Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 112 JANUARY 1963 No. 1

SECTION MEETINGS

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Hypothalamic Stimulation and Ascorbic Acid Content of Endocrine Glands of the Albino Rat.* (27931)

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Despite the fact that the adrenal cortex, hypophysis and ovary contain relatively high concentrations of ascorbic acid, a specific role of this substance in endocrine function has not been clearly demonstrated. The depletion of ascorbic acid in adrenal and ovary after stimulation with ACTH and LH respectively has provided a basis for bioassay of these adenohypophyseal hormones but the precise relationship between the trophic principles, ascorbic acid and steroidogenesis is conjectural. The functional significance of the vitamin in the adenohypophysis itself is also obscure. Little or no change occurs in pituitary ascorbic acid level after experimentally-induced alterations in trophic hormone secretion(1,2). Schreiber and Kmentova(3) reported, however, that suspensions of rat hypothalamus depleted ascorbic acid from the pituitary *in vitro,* suggesting that this vitamin may be importantly involved in hypophysiotrophic activity. The present work attempts to clarify the possible relationship of ascorbic acid to hypothalamo-hypophyseal interaction by determining ascorbic acid levels in the pituitary and target glands after electrical stimulation of the hypothalamus and other brain areas. A preliminary account of the findings has been given(4).

Materials and methods. Albino female rats (Wistar) weighing 150-300 g and displaying typical 4-5-day vaginal cycles were used. Animals were given food (Purina Dog Checkers) and tap water in unrestricted amounts. Brain excitation was made through a Grass S-4 Stimulator using a specially constructed concentric stainless steel electrode, about .4 mm in diameter and insulated with

* This work was supported by grant from Nat. Inst. Health.

TABLE I. Ascorbic Acid Concentration in Endocrine Glands After Electrocautery or Electrical Stimulation of the Hypothalamus.

Treatment, (No. of rats)	Final body wt, g	Adenohypophysis Wt, mg	Adenohypophysis Asc. acid, mg/100g	Adrenal Wt, mg	Adrenal Asc. acid, mg/100g	Ovary Wt, mg	Ovary Asc. acid, mg/100g
Untreated controls (14)	261 ± 6*	10.7 ± .5	220 ± 11	61.7 ± 3.4	407 ± 18	68.4 ± 2.9	82.7 ± 4.8
Hypothalamic† stimulation (10)	227 ± 4	9.9 ± .2	232 ± 9	73.9 ± 2.6§	435 ± 13	67.4 ± 4.7	89.4 ± 4.0
Hypothalamic lesion (8)	280 ± 25	13.5 ± .8§	243 ± 12	53.5 ± 6.0	417 ± 17	70.1 ± 9.7	95.0 ± 8.6
Lesion plus stimulation‡ (10)	248 ± 18	10.4 ± .5	217 ± 7	45.8 ± 3.6§	508 ± 12§	31.2 ± 1.7§	80.1 ± 5.4

* In all columns, refers to mean ± standard error of mean.
† Body weight loss in this group approximated 10%.
‡ Group consisted of persistent estrous rats receiving electrical stimulation in the previously electrocauterized pre-optic and anterior hypothalamic zones.
§ Values are significantly different ($p = .05$ or less) from those of untreated controls.

resin except for the tip.† The electrode was inserted into the pre-optic and supra-optic regions of the hypothalamus by means of a stereotaxic instrument utilizing coordinates (7-7.5 mm rostral to external auditory meatus; .5 mm from the midline and 1 mm from skull base) which characteristically produce persistent estrus after electrocoagulation(2). The electrode was not permanently implanted but was reinserted in those animals receiving more than one stimulation. Parameters of electrical excitation were as follows: monophasic square waves in 1 msec pulses, with a frequency of 100 cycles per sec at 1.5 V. Stimulation was done bilaterally, under light ether anesthesia, with current applied alternately 15 secs on and off over a 10-minute period. In acute experiments, electrodes were introduced into preoptic, amygdala, and cortical areas of the brain and animals killed one hour after a single period of stimulation. Rats given multiple stimulations (20 minutes daily, 4 successive days) were killed 20-24 hours after the last excitation period. Two types of control animals were used; unoperated intact animals showing normal vaginal cycles, and rats with persistent vaginal estrus, rendered so by electrocautery of the rostral hypothalamus 40-50 days previously. Some rats of the latter group also received a series of electrical stimulations with electrodes inserted directly into the coagulated area. Other rats were "sham-stimulated" (electrodes in place without current). Vaginal smears were taken daily throughout the experimental period. Animals were killed by exsanguination. The anterior pituitary, adrenals, and ovaries were quickly dissected, weighed on a torsion balance, placed in 6% trichloroacetic acid solution and frozen. Ascorbic acid content of the adenohypophysis, left adrenal and left ovary was determined by the Roe-Keuther method(5). Localization of the lesion and position of the electrode in the brains of experimental animals were checked by histological examination of formalin fixed, toluidine blue stained sections. The right adrenal and ovary were also studied histologically.

Results and discussion. Ascorbic acid concentration in the anterior pituitary remained normal despite significant functional change in the pituitary-adrenal and ovarian systems induced by electrocautery or electrical stimulation of the hypothalamus (Table I). This accords with previous observations made by Salhanick *et al.*(1) who found constant levels in hypophyses of rats and rabbits with experimental procedures designed to alter secretion of TSH, ACTH, gonadotrophins and neurohypophyseal hormones. The results also extend findings by D'Angelo(2) with respect to ascorbic acid content of endocrine glands in hypothalamic lesioned, persistent estrous rats exposed to cold. The failure of the adenohypophysis to respond with change in ascorbic acid level after either hypothalamic electrocautery or excitation does not support the belief by Schreiber and Kmentova(3) that

† Obtained from the Meditron Co., El Monte, Calif.

TABLE II. Acute Effects of Brain Stimulation on Ascorbic Acid Levels in Endocrine Glands. Mean Ascorbic Acid—mg/100g ± S.E.

Brain area (No. of rats)	Adeno-hypophysis	Adrenal	Ovary
Hypothalamus (4)	195 ± 7	289 ± 22	62.6 ± 4.0
Amygdala (5)	199 ± 14	346 ± 22	73.2 ± 6.6
Sham stimulation (6)	178 ± 11	315 ± 10	87.9 ± 3.4

the vitamin is specifically implicated in hypothalamo-hypophyseal interaction.

No indication was seen that pituitary ascorbic acid is rapidly mobilized in response to acute stress as is that in the adrenal. Ascorbic acid levels in the adrenal were substantially lower in rats killed within 1-2 hours of treatment (Table II), than in those receiving chronic treatment. The slightly lower values for pituitary ascorbic acid concentration in the acute experiments reflected the use of younger rats (150-200 g), a finding which strengthens a previous suggestion that Vit. C content of the hypophysis increases with age(1). The relatively high and fixed content of ascorbic acid in the pituitary is difficult to explain. Concentrations are well maintained even after severe starvation but some success has been achieved in depleting ascorbic acid from the pituitary of rats with exogenous thyroid hormone (unpublished experiments). The effects are not specific, however, and the functional significance of the vitamin in pituitary secretion remains unknown.

Pituitary secretion was definitely altered by experimental manipulation at the hypothalamic level. Excitation of the rostral hypothalamus at the junction of pre-optic and supra-optic areas produced significant adrenal hypertrophy. This enlargement could not be elicited by electrical stimulation of the same area if previously damaged by electrocoagulation. Although adrenal weight changes after destruction of pre-optic and supra-optic zones are inconsistent, the hypertrophy of the adrenal cortex after repeated electrical stimulation of these regions provides additional evidence that the rostral hypothalamus is somehow involved in the regulation of ACTH secretion(2).

The induction of persistent estrus in the rat following placement of electrolytic lesions in the hypothalamus has been studied often. The change in reproductive cyclicity is accompanied by inhibition of corpus luteum formation and extensive folliculation in the ovary, uterine hypertrophy and pituitary enlargement. All of these phenomena were observed here. Hypothalamic excitation in normal cycling rats consistently delayed the onset of estrus. Release of ovulating hormone from the adenohypophysis and ovulation must have occurred as evidenced by formation of fresh corpora lutea in gonads of stimulated rats. Ovarian ascorbic acid concentration nevertheless remained normal. Vaginal cornification was replaced by a smear of the diestrous type in those persistent estrous rats receiving electrical stimulation of the previously damaged hypothalamic areas. Ovarian and uterine weights were also markedly reduced. This pattern of reproductive tract atrophy, typical of that which follows arcuatotuberal hypothalamic lesions (6), could have resulted from repeated insertions of the electrode expanding the original lesion, and effecting further reduction of gonadotrophin secretion. The significant rise in adrenal ascorbic acid concentration with reduction in weight of the gland is also observed in rats with median eminence lesions. What influence electrolytically deposited iron from stimulating electrodes may have had in these experiments was not assessed. It has been established in acute experiments that irritative foci of iron deposits exert actions, hours after cessation of the electrical stimulus, which may facilitate ovulation in the rat (7) or delay it in the hen(8). This aspect of the problem is being studied further in chronic experiments using rats with permanently implanted electrodes.

Summary. Electrical stimulation of preoptic and anterior hypothalamic areas of the brain in adult female rats resulted in activation of the pituitary-adrenal and ovarian systems, as evidenced by adrenal hypertrophy, alterations in reproductive cyclicity and by corpus luteum formation. Neither electrical excitation nor electrocautery of the rostral hypothalamus had any significant effect on

ascorbic acid levels in the adenohypophysis.

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Received September 24, 1962. P.S.E.B.M., 1963, v112.

Therapeutic Anti-Viral Activity in Tissue Culture.* (27932)

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5-Iodo and 5-bromo-2′-deoxyuridine have been found to cure corneal infection caused by herpes simplex and vaccinia virus and eradicate culturable virus even when these infections were clinically far advanced. In addition to being therapeutically effective in experimental herpes keratitis in rabbits, these agents appear beneficial in herpetic keratitis in man(1,2).

Although the unique ability to eradicate virus infection of the cornea has made it possible to find several agents active against both herpes simplex and vaccinia, many limitations are inherent in restricting the study of virus chemotherapy to the eye. The need for an *in vitro* correlate of *in vivo* anti-viral activity is apparent. This communication will describe therapeutic arrest of cytopathogenic changes in tissue culture and complete elimination of virus from the culture following herpes simplex and vaccinia infection even after cytopathogenic changes in the culture are obvious.

Methods. Primary rabbit kidney cell cultures were maintained in Hanks' balanced salt solution with 0.5% lactalbumin hydrolysate and 2% calf serum, and primary human amnion cell cultures were maintained in Eagle's minimum essential medium with 10% calf serum. Herpes simplex virus (10^8 infective doses) previously isolated from a patient with dendritic keratitis and vaccinia virus (10^8 infective doses) were used for infection. 5-Iodo-2′-deoxyuridine (IDU) was dissolved in media and the *drug containing media was changed daily.* Control tubes were handled in an identical manner with medium not containing IDU. Membranes of the roller tubes were prepared at intervals by the collodion membrane technic(3) and were stained with hematoxylin and eosin to detect giant cells and cells with virus inclusions. Intracellular virus titers were determined on cells disrupted by ultrasound to yield the highest virus titer and titers so obtained were higher than those found by multiple freezing and thawing(4).

Results. As early as 12 hours after infection cells exhibiting specific cytopathogenic changes could be seen, but these, which consisted of giant cells and inclusion containing cells, were rare. By 24 hours micro-plaques were common and single giant cells as well as cells with both acidophilic and basophilic inclusion bodies were abundant. Shortly thereafter, in untreated cultures degeneration became so extensive that evaluation of the accelerating process of degeneration was difficult, and by 48 hours after infection most cell sheets were completely destroyed. Aliquots of media at this time when diluted 10^5 were all shown to be infectious *in vitro* (Fig. 1).

When IDU was added 24 hours after infection, although many giant cells and inclusion containing cells were visible, by 48 hours (24 hours of treatment) their number had diminished. Following treatment the number of visible giant and refractile cells

* This work was supported in part by grants from Nat. Inst. of Neurol. Dis. and Blindness and from Nat. Cancer Inst., N.I.H., U.S.P.H.S.