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Central Cholinergic Mechanisms in Electrical Self-stimulation and in Drug-induced Tremor in Rats[1]

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WISHART, T. B. AND L. J. HERBERG. *Central cholinergic mechanisms in electrical self-stimulation and in drug-induced tremor in rats.* PHARMAC. BIOCHEM. BEHAV. 11(6) 625–629, 1979.—Oxotremorine, a specific stimulant of central muscarinic acetylcholine receptors, inhibited lateral hypothalamic self-stimulation at a dose-level less than one-tenth of that necessary to produce body tremor. Tremor induced by oxotremorine (0.5 mg/kg) was inhibited by pretreatment with hyoscine (scopolamine) (0.3 mg/kg) or propranolol (20 mg/kg) but not by methylhyoscine (0.3 mg/kg) or apomorphine (0.3 mg/kg). Inhibition of self-stimulation by oxotremorine (.03 mg/kg) was prevented by hyoscine (0.3 mg/kg) but not by any other of the drugs tested and thus constitutes a uniquely specific *in vivo* model for assessing central antimuscarinic activity. The results confirm the presence of centrally situated ACh receptors eleciting tremor and inhibiting self-stimulation but provide no evidence of an effect on tremor by central adrenergic β-receptors.

Acetylcholine Apomorphine Hyoscine Methylscopolamine Oxotremorine Propranolol
Self-stimulation Scopolamine Tremor

CENTRAL cholinergic function has become increasingly important in the study of antischizophrenic [17,20], antidepressant [19], and antiparkinsonian [16] drugs but there is no entirely adequate method for assessing drug effects on central cholinergic transmission. The interpretation of *in vitro* data has been queried on clinical and pharmacological grounds [3,6], electrophysiological and iontophoretic techniques are difficult to apply, and peripheral measurements e.g. on isolated guinea-pig ileum do not necessarily reflect the properties of central receptors [18].

Another widely used technique is based on the ability of anticholinergic agents to suppress the tremor-inducing effects of central cholinergic agonists such as oxotremorine [8,10]. Unfortunately this seems an equally dubious procedure since several unrelated drugs [8, 14, 21], including propranolol and other adrenergic-receptor blocking agents [1,8] are similarly effective in preventing oxotremorine tremor, whether by a supposed central antagonism [2] or by acting directly on muscle [4,15].

Recent findings have raised the possibility that self-stimulation of 'reward' areas of the rat's brain may provide a sensitive and more specific measure of central cholinergic activity. In this procedure response rats for hypothalamic stimulation have been shown to reflect a balance between brain dopamine and brain ACh [22] but to be relatively insensitive to all except extreme changes in brain serotonin [12] or brain noradrenaline [7,11].

In the present investigation we have applied this procedure as a test for central cholinergic activity and we report firstly that the administration of oxotremorine in doses far smaller than those needed to elicit tremor cause a prompt and dose-related inhibition of hypothalamic self-stimulation, and secondly that the ability of various agents to prevent the effects of oxotremorine on self-stimulation provides a more specific and more reliable indication of their central anticholinergic activity than does their ability to suppress oxotremorine-induced tremor.

METHOD

Tremor

Subjects and apparatus. Six adult male Wistar rats weighing 450 to 600 g were housed individually in transparent acrylic cages with free access to food and water. Drug-induced coarse tremor was measured by removing the food and water-bottle from the top of the cage and placing the caged rat on the platform of a TechServ jiggle counter. Movement of the jiggle platform relative to a suspended pendulum activated the pens of a Gerbrands event recorder and advanced a bank of electromagnetic counters which were automatically cumulated at 2.5-min intervals. The suspension of the pendulum was adjusted to be maximally sensitive to coarse tremor of the rat's trunk but not to fine tremor, and it was relatively insensitive to slow postural movements.

Procedure. Tests were administered between 12 noon and

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4:00 p.m. on two days a week. The caged rat was placed on the jiggle platform and left undisturbed for at least 10 min. until spontaneous movements had subsided to less than 50 counts in a 5-min period. A pre-treatment injection of hyoscine, methylhyoscine, apomorphine, propranolol or NaCl was then given, and was followed 12 to 15 min later by a test injection of either oxotremorine (0.5 mg/kg) or NaCl. Tremor recording was started 1 min after the test injection and continued for 10 min. At the same time the rat's behaviour was continuously observed and scored on a 3-point scale:-

0 No observable tremor
1 Intermittent bursts of tremor
2 Sustained (>10-sec) tremor.

Each pretreatment was given to each rat twice, once followed by oxotremorine and once by NaCl. Drugs were given in random order at intervals of not less than 72 hr, with at least 7 days between successive injections of oxotremorine. Tremor was scored by subtracting the count recorded after pretreatment-plus-NaCl from that after pretreatment-plus-oxotremorine. At the end of the series, NaCl plus two additional doses of oxotremorine (0.03 and 0.1 mg/kg) were given in a balanced order.

Drugs. Oxotremorine (Aldrich) was dissolved in isotonic saline under nitrogen and stored frozen until required. The test dose (0.5 mg/kg) had previously been shown to produce strong but submaximal tremor [8]. Apomorphine hydrochloride (McFarlane Smith) was dissolved in isotonic saline containing 0.1% sodium metabisulphite and administered in a dose (0.3 mg/kg) reported to be selective for postsynaptic or stimulant effects rather than presynaptic or depressant effects in mice and rats [12,23]. Propranolol hydrochloride (ICI) was dissolved in saline and dosage (20 mg/kg) was selected after preliminary trials. *l*-Hyscine hydrobromide (0.3 mg/kg) ((−)-scopolamine methylbromide, Sigma) and *l*-methylhyoscine bromide (0.3 mg/kg) ((−)-scopolamine methylbromide, Sigma) were dissolved in saline. This dose of hyoscine reduces neuroleptic-induced depression of self-stimulation but has no pronounced effects in rats not treated with neuroleptics [22]. All solutions were injected intraperitoneally in a volume of 1.0 ml/kg.

Self-stimulation

Subjects. Twisted bipolar stainless steel electrodes were implanted unilaterally in the mid-lateral hypothalamus of 14 male Wistar rats weighing 250–350 g. De Groot [9] stereotaxic coordinates were A4.3, 1.6, 8.5 and at the end of the experiment the location of the electrodes was verified on 10 × enlarged photographic projections of unstained frozen sections. After recovery from surgery the rats were trained to operate a lever for 0.5-sec sine-wave reinforcing pulses made available through the electrodes at randomly varied intervals of 10 sec mean duration (VI 10 sec). Use of this reinforcement schedule ensures a steady, seizure-free rate of responding on which strong stimulant or depressant effects can be imposed without causing appreciable change in the rate at which the reinforcing shocks are received. The stimulating current for each rat was adjusted to the lowest intensity (range 30–175 μA) that elicited sustained responding at a rate between 10 and 20 responses per min, and regular training continued until response rates were stable.

Procedure. Three series of drug tests were administered to each rat:—(i) A dose-response curve for oxotremorine was determined by comparing self-stimulation rates in two 30-min periods immediately before and after injections of oxotremorine in doses of 0.0 (=NaCl), 0.003, 0.01 and 0.3 mg/kg given in balanced sequence at 3- or 4-day intervals. (ii) Each rat was allowed to self-stimulate for 30 min to provide preinjection baselines and injected with propranolol (0.0, 1.0, 3.0, 10 or 20 mg/kg), hyoscine (0.3 mg/kg) or methylhyoscine (0.3 mg/kg). Injections were given on different days in a random sequence and each was followed 30 min later by a test-dose of oxotremorine (0.03 mg/kg), and a further 30 min of self-stimulation. (iii) The effect of a apomorphine (0.3 mg/kg) pretreatment was investigated in the same way except that rats were returned to the self-stimulation chamber immediately after injection of apomorphine to allow for its rapid action, and oxotremorine (0.3 mg/kg) was injected after 15 min. Self-stimulation scores for apomorphine were derived from the third 5-min period after its injection, and from a 10-min period starting 5 min after oxotremorine. Drug solutions were prepared as described in the previous section, and test injections were separated by intervals of at least 72 hr.

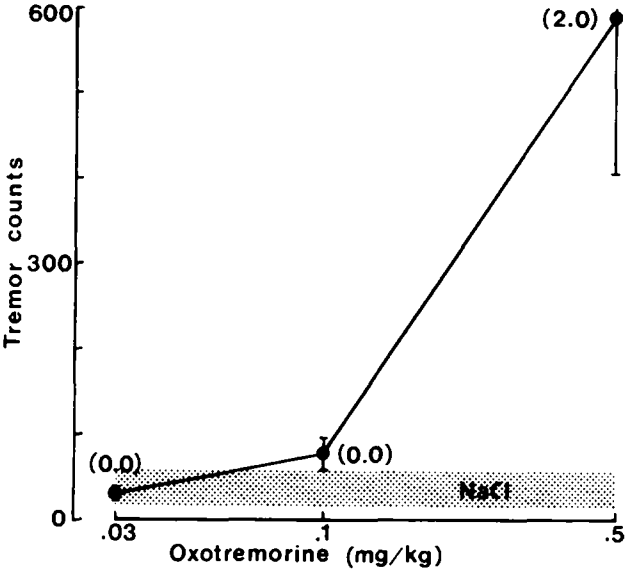

FIG. 1. Tremor counts in arbitrary units after different doses of oxotremorine plotted on a logarithmic scale. Shading indicates tremor counts after NaCl. Ranges are standard errors. Figures in parenthesis are visual estimates of tremor on a 3-point scale (0–2) that were made at the same time.

RESULTS

Tremor

The lower doses of oxotremorine (0.1 and 0.3 mg/kg) elicited strong parasympathetic signs, particularly salivation and frequent pungent faeces, but no tremor was detected on visual inspection or on the jiggle recordings (Fig. 1). Tremor occurred only after the 0.5-mg/kg dose. It developed rapidly to involve head and trunk within 1 or 2 min of injection, reached a maximum within 5 min and subsided during the ensuing 15 min as shown in the experimental record reproduced in Fig. 2.

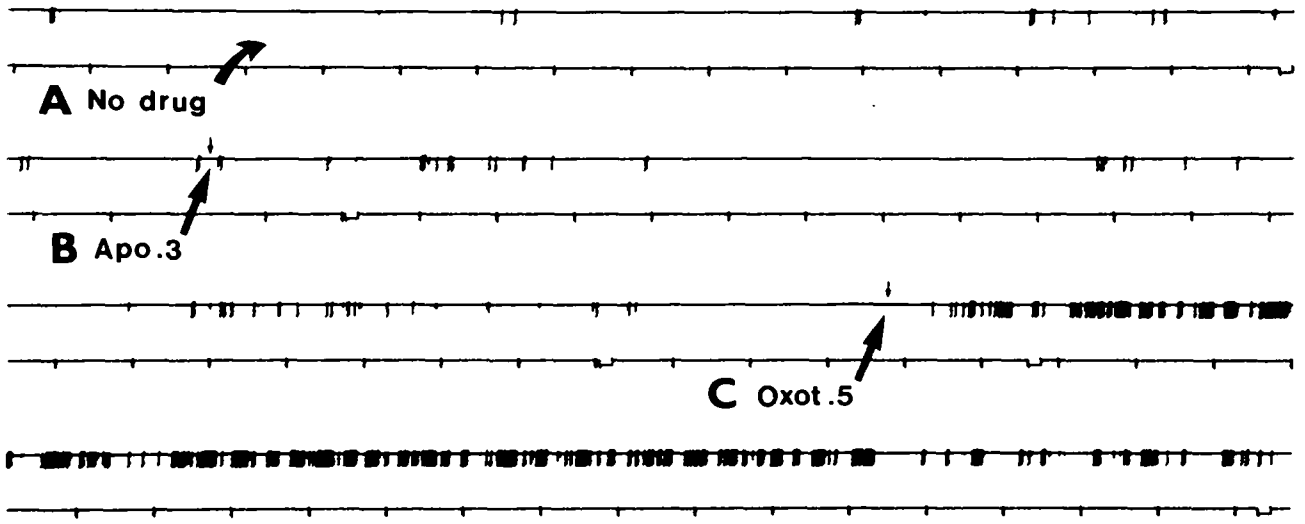

FIG. 2. Continuous recording of stabilimetric counts after No treatment (A), after apomorphine (0.3 mg/kg) injected at (B), and after oxotremorine (0.5 mg/kg) at (C). Scattered counts after apomorphine were caused by locomotor activity. Oxotremorine record shows frequent bursts of sustained tremor that commenced within a minute of injection and began to subside after 8 min. Time marker = 30 sec. Square signals on timer trace denote Counter on/off.

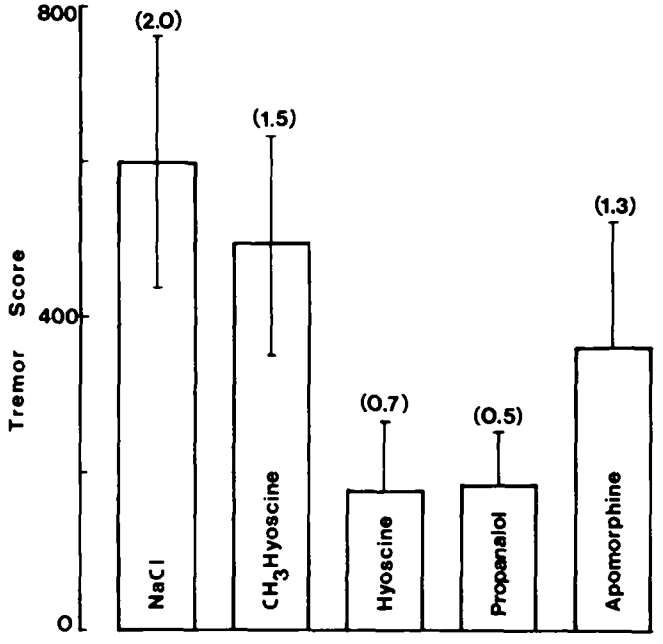

FIG. 3. Oxotremorine-induced tremor in arbitrary units after pretreatments as indicated. Tremor scores are counts recorded after pretreatment-plus-oxotremorine (0.5 mg/kg) less counts after pretreatment-plus-NaCl. Ranges are standard errors. Figures in parenthesis are visual estimates of tremor on a 3-point scale (0–2).

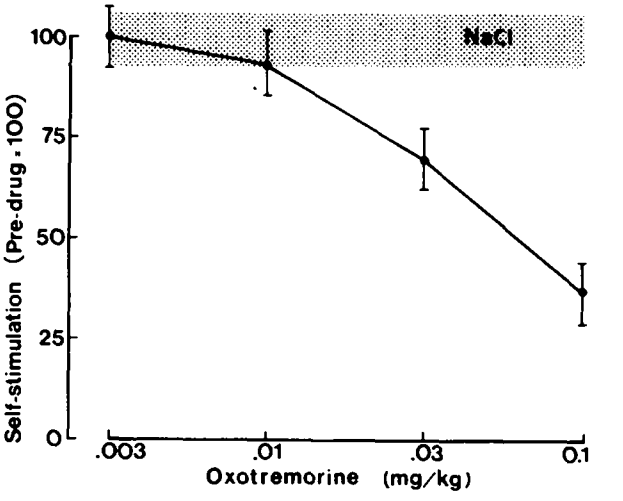

FIG. 4. Self-stimulation rates after NaCl and different doses of oxotremorine plotted on a logarithmic scale. Self-stimulation is expressed as a percentage of the pre-injection response rates. Shading indicates the standard error of the response rate after NaCl.

Figure 3 shows the effects on tremor of drug pretreatments. Tremor was strongly inhibited by hyoscine ($t(10)=2.3$, $p<0.05$) and by propranolol ($t(10)=2.4$, $p<0.05$) but not by methylhyoscine or apomorphine ($t(10)\leq 1.0$, $p>0.3$). Parasympathetic effects of oxotremorine including salivation, defecation and lacrimation (chromodacryorrhoea) [10] were much reduced by hyoscine and methylhyoscine but were unaffected by other pretreatments.

Visual estimates of tremor after oxotremorine and different pretreatments are summarised in Figs. 1 and 3. Scores on the 3-point scale showed good agreement with jiggle counts (Spearman rho = .90, $p=0.05$) although jiggle counts after propranolol-plus-oxotremorine were somewhat inflated by sudden jerks that were a feature of this combination.

Self-stimulation

Oxotremorine produced a dose-dependent depression of self-stimulation (Friedman $\chi^2=362$, $p<0.001$) which reached significance at 0.03 mg/kg ($t(12)=5.6$ and 7.0, $p<0.01$) (Fig. 4). The ID_{50} dose producing a 50% inhibition of self-stimulation) calculated from these data by linear regression was .075 ± .005 mg/kg. After a dose of 0.1 mg/kg, slowing of

TABLE 1

SELF-STIMULATION RATES (PRE-DRUG=100) AFTER OXOTREMORINE OR NaCl, FOLLOWING PRETREATMENTS WITH HYOSCINE, METHYLHYOSCINE, APOMORPHINE OR NaCl

	Pretreatment			
Test Treatment	NaCl	Hyoscine	CH_3-Hyoscine	Apomorphine
NaCl	98.6 ± 7.8	117.6 ± 8.6*	89.4 ± 8.6	139.1 ± 23.5†
Oxotremorine .03	63.7 ± 6.8*	117.8 ± 14.9*	62.9 ± 7.8*	65.6 ± 18.8

*Significantly different ($t(12)>2.2$, $p<0.05$) from NaCl+NaCl.
†NaCl was omitted after apomorphine and the control value is a no-injection control (see text).

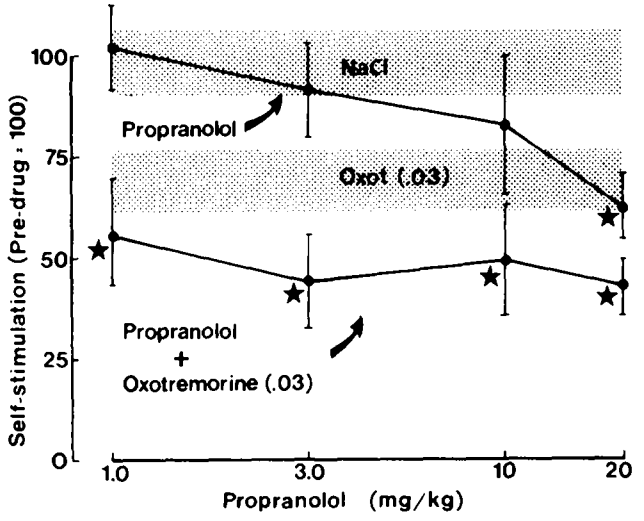

FIG. 5. Self-stimulation rates after different doses of propranolol, either alone or followed by oxotremorine (0.03 mg/kg). Response rates are expressed as a percentage of pre-drug response rates. Ranges are standard errors. Shaded areas indicate response rates after NaCl, and after oxotremorine (0.03 mg/kg) given alone. *Different from Pre-drug response rate ($p<0.05$).

self-stimulation usually became obvious within 5 min of injection, reached a peak in 20 min and was still apparent at the end of 30 min.

Figure 5 shows that propranolol alone (1.0, 3.0, 10 and 20 mg/kg) had a mildly depressant effect on response rate (Friedman $\chi^2=148$, $p<0.001$), reaching significance at the highest dose tested (−38%: $t(12)=4.9$, $p<0.001$; $ID_{30}=26.6$ mg/kg ± 1.3). None of the doses of propranolol in the range tested counteracted the action of oxotremorine; in fact Fig. 5 indicates their effects were simply additive, the propranolol+oxotremorine combinations being slightly more depressant than the same dose of oxotremorine given alone.

Table 1 shows the effects of pretreatments with hyoscine, methylhyoscine and apomorphine. Hyoscine (0.3 mg/kg) produced a small but significant increase in response rates when given alone (+18%: $t(12)=3.5$, $p<0.05$), and the same dose was sufficient to eliminate completely the depression of self-stimulation by oxotremorine. Methylhyoscine, on the other hand, did not affect response rates (−11%; $t(12)=0.23$, $p>0.1$) and did not affect the depression of self-stimulation by oxotremorine ($t(12)=0.62$, $p>0.1$).

Apomorphine (0.3 mg/kg) enhanced responding in some rats and depressed it in others and its effects overall were not significant (+39%; $t(12)=1.67$, $0.2>p>0.1$). Nor did it prevent inhibition of self-stimulation by oxotremorine (0.03 mg/kg) injected 15 min later (−54%; $t(12)=6.2$, $p<0.001$). The response rate after apomorphine-plus-oxotremorine was 65.6% of the pre-drug rate, very similar to that after oxotremorine alone (69.3%).

DISCUSSION

Comparison of Figs. 1 and 4 shows that the dose of oxotremorine needed to produce a 30% change in self-stimulation was only about one-tenth of that needed to produce tremor. Furthermore, the inhibition of self-stimulation by oxotremorine was shown to constitute a more specific model of central cholinergic activity than does oxotremorine tremor. This was illustrated by the results with propranolol which strongly inhibited oxotremorine tremor (Fig. 3) but did not reverse or reduce oxotremorine-induced depression of self-stimulation in any of the four doses tested (Fig. 5). The differing effects or propranolol on tremor and on self-stimulation is accounted for by recent reports that adrenergic β-receptor blocking agents such as propranolol may relieve tremor by acting directly on muscle rather than by antagonising the action of ACh within the brain[4,15].

Hyoscine, unlike propranolol, antagonises oxotremorine by blocking its action on the ACh receptor; hyoscine would thus be expected to be effective against the central effects of oxotremorine. This was confirmed by the finding that hyoscine was fully as effective in restoring self-stimulation after oxotremorine (Table 1) as it was in relieving oxotremorine-induced tremor (Fig. 3).

Methylated derivatives of hyoscine are less freely permeable from the circulation into the brain [5] and the ineffectiveness of methylhyoscine in restoring self-stimulation (Table 1) or in inhibiting tremor (Fig. 3) confirms that the effectiveness of hyoscine depends on blockade of receptors situated within the brain.

Apomorphine is a stimulant of central DA receptors and to this extent its failure to antagonise oxotremorine was unexpected. A possible explanation for this finding is that the action of apomorphine on self-stimulation sites in the hypothalamus is strongly biphasic: doses slightly less than optimal (e.g. 0.1 instead of 0.3 mg/kg) do not enhance self-stimulation but depress it[13]. Mutual antagonism between apomorphine and oxotremorine would therefore not only attenuate the depressant effect of oxotremorine but could

also invert the actual effect of apomorphine, making the direction of the nett effect of the combination difficult to predict.

These findings confirm the role of central cholinergic mechanisms in certain forms of tremor. They do not support a recent suggestion[2] that central cholinergic activity may be antagonised by central β-adrenergic blockade. Hyoscine, a specific ACh-receptor blocking agent, represented the only class of agent of those tested able to prevent the depression of self-stimulation by oxotremorine, and the specificity of this effect has furnished an opportunity to reassess the disputed role of anticholinergic activity[3,6] in the action of noncataleptogenic neuroleptic drugs.

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