewska, A.; Schlicker, E.; Malinowska, B. Cross-Talk between the (Endo)Cannabinoid and Renin-Angiotensin Systems: Basic Evidence and Potential Therapeutic Significance. Int. J. Mol. Sci. 2022, 23, 6350. https://doi.org/10.3390/ijms23116350*

wchodzącej w skład rozprawy doktorskiej Pana mgr inż. Krzysztofa Mińczuka polegał na współuczestniczeniu w opracowaniu koncepcji manuskryptu oraz jego edytowaniu.*

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Pana mgr inż. Krzysztofa Mińczuka jako części rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Marta Baranowska-Kuczko

.....

Podpis (czytelny)

W przypadku prac dwu- lub wieloautorskich zaleca się złożenie oświadczenia **przez współautora wskazujące na jego merytoryczny (a NIE procentowy) wkład w powstanie pracy [np. twórca hipotezy badawczej, pomysłodawca badań, wykonanie specyficznych badań (np. przeprowadzenie konkretnych doświadczeń, opracowanie i zebranie danych, wykonanie zestawień statystycznych itp.), wykonanie analizy wyników, przygotowanie manuskryptu artykułu, i inne]. Określenie wkładu danego współautora powinno być na tyle precyzyjne, aby umożliwić dokładną ocenę jego udziału i roli w powstaniu każdej pracy.*

Białystok, 08.06.2022

Anna Krzyżewska

Uniwersytet Medyczny w Białymstoku
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Oświadczenie współautora

Oświadczam, iż mój udział w przygotowaniu publikacji:

2. *Mińczuk, K.; Baranowska-Kuczko, M.; Krzyżewska, A.; Schlicker, E.; Malinowska, B. Cross-Talk between the (Endo)Cannabinoid and Renin-Angiotensin Systems: Basic Evidence and Potential Therapeutic Significance. Int. J. Mol. Sci. 2022, 23, 6350. https://doi.org/10.3390/ijms23116350*

wchodzącej w skład rozprawy doktorskiej Pana mgr inż. Krzysztofa Mińczuka polegał na współuczestniczeniu w pisaniu i edytowaniu manuskryptu.*

Jednocześnie wyrażam zgodę na przedłożenie wyżej wymienionej pracy przez Pana mgr inż. Krzysztofa Mińczuka jako części rozprawy doktorskiej w formie spójnego tematycznie cyklu prac opublikowanych w czasopismach naukowych.

Anna Krzyżewska
.....
Podpis (czytelny)

W przypadku prac dwu- lub wieloautorskich zaleca się złożenie oświadczenia **przez współautora wskazujące na jego merytoryczny (a NIE procentowy) wkład w powstanie pracy [np. twórca hipotezy badawczej, pomysłodawca badań, wykonanie specyficznych badań (np. przeprowadzenie konkretnych doświadczeń, opracowanie i zebranie danych, wykonanie zestawień statystycznych itp.), wykonanie analizy wyników, przygotowanie manuskryptu artykułu, i inne]. Określenie wkładu danego współautora powinno być na tyle precyzyjne, aby umożliwić dokładną ocenę jego udziału i roli w powstaniu każdej pracy.*

Rozdział 13. Zgoda Komisji Bioetycznej lub Lokalnej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach

UCHWAŁA NR 77/2019

z dnia 29.10.2019 r.

Lokalnej Komisji Etycznej do spraw doświadczeń na zwierzętach w Olsztynie

§ 1

Na podstawie art. 48 ust. 1 pkt. 1 / ~~art. 48 ust. 1 pkt. 2~~[1] ustawy z dnia 15 stycznia 2015r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych (Dz. U. poz. 266), zwanej dalej „ustawą" po rozpatrzeniu wniosku pt.: **„Interakcja pomiędzy mechanizmami kannabinoidowymi i angiotensynowymi w jądrze przykomorowym podwzgórza a regulacja ciśnienia krwi u szczurów z nadciśnieniem pierwotnym"** z dnia 15.10.2019 r., złożonego przez **Uniwersytet Medyczny w Białymstoku, Wydział Farmaceutyczny z Oddziałem Medycyny Laboratoryjnej (0019)**, adres: **ul. A. Mickiewicza 2D, 15-222 Białystok**, zaplanowanego przez **Barbara Malinowska**[2], przy udziale[3] (nie dotyczy) Lokalna Komisja Etyczna:

WYRAŻA ZGODĘ[4]

Na przeprowadzenie doświadczeń na zwierzętach w zakresie wniosku **75/2019**.

§ 2

W wyniku rozpatrzenia wniosku o którym mowa w § , Lokalna Komisja Etyczna ustaliła, że:

1. Wniosek należy przypisać do kategorii: **Badania podstawowe (A), kategoria: sercowo-naczyniowy układ krążenia krwi i limfy.**
2. Najwyższy stopień dotkliwości proponowanych procedur to: **umiarkowana.**
3. Doświadczenia będą przeprowadzane na gatunkach lub grupach gatunków: **125 szt., szczur wędrowny (*Rattus norvegicus*), szczep SHR/NHsd. 125 szt., szczur wędrowny (*Rattus norvegicus*), szczep WKY/NCrl; wszystkie szczury: 9-10 tygodni, 220-250 g.**
4. Doświadczenia będą przeprowadzane przez: **Malinowska Barbara, Mińczuk Krzysztof, Toczek Marek, Malinowska Irena.**
5. Doświadczenie będzie przeprowadzane w terminie[5] **od 15.11.2019 do 31.12.2021**.
6. Doświadczenie będzie przeprowadzone w ośrodku[6]: **nie dotyczy**
7. Doświadczenie będzie przeprowadzone poza ośrodkiem w: **nie dotyczy**
8. Użyte do procedur zwierzęta dzikie zostaną odłowione przez ....., w sposób: nie dotyczy
9. Doświadczenie **nie zostanie** poddane ocenie retrospektywnej.

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[1] Niewłaściwy zapis usunąć
[2] imię i nazwisko osoby, która zaplanowała i jest odpowiedzialna za przeprowadzenie doświadczenia
[3] Wypełnić w przypadku dopuszczenia do postępowania organizacji społecznej.
[4] Niewłaściwy zapis usunąć
[5] Nie dłużej niż 5 lat
[6] Podać jeśli jest to inny ośrodek niż użytkownik

§ 3

Uzasadnienie: Po dokonaniu oceny wniosku zgodnie z art. 47 ust. 1 i 2 ustawy z dnia 15 stycznia 2015 r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych (Dz. U. poz. 266) Lokalna Komisja Etyczna w Olsztynie stwierdza, że projekt nie budzi zastrzeżeń pod względem celowości jego wykonania, liczby użytych zwierząt oraz zasadności i klasyfikacji procedur objętych wnioskiem i wyraża zgodę na przeprowadzenie doświadczenia. Osobą odpowiedzialną za przeprowadzenie badań zgodnie z procedurami opisanymi we wniosku jest **Barbara Malinowska.**

§ 4

Integralną część niniejszej uchwały stanowi kopia wniosku, o którym mowa w § 1.

(Pieczęć lokalnej komisji etycznej)

UNIWERSYTET WARMIŃSKO-MAZURSKI
w Olsztynie
LOKALNA KOMISJA ETYCZNA
do Spraw Doświadczeń na Zwierzętach
10-718 Olsztyn, ul. Oczapowskiego 13/4

Podpis przewodniczącego komisji

PRZEWODNICZĄCY
Lokalnej Komisji Etycznej
do Spraw Doświadczeń na Zwierzętach
prof. dr hab. Jerzy Juśkiewicz

Pouczenie:

Zgodnie z art. 33 ust. 3 i art. 40 ustawy w zw. z art. 127 § 1 i 2 oraz 129 § 2 ustawy z dnia z dnia 14 czerwca 1960 r. Kodeks postępowania administracyjnego (Dz. U. 2017, poz. 1257 – t.j.; dalej KPA) od uchwały Lokalnej Komisji Etycznej strona może wnieść, za jej pośrednictwem, odwołanie do Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w terminie 14 od dnia doręczenia uchwały.

Na podstawie art. 127a KPA w trakcie biegu terminu do wniesienia odwołania strona może zrzec się prawa do jego wniesienia, co należy uczynić wobec Lokalnej Komisji Etycznej, która wydała uchwałę. Z dniem doręczenia Lokalnej Komisji Etycznej oświadczenia o zrzeczeniu się prawa do wniesienia odwołania przez ostatnią ze stron postępowania, decyzja staje się ostateczna i prawomocna.

Otrzymuje:

1) Użytkownik,
2) Organizacja społeczna dopuszczona do udziału w postępowaniu (jeśli dotyczy)
3) a/a

Użytkownik kopie przekazuje: Osoba planująca doświadczenie; Zespół ds. dobrostanu.

Rozdział 14. Dorobek naukowy

Łączna wartość Impact Factor: 46,427

Łączna liczba punktów MEiN: 1080

Lista publikacji stanowiących rozprawę doktorską:

1. **Mińczuk, K**., Schlicker, E., Malinowska, B. Cross-Talk between CB_1, AT_1, AT_2 and Mas Receptors Responsible for Blood Pressure Control in the Paraventricular Nucleus of Hypothalamus in Conscious Spontaneously Hypertensive Rats and Their Normotensive Controls. Cells 2022, 11, 1542. https://doi.org/10.3390/cells11091542
   IF = 6,600; MEiN = 140 pkt

2. **Mińczuk, K**., Baranowska-Kuczko, M., Krzyżewska, A., Schlicker, E., Malinowska, B. Cross-Talk between the (Endo)Cannabinoid and Renin-Angiotensin Systems: Basic Evidence and Potential Therapeutic Significance. Int. J. Mol. Sci. 2022, 23, 6350. https://doi.org/10.3390/ijms23116350
   IF = 5,924; MEiN = 140 pkt

Wykaz innych publikacji naukowych:

1. Li, J., Kemp, B. A., Howell, N. L., Massey, J., **Mińczuk, K**., Huang, Q., Chordia, M. D., Roy, R. J., Patrie, J. T., Davogustto, G. E., Kramer, C. M., Epstein, F. H., Carey, R. M., Taegtmeyer, H., Keller, S. R., Kundu, B. K. Metabolic Changes in Spontaneously Hypertensive Rat Hearts Precede Cardiac Dysfunction and Left Ventricular Hypertrophy. Journal of the American Heart Association, 2019, 8, e010926. https://doi.org/10.1161/JAHA.118.010926
   IF = 4.605; MEiN = 140

2. Molinos, C., Sasser, T., Salmon, P., Gsell, W., Viertl, D., Massey, J. C., **Mińczuk, K.**, Li, J., Kundu, B. K., Berr, S., Correcher, C., Bahadur, A., Attarwala, A. A., Stark, S., Junge, S., Himmelreich, U., Prior, J. O., Laperre, K., Van Wyk, S., Heidenreich, M. Low-Dose Imaging in a New Preclinical Total-Body PET/CT Scanner. Frontiers in medicine, 2019, 6, 88. https://doi.org/10.3389/fmed.2019.00088
   IF = 3,297; MEiN = 70

3. Huang, Q., Massey, J. C., **Mińczuk, K.**, Li, J., Kundu, B. K. Non-invasive determination of blood input function to compute rate of myocardial glucose uptake from dynamic FDG PET images of rat heart in vivo: comparative study between the inferior vena cava and the left ventricular blood pool with spill over and partial volume corrections. Physics in medicine and biology, 2019, 64, 165010. https://doi.org/10.1088/1361-6560/ab3238
   IF = 2,883; MEiN = 100

4. Li, J., **Mińczuk, K.**, Massey, J. C., Howell, N. L., Roy, R. J., Paul, S., Patrie, J. T., Kramer, C. M., Epstein, F. H., Carey, R. M., Taegtmeyer, H., Keller, S. R., Kundu, B. K. Metformin Improves Cardiac Metabolism and Function, and

Prevents Left Ventricular Hypertrophy in Spontaneously Hypertensive Rats. Journal of the American Heart Association, 2020, 9, e015154. https://doi.org/10.1161/JAHA.119.015154
IF = 5,501; MEiN = 140

5. Massey, J. C., Seshadri, V., Paul, S., **Mińczuk, K**., Molinos, C., Li, J., Kundu, B. K. Model Corrected Blood Input Function to Compute Cerebral FDG Uptake Rates From Dynamic Total-Body PET Images of Rats in vivo. Frontiers in medicine, 2021, 8, 618645. https://doi.org/10.3389/fmed.2021.618645
IF = 5,093; MEiN = 70

6. Krzyżewska, A., Baranowska-Kuczko, M., **Mińczuk, K**., Kozłowska, H. Cannabinoids-A New Perspective in Adjuvant Therapy for Pulmonary Hypertension. International journal of molecular sciences, 2021, 22, 10048. https://doi.org/10.3390/ijms221810048
IF = 5,924; MEiN = 140

7. Weresa, J., Pędzińska-Betiuk, A., **Mińczuk, K.**, Malinowska, B., Schlicker, E. Why Do Marijuana and Synthetic Cannabimimetics Induce Acute Myocardial Infarction in Healthy Young People?. Cells, 2022, 11, 1142. https://doi.org/10.3390/cells11071142
IF = 6,600; MEiN = 140

Wykaz doniesień zjazdowych:

1. Toczek, M., Grzęda, E., **Mińczuk, K**., Kossakowski, R., Malinowska, B. Wpływ jednorazowego podania inhibitora rozkładu endokannabinoidów URB597 na układ krążenia u szczurów z nadciśnieniem DOCA-salt. XXII Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego oraz Komitetu Nauk Fizjologicznych i Farmakologicznych Polskiej Akademii Nauk, Gdańsk, 26-28 października 2017.

2. **Mińczuk, K.**, Li, J., Massey, J., Roy, J., Soumen, P., Patrie, J., Carey, R., Taegtmeyer, H., Keller, S., Kundu, B., Malinowska, B. Metformina poprawia metabolizm, funkcje i zapobiega zmianom strukturalnym w sercach szczurów SHR. XXIV Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego, Tomaszowice k. Krakowa, 28-30.11.2019 (I miejsce za najlepszy poster).

3. **Mińczuk, K.**, Malinowska, B. The CB_1 receptor antagonist reduces the pressor response of angiotensin II and angiotensin 1-7 injected into paraventricular nucleus of the hypothalamus in conscious normotensive rats. 28th Congress of the Polish Physiological Society. Gdansk, Poland (Online). September 15-17, 2021.

4. **Mińczuk, K.**, Malinowska, B. Receptory kannabinoidowe CB_1 modyfikują odpowiedź presyjną Ang II i Ang 1-7 podanych do jądra przykomorowego podwzgórza u czuwających szczurów z nadciśnieniem pierwotnym i normotensyjnych. XXV Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego oraz Komitetu Nauk Fizjologicznych i Farmakologicznych PAN, Kazimierz Dolny, 2021.10.21-23.

5. Remiszewski, P., Pędzińska-Betiuk, A., **Mińczuk, K**., Weresa, J., Krzyżewska, A., Malinowska, B. Effects of peripheral cannabinoid cb1 receptor inverse agonist jd5037 in mono- and polytherapy with metformin in a monocrotaline-induced rat model of pulmonary arterial hypertension. 28th Congress of the Polish Physiological Society. Gdansk, Poland (Online). September 15-17, 2021.

6. Remiszewski, P., Pędzińska-Betiuk, A., **Mińczuk, K**., Weresa, J., Krzyżewska, A., Malinowska, B. Combined AMPK activation and CB_1 receptor blockade as a new target in pulmonary arterial hypertension treatment. 3rd Baltic Pulmonary Hypertension and Circulation Conference. Tallinn, Estonia, 01.10.2021.

Wykaz innych aktywności naukowych (np.: granty, staże naukowe, nagrody naukowe):

Wyjazd do USA w ramach programu BioLAB, koordynowanego przez Komisję Fulbrighta: roczny staż w Zakładzie Radiologii i Obrazowania Medycznego w University of Virginia, Charlottesville (lipiec 2018 – wrzesień 2019).