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Corticotropin Releasing Activity of a Pepsin Labile Factor in the Hypothalamus.* (24149)

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Acid extracts of calf hypothalamus elevate the level of blood ACTH in the decerebrate rat(1). The possibility was entertained that these extracts contained a corticotropin releasing factor (CRF), *i.e.*, a factor which travels from the hypothalamus *via* the portal venous system to the adenohypophysis to release ACTH. The present study was designed to evaluate the activity of hypothalamic extracts in a more specific test object, namely, the median eminence lesioned rat(2) and to determine whether the activity could be accounted for by the presence of ACTH and vasopressin. The results indicate that a third and as yet unidentified factor is in part responsible for ability of the hypothalamic extracts to induce adrenal ascorbic acid depletion in the median eminence lesioned rat.

Methods. Calf brains were dissected at the slaughter house. The pituitary stalk was transected immediately dorsal to the diaphragm sella. The median eminence area with attached pituitary stalk was separated from the rest of the hypothalamus, placed on dry ice and transported to the laboratory. Each piece of tissue has been designated a stalk median eminence unit (SME unit). The frozen tissue was placed in a Waring blendor which contained 2 ml of 0.2 M acetic acid per SME unit added, usually 20 SME units in 40

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TABLE I. Adrenal Ascorbic Acid Depletion in Acute Median Eminence (ME) Lesioned Rats Following ACTH, Pitressin or Stalk-Median Eminence (SME) Extracts.

Exp. No.	Material inj.	Dose in fraction of SME unit	Pressor activity, mu	Adrenal ascorbic depletion, mg/100 g	
				Hypox.	ME lesioned
1	Acid saline or polymixin B				18 ± 13*(13)†
2	a. ACTH, .5 mu			58 ± 6*(20)†	50 ± 12 (13)
	b. " , 2.0 "			139 ± 5 (14)	150 ± 7 (13)
3	a. Pitressin		5,000	77 ± 11 (9)	172 ± 8 (7)
	b. "		500		144 ± 11 (5)
	c. "		100	5 ± 13 (5)	90 ± 15 (8)
	d. "		20		14 ± 8 (9)
4	a. SME extract, A-23	.4	670 (560 to 800)‡	201 ± 15 (6)	203 ± 12 (5)
	b. *Idem*	.025	42 (35 to 50)‡	39 ± 12 (11)	117 ± 6 (15)
5	a. SME extract, M-7 oxyc. eluate	.1	100§		
	b. SME extract, M-7 oxyc. supernatant	.1	10§	26 ± 10 (11)	148 ± 14 (8)
	c. SME extract, M-29 oxyc. eluate	.1	80§	204 ± 17 (6)	191 ± 10 (9)
	d. SME extract, M-29 oxyc. supernatant	.1	12§	61 ± 14 (5)	154 ± 10 (15)
6	a. SME extract, M-29 oxyc. supernatant + pepsin	.1			42 ± 7 (6)
	b. SME extract, A-23	.025	42 (35 to 50)‡	39 ± 12 (11)	117 ± 6 (15)
	c. *Idem* + pepsin	"	46 (34 to 63)‡	31 ± 10 (6)	49 ± 9 (11)
	d. *Idem* + ACTH, 1.0 mu	"		141 ± 14 (5)	
	e. *Idem* + pepsin	"		171 ± 7 (5)	

* Mean and stand. error. † No. of animals. ‡ Potency with 95% fiducial limits.
§ Estimate based on 2 or more responses each of standard and of unknown.

ml of 0.2 M acetic acid. The mixture was homogenized at full speed for 120 seconds and immersed in a 100°C water bath, refluxed for 10 minutes, and centrifuged for one hour at 16,000 r.p.m. in a preparative head (SW-25.1) of a Spinco ultracentrifuge. The clear aqueous phase (SME extract) was decanted and stored at 4°C; activity was undiminished after 4 weeks of storage. Washed oxycellulose(3) in a quantity of 250 mg per 10 SME units was added to the crude extract, the pH adjusted to 3.0 with glacial acetic acid and the mixture stirred intermittently for 12 hours. The oxycellulose was filtered on a sintered glass funnel. The fraction unadsorbed by oxycellulose was designated the SME oxycellulose supernatant and the material eluted from the oxycellulose with 0.1 M HCl (2 times with 10 ml) designated the SME oxycellulose eluate. In the pepsin experiments, 4 ml of a solution which contained NaCl (0.9%), HCl (0.01 M) and USP pepsin (5 μg/ml) were incubated for 2 hours at 37°C with 1 ml of SME extract which contained the equivalent of 0.5 SME unit. Crude extracts, oxycellulose fractions and pepsin treated extracts were appropriately diluted for bioassay with a solution which contained NaCl (0.9%) and HCl (0.01 M). Doses in column 3 of Table I are expressed as fractions of an SME unit.

Assays for CRF activity were performed in the acute hypothalamic lesioned rat (250 to 300 g)(4). Immediately after lesioning the rats were placed in metabolic cages. Rats with 24-hour urine volumes greater than 75 ml and water consumptions greater than 110 ml were used for assay 48 hours after lesioning. Extracts were infused intravenously over 5 minutes following left adrenalectomy. Thirty minutes later the right adrenal was removed. The difference between concentrations of adrenal ascorbic acid in the left and right adrenals is an index of ACTH action during the period between removal of the two glands. The response may represent ACTH

release and or ACTH injected. ACTH activity of the extracts was determined by assay in 24-hour hypophysectomized rats (110 to 135 g)(5). Vasopressin content was estimated by pressor response of rats pretreated with phenoxybenzamine (U.S.P. XV method).

Results. Acid saline alone or with addition of polymyxin B, up to and including toxic amounts, does not induce adrenal ascorbic acid depletion in the acute lesioned rat (Exp. 1, Table I). Lack of response is not attributable to hyposensitivity of the adrenal to ACTH; 0.5 and 2.0 milliunits (mu) ACTH evoked adrenal ascorbic acid depletion of a magnitude similar to that observed in the 24-hour hypophysectomized rat (Exp. 2). Significant depletion was observed with quantities of Pitressin as low as 100 milliunits (mu) of pressor activity (Exp. 3). The observed response was not entirely due to release of pituitary ACTH as Pitressin also induced adrenal ascorbic acid depletion in hypophysectomized rats. The greater response in presence of the pituitary may be interpreted as indicating a direct effect of Pitressin on the adenohypophysis. McCann's claim(6) of direct action of vasopressin on the adenohypophysis is confirmed.

An acetic acid extract (SME, A-23), prepared from calf stalk-median eminence tissue depletes adrenal ascorbic acid in the lesioned assay animals. At a dose of 0.4 of an SME unit, degree of adrenal ascorbic acid depletion was equal and near maximum in lesioned and in hypophysectomized rats (Exp. 4a). However, at a dose of 0.025 SME unit, response was much greater in the lesioned than in the hypophysectomized animal (Exp. 4b). This suggests that there is an insufficient quantity of ACTH in the extracts to account for the activity in the lesioned rat. 0.025 SME unit contained 42 mu of pressor activity, a quantity which acting alone is insufficient to account for the adrenal ascorbic acid depletion in the lesioned rat. The possibility is not ruled out that small quantities of ACTH acting in conjunction with vasopressin potentiate (7) to yield the result observed in the lesioned rat. CRF activity was retained by the extracts after adsorption of most of the ACTH-like and vasopressin-like activity on oxycellulose. Approximately 90% of the pressor activity of extract M-7 was adsorbed on oxycellulose (Exp. 5a and 5b). The pressor activity of SME, M-7, oxycellulose supernatant was insufficient to account for the adrenal ascorbic acid depletion observed in the lesioned rat (cf. Exp. 3d with Exp. 5b). The quantity of ACTH in the oxycellulose supernatant was barely detectable. Adsorption of a second extract (SME, M-29) on oxycellulose eliminated approximately 85% of the pressor activity from the supernatant and led to a striking reduction of response in the hypophysectomized rat (Exp. 5c and 5d). CRF activity was not proportionately reduced. Again, the possibility that a small amount of ACTH plus a small amount of vasopressin induced a response in the lesioned rat which represented potentiation cannot be ruled out.

More definitive evidence for presence of a factor in SME, distinct from ACTH and from vasopressin, which induces adrenal ascorbic acid depletion in the lesioned rat, was derived from experiments with pepsin. The factor largely responsible for adrenal ascorbic acid depletion in the lesioned rat was pepsin labile (Exp. 5d, cf. 6a; 6b, cf. 6c), whereas inherent pressor activity and activity of added ACTH were unaffected by pepsin (Exp. 6b, c, d, e). Incubation of SME, A-23 in absence of pepsin resulted in no significant change in activity in lesioned rats.

Discussion. Ablation of the median eminence area of the hypothalamus results in failure of ACTH release in response to a variety of otherwise effective stimuli(4) (ether anesthesia plus unilateral adrenalectomy, histamine, epinephrine, toxic doses of polymyxin B). Heretofore, only 2 agents, vasopressin and ACTH, have been demonstrated to induce adrenal ascorbic acid depletion in the lesioned rat(4). The present study demonstrates that extracts prepared from tissue composed of calf pituitary stalk and median eminence area are also effective. The extracts contain both vasopressin-like activity and ACTH-like activity. Assays of diluted extracts and of oxycellulose fractions suggest that these 2 agents are only in part respon-

sible for the activity in the lesioned rat. Resistance of ACTH and of vasopressin to pepsin digestion has been previously reported and is confirmed in the present study(8,9). Loss of CRF activity of the SME extracts following incubation with pepsin constitutes definitive evidence for a CRF distinct from vasopressin.

Pitressin (Exp. 3), purified natural vasopressin and synthetic vasopressin(7) have been demonstrated to deplete adrenal ascorbic acid in the hypophysectomized rat. The pepsin labile factor may also have an extrapituitary action. The fact that Pitressin and the pepsin labile factor induce a much greater degree of adrenal ascorbic acid depletion in the lesioned than in the hypophysectomized rat suggests that the major action of both agents is on the adenohypophysis.

A working concept for action of vasopressin in inducing adrenal ascorbic acid depletion has been presented(7). Vasopressin may release ACTH from the adenohypophysis, from the kidney, and possibly from other tissues by competing for polypeptide binding sites. The pepsin labile factor may act in a similar fashion. That the release of ACTH is relatively specific is indicated by failure of oxytocin(6), glucagon (unpublished observation) and polymyxin B to deplete adrenal ascorbic acid in the lesioned rat.

Both the pepsin labile factor and vasopressin satisfy the classical criteria for endocrine roles. They are present in an area whose ablation results in an endocrine deficiency (failure of ACTH release in response to acute stimuli) and administration of either agent to the lesioned rat corrects the deficiency (release of ACTH). Demonstration of the pepsin labile factor at site of origin of the portal venous system lends support to the concept that a substance elaborated in the hypothalamus is carried *via a* vascular link to the adenohypophysis where it initiates ACTH release.

Summary. Ability of acid extracts of calf hypothalamus (stalk-median eminence area) to induce adrenal ascorbic acid depletion in the median eminence lesioned rat is attributable in large measure to a pepsin labile factor distinct from ACTH and from vasopressin.

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Species Differences in Cholesterol Biosynthesis by Arterial Tissue.* (24150)

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The role of the artery in production or maintenance of atherosclerosis is unknown. Since the artery itself is the primary target of the disease, its own metabolic activity may be of great importance in formation of atheroma. Until recently the evidence available(1,2,3) seemed to indicate that the lipids found in atheromata are deposited directly from the plasma. However, studies with P^{32} indicate that at least part of the phospholipid moiety

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