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Electrophysiological Investigation of Sympathetic Cardiac Afferent Fibers (40830)[1]

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Afferent nerve activity of cardiac origin has been recorded from canine thoracic sympathetic nerves, including the close cardiac nerves and the upper thoracic white rami communicantes (3, 9, 15). These nerve recordings have indicated two types of afferent fibers. Most recordings have been obtained from myelinated Aδ fibers, with conduction velocities between 2 and 25 m/sec, while fewer potentials have been obtained from smaller, nonmyelinated C fibers, with conduction velocities below 2 m/sec. In contrast, an ultrastructural study, which employed Wallerian degeneration of sympathetic afferent fibers initiated by dorsal root ganglionectomy, has found that the majority of afferent fibers were really the C fibers, with a smaller population of Aδ fibers (14). The discrepancy over relative fiber numbers between recorded and anatomical findings may be due to the technical difficulties in obtaining C fiber recordings, or to the lack of adequate stimulation of the C fibers.

To resolve the differences in results between recorded and anatomical studies, this study was performed to electrophysiologically determine the relative percentages of afferent Aδ and C fibers in three close cardiac nerves—the ventrolateral (VLCN), ventromedial (VMCN), and stellate (SCN) cardiac nerves. Systematic stimulation of each nerve to evoke cardiopulmonary afferent fibers was employed to semiquantitatively determine the relative number of fibers of each type entering the upper thoracic spinal cord.

Methods. To activate the cardiac sympathetic afferent fibers, the close cardiac nerves were each individually stimulated distal to the stellate ganglion. Evoked activity was recorded from either the T_3 white ramus or the sympathetic chain below T_3. Stimulation distal to the stellate ganglion excited both sympathetic and parasympathetic (vagal) afferent fibers and efferent sympathetic postganglionic fibers, but placement of the recording electrodes central to the stellate ganglion restricted recording to afferent sympathetic fibers only. The presence of the stellate ganglion between the stimulating and recording electrodes eliminated recording retrograde excitation of sympathetic preganglionic fibers. While the possibility of long preganglionic fibers that extend beyond the stellate ganglion exists, the number of these are not thought to be of a sufficient amount to alter the results of the study (4).

Ten mongrel dogs were anesthetized with sodium pentobarbital (35 mg/kg), intubated with a cuffed endotracheal tube, and placed on positive pressure ventilation (60% O_2, 40% room air). The left second through fifth ribs and adjoining sternum were removed to expose the left thoracic nerves, stellate ganglion and sympathetic chain. The left close cardiac nerves—the ventrolateral (VLCN), ventromedial (VMCN), and stellate (SCN) cardiac nerves (Fig. 1)—were isolated from the surrounding connective tissue and sectioned near the heart. Their central ends were then placed in a nerve tank, covered with warm mineral oil and desheathed to facilitate stimulation. The left sympathetic chain was isolated and sectioned at T_4. The left T_3 white ramus communcans was also isolated and sectioned at its junction with the spinal nerve, and along with adjoining chain, was drawn into a nerve tank and covered with warm mineral oil. The T_3 white ramus and chain were used as the recording sites for the T_1 and T_2 white rami were generally too short to permit their use. The T_3 white ramus was thought to be a representative site, for other

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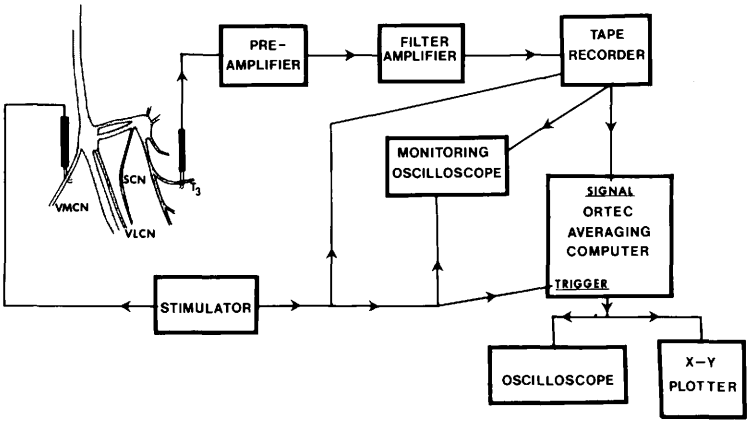

FIG. 1. Diagram showing nerves of the left cardiac region of the dog and the experimental design for stimulating and recording evoked activity from the sympathetic afferent fibers of the close cardiac nerves. The afferent fiber populations of the ventromedial (VMCN), ventrolateral (VLCN), and left stellate (SCN) cardiac nerves were evoked by electrical stimulation close to the heart, while the evoked potentials were recorded from the T_3 white ramus (T_3) or the sympathetic chain below T_3. Nerve activity was entered via a high gain preamplifier–amplifier system into a computer of averaged transients, which reproduced the average nerve tracing on an $X-Y$ plotter.

studies have indicated that almost 50% of the sympathetic afferent fibers enter the cord through this white ramus.

The chain and ramus were desheathed and dissected into small bundles of fibers to facilitate recording. As indicated in Fig. 1, each slip of chain or ramus was then tested for afferent activity during sequential stimulation of the close cardiac nerves at 3 Hz, 0.5 msec duration, and 9–11 mA, parameters sufficient to excite both Aδ and C fibers. The low frequency was chosen to permit sufficient time for complete recording of the relatively slow C fiber activity prior to the next stimulus. The nerves were stimulated via tungsten carbide electrodes connected to an isolated output, constant current stimulator. Nerve recordings were made by sequentially placing the small bundles of chain or ramus fibers on tungsten carbide electrodes connected via a high gain preamplifier-filter amplifier system to a Tandberg FM tape recorder and Ortec computer of averaged transients. The stimulator was used to trigger both the averaging computer and the monitoring oscilloscope. The result of 128 computer-averaged nerve traces for each stimulation procedure was printed out by an $X-Y$ plotter and used to characterize the fiber populations of each stimulated nerve. Following sacrifice of the animal with intravenous saturated KCl, the distance between stimulating and recording electrodes was measured and divided by conduction time to the initial deflections of the recorded potentials in order to calculate conduction velocity for each evoked potential. The average distance between electrodes ranged from 15 to 20 cm. Depending on the polarity of the recording electrodes, the initial deflections of the evoked potentials were in either a positive or negative direction.

All potentials obtained in this study could be ascribed to either Aδ fibers or C fibers, based on conduction velocities ($C < 2$ m/sec, $A\delta > 2$ m/sec). The actual number of individual fibers could not be determined by examination of the large recorded evoked potentials. Each large deflection, although ascribed to a single conduction velocity, probably consisted of a grouping of potentials from a number of Aδ or C fibers with similar conduction velocities. Therefore, in the discussion of the results for this study, each large recorded evoked potential should be considered as representing a group of individual evoked fibers. Only the large deflections that were consistently present following several series of

128 averages were considered to be actual evoked potentials. Not every small bundle of rami or chain examined yielded evoked potentials. In general, only 60% or less of the multifiber preparations contained evoked potentials.

Results. All close cardiac nerves examined contained both Aδ and C afferent fibers, but the numbers of potentials for each type obtained varied from nerve to nerve. The presence of a left SCN was variable, for it was found in only 40% of the dogs examined. From the results explained below, it appears that the SCN is preferentially incorporated in the VLCN of the other 60% of the dogs. Therefore, the presence of this nerve appeared to dictate the distribution of the afferent fibers to the VLCN, but not to the VMCN.

Stimulation of the VLCN in dogs that lacked a SCN produced a large range of potentials, varying from 17.8 to 0.55 m/sec (Fig. 2). Most potential groupings were obtained from the T_3 white ramus, with few found in the sympathetic chain below that level (Fig. 2). The majority of fibers in these two nerves, based on the evoked potential obtained, had conduction velocities less than 2 m/sec, indicating a large C fiber population (Table I).

While absolute numbers of Aδ and C fibers were not determined in this study, the work of Erlanger and Gasser (6) indicates that a proportional relationship exists between the areas of evoked potential components and the number of nerve fibers that contribute to them. Based on this assumption, the preponderance of evoked potential groupings with conduction velocities below 2 m/sec indicate the large C fiber population.

Stimulation of the VMCN in the dogs that lacked a SCN produced similar distributions of potentials from dog to dog. Again, a mixture of Aδ (10 groupings) and C (32 groupings) fiber potential groupings were obtained (Table I). The tracing seen in Fig. 3, with a high percentage of C fibers, was

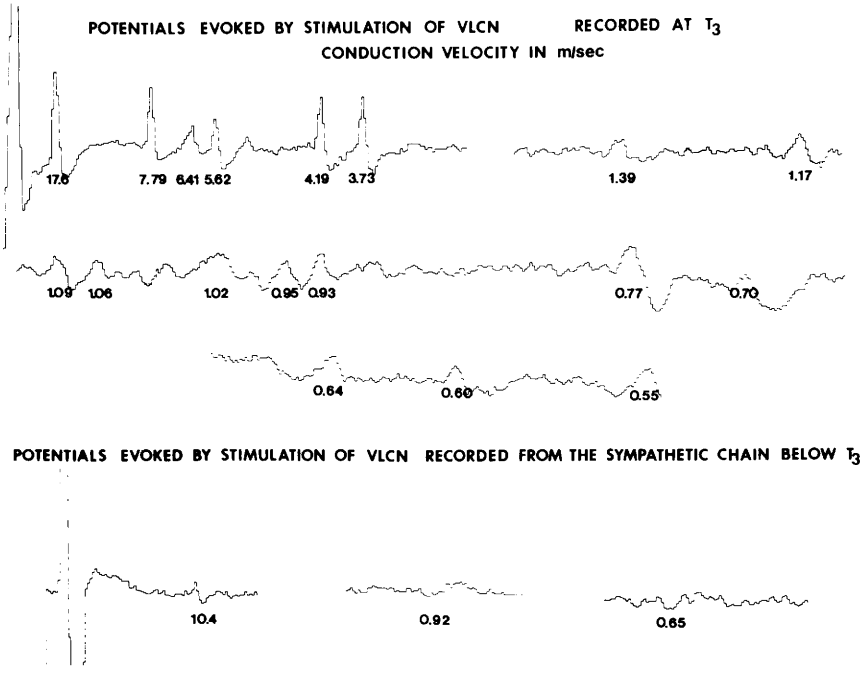

FIG. 2. Averaged nerve tracing of afferent evoked potentials produced by stimulation of the VLCN and recorded from both the left T_3 white ramus and the left sympathetic chain below T_3. Conduction velocities in m/sec are shown under each potential. A greater percentage of slow C-fibers (<2 m/sec) versus faster Aδ fibers was recorded from each site, with a marked reduction in the total number of potentials obtained from the chain. Results of stimulation of the VMCN in this dog are seen in Fig. 3.

TABLE I. COMPARISON OF ULTRASTRUCTURAL[a] AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF SYMPATHETIC AFFERENT FIBERS IN THE LEFT CLOSE CARDIAC NERVES OF DOGS

Close cardiac nerve	Total No.[a] of Fibers in each Nerve	Percentage[a] of afferent fibers in each nerve	Relative percentage[a] of afferent fibers of each type	Evoked potential[b] distribution of afferent fibers in each nerve based on conduction velocities	
				Four dogs with SCN	Six dogs without SCN
VMCN	103 myelinated 7,214 nonmyelinated	5–10%	5% myelinated 95% nonmyelinated	7-Aδ 16-C	10-Aδ 32-C
VLCN	8 myelinated 29,284 nonmyelinated	<5%	No myelinated 100% nonmyelinated	10-Aδ 11-C	23-Aδ 35-C
SCN	Over 95% of the area[c] large myelinated fibers Small numbers of nonmyelinated fibers	Primarily afferent fibers[d]		24-Aδ 47-C	

[a] Unltrastructural data from Seagard *et al.*, 1978 (14).
[b] Normal conduction velocities, Aδ > 2–25 m/sec, C < 2 m/sec. Potentials are the result of one trial from each dog (consisting of 128 averaged consecutive traces).
[c] Data from Armour and Randall, 1975 (2).
[d] Data from Randall *et al.*, 1972 (13).

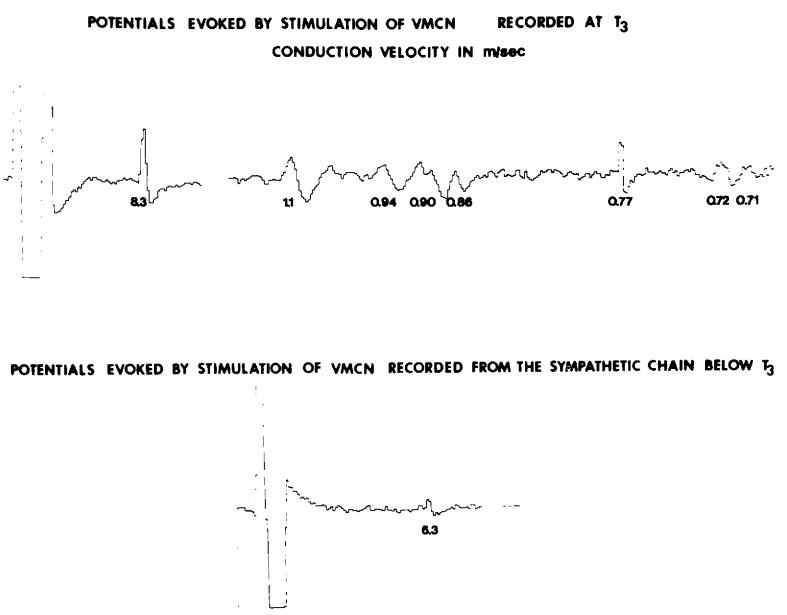

FIG. 3. Averaged nerve tracing of afferent evoked potentials produced by stimulation of the VMCN and recorded from the left T_3 white ramus and the sympathetic chain below T_3. Conduction velocities in m/sec are shown under each potential. A larger number of C-fiber potentials (<2 m/sec) versus Aδ fibers (>2 m/sec) were recorded from T_3, with only a single Aδ potential obtained from the lower sympathetic chain. Results of stimulation of the VLCN in this dog are seen in Fig. 2.

the more typical of those obtained from all dogs, although the VMCN from two dogs had a greater than 45% Aδ component. Again, as with the VLCN, the number of potentials obtained from the chain below T_3 was markedly reduced compared to the number obtained from the T_3 white ramus (Fig. 3).

In dogs where all three cardiac nerves were present, a different distribution of fibers was obtained. Stimulation of VLCN in these dogs (Fig. 4) showed a marked reduction in the number of potential groupings, while the distributions of potential groupings obtained from the VMCN (Fig. 4) were not markedly different from those seen in dogs without a SCN (Table 1). Stimulation of the SCN (Fig. 5) produced a number of large potential groupings in both the Aδ (3) and C (6) fiber range. The combination of potentials obtained from the VLCN and SCN in these dogs would produce tracings similar to the VLCN tracing of Fig. 2, obtained from a dog without a SCN.

One dog studied contained only a large, prominent left SCN, without either a VLCN or VMCN. Stimulation of this nerve produced an extremely large number of potential groupings in both the T_3 white ramus and the sympathetic chain (Fig. 6). This was the only dog studied that contained a relatively large number of potentials in the sympathetic chain below T_3. Of the potentials obtained in this large SCN, only six groupings could be described as containing Aδ fibers (conduction velocity > 2 m/sec), while 26 appeared to be in the range to belong to C fibers. The large number of C fibers resulted in some C potentials with an almost spike-like appearance, unlike the more typical widened and rounded appearance (Fig. 5).

Discussion. The results of this study indicate that the majority of cardiac sympathetic afferent fibers from the close cardiac nerves examined are C fibers. This finding is in accord with the results obtained from the earlier ultrastructural study (14) (Table I). Electron microscopic identification of sympathetic afferent fibers in the VLCN and VMCN had previously indicated that the overwhelming majority of these fibers

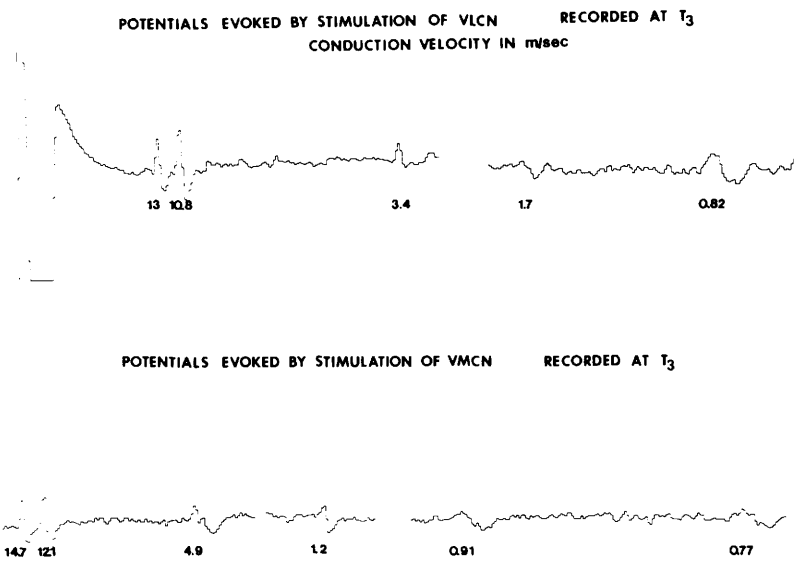

FIG. 4. Averaged nerve tracings produced by stimulation of the VLCN and VMCN and recorded from the left T_3 white ramus. These were obtained from a dog with a left SCN (Fig. 5). Conduction velocities in m/sec are shown under each potential. A similar number of Aδ (>2 m/sec) and C (<2 m/sec) fiber potentials were obtained.

were nonmyelinated C fibers. This relative dominance of afferent C fibers reflects the overall majority of C fibers in the total composition of the nerves. The distribution of potentials obtained from each nerve in this study correlates well with the anatomical distribution of afferent fibers in that nerve in all instances except the VLCN. The distribution of potentials obtained from this nerve were not predictable, based on earlier anatomical studies.

Nerve recordings (1) and anatomical studies (1, 2, 14) have previously indicated little afferent activity in the VLCN. In the

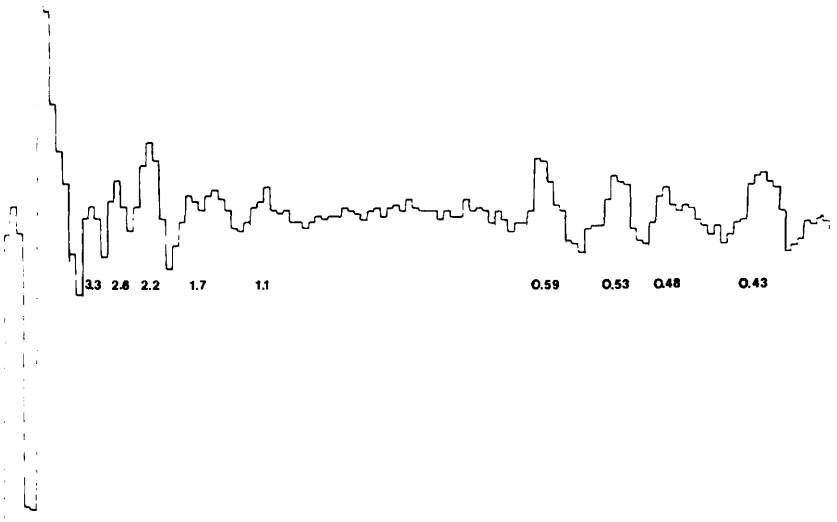

FIG. 5. Averaged nerve tracing produced by stimulation of the left SCN and recorded from the left T_3 white ramus. These data and those of Fig. 4 were obtained from the same dog. Conduction velocities in m/sec are shown under each potential. A larger number of C-fiber potentials (<2 m/sec) than Aδ fiber (>2 m/sec) potentials were obtained.

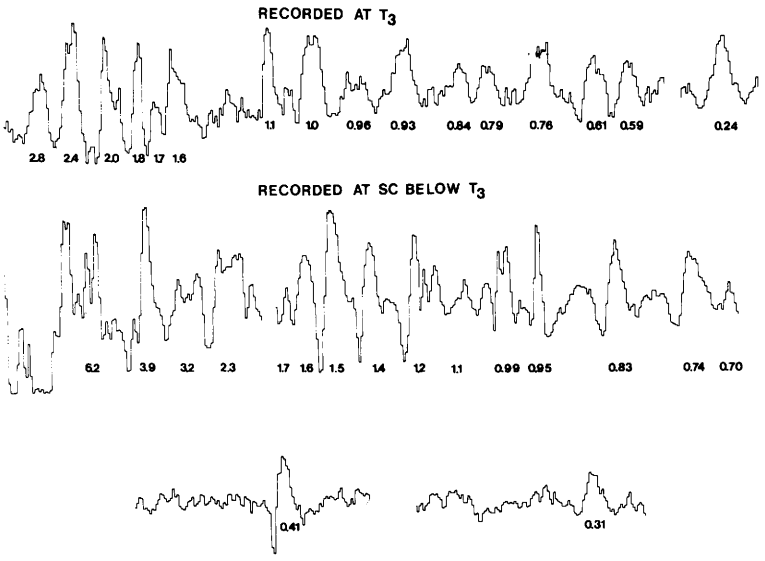

FIG. 6. Averaged nerve tracings produced by stimulation of the left SCN and recorded from the left T_3 white ramus and the sympathetic chain below T_3. This data was obtained from a large prominent SCN in a dog that lacked both a VLCN and a VMCN. Conduction velocities in m/sec are shown under each potential. A much larger number of C fiber potentials versus Aδ fiber potentials were obtained from both sites, indicating a large afferent C-fiber population.

present study, regardless of the distribution of fibers to other nerves, some evoked potentials were consistently produced through stimulation of the VLCN. Some fast potentials ascribable to Aδ fibers were always obtained. Yet the anatomical and functional evidence for myelinated afferent fibers in this nerve is lacking (2, 11, 14) (Table I). The presence of a limited number of afferent C fibers in this nerve has some foundation in earlier studies (1, 14) and therefore the ranges of C potentials obtained in this study were not unexpected. The large number of C fiber potentials obtained from every VLCN studied indicated an afferent input that had not previously been described for the VLCN.

The potentials obtained by stimulation of the isolated SCN again indicated both Aδ and C afferent fibers, with the majority in the C fiber range. In the one dog which contained only a SCN, this relative dominance of C fibers was magnified. The importance of the presence or absence of the SCN on the distribution of afferent fibers to the VLCN previously had not been functionally examined, but some anatomical basis exists for this finding. Previous investigators have described the anatomical junction of these two nerves in dogs (2, 11) which could lead to the distribution of potentials observed in the present experiment. The presence of the SCN appears to preferentially reduce the number of afferent fibers in the VLCN, compared to the VMCN.

Stimulation of the VMCN in all dogs produced a combination of Aδ and C fiber potentials which in most dogs showed a majority of C fibers. The distribution of fibers in the VMCN was relatively unaffected by the presence or absence of the SCN. This distribution of fiber types in the VMCN corroborated the anatomical distribution reported previously (2, 11, 14) (Table I). The consistent input of afferent information via the VMCN has also been indicated by nerve recordings obtained when nerve activity was evoked by mechanical distention of cardiac chambers or the aorta (2). In these studies, the VMCN contained relatively large numbers of active sympathetic afferent fibers.

The lack of fibers entering below T_3 was consistent and unexpected. Cardiac afferent fibers are known to enter between T_1 and T_5 (18) but the relative number entering at each level was not known. The anatomical structure of most dogs precludes the

recording from rami above T_3, but a recent report (17) which employed axonal transport of a labeled marker indicates that T_2 and T_3 white rami transmit approximately equally almost 90% of the cardiac sympathetic afferent fibers, with only a small percentage entering at the T_4 level. This would agree with the results of the present study.

The physiological functions of the cardiac sympathetic afferent fibers has not been determined. While their stimulation generally produces a pressor response (12), and contributes to positive inotropic and chronotropic cardiac effects (5, 8, 9), careful selective electrical stimulation may produce a depressor response (7). Earlier work indicated that the Aδ fibers were mechanically stimulated by distention or stretch of the cardiac chambers, while C fibers carried activity from chemoreceptors near the coronary arteries (15). Evidence has been found to contradict this theory (1), indicating C fibers may also arise from mechanoreceptors, but there is no way of determining the degree of C fiber activation in different experiments which employed a variety of stimuli. This study has provided electrophysiological evidence to indicate that the majority of sympathetic afferent fibers are the C fibers, for which the function has not yet been determined. The anatomical predominance of the C fibers over Aδ fibers was seen in all nerves examined, but it is not known whether there is a corresponding functional domination of C over Aδ fibers. Until the respective roles of each fiber type can be determined, the importance of actual numbers of each can not be assessed. The large population of sympathetic afferent C fibers described in the present study may play a prominant, but as yet, undertermined role in neural regulation of the heart.

Summary. Cardiac sympathetic afferent activity was electrically evoked from several close cardiac nerves and recorded from the left T_3 white ramus and sympathetic chain below T_3 in anesthetized dogs. Electrical stimulation of sympathetic afferent fibers in the ventrolateral, ventromedial, and left stellate close cardiac nerves distal to the stellate ganglion resulted in the evoking of Aδ and C fibers potentials for each nerve stimulated. A predominance of C-fiber potentials in comparison to Aδ potentials was observed for each nerve, from dog to dog. This result correlates well with earlier anatomical evidence. Most potentials were recorded from the T_3 white ramus, with little activity obtained from the sympathetic chain below that level. The presence of the left stellate cardiac nerve, which was found in only 40% of the dogs, appeared to dictate the distribution of afferent fibers to the VLCN. In the absence of the SCN, the number of potentials obtained from the VLCN was greatly increased relative to that obtained from the VLCN of dogs which contained a SCN. The distribution of potentials from the VMCN was the most consistent and reflected the larger population of C fibers seen in all nerves.

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