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Assessment of the Adrenergic Effects of Orphenadrine in Rat Vas Deferens

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Abstract

The peripheral adrenergic effects of orphenadrine, an antiparkinsonian drug, have been evaluated in the rat vas deferens to investigate whether these properties are the same as those of other phencyclidine ligands.

In the low micromolar range, orphenadrine enhanced electrically-evoked and exogenous noradrenaline contractile responses in the epididymal portion of rat vas deferens. It also induced spontaneous activity that was inhibited by prazosin (1 μM) but not by atropine (20 nM). It inhibited accumulation of [^{3}H]noradrenaline in rat vas deferens (IC50 = 14·2 ± 2·3 μM). Orphenadrine competitively inhibited [^{3}H]nisoxetine binding in rat vas deferens membranes (K_i = 1·05 ± 0·20 μM).

It can be concluded that orphenadrine, at low micromolar concentrations, interacts with the noradrenaline reuptake system inhibiting its functionality and thus potentiating the effect of noradrenaline.

Orphenadrine (*N*,*N*-dimethyl-2(*o*-methyl-α-phenylbenzyloxy) ethylamine) has been used in the treatment of Parkinson's disease and acute phases of drug-induced parkinsonism (Midham et al 1972; Bassi et al 1986). Its therapeutic efficacy has been attributed to its anticholinergic properties (Danysz et al 1994). Moreover, Kornhuber et al (1995) demonstrated, in cultured superior colliculus neurons, that orphenadrine is active at the phencyclidine (PCP) binding site of the *N*-methyl-D-aspartate (NMDA) receptor at therapeutic concentrations. This raises the possibility that some of the antiparkinsonian effects of orphenadrine could also be mediated by blockade of hyperactive glutamate pathways in the basal ganglia (Klockgether & Turski 1993; Danysz et al 1995).

In the central nervous system (CNS), glutamate could act as a neuromodulator of catecholamine neurons, thus involving a variety of functions in which catecholamines participate (Rudolph et al 1983; Olney 1986). Lara & Bastos-Ramos (1988) described the interaction of glutamate with noradrenergic neurons in rat vas deferens and suggested it was involved in presynaptic control of the noradrenaline release or had a direct effect on the smooth muscle cells.

The non-competitive antagonists of the NMDA receptor such as MK-801 (5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine) and PCP possess anticonvulsant and neuroprotective properties and bind to a specific site in the receptor's channel, referred to as the PCP site. PCP inhibits catecholamine uptake in rat brain synaptosomes and in adrenomedullary chromaffin cells (Rogers & Lemaire 1991). PCP can also inhibit [^{3}H]desmethylimipramine binding to rat brain preparations (Massamiri & Duckles 1991; Rogers & Lemaire 1992), which indicates direct interaction with the noradrenaline carrier.

Recently, we have reported that MK-801, a PCP ligand, potentiates the response to noradrenaline in the rat vas deferens by inhibiting the noradrenaline uptake (Pubill et al 1996). In addition, a direct interaction of the NMDA receptor ligands with the noradrenaline uptake system in the rat vas deferens has been demonstrated (Pubill et al 1998).

The aim of this study was to assess whether orphenadrine, as a non-competitive NMDA receptor antagonist (Kornhuber et al 1995), has the same peripheral adrenergic properties as the other PCP ligands.

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Materials and Methods

Male Sprague–Dawley rats (200–250 g, Harlan, BCN, Spain), were used. After decapitation, vasa deferentia were carefully removed and placed in Krebs–Henseleit solution (composition in mM: NaCl 119, KCl 4·6, $CaCl_2$ 2·5, $NaHCO_3$ 25, KH_2PO_4 1·2, $MgSO_4 7H_2O$ 1·2 and glucose 11·1) previously gassed with 95% O_2/5% CO_2 for the isolated organ and uptake experiments, and in Tris–HCl 50 mM buffer for binding experiments. Tissues were cleaned of connective tissue and adhering fat, and bisected into the epididymal and the prostatic end.

Isolated organ experiments

For the organ bath experiments, the epididymal portions were mounted in 20-mL tissue chambers and attached to isometric strain-gauge transducers in order to record changes in tension. The organs were suspended under a resting tension of 400 mg at 37°C and allowed to equilibrate for 60 min. Tension was amplified, recorded and sent to a computerised system running PROTO5 software (Letica Software Dept, BCN, Spain) via a Letica LE 60-100 interface.

Electrically-induced responses

For these experiments vasa deferentia were mounted between platinum field electrodes. After the equilibration period, contractions were elicited, for 5 min, by single pulses (0·05 Hz, 0·5 ms, Grass S88 stimulator) at supramaximal voltage. Orphenadrine was then added at the appropriate concentration and the response was recorded for a further 5 min. Potentiation was measured as the percentage increase in twitches in relation to the response recorded immediately before addition of the drug. A concentration(log)–effect (% potentiation) graph was constructed and the ED100 value was calculated by least-square linear regression (concentration of orphenadrine leading to 100% potentiation). The concentration range tested for orphenadrine was 0·3–30 μM.

Noradrenaline-induced contractile responses

Vasa deferentia contractions in response to exogenous noradrenaline (0·2 μM) were measured in the absence (control curves) and in the presence of different concentrations of orphenadrine (0·5–10 μM). The contractions elicited by higher concentrations were too vigorous to allow accurate measurement. Drugs (described below) were added to the bath 10 min before the addition of noradrenaline and the ED100 was calculated as described above for electrically-induced responses.

Data analysis

Results are expressed as mean ± s.e.m. from n experiments. A P value of $< 0{\cdot}05$ was considered as significant. Differences between values were estimated using analysis of variance.

[^{3}H]Noradrenaline uptake

The effect of orphenadrine on noradrenaline uptake, was measured as described elsewhere (Pubill et al 1996). Briefly, vasa deferentia were obtained as described above, cleaned and bisected transversally. Slices of the epididymal portions were gently blotted, weighed and placed in glass vials containing 10 mL of Krebs–Henseleit solution (with ascorbic acid 0·1 mM) gassed with 95% O_2/5% CO_2. The tissues were preincubated for 25 min at 37°C (or at 0°C and 3 mM noradrenaline when the non-specific incorporation was measured). After pre-incubation, the slices were transferred to tubes containing 0·95 mL of gassed Krebs–Henseleit solution at 37°C. For the inhibition studies, the tissues were kept for 10 min in 0·95 mL of medium containing the drugs at various concentrations. The incubation was started by the addition of 50 mL of a solution of [^{3}H]noradrenaline to make a final concentration of 0·1 μM.

Incubation was continued for a further 3 min, after which the tissues were rapidly removed, rinsed in about 200 mL of ice-cold Krebs–Henseleit solution and transferred to scintillation vials containing 0·5 mL Solvable (DuPont, Germany). After 3-h solubilization at 50°C, the vials were allowed to reach room temperature and 10 mL of Ecolite (+) scintillation fluid (ICN Biomedicals) was added. Tritium content was estimated by liquid scintillation counting using a Beckman LS 1800 counter. The efficiency of counting was 40%. The total uptake of noradrenaline was determined by subtracting the uptake at 0°C from that obtained at 37°C. The uptake of noradrenaline in the presence of various concentrations of orphenadrine was expressed as a percentage of the uptake in the absence of the drug.

The IC50 value (the concentration of orphenadrine that inhibits 50% of maximal [^{3}H]noradrenaline accumulation) was calculated by non-linear regression analysis using GraphPAD InPlot software (GraphPAD Software, San Diego, CA), setting the maximum inhibition value as variable.

[^{3}H]Nisoxetine binding assays

The interaction of orphenadrine with the noradrenaline carrier was evaluated by competition binding assays using [^{3}H]nisoxetine ([^{3}H]NIS) as

radioligand. All the experiments were performed as described elsewhere (Pubill et al 1998). In brief, vasa deferentia were removed and homogenized in 20 vol. of ice-cold Tris–HCl buffer (in mM: Tris–HCl 50, sucrose 250 mM, KCl 5, NaCl 300; pH 7·4) with a Polytron (Kinematica) twice at setting 10 for 30 s. The homogenate was filtered through three layers of surgical gauze and centrifuged at 700 × *g* for 10 min. The supernatant was decanted and centrifuged at 17 500 × *g* for 10 min. Finally, the resulting supernatant was centrifuged at 100 000 × *g* for 45 min. The membrane pellets were suspended in Tris–HCl buffer without sucrose. The concentration of protein was determined according to the method of Bradford (1976). The membrane preparations were stored at −70°C until analysis.

Competition studies with orphenadrine (10 nM–1 mM) were carried out with 125 μL of membrane suspension (containing 50 μg of protein) incubated with 125 μL of [^{3}H]nisoxetine (1 nM final concentration) in the absence (total binding) and presence (non-specific binding) of desipramine (10 μM). After incubation for 3 h at 0–4°C, the samples were passed under vacuum through Whatman GF/B filters presoaked in 0·5% polyethylenimine. Tubes and filters were washed twice with 4 mL of ice-cold Tris–HCl buffer. The filters were placed in vials containing 10 mL of Biogreen-1 scintillation fluid (Scharlau) and radioactivity was measured in a Beckman LS 1800 counter after 1 h with an efficiency of 40%.

The data from competition experiments were analysed with RADLIG version 4·0 (McPherson 1994).

Drugs and chemicals

Atropine, desipramine, noradrenaline bitartrate, orphenadrine HCl, prazosin HCl, Tris–HCl and yohimbine were from Sigma Chem. Co. (St Louis, MO); *levo*-[7-^{3}H]noradrenaline (specific activity 13·1 Ci mmol^{-1}) and [*N*-methyl-^{3}H]nisoxetine (specific activity 80·6 Ci mmol^{-1}) were from New England Nuclear (Dreiech, Germany). All other reagents were of analytical grade and were purchased from Panreac (BCN, Spain).

Results

Effect of orphenadrine on electrically-induced responses

Orphenadrine (0·3–30 μM) induced a significant concentration-dependent increase in electrically-induced responses in the vas deferens (Figure 1a), with an ED100 value of 4·92 ± 0·5 μM (Figure 1b). At 30 μM there was also an increase in resting tension (see Figure 1a).

Effect of orphenadrine on resting tension

Throughout the range of concentrations tested (0·3–30 μM) orphenadrine induced spontaneous contractions in the vasa deferentia ranging from 0·15 to 0·95 g of tension. This spontaneous activity was more marked at higher concentrations of orphenadrine. This effect was completely abolished by prazosin (1 μM) but not by atropine (20 nM).

Effect of orphenadrine on noradrenaline-induced contractions

Orphenadrine produced a concentration-dependent increase in the effect of noradrenaline 0·2 μM (Figure 2) (ED100 = 1·77 ± 0·2 μM).

Neither atropine (20 nM) nor yohimbine (12 nM) modified the effect of orphenadrine on noradrenaline-induced contractions.

[^{3}H]Noradrenaline uptake

In order to study the effect of orphenadrine on noradrenaline uptake, we measured the influence of this drug on the accumulation of [^{3}H]noradrenaline

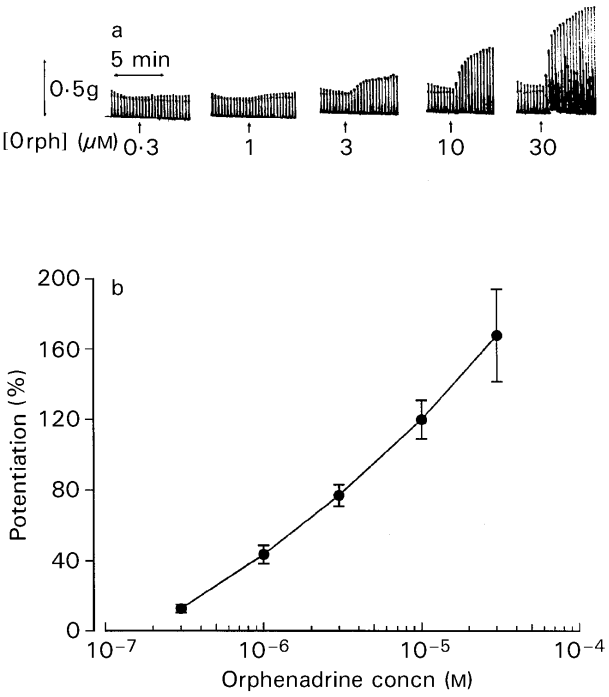

Figure 1. a. Representative tracings of twitch response potentiation by different concentrations of orphenadrine (Orph) in rat vas deferens. b. Concentration–response curve of orphenadrine on electrically-induced contractions in isolated rat vas deferens. Points are the means ± s.e.m. of n = 3–5 different experiments.

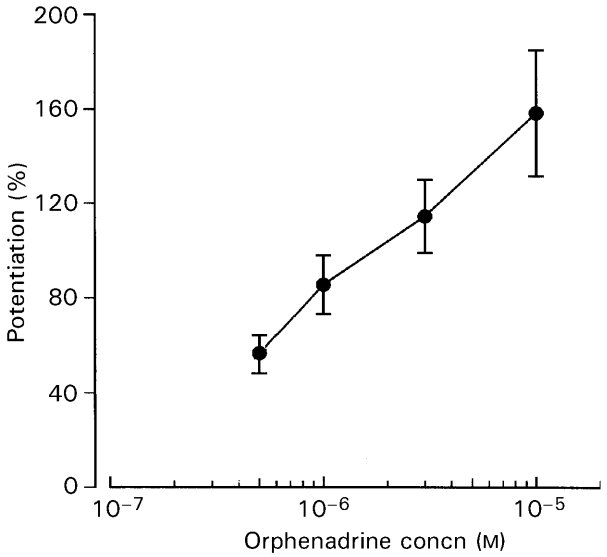

Figure 2. Concentration–response curve of orphenadrine on noradrenaline-induced contractions in rat vas deferens. Points are the means ± s.e.m. of n = 3–5 different experiments.

into rat vas deferens. In our study, a 3-min period of incubation was chosen for measuring the initial rate of uptake (Finberg et al 1992).

Orphenadrine caused a 70% inhibition of the uptake of [^{3}H]noradrenaline into the epididymal portion of rat vas deferens at the highest concentration tested (100 μM), with an IC50 value of 14·2 ± 2·3 μM (Figure 3).

[^{3}H]Nisoxetine competition studies

Orphenadrine produced a complete and competitive inhibition (Hill slope $n_H = 1{\cdot}02 \pm 0{\cdot}02$; $P > 0{\cdot}05$ vs the unity) of [^{3}H]nisoxetine binding to rat vas deferens membranes, with a K_i value (the concentration of orphenadrine that occupies 50% of total binding sites) of 1·05 ± 0·20 μM (n = 3) (Figure 4).

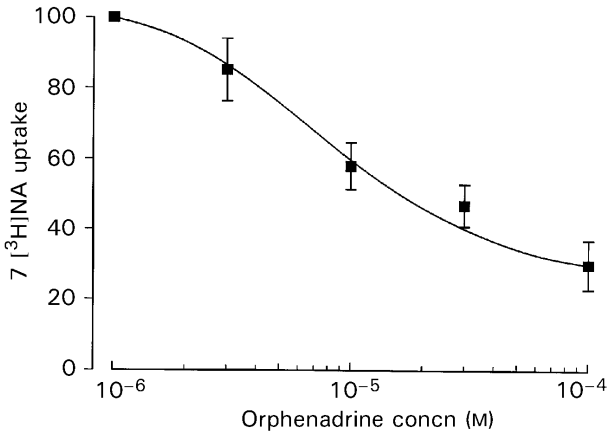

Figure 3. Concentration-dependent inhibition of [^{3}H]noradrenaline (0·1 μM) uptake by orphenadrine in rat vas deferens. Points are the means ± s.e.m. of three different experiments carried out in duplicate.

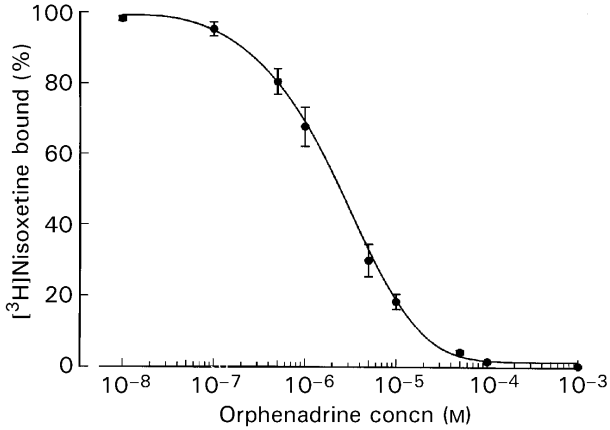

Figure 4. Inhibition of [^{3}H]nisoxetine (1 nM) binding by orphenadrine in rat vas deferens membranes. Points are the mean ± s.e.m. of three different experiments carried out in duplicate.

Discussion

These results indicate that orphenadrine has similar pharmacological properties to non-competitive NMDA receptor antagonists such as PCP ligands (Pubill et al 1998).

Orphenadrine, an anticholinergic drug used in the treatment of Parkinson's disease (Klockgether & Turski 1993; Danysz et al 1994, 1995), enhances the contractile responses in the epididymal portion of rat vas deferens. The spontaneous activity intensity varies among different organs when tested. The pattern of spontaneous contractions observed is different to that of a direct contractile effect. The potentiation can be observed in electrically-evoked contractions and also when noradrenaline is added to the organ bath. Moreover, orphenadrine alone induces spontaneous activity that is abolished by prazosin, indicating that the contractile effect is due to the noradrenaline released by the organ.

As rat vas deferens has only minor cholinergic innervation, a possible anticholinergic effect can be ruled out. Moreover, the presence of atropine in the medium did not modify the potentiation induced by orphenadrine. In addition, a presynaptic α_2-effect is ruled out by the lack of effect of yohimbine.

Since the epididymal portion of rat vas deferens contains predominantly adrenergic innervation, the molecular mechanisms involved in orphenadrine potentiation could be due to an indirect adrenergic effect.

The results obtained in [^{3}H]noradrenaline experiments showed that orphenadrine strongly inhibited noradrenaline accumulation. These results confirms the findings of previous studies on atria and rat brain slices (Story & Story 1969; Zee & Hespe 1973).

Nisoxetine has been reported to be the most suitable ligand for labelling the noradrenaline transporter. Its binding profile in rat vas deferens membranes has been previously characterized (concentration of [^{3}H]nisixetine that occupies 50% of total binding sites $K_D = 1{\cdot}6$ nM and maximal density of binding sites $B_{max} = 1600$ fmol (mg protein)$^{-1}$) (Pubill et al 1998). In the experiments reported in the present paper, orphenadrine competitively inhibited the binding of [^{3}H]nisoxetine in membranes obtained from rat vas deferens with a K_i value in the low micromolar range.

The present study demonstrates the enhancement of noradrenaline responses by orphenadrine in the epididymal portion of the rat vas deferens, a typical adrenergic tissue. This effect is evidenced by a potentiation of the contractile responses in isolated organ (noradrenaline-induced and neurogenic) and by inhibition of the [^{3}H]noradrenaline reuptake. The interaction with the noradrenaline carrier system is corroborated by results from the competition binding assays.

From all these results we can conclude that, at low micromolar concentrations, orphenadrine interacts with the noradrenaline reuptake system inhibiting its functionality and thus potentiating the effects of noradrenaline. Although the side effects of orphenadrine include typical anticholinergic-mediated actions, the present results show peripheral adrenergic potentiation at concentrations in the micromolar range that correlate with serum levels of orphenadrine in therapeutic conditions (Altamura et al 1986; Contin et al 1987). It would thus be of interest to evaluate the presence of some of these peripheral effects in patients receiving long-term treatment with orphenadrine.

It is well known that many drugs can interact with the noradrenaline transporter. The non-competitive NMDA receptor antagonists (such as PCP and MK-801) and sigma receptor ligands have been found to elicit this interaction (Massamiri & Duckles 1991; Rogers & Lemaire 1992; Pubill et al 1996). Orphenadrine has recently been reported to bind to the NMDA receptor-coupled ion channel (Kornhuber et al 1995) at micromolar concentrations and thus to behave as a PCP-like ligand. Therefore orphenadrine could also have in its structure a part able to interact with the noradrenaline transporter. Such interaction has been demonstrated, in the work reported here, in the rat vas deferens by binding studies.

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