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ANTIDIURETIC ACTIVITY OF POSTERIOR PITUITARY PREPARATIONS

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Biological assays depend on the quantitative comparison between the effects of unknown amounts of a biologically active principle and those of a standard preparation. One requirement for a valid assay is that the unknown principle should be qualitatively identical with the standard active principle (Wood, 1946; Finney, 1952). In assays of neurohypophysial activities, commercial preparations from beef pituitaries are usually employed as standards. Such preparations are assayed by the manufacturers and their potencies are expressed in terms of the activity of the international standard pituitary (posterior lobe) powder. In some assays oxytocic activity is used (e.g., in the assay of Pitocin, a preparation of the differentiated oxytocic principle, and of Pituitrin, the undifferentiated extract with both ocytocic and pressor effects), whereas, in other assays, pressor activity is used (e.g., in the assay of Pitressin, a preparation of the differentiated vasopressor and antidiuretic principle).

In a study of the effect of haemorrhage on the active contents of the rat neurohypophysis, extracts of rat posterior pituitaries were assayed for oxytocic, vasopressor, and antidiuretic activity. Since none of the commercial preparations are assayed by antidiuretic activity, and since the extracts to be tested had both vasopressor and oxytocic activities, it was decided, as a precaution, to perform two antidiuretic assays on each extract, one using pitressin as the standard and one with pituitrin. The results of the experiments are described, and the discrepancies observed are discussed, in the present paper.

METHODS

White albino rats of the Wistar strain (180–220 g.) were used in all experiments. Antidiuretic potency was assayed by intravenous injection into unanaesthetized water-loaded rats following the technique of Ginsburg and Heller (1953) as modified by Ginsburg and Brown (1956). Pressor activity was assayed on the blood pressure of rats anaesthetized with urethane and treated with dibenamine as described by Dekanski (1952). Holton's (1948) method was used for the assay of oxytocic activity on the isolated rat uterus.

Rat neurohypophysial extracts were prepared by heating the homogenized glands in a boiling water-bath in 0.025% (w/v) acetic acid for 5 min. Extracts were prepared from the international standard pituitary (posterior lobe) powder following the instructions in the British Pharmacopœia (1953).

Messrs. Parke Davis & Co.'s Pitressin, Pituitrin and Pitocin were also used.

RESULTS

Fig. 1 shows the antidiuretic potencies of rat neurohypophysial extracts compared with their pressor potencies. When pitressin was used as the standard in the antidiuretic assay the antidiuretic potencies were, on average, about $1\frac{1}{2}$ times greater than the pressor potencies, and there was a good statistical correlation between the pressor and antidiuretic activities as they varied from extract to extract ($r = +0.83$; $P<0.01$). When pituitrin was used as the standard some of the results were in good agreement with those of antidiuretic assays using pitressin as standard, whereas others were 4–6 times greater; the antidiuretic activities of the extracts did not appear to be correlated with the pressor potencies ($r = +0.50$; $P>0.1$).

The antidiuretic effects of pitressin and pituitrin were then compared directly on the same rats. In ten rats out of sixteen, the difference between the antidiuretic activities of pitressin and pituitrin was within the large error of the method of assay (approx. $\pm 40\%$; Ginsburg and Heller, 1953). In the remaining six rats the antidiuretic potency of pituitrin varied from one half to less than one tenth of the potency of pitressin. Fig. 2 shows an experiment in a rat in which pituitrin had only one ninth of the antidiuretic activity of pitressin. This difference cannot be attributed to the oxytocin content of pituitrin as suggested by Fraser (1937, 1942) and Selye (1949), since the addition of pitocin to pitressin did not affect the antidiuretic response.

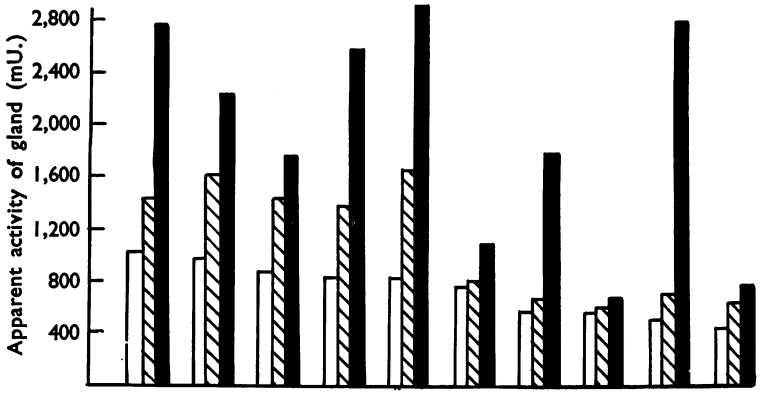

FIG. 1.—Estimated antidiuretic and pressor potencies of rat neurohypophyses. Unshaded columns, pressor potency–pitressin standard. Hatched columns, antidiuretic potency–pitressin standard. Solid columns, antidiuretic potency–pituitrin standard.

In further experiments, pitressin and pituitrin were compared with international standard pituitary (posterior lobe) powder (Fig. 3). The oxytocic potency found for pituitrin agreed well with the manufacturers' estimate, and its vasopressor activity (per unit oxytocin potency) was equal to that of the international standard. However, the antidiuretic activity of pituitrin (compared with the international standard) did not correspond with its oxytocic and vasopressor potencies. In two of the three rats used for these antidiuretic assays pituitrin had less than one third of the activity of the international standard; in the other rat the difference between the antidiuretic potency of pituitrin and the international standard could be within the error of the antidiuretic assay.

Pitressin had the pressor activity claimed by the manufacturers, and, although its antidiuretic potency was less than that of the international standard, the differences were within the error of the antidiuretic assay. These assays were performed on the same rats as had been used for comparing the antidiuretic potency of the international standard and pituitrin. In the two rats in which the antidiuretic effect of pituitrin did not correspond with its vasopressor activity, the antidiuretic potency of pitressin was not less than was expected from its vasopressor activity.

It is well known that corticotrophin preparations are frequently contaminated with posterior pituitary hormones. If corticotrophin were to interfere with the antidiuretic action but not the vasopressor action of posterior pituitary hormone, it is possible that corticotrophin contamination of pituitrin could account for the relatively small antidiuretic effect of pituitrin in some rats. A preparation containing 12.6 units corticotrophin/mg. supplied by Organon Ltd. was tested for antidiuretic and pressor potency. The rats used in the antidiuretic assay had been previously shown to be approximately three times more sensitive to the international standard than to pituitrin. There was good agreement between the results of the pressor and antidiuretic assays, there being 9.6 mU. of vasopressin activity and 8.9 mU. antidiuretic activity in each mg.

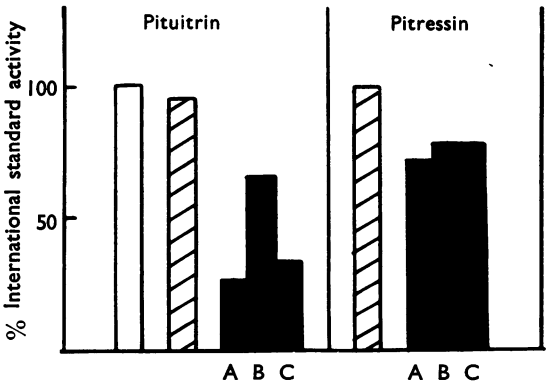

FIG. 3.—Oxytocic, pressor and antidiuretic activities of pituitrin and pitressin expressed as % of the activity of international standard extract. In the antidiuretic tests the same rats, A, B, and C, were used in experiments with pituitrin and pitressin. Unshaded columns, oxytocic activity. Hatched columns, pressor activity. Solid columns, antidiuretic activity.

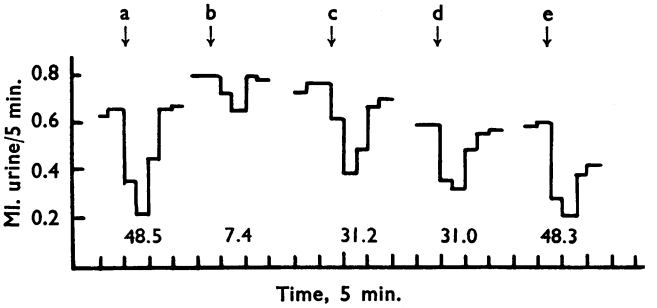

FIG. 2.—Antidiuretic effects of intravenous pitressin and pituitrin in an unanaesthetized rat (wt. 220 g.); a, 100 μU. pitressin; b, 100 μU. pituitrin; c, 33 μU. pitressin; d, 300 μU. pituitrin; e, 100 μU. pitressin+100 μU. pitocin. The numbers under each response are the "percentage antidiuresis."

(international standard was used as standard in these experiments).

Discussion

The problem which prompted this small investigation was to explain why antidiuretic assays of rat neurohypophysial extracts gave higher values when the standard was pituitrin than when pitressin was the standard. The explanation found from the present experiments is that pituitrin seems to have less antidiuretic activity relative to its oxytocic and vasopressor potencies than does either international standard pituitary (posterior lobe) extract or pitressin, a preparation of the differentiated vasopressor principle. This difference between antidiuretic action of pituitrin on one hand, and pitressin and international standard on the other, seems to depend on the rat used for the antidiuretic assay. In about 60% of the rats, the difference was not greater than could be accounted for by the error of the assay ($\pm 40\%$), but in others the difference was sometimes as much as (or more than) tenfold.

These results suggest that there is some factor in pituitrin which can antagonize the antidiuretic effect, and to which rats have varying sensitivities. Oxytocin preparations antagonize antidiuretic effects of vasopressin preparations in some amphibia (Barker-Jorgenson, 1950; Ewer, 1951) and it has been suggested that this may also occur in mammals (Fraser, 1937 and 1942; Selye, 1949). However, in the present experiments, addition of pitocin to pitressin did not affect the antidiuretic response, and international standard (which contains the oxytocic principle) had greater antidiuretic effects than pituitrin. The other possible contaminant excluded by these experiments is adrenocorticotrophic hormone, and it therefore seems unlikely that the corticotrophin releasing activity which contaminates commercial pituitary preparations (Guillemin and Hearn, 1955) can be involved.

Heller and Stephenson (1950) found that in rats pituitrin increased urinary excretion of Na and Cl to a greater extent than could be explained by regarding it as a simple mixture of pitocin and pitressin. Increased excretion of electrolytes could interfere with antidiuretic action, and this effect of pituitrin might account for its relative lack of antidiuretic effect in some rats.

In the present experiments the only commercial neurohypophysial preparations used were pitressin and pituitrin and only one method of testing for antidiuretic activity was employed. It is possible that the discrepancies appear only under these conditions. Nevertheless the implications of these experiments carry the warning that, before a commercial preparation, which may have been assayed by the manufacturer for oxytocic and vasopressor potencies, is used as the standard in antidiuretic assays, it is necessary to ensure that its antidiuretic potency relative to vasopressor potency is the same as in the international standard.

To insist on complete qualitative identity between active principles in standard and unknown in biological assays must be regarded as a counsel of perfection which is often unattainable. In view of the different molecular structures of beef and pig vasopressin (Popenoe, Lawler, and du Vigneaud, 1952) it is unlikely that the active neurohypophysial principles extracted from the pituitaries, other tissues or body fluids in other species are qualitatively identical with beef vasopressin, which is the active principle in international standard and in the commercial preparations. Although there is no reasonable alternative to the use of these beef pituitary extracts as standards it should not be forgotten that, strictly speaking, many assays may be invalid.

Summary

1. Extracts of rat neurohypophysis had greater antidiuretic potencies when pituitrin was the standard in antidiuretic assays than when pitressin was the standard.

2. In 40% of rats used for antidiuretic assays the activity of pituitrin was less than half that of pitressin or international standard.

3. Estimates of the pressor and oxytocic potencies of pituitrin in comparison with international standard and pitressin were in good agreement with those of the manufacturers.

4. The lower antidiuretic potency of pituitrin cannot be attributed to its oxytocin content or to corticotrophin or to corticotrophin-releasing activity contaminating pituitrin.

References

Barker-Jorgenson, C. (1950). *Acta physiol. scand.*, **22**, Supp. 78.
Dekanski, G. (1952). *Brit. J. Pharmacol.*, **7**, 567.
Ewer, R. F. (1951). *J. exp. Biol.*, **28**, 374.
Finney, D. (1952). *Statistical Method in Biological Assay*, p. 60. London: Griffin.
Fraser, A. M. (1937). *J. Physiol.*, **60**, 89.
—— (1942). Ibid., **101**, 236.
Ginsburg, M., and Brown, L. M. (1956). *Brit. J. Pharmacol.*, **11**, 236.
—— and Heller, H. (1953). *J. Endocr.*, **9**, 267.
Guillemin, R., and Hearn, W. R. (1955). *Proc. Soc. exp. Biol., N.Y.*, **89**, 365.
Heller, H., and Stephenson, R. P. (1950). *Nature*, **165**, 189.
Holton, P. (1948). *Brit. J. Pharmacol.*, **3**, 328.
Popenoe, E. A., Lawler, H. C., and du Vigneaud, V. (1952). *J. Amer. chem. Soc.*, **74**, 3713.
Selye, H. (1949). *Textbook of Endocrinology*, p. 219, 2nd ed. Montreal: Acta Endocrinologica Inc.
Wood, E. C. (1946). *Analyst*, **71**, 1.