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Renal Response to Unilateral Vagotomy During Water Diuresis.* (30730)

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Bilateral cervical vagotomy promotes prolonged inhibition of mild water diuresis(1) and increased antidiuretic activity of blood (2,3). These reports support the concept of vagal afferent control of antidiuretic hormone secretion, which has evolved from the observation that left atrial stretch induces diuresis in dogs with intact vagus nerves, but has no effect when vagal transmission is blocked(4). Since vagal afferents responsible for the induction of certain respiratory and cardiovascular phenomena are confined to or predominate in a particular vagus nerve (*e.g.*, 5,6), the present experiments were performed to determine the influence on renal function of unilateral vagal section during mild water diuresis.

Methods. Mongrel dogs (12.7-21.8 kg) of both sexes were anesthetized with pentobarbital sodium (30 mg/kg, i.v., with supplementation as required). After a priming intravenous injection of creatinine and sodium para-aminohippurate (PAH), warm 5% glucose in water, containing appropriate amounts of these substances, was infused continuously by pump at the rate of 2.5 ml/min throughout the experiments. The ureters were exposed through a mid-ventral incision, and were cannulated with polyethylene tubing. Short segments of the cervical vagus nerves (vagosympathetic trunks) were isolated approximately 5-7 cm below the lower border of the larynx, and double loose ligatures were placed around each nerve. Parafilm was placed between the cut surfaces of the skin to prevent adherence and the wound was closed with a towel clip. A femoral artery was cannulated for determining mean blood pressure with a mercury manometer, and an indwelling needle was inserted into an external jugular vein for obtaining blood samples at 30-minute intervals during subsequent clearance studies. An animal was considered suitable for an experiment if urine flow was reasonably stable during three 15-minute control periods and the urine was hypotonic; during the control phase, the mean $\pm$ S.E. for urine osmolality was 165 $\pm$ 15 mOs/kg H_2O. At the end of the last control period, the neck wound was opened and one vagus nerve was gently lifted and cut with scissors; manipulation of the nerve preparatory to sectioning was not accompanied by bodily movements or changes in blood pressure. It was found previously(1) that sectioning the vagi with or without ligation and in the presence or absence of a locally applied anesthetic did not alter the results. Right vagotomy was performed in 5 dogs and the left nerve was sectioned in a second group of 5 dogs. Upon completion of urine collections (9-16 fifteen-minute periods) after unilateral vagotomy, the contralateral vagus was sec-

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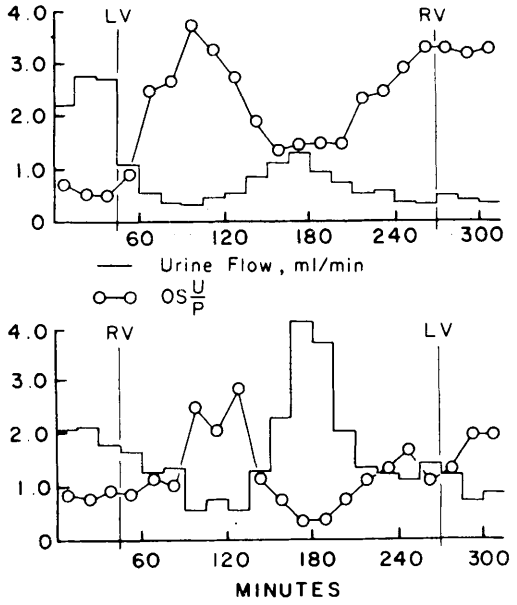

FIG. 1. Effect of unilateral vagotomy and subsequent contralateral vagotomy on urine flow and osmolal U/P ratio (os U/P) for an animal on each procedure. LV: left vagotomy; RV: right vagotomy.

tioned and urine collections were continued for an additional 3-7 fifteen-minute periods. The stability of renal function in dogs with intact vagus nerves and subjected to present hydration and surgical procedures has been reported(1).

Conventional renal clearances were performed before and after vagotomy. Exogenous creatinine and PAH clearance were used as estimates of glomerular filtration rate (GFR) and effective renal plasma flow (RPF), respectively. Methods used previously(7) were employed for analyses of creatinine, PAH, osmolality, Na and K in plasma and urine. Osmolal clearance (C_{os}) and free-water clearance (C_{H_2O}) were calculated as follows: $C_{os} = U_{os}/P_{os} \times V$, where U_{os} and P_{os} and the urine and plasma osmolalities in mOs/kg H_2O, respectively, and V is urine flow in ml/min; C_{H_2O}, $= V - C_{os}$.

Results. After unilateral section of either vagus nerve during mild water diuresis, urine flow and free-water clearance (C_{H_2O}) declined, then rose, and declined again, while changes in osmolal urine-to-plasma ratio (os U/P) were of the converse order. Although patterns for these parameters were the same for the two groups, overall inhibition of water diuresis was more profound after left than after right vagotomy. Certain significant changes within each group and significant differences in the magnitude of the phasic responses between the two groups occurred.

Within groups. Complete data for urine flow and os U/P for a representative animal in each group are plotted in Fig. 1. Mean values for urine flow, concomitant os U/P, C_{H_2O}, and osmolal clearance (C_{os}) for each group are shown in Fig. 2 before vagotomy (C) and for the following phases after vagotomy: I—lowest urine flow during the initial decline after unilateral vagal section; II—peak urine flow after phase I; III—three consecutive 15-minute periods just prior to contralateral vagal section; and IV—last two 15-minute periods after contralateral vagotomy. Within each group after unilateral vagotomy, differences between the means for urine flow, os U/P, and C_{H_2O} for the successive phases are statistically significant ($P<0.05$ to <0.001). Left vagotomy in the right vagotomized group promoted a further significant decline in urine flow and C_{H_2O} ($P<0.05$) and a rise in os U/P ($P<0.02$), whereas right vagotomy after left

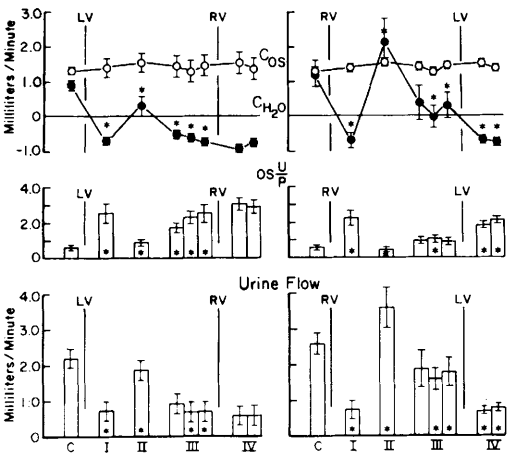

FIG. 2. Mean values (± S.E.) for urine flow, osmolal U/P ratio (os U/P), free-water clearance (C_{H_2O}), and osmolal clearance (C_{os}) before unilateral vagotomy (C: mean of average values for three 15-min control periods) and during post-vagotomy phases (see text). Asterisk denotes statistically significant difference from mean of preceding phase ($P<0.05$ to <0.001). LV: left vagotomy; RV: right vagotomy.

TABLE I. Glomerular Filtration Rate (GFR) and Effective Renal Plasma Flow (RPF) Before and During Post-Vagotomy Phases of Altered Urine Flow.

Procedure	Control*	Post-vagotomy phases† I	II	III	IV
		GFR, ml/min/100 g kidney			
LV-RV	82 ± 2	81 ± 3	81 ± 5	80 ± 7	79 ± 3
RV-LV	78 ± 2	77 ± 4	77 ± 2	79 ± 4	74 ± 1
		RPF, ml/min/100 g kidney			
LV-RV	219 ± 14	221 ± 19	220 ± 17	221 ± 31	207 ± 9
RV-LV	204 ± 19	201 ± 22	209 ± 25	200 ± 18	202 ± 18

LV: left vagotomy; RV: right vagotomy.
* Mean ± S.E. of mean for three 15-min clearance periods before vagal section.
† Mean ± S.E. for post-vagotomy phases presented in Fig. 2, except III represents last period before and IV last period after contralateral vagotomy.

vagotomy had no further significant effect. During phase II urine flow for the right vagotomized group exceeded control values significantly in 4 of the 5 animals ($P<0.01$), whereas urine flow in the other group was somewhat below, but not significantly different from, control values in 4 of the 5 animals. During phase III, urine flow, os U/P, and C_{H_2O} in the right vagotomized group stabilized at values that did not differ significantly from control values, whereas in the other group these functions returned to practically the same mean values as those during the initial urine flow depression (*cf* phases III and I, Fig. 2).

No significant changes in C_{os} (Fig. 2) or K excretion occurred within either group after unilateral vagotomy. Within each group, the maximum net increase over control values for Na excretion was 35 μEq/min, a value within the range of increase for intact animals subjected to the same surgical and hydration procedures(1).

During initial drastic changes in urine flow, GFR and RPF fluctuated, probably as a result of dead space errors(8). However, when urine flow remained reasonably stable at the altered flow rate, then the mean values for GFR and RPF during all post-vagotomy phases were not significantly different from control values (Table I).

Average mean arterial blood pressure did not change significantly during any of the phases of altered urine flow in either group. A transient rise in blood pressure occurred after section of each nerve.

Between groups. Differences in mean values between the two groups for urine flow, C_{H_2O}, and os U/P during the control period and during phase I (Fig. 2) are not statistically significant. During phase II (Fig. 2) mean urine flow and C_{H_2O} increased to significantly higher values, while mean os U/P decreased to a significantly lower value in the right than in the left vagotomized group ($P<0.05$). During the last period of phase III (Fig. 2), urine flow and C_{H_2O} were significantly higher ($P<0.05$) and os U/P was significantly lower ($P<0.02$) in the right than in the left vagotomized group. Differences in values for these functions during phase IV (Fig. 2) are not statistically significant. Differences in values between the two groups for C_{os} (Fig. 2), GFR and RPF (Table I) are statistically insignificant during the control period and during all post-vagotomy phases.

Discussion. In contrast to an uninterrupted inhibition of mild water diuresis after bilateral vagotomy(1), the renal response to unilateral section of either vagus nerve was triphasic. Urine flow and free-water clearance declined, then rose, and declined again, while changes in osmolal U/P ratio were of the reverse order. During the phases of altered urine flow, there were no significant changes in glomerular filtration rate, effective renal plasma flow, osmolal clearance, or mean arterial blood pressure. Such renal responses are consistent with the possibility of fluctuating blood levels of antidiuretic hormone (ADH). Since plasma osmolality in both groups declined gradually throughout the experiments (from 280 during the control period to 268 mOs/kg H_2O at the end), the

"osmoreceptor" mechanism cannot account for the phasic response. If vagal afferent fibers control ADH secretion(4), then the present results suggest that "ADH inhibitory" fibers course through both vagus nerves. Provided such fibers are carried in both vagus nerves, unilateral vagotomy should conceivably lead to some expression of the degree of activity of the remaining fibers, analogous to the reflex control of the cardiovascular system.

The initial fall in urine flow and free-water clearance and the rise in osmolal U/P ratio were equivalent after left or right vagotomy (phase I, Fig. 2). After this interval of increased renal reabsorption of water, presumably inhibitory activity in the contralateral nerve resumed and could account for the subsequent temporary resumption of diuresis (phase II, Fig. 2). Urine flow and free-water clearance increased significantly more in the right than in the left vagotomized group. Loss of fluid volume during the diuretic phase possibly promoted decreased activity of "ADH inhibitory" afferents thereby inducing the secondary decline in urine flow (phase III, Fig. 2). During this phase, urine flow and free-water clearance were significantly lower in the left than in the right vagotomized group. Subsequent left vagotomy in the right vagotomized animals resulted in further significant depression of urine flow and free-water clearance, whereas the reverse procedure was ineffective (phase IV, Fig. 2). These findings suggest either greater activity or more "ADH inhibitory" fibers in the left than in the right vagus nerve.

Present data indicate that the mechanism (presumably ADH secretion) which ultimately influences the renal reabsorption of solute-free water is more profoundly affected by the left than by the right vagus nerve. Although left atrial stretch receptors are the likely origin of the responsible vagal fibers(4), the above data suggest either the presence of similar receptors in the right side of the heart or distribution of fibers from left atrial receptors to the right vagus nerve. It is unlikely that vagal afferents from abdominal viscera are involved, since bilateral cervical vagotomy promotes antidiuresis even after previous bilateral sub-cardiac vagotomy(1). However, the following possibilities cannot be excluded: a) participation of vagal afferent fibers originating in other cardiovascular receptors or in pulmonary receptors(9); b) participation of non-vagal afferents(3,7,10); and c) phasic changes in the removal mechanisms for circulating ADH(11).

In the overall regulation of body fluid volume under normal conditions, the vagal mechanism conceivably responds to changes in some hemodynamic parameter induced by fluctuations in the effective circulating blood volume. However, when fluid volume is expanded excessively, other reflex mechanisms affecting renal function are probably activated (10,12).

Summary. After section of the left or right vagus nerve in two groups of dogs in mild water diuresis, triphasic responses in urine flow, free-water clearance, and osmolal U/P ratio occurred. However, significant quantitative differences in these responses for the two groups indicate a superior influence of the left vagus nerve on the mechanism controlling the renal reabsorption of solute-free water. During the various phases, glomerular filtration rate, effective renal plasma flow, or osmolal clearance did not change significantly from control values.

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Effect of Puromycin on Virus and Endotoxin-Induced Interferonlike Inhibitors in Rabbits.* (30731)

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Since the discovery that bacterial endotoxins as well as viruses may induce interferonlike viral inhibitors in experimental animals(1,2), there have been indications that the endotoxin-induced inhibitor (EII) and virus-induced interferon (VII) are not identical in physical properties and that the processes of their intracellular formation are different. Thus crude EII has been shown to be different from VII in being more labile to heat and acid(2), and the molecular weight of EII has been estimated to be considerably greater than that of VII(3).

In addition, it has been reported that the induction of interferon by viruses in cell cultures(4,5) and in rabbits(6) was found to be inhibited by actinomycin D and hence at least partially dependent on the transcriptive function of cell DNA. On the other hand, the induction of EII in rabbits was not found to be affected by actinomycin D (6). These results were interpreted to mean that endotoxin and virus induced the production of interferons in a different manner and that perhaps EII was largely preformed.

This paper reports further efforts to test this hypothesis. The production of EII and VII following inoculation of endotoxin and virus was measured in rabbits in which protein synthesis had been largely blocked by puromycin(7). It was thought that if the production of VII was inhibited by puromycin, as has been shown in cell cultures(8), whereas the production of EII was not inhibited, further evidence would be provided that EII is largely preformed, in contrast to VII.

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Materials and methods. Details on materials and methods used, unless hereafter specified, were as described elsewhere(6). Small albino rabbits weighing 320-470 g were used because of the cost of puromycin.† These were fasted for 24 hours prior to experimentation. Puromycin was dissolved in phosphate-buffered saline (PBS, pH = 7.2) and administered intraperitoneally (i.p.). Preliminary experiments showed that 13.3 mg of the drug per 100 g weight of the animal was the minimum dose necessary to maintain more than 95% inhibition of protein synthesis for at least 2 hours. Therefore, a total dose of 40 mg per 100 g of body weight divided into bihourly subdoses was used in experiments lasting 2.5 and 4.5 hours (*i.e.*, 2 injections of 20 mg per 100 g of body weight and 3 injections of 13.3 mg per 100 g of body weight, respectively). In the case of experiments lasting 7.5 hours, 13.3 mg per 100 g of rabbit was administered bihourly for as long as required (*i.e.*, 4 injections). To induce EII and VII, *E. coli* (0113) endotoxin‡ (0.5 μg in PBS) or Sindbis virus pellet suspended in PBS (about 10^9 p.f.u.) was injected intravenously (i.v.) 0.5 hour after the first injection of puromycin(6). To monitor protein synthesis, C^{14}-leucine,§ in dosages of 10 μc/100 g rabbit weight was injected i.v. 0.5 hour before sacrifice. Control animals received PBS rather than puromycin.

To measure EII or VII, serum samples were obtained by cardiac puncture. Serial 2-fold dilutions were made and 0.3 ml was

† Nutritional Biochemicals Corp.

‡ Provided through courtesy of Dr. A. I. Braude.

§ New England Nuclear Corp., uniformly labeled, specific activity > 200 mc/mM.