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Centpropazine affinity to cortical noradrenergic receptors and effect on their responsiveness in the rat*

M. DIKSHIT†, E. CHALECKA-FRANASZEK, I. NALEPA, *Department of Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, Smetna 12, 31-343 Krakow, Poland, †Division of Pharmacology, Central Drug Research Institute, Chattar Manzil, Lucknow 26601, India*

Abstract—We have studied the in-vitro effect of centpropazine on cerebral cortical noradrenergic receptors measured as the accumulation of second messengers, cyclic AMP and inositol phosphate, stimulated by noradrenaline, and the binding to α_1- and β-adrenoceptors. Centpropazine inhibited inositol phosphate, but not the cyclic AMP accumulation in the cerebral cortical slices of the rat. It moderately antagonized the specific binding of [^{3}H]prazosin, but did not affect the specific binding of the β-adrenoceptor ligand, [^{3}H]CGP 12177, to cerebral cortical membranes.

Centpropazine, 1-(*p*-propionylphenoxy)-3-(N^4-phenylpiperazynyl)-propan-2-ol, has been described as a putative antidepressant with a pharmacological profile resembling that of amitriptyline and imipramine (Rastogi et al 1972). In male volunteers centpropazine given in single oral doses of 10–160 mg was well tolerated and did not affect vital parameters (Gupta et al 1989). Phase II clinical studies with depressed patients gave promising results (Srivastava et al 1991).

In animal experiments, centpropazine counteracted reserpine-induced depression, potentiated amphetamine-induced activity in mice and rats, and augmented amphetamine toxicity in mice. In higher doses the drug displayed anti-amphetamine properties (Rastogi et al 1972).

Given chronically to rats, centpropazine, like imipramine, depressed the density of 5-HT_1 and 5-HT_2 receptors in the cortex, but differed from imipramine in producing no β-down-regulation (Hussain et al 1988). As β-down-regulation is regarded as one of the important characteristics of antidepressant drugs (Vetulani 1984), further studies on effects of centpropazine on adrenergic receptor responses were warranted.

No biochemical data concerning the action of centpropazine on the second messenger systems related to adrenergic receptors

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Correspondence: I. Nalepa, Department of Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, Smetna 12, 31-343 Krakow, Poland.

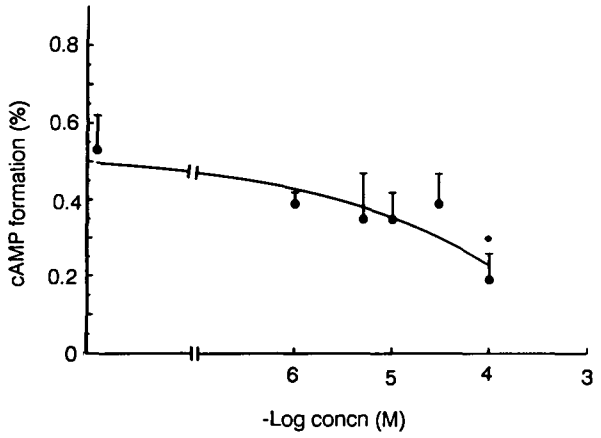

FIG. 1. The effect of centpropazine on noradrenaline-induced cyclic cAMP accumulation in rat cortical slices in-vitro. The data (%[^{3}H]adenine conversion) represent net stimulation (increase over the basal level, which was 0·095±0·018%, n=6). The points are means of 4–6 separate experiments carried out in duplicate. The bars represent s.e.m. Analysis of variance demonstrated no significant differences between means (F=2·18, df 5/24). Ex post LSD test demonstrated a significant inhibitory effect ($P<0·01$) of 10^{-4} M centpropazine.

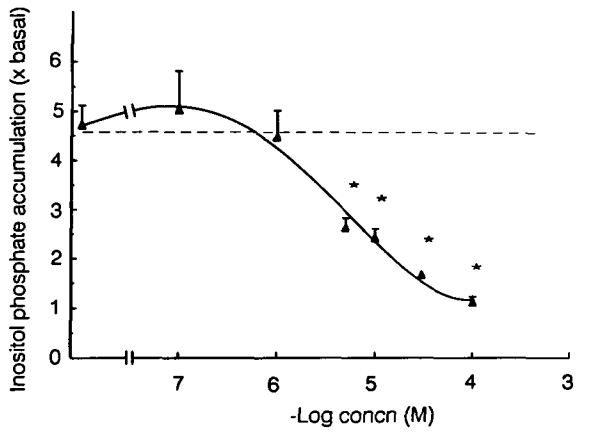

FIG. 2. The effect of centpropazine on noradrenaline-induced inositol phosphate accumulation in the rat cortical slices in-vitro. The data (folds) are the multiples of the basal level (d min^{-1} stimulated/d min^{-1} basal); the basal level was 1115±104 d min^{-1} (n=6). The points are means of six separate experiments carried out in triplicate. The bars represent s.e.m. Analysis of variance showed significant differences between means (F=17·1, df 6/30, $P<0·01$) (difference between the response in the presence of noradrenaline alone or together with centpropazine, LSD test). IC50 for centpropazine calculated from the sigmoid curve by nonlinear regression analysis was 4·8 μM.

are available. Therefore, in the present experiment we investigated the affinity of centpropazine to α_1- and β-adrenoceptors and on noradrenergic receptor responsiveness to noradrenaline as reflected by generation of second messengers, cyclic AMP (cAMP) and inositol monophosphate.

The adrenergic receptors are linked to two different second messenger systems. The stimulation of the β-receptor is followed by cAMP increase (Daly et al 1981), while the α_1-receptor causes phosphatidylinositol breakdown from which diacylglycerol and inositol trisphosphate are produced. The latter is finally degraded to inositol monophosphate (Berridge & Irvine 1989). While the response of the cAMP system evoked through β-adrenoceptors is modulated by α-adrenoceptors (Daly et al 1980), the stimulation of β-adrenoceptors does not affect inositol monophosphate responses from α-adrenoceptors (Nalepa & Vetulani unpublished data). Studies on the adrenergic second messenger systems usually employ noradrenaline, the natural neurotransmitter in the central nervous system (CNS). Although noradrenaline affects both α- and β-adrenoceptors, it has the advantage of producing much more potent responses than synthetic, more specific agonists.

Materials and methods

The experiment was carried out on a cortical membrane preparation (receptor binding studies) or cortical cerebral slices (second messenger assay) obtained from male Wistar rats, 200–220 g, kept in standard animal room conditions. The rats were killed by guillotine, their brains were excised, and cortices were dissected.

The membrane preparation (P_2 fraction) and tissue slices (350 μm prisms) were prepared as described previously (Nalepa & Vetulani 1991).

α_1-Adrenergic binding sites were characterized with [^{3}H]prazosin (0·07–3·5 nM) using 10 μM phentolamine as a displacer. β-Adrenoceptor sites were labelled with [^{3}H]CGP 12177 (0·06–2·33 nM), and propranolol (10 μM) was used to define nonspecific binding in this assay. The incubations were carried out in a standard manner (Nalepa & Vetulani 1991) and the B_{max} and K_D values were calculated from the binding isotherm. The K_D values were used for calculation of K_i values in the subsequent experiments.

To characterize the interaction of centpropazine with α_1-adrenergic receptors, nine concentrations of the drug (10^{-11}–10^{-4} M) were incubated in the presence of 0·163 nM [^{3}H]prazosin. In a parallel experiment, nine concentrations of phentolamine were tested. To study centpropazine interaction with β-adrenergic receptors, nine concentrations of centpropazine and of propranolol (10^{-11}–10^{-4} M) were incubated in the presence of 0·243 nM [^{3}H]CGP 12177. The results were calculated from a sigmoid curve using a GraphPad program. K_i was calculated from the formula

$$K_i = EC50/(1 + L/K_D)$$

where L is the concentration of radioligand and K_D is its dissociation constant.

For second messenger assay the slices were suspended in glucose-containing modified Krebs–Henseleit medium (in mM NaCl 118, KCl 5, $CaCl_2$ 1·3, $MgSO_4$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25, glucose 11·7, pH 7·4) gassed with 95% O_2–5% CO_2 at 37°C, which was used throughout all incubations.

cAMP was assayed by the method of Shimizu et al (1969) and inositol monophosphate by the method of Brown et al (1984) with modifications (Nalepa & Vetulani 1991).

Centpropazine was obtained from Central Drug Research Institute, Lucknow, India, and propranolol from Polfa, Kraków. Noradrenaline and unlabelled cAMP were obtained from Sigma Chemical Company (St Louis, MO, USA), phentolamine was obtained from Ciba-Geigy. [^{3}H]Adenine (sp. act. 20·7 Ci $mmol^{-1}$), [^{14}C]cAMP (sp. act. 261 mCi $mmol^{-1}$), [^{3}H]myo-inositol (sp. act. 20 Ci $mmol^{-1}$) and [^{3}H]prazosin (sp. act. 22 Ci $mmol^{-1}$) were purchased from New England Nuclear, [^{3}H]CGP 12177 (sp. act. 73·9 Ci $mmol^{-1}$) was from the Radiochemical Centre, Amersham.

Results and discussion

Receptor binding studies demonstrated specific binding of [^{3}H]prazosin with a K_D of 0·09 nM and B_{max} of 75·5 fmol (mg protein)$^{-1}$. The specific binding was also determined for [^{3}H]CGP 12177 (K_D = 0·36 nM, B_{max} = 37·6 fmol (mg protein)$^{-1}$). Our data were of the same order of magnitude as those reported by others (Greengrass & Bremner 1979; Riva & Creese 1989). Centpropazine interfered with [^{3}H]prazosin binding with a K_i of 3036 nM, while the K_i for the specific α_1-adrenergic receptor

antagonist phentolamine was 19·3 nM. Centpropazine, even at a concentration of 100 μM, did not inhibit the [^{3}H]CGP 12177 binding by more than 43%, while the specific β-adrenergic receptor antagonist, propranolol, inhibited the binding with a K_i value of 30·2 nM.

Noradrenaline stimulates the cAMP and inositol monophosphate formation in dose-dependent manner (Nalepa et al 1988; Chalecka-Franaszek et al 1990). In the following experiments, a supramaximal concentration of 100 μM noradrenaline was used. Centpropazine did not affect the basal level of cAMP, and in concentrations up to 30 μM did not produce a clear inhibitory effect on cAMP accumulation induced by noradrenaline. A trend suggesting a moderate inhibitory action of the drug could be recognized and at the highest concentration used (100 μM) centpropazine significantly inhibited the response to noradrenaline (Fig. 1).

Centpropazine did not affect the basal level of inositol monophosphate, but the response of this second messenger to noradrenaline was concentration-dependently inhibited by the drug (Fig. 2). The effects of lower concentrations of centpropazine suggested a biphasic mode of action, but the enhancement of the noradrenaline-stimulated inositol monophosphate accumulation by 0·1 μM centpropazine did not reach the level of statistical significance ($0·1 < P < 0·05$). The biphasic response may reflect the possible opposite effects of centpropazine resulting from simultaneous blocking of noradrenaline uptake (thus increasing neurotransmitter concentration in the synaptic cleft) and blocking of postsynaptic α_1-adrenoceptors.

The observed effects of centpropazine on the adrenergic receptor system in the rat cerebral cortex demonstrate the similarities between the action profile of that compound and of imipramine, which was investigated previously (Nalepa & Vetulani 1991). Thus, neither compound, even at concentrations of 100 μM showed a significant affinity to β-adrenoceptors, and in concentrations up to 30 μM did not inhibit noradrenaline-induced cAMP accumulation. At 100 μM, centpropazine inhibits significantly the response, while the effect of imipramine did not reach the level of statistical significance, but the pattern of response was similar. Both compounds showed a similar pattern of action on inositol monophosphate formation, the lower concentration showing a stimulatory effect while higher effectively and dose-dependently inhibited the response, although only in the case of imipramine, in which the biphasic effect was more accentuated, did the initial increase in inositol monophosphate accumulation reach statistical significance.

The only in-vitro effect of centpropazine which differed from that of imipramine was the displacement of the α_1-adrenoceptor ligand, [^{3}H]prazosin. While imipramine has a considerable affinity to α_1-adrenoceptors (K_i of 38·1 nM) (Nalepa & Vetulani 1991), the affinity of centpropazine was more than two orders of magnitude lower.

A question arises as to whether the present in-vitro data are relevant to the clinical situation, and in particular whether concentrations of centpropazine occurring in the human brain during drug therapy are comparable with those used in the present experiment. However, no data on centpropazine blood or brain levels after clinical dosage are available. In the clinic, centpropazine is given in doses comparable with those of imipramine. The blood concentrations of imipramine and desipramine at steady-state are 100–300 ng mL^{-1} i.e. approximately 0·25–0·8 μmol L^{-1} (Giardina et al 1979). The brain concentrations in the rat are reported to be ca. 5 (Daniel et al 1981) or 6–10 (Nagy & Johansson 1975) times higher than in blood. Therefore, it could be assumed that the concentrations of imipramine (+desipramine) in the brain of treated patients are approximately 3–8 μM. If one assumes that the pharmacokinetics of centpropazine are similar, the drug concentrations in the brain would be in the lower part of our concentration-response curves. Therefore, the observed effects may be meaningful for the clinical situation.

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