Effect of Ethyleneimine on Renal Action of Vasopressin.* (31620)

EDWARD L. GOLDBLATT, MICHAEL L. KAUKER, RUTH S. HARE, AND KENDRICK HARE
Department of Pharmacology, University of Alabama Medical Center, Birmingham

When Jackson and James(1) were studying effects of some antitumor drugs, they observed that ethyleneimine caused an increase in water exchange of rats. They synthesized a series of related compounds and, after testing them for their diuretic effectiveness, suggested that these drugs might interfere with the action of the antidiuretic hormone. We have confirmed this hypothesis.

Methods. Young, adult female dogs with experimental diabetes insipidus in the permanent polyuria phase following stalk transection were anesthetized with pentobarbital and the urinary bladder catheterized. Water was given by stomach tube to maintain the polyuria. After a few 10-minute control periods, a series of intravenous injections of vasopressin in doses of 1-5 milliunits was given. Full recovery of the diuresis was allowed before subsequent injections were made. Serial blood samples were drawn from the femoral artery. Serum and urine analyses included urea(2), endogenous creatinine(3), and osmolarity with a Mechrolab vapor pressure osmometer. From these were calculated glomerular filtration rate, percent excretion of filtered water and urea and the osmotic U/P ratio. A week or more later, the dogs were given 1.5 mg/kg of ethyleneimine (K & K Laboratories, Inc.) in freshly prepared solution by vein. The next morning the above procedure was repeated.

The vasopressin was from a single ampoule of Pitressin; it was diluted 100-fold and stored in small sealed vials at −25°C until used. To insure that loss of potency was not the cause of the diminished response after ethyleneimine, the experimental procedure was reversed in two instances: the controls were run after the dog had recovered from the drug. The interval between the observations was prolonged since Jackson and James(1) reported that the polyuria following a single injection could persist for many weeks.

Results. Ethyleneimine impaired the concentrating mechanism which forms a hypertonic urine (Fig. 1). In the untreated dog with a permanent polyuria, one milliunit of vasopressin regularly produced a urine of an osmolarity greater than that of plasma while 5 mU elevated the osmotic U/P of less than one to 2-3. After ethyleneimine, vasopressin in doses up to 25 milliunits failed to make the urine as concentrated as the plasma (lower line in Fig. 1) except in 2 experiments in which 25 mU of vasopressin produced an osmotic U/P of 1.1. An approximation of the impairment of the renal concentrating mechanism can be obtained by comparing the increase of the osmotic U/P in the untreated dog with diabetes insipidus in response to 1 mU of vasopressin to the smaller response to 25 mU after treatment of the same dog with ethyleneimine. There is more than a 95% reduction in the kidney's capacity to produce a hypertonic urine in response to a known dose of exogenous vasopressin.

The antidiuretic response was also diminished by ethyleneimine (Fig. 2), but the influence of ethyleneimine on the antidiuretic response to vasopressin varied extensively from one dog to another. Reductions in urine flow as high as 80% in response to 10-25 mU of vasopressin were recorded, but even with these degrees of antidiuresis, the urine remained hypotonic except in the two experiments noted above.

The urea transport sensitive to vasopressin was more depressed by ethyleneimine than the water transfer mechanism. In several instances it was completely paralyzed (Fig. 2) even at the highest dose of 25 mU of vasopressin. In other experiments, the urea transport was only moderately affected by vasopressin after ethyleneimine as compared to the control response, despite a good antidiuresis. This is illustrated in Fig. 3 which includes responses to 5 mU of vasopressin before and after ethyleneimine.

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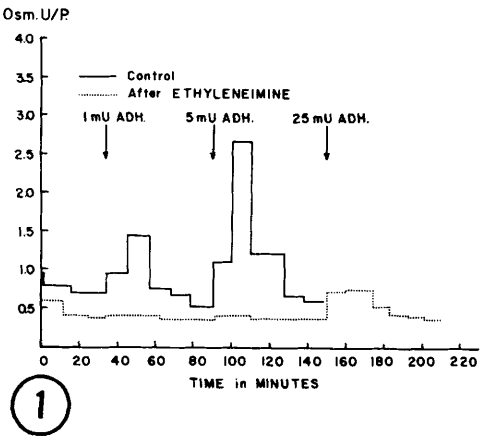

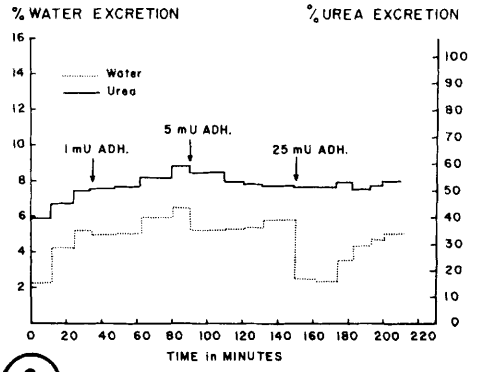

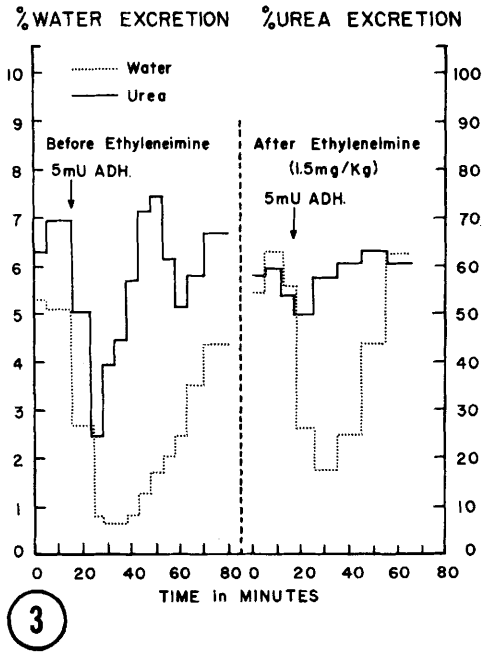

FIG. 1. The osmotic U/P ratio is plotted against time. Solid line indicates response of a dog with experimental polyuria to injections of 1 and of 5 mU vasopressin. Broken line represents responses of the same dog to 1, 5 and 25 mU of vasopressin after treatment with ethyleneimine.

FIG. 2. Percent of filtered water and urea excreted in urine are plotted against time. Injection of vasopressin, indicated by arrows, produced minimal reductions in water excretion, but no reduction in urea excretion even at a dose of 25 mU. The subject was a dog with a pituitary stalk section pretreated 18 hours before the experiment with 1.5 mg/kg of ethyleneimine.

FIG. 3. Percent of filtered water and urea excreted in urine are plotted against time. Reduction in excretion of urea and water in the untreated diabetes insipidus dog in response to 5 mU of vasopressin is shown on left. After treatment of the same dog with ethyleneimine, the same dose of vasopressin produced a smaller response of shorter duration.

Discussion. Since the greatest effect of ethyleneimine observed in these experiments was on the vasopressin sensitive system for transport of urea it is suggested that this is the primary action of the drug. It is now well established that in water diuresis, the concentration of medullary solutes is reduced far below that of the hydropenic animal(4), and that vasopressin quickly restores the marked hypertonicity of the medullary interstitium(5,6). We suggest, therefore, that the impairment in renal concentrating ability is indicated by the low osmolarity of the urine and the diminished antidiuresis to vasopressin is secondary to the greatly reduced capacity of the renal tubules to transport urea against a concentration gradient.

Summary. In dogs with experimental diabetes insipidus intravenous injections of vasopressin in doses of 1-5 mU produced markedly hypertonic urine, a sharp antidiuresis and decrease in the urinary excretion of urea. For at least 25 hours after the intravenous administration of 1.5 mg/kg of body weight of ethyleneimine, these responses to vasopressin are reduced. Ethyleneimine interferes with the renal effects of exogenous vasopressin.

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Effect of Temperature (and Substrate Concentration) on Chick Lactate Dehydrogenase Activity.* (31621)

H. MAISEL AND M. KERRIGAN (Introduced by A. C. Kuyper)
Department of Anatomy, Wayne State University School of Medicine, Detroit, Mich.

It has been reported that total lactate dehydrogenase (LDH) activity of several human tissues reacts more similarly toward pyruvate and lactate at 25°C than expected from marked differences in substrate inhibition at this temperature between isolated and purified LDH 1 and 5(1). Furthermore, at 37°C LDH 5 closely resembled LDH 1 in extent of substrate inhibition. It was concluded that these results are incompatible with the theory that differences in the degree of isozyme inhibition have resulted in the predominance of LDH 5 in anaerobic tissues and LDH 1 in aerobic tissues.

Previously we have reported that at 30°C it was possible to distinguish between the activities of chick lens LDH isozymes at high and low pyruvate concentrations, and that the results were in agreement with the starch gel data(2). The purpose of the present experiment was to test the effect of temperature on LDH activities of chicken heart, breast muscle, and LDH 1 and LDH 5 isolated from these tissues respectively.

Heart and breast muscle of 1 year old chickens was obtained immediately after death, and homogenized in a Waring blendor at 4°C using one gram of tissue to 3 ml of 0.9% saline. The homogenates were centrifuged at 20,000 *g* for 30 minutes. Enzyme activity in the supernatant was assayed on a Beckman DB spectrophotometer at 340 mμ at 25°C, 30°C and 40°C. The temperature of the reaction chamber was stabilized by the use of a temperature controlled water bath. The final dilution of heart muscle supernatant was 1:600 and 1:1200 for breast muscle.

The assay mixtures, as described previously, were at pyruvate concentrations of 3.3×10^{-4} M and 6.6×10^{-3} M respectively (2). Sufficient activity was obtained by using .05 ml of the diluted supernatant, and the assay volume was kept constant at 3 ml. To isolate LDH 1 and LDH 5, 8 samples of heart muscle supernatant and breast muscle supernatant (1:4 dilution) were subjected to electrophoresis on starch gel for 16 hours. The section of the gel containing LDH 1 and that containing LDH 5 was cut out and the enzyme eluted.

The effect of temperature on the activity of heart and breast muscle supernatant measured at high and low pyruvate concentrations is shown in Fig. 1. At all temperatures breast muscle showed greater activity at high pyruvate concentration than at low. With increasing temperatures there was greater activity at both pyruvate concentrations, but this increase was especially marked at high pyruvate concentration measured at 40°C over that at 25°C, while at low pyruvate concentration the increase was small. Heart muscle was also more active at higher temperatures, but the increase was slight compared to that of the breast muscle. Furthermore, the difference between activity at high and low pyruvate concentrations was more marked at 25°C than at 40°C for heart muscle. The data obtained for isolated LDH 1 and LDH 5 (Fig. 2) corresponded to that obtained for the heart and breast muscle respectively.

The results of this experiment show that the LDH in the supernatant of chicken breast

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