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Short Communication

The physiological and anatomical demonstration of functionally selective parasympathetic ganglia located in discrete fat pads on the feline myocardium

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Abstract

Experiments utilizing surgical parasympathectomy of discrete fat pad ganglia on the surface of the heart have suggested that there are two anatomically segregated and physiologically independent parasympathetic intracardiac ganglia which are capable of selective control of sino-atrial (SA) rate and atrio-ventricular (AV) conduction. Some pharmacological data, however, are inconsistent with these conclusions. We have examined the cardiodynamic effects of discrete injections of a ganglionic blocking drug into two fat pads on the surface of the cat heart. These fat pads were shown to contain ganglion cells histologically. It was observed that vagal effects upon cardiac rate are selectively mediated by neurons located in ganglia overlying the right pulmonary veins at the junction of the right atrium and superior vena cava. On the other hand, vagal effects upon AV conduction were selectively mediated by neurons located in a fat pad at the junction of the inferior vena cava and the inferior left atrium. These pharmacological data support the concept that specific intracardiac ganglia are capable of selective control of SA rate and AV conduction.

Keywords: Parasympathetic ganglion; Fat pad; Myocardium; (Cat)

It has been known since the turn of the century that activation of the parasympathetic input to the heart by stimulation of the vagus nerves results in bradycardia, AV block and reduced myocardial contractility [2,12]. During the past decade, Randall and collaborators have intensively studied the physiological effects of selective parasympathectomy on the heart [1,5–10]. Their data indicate that there are anatomically segregated and physiologically independent parasympathetic ganglia on the surface of the heart which are capable of selective control of sino-atrial (SA) rate, atrio-ventricular (AV) conduction, and atrial contractility. They have proposed that vagal effects upon cardiac rate are mediated by a group of ganglia located in a fat pad overlying the right pulmonary veins at the junction of the right atrium

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and superior vena cava (the RPV fat pad ganglion). Surgical excision [7–10] of the RPV fat pad was shown to block selectively the negative chronotropic effect of vagal stimulation without influencing AV conduction or atrial contractility. Conversely, when ganglia located at the junction of the inferior vena cava and the inferior left atrium were excised (the IVC-ILA fat pad ganglion), the negative dromotropic effects of vagal stimulation were selectively blocked without influencing the negative chronotropic or inotropic effects of vagal stimulation. It was concluded from these data that the RPV fat pad ganglion is the major source of post-ganglionic neurons innervating the SA node, while the IVC-ILA fat pad ganglion is the major source of post-ganglionic neurons innervating the AV node.

Randall's observations have also been confirmed in primates [1], and by an independent group not associated with Randall in the dog [11]. On the other hand, Fee et al. [3] observed a statistically significant attenuation in AV nodal responses when the drugs hexamethonium or lidocaine were injected into the RPV fat pad. Furthermore, they noted comparably diminished SA nodal responses when these drugs were injected into the IVC-ILA fat pad. The effects of pharmacological blockade, therefore, do not support the concept that these ganglia are physiologically independent and exert selective control over SA rate and AV conduction. We were surprised by this apparent discrepancy. Therefore, we have reexamined this issue in the present report. A preliminary account of this report has been published elsewhere [4].

Six adult cats (2.5–4.0 kg) were anesthetized with pentobarbital (30 mg/kg, i.v.). The animal was intubated, and a thoracotomy was performed via the right fifth intercostal space. The cat was artificially respired at this point with room air. Body temperature was maintained with heating pads. The pericardium was incised. Limb leads were attached to record lead II of the electrocardiogram (ECG). HR was obtained from the R-R interval, and A-V conduction was obtained from the P-R interval when the heart was not paced. A pacing wire was sewn onto the right atrial appendage, and another wire was sewn onto the left ventricle to obtain the ventricular electrogram. When the heart was paced, AV conduction was obtained from the ventricular electrogram, because pacing obscured the measurement of the P-R interval. Slowing of the ventricular rate while pacing the heart is an indicator of diminished AV conduction. Subsequently, the right cervical vagus nerve was isolated, placed on stimulating electrodes, and bathed in mineral oil. The vagus was stimulated at 10–20 Hz, 1.0 ms duration, for 15-s intervals using a stimulus isolation unit. Stimulus parameters were varied until HR was reduced approximately by half. These parameters remained constant for the remainder of each experiment. Subsequently, the vagus nerve was stimulated 3 times, allowing 1–2 min between cycles of stimulation, to determine control values of bradycardia and AV blockade. The RPV fat pad was then identified, and trimethaphan, a ganglionic blocker with a short half-life (5 μl/10 μl) was injected into it using a 10-μl Hamilton syringe. The vagus was restimulated 5 min later, and drug induced changes in vagal effects were noted. After 60–90 min the vagus was again stimulated, and it was observed that responses had returned to control levels. The right atrium was then paced at 10% above sinus rate and the vagus was restimulated in order to obtain control values for AV blockade. The IVC-ILA fat pad was then identified visually by its relationship to defining anatomical landmarks and trimethaphan (50–100 μg/10–20 μl) was injected into this fat pad. Since the IVC-ILA fat pad is larger than the RPV fat pad, a larger volume of trimethaphan was required to achieve ganglionic blockade. The vagus was then restimulated and drug induced changes in vagal effects were again noted. Pacing was then discontinued and the vagus restimulated in order to assess whether ganglionic blockade of the IVC-ILA fat pad ganglion influenced SA rate. Data are reported as the mean $\pm$ SEM. Statistics include the paired Student's t-test using a criterion of $P < 0.05$ for statistical significance. Three adult cats (2–4 kg) were used in the histological analysis of the intracardiac ganglia. The animals were deeply anesthetized with pentobarbital (35 mg/kg, i.v.). The animals were perfused sequentially via a cannula inserted retrogradely in

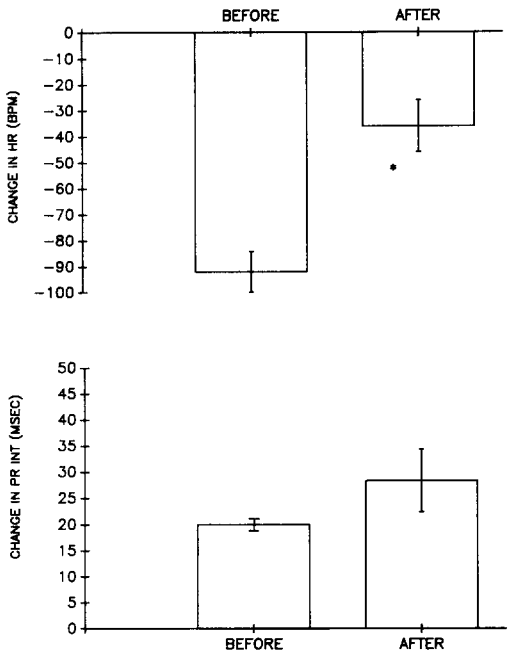

Fig. 1. (Top) The effect of vagal stimulation on heart rate (HR) before and after injection of trimethaphan (5 μg/10 μl) into the RPV fat pad ($n = 5$). Trimethaphan significantly blocked the vagally-induced bradycardia (* $P < 0.05$). (Bottom) The effect of vagal stimulation on P-R interval (P-R int) before and after injection of trimethaphan (5 μg/10 μl) into the RPV fat pad in these same animals. Trimethaphan did not affect the vagally-induced decrease in AV conduction.

the abdominal aorta with (1) 1 liter of 0.1 M phosphate-buffered saline (pH 7.4) containing heparin sulfate (2500 Units) and (2) 3 liters of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The hearts were removed, postfixed in the same fixative for 1 h and then transferred to 20% sucrose in phosphate buffer for 48 h. Blocks of cardiac tissue including the RPV and IVC-ILA fat pads were removed, embedded in O.C.T. compound and cut in a cryostat into serial 16-μm sections. The sections were mounted onto gelatinized slides, stained with hematoxylin and eosin, dehydrated with alcohols, cleared in xylene and cover slipped with Permount. Tissues were examined in a Nikon FXA photomicroscope.

During control intervals, right cervical vagal stimulation decreased SA rate by 92 ± 8 bpm and increased the P-R interval by 20 ms (Fig. 1). These effects could be repeated every minute with very little variation. After injection of trimethaphan into the RPV fat pad ganglion, there was a significant blockade of vagally induced bradycardia. However, trimethaphan did not effect the vagally induced decrease in AV conduction. 90 min following the injection, control responses to vagal stimulation were again observed.

In 4 cats vagal stimulation decreased HR by 97 ± 8 bpm prior to pacing the right atrium. While pacing, vagal stimulation decreased the ventricular rate by 79 ± 22 bpm, indicating that A-V block was produced by vagal stimulation (Fig. 2).

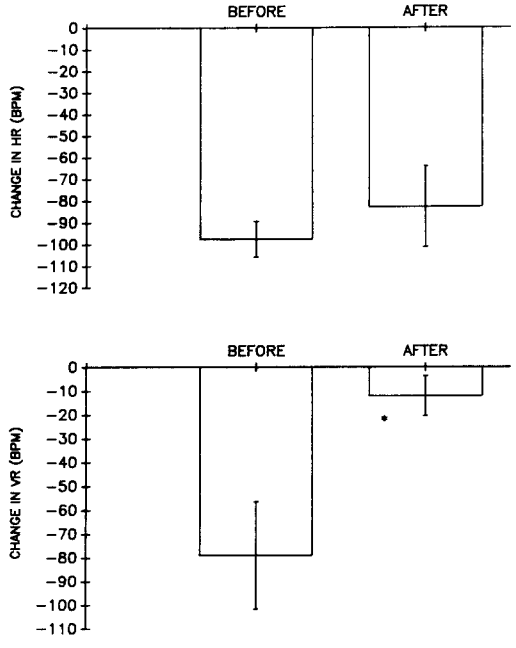

Fig. 2. (Top) The effect of vagal stimulation on SA rate (HR) before and after injection of trimethaphan (50–100 μg/10–20 μl) into the IVC-ILA fat pad ($n = 4$). Trimethaphan did not significantly affect the vagally-induced bradycardia. Bottom: The effect of vagal stimulation on AV conduction (VR) before and after injection of trimethaphan (10–20 μg/10–20 μl) into the IVC-ILA fat pad in these same animals. Trimethaphan blocked the vagally-induced decrease in conduction through the A-V node (* $P < 0.05$) but did not effect SA rate. Animals were paced at 10% above resting heart rate.

After injections of trimethaphan into the IVC-ILA fat pad, trimethaphan blocked the vagally-induced decrease in conduction through the AV node, but did not significantly affect vagally induced bradycardia. Histological examination of the atrial injection sites revealed the presence of multiple ganglia (Fig. 3). Both the RPV and IVC-ILA fat pads contained several groups of loosely organized but large (30–50 μm) basophilic neurons which were embedded within adipose tissue on the surface of the heart.

In the present study we have observed the presence of ganglia (the RPV and IVC-ILA fat pad ganglia) in the cat heart in injection sites which are analagous to those described in dog and monkey hearts [1,8,9]. The results of our pharmacological experiments in the cat are in agreement with the conclusions of studies which have examined the physiological effects of surgical parasympathectomy on the heart [1,5–10] in other species. That is, we have observed that vagal effects upon cardiac rate are selectively

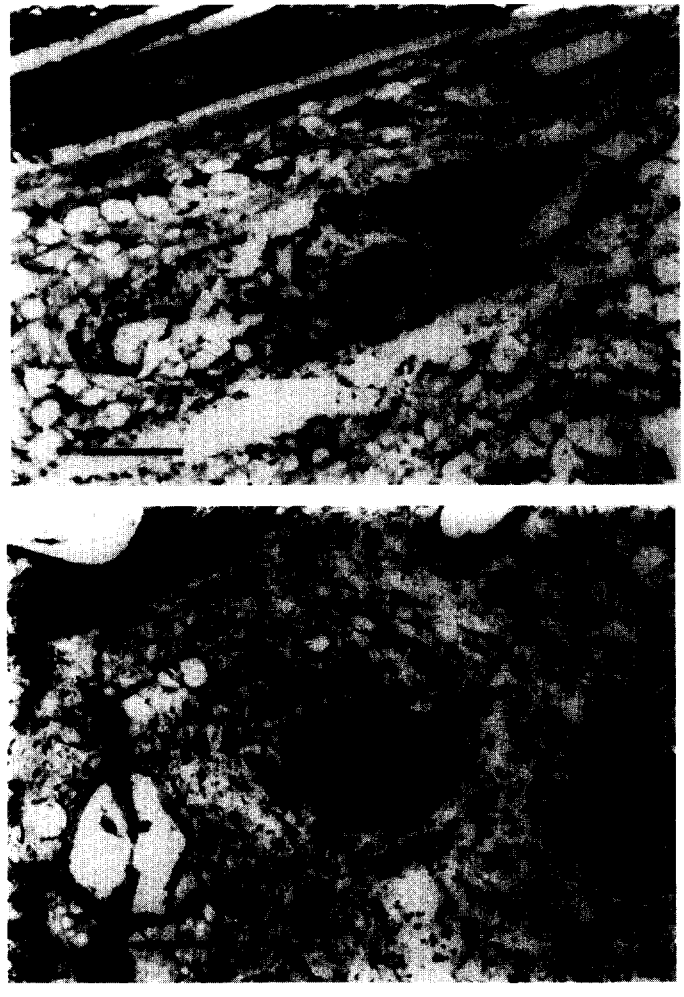

Fig. 3. (A) The RPV fat pad ganglion. (B) The IVC-ILA fat pad ganglion. Note the presence of loosely organized, but fairly large groups of basophilic neurons (arrows) which are embedded within the fat pads on the surface of the atria. CM, cardiac muscle; FP, fat pad. Original magnification 125 ×. Bar = 150 μm.

mediated by neurons located in the RPV fat pad. On the other hand, vagal effects upon AV conduction are selectively mediated by neurons located in the IVC-ILA fat pad. The degree of physiological selectivity of the RPV and IVC-ILA ganglia may have to be reevaluated in the future, as more data become available on the regional effects of individual intracardiac ganglia upon various parameters of cardiac performance.

Fee et al. [3], in the dog, observed a statistically significant attenuation in AV nodal responses when the ganglionic blocking drug hexamethonium was injected into the RPV fat pad. Furthermore, they noted comparably diminished SA nodal responses when hexamethonium was injected into the IVC-ILA fat pad. These data suggested the possibility that these two ganglia were not physiologically selective, but these pharmacological data were at odds with the results of numerous studies using a surgical (as opposed to pharmacological) approach to inducing ganglionic blockade. The present pharmacological data in the cat, however, do not agree with the results of Fee et al. [3]. We believe that the observation of non-selective effects by these authors may represent: (1) the diffusion of relatively large volumes of injected drugs outside of the individual ganglia being examined, or (2) systemic effects of injected hexamethonium following diffusion into the general circulation. In the present experiments, injection volumes were 10–20-times smaller than those used by Fee et al. [3] and we saw no indication of non-selective cardiac effects.

Acknowledgements

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