Effect of Leucine-Vasopressin (Phenylalanine-Oxytocin) on Renal Excretion of Na and K in Hydrated Rats and Dogs.* (24595)

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Oxytocin has a powerful naturetic effect in dehydrated† dogs(1,2) and rats(3) whereas physiological doses of arginine-vasopressin or lysine-vasopressin have no effect on sodium or potassium excretion in these species. Several analogues of oxytocin have recently been synthesized(4). One of them, the phenylalanine-analogue (du Vigneaud's "oxypressin") is remarkable in having both some antidiuretic and oxytocic activities. This substance which derives its name from having phenylalanine instead of isoleucine in the ring-part of the structure (Fig. 1), and may equally well be called leucine-vasopressin, combines in its molecule the characteristics of vasopressin and oxytocin. It therefore seemed of interest to study its effect on renal excretion of sodium and potassium in hydrated animals.

Methods. Experiments were performed as assays for antidiuretic potency of leucine-vasopressin in dogs and rats, with simultaneous determinations of rates of urinary excretion of sodium and potassium. In dog experiments the method of Ames, Moore and van Dyke(5) using trained, hydrated dogs was employed with the modification that 8% overhydration initially obtained was maintained by administering tap water by stomach tube in 200 ml portions as soon as this volume of urine had been excreted. As index of antidiuretic activity changes in urine osmolality (determined by Fiske osmometer) were used. Assays in rats were done by the method of Thorn(6) with the modification that changes in urine osmolality were used as an index of antidiuretic response. For these determinations the microadaptation unit of the Fiske osmometer was employed. Sodium and potassium concentrations of urine samples were determined by flamephotometry using lithium as an internal standard. A sample of leucine-vasopressin (phenylalanine-analogue of oxytocin) "OTP 79" was kindly supplied by Drs. Berde and Cerletti of Sandoz, Basle. One ml of this solution was stated to contain 1 pressor unit (tested on spinal cat) and 6 antidiuretic units (tested on non-anaesthetized rats by subcutaneous injection). Doses employed were such as should be considered physiological from the vasopressin action (submaximal in antidiuretic effect). All injections were done i.v. through indwelling polyethylene catheters.

Results. Dog Experiments. In 4 experiments with 3 dogs (body weights 13, 15, and 16.5 kg) a total of 16 injections of p-analogue were made. Doses varied from 0.11-0.77 millipressorunits/kg. Results of a typical experiment are illustrated in Fig. 2. Rates of excretion of sodium and potassium rose after each injection. Baseline rates of excretion

CYS • TYR • PHE • GLU (NH_2) • ASP (NH_2) • CYS • PRO • ARG • GLY (NH_2)
Arginine-vasopressin

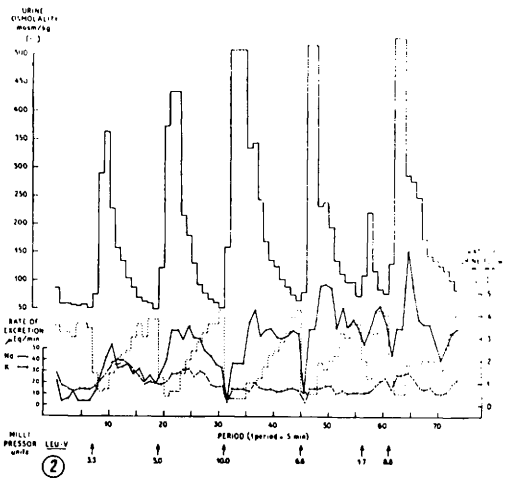
CYS • TYR • PHE • GLU (NH_2) • ASP (NH_2) • CYS • PRO • LEU • GLY (NH_2)
Leucine-vasopressin
(phenylalanine-oxytocin)

CYS • TYR • ILEU • GLU (NH_2) • ASP (NH_2) • CYS • PRO • LEU • GLY (NH_2)
Oxytocin

FIG. 1. Structures of arginine-vasopressin, leucine-vasopressin and oxytocin.

* Aided by research grant from Eli Lilly & Co.

† In hydrated animals oxytocin is naturetic only if given together with vasopressin.



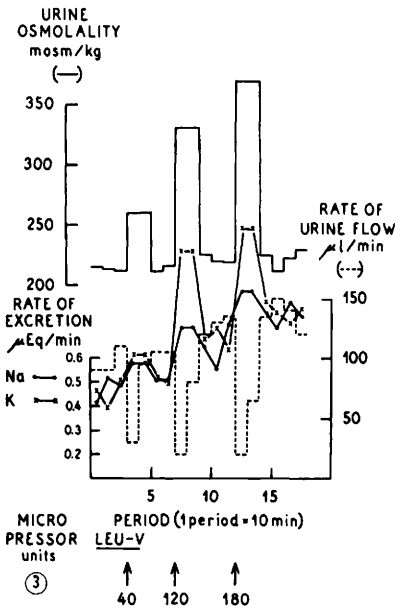


FIG. 2. Effect of leucine-vasopressin (LEU-V) on rate of urine flow, urine osmolality and rates of urinary excretion of sodium and potassium in a hydrated, conscious dog (wt 16.5 kg).

FIG. 3. Effect of leucine-vasopressin (LEU-V) on rate of urine flow, urine osmolality and rates of urinary excretion of sodium and potassium in a hydrated, ethanol anaesthetized rat (wt 250 g).

were 5-70 µeq/min for sodium, 5-15 µeq/min for potassium. Minimum increase in sodium excretion was from 5 to 9 µeq/min after 3.3 mU, in potassium excretion from 4 to 7 µeq min after 3.3 mU. Maximum increase in sodium excretion (from 20 to 110 µeq/min) and in potassium excretion (from 10 to 60 µeq/min) occurred after injection of 10 mU. A dose-response relation was evident from inspection of the data. The effects on electrolyte excretions were generally of a duration similar to that of the antidiuretic effect.

Rat experiments. In 6 experiments in different rats (average body wt 200 g), a total of 20 injections were made. Doses varied from 10-90 µU/100 g rat. In one experiment 0.5 and 2.5 mU/100 g rat were injected. Results of a typical experiment are shown in Fig. 3. Here also the p-analogue caused an increase in rates of excretion of sodium and potassium. (This for sodium occurred in 16 of the 20 injections, for potassium in 14). Baseline rates of excretion were 0.4-0.7 µeq/min for sodium, 0.3-1.0 µeq/min for potassium. Minimum effect on sodium excretion was a rise from 0.7 to 0.8 µeq/min after 20 µU, on potassium excretion a rise from 0.5 to 0.6 µeq/min after 20 µU. Maximum effect on sodium excretion (from 0.5 to 1.2 µeq/min) was seen after 120 µU, for potassium (1.0 to 2.9 µeq/min) after 5 mU. Also in rats a dose-response relation could be seen. Duration of the effects on electrolyte excretion was roughly similar to that on urine osmolality.

Discussion. The mechanism of action of leucine-vasopressin in increasing the rates of renal excretion of sodium and potassium is obscure. That the mechanism is not one of interference with renal tubular actions of aldosterone or other adrenocortical steroids is suggested by the fact that the rate of excretion of potassium also rose.

It is interesting that leucine-vasopressin, which can be regarded as a structural intermediate between natural vasopressin and oxytocin, given alone has an effect equal to the combined effects of both of these hormones. It appears that a basic amino acid residue in the side chain is essential for strong antidiuretic activity. The leucine residue in the side chain may be of significance for the effect on urinary excretion of sodium and potassium.

Summary. Leucine-vasopressin (phenylalanine-analogue of oxytocin) injected i.v. in submaximal antidiuretic doses to hydrated dogs and rats produced an increase in rate of urinary excretion of both sodium and potassium.

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Received October 30, 1958. P.S.E.B.M., 1959, v100.

Pb^{212}(ThB) Tracer Studies in Adult Beagle Dogs.* (24596)

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Interest in metabolism of Pb^{212}(ThB) developed from studies of chronic toxicities of Th^{228}(RdTh) and Ra^{228}(MsTh) in adult beagle dogs. In decay of these isotopes the inert gas thoron (Em^{220}) is formed, and, when decay occurs *in vivo*, a significant fraction of Em^{220} reaches the blood. Part is transported to the lungs and exhaled(1); the remainder decays to give Pb^{212}. Some Pb^{212} leaves the blood, but a significant amount binds to red cells to maintain a high concentration (relative to other activities in blood). For dosimetric purposes we need to know what fraction of Em^{220} decays in blood. This can be determined from measurements of Pb^{212} in blood, if we have a knowledge of Pb^{212} metabolism. There is an abundant literature on lead metabolism, but it relates mainly to chemical toxicity of lead, and includes only a limited number of studies in which carrier-free tracers were used. The affinity of red cells for tracer amounts of lead was reported as early as 1927(2) and the literature includes studies with rats(3) rabbits(4) and humans (5).

Methods. Two healthy young male adult beagle dogs from a large, well controlled colony were used. In Experiment A they received Pb^{212} by intravenous injection; in B, blood was drawn from each dog, tagged with Pb^{212} and reinjected into the dog from which it was drawn. Pb^{212} occurs in the Th^{228} decay chain and can be separated in high purity by de-emanation.

* Supported by U. S. Atomic Energy Comm.

$$Th^{228} \xrightarrow[1.9\ yr]{\alpha} Ra^{224} \xrightarrow[3.64\ d]{\alpha} Em^{220} \xrightarrow[54.5\ s]{\alpha} \xrightarrow{\alpha}$$

$$Pb^{212} \xrightarrow[10.6\ hr]{\beta,\gamma} Bi^{212} \xrightarrow[60.5\ m]{\beta,\gamma} \xrightarrow{\alpha} Pb^{208}$$

Air is bubbled through 1 to 2 ml Th^{228} solution in a small culture tube. The inert gas Em^{220} is carried by the effluent air through a short length of capillary tubing into a 1 L. flask. There it decays to Pb^{212} which deposits on the flask walls. As shown by Evans(6), a good yield of Pb^{212} is obtained if volume of the Th^{228} container plus connecting tubing (V_1) is small compared with the receiving flask (V_2), and if the air flow rate is such that V_1 is displaced in a time short compared with the half-period of Em^{220} (54.5 sec), but displacement of V_2 requires several half-periods. To prepare the injection solution for Exp. A, the flask was rinsed with 50 ml citric acid-sodium citrate buffer, pH = 3.5 and total citrate 0.08 M. In B, Em^{220} was bubbled through 10 ml heparinized blood for 1 hour, followed by 30 minutes incubation at 37°C. Since Pb^{212} was determined by gamma measurement, no chemical concentration or ashing of samples was necessary. Blood cell, plasma, and urine samples were measured in a well type liquid scintillation counter; the whole dog counter(7) was used for *in vivo* retention and feces measurements. In both cases sample radioactivity was compared with that of an aliquot of the injection standard. (This procedure automatically corrects for radioactive decay.) Each sample was measured over a period of time sufficient to demonstrate Pb^{212} - Bi^{212} equilibrium.