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Strain-Related Differences in Adenosine Receptor Density and in Behavioral Sensitivity to Adenosine Analogs in Mice

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FLORIO, C., A. M. ROSATI, U. TRAVERSA AND R. VERTUA. *Strain-related differences in adenosine receptor density and in behavioral sensitivity to adenosine analogs in mice.* PHARMACOL BIOCHEM BEHAV **49**(2) 271-276, 1994. - The behavioral effects of the adenosine agonists 5'-*N*-ethylcarboxamidoadenosine (NECA) was investigated in two strains of inbred mice, CD1 and CBA. NECA dose dependently reduced spontaneous locomotor activity with similar potency (ED_{50} = 36 ± 1.5 and 36 ± 1.1 nmol/kg IP for CBA and CD1 mice, respectively) and efficacy (>90% at 100 nmol/kg) in the two strains. One nmol/kg NECA, an ineffective dose in CBA mice, exerted a significant stimulant action in CD1 mice. In saturation experiments, no differences were found in the density or in the affinity of striatal A_{2a} receptors labeled with [^{3}H]NECA. A strain-related difference was found in the density of striatal A_1 receptors labeled with [^{3}H]CCPA. In CBA mice, the B_{max} value was 32% less than in CD1 mice (0.646 ± 0.037 and 0.951 ± 0.073 pmol bound/mg protein, respectively, $p < 0.05$). No differences in [^{3}H]CCPA binding parameters were found in cortical and hippocampal membranes obtained from the two strains, whereas a higher density of A_1 binding sites was found in the cerebellum of CBA mice. The present results show a close correlation between binding studies and the depressant action of NECA and present evidence for strain-related differences in regional distribution of central adenosine receptors and in behavioral response to purinergic drugs.

Adenosine NECA CPT Adenosine A_1 and A_2 receptors Locomotor activity Inbred mice Radioligand binding

ADENOSINE elicits a wide range of behavioral responses in mammals including sedation, depression of locomotor activity, anticonvulsant actions, analgesia, and hypothermia (2, 24). The central actions of adenosine appear to be mediated by at least two binding sites, named A_1 and A_2 receptors. Activation of A_2 receptors leads to activation of adenylate cyclase, whereas activation of A_1 receptors may result in a variety of cellular responses, including inhibition of cAMP formation, modulation of potassium and calcium channels, and/or stimulation of phosphatidil inositol hydrolysis. Within the central nervous system, adenosine A_1 and A_2 receptors are unevenly distributed, showing distinct regional differences (20). A_2 receptors are primarily associated with the striatum, nucleus accumbens, globus pallidus, and olfactory tubercle (16). A_1 receptors are more widely distributed, being present in the cortex, hippocampus, cerebellum, thalamus, and striatum (21). The region-specific organization of adenosine receptors implies that the selective distribution of A_1 and A_2 receptor subtypes may confer distinct functions to the adenosine system, depending on its localization. It is generally assumed that A_1 receptors modulate the excitability of the central nervous system by inhibiting the release of several neurotransmitters (13), and recent findings strongly support the hypothesis of an involvement of striatal A_2 receptors in mediating the depres-

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sion of locomotor activity induced by adenosine agonists (3,14). However, at the present, the physiological significance of the adenosine receptor organization at the level of distinct structures within the brain is largely unknown.

Highly inbred strains of mice have been reported to show great variations in the behavioral sensitivity to methylxanthines, stimulant drugs that can act as antagonists at adenosine A_1 and A_2 receptors (4,22). The strain-specific differences in the sensitivity to the stimulant effects of methylxanthines have, at least in part, been ascribed to selective differences in the number of purinergic receptors in discrete brain regions (15). In the present study, we present evidence that the behavioral responses to the adenosine agonist 5′-*N*-ethylcarboxamide adenosine (NECA) and to the xanthine derivative 8-cyclopentyl-theophylline (CPT) differ in mice of two inbred strains, the CD1 and CBA mice. Concurrently, different densities of adenosine receptors between the two strains are shown by radioligand binding studies on membranes obtained from separate brain areas. The role of striatal adenosine A_1 and A_2 receptors in mediating variations in spontaneous locomotor activity in mice is discussed.

METHOD

Subjects

Adult male mice of two inbred strains, Swiss CD1 (Nossan, Corezzana, Italy) and CBA/lac (belonging to a conventional breeding colony established locally) were housed in groups of 10 animals per cage and kept on a 12 L : 12 D cycle (on at 0700 h, off at 1900 h). The animals had free access to standard pellet food and water. Twenty-four hours before locomotor activity testing, mice were put in groups of five per cage in the experimental room. Each animal was drug naive and was used only once. All experiments were carried out between 0900 h and 1400 h.

Drug Sources and Administration

Cyclopentyl adenosine (CPT) and 5′-*N*-ethylcarboxamide adenosine (NECA, both from Research Biochemicals International, Natick, MA) were dissolved in 0.9% NaCl saline solution and administered intraperitoneally (IP) in a volume of 1 ml/kg body weight. For dose-response curves, the choice of the dose was made randomly.

Locomotor Activity

The effect of parenteral administration of adenosine analogs on spontaneous locomotor activity was studied in two activity cages (Basile, Varese, Italy). Each activity chamber of 38 × 24 × 24 cm consisted of opaque perspex walls, a transparent perspex lid, and a floor consisting of 30 spaced stainless steel bars. The odd bars were earthed and the even bars were active and connected with a few microampere energy source. The paws of the animals connected or disconnected the active bars producing random configurations that were converted into pulses. The pulses, proportional to the spontaneous locomotor activity, were printed out as cumulative total counts for a selected minute period. Animals were placed singly in the activity cages immediately after injection. Data were collected at consecutive intervals of 5 min each for 60 min, analyzed as a group for a 60-min sampling period and reported as mean ± SE of percent changes relative to saline controls, except for saline-injected control groups, where the results are reported as mean ± SE of counts for each point (Fig. 1). Every experimental group consisted of 5–12 mice each.

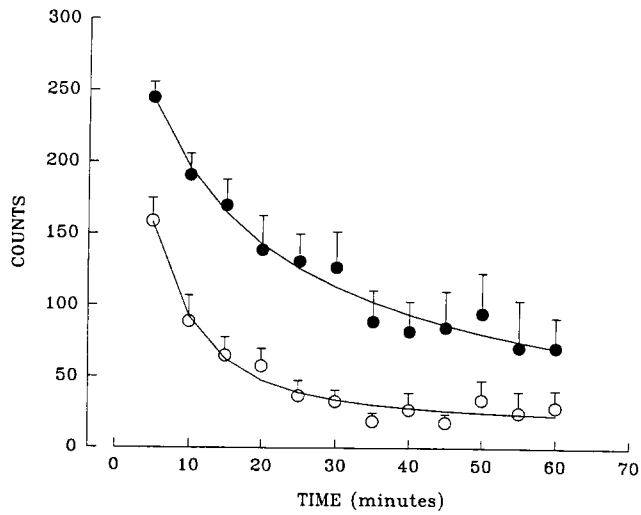

FIG. 1. Temporal pattern of spontaneous locomotor activity of CD1 (○) and CBA (●) mice. Mice were injected with saline and immediately placed in the activity cage. Counts registered within 5 min intervals are presented as mean ± SE. The number of experimental groups was $n = 7$. The level of activity of the CBA strain was significantly higher than CD1 strain ($p < 0.005$, Mann-Whitney test).

Preparation of Membranes

Mice were killed by decapitation, brains were removed and carefully dissected on ice. For each preparation of membranes, the following brain areas from 10 animals were dissected and pooled: cerebral cortex, striatum, hippocampus, and cerebellum. Membranes from the different brain areas were prepared as described earlier (11) and stored at −80°C. On the day of the assay, membranes were thawed and incubated for 30 min at 25°C with 2 U/ml adenosine deaminase (Sigma Chemical Co., St. Louis, MO), diluted, and immediately used in the binding assays.

Proteins were determined by the method of Lowry (23), using bovine serum albumin as a standard.

Radioligand Binding Studies

Chloro-N^6-2-[cyclopentyl 2,3,4,5 ^{3}H] adenosine (CCPA) and 5′-*N*-ethylcarboxamido[8(n)-^{3}H] adenosine (NECA) were used to label A_1 and A_2 receptors, respectively.

[^{3}H]CCPA radioligand binding assays were performed according to Klotz et al. (19). In saturation experiments, [^{3}H]CCPA (42.8 Ci/mmol, New England Nuclear, Boston, MA) ranging from 0.025 to 2 nM (in triplicate) was incubated in 0.5 ml (total volume) of 50 mM Tris-HCl buffer, pH 7.4. One mM theophylline was used to define the nonspecific binding. The incubation was started by the addition of 0.05–0.1 mg protein, carried out for 180 min at 25°C and stopped by vacuum filtration through Whatman GF-B glass-fiber filters, previously soaked in Tris-HCl buffer. The filters were washed four times with 3 ml of ice-cold Tris-HCl, dried, and placed in 5 ml scintillation vials containing Filter Count cocktail and counted by scintillation spectrometry (Tri-Carb 300 CD, Packard Instrument Co., USA).

Binding of [^{3}H]NECA to striatal A_2 receptors was performed according to Bruns et al. (5). In saturation experiments, [^{3}H]NECA (26 Ci/mmol, Amersham International plc., UK) ranging from 0.5 to 50 nM (in triplicate) was incu-

bated in 1 ml (total volume) of 50 mM Tris-HCl buffer, pH 7.4 containing 10 mM $MgCl_2$. Cyclopentyladenosine (CPA) (1 mM) was used to define the nonspecific binding. CPA (50 mM) was included in the total binding to eliminate the binding of [^{3}H]NECA to A_1 receptors. The incubation was started by the addition of 0.200–0.250 mg protein, carried out for 60 min at 25°C, and stopped by vacuum filtration, as described above.

Statistic and Analysis of Binding Data

Statistical analysis was performed by using the nonparametric Mann–Whitney test with the aid of the computerized program PHARM/PCS (35). Dunnett's test was used to compare control groups with NECA-treated groups for dose–response curves.

Saturation data were resolved by the computerized program LIGAND (25).

RESULTS

Locomotor Activity

Figure 1 shows the temporal pattern of spontaneous locomotor activity in CBA and CD1 mice. In both strains, locomotion was high during the first 15 min and declined progressively over the 60 min of the recording period. The level of activity of the CBA strain was significantly higher than CD1 strain ($p < 0.005$, Mann–Whitney test).

Figure 2 shows the behavioral effect of the adenosine agonist NECA in the two strains of mice. NECA reduced locomotion in a dose-related way in mice of the CBA strain with an ED_{50} of 36 ± 1.5 nmol/kg, similar to the value reported by Seale et al. (29). In mice of the CD1 strain, low doses of NECA exerted a stimulant effect that was 42 ± 12% and 31 ± 9% at 1 nmol/kg and 3 nmol/kg, respectively, with respect to control values. Higher doses gradually decreased locomotor activity. The calculated ED_{50} value was 36 ± 1.1 nmol/kg (10–100 nmol/kg range). The efficacy of NECA for the inhibition of locomotor activity was equal in the two strains at the final points of the dose–response curve. NECA (30, 50, and 100 nmol/kg) reduced locomotor activity by 50 ± 4, 63 ± 4, and 94 ± 3%, respectively, in mice of the CBA strain and by 51 ± 5, 60 ± 8, and 92 ± 5%, respectively, in mice of the CD1 strain.

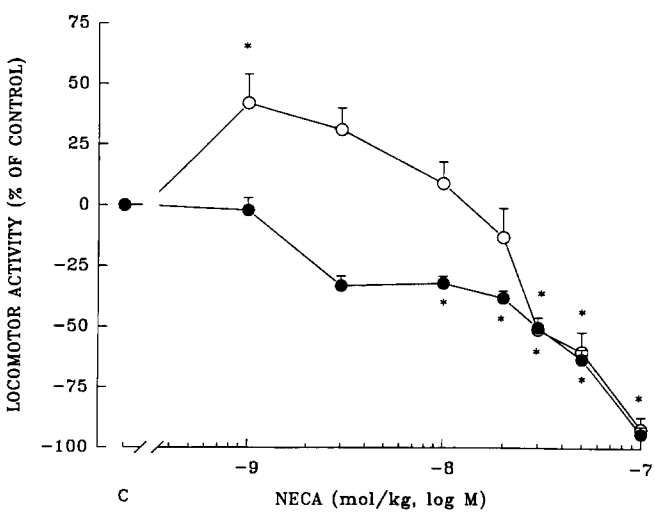

FIG. 2. Effects of different doses of the adenosine agonist NECA on spontaneous locomotor activity of mice. CD1 (○) and CBA (●) mice were injected with NECA and immediately placed in the activity cage. Data were analyzed as a group for a 60-min sampling period and reported as mean ± SE of percent changes relative to saline controls. The number of experimental groups was n = 5–12. Significance levels determined by comparisons with saline-treated controls are shown. *p < 0.05, Dunnett's test.

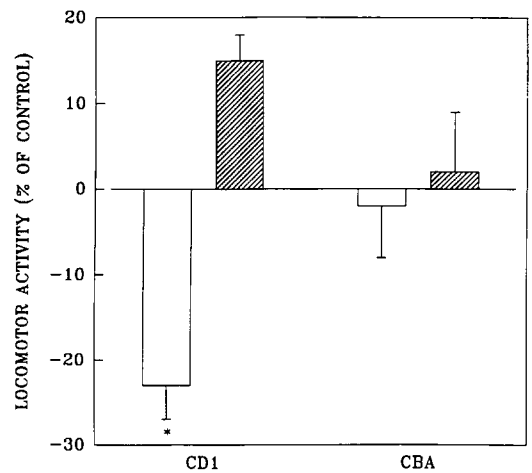

FIG. 3. Effects of the adenosine antagonist CPT on spontaneous locomotor activity in CD1 and CBA mice. Mice were injected with 100 nmol/kg CPT (open bar) or 40 μmol/kg CPT (hatched bars) and immediately placed in the activity cage. Data were analyzed as a group for a 60-min sampling period and reported as mean ± SE of percent changes relative to vehicle-treated controls. The number of experimental groups was n = 6–7. Significance levels determined by comparisons with saline-treated controls are shown. *p < 0.05, Mann–Whitney test.

Previous studies in our laboratory have shown a biphasic effect of adenosine analogs on spontaneous locomotor activity in mice of the CD1 strain (unpublished results). Those results showed that, whereas the agonist NECA dose dependently firstly increased and subsequently decreased locomotion, the antagonists CPT, PACPX, and PD 115,199 dose dependently firstly decreased and subsequently increased locomotion. Because in mice of the CBA strain NECA exhibited a consistent depressant action at all the tested doses, without any stimulant effect, we investigated whether the two strains were differently affected by the antagonist CPT. Figure 3 shows the behavioral effect of two doses of CPT in the two strains of mice. CPT (100 nmol/kg) significantly reduced locomotor activity in mice of the CD1 strain (25 ± 7% with respect to control values; p < 0.05) but was ineffective in mice of the CBA strain (2 ± 6% with respect to control values). Neither strain showed a significant increase in locomotor activity after administration of 40 μmol/kg CPT (15 ± 3% and 2 ± 7% for CD1 and CBA mice, respectively).

Binding Studies

To investigate whether the difference in responsiveness to adenosine analogs of the two strains of mice corresponded to difference in density of adenosine central receptors, saturation experiments were carried out in membranes obtained from separate areas of the brain. [^{3}H]CCPA was employed to label

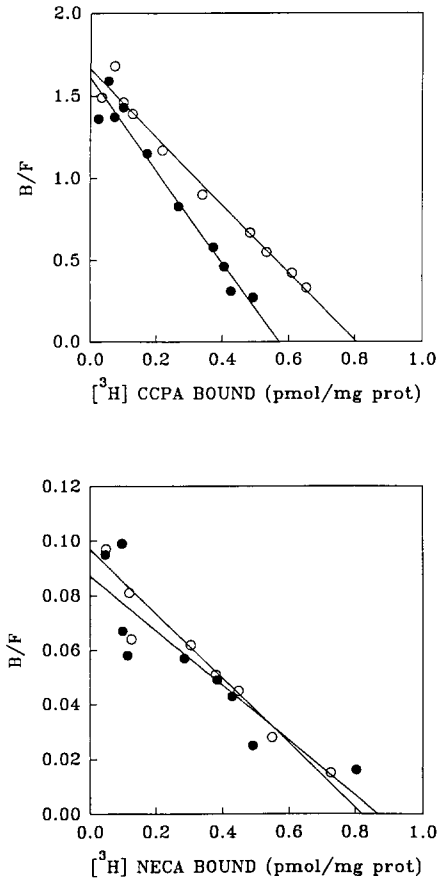

FIG. 4. Representative Scatchard plots of specific [^{3}H]CCPA and [^{3}H]NECA binding to A_1 and A_{2a} striatal receptors, respectively. (●) Represent data from CBA mice and (○) represent data from CD1 mice.

A_1 receptors in cerebral cortex, striatum, cerebellum, and hippocampus and [^{3}H]NECA was used to label striatal A_{2a} receptors. For both radioligands, the binding data were best fit to a one-site model in all brain regions.

The two strains of mice had a similar density of [^{3}H]CCPA binding sites in the cerebral cortex and in the hippocampus. In the cerebral cortex, B_{max} values of 1.34 ± 0.087 and 0.950 ± 0.047 pmol bound/mg protein were found for CD1 and CBA mice, respectively. The saturation parameters (K_d and B_{max}) for [^{3}H]CCPA binding agree with binding data reported elsewhere (19). In the hippocampus, maximal number of binding sites were 0.924 ± 0.045 and 0.858 ± 0.044 pmol bound/mg protein for CD1 and CBA mice, respectively. Significant differences in densities of A_1 receptors between the two strains were observed in cerebellum and striatum. In the cerebellum of CD1 mice, less than 30% of [^{3}H]CCPA binding sites were found, with respect to the cerebellum of CBA mice (0.318 ± 0.011 and 0.421 ± 0.040 pmol bound/mg protein, respectively). On the other hand, the striatum of CD1 mice contained an approximately 32% greater density of [^{3}H]CCPA binding sites compared to striatum of CBA mice (0.951 ± 0.073 and 0.646 ± 0.037 pmol bound/mg protein, respectively). The affinity of [^{3}H]CCPA did not differ across the brain regions examined or between strains. With respect to adenosine A_{2a} receptors, no strain-related differences in the density of [^{3}H]NECA binding sites or receptor affinity were found in the striatum (0.823 ± 0.069 and 0.923 ± 0.101 pmol bound/mg protein). In Figure 4, representative Scatchard plots of specific [^{3}H]CCPA and ^{3}H]NECA binding to striatal A_1 and A_{2a} receptors are given. Results of binding studies are summarized in Table 1.

DISCUSSION

The behavioral responses to the administration of centrally active drugs have been shown to differ among highly inbred strains of rodents (26,32,34). The genetically determined traits subserving such variability can be inherited in a simplex (27) or complex (28) way and may consist on differences in the content of neurotransmitters and their metabolites (30) or in the receptor sensitivity or density (15).

Several reports have been focussed on the different behavioral responses to methylxanthines among inbred mice (7, 22,28). Although methylxanthines exert a variety of effects not related to the antagonism at adenosine receptors (10,17, 18), the stimulant effect is believed to be principally due to the blockade of purinergic binding sites (1,4,9). Differences in adenosine A_1 and A_2 density have been reported that correlated to the different sensitivity to methylxanthine between CBA/J and SWR/J mice (15). Furthermore, a relationship between the number of [^{3}H]R-phenylisopropyl adenosine

TABLE 1

SATURATION PARAMETERS OF [^{3}H]CCPA AND [^{3}H]NECA BINDING IN CD1 AND CBA MICE

	K_d (nM)		B_{max} (pmol/mg protein)	
Brain region	CD1	CBA	CD1	CBA
[^{3}H]CCPA (A_1 receptors)				
Cortex	0.44 ± 0.12	0.33 ± 0.08	1.340 ± 0.087	0.950 ± 0.047
Hippocampus	0.37 ± 0.07	0.43 ± 0.15	0.924 ± 0.045	0.858 ± 0.044
Striatum	0.43 ± 0.09	0.36 ± 0.02	0.951 ± 0.073*	0.646 ± 0.037
Cerebellum	0.34 ± 0.01	0.55 ± 0.23	0.318 ± 0.011*	0.421 ± 0.040
[^{3}H]NECA (A_2 receptors)				
Striatum	9.3 ± 1.4	12.1 ± 1.5	0.823 ± 0.069	0.923 ± 0.101

Values represent K_d and B_{max} (mean ± SE) from three separate experiments done in triplicate on different preparations of membranes. Each preparation of membranes consisted of pooled brain regions from 10 animals.
*Represent significant strain differences determined by Mann-Whitney test, $p < 0.05$.

(R-PIA) binding sites and the behavioral and hypotermic effects of the same analog was found in mice with different sensitivity to ethanol (12). In the present study, we present evidence that the responses to the adenosine analogs NECA and CPT in CBA and CD1 mice may be related to the density of striatal A_1 and A_2 receptors.

The adenosine agonist NECA decreased locomotion with the same potency in mice of the CBA and CD1 strains and [^{3}H]NECA bound with equal affinity to membranes obtained from the striatum of CD1 and CBA mice. In addition, NECA had identical efficacy in inhibiting locomotor activity in both strains (>90% at 100 nmol/kg) and no difference in the density of [^{3}H]NECA-labeled binding sites was found between the two strains of mice. In binding studies, a concentration range of [^{3}H]NECA was used that labeled only high affinity A_{2a} receptors, with the exclusion of low affinity A_{2b} receptors (11, 37), and 50 nM CPA was utilized to eliminate [^{3}H]NECA binding to A_1 receptors (5). Thus, the high correlation between behavioral and binding studies adds new evidence to the opinion that striatal receptors of the A_{2a} subtype are involved in mediating the hypomotility induced by adenosine analogs, in agreement with recent reports (3,14).

A strain-related difference was found in the behavioral response to low doses of NECA and to CPT, a selective A_1 receptor antagonist. NECA (1 and 3 nmol/kg), that exhibited a stimulating effect in CD1 mice, failed to increase locomotor activity in mice of the CBA strain. Moreover, a low dose of CPT, which inhibited locomotion in mice of the CD1 strain, did not affect spontaneous activity in CBA mice. It has been suggested that the paradoxical stimulant effect of adenosine analogs may be due to the presence of a heterogeneous population of adenosine receptors, some of which produce sedation and other cause stimulation (2,17). On the other hand, the hypomotility observed after administration of adenosine antagonists has been related to inhibition of phosphodiesterase activity (8,31). It is, thus, noteworthy that a low dose of CPT, which is about 100-fold more potent as an adenosine receptor antagonist than as phosphodiesterase inhibitor (36), exhibited a depressant effect in CD1 mice.

In the present study, a strain-related difference was found in the density of striatal A_1 receptors. In mice of the CBA strain, the B_{max} of [^{3}H]CCPA striatal binding was significantly reduced by 32% with respect to the CD1 strain. The striatum plays an important role in regulating locomotor behavior. It is, thus, possible that the different responsiveness to low doses of CPT and NECA may be due to strain-specific differences in density or distribution of striatal A_1 receptors at pre- and/or postsynaptic level. However, because the contribution of pre- and postsynaptic striatal A_1 receptors in mediating the behavioral effect of adenosine derivatives has not been conclusively established, the present results can only be taken as evidence of two phenomena that may not necessarily be related, the strain-related differences in the density of A_1 receptors and in the behavioral response to drug administration.

Beside the striatum, a difference in the density of A_1 receptors was found in the cerebellum, a further evidence for the genetically determined intrastrain variability of the adenosine system. Interestingly, the density of cerebellar A_1 receptors was higher in mice of the CBA strain, an opposite condition with respect to the striatal A_1 receptors. At the present, we have no proposal for a functional significance related to the different density of cerebellar A_1 receptors. Nonetheless, the finding that the adenosine agonist R-PIA differentially affected the latency of seizures induced by pentylentetrazol in CBA and CD1 mice (unpublished results) indicates that strain-related differences are present in the purinergic system, involving both separate aspects of behavior and the distribution of adenosine receptors in specific regions of the brain.

In conclusion, the data reported here support the notion of an involvement of striatal A_{2a} receptors in mediating locomotor depression in rodents, whereas additional work is required to define the mechanism(s) at the basis of the stimulant action of adenosine agonist NECA and the depressant action of adenosine antagonists. The present results emphasize the usefulness of inbred strains of mice as a tool to investigate the physiological functions of the adenosine system in the central nervous system and the relevance of the subtypes of adenosine receptors in mediating the behavioral responses to adenosine agonists and antagonists.

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