Brit. J. Pharmacol. (1958), **13**, 131.

A COMPARISON OF SOME ACTIVITIES OF ARGININE VASOPRESSIN AND LYSINE VASOPRESSIN ON KIDNEY FUNCTION IN CONSCIOUS DOGS

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(RECEIVED DECEMBER 3, 1957)

Comparison was made on conscious trained dogs of the effect of highly purified arginine vasopressin and lysine vasopressin on the rate of urine flow, the excretion rate of Na and K and the renal clearances of creatinine and diodone, and of the actions of the vasopressins when administered together with oxytocin. The vasopressins were used in doses causing a maximal antidiuresis.

The results showed, first, that the antidiuretic potency of arginine vasopressin was greater than that of lysine vasopressin and that its action lasted longer; second, that during water diuresis arginine vasopressin increased only Na excretion while lysine vasopressin increased both Na and K excretion; third, that arginine vasopressin had a potency about 5 to 6 times that of lysine vasopressin in antagonizing the augmentor effect of oxytocin on glomerular filtration rate and renal plasma flow.

It has been shown that at least two vasopressins can be found in mammals, arginine vasopressin from beef and lysine vasopressin from hog (Turner, Pierce, and du Vigneaud, 1951; Poponoe, Lawler, and du Vigneaud, 1952). Van Dyke, Engel, and Adamsons (1956) compared a number of the activities of these two vasopressins and found that the ratio of pressor potency to antidiuretic potency is 1.0 for arginine vasopressin and 6 for lysine vasopressin when the antidiuretic potency is determined by intravenous injection in hydrated unanaesthetized dogs. On the other hand, as compared with the USP standard, lysine vasopressin and arginine vasopressin on intravenous injection were equally potent as pressor agents in anaesthetized rats and dogs. This difference in the potency ratio of the two substances suggested the possibility that the activities considered were related to different parts of the molecular configuration. It was not known whether lysine vasopressin had certain of the other activities of arginine vasopressin, and it seemed of interest to make a further comparison of the biological properties and potency ratios of the two vasopressins in the hope that ultimately some clue might be obtained as to the chemical nature of the processes involved in their actions. A comparison was made of the power of the two vasopressins to affect the excretion rate of Na and K during diuresis and at low rates of urine flow, and of their relative capacity to antagonize the augmentor effect of oxytocin on glomerular filtration rate and renal plasma flow. The first of these activities was investigated because Anslow and Wesson (1955) and Brooks and Pickford (1957) found that in dogs arginine vasopressin increased electrolyte excretion during water diuresis, but was ineffective at low rates of urine flow. The second comparison was made since Brooks and Pickford (1957) found that arginine vasopressin was highly effective at antagonizing the increased renal plasma flow induced by the administration of oxytocin.

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METHODS

A total of rather more than 90 observations were made. Most of the observations were made on two trained conscious bitches, Theone (21 kg.) and Sophie (18 kg.), but a few were also made on 3 other dogs. They were given 15 to 20 ml. tap water/kg. body weight 2 to 2½ hr. before each experiment, and observations were made at about the same time in the afternoon. Diuresis was induced by a second dose of 20 ml./kg. warm tap water given by stomach tube. A number of observations were made at a "resting

rate" of urine flow when the dogs were hydrated as usual but were not given the second dose of water at the time observations were begun. The urine was collected through a self-retaining catheter into dry graduated tubes which had been carefully washed in Na and K free water. In both dogs the dorsal perineum was slit to facilitate catheterization. The urine collection periods were of 10 to 20 min. duration, usually 10 min. during diuresis and 20 min. during resting rates of flow. The dead space of the urine collecting system was reduced as much as possible by keeping the external part of the catheter short. Intravenous injections were made into the saphenous vein and infusions into the cubital vein. When blood samples were needed these were obtained from the saphenous vein.

For the clearance experiments diodone (0.4 to 0.6 ml. of a 35% w/v solution/kg. body weight) and creatinine (0.2 to 0.3 g./kg.) were given subcutaneously 30 to 40 min. before the induction of diuresis. This subcutaneous administration was found to maintain for a period an adequate and fairly steady plasma concentration of both substances; consequently in calculating the clearances little adjustment was necessary to allow for dead space (Chinard, 1955).

Vasopressins and oxytocin were given either as single intravenous injections or as intravenous infusions. The single injections were of 1 ml. 0.9% NaCl solution containing the required amount of hormone(s). Infusions were given at a constant rate of 3 ml./hr. Highly purified natural vasopressins were used throughout. These were available owing to the kindness of Dr. du Vigneaud and Dr. van Dyke, and were standardized in pressor units. The oxytocin used was synthetic and this has been shown to be identical in its activities with natural oxytocin. On a few occasions highly purified natural oxytocin was used.

Diodone iodine of plasma and urine was estimated by the method of Alpert (1941). Creatinine in plasma and urine was estimated as suggested by Rehberg (1926) and by the colorimetric method of Folin (1914) respectively. Sodium and potassium were estimated by flame photometer.

RESULTS

Antidiuresis and Electrolyte Excretion Following Injection of the Vasopressins

Preliminary tests on the two dogs chiefly used showed that, despite their different body weights, a single intravenous injection of 2 mU. arginine vasopressin produced a maximal antidiuresis, and that this was matched in degree by the administration of 7 mU. lysine vasopressin (Fig. 1), but recovery from antidiuresis caused by lysine vasopressin was more rapid than that from arginine vasopressin. Further comparison of the actions of the two hormones was based on the use of these equi-antidiuretic doses.

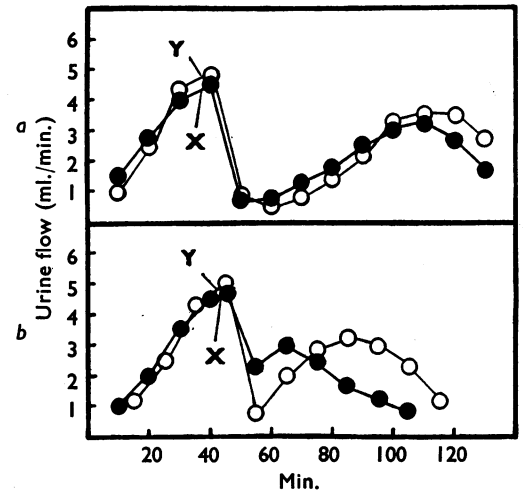

FIG. 1.—Antidiuretic activities of arginine and lysine vasopressins. *a*, (Theone), 400 ml. water by mouth at zero time. Arginine vasopressin injected intravenously, 2 mU. at *X* (●); 3 mU. at *Y* (○). *b*, Curve ● (Sophie), 350 ml. water by mouth at zero time; 5 mU. lysine vasopressin intravenously at *X*. Curve ○ (Theone), 400 ml. water by mouth at zero time; 7 mU. lysine vasopressin injected intravenously at *Y*.

Before considering the effect of the hormones on electrolyte excretion it is as well to make it clear that no significance was attached to a rise in excretion rate in a single immediately post-injection sample. A rise in two consecutive samples was possibly significant. No rise in electrolyte excretion ever followed control injections. When it is stated that the injection of hormone caused an increase in electrolyte loss this implies that the increased loss always occurred. Fig. 2 shows the rate of Na and K excretion during control observations when 1 ml. 0.9% NaCl solution was injected intravenously. This may be compared with the typical effect on Na and K excretion of arginine vasopressin and lysine vasopressin injected during diuresis (Fig. 3). Both vasopressins caused a considerable, though short-lived, rise in Na excretion. The effect of 2 mU. arginine vasopressin on K loss was of no significance on this occasion, though it was found that 3 mU. arginine vasopressin moderately increased the excretion rate of K in the two 10 min. samples following on those injections. However, in terms of antidiuresis 3 mU. arginine vasopressin was a supramaximal dose and therefore not comparable with the effect of 7 mU. lysine vasopressin which regularly raised the excretion rate of K, sometimes for as long as 40 to 50 min. Lysine vasopressin infused during diuresis at a rate of 0.01 to 0.02 mU./kg./min. also caused an increase in the

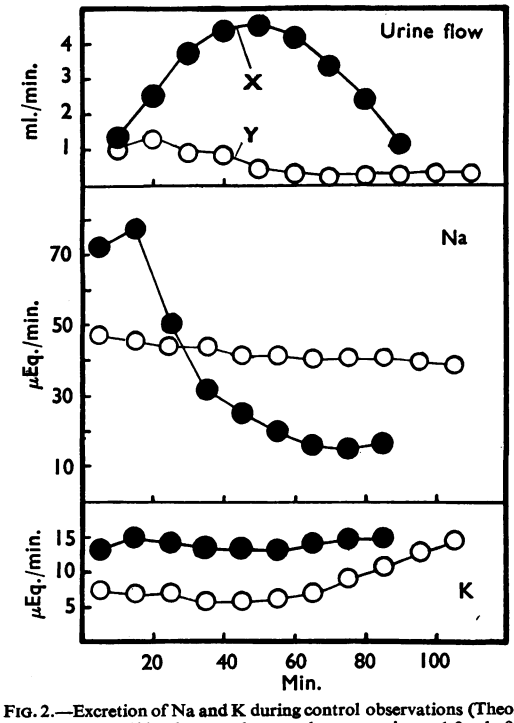

FIG. 2.—Excretion of Na and K during control observations (Theone). Curves ●, 400 ml. water by mouth at zero time: 1.0 ml. 0.9% NaCl solution injected intravenously at *X*. Curves ○, no water at zero time, 1.0 ml. 0.9% NaCl solution intravenously at *Y*.

excretion of both Na and K, the increase being maintained for the period of infusion.

At a resting rate of urine flow (when the dogs had been hydrated but were not diuretic at the time of observation) it was found that neither single injections nor infusions of arginine vasopressin appreciably nor consistently increased Na or K loss (Fig. 4). This is in agreement with the findings of Anslow and Wesson (1955) and Brooks and Pickford (1957). At the low rate of urine flow lysine vasopressin similarly had little action on the excretion rate of Na, but it caused an augmented loss of K both after single injections (Fig. 4) and more clearly during infusions.

Antidiuresis and Electrolyte Excretion Following Injection of the Vasopressins Together with Oxytocin

It will be shown later that both vasopressins antagonized the action of oxytocin on the renal blood vessels. For this reason it was thought as well to test if they also antagonized the effect of oxytocin on electrolyte excretion. Observations were made on the excretory response, at both high and low rates of urine flow, to the injection of oxytocin simultaneously with either arginine or

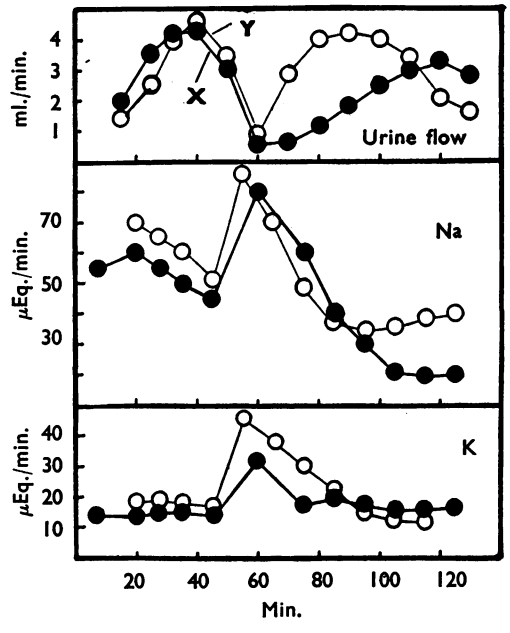

FIG. 3.—The effect of arginine vasopressin and lysine vasopressin on urine flow and on the excretion of Na and K during water diuresis. (Theone), 400 ml. water by mouth at zero time. Intravenous injection of 2 mU. arginine vasopressin at *X* (●); 7 mU. lysine vasopressin at *Y* (○).

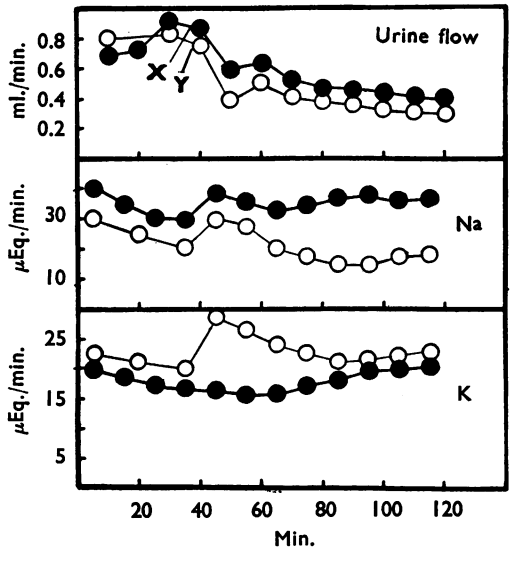

FIG. 4.—The effect of arginine and lysine vasopressins on urine flow and on Na and K excretion at a low rate of urine flow. (Theone), no water at zero time. Intravenous injection of 2 mU. arginine vasopressin at *X* (●); 7 mU. lysine vasopressin at *Y* (○).

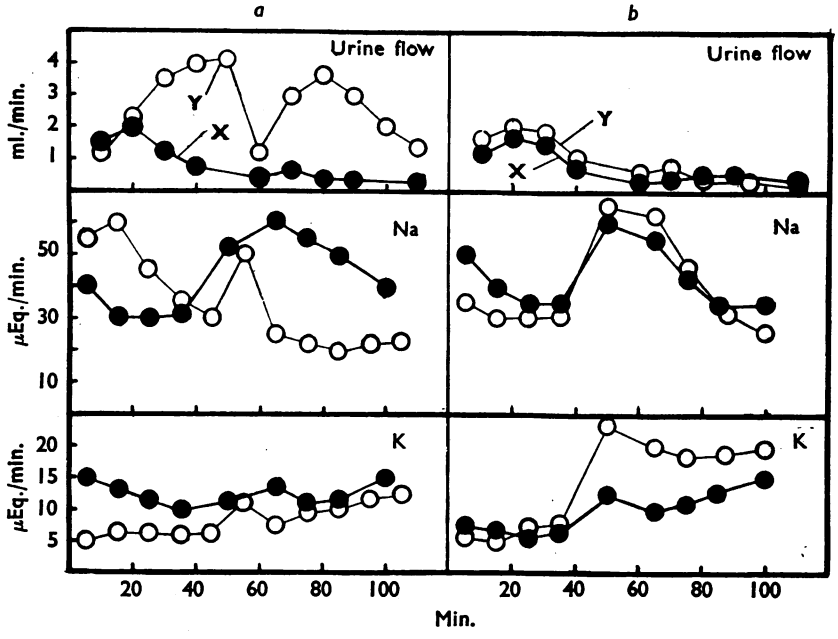

FIG. 5.—(*a*) The effect of oxytocin on urine flow and on Na and K excretion. Curves ○, water diuresis; curves ●, "resting" rate of urine flow. On both occasions 150 mU. oxytocin injected intravenously at times marked *X* and *Y*. (*b*) The effect of oxytocin plus arginine vasopressin and of oxytocin plus lysine vasopressin on urine flow and on Na and K excretion during "resting" rate of urine flow. Intravenous injections: 150 mU. oxytocin plus 2 mU. arginine vasopressin at *X* (●); 150 mU. oxytocin plus 7 mU. lysine vasopressin (○).

lysine vasopressin. The doses of oxytocin given were the same as those used below for testing the activity of the two vasopressins on the renal blood vessels. Confirming the results of Brooks and Pickford (1957) it was found that oxytocin, given either by infusion or as a single intravenous injection, raised the excretion rate of Na at a resting rate of urine flow, but was ineffective during diuresis (Fig. 5*a*). Following the administration, at a resting rate of urine flow, of oxytocin together with arginine vasopressin, the period of increased Na loss was shorter than that following oxytocin alone, but K excretion was not consistently changed (Fig. 5*b*). Lysine vasopressin, like arginine vasopressin, appeared to shorten the period of increased Na loss which followed oxytocin alone (Fig. 5*b*); on the other hand, lysine vasopressin and oxytocin given together always clearly enhanced the rate of K excretion (Fig. 5*b*) as compared with the action of either substance given alone (cf. Fig. 5*a* and Fig. 3). During water diuresis the administration of oxytocin together with arginine vasopressin shortened the duration of the expected antidiuresis and resulted in a sharper increase in loss of Na than followed arginine vasopressin alone (cf. Figs. 3 and 6). K excre-

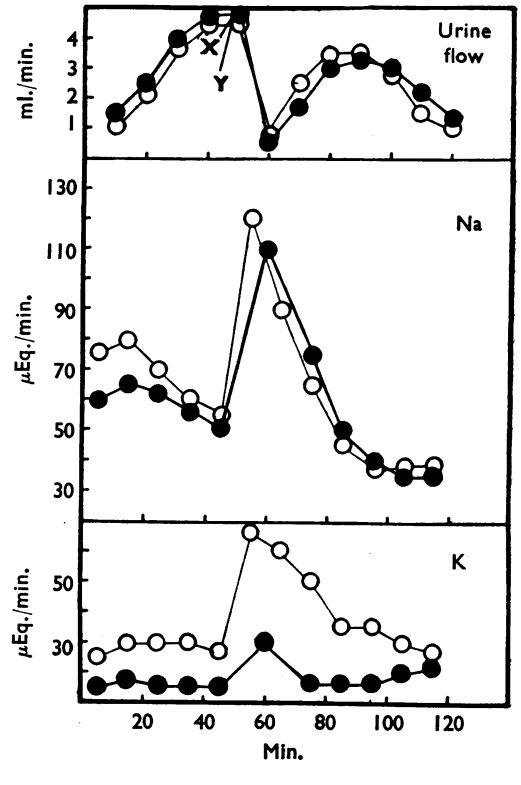

FIG. 6.—The effect of oxytocin plus arginine vasopressin and of oxytocin plus lysine vasopressin on urine flow and on Na and K excretion during water diuresis. Intravenous injections; 150 mU. oxytocin plus 2 mU. arginine vasopressin at *X* (●); 150 mU. oxytocin plus 7 mU. lysine vasopressin at *Y* (○).

FIG. 6

tion was not significantly altered by the simultaneous injection of arginine vasopressin and oxytocin (Fig. 6). When oxytocin was injected together with lysine vasopressin, again Na and possibly also K loss was enhanced as compared with the action of lysine vasopressin alone.

Effect of the Vasopressins Alone and Together with Oxytocin on Glomerular Filtration Rate and Renal Plasma Flow

It is generally agreed that Pitressin given in a dose just sufficient to cause maximal inhibition of water diuresis does not consistently alter either the glomerular filtration rate or renal plasma flow (Maxwell and Breed, 1951 ; Sellwood and Verney, 1955). Brooks and Pickford (unpublished observation) found that highly purified arginine vasopressin behaved in this respect like Pitressin. Nevertheless, these small doses of vasopressin abolish the increase of renal plasma flow caused by oxytocin (Brooks and Pickford, 1957), and it will be shown below that lysine vasopressin has to some extent the same property. Since the object was to compare the renal vascular actions of the two vasopressins, oxytocin was used in almost certainly unphysiological doses in order to ensure a large rise in renal plasma flow, and thus test to the full the power of the two vasopressins to antagonize this rise.

Control measurements during water diuresis in Theone showed that the clearance rates of creatinine and diodone increased by approximately 10 and 15% respectively with the onset of diuresis and declined as diuresis subsided. These clearance changes are of the same order as those found by Sellwood and Verney (1955). Brooks and Pickford (1957) using oxytocin in intravenous doses of 5 mU./kg. found that it caused an increase in renal plasma flow. In the present work doses of 7 to 10 mU./kg. were given and it was found that both glomerular filtration rate and renal plasma flow increased ; in Theone the former rose by 50 to 80% with a return to the initial value in 30 to 40 min., and the latter by 25 to 50% for a short time only. Fig. 7*a* records the results of one such

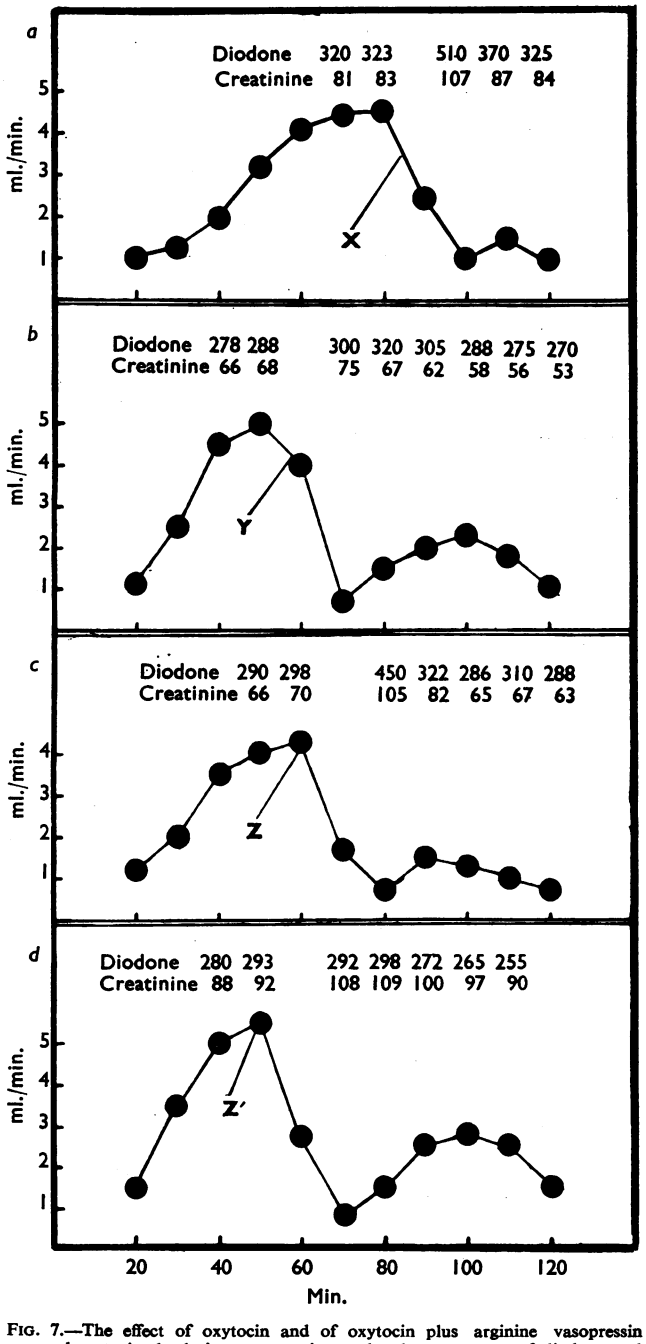

FIG. 7.—The effect of oxytocin and of oxytocin plus arginine vasopressin and oxytocin plus lysine vasopressin on the clearance rates of diodone and creatinine during water diuresis. All drugs were given by intravenous injections. *a*, 150 mU. oxytocin at *X*; *b*, 150 mU. oxytocin plus 2 mU. arginine vasopressin at *Y*; *c*, 150 mU. oxytocin plus 7 mU. lysine vasopressin at *Z*; *d*, 150 mU. oxytocin plus 10.5 mU. lysine vasopressin at *Z'*. The related clearance values of diodone and creatinine are indicated above each curve in ml./min. Ordinates, urine flow in ml./min.; abscissae, time in min.

observation. This is similar to the findings of Dicker and Heller (1946) on rats. When 2 mU. arginine vasopressin was injected together with 150 mU. oxytocin the increase in clearance values was far less than when oxytocin was given alone (Fig. 7*b*). On the other hand, 7 mU. lysine vasopressin, that is, the antidiuretic dose-equivalent of 2 mU. arginine vasopressin, only slightly antagonized the action of oxytocin (Fig. 7*c*) and it was necessary to use 10.5 mU. lysine vasopressin to prevent the effect of oxytocin on renal plasma flow. Even this dose did not fully counteract the increase in glomerular filtration rate (Fig. 7*d*). On two occasions 7 mU. lysine vasopressin was injected 3 to 4 min. before the 150 mU. oxytocin; but this procedure did not increase the power of lysine vasopressin to antagonize the action of oxytocin.

Discussion

Under the conditions of the present observations 2 mU. arginine vasopressin and 7 mU. lysine vasopressin caused a maximal antidiuresis, but that due to lysine vasopressin lasted for a shorter time. The 1/3.5 ratio of antidiuretic potency of arginine/lysine vasopressin is lower than that (1/6) found by van Dyke, Engel, and Adamsons (1956), but this may perhaps be due to certain differences in procedure. Both sets of results show that in the conscious dog lysine vasopressin is a weaker antidiuretic than arginine vasopressin. As far as the electrolyte excreting properties of the vasopressins are concerned, when given in the maximal antidiuretic dose both increased Na excretion to the same extent during water diuresis, but lysine vasopressin also raised the excretion of K. Arginine vasopressin only increased K loss in addition to that of Na if given in supramaximal antidiuretic dose. The same results were seen following infusions, namely that lysine vasopressin was the more effective at increasing K loss.

In the present work oxytocin was used in fairly large doses, namely 7 to 10 mU./kg. According to an assay made in dogs by Abrahams and Pickford (1954), an osmotic stimulus which caused maximal antidiuresis brought about the liberation of 80 to 100 mU. oxytocin (O) and less than 5 mU. vasopressin (V), so that the V/O ratio was approximately 1/20. The doses used in the present observations gave an arginine vasopressin/oxytocin ratio of 1/75 and a lysine vasopressin/oxytocin ratio of 1/20 or 1/30. In water diuresis 2 mU. arginine vasopressin and 7 mU. lysine vasopressin caused antidiuresis of equal degree, and this degree was not changed by the injection of oxytocin with the vasopressins; but the duration of the antidiuresis due to arginine vasopressin was shortened by the 75 times greater dose of oxytocin, whereas the already short antidiuresis caused by lysine vasopressin was unchanged by 20 times as much oxytocin. With regard to Na, at a low rate of urine flow both vasopressins showed antagonism to oxytocin, since when given together with oxytocin they cut short the increased Na loss caused by oxytocin alone. On the other hand, during water diuresis no antagonism appeared since a sharper, though not longer, rise in Na loss followed the injection of either vasopressin together with oxytocin. Since the vasopressins either fully or partially antagonized the action of oxytocin on glomerular filtration rate and renal plasma flow it is not possible to explain the enhanced Na loss in diuresis when both oxytocin and vasopressin were injected, as due to the specific action of vasopressin summed with an increase in filtered Na load. Obviously some other factor has to be considered, perhaps the rate of flow of tubular urine.

Regarding K excretion and comparing the two vasopressins from the standpoint of equi-antidiuretic doses, lysine vasopressin always increased the rate of loss of this ion at high or low rate of urine flow, whereas arginine vasopressin had little effect. When lysine vasopressin was given together with oxytocin there seemed to be a rather greater loss of K, but following arginine vasopressin plus oxytocin the loss of K was still small and inconstant. In general, then, as far as electrolyte excretion was concerned, the two vasopressins were alike in their behaviour towards Na, but lysine vasopressin had a greater power to raise the rate of K loss.

Measurements of glomerular filtration rate and renal plasma flow showed that 2 mU. of arginine vasopressin was able nearly completely to antagonize the effects of 150 mU. oxytocin on the renal blood vessels, that is, this small dose of arginine vasopressin was able almost to counteract 75 times as much oxytocin, or about twice the presumed physiological dose (Abrahams and Pickford, 1954). The dose of lysine vasopressin (7 mU.) equivalent in terms of antidiuresis to 2 mU. arginine vasopressin only partially antagonized the action of oxytocin on glomerular filtration rate and renal plasma flow, and for complete antagonism 10.5 mU. lysine vasopressin had to be used, that is, a lysine vasopressin/oxytocin ratio of 1/15. It seems, then, that although arginine vasopressin and lysine vasopressin are equally potent in raising the systemic blood pressure in anaesthetized dogs, lysine vasopressin has a feebler action than

arginine vasopressin on renal blood vessels dilated by oxytocin. The physiological significance of these results is uncertain since there is no direct evidence as to the variety of vasopressin occurring naturally in dogs, but from the results of their own observations van Dyke, Engel, and Adamsons (1956) concluded that it is arginine vasopressin.

The main pharmacological points emerging from these experiments are, first, that in the dog both arginine vasopressin and lysine vasopressin behave in parallel manner towards Na excretion, and somewhat alike towards water, though lysine vasopressin must be used in a larger dose. Second, that relative to its antidiuretic and Na excreting potency, lysine vasopressin is more active than arginine vasopressin in augmenting K loss; third, arginine vasopressin, in the circumstances considered, is far more efficient than lysine vasopressin at restoring to normal glomerular filtration rates and renal plasma flows which have been raised by oxytocin. The potencies of the two vasopressins vary in such a manner that it is unlikely that their effects on Na and K excretion are related to their vascular activities.

The results offer further evidence that there can be independent regulation of the excretion of water, sodium, and potassium.

Grateful thanks are due to Dr. V. du Vigneaud and Dr. H. B. van Dyke for their generous supplies of purified natural vasopressin and oxytocin, and to Dr. Holgate of Messrs. Sandoz Ltd. for supplies of synthetic oxytocin. Sincere thanks are due to Dr. M. Pickford for valuable advice and suggestions and for operative preparation of the dogs. The work was undertaken during the tenure of a scholarship sponsored by the Government of Pakistan. This work was part of that presented in a thesis for the degree of Master of Science and accepted by the University of Edinburgh.

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