Brit. J. Pharmacol. (1958), **13**, 315.

ABSENCE OF RENAL ACTION OF PURE INTERMEDIATE LOBE HORMONE

BY

R. B. CROSS

From the Department of Physiology, the University, Edinburgh

(RECEIVED MAY 9, 1958)

The action of melanophore expanding hormone of the intermediate lobe of the pituitary (MSH) on the urinary excretion of water, Na, K and Cl was studied in the conscious dog both during water diuresis and in the non-diuretic state. Doses of up to 2.3 IU MSH did not influence renal function. MSH did not modify the renal actions of vasopressin or oxytocin. The antidiuretic action of a massive dose of MSH was attributed to contamination by vasopressin.

Commercial preparations of oxytocin have been observed to have an action on renal function which is not exhibited by highly purified natural oxytocin. Working with the conscious dog, Brooks and Pickford (1957) reported that, during water diuresis, highly purified oxytocin exerted no effect other than to cause a moderate antidiuresis while the same dose of commercial extract in about 60% of cases interrupted the diuresis and caused a simultaneous increase in the absolute quantities of Na, K and Cl present in the urine. This electrolyte response was similar to, but of greater magnitude than, that caused by an antidiuretic dose of vasopressin. Brooks and Pickford were unable to reproduce this action of commercial oxytocin by contaminating purified natural oxytocin with purified natural vasopressin. This suggested the existence of a third substance, present as a contaminant which either increased electrolyte excretion by itself or modified the effect of one or both of the known posterior lobe hormones.

The melanophore expanding hormone of the intermediate lobe (MSH) is a possible contaminant of all pituitary extracts and, when first extracted, was thought to possess potent antidiuretic activity (Sulzberger, 1933). This property was, however, later attributed to the presence of residual posterior pituitary hormone and purified MSH extracts were declared to be without antidiuretic activity (Fraser, 1937; Landgrebe and Waring, 1941). As there do not appear to have been any extensive investigations into the actions of MSH on electrolyte excretion, this present study was undertaken to determine whether MSH was the active substance which occasionally contaminates commercial oxytocin.

METHODS

A total of 48 observations were made on a young conscious adult bitch of 24 kg. At a preliminary operation the perineum was slit to facilitate catheterization of the bladder. During observations she rested quietly on her side while urine drained into an open measuring cylinder. A T-piece in the catheter close to the vulva enabled the external collecting system to be drained at the end of each collecting period. The dog was trained to contract the bladder when the tail was manipulated. Urinary dead space was therefore minimal.

At 11.45 a.m. the dog was given 250 ml. water by stomach tube and all further food and water withheld until 2.15 p.m. when a water diuresis was provoked by the administration of 300 ml. water by mouth. The bladder was drained subsequently at 10 min. intervals for 2 hr. The test substances were made up to a volume of 1.5 ml. in 0.9% NaCl, and administered intravenously about 45 min. after the water loading. When observations were to be made on the non-diuretic state, the foregoing timetable was adhered to but with the omission of the water loading at 2.15 p.m. Na and K were measured by an EEL flame photometer and Cl was estimated by the method of Prout Winter (Cole, 1919).

The MSH used was from a sample prepared by Dr. J. Porath, using the method of Porath, Roos, Landgrebe, and Mitchell (1955). Subdivisions of this sample were made by first dissolving in distilled water, dividing by volume, and as rapidly as possible freeze-drying and sealing in a vacuum. Each batch of subdivisions was tested for activity on an isolated frog skin and at the end of the series of experiments a sample was submitted to Dr. B. Hobson for accurate assay by the method of Landgrebe and Waring (1944). The vasopressin used was highly purified arginine vasopressin prepared by Dr. V. du Vigneaud. The oxytocin used was the synthetic product Syntocinon

made by Messrs. Sandoz and the commercial extract was the Pitocin of Messrs. Parke, Davis & Co. The doses of vasopressin and oxytocin used were of the same order as those used by Brooks and Pickford (1957). The dose of MSH was estimated as something grossly in excess of any contaminating level in commercial pituitary extracts.

RESULTS

Throughout the experiments the pattern of electrolyte excretion remained fairly uniform. The initial level of both Na and K excretion usually lay between 30 μ equiv./min. and 50 μ equiv./min. with the K level usually the lower. Both levels fell progressively during the diuresis in the manner reported by Ali, Cross, and Pickford (1958). The Cl level usually began about 20 μ equiv./min. above the Na level but fell more rapidly during the early stages of the diuresis so that the subsequent course of the Cl excretion often corresponded fairly closely with that of the Na excretion. Initial levels of Na excretion as low as 15 μ equiv./min. and as high as 100 μ equiv./min. were observed, but these were due to known errors of diet. Results obtained from experiments carried out at these extreme levels of electrolyte excretion did not differ from those obtained when the experiments were repeated at the intermediate levels of electrolyte excretion.

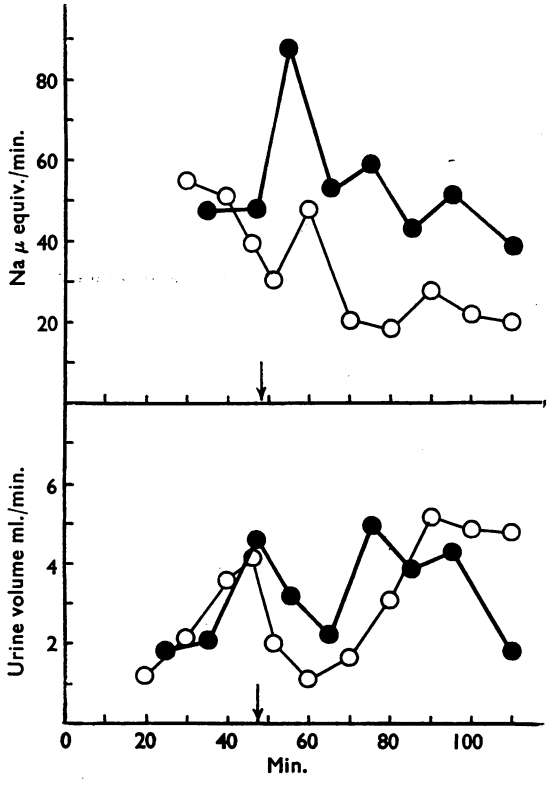

FIG. 1.—Comparison of the effects of synthetic oxytocin and commercial oxytocin upon water and Na excretion during water diuresis in a dog. 300 ml. water was given at 0 time. Intravenous injections were made at 48 min. (arrow). Solid circles, Pitocin 150 mU., and open circles, synthetic oxytocin 150 mU.

Oxytocin Alone.—During the non-diuretic state both synthetic oxytocin and Pitocin caused an increase in electrolyte excretion without any corresponding alteration in urine flow. During a water diuresis a dose of 150 mU. synthetic oxytocin was found to decrease slightly the urine output and to cause a transitory interruption in the downward trend in electrolyte excretion. The same dose of Pitocin caused an inhibition of diuresis with a simultaneous increase in Na and Cl excretion (Fig. 1).

MSH Alone.—MSH was administered alone by single injection in doses of 10 mU., 70 mU., 140 mU., and 2.3 IU both in the diuretic and non-diuretic state and did not alter the expected pattern of water and electrolyte excretion. A priming dose of 70 mU., followed by 100 mU. infused during 15 min., was also without effect during a diuresis. On one occasion a massive dose of 100 IU administered during a diuresis inhibited the diuresis and increased the rate of electrolyte excretion. This response was similar to that expected from a dose of vasopressin of the order of 2 mU.

MSH plus Vasopressin.—A dose of 2 mU. vasopressin administered during a water diuresis was found to cause a transient fall in urine volume with a simultaneous increase in electrolyte excretion. This response was not altered by the addition of either 70 mU. or 2.3 IU MSH to the vasopressin. During the non-diuretic state, vasopressin neither alone nor in combination with MSH altered the pattern of excretion of water and electrolytes.

MSH plus Oxytocin.—MSH in doses of 70 mU. and 2.3 IU administered in combination with 150 mU. synthetic oxytocin did not alter the action of the oxytocin either in the diuretic or non-diuretic state.

MSH plus Vasopressin and Oxytocin.—A mixture of 0.6 mU. vasopressin and 80 mU. synthetic oxytocin was found to inhibit the diuresis and to increase the Na excretion by 15/20 μ equiv./min. for 15 min. The action of this mixture was not modified by the addition to it of either 10 mU. or 2.3 IU MSH.

Discussion

The above findings would appear to dispose of the suspicion that MSH was responsible for the unexpected actions of commercial oxytocin observed by Brooks and Pickford (1957). The doses of MSH used in this study varied from small ones to those grossly in excess of any expected level of contamination in commercial extracts. Indeed, the preparation used by Brooks and Pickford (Pitocin) was reported by Landgrebe and Waring (1950) to be free of MSH, and a more recent assay using a method sensitive to 1 mU. failed to detect any MSH in a dose of Pitocin equivalent to 300 mU. oxytocin (Hobson, personal communication).

There is also no evidence that MSH exerts any direct influence on the excretion of water and electrolytes. The one occasion on which a massive dose of 100 IU MSH inhibited a diuresis and increased the output of electrolytes can probably be attributed to the presence of contaminating vasopressin. This dose was administered during studies into the excretion of MSH, and when compared with the dose levels required for pigment change in amphibia appears to be well beyond physiological limits. Although the extract was stated to contain less than 0.001% vasopressin (Porath, personal communication), such a level of contamination might explain the findings in this case.

It may be that some action of MSH on renal function would have appeared had the observations been continued beyond the 60 to 90 min. periods used in this study, but short as this time is it represents at least three times the circulating life of an injected dose of MSH (Landgrebe and Waring, 1941). Lerner, Shizume and Bunding (1954) administered massive doses of MSH to human subjects daily for 36 days. Unfortunately they did not investigate urinary excretion of electrolytes, but their studies of serum electrolytes did not reveal a significant deviation from normal. Since any gross changes in the urinary excretion of electrolytes if maintained for 36 days might be expected to cause some secondary alteration in serum electrolyte levels, the failure to observe these changes suggests that the prolonged administration of MSH did not alter renal function to any great degree.

I desire to thank Dr. M. Pickford, who made available the samples of MSH and vasopressin from supplies kindly provided by Dr. J. Porath and Dr. V. du Vigneaud. Dr. Pickford also prepared the perineum of the dog. I also wish to thank Dr. B. Hobson, of the Pregnancy Diagnosis Laboratory of the University of Edinburgh, who carried out the assay of MSH. This work was undertaken while holding the Wilkie Research Fellowship of the University of Edinburgh.

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