References and Notes

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4-Leucine-Oxytocin: Natriuretic, Diuretic, and Antivasopressin Polypeptide

Abstract. *During water diuresis in anesthetized rats, 4-leucine-oxytocin increased the urine output and the rates of sodium and chloride excretion. The potassium excretion rate was only slightly increased. During vasopressin-suppressed water diuresis, 4-leucine-oxytocin produced similar effects on urine and electrolyte excretions. In addition, it inhibited the vasopressin-induced free-water reabsorption, and it could reverse reabsorption to free-water clearance.*

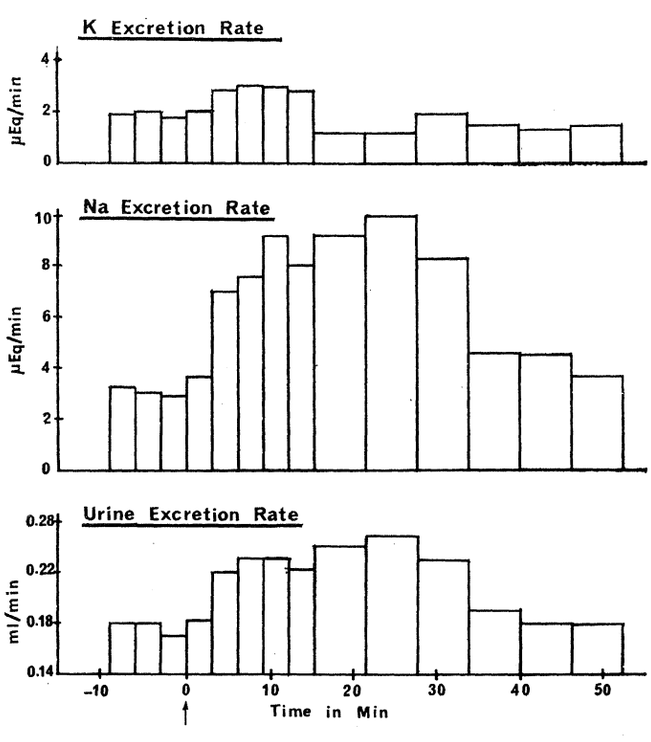
4-Leucine-oxytocin differs from the hormone oxytocin only in that it has a leucine residue in the 4-position instead of the glutamine residue of the oxytocin molecule. This oxytocin analog was synthesized (*1*) as part of a study to determine the molecular requirement for the biological activity of oxytocin.

Bioassays of this analog on rats showed that 4-leucine-oxytocin has approximately $1/50$ of the uterotonic activity of oxytocin. It also has a weak and inconsistent depressor effect on the blood pressure of the rat. It has no antidiuretic activity. On the contrary, it has a potent natriuretic and diuretic effect. Furthermore, it inhibits antidiuretic hormone activity and can reverse the free-water reabsorption induced by vasopressin (ADH) to free-water clearance in rats during vasopressin-suppressed water diuresis. More extensive studies on the renal pharmacology of 4-leucine-oxytocin are being conducted, but we believe that the renal actions of this analog are of sufficient significance and interest that a report at this stage is warranted.

Male Sherman albino rats weighing between 200 and 350 g were used. They were anesthetized with ethanol and prepared for recording and collection of urine as described by Chan (*2*). Water diuresis was induced by an oral water load maintained at a constant 8 percent of the body weight as described by Sawyer (*3*). The hydrating solution was a mixture of 2 percent ethanol, 0.5 percent dextrose, and 0.3 percent NaCl; the ethanol was incorporated to maintain anesthesia.

Urinary and plasma sodium and potassium concentrations were determined by a Baird-Atomic flame photometer, with Li_2SO_4 as an internal standard. Chloride was determined by a Buchler-Cotlove direct-readout chloridometer. Osmolality of urine and plasma was determined by the freezing-point depression method with a Fiske osmometer. The osmolality thus determined is slightly overestimated because of the alcohol content in the samples. This, however, does not greatly affect the calculation for free-water clearance.

In rats during water diuresis, when doses of 4-leucine-oxytocin less than 0.5 μg per 100 g of body weight were injected intravenously, they produced no detectable effect on the rate of urine excretion. This dose is 100 times larger than an effective antidiuretic dose of oxytocin and more than 5000 times larger than that of arginine-vasopressin. Higher doses produced a diuresis which was a consequence of marked natriuresis. The percentage increase in the rate of sodium excretion was greater



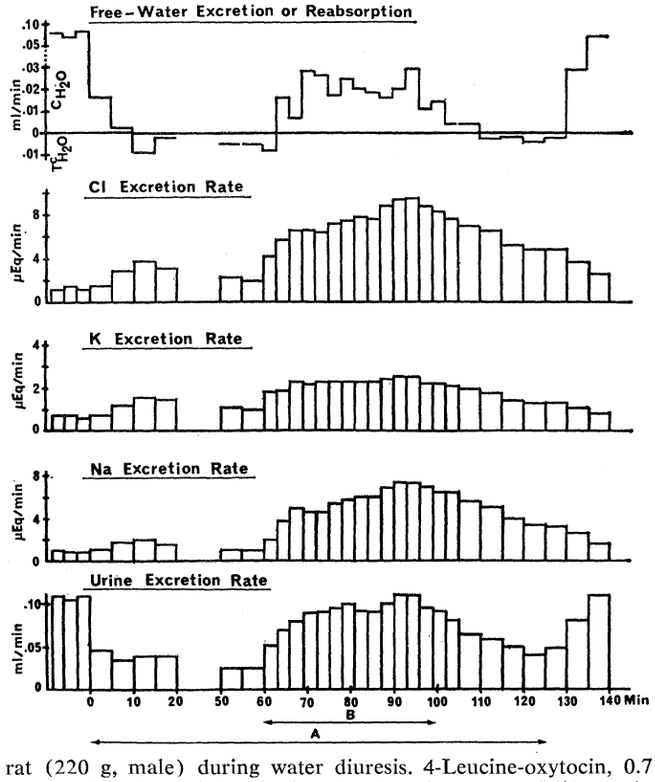


Fig. 1 (left). Natriuretic-diuretic effect of 4-leucine-oxytocin in a rat (220 g, male) during water diuresis. 4-Leucine-oxytocin, 0.7 μg per 100 g of body weight was injected intravenously at zero time. Fig. 2 (right). Natriuretic, diuretic, and antivasopressin effects of 4-leucine-oxytocin in a rat (200 g, male) during vasopressin-suppressed water diuresis. During period *A*, infusion of arginine-vasopressin, priming dose 0.025 milliunit, infusion rate 0.005 milliunit/min. During period *B*, infusion of 4-leucine-oxytocin, priming does 1.0 μg, infusion rate 0.2 μg/min.

than the percentage increase in the rate of urine excretion. The rate of potassium excretion was only slightly increased. These renal responses to 4-leucine-oxytocin were consistently demonstrated in four experiments with a dose range from 0.5 μg per 100 g to 0.7 μg per 100 g of body weight. Figure 1 shows the results from one typical experiment.

In three other experiments, rats with marked water diuresis were given a continuous infusion of arginine-vasopressin (synthetic) to suppress water diuresis. The infusion rate was adjusted to produce and maintain a 50- to 60-percent inhibition of water diuresis. Under our experimental conditions, the priming dose of arginine-vasopressin was between 0.025 and 0.04 milliunit. The infusion rate was between 0.0035 and 0.006 milliunit/min, with a flow rate always less than 0.05 ml/min delivered by a Harvard infusion pump.

Arginine-vasopressin infusion reduced the output of urine, increased the urinary osmolality to hyperosmotic levels, and reversed clearance of free water to reabsorption of free water. It had either no effect or caused a slight increase in osmolar clearance (C_{osm}) and excretion rates of sodium, potassium, and chloride.

When, under the influence of vasopressin infusion, a steady state was achieved, simultaneous infusion of 4-leucine-oxytocin (priming dose 0.5 μg per 100 g of body weight; an infusion rate of 0.05 to 0.20 μg per 100 g per minute) caused a marked diuresis. Urine output was increased to or above the initial level of water diuresis before vasopressin infusion. Osmolality of the urine decreased, and the urine became hypoosmotic. Free-water reabsorption was decreased in all three experiments, and in two of these experiments, it was reversed to free-water clearance; in effect the antidiuretic action of vasopressin was inhibited. Since the unavoidable effect of alcohol on the determination of osmolality tends to minimize the calculated reduction in free-water reabsorption, the absolute effect of 4-leucine-oxytocin on this is even greater. Osmolar clearance and excretion rates for sodium, potassium, and chloride were all increased. Natriuresis and chloruresis were the most prominent features of the response to 4-leucine-oxytocin infusion. When 4-leucine-oxytocin infusion was stopped, the urinary excretion pattern returned to that seen under vasopressin infusion. The duration of 4-leucine-oxytocin infusion varied from 15 to 60 minutes, and after its cessation, the anti-ADH effect subsided accordingly in 10 to 30 minutes. Figure 2 shows the results of one experiment.

Thus 4-leucine-oxytocin is a potent natriuretic-diuretic polypeptide and also has an antivasopressin activity. It has only a slight kaliuretic activity. The natriuretic-diuretic property of 4-leucine-oxytocin is not unique. Oxytocin at low doses can produce natriuresis and diuresis in rats (*2, 4, 5*); but at high doses, it is definitely antidiuretic. The uniqueness of 4-leucine-oxytocin is its lack of antidiuretic activity and its antivasopressin activity.

Brunner *et al.* (*5*) and Sawyer and Valtin (*6*) have shown that at certain doses oxytocin inhibits the antidiuretic response of rats to vasopressin. In these experiments, the inhibition was partial, and the free-water reabsorption was not measured. Therefore, Sawyer and Valtin (*6*) could not ascertain whether the inhibition is a specific antagonism or is simply the impact of oxytocin natriuresis-diuresis superimposing on the vasopressin antidiuresis.

Although the experiments reported herein are not extensive, we do have sufficient data to provide a meaningful discussion of the mechanism of the renal actions of 4-leucine-oxytocin.

First, it can be concluded that the natriuretic-diuretic activity of 4-leucine-oxytocin is not vasopressin-dependent, since this effect was demonstrable during water diuresis in water-loaded rats anesthetized with ethanol. It can be assumed with reasonable certainty that the release of endogenous vasopressin in rats during water diuresis was effectively blocked by the water load and the added ethanol.

Second, 4-leucine-oxytocin has an antivasopressin activity. This is shown by the ability of 4-leucine-oxytocin to reduce the vasopressin-induced free-water reabsorption and to reverse it to free-water clearance. The present experiments, however, do not provide data to indicate whether the 4-leucine-oxytocin inhibition of vasopressin is at a receptor level or is due to the inhibitory activity of 4-leucine-oxytocin on renal sodium reabsorption at a certain segment or segments of the nephron which, in turn, affects the concentrating capacity of the kidney.

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Carbon Dioxide Exchange in Cotton: Some Anomalous Fluctuations

Abstract. *Anomalous depressions in carbon dioxide exchange were observed in cotton leaves that were exhibiting oscillations in transpiration under controlled conditions of environment. The depressions occurred only when leaf temperature exceeded 37.5°C and when the leaf diffusive resistance was minimum. Stomatal control of the supply of carbon dioxide to the leaf does not seem to be implicated in the effect.*

Several observers have noted oscillations, with periods of 20 to 30 minutes, in the rate of transpiration and carbon dioxide exchange in various species of plants (*1*). It has been confirmed that these oscillations are associated with fluctuations in the diffusive resistance of the stomata. This suggests that the net CO_2 exchange during the oscillations is normally controlled by the supply of CO_2 to the chloroplasts. We report fluctuations in the CO_2 exchange which are not directly related to the oscillations in diffusive resistance.

Measurements were made of CO_2 exchange (by differential infrared gas