Effect of Histamine, Hog Vasopressin, and Corticotropin-Releasing Factor (CRF) on ACTH Release *in vitro*. (22568)

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Both vasopressin and histamine have been suggested as neurohumoral mediators in the release of ACTH(1-3). Recently, Swingle *et al.*(4) reported that an acid extract of pitressin contained a histamine-like substance and was active in releasing ACTH *in vitro*. Martini *et al.*(5) claimed that both oxytocin and vasopressin can induce a discharge of ACTH if injected into normal rats. However, Guillemin and Hearn(6) demonstrated that highly purified arginine (beef) vasopressin was without effect on the release of ACTH by tissue cultures of rat pituitaries. Our own work(7) has shown that corticotropin-releasing-factor (CRF) is distinct from oxytocin and beef vasopressin. This paper describes the effects of histamine, purified lysine (hog) vasopressin and purified CRF on the release of ACTH *in vitro*.

Materials and methods. Histamine dihydrochloride (Fisher Reagent); the amounts added are expressed as μg of histamine base per incubation flask. Vasopressin, from hog sources, was prepared according to the method of Stehle and Fraser(8) and subjected to paper chromatography in butanol:acetic acid: water by the procedure of Benfey*(9). This preparation, possessing a pressor activity of 200 U./mg, was assayed for CRF activity and then further purified by paper chromatography in m-cresol(10). Purified CRF was prepared from Protopituitrin† (Parke, Davis, and Co.), which corresponds to fraction C of Kamm(11). The purification procedure for CRF will be reported elsewhere(10). The detection of CRF activity was made according to the method of Saffran and Schally(12) using isolated rat pituitaries and the *in vitro* bioassay of ACTH by the method of Saffran and Schally(13). The results are expressed as the ratio of the ACTH released in the presence of the substance tested to the ACTH released by the control, accompanied by the 95% confidence limits. A significant stimulation of ACTH-release is denoted by a lower confidence limit greater than 1.0.

TABLE I. CRF and ACTH-Release.

CRF preparation	l-Arterenol .0002M	Dose CRF, μg/flask	Ratio, exp. to control	95% limits
Purified 40×	Present	.26	2.1	1.6→2.6
Idem	"	.26	2.15	1.3→3.5
"	Absent	.26	2.5	1.7→3.6
"	"	2.6	3.4	2.9→4.0
Purified 110×	"	.09	2.3	1.3→4.1

Results. Table I shows the results obtained with CRF preparations at various purification stages, with or without arterenol. Small doses of purified CRF significantly double the release of ACTH. This was the case whether arterenol was present or not. The resuts obtained with histamine are shown in Table II. Arterenol was omitted in tests with histamine. Histamine did not significantly increase the release of ACTH *in vitro*, whereas in one experiment a dose of 0.01 μg significantly inhibited the release of ACTH. Results obtained with hog vasopressin are shown in Table III. There was a slight, but not significant increase in the release of ACTH. After further purification in m-cresol, in which vasopressin and CRF have distinctly different R_f's, CRF activity in vasopressin was not detectable.

Discussion. Harris *et al.*(14) were able to extract histamine from hypothalamus and pos-

TABLE II. Histamine and ACTH-Release.

Dose, histamine/flask (μg)	Ratio of experimental to control	95% confidence limits
.01	.5	.4 → .8
.1	.5	.2 → 1.3
.1	1.1	.8 → 1.4
.5	1.5	.6 → 3.9

* We are indebted to Dr. B. G. Benfey for his gift of purified lysine vasopressin.

†We are grateful to Dr. D. A. McGinty of Parke, Davis, and Co. for generous gifts of posterior pituitary preparations.

TABLE III. Hog Vasopressin and ACTH-Release.

Preparation	l-Arterenol .0002M	Dose vasopressin per flask, μg	Ratio, exp. to control	95% limits
Benfey	Present	.5	1.5	.9→2.6
"	"	.5	1.4	.8→2.6
m-cresol purified	Absent	.2	1.3	.9→1.7
Idem	"	.5	.9	.8→1.2

terior pituitary tissue. The results reported here indicate that free histamine is not the neurohumour responsible for the release of ACTH. If histamine in pitressin is bound in a peptide linkage, as Swingle *et al.*(4) suggest, then it should have been liberated by their extraction procedure. In our hands posterior pituitary preparations lose their CRF activity when subjected to overnight hydrolysis in 6N HCl at 110°C(15), a procedure which would not destroy histamine. Similarly, we cannot support the claims of Martini *et al.*(5) that vasopressin may stimulate the anterior lobe to release ACTH. Their results were obtained with impure preparations which could contain CRF(7). The same conclusion, with respect to the absence of CRF activity in histamine, was reached independently by Guillemin(16).

Summary. Histamine and purified lysine (hog) vasopressin did not accelerate the release of ACTH by isolated rat pituitaries. In contrast, purified CRF preparations in minute doses significantly increased the release of ACTH.

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Received June 25, 1956. P.S.E.B.M., 1956, v92.

Sulfamethoxypyridazine: Preliminary Observations on Absorption and Excretion of a New, Long-Acting Antibacterial Sulfonamide.* (22569)

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Antimicrobial agents that have a prolonged action have potential advantages, particularly if they are effective in prophylaxis, or in the prolonged treatment of chronic or subacute infections, or in the suppression of chronic infections that are difficult to eradicate. A new antibacterial sulfonamide, sulfamethoxypyridazine (3-sulfonamido-6-methoxypyridazine)† was found to have the following properties, judged from experiments in dogs, rabbits and mice: high solubility in urine, good absorp-

* Aided by a grant from the National Institutes of Health.

† Generously provided by Lederle Laboratories Division, American Cyanamid Co. under the trademark Kynex (Lederle).