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α-FLUPENTHIXOL: AN ANTAGONIST OF DOPAMINE-EVOKED FLUID SECRETION BY AN INSECT SALIVARY GLAND PREPARATION

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1 We have demonstrated inhibition of secretory responses of cockroach salivary glands to dopamine, adrenaline, noradrenaline and neurotransmitter by α-flupenthixol. This inhibition was slow in onset (60 min) and in reversal (> 2 h).

2 Inhibition of responses to adrenaline and noradrenaline was non-competitive, since the maxima and slopes of dose-response curves of these agonists were reduced.

3 Although at low concentrations (< 3 μM) the antagonism of responses to dopamine showed some characteristics of competitive inhibition, at higher doses non-competitive inhibition was clearly demonstrated.

4 These results are explained in terms of different efficacies of the agonists for the receptors antagonized by α-flupenthixol.

5 β-Flupenthixol was shown to antagonize responses to dopamine; however it was 10 to 100 times less potent than α-flupenthixol.

Introduction

There is considerable interest in the ability of neuroleptic drugs to inhibit dopamine-evoked responses in the mammalian brain. It has been shown that many neuroleptics are potent antagonists of dopamine-sensitive production of cyclic adenosine 3',5'-monophosphate (cyclic AMP) by homogenates of certain regions of rat brain (Clement-Cormier, Kebabian, Petzold & Greengard, 1974). Among the most potent are the thioxanthenes which exhibit geometric isomerism, the *cis* isomers being more effective than the *trans* isomers (Miller, Horn & Iversen, 1974). The difference in potency is particularly marked in the compound flupenthixol; the *cis* isomer, α-flupenthixol, is a powerful antipsychotic drug (Reiter, 1969; Gottfries, 1971) and inhibitor of dopamine-stimulated cyclic AMP formation whereas the *trans* isomer, β-flupenthixol, is virtually ineffective.

Behavioural (Møller Nielsen, Pedersen, Nymark, Franck, Boeck, Fjalland & Christensen, 1973) and biochemical studies (Miller *et al.*, 1974) suggest that it would be productive to examine the actions of α-flupenthixol on cellular preparations responsive to dopamine. House & Ginsborg (1976), for example, have shown that the electrical responses to dopamine and nerve stimulation of acinar cells in the cockroach salivary gland are suppressed by α-flupenthixol. Isolated salivary glands of cockroaches also secrete fluid when stimulated by nervous activity or bath applications of adrenaline, noradrenaline and dopamine (House & Smith, 1978). The aim of the present investigation was to study the mechanism of flupenthixol's inhibition of the secretory responses to these agonists.

Methods

Experiments were carried out at room temperature on the isolated salivary glands of adult cockroaches, *Nauphoeta cinerea* Olivier, of either sex allowed free access to food and water. The preparation consisted of the paired glands, ducts and reservoirs mounted in a perspex chamber as described previously (Smith & House. 1977). The composition of the bathing fluid (pH 7.6) was (mM): NaCl 160, KCl 10, $CaCl_2$ 5, Tris 5, HCl 4 and glucose 20. One of the salivary ducts was freed from its adherent reservoir duct, ligatured near its cut end with enamelled Ag wire and pulled through an orifice in a celluloid barrier into an adjacent paraffin pool. Fluid secreted at the cut end of the salivary duct was drawn into a siliconized glass micropipette and transferred to a paraffin pool where its diameter was measured by a microscope with a micrometer eyepiece (House & Smith, 1978).

The reservoir duct bearing the salivary nerve on the same side as the ligatured duct was drawn into a suction electrode for electrical stimulation. Rectangu-

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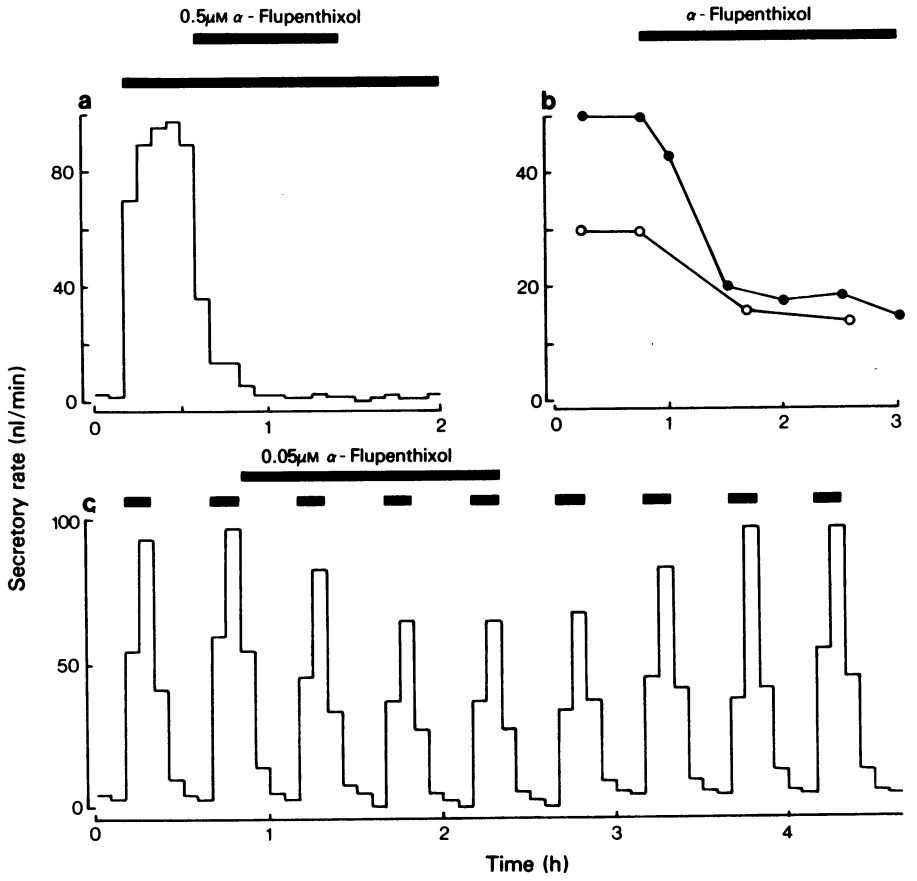

Figure 1 Time course of antagonism produced by α-flupenthixol. In this figure and all others the horizontal bars indicate periods of drug application. (a) Antagonism of secretory response to prolonged stimulation by 0.05 μM dopamine (unlabelled bar). (b) Antagonism of secretory response to 10 min applications of 10 μM adrenaline (●) and 600 nerve stimuli (○) by 0.3 and 0.1 μM α-flupenthixol respectively. (c) Inhibition and recovery of secretory responses to 10 min exposures to 0.1 μM dopamine (unlabelled bars); α-flupenthixol concentration was 0.05 μM.

lar pulses (0.5 ms duration, 50 V) were delivered at 5 or 10 Hz to the nerve from either a Grass stimulator (SD 5) or an isolated stimulator (Devices Ltd.) driven by a gated pulse generator (Devices Type 2533) and a Digitimer.

Concentrated stock solutions of drugs were made before each experiment, stored at 4°C and diluted into volumes of the bathing fluid immediately before perfusion. The drugs used were: (−)-adrenaline bitartrate, (−)-noradrenaline hydrochloride, dopamine hydrochloride (Sigma), *cis* and *trans* isomers of flupenthixol (Lundbeck).

The chamber (volume 1.5 ml) was normally perfused at a rate of about 2 ml/min by a Watson-Marlow flow inducer (MHRE 200); this was increased to 20 ml/min for perfusion of agonist solutions.

When solutions containing flupenthixol were applied to the preparation these were perfused initially at 20 ml/min for about 2 min to ensure rapid mixing in the chambers and thereafter at 2 ml/min. Because the inhibition of agonist responses by flupenthixol was slow it was found necessary to remake solutions at high dilutions (<0.1 μM) at frequent intervals (10 min) to avoid loss of potency.

Results

Inhibition of responses to nerve and dopamine stimulation

In previous electrophysiological experiments (House

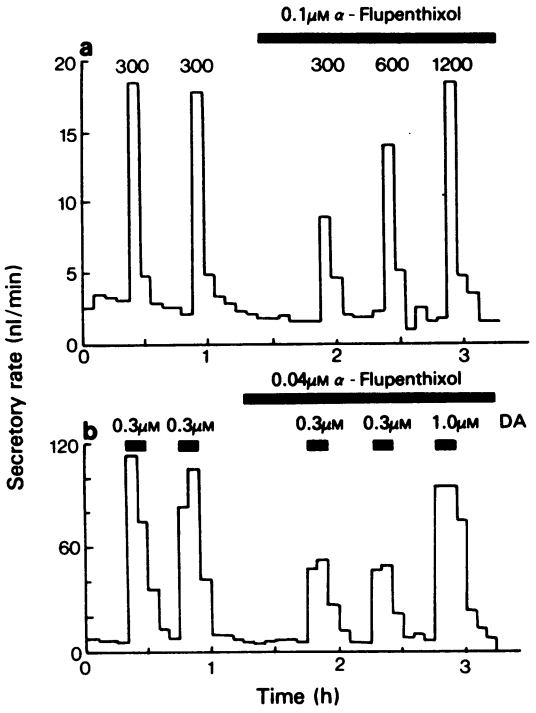

Figure 2 Surmountability of antagonism produced by α-flupenthixol. (a) Responses to nerve stimulation; number of stimuli given above each response. (b) Responses to bath applications of dopamine (DA) at concentrations shown.

& Ginsborg, 1976) on cockroach salivary gland it was noted that α-flupenthixol acted slowly and that its inhibition of the intracellular responses of acinar cells to nerve and dopamine stimulation had not reached a maximum within a 30 min period of exposure. Therefore, in the present study it was necessary to determine the exact time course of flupenthixol's antagonism. Because the rate of development of this inhibition was slow, solutions of flupenthixol had to be remade frequently especially at low concentrations (see Methods).

α-Flupenthixol Prolonged exposure to dopamine evokes fluid secretion at a steady rate which is not markedly reduced by fatigue or desensitization (House & Smith, 1978). When α-flupenthixol was added to the bathing solution during a maintained application of dopamine the secretory rate fell slowly (Figure 1a). A slow onset of inhibition was also found when the antagonist was applied to glands responding to nerve stimulation or relatively short (10 min) exposures to agonists, such as adrenaline (Figure 1b). These results indicate that α-flupenthixol requires about 30 to 60 min to obtain its maximal blocking action.

Some experiments were done to determine whether the antagonism caused by α-flupenthixol was reversible. Many of these necessarily lasted more than 6 h and hence their interpretation was complicated by the possible presence of fatigue of the secretory system. Figure 1c shows the results of an experiment where recovery from the suppression caused by 0.05 μM α-flupenthixol was complete within 2 h of washout. In three other experiments, however, with higher concentrations of the antagonist the recovery was only partial even after 4 h, nor was there satisfactory recovery of responses to nerve stimulation. It may be concluded that recovery is generally very slow.

The possibility that α-flupenthixol intervenes directly in the transport systems underlying salivary secretion seems to be ruled out because the block it produces can be surmounted by increasing the number of nerve stimuli or the concentration of agonist. Figure 2 illustrates two examples where matching responses before and during application of the antagonist were achieved by increasing the number of nerve stimuli four fold and by raising the agonist concentration from 0.3 to 1 μM. Thus it seems highly likely that α-flupenthixol inhibits fluid secretion by combining with postjunctional receptors for the transmitter and dopamine.

A quantitative study of the suppression of the secretory response by α-flupenthixol was attempted by monitoring the displacement of the log dose-response curves for dopamine. At concentrations of α-flupenthixol up to 3.0 μM there was a parallel shift of the curve and the maximum response to dopamine could be matched. The initial experiments at low antagonist concentrations (<0.3 μM) gave the misleading impression that the antagonism was competitive since the size of the displacement of the dose-response curve appeared to be closely related to the inhibitor concentration. In a typical experiment (Figure 3), where the α-flupenthixol concentration was 0.01 μM, the equipotent dose-ratio was estimated to be about 4. In addition to this series (over the range 0.01 to 3.0 μM) (Figure 4a) two further experiments were carried out with 5 μM α-flupenthixol. In neither case could the maximum response to dopamine be attained in the presence of the antagonist, and in each case the slope of the linear portion of the dose-response curve was depressed. One of these experiments is illustrated (Figure 4b). These results suggest that α-flupenthixol may be a non-competitive antagonist of dopamine-evoked fluid secretion.

β-Flupenthixol Like the *cis* isomer the *trans* form, β-flupenthixol, also slowly blocked secretory responses to nerve and dopamine stimulation. However,

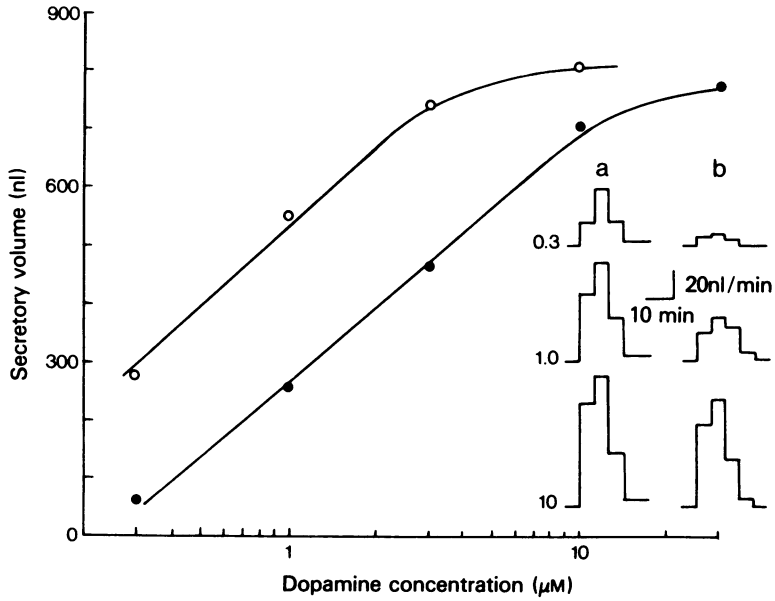

Figure 3 Antagonism by α-flupenthixol of secretory responses to dopamine. Typical responses to 10 min exposures of dopamine before (a) and during (b) the application of 0.01 μM α-flupenthixol are illustrated in the inset; the dopamine concentrations (μM) used are given on left. Note that in this figure the results are plotted as log dose-response curves where the response was measured as the secretory volume produced during exposure to dopamine (see House & Smith, 1978).

the *trans* isomer is considerably less potent than α-flupenthixol. Concentrations of β-flupenthixol exceeding 0.1 μM were required to inhibit responses to nerve stimulation but as with the more potent *cis* isomer the block could be overcome by delivering more nerve stimuli (Figure 5a). Moreover, β-flupenthixol produced a parallel shift of the log dose-response curve for dopamine (Figure 5b), the equipotent dose-ratio being about 6. Thus β-flupenthixol is about 10 to 100 times less potent as an antagonist than α-flupenthixol but a detailed study of this difference in potency has not been made.

Inhibition of responses to noradrenaline and adrenaline

Isolated cockroach salivary glands also secrete fluid in response to noradrenaline and adrenaline. These agonists are about 50 times less potent than dopamine and moreover there is evidence suggesting that they interact with receptors distinct from those combining with dopamine (House & Smith, 1978). It was, therefore, of interest to examine the ability of α-flupenthixol to inhibit responses to adrenaline and noradrenaline.

Adrenaline The antagonism by α-flupenthixol of responses to adrenaline was quantitatively different from that exerted on dopamine responses. In only one experiment out of six was there a parallel shift of the log dose-response curve, corresponding to an equipotent dose-ratio of 7.6 for 0.2 μM α-flupenthixol. The other experiments indicated a pronounced decrease in the slope of the log dose-response curve and in four cases there was also a marked reduction in the maximal response during application of α-flupenthixol. Two examples of these changes in the adrenaline curves are illustrated in Figure 6.

Noradrenaline α-Flupenthixol caused a parallel shift of the log dose-response curve in only two experiments out of nine. An example is shown in Figure 7a. The gland was exposed to 6 μM α-flupenthixol and the estimated equipotent dose-ratio was 37. In the other experiment where the antagonist concentration was 3 μM, the equipotent dose-ratio was 31. All of the other experiments with noradrenaline showed a significant reduction in the slope of the log dose-response curve in the presence of α-flupenthixol at concentrations in the range 0.1 to 10 μM. In several cases it was not possible to match maximal responses; an example is illustrated in Figure 7b. However, failure to achieve a maximal response was not an invariable finding (Figure 7c).

Evidently there is considerable variation in the sen-

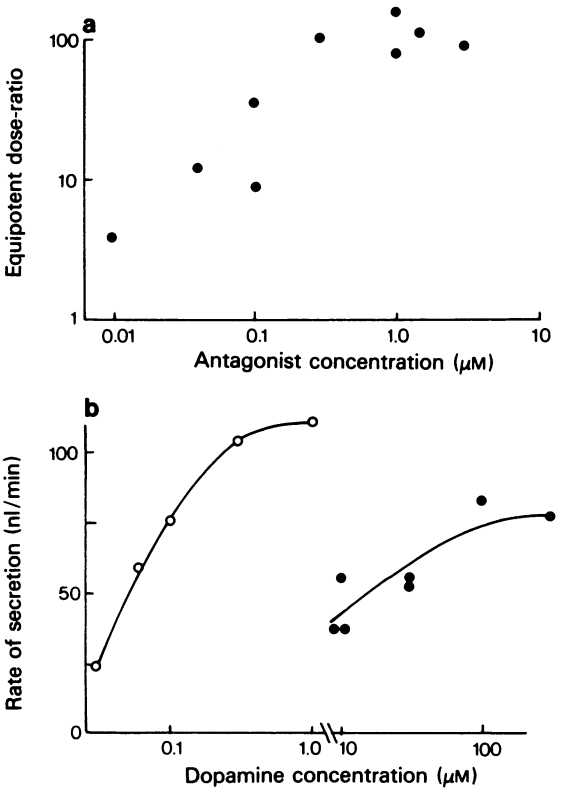

Figure 4 (a) The effect of α-flupenthixol concentration on the equipotent dose-ratio for responses to dopamine. (b) Antagonism of secretory responses to dopamine by 5 μM α-flupenthixol. Log dose-response curves before (○) and during (●) the presence of the antagonist.

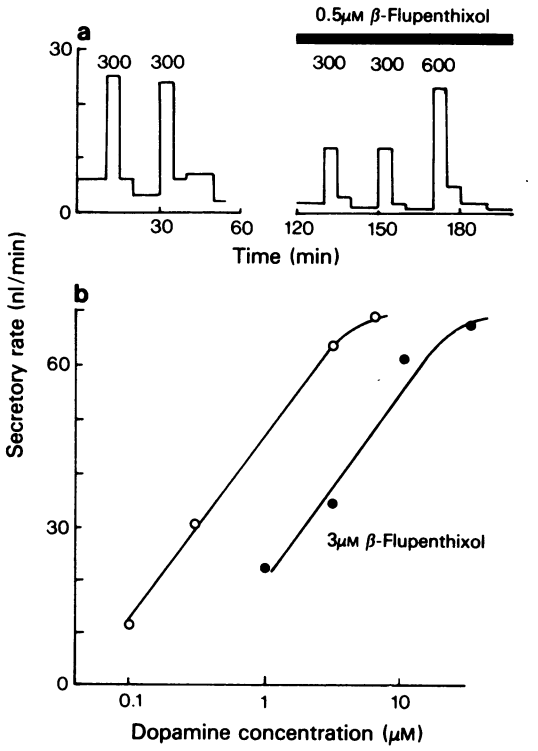

Figure 5 Antagonism by β-flupenthixol of responses to nerve and dopamine stimulation. (a) Responses to nerve stimulation; number of stimuli given above response. (b) Log dose-response curves before (○) and during (●) the presence of 3 μM β-flupenthixol. The response was measured as the peak secretory rate produced during exposure to dopamine.

sitivity of glands to α-flupenthixol. For example in Figure 7a the antagonist at 6 μM caused a parallel displacement of the dose-response curve whereas in another experiment 0.5 μM produced a pronounced alteration in the shape of the curve (Figure 7b). Similar variations occurred when adrenaline was the agonist (cf. Figure 6). Although differences in sensitivity to α-flupenthixol were not so obvious when dopamine was applied the scatter of points in Figure 4a may be due to such a source. It seems unlikely that the variability is caused by some feature of the methods since a corresponding study of the antagonist phentolamine gave no similar discrepancies (Bowser-Riley, House & Smith, 1978).

Recovery of responses to noradrenaline was not observed even several hours after washout of the antagonist. In contrast the responses to dopamine showed partial recovery over a similar period.

Discussion

The present results add further support to the accumulated evidence that flupenthixol is a dopamine antagonist. In disparate circumstances, where responses to dopamine reflect changes in conditioned behaviour (Møller Nielsen *et al.*, 1973), neuronal firing rate (Ben-Ari & Kelly, 1976), formation of cyclic AMP (Miller *et al.*, 1974), prolactin secretion (Meltzer, Paul & Fang, 1977), membrane potential (House & Ginsborg, 1976) and fluid secretion (present study), the *cis* isomer is markedly more effective than the *trans* isomer. Evidently the onset of α-flupenthixol's antagonism of the electrical and secretory responses of cockroach salivary glands is slow and the inhibition of the secretory response is poorly reversible. These findings may be relevant to the prolonged reduction in dopamine sensitivity which has been observed in

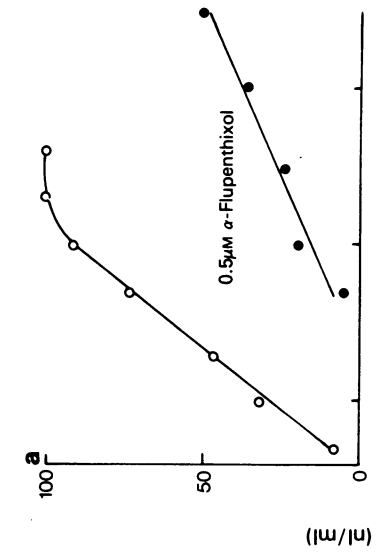

Figure 6 Antagonism by α-flupenthixol of responses to adrenaline: (a) and (b) show typical log dose-responses curves before (○) and during (●) presence of α-flupenthixol applied at concentrations shown.

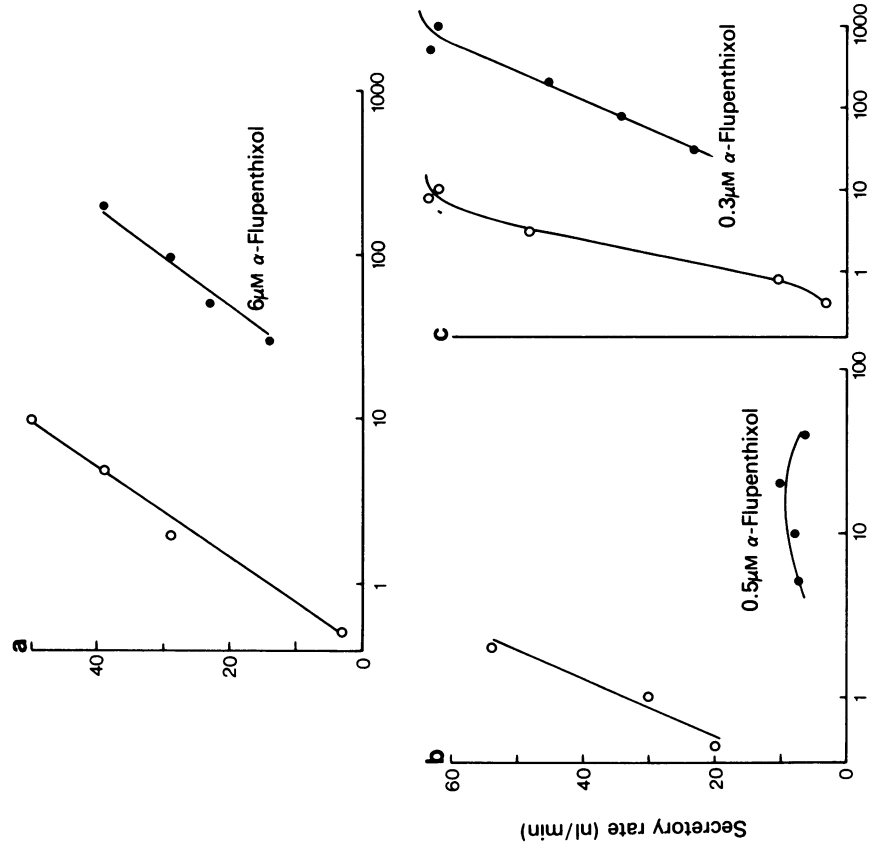

Figure 7 Antagonism by α-flupenthixol of responses to noradrenaline: (a), (b) and (c) show examples of log dose-response curves before (○) and during (●) presence of α-flupenthixol applied at concentrations shown.

animal behaviour testing after administration of α-flupenthixol (Møller Nielsen *et al.*, 1973).

α-Flupenthixol inhibits dopamine-evoked fluid secretion by cockroach salivary glands probably in a non-competitive manner by combining with post-junctional receptors. However, the possibility that this antagonist also acts presynaptically to reduce transmitter output (cf. Seeman & Lee, 1975) has not been excluded. Its high potency as an antagonist of responses to dopamine is similar to that found for inhibition of cyclic AMP formation by brain homogenates (Miller *et al.*, 1974). These values may be relevant to the empirical observation that α-flupenthixol is one of the most powerful antipsychotic drugs (cf. Seeman & Lee, 1975).

An interesting feature of the present results is that there is a quantitative difference in the blocking action of α-flupenthixol against responses to dopamine and the other agonists. It is clear that at concentrations sufficient to produce parallel shifts of the dopamine dose-response curves there are usually striking changes in slopes and maxima of the curves for adrenaline and noradrenaline. This differential effect may be due to very low efficacies of noradrenaline and adrenaline compared with that of dopamine. Thus responses to the former agonists would be more sensitive to the presence of a non-competitive antagonist (Stephenson, 1956). A further consequence, compatible with our results, would be that sufficiently high concentrations of α-flupenthixol would change the slope and depress the maximum of the dose-response curve for the most potent agonist, dopamine.

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References

BEN-ARI, Y. & KELLY, J.S. (1976). Iontophoretic and intravenous effects of the neuroleptic, alpha-flupenthixol, on dopamine-evoked inhibition. *J. Physiol.*, **242**, 66–67P.

BOWSER-RILEY, F., HOUSE, C.R. & SMITH, R.K. (1978). Competitive antagonism by phentolamine of responses to biogenic amines and the transmitter at a neuroglandular junction. *J. Physiol.*, **279**, 473–489.

CLEMENT-CORMIER, Y.C., KEBABIAN, J.W., PETZGOLD, G.L. & GREENGARD, P. (1974). Dopamine-sensitive adenylate cyclase in mammalian brain: a possible site of action of antipsychotic drugs. *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1113–1117.

GOTTFRIES, C.G. (1971). Flupenthixol and trifluoparazine: a double-blind investigation in the treatment of schizophrenia. *Br. J. Psychiat.*, **119**, 547–548.

HOUSE, C.R. & GINSBORG, B.L. (1976). Actions of a dopamine analogue and a neuroleptic at a neuroglandular synapse. *Nature*, **261**, 332–333.

HOUSE, C.R. & SMITH, R.K. (1978). On the receptors involved in the nervous control of salivary secretion by *Nauphoeta cinerea* Olivier. *J. Physiol.*, **279**, 457–471.

MELTZER, H.Y., PAUL, S.M. & FANG, V.S. (1977). Effect of flupenthixol and butaclamol isomers on prolactin secretion in rats. *Psychopharmacology*, **51**, 181–183.

MILLER, R.J., HORN, A.S. & IVERSEN, L.L. (1974). The action of neuroleptic drugs on dopamine-stimulated adenosine cyclic 3′,5′-monophosphate production in rat neostriatum and limbic forebrain. *Molec. Pharmac.*, **10**, 759–766.

MØLLER NIELSEN, I., PEDERSEN, V., NYMARK, M., FRANCK, K.F., BOECK, V., FJALLAND, B. & CHRISTENSEN, A.V. (1973). The comparative pharmacology of flupenthixol and some reference neuroleptics. *Acta. Pharmac. Tox.*, **33**, 353–362.

REITER, P.J. (1969). On flupenthixol, an anti-depressant of a new chemical group. *Br. J. Psychiat.*, **115**, 1399–1402.

SEEMAN, P. & LEE, T. (1975). Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurones. *Science, N.Y.*, **188**, 1217–1219.

SMITH, R.K. & HOUSE, C.R. (1977). Fluid secretion by isolated cockroach salivary glands. *Experientia*, **33**, 1182–1184.

STEPHENSON, R.P. (1956). A modification of receptor theory. *Br. J. Pharmac. Chemother.*, **11**, 379–393.

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