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Direct effect of a nomifensine derivative on dopamine receptors

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A study was made of the effects of nomifensine, 4′-hydroxynomifensine and 3′,4′-dihydroxynomifensine on dopamine receptors in rat striatum and nucleus accumbens, using the dopamine-sensitive adenylate cyclase assay. Nomifensine and its 4′-hydroxy metabolite were both inactive as dopaminergic agonists. 3′,4′-dihydroxynomifensine was, however, a potent agonist, being approximately 2 to 4 times less active than dopamine. The effects of dopamine and of 3′,4′-dihydroxynomifensine were blocked by fluphenazine. It is concluded that the dopaminergic activity of 3′,4′-dihydroxynomifensine is dependent upon the presence of the two hydroxyl groups.

The results of behavioural studies in rats indicate that nomifensine is a potent agonist at dopamine receptors (Braestrup & Scheel-Kruger, 1976; Costall, Kelly & Naylor, 1975). In addition to its known ability to inhibit the uptake of noradrenaline and dopamine (Hunt, Kannengiesser & Raynaud, 1974; Schact & Heptner, 1974), it has been suggested that nomifensine is 'able to influence both pre- and post-synaptic dopamine mechanisms' (Costall & Naylor, 1977), although an effect of nomifensine on post-synaptic dopamine receptors has been questioned (Bedard, Parkes & Marsden, 1977).

Most compounds able to directly stimulate post-synaptic dopamine receptors contain hydroxyl groups in positions equivalent to the 3 and 4 positions of the ring of dopamine (reviews by Goldberg, 1972; Woodruff, 1971, 1978; Iversen, 1975). For this reason we have recently considered the possibiity (Woodruff, 1977) that some of the dopaminergic actions of nomifensine could be mediated by a dihydroxy metabolite of nomifensine (Fig. 1). This compound has now been made available to us and in the present study we have compared the direct effects of this compound with those of nomifensine and one of its known metabolites on dopamine receptors in rat brain, using the dopamine-sensitive adenylate cyclase system to test for dopaminergic activity.

METHODS

The brains were removed from freshly killed male or female Wistar rats (about 300 g) and the striatum and nucleus accumbens was removed from each side, using the methods of Kebabian, Petzhold & Greengard, (1972) and Horn, Cuello & Miller (1974).

The activity of the dopamine-sensitive adenylate cyclase in the homogenates from the two regions of the brain was estimated according to the method of Kebabian & others (1972). All drugs were dissolved in 5 mM tartaric acid and added to the assay mixture in a volume of 10 μl. The reaction was started by the addition of ATP (final concentration 0·5 mM). Incubations were carried out for 2·5 min (striatum) or 3 min (n. accumbens) at 30°; the reaction was, terminated by placing the tubes in a boiling water bath for 2·5 min. The mixture was centrifuged and the cyclic (c) AMP formed was assayed using the method of Brown, Ekins & Albano (1972).

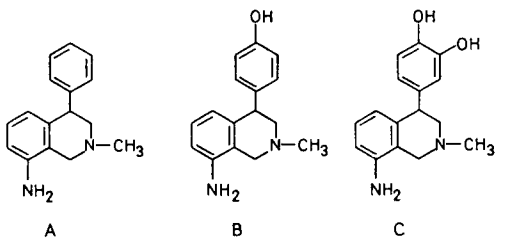

FIG. 1. Structures of (A) nomifensine (B) 8-amino-2-methyl-4-(4′-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline (4′-hydroxynomifensine) and (C) 8-amino-2-methyl-4-(3′,4′-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline (3′,4′-dihydroxynomifensine).

The following drugs were used: dopamine HCl; nomifensine; 8-amino-2-methyl-4-(3′,4′-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline, (3′,4′-dihydroxynomifensine); 8-amino-2-methyl-4-(4′-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline, (4′-hydroxynomifensine) and fluphenazine HCl.

* Correspondence.

RESULTS

The basal concentrations of cAMP production, in the absence of any added dopamine were 42·6 ± 4·5 (20) pmol/assay tube in striatal homogenates and 51·8 ± 9·4 (14) pmol/tube in homogenates of nucleus accumbens. In the presence of 100 μM dopamine, cAMP production increased to 76·8 ± 5·6 (12) pmol/tube and 81·3 ± 9·4 (12) pmol/tube in homogenates of striatum and nucleus accumbens respectively. The EC50 values (concentrations causing 50% of maximum response) were 3 μM in the striatum and 6 μm in homogenates of nucleus accumbens.

Nomifensine (200 μM) had no effect on cAMP production in homogenates of either striatum or nucleus accumbens (Fig. 2). 4'-Hydroxynomifensine was similarly ineffective in activating adenylate cyclase in striatal homogenates in concentrations of up to 200 μM.

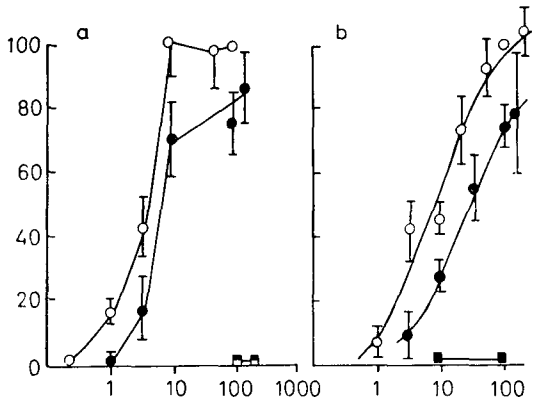

FIG. 2. Dose-response relations for the stimulation of adenylate cyclase by dopamine, nomifensine and two nomifensine derivatives in homogenates of (a) rat striatum and (b) nucleus accumbens. Results are expressed as a percentage of the maximum response which was taken as that produced by 100 μm dopamine. ○ dopamine. ● 3',4'-dihydroxynomifensine, ■ nomifensine, □ 4'-hydroxynomifensine. Each point represents the mean of 4-10 observations and is given with s.e.m. Ordinate: Increased cAMP formation (% maximum). Abscissa: Agonist concentration (μM).

3',4'-Dihydroxynomifensine was, however, an effective agonist in homogenates from both regions of the brain (Fig. 2). The maximum response produced by this compound was identical to that produced by dopamine. The EC50 values for 3',4'-dihydroxynomifensine were 6 μM (striatum) and 24 μM (n. accumbens).

The effects of 3',4'-dihydroxynomifensine on dopamine-sensitive adenylate cyclase were blocked by fluphenazine (Table 1). Fluphenazine is a potent dopamine antagonist on striatal dopamine-sensitive adenylate cyclase (Miller, Horn & Iversen, 1974).

DISCUSSION

The dopamine-sensitive adenylate cyclase, first shown to be present in homogenates of rat striatum (Kebabian & others, 1972), and now known to be present in several other regions of the brain, has proved to be a useful model of postsynaptic dopamine receptors (Iversen, 1975). Structure-activity studies on dopamine receptors in a range of tissues, including homogenates of rat striatum and nucleus accumbens, have shown that the most active dopamine agonists are phenethylamine derivatives containing hydroxyl groups in the 3 and 4 positions and 2-amino-1,2,3,4-tetrahydronaphthalene derivatives containing hydroxyl groups in the equivalent positions. Indeed, the presence of two hydroxyl groups is essential for dopamine-like activity in such molecules (Woodruff, 1971; Iversen, 1975).

In addition, there are certain derivatives of lysergic acid, such as ergometrine and LSD, which contain no phenolic hydroxyl groups, but which are able to stimulate dopamine receptors. On the dopamine-sensitive adenylate cyclase, such compounds behave as partial agonists, producing maximum responses of about 50% of that produced by dopamine (Munday, Poat & Woodruff, 1976; Giovoni, Iuliano, & others, 1977).

Our study indicates that nomifensine and its derivatives are similar to phenethylamines and to 2-amino-1,2,3,4-tetrahydronaphthalenes, rather than

Table 1. *Effect of fluphenazine* (1 μM) *on stimulation of cAMP production caused by dopamine* (*I*) *and* 3',4'-*dihydroxynomifensine* (*II*) *in striatal* (*s*) *and nucleus accumbens* (*n.a.*) *homogenates. Responses are expressed as the % of the maximum response produced by* 100 μM *dopamine which was tested in parallel in every experiment and are given with the s.e.m. The numbers in parentheses refer to the number of observations.*

	Response in absence of fluphenazine		% max in presence of fluphenazine		% inhibition produced by fluphenazine	
	s	n.a.	s	n.a.	s	n.a.
I	100	100	28·1 ± 7·8	29·5 ± 2·95(8)	71·8 ± 7·23	70·5 ± 7·6(8)
II (100 μM)	83·2 ± 16·7(4)	60·7 ± 25·3(6)	3·5 ± 3·5	0(4)	96·5 ± 3·5	100(4)

to ergot alkaloids, in terms of their actions on the dopamine-sensitive adenylate cyclase. Thus, nomifensine, which contains no hydroxyl group, and 4'-hydroxynomifensine which contains one hydroxyl group, were both inactive on the dopamine-sensitive adenylate cyclase. The dihydroxy-analogue of nomifensine was, however, a dopamine agonist on dopamine receptors associated with the adenylate cyclase from both the nucleus accumbens and the striatum, the effects of the compound being blocked by fluphenazine. 3',4'-Dihydroxynomifensine produced a similar maximum response to dopamine and had a potency, calculated from EC50 values, of between 2 and 4 times less than dopamine.

Kellner, Baeder & others (1977) have studied the metabolism of nomifensine in rats, dogs and monkeys. The major metabolites of nomifensine so far reported are 4'-hydroxynomifensine, 4'-hydroxy-3'-methoxynomifensine and 3'-hydroxy-4'-methoxynomifensine. The compound studied in the present paper, 3',4'-dihydroxynomifensine, has not been reported as a metabolite, although it is possible that it could be a precursor of one or both of the two methoxy derivatives. If any of the actions of nomifensine can be attributed to a direct action on postsynaptic dopamine receptors, then the possibility should be born in mind that the 3',4-dihydroxy analogue might mediate these actions.

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REFERENCES

BEDARD, P., PARKES, J. D. & MARSDEN, C. D. (1977). *Br. J. clin. Pharmac.*, **4**, 1855–1865.

BRAESTRUP, C. & SCHEEL-KRUGER, J. (1976). *Eur. J. Pharmac.*, **38**, 305–312.

BROWN, B. L., EKINS, R. P. & ALBANO, J. D. M. (1972). In: *Advances in cyclic nucleotide research*, Vol. 2, pp. 25–40. Editors: Greengard, P. & Robinson, G. A. New York: Raven Press.

COSTALL, B., KELLY, D. M. & NAYLOR, R. J. (1975). *Psychopharmacologia Berl.*, **41** (2), 153–164.

COSTALL, B. & NAYLOR, R. J. (1977). *Br. J. clin. Pharmac.*, **4**, 89S–99S.

GOLDBERG, L. I. (1972). *Pharmac. Rev.*, **24**, 1–29.

GIOVONI, S., IULIANO, E., SPANO, P. F. & TRABUCCHI, M. (1977). *J. Pharm. Pharmac.*, **29**, 45–47.

HORN, A. S., CUELLO, A. C. & MILLER, R. J. (1974). *J. Neurochem.*, **22**, 265–270.

HUNT, P., KANNENGIESSER, M. H. & RAYNAUD, J. P. (1974). *J. Pharm. Pharmac.*, **26**, 370–371.

IVERSEN, L. L. (1975). *Science*, **188**, 1084–1089.

KEBABIAN, J. W., PETZHOLD, G. L. & GREENGARD, P. (1972). *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2145–2149.

KELLNER, H. M., BAEDER, C., CHRIST, O., HEPTNER, W., HORNKE, I. & INGS, R. M. J. (1977). *Br. J. clin. Pharmac.*, **4**, 109S–116S.

MILLER, R. J., HORN, A. S. & IVERSEN, L. L. (1974). *Mol. Pharmac.*, **10**, 759–766.

MUNDAY, K. A., POAT, J. A. & WOODRUFF, G. N. (1976). *Br. J. Pharmac.*, **57**, 452P–453P.

SCHACT, U. & HEPTNER, W. (1974). *Biochem. Pharmac.*, **23**, 3413–3422.

WOODRUFF, G. N. (1971). *Comp. gen. Pharmac.*, **2**, 439–455.

WOODRUFF, G. N. (1977). *Br. J. clin. Pharmac.*, **4**, 105S.

WOODRUFF, G. N. (1978). In: *Dopamine* (*Adv. Biochem. Psychopharmac.*, Vol. 19). pp. 89–119. Editors: Roberts, P. J., Woodruff, G. N. & Iversen, L. L. New York: Raven Press.