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Lack of effect of chronic haloperidol administration on the prolactin-lowering actions of piribedil

A. J. RAVITZ, K. E. MOORE*, *Department of Pharmacology, Michigan State University, East Lansing, Michigan 48824, U.S.A.*

If impulse traffic along a neuronal tract is interrupted either surgically or pharmacologically for a prolonged time, post-synaptic supersensitivity can develop to the neurotransmitter involved (Trendelenburg, 1966; Fleming, McPhillips & Westfall, 1973). This phenomenon has been demonstrated in central dopaminergic systems after a variety of treatments. Ungerstedt (1971) reported that 6-hydroxydopamine-induced destruction of nigrostriatal dopamine neurons in rats causes supersensitivity to the behavioural effects of apomorphine, a drug believed to act directly on dopamine receptors (Andén, Rubenson & others, 1967). Withdrawal of a chronic diet of α-methyltyrosine, a tyrosine hydroxylase inhibitor, in mice leads to an enhanced response to the locomotor stimulant effects of (+)-amphetamine, which is thought to exert these effects by increasing dopamine concentrations at central receptors (Dominic & Moore, 1969) and of apomorphine, a direct acting dopamine agonist (Gudelsky, Thornburg & Moore, 1975). Finally, prolonged treatment of rats with a variety of neuroleptics induces supersensitivity to the behavioural actions of apomorphine (Gianutsos, Drawbaugh & others, 1974; Tarsy & Baldessarini, 1974). Electrophysiological evidence for the development of supersensitivity of dopamine receptors in the caudate nucleus after chronic impulse interruption has also been presented (Siggins, Hoffer & Ungerstedt, 1974; Yarbrough, 1975).

Since prolactin release from the anterior pituitary is under tonic inhibitory control by tuberoinfundibular dopamine neurons (MacLeod, 1976), the measurement of serum prolactin concentrations is an easily accessible, easily quantifiable estimate of tuberoinfundibular dopamine activity. Agents which increase central dopamine activity lower circulating prolactin concentrations, while agents which reduce this activity increase serum prolactin (Neill, 1974; MacLeod, 1976; Mueller, Simpkins & others, 1976).

* Correspondence.

We set out to determine whether supersensitivity to the prolactin-lowering effects of piribedil (Mueller & others, 1976), a direct-acting dopamine agonist (Corrodi, Farnebo & others, 1972), occurs after chronic dopamine receptor blockade by haloperidol. If supersensitivity does develop, the dose-response curve for piribedil-induced lowering of prolactin in neuroleptic-treated animals will show a shift to the left as compared with animals receiving vehicle chronically. This would be presumptive evidence of similarity between dopamine receptors involved in the control of prolactin secretion and other central dopamine receptors.

A problem encountered when measuring decreases in prolactin concentrations in male rats is that basal hormone concentrations are already so low that dose-related decreases cannot accurately be determined using the prolactin radioimmunoassay. This problem was avoided by pretreating all animals with α-methyltyrosine 30 min before injecting piribedil. This tyrosine hydroxylase inhibitor effectively increases circulating prolactin concentrations by decreasing endogenous dopamine without blocking postsynaptic dopamine receptors and therefore would not be expected to interfere with the prolactin-lowering actions of piribedil, as these actions are exerted through direct stimulation of the postsynaptic dopamine receptors.

Male Sprague-Dawley rats (Spartan Research Animals, Haslett, MI) 150–175 g, received haloperidol (McNeil Laboratories; 2·5 mg kg^{-1}, s.c.) every 12 h for 7 consecutive days, then haloperidol (5·0 mg kg^{-1}, s.c.) every 12 h for an additional 7 days. This schedule of haloperidol administration is more intensive than those used previously to demonstrate an enhanced response to apomorphine (Tarsy & Baldessarini, 1974; Yarbrough, 1975), a drug having dopaminergic agonistic properties which are similar to those of piribedil (Corrodi & others, 1972; Thornburg & Moore, 1974, 1975). A second group of rats received vehicle (0·3 % tartaric acid) twice daily for 14 consecutive days. 72 h after the last injection, rats received DL-α-methyltyrosine

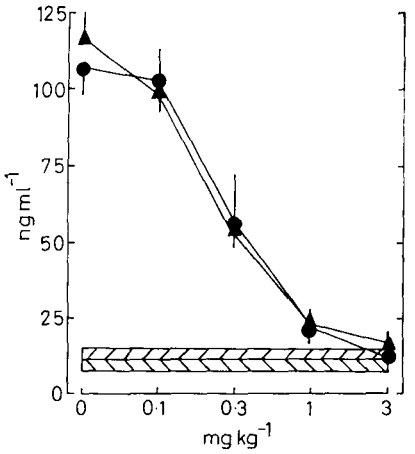

FIG. 1. Reduction in serum prolactin concentrations (ng ml^{-1}) by piribedil (mg kg^{-1}) in rats chronically treated with haloperidol. Rats received chronic haloperidol or vehicle (0·3% tartaric acid) as described in text. 72 h after the last injection, rats received α-methyltyrosine methylester HCl (250 mg kg^{-1}, i.p.) or vehicle (saline) 30 min before receiving piribedil monomethane sulphonate (s.c.) or vehicle (water). Rats were killed 1 h after last injection. Symbols represent mean ±s.e. of 8 determinations. ●—chronic vehicle treated rats; ▲—chronic haloperidol treated rats. Prolactin concentrations of animals treated chronically with vehicle (9±1) or haloperidol (12 ± 2) and then with appropriate vehicles were not significantly different ($P<0·05$). Thus, these values were combined and mean ±s.e. represented by the horizontal line and hatched area.

methylester HCl (Regis Chemical Co.; 250 mg kg^{-1}, i.p.) or vehicle (0·9% saline) 30 min before receiving various doses of piribedil monomethane sulfonate (ET 495, Les Laboratoires Servier; s.c.) or vehicle (water). One h after the last injection, rats were decapitated and blood collected from the trunk, centrifuged, and the serum assayed for prolactin content by the double antibody radioimmunoassay of Niswender, Chen & others (1969). Values are expressed in terms of NIAMDD-rat prolactin-RP-1. At each dose of piribedil, group means of animals which received haloperidol or vehicle chronically were compared using Student's *t*-test.

Piribedil caused a dose-related reversal of the α-methyltyrosine-induced elevation of serum prolactin concentrations (Fig. 1). Prior chronic treatment with haloperidol did not alter this response to piribedil.

As there was no shift to the left in the dose-response curve, it must be concluded that chronic treatment with haloperidol does not induce supersensitivity to the prolactin-lowering effects of piribedil. It appears, then, that in the development of supersensitivity after chronic neuroleptic blockade the dopamine receptors that mediate the inhibition of prolactin secretion differ from those of the mesolimbic and nigrostriatal systems which mediate locomotor and stereotyped activity.

These results are in agreement with a preliminary report by Meltzer, Goade & others (1976) who found that chronic treatment with chlorpromazine did not affect the prolactin-lowering action of apomorphine in rats. There is also some clinical evidence for a difference between tuberoinfundibular and other central dopamine neuronal systems. It has been shown that the ability of neuroleptics to induce extrapyramidal side effects decreases after chronic treatment with these agents (Byck, 1975). As extrapyramidal signs are thought to be due to blockade of dopamine receptors in the neostriatum (Hornykiewicz, 1973), tolerance to these side effects has been postulated to be due to the development of supersensitivity of these receptors. Meltzer & Fang (1976), however, have shown that serum prolactin concentrations are elevated in patients receiving neuroleptic therapy even after 3 months of continuous treatment. This would indicate that tolerance does not develop to the prolactin-increasing actions of neuroleptics, and by analogy, that dopamine receptors are not capable of modifying their sensitivity to the prolactin-lowering effects of dopamine in order to normalize prolactin concentrations.

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LETTERS TO THE EDITOR

Effect of sucrose on the spectrophotometric determination of cholinesterase activities

C. K. ATTERWILL*, M. J. NEAL, *Department of Pharmacology, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, U.K.*

In studies of the subcellular distribution of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) in sucrose fractions prepared from homogenates of retina we found (unpublished) that the apparent enzyme activities were significantly increased by the presence of sucrose when assayed by the spectrophotometric Ellman procedure (Ellman, Courtney & others, 1961). As we were unaware of any mention of this phenomenon in the literature it was examined in more detail.

Test cuvettes were prepared containing 0·1 ml 5,5-dithiobis-2-nitrobenzoic acid (DTNB—final concentration = 3×10^{-4} M); 0·2–0·5 ml of sucrose samples; potassium phosphate buffer to give a final volume of 3·1 ml (final concentration = 0·1 M, pH 8·0). Specific inhibitors of either AChE or BuChE (1,5-bis(*p*-allyldimethylammonium-phenyl)-pentan-3-one dibromide (BW284C51), or ethopropazine hydrochloride (Parsidol) respectively) were included for the differential assay of the enzymes in tissue fractions. After a preincubation period of 10 min at 37° the reaction was initiated by the addition of substrate: either 0·1 ml acetylthiocholine iodide (final concentration = 2·5 mM) or 0·2 ml butyrylthiocholine iodide (final concentration = 10 mM). The reaction mixtures were then incubated for further periods of up to 10 min (37°) and the initial rate of change of absorbance, due to the production of the yellow anion of 5-thio-2-nitrobenzoic acid, measured at 412 nm on a spectrophotometer.

When test cuvettes containing substrate only were run against air as the blank, the rates of hydrolysis were 0·012 $\Delta A\ min^{-1}$ (2·73 nmol min^{-1}) and 0·029 $\Delta A\ min^{-1}$ (6·65 nmol min^{-1}) for acetylthiocholine and butyrylthiocholine respectively.

To examine the effects of sucrose on the assay, 0·2 ml (acetylthiocholine present) or 0·5 ml (butyrylthiocholine present) of sucrose solutions (BDH Analar grade sucrose) ranging from 0·4 to 1·8 M were included in the reaction mixtures containing the substrate indicated. These volumes were those normally assayed for the respective enzyme activity (unpublished). Inclusion of sucrose did not significantly alter the pH of the reaction mixtures. Sucrose was replaced with an equal volume of distilled water in the blank cuvettes. Thus the overall rate of reaction due to utilization of substrate in the test cuvettes was automatically corrected for buffer-mediated hydrolysis of substrate.

The results from such an experiment are depicted in Fig. 1. It can be seen that the presence of sucrose led to an increase in the rate of formation of product, 5-thio-2-nitrobenzoate ion. The rates of reaction increased with increasing sucrose concentration.

The omission of the specific inhibitors (employed in the assay of the enzymes in tissue fractions) had no effect on this phenomenon. In the absence of substrate no reaction was observed between sucrose and DTNB. Furthermore, these effects were consistently observed for several batches of sucrose. It was also found, using

* Present address and correspondence: MRC Clinical Pharmacology Unit, University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE, U.K.