Influence of Enteramine (5 Hydroxytryptamine) on Renal Function of the Dog. (20196)

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Among the hormonal factors controlling water excretion by the kidney, enteramine is to be listed. Enteramine is the specific hormonal product of the enterochromaffin cell system which is widely distributed in vertebrates and invertebrates(1). It was isolated from beef serum by Rapport *et al.*(2), and tentatively identified as 5-hydroxytryptamine and named serotonin. Enteramine was isolated from the extracts of *Octopus vulgaris* and *Discoglossus pictus* by Erspamer and Asero(3); it has been simultaneously and independently synthesized in 3 different laboratories(3-5,9). 5 hydroxytryptamine was said to be vasoconstrictor and pressor(2,6), general smooth muscle stimulator(7), to play an important role in hemostasis(8). Erspamer claims that the physiological and main activity of the substance is exerted on the kidney, where it promotes water retention by reducing the GFR through the increased tone of the glomerular afferent vascular bed: extrarenal vascular and spasmogenic effects are to be considered only as pharmacological actions(3,14).

The present study was undertaken to investigate the effect of different doses of enteramine on the urine volume and the renal function of the hydrated dog.

Methods. Assays on the antidiuretic activity of enteramine were performed on trained, unanaesthetised and colpotectomized female dogs averaging 15 kilos in weight. The dogs were maintained for 16-20 hours prior to the experiment without food but with free access to water. For oral hydration, 50 ml per kilo of tap water were administered by stomach tube half an hour before the start of the experiment; for intravenous hydration, distilled water or saline were infused at a constant rate of 3-5 ml per minute. During clearance periods, lasting 10-20 minutes, the dogs were restrained loosely on a comfortable animal table. Blood samples were collected midway in the periods, no correction being made for delay time. Catheterisation was performed with a rubber catheter and each period was terminated by a 20 ml saline washout. The clearance of sodium thiosulfate was used as a measure of filtration rate, a quantity of thiosulfate being infused necessary to sustain plasma levels over 20 mg %; the clearance of sodium-aminohyppurate was used as a measure of the renal plasma flow, the plasma levels of PAH being maintained between 1 and 3 mg %. Thiosulfate and PAH were determined in the urine and plasma according to Brun(10) and Finkelstein and Aliminosa(11), respectively. Enteramine picrate was dissolved into hot water and injected subcutaneously or, intravenously; the quantity used is expressed as picrate. The picrate contains 41% of the free base 5 hydroxytryptamine.

Results. The effect of enteramine was studied in a series of 30 dogs; the results of 3 representative experiments are summarized in Fig. 1, 2, 3.

Urine volume was reduced according with the pre-injection values; at initial rates of 7-9 ml per minute, doses of 0, 1-0, 3 mg per kilo of enteramine decreased the urine flow to 1-0, 8 ml per minute, while at initial values of 1-3 ml per minute, enteramine even in doses of 0, 5-1 mg per kilo was unable to reduce the

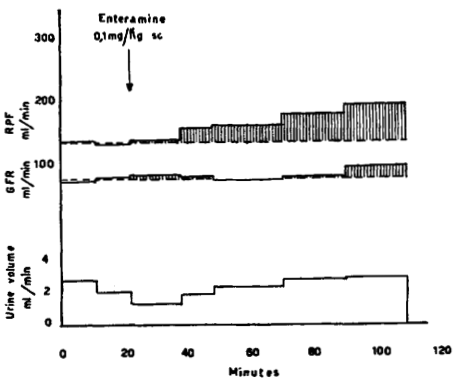

FIG. 1. Effect of 0,1 mg/kg of enteramine on urine volume, GFR and RPF of the hydrated dog.

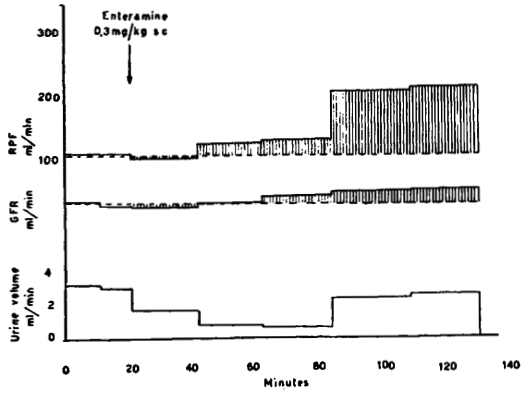

FIG. 2. Effect of 0,3 mg/kg of enteramine on urine volume, GFR and RPF of the hydrated dog.

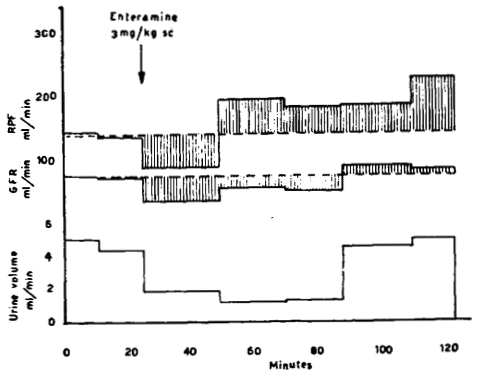

FIG. 3. Effect of 3 mg/kg of enteramine on urine volume, GFR and RPF of the hydrated dog.

urine flow under 1-0, 8 ml per minute. At constant initial urine flows, the higher doses of enteramine generally cause a stronger decrease of urine volume, but the antidiuretic effect is not directly correlated with the dose employed. The antidiuretic effect begins within 10 minutes after the subcutaneous or intravenous injection and lasts 40-60 min; successively the antidiuretic effect stops and the urine volume increases to, or even more, the preinjection values. Intavenously continuous infusion of enteramine (2-3 mg/kilo/hour) results in a more strong antidiuretic action.

Glomerular filtration rate shows a different behaviour according to the doses; in fact while the lower doses of enteramine (0, 1-0, 3 mg per kilo) do not significantly affect the filtration rate (Fig. 1, 2), the higher doses (0, 5-1-3 mg per kilo) are likely to reduce the GFR, (Fig. 3), the decrease being proportional to the dose employed. In this last case the decreased filtration rate, however, is not responsible by itself for the antidiuretic activity, inasmuch as even now the thiosulfate ratio of urine to plasma concentration (U thios./P thios.) increases markedly and more significantly than the change in GFR and the water to thiosulfate clearance (V/thios. Cl) constantly decreases, clearly indicating that enteramine determines an increase in the percentage of filtered water reabsorbed.

Renal plasma flow generally increases. The increase begins 20-30 minutes after the administration of enteramine and reaches its maximal rate during the diuretic phase which follows the reduction of the urine volume. It is manifest both in dogs infused with saline and distilled water and is present for doses as high as 1 mg per kilo even in those periods characterized by a slight decrease of GFR (Fig. 2). At higher doses the strong reduction of GFR meets with a concomitant decrease of PAH clearance; even in these high doses the diuretic phase is accompanied by a sharp increase of RPF (Fig. 3). The *filtration fraction* consequently decreases, this effect being obviously more evident in those periods when the filtration rate remains immodified and the plasma flow increases.

Discussion. The experiments here reported clearly emphasize the antidiuretic effect of enteramine, claimed by Erspamer *et al.*(3,14). The decrease of the urine flow in hydrated dogs is definite and the doses necessary to this effect are very low (in the order of 0, 1-0, 3 mg per kilo), certainly lower than those affecting the systemic blood pressure or those stimulating the smooth muscle. As regards the causal mechanism of the antidiuretic activity of enteramine, 3 main possibilities must be taken into account: a) the spasmogenic effect on smooth muscle of the bladder and the ureters b) the constriction of the afferent glomerular arteriole with reduction of GFR; c) the primitive increase of tubular reabsorption of water.

It has been demonstrated by Erspamer(12) and confirmed by subsequent trials, that enteramine is a spasmogenic substance and that the urinary excretory territory may become constricted under its action. What is to be stressed, however, is: 1) that the anti-

diuretic effect is already obtainable with doses which by themselves are not spasmogenic; 2) that it is generally assumed that the increased ureteral pressure meets with a decrease of GFR and a total reduction of the renal vascular bed(13). This second alternative will be discussed later.

The reduction of GFR which enteramine provokes in the rat led Erspamer to refer the antidiuretic effect of the substance to a constriction of the afferent bed of the glomerule. It is a matter of fact that the higher doses of enteramine cause in the dog a reduction of the filtration rate with consensual, though less evident, reduction of RPF, but these hemodynamic changes alone cannot explain the antidiuretic effect, insofar as the percentage of the filtered water reabsorbed is constantly increased by enteramine. Moreover enteramine may reduce urine volume without decreasing the GFR. and this effect occurs for the lower physiological doses.

The RPF consistently increased in the majority of the cases, only the higher doses of enteramine tending to determine a slight initial decrease of PAH clearance. In our opinion this fact leads again to cast some doubt on the prevailing role of the decrease of GFR in the mechanism of the antidiuretic effect of enteramine. Consequently, the antidiuretic effect of enteramine is to be referred to an increase of tubular reabsorption of water. We are now investigating the possible role of activation of the posterior pituitary, the relations between enteramine and the other antidiuretic substances in the blood, and the possible interference of Enteramine on water metabolism of the man in physiological and pathological conditions.

Summary. 1. Enteramine (5 hydroxytryptamine) decreases the urine volume of hydrated dogs at doses which do not stimulate the smooth muscle and do not modify the systemic blood pressure. The lower doses (0, 1-0, 3 mg per kilo) subcutaneously or intravenously decrease the urine volume without affecting the GFR, while higher doses reduce also the filtration rate; both lower and higher doses increase the RPF. 2. The above described experiments suggest that the antidiuretic effect of enteramine in the dog derives principally from an increased reabsorption of water from the renal tubule.

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