and pathological interest in the colon.

*Summary.* A technic is described for estimating regional blood flow in the gastrointestinal tract. This method utilizes the short range of the beta particle in tissue to limit the blood volume monitored by a special Geiger-Muller tube placed in the intestinal lumen.

Received March 10, 1961. P.S.E.B.M., 1961, v107.

## Corticotropin Releasing Activity of Synthetic Lysine Vasopressin.* (26672)

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Our attempts to isolate a corticotropin releasing factor (CRF) from extracts of porcine posterior pituitary have been complicated by the facts that CRF is apparently quite difficult to separate from vasopressin, and that vasopressin itself has been shown to possess corticotropin (ACTH) releasing activity. When doing CRF bio-assays on fractions containing vasopressin it is necessary to take into account any "CRF" activity due to the vasopressin present. In this paper we report our values for ACTH-releasing activity of synthetic lysine vasopressin, using the same *in vivo* CRF bio-assay(1) used in our isolation work.

*Materials and methods.* Synthetic lysine vasopressin was kindly provided by Dr. V. du Vigneaud, Dept. of Biochemistry, Cornell Univ. Medical College. The sample used in these experiments (JM V 68/16) was reported to contain 260 International Units (U)/mg. Two assays of this sample *vs* USP Posterior Lobe Reference Standard in dibenamine treated rat by method of Dekanski(2) gave us 258 (95% fiducial limits: 228-291) and 267 (95% fiducial limits: 256-279) U/mg, respectively; the mean of these 2 assays, 262 U/mg, was taken as the pressor activity of the sample. The entire solid sample as received was dissolved in deionized water for pressor assay, the solution then divided into aliquots and each of these lyophilized and stored in deep freeze; assays for CRF could then be carried out on a solid sample freshly diluted in physiological saline.

For CRF assays, 150-170 g male rats from Holtzman Co., Madison, Wisc. were used. Only modification of the *in vivo* assay procedure of Guillemin(1) was that morphine was injected 35 min after nembutal injection instead of 25 min. Uninjected rats and rats injected with 0.3 ml physiological saline instead of vasopressin were introducd randomly throughout each assay as controls. Release of ACTH was determined by measuring concentration of free corticosteroids in 2 ml of plasma by the method of Silber as modified by Guillemin(3). In rats, corticosterone (cpd B) is the principal steroid determined.

In several experiments, male Holtzman rats of the same weight range were hypophysectomized and 24 hrs later injected with the vasopressin sample; these rats were not treated with nembutal-morphine but were anesthetized with ether before injection of sample. After 15 min, blood was drawn and plasma corticosteroids were determined as in the standard CRF assay.

*Results* are shown in Table I. Controls in each case include all values from both uninjected and saline injected rats, since statistical analysis of the two types of control values showed no significant difference between them. Data for the 3 dose levels of vasopressin giving significant increases of

* This investigation has been supported in part by a grant-in-aid from Eli Lilly & Co. and by research grant from Nat. Inst. of Neurol. Dis. and Blindness, U.S.P.H.S. Journal Paper No. J-4022 of Iowa Agri. and Home Economics Exp. Station, Ames. Project No. 1304.

TABLE I. Effect of Synthetic Lysine Vasopressin on Plasma Corticosterone of Rats.

| Pressor amt of LVP in mU | Controls: No. of rats | Controls: Plasma-cpd B, μg/100 ml | Inj. rats: No. of rats | Inj. rats: Plasma-cpd B, μg/100 ml | p value† | % increase over controls |
|---|---|---|---|---|---|---|
| 8.4 | 7 | 8.0 ± .8* | 10 | 8.5 ± 1.1 | .8 > p > .7 | — |
| 16.8 | 18 | 8.2 ± .7 | 19 | 11.4 ± .7 | .01 > p > .001 | 39 |
| 33.5 | 10 | 8.1 ± .8 | 9 | 14.7 ± 1.4 | < .001 | 81 |
| 67 | 7 | 8.1 ± 1.4 | 10 | 18.2 ± 1.4 | < .001 | 125 |
| | | | Hypophysectomized rats | | | |
| 67 | 11 | 2.4 ± .1 | 9 | 2.5 ± .1 | .8 > p > .7 | — |

* Mean ± stand. error.
† p calculated from t on values for plasma-cpd B in controls and in sample-injected animals.

plasma corticosteroids over controls were also submitted to analysis of variance, giving a value of 11.4 for F, significant at the 0.1% level. Fig. 1 shows the regression line for percent increase of plasma corticosteroids *vs* log of dose of vasopressin and the 3 experimental points with standard errors of the means. The regression equation is: $y = -134 + 141.5\ (x)$.

In the hypophysectomized animals, control values were lower than in nembutal-morphine treated animals, but injection of as much as 67 mU of synthetic lysine vasopressin caused no increase whatever over controls.

*Discussion.* McCann and Fruit(4) have also investigated the ACTH releasing activity of synthetic lysine vasopressin, using rats with acute hypothalamic lesions as experimental animals and depletion of adrenal ascorbic acid as index of ACTH release. Synthetic vasopressin was found to give the same log dose *vs* response curve given by commercial Pitressin in the range of 62 to 1,000 mU of pressor activity. Minimal effective dose was of the order of 100 mU in McCann's system.

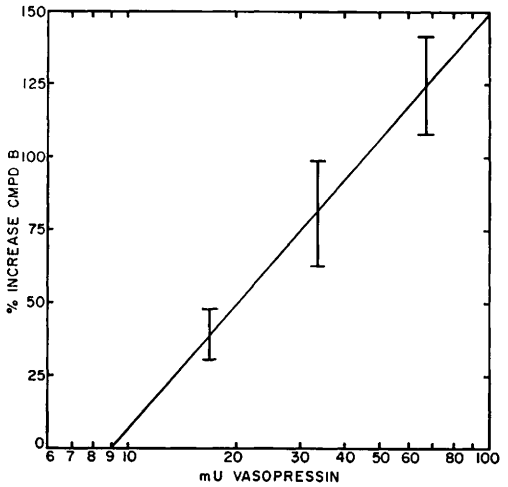


FIG. 1. Increase of plasma corticosterone (± S.E. of mean) in nembutal-morphine blocked rats *vs* mU synthetic lysine vasopressin injected; (logarithmic abscissa).

Guillemin *et al.*(1) more recently have examined ACTH releasing activity of lysine vasopressin in the nembutal-morphine blocked rat, using a sample of vasopressin isolated from porcine pituitary preparations. Their sample had been purified by the method of Ward and Guillemin(5) and further purified by paper chromatography in *m*-cresol/water to a pressor activity of 287 U/mg. They used much lower doses than McCann and Fruit, down to 6 mU, but concluded that there is a threshold for ACTH release by vasopressin at approximately 60-80 mU of pressor activity. That is, above about 80 mU a large discharge of ACTH was observed, increasing linearly with log dose of vasopressin, but below 80 mU, no statistically significant release of ACTH could be detected. Guillemin used increase of plasma corticosteroids determined fluorometrically as his index of ACTH release and found this to be a more sensitive index than adrenal ascorbic acid depletion when both determinations were done on the same animal.

In contrast to Guillemin's report, our results, using synthetic lysine vasopressin, show an excellent linear log dose *vs* response relationship all the way down to about 8-10 mU of pressor activity, using essentially Guillemin's experimental conditions. From our results it appears necessary for us to dilute unknown samples down to a pressor dose

of about 10 mU before assaying for CRF in the nembutal-morphine treated rat, instead of the 80-100 mU threshold indicated by Guillemin(6); in his laboratory, samples are actually diluted down to 30 mU for CRF assay(7). Alternatively, it may be legitimate to subtract the ACTH releasing activity corresponding to the amount of vasopressin injected (as determined by pressor assay). When assaying for CRF near a pressor peak in fractionations by chromatography, electrophoresis, or counter current distribution, the value taken for the ACTH releasing activity intrinsic in vasopressin assumes critical importance.

It is always possible to confuse ACTH releasing activity with activity of ACTH itself unless the latter is specifically ruled out. Different investigators, using a variety of vasopressin preparations, experimental animals, and indices of "ACTH-like" activity, have come to opposite conclusions about direct action of vasopressin on the adrenal cortex. Thus, Sobel *et al.*(8) found that urinary corticosteroids increased after injection of commercial Pitressin in normal guinea pigs, but not in hypophysectomized guinea pigs. Several figures in a paper by Schally and Guillemin(9) indicate that chromatographically purified arginine vasopressin and lysine vasopressin did not increase plasma corticosteroids on injection in hypophysectomized rats. On the other hand, Royce and Sayers(10) found that large doses (2.5-5.0 U) of purified arginine vasopressin or of commercial Pitressin depleted adrenal ascorbic acid in hypophysectomized rats, and Hume and Nelson reported that *i.v.* injection of vasopressin increased 17-hydroxycorticoids in adrenal venous blood of hypophysectomized dogs(11). Hilton *et al.*(12) recently described a direct ACTH-like effect of synthetic vasopressins, measured by increase of adrenal venous hydrocortisone during direct arterial perfusion of adrenals of hypophysectomized dogs. Our negative results with injection of synthetic vasopressin in hypophysectomized rats indicate that the effect we have observed is not "ACTH-like" but rather a true "CRF-like" effect.

*Summary.* Synthetic lysine vasopressin was assayed for ACTH releasing activity in nembutal-morphine treated rats using fluorometric determination of plasma corticosteroids as index of ACTH release. A linear log dose *vs* response relationship was established between pressor doses of 8 to 67 mU. This activity was shown to be "CRF-like" rather than "ACTH-like" by its absence in hypophysectomized rats injected with 67 mU of pressor activity.

The authors wish to express appreciation to Dr. Stephen Mandel, Dept. of Statistics, I.S.U., for many helpful discussions during this work.

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Received March 22, 1961. P.S.E.B.M., 1961, v107.