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Review Article

Caffeine and the Control of Cerebral Hemodynamics

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Abstract. While the influence of caffeine on the regulation of brain perfusion has been the subject of multiple publications, the mechanisms involved in that regulation remain unclear. To some extent, that uncertainty is a function of a complex interplay of processes arising from multiple targets of caffeine located on a variety of different cells, many of which have influence, either directly or indirectly, on cerebral vascular smooth muscle tone. Adding to that complexity are the target-specific functional changes that may occur when comparing acute and chronic caffeine exposure. In the present review, we discuss some of the mechanisms behind caffeine influences on cerebrovascular function. The major effects of caffeine on the cerebral circulation can largely be ascribed to its inhibitory effects on adenosine receptors. Herein, we focus mostly on the A_1, A_{2A}, and A_{2B} subtypes located in cells comprising the neurovascular unit (neurons, astrocytes, vascular smooth muscle); their roles in the coupling of increased neuronal (synaptic) activity to vasodilation; how caffeine, through blockade of these receptors, may interfere with the "neurovascular coupling" process; and receptor-linked changes that may occur in cerebrovascular regulation when comparing acute to chronic caffeine intake.

Keywords: Adenosine, arteriole, astrocyte, calcium, neurovascular coupling, synapse, vasodilation

INTRODUCTION

The widely-consumed psychostimulant, caffeine (1,3,7-trimethylxanthine), displays a broad array of actions on the brain. There are multiple targets of caffeine, giving rise to a high level of complexity that can confound experimental efforts to understand caffeine influences in the brain. Despite such "impediments", a substantial body of literature has been accumulated in recent years that has provided some insights into the mechanisms of caffeine influence in the brain, with important clinical/translational implications. One example of the latter would be Alzheimer's disease (AD), as supported by evidence suggesting a positive influence of long-term caffeine intake on cognitive function in patients, especially memory and learning (e.g. [1]). One possible contributing factor to the onset and progression of cognitive impairment in AD, is vascular dysfunction. In particular, this relates to pathologic changes in cerebral vascular tissue linked to accumulation of amyloid-β peptide (Aβ) in and around cerebral blood vessels, resulting in a condition labeled cerebral amyloid angiopathy (CAA). Some of the characteristics of CAA that are thought to contribute to exacerbation of AD pathology include impaired neurovascular coupling, cerebral hypoperfusion and hypercontractility, loss of cholinergic innervation, blood-brain-barrier damage, and microvascular ruptures [2–5]. Of some relevance to the present discussion, Arendash and coworkers [6] recently published compelling data showing that chronic caffeine consumption was associated with reductions in cerebral Aβ levels, and cognitive improvement in AD mice. Since increased presence of Aβ may impair cerebral vascular function (see above),

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it is tempting to postulate that caffeine could limit the severity of CAA and, perhaps, mitigate cognitive decline, via a vasculoprotective effect. However, such a mechanism remains to be established. Thus, in the remainder of this review, no further consideration will be given to caffeine-modulated vascular-AD linkages. Instead, we will focus primarily on acute and chronic caffeine influences on hemodynamic function, with some emphasis on neurovascular coupling (functional hyperemia).

CAFFEINE TARGETS RELATED TO CEREBRAL VASCULAR CONTROL

Caffeine influence on cerebral perfusion is likely to involve its interactions with targets in vascular cells (e.g., smooth muscle; endothelium) as well as non-vascular cells (e.g., neurons; astrocytes; microglia). The literature points to at least 4 targets of caffeine in the brain – adenosine receptors, cyclic nucleotide phosphodiesterases, ryanodine receptors, and $GABA_A$ receptors. With exception of the ryanodine receptors (activation), caffeine's influence on the above targets is inhibitory. Realistically, within the range of daily human caffeine consumption, among the caffeine-sensitive entities listed above, only the adenosine receptors may have any relevance, since caffeine possesses 1–2 orders of magnitude less potency toward the other targets listed [7]. Thus, the present review will focus only on caffeine effects on adenosine receptors and the implications of that interaction with respect to neurovascular coupling in particular.

NEUROVASCULAR COUPLING AND THE CONCEPT OF THE NEUROVASCULAR UNIT

The tight coupling between neuronal activity and blood flow is fundamental to brain function. When a specific brain region is activated, cerebral blood flow increases in a temporally and spatially coordinated manner, thereby improving substrate (glucose, O_2) delivery to meet local metabolic demands. Multiple signaling pathways have been shown to contribute to neurovascular coupling (reviewed in [8–11]). However, there appears to be a considerable degree of overlap in the mechanisms promoting the vasodilation accompanying increased neural activity, suggesting interactions among the signaling pathways. That complexity of the neurovascular signaling process, to a large degree, arises from the participation of multiple cells, principally neurons, astrocytes, and vascular cells (smooth muscle and endothelium), collectively termed the "neurovascular unit". Astrocytes, in fact, may represent the linchpin in transducing increased synaptic activity to local vasodilation. The important physiologic function of astrocytes in sensing neuronal activity and in turn regulating the tone of cerebral arterioles has been addressed by a number of investigators [8–11]. Astrocytic endfeet extensively ensheath cerebral microvessels, to the extent that direct neuronal contacts to cerebral arterioles are sparse. Thus, astrocytes physically link neurons and their synapses with the vasculature, and are in a strategic position to convey neuronal signals to the blood vessels. As an example, neuronal activation can evoke increases in astrocytic $[Ca^{2+}]$, which in turn may trigger certain enzymes or cell membrane channels to generate or release vasoactive compounds. In this review, owing to its relevance to caffeine influences, we consider only one such vasodilating pathway, one that is related to adenosine. Clearly, there are other astrocytic Ca^{2+}-linked vasodilating mechanisms, for example, involving K^+ or arachidonic acid-derived mediators [8–11]. The overall importance of astrocyte Ca^{2+} regulation to the neurovascular coupling process is reflected in the impairment of that process following inhibition of certain astrocyte Ca^{2+}-related pathways [12, 13].

Increased synaptic activity has been linked to initiation of a propagated signal between astrocytes. Thus, a signal originating at the site of increased neural (synaptic) activity can be transmitted, over multiple astrocytes, to perivascular glial endfeet and elicit changes in arteriolar tone. Calcium, as reflected in the appearance of a "Ca^{2+} wave" capable of traversing multiple astrocytes, appears to be a vital component in this signaling process. Other factors that may play important roles in the transmission of a neuronally-initiated vasodilating signal, via astrocytes, include excitatory neurotransmitters (e.g., glutamate), K^+, and ATP released by activated neurons (see [11]). There is evidence that ATP binds to purinergic receptors on neighboring astrocytes. These receptors are termed metabotropic purinergic receptors (labeled P_{2Y} in Fig. 1) by virtue of their linkage to phospholipase C activation, inositol trisphosphate generation, and the well-described release of Ca^{2+} from intracellular stores (e.g. [14]). This appears to play an important role in initiating and propagating the Ca^{2+} wave. The appearance of ATP in the extracellular compartment also leads to rapid formation of adenosine. This

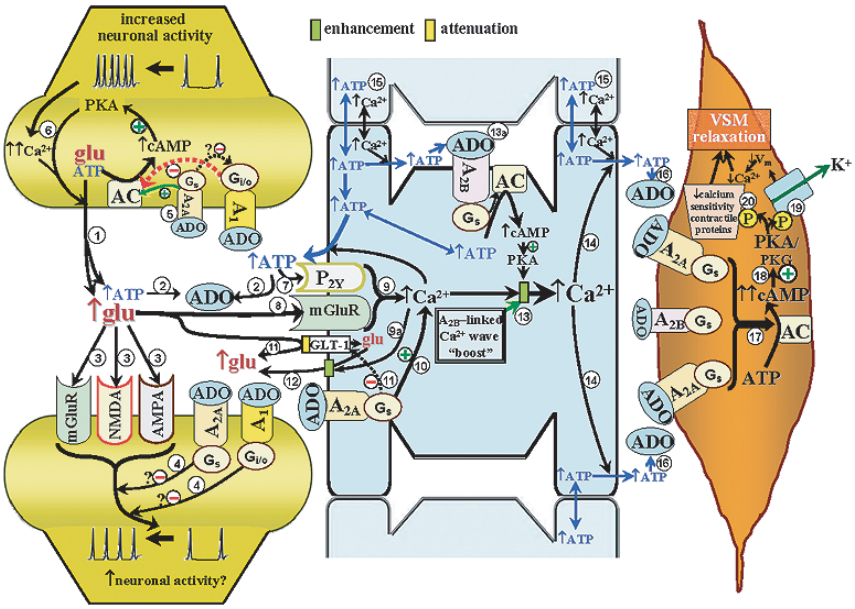

Fig. 1. The neurovascular unit (represented by a synaptic, astrocytic, and vascular component) and the role of adenosine (ADO), via its receptors, in the coupling of enhanced neural activity to arteriolar vascular smooth muscle (VSM) relaxation. *Synaptic Component.* Increased axonal activity is characterized by enhanced Ca^{2+} entry and accumulation in presynaptic nerve terminals, promoting a Ca^{2+}-dependent vesicular release of, for example, glutamate (glu) and ATP from the presynaptic terminal into the synaptic cleft①. The released ATP can be rapidly converted to adenosine (ADO) via ectonucleotidases②. The increased ADO can engage A_1 and A_{2A} receptors on pre- and post-synaptic membranes, and it can interact with A_{2A} and A_{2B} receptors on adjacent astrocytes (see below). Although A_1 receptors may be expressed on astrocytes and blood vessels, in the Fig. 1 model, those sites are not assigned any functional significance (see Table 1). The glutamate released from the presynaptic terminal can effect post-synaptic activation via engaging metabotropic (mGluR) or ionotropic (NMDA and AMPA) receptors on post-synaptic dendrites③. Post-synaptic activation of A_1 and A_{2A} receptors has been associated with repression of glutamate-linked post-synaptic function [22]. This could act as a "brake" on trans-synaptic signaling④. The patterns of A_1 and A_{2A} receptor expression, as well as the neurotransmitters they modulate, vary among brain structures. Based upon information obtained from cerebrocortical synaptosomes (where evidence indicates the presence of both A_1 and A_{2A} receptor-mediated modulation of glutamate release [32,34]), the model depicted in Fig. 1 (and Fig. 2) could be taken to represent cerebral cortex. The figure depicts the presence of A_1 and A_{2A} receptors in close association with one another in the presynaptic nerve ending. This "heteromeric" arrangement represents one of several possibilities, including scenarios where the A_1 or A_{2A} receptor subtype predominates. In the heteromeric arrangement, it has been postulated that the G_s-linked A_{2A} receptor not only will activate adenylyl cyclase (AC), but also, via a PKA-independent mechanism [32,34], prevent the $G_{i/o}$-linked A_1 receptor from inhibiting AC, especially under conditions of increased neuronal activity and ADO availability [35]⑤. One consequence of this will be a PKA-driven increased Ca^{2+} influx at the presynaptic membrane, overcoming A_1 receptor-linked depression of voltage-dependent Ca^{2+} entry [34], thereby potentiating Ca^{2+}-dependent glutamate/ATP release and extracellular ADO generation⑥. *Astrocytic Component.* The released ATP and glutamate can interact with astrocyte metabotropic P_{2Y} receptors⑦ and mGluR's⑧, respectively, leading to mobilization of Ca^{2+} from intracellular storage sites within astrocytes⑨. In addition, the increased presence of ADO, arising from the released ATP, activates A_{2A} receptors on astrocytes leading to cAMP/PKA-dependent mobilization of intracellular Ca^{2+} from cellular stores⑩. Adenosine interaction with astrocytic A_{2A} receptors also can contribute to blockade of the astrocytic glutamate import protein, GLT-1⑪; and promote Ca^{2+}-dependent ⑨enhancement of glutamate efflux⑫. This should result in further elevations in glutamate levels in the synaptic cleft, as well as contributing to the astrocytic "Ca^{2+} wave". The figure also speculates that a PKA-linked "boost" to the astrocytic Ca^{2+} mobilization⑬ may arise from ADO binding to G_s-linked A_{2B} receptors ⑬. The "wave" of Ca^{2+} generated by the combined influences of glutamatergic, purinergic P_{2Y}, and adenosinergic mechanisms will ultimately promote ATP release from astrocytes, including remote sites⑭. ATP represents an important signaling molecule in astrocytes. It arises from cellular glucose and O_2 metabolism and can diffuse (along with Ca^{2+}) from astrocyte to astrocyte through gap junctions⑮. Additionally, ATP represents perhaps the most important molecule involved in inter-astrocytic communication. Thus, Ca^{2+}-dependent release of ATP from one astrocyte interacts with P_{2Y} receptors on adjacent astrocytes, contributing to the spread of the Ca^{2+} wave. *Arteriolar Component.* The release of ATP in the vicinity of arterioles is likely to result in rapid formation of ADO⑯ and interactions with smooth muscle A_2 receptors. There is little doubt that cerebral arterioles are well-endowed with A_2 receptors. Both A_2 subtypes are likely to be present on cerebral resistance vessels; although the literature seems to favor the A_{2A} receptor, especially in intraparenchymal and pial arterioles [24,50]. This is reflected in the figure. Principally, A_2 activation generates cAMP⑰, which is not only capable of activating PKA, but cGMP-dependent protein kinase (PKG) as well [46,70]⑱. The increased kinase function is associated with phosphorylation and opening of K^+ channels⑲, leading to smooth muscle cell hyperpolarization ($\downarrow V_m$). This lowers intracellular Ca^{2+} levels through a reduction in Ca^{2+} influx via voltage-operated Ca^{2+} channels. Elevated PKA/PKG function also is accompanied by a reduction in the Ca^{2+}-sensitivity of contractile proteins (e.g., myosin⑳). The combination of reduced VSM Ca^{2+} levels and diminished sensitivity to Ca^{2+} leads to relaxation. See text for further discussion and additional citations.

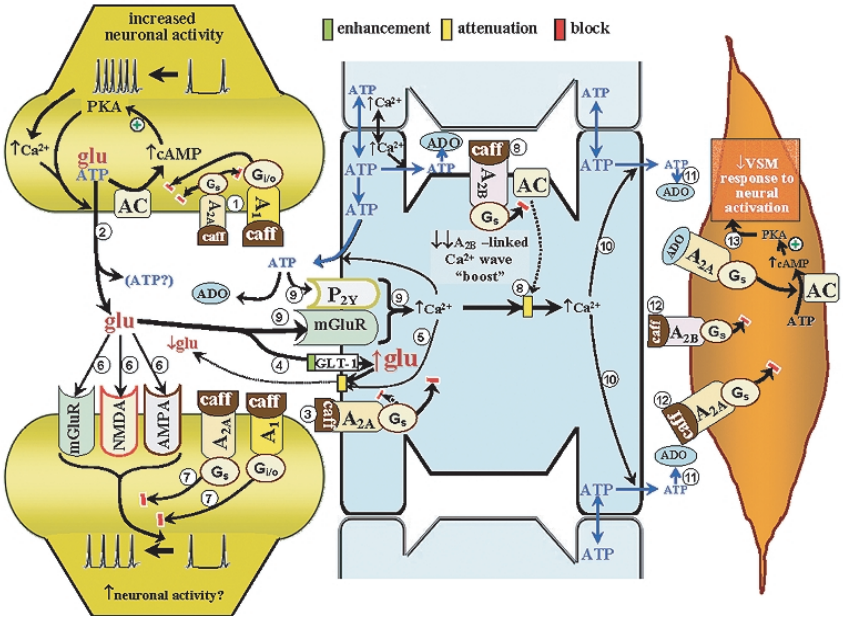

Fig. 2. Scheme depicting diminished neurovascular coupling during *acute* caffeine exposure. There are several potential manifestations related to caffeine restriction of ADO interactions with A_1 and A_{2A} receptors in presynaptic nerve endings. The net effect of that "multi-receptor blockade" on glutamate (and ATP) release during increased synaptic activity depends on the A_1/A_{2A} configuration. In an A_1 receptor-dominant presynaptic configuration, the presence of caffeine should remove the postulated A_1 receptor-related block of voltage-dependent Ca^{2+} entry (e.g. [71]), thus increasing Ca^{2+}-mediated glutamate and ATP release. In an A_{2A} receptor-dominant presynaptic scenario (probably the least likely), caffeine blockade is likely to attenuate glutamate release during states of increased neuronal activity. In Fig. 2, the heteromeric A_1/A_{2A} receptor arrangement is represented①. Although precise predictions are not possible, caffeine-related blockade of both receptors might be associated with modest or no changes in activity-evoked glutamate and ATP release②. Further reductions in glutamate presence in the synaptic cleft may arise from caffeine blockade of astrocytic A_{2A} receptors③ – leading to disinhibition of GLT-1 (permitting greater uptake of glutamate from the synaptic cleft)④ and diminished A_{2A} receptor-mediated Ca^{2+}-dependent glutamate efflux⑤. A net reduction in glutamate levels in the synaptic cleft could restrict the generation of glutamate receptor-mediated post-synaptic activation⑥. Yet, the presence of caffeine will also prevent ADO-linked post-synaptic depression mediated through activation of A_1 and A_{2A} receptors⑦. This may permit some post-synaptic activation to occur. Moreover, caffeine blockade of astrocytic A_{2A} and A_{2B} receptors③⑧, along with the diminished contributions from metabotropic purinergic and glutamatergic receptors (arising from reduced extracellular ATP and glutamate levels⑨), a large reduction in the capacity to generate a Ca^{2+} wave might be expected⑩, resulting in less ADO being "presented" to VSM cells⑪. The diminished ADO exposure, combined with caffeine blockade of VSM A_2 receptors⑫, leaves little capacity remaining for ADO-mediated vasodilation. The potentially diminished capacity to effect "remote" increases in extracellular ADO could also interfere with heterosynaptic influences (see Fig. 1 legend); although the lack of relevant information does not permit any further discussion of this matter. The above speculation provides some (although not the only) possible explanations for the finding that *acute* caffeine administration profoundly attenuates the *in vivo* dilation of pial arterioles⑬ accompanying somatosensory activation in rats [54].

is due to the ubiquitous presence of ecto-nucleotidases on astrocytic, vascular, and neuronal surfaces in the brain [15–19], particularly ecto-nucleoside triphosphate diphosphohydrolase-1 (E-NTPDase-1) and ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP), which mediate direct ATP to AMP conversions; and ecto-5'-nucleotidase, which catalyzes the subsequent formation of adenosine from AMP. In considering neurovascular coupling and the neurovascular unit, one cannot ignore the contributions from the pial arterioles that lie upstream from the parenchymal arterioles. Thus, although local dilation of parenchymal arterioles is important in adjusting nutrient supply to neuronal needs, that response could be ineffective in the absence of dilation in the pial arterioles. Indeed, during increased activity of cortical neurons, overlying pial arterioles dilate, despite the lack of any direct contact with activated neurons. In a recent report, we showed that astrocytes provide a key link between increased neuronal activity in the brain parenchyma and the remote arteriolar relaxation represented by pial arterioles [20].

ADENOSINE RECEPTORS AND NEUROVASCULAR COUPLING

The role of adenosine and its receptors in the coupling of increased neuronal/synaptic activity to dilation

Table 1
Explanation of simplifying assumptions relevant to Figs 1 and 2

Simplifying assumption	Rationale/ supporting evidence/caveats
A_1, A_{2A}, and A_{2B} receptors possess similar sensitivities to caffeine blockade.	Only applies to conditions of enhanced synaptic activity, where endogenous adenosine rises to levels sufficient to activate low-affinity A_{2B} receptors [7].
A_3 receptors are not considered.	Limited cerebral A_3 expression [27]; no direct vascular actions of A_3 receptor ligands [24]; order-of-magnitude less sensitive to caffeine vs A_1, A_{2A}, and A_{2B} receptors [7].
Caffeine blockade of A_1 receptors primarily reflects actions toward presynaptic sites; although post-synaptic sites cannot be ignored.	Although A_1 receptors exist on post-synaptic membranes, presynaptic expression may predominate [21]. A major consequence of presynaptic A_1 receptor activation is inhibition of neurotransmitter release [22]. Post-synaptic A_1 receptor activation may hyperpolarize post-synaptic elements, further restricting synaptic signaling.
Astrocytic A_1 receptors are not considered.	Synaptic A_1 receptor expression exceeds astrocytic expression [21]; reports are inconsistent regarding the manifestations of astrocytic A_1 receptor activation (e.g., intracellular Ca^{2+} regulation [25,26]).
Cerebral arterioles are devoid of functional A_1 receptor activity.	Direct applications of A_1 receptor ligands are without affect on cerebral arterial/arteriolar tone [24].
Caffeine influence on neurovascular coupling is likely to involve blockade of A_{2A} receptors expressed on pre- and post-synaptic membranes, astrocytes, and vascular cells.	Presynaptic A_{2A} receptor engagement can promote glutamate release both directly and via restricting A_1 receptor effects. Adenosine A_{2A} (and A_1) receptors on post-synaptic membranes are thought to depress glutamate receptor-mediated post-synaptic activation [22]. Post-synaptic expression of A_{2A} receptors may be of some importance in "heterosynaptic" regulation (see text), but is not assigned a major role in the synapse $\rightarrow$ astrocyte $\rightarrow$ arteriole signaling pathway.
Caffeine blockade of A_{2B} receptors, during synaptic activation states, primarily reflects actions toward astrocytic and vascular sites.	Neuronal A_{2B} receptor expression is sparse [38,39]. There is good evidence of A_{2B} receptor expression in astrocytes [22]. Although controversial, cerebral artery/arteriolar A_{2B} receptor expression and function may be less extensive than A_{2A} receptors [8,24, 50].

of arterioles and arteries, and how caffeine might influence that important physiologic response, is the central theme of this review. To facilitate the following discussion of this rather complex process, we have created two cartoons representing neurovascular coupling in the absence (Fig. 1) and presence (Fig. 2) of acute caffeine exposure. In constructing these cartoons, a number of simplifying assumptions were made regarding the interplay among the major components of the neurovascular unit (synapse, astrocyte, and arteriole), adenosinergic mechanisms, and the targets of caffeine. Those assumptions, along with their rationale, supporting evidence, and caveats are summarized in Table 1.

Adenosine receptors, also referred to as purinergic P_1 receptors, consist of four known subtypes (A_1, A_{2A}, A_{2B}, and A_3). These receptors are coupled to either $G_{i/o}$ (A_1 and A_3) or G_s (A_{2A} and A_{2B}) proteins, which, upon engagement with adenosine, results in inhibition or enhancement of adenylyl cyclase activity, respectively. Among the adenosine receptors, the A_1 receptor is the most regionally widespread in the brain. Evidence indicates that the A_1 receptor is enriched in synaptic membranes in relation to other cellular elements [21], which is consistent with its purported neuromodulatory role. A prime example of that role is the ability of A_1 receptor activation to restrict presynaptic neurotransmitter release [22]. Furthermore, A_1 receptor agonists have been reported to have a far greater effect toward restricting glutamate vs GABA release, indicating that presynaptic A_1 activation is primarily anti-excitatory [22]. Although some cerebrovascular presence of A_1 receptors has been documented [23], its functional significance is unclear, since direct applications of A_1 ligands do not affect cerebral arterial/arteriolar tone [24]. The A_1 receptor is expressed in astrocytes [25,26]. However, results from *in vitro* models (primary cultures, acutely isolated astrocytes, or brain slices) have not yielded consistent findings. That is, A_1 receptor activation has been associated with PLC-linked intracellular Ca^{2+} elevations in some situations [25], but repression of Ca^{2+} influx in others (e.g. [26]). The A_3 receptor also is expressed in brain tissue, but with a limited distribution [27]. Although some expression of A_3 receptors has been found in brain blood vessels [23], like the A_1 receptor, it does not appear to be associated with any direct hemodynamic function [24]. The A_3 receptor has also been detected in astrocyte *in vitro* preparations [25], and may be involved in the regulation of astrocyte intracellular Ca^{2+} [28].

The high affinity, A_{2A}, and low affinity, A_{2B}, receptors are expressed in cerebral resistance vessels (see, for example [24]). Despite its lower adenosine affinity, recent findings [29] suggested that the A_{2B} receptor

plays a significant role in physiologic cerebral vasodilation. Some have speculated that the A_{2A} and A_{2B} receptors are differentially distributed within the cerebral arterial/arteriolar system, with A_{2A} receptors predominating in the upstream pial arterial/arteriolar segments and A_{2B} having greater influence in the downstream intraparenchymal segments [8]. However, others have reported a substantial A_{2A} receptor participation in adenosine-induced dilations in rat brain parenchymal arterioles [24]. Irrespective of segmental localization, adenosine engagement of the vascular A_2 receptors promotes vasodilation. Like A_1 receptors, A_{2A} receptors are found in presynaptic structures, but with a more limited regional distribution compared to A_1 receptors [30]. In rodents, the A_{2A} subtype exhibits the highest expression in the striatum, in keeping with its association with dopamine-rich structures, with comparatively low abundance (relative to A_1) in the cortex and hippocampus (e.g. [31]). Nevertheless, the existence of A_{2A} receptors in the cortex is not insignificant, in light of evidence that glutamate release in rat cortical synaptosomes is enhanced by A_{2A} receptor activation [32]. In contrast to the hippocampus [30] and striatum [33], the possibility of a *cortical* A_1/A_{2A} colocalization has not been confirmed via immunohistochemistry. Nevertheless, the presence of both A_1 and A_{2A} receptors in cortical synaptic structures can be inferred from findings that evoked glutamate release from cortical synaptosomes exhibits a marked sensitivity to manipulations of A_1 and A_{2A} receptor function [32,34]. One postulated manifestation of colocalization is the "heteromeric" A_1/A_{2A} arrangement [31]. Thus, the higher levels of extracellular adenosine that are likely to be associated with conditions of elevated neuronal activity are thought to favor increased A_{2A} receptor function [35]. The activated A_{2A} receptor can, in turn, effect an inhibition of A_1 receptor function, via a mechanism involving protein kinase C, rather than cAMP-dependent protein kinase (PKA) [36,37]. The A_{2A} receptor effect could also include a more conventional PKA-dependent mechanism that involves elevated presynaptic Ca^{2+} entry [22]. The end result of these A_{2A} receptor-related actions is enhancement of glutamate release. Experimental evidence, obtained in synaptosomal preparations of rat cortex, hippocampus, and striatum, support an A_{2A} receptor crosstalk repression of A_1 receptor synaptic function that occurs across multiple cerebral structures [36,37].

With respect to A_{2B} receptors, evidence indicates a rather sparse neuronal presence in the brain [38,39]. Although A_{2B} receptors are reasonably well-expressed in astrocytes and cerebral vascular cells [8,22,24,40], in view of the low affinity of A_{2B} receptors toward adenosine, any functional relevance may be limited to conditions of elevated extracellular adenosine presence, as may be found during higher neuronal activity states. The model depicted in Fig. 1 represents such a state. Therefore, A_{2B} receptors on astrocytes and vascular smooth muscle are given consideration (see also Table 1).

In astrocytes, at least two functions for A_{2A} receptors have been identified (see Fig. 1). Both of these functions are likely linked to activation of adenylyl cyclase and enhanced PKA-mediated phosphorylation. The first involves restriction of glutamate uptake, via inhibition of the astrocytic GLT-1 transporter [41]. The second A_{2A} receptor function relates to PKA-mediated release of Ca^{2+} from astrocytic stores [42]. This not only can contribute to trans-astrocytic signaling (i.e., the "Ca^{2+} wave"), but also promote Ca^{2+}-dependent glutamate and ATP efflux (e.g. [41,43–45]). This will yield further elevations of glutamate and ATP in the synaptic cleft, potentiating neurotransmission [25,40] and providing more ATP for extracellular adenosine generation. It also has been suggested that adenosine engagement of astrocytic A_{2B} receptors may provide a "boost" for the astrocytic Ca^{2+} wave during neural activation states (see review by Koehler et al. [8]). The A_2 receptor-associated potentiation of astrocytic signaling, provided by a more robust Ca^{2+} wave, will ultimately enhance release of astrocytic ATP in the vicinity of arterioles. The ATP can then be rapidly converted to adenosine, which interacts with arteriolar smooth muscle A_2 receptors. The subsequent G_s-mediated activation of smooth muscle adenylyl cyclase will increase intracellular cAMP levels and activate PKA (and, perhaps, cGMP-dependent protein kinase [PKG] [46]), which in turn, can phosphorylate targets within the smooth muscle cells. One target is K^+ channels [47], leading to K^+ efflux, thereby promoting smooth muscle hyperpolarization and reduced intracellular Ca^{2+} levels. Another key group of phosphorylatable targets are myosin light chain phosphatase, myosin light chain kinase, and RhoA, with the result being a reduced sensitivity of contractile proteins to Ca^{2+} [46,48]. It should be emphasized that the signaling scheme provided by Fig. 1 applies both to local dilation of parenchymal arterioles, involving a single astrocyte, or upstream pial arteriolar dilation involving Ca^{2+}/ATP (and perhaps K^+) – linked communication over multiple astrocytes (see [11,20]).

It is of some interest to note that if one replaces the vascular smooth muscle cell in Fig. 1 with another

synapse, interactions of adenosine with its receptors in that synapse could lead to synaptic depression. Such a process might be found in the "heterosynaptic depression" model proposed by Cunha [31]. Thus, an astrocytic Ca^{2+} wave, arising as the result of synaptic activation at one locus, will ultimately result in the release of ATP and subsequent generation of adenosine at a remote astrocytic site. If that adenosine contacts an arteriole, vasodilation (neurovascular coupling) will occur. If another synapse is contacted instead, synaptic depression could occur. One might speculate that a possible contribution to that heterosynaptic depression could arise not only from the presence of A_1 receptors presynaptically, but also from the presence of A_1 and A_{2A} receptors on post-synaptic membranes of these distal synapses. Post-synaptic A_1 and A_{2A} receptors are thought, if anything, to be associated with attenuation of post-synaptic excitation (e.g., glutamatergic [22,49]). Thus, adenosine-related attenuation of post-synaptic function at remote (and even proximal) synapses could act to limit the spread of electrical activity from extending much beyond the area of initial activation. This might be viewed as an efficient way to ensure sufficient perfusion and substrate delivery to active neurons while limiting the amount of electrical "noise" (see Table 1). However, it merits mention that distribution of adenosine receptors between pre-and post-synaptic membranes may exhibit regional variations. For example, for A_{2A} receptors, evidence suggests post-synaptic localization being favored in the striatum, while a distribution favoring the presynaptic membrane appears to be the case in the cortex [49]. How such differences might affect heterosynaptic depression remains to be determined.

ACUTE CAFFEINE EFFECTS ON NEUROVASCULAR COUPLING

A scheme summarizing acute caffeine effects on neural activation-evoked arteriolar dilation is presented in Fig. 2. A number of the simplifying assumptions listed in Table 1 relate specifically to caffeine influences. First, we assumed a roughly equivalent caffeine potency toward blocking A_1, A_{2A}, and A_{2B} receptors [7]. However, despite that similarity, the A_{2B} receptors, owing to their low adenosine affinity, may be affected by caffeine only under conditions of enhanced neuronal (synaptic) activity, where endogenous adenosine levels are likely to be elevated. Since Fig. 2, like Fig. 1, depicts an increased synaptic activity state, caffeine influences on A_{2B} receptors are included. Second, possible contributions from A_3 receptors are not considered. This is due to limited A_3 receptor distribution in the brain [27] and an apparent caffeine inhibitory potency toward the A_3 receptor that is lower by approximately one order of magnitude, in relation to the other 3 receptors [7]. Third, we postulated that caffeine effects toward A_1 receptors primarily involve preventing presynaptic manifestations of A_1 receptor activation (i.e., the capacity to restrict neurotransmitter release); although post-synaptic A_1 receptor blockade is given some consideration [49]. Fourth, when examining the effects of caffeine-mediated blockade of A_{2A} receptors on arteriolar responses during neuronal activation states, we took into account pre- and post-synaptic, astrocytic, and vascular smooth muscle A_{2A} receptor sites (see earlier). For A_{2B} receptor-related influences of caffeine on arteriolar tone, under conditions of increased synaptic activity, it seems reasonable to limit the focus to blockade of astrocytic and smooth muscle A_{2B} receptors.

Nevertheless, in the context of neurovascular coupling, the assumption of an A_1 receptor effect of caffeine being largely confined to presynaptic elements is bolstered by findings that topical application of the A_1 receptor antagonist, DPCPX, actually potentiates pial arteriolar dilations evoked by sciatic nerve stimulation in rats [50]. This might be explained on the basis of an A_1 receptor-linked repression of neurotransmitter (e.g., glutamate) release, during increased axonal activity, acting as a "brake" on synaptic function. The upshot of this is that A_1 receptor blockade will be accompanied by *enhanced* synaptic activity. In fact, there appears to be a significant A_1 receptor-related tonic inhibition of synaptic transmission [51]. This could account for the well-known arousal effects associated with caffeine consumption. However, caffeine-induced arousal was reported to be absent in A_{2A} receptor knockout mice [52]. Although this seems to imply an important role for A_{2A} receptor inhibition in the arousal response to caffeine, those findings have been questioned in a recent report [53]. On the other hand, acute caffeine administration in rats constricts pial arterioles [54] and lowers CBF in rats [55] and human subjects [56,57]. This could be explained by the caffeine-induced loss of a basal A_2 receptor-associated vasodilating "tone". The importance of a vascular (presumably A_2 receptor) site for the vasoconstrictive actions of caffeine is underscored by findings showing that direct application of caffeine was accompanied by a $\sim$70% reduction in adenosine-induced dilation of iso-

lated rat brain arterioles [54]. The acute effects of caffeine on vascular tone and CBF not only may be related to the presence of A_{2A} and A_{2B} receptors on vascular smooth muscle, but also on astrocytes. Thus, especially under conditions of elevated synaptic activity, blockade of astrocytic A_2 receptors could disrupt Ca^{2+} signaling mechanisms, diminishing ATP release, and reducing the amount of adenosine to which arterioles are exposed. Furthermore, one also cannot ignore effects arising from caffeine blockade of A_{2A} (and A_1) receptors on synaptic membranes. During enhanced axonal activity, A_{2A} receptor-mediated facilitation may override A_1 receptor-mediated repression of glutamate release from nerve endings [31]. In the presence of caffeine, which blocks both receptors, it is difficult to predict what the net effect on glutamate release will be. Caffeine-associated blockade of astrocytic A_{2A} receptors, as depicted in Fig. 2, may favor net glutamate uptake into astrocytes, reducing glutamate levels in the synaptic cleft. Thus, there is a measure of uncertainty regarding the net effect of caffeine on activation of post-synaptic glutamate receptors. Adding to the uncertainty, caffeine will also block post-synaptic A_1 and A_{2A} receptors [22]. Since this could remove adenosine-associated inhibitory influences on glutamate-related post-synaptic function, the overall net effect could be enhanced post-synaptic activity. It is tempting to speculate that caffeine blockade of post-synaptic adenosine receptors might enhance the post-synaptic electrical response to glutamate during states of elevated neural activity. This might lead to a greater spread of electrical "noise" beyond the initial site of increased axonal firing, perhaps contributing to increased heterosynaptic activity and caffeine-related arousal. Thus, the ability to maintain or increase neural activity, in the face of direct vasoconstrictive actions (caffeine blockade of VSM A_2 receptors) is consistent with findings showing neurovascular "uncoupling" in rats and humans given acute caffeine treatment [54,56,64].

CHRONIC CAFFEINE EXPOSURE AND CEREBRAL PERFUSION

Although there is agreement among studies, with respect to the vasoconstrictive, CBF-lowering actions of acute caffeine treatments, the effects of chronic caffeine exposure on cerebral perfusion cannot be easily generalized. There are a number of factors one might consider regarding cerebrovascular regulation in conjunction with chronic caffeine use. Certainly, adenosine-linked factors merit prime consideration. This includes (but is not limited to), changes in adenosine receptor expression and agonist sensitivity associated with caffeine tolerance. One example is the upregulation of platelet A_{2A} receptor expression observed in human subjects following chronic caffeine exposure [58]. One could also consider how variations in adenosine receptor distribution, within the cerebral arterial system and the other components of the neurovascular unit, may impact on caffeine effects. A number of publications appearing over the past two decades point to cerebral A_1 (but not A_{2A}) receptor upregulation and sensitization in rodents in association with chronic caffeine intake (reviewed in [59]). Consistent with a lack of influence of chronic caffeine intake on A_{2A} receptor function, one study in rats revealed that the tolerance that develops toward the striatal motor stimulant effects of caffeine, with chronic caffeine intake, could not be recapitulated with prolonged administration of a selective antagonist of A_{2A} receptors [60]. However, the results of other investigations showed seemingly opposite effects of chronic caffeine treatment. Thus, an enhanced A_{2A} receptor ligand binding in the striatum was reported in one study [61], while a downregulation of striatal A_{2A} mRNA and protein expression was observed in another [62]. Adding to the uncertainty, Arendash and co-workers [63] reported no changes in A_{2A} and A_1 expression in hippocampus and frontal cortex of mice following chronic caffeine intake. While this might reflect regional selectivity in adenosine receptor responses to chronic caffeine exposure, it should be noted that the results in the latter study were obtained in amyloid precursor protein transgenic mice, and, thus, should be viewed with some caution.

Another potentially interesting aspect of the role of adenosine receptors in caffeine tolerance development relates to the postulated presence of presynaptic A_1-A_{2A} receptor heterodimers in the brain [31], as touched upon earlier. In one postulated scenario [35], at least in the striatum, receptor tolerance to chronic caffeine exposure affects A_{2A} more than A_1 receptors. This relative enhancement of A_{2A} receptor function could effect further inhibition of caffeine-repressed A_1 receptors. In this way, chronic caffeine exposure would favor a greater A_{2A} over A_1 receptor function and enhanced presynaptic activity, as opposed to caffeine-naïve rats. It might be speculated that such a scheme could be associated with a partial recovery of the repressed neurovascular coupling accompanying acute caffeine exposure. However, this co-localization of adenosine receptors is not uniformly distributed among brain regions and may

simply function as a limited local "fine-tuning" mechanism [30,31,33]. Furthermore, the potential impact of A_1-A_{2A} receptor heterodimerization as a facilitator of neurovascular coupling may only be manifested in higher brain activation states [31]. In summary, there remains a high level of uncertainty surrounding this issue, and further study is clearly warranted.

However, none of the studies cited above provided evidence regarding expression or functional changes in vascular-specific adenosine receptor populations. Based upon the earlier discussion, it is difficult to envisage chronic caffeine effects that do not involve vascular smooth muscle A_2 receptors (see [54]). Indeed, it has been suggested that the increased CBF that occurs in human subjects within 24 h of caffeine withdrawal results from adenosine binding to a more sensitized population of receptors or a greater abundance of cerebral arterial receptors (see [65,66] for references). Due to the lack of evidence supporting any direct adenosine A_1 receptor effects on cerebral arteriolar tone [54], one might ascribe the apparent "upregulation" of adenosine receptor function to vascular A_2 receptors. Furthermore, in human subjects, a partial (or incomplete) tolerance to acute caffeine-related CBF reductions was found in association with chronic caffeine intake [66, 67]. While this could be a reflection of A_{2A} receptor upregulation (as postulated by Addicott et al. [65]), in the absence of any data regarding A_2 receptor expression or ligand affinity in cerebral arteries and arterioles, one cannot eliminate the possibility that these chronic caffeine effects may be related to adenosine receptors residing in astrocytes or neurons. One also cannot ignore the role of altered abundance of non-adenosinergic receptor populations. For example, increased expression of brain tissue acetylcholine, serotonin, and $GABA_A$ receptors has been documented in the mouse in conjunction with chronic caffeine ingestion [59,68]. As such, there is the possibility that chronic caffeine effects on neurovascular coupling (or vascular function in general) may arise from altered expression of non-adenosinergic receptors, or even ion channels [59,68], residing in one or more of the cellular elements of the neurovascular unit. These and related issues will make interesting subjects for future experimentation.

CONCLUDING REMARKS

In conclusion, caffeine can have multiple effects on cerebral arteries and arterioles, resulting in a complex influence on brain perfusion both under resting (basal) conditions and during states of increased synaptic activity (neural activation-induced hyperemia). At the levels of caffeine achieved in the circulation and brain during normal human consumption, caffeine is known to exert measurable effects on CBF. Those effects can be largely attributed to actions toward adenosine receptors and not other potential targets of caffeine, such as phosphodiesterases and ryanodine receptors. Both animal and human studies have indicated that acute caffeine exposure leads to constriction of cerebral vessels and reduced CBF, as well as diminished neurovascular coupling. It can be postulated that those effects of caffeine likely arise from inhibitory actions on A_1, A_{2A}, and A_{2B} receptors distributed among the cellular components of the neurovascular unit – namely, neurons, astrocytes and vascular smooth muscle. The principal site of acute caffeine influence on A_1 receptors is likely presynaptic nerve endings. Yet, if this were the only target of caffeine to affect neurovascular coupling, one should observe an enhanced cerebral vasodilating response to neural/synaptic activation, as was reported by Meno et al. [50] in the presence of an A_1 receptor antagonist. Since caffeine suppresses neurovascular coupling (e.g., in the cortex [54]), it is probable that this reflects caffeine actions toward A_{2A} receptors, and, perhaps to a lesser extent, A_{2B} receptors. The A_2 receptors contributing to the caffeine effect are likely to be found in multiple cellular sites. Indeed, A_{2A} receptors have been identified in presynaptic, post-synaptic, astrocytic, and vascular smooth muscle sites; whereas A_{2B} receptors are, at the least, expressed in astrocytes and vascular smooth muscle cells.

Attempts to understand the effects of chronic caffeine intake on cerebral hemodynamics have been confronted with more uncertainty. A principal problem here arises from a lack of consensus with respect to changes in the expression and/or adenosine sensitivity of A_1 vs A_2 receptors in association with prolonged exposure to an antagonist, like caffeine. While we cannot ignore A_1 receptor changes, there is some indirect evidence to suggest that vascular A_{2A} receptors might selectively upregulate during chronic caffeine exposure. This could explain the repeated observation that acute caffeine withdrawal from chronic caffeine ingestion is associated with an increase in CBF – one that is directly proportional to the daily caffeine intake immediately preceding the onset of abstinence (e.g. [69]). However, further experimentation is clearly warranted. Such experimentation could include, in animal models, a simple verification of whether there is at least a trend toward normalization of an acutely depressed neurovas-

cular coupling in the presence of continuous caffeine intake. In association with such studies, one might consider the use of conditional knockouts to characterize the participation of adenosine receptors associated with specific cellular elements within the neurovascular unit.

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