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Effect of Yohimbine on Rat Prolactin Secretion[1]

HERBERT Y. MELTZER,[2] MILJANA SIMONOVIC and GARY A. GUDELSKY

Department of Psychiatry, University of Chicago Pritzker School of Medicine and the Illinois State Psychiatric Institute, Chicago, Illinois

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ABSTRACT

It was recently proposed that yohimbine (YOH), an indole alkaloid with multiple pharmacological effects, is an antagonist of the D_2 dopamine (DA) receptor. Because the pituitary DA receptor involved in the inhibition of prolactin (PRL) secretion is the prototypic D_2 receptor, we examined the effect of YOH on PRL secretion in male rats. YOH produced marked, dose-dependent and sustained increases in plasma PRL levels. However, YOH did not block the inhibitory effect of DA on PRL release from rat pituitary glands *in vitro*, did not displace [^{3}H] spiperone from bovine pituitary membranes and had no effect on the concentration of DA in pituitary stalk plasma of anesthetized rats, suggesting that the stimulation of PRL release by YOH is not due to its antidopaminergic effects. Clonidine, an *alpha*-2 adrenergic agonist, produced a partial, non-dose-dependent inhibition of the YOH-induced rise in serum PRL levels. Two antagonists of the H_1 histamine receptor, diphenhydramine and promethazine, markedly antagonized the PRL-releasing effect of YOH, but another H_1 blocker, chlorpheniramine, and an H_2 antagonist, metiamide, had no effect. Serotonin receptor blockers, cyproheptadine, mianserin and pizotifen, and the opiate antagonist, naloxone, also had no effect on the PRL response to YOH. Nevertheless, the PRL-releasing effect of YOH was potentiated 24 hr after the administration of reserpine or *para*-chlorophenylalanine, an inhibitor of serotonin synthesis. Thus, the mechanisms by which YOH stimulates rat PRL secretion has has not been fully elucidated. It is possible that YOH may stimulate PRL secretion by a novel mechanism, possibly through the intervention of a PRL-releasing factor.

YOH, an indole alkaloid structurally related to reserpine, has a complex pharmacology. Among its reported actions are: 1) selective blockade of *alpha*-2 adrenergic receptors (Starke *et al.*, 1975); 2) stimulation of 5-HT receptors (Sanghvi and Gershon, 1970; Papeschi *et al.*, 1971); 3) blockade of 5-HT receptors (Gyermek, 1961); 4) increased catabolism of newly synthesized striatal DA (Papeschi and Theiss, 1975); and 5) blockade of DA receptors (Scatton *et al.*, 1980). Scatton *et al.* (1980) reported that YOH markedly increased DA turnover in the rat striatum and limbic region and that this increase could not be blocked by clonidine, an *alpha* adrenergic agonist. They also reported that YOH had a number of other effects consistent with a neuroleptic-like action and concluded that it blocks DA receptor of the D_2 type, that is, the type of DA receptor which does not mediate the stimulation of a DA-sensitive adenylate cyclase (Kebabian and Calne, 1979).

The pituitary DA receptor involved in the inhibition of PRL release is considered the prototype of the D_2 receptor. The effect of YOH on rat PRL secretion was therefore examined. YOH has previously been reported to stimulate PRL secretion in monkeys (Gold *et al.*, 1979). This was attributed to its *alpha* adrenoreceptor blocking properties because piperoxane, another *alpha* adrenergic receptor blocker, also stimulated PRL secretion in the monkey and this effect was blocked by clonidine (Gold *et al.*, 1978). However, we have found no effect of piperoxane or clonidine on plasma PRL concentrations in the rat (Meltzer *et al.*, 1978a). Other investigators have found: 1) that the infusion of NE into a pituitary portal vessel either has no effect (Kamberi *et al.*, 1971) or inhibits (Takahara *et al.*, 1974) PRL secretion in anesthetized male rats; 2) that either NE depletion (Donoso *et al.*, 1971; Carr *et al.*, 1977) or i.v. administration of NE (Blake, 1976) inhibits PRL secretion in female rats; 3) that clonidine increases plasma PRL levels in ovariectomized (Stevens and Lawson, 1977), reserpine-treated (Meltzer *et al.*, 1979a) or α-methylparatyrosine-treated male rats (Durand *et al.*, 1977); and 4) that two *alpha* adrenergic antagonists, phenoxybenzamine and phentolamine, increase rat plasma PRL levels in ovariectomized, estrogen-treated rats (Lawson and Gala, 1975). Thus, there is conflicting evidence for a wide range of effects of *alpha* adrenergic agonists and antagonists on rat PRL secretion which may be modified by other factors such as catecholamine depletion or gonadal hormones.

It has been shown that YOH can stimulate rat PRL secretion (Meltzer *et al.*, 1981). The current paper examines the possibility that YOH stimulates rat PRL secretion through DA receptor blockade, *alpha*-2 adrenoreceptor blockade, a reserpine-like mechanism, a 5-HT agonist action, a morphine-like mechanism or a direct effect on the anterior pituitary gland.

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ABBREVIATIONS: YOH, yohimbine; 5-HT, serotonin; DA, dopamine; PRL, prolactin; NE, norepinephrine; PCPA, *para*-chlorophenylalanine.

Materials and Methods

Animals. Sprague-Dawley rats of both sexes, purchased from Sprague-Dawley Inc. (Madison, WI), weighing 175 to 225 g, were used in these studies. They were housed six per cage in temperature (21 ± 2°C) and humidity (60 ± 5%) regulated room with a 12-hr light/dark cycle (lights on at 7:00 A.M.). The animals had free access to Purina Rat Chow and water at all times.

Drugs. The following drugs were gifts: chlorpromazine HCl, metiamide and phenoxybenzamine HCl (Smith Kline and French Laboratories, Philadelphia, PA); chlorpheniramine (Schering Corporation, Kenilworth, NJ); clonidine HCl (Boehringer Ingelheim Ltd., Elmsford, NY); cyproheptadine HCl (Merck Sharp & Dohme Research Laboratories, Rahway, NJ); mianserin HCl (Organon, Inc., West Orange, NJ); naloxone HCl (Endo Laboratories, Inc., Garden City, NY); piperoxane HCl (Rhône-Poulenc, Paris, France); pizotifen-HMA (Sandox Pharmaceuticals, Hanover, NJ); promethazine HCl (Phenergan; Wyeth Laboratories, Philadelphia, PA); propranolol HCl (Ayerst Laboratories, New York, NY); and reserpine (Serpasil; Ciba-Geigy Corp., Summit, NJ). The other drugs were purchased: dopamine HCl, PCPA methyl ester HCl and yohimbine HCl (Sigma Chemical Co. St. Louis, MO); haloperidol (Haldol; McNeil Laboratories, Inc., Fort Washington, PA); and diphenhydramine HCl (Benadryl; Parke, Davis and Company, Detroit, MI).

Doses of basic compounds refer to the salt form. All drugs were administered by the i.p. route in a volume of 1 ml/kg. Control rats always received the appropriate number of saline injections.

***In vivo* studies.** To determine the effect of YOH on plasma PRL levels over time, indwelling catheters were placed into the right jugular vein of 10 male rats according to the method of Weeks and Davis (1964). All operative procedures were performed under pentobarbital anesthesia at least 2 weeks before the experimental session. During this period, rats were housed in individual cages and their catheters were flushed with saline every other day to accustom the rats to the bleeding procedure. Blood samples were obtained from catheterized rats by withdrawing 0.3 to 0.4 ml of blood at designated times. After each bleeding, plasma volume was restored by infusing the appropriate quantity of saline. Each blood sample was immediately transferred into a heparinized 1.5-ml plastic centrifuge tube. In all other experiments, trunk blood was collected after decapitation. Each experimental and control group consisted of five rats.

Prolactin release *in vitro*. To determine the ability of YOH to block the DA-induced inhibition of PRL release, male rats were decapitated and their anterior pituitary glands were removed and bisected in the midsagittal plane. Each hemipituitary was placed in a tube containing 1 ml of a Krebs-Ringer-bicarbonate buffer (118 mM NaCl, 5.0 mM KCl, 1.15 mM $MgSO_4$, 1.15 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 11.1 mM D-glucose). This buffer was equalibrated with 95% O_2-5% CO_2 and adjusted to pH 7.4 immediately before use. The tissue was preincubated for 60 min at 37°C in a shaking water bath in an atmosphere of 95% O_2-5% CO_2. At the end of the preincubation period, the medium was replaced with fresh medium to which the appropriate drug(s) was added, and the incubation was continued for 90 min. At the end of the incubation period the medium from each tube was removed and frozen until assayed for PRL. The anterior pituitary tissue was then blotted and weighed.

Prolactin assay. Plasma (serum) was separated, frozen and later assayed for PRL in duplicate by the double antibody radioimmunoassay as previously described (Meltzer *et al.*, 1979b). The results are expressed as nanograms per milliliter of National Institutes of Health rat PRL-RP-1. The sensitivity of the assay was 1 ng/ml. The intra-assay variance was 5% and the interassay variance less than 10%. All samples from the same experiment were analyzed together.

DA in pituitary stalk plasma. To determine the effect of YOH on the release of DA from tuberoinfundibular neurons, pituitary stalk blood was collected from ovariectomized female rats under pentobarbital-induced anesthesia according to the procedure of Porter and Smith (1967). Stalk blood was collected through a polyethylene cannula placed over the transected stalk and was kept cold (2–4°C) throughout the collection period. The blood samples were centrifuged for 1 min at 10,000 × *g* in a Beckman Microfuge, and the plasma was acidified with an equal volume of 0.6 M perchloric acid containing 0.1% ethylene glycol bis-(β-aminoethyl ether)-N,N'-tetraacetic acid. The acidified plasma samples were centrifuged, and the resulting supernatant fluids were stored at −20°C until analyzed for DA by a radioenzymatic procedure similar to that described by Ben-Jonathan and Porter (1976).

[^{3}H]Spiperone binding. Bovine anterior pituitary glands or striata were obtained within 2 hr of slaughter. They were immediately frozen on Dry Ice and stored at −80°C until used in the binding assay. Pituitary or striatal membranes were prepared and the binding assay was performed as previously described (Meltzer *et al.*, 1979b). Briefly, incubation tubes contained 50 mM Tris-HCl (pH 7.4 at 25°C), 0.05% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.13 nM (for striatal samples) or 0.26 nM (for pituitary samples) [^{3}H] spiperone (23.0 Ci/mmol; New England Nuclear, Boston, MA), 1 μM *d*-butaclamol, approximately 0.5 mg of protein and one of six different concentrations of YOH (from 10^{-8} to 10^{-4} M) or chlorpromazine (from 10^{-9} to 10^{-6} M). The samples were incubated at 37°C for 10 min and then rapidly filtered under vacuum through Whatman GF/B glass fiber filters. The filters were washed and the radioactivity was counted as described (Meltzer *et al.*, 1979b). Specific [^{3}H]spiperone binding was defined as the difference between binding in the absence and in the presence of 1 μM *d*-butaclamol and represented 65 to 70% of the total binding. Each drug concentration was tested in triplicate and the experiment was repeated twice.

Statistics. Statistical analysis of the results was carried out by using two-tailed Student's *t* test.

Results

The results summarized in figure 1 illustrate that 30 min after the administration of YOH to male rats, there was a dose-related increase in serum PRL levels. The lowest dose of YOH

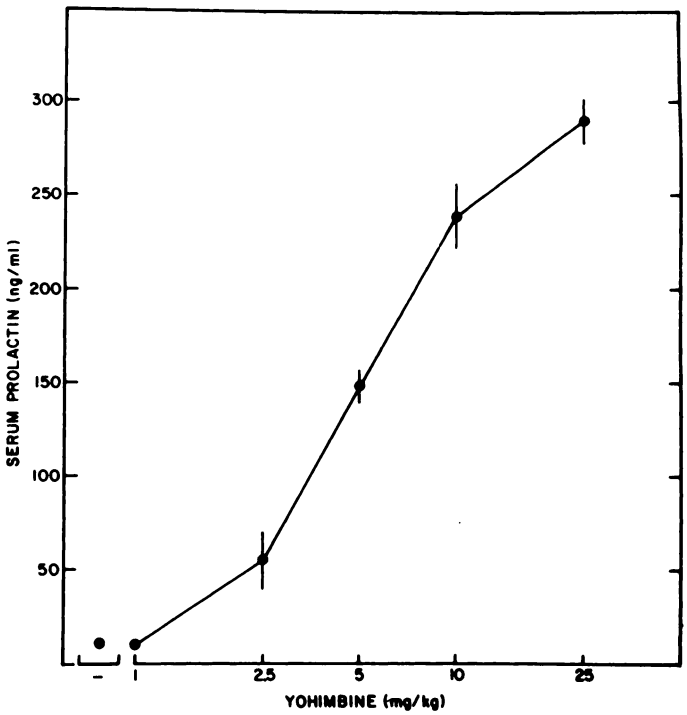

Fig. 1. The dose-related effect of YOH on rat serum PRL levels. Rats received YOH (1, 2.5, 5, 10 or 25 mg/kg) or saline and were decapitated 30 min later. Each value represents mean serum PRL levels for a group of five rats. Vertical bars represent 1 S.E.

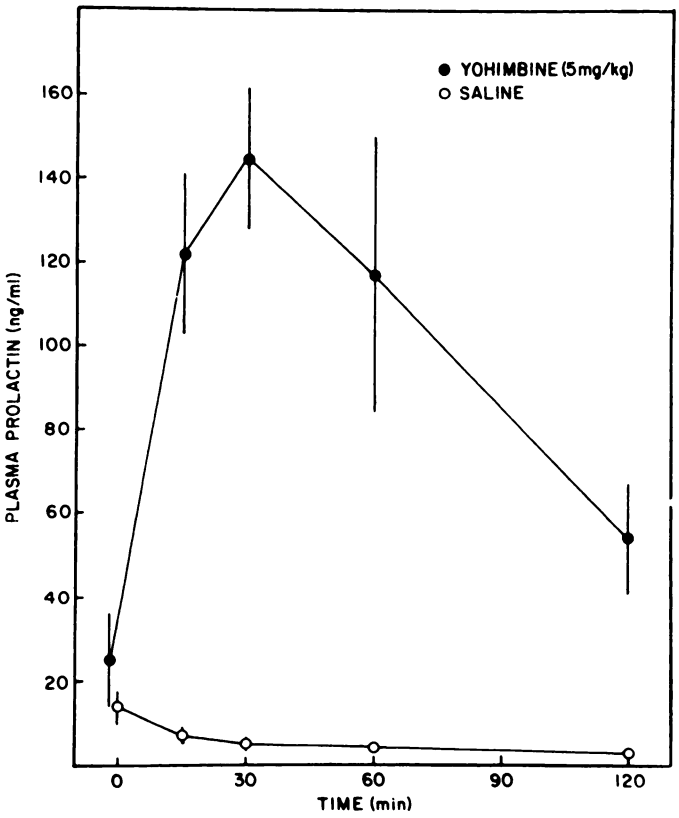

Fig. 2. Time-dependent effect of YOH on rat plasma PRL levels. Groups of five rats received YOH (5 mg/kg) or saline immediately after the first bleeding. Four subsequent blood samples were obtained from each rat at designated times. Shown are means ± S.E.

TABLE 1

Antagonism of the DA-induced inhibition of PRL secretion *in vitro*

Anterior pituitary tissue from male rats was incubated for 90 min at 37°C in the presence of the indicated drugs. Tissue incubated in drug-free medium served as control. Numbers in parentheses indicate the number of hemipituitaries in each group. Values are expressed as percentage of control which was 237 ± 40 ng of PRL per mg of tissue.

Treatment	PRL Release
	% of control
Control	100 ± 17 (6)
YOH (10^{-6} M)	100 + 11 (6)
DA (10^{-6} M)	34 ± 4 (6)
YOH (10^{-7} M) + DA	43 ± 9 (6)
YOH (10^{-6} M) + DA	30 ± 3 (6)
Haloperidol (10^{-7} M) + DA	100 ± 9 (6)

tested (*i.e.*, 1 mg/kg), had no effect, whereas each of the four higher doses (*i.e.*, 2.5, 5, 10 and 25 mg/kg) significantly ($P < .01$) increased serum PRL levels (fig. 1). The maximal increase in serum PRL levels ranged from 25- to 30-fold above the base line. The effect of 50 mg/kg YOH on PRL secretion could not be determined as this dose was lethal in five out of five rats.

The time course for the stimulation of PRL secretion by YOH (5 mg/kg) is presented in figure 2. Plasma PRL concentrations were significantly ($P < .05$) increased within 15 min of YOH administration and remained elevated for more than 2 hr.

Yohimbine (10^{-6} M) had no effect on the release of PRL from rat pituitary glands *in vitro* (table 1). DA (10^{-6} M) produced a 66% inhibition of PRL release, and this inhibitory effect was markedly attenuated by haloperidol (10^{-7} M). However, YOH (10^{-7} or 10^{-6} M) had no effect on the DA-induced inhibition of PRL release.

The effects of reserpine or YOH on the concentration of DA in pituitary stalk plasma of anesthetized female rats are shown in figure 3. The mean concentration of DA in pituitary stalk plasma of rats treated with reserpine (2.5 mg/kg) was significantly ($P < .001$) lower than that of control rats. In contrast, YOH (10 mg/kg) had no effect on the concentration of DA in portal plasma.

The ability of YOH and chlorpromazine, which is as potent as YOH in stimulating PRL secretion *in vivo*, to displace [^{3}H] spiperone from bovine striatal and pituitary membrane preparations were compared. The IC_{50} values for YOH and chlorpromazine to displace [^{3}H]spiperone from bovine striatal membranes were 2732 ± S.E. 220 nM and 37 ± 7 nM, respectively. YOH, at concentrations of 10^{-4} to 10^{-7} M, did not displace [^{3}H] spiperone from pituitary membranes, whereas the IC_{50} for chlorpromazine was 178 ± 4 nM. Thus, chlorpromazine was only slightly less effective in displacing [^{3}H]spiperone in the pituitary than in the striatum, whereas there was an important difference between the effects of YOH at these two DA receptors.

As can be seen in figure 4, the increase in PRL levels produced by YOH (5 mg/kg) was partially inhibited by clonidine, from 0.1 to 1.0 mg/kg, but the inhibition was not dose-related. Higher doses of clonidine (*i.e.*, 2.5 and 5.0 mg/kg) did not inhibit the

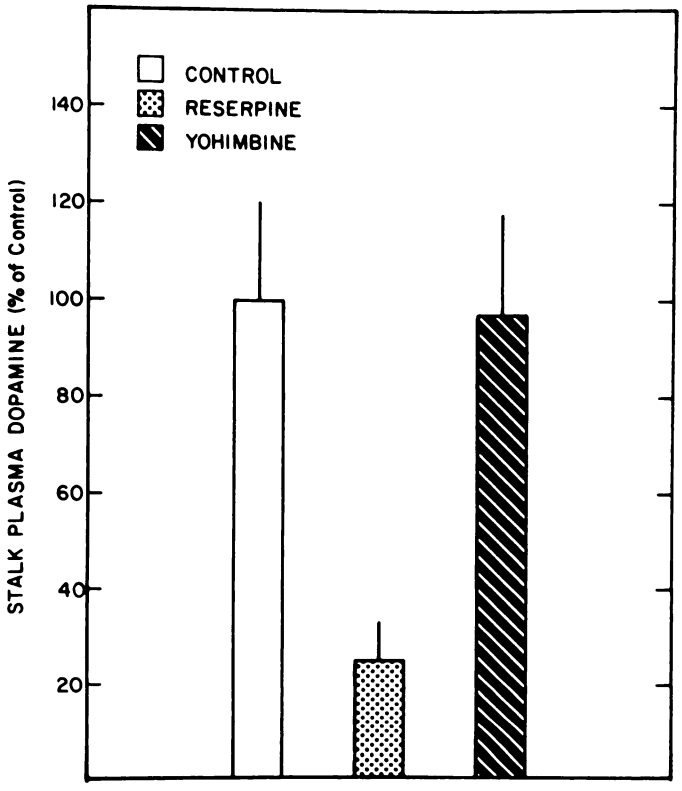

Fig. 3. The effect of YOH or reserpine on the concentration of DA in pituitary stalk plasma. Pituitary stalk blood was collected for 60 min from five ovariectomized female rats (control) and from five ovariectomized rats given reserpine (2.5 mg/kg) 2 hr before the start of the collection. In another experiment, stalk blood was obtained from five ovariectomized rats during the 40-min period before (control) and after the administration of YOH (10 mg/kg). The DA values for the control groups were pooled. Shown are mean levels of DA in pituitary stalk plasma. Vertical lines represent 1 S.E. Values are expressed as percentage of control which was 1.0 ± 0.2 ng of DA per ml of plasma.

increase in PRL secretion produced by this dose of YOH (data not presented). Two other *alpha* adrenergic blockers, phenoxybenzamine (10 mg/kg) and piperoxane (0.5 and 10 mg/kg), and the *beta* adrenergic blocker, propranolol (10 mg/kg), given 60 min before YOH (5 mg/kg), had no effect on the YOH-induced increase in PRL secretion (data not presented).

Cyproheptadine (10 mg/kg), pizotifen (2.5 mg/kg), mianserin (10 mg/kg) and naloxone (10 mg/kg) had no effect on the increase in serum PRL levels produced by YOH, 10 mg/kg (data not presented). However, the stimulation of PRL secretion by YOH was significantly enhanced 24 hr after PCPA (300 mg/kg) treatment. The mean (±1 S.E.) serum PRL levels produced by YOH (10 mg/kg) in groups of five rats pretreated with saline or PCPA were, respectively, 233 ± 26 and 352 ± 26 ng/ml ($P < .05$).

The effects of antihistamines on the stimulation of PRL secretion by YOH are summarized in table 2. Two antagonists of the H_1 histamine receptor, diphenydramine (10 and 25 mg/kg) and promethazine (10 mg/kg), markedly reduced the PRL response to YOH (5 mg/kg); the effect of the former drug was dose-related (table 2). In contrast, chlorpheniramine (10 mg/kg), another H_1 blocker, and the H_2 antagonist, metiamide (10 mg/kg), had no effect on the YOH-induced rise in serum PRL levels (table 2).

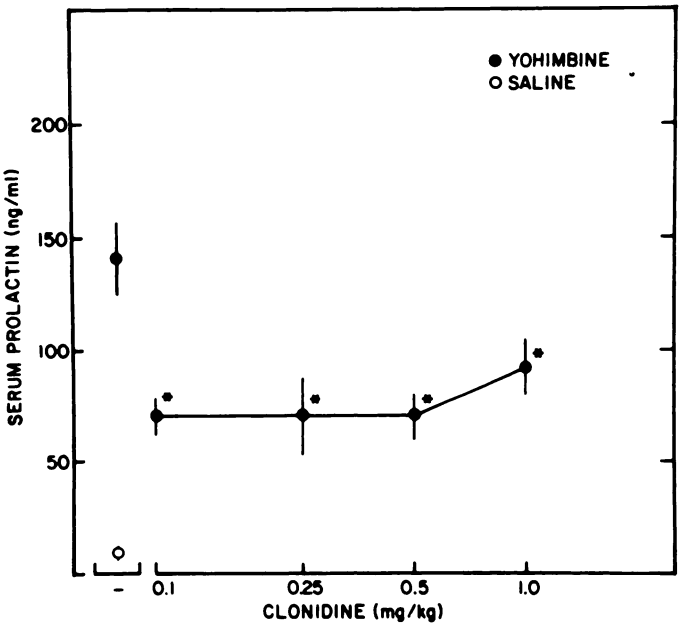

Fig. 4. The effect of clonidine on the YOH-induced stimulation of PRL secretion. Rats received the designated doses of clonidine or saline 60 min and YOH (5 mg/kg) or saline 30 min before decapitation. Shown are mean serum PRL levels for groups of five rats. Vertical bars represent 1 S.E. *Significantly different from the saline + YOH group, $P < .05$.

We have also tested the effect of reserpine pretreatment on the stimulation of PRL release by YOH. Serum PRL levels are significantly ($P < .05$) elevated from 30 min to at least 24 hr after reserpine administration (results not shown). The effect of YOH in reserpine-treated rats differed with the length of the pretreatment interval (fig. 5). The PRL-releasing effect of YOH (5 mg/kg) was blocked 6 hr after, but was potentiated 24 hr after the administration of reserpine. In contrast, the PRL-releasing effect of a second injection of reserpine (5 mg/kg) was significantly attenuated both 6 and 24 hr after the initial administration of reserpine (fig. 5). Unlike YOH, the *alpha* adrenergic antagonists, piperoxane (2 mg/kg) and phenoxybenzamine (10 mg/kg), did not stimulate PRL secretion in rats given reserpine 24 hr previously (results not shown).

Discussion

YOH produced a rapid, sustained and dose-related increase in plasma or serum PRL levels in male rats. Because of the complex pharmacology of YOH and the numerous factors which can affect PRL secretion, there are many possible mechanisms through which YOH may increase circulating PRL levels.

We first considered the possibility that YOH stimulates PRL secretion by interfering with the tonic dopaminergic inhibition of PRL release. The observation that YOH increases rat striatal DA turnover (Anden and Grabowska, 1976; Scatton *et al.*, 1980; Hedler *et al.*, 1981), but has no effect on DA-sensitive adenylate cyclase (Scatton *et al.*, 1980), led to the hypothesis that it selectively blocks the subpopulation of DA receptors known as the D_2 receptors (Scatton *et al.*, 1980). Yohimbine may also interfere with the availability of newly synthesized DA in the striatum (Papeschi and Theiss, 1975). If similar effects occurred at pituitary DA receptors and/or the tuberoinfundibular dopaminergic neurons, the decrease in dopaminergic transmission would lead to increased PRL secretion. As the pituitary DA receptor involved in the inhibition of PRL release is of the D_2 type (Kebabian and Calne, 1979), our finding that YOH elevates rat serum PRL levels is consistent with the hypothesis that it blocks D_2 receptors. However, the failure of YOH to antagonize the inhibitory effect of DA on PRL secretion *in vitro* (table 1), as do all other D_2 receptor blockers, or to inhibit [^{3}H]spiperone binding to bovine pituitary membrane preparations, constitutes strong evidence against such a hypothesis.

The inability of YOH to enhance the release of PRL from the anterior pituitary glands *in vitro* (table 1) rules out the possibility that it stimulates PRL secretion by a direct pituitary action. The possibility that YOH stimulates PRL secretion through a reserpine-like mechanism is equally doubtful in view

TABLE 2

The effect of antihistamines on YOH-induced increase in serum PRL levels

Rats received antihistamines or saline 60 min and YOH 30 min before decapitation. Each value represents mean (±S.E.) serum PRL levels for a group of five rats.

Group	Drug 1	Dose	Drug 2	Dose	Serum PRL	Comparison	P
		mg/kg		mg/kg	ng/ml		
1	Saline		YOH	5	142 ± 13		
2	Metiamide	10	YOH	5	112 ± 11	1-2	N.S.
3	Chlorpheniramine	10	YOH	5	148 ± 17	1-3	N.S.
4	Promethazine	10	YOH	5	36 ± 13	1-4	<.001
5	Diphenhydramine	5	YOH	5	126 ± 5	1-5	N.S.
6	Diphenhydramine	10	YOH	5	64 ± 10	1-6	<.001
7	Diphenhydramine	25	YOH	5	31 ± 7	1-7	<.001

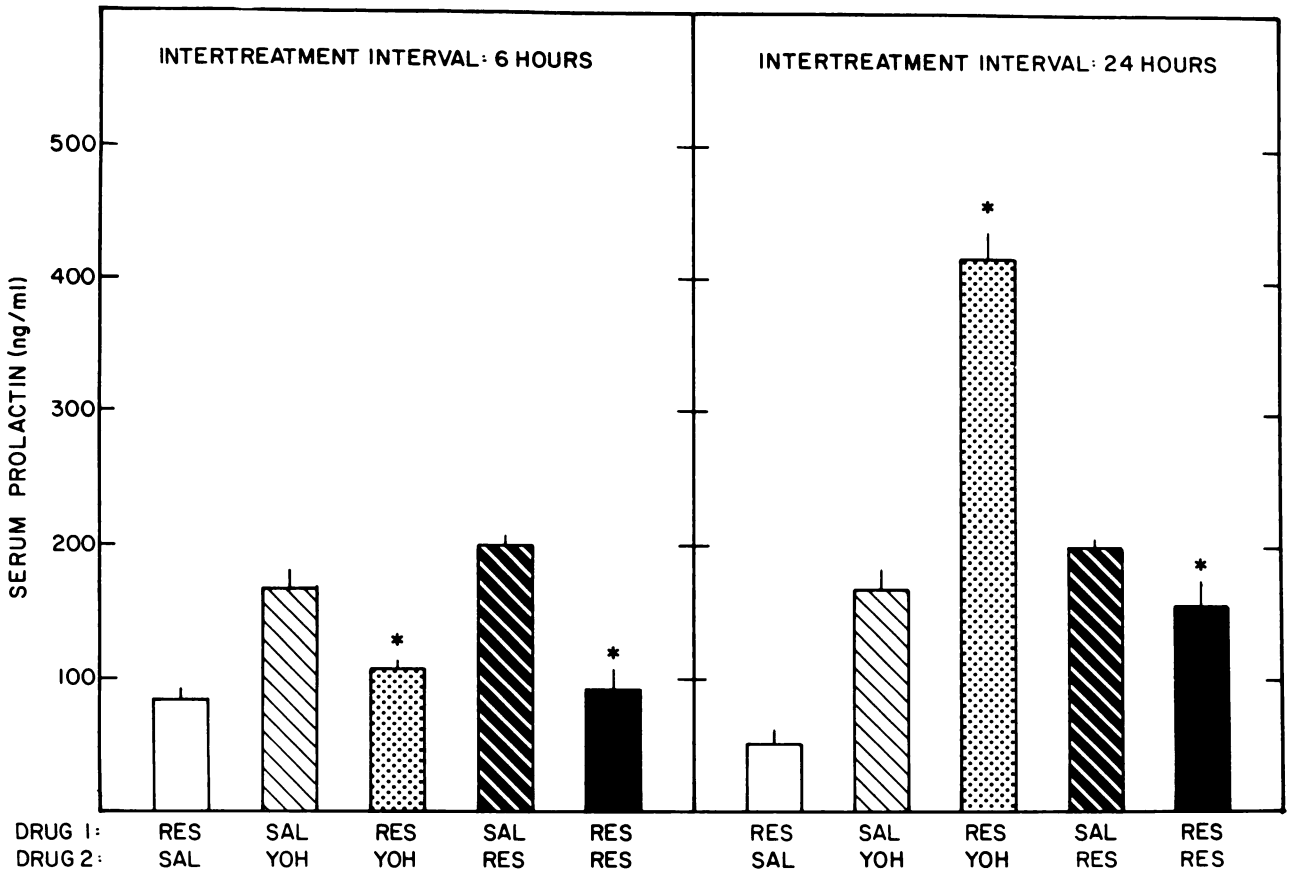

Fig. 5. The effect of reserpine (RES) pretreatment on YOH- or RES-induced increase in serum PRL levels. Rats were treated with RES (5 mg/kg) or saline (SAL); 6 or 24 hr later they received YOH (5 mg/kg), RES (5 mg/kg) or SAL. Rats were decapitated 30 min after YOH or 60 min after the second RES injection. Shown are mean serum PRL levels for groups of five rats. Vertical bars represent 1 S.E. *Significantly different from the appropriate control, $P < .05$.

of its inability to decrease DA concentrations in pituitary stalk plasma as reserpine decreases stalk plasma concentrations of DA by more than 50% (fig. 3) (Gudelsky and Porter, 1979).

We next considered whether YOH produces its effect on rat PRL secretion by blocking *alpha* adrenergic receptors. We have previously reported that piperoxane, a selective *alpha*-2 adrenergic antagonist, has no effect on PRL secretion in male rats (Meltzer *et al.*, 1978a). In this study, neither piperoxane nor phenoxybenzamine, another *alpha* adrenergic antagonist, had any effect on PRL secretion in naive or reserpine-treated rats. In addition, clonidine, an *alpha* adrenergic agonist, produced only a partial inhibition of YOH induced rise in serum PRL levels, but this effect was not dose-related. The inability of clonidine to block YOH-induced stimulation of PRL release in doses that completely antagonize the YOH-induced increase in NE turnover (Scatton *et al.*, 1980), as well as the failure of two other *alpha* adrenergic blockers to elevate serum PRL levels in male rats, suggest that *alpha* adrenergic receptors are not primarily involved in the action of YOH on rat PRL secretion, although there is evidence for such a mechanism in monkeys (Gold *et al.*, 1979). It is of interest that YOH stimulates PRL secretion in man (Charney *et al.*, 1981), whereas *alpha* adrenergic blockade has no effect on basal or stimulated human PRL secretion (Board *et al.*, 1977; Lauridsen *et al.*, 1978; Lorenzi *et al.*, 1979).

The lack of an effect of YOH on PRL secretion in rats given reserpine 6 hr before is similar to the inability of reserpine itself to further elevate serum PRL levels 6 hr after the first reserpine injection. However, reserpine, given 24 hr before, *potentiated* the YOH-induced increase in PRL secretion, but *reduced* the effect of reserpine. These results also suggest that the stimulation of PRL release by YOH depends on a mechanism depleted by or blocked by reserpine and that YOH and reserpine elevate PRL through different mechanisms, as the effect of YOH, but not that of reserpine, is potentiated 24 hr after the reserpine pretreatment.

The inhibitory effect of reserpine on the stimulation of PRL secretion by YOH or by a second injection of reserpine may be related to the recent finding that reserpine inhibits PRL release from the pituitary gland *in vitro* by interfering with the calcium-dependent release of this hormone (Login and MacLeod, 1981). The observation that YOH-induced rise in serum PRL levels is enhanced 24 hr after reserpine may be related to the similar effect of PCPA pretreatment (see below).

The possibility that YOH stimulates PRL secretion *in vivo* by stimulating 5-HT receptors is supported by the marked enhancement of the release of PRL in PCPA-pretreated rats. We have previously reported that the depletion of brain 5-HT with PCPA potentiates the PRL-releasing effect of quipazine, N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine, mescaline and other 5-HT agonists (Meltzer *et al.*, 1976, 1978b). This effect was attributed to the supersensitivity of 5-HT receptors secondary to 5-HT depletion. The lack of effect of the 5-HT receptor blockers, cyproheptadine, mianserin and

pizotifen, on the YOH-induced increase in PRL secretion does not rule out a serotonergic mechanism. Although these drugs can block the increase in PRL secretion produced by 5 hydroxytryptophan and quipazine, they are not effective antagonists of two other 5-HT agonists, 5-methoxy-N,N-dimethyltryptamine and N,N-dimethyltryptamine (H. Y. Meltzer, M. Simonovic and V. S. Fang, unpublished data). Conceivably, YOH and the indole hallucinogens act at 5-HT receptors which are not blocked by these agents.

The inability of naloxone to inhibit the YOH-induced increase in serum PRL levels argues against the possibility of an opiate-like mechanism, as naloxone can inhibit the increase in PRL secretion due to morphine and endogenous opiate-like substances (Meites *et al.*, 1979). Moreover, the stimulation of PRL release by morphine is blocked by cyproheptadine, and by PCPA pretreatment (Koenig et al., 1979). Thus, a morphine-like action of YOH is also unlikely on these grounds.

There is some evidence that histamine-containing neurons may be involved in the regulation of PRL secretion. Intraventricular administration of histamine stimulates PRL secretion in male rats, perhaps through the activation of H_1 receptors, as this effect is blocked by chlorpheniramine (Donoso and Bannza, 1976). The observation that YOH-induced stimulation of PRL release is markedly antagonized by diphenydramine and promethazine suggests that a histaminergic mechanism may be involved in this effect of YOH. Moreover, there is evidence that some drugs, including clonidine, can stimulate *alpha*-2 adrenergic and histamine receptors (Finch *et al.*, 1978; Braunwalder *et al.*, 1981). We have found that promethazine, but not diphenhydramine, weakly antagonized the specific binding of [^{3}H] YOH to rat brain membranes (M. Mijuni and H. Y. Meltzer, unpublished data). The inhibitory effect of reserpine (6 hr after its administration) on the YOH-induced stimulation of PRL release may also be consistent with the hypothesis that YOH stimulates PRL secretion through a histaminergic mechanism, as it has been shown that reserpine depletes hypothalamic histamine stores (Snyder *et al.*, 1966). However, the fact that chlorpheniramine, cyproheptadine and mianserin, all of which are potent blockers of H_1 receptors (Peroutka and Snyder, 1981), had no effect on the YOH-induced rise in serum PRL levels tends to argue against such a possibility. The inhibitory effect of diphenydramine and promethazine on the PRL-stimulating effects of YOH may be due to some action of these agents other than their ability to block H_1 receptors.

It is unlikely that YOH stimulates rat PRL secretion *via* release of thyrotropin-releasing hormone, which is a potent PRL releaser (Mueller *et al.*, 1973), as Krulich *et al.* (1982) reported that systemic YOH, 0.5 and 2.5 mg/kg, significantly decreased thyroid-stimulating hormone levels in rat and inhibited the clonidine-induced increase in thyroid-stimulating hormone secretion.

The results of these and other studies demonstrate that the stimulation of rat PRL secretion by YOH is not mediated by: 1) the blockade of the pituitary DA receptor; 2) a direct effect on the pituitary gland; 3) the inhibition of DA release from tuberoinfundibular neurons; 4) the activation of opiate receptors; or 5) the release of thyrotropin-releasing hormone. Partial blockade of the PRL response to YOH by clonidine, diphenhydramine and promethazine and potentiation by PCPA suggest that adrenergic, histaminergic and serotonergic mechanisms may be factors, but there is considerable evidence inconsistent with these hypotheses. There are still other mechanisms by which YOH could stimulate PRL secretion: *e.g.*, by increasing the release of vasoactive intestinal peptide or other, as yet unidentified, PRL-releasing factors. It is also possible that YOH might decrease the clearance of PRL from plasma. Further studies are needed to explore the above-mentioned possibilities.

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Send reprint requests to: Herbert Y. Meltzer, M.D., Department of Psychiatry, University of Chicago, Pritzker School of Medicine, 950 E. 59th St., Chicago, IL 60637.