



REVIEW

Cannabinoid receptor 1 signaling in cardiovascular regulating nuclei in the brainstem: A review



Badr M. Ibrahim, Abdel A. Abdel-Rahman *

Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC 27858, USA

ARTICLE INFO

Article history:

Received 24 December 2012
Received in revised form 11 March 2013
Accepted 26 March 2013
Available online 3 April 2013

Keywords:

Cannabinoids
ERK1/2
nNOS
PI3K
Cardiovascular
RVLM

ABSTRACT

Cannabinoids elicit complex hemodynamic responses in experimental animals that involve both peripheral and central sites. Centrally administered cannabinoids have been shown to predominantly cause pressor response. However, very little is known about the mechanism of the cannabinoid receptor 1 (CB₁R)-centrally evoked pressor response. In this review, we provided an overview of the contemporary knowledge regarding the cannabinoids centrally elicited cardiovascular responses and the possible underlying signaling mechanisms. The current review focuses on the rostral ventrolateral medulla (RVLM) as the primary brainstem nucleus implicated in CB₁R-evoked pressor response.

© 2013 Cairo University. Production and hosting by Elsevier B.V. All rights reserved.

Cannabinoids

Cannabinoids are heterogeneous group of compounds that target cannabinoid receptors: CB₁ and CB₂. These compounds include the naturally occurring Δ^9 -tetra-hydrocannabinol (Δ^9 -THC), isolated from the plant *Cannabis sativa* (marijuana), endogenous compounds known as endocannabinoids (ECs), as well as other synthetic compounds. Since at least 2000 B.C., the plant *Cannabis* has been long used for recreational

* Corresponding author. Tel.: +1 252 744 3470; fax: +1 252 744 3203.
E-mail address: ABDELRAHMANA@ecu.edu (A.A. Abdel-Rahman).

Peer review under responsibility of Cairo University.



Production and hosting by Elsevier

and medical purposes. Δ^9 -THC, Cannabidiol (CBD), and cannabinol are the most abundant natural cannabinoids active at CB₁ and CB₂ receptors, but only Δ^9 -THC has an equal affinity for both CB₁ and CB₂ receptors [1,2]. The first endogenous ligand for both cannabinoid receptors [2], anandamide, is a derivative of arachidonic acid (arachidonoyl ethanolamide; AEA), which was isolated from pig brain in 1992 [3], and 2-arachidonoyl glycerol (2-AG) is another abundant ECs [4]. Most of the endogenous cannabinoids discovered so far are agonists except the inverse agonist virodhamine [5]. The high affinity non-eicosanoid cannabinoids CP55940 and the amino-alkyl-indole cannabinoid WIN55,212-2 were developed by Pfizer and Sterling Winthrop, respectively. SR141716A and AM251 are selective antagonists for the CB₁R, while SR144528 is selective for the CB₂R [2,6]. Notably, most of the synthetic compounds are highly lipophilic and water insoluble except for O-1057, which is highly water soluble and possesses comparable potency as CP55940 [7]. Hemopressin, a

short peptide identified in rat brain, has been recently categorized as inverse cannabinoid agonist [8,9].

Cannabinoid receptor 1

It is now known that cannabinoids exert their actions mainly via two subtypes of G-protein-coupled receptors (GPCRs): CB₁ and CB₂. Additional non-CB₁, non-CB₂ established GPCRs, such as GPR55 and GPR18, are also targeted by these compounds (e.g. anandamide, virodhamine, CP559440, and AM251 but not WIN55,212-2) [10–14]. Our review focuses on the CB₁R, which is found primarily in the CNS, including the cardiovascular regulatory nuclei in the brainstem. The CB₁ receptor, a 473-amino-acid protein, was first cloned from a rat cerebral cortex cDNA library [15] and a human brainstem library [16], which maintains the essential topographical features for a G-protein-coupled receptor (GPCR) of (i) seven hydrophobic transmembrane domain regions that extend through the plasma membrane; (ii) three extracellular loops; (iii) three intracellular loops; (iv) an extracellular N-terminal; (v) and an intracellular C-terminal [17].

CB₁R signaling

Activation of CB₁R triggers several downstream effectors including inhibition of adenylyl cyclase, stimulation of inwardly rectifying potassium channels, inhibition of N- and P/Q-type voltage-dependent calcium channels, and activation of mitogen-activated protein kinase (MAPK) pathway. Cannabinoids acting via CB₁R reduce cAMP production by inhibiting adenylyl cyclase [18–20] which is antagonized by cannabinoid antagonists SR141716A and LY320135 [21]. These effects are mediated via inhibitory G-protein (G $\alpha_{i/o}$) because they were blocked by G $\alpha_{i/o}$ -selective pertussis toxin in mammalian brain and in cultured neuronal cells [18–20]. Many other CB₁R-mediated physiological functions are G-protein G $\alpha_{i/o}$ mediated [19,22,23]. However, the diverse, sometimes opposing, CB₁R-evoked physiological functions that are not completely attributable to simply lowering intracellular cAMP levels, have led to investigations of the role of other non-G $\alpha_{i/o}$ signaling mechanisms [24]. In this line, recent studies have linked CB₁R coupling to activation of G $\alpha_{q/11}$ or G α_s . It is possible that heterodimerization between the CB₁R and other receptor(s) contribute, at least partly, to this divergent signal transduction. This notion is supported by the reported interaction between CB₁R and other co-localized receptors e.g. dopamine D₂R, which resulted in accumulation of cAMP [25,26]. Second, CB₁R behaves as a G $\alpha_{q/11}$ -G-protein-coupled receptor in cultured hippocampal neurons and trabecular meshwork cells [24,27]. Further, the findings that heterodimerization between CB₁R and OX₁R resulted in enhanced G $\alpha_{q/11}$ -dependent OX₁R signaling in presence of CB₁R [28].

Retrograde CB₁R-mediated signaling

CB₁R is located mostly presynaptically, thus playing crucial roles in controlling the release of neurotransmitters at both excitatory and inhibitory synapses. Upon depolarization, the postsynaptically released endocannabinoids activate presynaptic CB₁R, which in turn modulates the release of various

neurotransmitters [23,29]. For example, WIN55,212-2 inhibited GABA release from presynaptic terminals in cultured hippocampal or ventromedial medulla (RVM) neurons following postsynaptic depolarization [30,31]. The latter effect was completely abolished in presence of selective CB₁ receptor antagonists. This phenomenon is termed depolarization-induced suppression of inhibition (DSI). Findings from cerebellar Purkinje cells support the possibility that postsynaptically released endocannabinoids act as retrograde secondary messengers at both inhibitory as well as excitatory synapses because following depolarization, the released endocannabinoids, which stimulate presynaptic CB₁R, ultimately suppress presynaptic calcium-induced glutamate release [32]. The latter phenomenon is termed depolarization-induced suppression of excitation or (DSE). Both CB₁R mediated DSE and DSI are considered key mechanisms for many of the central effects of endogenous and exogenous cannabinoids.

Cardiovascular effects of cannabinoids

The cardiovascular responses to cannabinoids are complex and are dependent on the state of the studied animals (conscious vs. anaesthetized) and the route of administration (systemic vs. central) [33–38].

Systemic CB₁R-evoked cardiovascular effects

In anesthetized animals, systemically administered cannabinoids elicit predominantly hypotension and bradycardia. These effects are mediated peripherally through prejunctional inhibition of sympathetic outflow and vagal stimulation resulting in reduction in BP and HR, respectively [39–42]. Systemic administration of THC, anandamide, or WIN55,212-2 elicited tri-phasic effects on BP in anesthetized rats: (i) an initial brief hypotensive phase, secondary to a bradycardic response, which was blocked by atropine pretreatment or vagotomy; (ii) a transient pressor response due to direct vasoconstriction; (iii) a more predominant depressor phase. The prolonged depressor phase was mediated via peripheral sympathoinhibition because it was attenuated by cervical spinal transection and blockade of α -adrenoceptors [39–43]. Interestingly, recent studies have suggested that, in addition to the direct vasoconstrictor action discussed above, the transient pressor response evoked by systemic cannabinoids in anaesthetized animals might involve central mechanisms [44,45]. However, the cardiovascular responses of systemically administered cannabinoids in conscious animals are quite different. The prolonged depressor response (phase III) is absent following systemically injected anandamide or WIN55,212-2 which, in contrast, cause predominate pressor responses along with bradycardia in conscious rats [36,37]. The elicited pressor response by systemic WIN55,212-2 in conscious animals is centrally mediated because it was attenuated by ganglion blockade [37]. Importantly, in humans, acute administration of cannabinoid is associated with tachycardia and a pressor response [46–48].

Central CB₁R-evoked cardiovascular effects

Centrally administered cannabinoids predominantly elicit sympathoexcitation/pressor responses. Studies have elucidated the

involvement of various brainstem nuclei in the cardiovascular responses elicited by central CB₁R activation, e.g. Nucleus Tractus Solitarius (NTS) and the rostral ventrolateral medulla (RVLM) [39,49–52].

The NTS

The NTS is located in the brainstem flanked on each side of the fourth ventricle and consists of groups of cells in a column-like structure dorsal to the RVLM and represents the first relay station in the baroreflex arc. Upon stimulation, the NTS elicits a reduction in the BP, HR, and sympathetic outflow [53,54]. The most cardiovascular-relevant part of the NTS is located at the most caudal part of the NTS, which contains synapses from chemo and aortic baroreceptor processes that contact with secondary order neurons within the NTS [55,56]. The latter communicate either directly or indirectly through third order neurons with other nuclei including RVLM, hypothalamus or CVLM [57–60]. Functionally, activation of cardiovascular afferents (chemo or baroreceptors) enhances the release of excitatory amino-acid L-glutamate within the NTS [54], which prompts the excitation of NTS-projections to other baroreflex arc nuclei e.g. RVLM and CVLM. Several reports have shown important roles for activation of CB₁R in the NTS in blood pressure regulation [50–52,61]. For examples, activation of NTS cannabinoid receptors by anandamide enhanced baroreflex-mediated sympathoinhibition, at least partly, via presynaptic inhibition of GABA release [52,62].

The RVLM

In this review, attention has been focused on the RVLM, which plays pivotal role in central control of cardiovascular function [63–65]. The RVLM is the final supraspinal site within the central nervous system that integrates multitudes of influences on blood pressure (BP) from higher brain regions such as paraventricular nucleus, lateral hypothalamus, and periaqueductal gray [64,66]. The RVLM is of high significance in controlling BP since bilateral lesioning of the RVLM leads to a profound fall in BP [59]. The RVLM is located in the ventral part of the brainstem, lateral to the inferior olive, caudal to the facial nucleus, and ventral to the nucleus ambiguus [59,67]. It is heterogeneous in composition and contains multiple cell groups that are different in their neurochemical phenotype (e.g. rostroventrolateralis, gigantocellular nucleus, and paragigantocellularis lateralis [68–71]). Within the RVLM, the adrenergic group C1 neurons, alternatively known as adrenergic neurons, are defined based on their expression of phenylethanolamine-n-methyltransferase (PNMT) [72,73]. The rostral C1 subgroup contains barosensitive neurons which project to the spinal cord [74,75] and provides tonic excitatory inputs to the sympathetic preganglionic neurons [76,77]. Beside catecholamine-containing neurons in RVLM [78], a wide variety of neurotransmitters and receptors are present in the RVLM including substance P [79], neuropeptide Y [80], enkephalin [80,81], adenosine receptors (A_{2A}) [82], P₂X receptors [83], Angiotensin II AT₁ receptors [84], imidazoline I₁

receptors [85,86], α_{2A} adrenergic receptors [87,88], cannabinoid CB₁ receptors [89,90], CB₂ receptors [91], and mu-opioid receptors [92,93]. The RVLM is a crucial brainstem nucleus for the tonic generation of sympathetic nerve activity [59,60]. Activation of specific neurons within the RVLM causes an increase in BP by increasing peripheral resistance and cardiac output via released catecholamines [94–97]. In addition to cardiovascular control, specific neurons within the RVLM are involved in nociception [98,99] and breathing [100]. Intracisternal (i.c.) administration [101–103] or intra-RVLM microinjection [90,104] of cannabinoids such as WIN55,212-2 or CP-55940 elicited a pressor response and caused increases in sympathetic nerve activity, plasma norepinephrine and blood pressure, in conscious and anesthetized animals, and these responses were attenuated by pretreatment with the CB₁R antagonists SR171416A or AM251. The significant increase in tyrosine hydroxylase immunoreactive neurons (TH-ir) expressing c-Fos, a marker of neuronal activity, following i.c. WIN55,212-2 provided direct *in vivo* evidence that central CB₁R-evoked pressor response involves activation of RVLM-catecholaminergic neurons [102], which was abrogated by CB₁R antagonist AM251.

Centrally elicited hemodynamic effects of CB₁R in conscious Sprague Dawley rats

In our recent studies, we sought to elucidate the mechanisms implicated in the central CB₁R-evoked sympathoexcitation/pressor response [102,104,105]. In pursuit of this goal, we characterized the centrally mediated cardiovascular effects of central CB₁R activation in conscious Sprague Dawley rats. We have confirmed the expression of CB₁R (protein) in the RVLM by detecting the two bands at 64 and 53 kDa, which represent the N-glycosylated and non-glycosylated forms of CB₁R, respectively (unpublished data) [106].

We reported that i.c. administration of WIN55,212-2 elicited dose-dependent pressor responses and increased NE plasma levels, denoting an increase in central sympathetic tone in conscious rats [102], which agrees with findings in experimental animals discussed above [39,101,103], and reflects similar responses observed in humans [47,48]. Similar pressor response was observed following microinjection of WIN55,212-2, for the first time, in the RVLM of conscious freely moving rats [104]. These studies were conducted in conscious rats to circumvent the negative impact of anesthesia that was shown to dramatically compromise cannabinoid-evoked hemodynamic responses [36–38].

We demonstrated in our studies that the cardiovascular, biochemical, and molecular responses elicited by WIN55,212-2 were CB₁R mediated. This is important because (i) WIN55,212-2, which is routinely used in cannabinoid research, can also bind to CB₂R [107,108]; (ii) both CBR subtypes are expressed in the brain [89,109], including the brainstem [90]. The ability of the selective CB₁R antagonist AM251 [39,101,103] to virtually abolish the pressor, biochemical and neurochemical responses elicited by i.c. WIN55,212-2 clearly implicates the CB₁R in the observed responses. It is important to note, however, that the lack of change in blood pressure, as well as other neurochemical

responses, following AM251 administration argues against the involvement of central CB₁R signaling in tonic control of blood pressure in conscious rats [102,104,105].

Signaling mechanisms involved in CB₁R-evoked pressor response in the RVLM

Role of ERK1/2-PI3K/Akt signaling pathway

Cannabinoids are highly potent activators of extracellular-signal regulated kinase 1/2 (ERK1/2), which was evident in stably transfected Chinese hamster ovary cells expressing human CB₁R. This effect was (i) abrogated by SR141716A; (ii) sensitive to pertussis toxin; (iii) and independent of the cannabinoid-induced inhibition of cAMP production [110]. The pivotal role of PI3K/Akt and ERK1/2 as potential downstream molecular mediators of the central CB₁R-mediated sympathoexcitation/pressor response as suggested by multiple lines of evidence was demonstrated recently [105]. Central administration of WIN55,212-2 (i.c.) significantly elevated pERK1/2 in the NTS and RVLM [105]. The involvement of any CB₂R role in these responses was precluded because of the abrogation of the WIN55,212-2-mediated cardiovascular and neurochemical responses by MEK-ERK1/2 inhibition (PD98059) and attenuation of the concomitant activation of ERK1/2 pathway by pretreatment with the selective CB₁R antagonist AM251 (i.c.). In view of the crucial role of brainstem pERK1/2 signaling in central control of blood pressure, previous studies from our laboratory [82,86] and others [111–113] suggest that brainstem ERK1/2 plays a bi-directional role in central regulation of blood pressure. For example, in both normotensive and hypertensive rats, inhibition of RVLM ERK1/2 phosphorylation gradually lowered blood pressure [111], and its rapid activation plays pivotal role in the angiotensin II-mediated pressor response [113,114]. In contrast, we have previously shown that RVLM MEK-ERK1/2 signaling activation underlies the central α_2A adrenergic or imidazoline evoked acute hypotensive response [82,86].

Studies on the neuroprotective and/or anti-oncogenic effects of cannabinoids via PI3K/Akt signaling pathway have yielded controversial results. First, intraperitoneal injection of Δ^9 -THC activated PI3K/Akt pathway in mouse hippocampus, striatum, and cerebellum via a mechanism that was ERK1/2-independent [115]. Second, THC-mediated anti-cancer effect in human prostate cells involved PI3K/AKT and ERK1/2 signaling pathway activation [116]. On the other hand, it was demonstrated in multiple cancer cell lines that CB₁R activation down regulates both PI3K/Akt and ERK1/2 signaling pathway [117,118]. Based on the molecular findings from our studies, we concluded that the effect of WIN55,212-2 on PI3K/Akt may contribute to the enhancement of ERK1/2 phosphorylation because in the presence of the PI3K/Akt inhibitor wortmannin, WIN55,212-2-induced ERK1/2 phosphorylation was exacerbated [105]. Additionally, PD98059, MEK-ERK1/2 inhibitor, alone or in the presence of WIN55,212-2 had no effect on brainstem pAkt phosphorylation levels.

Consistent with a diverse physiological role of PI3K/Akt-ERK1/2 pathway, we showed that a dose-related reduction in pAkt phosphorylation levels in the NTS and RVLM con-

tributes to the i.c. WIN55,212-2-evoked pressor response [105]. In support of this conclusion are the findings that the inhibition of Akt phosphorylation in the NTS and RVLM preceded the peak WIN55,212-2-evoked pressor response (5 min). Our Western blot findings are consistent with reported findings that CB₁R activation resulted in down-regulation of the PI3K/Akt signaling [105,117,118]. However, others have shown that CB₁R activation up-regulated PI3K/Akt signaling in U373 MG human astrocytoma cells [119], hippocampal slices [120], and *in vivo* [115]. Nonetheless, further support for a causal role for the observed inhibition in Akt phosphorylation in the brainstem in the central CB₁R-mediated pressor response are the findings that pharmacological inhibition of brainstem PI3K-Akt signaling (wortmannin) significantly enhanced the WIN55,212-2 evoked dose-related pressor response [105]. Interestingly, the latter study reported an increase in Akt phosphorylation elicited by WIN55,212-2 following CB₁R blockade with AM251 in the NTS but not in the RVLM. This finding clearly highlights differences between neurochemical responses elicited by CB₁R activation in the RVLM vs. NTS.

CB₁R enhances RVLM nNOS-NO signaling pathway

The well-documented role of NOS-NO signaling in the RVLM regulation of autonomic function has led us to investigate whether nNOS-NO plays a significant role in the central CB₁R-mediated pressor response [104,121–123]. We reported that intra-RVLM WIN55,212-2 microinjection elicited dose-dependent increases in real-time RVLM NO and blood pressure; NO was measured by *in vivo* electrochemistry and is possibly nNOS-generated because: (i) parallel to the WIN55,212-2 dose-dependent enhancement of NO release, we detected a significant increase in nNOS phosphorylation in the WIN55,212-2-treated RVLM compared to the contra-lateral side (control); (ii) i.c. WIN55,212-2 increased the number of nNOS-ir neurons expressing c-Fos, denoting an increase in the activity of nNOS expressing neurons; (iii) these neurochemical responses were abolished following selective CB₁R blockade (AM251) or prior inhibition of nNOS phosphorylation (NPLA) [104]; (iv) only RVLM nNOS, but not eNOS or iNOS, derived NO is implicated in centrally evoked hypertension [123]. Because ERK1/2 dependent phosphorylation of RVLM nNOS is implicated in sympathoexcitation [124–126], the interesting possibility exists that CB₁R-mediated nNOS activation might be downstream to MEK-ERK1/2 activation, which ultimately results in CB₁R-mediated pressor response.

CB₁R downregulates brainstem GABAergic transmission

It is highly likely that central CB₁R-elicited sympathoexcitation is mediated via indirect modulation of presympathetic neurons in the brainstem whose activity is regulated by an array of tonic excitatory and inhibitory inputs [90,127]. Notably, CB₁R modulates synaptic transmission of both inhibitory (GABA) and excitatory (glutamate) neurotransmitters [23,29,128,129]. Interestingly, stimulation of central GABA_A receptors (muscimol) caused the following: (i) abolished the CB₁R-evoked pressor response and the elevation in plasma NE; (ii) attenuated the WIN55,212-2 evoked increase in the

activity (c-Fos) of catecholamine (TH-ir) [102]. These findings are consistent with reported *in vitro* findings that demonstrated CB₁R-evoked inhibition of GABAergic transmission in cultured rostral ventromedial medulla (RVM) neurons [31]. Yet, in the NTS, studies have demonstrated a controversial role for CB₁R-mediated presynaptic modulation of excitatory (glutamate) and inhibitory (GABA) neurotransmitters. Anandamide increased baroreflex-mediated sympathoinhibition in the NTS, presumably, via presynaptic inhibition of GABA release because the response was reversed in presence of the GABA_AR antagonist [52].

Conclusions

As summarized in Fig. 1, the present review highlights the molecular mechanisms implicated in the predominant sympathoexcitatory effect of brainstem CB₁R activation in conscious rats. CB₁R stimulation enhanced neuronal activity of presympathetic neurons in the RVLM (c-Fos/TH-ir ratio). Furthermore, PI3K/Akt-ERK1/2 signaling in the brainstem seems to differentially contribute, at least in part, to the sympathoexcitatory responses elicited by the central CB₁R activation in conscious rats. The discussed studies demonstrated that CB₁R

activation in the RVLM elicits down-regulation of PI3K/Akt pathway along with the pressor response, which was supported by the exacerbation of WIN55,212-2 evoked hemodynamic responses when PI3K/Akt was inhibited by wortmannin. By contrast, the CB₁R-evoked sympathoexcitation was associated with enhanced ERK1/2 activity in the brainstem. Further, suppressing ERK1/2 signaling abolished the central CB₁R-evoked pressor response. Finally, CB₁R activation in the RVLM enhanced neuronal nitroxidergic activity (nNOS-NO) essential for the central regulation of cardiovascular function. These latter neuronal responses may be linked to the modulation of brainstem GABAergic neurotransmission and subsequently to the central CB₁R-evoked sympathoexcitatory and pressor response. It is imperative to note that this overview highlights important signaling networks implicated in the modulation of blood pressure caused by central CB₁R activation in normotensive rats. The neurochemical and molecular responses discussed above might be different under pathophysiological conditions and might, therefore, lead to different cardiovascular outcomes. Therefore, future studies on the role of central CB₁R signaling in animal models of human diseases are warranted.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgments

The studies conducted in the authors' lab were supported, in part, by NIH Grant 2R01 AA07839-19 (Abdel A. Abdel-Rahman), and by predoctoral fellowship provided by the Egyptian Cultural and Educational Bureau (Badr M. Ibrahim).

References

- [1] Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993;365(6441): 61–5.
- [2] Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, et al. Comparison of the pharmacology and signal transduction of the human cannabinoid CB₁ and CB₂ receptors. *Mol Pharmacol* 1995;48(3):443–50.
- [3] Devane W, Hanus L, Breuer A, Pertwee R, Stevenson L, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992;258(5090):1946–9.
- [4] Sugiura T, Kodaka T, Nakane S, Miyashita T, Kondo S, Suhara Y, et al. Evidence that the cannabinoid CB₁ receptor is a 2-arachidonoylglycerol receptor. *J Biol Chem* 1999;274(5): 2794–801.
- [5] Porter AC, Sauer J-M, Knierman MD, Becker GW, Berna MJ, Bao J, et al. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB₁ receptor. *J Pharmacol Exp Ther* 2002;301(3):1020–4.
- [6] Rinaldi-Carmona M, Barth F, Millan J, Derocq J-M, Casellas P, Congy C, et al. SR 144528, the first potent and selective antagonist of the CB₂ cannabinoid receptor. *J Pharmacol Exp Ther* 1998;284(2):644–50.
- [7] Pertwee RG, Gibson TM, Stevenson LA, Ross RA, Banner WK, Saha B, et al. O-1057, a potent water-soluble

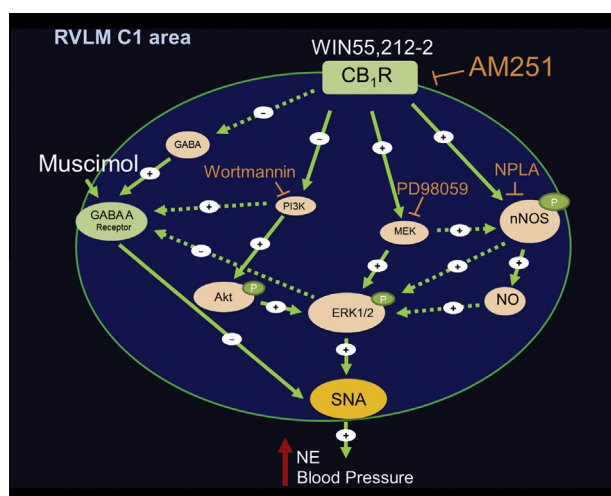


Fig. 1 Schematic presentation of signaling mechanisms in the rostral ventrolateral medulla (RVLM) catecholaminergic C1 area, underlying central CB₁R-mediated pressor response. In conscious freely moving rats, central CB₁R activation (WIN55,212-2) increases blood pressure, plasma norepinephrine (NE), sympathetic neuronal activity (SNA) [102], enhances ERK1/2 and nNOS phosphorylation (NO production) and reduces Akt phosphorylation in the RVLM [104,105]. AM251 (CB₁R antagonist); NPLA (nNOS inhibitor); PD98059 (MEK-ERK1/2 inhibitor) or muscimol (GABA_A receptor agonist) attenuated CB₁R (WIN55,212-2)-evoked pressor response [102,104,105]. In contrast, wortmannin (PI3K-Akt inhibitor) exaggerated WIN55,212-2 response [105]. The proposed model system is further supported by our neurochemical and pharmacological findings following intracisternal or intra-RVLM microinjection of the CB₁R agonist WIN55,212-2 [104,105]. Solid arrows indicate signaling based on reported *in vivo* findings, while dashed arrows indicate proposed signaling based on reported *in vitro* findings, but not tested in this model (see text for details).

- cannabinoid receptor agonist with antinociceptive properties. *Br J Pharmacol* 2000;129(8):1577–84.
- [8] Dodd GT, Mancini G, Lutz B, Luckman SM. The peptide hemopressin acts through CB1 cannabinoid receptors to reduce food intake in rats and mice. *J Neurosci* 2010;30(21):7369–76.
- [9] Heimann AS, Gomes I, Dale CS, Pagano RL, Gupta A, de Souza LL, et al. Hemopressin is an inverse agonist of CB1 cannabinoid receptors. *Proc Natl Acad Sci* 2007;104(51):20588–93.
- [10] Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Natl Acad Sci USA* 2008;105(7):2699–704.
- [11] Jarai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR, et al. Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc Natl Acad Sci USA* 1999;96(24):14136–41.
- [12] Kapur A, Zhao PW, Sharif H, Bai YS, Caron MG, Barak LS, et al. Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *J Biol Chem* 2009;284(43):29817–27.
- [13] Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007;152(7):1092–101.
- [14] Waldeck-Weiermair M, Zoratti C, Osibow K, Balenga N, Goessnitzer E, Waldhoer M, et al. Integrin clustering enables anandamide-induced Ca(2+) signaling in endothelial cells via GPR55 by protection against CB1-receptor-triggered repression. *J Cell Sci* 2008;121(10):1704–17.
- [15] Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990;346(6284):561–4.
- [16] Gerard CM, Mollereau C, Vassart G, Parmentier M. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem J* 1991;279(Pt 1):129–34.
- [17] Howlett AC. The CB1 cannabinoid receptor in the brain. *Neurobiol Dis* 1998;5(6):405–16.
- [18] Bidaut-Russell M, Devane WA, Howlett AC. Cannabinoid receptors and modulation of cyclic AMP accumulation in the rat brain. *J Neurochem* 1990;55(1):21–6.
- [19] Howlett AC, Qualy JM, Khachatrian LL. Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol Pharmacol* 1986;29(3):307–13.
- [20] Childers SR, Fleming L, Konkoy C, Marckel D, Pacheco M, Sexton T, et al. Opioid and cannabinoid receptor inhibition of adenylyl cyclase in brain. *Ann NY Acad Sci* 1992;654(1):33–51.
- [21] Felder CC, Joyce KE, Briley EM, Glass M, Mackie KP, Fahey KJ, et al. LY320135, a novel cannabinoid CB1 receptor antagonist, unmasks coupling of the CB1 receptor to stimulation of cAMP accumulation. *J Pharmacol Exp Ther* 1998;284(1):291–7.
- [22] Howlett AC, Mukhopadhyay S. Cellular signal transduction by anandamide and 2-arachidonoylglycerol. *Chem Phys Lipids* 2000;108(1–2):53–70.
- [23] Piomelli D. The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* 2003;4(11):873–84.
- [24] Lauckner JE, Hille B, Mackie K. The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB1 receptor coupling to Gq/11 G proteins. *Proc Natl Acad Sci USA* 2005;102(52):19144–9.
- [25] Kearn CS, Blake-Palmer K, Daniel E, Mackie K, Glass M. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Mol Pharmacol* 2005;67(5):1697–704.
- [26] Glass M, Felder CC. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J Neurosci* 1997;17(14):5327–33.
- [27] McIntosh BT, Hudson B, Yegorova S, Jollimore CAB, Kelly MEM. Agonist-dependent cannabinoid receptor signalling in human trabecular meshwork cells. *Br J Pharmacol* 2007;152(7):1111–20.
- [28] Ellis J, Pediani JD, Canals M, Milasta S, Milligan G. Orexin-1 receptor-cannabinoid CB1 receptor heterodimerization results in both ligand-dependent and -independent coordinated alterations of receptor localization and function. *J Biol Chem* 2006;281(50):38812–24.
- [29] Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* 2003;83(3):1017–66.
- [30] Ohno-Shosaku T, Maejima T, Kano M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 2001;29(3):729–38.
- [31] Vaughan CW, McGregor IS, Christie MJ. Cannabinoid receptor activation inhibits GABAergic neurotransmission in rostral ventromedial medulla neurons *in vitro*. *Br J Pharmacol* 1999;127(4):935–40.
- [32] Kreitzer AC, Regehr WG. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto purkinje cells. *Neuron* 2001;29(3):717–27.
- [33] Mendizabal VE, Adler-Graschinsky E. Cannabinoids as therapeutic agents in cardiovascular disease: a tale of passions and illusions. *Br J Pharmacol* 2007;151(4):427–40.
- [34] Randall MD, Kendall DA, O'Sullivan S. The complexities of the cardiovascular actions of cannabinoids. *Br J Pharmacol* 2004;142(1):20–6.
- [35] Randall MD, Harris D, Kendall DA, Ralevic V. Cardiovascular effects of cannabinoids. *Pharmacol Ther* 2002;95(2):191–202.
- [36] Stein EA, Fuller SA, Edgemond WS, Campbell WB. Physiological and behavioural effects of the endogenous cannabinoid, arachidonyl ethanolamide (anandamide), in the rat. *Br J Pharmacol* 1996;119(1):107–14.
- [37] Gardiner SM, March JE, Kemp PA, Bennett T. Regional haemodynamic responses to the cannabinoid agonist, WIN 55212-2, in conscious, normotensive rats, and in hypertensive, transgenic rats. *Br J Pharmacol* 2001;133(3):445–53.
- [38] Lake KD, Martin BR, Kunos G, Varga K. Cardiovascular effects of anandamide in anesthetized and conscious normotensive and hypertensive rats. *Hypertension* 1997;29(5):1204–10.
- [39] Niederhoffer N, Szabo B. Effect of the cannabinoid receptor agonist WIN55212-2 on sympathetic cardiovascular regulation. *Br J Pharmacol* 1999;126(2):457–66.
- [40] Varga K, Lake KD, Huangfu D, Guyenet PG, Kunos G. Mechanism of the hypotensive action of anandamide in anesthetized rats. *Hypertension* 1996;28(4):682–6.
- [41] Lake KD, Compton DR, Varga K, Martin BR, Kunos G. Cannabinoid-induced hypotension and bradycardia in rats mediated by CB1-like cannabinoid receptors. *J Pharmacol Exp Ther* 1997;281(3):1030–7.
- [42] Varga K, Lake K, Martin BR, Kunos G. Novel antagonist implicates the CB1 cannabinoid receptor in the hypotensive action of anandamide. *Eur J Pharmacol* 1995;278(3):279–83.
- [43] Siqueira SW, Lapa AJ, Ribeiro do Valle J. The triple effect induced by delta 9-tetrahydrocannabinol on the rat blood pressure. *Eur J Pharmacol* 1979;58(4):351–7.
- [44] Kwolek G, Zakrzeska A, Schlicker E, Gothert M, Godlewski G, Malinowska B. Central and peripheral components of the pressor effect of anandamide in urethane-anesthetized rats.[see comment]. *Br J Pharmacol* 2005;145(5):567–75.
- [45] Malinowska B, Zakrzeska A, Kurz C, Göthert M, Kwolek G, Wielgat P, et al. Involvement of central β_2 adrenergic, NMDA

- and thromboxane A₂ receptors in the pressor effect of anandamide in rats. *Naunyn Schmiedebergs Arch Pharmacol* 2010;381(4):349–60.
- [46] Benowitz NL, Rosenberg J, Rogers W, Bachman J, Jones RT. Cardiovascular effects of intravenous delta-9-tetrahydrocannabinol: autonomic nervous mechanisms. *Clin Pharmacol Ther* 1979;25(4):440–6.
- [47] Foltin RW, Fischman MW, Pedrosa JJ, Pearlson GD. Marijuana and cocaine interactions in humans: cardiovascular consequences. *Pharmacol Biochem Behav* 1987;28(4):459–64.
- [48] Sidney S. Cardiovascular consequences of marijuana use. *J Clin Pharmacol* 2002;42(90110):S64–70.
- [49] Dean C. Cannabinoid and GABA modulation of sympathetic nerve activity and blood pressure in the dorsal periaqueductal gray of the rat. *Am J Physiol Regul Integr Comp Physiol* 2011;301(6):R1765–72.
- [50] Seagard JL, Hopp FA, Hillard CJ, Dean C. Effects of endocannabinoids on discharge of baroreceptive NTS neurons. *Neurosci Lett* 2005;381(3):334–9.
- [51] Rademacher DJ, Patel S, Hopp FA, Dean C, Hillard CJ, Seagard JL. Microinjection of a cannabinoid receptor antagonist into the NTS increases baroreflex duration in dogs. *Am J Physiol – Heart Circulat Physiol* 2003;284(5):H1570–6.
- [52] Seagard JL, Dean C, Patel S, Rademacher DJ, Hopp FA, Schmeling WT, et al. Anandamide content and interaction of endocannabinoid/GABA modulatory effects in the NTS on baroreflex-evoked sympathoinhibition. *Am J Physiol – Heart Circulat Physiol* 2004;286(3):H992–1000.
- [53] Aicher SA, Randich A. Antinociception and cardiovascular responses produced by electrical stimulation in the nucleus tractus solitarius, nucleus reticularis ventralis, and the caudal medulla. *Pain* 1990;42(1):103–19.
- [54] Miura M, Reis DJ. The role of the solitary and paramedian reticular nuclei in mediating cardiovascular reflex responses from carotid baro- and chemoreceptors. *J Physiol* 1972;223(2):525–48.
- [55] Nomura S, Mizuno N. Central distribution of afferent and efferent components of the glossopharyngeal nerve: an HRP study in the cat. *Brain Res* 1982;236(1):1–13.
- [56] Seiders EP, Stuesse SL. A horseradish peroxidase investigation of carotid sinus nerve components in the rat. *Neurosci Lett* 1984;46(1):13–8.
- [57] Aicher SA, Saravay RH, Cravo S, Jeske I, Morrison SF, Reis DJ, et al. Monosynaptic projections from the nucleus tractus solitarius to C1 adrenergic neurons in the rostral ventrolateral medulla: comparison with input from the caudal ventrolateral medulla. *J Comp Neurol* 1996;373(1):62–75.
- [58] Aicher SA, Kurucz OS, Reis DJ, Milner TA. Nucleus tractus solitarius efferent terminals synapse on neurons in the caudal ventrolateral medulla that project to the rostral ventrolateral medulla. *Brain Res* 1995;693(1–2):51–63.
- [59] Dampney RA, Czachurski J, Dembowski K, Goodchild AK, Seller H. Afferent connections and spinal projections of the pressor region in the rostral ventrolateral medulla of the cat. *J Auton Nerv Syst* 1987;20(1):73–86.
- [60] Ross CA, Ruggiero DA, Reis DJ. Projections from the nucleus tractus solitarius to the rostral ventrolateral medulla. *J Comp Neurol* 1985;242(4):511–34.
- [61] Van Sickle MD, Oland LD, Ho W, Hillard CJ, Mackie K, Davison JS, et al. Cannabinoids inhibit emesis through CB₁ receptors in the brainstem of the ferret. *Gastroenterology* 2001;121(4):767–74 [see comment].
- [62] Chen C-Y, Bonham AC, Dean C, Hopp FA, Hillard CJ, Seagard JL. Retrograde release of endocannabinoids inhibits presynaptic GABA release to second-order baroreceptive neurons in NTS. *Auton Neurosci* 2010;158(1–2):44–50.
- [63] Nassar N, Abdel-Rahman AA. Central adenosine signaling plays a key role in centrally mediated hypotension in conscious aortic barodenervated rats. *J Pharmacol Exp Ther* 2006;318(1):255–61.
- [64] Dampney RA, Polson JW, Potts PD, Hirooka Y, Horiuchi J. Functional organization of brain pathways subserving the baroreceptor reflex: studies in conscious animals using immediate early gene expression. *Cell Mol Neurobiol* 2003;23(4–5):597–616.
- [65] Strack AM, Sawyer WB, Hughes JH, Platt KB, Loewy AD. A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. *Brain Res* 1989;491(1):156–62.
- [66] Guyenet PG, Haselton JR, Sun MK. Sympathoexcitatory neurons of the rostroventrolateral medulla and the origin of the sympathetic vasomotor tone. *Prog Brain Res* 1989;81:105–16.
- [67] Farlow DM, Goodchild AK, Dampney RA. Evidence that vasomotor neurons in the rostral ventrolateral medulla project to the spinal sympathetic outflow via the dorsomedial pressor area. *Brain Res* 1984;298(2):313–20.
- [68] Andrezik JA, Chan-Palay V, Palay SL. The nucleus paragigantocellularis lateralis in the rat. *Anat Embryol* 1981;161(4):355–71.
- [69] Villanueva L, de Pommery J, Menétrey D, Le Bars D. Spinal afferent projections to subnucleus reticularis dorsalis in the rat. *Neurosci Lett* 1991;134(1):98–102.
- [70] Watanabe S, Kitamura T, Watanabe L, Sato H, Yamada J. Projections from the nucleus reticularis magnocellularis to the rat cervical cord using electrical stimulation and iontophoretic injection methods. *Anatom Sci Int* 2003;78(1):42–52.
- [71] Ruggiero DA, Cravo SL, Arango V, Reis DJ. Central control of the circulation by the rostral ventrolateral reticular nucleus: anatomical substrates. *Prog Brain Res* 1989;81:49–79.
- [72] Ross CA, Ruggiero DA, Joh TH, Park DH, Reis DJ. Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons. *J Comp Neurol* 1984;228(2):168–85.
- [73] Jeske I, McKenna KE. Quantitative analysis of bulbospinal projections from the rostral ventrolateral medulla: contribution of C1-adrenergic and nonadrenergic neurons. *J Comp Neurol* 1992;324(1):1–13.
- [74] Kanjhan R, Lipski J, Kruszezka B, Rong W. A comparative study of pre-sympathetic and Bötzing neurons in the rostral ventrolateral medulla (RVLM) of the rat. *Brain Res* 1995;699(1):19–32.
- [75] Schreihofer AM, Guyenet PG. Identification of C1 presympathetic neurons in rat rostral ventrolateral medulla by juxtacellular labeling *in vivo*. *J Comp Neurol* 1997;387(4):524–36.
- [76] Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci* 2006;7(5):335–46.
- [77] Guyenet PG, Koshiya N, Huangfu D, Baraban SC, Stornetta RL, Li Y-W. Chapter 8 role of medulla oblongata in generation of sympathetic and vagal outflows. In: Holstege RBG, Saper CB, editors. *Progress in brain research*. Elsevier; 1996. p. 127–44.
- [78] Goodchild AK, Phillips JK, Lipski J, Pilowsky PM. Differential expression of catecholamine synthetic enzymes in the caudal ventral pons. *J Comp Neurol* 2001;438(4):457–67.
- [79] Pilowsky P, Minson J, Hodgson A, Howe P, Chalmers J. Does substance P coexist with adrenaline in neurones of the rostral ventrolateral medulla in the rat? *Neurosci Lett* 1986;71(3):293–8.
- [80] Polson JW, Halliday GM, McAllen RM, Coleman MJ, Dampney RA. Rostrocaudal differences in morphology and neurotransmitter content of cells in the subretrofacial vasomotor nucleus. *J Auton Nerv Syst* 1992;38(2):117–37.

- [81] Boone Jr JB, Corry JM. Proenkephalin gene expression in the brainstem regulates post-exercise hypotension. *Brain Res Mol Brain Res* 1996;42(1):31–8.
- [82] Nassar N, Abdel-Rahman AA. Brainstem phosphorylated extracellular signal-regulated kinase 1/2-nitric-oxide synthase signaling mediates the adenosine A2A-dependent hypotensive action of clonidine in conscious aortic barodenervated rats. *J Pharmacol Exp Ther* 2008;324(1):79–85.
- [83] Ralevic V. P2 receptors in the central and peripheral nervous systems modulating sympathetic vasomotor tone. *J Auton Nerv Syst* 2000;81(1–3):205–11.
- [84] Potts PD, Allen AM, Horiuchi J, Dampney RA. Does angiotensin II have a significant tonic action on cardiovascular neurons in the rostral and caudal VLM? *Am J Physiol – Regulat Integrat Comp Physiol* 2000;279(4):R1392–402.
- [85] Zhang J, Abdel-Rahman AA. The hypotensive action of rilmenidine is dependent on functional N-methyl-D-aspartate receptor in the rostral ventrolateral medulla of consciously spontaneously hypertensive rats. *J Pharmacol Exp Ther* 2002;303(1):204–10.
- [86] Zhang J, Abdel-Rahman AA. Mitogen-activated protein kinase phosphorylation in the rostral ventrolateral medulla plays a key role in imidazoline (i1)-receptor-mediated hypotension. *J Pharmacol Exp Ther* 2005;314(3):945–52.
- [87] El-Mas MM, Abdel-Rahman AA. Differential modulation by estrogen of alpha2-adrenergic and II-imidazoline receptor-mediated hypotension in female rats. *J Appl Physiol* 2004;97(4):1237–44.
- [88] Li G, Wang X, Abdel-Rahman AA. Neuronal norepinephrine responses of the rostral ventrolateral medulla and nucleus tractus solitarius neurons distinguish the II- from the alpha2-receptor-mediated hypotension in conscious SHR. *J Cardiovasc Pharmacol* 2005;46(1):52–62.
- [89] Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J Neurosci* 1991;11(2):563–83.
- [90] Padley JR, Li Q, Pilowsky PM, Goodchild AK. Cannabinoid receptor activation in the rostral ventrolateral medulla oblongata evokes cardiorespiratory effects in anaesthetized rats. *Br J Pharmacol* 2003;140(2):384–94.
- [91] Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, et al. Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 2005;310(5746):329–32.
- [92] Drake CT, Aicher SA, Montalmant FL, Milner TA. Redistribution of mu-opioid receptors in C1 adrenergic neurons following chronic administration of morphine. *Exp Neurol* 2005;196(2):365–72.
- [93] Aicher SA, Kraus JA, Sharma S, Patel A, Milner TA. Selective distribution of mu-opioid receptors in C1 adrenergic neurons and their afferents. *J Comp Neurol* 2001;433(1):23–33.
- [94] Guertzenstein PG, Silver A. Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. *J Physiol* 1974;242(2):489–503.
- [95] Feldberg W, Guertzenstein PG. Vasodepressor effects obtained by drugs acting on the ventral surface of the brain stem. *J Physiol* 1976;258(2):337–55.
- [96] Guertzenstein PG. Vasodepressor and pressor responses to drugs topically applied to the ventral surface of the brain stem. *J Physiol* 1972;224(2):84P–5P.
- [97] McAllen RM, Dampney RA. The selectivity of descending vasomotor control by subretrofacial neurons. *Prog Brain Res* 1989;81:233–42.
- [98] Karlsson GA, Preuss CV, Chaitoff KA, Maher TJ, Ally A. Medullary monoamines and NMDA-receptor regulation of cardiovascular responses during peripheral nociceptive stimuli. *Neurosci Res* 2006;55(3):316–26.
- [99] Javanmardi K, Parviz M, Sadr Ss, Keshavarz M, Minaei B, Dehpour AR. Involvement of N-methyl-D-aspartate receptors and nitric oxide in the rostra ventromedial medulla in modulating morphine pain-inhibitory signals from the periaqueductal grey matter in rats. *Clin Exp Pharmacol Physiol* 2005;32(7):585–9.
- [100] Nattie EE, Li AH. Fluorescence location of RVLM kainate microinjections that alter the control of breathing. *J Appl Physiol* 1990;68(3):1157–66.
- [101] Niederhoffer N, Szabo B. Cannabinoids cause central sympathoexcitation and bradycardia in rabbits. *J Pharmacol Exp Ther* 2000;294(2):707–13.
- [102] Ibrahim BM, Abdel-Rahman AA. Role of brainstem GABAergic signaling in central cannabinoid receptor evoked sympathoexcitation and pressor responses in conscious rats. *Brain Res* 2011;1414:1–9.
- [103] Pfitzer T, Niederhoffer N, Szabo B. Central effects of the cannabinoid receptor agonist WIN55212-2 on respiratory and cardiovascular regulation in anaesthetized rats. *Br J Pharmacol* 2004;142(6):943–52.
- [104] Ibrahim BM, Abdel-Rahman AA. Enhancement of rostral ventrolateral medulla neuronal nitric-oxide synthase-nitric-oxide signaling mediates the central cannabinoid receptor I-evoked pressor response in conscious rats. *J Pharmacol Exp Ther* 2012;341(3):579–86.
- [105] Ibrahim BM, Abdel-Rahman AA. Differential modulation of brainstem PI3K/Akt and ERK1/2 signaling underlies WIN55,212-2 centrally-mediated pressor response in conscious rats. *J Pharmacol Exp Ther* 2012;340(1):11–8.
- [106] Song C, Howlett A. Rat brain cannabinoid receptors are N-linked glycosylated proteins. *Life Sci* 1995;56(23–24):1983–9.
- [107] Griffin G, Atkinson PJ, Showalter VM, Martin BR, Abood ME. Evaluation of cannabinoid receptor agonists and antagonists using the guanosine-5'-O-(3-[35S]thio)-triphosphate binding assay in rat cerebellar membranes. *J Pharmacol Exp Ther* 1998;285(2):553–60.
- [108] Showalter VM, Compton DR, Martin BR, Abood ME. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J Pharmacol Exp Ther* 1996;278(3):989–99.
- [109] Viscomi MT, Oddi S, Latini L, Pasquariello N, Florenzano F, Bernardi G, et al. Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway. *J Neurosci* 2009;29(14):4564–70.
- [110] Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi-Carmona M, et al. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J* 1995;312(Pt 2):637–41.
- [111] Seyedabadi M, Goodchild AK, Pilowsky PM. Differential role of kinases in brain stem of hypertensive and normotensive rats. *Hypertension* 2001;38(5):1087–92.
- [112] Lin YZ, Matsumura K, Tsuchihashi T, Fukuhara M, Fujii K, Iida M. Role of ERK and Rho kinase pathways in central pressor action of urotensin II. *J Hypertens* 2004;22(5):983–8.
- [113] Chan SH, Wang L-L, Tseng H-L, Chan JY. Upregulation of ATI receptor gene on activation of protein kinase C[beta]/nicotinamide adenine dinucleotide diphosphate oxidase/ERK1/2/c-fos signaling cascade mediates long-term pressor effect of angiotensin II in rostral ventrolateral medulla. *J Hypertens* 2007;25(9):1845–61.
- [114] Chan SHH, Hsu K-S, Huang C-C, Wang L-L, Ou C-C, Chan JYH. NADPH oxidase-derived superoxide anion mediates angiotensin II-induced pressor effect via activation of p38

- mitogen-activated protein kinase in the rostral ventrolateral medulla. *Circ Res* 2005;97(8):772–80.
- [115] Ozaita A, Puighermanal E, Maldonado R. Regulation of PI3K/Akt/GSK-3 pathway by cannabinoids in the brain. *J Neurochem* 2007;102(4):1105–14.
- [116] Sanchez MG, Ruiz-Llorente L, Sanchez AM, Diaz-Laviada I. Activation of phosphoinositide 3-kinase/PKB pathway by CB(1) and CB(2) cannabinoid receptors expressed in prostate PC-3 cells. Involvement in Raf-1 stimulation and NGF induction. *Cell Signal* 2003;15(9):851–9.
- [117] Ellert-Miklaszewska A, Kaminska B, Konarska L. Cannabinoids down-regulate PI3K/Akt and Erk signalling pathways and activate proapoptotic function of Bad protein. *Cell Signal* 2005;17(1):25–37.
- [118] Greenhough A, Patsos HA, Williams AC, Paraskeva C. The cannabinoid delta(9)-tetrahydrocannabinol inhibits RAS-MAPK and PI3K-AKT survival signalling and induces BAD-mediated apoptosis in colorectal cancer cells. *Int J Cancer* 2007;121(10):2172–80.
- [119] Galve-Roperh I, Rueda D, Gomez del Pulgar T, Velasco G, Guzman M. Mechanism of extracellular signal-regulated kinase activation by the CB(1) cannabinoid receptor. *Mol Pharmacol* 2002;62(6):1385–92.
- [120] Derkinderen P, Valjent E, Toutant M, Corvol JC, Enslen H, Ledent C, et al. Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. *J Neurosci* 2003;23(6):2371–82.
- [121] Martins-Pinge MC, Araujo GC, Lopes OU. Nitric oxide-dependent guanylyl cyclase participates in the glutamatergic neurotransmission within the rostral ventrolateral medulla of awake rats. *Hypertension* 1999;34(4):748–51.
- [122] Mayorov DN. Nitric oxide synthase inhibition in rostral ventrolateral medulla attenuates pressor response to psychological stress in rabbits. *Neurosci Lett* 2007;424(2):89–93.
- [123] Martins-Pinge MC, Garcia MR, Zoccal DB, Crestani CC, Pinge-Filho P. Differential influence of iNOS and nNOS inhibitors on rostral ventrolateral medullary mediated cardiovascular control in conscious rats. *Auton Neurosci-Basic Clin* 2007;131(1–2):65–9.
- [124] Chan JYH, Chan SHH, Chang AYW. Differential contributions of NOS isoforms in the rostral ventrolateral medulla to cardiovascular responses associated with mevinphos intoxication in the rat. *Neuropharmacology* 2004;46(8):1184–94.
- [125] Chan SH, Sun EY, Chang AY. Extracellular signal-regulated kinase 1/2 plays a pro-life role in experimental brain stem death via MAPK signal-interacting kinase at rostral ventrolateral medulla. *J Biomed Sci* 2010;17:17.
- [126] Chan JYH, Chan SHH, Li FCH, Tsai CY, Cheng HL, Chang AYW. Phasic cardiovascular responses to mevinphos are mediated through differential activation of cGMP/PKG cascade and peroxynitrite via nitric oxide generated in the rat rostral ventrolateral medulla by NOS I and II isoforms. *Neuropharmacology* 2005;48(1):161–72.
- [127] Pilowsky PM, Goodchild AK. Baroreceptor reflex pathways and neurotransmitters: 10 years on. *J Hypertens* 2002;20(9):1675–88.

[128] Drew GM, Mitchell VA, Vaughan CW. Glutamate spillover modulates GABAergic synaptic transmission in the rat midbrain periaqueductal grey via metabotropic glutamate receptors and endocannabinoid signaling. *J Neurosci* 2008;28(4):808–15.

[129] Jelsing J, Galzin A-M, Guillot E, Pruniaux M-P, Larsen PJ, Vrang N. Localization and phenotypic characterization of brainstem neurons activated by rimonabant and WIN55,212-2. *Brain Res Bull* 2009;78(4–5):202–10.



Dr. Badr Ibrahim is an assistant professor of Pharmacology and Toxicology at El-Minia Faculty of Pharmacy, Egypt. He has completed his postdoctoral training and earned his PhD from the Department of Pharmacology and Toxicology, at East Carolina University, North Carolina, USA. Dr. Ibrahim's research focused on elucidating the cellular and molecular mechanisms underlying the central cannabinoid receptor-mediated hypertensive effect in unrestrained conscious rats. The

numerous national and international awards Dr. Ibrahim had received, as a graduate student, lend further support to his contributions to the field of cardiovascular neuropharmacology.



Dr. Abdel-Rahman is Distinguished Professor of Pharmacology and Vice Chair of the Department of Pharmacology and Toxicology, Brody School of Medicine at East Carolina University, Greenville, NC, USA. He published over 120 refereed scientific papers in addition to 10 education-related articles. His research findings have been published in top journals in his discipline and cited hundreds of times in scientific literature. Dr. Abdel-Rahman research deals with neural control of

circulation and neurobiology of hypertension. Two National Institutes of Health grants fund his research. In the first project his research team investigates the effect of ethanol on neuronal pathways that control blood pressure and cardiac reflexes. The second project deals with the neuroprotective and cardioprotective actions of estrogen and how concurrent alcohol use might compromise these beneficial physiological effects of estrogen.

In addition to his contributions to research, Dr. Abdel-Rahman has been active as a member of many scientific societies for the past 30 years and has been named a Fellow of the American Heart Association. Dr. Abdel-Rahman also served as President of the East Carolina University Neuroscience Chapter in addition to his services editor/associate editor and reviewer for a number of scientific journals. He has also served as a member of review boards (study sections) of the National Institutes of Health and the American Heart Association.