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Influence of Neurohumors on Anterior Pituitary Lactogen Production *in vitro*.* (30491)

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In summarizing the literature on characteristics of a neurohumor, Harris(1) concluded that in all probability a neurohumor would be of low molecular weight. *In vivo* experiments suggest that neurohumors may also be involved in control of pituitary lactogen production. Epinephrine administration induces deciduomata formation(2), initiates lactation(3,4,5), retards mammary involution (3,4), and decreases pituitary lactogen content(6). Similar results have been obtained with acetylcholine(3,5) and serotonin(5,6,7). The results of some experiments(8,9) have been interpreted as indicating that oxytocin stimulated lactogen release, whereas results of lactogen assays(10,11), both *in vivo* and *in vitro,* indicate that oxytocin does not stimulate lactogen release. The investigation reported herein was conducted to determine the effect of various neurohumors on lactogen production *in vitro* and a preliminary report appeared elsewhere(12). Neurohumor is used as a general term to describe chemical entities produced by neural tissue that may act either in their immediate area of production or pass into circulation and act at some area distant to site of synthesis.

Materials and methods. Anterior pituitaries (AP) were removed aseptically from female, hooded Norway rats weighing 165 to 175 g and divided into 4 pieces. A piece from each AP was placed in each of 4 groups until a total of one AP was present per culture dish. The culture techniques were similar to those previously reported(13) and AP were cultured at 35 ± 1.0°C for 3 days. The neurohumors, serotonin, acetylcholine, epinephrine, and norepinephrine were added at a level of 1, 10, and 100 μg/ml; Pitocin and Pitressin were added to the medium at a level of 1, 10, and 100 mU/ml. Control cultures were run simultaneously with all neurohumors, with 6 to 8 culture dishes per group.

The lactogen content of the medium was determined as previously described(14),† and representative pituitary fragments from each group were examined histologically and the degree of viable tissue rated from zero to 4 (13). Differences between means were assessed statistically using Student's *t* test(15).

Results. Addition of serotonin to the medium did not alter lactogen production from that of the control medium. The lactogen content of cultured tissue, however, was significantly increased by 1 μg of serotonin but not by 10 or 100 μg. Neither acetylcholine nor norepinephrine significantly altered lactogen content of the medium, lactogen content of cultured AP tissue, or AP viability ratings. Epinephrine at 1 μg/ml increased lactogen production 43%, a significant increase, but the other 2 levels of epinephrine did not significantly alter lactogen production. Lactogen content of cultured AP tissue was significantly decreased by the 100 μg level of epinephrine but not by 1 or 10 μg. Addition of Pitressin to the medium did not alter the parameters examined. The addition of either 1 or 10 mU of Pitocin did not influence lactogen production or the lactogen content of cultured AP. Addition of 100 mU, however, significantly increased (53%) lactogen production (Table I).

Discussion. Although serotonin adminis-

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† The prolactin used in this study was a gift from the Endocrinology Study Section, N.I.H.

TABLE I. Influence of Neurohumors on Anterior Pituitaries Cultured *in vitro*.

Neurohumor	Level of neuro-humor	Avg AP wt post-culture (mg)*†	Lactogen/ 100 ml medium (IU)*	Lactogen in medium/100 mg AP (IU)*	Lactogen in 100 mg cultured AP (IU)*	AP viability rating
Serotonin	Control	4.13 ±.13	49.0 ± 6.9	32.8 ± 4.7	1.88 ± .38	3.25
	1 μg	4.33 ±.23‡	58.9 ± 7.5	37.6 ± 4.8‡	3.38 ± .57§	2.75
	10 "	4.38 ±.14‡	42.8 ± 4.6	26.7 ± 2.9‡	2.37 ± .32‡	2.50
	100 "	4.20 ±.18‡	41.5 ± 3.4	27.3 ± 2.2‡	2.50 ± .29‡	2.50
Acetylcholine	Control	4.23 ±.17	49.7 ± 4.2	31.9 ± 2.7	2.13 ± .45	2.00
	1 μg	3.83 ±.24‡	44.5 ± 5.5	31.3 ± 3.9‡	2.57 ± .07‡	2.00
	10 "	4.02 ±.22‡	54.3 ± 7.6	36.5 ± 5.1‡	1.92 ± .74‡	2.25
	100 "	3.98 ±.17‡	45.4 ± 5.2	30.6 ± 3.5‡	1.32 ± .19‡	2.50
Norepinephrine	Control	4.18 ±.18	42.2 ± 6.8	27.7 ± 4.4	2.63 ± .65	2.75
	1 μg	4.53 ±.15‡	54.1 ± 7.1	32.7 ± 4.3‡	2.60 ± .73‡	2.00
	10 "	4.35 ±.23‡	44.7 ± 6.1	27.6 ± 3.8‡	3.38 ±1.12‡	2.50
	100 "	4.48 ±.15‡	44.9 ± 7.9	27.5 ± 4.8‡	1.46 ± .32‡	2.50
Epinephrine	Control	4.63 ±.21	43.4 ± 7.9	25.6 ± 4.5	2.38 ± .33	2.75
	1 μg	4.25 ±.21‡	58.5 ± 5.6	36.5 ± 3.5§	3.13 ±1.19‡	2.50
	10 "	4.43 ±.18‡	39.8 ± 3.8	23.9 ± 2.3‡	1.67 ± .44‡	2.50
	100 "	4.97 ±.20‡	46.3 ± 5.6	25.2 ± 3.1‡	1.21 ± .15¶	2.75
Pitressin	Control	4.30 ±.15	48.0 ± 7.5	30.1 ± 4.7	2.19 ± .49	3.00
	1 mU	4.19 ±.29‡	45.4 ± 4.9	29.2 ± 3.2‡	1.97 ± .72‡	2.50
	10 "	4.14 ±.22‡	39.6 ± 6.8	25.4 ± 4.4‡	1.69 ± .19‡	3.25
	100 "	4.48 ±.31‡	47.3 ± 6.7	28.0 ± 4.0‡	2.06 ± .83‡	3.00
Pitocin**	Control	4.38 ±.13	39.3 ± 4.4	23.6 ± 2.5	2.24 ± .37	2.88
	1 mU	4.24 ±.14‡	41.7 ± 4.0	26.4 ± 2.5‡	2.01 ± .31‡	2.25
	10 "	4.19 ±.13‡	42.5 ± 4.4	27.0 ± 2.8‡	1.93 ± .31‡	2.38
	100 "	4.39 ±.14‡	57.5 ± 7.5	35.1 ± 4.4‖	2.73 ± .36‡	2.88

* Mean and standard error of mean. † Six to eight AP cultured per group. ‡ Not significantly different ($P > .10$) from control. § Significantly higher ($P < .10$) than control. ‖ Significantly higher ($P < .05$) than control. ¶ Significantly lower ($P < .05$) than control.
** Combined results of 2 experiments.

tration *in vivo* has been reported to initiate mammary gland secretory activity, retard mammary gland involution after weaning, and decrease pituitary lactogen content, it did not alter lactogen production when added to the medium of pituitaries cultured *in vitro*. The possibility that serotonin may be acting indirectly on lactogen production, *via* the hypothalamus, is not supported by the experiments of Sawyer(16), who failed to initiate lactation in rabbits with intraventricular injections of serotonin. Sawyer(16) also failed to initiate lactation with intrahypophyseal injections of serotonin. Our *in vitro* results confirm Sawyer's latter observation that serotonin does not have a direct effect on pituitary lactogen production.

Epinephrine administration *in vivo* has an effect similar to serotonin. The 43% increase in lactogen production by pituitaries cultured with 1 μg epinephrine indicates a direct action on the AP. Talwalker *et al*(17), however, did not observe an increase in lactogen production when epinephrine was added *in vitro*, but the amount added was 40 μg/ml. Other investigators(18) have found that epinephrine had a direct effect on increasing AP oxygen consumption and glucose utilization *in vitro*. It was also noted that higher levels of epinephrine did not have an effect on the metabolic parameters examined, an observation which we have noted for lactogen production. The significant decrease in lactogen content of pituitaries cultured with the higher epinephrine level indicates that the decrease in pituitary lactogen content observed by Meites *et al*(6) after *in vivo* administration may have been due to a direct effect on AP tissue.

Nicoll and Meites(11) reported that Pitressin had no effect on pituitary lactogen production *in vitro*, an observation that we have also noted. They observed no effect of Pitocin whereas we noted an increase in lactogen production at the 100 mU level. Hawker(19) reported 0.21 mU oxytocin/ml

blood before hypothalamic stimulation and 0.66 mU after hypothalamic stimulation, and these values may represent the physiological limits of oxytocin in direct contact with tissues. The lactogen-stimulatory effect of 100 mU Pitocin *in vitro* was therefore obtained with an unphysiological level of oxytocin. The Pitocin used in this study was commercially prepared by extraction of posterior pituitaries and, since epinephrine has been identified in the hypothalamus and other neural tissue(20), it is possible that epinephrine might have been present in the preparation. Epinephrine then would account for the increase in lactogen production by the highest Pitocin level.

Summary. Anterior pituitaries from female, hooded Norway rats were cultured in Mixture 199 for 3 days at 35 ± 1°C in 95% O_2-5% CO_2. Neurohumors serotonin, acetylcholine, epinephrine, and norepinephrine were added to the medium at a level of 1, 10, and 100 μg/ml; Pitocin and Pitressin were added at levels of 1, 10, and 100 mU/ml. Serotonin, acetylcholine, norepinephrine, and Pitressin did not alter lactogen production from that of controls. Epinephrine, at a level of 1 μg/ml, increased lactogen production 43% and Pitocin at a level of 100 mU increased lactogen production 53%.

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Influence of Oligodeoxyribonucleotides on Phagocytic Activity of the Reticuloendothelial System.* (30492)

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Enzymatic digests of deoxyribonucleic acid stimulate multiplication and DNA synthesis of Gram-positive bacteria and also enhance antibody formation in animals(1-3). The activity of these digests, which is independent of the source of DNA, is attributable to oligodeoxyribonucleotides and cannot be duplicated by monodeoxyribonucleotides or -sides. The activity of the oligonucleotides can be attributed, at least in part, to a stimulation of kinases involved in nucleoside synthesis (4). Administration of DNA digest with

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