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 β-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 β-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 disease 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 adenylyl 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 μM for dopamine and 150 μM 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 ± 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 ± 0.3, 6.7 ± 0.5, and 6.0 ± 0.6 pmole per milligram of protein, respectively, for the dopamine, apomorphine, and isoproterenol-norepinephrine curves. (C) Blockade by 100 μM fluphenazine (FLU) or 100 μM 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 μM dopamine (DA, top), 1 μM L-isoproterenol (ISO, center), and 100 μM L-norepinephrine (NE, bottom) were 4.2 ± 0.2, 15.4 ± 0.8, and 12.3 ± 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 ± 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 ± 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 pM. 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 μM. 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 μM (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 benztrypine, 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 μM dopamine, 1 μM isoproterenol, and 100 μM norepinephrine (Fig. 1C). Fluphenazine (100 μM), an antipsychotic agent of the phenothiazine class which has been found to be a potent inhibitor of the dopaminergic adenylate cyclase 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 tranquilizer 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 adenylate cyclase in homogenates of rat caudate nucleus (6). Stimulation by norepinephrine was inhibited 25 percent by fluphenazine. The β-adrenergic antagonist dl-propranolol (100 μM) completely blocked the increase in cyclic AMP 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 μM phenolamine, an α-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 adenylate cyclase sensitive to β-adrenergic agonists is present in the caudate nucleus. Since it has not been possible to demonstrate isoproterenol-sensitive adenylate cyclase activity in homogenates of the caudate nucleus (5), it would appear that this cyclase 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 adenylate 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 adenylate cyclase 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 adenylate cyclase may be the receptor for dopamine in mammalian brain.

JAVIER FORN, BRUCE K. KRUÉGER
PAUL GREENGARD

Department of Pharmacology,
Yale University School of Medicine,
New Haven, Connecticut 06510
slices were added to tubes containing antago-
nists after incubation for 50 minutes and the other agents were added 10 minutes later. Incubations were terminated by placing the tubes containing samples for 10 minutes. The samples were then centrifuged at low speed, and duplicate 50-μL portions of each superna-
tant were analyzed for cyclic AMP as described by B. L. Brown, J. D. M. Albano, R. P. Ekton, and A. M. Siglerzi [Biochem. (Wash.) 121, 561 (1971)]. The protein content of the samples was determined by the method of O. H. Lowry, N. J. Roseborough, A. L. Farr, and R. J. Randall [J. Biol. Chem. 193, 265 (1951)]. The cyclic AMP content was pro-
portional to the size of the portion tested, and known amounts of authentic cyclic AMP added as an internal standard were quantita-
tively recovered. Cyclic AMP content mea-
sured by this method was the same as that found by homogenizing the tissue in a mixture of ethanol (96 percent) and HCl (0.3M), centrifuging, evaporating the supernatant to dryness, and redissolving the residue for de-
termination of cyclic AMP as described previously (11). In experiments where the cyclic AMP content of the slices and the incubation medium were determined separate-
ly, virtually all the cyclic AMP was found in the slices. The data are expressed as pic-o-
moles of cyclic AMP per milligram of pro-
tein. Each point represents the mean ± the standard error of the mean (S.E.M.) for six or more replicative samples.

Attempts to establish a dose-response relationship between environmental lead concentrations and subtle changes in human health have met with only lim-
ited success, largely because of the multiplicity of sources and the conse-
quent 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 lo-
cated 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, respective-
ly. Procedures for the collection, prepara-
tion, 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 concentra-
tions indicated that an exponential de-
crease with distance, from values of 40,000 and 16,000 μg per gram of soil close to smelter A and smelter B, re-
spectively, to an urban background of 100 to 500 μg per gram of soil, ac-

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 pres-
ence of two other phosphodiesterase inhibitors structurally unrelated to 3-isobutyl-1-methyl-
xanthine: 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 ab-

15. L. L. Iverson, The Update and Storage of Noradrenaline in Sympathetic Nerves (Cam-


1120 SCIENCE, VOL. 186

August 1974

Scientific Literature

As a high rate of lead fallout around two secondary lead smelters origi-

Abstract. A high rate of lead fallout around two secondary lead smelters origi-
nated mainly from episodal large-particle emissions from low-level fugitive sources rather than from stack fumes. The lead content of dustfall, and conse-
sequently of soil, vegetation, and outdoor dust, decreased exponentially with distance from the two smelters. Between 13 and 30 percent of the children examined. It seemed that the ingestion of con-
taminated areas had absorbed excessive amounts of lead (more than 40 micro-
grams 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 con-
taminated dirt and dusts rather than "paint pica" was the major route of lead absorption; 10 to 15 percent of this group showed subtle

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.

The lead aerosol was predominantly submicron in size, hav-
ing a mass median diameter (MMD) of 0.8 ± 0.2 μm. This was also the dis-
tribution at sites close to the smelters except for episodal days when, for ex-


1120 SCIENCE, VOL. 186

August 1974

Scientific Literature