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Reserpine Inhibits Rat Anterior Pituitary Hormone Secretion *in Vitro*: Effects on GH, TSH, and LH (41506)

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Abstract. We have extended our observations on the ability of reserpine to inhibit secretion of anterior pituitary hormones *in vitro*. The release of newly synthesized [^{3}H]GH and radioimmunoassayable TSH and LH from female rat anterior hemipituitary glands was measured after 5 hr incubation in [^{3}H]leucine in the presence or absence of reserpine. Reserpine, 9 μM, blocked the release of each hormone stimulated by 50 mM K^+ but not basal hormone secretion. Stimulation of [^{3}H]GH release by 10 μM PGE_1, or 5 mM dbcAMP was not affected by 5 μM reserpine, while stimulation of TSH release by 70 nM TRH was significantly blunted with 5 μM reserpine. The influence of reserpine on pituitary hormone secretion may define a pattern suggesting that the drug interferes with utilization of the extracellular but not the intracellular calcium mobilized during the secretory process.

Reserpine is usually administered *in vivo* to effect biogenic amine depletion through blockade of aminergic reuptake mechanisms. Used in this manner, reserpine causes hyperprolactinemia indirectly through dopamine depletion and thus reduction of the inhibitory tone exerted on the lactotroph (1). Reserpine *in vitro*, however, inhibits prolactin secretion from rat anterior pituitary glands (2, 3). This unexpected effect was dose-related (between 0.09 and 9 μM) and apparently independent of the traditional aminergic interactions of reserpine (4). The present study was designed to explore further the effects of reserpine *in vitro* on the secretion of other pituitary hormones. We have investigated basal and stimulated secretion of newly synthesized ^{3}H-labeled growth hormone ([^{3}H]GH) and radioimmunoassayable LH and TSH from female rat anterior pituitary glands in the presence or absence of reserpine.

Methods. Hormone secretion was evaluated by established techniques (5). Briefly, anterior pituitary glands from adult female Sprague–Dawley rats of 200–220 g (Dominion Laboratories, Dublin, Va.) were removed and bisected prior to 1000 hr. Three hemipituitaries from three different rats were pooled, weighed, and placed into an incubation flask containing 1 ml of tissue culture medium M-199 (M.A. Bioproducts, Walkersville, Md.) or Earle's MEM (GIBCO Labs, Grand Island, N.Y.) and 10 μCi [^{3}H]leucine (40–50 Ci/mmole, Amersham, Arlington Heights, Ill.). Each experimental group had four flasks. The flasks were then incubated for 5 hr under 95% O_2–5% CO_2 on a Dubnoff shaker at 37°.

Samples of incubation medium were assayed for [^{3}H]GH, TSH, and LH. Secretion of newly synthesized [^{3}H]GH was measured by polyacrylamide gel electrophoresis and liquid scintillation spectrometry with the incorporated radioactivity expressed relative to anterior pituitary wet weight as counts per minute [^{3}H]GH per milligram pituitary (5). Standard double antibody radioimmunoassay was used to measure TSH and LH with reagents kindly supplied by Dr. A. F. Parlow and the NIAMDD. These results were expressed as micrograms hormone per milligram pituitary relative to the reference preparations TSH RP-1 and LH RP-1. The intraassay coefficients of variation for TSH and LH were 10 and 9.8%, respectively.

Experimental agents were added to the

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incubation flasks at the start of each incubation. Reserpine (kindly supplied by Ciba, Summit, N.J.) was dissolved and diluted in ethanol and control groups contained the appropriate solvent (1% EtOH). We found the effects of 50 m*M* K^+ on hormone secretion were independent of the medium osmolarity. Results were similar using medium M-199 with K^+ added to make 50 m*M* K^+ or using Earle's MEM formulated to provide a 50 m*M* K^+ solution with isotonicity achieved by lowering the NaCl concentration.

The results were statistically analyzed by comparing the mean ± SE of the experimental groups using analysis of variance. A value of $P < 0.05$ suggested a significant difference existed in the hormone values.

Results. The effect of reserpine on K^+-stimulated hormone secretion is demonstrated in Table I. The basal secretion of [^{3}H]GH, TSH, and LH from female rat anterior hemipituitary glands during a 5-hr incubation was not altered by 9 μM reserpine. In contrast the large stimulation of secretion of each hormone by 50 m*M* K^+ was significantly impaired by simultaneous exposure to 9 μM reserpine. Although always statistically significant, the magnitude of the reserpine inhibition did vary among different experiments. Very similar results were obtained when Earle's MEM was used which contained 50 m*M* K^+ but was isotonic as described under Methods (results not shown).

Secretion of [^{3}H]GH from female rat pituitary glands was also stimulated by 10 μM PGE_1 or by 5 m*M* dibutyryl cAMP in two separate experiments (Table II). Unlike the K^+-stimulated [^{3}H]GH release, reserpine did not influence [^{3}H]GH secretion by these two agents.

The stimulatory effect of TRH on TSH secretion was evaluated in the presence or absence of reserpine (Fig. 1). Release of TSH was stimulated by 70 n*M* TRH ($P <$ 0.01 vs control) but in the presence of 5 μM reserpine the TRH action was blunted ($P <$ 0.01 vs TRH alone).

Discussion. Previously we observed that under *in vitro* conditions reserpine inhibited secretion of newly synthesized prolactin and speculated that the drug altered cellular calcium kinetics to achieve this effect (3, 4). Now we report that reserpine selectively inhibits the secretion of other pituitary hormones under a variety of specific conditions. Stimuli such as K^+, PGE_1, dbcAMP, and TRH each have selective effects on the secretion of pituitary hormones and they produce their enhanced hormone release through specific mechanisms.

Extracellular calcium is required for both the K^+-stimulated release of hormones and neurotransmitters (6) and pituitary prolac-

TABLE I. THE EFFECT OF RESERPINE ON K^+-STIMULATED RELEASE OF [^{3}H]GH, TSH, AND LH FROM FEMALE RAT ANTERIOR HEMIPITUITARY GLANDS

Incubation parameter	Hormone released into the incubation medium		
	[^{3}H]GH (cpm/mg pituitary)	TSH (μg/mg pituitary)	LH (μg/mg pituitary)
Control	588 ± 114	14.74 ± 0.32	0.69 ± 0.02
9 μM reserpine	402 ± 78[a]	13.83 ± 2.12[a]	0.71 ± 0.12[a]
50 m*M* K^+	1471 ± 172[b]	30.03 ± 2.34[b]	2.61 ± 0.18[b]
Reserpine and K^+	385 ± 76[c]	19.52 ± 2.11[d]	1.46 ± 0.07[c]

Note. Each experimental group contained four incubation flasks containing Medium 199 to which reserpine or additional KCl was added and hemipituitary glands taken from six rats. Each value represents the hormone secreted during the 5-hr incubation as the mean ± SE for the group. Newly synthesized [^{3}H]GH was measured by polyacrylamide gel electrophoresis and liquid scintillation techniques, while TSH and LH were quantitated by radioimmunoassay.

[a] $P > 0.05$ compared to the respective control group.
[b] $P < 0.01$ compared to the respective control group.
[c] $P < 0.01$ compared to the respective K^+ group.
[d] $P < 0.05$ compared to the respective K^+ group.

TABLE II. THE EFFECT OF RESERPINE ON [^{3}H]GH SECRETION CAUSED BY PGE_1 OR DIBUTYRYL cAMP (dbcAMP)

Treatment groups	[^{3}H]GH released into the incubation medium (cpm/mg pituitary)
Experiment 1	
Control	222 ± 50
10 μM PGE_1	734 ± 88[a]
10 μM PGE_1 + 5 μM reserpine	609 ± 46[b]
Experiment 2	
Control	800 ± 129
5 mM dbcAMP	2555 ± 191[a]
5 mM dbcAMP + 5 μM reserpine	2114 ± 346[b]

Note. The incubation parameters were as described in Table 1.
[a] $P < 0.01$ compared to the respective control.
[b] $P > 0.05$ compared to treatment without reserpine.

tin secretion (7). The present findings show that reserpine caused a partial to complete blockade of the K^+-mediated stimulation of [^{3}H]GH, TSH, and LH (Table I), and in previous studies the compound inhibited prolactin secretion (2, 3). Using other systems reserpine blocked the release of [^{3}H]DA from rat striatum (8) and norepinephrine from rabbit heart (9). These interactions suggest that reserpine may prevent effective utilization of extracellular calcium.

Stimulation of GH secretion by PGE_1 and dbcAMP was not influenced by reserpine (Table II). The action of these two secretagogues, unlike K^+-mediated secretion, is associated with intracellular calcium mobilization but not influx of extracellular calcium (10–12).

Stimulation of TSH secretion by TRH may depend on both transport of extracellular and mobilization of membrane-bound intracellular calcium (13, 14). We found that during a 5-hr incubation the TRH effect was significantly but only partially inhibited by reserpine (Fig. 1).

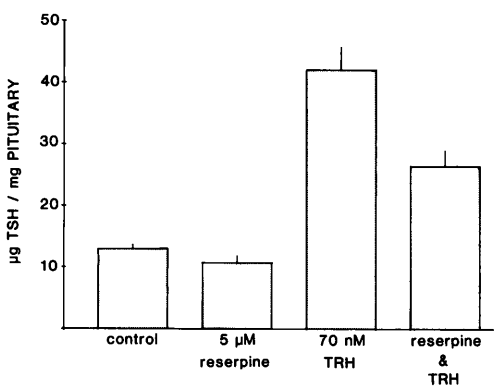

FIG. 1. The effect of reserpine on TRH-stimulated secretion of TSH *in vitro*. This experiment included eight flasks in each group, and the ordinate represents μg TSH/mg pituitary as the mean ± SE. Reserpine reduced the stimulatory effect of TRH on TSH release ($P < 0.01$ vs TRH alone).

Hormone secretion in the presence of reserpine seems to define a pattern from which some inferences may be drawn, although we have no direct information on the mechanism(s) involved. It is unlikely that reserpine is altering membrane properties in a general manner to inhibit secretion since (1) the effects are selective and (2) hormone release can still be stimulated by certain secretagogues. The inhibitory action of reserpine is most pronounced in those secretory events which are strongly dependent on extracellular calcium, such as basal prolactin and K^+-mediated hormone secretion. Reserpine is much less active with secretory events dependent largely on intracellular calcium. Studies are in progress to evaluate the hypothesis that reserpine interrupts calcium uptake in its action to inhibit hormone secretion.

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