Effect of Human Serum Oxytocinase on the Antidiuretic Action of Lysine Vasopressin and Oxytocin in the Rat

Increased antidiuretic activity in the plasma has been noted in a variety of pathological states such as pre-eclampsia[1], bronchogenic 'oat cell' carcinoma[2–4], pulmonary tuberculosis[5], bilharzial hepatic fibrosis[6] and other liver diseases[7], intermittent porphyria[8] and myxedema[9]. This increase in antidiuretic activity, which may be due to excessive secretion of the antidiuretic hormone or its decreased inactivation, is made responsible for the disturbances in water and electrolyte metabolism observed in these pathological states. Attempts to lower the plasma concentration of antidiuretic hormone to normal levels may therefore be regarded as therapeutically legitimate.

In the case of the peptide kinins, a decrease in the level of circulating hormone by parenteral administration of inactivating enzymes has been achieved[10,11]. To be a candidate for clinical use, an inactivating enzyme should be relatively specific for the peptide concerned, free from the danger of anaphylactic shock, and have a long half-life in the circulation. In relation to the neurohypophysial hormones the first two conditions appear to be met by 'serum oxytocinase', an aminopeptidase appearing in human pregnancy plasma. The effect of this enzyme on the antidiuretic response to oxytocin and vasopressin has now been tested in rats.

Female rats of the Wistar strain were hydrated[12] and infused at a constant rate (250 μU antidiuretic activity in 0.65 ml/h) into the femoral vein with solutions of purified synthetic oxytocin or lysine vasopressin in 0.45% saline. The antidiuretic response was measured by both the rate of urine flow and the urine conductivity and continuously recorded[12]. When the antidiuretic response to the infusion had reached a steady state a solution (1 ml) containing 0.2–2.5 milliunits of serum oxytocinase (5–10 mg protein) was injected into the other femoral vein; 1 unit of oxytocinase is defined as that amount of enzyme which inactivates 1 μmole of oxytocin in 1 min at 37 °C[13,14]. The enzyme was prepared from the globulin fraction of human retroplacental serum by alcohol fractionation and chromatography on DEAE-cellulose, and freed of salts by dialysis[15,16]. A solution of normal γ-globulin of about the same protein concentration served as control.

Rats of the same strain and age were used to study the change in the enzyme blood level with time. The rats were anaesthetized with ether and a hypodermic needle was introduced into the jugular vein (exposed by a small incision) through the major pectoral muscle without damaging the outer vessel wall; this technique could be used repeatedly, both for the initial injection of the enzyme and for sampling the blood (0.2 ml portions) with heparinized syringes at the time intervals indicated in Figure 2. The total amount of blood taken was 1.2 ml during the first day and 0.2 ml on each subsequent day. Vitamin B_{12} (Spofa; 1 μg daily, i.m.) and ferridextran (Léčiva, Praha) (5 mg Fe the first day and 1 mg Fe on each subsequent day i.m.) were given to stimulate hemopoesis. The oxytocinase activity of the plasma was determined after fivefold dilution with phosphate buffer (final concentration 0.04 M, pH 6.8) and addition of oxytocin to a final concentration of 10 μM; the residual oxytocin was assayed by its antidiuretic activity[12] after 30 and 60 min incubation at 37 °C.

When the control samples of γ-globulin were injected into hydrated rats in steady-state antidiuresis during infusions of oxytocin or vasopressin, there was a slight increase (mean: 1.9%) in the rate of urine flow together with a slight increase (mean: 2.3%) in the conductivity. This effect was not statistically significant at the 1% probability level and is presumably due to the expansion

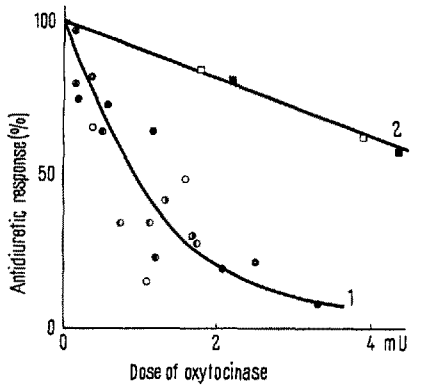

Fig. 1. Effect of oxytocinase injection on steady-state antidiuresis. 1 = oxytocin; 2 = vasopressin. Points coded by different symbols correspond to experiments on different rats.

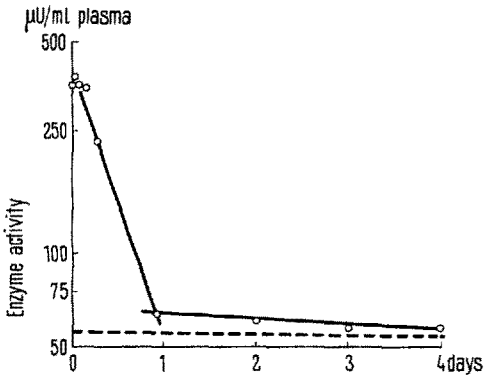

Fig. 2. Decay of circulating oxytocinase activity. The dashed line shows the base-line level of activity (control value).

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of the extracellular volume by the hypo-oncotic protein solution; an effect on the hormone level would have been expected to show opposite movement in the urine flow and urine conductivity. Injection of the oxytocinase preparation did, indeed, cause an increase in the urine flow and a decrease in the urine conductivity, the effect being dependent on the dose of the enzyme (Figure 1). The changes in the 2 parameters were approximately matched, as they would be if the rate of infusion of the hormone had been decreased at the moment the enzyme was injected. For 2 h after the injection there was little further change in the antidiuretic response, indicating that the enzyme activity was practically unchanged over this period. The effect of a given dose of the enzyme on the response to oxytocin was much greater than on the response to lysine vasopressin. Since serum oxytocinase[17] or pregnancy serum[18] inactivate both oxytocin and vasopressin at about the same rate in vitro, it is unlikely that this difference reflects the specificity of the enzyme; it may be a consequence of different distribution patterns for the 2 hormones in the organism.

The change with time in the level of exogenous oxytocinase activity in rats is shown in Figure 2. A first, relatively rapid, exponential phase (half-time 7.16 h) during which the activity decreases over 24 h to only about twice the control value, is followed by a second, slow, exponential phase. The half-life of the enzyme in the circulation is thus higher than that of other exopeptidases[10] but still considerably lower than that of the globulins[19].

The highest dosages of enzyme used in our experiments were calculated so as to increase oxytocinase activity in the rat plasma (assumed to be 4% of body weight) to approximately the same level as that found in the plasma of pregnant women nearing term (about 0.12 milliunits/ml). The actual maximal enzyme level found was only $^1/_2$ to $^1/_5$ of this value, and rapidly decreased with time. If the inactivation rate and distribution pattern of exogenous enzyme in humans is similar to that observed in our experiments, repeated doses of the enzyme would be required in clinical use to maintain a useful level of activity in the blood, e.g. in toxemia of pregnancy associated with low levels of endogenous oxytocinase activity[20,21]. The behaviour of exogenous enzyme in human patients would appear to be the most important consideration for its use at the present time[22].

Zusammenfassung. Nach i.v. Dauerinfusion von Lysin-Vasopressin oder Oxytocin wird bei der experimentellen Analyse der Wirkung menschlicher Oxytocinase eine Erniedrigung des antidiuretischen Effekts gefunden; diese rührt vom Absinken der Peptidkonzentration sowohl im Blutplasma als auch am Wirkort her.

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Extravascular Short-Circuiting of Oxygen Indicating Countercurrent Exchange in the Intestinal Villi of the Cat

Countercurrent exchange of materials between arterial and venous segments of capillaries is firmly established in the mammalian kidney[1] and in the swimbladder of many fishes[2]. The countercurrent mechanism is dependent upon the close association of the venous and arterial limbs of a vascular loop. Such a special vascular anatomy is present in the intestinal villus of many mammals, in which ascending non-branching central arterioles are surrounded by a descending dense subepithelial capillary network[3]. The distance between the arterial and venous parts of this vascular loop is estimated to be 10–30 μ and can be traversed by easily diffusible lipid-soluble agents, such as oxygen, in a fraction of a second. Whether water-soluble materials will pass depends upon the presence of endothelial 'pores' in the ascending arteriolar limb. Anyhow, anatomical prerequisites should exist for an extravascular diffusion of oxygen from ascending to descending limbs of the loop. Experiments were performed to determine whether such an extravascular shunting of oxygen existed, by comparing venous appearance-time of oxygen with that of red cells after injection into the superior mesenteric artery.

Methods. 5 cats (2–4 kg) were fasted for at least 24 h and anaesthetized with chloralose (50–70 mg/kg). Venous outflow from jejunal sections weighing 40–60 g was recorded by means of a drop recorder unit operating an ordinate writer. Arterial blood pressure was monitored from the left femoral artery by a mercury manometer. Atropine (1 mg/kg) was given and the splanchnic nerves cut bilaterally.

3 different types of blood mixtures were administered to the experimental animals via a thin polyethylene catheter (PE 10) inserted into a branch of the superior mesenteric artery: (1) blood equilibrated with pure oxygen ('oxygenated blood'); (2) blood treated with 1% sodium nitrite and washed with isotonic saline to produce

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