Oxytocic Activity of Purified Vasopressin. (19924)

EDWIN A. POPENOE,* JOHN G. PIERCE, VINCENT DU VIGNEAUD,† AND H. B. VAN DYKE.‡

From the Department of Biochemistry, Cornell University Medical College, and the Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York City.

In the fractionation of aqueous extracts of posterior pituitary glands, Kamm and coworkers(1) obtained preparations high in oxytocic activity, with small amounts of pressor and antidiuretic activities. They also obtained preparations high in pressor and antidiuretic activities with low oxytocic activity. Relatively, however, oxytocin preparations were freer of pressor activity than *vice versa*. Since their most potent pressor fractions still possessed considerable oxytocic activity, they suggested that the pressor hormone might possess inherent oxytocic activity. Following the appearance of the work of Kamm and coworkers, several alternative procedures for the separation and purification of the active principles from the posterior pituitary have been employed. These procedures have been reviewed extensively(2). None of the methods has yielded a pressor preparation completely devoid of oxytocic activity. However, on these preparations there was no analytical data which could exclude the possibility of contamination with oxytocin.

With the more refined technics recently available—countercurrent distribution(3) for purification and starch column chromatography(4) for analysis of the constituent amino acids—it has been possible to prepare and to analyze high potency oxytocic material(5,6) and high potency vasopressor material(7). The present paper reports the pharmacological testing of such preparations for oxytocic, pressor, and antidiuretic activities.

Experimental. The vasopressin preparation used in this study was prepared from desiccated beef posterior pituitary lobes by the procedure of Turner, Pierce, and du Vigneaud(7), except that a greater number of transfers was used in the countercurrent distribution procedure and in the later stages the distribution was carried out at 10°. Analysis of a hydrolysate of this material by the starch column chromatographic technic of Moore and Stein(4) showed the presence of the 8 constituent amino acids of high potency beef vasopressin preparations(7)—phenylalanine, tyrosine, proline, glutamic acid, aspartic acid, glycine, arginine, and cystine—in molar ratios approximating 1:1, plus 3 moles of ammonia per mole of any one amino acid. No other amino acids could be detected. Since the presence of phenylalanine in vasopressin preparations has been questioned and the presence of isoleucine has been suggested (8), we would add that microbiological assay of a hydrolysate of a vasopressin preparation for L-phenylalanine, performed in the Cornell Laboratories by Dr. Dorothy S. Genghof using *L. arabinosus*(9), gave results in good agreement with the starch column analysis. Furthermore, when isoleucine was added to a hydrolysate of vasopressin prior to starch column analysis a new peak appeared at the isoleucine position which was clearly distinguishable from the phenylalanine peak.

Pressor assays were made on the preparation by comparing its effect with that of U. S. P. standard posterior pituitary powder on the arterial blood pressure of cats under phenobarbital anesthesia. Operative and mechanical procedures were similar to those described by Kamm and coworkers(1) for assays using dogs. The preparation consistently showed 600-650 pressor units per mg by this method. Assayed for oxytocic activity by the chicken blood pressure method of Coon(10), the preparation appeared to possess an activity of 80-90 oxytocic units per mg. Oxytocic

* Public Health Service Postdoctorate Research Fellow of the National Institutes of Health.

† Appreciation is expressed to Lederle Laboratories, American Cyanamid Co., for a grant-in-aid and to the Eli Lilly Co., Parke, Davis and Co., and Armour and Co., for gifts of posterior pituitary material.

‡ A portion of the experiments was completed in the National Institute for Medical Research, Mill Hill, London, through the kind hospitality of Sir Charles Harington and Dr. W. Feldberg.

assays were then performed on the rat uterus immersed in de Jalon solution(11) at 32.5°. An oxytocic activity of approximately 30 units per mg was found for this preparation. This relationship of 1 oxytocic unit to each 20 vasopressor units even held after a considerable loss of activity had been encountered, when some of the preparation was dissolved in 0.25% acetic acid and distributed in ampoules which were sealed and sterilized. The activity of the contents in this instance happened to fall from 20 to 10 vasopressor units per ml. Coincidentaly the oxytocic potency for the rat uterus fell from 1 unit per ml to 0.44 unit per ml.

An antidiuretic assay on the 600-650 unit per mg vasopressin preparation, using rats and diabetes insipidus dogs, also gave 600-650 antidiuretic units per mg, in reference to the U. S. P. standard posterior pituitary powder. Another preparation showing 450 units per mg in the cat pressor assay also showed 450 units per mg in the antidiuretic assays.§

A purified preparation of oxytocin(6,13) assaying approximately 500 oxytocic units per mg by the chicken blood pressure method and showing no detectable pressor activity in the cat, showed an antidiuretic activity of less than 0.5 unit per mg in the diabetes insipidus dog.

Discussion. Of the 8 amino acids present as constituents of high potency vasopressin preparations, 6 are also found in hydrolysates of highly purified oxytocin preparations(6), that is tyrosine, proline, glutamic acid, aspartic acid, glycine, and cystine. The remaining 2, arginine and phenylalanine, are absent in oxytocin preparations while leucine and isoleucine are present. Since there is good reason to believe that these oxytocin preparations are pure or nearly pure samples of the oxytocic principle, the leucine/isoleucine content of vasopressin preparations is on this basis a measure of the maximum contamination by oxytocin. In the analysis of the 2 mg sample of vasopressin by starch column chromatography, it would be possible to detect 0.7% contamination by oxytocin by the appearance of a leucine/isoleucine peak in the chromatogram. Since the vasopressin preparation used in the present study contained no detectable leucine or isoleucine, the contamination by oxytocin (500 units per mg) must be less than 0.7%, or less than 3.5 oxytocic units per mg. From the oxytocic assay of 30 units per mg, one is led to conclude that vasopressin has intrinsic oxytocic activity as one of its biological properties, unless there is another oxytocic contaminant, which at the moment we have no reason to suspect. The finding that with decline of vasopressor activity in a dilute solution of vasopressin there was a corresponding and proportional loss of oxytocic activity supports the conclusion made.

The fact that the most highly purified vasopressin preparation shows the same number of units per mg in the antidiuretic assay as in the pressor assay offers convincing evidence that the antidiuretic and pressor activities are truly due to the same chemical compound, which has long been suspected. The fact that highly purified oxytocin preparations show no pressor activity by the blood pressure method and only a slight trace of activity in the extremely sensitive antidiuretic assay leaves no doubt that oxytocin does not possess pressor or antidiuretic activity.

It should be recalled that Coon(10) found that when pressor activity strongly predominated in various preparations, exaggerated responses were obtained by the chicken blood pressure method for oxytocic activity in reference to the uterine strip method. For example, he reports, for a solution possessing 1.5 units per cc by the uterine method and an oxytocic/pressor ratio of 0.06, that the chicken depressor method gave 5 units per cc, 3.5 times the uterine assay. This observation compares well with the results of the present study on high potency vasopressin. Here the oxytocic/pressor ratio is 0.05 and the chicken depressor assay is 2.7-3.0 times the uterine assay.

The recent experiments with lactating sows

§ Preliminary assays of antidiuretic potency using rats according to Burn(12), carried out with the capable assistance of Mrs. Jacqueline E. Parton, had indicated that a vasopressin preparation of about 400 pressor units per mg possessed approximately 400 antidiuretic units per mg in reference to the U. S. P. standard powder.

reported by Whittlestone(14) on milk-ejecting activity of high potency preparations of both oxytocin and vasopressin, prepared in the Cornell Laboratories, are of interest in connection with the question of the inherent oxytocic activity of vasopressin. He found that an oxytocic preparation of approximately 500 units per mg(6.13) showed a milk-ejecting activity equivalent to its oxytocic activity and that a vasopressin preparation of 400 pressor units per mg(7) showed a milk-ejecting activity equivalent to that which would be expected of approximately 80 units of oxytocin. Cross and van Dyke(15) have made similar comparisons in lactating rabbits by the method of Cross and Harris(16), using an oxytocin preparation with a potency of about 500 units per mg, and the 600 unit per mg vasopressin preparation of the present study, both of which had been prepared in the Cornell Laboratory. The results of Cross and van Dyke were in good agreement with those reported by Whittlestone.

Summary. Evidence has been obtained with a highly purified preparation that vasopressin, in addition to its pressor and antidiuretic activities, possesses intrinsic oxytocic action as one of its pharmacological properties, in contrast with purified oxytocin which appears not to possess pressor or antidiuretic activity. For each 100 units of vasopressor-antidiuretic activity there is intrinsic "oxytocic" activity represented by approximately 5 units as determined by the isolated rat uterus assay or by approximately 13-15 units by the chicken blood pressure depressor assay. The evidence that vasopressin possesses milk-ejection activity about one-fifth that of oxytocin has been discussed.

1. Kamm, O., Aldrich, T. B., Grote, I. W., Rowe, L. W., and Bugbee, E. P., *J. Am. Chem. Soc.*, 1928, v50, 573.

2. Irving, G. W., Jr., and du Vigneaud, V., *Ann. N. Y. Acad. Sci.*, 1943, v43, 273.

3. Craig, L. C., *J. Biol. Chem.*, 1944, v155, 519; *Anal. Chem.*, 1950, v22, 1346.

4. Moore, S., and Stein, W. H., *J. Biol. Chem.*, 1949, v178, 53.

5. Livermore, A. H., and du Vigneaud, V., *J. Biol. Chem.*, 1949, v180, 365.

6. Pierce, J. G., and du Vigneaud, V., *J. Biol. Chem.*, 1950, v186, 77.

7. Turner, R. A., Pierce, J. G., and du Vigneaud, V., *J. Biol. Chem.*, 1951, v191, 21.

8. Stehle, R. L., *Science*, 1952, v115, 610.

9. Henderson, L. M., and Snell, E. E., *J. Biol. Chem.*, 1948, v172, 15.

10. Coon, J. M., *Arch. internat. pharmacodynamie*, 1939, v62, 79.

11. Burn, J. H., Finney, D. J., and Goodwin, L. G., *Biological Standardization*, 2nd Edition, Oxford, 1950, p. 180.

12. Burn, J. H., *Quart. J. Pharm. Pharmacol.*, 1931, v4, 517.

13. Pierce, J. G., Gordon, S., and du Vigneaud, V., *J. Biol. Chem.*, in press.

14. Whittlestone, W. G., *J. Endocrinol.*, 1952, v8, 89.

15. Cross, B. A., and van Dyke, H. B., To be published.

16. Cross, B. A., and Harris, G. W., *J. Endocrinol.*, 1952, v8, 148.

Received October 24, 1952. P.S.E.B.M., 1952, v81.

Assay of Botulinum a Toxin with Goldfish.* (19925)

T. E. CARTWRIGHT AND MAX A. LAUFFER.

From the Department of Biophysics, University of Pittsburgh, Pittsburgh, Pa.

The purpose of this report is to describe the successful use of the common goldfish as an assay animal for botulinum toxin. Goldfish have the obvious advantage that they are inexpensive and can be maintained healthy in a very small space.

The goldfish has been used extensively as a laboratory animal in toxicity tests. Pittenger and Vanderkleed(1), Pittenger(2), and Lapenta(3) observed specific reactions in

* Publication of the Department of Biophysics.