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Effect of Intraventricular Adenosine on Food Intake in Rats

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LEVINE, A. S. AND J. E. MORLEY. *Effect of intraventricular adenosine on food intake in rats.* PHARMACOL BIOCHEM BEHAV 19(1) 23–26, 1983.—Previous studies have shown that peripherally administered purines suppress food intake in rats. In this study we show that central administration of adenosine, adenine and AMP potently suppressed food intake in rats. Intraperitoneal adenosine suppressed feeding at the 100 and 50 mg/kg dose whereas 100, 50 and 10 μg of intraventricular adenosine suppressed feeding after intracerebroventricular injection at 30 minutes and up to 120 minutes at the high doses. Inosine, 2-deoxyinosine, 7-methyl-inosine and 2-deoxyguanosine all failed to suppress food intake when given intraventricularly at the same doses used for adenosine, adenine and AMP. Adenosine, 10 μg ICV, also decreases water uptake. The effect of adenosine was specific for ingestive behaviors as it did not significantly decrease spontaneous movement or grooming. These results suggest that adenosine suppresses feeding via a central mechanism and that this suppressive effect is not dependent on deamination of adenosine to inosine. The central adenosine effect appears to work by a different mechanism to the satiety effect of peripherally administered inosine.

Adenosine Inosine Appetite Purines Food intake Diazepam

CURRENTLY there is a great deal of interest in the role of purines as modulators of neurotransmission. Evidence for the release of ATP from sensory nerve endings was first reported by Holton and Holton in 1953 [18] and the release of ATP from central nervous structures both *in vivo* [15, 19, 31, 50] and *in vitro* [36, 37, 38, 46, 51] was later reported. The release of purines following nerve stimulation is a general phenomenon as nerve stimulation releases purines in the rat stomach [34], kidney [12], blood vessels [44], vas deferens [48] and the urinary bladder [4]. Burnstock [2, 3, 4] has proposed that ATP is a neurotransmitter which may have developed early in the evolution of nervous communication systems and that purinergic nerves exist in addition to cholinergic and adrenergic nerves. Adenosine and adenine nucleotides inhibit release of transmitters such as norepinephrine [10, 45, 47], serotonin [17], dopamine [17,22] and acetylcholine [17] probably by acting on the cell membrane [13]. Adenosine in general appears to have a depressant action on the discharge of cortical neurons [30]. Such a depressant action of adenosine on cortical neurons is shared by adenosine nucleotides but not by degradative products such as inosine, hypoxanthine, xanthine or adenine [30]. Many of the actions of the methylxanthines may be due to their inhibitory actions on adenosine and adenine nucleotides (and antagonism of adenosine receptors) [11, 32, 35).

It is well known that both the central and peripheral nervous systems are involved in the regulation of food intake with a balance between multiple neurotransmitters carefully regulating the ingestion of food [23]. Recent reports indicate that peripherally administered purines may be neuroregulators of consummatory behavior [6,21]. Two separate lines of evidence have stimulated the investigation of the role of purines in feeding behavior. In one case the role of adenosine in feeding was examined since adipocytes release adenosine [39], adenosine has antilipolytic action [14,42] and large fat cells contain greater amounts of ATP and cyclic AMP. In the other case, inosine and 2-deoxyinosine were examined as possible regulators of food intake since these compounds have been suggested to be endogenous ligands of the benzodiazepine receptor [1,41], a receptor reported to be involved in the initiation of feeding [7,23]. In the present study, we report for the first time that centrally administered adenine, adenosine and AMP markedly suppress food intake in rats.

METHOD

Forty-eight male Sprague-Dawley rats (125–250 g) given free access to Purina lab chow and tap water and housed in individual cages under conditions of controlled temperature and illumination (0600 to 1800 hours) were used in all studies. In the rats receiving intraventricular purines or vehicle, stainless steel guide tubes were stereotactically implanted into the lateral ventricle under nembutal anesthesia at least five days prior to the commencement of the experiments [24]. All agents were administered in a 10 μl volume of vehicle when given intraventricularly, or in a 0.2 cc volume of vehicle subcutaneously. All testing was done in the home cage.

All substances were purchased commercially: adenosine, adenine, AMP, inosine, 2-deoxyinosine, 7 methylinosine and 2-deoxyguanosine (Sigma Chemical Company, St. Louis, MO).

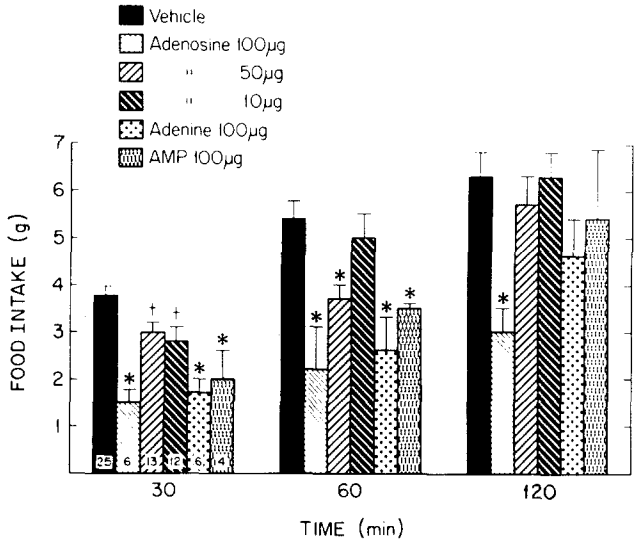

FIG. 1. Effect of peripheral administration of adenosine, adenine and AMP on food intake in rats. *$p<0.01$, †$p<0.05$, F(30 min)=7.22, $p<0.005$, F(60 min)=5.05, $p<0.005$, F(120 min)=18.71, $p<0.005$.

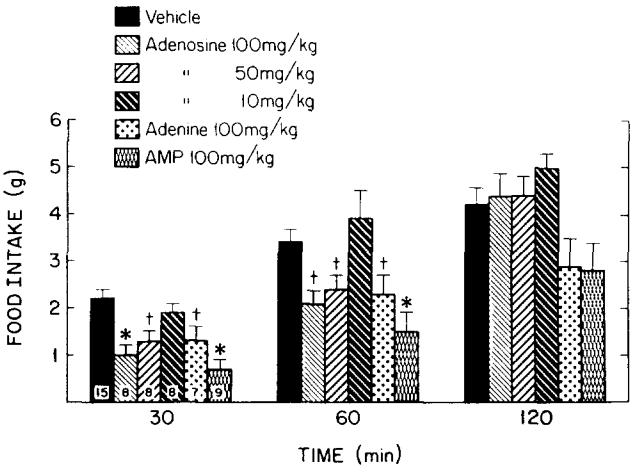

FIG. 2. Effect of intracerebroventricular administration of adenosine, adenine and AMP on food intake in rats. *$p<0.01$, †$p<0.05$, F(30 min)=9.2, $p<0.005$, F(60 min)=5.38, $p<0.005$, F(120 min)=3.12, $p<0.05$.

Feeding was induced by a 24 hour starvation period (all rats received a training period prior to experimentation to insure consistant food intake). Rats were injected with purines or vehicle (saline pH 5.5 or 9.0) intracerebroventricularly (ICV) or intraperitoneally following the starvation period. Rats were immediately placed back in their home cage and given 7–10 g of Purina lab chow and water ad lib. Food was removed and weighed at 30, 60 and 120 minutes and replaced with pre-weighed lab chow. Animals were utilized in a cross over manner with a control group included in each experiment. Animals were given a 3–4 day rest period between experiments.

In a separate experiment, 16 animals were water but not food deprived, for 18 hours and water intake was then measured for 30 minutes after ICV injection of 10 μg adenosine or vehicle. In this experiment we also noted the time the animals spent in spontaneous locomotion, resting, eating, drinking and grooming by observing the behavior of each animal once every minute for 30 minutes.

All results are expressed as the mean±SEM. Results were compared by a one-way analysis of variance at each time point followed by the two-tailed unpaired Student's *t*-test.

RESULTS

Peripheral administration of adenosine resulted in a marked decrease in food intake for up to 60 minutes at the 100 and 50 mg/kg doses, but not at the 10 mg/kg dose (Fig. 1). Animals did not appear to be sedated and behavior patterns appeared to be normal. Adenine and AMP also suppressed feeding when administered intraperitoneally. ICV adenosine suppressed food intake in a dose related manner. ICV administration of adenosine, adenine and AMP all suppressed food intake at doses about 200 times as small as those given peripherally (Fig. 2). Central administration of inosine, 2-deoxyinosine, 7-methylinosine and 2-deoxyguanosine (100 μg) all failed to suppress food intake (Table 1). Central administration of 10 μg adenosine also suppressed water intake

TABLE 1

EFFECT OF INTRAVENTRICULAR ADMINISTRATION OF INOSINE, 2-DEOXYINOSINE, 7-METHYLINOSINE AND 2-DEOXYGUANOSINE ON FOOD INTAKE

		Food Intake (g)		
	n	30 min	60 min	120 min
Vehicle (100 μg)	7	3.3 ± 0.7	4.4 ± 0.8	5.9 ± 1.1
Inosine (100 μg)	6	3.5 ± 0.4	4.9 ± 0.7	6.1 ± 0.6
2 Deoxyinosine (100 μg)	6	2.9 ± 0.4	4.4 ± 0.7	5.6 ± 1.0
7 Methylinosine (100 μg)	6	3.1 ± 0.5	4.5 ± 0.8	5.7 ± 1.0
2 Deoxyguanosine (100 μg)	6	3.8 ± 0.5	5.4 ± 0.6	6.3 ± 0.6

(Table 2). At this dose the effect of adenosine appeared to be specific for ingestive behaviors as there was no significant decrease in spontaneous locomotion or grooming behavior (Table 1).

DISCUSSION

In the present study we have demonstrated that adenosine potently suppresses feeding when administered both centrally and peripherally. Intraventricular administration of 10 μg of adenosine suppressed feeding during the first 30 minutes of the study whereas 10,000 μg of adenosine administered peripherally (to a 200 g rat) was necessary to suppress feeding during this same time period. Adenine and adenosine have previously been demonstrated to cross the blood brain barrier [8]. However, AMP can be converted to adenosine and adenine and can therefore indirectly be transported to the central nervous system [49]. These observations suggest that adenosine may act via a central mechanism. The purine base adenine and the nucleotide AMP also suppressed feeding markedly when given in much smaller quantities centrally compared with peripheral administra-

TABLE 2

EFFECT OF CENTRAL ADMINISTRATION OF ADENOSINE (10 μg) ON WATER INTAKE AND BEHAVIOR

	Adenosine (10 μg ICV)	Saline	p
Drinking (ml)	3.0 ± 1.4	11.2 ± 1.6	<0.01
Ingestive behaviors (% total)	8 ± 3	25 ± 1	<0.01
Drinking (% total)	8 ± 3	18 ± 3	<0.05
Eating (% total)	0	8 ± 3	<0.05
Grooming (% total)	15 ± 6	15 ± 3	NS
Spontaneous movement (% total)	25 ± 4	31 ± 4	NS
Resting (% total)	52 ± 8	28 ± 7	<0.05

tion. Centrally administered adenosine also suppresses drinking. The effect of adenosine appears to be relatively specific for ingestive behaviors as there was no significant decrease in spontaneous locomotion or grooming behavior after central adenosine administration.

We and others [6,21] have shown that peripheral administration of inosine also suppresses food intake in rats. This effect did not seem to be to an aversive phenomenon as both peripherally administered inosine and adenosine did not suppress water intake and the animals did not show any unusual behaviors [6,21]. Also the doses of adenosine and inosine were well below the tranquilizing dose of these purines [9]. Capogrossi *et al.* [6] found that adenosine more potently suppressed feeding during the first hour of their study (86% reduction) when compared to inosine (69%). In the present study, after ICV administration, inosine, 2-deoxyinosine and 7-methylinosine all failed to suppress feeding when given at the same dosage as adenosine. Thus, although adenosine deaminase activity is high in the brain [40] it does not appear that conversion of adenosine to inosine is the mechanism by which purines suppress food intake centrally. This is in contrast to the suggestion by Skolnick *et al.* [41] that the anticonvulsant activities of adenosine may result from deamination of adenosine to inosine. In the case of food suppression it appears that adenosine is more potent than inosine both centrally and peripherally. Since inosine can be converted to adenosine by means of a salvage pathway [49] it is possible that conversion to adenosine is necessary for suppression of food intake to occur.

The inhibitory effects of adenosine on the release of neurotransmitters indicates that adenosine may act centrally by inhibiting norepinephrine, GABA and dopamine, all known to be involved in the initiation of feeding [16, 20, 26, 27, 28]. In addition, a role has been reported [29,43] for adenosine in certain central actions of opiates, pharmacologic agents known to induce feeding in sated rats [25,33]. Thus, purines may be involved in short term regulation of feeding by interacting with the intricate network of peptides and monoamines involved in the modulation of food intake [23].

Since adenosine has antilipolytic activity [14,42] and is also released by fat cells [39] it is tempting to postulate that purines may also be involved in long term regulation of feeding. Capogrossi *et al.* [6] suggested adenosine may act as a signal between the adipose tissue and the hypothalamus. The fact that the suppression of food intake is more marked when adenosine is administered centrally than when given peripherally suggests that purines may indeed act as messengers between the peripheral and the central nervous system. However, the fact that centrally administered adenosine suppressed both water and food intake, in contrast to peripherally administered purines which only decrease food intake, suggests that the peripheral satiety effects of inosine and adenosine may work through a different mechanism to the central effect of adenosine.

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