Localization of the Cardiac Sympathetic Synapses in the Dog* (33872)

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Recently we reported (1) that in the dog the cardiac effects produced by angiotensin were caused by stimulation of ganglia in the vicinity of the caudal cervical ganglia. Since it is generally assumed that the stellate ganglia and not the caudal cervical ganglia are the principal cardiac sympathetic ganglia, we devised experiments to verify our contention and to locate more precisely the major synapse of the sympathetic fibers in the dog that affect heart rate and right ventricular contractile force. These experiments consisted of stimulation of the sympathetic fibers at several sites along their pathway from the thoracic sympathetic trunk to a location near the heart, before and after complete ganglionic blackade.

Since Flacke and Gillis (2), and Trendelenburg (3), showed that nicotinic *and* muscarinic blocking agents are necessary to completely block ganglionic transmission, we also tested the effects of hexamethonium and atropine, alone or together, on ganglionic transmission in the intact open-chest dog preparation.

Methods. We conducted open-chest experiments in 14 dogs weighing 5–20 kg anesthetized with 30 mg/kg of pentobarbital sodium. Contractile force was measured with a Walton strain-gauge arch sutured to the anterior wall of the right ventricle. We stretched the muscle between the feet of the arch to such an extent that the greatest active tension was recorded. Heart rate was recorded by tachography and blood pressure was recorded with a Statham pressure transducer.

In all experiments, the thoracic sympathetic trunks, stellate ganglia, ansae subclavia, caudal cervical ganglia and the nerve plexus just distal to the caudal cervical ganglia were dissected. Both vagi were cut in the neck and all fibers, sympathetic and other, entering the stellate ganglia were cut centrally. Shielded platinum electrodes were placed around the sympathetic trunk indicated in Fig. 1 as prestellate Site 1, the ansa subclavia just distal to the stellate ganglion, but proximal to the caudal cervical ganglion, Site 2, and the nerve trunks of the cardiac plexus, postcaudal cervical Site 3. "R" and "L" in the paper refer to the nerves of the right and left side.

The nerves were stimulated with shocks of supramaximal voltage which was determined in each experiment (usually 12–15 V), 2 msec duration and a frequency between 10 and 20 cps. Mineral oil was placed around the electrodes in order to reduce the spread of current to the surrounding structures. The drugs used in this study were hexamethonium bromide and atropine sulfate.

Results. I. The use of hexamethonium and atropine to produce ganglionic blockade. In order to test whether both a nicotinic blocking agent and an anti-muscarinic agent are required for complete ganglionic blockade, we injected in 3 dogs hexamethonium bromide first and then atropine sulfate and in 2 dogs atropine first, then hexamethonium. The stimulations were done either in R1 or R2. In the experiments (Fig. 2, left side) in which we gave 20 mg/kg of hexamethonium first, the ganglionic transmission of the heart rate response produced by sympathetic stimulation of the right side was decreased by about 65%. Addition of 1 mg/kg of atropine abolished the response in 5 out of 6 experiments and effected full ganglionic blockade. The increase in contractile force produced by sympathetic stimulation was also decreased by more than 70% after injection of hexamethonium, and this response was abolished after the addition of atropine. In the second set of experiments (Fig. 2, right side) we gave the same dose of atropine first without much

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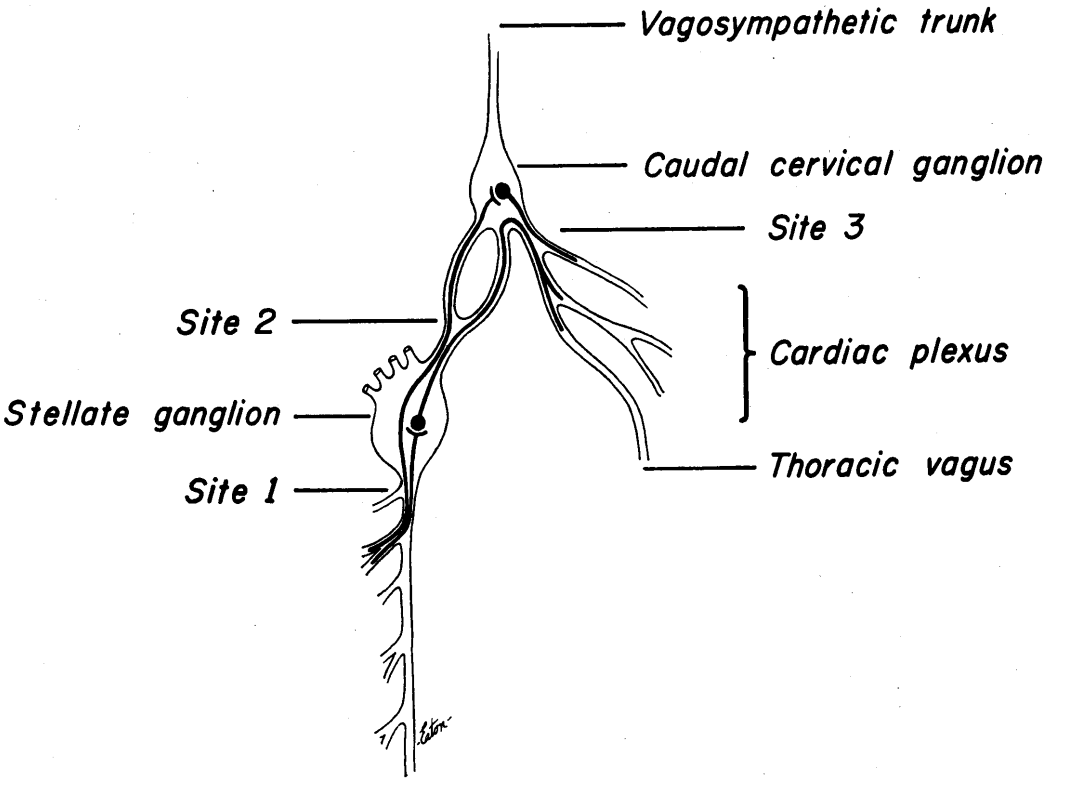

FIG. 1. Diagram of autonomic innervation of the dog heart (right side) showing sites of stimulation.

effects. However the subsequent injection of 20 mg/kg of hexamethonium now produced complete ganglionic blockade, *i.e.*, the increase in heart rate was now completely abolished. The contractile force response followed a similar course being minimally affected with injection of atropine alone, but becoming abolished after the addition of hexamethonium. Similar results were obtained on the left side in our other experiments.

II. Localization of cardiac sympathetic ganglia. In 14 dogs control stimulation of the cardiac sympathetic nerves at Sites 1 and 2 were performed, 10 on the right side, 4 on the left side. This produced an increase in heart rate (Table I) and right ventricular contractile force. Right-sided stimulation increased heart rate more than stimulation of the left. Ganglionic blockade was then induced by intravenous injection of hexamethonium, 20 mg/kg, plus atropine, 1 mg/kg. After stabilization, Sites 1 and 2 were restimulated and the responses were now absent or markedly depressed (Table I). At this time in 6 dogs stimulation of Site 3 (postganglionic site) still produced the same response

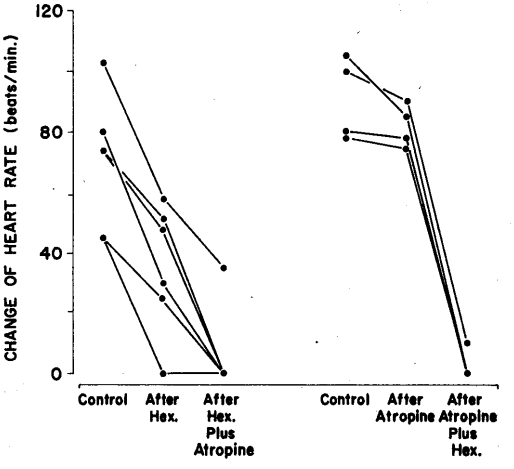

FIG. 2. Heart rate response to stimulation of R1 or R2 before and after giving either hexamethonium first and then atropine (left side of graph) or atropine first and then hexamethonium (right side of graph).

TABLE I. Effect of Sympathetic Nerve Stimulation on Heart Rate before and after Ganglionic Blockade.[a]

Site stimulated	Before ganglionic blockade			After ganglionic blockade		
	Heart rate (beats/min)		Amount increased	Heart rate (beats/min)		Amount increased
	Basal	Maximal		Basal	Maximal	
R1	141 ± 6.3 (10)	220 ± 7.3 (10)	79	135 ± 6.3 (10)	136 ± 6.4 (10)	1
R2	146 ± 7.1 (10)	225 ± 9.1 (10)	79	136 ± 7.1 (10)	153 ±11.9 (10)	17
R3	145 (1)	205 (1)	60	129 ± 7.5 (6)	206 ± 5.9 (6)	77
L1	140 ± 9.8 (4)	181 ±15.9 (4)	41	126 ± 9.3 (4)	129 ± 9.3 (4)	0
L2	140 ± 9.8 (4)	187 ±14.2 (4)	47	128 ± 8.3 (4)	128 ± 8.0 (4)	0
L3	144 (2)	202 (2)	58	127 ± 9.2 (4)	187 ±23 (4)	60

[a] Values represent the mean ± the SE; number of dogs given in parentheses.

as before ganglionic blockade. On the left side of Table I under R3 and L3 (before ganglionic blockade) we list responses to postganglionic stimulation after administration of atropine. We did not routinely stimulate R3 and L3 before ganglionic blockade as these fibers are composed of both sympathetic and parasympathetic fibers and the stimulation of these without blockade of parasympathetic pathways would show consistent bradycardia and negative inotropic effects.

The response of right ventricular contractile force to stimulation of Sites 1, 2, and 3 was observed before and after ganglionic blockade. After blockade this response was markedly depressed during Site 1 and 2 stimulation, but it was still present during Site stimulation when the ganglionic transmission was completely blocked. From these results, we conclude that the major ganglionic junction for heart rate and right ventricular contractile force is located between Sites 2 and 3.

Discussion. We observed in these experiments, conducted in intact and open-chest dog preparations, that complete ganglionic blockade required a nicotinic and a muscarinic blocking agent. Hexamethonium alone (a nicotinic blocking agent) did reduce, but did not abolish ganglionic transmission. The addition of atropine (an antimuscarinic agent) was required to affect complete blockade. On the other hand, atropine alone had little effect but the addition of hexamethonium now caused complete blockade. These findings are in keeping with those of Flacke and Gillis (2), who reported that there are muscarinic receptors involved in ganglionic transmission. These receptors only become obvious after previous administration of a nicotinic blocking agent (2). Functional nicotinic receptors prevent the observation of the effects of muscarinic blocking agents on ganglionic transmission.

We confirmed our previous deduction (1) that the major site of cardiac sympathetic synapse in the dog is not located in the stellate ganglia. After complete ganglionic blockade the response to stimulation of Site 3 (which is distal to the caudal cervical ganglia) is not influenced at all. Therefore, we conclude that in the dog, the major cardiac sympathetic junction affecting heart rate and right ventricular contractile force is between the ansa subclavia and the post ganglionic fibers to the heart (Sites 2 and 3), namely in the area of the caudal cervical ganglia. It is of interest that Aiken and Reit (4) recently showed that the cardiac sympathetic junctions in the cat are indeed located in the stellate ganglia. We must, therefore, consider a species difference with respect to the cardiac sympathetic ganglia. There is no information as yet what pattern of sympathetic ganglionic transmission man follows, but there is no reason to accept without challenge the stellate ganglia as the cardiac sympathetic ganglia in man.

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Biochemical Characterization of the Antimicrobial Histone Released by Deoxyribonuclease and Lactic Acid* (33873)

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In 1965 Miller and Watson (1) proposed a model which may be determinative in the host–parasite interaction. It was shown that intact nuclei or deoxyribonucleohistone complexes (DNH) manifested little or no bactericidal activity, but the addition of deoxyribonuclease (DNase) liberated cationic bactericidal histones. The DNase contained in the lysosomes of the inflammatory leukocytes or paradoxically, the DNase produced by various pathogenic bacteria (2), may effectively liberate this bactericidal histone in an inflammatory area. Also, since lactic acid is produced by the glycolytic metabolism of the inflammatory cells (3), this lactic acid may also effectively liberate a bactericidal histone from the nucleus. The present paper describes the characterization of the type of histones released from DNH by treatment with DNase or lactic acid.

Materials and Methods. DNase I was obtained from Worthington Biochemical Corporation, Freehold, N.J. and DNH was isolated from calf thymus according to the method of Crampton *et al.* (4). The histones were extracted from the DNH by diluting it with a more concentrated acid solution until the desired acid concentration was achieved. The acidic solution was then stirred at 4° for 1 hr and centrifuged at 2000*g* for 10 min. The supernatant fluid was then dialyzed for 24 hr, pervaporated and lyophilized. Three acids were used for the extractions: 0.2 *N* HCl was used for the extraction of the "reference" total histones (5); 5% trichloracetic acid (TCA) was used to extract the "reference" lysine-rich histone (6), and 5% lactic acid (LA) was used experimentally to determine what type of histone might be released.

To release the histones, DNase at a concentration of 10 μg/ml was used to degrade the DNH during a 1-hr incubation at 37°. The undegraded DNH was precipitated out at 4° by making the solutions 0.85% with NaCl. The supernatant fraction from a 2000*g*, 10-min centrifugation was then dialyzed to remove the soluble nucleotides and then lyophilized. The precipitated DNH was then re-extracted with 5% TCA to remove the lysine-rich histones. DNase was omitted from the control tube.

For amino acid analysis, the proteins were hydrolyzed for 24 hr in sealed evacuated then lyophilized. The precipitated DNH was removed, *in vacuo*, in a desiccator over a desiccant and NaOH pellets. A Beckman model 120 B amino acid analyzer was then used for the analyses.

The electrophoretic separations were made in the E-C vertical acrylamide gel electrophoresis cell. A 5% concentration of acrylamide gel was prepared with 0.1 *M* acetate buffer at pH 4.2. Twenty μl of a 5% solution

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