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The role of adenosine receptors in the central action of caffeine

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Abstract

The behavioral effects of caffeine appear likely to be due in large measure to antagonism of the action of endogenous adenosine at A_1- and A_{2a}-receptors in the central nervous system. Other biochemical mechanisms of action of caffeine, such as release of intracellular calcium, inhibition of phosphodiesterases and blockade of regulatory sites of $GABA_A$-reccptors, would require much higher concentrations than the micromolar concentrations of caffeine associated with behavioral stimulation. However, micromolar concentrations of caffeine also would be expected to cause only a modest blockade of adenosine receptors. Selective adenosine agonists and xanthine antagonists have provided some insights into central roles for adenosine receptor subtypes. Thus, behavioral stimulation by xanthines appears to require blockade of both A_1- and A_{2a}-receptors. Chronic blockade of adenosine receptors by caffeine would be expected to result in alterations in the central receptors and pathways that are regulated by adenosine through A_1- and A_{2a}-receptors. Indeed, chronic caffeine docs alter the density not only of adenosine receptors, but also of adrenergic, cholinergic, GABAergic and serotonergic receptors. Behavioral responses to agents acting through dopaminergic and cholinergic pathways arc altered. As yet, a coherent explanation of the acute and chronic effects of caffeine in terms of blockade of adenosine receptors has not emerged. Interactions between pathways subserved by A_1 - and A_{2a}-adcnosine receptors complicate attempts to interpret caffeine pharmacology, as does the complex control by adenosine receptors of dopaminergic, cholinergic and other central pathways.

Keywords

Adenosine receptors; Calcium storage; Phosphodiesterase; Dopamine; Cocaine; Amphetamine; Nicotine; Muscarinic antagonists

The widespread usc of caffeine-containing beverages has focused research on the mechanisms underlying the central effects of caffeine (Nehlig et al., 1992; Daly, 1993). While the effects of moderate doses of caffeine on behavior arc complex, it appears likely that blockade of A_1- and/or A_2-adenosine receptors, are the primary molecular site of action

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for caffeine. There are at lea st four types of adenosine receptors in brain (Jacobson et al., 1992; Daly & Jacobson, 1994, and ref. therein). The A_1-class can be inhibitory of adenylate cyclase, stimulatory to potassium channels, inhibitory to calcium channels, and stimulatory to phosphoinositide breakdown. Selective agonists and selective xanthine and nonxanthine antagonists are available for A_1-receptors. The A_{2a}- and A_{2b}-Subclasses are stimulatory to adenylate cyclase. The A_{2a}- and A_{2b}-receptors differ in affinity and in agonist selectivity. Selective agonists and antagonists for A_{2a}-rcceptors are available. Selective agents for A_{2b}-reccptors arc not available. Caffeine is not selective for A_1- or A_2-receptors. The A_3-rcceptor also occurs in brain, is inhibitory to adenylate cyclase, and is remarkable in being insensitive to block ade by xanthines, at least in rodents.

Direct effects of caffeine on receptors, other than adenosine receptors, have not been reported. Indirect effects of caffeine on systems served by receptors other than the adenosine receptor will occur due to the blockade by caffeine of the tonic inhibitory input by adenosine through A_1-receptors on release of norepinephrine, dopamine, serotonin, acetylcholine, GABA, glutamate, and perhaps even ncuropeptides. Since A_2-adcnosinc receptors can modulate responses of brain second messenger systems to norepinephrine, serotonin, histamine (Daly, 1977), both the cyclic AMP-generation and the phosphoinositide-breakdown mediated by receptors for those neurotransmitters would be expected to be altered by caffeine. Activation of A_{2a}-adenosine receptors has been shown to reduce affinity of dopaminergic agonists for D_2-receptors (Ferré et al., 1991) and caffeine, thus, could affect dopamine function through blockade of tonic adenosine input to such A_{2a}-adenosine receptors. Thus, caffeine, through blockade of adenosine receptors, would be expected to indirectly influence the function of most neuronal pathways in the brain. There is evidence for central effects of caffeine *in vivo* and *in vitro* on noradrenergic, dopaminergic, serotonergic, cholinergic, GABAergic, and glutaminergic systems (Daly, 1993), but current research is focused on the interrelated adenosine-dopamine-acetylcholine systems of the basal ganglia, in particular the striatum (Ferré et al., 1992).

Molecular sites of action other than adenosine receptors are known for caffeine. Historically, the first site of action of caffeine to be identified was stimulation of release of calcium from intracellular storage sites. Caffeine binds to a site on a calcium-channel, which is associated with the intracellular, so-called calcium-sensitive pool of calcium, and thereby enhances calcium-dependent activation of the channel (McPherson et at., 1991). This calcium-channel is the one blocked by ryanodine. Caffeine is now a widely-used tool for studies of the role of this pool in nerve and muscle function, particularly with regard to oscillations in membrane potentials and calcium levels. Caffeine, however, has a very low affinity for such sites with thresholds for effects on release of intracellular calcium at about 250 μM, while 5 to 20 mM concentrations are required for robust effects. This is in contrast to the higher affinities of caffeine as an antagonist for adenosine receptors, where thresholds are less than 10 μM and Ki values arc 40 to 50 μM, well within plasma and brain levels attained by htlmans and ani mals with behaviorally effective doses of caffeine. Certain xanthines that arc more potent than caffeine as calcium releasing agents (Muller & Daly, 1993) may prove to be valuable in probing the role of the intracellular calcium-sensitive calcium channels in the behavioral pharmacology of caffeine.

Historically, the second site of action of caffeine to be identified was inhibition of phosphodiesterases. Other xanthines that are much more potent than caffeine as phosphodiesterase inhibitors have been developed and most have proved rather nonspecific as inhibitors of various phosphodiesterase isozymes. Caffeine itself has IC_{50} values for phosphodiesterase isozymes ranging from 500 μM to 1 mM, again well above the range at which caffeine blocks adenosine receptors. Xanthines that are potent phosphodiesterase inhibitors, in particular towards a brain calcium-independent cyclic AMP phosphodiesterase (rolipram-sensitive type IV isozyme) are behavioral “depressants”. in contrast to the behavioral stimulant activity of caffeine and other xanthines that are weak inhibitors of that isozyme (Choi et al., 1988). The “depressant” part of the bell-shaped dose response curve of caffeine with respect to open-field locomotor activity (Fig. 1) may be due to inhibition of the calcium-independent phosphodiesterase, which would become significant only at the highest doses of caffeine.

In the late 1970’s, caffeine was found to inhibit binding of benzodiazepines to sites on the GABAAreceptor channel (Marangos et al., 1979). Although exciting from the standpoint of the anxiogenic properties of caffeine, the affinity of caffeine (K_i 280 μM) was several-fold higher than *in vivo* concentrations of caffeine that would be reached at non-toxic doses of caffeine. Interaction s at $GABA_A$-receptors may be relevant to the convulsant activity of caffeine. Xanthines more potent than caffeine at benzodiazepine sites have not been developed.

Thus, in spite of extensive studies on possible biochemical sites of action for caffeine *in vivo*, only adenosine receptors have the requisite 10-50 μM affinities for caffeine. Other sites, such as intracellular calcium-sensitive calcium release channels, phosphodiesterases and $GABA_A$ receptors require> 200 μM concentrations of caffeine. At such concentrations caffeine is a convulsant *in vivo*.

Chronic treatment of animals with caffeine, not surprisingly, results in an up-regulation of A_1-adenosine receptors as first reported by Fredholm (1982) for caffeine, and by Murray (1982) for theophylline. Almost all s ub sequent studies on chronic caffeine or theophylline have documented an increase in cortical A_1-adenosine receptors, except for ene study with rats (Holtzman et al., 1991). The A_{2a}-adenosine receptors do not appear to be up-regulated (Johansson et al., 1993; Shi et al., 1993), although there is one report of an increase in levels of A_{2a}-aclenosine receptors in striatum after chronic ingestion of caffeine by mice (Hawkins et at., 1988). The A_{2b}-receptor-mediated stimulation of cyclic AMP in rat brain slices does not appear altered after chronic caffeine (Fredholm, 1982; Zielke & Zielke, 1987).

Most chronic studies related to levels or function of adenosine receptors have been caffeine or theophylline. In the 1980’s, chronic administration of N^6-R-phenylisopropyladenosine was shown to reduce the analgetic and locomotor depressant effects of caffeine (Ahlijanian & Takemori, 1986). Levels of A_1-adenosine receptors were unaltered. Recently, chronic injections of A_1-selective agents, 8-cyclopentyl-1, 3-dipropylxanthine or N^6-cyclopentyladenosine were shown to have opposite effects on NMDA-induced seizures (Von Lubitz et al., 1994). Chronic xanthine treatment greatly reduces the NMDA-effects, while

chronic treatment with the adenosine analog enhances the NMDAelicited seizures. Levels of A_1-adenosine receptors were unaltered.

Adenosine receptors are not the only central receptors, whose levels are altered after chronic caffeine ingestion. This is not surprising, since removal of tonic adenosine inhibition of neurotransmitter release might be expected to increase neurotransmitter release and lead to a downregulation 0f the relevant neurotransmitter receptor. Hewever, in most cases an up-regulation rather than a down-regulation of receptors occurs. There has been only one broad study of effects of chronic caffeine on levels of central receptors (Shi et al., 1993). Chmnic caffeine ingestion in male Swiss strain mice was found to affect the density of receptors subserving noradrenergic, serotonergic, cholinergic and GABAergic pathways (Table 1). Remarkably, since a variety of evidence indicates that caffeine affects dopaminergic function (Ferré et al., 1992), the levels of dopaminergic receptors appear unaffected. The levels of cortical and striatal At adenosine receptors are increased by 15-20% by chronic caffeine, while the level of striatal A_{2a}-adenosine receptors is unaltered. The levels of cortical ß$_1$- and cerebellar ß$_2$-adrenergic receptors are reduced by about 25%, while the levels of cortical α_1 and α_2-adrenergic receptors are not significanLly altered. The levels of striatal D_1- and D_2-dopaminergic receptors are not altered. Levels of cortical muscarinic and nicotinic receptors are increased by 40-50%. The apparent up-regulation of nicotinic receptors may actually represent conversion of nicotinic receptors to a desensitized state. The level of cortical benzodiazepine-binding sites associ-ated with $GABA_A$-receptors is increased by 65% anci in this case the affinity for diazepam appears slightly decreased. The level of cortical MK-801 binding sites associated with NMDA-glutaminergjc receptors appear unaltered. The level of cortical delta-opioid receptors is increased by 25%, while the levels of cortical mu- and kappa-opioid receptors are unchanged. The level of cortical sigma receptors is unchanged. The density of cortical nitrendipine-binding sites associated with L-type calcium channels is increased by 18%. Thus, there is an incredible array of alterations in levels of central receptors elicited by chronic caffeine ingestion in NIH Swiss strain mice. In addition, basal levels of striatal adenylate cyclase are decreased after chronic caffeine, while stimulations *via* D_2-dopamine receptors or A_{2a}-adenosine receptors are unaltered (Shi et al., 1994). In rats, the up-regulation of adenosine receptors (see Daly, 1993), the down-regulation of ß-adrenergic receptors (Goldberg et al., 1982; Fredholm et al., 1984; Green & Stiles, 1986) and an up-regulation of benzodiazepine sites, associated with $GABA_A$-receptors (Wu & Coffin, l984; Wu & Phillis, 1986) have been reported after chronic caffeine or theophylline. Effects on other receptors do not appear to have been examined systematically in rats. The levels of forskolin-binding sites, associated with adenylate cyclase, have been reported to be increased in rat cerebral cortex after chronic caffehiC (Daval et al., 1989).

Clearly, the plethora of biochemical alterations in mice after chronic caffeine will make difficult interpretations of behavioral alterations in the chronically caffeine-treated animal. The most studied behavioml alteration has been tolerance to caffeine. An “insurmountable tolerance” has been reported in rats (Holtzman, 1983; Holtzman et al., 1988, 1991). An explanation as to how up-regulation of A_1-adenosine receptors could lead to an “insurmountable” tolerance to an agent, caffeine, that acts as an antagonist has not been forthcoming. The answer may lie in the biphasic dose-response curve to caffeine (see Fig. 1) where low doses of caffeine cause stimulation, while higher doses cause depression of

locomotor activity. Thus, after chronic caffeine the depressant effects may predominate, leading to the appearance of an “insurmountable tolerance” with respect to behavioral stimulation. The effects of chronic caffeine on open-field locomotor activity have been also evaluated thoroughly, not in Sprague-Dawley rats, but recently in NIH Swiss strain mice. Tolerance does not occur in these mice and indeed the threshold for stimulatory effects of caffeine is significantly lowered (Fig. 2, Nikodijevic et al., 1993a). Sensitization to behavioral effects of caffeine after chronic caffeine has also been reported in rats (Meliska et al., 1990). Behavioral depression by high doses of caffeine is perhaps slightly enhanced after chronic caffeine ingestion by mice (Nikodijevic et al., 1993a). The choreiform (dance-like) movements elicited in mice by high doses of caffeine are significantly reduced after chronic caffeine ingestion (Nikodijevic et al., 1993c).

More consonant with the up-regulation of A_1-receptors is the observation that the behavioral depressant effects of an A_1-selective adenosine analog, N^6-cyclohexyladenosine (CHA) are slightly enhanced after chronic caffeine ingestion in mice (Nikodijevic et al., 1993ab). However, the behavioral depressant effects of an A_{2a}-selective adenosine analog, APEC, arc also slightly enhanced, as are those of a potent mixed A_1/A_2-adenosine analog, NECA. A simple interpretation of these results is complicated by the fact that there appea rs to be a synergism between the behavioral depressant effects of activation of A1-receptors and A_{2a}-receptors by selective adenosine analogs in mice (Nikodijevic et al., 1991). This may explain the high potency of the mixed A_1/A_2 agonist NECA as a behavioral depressant, and might explain the enhanced depressant effects of all adenosine analogs after the chronic caffeine-elicited up-regulation of A_1-adenosine receptors.

The converse to synergism s for agonists appears to apply with respect to the behavioral stimulation elicited by xanthines. Thus, 8-cyclopentyltheophylline (CPT), an A_1-selective antagonist, is a weak behavioral stimulant, 8-cyclopentyl-1, 3-dipropylxanthine (CPX), an even more A1-selective antagonist, is not a behavioral stimulant (Nikodijevic et al., 1991, 1993b) and 8-(3-chlorostyryl) caffeine (CSC), an A_{2a}-seleclive antagonist, is a very weak behavioral stimulant (Jacobson et al., 1993). However, a combination of CPX and CSC results in a synergistic stimulation of open-field locomotor activity (Fig. 3). Thus, caffeine and other xanthines that are relatively non-selective as adenosine receptor afltagonists may owe their effectiveness as behavioral stimulants to blockade of both A_1- and A_{2a}-adenosine receptors.

Geminal studies in the early 1980’s proposed a correlation of A_1-receptor affinity and behavioral stimulation for xanthines (Snyder et al., 1981; Katims et al., 1983). This no longer appears true and indeed A_{2a}-receptors have been proposed to have a more dominant role in regulation of behavioral activity (Durcan & Morgan, 1989). Recent studies suggest that stimulation of locomotor activity by caffeine and development of tolerance to caffeine arc more closely related to blockade of A_1-adenosine receptors (Kaplan et al., 1992, 1993). The demonstration of synergistic interactions of A_1- and A_{2a}-adenosine receptors in control of locomotor activity (Nikodijevic et al., 1991, Jacobson et al., 1993), suggests that questions as to relative importance of blockade of A_1- versus A_2-adenosine receptors to the effects of caffeine will be difficult to answer. It is noteworthy that the effectiveness with which caffeine and other xanthines reverse the depressant effects of adenosine analogs is

usually greater than their ability to cause behavioral stimulation alone (Katims et al., 1983; Coffin et al., 1984; Holtzman et al., 1991).

One other aspect of behavioral effects of adenosine analogs and xanthines further iiluslrates the complexity of interactions of adenosinc/xanthines. In the early 1980's it was noted that the combination of caffeine with N^6-Rphcnylisopropyladenosinc not only reversed the behavioral depressant effects of the adenosine analog, bllt actually caused a behavioral stimulation greater than that elicited by the xanthines alone (Snyder et al., 1981; Katims et al., 1983, see also Phillis et al., 1986). This occurs with caffeine and theophylline and even with a non-stimulatory xanthine, isobutylmethylxanthine (Fig. 4). An explanation was not apparent in 1980, nor has one been forthcoming. However, on examining dose-response effects on open-field locomotor activity for the adenosine analogs CHA, NECA and APEC in combination with caffeine, it appears that the synergistic stimulatory effect of xanthine-adenosine analog combinations manifests itself as a stimulatory "bump" or at least a plateau in the behavioral dose-response curve as illustrated for NECA in Fig. 5A. It is noteworthy that the stimulatory "bump" is diminished after chronic caffeine ingestion (Fig. 5A and Nikodijevic et al., 1993b) as are the synergistic depressant effects of A_1- and A_{2a}-agonists (Nikodijevic et al., 1993b). Such "bumps" or plateaus in the dose-response curves for NECA have now been observed when NECA is administered in combination with central stimulants other than ca ffeine (see below).

Since dopaminergic systems are strongly linked to caffeine pharmacology, it was reasonable to examine alterations in behavior subserved by dopaminergic pathways after chronic caffeine. Behaviorally, the stimulation of open-field locomotor activity by amphetamine, which releases dopamine, and cocaine which blocks reuptake of dopamine, are liule affected by chronic caffeine ingestion in mice (Nikodijevic et al., 1993a). This is at least consonant with the lack of change in density of dopamine receptors. However, it should be noted that a 1 mg/kg dose of amphetamine has significantly less effect after chronic caffeine ing estion {Nikodijevic et al., 1993a, sec Fig. 5B). Further studies are needed, since a large body of evidence suggests that chronic caffeine ought to affect, via blockade of striatal adenosine receptors, the function of dopaminergic receptors and/or dopaminergic sensitivity (Ferré et al., 1992 and ref. therein). Recent studies, demonstrating that antagonists for Dt and Indopamine receptors block caffeine-induced stimulation of locomotor activity in rats (Garrett & Holtzman, 1994) provide further evidence for the importance of dopamine systems to the behavioral pharmacology of caffeine. Dopamine systems do undergo hom eostatic changes as a result of denervation, chronic receptor activation, or chronic receptor-blockade. Chronic treatment with adenosine analogs has been shown to result in an attenuation of both A_{2a}-adenosine and Dt-dopamine receptor-mediated stimulation of striatal adenylate cyclase (Porter et al., 1988). Dopamine denervation was reported to enhance A_{2a}-adenosine and Indopamine receptor interactions in rat striatum (Ferré & Fuxe, 1992). Thus, it is clear that dopamine and A_{2a}-adenosine systems are subject to homeostatic regulation, and the lack of major changes after chronic caffeine remains puzzling. It is of mechanistic interest that the dose-response curves for effects of NECA on open-field locomotor activity can exhibit a stimulatory "bump" not only when assessed in the presence of a stimulatory dose of caffeine (Fig. 5A), but also with a stimulatory dose of amphetamine or cocaine (Fig. 5BC). The "bump" for amphetamine is diminished after chronic caffeine ingestion.

Cholinergic systems also appear intimately linked to function of striatal dopaminergic systems and to caffeine-sensitive adenosine systems. Chronic caffeine ingestion does cause alterations in levels of both muscarinic and nicotinic receptors (Shi et al., 1993). Nicotine/caffeine interacti ons have been extensively studied {White, 1988; Cohen et al., 1991 and ref. therein). Behaviorally, the stimulation of open-field locomotor activity by the muscarinic agonist scopolamine in mice is significantly changed after chronic ca ffeine ingestion (Nikodijevic et al., 1993a). Higher doses of scopolamine are required for the same degree of locomotor stimulation suggesting an increased tonic input of ace tylcholine to muscarinic receptors. The dep ressant effects of a muscarinic agonist, oxotremorine, appear only somewhat diminished after chronic caffeine ingestion. The dose-response curves for effects of NECA on locomotor activity in the presence of a sti mulatory dose of scopolamine arc only ma rginally biphasic (Fig. 5D). Behavioral depressant effects of a nicotinic agonist, nicotine, arc absent after chronic caffeine ingestion (Nikodijevic et al., 1993a, Shi et al., 1994) in spite of an apparent up-regulation of nicotine receptors. Probably, as is the case for tolerance to nicotine elicited by chronic nicotine, these “up-regulated” receptors are actually desensitized and non-functional (Marks et al., 1993). Nicotine, in combination with caffeine, has little effect in control mice, but can cause behavioral stimulation after chronic caffeine (Shi et at., 1994). It should be noted that the open-field locomotor activity of the NIH Swiss strain mice has been reduced by nearly 40% as a result of chronic ingestion of caffeine, and it was postulated that this might be due in part to enhanced cholinergic function (Nikodijevic et al., 1993a). Mecamylamine, a nicotinic antagonist causes somewhat greater depression in mice after caffeine ingestion (Shi et at, 1994).

Behavioral studies on noradrenergic, serotoninergic, $GABA_A$, and calcium channel faneLion after chronic caffeine ingestion also are neeelecl, since levels of receptors subserving Stich systems are altered after chronic treatment of male NIH Swiss strain mice (Shi et at, 1993).

In summary, a large body of evidence suggests that A_1- and A_{2a}-adcnosine receptors arc the most likely targets for pharmacological actions of caffeine in the central system. The A_1- and A_2-adenosinc receptor-regulated pathways are not independent and seem to interact synergistically to cause betiavioral depression, while blockade by receptor-selective xanthines interacts synergistically to cause behavioral stimulation. Thus, caffeine may owe many central-mediated behavioral effects to its nom-selective ability to block A_1- and A_{2a}-adenosine receptors. Chronic ingestion of caffeine by NIH Swiss strain mice results in a wide range of biochemical alterations in the central nervous system (Table 1). A depression in basal open-field locomotor activity occurs, accompanied by changes in respoasi-vcness to caffeine and other xanthines, to adenosine analogs, and to cholinergic agents. Changes in behavioral responsiveness to dopaminergic agents are minimal. Combinations of caffeine with an adenosine analog reveal interesting multiphasic effects of the adenosine analog on locomotor activity. Such multiphasic effects also pertain for combinations of the adenosine analog with doparninergic (cocaine, amphetamine) agents, but not with muscarinic (scopolamine) agents. Chronic caffeine ingestion reduces the depressant effects of ethanol, but chronic ethanol ingestion has no effect on the locomotor stimulation evoked by caffeine (Daly et at, 1994).

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References

Ahlijanian MK, Takemori AE. Cross-tolerance studies between caffeine and (−)-N^6-(phenylisopropyl)-adenosine (PIA) in mice. Life Sciences. 1986; 38: 577–588. [PubMed: 3003486]

Choi OH, Shamim MT, Padgett WL, Daly JW. Caffeine and theophylline analogues: Correlation of behavioral effects with activity as adenosine receptor antagonists and as phosphodiesterase inhibitors. Life Sciences. 1988; 43: 387–398. [PubMed: 2456442]

Coffin VL, Taylor JA, Phillis JW, Altman HJ, Barraco RA. Behavioral interaction of adenosine and methylxanthines on central purinergic systems. Neuroscience Leuers. 1984; 47: 91–98.

Cohen C, Welzl H, Bauig K. Effects of nicotine, caffeine, and their combination on locomotor activity in rats. Pharmacology Biochemistry and Behavior. 1991; 40: 121–123. [PubMed: 1780334]

Daly, JW. Mechanism of action of caffeine. In: Garauini, S, editor. Caffeine, Coffee and Heallh. Raven Press; New York: 1993. 97–150.

Daly, JW. Cyclic Nucleotides in the Nervous System. Plenum Press; New York: 1977.

Daly, JW, Jacobson, KA. Adenosine receptors: Selective agonists and antagonists. In: Pelleg, Belardinelli L., editor. Adenosine and Adenine Nucleotides: Molecular Biology to Integrative Physiology. Kluwer Academic Publishers; Norwall MA: 1994. in press

Daly JW, Shi D, Wong V, Nikodijevic O. Chronic effects of ethanol on central adenosine function of mice. Brain Research. 1994; 650: 153–156. [PubMed: 7953667]

Daval JL, Deckert J, Weiss SRB, Post RM, Marangos PJ. Up-regulation of adenosine A_1-receptors and forskolin binding sites following chronic treatment with caffeine or carbamazepine: a quantitative autoradiographic study. Epilepsia. 1989; 30: 26–33. [PubMed: 2912715]

Durcan M, Morgan PF. Evidence for A_2 receptor involvement in the hypomotility effects of adenosine analogs in mice. European Journal of Pharmacology. 1989; 168: 285–290. [PubMed: 2583238]

Ferré S, Fuxe K. Dopamine denervation leads to an increase in the membrane interaction between adenosine A_2 and dopamine D_2 receptors in the neostriatum. Brain Research. 1992; 594: 124–130. [PubMed: 1467931]

Ferré S, von Euler G, Johansson B, Fredholm B, Fuxe K. Stimulation of high affinity A-2 receptors decreases the affnity of dopamine D-2 receptors in rat striatal membranes. Proceedings of the National Academy of Sciences. 1991; 88: 7237–7241.

Ferré S, Fuxe K, von Euler G, Johansson B, Fredholm BB. Adenosine-dopamine interactions in the brain. Neuroscience. 1992; 51: 501–512. [PubMed: 1488111]

Fredholm BB. Adenosine actions and adenosine receptors after 1 week treatment with caffeine. Acta Physiologica Scandinavica. 1982; 115: 283–286. [PubMed: 6291335]

Fredholm BB, Jonzon B, Lindgren E. Changes in noradrenaline release and in beta receptor number in rat hippocampus following long-term treatment with theophylline or L-phenylisopropyladenosine. Acta Physiologica Scandinavica. 1984; 122: 55–59. [PubMed: 6095597]

Garrett BE, Holtzman SG. D_1 and D_2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor, activity in rats. Pharmacology Biochemistry and Behavior. 1994; 47: 89–94. [PubMed: 7906891]

Goldberg MR, Curatolo PW, Tung CS, Robertson D. Caffeine down-regulates 13 adrcnoreceptors in rat forebrain. Neuroscience Letters. 1982; 31: 47–52. [PubMed: 6289200]

Green RM, Stiles GL. Chronic caffeine ingestion sensitizes the A_1 adenosine receptor-adenylate cyclase system in rat cerebral cortex. Journal of Clinical Investment. 1986; 77: 222–227.

Hawkins M, Dugich MM, Porter NM, Urbancic M, Radulovacki M. Effects of chronic administration of caffeine on adenosine A_1 and A_2 receptors in rat brain. Brain Research Bulletin. 1988; 21: 479–482. [PubMed: 3214753]

Holtzman SG. Complete, reversible drug-specific tolerance to stimulation of locomotor activity by caffeine. Life Sciences. 1983; 33: 779–787. [PubMed: 6888193]

Holtzman SG, Finn IB. Tolerance to behavioral effects of caffeine in rats. Pharmacology Biochemistry and Behavior. 1988; 29: 411–418. [PubMed: 3362935]

Holtzman SG, Mante S, Minncman KP. Role of adenosine receptors in caffeine tolerance. Journal of Pharmacology and Experimental Therapeutics. 1991; 256: 62–68. [PubMed: 1846425]

Jacobson KA, van Galen PJM, Williams M. Adenosine receptors: Pharmacology. structure-activity relationships and therapeutic potential. Journal of Medicinal Chemistry. 1992; 35: 407–422. [PubMed: 1738138]

Jacobson KA, Nikodijevic O, Padgett WL, GalloRodriguez C, Maillard M, Daly JW. 8-(3-Chlorostyryl) caffeine (CSC) is a selective A_2-adenosine antagonist *in vitro* and *in vivo*. FEBS Letters. 1993; 323: 141–144. [PubMed: 8495727]

Johansson B, Ahlberg S, van der Ploeg I, Brene S, Lindefors N, Persson H, Fredholm BB. Effect on long term caffeine treatment on A_1 and A_2 adenosine receptor binding and on mRNA levels in rat brain. Naunyn-Schmideberg's Archives of Pharmacology. 1993; 347: 407–414. [PubMed: 8510768]

Kaplan GB, Greenblatt OJ, Kent MA, Cotreau MM, Arcelin G, Shader RJ. Caffeine-induced behavioral stimulation is dose-dependent and associated with At adenosine receptor occupancy. Neuropsychopharmacology. 1992; 6: 145–153. [PubMed: 1599605]

Kaplan GB, Greenblat OJ, Kent MA, Cotreau-Bibbo MM. Caffeine treatment and withdrawal in mice: Relationships between dosage, concentrations, locomotor activity and A_1 adenosine receptor binding. Journal of Pharmacology and Experimental Therapeutics. 1993; 266: 1563–1572. [PubMed: 8371158]

Katims JJ, Annau Z, Snyder SH. Interactions in the behavioral effects of methylxanthines and adenosine derivatives. Journal of Pharmacology and Experimental Therapewics. 1983; 227: 167–173.

Marangos PJ, Paul SM, Parma AM, Goodwin FK, Synapin P, Skolnick P. Purinergic inhibition of diazepam binding to rat brain (*in vitro*). Life Sciences. 1979; 24: 851–858. [PubMed: 449624]

Marks MJ, Grady SR, Collins AC. Down regulation of nicotinic receptor function after chronic nicotine infusion. Journal of Pharmacology and Experimental Therapeutics. 1993; 266: 1268–1276. [PubMed: 8371136]

McPherson PS, Kim YK, Valdivia H, Knudson CM, Takekura H, Franzini-Armstrong C, Coronado R, Campbell KP. The brain ryanodine receptor: a caffeine-sensitive calcium release channel. Neuron. 1991; 7: 17–25. [PubMed: 1648939]

Meliska CJ, Landrum RE, Landrum TA. Tolerance and sensitization to chronic and subchronic oral caffeine: Effects on wheel running in rats. Pharmacology Biochemistry and Behavior. 1990; 35: 477–479. [PubMed: 2320659]

Muller CE, Daly JW. Stimulation of calcium release by caffeine analogs in pheochromocytoma cells. Biochemistry and Pharmacology. 1993; 46: 1825–1829.

Murray TF. Up-regulation of rat cortical adenosine receptors following chronic administration of theophylline. European Journal of Pharmacology. 1982; 82: 113–114. [PubMed: 6290230]

Nehlig A, Daval JL, Dcbry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Research Reviews. 1992; 17: 139–170. [PubMed: 1356551]

Nikodijevic O, Jacobson KA, Daly JW. Locomotor activity in mice during chronic treatment with caffeine and withdrawal. Pharmacology Biochemistry and Behavior. 1993a; 44: 199–216. [PubMed: 7679219]

Nikodijevic O, Jacobson KA, Daly JW. Effects of combinations of methylxanthines and adenosine analogs on locomotor activity in control and chronic caffeine-treated mice. Drug Development Research. 1993b; 30: 104–110.

Nikodijevic O, Jacobson KA, DaJy JW. Acute treatment of mice with high doses of caffeine: An animal model for choreiform movement. Drug Development Research. 1993c; 30: 121–128.

Nikodijevic O, Sarges R, Daly JW, Jacobson KA. Behavioral effects of A_1- and A_2-selective adenosine agonists and antagonists: Evidence for synergism and antagonism. Journal of Pharmacology and Experimental Therapeutics. 1991; 259: 286–294. [PubMed: 1920121]

Phillis JW, Barraco RA, Delong RE, Washington DO. Behavioral characteristics of centrally administered adenosine analogs. Pharmacology Biochemistry and Behavior. 1986; 24: 263–270. [PubMed: 2419924]

Porter NM, Radulovacki M, Green RD. Dcnscnsitization of adenosine and dopamine receptors in rat brain after treatment with adenosine analogs. Journal of Pharmacology and Experimental Therapeutics. 1988; 244: 218–225. [PubMed: 3335999]

Shi D, Nikodijevic O, Jacobson KA, Daly JW. Chronic caffeine alters the density of A_1-adenosine, ß-adrenergic, serotonin, cholinergic, and GABAA receptors and calcium channels in mouse brain. Cellular Molecular Neurobiology. 1993; 13: 247–261. [PubMed: 8242688]

Shi D, Nikodijevic O, Jacobson KA, Daly JW. Effects of chronic caffeine on adenosine, dopamine and acetylcholine systems in mice. Archives lnternationale Pharmacodynamie et Therapie. 1994.

Snyder SH, Katims JJ, Annau Z, Burns RF, Daly JW. Adenosine receptors and behavioral actions of methylxanthines. Proceedings of the National Academy of Sciences. 1981; 78: 3260–3264.

Von Lubitz DKJE, Paul lA, Ji XD, Carter M, Jacobson KA. Chronic adenosine At receptor agonist and antagonist: Effect on receptor density and Nmethyl-D-aspartate-induced seizures in mice. European Journal of Pharmacology. 1994; 253: 95–99. [PubMed: 8013554]

White JM. Behavioral interactions between nicotine and caffeine. Pharmacology Biochemis1ry and Behavior. 1988; 29: 63–66.

Wu PH, Coffin VL. Up-regulation of brain [^{3H}]diazepam binding sites in chronic caffeine-treated rats. Brain Research. 1984; 294: 186–189. [PubMed: 6697237]

Wu PH, Phillis JW. Up-regulation of brain [^{3H}]diazepam binding sites in cluonic caffeine-ueated rats. General Pharmacology. 1986; 17: 501–503. [PubMed: 3019827]

Zielke CL, Zielke HR. Cluonic exposure to sub-cutaneously implanted methylxanthines. Differential elevation of A_1-adenosine receptors in mouse cerebellar and cerebral cortical membranes. Biochemistry and Pharmacology. 1987; 36: 2533–2538.

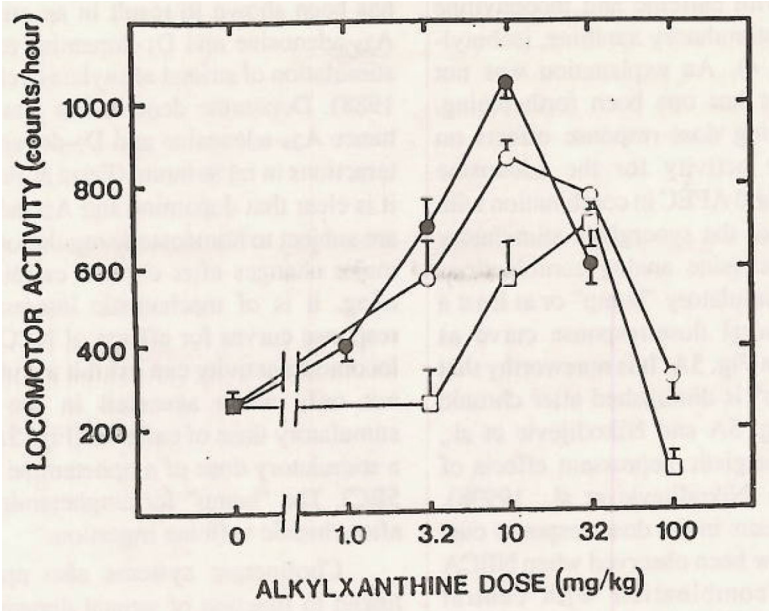


Fig. 1.

Typical Bell-shaped Dose-Response Curves for Effects of Xanthines on Open-Field Locomotor Activity in Mice (Daly, 1993). Caffeine (●), theophylline (□), 3,7-dimethyl-1-propargylxamhine (○). Activity measured for 60 min in a circular arena after intraperitoncal injection of xanthine to male NIH Swiss strain mice.

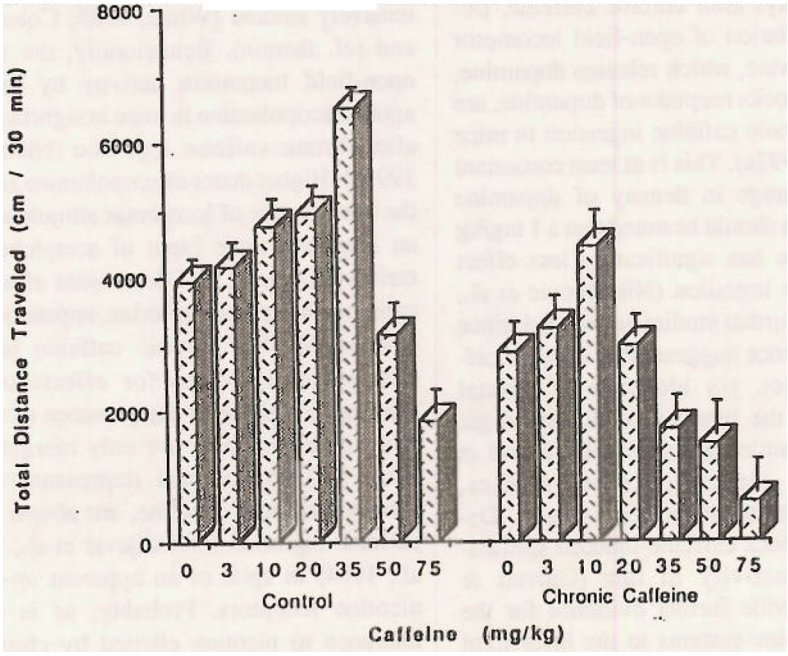


Fig. 2.

Dose-Response Relationships for Effects of Caffeine on Locomotor Activity in Mice (Nikodijevic et al., 1993a). Activity was measured for 30 min in a circular arena after intraperitoneal injection of caffeine in control and chronic caffeine male NIH Swiss strain mice. Caffeine ingestion (100 mg/kg/day) was for 7 days. followed by 2-4 hr withdrawal for caffeine clearance.

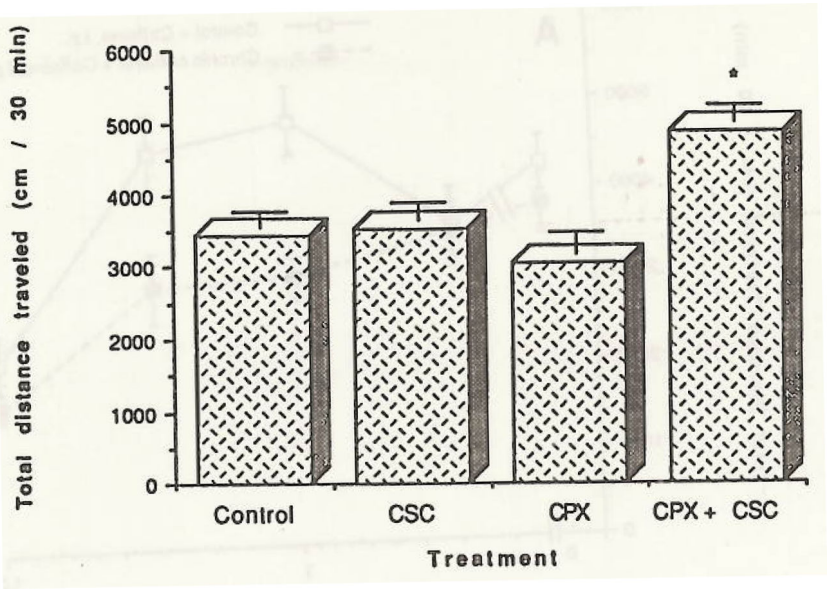


Fig. 3.

Effects of an A_1-Selective Antagonist (CPX) and an A_{2a}-Selective Antagonist (CSC) on Locomotor Activity in Mice (Jacobson et al., 1993). Activity was measured for 30 min after intraperitoneal injection of 8-cyclopentyl-1, 3-dipropyll(anthine (CPX, 0.25 mg/kg), and/or 8-(3-chlorostyryl) xanthine (CSC, 1.0 mg/kg) in male NIH Swiss strain mice.

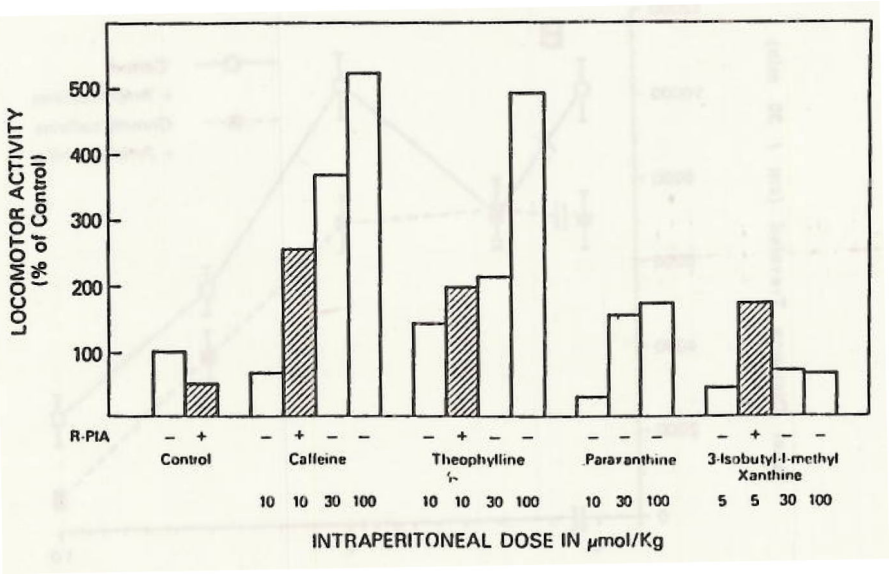
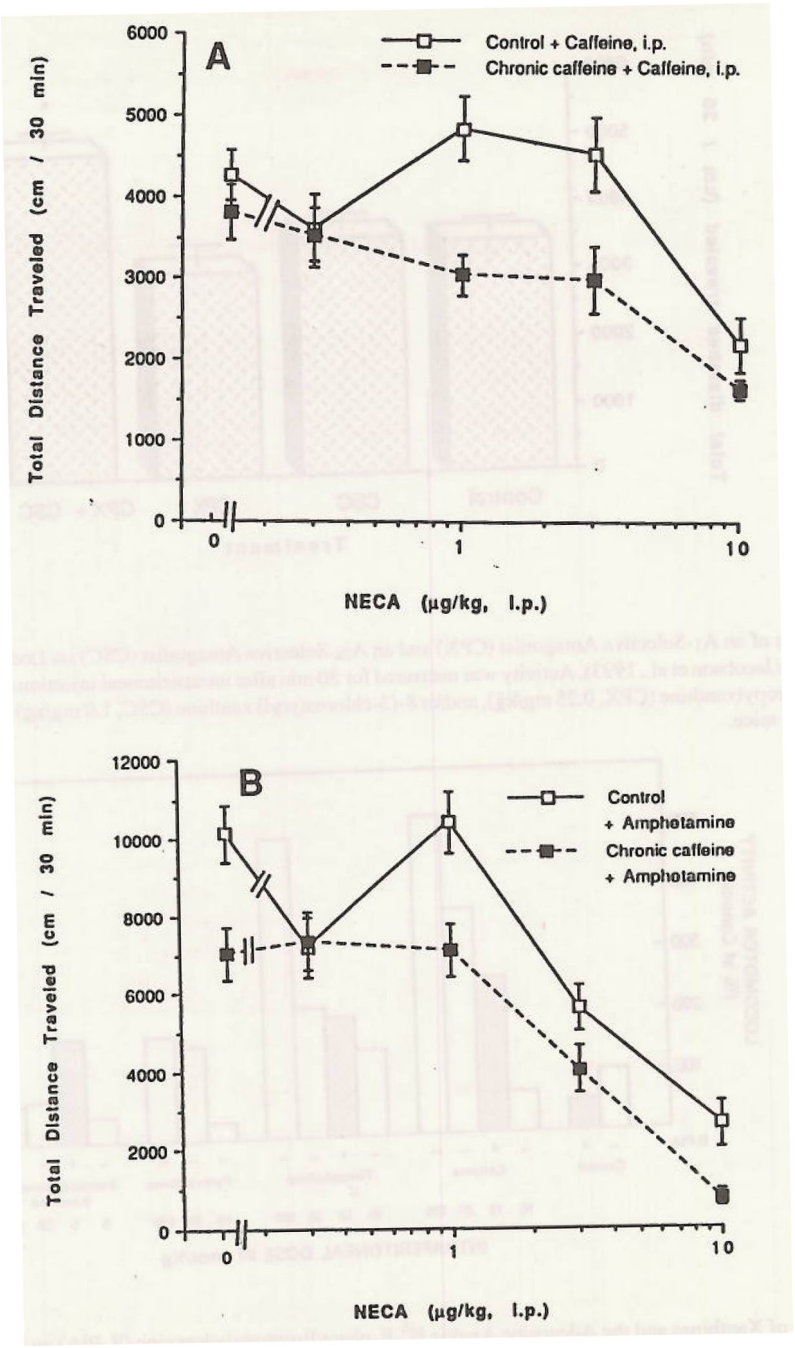


Fig. 4.

Effects of Xanthines and the Adenosine Analog N^6-R-phenylisopropyladenosine (R-PIA) on locomotor activity in mice (Daly, 1993). Open-field activity is for the second thirty minute period after intraperitoneal injections to Male ICR mice of xanthines alone or in combination with R-PIA (0.2 μmol/kg). Note the depressant effect of lowest dose of caffeine and stimulatory effect of R-PIA in combination with that dose of caffeine.



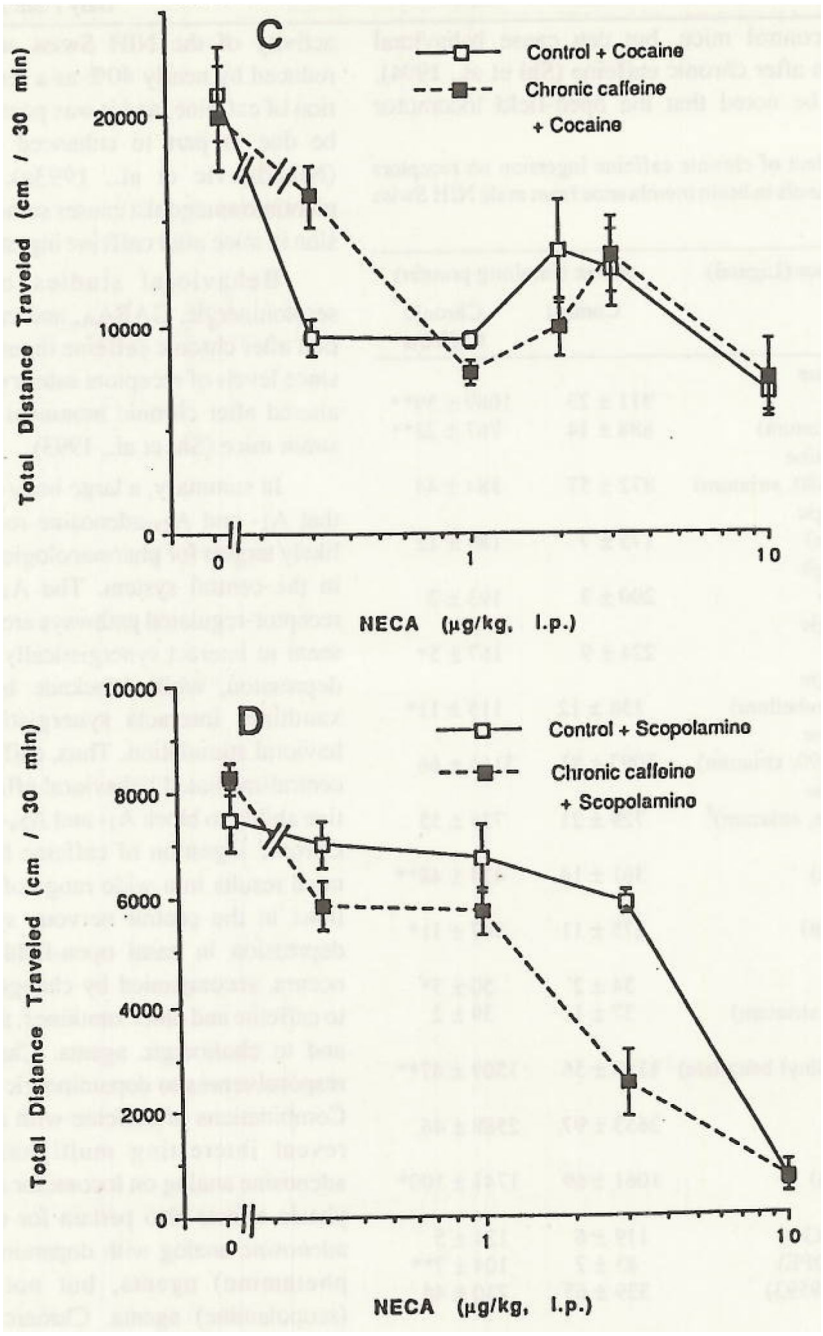


Fig. 5.

Dose-Dependent Effects of NECA on Open-Field Locomotor Activity of Mice in the Presence of a Central Stimulant (Nikodijevic et al., 1993b; Shi et al., 1994). *A*. Caffeine (5 mg/kg). *B*. Amphetamine (1 mg/kg). *C*. Cocaine (20 mg/kg}. *D*. Scopolamine (1 mg/kg}. Male NIH Swiss strain mice were injected intrapcritoneally with a central stimulant (caffeine, amphetamine, cocaine, scopolamine) and varying doses of the adenosine analog NECA and open-field locomotor activity was measured for a 30 minute period. Caffeine

ingestion (100 mg/kg/day) was for 4 days, followed by 2-4 hr withdrawal for caffeine clearance.

Table 1

Effect of chronic caffeine ingestion on receptors and ion channels in brain membrance from male NIH Swiss Strain mice.

Receptor (Ligand)	B_{max} (fmol/mg protein)	
	Control	Chronic Caffeine
A_1-Adenosine		
(CHA)	911 ± 23	1089 ± 39**
(CHA, striatum)	688 ± 14	767 ± 28**
A_{2A}-Adenosine		
(CGS 21680, striatum)	872 ±57	884 ± 44
α_1-Adrenergic		
(Cionidinc)	175 ± 7	189 ± 12
α_2-Adrenergic		
(Prazosin)	200± 3	193 ± 2
β_1-Adrenergic		
(DHA)	224± 9	167 ± 5*
β_1-Adrenergic		
(DHA, cerebellum)	158 ± 12	115 ± 11*
D_1-Dopamine		
(SCH 23390, striatum)	3097 ± 81	3165 ± 66
D_2-Dopamine		
(Spipcrone, striatum)≠	729 ± 21	725 ±55
5-HT_1		
(Serotonin)	361 ± 14	474 ± 48**
5-HT_2		
(Ketanserin)	275 ± 11	347 ±11*
Nicotinic		
(Nicotine)	34 ± 2	50 ± 3*
(Nicotine, striatum)	37 ± 1	39 ± 2
Muscarinic		
(Quinuclidinyl benzilate)	1153 ±56	1509 ± 47**
NMDA		
(MK-801)	2653 ± 97	2588 ± 46
$GABA_A$		
(Diazepam)	1061 ± 69	1741 ± 100*
Opioid		
mu (DAMGO)	119 ± 6	134 ± 5
delta (DPDPE)	83 ±2	104 ± 7**

Receptor (Ligand)	B_{max} (fmol/mg protein)	
	Control	Chronic Caffeine
kappa (U69593)	339 ± 65	230± 48
Sigma		
(DTG)	2580± 90	2560± 170
Ca^{2+} Channel		
(Nitredinine}	314 ± 6	369 ± 12 **

Binding of radioligands to conical membranccs or as noted to cerebellar or striatal mcmbr.ances from control mice and chronic caffeine mice. Values for B_{max} are means ± S.E.M. (Shi et al., 1993, 1994).

* $p<0.01$

** $p<0.05$.

≠ no significant change when assayed with [3H] raclopride.