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DOPAMINE-SENSITIVE ADENYLATE CYCLASE OF THE CAUDATE NUCLEUS OF RATS TREATED WITH MORPHINE OR HALOPERIDOL

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Abstract—The effects of morphine and haloperidol were compared on dopamine-sensitive adenylate cyclase activity of the rat caudate nucleus, an enzyme activity which has been related to the "dopamine receptor." The response of adenylate cyclase activity to dopamine was measured in a hypo-osmotically shocked mitochondrial-synaptosomal fraction. The addition of 3–300 μM morphine, levorphanol, dextrorphan, *d*- and *l*-methadone, nalorphine and naloxone *in vitro* caused no significant changes in the response to dopamine, while addition of haloperidol *in vitro* completely inhibited the dopamine response at a concentration of 3 μM or higher. When morphine was administered subcutaneously at a dose of 60 mg/kg, significant increases were found in basal and dopamine-sensitive adenylate cyclase activity from the rat caudate from 15 to 120 min after the injection. Twenty to sixty min after the subcutaneous injection of 20 mg/kg of haloperidol, 1·7 to 2·0-fold increases were found in the dopamine-stimulated activity. The increased dopamine-sensitive cyclase activity found after acute administration of haloperidol or morphine returned to control values 4 hr after the injection. Implantation of two morphine pellets for 2 or 3 days produced a significant increase in the dopamine sensitivity of the cyclase activity without altering basal activity. These results from experiments *in vitro* and *in vivo* suggest that, while haloperidol has a direct effect on the dopamine receptor-associated cyclase activity, morphine must act by another mechanism, and that chronic use of either drug produces enhanced dopamine sensitivity.

Opiates and neuroleptics are known to interfere with dopaminergic neurons in the central nervous system: after the administration of morphine [1–3], methadone [4], haloperidol and chlorpromazine [5, 6], an increased synthesis of dopamine or an accelerated formation of dopamine catabolites, or an accelerated depletion of dopamine in the brain by the concomitant treatment with an inhibitor of tyrosine hydroxylase, has been observed, suggesting that dopamine turnover is increased. A major increase in dopamine turnover after the administration of narcotic analgesics or neuroleptics has been shown in a tissue rich in dopamine neurons, the caudate nucleus of the striatum [1, 7, 8]. Catalepsy in the rat has been related to an increase in dopamine catabolites in the striatum after the administration of an opiate [4, 9, 10] or a neuroleptic [9, 11]. One possibility is that an increased dopamine turnover may be a result of a compensatory activation mechanism after dopamine receptor blockade [11], and that dopamine receptor supersensitivity explains the behavioral responses after chronic administration of neuroleptics or opiates [12, 13].

A dopamine-sensitive adenylate cyclase, found in the caudate nucleus, has been suggested as the "dopamine receptor" by Kebabian *et al.* [14]. The stimulation of adenylate cyclase activity by dopamine is blocked by antipsychotic drugs added *in vitro*. In order to determine whether active opiates inhibit dopamine receptor activity as do the neuroleptics, and whether the receptor activity is under the influence of a compensatory feedback mechanism as is the turnover of the transmitter when drug of both classes are administered *in vivo*, we have measured dopamine-sensitive adenylate cyclase activity in ruptured nerve-ending preparations from rat caudate nucleus, and the effects of opiates and related compounds on dopamine stimulation of the enzyme activity.

METHODS

Preparation of the ruptured nerve-ending fraction of caudate nucleus. Wistar male rats (130–160 g) were killed by decapitation. Bilateral caudate nuclei were separated from the internal capsule and removed. The tissues were lightly homogenized in 50 vol. of 0·32 M sucrose and centrifuged at 1000 *g* for 10 min. The supernatant was centrifuged at 10,000 *g* for 15 min to sediment the crude mitochondrial-synaptosomal fraction. The nerve endings were ruptured by hypo-osmotic shock by suspending the sedimented particulate fraction in 2 mM Tris-maleate buffer, pH 7·5, containing 2 mM EGTA, to give a final protein concentration of 35–50 μg/50 μl. The protein content was measured by the method of Lowry *et al.* [15].

Adenylate cyclase assay. Fifty μl of the tissue suspension was incubated for 3 min at 30° with 200 μl of medium containing 0·2 to 0·3 μCi α-[^{32}P]-ATP (0·5 mM), Tris-maleate buffer, pH 7·5 (50 mM), $MgSO_4$ (2·0 mM), EGTA (0·2 mM), theophylline (10 mM), cAMP (1·0 mM), phosphatidylserine (10 μg/200 μl) and drugs tested, to give final concentrations as indicated. The reaction was terminated by heating to

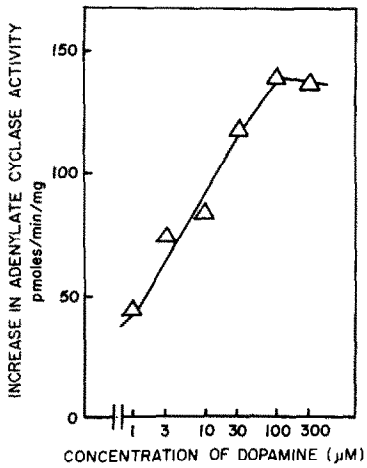

Fig. 1. Effect of dopamine on adenylate cyclase activity in ruptured nerve-ending preparations from rat caudate nucleus. Dopamine was added to the assay in the concentrations shown on the abscissa. The ordinate shows the increase above basal adenylate cyclase activity (336 ± 21 pmoles/min/mg of protein).

100° for 2 min in a boiling water bath and samples with heat-denatured enzyme solution were run as blanks. One ml of 5 mM Tris-maleate buffer, pH 7·5, containing approximately 10,000 cpm of [^{3}H]-cAMP was added before centrifugation in order to calculate recovery. The entire supernatant was placed on a column of neutral aluminum oxide (4·0 × 0·4 cm) prepared according to the method of White and Zenser [16], and the effluent was discarded. The fraction containing cAMP was eluted with 2 ml of 50 mM Tris buffer, pH 7·5, and then mixed with 0·2 ml of 0·17 M $ZnSO_4$ and 0·2 ml of 0·15 M $Ba(OH)_2$ and centrifuged. The precipitation was repeated [17]. The [^{3}H] and [^{32}P] radioactivities of the supernatant fractions were determined in a Nuclear-Chicago liquid scintillation counter.

The ruptured nerve-ending preparation, which included synaptic membranes, mitochondria and cytoplasmic components from within the nerve endings, contained about half of the basal adenylate cyclase activity of striatal homogenates.*

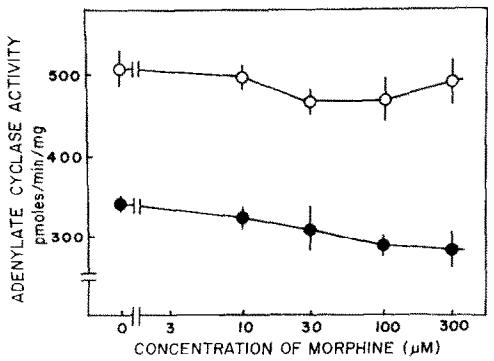

Fig. 2. Effect of various concentrations of morphine on adenylate cyclase activity, in the presence and absence of dopamine, in ruptured nerve-ending preparations from rat caudate nucleus. Each value represents the mean ±S.E. of three determinations. Closed circle: adenylate cyclase activity in the absence of dopamine. Open cirlce: adenylate cyclase activity in the presence of 100 μM dopamine.

* Unpublished observation.

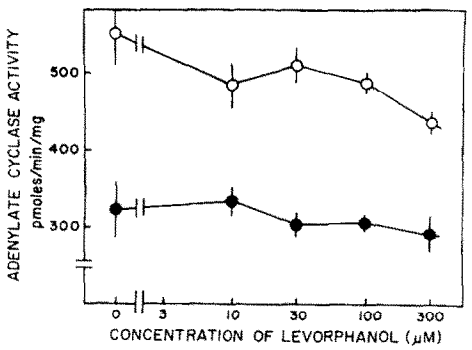

Fig. 3. Effect of various concentrations of levorphanol on adenylate cyclase activity, in the presence and absence of dopamine. Each value represents the mean ±S.E. of three determinations. Closed circle: adenylate cyclase activity in the absence of dopamine. Open circle: adenylate cyclase activity in the presence of 100 μM dopamine.

Materials. Cyclic [^{3}H]-AMP (38·2 Ci/m-mole) and α-[^{32}P]-ATP (8–12 Ci/m-mole) were obtained from New England Nuclear Corp. Haloperidol was purchased from McNeil Laboratories Inc. Morphine hydrochloride, levorphanol and dextrorphan tartrates, *levo-* and *dextro*-methadone hydrochlorides, nalorphine hydrochloride and naloxone hydrochloride were obtained from commercial sources. (When drugs were injected, the doses were calculated as the base.) Pellets of morphine (75 mg morphine base/tablet), prepared as described by Way *et al.* [18], were implanted subcutaneously in the back near the neck.

RESULTS

Effect of dopamine on adenylate cyclase activity in the ruptured nerve-ending preparation from the rat caudate nucleus. When 1–300 μM dopamine was incubated with the ruptured nerve-ending preparation from the caudate nucleus, a dose-dependent increase in adenylate cyclase activity was observed (Fig. 1).

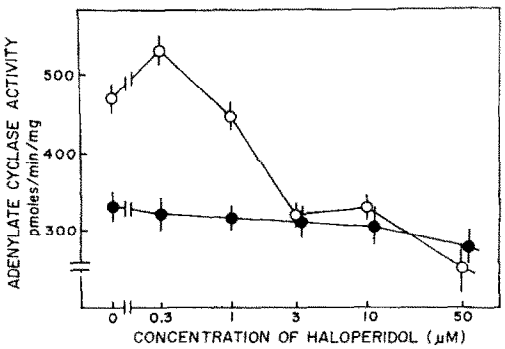

Fig. 4. Effect of various concentrations of haloperidol on adenylate cyclase activity, in the presence and absence of dopamine. Each value represents the mean ±S.E. of three determinations. Closed circle: adenylate cyclase activity in the absence of dopamine. Open circle: adenylate cyclase activity in the presence of 100 μM dopamine.

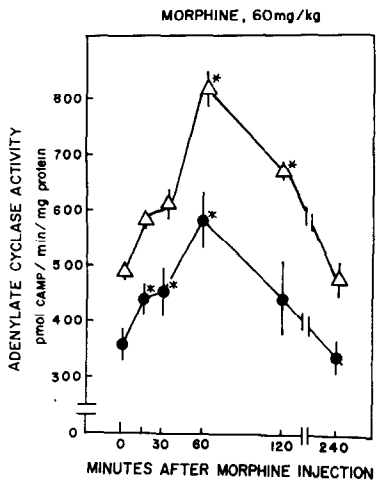

Fig. 5. Effect of a single dose of morphine on dopamine-sensitive adenylate cyclase activity in ruptured nerve-ending preparations from caudate nucleus. Morphine (60 mg/kg) was administered subcutaneously at various times before sacrifice. Each value represents the mean ±S.E. of four rats assayed individually. Closed circle: adenylate cyclase activity assayed in the absence of dopamine. Open triangle: adenylate cyclase activity assayed in the presence of 100 μM dopamine. Starred points are significantly different from respective zero time values ($P < 0.05$).

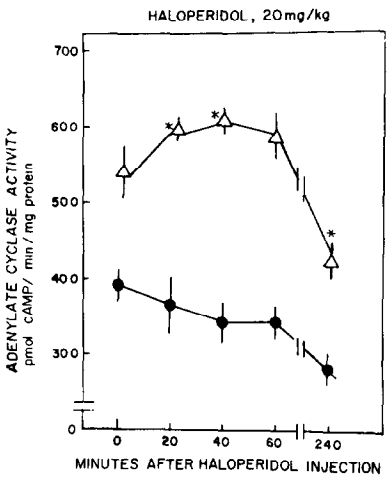

Fig. 6. Effect of a single dose of haloperidol on dopamine-sensitive adenylate cyclase activity in ruptured nerve-ending preparations from rat caudate nucleus. Haloperidol (20 mg/kg) was administered subcutaneously at various times before sacrifice. Each value represents the mean ±S.E. of four rats assayed separately. Closed circle: adenylate cyclase activity assayed in the absence of dopamine. Open triangle: adenylate cyclase activity assayed in the presence of 100 μM dopamine. Starred points are significantly different from respective zero time values ($P < 0.05$).

Maximal stimulation found at 100 μM dopamine concentration increased activity 40–50 per cent above the basal activity.

Effect of opiate agonists and antagonists or haloperidol in vitro *on dopamine-sensitive adenylate cyclase activity.* Addition of 3–300 μM morphine or levorphanol *in vitro* to the dopamine-sensitive adenylate cyclase caused no significant changes of the response to 100 μM dopamine (Figs. 2 and 3). Besides these opiates, dextrorphan, *d* and *l*-methadone, nalorphine and naloxone also had no influence on the dopamine response of adenylate cyclase. At high concentrations of some of the opioids, both the basal activity and the dopamine-dependent activity were slightly inhibited. On the other hand, the addition of haloperidol produced complete inhibition of dopamine-sensitive adenylate cyclase activity at concentrations of 3 μM or higher, with a slight change in the basal activity only at a high concentration (Fig. 4).

Effect of single administration of morphine or haloperidol on dopamine-sensitive adenylate cyclase activity. From 15 to 120 min after the subcutaneous injection of 60 mg/kg of morphine (a dose which produced deep sedation and catalepsy, but with positive righting reflex in rats), dopamine-sensitive adenylate cyclase activity was increased when compared with the zero time values, with a return to control levels by 240 min after the drug administration (Fig. 5). Since the basal activities were also elevated by the acute dose of morphine (with significant increases from 15 to 60 min after drug administration), the per cent stimulation by dopamine was unaffected (Fig. 5). Subcutaneous injection of 20 mg/kg of haloperidol (a dose which produced profound sedation and caused loss of righting reflex from 20 to 30 min after injection) also produced increases in dopamine-sensitive adenylate cyclase activity 20–60 min after the drug administration (Fig. 6). After 240 min, the increased activity had returned to control value. The basal activity decreased gradually with significant lowering of activity at 240 min (Fig. 6).

Effect of chronic administration of morphine or haloperidol on dopamine-sensitive adenylate cyclase activity. The treated rats were implanted with two mor-

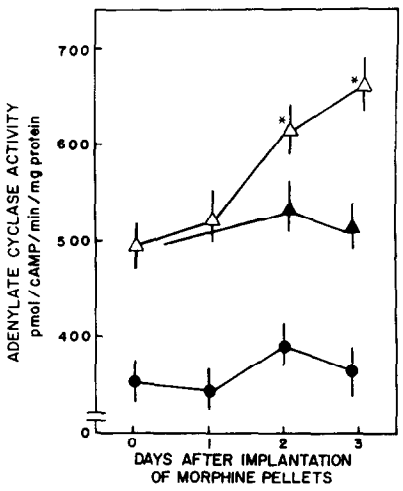

Fig. 7. Effect of implantation of morphine pellets on dopamine-sensitive adenylate cyclase activity in ruptured nerve-ending preparations from rat caudate nucleus. Each value represents the mean ±S.E. from four rats. Closed circle: adenylate cyclase activity assayed in the absence of dopamine. Closed triangle: adenylate cyclase activity assayed in the presence of 100 μM dopamine in sham-operated rats. Open triangle: adenylate cyclase activity assayed in the presence of 100 μM dopamine in rats implanted with morphine pellets. Starred points show significant difference between values of sham and pellet-implanted rats ($P < 0.05$).

Table 1. Effect of repeated administration of haloperidol on dopamine-sensitive adenylate cyclase activity of caudate nucleus*

Treatment	Adenylate cyclase activity (pmoles cAMP/min/mg protein) Basal activity	Difference between the activities with and without dopamine
Saline	313·5 ± 27·8	132·0 ± 12·4
Haloperidol	352·9 ± 28·9	208·3 ± 16·3
	P < 0·7	P < 0·02

* Rats were injected subcutaneously either with saline (2·0 ml/kg) or with haloperidol 20 mg/kg twice daily for 3 days and sacrificed 21 hr after the last injection. The ruptured nerve-ending preparation from caudate nucleus was assayed for cyclase activity in the presence or absence of 100 μM dopamine. Each value represents the mean ± S.E. of four animals. The significance of differences between treatment groups is shown.

phine pellets and the control animals were sham-operated. Two and three days after pellet implantation, there were marked increases in dopamine-sensitive adenylate cyclase activity of ruptured nerve-ending preparations from the rat caudate nucleus (Fig. 7). Basal activity, which was elevated by an acute dose of morphine, was not altered by this chronic treatment.

Groups of rats were injected with 20 mg/kg of haloperidol, twice daily for 3 days. The animals were sacrificed 21 hr after the last injection. As shown in Table 1, there was a significant increase in dopamine-sensitive adenylate cyclase activity, but not in the basal activity.

DISCUSSION

Histfluorescence studies [19], perfusion studies [20] and electrophysiological studies [21] have provided evidence that the nigrostriatal pathway is associated with dopaminergic mechanisms: dopaminergic axons originating in the substantia nigra terminate on caudate neurons; dopamine released upon excitation of neurons in the substantia nigra has an inhibitory effect on caudate neurons. Biochemical studies of Kebabian *et al.* [14] and Clement-Cormier *et al.* [22] have provided evidence that dopamine-sensitive adenylate cyclase in the dopaminergic tissues is closely related to the dopamine receptor; adenylate cyclase activity is stimulated by a low concentration of dopamine and this response to dopamine is blocked by a low concentration of antipsychotic drugs, which are compounds known to block the dopamine receptor [7].

It is uncertain, however, that narcotic analgesic drugs block the dopamine receptor. In favor of the hypothesis that opiates block the striatal dopamine receptor are the following: (1) the cataleptic action of methadone was potentiated by α-methyl-tyrosine, and reversed by apomorphine [4]; (2) morphine blocked the stereotyped behavior induced by apomorphine or amphetamine, as did haloperidol [10]; and (3) methadone-induced accumulation of dopamine catabolites was prevented by apomorphine [4]. On the other hand, Kuschinsky and Hornykiewicz [9] and McKenzie and Sadof [23] have suggested that opiates act to inhibit dopaminergic transmission by some other mechanism, such as a "diversion" of newly synthesized dopamine from storage sites to catabolism, because: (1) morphine did not block stereotyped behavior induced by apomorphine [23]; and (2) morphine-induced catalepsy was more readily reversed by DOPA than was chlorpromazine-induced catalepsy [9].

Direct evidence on this point was obtained in the present experiments. Narcotic analgesics (or inactive isomers or opiate antagonists) added *in vitro* did not inhibit dopamine-sensitive adenylate cyclase activity (dopamine receptor activity) in ruptured nerve-ending preparations from rat caudate nucleus. Haloperidol, however, strongly inhibited the dopamine response in the same particulate preparation, in agreement with observed effects in the homogenates of dopaminergic tissues [14, 22].

When single doses of haloperidol or morphine were administered to rats, we found significant increases in dopamine-sensitive adenylate cyclase activity in the ruptured nerve-ending preparation isolated from the caudate. This increased dopamine sensitivity was transient, peaking at 40 and 60 min after the injections of haloperidol or morphine respectively. The basal activity of adenylate cyclase was also enhanced after an acute dose of morphine. Similar increases after the administration of various doses of morphine in basal adenylate cyclase activity in the ruptured nerve-ending preparations, but not in whole tissue homogenates, have been observed previously by us [24]. This observation, and the fact that the per cent stimulation by dopamine remained constant although the absolute basal and dopamine-stimulated adenylate cyclase activities were significantly increased, suggests that, after an acute dose of morphine, there was more active cyclase in the nerve endings, possibly due to an activation of the enzyme *in situ*, or to an acceleration of the transport from the sites of cyclase biosynthesis.

Chronic administration of haloperidol or morphine also produced enhancement of "dopamine receptor activity." Dopamine receptor supersensitivity has been suggested as responsible in animals treated chronically by either class of drugs for enhanced behavioral responses induced by the administration of apomorphine [12, 25] or methylphenidate [13], which are known to stimulate the dopamine receptor. The biochemical data obtained in the present chronic studies support these hypotheses.

Brain phosphodiesterase is not inhibited by haloperidol [26] or morphine [27], so that the apparent

alteration of both basal and dopamine-sensitive adenylate cyclase activity is probably not due to changes in phosphodiesterase activity *in vivo*. The inclusion of 10 mM theophylline in the incubation medium insured that cAMP formed in the assay was not metabolized by phosphodiesterase activity.

Results obtained from the present experiments *in vitro* and *in vivo* support the hypothesis that while neuroleptics block "the dopamine receptor" directly, morphine blocks dopaminergic transmission by some other mechanism, and that the blockade of transmission produces enhanced receptor activity as well as increased dopamine turnover in the caudate nucleus.

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