REVIEW

Brain stem adenosine receptors modulate centrally mediated hypotensive responses in conscious rats: A review

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GRAPHICAL ABSTRACT

Abbreviations: A2A, adenosine subtype A2A receptor; A1, adenosine subtype A1 receptor; ABC, avidin biotin complex; ABD rat, aortic barodenervated rat; zMNE, alpha methyl norepinephrine; ATP, adenosine triphosphate; BP, blood pressure; cAMP, cyclic adenosine monophosphate; CGS21680, 2-[4-[(2-carboxyethyl)phenyl]ethylaminophenyl]ethylaminol-5'-N-ethylcarboxamidoadenosine. Selective A2A receptor agonist; CNS, central nervous system; CPA, N6-cyclopentyladenosine. Selective A1 receptor agonist; DAG, diacylglycerol; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine. Selective A1 receptor antagonist; I1, imidazoline subtype 1 receptor; I.C., intracisternal; IP3, Inositol Triphosphate; I.V., intravenous; JNK, C-Jun N-terminal kinase; L-NAME, Nω-nitro-l-arginine methyl ester hydrochloride. Non-selective nitric oxide synthase inhibitor; NOS, nitric oxide synthase; NO, nitric oxide; NTS, nucleus tractus solitarius; PC-PLC, phosphatidyl choline-selective phospholipase C; PC12 cells, pheochromocytoma cells; PD98059, selective extracellular signal regulated kinase inhibitor; ERK1/2, extracellular signal regulated kinase; PDE, phosphodiesterase; PKA, protein kinase A; RVLM, rostral ventrolateral medulla; SAPK, stress activated protein kinase; SCH58261, 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-ε]-1,2,4-triazolo[1,5-c]pyrimidine. Selective adenosine A2A antagonist; SHR, spontaneously hypertensive rat; SND, sympathetic neuronal discharge; SO, sham operated = conscious normotensive rats; 8-SPT, 8-(p-sulfophenyl)-theophylline. Non-selective adenosine receptor blocker; WKY, Wistar Kyoto rat.

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Adenosine is implicated in the modulation of cardiovascular responses either at the peripheral or at central level in experimental animals. However, there are no dedicated reviews on the involvement of adenosine in mediating the hypotensive response of centrally administered clonidine in general and specifically in aortically barodenervated rats (ABD). The conscious ABD rat model exhibits surgically induced baroreflex dysfunction and exaggerated hypotensive response, compared with conscious sham-operated (SO) rats. The current review focuses on the role of adenosine receptors in blood pressure (BP) regulation and their possible crosstalk with other receptors e.g. imidazoline (I1) and alpha (α2A) adrenergic receptor (AR). The former receptor is a molecular target for clonidine, whose hypotensive effect is enhanced approx. 3-fold in conscious ABD rats. We also discussed how the balance between the brain stem adenosine A1 and α2A receptors is regulated by baroreceptors and how such balance influences the centrally mediated hypotensive responses. The use of the ABD rat model yielded insight into the downstream signaling cascades following clonidine-evoked hypotension in a surgical model of baroreflex dysfunction.

Introduction

The current review focuses on, the role of adenosine receptors in BP regulation and their possible crosstalk with other receptors e.g. imidazoline (I1) and α2A AR. The former receptor is a molecular target for clonidine, whose hypotensive effect is enhanced approx. 3-fold in conscious ABD rats. Furthermore, the current review delineates the role of the downstream adenosinergic-signaling pathway in mediating the centrally evoked hypotension elicited by clonidine and clonidine such as drugs. The review covers data generated in our laboratory and reported pertinent studies over the past 10 years, which covered the following: (i) imidazoline I1-receptor and centrally mediated hypotension; (ii) clonidine and aortic barodenervation; (iii) clonidine and SHR rats; (iv) clonidine effects in the RVLM; (v) clonidine effects in the NTS; (vi) central adenosine receptor signaling; and (vii) central MAPK-NOS signaling.

The nucleus of the solitary tract (NTS)

The NTS mediates inhibitory actions of baroreceptors on sympathetic discharge and is considered the main site of termination of the baroreceptor afferent fibers via both the aortic depressor nerve and the glossopharyngeal (IX) from the carotid sinus [1–3]. Notably, lesions to the NTS abolish the baroreflex responses [3]. Several reports have shown important roles for activation of NTS glutamate [1,3] as well as adenosine receptors in BP regulation [1,2,4–7].

Rostral and caudal ventrolateral medulla

A large body of evidence supports the view that the RVLM is the major brain stem area that controls sympathetic drive by projecting directly to the spinal cord [1,8–10]. Neuronal activation in the RVLM causes an increase in arterial pressure mediated by an increase in peripheral resistance, cardiac output, and secretion of catecholamines [1]. Electrical and chemical stimulation of the RVLM produces immediate and marked increases in arterial pressure. The direct connection with the
sympathetic preganglionic neurons explains why an alteration in the RVLM neuronal activity dramatically influences sympathetic neuronal discharge (SND) and arterial pressure (AP) [1,8–10]. The RVLM-spinal neuronal connection plays at least two important roles in sympathetic and cardiovascular control. First, RVLM-spinal neurons set the tone for AP by providing a basal SND. This tone generating ability explains why chemical inhibition or lesioning of the RVLM causes a dramatic fall in arterial pressure [1]. Second, a dominant aspect of the RVLM neurons is the control of the baroreflex response. By serving as a major neuroanatomical target for centrally acting antihypertensive agents including clonidine, moxonidine, and rilmenidine [3], the RVLM plays a fundamental role in BP regulation and in the control of BP in treated hypertensives. Similar to the NTS, the RVLM expresses receptors including the adenosine, α2A adrenergic and imidazoline receptors [11,12]. It is not surprising that the RVLM shares with the NTS a similar receptor population since it receives inhibitory projections from the NTS and is involved in mediating baroreceptor efferent response via the sympathetic nervous system [1]. It must also be remembered that the caudal ventrolateral medulla (CVLM) plays important intermediate role between the NTS and RVLM, particularly in regulating the baroreflex function [3]. Unlike the anatomic ally and functionally (sympathoexcitatory) well defined neurons of the RVLM, the CVLM neurons are more heterogeneous and scattered [3]. However, functional and retrograde studies revealed projections from the NTS to the CVLM, which sends tonic sympathoinhibitory projections to the RVLM [3].

**The aortic barodenervated (ABD) rat model**

Various genetic models of hypertension, knockout mice, pheochromocytoma (PC12) cells and anesthetized animals have been used extensively to outline the signaling cascades triggered by adenosine, imidazoline (I1) and α2A adrenergic receptor activation [13–26]. However, little is known about the role of these receptors in BP control or BP responses to centrally acting drugs in conscious rats. Notably, clonidine-evoked hypotension is evident in conscious or anesthetized hypertensive rats [25,30]. In conscious intact rats, the hypotensive response elicited by clonidine is virtually absent in marked contrast to the case in the conscious aortic barodenervated rats. Following denervation, acute rises in BP, heart rate, and peripheral resistance are apparent in the ABD rat while cardiac index and stroke volume were not altered. Forty-eight hours later, when cardiovascular measurements were conducted in the absence of anesthesia, the reductions in cardiac index and stroke volume were paralleled by a return of the BP of conscious ABD rats to sham-operated levels while the peripheral resistance remained significantly elevated. Compared to sham operated rats, clonidine (30 μg/kg, i.v.) elicited greater decreases in BP in ABD rats via decreases in cardiac index and stroke volume because peripheral resistance did not change [31–33]. However, these studies focused on the role of baroreceptor dysfunction and sympathetic nervous system over-activity as underlying causes for the enhanced response to some centrally acting hypotensive drugs [31,34]. Other reported studies built on these findings to delineate the central pathways and cellular mechanisms implicated in this response in the ABD rat. Specifically, this review focuses on studies that elucidated the role of central adenosine receptor signaling in the conscious ABD rat model and their involvement in centrally mediated hypotension.

**Adenosine receptors in the CNS**

The high affinity A1 and the A2A receptors in the brain are tonically activated by extracellular adenosine, which set the basal “purinergic” tone seen in most systems. This notion is supported by the ability of caffeine to antagonize the actions of endogenous adenosine and reversing the tonic inhibition [35]. Four different adenosine receptors have been characterized pharmacologically, structurally and functionally and are denoted A1, A2A, A2B and A3 [35–37].

**Role of central adenosine receptors in blood pressure control**

The primary neurons that regulate sympathetic outflow located in the NTS and the RVLM, express adenosine receptors [1,2,12,13,23,38–43]. While activation of the A1 receptor by adenosine, or by the more selective agonist N6-cyclopentyl-adenosine, causes a pressor response, adenosine A2A receptor activation by adenosine, or by the more selective agonist, 2-p-(2-carboxyethyl) phenylthethylamino-5-N-ethylcarboxamidoadenosine (CGS21680), causes a depressor response [2,44–47].

**Adenosine receptor signal transduction mechanisms**

The original delineation of adenosine receptors is based on their regulation of cyclic adenosine monophosphate (cAMP) levels. The A1 and A2 receptors mediate a reduction in cAMP via Gαi/o whereas the A2A receptor mediates elevation in cAMP via Gαs [20,48–50]. Notably, the A2A and A2B are also linked to Gαq and the activation of PKC [20,21,51]. Contrary to previous views where receptor activation leads to a sequential downstream signaling paradigm, recent evidence suggests that single receptor activation may converge on a multitude of downstream signaling cascades. In line with this concept, adenosine receptor activation results in the phosphorylation of the mitogen-activated protein kinase (MAPK p44/42), also known as pERK1/2, through either PLC-DAG or the PKA pathways [20]. The well-conserved and diverse MAPK family, which covers three main groups, the extracellular signal-regulated protein kinases (ERK), the stress-activated protein kinases (SAPK; p38) and the c-Jun N-terminal kinases (JNK), is involved in cell cycle progression, proliferation and differentiation in all organisms including mammals. Adenosine receptor signaling may enhance or inhibit proliferation of a variety of cell types depending on the adenosine receptor (or combination of adenosine receptor) subtypes and the tissue type. All adenosine receptors activate at least one MAPK. For example, the Gαi-coupled adenosine A2A receptor activation enhances ERK1/2 phosphorylation as summarized in Fig. 1. Reported studies including ours implicated central adenosine receptors in BP modulation in at least some forms of hypertension. Microinjection of adenosine into the nucleus tractus solitarius (NTS) elicited enhanced depressor and reduced pressor responses in the SHR compared to its
not reverse the hypotension elicited by local imidazoline I1 receptor activation [58,59]. On the contrary, the hypotensive action of clonidine analogues was attenuated by microinjections of idoxozan or efaxoran, antagonists with imidazoline structures, into RVLM [11,60–62]. Several imidazoline preferring compounds such as rilmenidine and moxonidine possess preferential binding to the I1 receptor over the 2A AR compared to clonidine, which is a mixed I1/2A AR agonist [11,55,63–65]. However, functional studies in 2A AR knockouts have shown that despite rilmenidine and moxonidine I1R selectivity, the 2A AR is an important mediator of their hypotensive action [58,66–69]. Other studies have suggested synergy between the 2A AR and the I1 receptor signaling pathways [14,15]. The imidazoline binding site is a separate entity based on binding and functional studies that demonstrated the ability of selective I1 receptor agonists (LNP509) to lower BP when microinjected into the brain stem of D79N mice [14,68,70]. D79N mice constitute a functional 2A AR knockout model, which has been useful in elucidating the role of 2A AR in several functions including hypotension and sedation [71].

Although it is not known whether the I1 and 2A AR are operating in parallel or in series, there is evidence that the I1 receptor downstream signaling is distinct from that of the 2A AR receptor. Several reports have shown that in PC12 cells, which exhibit neuronal phenotype when differentiated, activation of the I1 receptor involves the phosphatidylycholine-selective phospholipase-C (PC-PLC) and PKC (β, γ isoforms) pathway and the increased formation of the second messenger diacylglycerol (DAG). As a consequence of the activation of PKC, ERK1/2 phosphorylation is increased [19,72–74]. These cellular events contribute to I1 (rilmenidine) mediated hypotension because similar to I1 receptor blockade (efaxoran), PC-PLC (D609), or pERK1/2 (PD98059) inhibition abrogated the hypertensive response and the corresponding cellular events elicited by the I1 receptor activation [18,19,22,72,74]. Noteworthy, other neuromodulators in the CNS, including l-glutamate and adenosine, which also enhance ERK1/2 phosphorylation [20,75] might be implicated in I1 receptor signaling. In support of this notion, l-glutamate release increases following clonidine or rilmenidine administration [17,76–78] and l-glutamate releases adenosine [79,80] (Fig. 2).

**Crosstalk between adenosine and imidazoline receptors signaling underlies clonidine-evoked hypotension in conscious ABD rats**

Evidence for the involvement of central adenosine receptors in clonidine-evoked hypotension is supported by a number of pharmacological studies. The finding that systemic administration of theophylline virtually abolished the hypotensive effect of clonidine inferred a central interaction of these two drugs because clonidine lowers BP via a central mechanism of action [81], and theophylline gains access to the CNS to block central adenosine receptors [13]. This finding was consolidated by the observation that intracisternal, but not systemic, administration, of the water-soluble adenosine receptor blocker 8-p-sulphophenyl-theophylline (8-SPT) attenuated clonidine-evoked hypotension. The inability of systemic 8-SPT, which blocks peripheral, but not central, adenosine receptors [13,23] to influence clonidine-evoked hypotension [82] bolsters the conclusion that central adenosine receptors are implicated in clonidine-evoked hypotension.
evoked hypotension. Further, central administration of SCH58261, a selective A2A receptor blocker [83,84], virtually abolished the clonidine-evoked hypotension [82]. Together, these findings suggest the dependence of clonidine-evoked hypotension on central adenosine A2A receptor.

Although Bousquet et al. [85] classified clonidine as ligand at the imidazoline-binding site, clonidine is still considered a mixed I1/2A AR agonist [27,85]. Therefore, it was difficult to ascertain the type of receptor, I1 or 2A, whose activation triggers central adenosine signaling. Findings from our laboratory indicate that the central hypotensive response elicited by selective activation of the central I1 (rilmenidine) or 2A (a-MNE) receptor was attenuated by central adenosine receptor blockade [82]. It is imperative to note that although a-MNE is considered a “pure” 2A receptor agonist [65], the selective I1 agonist rilmenidine also exhibits 2A agonist activity [27,55]. Together, these findings raise the interesting possibility that 2A receptor activation might also trigger central adenosine receptor signaling [82]. However, an alternative explanation is that I1 activation by rilmenidine might depend on a downstream 2A AR activation as proposed by Head [66]. Collectively, these findings suggest that the adenosinergic system plays a critical role in mediating centrally mediated hypotension. However, the use of non-selective adenosine receptor blockers (theophylline or 8-SPT) in these earlier studies precluded ascertaining the adenosine receptor subtype implicated in the mediation of clonidine-evoked hypotension. Building on the A2A receptor as a viable candidate because its activation within the brain stem leads to hypotension [4], data from our laboratory confirmed A2A involvement because the selective A2A receptor antagonist SCH58261 virtually abolished clonidine-evoked hypotension in conscious ABD rats [82].

Reciprocal roles for central A1 and A2A in blood pressure regulation

A number of studies including ours demonstrated functionally opposite roles for central A2A and A1 adenosine receptors in BP regulation because they mediate depressor, and pressor responses, respectively [2,4,6,47]. These findings lead to the postulate that concomitant activation of the adenosine A1 receptor might counterbalance (mask) the adenosine A2A-dependent hypotensive action of clonidine, as discussed above.

Our laboratory showed that upregulations of 2A AR and I1 receptors were paralleled with similar A2A receptor upregulation in the same brain stem areas of the ABD rat model [29]. The latter confirms and extends earlier findings, which demonstrated the upregulation of 2A and I1 receptors in the same animal model [32,86]. It might be argued that aortic baroreception caused nonspecific upregulation of adenosine A2A as well as the 2A AR and I1 receptors because they followed the same pattern in the investigated brain stem nuclei. However, such parallel upregulation might be physiologically relevant because: (i) the A2A receptor, the 2A and the I1 receptors in the NTS and RVLM are spatially associated, (ii) all three receptors mediate hypotension [2,55,65,86], and (iii) their shared signaling pathways make it highly likely that these receptors physiologically interact [18–20]. These findings are consistent with a key role for central adenosine A2A in clonidine-evoked hypotension in conscious ABD rats [82].

Overexpressed adenosine A2A receptor in brain stem is functionally relevant

Immunohistochemical evidence demonstrated approximately twofold increase in the number of A2A receptors in the NTS and RVLM of ABD, compared to SO, rats [87]. These findings were functionally relevant because the selective adenosine A2A agonist CGS21680 elicited significantly greater dose-dependent hypotensive responses in the ABD, compared to SO, rats [29]. Notably, particularly in the NTS and RVLM, the A2A receptor activation produces sympathoinhibition and hypotension [2,12,47], which are shared by clonidine and similar drugs [18,31,82,86]. Together, these findings establish a link between the anatomical and functional upregulation of brain stem adenosine A2A receptor in the ABD rat [31,32]. Equally important, these findings might explain, at least partly, the enhanced hypotensive response elicited by clonidine in ABD rats [31,82] and its dependence on central adenosine A2A receptor signaling [82].

ERK1/2-NOS activation underlies centrally mediated hypotension

As discussed earlier, ERK1/2 phosphorylation constitutes important signaling event in clonidine-evoked hypotension. Noteworthy, pERK1/2 involvement in I1 receptor-evoked
hypotension has been based on two findings: (i) pERK1/2 expression in the RVLM is enhanced in association with centrally mediated hypotension elicited by rilmenidine, but not by z-methylnorepinephrine [18] and (ii) the ERK1/2 phosphorylation inhibitor PD98059 significantly attenuated rilmenidine-evoked hypotension [18]. By the same token, the exaggerated hypotensive response elicited by central A2A receptor activation with i.c. CGS21680 in ABD rats might involve enhancement of ERK1/2 phosphorylation [87]. Further, central A2A receptor blockade, which virtually abolished clonidine-evoked hypotension [82], abrogated the associated increase in brain stem ERK1/2 phosphorylation (pERK1/2). The latter findings suggest the involvement of the A2A receptor signaling in the centrally evoked hypotensive response elicited by clonidine and other I1R agonists. It was reasoned that NO activation (phosphorylation) is triggered by pERK1/2 based on an established signaling pathway in cultured cells [88,89], and because NOS-derived NO causes sympathoinhibition and hypotension [90]. This intriguing possibility is supported: (i) by pharmacologic inhibition of ERK1/2 phosphorylation attenuated clonidine-evoked hypotension and ERK1/2 and NOS phosphorylation in the RVLM and (ii) while L-NAME abrogated clonidine-evoked hypotension without affecting the enhanced ERK1/2 phosphorylation in the RVLM [87]. These findings are consistent with a role for pERK1/2 as an upstream activator of NOS [87,91] and bolster the conclusion that pERK1/2 plays a pivotal role in centrally-mediated hypotension via downstream NOS activation (enhanced NO production). Further, these reported findings rule out the possibility that ERK1/2 phosphorylation was consequence of clonidine-evoked hypotension in the ABD model system. Together, these findings delineate the molecular events in the brain stem triggered by central adenosine A2A receptor activation and suggest a biological relevance for the pERK1/2-NOS pathway in-vivo. By contrast, we showed that the latter signaling pathway contributes to the central CB1R-mediated pressor response [92] via GABA dependent mechanisms. Future studies are needed to address this controversy because the adenosine A2 receptors are expressed on GABAergic neurons of the medulla oblongata of the developing rat brain.

Why clonidine fails to lower BP in conscious normotensive rats?

Many reported findings, including ours showed that clonidine does not lower BP [31,34,93,94] or influence ERK1/2 phosphorylation in the NTS and RVLM [87] in conscious normotensive rats. By contrast, as discussed above, clonidine enhances pERK1/2 expression and lowers BP in conscious ABD rats via adenosine A2A receptor dependent mechanisms. These findings set forth the postulate that concomitant adenosine A1 receptor activation serves a negative (counterbalancing) role against adenosine A2A receptor signaling triggered by clonidine in conscious normotensive rats. In support of this hypothesis are the findings that clonidine significantly reduced BP and increased brain stem pERK1/2 expression following central adenosine A1 receptor blockade (DPCPX) in conscious normotensive rats [29]. Interestingly, these molecular and BP responses were similar to those elicited by clonidine in ABD rats [31,82]. Collectively, these findings support a dampening role for central adenosine A1 receptor against clonidine-evoked hypotension and advance our knowledge in this area of research because central adenosine A1 receptor blockade (i) unmasked clonidine-evoked hypotension and the enhanced phosphorylation of brain stem pERK1/2 in conscious normotensive rats and (ii) had no effect on the neurochemical (pERK1/2) or the hypotensive response elicited by clonidine in ABD rats. These findings are consistent with opposite roles for central A1 (pressor) and A2A (depressor) receptor activation [2,6] and further support a pivotal role for brain stem pERK1/2 in the hypotensive action of clonidine and similar drugs [18].

Finally, it is imperative to comment on the differential expression of the adenosine A1 receptor in the NTS and RVLM of SO and ABD rats. We demonstrated an inverse relationship between the level of adenosine A1 receptor expression and the BP response to clonidine [29] in marked contrast to a direct relationship between A2A receptor expression in the same brain nuclei and the hypotensive effect of clonidine in ABD rats [29,87]. It is likely, therefore, that the balance between the A1 and the A2A adenosine receptor populations

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**Fig. 3** Conceptual overview of the major findings discussed in this review. Upregulation of A2A (large circle) and the molecular targets for clonidine (I1/x2A, large circles) are more evident in ABD rats (right hand side) compared to sham-operated, SO, rats (left hand side, small circles). Note the downregulation of A1 (small circle) in ABD compared to SO rats (large circle) in the NTS and RVLM. Direct (CGS21680) or indirect (clonidine) central A2A activation enhances pERK1/2 expression, which subsequently phosphorylates NOS (increased NO) and ultimately reduces BP. Blockade of central A2A receptor (SCH58261) or inhibition of NOS (L-NAME) abrogated clonidine-evoked hypotension, but only the former abrogated clonidine-evoked elevation in pERK1/2 expression. Intracisternal A1 receptor blockade (DPCPX) (large circle) unravels clonidine-evoked hypotension and enhances pERK1/2 expression in conscious normotensive rats. Central A1 receptor is downregulated in the NTS and RVLM (small circle) in ABD compared to SO rats (large circle), which is paralleled by an attenuated pressor response to adenosine A1 receptor activation (CPA) in ABD, compared to SO, rats.
in the brain stem determines the magnitude of the BP response elicited by clonidine and perhaps other centrally acting drugs. Tipping the balance toward adenosine A2A dominance might explain the enhanced clonidine-evoked hypotension in conscious ABD rats [82,87] and SHRs [13]. It is also important to discuss the role of the NTS adenosine A1 receptor in BP regulation and how it might be impacted by anesthesia. In general, anesthesia dampens the NTS A1-mediated pressor response because Machado and de Paula [95] showed that intra-NTS adenosine produced pressor response via activation of the local A1 receptor in conscious rats. These findings explain, at least partly, why systemic or intracerebral clonidine lowers BP in anesthetized, but not in conscious rats. Consistent with this knowledge, as discussed above, suppression of adenosine A1 and concomitant upregulation of A2 receptors in the brain stem occurs in the ABD rat and clonidine lowers BP in this animal model in the conscious state [82]. Nonetheless, the NTS neurons are heterogeneous because our reported studies showed that under the same experimental condition (anesthetized rats), microinjection of adenosine into the rostral and caudal NTS produced pressor and depressor responses, respectively [13]. Whether the adenosine A1/A2 ratios are different in these two subareas of the NTS remains to be elucidated.

Conclusions

The reviewed pharmacological and molecular findings support a differential role of adenosine A2A and A1 receptors in mediating and opposing clonidine-evoked hypotension, respectively. This review also provides a brief account on the role of pERK1/2-NOS-NO activation in brain stem nuclei as a molecular mechanism for the centrally mediated hypotension elicited by direct and indirect activation of the central A2A receptor by CGS21680 and clonidine, respectively. Further, the reviewed findings support the conclusion that pERK1/2 is a mediator and not a result of the hypotension elicited by direct or indirect A2A receptor activation. This is the first review that discussed the novel mechanism that central A1 receptor signaling masks clonidine-evoked hypotension in conscious normotensive rats (summarized in Fig. 3). Since clonidine is clinically used for the management of hypertension, possible drug interactions with the adenosine agonists and antagonists that cross the blood brain barrier might have clinical implications.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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