Functional Characteristics of Sinoatrial and Subsidiary Pacemaker Activity in the Canine Right Atrium

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SUMMARY A canine in vitro right atrial preparation was developed to study the functional characteristics of subsidiary atrial pacemaker (SAP) activity and to compare them with those of sinoatrial node (SAN) activity. Extracellular bipolar electrodes were used to estimate the site of earliest activation and monitor spontaneous rate. Ligation of the SAN artery at the midpoint of the sulcus terminalis suppressed SAN activity and usually (73.5%) elicited SAP activity in a well-defined region of the inferior atrium. SAP activity in this region required a "threshold" concentration of norepinephrine (10⁻⁶ M) in the Tyrode's perfusate. In response to all concentrations of norepinephrine tested, SAN activity attained a greater maximum spontaneous rate than SAP activity. Cholinergic stimulation with acetylcholine or eserine elicited a greater negative chronotropic response from SAP than SAN activity. Overdrive pacing suppressed SAP activity to a significantly greater extent than SAN activity. We conclude that this in vitro preparation can be useful for studying the pharmacology and electrophysiology of subsidiary atrial pacemakers that emerge after suppression of SAN activity. In contrast to SAN activity, SAP activity requires norepinephrine and is more sensitive than SAN activity to acetylcholine and overdrive pacing. Consequently, after loss of SAN function, autonomic modulation of SAP activity may result in atrial dysrythmias and prolonged periods of overdrive suppression.

FOR THE PAST several years, this laboratory has been studying subsidiary atrial pacemaker (SAP) activity that emerges in the absence of sinoatrial node (SAN) activity. There is extensive evidence that after excision of the SAN in vivo, SAP activity emerges in regions along the sulcus terminalis or inferior right atrium.¹⁻³ These subsidiary pacemakers are under autonomic control³⁻⁴ and assume pacemaker function in the alert, conscious animal in which the SAN has been excised.⁵⁻⁵

The existence of SAP activity is also supported by clinical⁶,⁷ and other experimental studies.⁸⁻¹¹ Sealy and co-workers¹⁰,¹¹ found that when the SAN of the dog was surgically excluded from the rest of the atrium, pacemaker sites in the lower right atrium assumed permanent dominance. Boineau and co-workers⁹ proposed that multiple pacemaker sites in the canine right atrium are part of a normal primary pacemaker complex.

Work from our laboratory¹ has shown that after excision of the sulcus terminalis of the canine right atrium, including the SAN region, SAP activity assumes control of the heart. Subsidiary pacemakers are consistently located within a specific region of the inferior right atrium at its junction with the inferior vena cava, centimeters from the SAN region. These pacemakers have been studied only with in vivo techniques. However, because of the limitations inherent in in vivo preparations, it is difficult to study the functional characteristics of these subsidiary pacemakers. Therefore, we have developed an in vitro right atrial preparation where SAP activity in this region of the right atrium can be studied under more controlled conditions. An in vitro preparation provides several advantages not shared by in vivo studies. For example, other regions of competitive subsidiary pacemaker activity, such as the coronary sinus, atrioventricular node and Bachmann's bundle, are excluded, autonomic reflex changes are not present, the influence of circulating catecholamines is eliminated, and the administration of drugs and the response to them can be quantitated more accurately. The purpose of this study was to develop an in vitro preparation for studying the functional characteristics of SAP activity and to compare these characteristics with those of SAN activity. Portions of this work have been reported in abstract form.¹²

Methods

Preparation

Seventy-four adult mongrel dogs of either sex that weighed 13–20 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and maintained by positive-pressure respiration. A right thoracotomy was performed through the fourth intercostal space and the heart suspended by a pericardial cradle. Fifty-seven atria were selected for in vitro preparation based on the presence of a major branch of the right coronary artery leading to the SAN region, i.e., the SAN artery. The SAN artery was cannulated using the technique of James and Nadeau.¹³ A 2-cm length of the right coronary artery was isolated near the origin of the SAN branch and ligatures were placed proximal and distal to this point. A fluid-filled cannula (PE 50) was intro-
duced into the proximal right coronary artery, advanced into the first 1 cm of the SAN artery and tied in place. The distribution of the cannulated artery to the SAN region was assessed by testing for injection bradycardia or by inspecting the distribution pattern after injecting 0.5 ml of indocyanine green dye.

After cannulation, the heart, with at least 2 cm of each vena cava, was quickly excised and immersed in room temperature Tyrode's solution equilibrated with 95% O₂, 5% CO₂. The composition of the Tyrode's solution used in all experiments was (in mM): NaCl 137, KC1 2.7, NaHCO₃ 11.9, NaH₂PO₄ 0.33, CaCl₂ 1.8, MgCl₂ 1.05 and glucose 11, pH 7.4 ± 0.02. Dissection was carried out according to the method described by Woods et al. during perfusion of the preparation through the SAN artery with oxygenated room temperature Tyrode's solution at 2–4 ml/min by a roller pump. Ventricular tissue was removed by a cut made 1–2 cm below the atrioventricular groove (coronary sulcus). The right atrium was then opened by an incision along the tricuspid valve and up along the superior vena cava. The atrioventricular node, coronary sinus and all left atrial tissue up to the interatrial septum were removed. The remaining preparation consisted of the anterior free wall of the right atrium, including the atrial appendage, a portion of the interatrial septum and a small rim of right ventricular tissue containing the isolated right coronary artery. This preparation was transferred to a 200-ml tissue bath and pinned to the Sylgard floor, with the epicardial side up.

The perfusion cannula was switched to a second pump, which delivered oxygenated Tyrode's solution (P₀, 550 ± 25.7 mm Hg) through a water-warmed thermostatic coil to yield a tissue temperature of 36 ± 1°C (YSI, model 47). Perfusion pressure was monitored by a transducer (Statham P23 Db) connected to the perfusion line by a T-junction and displayed on a polygraph (Grass, model 7). Flow was maintained constant throughout each experiment at a rate (4.7 ± 0.1 ml/min) that yielded a perfusion pressure of 100 mm Hg as determined at the start of each experiment. In addition to perfusion through the SAN artery, the atria were superfused with warmed Tyrode's solution at a rate of 10–12 ml/min.

Recording Technique

Four extracellular bipolar electrodes were used to estimate the epicardial site of earliest activation (SEA). Preliminary experiments indicated that in this preparation, pacemaker activity was invariably located along the sulcus terminalis. Therefore, one fixed reference electrode was positioned in each of the rostral, mid, and caudal regions of the sulcus terminalis (fig. 1). Each electrode lightly touched the surface of the tissue and was oriented with the lead axis parallel to the direction of the sulcus terminalis. A fourth bipolar electrode was mounted on a micromanipulator and used as a mobile electrode to record electrical activity from any epicardial site.

All four electrograms were amplified (Grass Dual P9 AC preamplifier) and displayed simultaneously on a storage oscilloscope (Tektronix, 5103N) to be photographed with a Polaroid camera (Tektronix, C-5). Preamplifier filter settings at 10 Hz and 40 kHz yielded electrogram deflections with minimal baseline fluctuation. Signals from the caudal reference electrode were recorded on a polygraph and also triggered a tachograph (Grass model 7P448), whose output was displayed for a continuous record of spontaneous rate.

The mobile electrode was used to estimate the site that depolarized before the regions beneath the reference electrodes. The SEA was defined as the location in which the mobile electrogram preceded the reference electrograms by the greatest time interval from the initial rapid deflection in each recording. Movement of the mobile electrode by as little as 2 mm in any direction from the site of earliest activation attenuated the maximal time interval. We assumed that the electrogram recording the earliest depolarization was closest to the origin of pacemaker activity. An example of the mapping is shown in figure 1.

Eliciting Subsidiary Atrial Pacemaker Activity

Maintenance of SAN pacemaker activity in this in vitro atrial preparation depended on continuous perfusion through the SAN artery, confirming similar findings by others. Interruption of perfusion resulted in suppression of SAN activity within minutes. This was exploited in the present study to suppress SAN activity and elicit SAP activity. A 1–3 mm length of the SAN artery was isolated at the midpoint of the sulcus terminalis, caudal to the control SEA and ligated with fine silk thread. Any small branches of the SAN artery arising from below the ligation site and distributed toward the SAN region were also ligated. This resulted in a continuous decline in spontaneous sinus rate and the emergence of SAP activity. Once a stable atrial rhythm resumed, it was apparent from the activation sequence of the stationary electrodes when pacemaker activity had shifted to regions below the ligation. Using the mobile electrode, the mapping procedure was repeated to approximate the subsidiary SEA (fig. 2).

Perfusion of regions containing SAP activity was confirmed by observing the distribution of Microfil injected into the SAN artery at the conclusion of three experiments and in three other preparations in which no ligation was performed. Furthermore, in all of the preparations in which SAP activity was elicited, perfusion of acetylcholine or norepinephrine (NE) into the SAN artery resulted in appropriate changes in subsidiary pacemaker rate.

Preliminary experiments with this preparation were more successful in eliciting subsidiary pacemaker activity when a background level of NE (10⁴ M) was added to the Tyrode's perfusate. Therefore, to compare SAN and SAP activities, all preparations were perfused from the start of the experiment with Tyrode's solutions containing 10⁴ NE. To avoid pacemaker activity that may have resulted from injured tissue at the point of ligation, all preparations that exhibited subsidiary pacemaker activity selected for...
this study had a subsidiary SEA located more than 6 mm or four passive space constants from the ligation.

Pacemaker Characterization
SAN and SAP activity were compared by their chronotropic responses to perfused neuromediators and to overdrive pacing. NE and acetylcholine were tested by perfusing varying concentrations of each for 30 seconds and 1 minute, respectively, while recording maximum changes in spontaneous rate. The concentration-response curves for acetylcholine were compared by the effective concentration that produced a half-maximum response (EC$_{50}$). Time was allowed for complete recovery of spontaneous rate between test perfusions.

The response to overdrive pacing was determined by driving SAN and SAP activity at rates 100%, 150% and 200% above their control spontaneous rates for 0.5, 1 or 2 minutes. Stimulation was achieved by applying rectangular pulses, 2 msec in duration and 50% above threshold voltage to the inferior right atrium through bipolar pin electrodes coupled to a stimulator (Pulsar 4i, Frederick Haer). The amount of suppression after each period of overdrive pacing was measured directly from the polygraph traces of the caudal reference electrogram. Corrected recovery time (CRT) was obtained by subtracting the control cycle length from the duration of pacemaker suppression, as measured from the last driven beat to the first spontaneous beat.

Drugs
All solutions were freshly prepared before each experiment. Acetylcholine chloride (Sigma Chemical Co.) was prepared from cold stock solutions. All NE solutions (levophed bitartrate, Breon) were prepared in Tyrode's solution containing $6 \times 10^{-5} M$ ascorbic acid to minimize oxidation. Other drugs used in this study were methoxamine hydrochloride (Vasoxyl, Burroughs Wellcome), phentolamine (Regitine HCl, Ciba-Geigy), DL-propranolol hydrochloride (Sigma Chemical Co.) and eserine salicylate (Sigma Chemical Co.).

Statistics
Values are expressed as mean $\pm$ SEM. Statistical comparisons were made using an unpaired $t$ test. Differences were considered statistically significant at $p < 0.05$.

Results
Control
Of the 74 dogs, 57 (77%) had a SAN artery originating from the right coronary artery and were therefore
used for this study. Under in vitro conditions (in the absence of background NE), the mean steady-state spontaneous rate of these atria was 82.9 ± 1.3 beats/min. Adding background NE (10⁻⁸ M) to the Tyrode’s perfusate increased the steady-state spontaneous rate to 101.5 ± 1.9 beats/min (p < 0.0005). In 93% of the atria, the control epicardialSEA was located within the SAN region (fig. 1). These sites were 2–20 mm caudal to the junction of the superior vena cava with the right atrial appendage, an area that corresponds well to the anatomic site of the canine SAN determined histologically.¹⁸ Although the addition of NE occasionally caused the SEA to shift within the sinus region initially, once stabilized, the steady-state control SEA and spontaneous rate remained stable for several hours. In seven preparations containing an SAN artery, there was no significant difference in spontaneous rate from the beginning of the experiment (103.6 ± 3.1 beats/min) compared with that after 4 hours (105 ± 2.7 beats/min) (p > 0.20).

**Subsidiary Pacemaker Activity**

Figure 2 is an illustration of the location of the subsidiary SEAs found in all 36 preparations in which SAP activity was elicited after ligation of the SAN artery. Ligation of the SAN artery caudal to the control SEA always resulted in a gradual decline in spontaneous sinus activity. After a variable period, SAP activity emerged in these preparations to maintain atrial excitation. The extracellular mapping procedure was used to estimate the location of the subsidiary SEA. The site beneath the mobile electrode depolarized earlier than any of the reference sites and the activation sequence of the reference electrograms was retrograde compared with control (fig. 1). Usually, conducted activity was recorded from regions of tissue above the ligation, probably because the superficial fibers were still viable as a result of the superfusion. In the presence of background NE (10⁻⁸ M), suppression of SAN activity resulted in the appearance of SAP activity in 36 of the 49 preparations tested (73.5%). Of the remaining 13 preparations, six showed no evidence of SAP activity and seven developed pacemaker activity in which the SEA was less than four space constants from the ligation site. Consequently, these preparations were not used in this study. All 36 subsidiary SEAs were concentrated in a well-defined region of the inferior right atrium at its junction with the inferior
vena cava (fig. 2). Collectively, these sites were 28.3 ± 3.1 mm caudal to their control SEA and 11.6 ± 0.6 mm caudal to the site of ligation. In the tissue bath, this region of the atrium was always paler than the surrounding pink atrial tissue, especially when viewed from the endocardial surface.

In 10 preparations, the subsidiary SEA was first mapped on the epicardial and then on the endocardial surface of the same preparation. The sites on both surfaces differed by 3.8 ± 0.9 mm, suggesting that the subsidiary SEA was close to the cells generating SAP activity. This was confirmed in preliminary experiments using intracellular microelectrodes to record pacemaker (phase 4 depolarization) action potentials from tissue excised from regions exhibiting SAP activity.

The mean steady-state spontaneous rate of SAP activity was 86.6 ± 3.5 beats/min, significantly less than the mean spontaneous sinus rate (101.5 ± 1.9 beats/min) with the same background level of NE. Once elicited, SAP activity was also stable for several hours. Measured in six preparations, the spontaneous rate at the onset of SAP activity (77.8 ± 4.7 beats/min) was not significantly different from that after 4 hours (80.5 ± 5.3 beats/min) (p > 0.20).

Fifty-seven of the 74 dogs used in this study had an SAN artery. Nine of the remaining 17 had an atrial artery too small to cannulate and eight had an artery that did not supply the SAN region. In these eight dogs, the artery was cannulated in vivo, as usual. Instead of supplying the SAN region, these arteries supplied the inferior regions of the right atrium and therefore were not considered SAN arteries. This was determined by visual inspection of the distribution of dye injected into the artery and lack of injection bradycardia. In the presence of 10^-8 M NE, five of these preparations exhibited SAP activity in vitro, in the same region of the inferior right atrium described above, without ligation of the artery (fig. 2). In addition, the steady-state spontaneous rate of these five preparations (87.8 ± 8.2 beats/min), was not different from the spontaneous rate of SAP activity (86.6 ± 3.5 beats/min) elicited in preparations by ligating the SAN artery.

**Norepinephrine**

The obligatory role of NE in SAP activity was tested in 26 preparations, in 17 by perfusing NE-free Tyrode’s solution and in nine propranolol (1–3 × 10^-7 M) in the presence of NE. Figure 3 shows the effects of propranolol (10^-7 M) on SAN and SAP activity. In the case of SAN activity, propranolol caused a gradual decline in spontaneous rate, which leveled off to a new steady state after a few minutes. However, the same concentration of propranolol resulted in a more rapid decline in the SAP spontaneous rate, with an abrupt cessation of recorded activity. Qualitatively similar results were obtained when NE-free Tyrode’s solution was perfused instead of propranolol. Of the preparations tested, 73% became quiescent or irregular when either propranolol or NE-free Tyrode’s solution was perfused. These results indicate that in most preparations, β-adrenergic stimulation was required to sustain SAP activity, in contrast to SAN activity.

In five preparations, the minimal NE concentration or “threshold” level required to maintain a stable SAP

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** The influence of β-adrenergic blockade on sinoatrial node (SAN) and subsidiary atrial pacemaker (SAP) activity. (top) A typical response of SAN activity to a continuous perfusion of propranolol (10^-7 M) in the presence of norepinephrine (10^-8 M). The bottom panel shows the response of SAP activity to the same concentration of propranolol in the presence of norepinephrine (10^-8 M). Note the abrupt cessation of recorded SAP activity after a few minutes of exposure. SAN and SAP activities were not recorded from the same preparations in these experiments because β-adrenergic activation was needed to elicit SAP activity. CST = caudal sulcus terminalis.
rhythm was determined. The procedure involved lowering the concentration of NE until SAP activity ceased or became irregular. The threshold was determined by increasing the concentration of NE in small steps until rhythmic activity resumed. This threshold concentration was $6.4 \pm 4.7 \times 10^{-9} \text{ M}$. In 85% of the SAP preparations, the background level of NE used ($10^{-8} \text{ M}$) was suprathreshold and therefore sufficient to sustain SAP activity. In the remaining 15%, SAP activity required NE concentrations greater than $5 \times 10^{-8} \text{ M}$.

Adrenergic Effects

Figure 4 shows the chronotropic response of SAN and SAP activities to concentrations of NE ranging from $5 \times 10^{-8}$ to $5 \times 10^{-6} \text{ M}$. Under control conditions, with constant background NE ($10^{-8} \text{ M}$), SAP activity had a significantly lower spontaneous rate than sinus activity. When the preparations were exposed for 30 seconds to increasing concentrations of NE of $10^{-7} \text{ M}$ or less, the maximum spontaneous rate of both SAN and SAP activity increased in a parallel fashion. However, at concentrations greater than $10^{-7} \text{ M}$, there was a significant difference in maximum spontaneous rate, resulting from a sharp increase in SAN activity. During these higher levels of adrenergic stimulation, there were small transient shifts in activation sequence, associated with abrupt increases in spontaneous rate. In contrast, the same concentrations of NE produced significantly smaller increases in SAP spontaneous rate and no detectable shifts in activation sequence.

The effect of $\alpha$-adrenergic stimulation was also tested by perfusing methoxamine for 1 minute while recording spontaneous rate. At $10^{-5} \text{ M}$, methoxamine increased SAN activity slightly, by $3.0 \pm 2.5$ beats/min (n = 5; p > 0.20) but increased SAP activity significantly, by $5.3 \pm 0.6$ beats/min (n = 3; p < 0.02). At $10^{-4} \text{ M}$, methoxamine decreased both SAN and SAP activity by $11.7 \pm 0.9$ (n = 3) and $15.5 \pm 1.7$ beats/min (n = 4), respectively (p < 0.01). Both the negative and positive chronotropic effects of methoxamine were blocked by phentolamine ($5 \times 10^{-7} \text{ M}$), which by itself had no effect on spontaneous rate of SAN or SAP activities.

Cholinergic Effects

Figure 5 shows the maximum chronotropic responses of SAN and SAP activity to concentrations of acetylcholine ranging from $10^{-8} \text{ M}$ to $7.5 \times 10^{-6} \text{ M}$. The curve describing the response of SAP activity is shifted far to the left, indicating that SAP activity is more sensitive to acetylcholine than to SAN activity. The EC$_{50}$ for the individual SAP experiments of $5.7 \pm 2.1 \times 10^{-8} \text{ M}$ is significantly less than the EC$_{50}$ obtained for the SAN experiments of $1.2 \pm 0.3 \times 10^{-6} \text{ M}$. This relationship was found whether acetylcholine
was perfused under constant flow (n = 4) or constant pressure (n = 2). Further experiments were done to determine the time course of the rate changes induced by acetylcholine on SAN and SAP activity. When the same concentration of acetylcholine (10^{-7} M) was infused for 1 minute, the time to peak response was not different between the two types of pacemaker activity. However, the duration of the negative chronotropic response was significantly longer for SAP activity (181.1 ± 16.1 second) than for SAN activity (109.5 ± 18.1 second) (p < 0.05).

In four additional experiments, eserine (3.6 × 10^{-5} M) was tested for its effects on SAN and SAP activity. When eserine was infused for 15 seconds, SAN rate decreased by 28.8 ± 7.0% and SAP rate by 44.0 ± 10.0% (p > 0.1). These results suggest that endogenous acetylcholine may be released from this tissue and that SAP activity is more sensitive than SAN activity to its negative chronotropic effects.

Overdrive Suppression

The response of SAN and SAP activity to overdrive pacing was tested by pacing the preparation at rates above spontaneous rate and for different durations. Figure 6 is a summary of the results of three pacing periods (0.5, 1 and 2 minutes) at three frequencies of pacing (100%, 150% and 200%) above control rate. The magnitude of suppression of SAN and SAP activity was a function of both the magnitude and duration of the overdrive period. However, the SAP relationship had a much greater positive slope. Furthermore, at each magnitude of overdrive tested, the CRT for SAP activity was significantly greater than that for SAN activity for each pacing duration (p < 0.005). Suppression of SAP activity was measured in seconds, compared with milliseconds for SAN activity.

Discussion

We have developed an in vitro model for studying SAP activity that emerges after suppression of SAN activity. Experiments using this preparation have found that (1) A well-defined region of SAP activity is located at the junction of the inferior right atrium with the inferior vena cava. (2) Unlike SAN activity, this SAP activity is dependent on background β-adrenergic stimulation. (3) In response to NE, SAN activity attains a significantly greater maximum spontaneous rate than SAP activity. (4) SAP activity is more sensitive than SAN activity to cholinergic stimulation, and (5) SAP activity is overdrive-suppressed to a significantly greater extent than SAN activity.

Under control spontaneous rhythm, the SEA was consistently found within the SAN region, i.e., at the junction of the superior vena cava and the right atrial appendage. This pacemaker activity remained stable for several hours, as long as perfusion through its nutritive vasculature (the SAN artery) was maintained. This confirms the findings of Woods et al.11 and Musgrave16 in their in vitro studies of the canine sinus node. With the mapping procedure used here, we found control SAN activity to be at a single site at any given time. This is in agreement with previous reports2, 20, 21 but not consistent with recent reports of a multicentric atrial pacemaker complex.8, 9 Difference between our results and those of Boineau et al.8, 9 may be attributed to the different mapping procedures or the preparations used. However, the present study was not designed to determine the functional organization of sinus node pacemakers. Our experiments were more concerned with characterizing the functional responses of SAN pacemaker activity. The mapping procedure was used primarily to approximate the location of SAN pacemaker activity and monitor shifts in activation sequence before and after ligation of the SAN artery. With the administration of high concentrations of neomediators we often monitored small transient shifts in SAN pacemaker activity. However, at no time did these interventions shift sinus pacemaker activity to the region of the inferior right atrium, found in this study to contain SAP activity.

By ligating the SAN artery at the midpoint of the sulcus terminalis, we selectively interrupted flow to the SAN region and thereby suppressed SAP activity. As sinus pacemaker activity decreased to lower rates, SAP activity usually (73.5%) emerged to maintain atrial excitation. The spontaneous activity of these
subsidary pacemakers was also stable for several hours. Extracellular mapping localized the subsidiary SEA to a well-defined region of tissue at the junction of the inferior right atrium with the inferior vena cava. In 36 preparations, the subsidiary SEAs were localized to a region 28.3 ± 3.1 mm caudal to their control site of SAN activity. Experiments using intracellular recordings have confirmed the presence of pacemaker (phase 4 depolarization) action potentials in tissues excised from this region. Moreover, the present experiments corroborate previous in vivo experiments that have found SAP activity in the same region, after acute or chronic excision of the SAN region.

Boineau et al. reported that under normal conditions, primary pacemaker activity may be accomplished through a multicentric organization of atrial pacemakers. This raises the question of whether the SAP activity in the present study is part of a possible atrial pacemaker complex. Evidence suggests that it is not. Thus, according to Boineau et al., our ligation of the SAN artery was just caudal to their most inferior point C and would, therefore, have suppressed pacemaker activity of a possible atrial pacemaker complex. Moreover, the site of SAP activity in the present study was located at the junction of the inferior right atrium with the inferior vena cava, approximately 1 cm caudal to their most inferior point C of the pacemaker complex. Furthermore, in the present study, SAP activity was more sensitive to cholinergic stimulation, while pacemaker activity at point C was reported to be least sensitive to parasympathetic stimulation. In the present study, β-receptor blockade with propranolol abolished SAP activity, while point C of the pacemaker complex was found least affected by β blockade. Finally, infusion of acetylcholine or NE concentrations that produced large changes in spontaneous cycle length, never shifted pacemaker activity from the SAN region to the SAP region described here. Apparently, the subsidiary pacemakers described in the present study differ in location and functional properties from those in the SAN or an atrial pacemaker complex. In addition, they emerge after supression of primary pacemaker activity.

Unlike sinus node activity, SAP activity depended on a background level of β-adrenergic stimulation. With this in vitro preparation, we could quantitatively determine the threshold concentration of NE required to sustain subsidiary activity. When the level of NE fell below this level or when propranolol was added to the perfusate, SAP activity became irregular or quiescent. These experiments correlate well with our previous in vivo findings that when SAP activity is elicited after excision of the SAN region, the administration of propranolol frequently shifts pacemaker dominance to the atrioventricular junctional region. Apparenty, SAP activity in the region of the inferior right atrium depends on β-receptor stimulation. Beta-receptor blockade or changes in sympathetic tone could, therefore, cause SAP activity to shift to regions less dependent on adrenergic stimulation.

In response to NE, SAN activity attained a maximum spontaneous rate significantly greater than SAP activity. This agrees with in vivo experiments by Jones et al., who studied SAP activity in dogs, where most of the criata terminals, including the SAN region, had been excised 3-4 months earlier. Maximal heart rates of the SAN (postoperative) in response to exercise, isoproterenol and stellate stimulation during anesthesia were significantly greater than the response of SAP activity. The SAP activity in these chronic animals was mapped and found within the same region of the inferior right atrium as the SAP activity found here. Although SAP activity requires background adrenergic stimulation to support atrial excitation, the chronotropic response to adrenergic stimulation is always less than that of the SAN. This may reflect the differential effects of adrenergic stimulation on SAP impulse conduction and automaticity, respectively.

Using this in vitro preparation, a direct comparison of chronotropic responses indicated that SAP activity is more sensitive than SAN activity to acetylcholine. Based on the concentration-response curves, the EC50 value for SAN activity was about 21 times greater than that for SAP activity. In addition, the fact that eserine elicited a greater negative chronotropic response from SAP than from SAN activity also supports the conclusion that SAP activity is more sensitive to acetylcholine. In dogs in which the SAN region was surgically excised or destroyed by embolization several months earlier, SAP activity was hypersensitive to parasympathetic stimulation or atropine administration. Similar to the present experiments with acetylcholine, the recovery of SAP after vagal stimulation was also prolonged.

The present experiments demonstrate that the SAP region is within the distribution of the SAN artery. Therefore, infusions of parasympathomimetic agents (acetylcholine and eserine) into the SAN artery were distributed to the SAN region as well as to sites containing SAP activity. Moreover, these agents inhibited SAP activity more than SAN activity. This becomes particularly important in the interpretation of data used to determine the functional hierarchy of SAP activity in the heart. Thus, Ural, James and co-workers injected parasympathomimetic agents into the dog SAN artery in vivo to "selectively" inhibit sinus node activity. This resulted in atrioventricular junctional rhythms, which they concluded to be the second most automatic pacemaker site after failure of SAN activity. However, in the present experiments, more discrete suppression of SAN activity resulted in the appearance of SAP activity in regions of the inferior or right atrium that are within the distribution of the SAN artery. These results are supported by the in situ experiments of Sealy et al. and Randall, Jones and co-workers, who have also identified SAP activity in the inferior right atrium after surgical exclusion or excision of the SAN region, respectively. Taken together, the present results support the idea that after failure of the sinus node, the second most automatic pacemaker site is located within the right atrium.

One important characteristic of SAP activity clearly
distinguishing it from SAN activity was the significantly longer periods of suppression in response to overdrive pacing. With comparable rates and durations of pacing, suppression of SAP activity was measured in seconds, in contrast to milliseconds for sinus activity. The degree of suppression was related to the rate and duration of the pacing periods imposed. These findings are consistent with in vivo studies of subsidiary activity, after destruction or excision of the SAN region. Thus, Lange showed with acute experiments that subsidiary pacemaker activity in various regions of the dog heart, including the lower right atrium, was more sensitive to overdrive pacing than SAN activity. These findings were extended by Euler et al. to SAP activity in the conscious, unanesthetized dog, in which the crista terminalis and SAN had been surgically excised several weeks earlier. Recent work by Randall et al. has expanded these results by systematically characterizing the initial and long-term responses of SAP activity to overdrive pacing in the conscious dog.

In conclusion, this study demonstrates an in vitro preparation for studying SAP activity that emerges after suppression of SAN activity. The location and functional characteristics of SAP activity elicited in this in vitro preparation are consistent with SAP activity found in vivo and therefore support the validity of this preparation as a useful model for future studies on the pharmacology and electrophysiology of SAP function. The data obtained with this preparation illustrates a number of advantages over in vivo techniques. However, some of these advantages may also be limitations when relating the present results to those in the intact preparation or in man. For example, other regions of competitive subsidiary pacemaker activity, such as the atrioventricular node and changes in autonomic tone, are not present, thereby eliminating the arrhythmic pacemaker activity seen after destruction of the SAN. Furthermore, it seems likely that the ligation technique used in these experiments would suppress SAN pacemaker activity as well as other sites of subsidiary pacemaker activity that may be present within the distribution of the ligated SAN artery. Consequently, SAP activity was found in a well-defined region of the inferior right atrium rather than over a more widely distributed region, like that reported with intact preparations.

The present experiments indicate that after failure of SAN activity, SAPs that have functional characteristics significantly different from those of the SAN, may assume pacemaker control of the heart. Moreover, due to their functional properties, SAP rhythms may be unstable and may respond to stimuli abnormally. For example, after loss of SAN function, possibly as a result of infarction of the SAN region, autonomic modulation of SAP activity may result in brady-tachy atrial arrhythmias. Likewise, SAPs that assume pacemaker control, may exhibit prolonged periods of suppression in response to overdrive pacing of the atria. These responses have, in fact, been documented experimentally in dogs where SAP activity has assumed control after surgical excision of the SAN region. Finally, these experiments provide additional evidence that SAP activity is an important component in the hierarchy of subsidiary pacemaker activity in the heart.

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