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Effects of Benztropine Mesylate on Haloperidol-Induced Prolactin Secretion and Serum Haloperidol Levels in Rats

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Abstract □ The effects of benztropine mesylate on haloperidol-induced prolactin secretion and serum haloperidol levels were investigated in 240 rats. Animals were pretreated with benztropine mesylate or saline 20 min prior to receiving haloperidol or saline. Serum prolactin and haloperidol levels were analyzed at six time periods over 150 min. There was no significant difference in prolactin levels of control animals, *i.e.*, saline pretreated/saline treated rats compared to benztropine mesylate pretreated/saline treated rats. Haloperidol caused a significant rise ($p < 0.0001$) in serum prolactin compared with controls. The prolactin concentration for the 30–150-min sampling period was significantly higher when the rats received benztropine mesylate prior to haloperidol ($p < 0.05$). There was a significant correlation ($r = 0.57, p < 0.001$) between serum haloperidol levels and serum prolactin levels in haloperidol-treated animals pretreated with either saline or benztropine mesylate. Additionally, serum haloperidol levels were not significantly different in animals pretreated with benztropine mesylate compared with those pretreated with saline. Thus, the enhancement of prolactin levels by benztropine mesylate was independent of any effect of haloperidol metabolism. This study appears to indicate that in the rat, cholinergic mechanisms exert a weak inhibitory effect on prolactin secretion under conditions of dopamine blockade.

Keyphrases □ Benztropine mesylate—prolactin secretion, serum haloperidol levels, rats □ Haloperidol—prolactin secretion, benztropine mesylate, serum levels, rats

The secretion of the anterior pituitary hormone, prolactin, appears to be dominantly regulated by a prolactin-inhibitory factor liberated by the hypothalamus as a result of afferent dopaminergic impulses (1–3). Dopamine is believed to be the major prolactin-inhibitory factor (4). In addition, prolactin release appears to be mediated to some extent by prolactin-releasing factors (5). Evidence also exists in animals that cholinergic and serotonergic mechanisms modulate the secretion of prolactin at the level of the pituitary (6–9). Although pilocarpine and physostigmine inhibited prolactin secretion in rats (8, 10, 11), no effect was observed on neuroleptic-induced prolactin secretion in this species (11). There are complex and conflicting reports concerning the effects of cholinergic blocking drugs on serum prolactin levels in humans and animals (6, 11, 12).

A fairly good correlation between the antipsychotic potency of various neuroleptics and their prolactin-stimulating effects has been demonstrated both in humans and rats (9, 13–15). Neuroleptics differ considerably in their intrinsic central anticholinergic properties (16). According to Lal *et al.* (17) such differences may account, at least in part, for variations in serum prolactin-stimulating properties found among antipsychotic agents. This suggestion was based on their demonstration in humans that intramuscularly administered benztropine mesylate, a muscarinic receptor-blocking agent, significantly enhanced the elevated prolactin levels induced by intramuscularly administered haloperidol (17). However, no attempt was made to determine if benztropine mesylate influenced prolactin secretion by altering haloperidol metabolism (17).

In this laboratory, comparison of the rank-order of reported central anticholinergic activity of nine antipsychotic drugs belonging to five chemical classes of neuroleptics appeared not to have significant effect upon either the magnitude or duration of prolactin stimulation in rats (18). However, there are no assurances that central anticholinergic threshold levels had been reached with the doses of drugs utilized.

In light of the above, it appeared desirable to further investigate the possible role of anticholinergic activity in enhancing neuroleptic-induced prolactin secretion in rats, to help clarify if a species difference exists between humans and rats in regard to this effect, and to determine if benztropine mesylate influences haloperidol plasma levels. The present investigation examined the effects of benztropine mesylate on haloperidol-induced prolactin secretion and serum haloperidol levels in rats.

EXPERIMENTAL SECTION

Two hundred and forty male Sprague–Dawley adult rats[1] (225–300 g) were divided into 24 equal groups. The rats were housed for 14 d prior to the study

[1] Taconic Farms, Germantown, N.Y.

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in a temperature-controlled (23 ± 3°C) artificially illuminated (lights on from 7:00 a.m. to 7:00 p.m. daily) room. The animals were given food[2] and water *ad libitum*. Between 12:30 p.m. and 2:00 p.m., each group was injected intraperitoneally with a 2-mg/kg dose of benztropine mesylate[3] [a dose previously shown to produce a rapid onset of central anticholinergic action (19) with a duration of action of ~24 h (20)] or an equal volume of normal saline. Twenty minutes following pretreatment, 12 groups of rats received 0.5 mg/kg of haloperidol injection[4] administered intraperitoneally and the remaining animals received an equal volume of normal saline. Haloperidol was chosen because of its weak central anticholinergic effects (16). The dose utilized was previously shown in this laboratory to produce a significant increase in serum prolactin secretion above baseline levels for a period of 180 min (15).

Blood samples were obtained by decapitation from the trunk portions at 0, 30, 60, 90, 120, and 150 min after haloperidol or normal saline administration. Blood samples were allowed to stand for 10 min at 4°C; the serum was separated and stored at −20°C until prolactin and haloperidol analyses could be performed. Serum samples were analyzed for prolactin by a double-antibody competitive binding assay. Iodinated rat prolactin, prepared by a modification of the Hunter-Greenwood method (21), was obtained commercially[5]. Rat prolactin antiserum and the reference prolactin standard were supplied by the Rat Pituitary Distribution Program[6]. The procedure for prolactin analysis was modified by the use of a precipitating second antibody suspended in polymer solution[7]. All results are expressed in terms of National Institute of Arthritis, Diabetes, and Digestive Diseases and Kidney (NIADDK) rat prolactin. Each serum sample was assayed in duplicate and the mean was taken as representative of true prolactin concentration. All test and control samples were determined concurrently; the intra-assay variation was 4.2%.

Serum haloperidol concentrations were determined utilizing a commercially available RIA kit[8] according to the instructions supplied. The procedure uses a tritiated label and the bound and free haloperidol are separated by adsorption on dextran-coated charcoal. All tests and control samples were determined concurrently and the results are expressed in μg/mL. The intra-assay variation was 4.7%. Statistical significance was determined by a two-way fixed analysis of variance and factorial analysis of variance with repeated measurements (22).

RESULTS

Evaluation of serum prolactin levels over 150 min, in rats pretreated with normal saline followed by intraperitoneal injection of haloperidol (Fig. 1), indicated a significant rise in serum prolactin compared with controls (saline pretreated/saline treated rats; $F_{(1,91)} = 53.51, p < 0.0001$). Likewise, there was a significant rise in serum prolactin in the 150-min period following intraperitoneal injection of haloperidol (Fig. 1) in rats pretreated with benztropine mesylate compared with controls (benztropine pretreated/saline treated rats; $F_{(1,98)} = 74.64, p < 0.0001$). There was no significant difference in the prolactin levels of the control animals (Fig. 1), *i.e.*, saline pretreated/saline treated rats *versus* benztropine pretreated/saline treated rats over the time period of this experiment ($F_{(1,87)} = <1.0$, not significant). Additionally, there were no significant differences in the mean prolactin levels of the haloperidol-treated rats pretreated with either saline or benztropine mesylate ($F_{(1,87)} = 3.80, p > 0.05$). However, when the data were analyzed by a factorial analysis of variance the prolactin concentrations for the 30–150-min sampling period were significantly higher when the rats received benztropine mesylate prior to haloperidol than when they received saline prior to haloperidol ($F_{(1,89)} = 4.36, p < 0.05$). This approach more fully captured the interrelations between the drugs and prolactin than standard straight baseline comparisons and permitted the use of simple main effects to isolate the locus of effect on prolactin due to haloperidol within benztropine mesylate.

Table I shows the effects of benztropine mesylate and normal saline pretreatments on serum haloperidol levels over a period of 150 min. Serum haloperidol levels were not significantly different in animals pretreated with benztropine mesylate compared with animals pretreated with saline ($F_{(1,89)} < 1.0$, not significant). There was a significant correlation ($r = 0.57, p < 0.001$) between the serum prolactin and serum haloperidol levels in haloperidol-treated animals pretreated with either benztropine mesylate or saline.

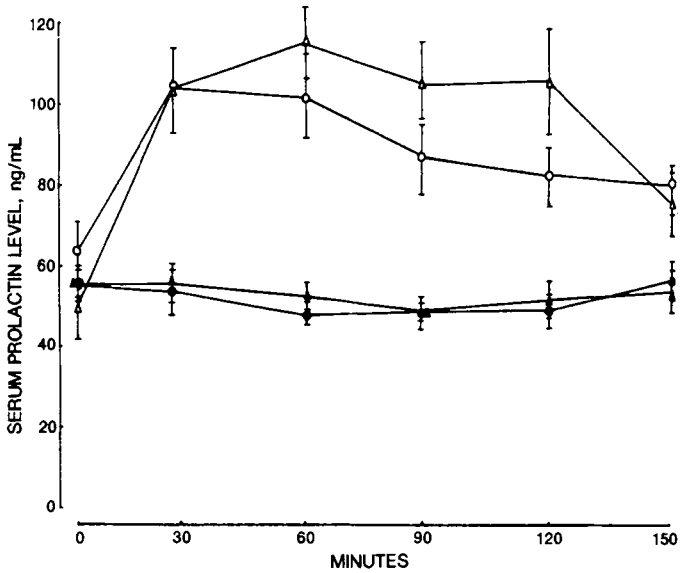

Figure 1—*Effects of pretreatment with benztropine mesylate or saline on the serum prolactin levels induced by treatment with haloperidol or saline. Each point represents a mean of 10 male rats; vertical lines show the* SEM. *Key: (●) saline pretreatment/saline treatment; (▲) benztropine mesylate pretreatment/saline treatment; (○) saline pretreatment/haloperidol treatment; (△) benztropine mesylate pretreatment/haloperidol treatment.*

DISCUSSION

Prolactin-elevating potency of neuroleptics in rats has previously been shown to correlate well with dopamine receptor blockade (23). The presence of a tuberoinfundibular cholinergic pathway has been described in rats (24). Adrenergic neurons regulating prolactin release have receptors for acetylcholine, and cholinergic neurons in the hypothalamus may terminate on adrenergic neurons (11). Pilocarpine has been shown to inhibit prolactin secretion in rats, and atropine reversed this effect (11). Atropine has been shown to potentiate neuroleptic-induced prolactin secretion in the monkey (6). These data are consistent with an inhibitory muscarinic mechanism on prolactin secretion. Although cholinergic drugs reduce prolactin secretion in the rat, after blockade of catecholamine receptors by chlorpromazine, haloperidol, or pimozide, no reduction in serum prolactin could be seen (11). Paradoxically, atropine may also reduce prolactin levels in the rat (8).

In humans it has been demonstrated that some patients who were receiving anticholinergic antiparkinsonian drugs in addition to chronic neuroleptic treatment had higher circulating prolactin concentrations than subjects on neuroleptics alone (25). However, in this laboratory previous comparisons of the rank-order of reported intrinsic central anticholinergic activity of nine antipsychotic drugs (belonging to five chemical classes of neuroleptics) with the experimentally determined rank-orders of time of maximum serum prolactin levels, magnitude of prolactin secretion at each sampling period, or the maximum serum prolactin elevation for each drug, demonstrated no significant correspondence (18). These comparisons suggested that reported intrinsic anticholinergic activity of neuroleptics did not have a significant effect upon either the magnitude or duration of prolactin stimulation produced by these drugs in rats. Although the doses of neuroleptics employed correspond well with human therapeutic dosages and produced marked prolactin stimulation, central anticholinergic activity was not experimentally determined. It is possible that anticholinergic threshold levels may not have been reached in rats with the doses utilized for some or all of the drugs; this requires further investigation.

In the present study the antimuscarinic agent, benztropine mesylate, had no effect on basal prolactin secretion in the rat but did significantly enhance the prolactin elevation promoted by haloperidol. This finding in the rat exactly corresponds to the observation in humans that intramuscularly administered benztropine mesylate did not significantly influence basal prolactin levels but did significantly enhance prolactin levels induced by intramuscularly administered haloperidol (17).

The present study also demonstrated that benztropine mesylate enhanced prolactin secretion independent of an effect on haloperidol metabolism, since pretreatment with benztropine did not significantly alter serum haloperidol levels. It appears that in both species cholinergic mechanisms have no effect on basal prolactin secretion but exert a weak inhibitory effect under conditions of dopamine receptor blockage.

[2] Purina Lab Chow; Ralston Purina Co., St. Louis, Mo.
[3] Merck Sharp and Dohme, Westport, Pa.
[4] McNeil Laboratories, Fort Washington, Pa.
[5] New England Nuclear, Boston, Mass.
[6] National Institute of Arthritis, Diabetes, Digestive Diseases and Kidney (NIADDK), Rat Pituitary Hormone Distribution Program, National Pituitary Agency, Baltimore, Md.
[7] Clinical Assays—Division of Travenol Laboratories, Cambridge, Mass.
[8] Damon Diagnostics, Needham Heights, Mass.

Table I—Effects of Benztropine Mesylate and Saline Pretreatments on Serum Haloperidol Levels

Pretreatment	Serum Haloperidol Levels, μg/mL[a]					
	0 min	30 min	60 min	90 min	120 min	150 min
Saline[b]	0.15 ± 0.05	16.92 ± 2.80	10.80 ± 1.41	8.47 ± 1.25	7.00 ± 0.78	6.60 ± 0.49
Benztropine Mesylate[c]	0.32 ± 0.30	15.71 ± 3.42	9.20 ± 1.17	8.38 ± 0.96	6.76 ± 1.04	6.20 ± 0.97

[a] Mean ± *SEM*; n = 10 animals per group. [b] 2 ml/kg ip 20 min prior to a 0.5-mg/kg ip injection of haloperidol. [c] 2 mg/kg ip 20 min prior to a 0.5-mg/kg ip injection of haloperidol.

It is unlikely that the antihistaminic properties of benztropine (26) or its inhibiting effect on the uptake of dopamine in certain brain regions (27) accounted for the reported observations. Libertun and McCann (28) found, in rats, that a dose-dependent rise in prolactin produced by histamine was blocked by the antihistamine diphenhydramine. It has also been reported that the inhibition of dopamine uptake by benztropine mesylate may potentiate the synaptic actions of dopamine (27), and dopamine has been shown to be a highly effective inhibitor of prolactin secretion (29). Lastly, although serum prolactin and serum haloperidol levels are statistically highly correlated, they should not be used interchangeably as the basis for experimental observations since >67% of the variance remains unknown.

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