# Failure of Beta-Adrenergic Receptor Blockade to Prevent Arrhythmias Induced by Sympathetic Nerve Stimulation

Abstract. *Cardiac arrhythmias produced by electrical stimulation of the ventrolateral cardiac sympathetic nerve in dogs were not blocked by the combined administration of propranolol and practolol in amounts that completely blocked cardiac beta-adrenergic receptors. Blockade of cardiac alpha-adrenergic receptors, as well as cardiac cholinergic receptors, also had no influence on the arrhythmias. These results suggest that the adrenergic neuroeffector junction is fundamentally different from any hitherto described, differing perhaps in the neurotransmitter involved or in the nature of the receptor.*

The traditional and current view regarding adrenergic synaptic transmission at the cardiac neuroeffector junction is that neural impulses release norepinephrine, which in turn interacts with beta-adrenergic receptors causing chronotropic and inotropic changes in cardiac function (*1–3*). Recently, Randall and his colleagues (*4, 5*) have obtained results that we felt might challenge this view. They reported that electrical stimulation of the ventrolateral cardiac sympathetic nerves (VLCN) in dogs produced arrhythmias which could not be prevented by pretreating with the beta-receptor blocking agent propranolol; they considered the dose inadequate to achieve beta-receptor blockade. The purpose of our study was to determine whether this explanation is plausible or whether these synapses differ from the usual sympathetic neuroeffector junctions in a more fundamental way.

Experiments were performed in mongrel dogs ranging from 11 to 16 kg in weight, anesthetized with intravenous (30 mg/kg) or intraperitoneal (35 mg/kg) pentobarbital sodium. All animals were respired artificially with room air. Carotid arterial pressure and electrocardiogram were monitored, and drugs were administered via the jugular vein.

The chest was opened in the midline, and lateral incisions were made between left fourth and fifth and right third and fourth ribs. Through a pericardial incision, stimulating electrodes were placed on the VLCN at the level of the inferior pulmonary vein and on the right postganglionic nerve trunk emerging from the sympathetic ganglia. Both nerve trunks were decentralized to eliminate effects occurring from stimulation of afferent fibers. Stimulation was performed with a Grass stimulator and impulses of 4 msec in duration. The VLCN was stimulated with 5 volts and 10 hertz for 10 seconds; the right sympathetic nerve, with 20 volts and 20 hertz for 20 to 30 seconds.

Stimulation was performed in nine dogs and an arrhythmia occurred in each, developing immediately with stimulation and usually disappearing immediately after it was terminated. The arrhythmia rate was faster than control, usually with an unchanged QRS configuration. Arrhythmias produced by this technique have been characterized with His bundle and multiple intracardiac electrode recordings as originating from low right atrial atrioventricular junctional, or bundle of His foci (*5*). A representative experiment illustrating the arrhythmia appears as Fig. 1. Administration of either 1 mg of propranolol per kilogram (intravenously) or 1 mg of atropine per kilogram (intravenously) failed to prevent the arrhythmia. These findings confirm the results of Randall and colleagues (*4, 5*).

The VLCN are anatomically sympathetic nerves emanating from the left caudal cervical ganglion (*6*). To determine if standard transmitters were involved in the end-organ response, we examined the influence of blockade of beta-adrenergic, cholinergic, and alpha-adrenergic receptors. We first attempted to block the arrhythmogenic effects of VLCN stimulation with supramaximal doses of beta-adrenergic blocking drugs. To confirm the presence of beta-blockade, we stimulated the right cardiac accelerator nerve, using increased sinus rate as an end point. Before drug administration, right sympathetic nerve stimulation increased the sinus rate by $75 \pm 8.8$ (standard error of the mean) beats per minute. After 1 and 1.5 mg of propranolol per kilogram, stimulation of this nerve increased the rate by only $30 \pm 6.5$ and by $10 \pm 3.3$ beats per minute, respectively. Despite this marked antagonism, VLCN stimulation still produced arrhythmias in six of six dogs.

Because larger doses of propranolol have nonspecific cardiodepressant effects (*7*), we next administered the new beta-adrenergic blocking agent, practolol, to these six dogs. This substance produces beta-adrenergic blockade in a dose of 1.0 mg/kg (intra-

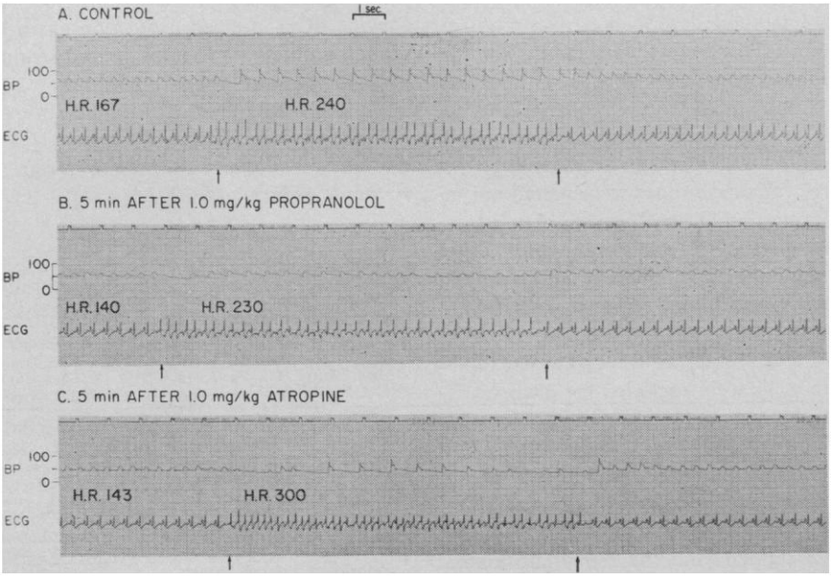



Fig. 1. Effects of propranolol and atropine on the cardiac rhythm disturbance produced by electrical stimulation of the ventrolateral cardiac sympathetic nerve (VLCN). (Panel A) Control tracings of the electrocardiogram (ECG) and blood pressure (BP) during stimulation (shown by arrows) of the VLCN. (Panel B) ECG and BP tracings during nerve stimulation (shown by arrows) and after the intravenous administration of propranolol. (Panel C) ECG and BP tracings during nerve stimulation (shown by arrows) and after the intravenous administration of atropine. H.R., heat rate.

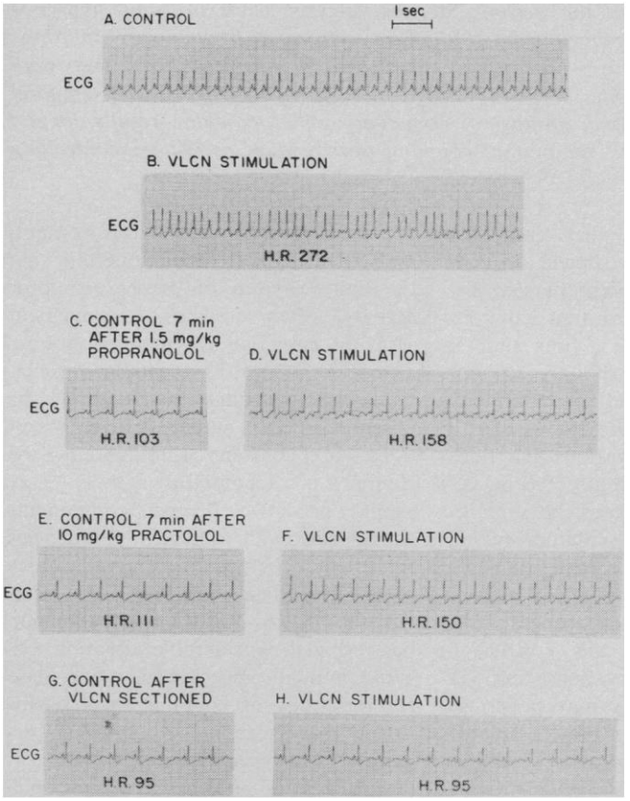
venously) (*8*) and does not possess the nonspecific membrane effects of propranolol (*9*). We employed a dose of 10 mg/kg (intravenously) and in six of six dogs found that right sympathetic nerve stimulation had no significant effect on sinus node rate ($+0.5 \pm 0.46$ beats per minute). In contrast, stimulation of the VLCN continued to produce arrhythmias in all six dogs. A representative experiment showing the failure of complete beta-receptor blockade to prevent arrhythmias induced by stimulation of the VLCN appears as Fig. 2; at the same time complete beta-blockade is demonstrated by the failure of right sympathetic nerve stimulation to elicit a response (Fig. 3).

To exclude cholinergic participation in the arrhythmia, atropine (1 mg/kg, intravenously) was administered to the six dogs after beta-receptor blockade. This drug also had no effect on the cardiac rhythm changes induced by VLCN stimulation. Although 1 mg/kg exceeds by 20 times the blocking dose for vagal-mediated chronotropic effects (*10*), we administered a larger dose of 3 mg/kg to one animal to exclude completely the possibility of a residual vagal effect. The arrhythmia was not prevented.

Since alpha-adrenergic receptors have been demonstrated in the heart (*11*), we attempted to prevent the arrhythmias in two animals with phentolamine in doses as high as 5 mg/kg. This drug was given after beta-receptor blockade and also failed to prevent the response to VLCN stimulation.

The failure of these standard blocking agents to prevent the rhythm disturbance caused us to examine whether current spread from our electrodes was directly stimulating the heart. This was ruled out in several ways: (i) relatively low voltages were used, thus minimizing current spread; (ii) the stimulating electrodes were immersed in mineral oil during stimulation; (iii) the cardiac tissue was pushed at least 2 cm away from the electrodes during stimulation; and (iv) either local application of lidocaine or section of the nerve distal to the electrode prevented the arrhythmia induced by VLCN stimulation in five of five dogs. An example of the effect of nerve section on the response is shown in panels G and H of Fig. 2.

Our results demonstrate that the arrhythmia produced by VLCN stimulation cannot be prevented by beta-blockade. The concentrations of beta-blockers employed far exceeded those reported by other investigators to produce blockade of cardiac beta-adre-



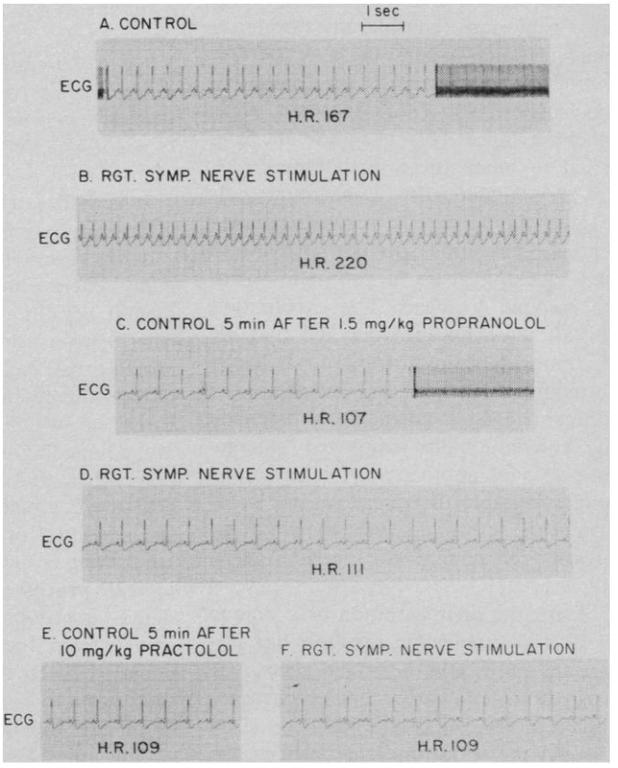




Fig. 2 (left). Effects of propranolol and practolol on the cardiac rhythm disturbance produced by electrical stimulation of the ventrolateral cardiac sympathetic nerve (VLCN). (Panel A) Control electrocardiogram (ECG) tracing before stimulation of the VLCN. (Panel B) ECG tracing during stimulation of the VLCN. (Panel C) ECG tracing after intravenous administration of propranolol and before stimulation of the VLCN. (Panel D) ECG tracing 5 seconds after tracing in panel C and during stimulation of the VLCN. (Panel E) ECG tracing after intravenous administration of practolol and before stimulation of the VLCN. (Panel F) ECG tracing 5 seconds after tracing in panel E and during stimulation of the VLCN. (Panel G) ECG tracing after sectioning the VLCN distal to the stimulating electrode and before stimulation of the VLCN. (Panel H) ECG tracing 2 minutes after tracing in panel G and during stimulation of the VLCN. H.R., heart rate. Fig. 3 (right). Effects of propranolol and practolol on sinus tachycardia produced by electrical stimulation of the right sympathetic accelerator nerve (RAN) in the same dog as described in Fig. 2. (Panel A) Control ECG tracing before stimulation of the RAN. (Panel B) ECG tracing during stimulation of the RAN. (Panel C) ECG tracing after intravenous administration of propranolol and before stimulation of the RAN. (Panel D) ECG tracing 20 seconds after tracing in panel C and during stimulation of the RAN. (Panel E) ECG tracing after intravenous administration of practolol and before stimulation of the RAN. (Panel F) ECG tracing 20 seconds after tracing in panel E and during stimulation of the RAN. RGT. SYMP., right sympathetic; H.R., heart rate.

nergic receptors in the intact dog (*8, 12*) and were sufficient to antagonize totally the effects of right accelerator nerve stimulation in the present study. Other less likely receptors that might have played a role in the response, such as alpha-adrenergic and cholinergic receptors, were excluded by utilizing supramaximal doses of the appropriate blocking agents. The most likely explanation for our results, therefore, is that the sympathetic neuroeffector junction involved is fundamentally different from any hitherto described, differing perhaps in the neurotransmitter involved or in the nature of the receptor.

A unique sympathetic neuroeffector junction with uncharacterized pharmacological properties might account for certain inconsistencies in experience with experimental and clinical arrhythmias. In experimentally induced arrhythmias, such as those seen with digitalis intoxication and coronary artery occlusion, considerable evidence indicates that hyperactivity of cardiac sympathetic nerves is a causative mechanism (*13, 14*). However, beta-blocking doses of propranolol have failed to block these arrhythmias (*13, 15, 16*). Clinically, Lown and colleagues (*17*) have described patients whose arrhythmias do not respond to beta-blocking doses of propranolol but do respond to sleep. The explanation for all these findings may be that arrhythmogenic stimuli travel to the heart through nerves whose neuroeffector junctions are not amenable to blockade by conventionally employed antagonists. Stated another way, it seems no longer possible to equate cardiac beta-adrenergic blockade with complete depression of sympathetic nervous activity.

Thus, the demonstration of a unique cardiac neuroeffector junction has important implications for the genesis of arrhythmias and for their pharmacologic therapy. Much may be gained by seeking the transmitter involved in the arrhythmogenic response and then developing antagonists to it. More fundamentally, it raises questions about the adequacy of present knowledge concerning the sympathetic nervous system.

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## Testosterone Induces "Splitting" of Circadian Locomotor Activity Rhythms in Birds

Abstract. *Under the influence of testosterone, the free-running circadian rhythm of locomotor activity of the starling,* Sturnus vulgaris, *tends to "split" into two components which temporarily run with different circadian frequencies: "splitting" occurred in intact birds whose testes grew, and in castrated birds that were injected with testosterone. Since "splitting" most probably reflects the temporal separation of two (or two groups of) circadian oscillators, these results suggest that testosterone affects the mutual coupling of circadian oscillators controlling locomotor activity.*

It is well established that many biological processes are subject to circadian variations which persist for many cycles under constant environmental conditions. For a long time it has been assumed, explicitly or implicitly, that such circadian rhythmicity reflects the action of *one* "physiological clock" to which the various functions are coupled. Only recently has it become clear that there must be a multiplicity of circadian oscillators within an individual organism, each oscillator controlling a particular set of physiological processes. The strongest evidence in support of this conclusion comes from the observations that (i) a circadian rhythmicity may persist in two or more organs or tissues isolated from one and the same individual (*1–4*) and that (ii) even in an intact organism different circadian rhythms may occasionally run with slightly different frequencies (*1–5*). Therefore, an organism can be considered a population of circadian oscillators, which, while normally synchronized with each other, may uncouple under certain conditions. These findings raise the question how mutual synchrony is normally maintained, that is, which factors are involved in coupling the various circadian oscillators to each other.

Several recent investigations in mammals have made it virtually certain that the daily rhythm of gross locomotor activity, often used as a convenient assay of circadian rhythmicity, is governed by at least two coupled oscillators. This is strongly suggested by the observation that under certain conditions of constant illumination the rhythm of locomotor activity may "split" into two distinct components which run with different frequencies for a while before they resynchronize with each other at a new phase relationship (*3, 6, 7*). This phenomenon has received considerable attention, because it might represent a model case for the study of coupling mechanisms within the circadian system of an organism. However, whereas some external conditions causing "splitting" have been identified, the underlying physiological mechanisms are unknown. The results communicated here suggest that a hormone, testosterone, may promote the "splitting" of circadian locomotor rhythms in the starling, *Sturnus vulgaris*.

The first evidence suggesting that splitting may result from changes in the hormonal state of these birds came from experiments in which groups of 6 to 16 male starlings were transferred