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Fractions of Commercial Pitressin which Release ACTH.*‡ (22537)

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Commercial Pitressin and certain fractions derived from it by chromatography, acid hydrolysis, Craig counter current distribution and other methods, are active ACTH releasing agents(1-9). In a footnote to an earlier communication(7), the writers called attention to the marked ACTH-releasing activity of hydrolysates of pitressin (Vasopressin Injection, Parke, Davis and Co.), in which the pressor activity had been destroyed by boiling for 1.5 hours in 2.2 N HCl. In addition to the releasing factor the acid hydrolysates contain a component indistinguishable in its activity from histamine when tested on the isolated ileum of the guinea pig and blood pressure of the etherized-atropinized cat.

Methods. A simple modification of the method of Saffran, *et al.*(5,6), was employed for determining the ACTH-releasing potency of pitressin† and its various fractions obtained by acid hydrolysis and Craig counter current distribution. Halved anterior pituitaries of adult rats were incubated in the Warburg apparatus for one hour with 0.67 mg of the test material. Small amounts of the incubation fluid (0.20 cc diluted to 0.5 cc), were then infused i.v. into 120-140 g male rats hypophysectomized 18-24 hours previously for adrenal ascorbic acid assay, as a measure of the efficacy of the test material for releasing ACTH.

Results. Table I-A gives the pertinent data obtained from experiments on the ACTH-releasing substance remaining in the pitressin hydrolysates after all pressor activity had been destroyed by boiling in strong acid(7).

Hydrolyzed pitressin exhibits potent releasing activity when incubated with halved pituitary glands *in vitro*, since the fall in adrenal ascorbic acid of the hypophysectomized rats averaged well over 100 mg %. The control halves of the same pituitary glands generally released small amounts of ACTH when compared with the quantities released by the experimental series. Arterenol added to the incubation fluid as originally recommended by Saffran, *et al.*, does not enhance releasing activity (Table I,A); therefore, it was not used in subsequent experiments. The hypophysectomized rat shows no depletion of adrenal ascorbic acid when infused i.v. with 300 units of pitressin boiled in 2.2 N HCl for 1.5 hours, despite the fact such material is highly active in releasing ACTH from pituitaries incubated in the Warburg.

Histamine, equivalent to 0.22 μg of the base, when added to incubation fluid containing halved pituitary glands did not induce release of greater amounts of ACTH than the corresponding control glands. These data agree with other reports of the ineffectiveness of this amine as an ACTH releaser(3,4) and make it unlikely that the histamine-like sub-

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stance which becomes evident in acid hydrolysates of pitressin is the ACTH-releasing factor.

Fractionation of pitressin by Craig counter current distribution. Non-hydrolyzed pitressin was fractionated in the Craig apparatus

TABLE I. ACTH-Releasing Activity of Pitressin.

		Sayers assay†	
		No. of rats	Ascorbic acid depletion, mg%*
A. Release of ACTH from ant. pituitaries *in vitro* by hydrolyzed pitressin, .67 mg in .55 cc incubation fluid/Warburg flask			
	Hypophysectomized		
Pitressin—hydrolyzed		37	−111 ± 18.6‡
Control		43	− 23 ± 6.6
Pitressin—hydrolyzed; arterenol added		5	−117 ± 32.8‡
Pitressin—hydrolyzed mildly in 0.6 N HCl for 30 min.		7	−103 ± 7.0‡
Control		8	− 18 ± 8.4
Histamine, 0.22 μg		6	− 31 ± 21.8
Control		6	− 36 ± 19.5
B. Release of ACTH *in vitro* by nonhydrolyzed pitressin fractionated by Craig counter current distribution, then hydrolyzed for testing			
Fraction (0.67 mg/flask)	Distribution coeff. (K)		
A_2–A_3	.59	8	−111 ± 12.0‡
Control		7	− 34 ± 16.8
B_1–B_2	1.33	8	−111 ± 15.2‖
Control		6	− 70 ± 7.9
C	4.24	7	−100 ± 21.7‡
Control		4	− 27 ± 28.0
B_3, nonhydrolyzed	1.33	5	−133 ± 15.0‡
Control		6	− 39 ± 10.0
C. Release of ACTH *in vitro* by pitressin hydrolyzed and then fractionated by Craig distribution			
(.67 mg/flask)	K		
Hydrolysate before Craig distribution		5	− 87 ± 12.1§
Control		6	− 39 ± 10.0
Hydrolysate after Craig distribution			
Fraction #1	1.33	5	−124 ± 16.1‡
#2	.75	5	−105 ± 14.7‡
Control		7	− 4 ± 12.3
Fraction #3	.42	5	− 5 ± 13.8
#4	4.24	5	− 40 ± 11.0‡
Control		4	− 29 ± 5.6
D. Release of ACTH *in vivo* by nonhydrolyzed pitressin in steroid-inhibited, intact rats			
	Dose, i.v.	Intact	
Pitressin nonhydrolyzed	3 units/rat	7	− 98 ± 8.4
Idem	5 "	5	−104 ± 19.1
Control (saline)	0.5 ml/rat	15	− 7 ± 12.8
E. Non-effect of i.v. injections of pitressin hydrolysate on adrenal ascorbic acid of hypophysectomized rats			
Pitressin—hydrolyzed	300 units/rat	8	− 8 ± 12.8

* Mean stand. error.

† Each rat intravenously infused with equivalent of .20 cc of incubation fluid from a Warburg flask containing 5 mg of anterior pituitary tissue.

‡ Differs significantly from controls ($P < .01$).

§ *Idem* ($P < .02$).

‖ " ($P < .05$).

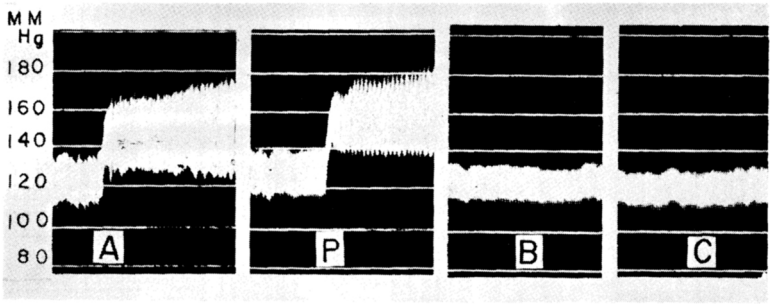

FIG. 1. Isolation of pressor factor in one fraction of pitressin by Craig counter current distribution. Dog, 11.4 kg, nembutal anesthesia, carotid blood pressure. A: Fraction A 0.05 unit/kg i.v. P: pitressin 0.05 unit/kg i.v. Fraction B: 1.0 unit/kg i.v. Fraction C: 1 unit/kg i.v. Fractions A, B and C are all active ACTH-releasing agents.

using secondary butanol and a 0.5% solution of trichloroacetic acid as a solvent system.§ One hundred transfers were made and the resulting fractions grouped on the basis of their distribution coefficients (Table I,B). Fractions A_2-A_3 administered i.v. to an anesthetized dog induced a sharp rise in the arterial pressure characteristic of the pressor action of commercial pitressin (Fig. 1). Fractions B and C were without effect on the blood pressure even in large doses, indicating that all of the pressor activity is confined to the A fraction. All fractions except B_3 were then hydrolyzed as usual and tested for ACTH-releasing activity. It should be noted, however, that the A, B, and C fractions all possessed ACTH-releasing activity, indicating that although the Craig counter current procedure, using the solvent system employed, was effective in concentrating the pressor factor in fraction A, it failed to isolate the releasing agent, which remained widely distributed. It was not difficult to rid the pitressin of all pressor activity by employing (1) strong acid hydrolysis; and (2) distribution in the Craig apparatus, but it remained to be demonstrated that the ACTH-releaser was definitely not the histamine-like component of the hydrolysate reported in earlier communications to be present in such material(7,8). Substantial evidence for complete separation of these activities was secured as follows: pitressin was hydrolyzed and then tested for ACTH-releasing activity by the *in vitro* Saffran technic and Sayers test and found to be active (Table I,C). The same material when tested on the isolated guinea pig ileum elicited typical histamine-like responses. The hydrolyzed pitressin was then distributed in the Craig apparatus and the various fractions tested by the usual method for determining ACTH-releasing activity and histamine-like response. Two fractions (Table I,C) of the hydrolyzed, Craig-distributed material were potent and resulted in sharp decline in the adrenal ascorbic acid of -124 ± 16.1 and -105 ± 14.7 mg %. One fraction, No. 4, was less potent and another fraction, No. 3, was completely inactive. However, negative results were obtained in repeated tests of the same material, both active and inactive as ACTH releasers, when tested on the guinea pig ileum. Thus, significant depletion of adrenal ascorbic acid occurs in hypophysectomized rats when these animals are infused with Warburg fluid obtained from incubation of halved anterior pituitaries with pitressin from which the histamine-like component has been removed by counter current distribution.

The corticotropin-releasing action of non-hydrolyzed pitressin can be readily demonstrated *in vivo* by i.v. infusion of 3-5 units into intact rats whose ACTH output has been inhibited by subcutaneous injections 16-20 hours previously of 10 mg each of microcrystalline DCA and the free alcohol of hydrocortisone suspended in 15% ethanol and Tween 80. Significant decline (98-104 mg %)

§ We are indebted to the Merck, Sharp and Dohme Research Laboratories for the counter current distributions recorded in this paper.

in adrenal ascorbic acid occurred in the experimental series whereas saline injected controls gave negative results (Table I,D), thus confirming the work of Porter and Rumsfeld (14).

Discussion. It is evident(1-9) that commercial pitressin contains a fraction active in releasing ACTH. The exact nature of this substance has not been definitely established although there is some evidence(3-6) that it is a peptide. It is apparent that the pressor factor in pitressin can be concentrated in one fraction by Craig counter current distribution. However, other fractions lacking the pressor agent retain their ACTH-releasing potency as do pitressin hydrolysates in which all pressor activity has been destroyed. The histamine-like component of commercial pitressin which appears in the acid hydrolysates is not to be confused with the ACTH releaser, since it, too, can be separated from the latter by counter current distribution.

It was recently announced(4,8) that Substance P, the smooth muscle stimulating factor originally described by von Euler and Gaddum(10), is an active ACTH-releasing agent. Data obtained by Guillemin and the writers afford evidence that the releasing factor in Substance P is a different entity from that commonly designated as P and can be separated from it by chromatography(4,9). It seems not improbable that the ACTH-releasing agents associated with both commercial pitressin and Substance P are identical substances. It is interesting to note that both occur in appreciable quantities in the hypothalamus and that Substance P is widely distributed throughout the nervous system(11, 12,13).

Summary. Commercial pitressin, hydrolyzed or nonhydrolyzed, exhibits ACTH-releasing activity when incubated with pituitaries in Warburg flasks and the incubation fluid infused i.v. into hypophysectomized rats. The ACTH-releasing agent of pitressin can be separated from the pressor component by Craig counter current distribution or by destroying the latter by acid hydrolysis. The hydrolysates when infused i.v. do not deplete the adrenal ascorbic acid of hypophysectomized rats. Unmodified pitressin will release ACTH *in vivo* from pituitaries of steroid inhibited rats. The histamine-like substance present in acid hydrolysates is not the ACTH-releasing agent and can be separated from it. Histamine added to the Warburg incubation fluid does not induce ACTH release.

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