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EFFECTS OF OXYPERTINE ON THE ISOLATED VAS DEFERENS OF THE RAT

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1 The isolated vas deferens of the rat was used to examine the peripheral action of oxypertine, a psychotropic-anxiolytic drug.

2 Oxypertine (4.4×10^{-10} M to 2.6×10^{-5} M) antagonized competitively the effects of noradrenaline ($pA_2 = 7.2$), 5-hydroxytryptamine ($pA_2 = 8.6$) and dopamine ($pA_2 = 9.8$).

3 Oxypertine (8.8×10^{-9} M to 2.6×10^{-5} M) antagonized the effects of low concentrations of acetylcholine and enhanced the contractions elicited by high concentrations of acetylcholine.

4 The contractions evoked by transmural stimulation of the vas deferens were reduced by oxypertine.

5 Oxypertine failed to antagonize the responses to potassium chloride.

6 These findings are compared with the effects of other antidepressant drugs.

Introduction

Actions of anxiolytic drugs may be mediated by functional changes of central neurones. While there is no doubt that dopaminergic neurones are selectively affected by anti-psychotic drugs (Vogt, 1973), all neuroleptics are competitive antagonists of dopamine, noradrenaline (NA) and adrenaline, acting as neurotransmitters in the peripheral and central nervous system (Janssen, 1967).

Oxypertine, 1-[2-(5,6 dimethoxy-2-methyl-3-indolyl)ethyl]-4-phenylpiperazine, is a highly effective agent in the treatment of anxiety (Bonn, Salkind & Rees, 1971). The drug reduces the levels of adrenaline and NA in the brain, but not that of 5-hydroxytryptamine (5-HT) and has little effect on dopamine levels in caudate nucleus and substantia nigra (Penn, 1972).

The present experiments were intended to study the effects of oxypertine on a peripheral autonomic synapse. For this purpose, the drug was applied *in vitro* to the isolated vas deferens of the rat and its interference with the contractile responses of this smooth muscle elicited by either chemical agents (ACh, dopamine, NA, 5-HT, KCl) or transmural stimulation was observed.

Methods

Sprague-Dawley rats, ranging in weight between 250 and 350 g, were used. Methods of isolation and mounting of vasa deferentia, composition of Tyrode solution and recording of contractions have been described before in detail (Miranda, 1976). Contractions were recorded isometrically from preparations suspended in a 30 ml bath and equilibrated for 1 h before the administration of drugs. They were added to the bath and washed out when the response to each dose reached a maximum.

The drugs used were: acetylcholine chloride (Sigma), dopamine hydrochloride (Sigma), 5-hydroxytryptamine creatinine sulphate (Sigma), (−)-noradrenaline (norepinephrine) bitartrate (Winthrop), oxypertine (Winthrop) and potassium chloride (Merck). Doses are expressed as the free bases.

Transmural stimulation of vasa deferentia was performed at supramaximal voltage with 0.5 ms pulses at frequencies of from 0.1 to 5 Hz, during 30 s, every 10 minutes.

Data are presented as mean values ± s.e. mean. Statistical differences were ascertained by Student's t test, $P < 0.05$ being regarded as significant. pA_2 values (Schild, 1947) were used for the study of competitive antagonism.

Results

None of the oxypertine concentrations used (from 4.4×10^{-10} M to 2.6×10^{-5} M) produced any contractile effect *per se*.

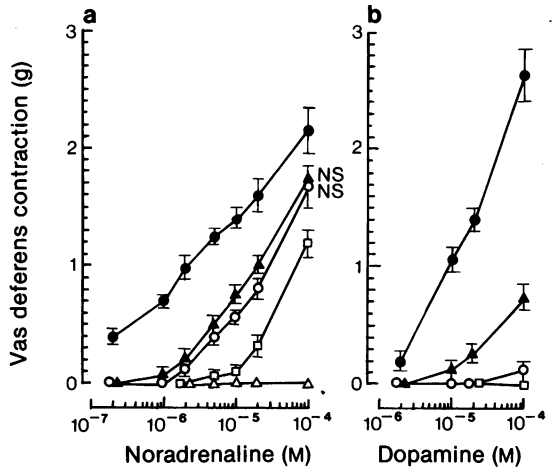

Figure 1 Responses of rat vas deferens to noradrenaline and dopamine, before and after application of oxypertine. (●) Controls; after oxypertine (▲) 4.4×10^{-10} M for 15 min; (○) 8.8×10^{-9} M for 15 min; (□) 8.8×10^{-8} M for 30 min; (△) 4.4×10^{-7} M for 30 min. Symbols show mean of 18 experiments, vertical bars indicate s.e.mean. All results are significantly different from controls ($P < 0.05$), except those denoted by NS.

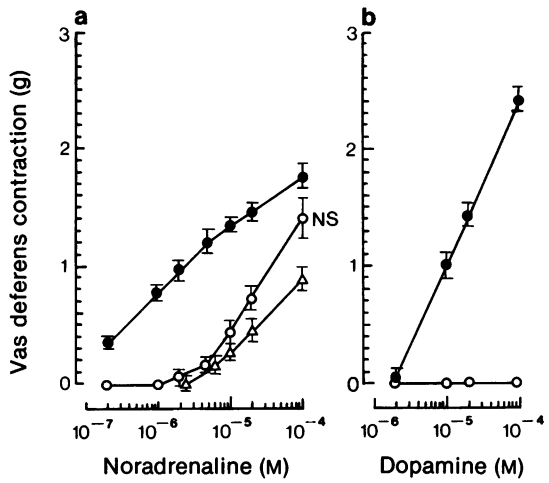

Figure 2 Responses of rat vas deferens responses to noradrenaline and dopamine before and during incubation with oxypertine. (●) Controls; (○) Oxypertine 2.6×10^{-6} M; (△) oxypertine 2.6×10^{-5} M. Symbols show means of 18 experiments; vertical bars indicate s.e.mean. All results are significantly different from controls ($P < 0.05$), except that denoted by NS.

Effect of oxypertine on responses to noradrenaline

Figure 1a illustrates responses of vasa deferentia to NA before and after treatment with oxypertine. After incubation of the preparation in Tyrode solution containing oxypertine (4.4×10^{-10} M) for 15 min followed by Tyrode solution alone for a further 30 min, the responses to all doses of NA were significantly reduced except for the largest one. Increasing the concentration 20 times did not significantly increase the inhibition, but increasing it 200 times and doubling the contact time to 30 min, resulted in a further shift to the right in the dose-response curve for NA. Contact with oxypertine (4.4×10^{-7} M) for 30 min produced complete blockade of the responses of the vas deferens to NA. The blockade persisted for at least 180 minutes.

Results obtained before and during incubation with Tyrode plus oxypertine 2.6×10^{-6} M and 2.6×10^{-5} M are presented in Figure 2a. The diminution in NA responses during incubation with oxypertine 2.6×10^{-6} M was similar to that seen when oxypertine 8.8×10^{-9} M was left in contact with the tissue for 15 min and then incubated in drug-free Tyrode solution for 30 min before determination of the dose-response curve to NA. A significant reduction in the response to the largest dose of NA was produced by oxypertine 2.6×10^{-5} M. The pA_2 value of oxypertine against NA was 7.2 ± 0.03.

Table 1 Effect of incubation with oxypertine (2.6×10^{-6} M) on responses of rat vas deferens to 5-hydroxytryptamine (5-HT), acetylcholine (ACh) and KCl

Agonist	*Concentration* (M)	*Control (g tension)*	*After oxypertine (g tension)*	*n*
5-HT	2.8×10^{-7}	0.30 ± 0.04	0*	12
	2.8×10^{-6}	1.20 ± 0.14	0*	12
ACh	1.7×10^{-7}	0.20 ± 0.04	0.04 ± 0.01*	12
	3.4×10^{-6}	0.48 ± 0.09	0.40 ± 0.01	12
KCl	2.5×10^{-3}	1.10 ± 0.16	1.04 ± 0.10	18

Results are given ±s.e. mean; *n:* number of experiments.
*Denotes significant difference from controls ($P < 0.05$).

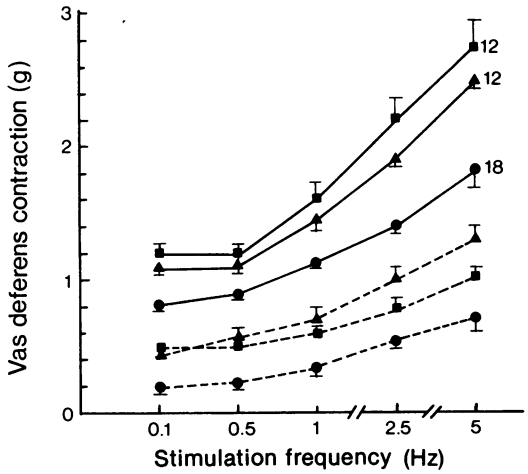

Figure 3 Effect of oxypertine on vas deferens responses to transmural stimulation at frequencies of 0.1, 0.5, 1, 2.5 and 5 Hz. Continuous lines show control responses; dashed lines responses after oxypertine. (●) After oxypertine 8.8×10^{-9} M for 15 min; (■) after 4.4×10^{-7} M for 30 min; (▲) during 2.6×10^{-5} M. Number of experiments shown beside control curves. All results are significantly different from controls ($P < 0.05$).

Effect of oxypertine on responses to dopamine

Results for dopamine-induced contractions are presented in Figures 1b and 2b. Application of the lowest concentration of oxypertine (4.4×10^{-10} M) for 15 min resulted in a pronounced reduction of responses to dopamine, complete blockade being obtained after 30 min application of oxypertine 8.8×10^{-8} M followed by 30 min bathing in drug-free Tyrode solution, or during incubation with oxypertine 2.6×10^{-6} M. The pA_2 value of oxypertine against dopamine was 9.8 ± 0.01.

Effect of oxypertine on responses to 5-hydroxytryptamine, acetylcholine and KCl

Table 1 shows that responses of the vas deferens to 5-HT were completely blocked in Tyrode solution containing oxypertine 2.6×10^{-6} M. Blockade also occurred when other concentrations of oxypertine were used (4.4×10^{-10} M to 2.6×10^{-5} M). The former concentration shifted the dose-response curve to the right by 1.6 log units and the latter by 2.8 log units. The pA_2 value for oxypertine against 5-HT was 8.6 ± 0.01.

Oxypertine had a dual effect upon ACh-elicited contractions. Responses to a low dose of ACh were greatly reduced in the presence of oxypertine (2.6×10^{-6} M), but contractions elicited by a high dose of ACh were not significantly modified by the same concentration as indicated in Table 1. High doses of ACh were potentiated during incubation with oxypertine (2.6×10^{-5} M) but low doses were still reduced.

Contraction of the vas deferens induced by KCl 2.5×10^{-3} M were not modified by oxypertine 2.6×10^{-6} M (Table 1) or 2.6×10^{-5} M.

Effect of oxypertine on responses to transmural stimulation

As illustrated in Figure 3, contractile responses of vasa deferentia to transmural stimulation at different frequencies (0.1, 0.5, 1, 2.5 and 5 Hz) were significantly reduced although not abolished by all concentrations of oxypertine used.

Discussion

The results show that oxypertine was able to antagonize competitively dopamine ($pA_2 = 9.8$) 5-HT ($pA_2 = 8.6$) and NA ($pA_2 = 7.2$)-induced contractions of the rat isolated vas deferens. It also reduced the effectiveness of transmural stimulation, but not the contractions evoked by KCl. Oxypertine antagonized the effects of low doses of ACh, but those of high doses were enhanced by oxypertine 2.6×10^{-5} M.

In contrast to the antagonism by tricyclic antidepressants of vas deferens responses to potassium (Westfall, 1973), the observation that contractile responses to KCl were unchanged even in the presence of large doses of oxypertine, indicates that excitability and contractility of smooth muscle cells are unimpaired by oxypertine. Thus, its blocking actions on responses to monoamines and transmural stimulation must be explained by an interference at a junctional level.

Although the rat vas deferens has a larger content of NA than dopamine, the latter has a higher turnover rate (Boadle-Biber & Roth, 1975). Furthermore, analysis of pharmacological blockade of vas deferens contractions elicited by electrical stimulation led Simon & van Maanen (1976) to suggest that dopamine is the functional transmitter and that when released from nerve terminals stimulates specific dopamine receptors in smooth muscle cells. Since oxypertine in low doses abolished responses to exogenous dopamine, results may provide evidence for a blocking effect of oxypertine on dopaminergic transmission, which might account for the reduction of the effects elicited by transmural stimulation. However, the fact that even large doses of the drug produced incomplete blockade of electrically induced responses, plus confirmation that NA is several times

more potent than dopamine as an agonist and less affected by oxypertine (compare dose-response curves in Figure 1 and 2), would suggest that nerve endings release both dopamine and NA, and that the smooth muscle cells are provided with both dopamine-receptors and α-adrenoceptors. Another possibility is that the inhibition of the responses to transmural stimulation could well be due to stimulation of presynaptic receptors limiting the output of the transmitter and having no relation to the blockade of postsynaptic receptors.

Since the action of 5-HT on the vas deferens is not caused by release of stored NA (Miranda, 1976), reduction of 5-HT-induced contractions by oxypertine may be ascribed to blockade of dopamine-receptors or specific 5-HT receptors.

Oxypertine-induced blockade of vas deferens responses to low doses of ACh could be an expression of a weak muscarinic receptor blocking action, but this does not explain the potentiation of the effect of ACh in high doses. Another possibility is that the effect of low doses of ACh is mediated by prejunctional release of dopamine or NA, and is thus susceptible to oxypertine blockade of catecholamine receptors, while that of high doses of ACh is a direct effect on smooth muscle cells.

Oxypertine reduction of NA and 5-HT responses described here differs from the potentiating action of low doses of tricyclic antidepressants (Westfall, 1973; Van Dorsser & Dresse, 1974) and suggests that their central actions are not necessarily related to their influence on the peripheral activity of biogenic amines (Benešová & Náhunek, 1971).

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