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Behavioral Characteristics of Centrally Administered Adenosine Analogs

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PHILLIS, J W, R A BARRACO, R E DeLONG AND D O WASHINGTON *Behavioral characteristics of centrally administered adenosine analogs* PHARMACOL BIOCHEM BEHAV **24**(2) 263–270, 1986 —Mice were implanted with chronic indwelling cannulae in the lateral cerebral ventricle A series of adenosine analogs and related compounds were injected into the lateral ventricle (ICVT) and their effects on spontaneous locomotor activity recorded All analogs produced dose-related decreases in locomotor activity 5′-N[6]-ethyl-carboxamidoadenosine (NECA) was the most potent compound tested, with a number of N[6]-substituted analogs also being effective depressants of activity Caffeine, administered either intracerebroventricularly or intraperitoneally, antagonized the depressant effects of the adenosine analogs 3-Isobutyl-1-methylxanthine, administered ICVT, depressed locomotor activity However, after caffeine, IBMX elicited behavioral stimulation Agents which inhibit the transport of adenosine (dipyridamole, dilazep, papaverine) depressed locomotor activity, as did erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), an inhibitor of adenosine deaminase The effects of dilazep, papaverine and EHNA, but not of dipyridamole, were antagonized by caffeine These results further substantiate the notion that endogenous adenosine is involved in the regulation of central nervous system excitability

Adenosine Intracerebroventricular injection Purines Methylxanthines Caffeine Dilazep Papaverine Erythro-9-(2-hydroxy-3-nonyl)adenine

EXPERIMENTS conducted during the past decade have done much to focus attention on adenosine and the adenine nucleotides as potential transmitters or neuromodulators in the central nervous system (CNS) Although much of the evidence implicating adenosine and adenosine triphosphate (ATP) in "purinergic transmission" has been forthcoming from the peripheral autonomic nervous system [1, 2, 27], there has been considerable interest in the potential role of these compounds in the CNS Adenosine and ATP meet many of the criteria for consideration as putative transmitters, they are widely distributed in the nervous system together with the enzymes for their synthesis and degradation [51,64], and are released *in vivo* in a calcium-dependent manner during neuronal activation [35, 59, 65, 72]

Adenosine and its analogs have potent inhibitory actions on neuronal firing at many levels of the neural axis [50,51] In experiments with *in vitro* preparations, adenosine depressed the amplitude of monosynaptic evoked responses [24, 37, 57, 58, 60] Purinergic inhibition of transmitter release from central and peripheral nerve terminals has been demonstrated in a number of laboratories [30, 51, 64] Perhaps the best characterized effect of adenosine is its capacity to modulate adenylate cyclase activity [41,67] A number of laboratories have provided evidence for more than one membrane-associated receptor involved in cyclic adenosine 5′-monophosphate (cAMP) regulation A high affinity receptor (A_1, R_i) is inhibitory to adenylate cyclase, while a lower affinity receptor (A_2, R_a) is stimulatory Responses at both sites can be competitively antagonized by methylxanthines such as theophylline or caffeine [21] Further, ligand binding studies on CNS tissues reveal a high affinity site that has tentatively been identified as the A_1 receptor [11] Overall, the brain contains amongst the highest concentration of adenosine receptor of any tissue examined [48]

In contrast to the electrophysiological and biochemical effects of adenosine in the central nervous system, relatively little is known about the behavioral actions of adenosine and its analogs It is known that adenosine has a marked effect on arousal levels Parenteral injections of adenosine and its analogs produce marked hypoactivity [18, 25, 62, 68], hypnogenic activity [56], hypothermia [5, 68, 71], anticonvulsant actions [3, 25, 42], muscle relaxation [5], and antinocispons-ive effects [34,68] Parenteral injections of adenosine and its analogs have also been shown to produce decreases in schedule-controlled operant behavior [15], and to suppress spontaneous and drug-induced food intake [14, 38, 71]

A number of studies have shown that intracerebroventricular administration of purines also produces similar effects on arousal levels and other behavioral responses For example, adenosine and ATP injected into the lateral ventricles of cats or rats produce muscular weakness, ataxia and sleepiness [12,28] Infusion of adenosine into the third ventricle or hypothalamus of the fowl caused behavioral and electrocortical sleep [44] Adenosine also had hypnogenic activity when administered into the lateral ventricle of dogs and rats [31,69] Administration of adenosine analogs into the rat lateral cerebral ventricle reduced the intensity and duration of kindled seizures [8] Adenosine nucleotides had a

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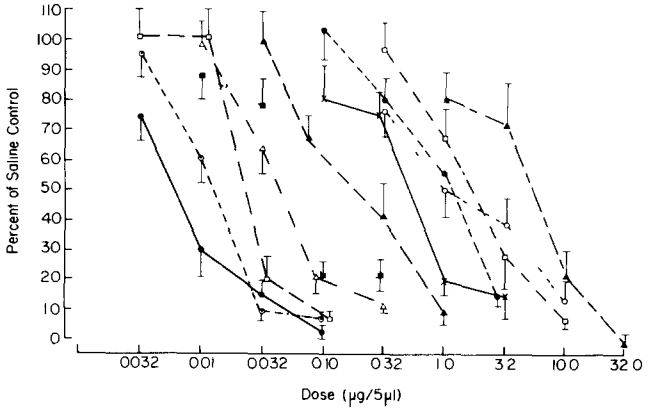

FIG 1 The effect on locomotor activity of ICVT injections of 11 adenosine analogs (●—●) N-ethylcarboxamidoadenosine, ○— —○ N^6-S-1-hydroxy-3-phenyl-2-propyladenosine □— —□ N^6-3-pentyladenosine, △— —△ N^6-R-1-phenyl-2-butyladenosine, ■ ■ N^6-R-1-phenylethyladenosine, ▲— —▲ 2-phenylaminoadenosine, ×——× N^6-R- phenylisopropyladenosine, ●— — —● N^6-S-1-phenyl-2-butyladenosine, ○— — —○ 2-chloradenosine, □— —□ N^6-S-1-phenylethyladenosine, ▲— — —▲ N^6-S-phenylisopropyladenosine) Values are expressed as percent of controls (mean±S E M) receiving saline ICVT

TABLE 1

LOCOMOTOR DEPRESSANT POTENCIES OF ADENOSINE AND RELATED COMPOUNDS

Compound	
Adenosine Analogs	ID_{50} (per mouse)
N^6-3-pentyladenosine	0 07 nmol
N^6-S-1-hydroxy-3-phenyl-2-propyladenosine	37 pmol
N^6-R-phenylisopropyladenosine*	1 8 nmol
N^6-S-phenylisopropyladenosine*	20 2 nmol
N^6-R-1-phenylethyladenosine	0 18 nmol
N^6-S-1-phenylethyladenosine	5 5 nmol
N^6-R-1-phenyl-2-butyladenosine	0 14 nmol
N^6-S-1-phenyl-2-butyladenosine	3 4 nmol
Adenosine*	67 4 nmol
2-chloroadenosine*	4 9 nmol
5′-N-ethylcarboxamidoadenosine*	22 pmol
2-phenylaminoadenosine	10 7 nmol
3-isobutyl-1-methylxanthine*	27 0 nmol
Transport Inhibitors	
Papaverine*	26 5 nmol
Dipyridamole	79 nmol
Dilazep	16 nmol
Deaminase Inhibitor	
Erythro-9-(2-hydroxy-3-nonyl)adenine	240 nmol

This table presents the doses of intracerebroventricularly administered adenosinergic ligands, and potentiators of adenosine required to elicit a 50% reduction in mouse locomotor activity

*ID_{50}'s for these compounds were previously reported in [17]

hypothermic action when injected into the cat anterior hypothalamus [22] Central administration of adenosine suppressed food and water intake by rats [38], and caused dose-related decreases in pain perception [32, 55, 73] The competitive blockade by methylxanthines in these studies suggests that the effects are mediated by adenosine receptors

Many of the behavioral effects of adenosine analogs can be mimicked by drugs which alter endogenous levels of brain adenosine For instance, adenosine uptake inhibitors can potentiate the sedative effects of adenosine in mice [19], and potentiate the antinocisponsive effects of adenosine in mice [73] Likewise, adenosine deaminase inhibitors such as 2′-deoxycoformycin produce sedative and hypnotic effects in humans [43] and rats [56], whereas erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), another adenosine deaminase inhibitor was shown to produce a profound decrease in spontaneous motor activity in mice and rats following parenteral administration [47]

In this paper we describe the behavioral effects of adenosine agonists, antagonists, and potentiators of adenosine on the locomotor activity of mice Drugs have been injected into the lateral cerebral ventricle (ICVT) to mitigate the hypotension associated with peripherally-administered purines The use of a standardized experimental paradigm made it possible for us to compare and characterize the actions of these drugs on a specific, but uniform, type of behavioral activity Two preliminary reports of these studies have appeared [7,17]

METHOD

Adult male Swiss Webster mice (ICR strain, Harlan Industries) approximately 10 weeks old (35–40 g) were implanted with permanent indwelling stainless steel guide cannulas for injection into the lateral ventricle of the brain The cannulae (26-gauge tubing cut to a length of 10 mm and blunted 90°) were implanted stereotaxically under sodium pentobarbital anesthesia using as coordinates AP 0 0 (bregma), ML, ±0 8 mm, and DV, −2 2 mm from the skull surface, with the skull surface in the horizontal plane [4] The cannulae were held in place with Fastcure dental repair material (Kerr) which was anchored to two small clips inserted through the bone A fine wire stilette was inserted into the cannula to prevent clogging The injector cannula was a 33-gauge tube, cut to a length of 10 2 mm, and attached by a length of PE 20 tubing to a Hamilton syringe All drugs were injected slowly, in a 5 μl volume of artificial cerebrospinal fluid, over a 30 sec period Control animals received 5 μl injections of artificial cerebrospinal fluid The correct placement of the cannulae was verified at the end of each study by sectioning the brain In some animals 5 μl of cresyl violet stain was injected through the guide cannula, after which the animal was sacrificed, the brain removed rapidly and sliced The presence of dye in the cerebral ventricular system was then assessed visually Animals in which the cannula placements was misdirected were excluded from the study

Animals were housed 6 per cage in 18×24×15 cm polypropylene cages on a 12 hr/12 hr light cycle and allowed ready access to food and water After surgery, the mice were allowed to recover and adapt to their housing for a minimum of 5 days After drug administration, mice were each placed in one of six testing chambers (18×24×15 cm) and the chambers were monitored simultaneously The test chambers

TABLE 2

MEAN ± S E M PERCENT LOCOMOTOR ACTIVITY OF SALINE CONTROLS‡ (μg/MOUSE)

	0 0032	0 01	0 032	0 10	1 0	3 2	5 6	10	17 8	32	100
NECA	72 ± 8	30 ± 9*	15 ± 4*	2 ± 1*							
NECA + Caffeine	77 ± 11	93 ± 22†	60 ± 14†	34 ± 8†							
IBMX					84 ± 7		58 ± 23	17 ± 5*	14 ± 3*		
IBMX + Caffeine					81 ± 14		100 ± 14†	127 ± 28†	249 ± 23†		
Adenosine						97 ± 18		65 ± 16		37 ± 8*	6 ± 1*
Adenosine + Caffeine								200 ± 23†		174 ± 32†	59 ± 1†

Effects of ICVT injections of NECA, IBMX and adenosine on spontaneous locomotor activity given alone and in combination with a single intraperitoneal injection of caffeine (32 mg/kg) At this dose caffeine had no significant effect on locomotor activity when given alone (mean ± S E M percent of saline controls = 94 ± 1 5)

*Indicates individual doses of drug alone differ from saline controls by $p<0$ 01

†Indicates caffeine (IP) in combination with drug (ICVT) differs from drug alone by $p<0$ 02

‡Data in this table taken from [17]

were located in a dark compartment equipped with a fan for ventilation and noise attenuation Locomotor activity was measured with a Stoelting 31410 Modular Electronic Activity Monitor with 6 sensors Each sensor was activated by a uniform, low power, radiofrequency field extending several inches above the surface Sensitivity of the sensors was adjusted to accumulate counts for gross movement All sensors were adjusted to equal sensitivity with a pendulum Immediately following ICVT injections, there was a 10-min pretest period, prior to the 30 min test period for locomotor activity Locomotor activity scores were analyzed by the Kruskal-Wallis ranked one-way analysis of variance and Mann-Whitney post hoc one-tailed paired comparisons

The drugs used in this study were 5′-N-ethylcarboxamidoadenosine (NECA), R- and S-N^6(phenylisopropyl)adenosine (R- and S-PIA) (gifts from Warner-Lambert), N^6-3-pentyladenosine (3-PA), N^6-S-1-hydroxy-3-phenyl-2-propyladenosine (S-HPPA), N^6-R- and S-1-phenylethyladenosine (R-PEA, S-PEA), N^6-R- and S-1-phenyl-2-butyladenosine (R-PBA, S-PBA), 2-phenylaminoadenosine (2-PAA) (gifts from Dr R A Olsson, University of South Florida), adenosine, 2-chloroadenosine (CADO), papaverine, 3-isobutyl-1-methylxanthine (IBMX) (Sigma), caffeine (Eastman Kodak), dipyridamole (C H Boehringer Sohn), dilazep (Asta-Werke), erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (Burroughs Wellcome and Co)

RESULTS

Adenosine and Adenosine Analogs

The effects of a series of adenosine analogs on locomotor activity (as percent of saline controls) at various doses are shown in Fig 1, and the calculated molar doses required to depress activity by fifty percent (ID_{50}) are given in Table 1 Analyses of variance on each compound indicated that all analogs produced highly significant dose-related decreases in locomotor activity NECA was the most potent analog tested, and many of the N^6-substituted compounds were also comparatively potent in depression of locomotor activity As little as 0 5 nmoles/kg of NECA is sufficient to reduce locomotor activity by fifty percent At higher dose levels, locomotor activity is even more markedly reduced and the animals generally lie on their ventral surface with the limbs splayed They are however capable of withdrawal responses when the tail is pinched and demonstrate righting and corneal reflexes

In order to demonstrate that these adenosine analogs were exerting their behavioral effects by activation of an adenosine receptor, caffeine was used as a receptor antagonist Caffeine was administered intraperitoneally 20 min before intracerebroventricular injections of adenosine or its analogs, at a dose which had no effect on locomotor activity when given alone (32 mg/kg, mean±SEM for the caffeine controls was 94±1 5%) Pretreatment with caffeine significantly antagonized the effects of NECA and adenosine on locomotor activity at all depressant doses, establishing that these effects are mediated by a specific adenosine receptor (Table 2) A paradoxical effect was observed with caffeine pretreatment and adenosine, in that low doses of adenosine (10 μg and 32 μg) now stimulated locomotor activity (ADO + caffeine vs caffeine alone H(3)=12 01) (Table 2) In contrast, when mice were given NECA with caffeine there were no dose combinations which produced stimulation

Intracerebroventricular Caffeine and IBMX

Caffeine was injected intracerebroventricularly at doses of 10 μg, 32 μg, 150 μg and 320 μg At the lowest dose (10 μg) caffeine reduced locomotor activity ($p<0$ 05), but it had no significant effect on locomotor activity at the higher doses At 150 μg, the locomotor scores of the caffeine-treated animals were identical to those of the saline controls However, when ICVT caffeine (150 μg) was administered prior to the injection of NECA, the effects of the latter were significantly reduced, H(4)=15 96, $p<0$ 01 Pretreatment with 150 μg caffeine completely antagonized the depressant effects of 0 01 μg NECA and reduced the effects of higher (0 032 μg, 0 01 μg) doses

IBMX (5 6 μg, 10 0 μg, 17 8 μg, ICVT) produced significant dose-related decreases in locomotor activity, H(4)=22 57, $p<0$ 001 IBMX was 1000-fold less potent than

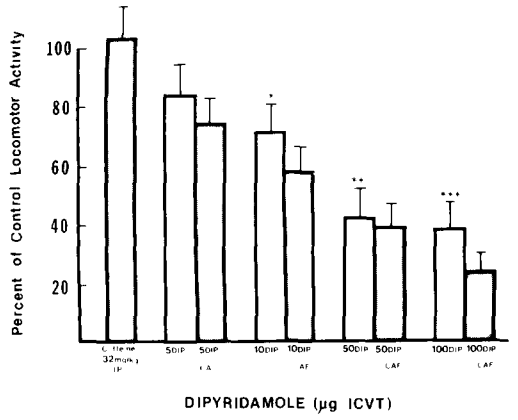

FIG 2 The effect on locomotor activity of ICVT injections of dipyridamole (DIP), IP injection of 32 mg/kg caffeine (CAF), and DIP doses in combination with CAF Values are expressed as the percent of controls (mean±S E M) receiving saline IP and ICVT Individual significance levels were determined by Mann-Whitney U one-tailed comparisons 10 μg ($p<0$ 05), 50 μg ($p<0$ 001), and 100 μg ($p<0$ 001) of DIP depressed locomotor activity relative to saline/saline controls, whereas DIP+CAF was not significantly different from DIP alone at any dose

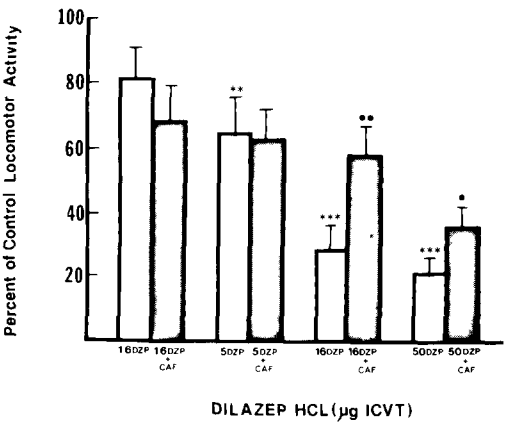

FIG 3 The effect on locomotor activity of ICVT injections of dilazep (DZP) and DZP doses in combination with 32 mg/kg of intraperitoneally-administered caffeine (CAF) Values are expressed as the percent of controls (mean±S E M) receiving saline IP and ICVT Individual significance levels were determined by Mann-Whitney U one-tailed comparisons 5 μg ($p<0$ 01), 16 μg ($p<0$ 001), and 50 μg ($p<0$ 001) of DZP depressed locomotor activity relative to saline/saline controls (*), and CAF (antagonized this depression at 16 μg ($p<0$ 01) and 50 μg ($p<0$ 05) of DZP (●)

NECA (Table 1) When ICVT IBMX was administered after pretreatment with intraperitoneal caffeine (32 mg/kg), a stimulant effect on locomotor activity became evident (IBMX + caffeine vs caffeine alone H(4)=19 45, $p<0$ 001) When IBMX (10 μg) and adenosine (22 μg) were administered in combination intracerebroventricularly, locomotor depression was not observed Rather, some stimulation became apparent (Table 2) In this situation, therefore, IBMX appears to exhibit antagonistic activity similar to that observed with caffeine

Studies with Inhibitors of Adenosine Uptake

The effects of adenosine transport inhibitors (papaverine, dipyridamole, dilazep) on locomotor activity were measured Papaverine produced a dose-related decrease in activity, H(4)=22 87, $p<0$ 001, and had an ID_{50} of 26 5 nmol When an ICVT combination of 10 μg of papaverine and 22 μg of adenosine were given, their effects were additive, producing a depression of locomotor activity similar in magnitude to 32 μg of either papaverine or adenosine given alone The depressant effects of papaverine were antagonized by caffeine (papaverine + caffeine vs caffeine alone, H(3)=15 29, $p<0$ 01)

The effect of dipyridamole, a potent inhibitor of adenosine uptake [52,53] on locomotor activity at various doses is shown in Fig 2 Intracerebroventricularly administered dipyridamole significantly depressed movement at doses of 10 μg, 50 μg and 100 μg (ID_{50}=79 nmol) In order to determine whether dipyridamole's effects were a result of the potentiation of endogenously released adenosine, caffeine (32 mg/kg) was administered intraperitoneally 20 min prior to the ICVT dipyridamole As shown in Fig 2, caffeine failed to antagonize the effects of dipyridamole

Dilazep is another potent inhibitor of adenosine uptake [52,53] Dilazep (5 0 μg, 16 μg, 50 μg, ICVT) significantly inhibited locomotor activity (Fig 3) with an ID_{50} of 16 nmol Caffeine significantly antagonized the depressant effects of the two higher doses of dilazep ($p<0$ 001)

Inhibition of Adenosine Deaminase

EHNA is a potent inhibitor of adenosine deaminase [61] This substance significantly inhibited locomotor activity (ID_{50}=240 nmol) at doses of 56 μg, 100 μg, 178 μg and 320 μg ICVT (Fig 4) Caffeine (32 mg/kg, IP) significantly antagonized the locomotor depressant effects of the three higher doses of EHNA

DISCUSSION

In the present study we have confirmed and extended our earlier observations [7,17] that intracerebroventricularly administered adenosine analogs exert a potent depressant action on mouse locomotor activity It has been known for many years [23,68] that adenosine and its derivatives can produce marked hypoactivity when administered peripherally, but these compounds also have multiple effects on cardiovascular function For example, adenosine analogs have direct negative actions on the heart which are blocked by the methylxanthines [13] and, when administered to rats intravenously or intraperitoneally, these compounds exert potent hypotensive actions [53,68] It is therefore conceivable that the central effects of these compounds are secondary to their peripheral, cardiovascular, actions [50,53] In an attempt to eliminate this problem as a consideration in the interpretation of behavioral data, the present experiments were conducted using intracerebroventricularly-administered drugs In a previous study on rats, we had demonstrated that the threshold dose for a hypotensive action of intracerebroventricularly administered NECA was 100-fold higher than the dose required to inhibit locomotor activity The dose of R-PIA necessary to elicit a significant reduction

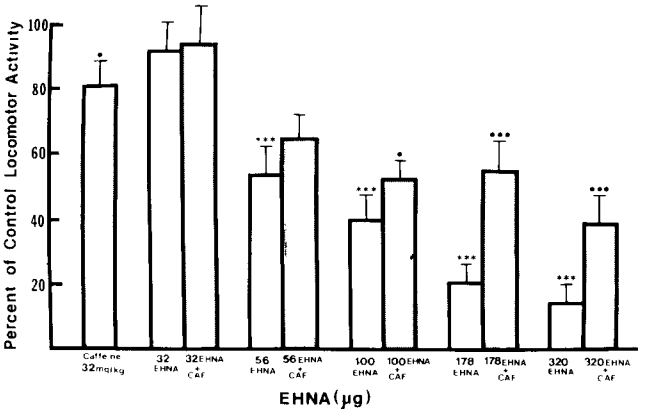

FIG 4 The effect on locomotor activity of ICVT injections of erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), IP injection of 32 mg/kg caffeine (CAF), and EHNA doses in combination with CAF Values are expressed as the percent of controls (mean±S E M) receiving saline IP and ICVT Individual significance levels were determined by Mann-Whitney U one-tailed comparisons the four highest doses of EHNA depressed locomotor activity (all $p<0\ 001$) relative to saline/saline controls (*), and CAF antagonized this depression at 100 μg ($p<0\ 05$), 178 μg ($p<0\ 001$), and 320 μg ($p<0\ 001$) of EHNA (●), although CAF lowered activity relative to saline/saline controls in this experiment ($p<0\ 05$)

in blood pressure was 10-fold higher than that for depression of locomotion [6] These results showed that the hypoactive and hypotensive effects of adenosine analogs can be dissociated by using an intracerebroventricular route of administration

A series of adenosine derivatives have been tested in the present experiments NECA was the most potent of these A number of N^6-substituted compounds were also very effective depressants of locomotor activity The R-diastereomers of PIA, PEA and PBA were 10–30 times more potent than the S-diastereomers 2-Phenylaminoadenosine and 2-CADO were also potent inhibitors of locomotor activity Adenosine, itself, was considerably less active, but this estimate was undoubtedly influenced by tissue uptake and metabolism

The order of agonist potency can be used to characterize receptor types if the agents mediate their effects via the same receptors The similarity of the dose response curves illustrated in Fig 1 suggests that this was, indeed, the case Both A_1/R_i and A_2/R_a-receptors are competitively antagonized by methylxanthines [11,40] In the absence of specific antagonists for these receptor subtypes, it cannot be conclusively established that the agonists interact solely with either A_1/R_i or A_2/R_a receptors in the brain However, antagonism by the methylxanthines does indicate that the effects observed were mediated via an adenosine (P_1) receptor and not via other purine sensitive sites (P_2-receptors or intracellular P site) [20]

The structure-activity relationships observed in these experiments are difficult to reconcile with the classic A_1/R_i A_2/R_a receptor profiles The very high potency of NECA is suggestive of an A_2/R_a receptor, but this conclusion is not supported by the greater than would be anticipated activity of many of the N^6-A_1/R_i ligands Data from the R_a receptor on dog coronary arteries indicates that 2-phenylaminoadenosine an A_2 ligand should have been considerably more effective than R-PIA [49] Still, the 10-fold potency difference between the R- and S-diastereomers of PIA is more indicative of an A_2/R_a, rather than an A_1/R_i receptor [11,40] It is difficult, therefore, to reach any definite conclusion about the classification of the receptor type involved in mediating these behaviorally depressant effects Our observation that NECA was the most potent analog tested is consistent with the results obtained in other behavioral studies in which peripherally administered NECA was a more potent depressant of schedule-controlled responding that R-PIA [16,39]

Recent reports demonstrating that lipophilic substances, such as R-PIA, are rapidly accumulated by cells [26,54] suggest that caution be applied to any comparisons of the potency of such agents with NECA Although R-PIA is non-metabolizable and apparently not transported by the adenosine transporter, it is able to equilibrate rapidly across the plasma membrane without carrier mediation consistent with its lipophilicity, and accumulates concentratively in cells due to partitioning into membrane lipids and binding to intracellular components In contrast, NECA is not accumulated by individual cells [26] The consequences of this accumulation are likely to be varied and could include a lag-phase before the agent reaches its maximal levels in the plasma membrane, concentrations in the plasma membrane in excess of those in the surrounding medium, and unanticipated effects on intracellular receptors and enzymes A recent report that nanomolar concentrations of R-PIA can stimulate a low K_m cyclic AMP phosphodiesterase in rat brain [45] may offer an alternative explanation for some of the effects of this agonist on brain cyclic AMP levels

Evidence for a neuromodulatory role of adenosine in the CNS was sought from experiments with inhibitors of adenosine transport and adenosine deaminase The action of adenosine appears to be terminated by its removal from the synaptic cleft by active neuronal uptake coupled to an intracellular enzyme, adenosine kinase, or by deamination to inosine via the enzyme adenosine deaminase [51] Previous studies have demonstrated that the transport inhibitors nitrobenzylthioinosine and nitrobenzylthioguanosine (administered IP) can potentiate the sedative effects of intraperitoneally-administered adenosine [19] However, nitrobenzylthioinosine did not have a sedative action of its own at doses of up to 60 mg/kg

In the present experiments we have administered two potent transport inhibitors [52,53] directly into the lateral cerebral ventricle Dipyridamole and dilazep both inhibited locomotor activity, but only the effects of dilazep were antagonized by caffeine The effects of dipyridamole were therefore likely due to some other aspect of its pharmacological activity, such as the inhibition of phosphodiesterase [46] and not a consequence of its potentiation of extracellular levels of adenosine The third transport inhibitor tested in this series was papaverine, a drug with recognized sedative properties [63] Papaverine also inhibited locomotor activity and, like dilazep, its effects were antagonized by caffeine

A decrease in spontaneous locomotor activity of mice and rats following the parenteral administration of EHNA has previously been reported [47] This compound competitively inhibits adenosine deaminase activity in brain homogenates with a K_i of approximately 75 nM [61] EHNA inhibited locomotor activity and its effects were antagonized by caffeine, confirming that endogenous adenosine was involved

CNS stimulation in animals due to methylxanthine administration has been repeatedly demonstrated using spontaneous locomotor activity measurement [10, 33, 62, 66] In a

report that evaluated ten methylxanthines for their locomotor activity effects and their affinities for adenosine receptors, a clear relationship was found between receptor affinity and stimulation of locomotor activity [62] The higher the affinity, the lower the stimulant dose Both caffeine and theophylline were able to reverse the reduction in locomotor activity caused by the peripheral administration of R-PIA At low doses (1–2 mg/kg) caffeine was observed to decrease locomotor activity, a finding which may be comparable to our observation that 10 μg of caffeine ICVT reduced locomotor activity in mice, whereas larger doses did not have such an effect Our finding that caffeine reversed the depressant action of low doses of adenosine into locomotor stimulation also finds a parallel in the earlier report [62] that, when administered with low doses of R-PIA, caffeine produces a marked stimulation In contrast, in the present study, when mice were given NECA with caffeine, there were no dose combinations which produced stimulation This might suggest the presence of a heterogeneous population of receptors mediating the behavioral effects of adenosine, some of which produce sedation while others cause stimulation At low concentrations, adenosine and R-PIA may have a higher affinity for the receptor which produces only stimulation Evidence for this concept is forthcoming from another study [36] in which it was demonstrated that low doses of R-PIA augmented locomotor activity Caffeine may compete more effectively with adenosine and R-PIA at the receptor subtype responsible for adenosine's depressant effects, thus uncovering actions at the stimulant receptor It is tempting to speculate that the lower affinity receptor is equivalent to the A_2/R_a receptor, through which adenosine stimulates cyclic adenosine 3',5'-monophosphate formation and that the higher affinity receptor is identical to the inhibitory site associated with adenylate cyclase Such an interpretation could explain the failure of caffeine to reverse the locomotor depressant actions of NECA into stimulation Indeed, Fredholm *et al* [29] were unable to demonstrate an inhibitory effect of NECA on the accumulation of cyclic AMP, whilst R-PIA inhibited the accumulation of cyclic AMP at low concentrations, but was stimulative at higher concentrations Finally, the possibility that the lower affinity receptor could mediate behavioral depression via elevated levels of cyclic AMP is also supported by findings that a variety of phosphodiesterase inhibitors produce behavioral depression in mice [70]

Somewhat paradoxical findings were seen with IBMX This methylxanthine has sedative effects on mice, even though it is a potent adenosine receptor antagonist ([62], present observations) Its failure to stimulate locomotor activity directly may reflect another activity, perhaps related to inhibition of adenosine uptake [52] or phosphodiesterase inhibition [9] Interestingly, caffeine was able to reverse the depressant effects of IBMX into a behavioral stimulation IBMX was itself able to antagonize the locomotor depressant actions of adenosine, with some stimulant activity becoming apparent In any case, it is evident that IBMX exerts complex effects on central purinergic systems

In summary, central adenosine systems appear to play an important role in regulating brain excitability Adenosine receptors can be activated by a variety of agonists, as well as by compounds which block adenosine uptake systems or adenosine deaminase and thus elevate endogenous levels of adenosine Adenosine receptor activation is antagonized by the dietary methylxanthines and this effect can account for the central stimulant action of these compounds

ACKNOWLEDGEMENTS

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