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Thyrotropin-Releasing Hormone Blockade of the Ergocryptine and Apomorphine Inhibition of Prolactin Release *in Vitro* (38839)

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Recently, a pattern has begun to emerge indicating that prolactin secretion from the anterior pituitary is a resultant of the interaction of factors stimulatory and inhibitory to prolactin release. It was suggested that dopamine or another catecholamine is a prolactin inhibiting factor (1–3) because it strongly inhibited the release of both newly synthesized ^{3}H-prolactin and of radioimmunoassayable prolactin from the rat anterior pituitary incubated *in vitro*. This inhibitory effect of dopamine can be completely blocked by perphenazine or haloperidol; these two α-adrenergic blocking agents can also prevent the inhibitory effects of apomorphine or ergocryptine on prolactin release (2). Likewise pimozide, a specific dopamine antagonist, can block the inhibitory effects of dopamine (4) or of apomorphine (2, 5) on prolactin release.

The physiological stimulus to prolactin release may be the thyrotropin-releasing hormone (TRH) (6, 7). This hypothalamic hormone rapidly stimulates prolactin release *in vivo* but has little effect upon the release of prolactin from normal pituitary tissue incubated *in vitro*. However, we have recently demonstrated that TRH can completely block the inhibitory effect of dopamine upon prolactin release from the rat anterior pituitary incubated *in vitro* (8). The results to be presented here demonstrate that TRH can also block the inhibition of prolactin release exerted by either apomorphine or ergocryptine.

Materials and Methods. Synthetic TRH was a gift from Abbott Laboratories, Chicago, IL. Apomorphine hydrochloride was obtained from Merck, and ergocryptine was a gift of Sandoz Pharmaceuticals. Rat prolactin, used as a standard in the polyacrylamide gel electrophoresis studies performed here, was a gift of NIAMDD Rat Pituitary Hormone Program.

Anterior hemipituitaries were obtained from 210- to 240-g Sprague–Dawley female rats (Flow Research Animals, Dublin, VA). Each incubation tube contained two weighed hemipituitaries (each from a separate animal) incubated in 0.50 ml Krebs–Ringer bicarbonate containing 0.2% glucose and a previously described (9) amino acid mixture. TRH and either ergocyrptine or apomorphine were present at concentrations indicated in the text, and [4,5-^{3}H]leucine (New England Nuclear, 50 Ci/mmole) was present at 10 μCi/ml. Incubations were carried out in a Dubnoff shaker at 37° under an atmosphere of 95% O_2–5% CO_2 for 5 hr, or for 6 hr after a prior 45-min incubation in Krebs–Ringer bicarbonate.

After incubation each two hemipituitaries were homogenized in 1.0 ml of 1 m*M* tetra Na EDTA (pH 10.5). Duplicate aliquots of each homogenate (25 μl) and incubation medium (50 μl) were subjected to polyacrylamide gel electrophoresis according to the Reisfeld *et al.* (10) modification of the method of Jones *et al.* (11). The protein bands on the gels were stained with amido black, and the prolactin band was identified by comparison with purified rat prolactin. The band corresponding to prolactin was cut from the gel, dissolved in 30% H_2O_2 at 105° and the radioactivity determined by liquid scintillation techniques.

Radioimmunoassay of incubation media was carried out using the reagents and protocol supplied by the NIAMDD Hormone Distribution Program. The prolactin was iodinated with ^{125}I obtained from Cambridge Nuclear Radiopharmaceutical Corp., Billerica, MA. Sheep anti-rabbit gamma globulin was obtained from Dr. Ann Johanson of the University of Virginia School of Medicine.

The data are presented as means ± SEM.

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Student's *t* test was used for statistical evaluation.

Results. The data in Table I show the effects of ergocryptine and apomorphine upon the release of prolactin labeled with [^{3}H]leucine during a 5-hr incubation. Ergocryptine at a concentration of 2×10^{-10} *M* caused a 50% inhibition in the release of labeled prolactin. When the concentration of the ergot alkaloid was doubled, it resulted in a greater inhibition in hormone release. We have previously reported that TRH has no *in vitro* effect to stimulate basal prolactin secretion (8). However, the inhibition of prolactin secretion caused by ergocryptine was largely overcome by co-incubation with 5 or 25 ng TRH/ml.

Apomorphine also inhibited the *in vitro* secretion of newly synthesized prolactin. Once again, co-incubation with TRH resulted in virtually complete restoration of the pituitary's ability to secrete prolactin.

Under circumstances where ^{3}H-prolactin release was inhibited either by apomorphine or ergocryptine, there was a significant accumulation of pituitary ^{3}H-prolactin (Table II). There was also a small inhibition of total incorporation into ^{3}H-prolactin (pituitary plus medium) in the presence of either inhibitor; however, this inhibition was statistically significant only in the case of ergocryptine. In the presence of either ergocryptine or apomorphine plus TRH, total incorporation (pituitary plus medium) was not significantly different from controls.

The TRH blockade of the inhibitory effects of both ergocryptine and apomorphine was also apparent when radioimmunoassayable prolactin released into the medium was measured. Both inhibitors significantly lowered medium levels, whereas the addition of TRH plus inhibitor led to prolactin values insignificantly lower than controls. Of interest is the fact that whereas ^{3}H-prolactin release in the presence of ergocryptine and apomorphine was 6.1% and 41.6%, respectively, of control values, radioimmunoassayable prolactin release was approximately 50% of control values in both cases. This indicates a greater sensitivity to ergocryptine of the mechanisms governing the secretion of newly synthesized prolactin as compared to the preformed prolactin.

As noted in both Tables I and II, ergocryptine inhibited prolactin release at concentrations 100-fold lower than those of apomorphine. Furthermore, TRH was able to completely block the inhibitory effects of apomorphine on ^{3}H-prolactin release, whereas in the presence of ergocryptine ^{3}H-prolactin release did not return to more than 73% of control values. However, as noted in Table II, TRH was equally effective in blocking both the ergocryptine and apomorphine inhibition of radioimmunoassayable prolactin release.

Neither TRH alone or TRH in conjunction with apomorphine or ergocryptine affected ^{3}H-growth hormone release.

Discussion. A recent report (8) from our laboratories showed that TRH, a proposed prolactin-releasing factor, can block the inhibitory effects of dopamine, a proposed prolactin-inhibiting factor. These data were consistent with TRH and dopamine having prolactin-releasing and prolactin-inhibiting activities, respectively. Both apomorphine and ergocryptine may exert their inhibition of prolactin secretion by binding to "dopaminergic" or α-adrenergic receptors (2, 5). The data presented here, demonstrating that TRH can block the inhibitory effect of these compounds as well as that of dopamine, support this conclusion.

We previously showed (8) that dopamine in a 5- or 6-hr incubation significantly inhibited incorporation of [^{3}H]leucine as well as inhibiting hormone release; addition of TRH (5 ng/ml) to the dopamine-containing solution returned total incorporation (pituitary plus medium) to levels not significantly lower than controls. However, this dopamine inhibition of incorporation had earlier been shown (1) to occur as an event secondary to the inhibition of prolactin release. In the investigation reported here, incorporation into ^{3}H-prolactin (pituitary plus medium) in the presence of 4×10^{-10} *M* ergocryptine and 5×10^{-8} *M* apomorphine remained at 78% and 84%, respectively, of control values during a 6-hr incubation. There was a resultant retention of ^{3}H-prolactin within the gland. This suggests that the initial effect of ergocryptine and apomorphine, like that of dopamine, is upon prolactin release with effects upon prolactin synthesis being sec-

TABLE I. TRH BLOCKADE OF THE ERGOCRYPTINE- AND APOMORPHINE-MEDIATED INHIBITION OF ^{3}H-PROLACTIN RELEASE.[a]

		^{3}H-Prolactin released into medium (cpm/mg tissue)	P[c]	Percentage of control release
Controls	(36)[b]	4158 ± 318		
Ergocryptine (2×10^{-10} M)	(3)	2101 ± 4	0.001	50.5
Ergocryptine (2×10^{-10} M, + 5 ng TRH/ml)	(4)	3051 ± 373[d]	0.05	73.4
Ergocryptine (4×10^{-10} M)	(8)	1018 ± 346	0.001	24.5
Ergocryptine (4×10^{-10} M, + 5 ng TRH/ml)	(4)	2340 ± 71[d]	0.001	56.3
Ergocryptine (4×10^{-10} M, + 25 ng TRH/ml)	(4)	2327 ± 232[d]	0.001	56.0
Apomorphine (1.5×10^{-8} M)	(3)	1253 ± 237	0.001	30.1
Apomorphine (1.5×10^{-8} M, + 5 ng TRH/ml)	(4)	4107 ± 1160[d]	n.s.	98.8

[a] Samples were incubated for 5 hr in Krebs-Ringer bicarbonate containing [^{3}H]leucine. Levels of ^{3}H-prolactin released into medium were assayed as described under Methods.

[b] Numbers in parentheses in both Tables I and II indicate total samples in each group.

[c] P Value compared to controls for both Tables I and II.

[d] Value significantly greater ($P < 0.05$) than value for inhibitor alone at concentration specified.

TABLE II. TRH BLOCKADE OF THE ERGOCRYPTINE AND APOMORPHINE EFFECTS ON PROLACTIN SECRETION.[a]

		Incorporation into prolactin (cpm/mg tissue)					
		Pituitary	P	Medium + pituitary	P	^{3}H-Prolactin released into medium	P
Control	(18)	6,266 ± 388		13,880 ± 896		7,615 ± 838	
Ergocryptine	(3)	10,326 ± 622	0.001	10,787 ± 741	0.02	462 ± 109	
Ergocryptine + TRH	(3)	11,164 ± 1097	0.001	13,908 ± 1482	n.s.	2,745 ± 415[b]	0.001
Apomorphine	(4)	8,911 ± 646	0.01	11,599 ± 1066	n.s.	2,614 ± 582	0.001
Apomorphine+TRH	(4)	7,459 ± 788	n.s.	13,477 ± 963	n.s.	6,017 ± 692[b]	n.s.
		Radioimmunoassayable prolactin released into medium, μg/mg tissue				P	
Control	(8)	5.58 ± 0.58					
Ergocryptine[b]	(3)	2.61 ± 0.39				0.01	
Ergocryptine + TRH	(3)	4.32 ± 0.21[b]				n.s.	
Apomorphine	(4)	2.75 ± 0.25				0.01	
Apomorphine + TRH	(4)	4.57 ± 0.40[b]				n.s.	

[a] Pituitaries were preincubated in Krebs-Ringer bicarbonate alone for 45 min before transferring to medium containing ^{3}H-leucine for a 6-hr incubation. Ergocryptine when present was added at 4×10^{-10} M, apomorphine at 5×10^{-8} M and TRH at 1.4×10^{-8} M (5 ng/ml).

[b] Value significantly greater ($P < 0.02$) than value for the inhibitor alone.

ondary. Greater inhibition of incorporation than that seen here was observed using 3×10^{-9} M ergocryptine and 6.4×10^{-8} M apomorphine (2).

TRH blockade of inhibitory effects upon prolactin release and synthesis may be more sensitive to inhibitor concentration than is the blockade exerted by the tranquilizing drugs. Either haloperidol or perphenazine, at 5×10^{-8} M, completely blocked the inhibitory effects of 5×10^{-7} M dopamine (2). However, in order to demonstrate a complete blockade by TRH of the dopamine effect, it was necessary to decrease the dopamine concentration to 7.5×10^{-8} M (8). Perphenazine (2.5×10^{-8} M) completely blocked the effect of 3×10^{-9} M ergocryptine (2). In the present studies, 5 or 25 ng/ml of TRH (1.4

$\times 10^{-8}$ *M* and 7×10^{-8}*M*) only partially blocked the 4×10^{-10} *M* ergocryptine inhibition of ^{3}H-prolactin release.

Interestingly, although 5 ng/ml of TRH only partially blocked the 4×10^{-10} *M* ergocryptine inhibition of ^{3}H-prolactin release, this TRH concentration completely restored radioimmunoassayable prolactin release. This may indicate that the newly synthesized ^{3}H-prolactin and the preformed prolactin are at least partially in separate pools. In the case of apomorphine, the effects of TRH and perphenazine more closely mimicked each other. Perphenazine (1.25×10^{-8} *M*) exerted a complete blockade of the effects of 6.4×10^{-8} *M* apomorphine (2). Likewise, TRH (1.4×10^{-8} *M*, 5 ng/ml) completely blocked the effects of 5×10^{-8} *M* apomorphine upon both ^{3}H-prolactin and radioimmunoassayable prolactin release (Table II).

The present data lend further support to the hypothesis that net prolactin release is a resultant of the interaction of factors stimulatory and inhibitory to release.

Summary. The interaction of thyrotropin-releasing hormone (TRH) and ergocryptine or apomorphine in affecting prolactin secretion was examined. Ergocryptine and apomorphine inhibited the release of ^{3}H-prolactin labeled in an *in vitro* incubation of the rat anterior pituitary and led to a retention within the gland of ^{3}H-prolactin. TRH (1.4×10^{-8} *M*) partially blocked the inhibitory effect of 4×10^{-10} *M* ergocryptine on ^{3}H-prolactin release and totally blocked the effect on radioimmunoassayable prolactin release. TRH also completely blocked the inhibitory effects of 5×10^{-8} *M* apomorphine on both ^{3}H-prolactin and radioimmunoassayable prolactin release. Since TRH can also completely block the inhibitory effects of dopamine upon prolactin secretion, these results are consistent with apomorphine and ergocryptine stimulating "dopaminergic" sites and also further support the role of TRH as a prolactin-releasing factor.

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