Biochemical Pharmacology, Vol. 29, pp. 2681–2683.

0006-2952/80/1001-2681 $02.00/0

SHORT COMMUNICATIONS

Interaction of oxypertine with rat brain monoamine receptors

(*Received* 18 *April* 1980; *accepted* 31 *May* 1980)

The most specific biochemical effect of oxypertine may be its depletory action on presynaptic storage of monoamines [1–4]. Thus, Hassler *et al.* [4] reported that at a low dose oxypertine depleted selectively norepinephrine in rat brain, while at a high dose it depleted dopamine and serotonin as well as norepinephrine. The depletory action of oxypertine on catecholamines have been explained by a blockade of the uptake–storage mechanism of amine granules [5]. On the other hand, it has been suggested that the immediate onset of behavioural effects induced by oxypertine may be due to an initial receptor blockade [5]. There is, however, little information concerning the biochemical effect of oxypertine on monoamine receptors. We describe here the effects of oxypertine on dopamine-sensitive adenylate cyclase [EC 4.6.1.1] activity and [^{3}H]spiroperidol binding in rat striatum and frontal cortex and on [^{3}H]WB-4101 binding in rat cerebral cortex.

Male, Wistar King rats (250–300 g) were decapitated and their brains were rapidly removed. The brains were cut into serial frontal slices of 300 μm thickness in a cryostat at −12°. Using a microknife, the nucleus caudatus putamen and medial field of prefrontal cortex [6] were dissected from the sections between A 7100 and 9650 μm and between A 10,300 and 11,500 μm planes, respectively, according to the atlas of König and Klippel [7]. Dopamine-sensitive adenylate cyclase was assayed as previously described [8]. The tissue homogenate were incubated in the assay mixture (final vol., 25 μl) containing 25 mM Tris-maleate buffer (pH 7.2 at 30°), 0.5 mM ATP, 1 mM $MgSO_4$, 0.6 mM EGTA, 40 U/ml creatine kinase, 20 mM creatine phosphate, 10 mM theophylline, 0.5–1 μCi [α-^{32}P]ATP, 0.005 μCi [^{3}H]cyclic AMP and test substances, at 30° for 5 min. [α-^{32}P]Cyclic AMP was isolated by the method of Salomon *et al.* [9]. Protein was measured according to Lowry *et al.* [10] using bovine serum albumin as standard. For [^{3}H]spiroperidol binding assay, the brains were cut into serial frontal slices of 500 μm thickness with a McIlwain tissue chopper. The anterior frontal lobe and nucleus caudatus putamen were dissected from the slices between A 12,000 and 10,000 μm and between A 9600 and 7000 μm, respectively. Special care was taken to prevent the preparations of frontal cortex tissue from contamination by the nucleus accumbens and striatum. The tissue preparation and binding assay were carried out essentially as described by Titeler *et al.* [11], except that EGTA was used instead of EDTA. The tissue suspension was incubated in 0.6 ml 10 mM Tris–HCl buffer (pH 7.7 at 25°), containing 1 mM EGTA, 1 mM ascorbic acid, 10 μM pargyline, 0.4 nM [^{3}H]spiroperidol and test drugs, at 37° for 15 min. The samples were filtered through Whatman GF/B filters and rinsed with 2 × 5 ml of ice-cold buffer. Specific binding of [^{3}H]spiroperidol was defined as that displaced by 1 μM (+)-butaclamol. The binding of [^{3}H]WB-4101 to rat cerebral cortex was measured by the method of U'Prichard *et al.* [12]. The tissue suspension prepared from whole cerebral cortex was incubated in 50 mM Tris–HCl buffer (pH 7.7 at 25°) containing 0.26 nM [^{3}H]WB-4101 and test drugs at 25° for 15 min. Specific binding of [^{3}H]WB-4101 was defined as that displaced by 100 μM (-)-norepinephrine. Dopamine, (-)-norepinephrine, cyclic AMP, ATP, creatine kinase and creatine phosphate were purchased from Sigma Chem. Co., St. Louis, MO, U.S.A. [α-^{32}P]ATP (20.8 Ci/mmole), [^{3}H]cyclic AMP (37.7 Ci/mmole), [^{3}H]spiroperidol (23.6 Ci/mmole) and [^{3}H]WB-4101 (24.4 Ci/mmole) were from New England Nuclear, Boston, MA, U.S.A. Chlorpromazine (Yoshitomi, Osaka, Japan), haloperidol (Dainihon, Osaka, Japan) and oxypertine (Daiichi, Tokyo, Japan) were obtained as gifts. (+)-Butaclamol was generously supplied by Dr. K. Saito, Osaka University, Japan.

Oxypertine was found to inhibit the dopamine-sensitive adenylate cyclase in the striatum and frontal cortex (Fig. 1C). There was no marked difference between the effects of oxypertine on the adenylate cyclase in the striatum and frontal cortex. However, oxypertine was 20- and 10-fold weaker than haloperidol (Fig. 1A) and chlorpromazine (Fig. 1B), respectively. These results suggest that oxyper-

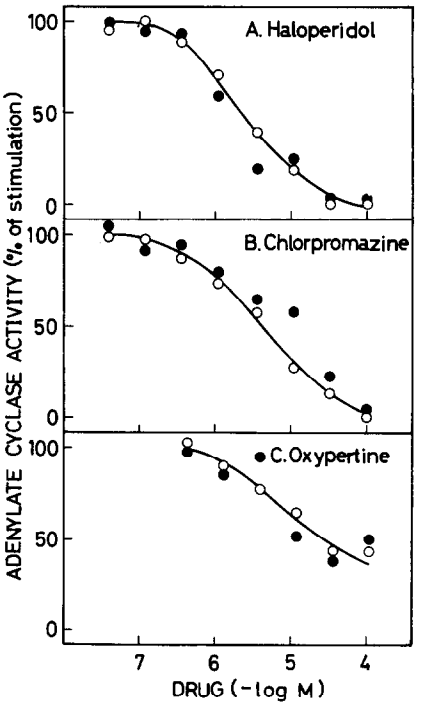

Fig. 1. Effect of haloperidol (A), chlorpromazine (B) and oxypertine (C) on the dopamine-sensitive adenylate cyclase in rat striatum (○) and frontal cortex (●). The striatal (76 μg protein) or frontal cortex (18 μg protein) homogenates were incubated in the presence of each drug. The results are expresssed as per cent of stimulation of the adenylate cyclase by 100 μM dopamine. The activities in the absence and in the presence of 100 μM dopamine were 116 and 270 pmoles·min^{-1}·mg protein^{-1} for striatum and 135 and 178 pmoles·min^{-1}·mg protein^{-1} for frontal cortex. Each point represents the mean of duplicate assays. The values of inhibition constant were: haloperidol, 0.11 μM; chlorpromazine, 0.23 μM; oxypertine, 2.2 μM. These values were calculated according to Cheng and Prusoff [13]. The concentration of dopamine required for half-maximal stimulation was 4.8 μM in both striatum and frontal cortex.

tine is a weak antagonist for the dopamine receptors coupled to the adenylate cyclase in both striatum and frontal cortex.

The specific binding of [^{3}H]spiroperidol (0.02–0.8 nM) to striatal preparations was saturable with a dissociation constant of 0.22 nM and a maximal number of binding sites of 17 pmoles/g tissue, whereas the [^{3}H]spiroperidol binding in the frontal cortex showed a non-linear Scatchard plot (data not shown). Oxypertine was less potent than haloperidol and slightly less potent than chlorpromazine in displacing [^{3}H]spiroperidol binding from striatal membranes (Fig. 2A and Table 1). In the frontal cortex, it was not simple to determine the potency of oxypertine relative to haloperidol because these drugs inhibited [^{3}H]spiroperidol binding in a biphasic manner (Fig. 2B). The inhibition of specific binding by haloperidol revealed two distinct components, a high affinity component (IC_{50}= 4.5 nM) constituting 35 per cent of total specific binding and a low affinity component (IC_{50}=150 nM). The inhibition curves of both chlorpromazine and oxypertine also revealed a less pronounced inflection at which 50–60 per cent of total specific binding was inhibited. In these cases, the IC_{50} values were calculated without distinction between these two components (Table 1). Oxypertine was 2-fold more potent than chlorpromazine in displacing [^{3}H]spiroperidol binding from frontal cortex membranes. Moreover, the IC_{50} value of oxypertine was eight times lower in the frontal cortex than in the striatum.

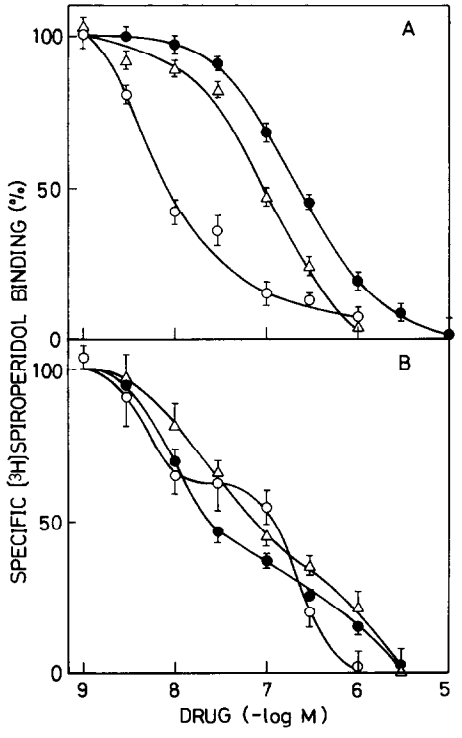

Fig. 2. Displacement of specific [^{3}H]spiroperidol binding in rat striatal and frontal cortex membranes. (A) Rat striatal (2.0 mg tissue) or (B) frontal cortex (3.5 mg tissue) membranes were incubated with 0.4 nM [^{3}H]spiroperidol in the presence of various concentrations of haloperidol (○), chlorpromazine (△) or oxypertine (●). The results are expressed as per cent of specific [^{3}H]spiroperidol binding defined as that displaced by 1 μM (+)-butaclamol. The specific binding constituted 70 per cent of total binding (1060 cpm) in the striatum, and 53 per cent of total binding (930 cpm) in the frontal cortex. Each point represents the mean ± S.E.M. of triplicate determinations.

Recent studies have suggested that [^{3}H]spiroperidol may bind to dopamine receptors in the striatum and serotonin receptors in the frontal cortex [14–16]. These studies also indicated that [^{3}H]spiroperidol labelled a heterogenous population of sites in the frontal cortex. In the present studies, displacement experiments showed two distinct components in the frontal cortex. Since the high affinity component of haloperidol displacement for [^{3}H]spiroperidol binding in the frontal cortex had an IC_{50} value similar to that observed in the striatum, the high affinity component may associate with dopamine receptors as described by Marchais *et al.* [17]. Haloperidol and oxypertine, at low concentrations (1–10 nM), exhibited a similar inhibition curve for [^{3}H]spiroperidol binding to frontal cortex membranes (Fig. 2B). However, these two drugs seemed to displace [^{3}H]spiroperidol from the different types of binding sites in the frontal cortex because haloperidol was much more potent than oxypertine in inhibiting [^{3}H]spiroperidol binding in the striatum. Therefore, oxypertine may displace [^{3}H]spiroperidol preferentially from serotonin receptors in the frontal cortex. These results suggest that oxypertine is considerably potent in blocking serotonin receptors but weak in blocking dopamine receptors.

Oxypertine was 5-fold less potent than chlorpromazine and as potent as haloperidol in displacing [^{3}H]WB-4101 binding from cerebral cortex membranes (Fig. 3 and Table 1). [^{3}H]WB-4101 has been found to label alpha-adrenergic receptors in brain membranes [12, 18]. The displacement studies of specific [^{3}H]WB-4101 binding in rat brain have shown that both chlorpromazine and haloperidol are as potent as classical alpha-adrenergic antagonists [12, 18]. In this respect, oxypertine also appeared to be a potent blocker of alpha-adrenergic receptors in the cerebral cortex.

In clinical studies, Praag and Korf [19] selected oxypertine as a selective blocker of noradrenergic transmission and suggested that it prevented exacerbation of delusion and hallucinations and increased the level of motivation. In the present studies, the ratios, IC_{50} for [^{3}H]spiroperidol in the frontal cortex/IC_{50} for [^{3}H]spiroperidol in the striatum, and IC_{50} for [^{3}H]WB-4101 in the cerebral cortex/IC_{50} for [^{3}H]spiroperidol in the striatum were: oxypertine, 0.12 and 0.26; chlorpromazine, 0.52 and 0.09. If a same extent

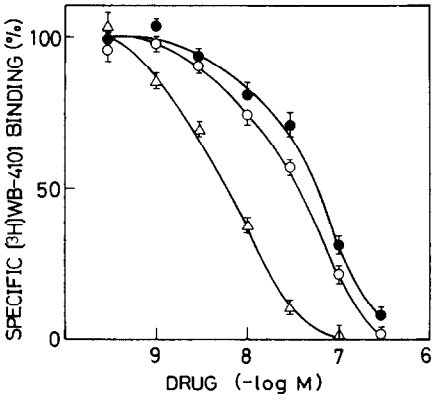

Fig. 3. Displacement of specific [^{3}H]WB-4101 binding in rat cerebral cortex. The cerebral cortex membranes (13 mg tissue) were incubated with 0.26 nM [^{3}H]WB-4101 in the presence of various concentrations of haloperidol (○), chlorpromazine (△) and oxypertine (●). The results are expressed as per cent of specific [^{3}H]WB-4101 binding defined as that displaced by 100 μM (-)-norepinephrine. The specific binding constituted 65 per cent of total binding (970 cpm). Each point represents the mean ± S.E.M. of triplicate determinations.

Table 1. Inhibition of [^{3}H]spiroperidol and [^{3}H]WB-4101 binding in rat striatal, frontal cortex and cerebral cortex membranes*

Drug	[^{3}H]Spiroperidol		[^{3}H]WB-4101
	Striatum	Frontal cortex	Cerebral cortex
		IC_{50} (nM)	
Oxypertine	210 ± 30 (4)	25 ± 7 (4)	54 ± 5 (3)
Chlorpromazine	120 ± 30 (3)	53 ± 21 (2)	11 ± 3 (3)
Haloperidol	15 ± 5 (4)	4.5 ± 1.4 (5)†, 150 ± 34 (5)	56 ± 13 (3)

* Inhibition studies of specific [^{3}H]ligand binding were performed as described in Figs. 2 and 3. The drug concentrations required for 50 per cent inhibition (IC_{50}) were determined by log probit analysis. The values are the means ± S.E.M. for the number of separate experiments indicated in parentheses.

† The high affinity component constituting 35 ± 4 (5) per cent of total specific binding.

of dopamine receptor blockade is attained at clinical doses of these two drugs, oxypertine may be about four times more potent than chlorpromazine in antagonizing serotonin receptors and three times less potent than chlorpromazine in antagonizing alpha-adrenergic receptors. These results may be useful to understand a detailed profile of therapeutic actions and side effects of oxypertine.

In conclusion, our results suggest that comparing with chlorpromazine, oxypertine is a weak blocker of dopamine and alpha-adrenergic receptors but a relatively potent blocker of serotonin receptors.

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