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NECA-Induced Hypomotility in Mice: Evidence for a Predominantly Central Site of Action

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DURCAN, M. J. AND P. F. MORGAN. *NECA-induced hypomotility in mice: Evidence for a predominantly central site of action.* PHARMACOL BIOCHEM BEHAV 32(2) 487–490, 1989.—The behavioral effects of four adenosine analogues (NECA, CHA, CPA and CV-1808) were investigated in mice using a holeboard test, which measures both directed exploration (head-dipping) and a locomotor activity. NECA, CHA and CPA showed significant dose-related reductions in all the holeboard measures (NECA >> CHA=CPA), whilst CV-1808 showed no significant effect on any of the measures over the dose range tested. In a subsequent experiment NECA-induced hypomotility was attenuated by the adenosine receptor antagonists, theophylline (which is both centrally and peripherally active) and, though to a lesser extent, by the adenosine receptor antagonist 8-(p-sulfophenyl)theophylline (8-pSPT), which poorly penetrates the blood-brain barrier. The results suggest that NECA-induced hypomotility may be predominantly mediated centrally since the centrally active antagonist was the most effective in reversing the effect, however, peripheral mechanisms may also play a role since equimolar concentrations of 8-pSPT elicit some reversal of NECA-induced hypomotility.

Adenosine N-ethylcarboxamidoadenosine (NECA) Cyclohexyladenosine (CHA) Cyclopentyladenosine (CPA) 2-(phenylamine) adenosine (CV-1808) Theophylline 8-(p-sulfophenyl)theophylline (8-pSPT) Locomotor activity Exploratory behavior Mice

ADENOSINE and a number of adenosine analogues, when administered IP, produced marked behavioral changes including sedation (7,15) which can be antagonized by methylxanthinines such as caffeine and theophylline (12,16). A similar effect is seen if these analogues are administered centrally (1, 3, 14). However, it remains to be established as to whether the hypoactivity caused by peripheral administration of these compounds is the result of a centrally mediated sedative effect or by actions in the periphery (e.g., cardiovascular actions).

In this study the relative behavioral effects of four adenosine analogues: 5'-N-ethylcarboxamidoadenosine (NECA), 2-(phenylamino) adenosine (CV-1808), N^6-cyclohexyladenosine (CHA), and N^6-cyclopentyladenosine (CPA), were compared in mice using a holeboard test. This test measures both locomotor activity and directed exploration (head dipping) and these measures have been shown to vary independent of one another (10,11).

In a subsequent experiment the behavioral effects of NECA were investigated in animals receiving pretreatment with either theophylline, 8(p-sulfophenyl)theophylline (8-pSPT), or vehicle. Both theophylline and 8-pSPT block adenosine receptors but whereas theophylline can cross the blood-brain barrier with relative ease, 8-pSPT penetrates the brain poorly (2), thus largely antagonizing only peripheral receptors. The aim of this experiment was therefore to compare the behavioral effects of NECA with central and peripheral receptors, or peripheral receptors only, antagonized: if the behavioral effects are predominantly peripheral then both antagonists should have similar effects whereas if they are predominantly central then theophylline, but not 8-pSPT, should antagonize NECA-induced hypomotility.

METHOD

Subjects

Naive NIH Swiss male mice were housed in groups of 10 on a 12:12 hour light:dark cycle with food and water available ad lib. The mice weighed between 21 and 25 g at the time of testing.

Drug Administration

In Experiment 1 NECA, CHA, CPA (all Research Biochemicals Inc., Wayland, MA) and CV-1808 (Takeda Chemical Industries, Osaka, Japan) were dissolved in a 3% DMSO saline vehicle. The following doses were administered IP at a volume of 10 ml/kg: NECA (10, 30, 100 μg/kg); CV-1808 (30,

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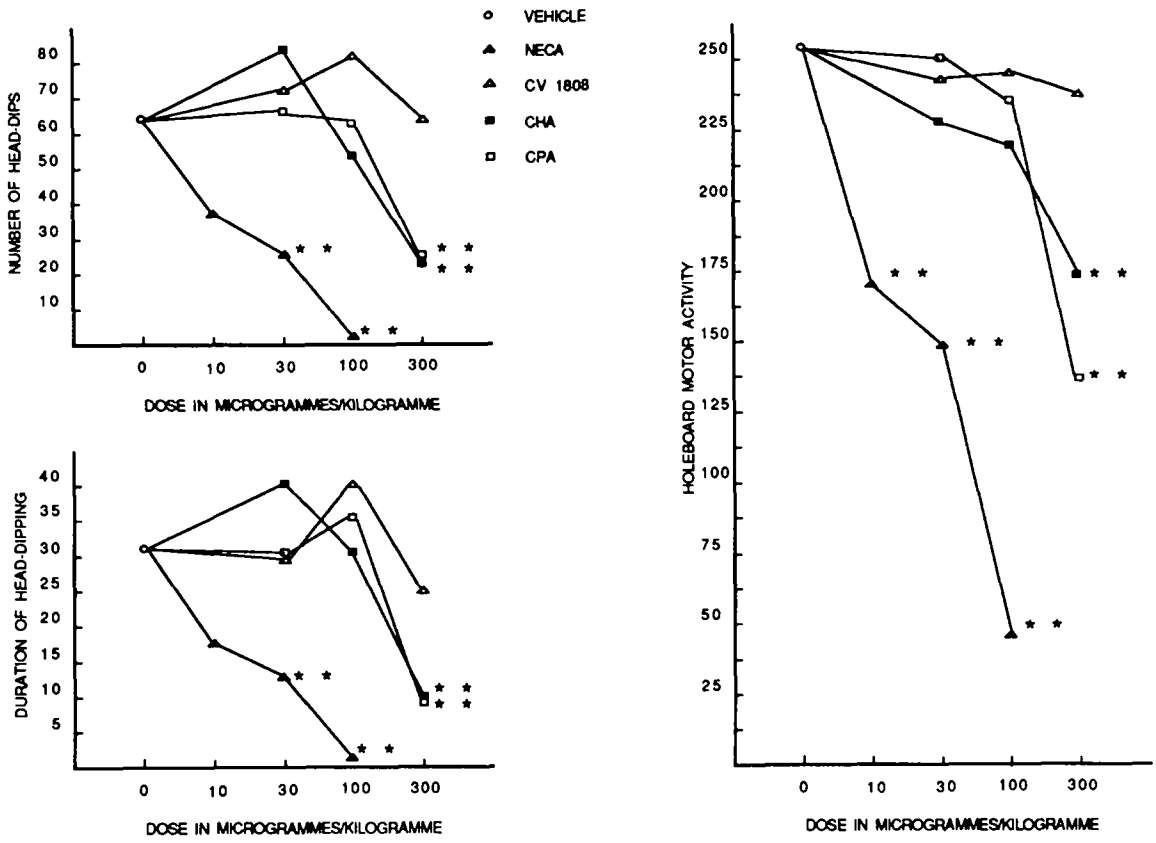

FIG. 1. Means for each dose of each of the adenosine analogues used in Experiment 1 for number of head-dips, duration of head-dipping (measured in seconds) and locomotor activity counts. $^{**}p<0.01$ vs. vehicle-treated control.

100, 300 μg/kg); CHA (30, 100, 300 μg/kg); CPA (30, 100, 300 μg/kg). Different groups of 9–11 mice were treated with each dose of each drug 18 minutes before testing in the holeboard apparatus.

In Experiment 2 groups of 9–11 mice were pretreated IP with 30 mg/kg theophylline (Research Biochemicals Inc., Wayland, MA) or the molar equivalent (mEq) dose of 8-pSPT (Research Biochemicals Inc., Wayland, MA) or distilled water vehicle (at a volume of 10 ml/kg) 45 minutes prior to testing. The same animals were then injected with either 0.0, 10 or 30 μg/kg NECA (in the same vehicle and the same volume as in Experiment 1) 18 minutes prior to testing.

Holeboard Testing

The holeboard consisted of a Plexiglas box (40×40×40 cm) the floor of which has four equally spaced holes (3 cm in diameter). In two opposite walls, 2 cm above the floor, were four equally spaced infrared photo-beams to measure movement in the box. There were also photo-beams beneath each hole to measure the number and duration of head-dips. The apparatus was interfaced with a PDP-11 computer running SKED-11 software (State Systems Inc., Kalamazoo, MI).

The holeboard testing, which took place in a dimly lit room, involved placing a mouse in the center of the floor and allowing it to explore for 8 min.

Statistical Analysis

The data were analyzed using a Kruskal-Wallis nonparametric analysis with differences between rank means being assessed using Mann-Whitney U-tests, the significance levels of which were adjusted using Bonferroni t procedure to avoid claiming differences as a result of multiple comparisons.

RESULTS

The means of the exploration and locomotor measures for the drugs used in Experiment 1 are plotted as Fig. 1. Kruskal-Wallis nonparametric analysis revealed significant group differences ($H=79.2$, $p<0.001$, for number of head-dips; $H=65.2$, $p<0.001$, for head-dip duration; $H=70.6$, $p<0.001$, for locomotor activity). Both the 30 and 100 μg/kg doses of NECA and the 300 μg/kg dose of both CPA and CHA were significantly different from vehicle controls for the head-dipping measures; all doses of NECA but only the highest dose of CHA and CPA significantly reduced locomotor activity (see Fig. 1). None of the doses of CV-1808 tested were found to differ significantly from vehicle-treated controls.

Kruskal-Wallis analysis of the data obtained from Experiment 2 also revealed significant group differences for both

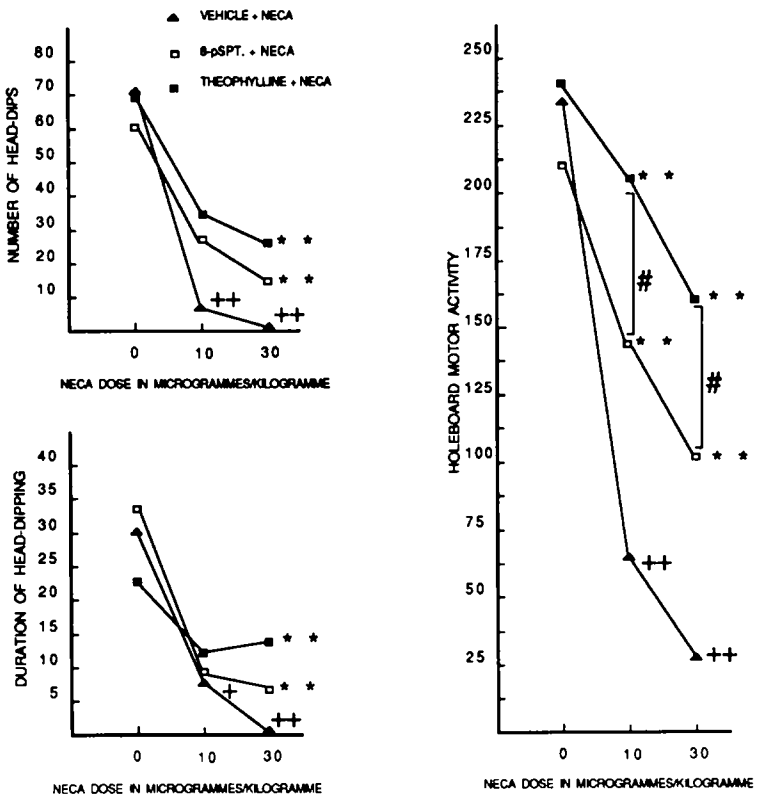

FIG. 2. Means for each pretreatment at each dose of NECA used in Experiment 2 for number of head-dips, duration of head-dipping (measured in seconds) and locomotor activity counts. $^{++}p<0.01$ vs. vehicle only control; $^{**}p<0.01$ vs. unpretreated control at the same dose of NECA; $\#p<0.05$ theophylline pretreatment vs. 8-pSPT pretreatment.

exploratory and locomotor measures ($H=72.0$, $p<0.001$, for number of head-dips; $H=56.9$, $p<0.001$, for duration of head-dipping; $H=73.1$, $p<0.001$ for locomotor activity). Comparison of the rank means revealed a significant ($p<0.01$) sedative effect for NECA on all three measures (see Fig. 2). In those groups pretreated with 30 mg/kg theophylline, significant antagonism of the NECA-induced sedation was seen. A significant antagonism was also seen as a result of 8-pSPT pretreatment though this was not of the same magnitude; for the locomotor activity measure there was a significant difference between the NECA-induced effect for these two pretreatments.

DISCUSSION

The results from Experiment 1 showed a marked dose-related depression of both exploration and locomotor activity for three of the compounds tested (NECA, CHA and CPA) in line with previous reports (9, 14, 17). This sedative profile was related to the relative affinities of these compounds at the A_2 adenosine receptor site (5), although alternative explanations are conceivable, for example, the much greater hydrophilicity of NECA as compared to the other compounds tested may also play a role (8). CV-1808, which is reported to be selective for A_2 receptors, showed no significant behavioral effect over the dose range tested. Subsequent reports (3,4) have shown a decrease in motor of activity but at doses much higher than those used in these experiments. It is, therefore, possible that an effective concentration at the site of action was not reached at the doses tested here. Due to the very poor aqueous solubility of CV-1808 it was not possible to test higher doses without having to use a vehicle with a percentage of DMSO which was itself sedative in these tests; other vehicles tested, such as propylene glycol or ethanol, presented similar problems. Additionally, CV-1808 has been reported to be a potent inhibitor of adenosine uptake (18) and such an effect may obscure A_2 actions.

It has previously been noted that subsedative doses of adenosine analogues cause an increase in motor activity (7, 9, 13). In this study both CPA and CHA showed an enhancement (albeit a nonsignificant one) in exploratory head-dipping at doses lower than those causing a reduction of this behavior; a similar enhancement was seen with CV-1808, although this did not show any reduction in exploration. This phenomenon was not seen at any of the NECA doses, however since all the NECA doses reduced activity it is possible that such an effect may occur at lower doses than those used in this study. None of the drugs tested however showed any increase in holeboard locomotor activity over the dose range used. The mechanism of the low dose enhancement of exploration is unclear, previous reports have shown that this effect

cannot be antagonized by caffeine and may possibly involve nonadenosinergic mediation (8,13).

A marked sedative effect of NECA was again seen in Experiment 2. This sedation could be reversed by pretreatment with either theophylline or 8-pSPT, however the antagonism was much greater for theophylline than 8-pSPT (significantly so for the locomotor activity measure). Since theophylline readily crosses the blood-brain barrier, whereas 8-pSPT does not, these results suggest that central mechanisms may largely mediate the sedative effects of NECA. This conclusion is further supported by reports that 8-pSPT has an equivalent or greater affinity for both the A_1 and A_2 adenosine receptors than theophylline (5) and, therefore, if the sedation was totally peripherally mediated, one might expect to show a stronger antagonism of NECA-induced effects than those seen with theophylline. Nonetheless, a significant antagonism of NECA's effects was seen with 8-pSPT pretreatment (although to a much lesser extent than that of theophylline). This further suggests that either some of the behavioral effects may be due to peripheral actions (e.g., cardiovascular) or that at least some 8-pSPT or an active metabolite may be able to cross the blood-brain barrier.

The conclusion that the sedative effects of NECA, and the other adenosine analogues tested, are mediated via a central action is further supported by the observation that central (intraventricular) administration of adenosine analogues elicits very similar behavioral actions to those observed after systemic administration (19). Such studies further reveal that, as seen in the present experiments, A_2-selective agonists (e.g., NECA) are more potent than A_1-selective ligands (e.g., CHA or PIA) clearly suggesting that A_2-receptors rather than A_1-receptors mediate these behavioral actions. However, since the agonists tested were not sufficiently adenosine receptor subtype selective, and solubility restrictions precluded higher doses of some of the compounds, the interpretation of the data cannot be unequivocal. The development of more selective ligands (particularly A_2-ligands) will better resolve this issue.

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REFERENCES

1. Barraco, R. A.; Coffin, V. L.; Altman, H. J.; Phillis, J. W. Central effects of adenosine analogs on locomotor activity in mice and antagonism of caffeine. Brain Res. 272:392–395; 1983.
2. Birch, B. D.; Louie, G. L.; Vickery, R. G.; Gaba, D. M.; Maze, M. L-phenylisopropyladenosine (L-PIA) diminishes halothane anesthetic requirements and decreases noradrenergic neurotransmission in rats. Life Sci. 42:1355–1360; 1988.
3. Bristol, J. A.; Bridges, A. J.; Bruns, R. F.; Downs, D. A.; Heffner, T. G.; Moos, W. H.; Ortwine, D. F.; Szotek, D. L.; Trivedi, B. K. The search for purine and ribose-substituted adenosine analogs with potential clinical application. In: Paton, D. M., ed. Adenosine and adenine nucleotides: Physiology and pharmacology. London: Francis and Taylor; 1988:17–26.
4. Bruns, R. F.; Davis, R. E.; Ninteman, F. W.; Poschel, B. P. H.; Wiley, J. N.; Heffner, T. G. Adenosine agonists as pharmacological tools. In: Paton, D. M., ed. Adenosine and adenine nucleotides: Physiology and pharmacology. London: Francis and Taylor; 1988:39–50.
5. Bruns, R. F.; Lu, G. H.; Pugsley, T. A. Characteristics of the A_2 adenosine receptor labelled by ^{3}H-NECA in rat striatal membranes. Mol. Pharmacol. 29:331–346; 1986.
6. Coffin, V. L.; Taylor, J. A.; Phillis, J. W.; Altman, H. J.; Barraco, R. A. Behavioral interaction of adenosine and methylxanthines on central puringergic systems. Neurosci. Lett. 47:91–98; 1984.
7. Crawley, J. N.; Patel, J.; Marangos, P. J. Behavioral characterization of two long lasting adenosine analogs: sedative properties and interaction with diazepam. Life Sci. 29:2623–2630; 1981.
8. Dunwiddie, T. V. The physiological role of adenosine in the CNS. Int. Rev. Neurobiol. 27:67–139; 1985.
9. Dunwiddie, T. V.; Worth, T. Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. J. Pharmacol. Exp. Ther. 220:70–76; 1982.
10. Durcan, M. J.; Lister, R. G. Does directed exploration influence locomotor activity in a holeboard test? Behav. Neural Biol. 51:121–125; 1989.
11. File, S. E.; Wardill, A. G. Validity of head-dipping as a measure of exploration in a modified holeboard. Psychopharmacologia 44:53–59; 1975.
12. Glowa, J. R.; Sobel, E.; Malaspina, S.; Dews, P. B. Behavioral effects of caffeine, (−)N-((R)-1-methyl-2-phenylethyl)-adenosine (PIA) and their combination in the mouse. Psychopharmacology (Berlin) 24:263–270; 1985.
13. Katims, J. S.; Annau, Z.; Snyder, S. H. Interactions of the behavioral effects of methylxanthines and adenosine derivatives. J. Pharmacol. Exp. Ther. 227:167–173; 1983.
14. Phillis, J. W.; Barraco, R. A.; DeLong, R. E.; Washington, D. O. Behavioral characteristics of centrally administered adenosine analogs. Pharmacol. Biochem. Behav. 24:263–270; 1986.
15. Snyder, S. H. Adenosine receptors and the actions of the methylxanthines. Trends Neurosci. 4:242–244; 1981.
16. Snyder, S. H.; Katims, J. S.; Annau, Z.; Bruns, R. F.; Daly, J. W. Adenosine receptors and the actions of the methylxanthines Proc. Natl. Acad. Sci. USA 78:3260–3264; 1981.
17. Spealman, R. D.; Coffin, V. L. Behavioral effects of adenosine analogs in squirrel monkeys: relation to adenosine A_2 receptors. Psychopharmacology (Berlin) 90:419–421; 1986.
18. Taylor, D. A.; Williams, M. Interactions of 2-phenylamino-adenosine (CV-1808) with adenosine systems in rat tissues. Eur. J. Pharmacol. 85:335–338; 1982.
19. Yarbrough, G. G.; McGuffin-Clineschmidt, T. C. In vivo behavioral assessment of central nervous system puringergic receptors. Eur. J. Pharmacol. 76:137–144; 1981.