TABLE III. Failure of Adjuvants to Alter Minimum Inhibitory Concentration of Tetracycline against *E. coli* Strains in M-9 Medium.

Organism	Medium	μg/ml of adjuvant		MIC of tetracycline
E. coli (tetracycline-sensitive)	M-9			1.56
	"	l-Histidine,	10	1.56
E. coli (tetracycline-resistant)	" + 10 μg. ml l-histidine			12.5
Idem	*Idem*	Urocanic acid,	100	12.5
			10	12.5
			1	12.5
"	"	Histidinol,	100	12.5
"	"	Histidinal,	100	12.5
"	"	l-Histidine,	100	12 5
			200	12.5
			400	12.5
"	"	Imidazole,	100	12.5

for tetracycline as determined by the 2-fold serial dilution technic in brain heart infusion broth. All the 20 strains received were able to grow in the synthetic medium approximately as well as the parent laboratory strain. Therefore, none of these tetracycline-resistant strains, which were developed naturally *in vivo,* had a requirement for l-histidine.

Summary. 1. A tetracycline-resistant *E.* coli, which had been selected by a laboratory technic, was found to have a specific requirement for l-histidine. 2. Urocanic acid, imidazole and 5 analogs, l-histidinol, and l-histidinal were unable to substitute for l-histidine, nor were they able to antagonize the activity of tetracycline against the parent *E. coli* (tetracycline-sensitive) in synthetic medium. 3. Twenty *E. coli* strains, all tetracycline-resistant and isolated from clinical sources were obtained. None had the specific requirement for l-histidine.

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On the Mechanism of Decrease of Aldosterone Secretion in the Dog. (26044)

DONALD S. GANN, IVOR H. MILLS, JUAN F. CRUZ, ALFRED G. T. CASPER AND FREDERIC C. BARTTER

Section on Clinical Endocrinology, National Heart Institute, Bethesda, Md.

Previous experiments(1) have suggested that the integrity of the vagus nerves is necessary for the decrease in secretion of aldosterone following release of constriction of the thoracic inferior vena cava, but not for the increase in secretion of aldosterone following caval constriction. Further, vagotomy alone has no effect on aldosterone secretion in the otherwise intact animal. This suggests that the vagal pathway is strictly an inhibitory one, and that release of this inhibition does not result in increased secretion of aldosterone. Other experiments(2,3) have indicated that stretch of the right atrium results in diminished secretion of aldosterone. These experiments suggest a possible mechanism for activation of the vagal pathway.

However, several questions concerning the function of the vagus nerve in control of secretion of aldosterone have remained unan-

swered. First, it is possible that efferent rather than afferent fibers in the vagi may be important. Second, it appeared not unlikely that the vagal system, if it were an afferent pathway, might also be able to lead to increased secretion of aldosterone if other pathways ordinarily involved in increase in secretion of aldosterone were inactivated. Recently, it has been shown(4) that the integrity of the baroceptor nerves arising from thyro-carotid arterial junctions is necessary for the increase in aldosterone secretion which normally follows caval(1) or carotid(4) constriction. It was not clear whether vagotomy would lead to increased aldosterone secretion in a dog with thyrocarotid areas denervated. In the third place, it has been shown(5) that bilateral constriction of the common carotid arteries produces an increase in right atrial pulse pressure without changing atrial mean pressure. Caval constriction, on the other hand, produces a decrease in atrial mean and pulse pressures(6). Thus, release of carotid constriction should not provide a means of activating atrial receptors similar to that present following release of caval constriction. Consequently, if aldosterone secretion decreases following release of carotid constriction, it should do so more slowly than it does following release of caval constriction. The present experiments were designed to answer these questions.

Methods. Experiments were performed on healthy mongrel dogs anesthetized with pentobarbital. Adrenal venous blood was collected intermittently by the method of Hume and Nelson(7). All collections were of one half hour's duration. Adrenal venous aldosterone was measured by Mills' modification (1,4) of the method of Neher and Wettstein (8). This method was further modified in some experiments by substitution of tritium-labelled aldosterone for C^{14} cortisone as the tracer. This added the theoretical advantage that the tracer used for correction of losses ran with the unknown aldosterone in all 3 chromatographic systems, rather than only the first two. Caval constriction(1), vagotomy(1) and carotid constriction(4) were all performed as reported previously. All blood lost through collection or incidentally was replaced by homologous transfusion. Arterial, venous and right atrial pressures were measured and recorded continuously.

Four experiments were performed on intact dogs with inflatable cuffs around the thoracic inferior vena cava. Following a control collection of adrenal venous blood, the cuff was inflated, and a second collection begun after one half hour. Following this collection, the cuff was deflated and a third collection made after one hour. The cuff was then reinflated and a fourth collection was made after one half hour. At the end of the second collection, each dog was injected with 2 mg of atropine intravenously (external jugular) and 2 mg intramuscularly.

Four experiments were performed on dogs in which both thyro-carotid arterial junctions had been denervated one to 2 weeks previously. The vagi were isolated low in the neck before experiment was begun. Following a control collection of adrenal venous blood, both vagi were sectioned. Further collections of adrenal venous blood were obtained at intervals from 30 to 240 minutes following vagotomy.

Five experiments were performed on intact dogs with silk loops passed about both carotid arteries. The design was similar to that in the dogs with caval constriction, except that carotid rather than caval constriction was used, and no atropine was given.

Results. The results of caval constriction and release in dogs given large doses of atropine are shown in Fig. 1A and Table I. Release of caval constriction in presence of massive amounts of atropine resulted in decreases of aldosterone secretion (mean −5.53 μg/hr $\pm$ 1.68 SEM) not different from those seen in normal dogs (mean −5.14 $\pm$ 1.32) and in marked contrast to the effect of release of

TABLE I. Effect of Caval Constriction and Release on Aldosterone Secretion (μg/Hr) in Normal Dogs Given Atropine (2 mg i.v. and 2 mg i.m.) at End of Second Collection.

Control	Constriction	Release	Constriction
1.8	5.2	2.5	3.8
1.5	8.2	2.3	6.2
14.0	18.0	8.6	3.4
5.0	11.4	7.3	12.0

Effect of release: mean fall 5.53 $\pm$ 1.68 S.E.M.

TABLE II. Effect of Vagotomy on Aldosterone Secretion (μg/Hr) in Dogs with Previous Denervation of the Thyro-carotid Arterial Junctions. Vagotomy performed at end of first collection.

	Time after section (min.)			
Control	30-60	120-150	180-210	210-240
1.5	1.0	1.1	1.6	
1.2	.6	1.4	1.4	
<1.4*	<1.4	<1.4	1.5	
4.5	4.6	4.1	—	<3.8

* When < is shown, no aldosterone was detected in the sample. The figure shown is calculated from sensitivity of the method, recovery rate, and dilution of sample. See ref.(4).

caval constriction in vagotomized dogs (mean -0.79 ± 1.86)(1). It is evident that the effect of vagotomy is not reproduced by atropine. Atropinization also did not prevent the rise in aldosterone secretion following caval constriction in 3 of the 4 dogs. The situation was complicated in the fourth dog by the onset of atrial fibrillation following injection of atropine, and reversion to normal rhythm with the second caval constriction. Thus, atrial pressure rose markedly in this animal after caval constriction.

The results of vagotomy in dogs with bilateral denervation of the thyro-carotid arterial junctions are shown in Fig. 1B and Table II. As is the case with intact dogs(1), vagotomy produces no change in aldosterone secretion.

The results of release of carotid constriction in normal dogs are shown in Table III and summarized and compared with the results of release of caval constriction in Fig. 2. The effect of carotid release is significantly less than that of caval release.

Discussion. These results suggest that the mechanism leading to decreases in aldosterone secretion mediated by the vagus nerves operates over afferent pathways and is exclusively

TABLE III. Effect of Release of Carotid Constriction on Aldosterone Secretion (μg/Hr) in Normal Dogs. Constriction had been on for one hr, and all dogs showed increase in aldosterone secretion above control levels.

Constriction	Release	Δ
11.9	10.8	−1.1
9.9	10.5	+ .6
8.6	6.9	−1.7
20.4	17.8	2.6
17.0	14.0	−3.0

Mean = $-1.56 \pm .72$ (S.E.M.) $t = 2.19$ $p > .05$

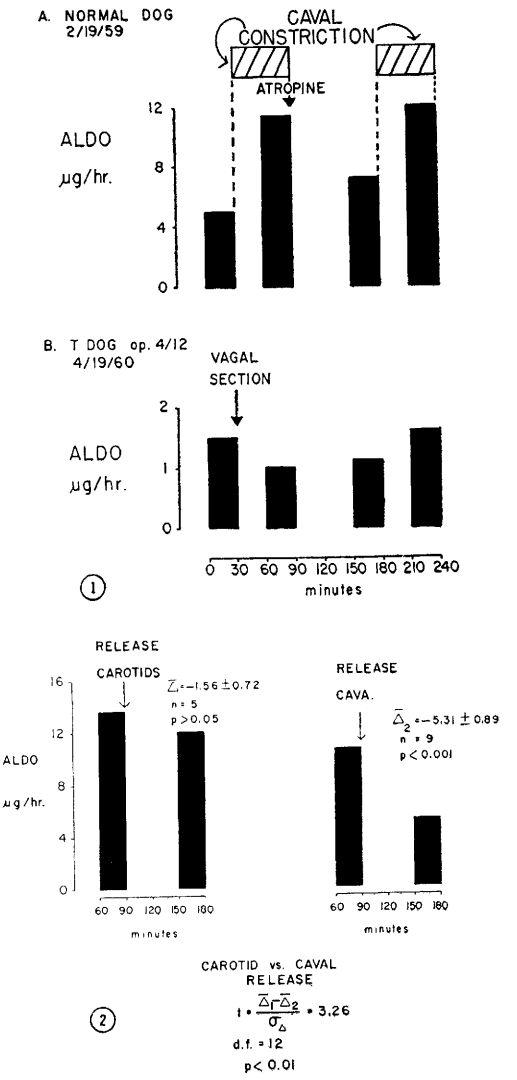

FIG. 1. A. Effect of caval release and subsequent caval constriction on aldosterone secretion in an atropinized dog. B. Effect of section of vagus nerves low in the neck upon aldosterone secretion in a dog one wk after thyrocarotid arterial junction denervation.

FIG. 2. Summary of experiments comparing aldosterone secretion after release of carotid arterial constriction with that following release of inferior vena cava constriction during acute experiments in the dog.

inhibitory in function. This situation, in which a reflex mechanism serves only to inhibit while a release of inhibition is not sufficient to stimulate, is unusual but is not unique in physiology. Alexander(9) has demonstrated a similar reflex, also utilizing afferent vagal pathways, in which venous tone

is diminished by increasing central venous pressure, but a fall in this pressure does not produce increased venous tone. This latter effect seems to be predominantly under the control of arterial baroceptors(10). The analogy between regulation of venous tone and that of aldosterone secretion is striking. It is of interest that an increase in secretion of aldosterone or in venous tone has the ultimate physiological effect of increasing the volume of blood available to the heart for perfusion of the arterial tree, and that both may be increased(11,12) in congestive heart failure. The difference between results of release of caval constriction, on the one hand, and of release of carotid constriction on the other, correlates with the different effects upon atrial pressure. This is consistent with the concept (1,2,3) that the vagal pathway is activated by atrial stretch, but does not exclude some other receptor area. Further support is provided by the fact that in dogs with prior denervation of the thyro-carotid arterial junctions, caval constriction produces no change, but carotid constriction produces a fall in aldosterone secretion(4). Thus, in the absence of the nerves arising from the thyro-carotid arterial junction, carotid constriction, which increases atrial pulse pressure(5) may activate the vagal mechanism. However, this evidence is not adequate to identify the hemodynamic parameter mediating decrease in aldosterone secretion or to distinguish whether type A or B atrial receptors may be involved(13).

Summary. Experiments were done in dogs to elucidate the mechanism by which the vagus nerves may influence aldosterone secretion. The results suggest that the vagus nerves form the afferent limb of a reflex activated by atrial stretch which may exert only an inhibitory effect upon aldosterone secretion. The role of efferent impulses was eliminated by atropinization, which did not prevent the fall of aldosterone secretion which follows caval release. The role of atrial stretch was suggested by the observation that caval release (which increased atrial pressure) lowered aldosterone secretion more than carotid release (which did not). The purely inhibitory role of the vagus was suggested by the finding that vagotomy did not stimulate aldosterone secretion even after denervation of the thyro-carotid arterial junctions.

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