J. Pharm. Pharmac., 1977, **29**, 422–427 *Received January* 21, 1977

Dopamine receptors in rat striatum and nucleus accumbens; conformational studies using rigid analogues of dopamine

G. N. WOODRUFF*, K. J. WATLING, C. D. ANDREWS, JUDITH A. POAT AND J. D. MCDERMED†

Department of Physiology & Biochemistry, University of Southampton, Southampton SO9 3TU, U.K. and †Wellcome Research Laboratories, North Carolina 27709, *U.S.A.*

A study was made of the actions of dopamine and of some 2-amino-1,2,3,4-tetrahydronaphthalenes on dopamine-sensitive adenylate cyclase in homogenates of rat striatum and nucleus accumbens. The compounds were also tested for their ability to stimulate motor activity following bilateral injection into the nucleus accumbens of conscious rats. The most active compounds on adenylate cyclase from both striatum and nucleus accumbens were dopamine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene(6,7-diOHATN). The 5,6-dihydroxy analogue (5,6-diOHATN) was 50 times less active than 6,7-diOHATN in striatal homogenates and 350 times less active in homogenates of nucleus accumbens. All dihydroxy compounds tested were active in causing stimulation of motor activity, the most active compounds being 6,7- and 5,6-diOHATN. Both dimethoxy derivatives tested were inactive on the adenylate cyclase and as locomotor stimulants.

In solution dopamine exists as *anti* and *gauche* conformers (Bustard & Egan, 1971; Rekker, Engel & Nys, 1972). Recently there has been considerable speculation as to the active conformation of dopamine at its receptor site. One approach to this problem is to study the actions of rigid dopamine analogues in which the side chain is locked in a fixed conformation. Thus, norsalsolinol (Fig. 1) contains the dopamine skeleton in a folded (corresponding to *gauche*) conformation, whereas the extended (*anti*) form of dopamine is contained in the 2-amino-1,2,3,4-tetrahydronaphthalene (ATN) ring system. The extended form of dopamine can itself exist as two rotameric extremes (see Cannon, 1975), which correspond to the two ATN compounds illustrated in Fig. 1.

As a result of studies on invertebrate dopamine receptors it was suggested (Woodruff, 1971) that the active conformation of dopamine was contained in the molecule of 2-amino-6,7-dihydroxy 1,2,3,4 tetrahydronaphthalene 6,7-diOHATN‡. This (Fig. 1) has since been shown to be a potent dopamine agonist in several different systems (see discussion). Compounds containing the 5,6-dihydroxy ATN residue have been shown by behavioural tests to have *in vivo* dopaminergic activity (McDermed, McKenzie & Phillips, 1975; McDermed, McKenzie & Freeman, 1976).

* Correspondence.
‡ ADTN used by us in previous papers.

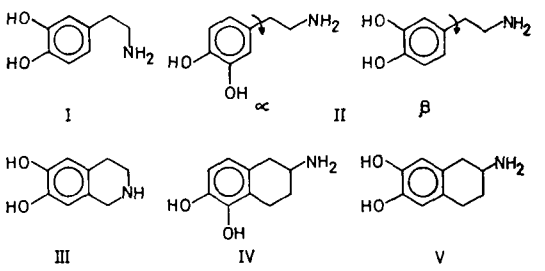


FIG. 1. Conformation of dopamine and the structure of three analogues. The dopamine residue in its folded conformation (I) is contained in the molecule norsalsolinol (III) (6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline). In its extended form (II) dopamine can exist as two rotameric extremes, α and β, 6,7-diOHATN (V) corresponds to the β-rotamer. (IV) 5,6-diOHATN.

The dopamine-sensitive adenylate cyclase, present in several regions of the central nervous system (Brown & Makman, 1972; Kebabian, Petzold & Greengard, 1972; Horn, Cuello & Miller, 1974; Clement-Cormier, Kebabian & others, 1974) has been used successfully as a model system on which to study the actions of drugs acting on mammalian dopamine receptors (review by Iversen, 1975). In the present study we have compared the actions of 6,7-diOHATN and the 5,6-dihydroxy analogue (5,6-diOHATN) on the adenylate cyclase from rat striatum and nucleus accumbens.

We have also examined the effects of both these drugs, following their injection into the nucleus accumbens, on the motor activity of conscious rats. In order to further delineate the structural requirements for dopamine-like activity we have tested some additional ATN compounds on the three systems.

MATERIALS AND METHODS

Adenylate cyclase assay

Male or female Wistar rats (about 300 g) were anaesthetized with ether and the brains were removed. The striata and nucleus accumbens were dissected according to Kebabian & others (1972) and Horn & others (1974) respectively.

The activity of adenylate cyclase in homogenates of the two brain areas was estimated according to Kebabian & others (1972). All drugs were freshly dissolved in 5 mM tartaric acid and added in a constant volume (10 μl) to the assay mixture. The reaction was started by the addition of ATP (final concentration 0·5 mM). Incubations were for 2·5 min (striatum) or 3 min (nucleus accumbens) at 30°; the reaction was terminated by placing the tube in a boiling water bath for 2·5 min. The mixture was centrifuged and the cyclic (c) AMP formed was assayed using either a muscle binding protein assay (Gilman, 1970) or a binding protein from adrenal cortex (Brown, Ekins & Albano, 1972).

Behavioural studies

Guide cannulae were implanted bilaterally in the brains of male Wistar rats (about 200 g) under pentobarbitone anaesthesia (50 mg kg^{-1}, i.p.). using previously described methods (Elkhawad & Woodruff, 1975). The cannulae were introduced at an angle of 13°, to avoid the lateral venticles, and were introduced in such a way that the injection sites were aimed at the following coordinates (Konig & Klippel, 1963): A 9·4; L 1·2; H −0·4. After recovery the animals were housed individually for at least one week before use.

Injections were given using a Hamilton 5 μl syringe, the needle of which protruded 0·5 mm below the tip of the guide cannula. Drugs were dissolved in 0·9% NaCl and were injected in a volume of 1 μl or occasionally 2 μl. After injections the rats were transferred individually to activity cages of the light beam type. Motor activity was measured for periods of 24 h after injection. Animals had free access to food and water. Motor activity was expressed as interruptions of the light beam/30 min (Elkhawad & Woodruff, 1975). Rats were not reused for at least 48 h.

Drugs

Adenosine-8-[^{3}H]-3′,5′-cyclic AMP was obtained from the Radiochemical Centre, Amersham, and had a specific activity of 26 Ci m mol^{-1}. The other drugs used were: dopamine HCl; 5,6-diOHATN HCl; 6,7-diOHATN HBr; 2-amino-5,6-dimethoxy-1,2,3,4-tetrahydronaphthalene HCl (5,6-diMeOATN); 2-amino-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalene HCl (6,7-diMeOATN); 2-dimethylamino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene HCl (*NN*-di-Me-5,6-diOHATN); 2-diethylamino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene HI (*NN*-diEt-5,6-di-OHATN); fluphenazine HCl.

RESULTS

Adenylate cyclase

Basal levels of cAMP production, obtained in the absence of dopamine, were 39·6 ± 3·5 (s.e.m., n = 14) p mol/assay tube in striatal homogenates and 49·9 ± 9·4 (n = 12) p mol/tube in homogenates of the nucleus accumbens. In the presence of 100 μM dopamine these values increased to 75·8 ± 2·7 p mol (n = 15) and 80·2 ± 9·4 p mol (n = 12) respectively.

The dose-response relations for dopamine-induced stimulation of cAMP production are illustrated in Fig. 2, from which it can be seen that dopamine was maximally effective at 10 μM in striatal homogenates and at 50 μ M in homogenates of nucleus accumbens. The EC50 values for dopamine (concentration caus-

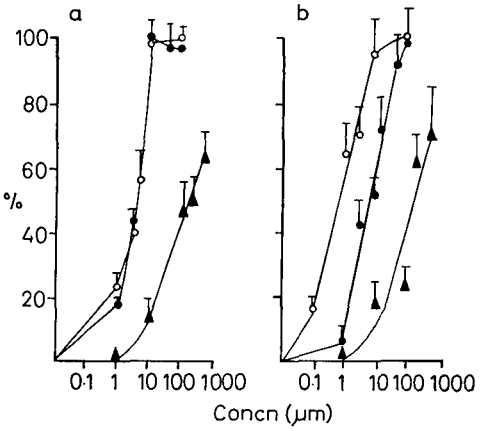


FIG. 2. Dose-response relations for the stimulation of adenylate cyclase by dopamine and some ATN derivatives in homogenates of (a) rat striatum and (b) nucleus accumbens. Results are expressed as a percentage of the maximum response which was taken as that produced by 100 μM dopamine. ● dopamine; ○ 6,7-diOHATN; ▲ 5,6-diOHATN. Each point represents the mean of not less than 8 observations and is given with s.e.m. Ordinate—Increased cAMP formation (% maximum). Abscissa—Agonist concentration (μM).

ing 50% of maximum response) were 3·5 μM for preparations from the striatum and 6·0 μM for those from the nucleus accumbens.

6,7-diOHATN mimicked the action of dopamine in homogenates from both regions of the brain. As can be seen from Fig. 2 and Table 1, it was approximately equipotent with or more potent than dopamine in terms of the EC50 values. The maximum responses produced by dopamine and 6,7-diOHATN were identical.

In contrast 5,6-diOHATN (compound 2, Table 1), which corresponds to the α-rotamer of dopamine, was considerably less active than dopamine on the adenylate cyclase from both nucleus accumbens and striatum (Fig. 2). The EC50 values for 5,6-diOHATN were $2{\cdot}0 \times 10^{-4}$ M in the striatum and $2{\cdot}4 \times 10^{-4}$ M in the nucleus accumbens. 6,7-diOHATN was thus more than 50 times more active than 5,6-diOHATN in the striatum and more than 350 times more active in homogenates of nucleus accumbens (Table 1).

To avoid non-specific interference with the binding assay we have not tested any compounds at concentrations higher than 500 μM; for this reason it was not possible to determine the maximum response produced by 5,6-diOHATN.

The results obtained with the other compounds tested are summarised in Table 1. Both the 5,6- and 6,7-diMeOATN compounds were inactive from 100 to 500 μM. The *NN*- diMe- and *NN*-diEt-5,6-diOHATN compounds (3,4 in Table 1) were both active on the adenylate cyclase, although they were considerably less active than either 6,7-diOHATN or dopamine in homogenates of either striatum or nucleus accumbens.

Evidence that the most active compounds were acting in a similar manner to dopamine was obtained in experiments using fluphenazine (1 μM). This neuroleptic was a potent antagonist of the increase in cAMP formation produced by ATN compounds in striatal homogenates (Table 2). Fluphenazine has previously been shown to be a potent dopamine antagonist on striatal dopamine-sensitive adenylate cyclase (Miller, Horn & Iversen, 1974a).

Table 2. *Effect of fluphenazine (1 μM) on stimulation of cAMP production caused by dopamine and some derivatives of ATN in striatal 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.

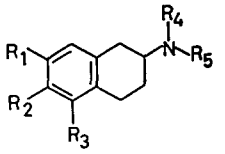
Compound†	Response (% maximum) Absence of fluphenazine	Presence of fluphenazine (1 μM)	% inhibition produced by fluphenazine
Dopamine	100	9·4 ± 4·9 (8)***	89
1 100 μM]	82·5 ± 8·5(4)	24·7 ± 11·3(4)**	70
2 (500 μM)	44·0 ± 4 (4)	1·0 ± 1·0 (6)***	98
4 (500 μM)	48·3 ± 16(4)	10·3 ± 4·2 (6)*	79

*$P<0.05$. **$P<0.01$. ***$P<0.001$. † See Table 1.

Locomotor stimulation

In accordance with previous reports (Elkhawad & Woodruff, 1975), bilateral injections of 6,7-diOHATN into the nucleus accumbens caused a lasting stimulation of motor activity. The duration of action of 6,7-diOHATN (100 nmol each side) was approximately 15 h (Fig. 3). The bilateral injection of 5,6-diOHATN (100 nmol on each side) was similarly effective in producing a powerful stimulation of motor activity (Fig. 3), although its duration of action (about 11 h) was slightly less than that of 6,7-diOHATN. Similar injections of the same dose of *NN*-diMe-5,6-diOHATN caused a briefer (about 7 h) hyperactivity response following a delay of about 3 h. However, *NN*-diEt-5,6-diOHATN, also at the

Table 1. *The potencies of some ATN derivatives in stimulating adenylate cyclase in rat striatum and nucleus accumbens.* Potencies are expressed as EC50 values (concentration producing 50% of maximum response which is taken as that produced by 100 μM dopamine). Each value is from dose response curves and is the mean of 4–6 observations. I = inactive from 100 to 500 μM. — = not tested.



Comp.	R_1	R_2	R_3	R_4	R_5	EC50 (M) Striatum	EC50(M) N. accumbens
1	HO	HO	H	H	H	$3{\cdot}5 \times 10^{-6}$	$6{\cdot}5 \times 10^{-7}$
2	H	HO	HO	H	H	$2{\cdot}0 \times 10^{-4}$	$2{\cdot}4 \times 10^{-4}$
3	H	HO	HO	CH_3	CH_3	$6{\cdot}0 \times 10^{-4}$	$4{\cdot}0 \times 10^{-4}$
4	H	HO	HO	CH_3CH_2	CH_3CH_2	$2{\cdot}6 \times 10^{-4}$	$1{\cdot}1 \times 10^{-4}$
5	CH_3O	CH_3O	H	H	H	I	—
6	H	CH_3O	CH_3O	H	H	I	I

1 = 6,7-diOHATN; 2 = 5,6-diOHATN; 3 = *NN*-diMe- 5,6-diOHATN; 4 = *NN*-diEt-5,6-diOHATN; 5 = 6,7-diMeOATN; 6 = 5,6-diMeATN

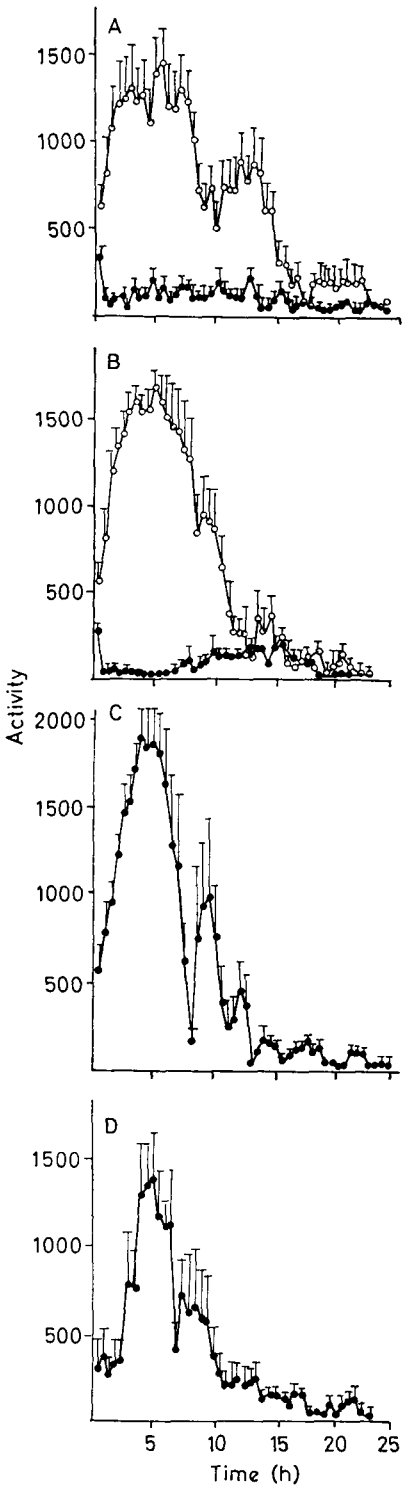


FIG. 3. Stimulation of motor activity produced by the bilateral injection of some ATN derivatives into the nucleus accumbens of conscious rats. Each compound was injected at a dose level of 100 nmol on each side. Each point is the mean of 4–6 observations and is given with the s.e.m.

A ○ 6,7-diOHATN; ● 0·9% NaCl control. B ○ 5,6-diOHATN; ● 5,6-diMeOATN. C *NN*-diEt-5,6-diOHATN. D *NN*-diMe-5,6-diOHATN. Ordinate—Activity (interruptions per 30 min).

same dose level, produced locomotor stimulation lasting for about 8–10 h (Fig. 3) and was thus only slightly less active than 5,6-diOHATN. 5,6-diMeOATN (100 nmol on each side) caused no significant stimulation of motor activity. We have previously shown (Elkhawad & Woodruff, 1975) that 6,7-diMeOATN is similarly devoid of locomotor stimulant action following injections in to the nucleus accumbens.

DISCUSSION

Previous work on dopamine receptors has shown that for maximum dopamine-like activity phenethylamine derivatives must have phenolic hydroxyl groups in the 3 and 4 positions of the benzene ring, together with a terminal nitrogen either unsubstituted or containing a single methyl group (Goldberg, Sonneville & McNay, 1968; Woodruff & Walker, 1969; Woodruff, 1971; Goldberg, 1972; Miller, Horn & others 1974b). The results that we obtained in the present study, using some ATN derivatives, are broadly in line with this earlier work. The only active compounds were those containing two free hydroxyl groups. Furthermore the introduction of two methyl groups onto the nitrogen led to a reduction of activity as measured on the adenylate cyclase system. However, *NN*-diEt-5,6-diOHATN was not less active than the *NN*-diMe- analogue on the adenylate cyclase. It might be that with the introduction of larger groups on to the nitrogen additional binding with regions adjacent to the receptor becomes a possibility. In this context it is noteworthy that ergometrine, a compound only distantly related to dopamine, is nevertheless capable of stimulating mammalian dopamine receptors (Munday, Poat & Woodruff, 1976; Woodruff, McCarthy & Walker, 1976).

It has been suggested that there might be two types of dopamine receptor (Cools & van Rossum, 1976), capable of accepting the two rotameric extremes of dopamine (Cannon, 1975). The results reported in the present paper provide no support for this hypothesis in terms of the dopamine receptors associated with adenylate cyclase in the nucleus accumbens or striatum. The close similarity between the relative potencies obtained in homogenates of the two areas

rather suggests that the receptors are similar in these two regions of the brain. Our results do not, of course, preclude the possible existence of additional dopamine receptors, the effects of which are mediated by a mechanism not involving cAMP.

As mentioned in the introduction, dopamine can exist in different conformations. It is unlikely that dopamine is active in the folded conformation since compounds like salsolinol and norsalsolinol, which both contain the dopamine skeleton fixed in a folded conformation, are weak agonists on dopamine receptors (Woodruff, 1971; Iversen, 1975). In the extended conformation rotation about the phenyl-carbon bond leads to the possibility of 2 rotameric extremes. Our results indicate that, in terms of the dopamine receptor involved in the stimulation of adenylate cyclase, it is the β-rotamer, which corresponds to 6,7-diOHATN (Fig. 1), that is of major importance rather than the α-rotamer, the skeleton of which is in 5,6-diOHATN. Thus whereas 6,7-diOHATN was equipotent with dopamine in stimulating the adenylate cyclase from the striatum and more active than dopamine in homogenates of the nucleus accumbens, 5,6-diOHATN was respectively 57 and 40 times less active than dopamine on the adenylate cyclases from the striatum and nucleus accumbens. It is of interest that Phillipson & Horn (1976) have shown that 6,7-diOHATN is more active than dopamine in stimulating the adenylate cyclase obtained from the rat substantia nigra.

The high potency of 6,7-diOHATN on all dopamine receptors examined would suggest that the β-rotamer of dopamine is of major importance at most peripheral and central dopamine receptors, together with those in invertebrates. In addition to its action on the adenylate cyclase system, 6,7-diOHATN is approximately equipotent with dopamine on dopamine receptors mediating hypotension in guinea-pigs (Woodruff, Elkhawad & Pinder, 1974a), renal vasodilation in dogs (Crumly, Pinder & others, 1976) hyperpolarization of cockroach salivary gland cells (House & Ginsborg, 1976), depression of firing of neurons in the rat caudate nucleus or nucleus accumbens (Woodruff & others, 1976) and in causing excitation or inhibition of specific snail neurons, the receptors of which closely resemble those of the striatal adenylate cyclase system (Munday & others, 1976). It also has powerful behavioural actions consistent with a direct activation of dopamine receptors. Thus following intracerebroventricular injection it causes strong turning towards the innervated side in rats with unilateral lesions of the nigrostriatal tract (Woodruff, Elkhawad & others, 1974a) and it causes a strong and long lasting stimulation of motor activity following injection into the nucleus accumbens of conscious rats (Elkhawad & Woodruff, 1975; Costall, Naylor & others, 1977; present investigation).

Notwithstanding its low activity on adenylate cyclase from either the striatum or the nucleus accumbens, 5,6-diOHATN was nevertheless a potent stimulant of locomotor activity, although its duration of action was less than that of 6,7-diOHATN. Costall & others (1977) have recently reported 5,6-diOHATN to be more potent than 6,7-diOHATN as a locomotor stimulant during the 6 h immediately following bilateral injection into the nucleus accumbens. In the results described in this paper we have shown that, in common with their activities on the adenylate cyclase, *NN*-diMe-5,6-diOHATN was less active than 5,6-diOHATN as a locomotor stimulant, whereas the *NN*-diEt-analogue was more active than *NN*-diMe-5,6-diOHATN. However, it should be emphasized that behavioural measures of this type have their limitations in attempts to elucidate receptor mechanisms. It is possible, for example, that at least a component of the locomotor stimulant action of some of the ATN compounds is produced following conversion to an active metabolite or by a mechanism not involving direct stimulation of dopamine receptors.

Acknowledgements

We are grateful to E. R. Squibb & Sons for a gift of fluphenazine and to the M.R.C. for a grant to K. J. Watling.

REFERENCES

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

BROWN, J. H. & MAKMAN, M. H. (1972). *Proc. Natn Acad. Sci. U.S.A.*, **69**, 539–543.

BUSTARD, T. M. & EGAN, R. S. (1971). *Tetrahedron*, **27**, 4457–4469.

CANNON, J. G. (1975). *Advances in Neurology*, Vol. 9, pp. 177–183. Editors: Calne, D. B., Chase, T. N. & Barbeau, A. New York: Raven Press.

CLEMENT-CORMIER, Y. C., KEBABIAN, J. W. PETZOLD, G. L. & GREENGARD, P. (1974). *Proc. Natn Acad. Sci. U.S.A.*, **71**, 1113–1117.

COOLS, A. R., & VAN ROSSUM, J. M. (1976). *Psychopharmacologia*, **45**, 243–254.
COSTALL, B., NAYLOR, R. J., CANNON, J. G. & LEE, T. (1977). *Eur. J. Pharmac.*, **41**, 307–319.
CRUMLY, H. J., PINDER, R. M., HINSHAW, W. B. & GOLDBERG, L. I. (1976). *Nature, London.*, **259**, 584–587.
ELKHAWAD, A. O. & WOODRUFF, G. N. (1975). *Br. J. Pharmac.*, **54**, 107–114.
GILMAN, A. G. (1970). *Proc. Natn Acad. Sci. U.S.A.*, **67**, 305–312.
GOLDBERG, L. I. (1972). *Pharmac. Rev.*, **24**, 1–29.
GOLDBERG, L. I., SONNEVILLE, P. F. & MCNAY, J. L. (1968). *J. Pharmac. exp. Ther.*, **163**, 188–197.
HORN, A. S., CUELLO, A. C. & MILLER, R. J. (1974). *J. Neurochem.*, **22**, 265–270.
HOUSE, C. R. & GINSBORG, B. L. (1976). *Nature, Lond.*, **261**, 332–333.
IVERSEN, L. L. (1975). *Science*, **188**, 1084–1089.
KEBABIAN, J. W., PETZOLD, G. L. & GREENGARD, P. (1972). *Proc. Natn Acad. Sci. U.S.A.*, **69**, 2145–2149.
KONIG, J. F. R. & KLIPPEL, R. A. (1963). *The Rat Brain*, Maryland: Williams & Wilkins.
MCDERMED, J. D., MCKENZIE, G. M. & FREEMAN, H. S. (1976). *J. medl Chem.*, **19**, 547–549.
MCDERMED, J. D., MCKENZIE, G. M. & PHILLIPS, A. P. (1975). *Ibid.*, **18**, 362–367.
MILLER, R. J., HORN, A. S. & IVERSEN, L. L. (1974a). *Molec. Pharmac.*, **10**, 759–766.
MILLER, R. J., HORN, A. S., IVERSEN, L. L. & PINDER, R. M. (1974b). *Nature, Lond.*, **250**, 238–241.
MUNDAY, K. A., POAT, J. A. & WOODRUFF, G. N. (1976). *Br. J. Pharmac.*, **57**, 452–453P.
PHILLIPSON, O. T. & HORN, A. S. (1976). *Nature, Lond.*, **261**, 418–420.
REKKER, R. F., ENGEL, D. J. C. & NYS, G. G. (1972). *J. Pharm. Pharmac.*, **24**, 589–591.
WOODRUFF, G. N. (1971). *Comp. gen. Pharmac.*, **2**, 439–455.
WOODRUFF, G. N., ELKHAWAD, A. O., CROSSMAN, A. R. & WALKER, R. J. (1974a). *J. Pharm. Pharmac.*, **26**, 740–741.
WOODRUFF, G. N. ELKHAWAD, A. O. & PINDER, R. M. (1974b). *Eur. J. Pharmac.*, **25**, 80–86.
WOODRUFF, G. N., MCCARTHY, P. S. & WALKER, R. J. (1976). *Brain Res.*, **115**, 233–242.
WOODRUFF, G. N. & WALKER, R. J. (1969). *Int. J. Neuropharmac.*, **8**, 279–289.