Concentration of Corticotropin Releasing Factor by Chromatography on Carboxymethylcellulose. (24551)

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Evidence has been obtained that minute doses of a corticotropin releasing factor (CRF) purified by paper chromatography and electrophoresis are active in stimulating release of corticotropin from the anterior pituitary tissue *in vitro*(1,2). The effect of these preparations to increase incorporation of P^{32} into the pituitary phospholipids, interpreted as an indication of active secretory process has also been described(3). Though it was possible to obtain amino acid composition of purified material(2) amounts obtained were not sufficient for homogeneity, degradation and structural studies. This communication describes large scale purification of CRF on carboxymethylcellulose (CMC) cation exchange adsorbent on the basis of an *in vivo* assay for CRF.

Methods and materials. CMC was prepared as described in Schally *et al.*(4) except for additional treatment with 8-hydroxyquinoline after synthesis. The operation of columns and other details of chromatography have also been described(4). Starting materials for chromatography were protopituitrin and pitressin intermediate (Parke, Davis and Co.) of posterior pituitary origin, which are fractions C and D in the Kamm procedure as outlined by Waring and Landgrebe(5).* Protopituitrin of hog origin was treated 3 X with oxycellulose to remove ACTH(6). Elution of peptide and protein material was effected by use of ammonium acetate buffers of progressively increasing ionic strength and pH (4). Bracketed assays for oxytocin were performed by vasodepressor assay of Coon(7) as modified by Thompson(8) and pressor activity was measured in the rat(9) against USP XV posterior pituitary reference standard. CRF activity as evidenced by stimulation of ACTH release was assessed by measurement of plasma free corticosteroids in the rat(10) after morphine-nembutal administration. All fractions were tested at a dose corresponding to 10 or 30 milliunits pressor activity as determined previously by assay for vasopressin (see above). This low dose of vasopressor activity, as highly purified lysine vasopressin (287 U/mg, obtained by separation on CMC(11) followed by paper partition chromatography in m-cresol/H_2O(2)) is devoid of hypophysiotropic activity in the nembutal-morphine preparation(12,13). The results are expressed in provisional CRF units using crude CRF preparation fraction D(1) as standard. Response of the nembutal-morphine blocked rat to fraction D is similar to that of the rat 20-24 hours after effective hypothalamic lesion(12). All fractions showing CRF activity are finally tested in the 24 hour hypophysectomized rat.

Results. Figs. 1 and 2 show the chromatographic pattern and biological activities obtained. It is apparent from Fig. 1 that pressor and CRF activities were closely related in their chromatographic behavior on the small column of carboxymethylcellulose. The maximal CRF activity, however, was found in the early tubes of the vasopressin peak. Fraction No. 610 was active to release ACTH at level of 0.06 μg and was 4-5 times as active as fraction No. 630, though both these fractions had the same pressor activity of 260 U/mg. Fraction No. 590, was more potent CRF-wise than fraction No. 630, though pressor activity of No. 590 was only half that of No. 630.

Fig. 2 shows that with a large column and different gradients a better separation of the 2 activities was obtained, even though specific activity of CRF fractions was lower in the second column than in the first, due to presence of large amounts of other contaminating peptides or proteins but different from vasopressin and oxytocin. Fractions No. 640-680 had most of the CRF activity and only 13-25

* Evidence has been reported that CRF is present in both hypothalamus tissue and commercial extracts of posterior pituitary(1,2).

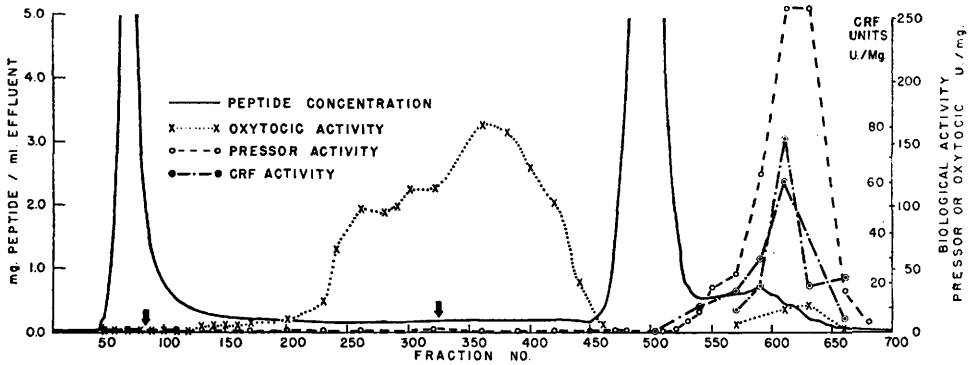

FIG. 1. 1 g protopituitrin applied on column 1.5 × 47 cm, equilibrated with 0.01 M, pH 4.5 ammonium acetate buffer. Hold-up vol 50 ml. Gradient to pH 6, 0.02 M buffer started at tube 85 through a 125 ml flask. Gradient to 0.2 M, pH 7 buffer started at tube 323 through a 250 ml flask. 1.2 ml fractions collected.

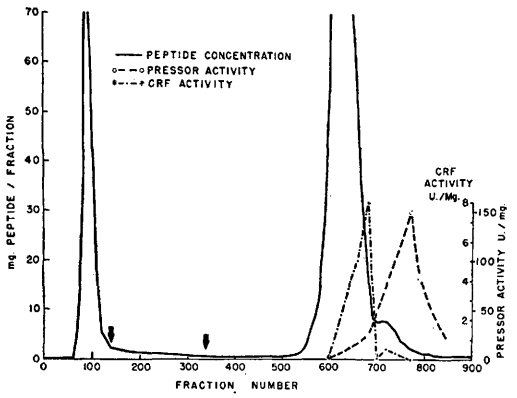

FIG. 2. 10 g pitressin intermediate applied on column 3.8 × 52 cm. Hold-up vol 300 ml. Gradient was 0.01 M, pH 4.5 to 0.02 M, pH 6 buffer through 500 ml flask. Second gradient was to 0.2 M, pH 7 buffer through 2000 ml flask started at tube 340. 5 ml fractions collected.

U/mg pressor activity. Fractions 700-770 had no CRF activity at a dose of 30 pressor milliunits.

The fractions containing CRF were tested in the 24 hour hypophysectomized rat for ACTH at doses twice those injected for the CRF assay. All were found to be inactive in the hypophysectomized rat.

Summary. 1) Neurohypophysial extracts of commercial origin were chromatographed on carboxymethylcellulose cation exchange adsorbent under conditions of pH and ionic strength gradient in view of separating CRF. An *in vivo* assay was used to assess CRF activity. 2) The method described is useful in large scale preparations. Fractions containing CRF were regularly found to emerge before lysine vasopressin and were well separated from oxytocin.

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