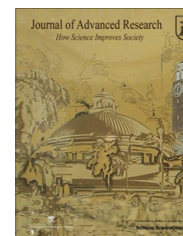Cairo University

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REVIEW

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

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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 (CB_1R)-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 CB_1R-evoked pressor response.

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Cannabinoids

Cannabinoids are heterogeneous group of compounds that target cannabinoid receptors: CB_1 and CB_2. 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 and medical purposes. Δ^9-THC, Cannabidiol (CBD), and cannabinol are the most abundant natural cannabinoids active at CB_1 and CB_2 receptors, but only Δ^9-THC has an equal affinity for both CB_1 and CB_2 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 CB_1R, while SR144528 is selective for the CB_2R [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

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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): CB_1 and CB_2. Additional non-CB_1, non-CB_2 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 CB_1R, which is found primarily in the CNS, including the cardiovascular regulatory nuclei in the brainstem. The CB_1 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].

CB_1R signaling

Activation of CB_1R 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 CB_1R 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 CB_1R-mediated physiological functions are G-protein $G\alpha i_{/o}$ mediated [19,22,23]. However, the diverse, sometimes opposing, CB_1R-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 CB_1R coupling to activation of $G\alpha q_{/11}$ or $G\alpha s$. It is possible that heterodimerization between the CB_1R and other receptor(s) contribute, at least partly, to this divergent signal transduction. This notion is supported by the reported interaction between CB_1R and other co-localized receptors e.g. dopamine D_2R, which resulted in accumulation of cAMP [25,26]. Second, CB_1R 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 CB_1R and OX_1R resulted in enhanced $G\alpha q_{/11}$-dependent OX_1R signaling in presence of CB_1R [28].

Retrograde CB_1R-mediated signaling

CB_1R 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 CB_1R, 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 CB_1 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 CB_1R, ultimately suppress presynaptic calcium-induced glutamate release [32]. The latter phenomenon is termed depolarization-induced suppression of excitation or (DSE). Both CB_1R 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 CB_1R-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 CB_1R-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 CB$_1$R activation, e.g. Nucleus Tractus Solitarii (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 CB$_1$R 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 ambiguous [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], P$_2$X receptors [83], Angiotensin II AT$_1$ receptors [84], imidazoline I$_1$ receptors [85,86], α_{2A} adrenergic receptors [87,88], cannabinoid CB$_1$ receptors [89,90], CB$_2$ 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 CB$_1$R 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 CB$_1$R-evoked pressor response involves activation of RVLM-catecholaminergic neurons [102], which was abrogated by CB$_1$R antagonist AM251.

Centrally elicited hemodynamic effects of CB$_1$R in conscious Sprague Dawley rats

In our recent studies, we sought to elucidate the mechanisms implicated in the central CB$_1$R-evoked sympathoexcitation/pressor response [102,104,105]. In pursuit of this goal, we characterized the centrally mediated cardiovascular effects of central CB$_1$R activation in conscious Sprague Dawley rats. We have confirmed the expression of CB$_1$R (protein) in the RVLM by detecting the two bands at 64 and 53 kDa, which represent the N-glycosylated and non-glycosylated forms of CB$_1$R, 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 CB$_1$R mediated. This is important because (i) WIN55,212-2, which is routinely used in cannabinoid research, can also bind to CB$_2$R [107,108]; (ii) both CBR subtypes are expressed in the brain [89,109], including the brainstem [90]. The ability of the selective CB$_1$R antagonist AM251 [39,101,103] to virtually abolish the pressor, biochemical and neurochemical responses elicited by i.c. WIN55,212-2 clearly implicates the CB$_1$R 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 CB_1R signaling in tonic control of blood pressure in conscious rats [102,104,105].

Signaling mechanisms involved in CB_1R-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 CB_1R. 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 CB_1R-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 CB_2R 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 CB_1R 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 CB_1R 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 CB_1R activation resulted in down-regulation of the PI3K/Akt signaling [105,117,118]. However, others have shown that CB_1R 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 CB_1R-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 CB_1R blockade with AM251 in the NTS but not in the RVLM. This finding clearly highlights differences between neurochemical responses elicited by CB_1R activation in the RVLM vs. NTS.

CB_1R 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 CB_1R-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 CB_1R 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 CB_1R-mediated nNOS activation might be downstream to MEK-ERK1/2 activation, which ultimately results in CB_1R-mediated pressor response.

CB_1R downregulates brainstem GABAergic transmission

It is highly likely that central CB_1R-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, CB_1R 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 CB_1R-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 CB$_1$R-evoked inhibition of GABAergic transmission in cultured rostral ventromedial medulla (RVM) neurons [31]. Yet, in the NTS, studies have demonstrated a controversial role for CB$_1$R-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$_A$R antagonist [52].

Conclusions

As summarized in Fig. 1, the present review highlights the molecular mechanisms implicated in the predominant sympathoexcitatory effect of brainstem CB$_1$R activation in conscious rats. CB$_1$R 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 CB$_1$R activation in conscious rats. The discussed studies demonstrated that CB$_1$R

Fig. 1 Schematic presentation of signaling mechanisms in the rostral ventrolateral medulla (RVLM) catecholaminergic C1 area, underlying central CB$_1$R-mediated pressor response. In conscious freely moving rats, central CB$_1$R 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 (CB$_1$R antagonist); NPLA (nNOS inhibitor); PD98059 (MEK-ERK1/2 inhibitor) or muscimol (GABA$_A$ receptor agonist) attenuated CB$_1$R (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 CB$_1$R 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).

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 CB$_1$R-evoked sympathoexcitation was associated with enhanced ERK1/2 activity in the brainstem. Further, suppressing ERK1/2 signaling abolished the central CB$_1$R-evoked pressor response. Finally, CB$_1$R 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 CB$_1$R-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 CB$_1$R 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 CB$_1$R signaling in animal models of human diseases are warranted.

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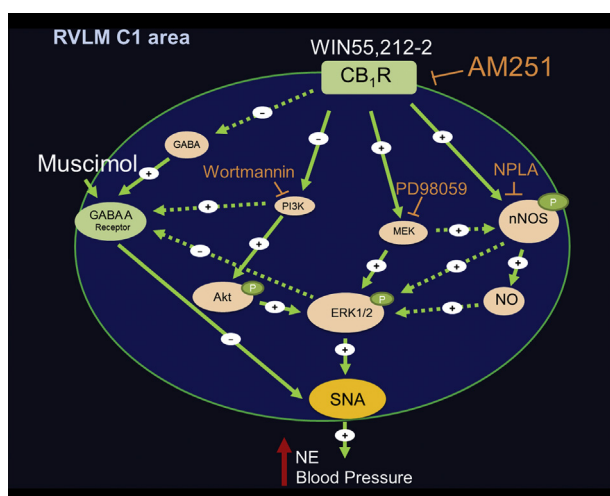[7] Pertwee RG, Gibson TM, Stevenson LA, Ross RA, Banner WK, Saha B, et al. O-1057, a potent water-soluble

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