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Studies on the Mechanism of the GABA-Mediated Inhibition of Prolactin Secretion[1] (40128)

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The secretion of prolactin by the anterior pituitary is normally under tonic inhibition by the hypothalamus. It has been shown that *in vitro*, dopamine acts directly on the pituitary gland to inhibit prolactin release (1). Recently Schally *et al.* (2) isolated GABA from pig hypothalami and demonstrated its prolactin release-inhibiting activity *in vivo* and *in vitro*. These findings are at variance with those of others in which no direct effect of GABA on prolactin secretion *in vitro* was reported (3), although these authors reported recently a PRL release inhibiting effect of high concentrations of GABA (4). Conversely, the parenteral administration was shown to stimulate the secretion of prolactin (3, 5). In this study we reinvestigated the mechanism of action of GABA on prolactin secretion.

Material and methods. Multiple flasks containing three hemipituitary glands from female or male rats (Wistar–Furth 200–220 g) were incubated in 1 ml tissue culture medium 199, for 5 hr in an atmosphere of 95% O_2 5% CO_2 in a Dubnoff shaker at 37°. In order to measure newly synthesized prolactin, the glands were incubated with 10 μCi (4, 5, ^{3}H)-Leucine (30 Ci/mmol) and aliquots of pituitary homogenate and/or incubation medium were subjected to polyacrylamide gel electrophoresis as described (1). ^{3}H-Labeled prolactin was identified on the gels by subjecting NIH reference prolactin to the same electrophoretic separation. The prolactin content of the incubation medium was measured by a double antibody radioimmunoassay using materials and protocols supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. The results are expressed in terms of NIAMDD rat prolactin RP-1. There were three or four flasks in each group. All results are expressed as mean ± SEM and statistical analysis was performed by analysis of variance.

Results. A direct effect of GABA on prolactin secretion by normal pituitary glands was investigated (Fig. 1). GABA (3×10^{-5} *M*) inhibited significantly the amount of prolactin released into the incubation medium. Prolactin secretion, as measured by radioimmunoassay, was inhibited 31% while the secretion of newly-synthesized ^{3}H-prolactin was inhibited 44%. Lesser concentrations of GABA did not consistently significantly inhibit the *in vitro* secretion of prolactin. Additionally, the inhibition of prolactin secretion by GABA was considerably less impressive than that elicited by 5×10^{-7} *M* dopamine. Although newly-synthesized ^{3}H-prolactin accumulated within the pituitary in the presence of dopamine, no increase of ^{3}H-prolactin was seen in response to GABA.

A possible relationship between the inhibiting action of both substances on prolactin secretion was studied (Fig. 2). Dopamine, in a concentration of 5×10^{-8} *M* did not have a measurable effect on prolactin synthesis or release, whereas GABA, 3×10^{-5} *M*, suppressed ^{3}H-prolactin release significantly. The combination of both substances produced a significantly greater inhibition of prolactin release than GABA alone. Haloperidol, 10^{-8} *M*, partially blocked the effect of dopamine and GABA.

The mechanism of action of GABA on prolactin secretion *in vitro* was studied. GABA, 5×10^{-5} *M*, inhibited prolactin release from male pituitary glands by more than 50% (Table I). The GABA receptor-blocking agent picrotoxin (10^{-6} *M*) was with-

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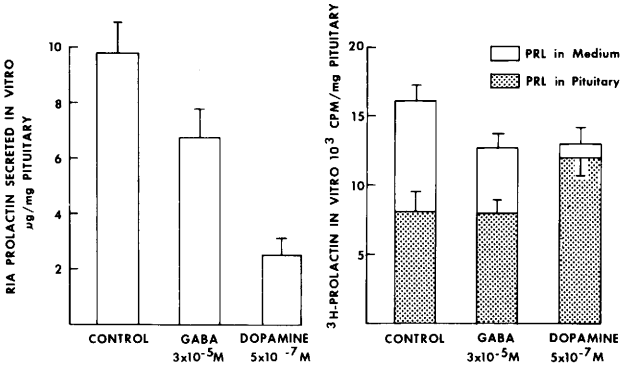

FIG. 1. *In vitro* comparison of the effect of GABA and dopamine on prolactin secretion. Pituitary glands incubated in 1 ml TC medium 199 containing ^{3}H-Leucine for 5 hr. Homogenates of female pituitary glands and incubation medium subjected to polyacrylamide gel electrophoresis. The bands containing ^{3}H-PRL were counted. PRL in incubation medium also measured by RIA.

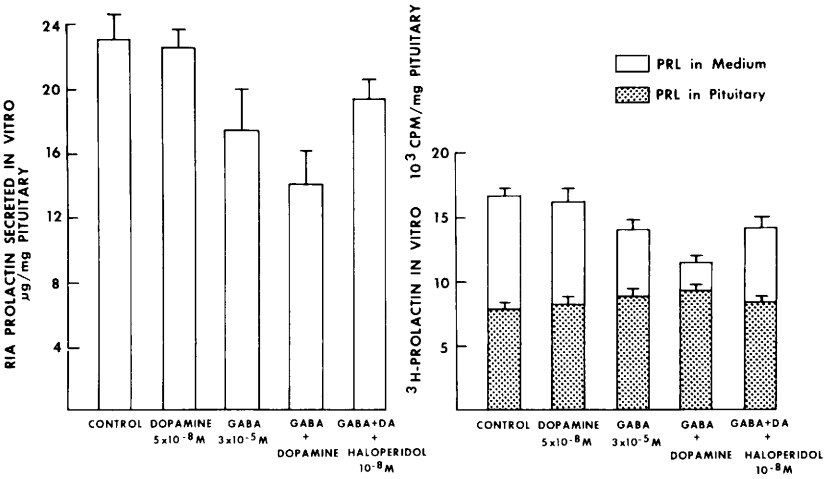

FIG. 2. *In vitro* comparison of the effect of dopamine, GABA, GABA + dopamine and GABA + dopamine + haloperidol on prolactin secretion. Female rat pituitary glands incubated as described in Fig. 1.

out effect on prolactin secretion and synthesis by the pituitary gland *in vitro* and did not influence the inhibition of prolactin release elicited by GABA. Larger amounts of picrotoxin (3.4×10^{-5} *M*) did not affect prolactin release nor did it affect the GABA mediated inhibition of prolactin release (data not shown).

The *in vivo* action of GABA on prolactin secretion was investigated. No effect on serum prolactin was observed 90 min after the ip administration of 25 mg/kg GABA to male rats (Fig. 3). The pituitary glands from these animals were incubated *in vitro*. The amount of radioimmunoassayable prolactin secreted was not affected, but the release of newly synthesized ^{3}H-prolactin was suppressed after GABA injection (Fig. 3c).

The administration of 5-hydroxytryptophan to rats is well known to increase serum prolactin (Table II). A marked suppression of the response to 5-hydroxytryptophan injection was observed, however, by the simultaneous administration of GABA.

Discussion. It has been shown that GABA is present in the hypothalamus of the rat (6). The observation that the amino acid is unevenly distributed in various hypothalamic areas (7) may suggest its possible role in the control of certain hypothalamic functions (8).

Recently, Schally *et al.* (2) found considerable quantities of GABA in porcine hy-

pothalami and suggested that the compound is a prolactin-inhibiting factor, which is active at the pituitary gland. Hall *et al.* (4) could only detect a prolactin release-inhibiting action of high concentrations of GABA on the pituitary *in vitro*, which was not found in previous investigations (3). Rivier and Vale (9) observed that large amounts of GABA (10^{-3} *M*) produced an 11% inhibition of prolactin secretion in normal rat pituitary cells in culture. In contrast, the intraventricular administration of GABA resulted in a significant increase of serum prolactin (3, 5), while the systemic injection of GABA (50–500 mg/kg) to hypophysectomized male rats with a pituitary transplant did not affect the levels of serum prolactin (3). The stimulative effects of agents such as perphenazine, haloperidol, chlorpromazine, sulpiride, neurotensin, histamine, bicuculline, clonidine and morphine on prolactin secretion were inhibited by the concomitant injection of GABA (2, 9).

TABLE I. THE EFFECT OF GABA AND PICROTOXIN ON PROLACTIN SECRETION BY NORMAL MALE RAT PITUITARY GLANDS *In Vitro*.[a]

	Incorporation of ^{3}H-leucine into prolactin (cpm/mg pituitary gland)		Prolactin measured by radioimmunoassay (μg/mg pituitary)
	Pituitary gland	Medium	Medium
Control	2770 ±430	1020 ±70	2.78 ±0.25
GABA (5×10^{-5} *M*)	2770 ±500	215 ±50[b]	1.07 ±0.17[b]
Picrotoxin (10^{-6} *M*)	2530 ±245	1030 ±50	2.37 ±0.12
GABA + Picrotoxin	3100 ±275	235 ±40[b]	1.02 ±0.16[b]

[a] Four flasks per group; mean ± SEM.
[b] $P < 0.01$ vs control.

Our observations of the systemic effect of GABA on prolactin secretion agree with most of the earlier observations. Although the 5-hydroxytryptophan induced increase in prolactin secretion was significantly inhibited by the injection of GABA, it did not affect the basal serum prolactin concentration. The findings, however, do not define whether GABA exerts its effect at the hypothalamic or pituitary level.

The direct *in vitro* prolactin release-inhibiting effect of high concentrations of GABA as shown by Schally *et al.* (2) is confirmed. The mechanism of action of GABA to inhibit prolactin secretion is probably different from that of dopamine (1). The effect on prolactin release is not mediated by a dopamine receptor because its inhibition is not blocked by low amounts of neuroleptics such as haloperidol and perphenazine (2). In addition, the specific GABA-receptor blocking agent picrotoxin (10) was also unable to block the GABA-mediated inhibition of prolactin secretion. Hence, the mechanism whereby GABA inhibits prolactin secretion is not clear. The concentration of GABA necessary to inhibit the release of newly-synthesized

TABLE II. THE EFFECT OF GABA AND/OR 5-HYDROXYTRYPTOPHAN (5-HTP) ON SERUM PROLACTIN IN NORMAL FEMALE RATS.[a]

minutes after injection	Serum prolactin (ng/ml)			GABA + 5-HTP
	control	GABA	5-HTP	
30 min	33.0 ±18.6	27.2 ±19.3	883.7 ±132.1	228.4 ±123.0*
120 min	34.0 ±8.2	32.4 ±14.9	162.0 ±63.0	33.2 ±10.2

[a] GABA (25 mg/kg), 5-HTP (25 mg/kg), or a simultaneous injection of both substances was given intraperitoneally to female rats.
[b] $P < 0.01$ versus 5-HTP induced increase in serum PRL.

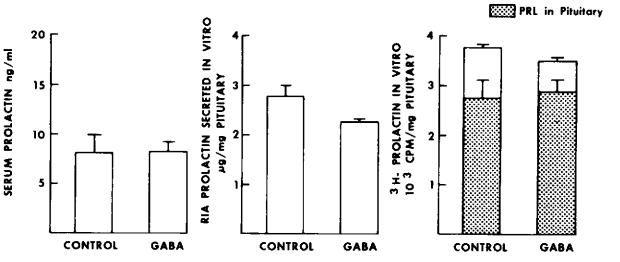

FIG. 3. The effect of GABA, 25 mg/kg body weight, administered ip to male rats and killed 90 min. later. Pituitary glands incubated as described in Fig. 1.

prolactin is very high and it appears to be at least 100-fold less potent than dopamine. These observations suggest that the direct prolactin secretion inhibiting effect of GABA *in vitro* is a metabolic or toxic effect rather than a neurotransmitter function of the amino acid.

Summary. GABA has a direct inhibiting effect on prolactin release by the pituitary *in vitro* and this effect is not blocked by coincubation with the specific GABA-receptor antagonist picrotoxin. GABA appears to be at least 100-fold less potent than dopamine as an inhibitor of prolactin secretion. Systemic administration of GABA did not affect basal serum prolactin levels, but inhibited the 5-hydroxytryptophan-induced secretion of prolactin. It is concluded that the direct effect of GABA on prolactin secretion by the pituitary is not mediated by well-defined neurotransmitter mechanisms.

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1. MacLeod, R. M., and Lehmeyer, J. E., Endocrinology **94,** 1077 (1974).
2. Schally, A. V., Redding, T. W., Arimura, A., Dupont, A., and Linthicum, G. L., Endocrinology **100,** 681 (1977).
3. Mioduszewski, R., Grandison, L., and Meites, J., Proc. Soc. Exp. Biol. Med. **151,** 44 (1976).
4. Hall, T. R., Mioduszewski, R., Campbell, G. A., Grandison, L., and Meites, J., IRCS J. of Med. Sci. **5,** 112 (1977).
5. Pass, K. A., and Ondo, J. A., Endocrinology **100,** 1437 (1977).
6. Enna, S. J., Kuhar, J., and Snyder, S., Brain Res. **93,** 168 (1975).
7. Kimura, H., and Kuriyama, K., J. Neurochem. **24,** 903 (1975).
8. Roberts, E., *in* "GABA in Nervous System Function" (E. Roberts, T. N. Chase and D. B. Tower, eds), p. 515. Raven Press, New York (1976).
9. Rivier, C., and Vale, W., Endocrinology **101,** 506 (1977).
10. Franz, D. N., *in* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman eds), 5th Edition, p. 361. McMillan Publishing Co., New York (1975).

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