Quantitative Response of Rat Mammary Glands to Mammogens. II. Anterior Pituitary Tissue Alone and with Estrogen.* (28165)

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Silver(1) was able to stimulate mammary growth in suckling, ovariectomized, hooded Norway rats with macerated anterior pituitary tissue and estrogen, but either one of these used alone had no influence on mammary area. Although anterior pituitary tissue alone has a mammogenic action in hypophysectomized rats, investigators are not in agreement regarding the mammogenic potency of pituitaries from non-injected rats and those from rats previously injected with estrogen. Initially, Silver's results suggested the possibility of using the suckling rat as an assay animal in determining the mammogenic potency of pituitary tissue, but our finding (2) that estrogen alone increased mammary area detracted somewhat from the idea. Mammary areas greater than those resulting from injecting 1.0 μg of estrogen are, nevertheless, obtainable, and the suckling, ovariectomized rat can, therefore, be used to determine the mammogenic potency of anterior pituitary tissue when injected with estrogen.

Methods. Assay animals, time sequences, and mammary area measurement were as previously described(2). Every other day, 1, 2.0, or 4.0 μg of 17β-estradiol dipropionate (ED) was injected (sbc) in 0.05 ml corn oil. Acetone-dry bovine anterior pituitary powder (BAP) was ground in an agate mortar, and 1.0, 2.0 or 4.0 mg in 0.1 ml distilled water (pH 3.5) was injected (sbc) daily. Hooded Norway female rats, 4 to 6 months of age and ranging in body weight from 200 to 300 g, provided rat anterior pituitary tissue. Pituitaries were collected from one group in diestrus and from 3 other groups which were paired according to body weight; one of each pair was injected with 6.6 μg estradiol benzoate (EB) for 8 days (multiparous group) or 15 days (2 groups of virgins). Following collection, pituitaries were weighed, frozen, and ground. One or 2.0 mg of anterior pituitary tissue from non-injected rats (RAP) was injected (sbc) in 0.1 ml water, whereas pituitaries from injected rats (ERAP) were placed in the same total volume as their respective controls and similarly administered.

Anterior pituitaries were collected from 30 ovariectomized, suckling rats, 75 ovariectomized, suckling rats injected with 1.0 μg ED (sbc) every other day from 11th through 19th day of age, and 45 ovariectomized suckling rats similarly injected with 2.0 μg ED. The glands were assayed for their lactogenic content by the Reece-Turner method(3). Ten pigeons were used to assay pituitaries from 30 control rats and 30 rats injected with 1.0 μg ED and 15 pigeons were used to assay pituitaries from 45 rats injected with 1.0 μg ED and 45 rats injected with 2.0 μg ED.

Results. One and 2.0 mg BAP significantly increased mammary area (mm^2 per 100 cm^2 of body surface area) over that of ovariectomized controls, 26.1 and 28.3 *vs* 17.4 respectively ($P<0.01$). These BAP doses plus 1.0 μg ED resulted in mammary areas that were similar to those induced by 2.0 μg ED alone, 47.7 and 45.3 *vs* 51.8, respectively (Table I). Increasing either BAP or ED dose, or both, did not enhance mammary response. All BAP plus ED treatments stimulated mammary growth, which was significantly greater than that induced by 1.0 μg ED alone ($P<0.05$).

In 2 experiments, when virgin ERAP was injected alone (2.08 and 2.28 mg, respectively) mammary areas (22.7 and 22.3) were significantly greater than those (17.6 and 18.5) of animals injected with 1.0 mg virgin RAP alone ($P<0.01$). No difference was seen between glands of non-injected pups and those of pups injected with 1.0 mg virgin RAP. Injection of 1.0 mg multiparous RAP and 1.0 μg ED resulted in mammary areas not significantly different from those of animals

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TABLE I. Mammary Growth Response of Suckling, Ovariectomized Rats to Anterior Pituitary Tissue Alone and with Estrogen.

Treatment*	No. of animals	Mammary area†
Control‡	11	17.4 ± 1.1
1.0 μg ED‡	10	37.5 ± 3.1
2.0 " " ‡	12	51.8 ± 2.7
4.0 " " ‡	11	51.7 ± 2.3
1.0 mg BAP	11	26.1 ± 1.4
2.0 *id.* m	11	28.3 ± 1.6
1.0 " + 1.0 μg ED	11	47.7 ± 3.4
2.0 " + 1.0 *id.* m	12	45.3 ± 2.3
1.0 " + 2.0 "	12	49.9 ± 2.0
1.0 " + 4.0 "	12	46.6 ± 2.4
2.0 " + 4.0 "	12	45.9 ± 3.8
1.0 mg virgin RAP	6	17.6 ± 1.5
2.08 " " ERAP	6	22.7 ± 1.6
1.0 " " RAP	6	18.5 ± 1.7
2.28 " " ERAP	14	22.3 ± 1.4
1.0 mg multiparous RAP + 1.0 μg ED	6	47.5 ± 3.1
1.62 mg multiparous ERAP + 1.0 μg ED	7	49.3 ± 4.2
1.0 mg diestrous RAP + 4.0 μg ED	12	50.2 ± 1.8
2.0 mg diestrous RAP + 1.0 μg ED	11	51.2 ± 2.4

* ED, 17β-estradiol dipropionate; BAP, bovine anterior pituitary; RAP, rat anterior pituitary; ERAP, experimental rat anterior pituitary.
† mm^2 per 100 cm^2 of body surface area ($W^{2/3} \times 10$) with S.E.
‡ Data from Macdonald and Reece(2).

receiving multiparous ERAP (1.62 mg) and 1.0 μg ED (47.5 *vs* 49.3). Glands from both groups of rats, however, were significantly larger than those of rats injected with 1.0 μg ED alone and similar to those of rats receiving 2.0 μg ED alone. Increasing ED dose to 4.0 μg or diestrous RAP to 2.0 mg did not result in mammary areas significantly greater than those observed following administration of 1.0 mg multiparous RAP with 1.0 μg ED (50.2 and 51.2 *vs* 47.5).

Anterior pituitaries of non-injected rats contained .13 of a Reece-Turner unit per anterior pituitary whereas those of rats injected with 1.0 μg ED contained .39 of a unit. The lactogen content of anterior pituitaries of rats injected with either 1.0 or 2.0 μg ED was similar, .37 and .34 R-T unit, respectively. The increased lactogen content of rats receiving estrogen was due to an increase in pituitary weight and an increase in lactogen concentration (Table II).

Discussion. Mammary gland area of the ovariectomized, suckling rat was increased by anterior pituitary tissue alone. When anterior pituitary tissue was injected either alone or with estrogen, above a stimulatory dose, it did not induce a further increase in mammary area. When 2 mammogens, ED (1.0 μg) and BAP, were injected simultaneously they induced mammary growth that was greater than that stimulated by 1.0 μg ED but essentially the same as that produced by either 2.0 or 4.0 μg ED alone or in combination with BAP. These observations suggest that 2.0 and 4.0 but not 1.0 μg of ED stimulated the secretion and release of pituitary mammogen. Similar results were also obtained when either RAP or ERAP was substituted for BAP. There was no detectable difference in mammogenic potency of pituitaries from non-injected rats and those from estrogen-injected rats when injected on a pituitary gland basis with 1.0 μg of ED. When RAP and ERAP were injected alone the former, at 1.0 mg dose, failed to increase mammary area whereas the latter did.

Anterior pituitary lactogenic hormone content was increased over control values by injecting 1.0 or 2.0 μg ED. The two ED doses increased lactogen concentration, suggesting

TABLE II. Lactogenic Hormone Content of Anterior Pituitaries from Suckling, Ovariectomized Rats Injected with Estrogen.

ED,* μg	No. of rats	3 AP/bird, mg	Reece-Turner units		
			Avg†	Per mg	Per AP
.0	30	3.8 } (10)‡	.38 ± .09	.10	.13
1.0	30	4.8 } (10)‡	1.18 ± .14	.24	.39
1.0	15	4.8 } (15)‡	1.11 ± .10	.23	.37
2.0	15	4.7 } (15)‡	1.02 ± .15	.22	.34

* ED, 17β-estradiol dipropionate/2 day, 5 injections.
† With S.E.
‡ No. of birds.

that they stimulated hormone synthesis. Mammary gland growth data suggest that less lactogenic hormone is released from the pituitary by 1.0 μg ED than by either 2.0 or 4.0 μg ED, which presumably are stimulating maximal secretion and release since only 1.0 μg ED could be potentiated with additional pituitary tissue. These results also indicate that the anterior pituitary is responsive to estrogen prior to weaning, in this case 21 days of age, and that the hypothalamic controlling mechanism(s) responsible for the synthesis and release of lactogen is either mature at this time or is matured by exogenous estrogen in suckling rats.

Summary. Anterior pituitary tissue alone and with estradiol dipropionate increased mammary area in suckling, ovariectomized, hooded Norway rats. Anterior pituitary tissue injected with 1.0 μg estrogen increased mammary area to a greater extent than did 1.0 μg estrogen alone and similar to that stimulated by 2.0 μg estrogen. When injected alone, anterior pituitaries from rats previously injected with estrogen increased mammary area to a greater extent than did anterior pituitaries from non-injected rats. Estrogen injections, either 1.0 or 2.0 μg, increased pituitary lactogen content.

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Renal Tubular Effect of Val$_5$-Angiotensin II Amide in Rats. (28166)

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The angiotensins are vasoconstrictor drugs. Their most evident effect on renal function in the dog(1,2,3), in man(4-6), and in the rat (7) is an increase in vascular resistance in the kidney which is apparently more pronounced than in other parts of the circulation(8). This vascular action of angiotensin appears to affect the afferent arterioles more than the efferent vessels and, therefore, causes a decrease in glomerular filtration rate(1,3,5,6). Since changes in glomerular filtration rate usually induce large changes in tubular function, any direct effect of angiotensin on renal tubules tends to be obscured by the effect on renal hemodynamics and glomerular filtration. In contrast to normal humans, patients with severe chronic hypertension do not respond to infusions of angiotensin by a major drop in effective renal blood flow: the clearance of p-aminohippurate (PAH) either remains unchanged or decreases only slightly(6,9-11). Glomerular filtration rate (GFR) remains unchanged. In such patients angiotensin induces an increase in urine flow and in sodium excretion which must be attributed to a tubular effect of the drug. A similar tubular effect in normal rats will be described here.

Methods. Male rats weighing 200-300 g, of mixed stock, were used. Renal clearance and water-electrolyte excretion was measured as described by Cotlove, with modifications suggested by the writer(14). The restraining cages used have been described(15). A 0.15 M NaCl solution, containing suitable amounts of inulin and Na-PAH, was infused into a tail vein at a rate of approximately 0.85 ml/kg • min. After an equilibration period of approximately one hour, renal functions were measured for 2 control periods of 15-30 minutes each. Blood samples were taken after each period, and were replaced by i.v. injections of normal donor blood, containing inulin and PAH. At the conclusion of the second control period, angiotensin II amide ("Hypertensin CIBA") was added to the infusion fluid in amounts calculated to result in a drug administration rate of 0.008-0.2 μg/kg • min. In control animals, the isotonic saline infu-