

## Adenosine 3',5'-Monophosphate Content in Rat Caudate Nucleus: Demonstration of Dopaminergic and Adrenergic Receptors

**Abstract.** Dopamine, apomorphine, isoproterenol, and norepinephrine each increased the concentration of adenosine 3',5'-monophosphate in slices of rat caudate nucleus. The concentrations of dopamine, apomorphine, isoproterenol, and norepinephrine causing half-maximal increases were 60, 150, 0.03, and 30 micromoles per liter, respectively. The effect of dopamine was blocked by fluphenazine, a dopamine receptor antagonist, but not by propranolol, a  $\beta$ -adrenergic receptor antagonist. Conversely, the effect of isoproterenol was blocked by propranolol but not by fluphenazine. The results suggest that in rat caudate nucleus there are two distinct catecholamine receptors capable of causing increased concentrations of adenosine 3',5'-monophosphate, one having the characteristics of a dopamine receptor, and the other having the characteristics of a  $\beta$ -adrenergic receptor.

Considerable evidence has accumulated implicating dopamine as a neurotransmitter in the mammalian brain (1). In addition, dopamine has been implicated in the etiology of two major types of clinical abnormality, Parkinson's dis-

ease and schizophrenia. Parkinson's disease has been shown to be associated with a deficiency of dopamine in the caudate nucleus (2), and Parkinsonian side effects of antipsychotic drugs have been attributed to a blockade of the

effect of dopamine at the receptor level in the caudate nucleus (3). Hyperactivity of dopaminergic pathways in the limbic region of the brain may be involved in the pathophysiology of schizophrenia, and there is evidence that antipsychotic drugs may achieve their therapeutic effects by virtue of blocking dopamine receptors in this region of the brain (4).

In homogenates of the caudate nucleus and of the limbic region of rat brain, an adenylate cyclase was recently found which is activated by low concentrations of dopamine and is specifically blocked by antipsychotic tranquilizers (5-7). The data suggested that the dopamine receptors of mammalian brain may be identical to the dopamine-binding moiety of dopamine-sensitive adenylate cyclase, and that the effects of dopamine in these regions of the brain may be attributable to dopamine-induced increases in adenosine 3',5'-monophosphate (cyclic AMP) in postsynaptic cells of these regions. Moreover, administration of L-dopa, a precursor of dopamine and norepinephrine, raises the cyclic AMP content in rat caudate nucleus in vivo (8). In addition, other evidence suggests that cyclic AMP may mediate the effects of dopamine in mammalian superior cervical ganglion (9) and retina (10), and in invertebrate thoracic ganglia (11). In view of the importance of dopamine as a possible neurotransmitter and of the substantial amount of evidence for its involvement in certain types of neurological and psychiatric illnesses, it seemed important to determine whether dopamine applied to intact neural tissue might raise the cyclic AMP content by interacting with a specific receptor. Therefore, we have examined the effects of dopamine and other catecholamines on cyclic AMP content in slices of rat caudate nucleus.

Slices of rat caudate nucleus were incubated for 15 minutes at 37°C in Krebs-Ringer bicarbonate buffer containing 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor, in the absence or presence of various test substances (12). Maximally effective concentrations of dopamine and of apomorphine, a dopamine agonist (13), increased cyclic AMP content 75 to 100 percent (Fig. 1A) (14). The concentrations causing half-maximal stimulation were approximately 60  $\mu$ M for dopamine and 150  $\mu$ M for apomorphine. The effects of *l*-isoproterenol and *l*-norepinephrine on cyclic AMP levels are shown in Fig. 1B. A maximally effective

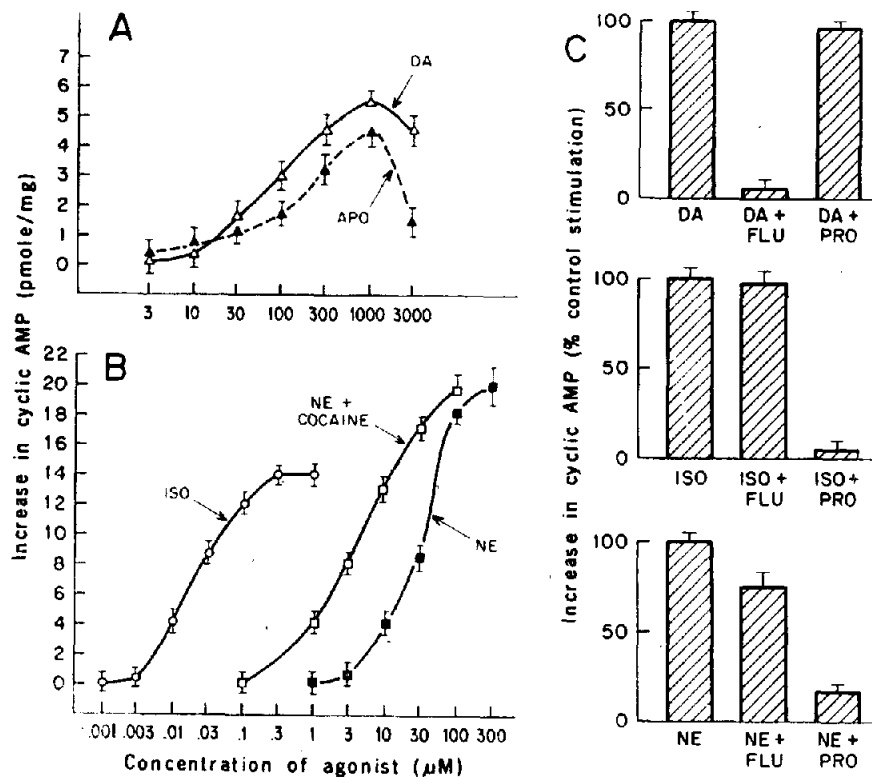


Fig. 1. (A and B) Increase in cyclic AMP in slices of rat caudate nucleus as a function of the concentration of (A) dopamine (DA) and apomorphine (APO) and (B) *l*-isoproterenol (ISO) and *l*-norepinephrine (NE). The concentration of cocaine was 0.1 mM. The data represent the mean  $\pm$  S.E.M. for 40 replicate samples (dopamine), 12 replicate samples (apomorphine), or 18 replicate samples (isoproterenol and norepinephrine). In the absence of added agonist, the cyclic AMP content was  $7.1 \pm 0.3$ ,  $6.7 \pm 0.5$ , and  $6.0 \pm 0.6$  pmole per milligram of protein, respectively, for the dopamine, apomorphine, and isoproterenol-norepinephrine curves. (C) Blockade by 100  $\mu$ M fluphenazine (FLU) or 100  $\mu$ M *dl*-propranolol (PRO) of catecholamine-induced increases in cyclic AMP in slices of rat caudate nucleus. In the absence of any blocking agent the increases in cyclic AMP induced by 100  $\mu$ M dopamine (DA, top), 1  $\mu$ M *l*-isoproterenol (ISO, center), and 100  $\mu$ M *l*-norepinephrine (NE, bottom) were  $4.2 \pm 0.2$ ,  $15.4 \pm 0.8$ , and  $12.3 \pm 0.6$  pmole per milligram of protein, respectively. The basal cyclic AMP content (in the absence of added agonist or blocking agent) was  $6.8 \pm 0.3$  pmole/mg. At the concentrations studied, none of the blocking agents significantly altered this basal level of cyclic AMP. Each determination represents the mean  $\pm$  S.E.M. for 8 to 12 replicate samples.

concentration of isoproterenol caused a two- to four-fold increase in cyclic AMP, and the concentration of isoproterenol causing half-maximal stimulation was 0.03  $\mu\text{M}$ . A maximally effective concentration of norepinephrine caused a three- to five-fold increase in cyclic AMP, and the concentration of norepinephrine causing half-maximal stimulation was 30  $\mu\text{M}$ . The maximal stimulation by norepinephrine was always greater than the maximal stimulation by isoproterenol.

Since uptake of norepinephrine and dopamine by presynaptic nerve endings is considered to be an important mechanism by which the action of these neurotransmitters is terminated (15), we have studied the effect of blockers of the uptake process on the norepinephrine- and dopamine-induced increases in cyclic AMP. When cocaine, a blocker of catecholamine uptake (16) was added to slices of caudate nucleus 10 minutes before the addition of catecholamines, the sensitivity to norepinephrine was increased, so that the concentration of norepinephrine required to give half-maximal stimulation decreased from 30 to 4  $\mu\text{M}$  (Fig. 1B). The maximal response to norepinephrine was not affected by cocaine. The dopamine dose-response curve was not affected by 0.1 or 1.0 mM cocaine, or by 0.1 mM benztropine, a specific blocker of dopamine uptake in synaptosomes from rat caudate nucleus (17, 18).

We have examined the effects of dopaminergic and adrenergic blocking agents on the increase in cyclic AMP due to 100  $\mu\text{M}$  dopamine, 1  $\mu\text{M}$  isoproterenol, and 100  $\mu\text{M}$  norepinephrine (Fig. 1C). Fluphenazine (100  $\mu\text{M}$ ), an antipsychotic agent of the phenothiazine class which has been found to be a potent inhibitor of the dopamine-sensitive adenylylase in homogenates of the rat caudate nucleus (6), completely blocked the dopamine-induced increase in cyclic AMP but did not affect the increase caused by isoproterenol. The antipsychotic tranquilizers chlorpromazine and haloperidol also inhibited the dopamine-induced increase in cyclic AMP, but were less potent than fluphenazine; these compounds were found previously to be less potent than fluphenazine as inhibitors of the dopamine-sensitive adenylylase in homogenates of the rat caudate nucleus (6). Stimulation by norepinephrine was inhibited 25 percent by fluphenazine. The  $\beta$ -adrenergic antagonist *dl*-propranolol (100  $\mu\text{M}$ ) completely blocked the increase in cyclic AMP

Table 1. Effect of dopamine, *l*-isoproterenol, and *l*-norepinephrine, individually and in combination, on amounts of cyclic AMP in slices of rat caudate nucleus. The cyclic AMP content is expressed as picomoles per milligram of protein; each value is the mean  $\pm$  S.E.M. for eight replicate samples.

Addition	Cyclic AMP (pmole/mg)
None	8.7 $\pm$ 0.7
Dopamine (300 $\mu\text{M}$ )	15.6 $\pm$ 1.0
Isoproterenol (10 $\mu\text{M}$ )	25.7 $\pm$ 0.8
Norepinephrine (300 $\mu\text{M}$ )	30.2 $\pm$ 1.5
Dopamine (300 $\mu\text{M}$ ) + isoproterenol (10 $\mu\text{M}$ )	31.0 $\pm$ 1.5*
Dopamine (300 $\mu\text{M}$ ) + norepinephrine (300 $\mu\text{M}$ )	30.9 $\pm$ 1.7†
Isoproterenol (10 $\mu\text{M}$ ) + norepinephrine (300 $\mu\text{M}$ )	29.7 $\pm$ 2.1†

\* Significantly different from isoproterenol alone at  $P < .005$ . † Not significantly different from norepinephrine alone.

induced by isoproterenol but had no effect on the increase due to dopamine. The stimulation by norepinephrine was inhibited 85 percent by propranolol. In other experiments it was found that 100  $\mu\text{M}$  phentolamine, an  $\alpha$ -adrenergic antagonist, caused a slight inhibition of the stimulation by dopamine and by norepinephrine, but had no effect on the stimulation by isoproterenol.

We have also examined the effects of combinations of catecholamines on amounts of cyclic AMP in slices of rat caudate nucleus (Table 1). In the presence of a maximally effective concentration of norepinephrine, neither dopamine nor isoproterenol caused a further increase in cyclic AMP. In the presence of a maximally effective concentration of isoproterenol, dopamine caused a significant ( $P < .005$ ) increase in cyclic AMP, such that stimulation by the combination of dopamine and isoproterenol was the same as that by norepinephrine alone.

The results with tissue slices reported here indicate that there are, in rat caudate nucleus, two distinct catecholamine receptors, the stimulation of which results in increased amounts of cyclic AMP. One receptor is activated by low concentrations of either isoproterenol or norepinephrine, but not by dopamine or apomorphine, and is blocked by propranolol but not by fluphenazine; the results suggest that an adenylylase sensitive to  $\beta$ -adrenergic agonists is present in the caudate nucleus. Since it has not been possible to demonstrate isoproterenol-sensitive adenylylase activity in homogenates of the caudate nucleus (5), it would appear that this caudate enzyme loses its hormonal sensitivity on homogenization of the tissue. Such a loss of hormonal sensitivity as a

result of homogenization has been observed for various types of adenylylase cyclases from a number of tissues (19). The other receptor is activated by dopamine, apomorphine, or norepinephrine, but not by isoproterenol, and is blocked by fluphenazine but not by propranolol. The properties of this receptor are similar to the properties of the dopamine-sensitive adenylylase found in homogenates of rat caudate nucleus, which is stimulated by dopamine and apomorphine, as well as by higher concentrations of norepinephrine; the results provide further support for the hypothesis that dopamine-sensitive adenylylase may be the receptor for dopamine in mammalian brain.

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- Caudate nuclei were removed from male Sprague-Dawley rats (150 to 200 g) as described previously (5) and cut into slices measuring 260 by 260  $\mu\text{m}$  with a McIlwain tissue chopper. Slices were suspended in Krebs-Ringer bicarbonate buffer (composition in millimoles per liter: NaCl, 124; KCl, 5.0; NaHCO<sub>3</sub>, 26; CaCl<sub>2</sub>, 0.8; MgCl<sub>2</sub>, 1.3; KH<sub>2</sub>PO<sub>4</sub>, 1.4; and glucose, 10) with a pH of 7.4, which was equilibrated with a mixture of O<sub>2</sub> (95 percent) and CO<sub>2</sub> (5 percent). The slices were incubated in a shaking water bath for 60 minutes at 37°C with two changes of medium. After 60 minutes, 0.3-ml portions containing 15 mg of tissue (wet weight) were added to tubes containing 3-isobutyl-1-methylxanthine (final concentration, 1 mM), a potent phosphodiesterase inhibitor [J. A. Beavo, N. L. Rogers, O. B. Crofford, J. G. Hardman, E. W. Sutherland, E. V. Newman, *Mol. Pharmacol.* **6**, 597 (1970)], and the various catecholamines to be tested. All catecholamines were added in Krebs-Ringer bicarbonate buffer containing 6 mM ascorbic acid such that the final concentration of ascorbic acid was 0.6 mM. The tubes were flushed with a mixture of O<sub>2</sub> (95 percent) and CO<sub>2</sub> (5 percent), sealed, and incubated with shaking for a further 15 minutes. In experiments with blocking agents,

- slices were added to tubes containing antagonists after incubation for 50 minutes and the other agents were added 10 minutes later. Incubations were terminated by placing the tubes in boiling water for 10 minutes. The samples were then centrifuged at low speed, and duplicate 50- $\mu$ l portions of each supernatant were analyzed for cyclic AMP as described by B. L. Brown, J. D. M. Albano, R. P. Ekins, and A. M. Sgherzi [*Biochem. J.* 121, 561 (1971)]. The protein content of the samples was determined by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* 193, 265 (1951)]. The cyclic AMP content was proportional to the size of the portion tested, and known amounts of authentic cyclic AMP added as an internal standard were quantitatively recovered. Cyclic AMP content measured by this method was the same as that found by homogenizing the tissue in a mixture of ethanol (98 percent) and HCl (0.2M), centrifuging, evaporating the supernatant to dryness, and redissolving the residue for determination of cyclic AMP as described previously (11). In experiments where the cyclic AMP content of the slices and the incubation medium were determined separately, virtually all the cyclic AMP was found in the slices. The data are expressed as picomoles of cyclic AMP per milligram of protein. Each point represents the mean  $\pm$  the standard error of the mean (S.E.M.) for six or more replicate samples.
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  14. Basal levels of cyclic AMP and dopamine-induced increases in cyclic AMP, similar to those observed in the presence of 3-isobutyl-1-methylxanthine, were observed in the presence of two other phosphodiesterase inhibitors structurally unrelated to 3-isobutyl-1-methylxanthine: SQ 20,009 (1 mM; Squibb) and RO 20,1724 (1 mM; Hoffmann-La Roche). Basal amounts of cyclic AMP were three- to four-fold higher in the presence than in the absence of each of the three phosphodiesterase inhibitors.
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  20. We are grateful to J. P. Perkins for suggesting the use of 3-isobutyl-1-methylxanthine as the phosphodiesterase inhibitor for this study. Supported by PHS grants MH-17387 and NS-08440. B.K.K. was the recipient of NIH predoctoral fellowship 5-F01-GM-48047. J.F. was the recipient of a Smith Kline and French postdoctoral fellowship.

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## Lead Contamination around Secondary Smelters: Estimation of Dispersal and Accumulation by Humans

**Abstract.** *A high rate of lead fallout around two secondary lead smelters originated mainly from episodal large-particulate emissions from low-level fugitive sources rather than from stack fumes. The lead content of dustfall, and consequently of soil, vegetation, and outdoor dust, decreased exponentially with distance from the two smelters. Between 13 and 30 percent of the children living in the contaminated areas had absorbed excessive amounts of lead (more than 40 micrograms per 100 milliliters of blood and more than 100 micrograms per gram of hair) as compared with less than 1 percent in a control group. A relationship between blood and hair was established which indicated that the absorption was fairly constant for most children examined. It seemed that the ingestion of contaminated dirt and dusts rather than "paint pica" was the major route of lead intake. Metabolic changes were found in most of 21 children selected from those with excessive lead absorption; 10 to 15 percent of this group showed subtle neurological dysfunctions and minor psychomotor abnormalities.*

Attempts to establish a dose-response relationship between environmental lead concentrations and subtle changes in human health have met with only limited success, largely because of the multiplicity of sources and the consequent difficulties of quantifying individual exposure. We report here the health effects of lead contamination around two secondary lead smelters.

Although smelters A and B are located in different parts of Toronto, each smokestack (in this report all distances are measured from the respective smokestack) is about 100 m south of a residential area and 100 to 200 m north of an elevated expressway (10 to 20 m high carrying 50,000 to 150,000

cars per day). Lead emissions from the two smelters were estimated to be 15,000 and 30,000 kg/year, respectively. Procedures for the collection, preparation, and analysis of materials have been described extensively elsewhere (1).

A mosaic of lead concentrations in the soil was found in each urban-industrial complex, but extremely high values were recorded in localized areas around each of the smelters (Fig. 1a). Regression analysis of the concentrations indicated that an exponential decrease with distance, from values of 40,000 and 16,000  $\mu$ g per gram of soil close to smelter A and smelter B, respectively, to an urban background of 100 to 500  $\mu$ g per gram of soil, ac-

counted for 60 to 80 percent of the variability in the data (2). The month-to-month variation in the lead content of dustfall was proportional to the mean, but regression analysis also indicated an exponential decrease with distance from each smelter (Fig. 1b). In this case the distance parameter accounted for only 40 to 60 percent of the data variability within the limit of influence. Because the lead fallout was very localized and consisted primarily of large particles, it appears that the emissions originated mainly from low-level, dust-producing operations rather than from stack fumes.

Unlike the extremely high soil and dustfall values, the monthly geometric means of the lead concentrations in suspended particles close to the smelters (1 to 5.3  $\mu$ g per cubic meter of air) were only double those for urban sites (0.8 to 2.4  $\mu$ g/m<sup>3</sup>). However, the range of daily concentrations was much greater, producing a marked log-normal distribution (Fig. 1c). The episodal peaks were correlated with winds from the smelters, but extensive monitoring was necessary to separate emissions from the smelters and auto emissions on the expressway when winds were from the south. At two sites the same distance from the expressway as samplers north of smelter A and smelter B (that is, 300 and 200 m, respectively), the lead concentrations averaged 1.0 and 1.6  $\mu$ g/m<sup>3</sup>, in agreement with calculations from values reported by Daines *et al.* (3). The lead aerosol was predominantly submicron in size, having a mass median diameter (MMD) of  $0.8 \pm 0.2 \mu$ m. This was also the distribution at sites close to the smelters except for episodal days when, for example, concentrations at one site 100 m north of smelter A averaged  $6.6 \pm 1.0 \mu$ g/m<sup>3</sup> and the MMD increased to  $4.6 \pm 1.3 \mu$ m. The average concentrations of antimony and arsenic, also associated with smelter fumes, increased from 20 ng/m<sup>3</sup> to 440 and 180 ng/m<sup>3</sup>, respectively, on episodal days, and the size distribution reflected similar changes between nonepisodal and episodal days. Bromine concentrations, associated with automobile emissions, were about 0.3  $\mu$ g/m<sup>3</sup>, irrespective of wind direction, with an MMD of  $0.4 \pm 0.2 \mu$ m. The average bromine/lead ratio at the control sites was  $0.32 \pm 0.04$  as compared to  $0.05 \pm 0.02$  close to smelter A on episodal days, which is a value similar to that found by Wesolowski *et al.* (4) for samples containing industrial lead emissions.