Effects of Intense Antecedent Sympathetic Stimulation on Sympathetic Neurotransmission in the Heart

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We studied the effects of intense sympathetic stimulation on the chronotropic responses of the heart to subsequent test stimulations of the cardiac autonomic nerves in dogs anesthetized with a-chloralose. Such intense sympathetic stimulations (which we refer to as “release stimulations”) are known to release neuropeptide Y as well as norepinephrine. The changes in cardiac cycle length evoked by vagal and sympathetic test stimulations were progressively more attenuated as we increased the frequency and duration of the antecedent sympathetic release stimulations. We found that 2.5 minutes after a maximal release stimulation (30 Hz for 5 minutes), the mean ± SEM chronotropic responses to the vagal and sympathetic test stimulations were diminished to 36.5 ± 1.6% and 54.7 ± 1.3%, respectively, of the prestimulation responses. The mean times for the chronotropic responses to the vagal and sympathetic test stimulations to recover to their control values were 52.0 ± 1.3 and 63.2 ± 2.9 minutes, respectively. This enduring effect suggests the action of a neuropeptide, such as neuropeptide Y. Phentolamine potentiated the inhibitory effects of the sympathetic release stimulations. The chronotropic responses to isoproterenol infusions were not affected appreciably by antecedent sympathetic release stimulation. We conclude, therefore, that the inhibitory effects of antecedent sympathetic release stimulation on cardiac sympathetic neurotransmission are mediated prejunctionally, probably via an inhibition of the neuronal release of norepinephrine by neuropeptide Y. (Circulation Research 1993;72:137–144)

KEY WORDS • autonomic nervous system • heart rate • neuropeptide Y • cardiac parasympathetic nerves • cardiac sympathetic nerves

Sympathetic nerve fibers, including those that innervate the heart, release not only norepinephrine (NE) but also certain neuropeptides, especially neuropeptide Y (NPY).1–3 NPY and NE are released together from sympathetic nerves in response to spontaneous neural activity,4,5 to electrical stimulation of the cardiac nerve trunks,6,7 or to field stimulation of the sympathetic nerve fibers in isolated blood vessels.8–10

NPY may act postjunctionally to affect the mechanical and electrophysiological properties of cardiac tissues.11–13 However, its principal cardiac action appears to be mediated prejunctionally; i.e., it modulates the release of various neurotransmitters from autonomic nerve endings. NPY attenuates the vagal effects on heart rate, atrioventricular conduction, and atrial contractility,7,14–18 presumably by inhibiting the release of acetylcholine from vagal nerve endings.15 NPY also modulates sympathetic neurotransmission, but its physiological role is not well established. Exogenous NPY diminishes the release of NE evoked by sympathetic stimulation in perfused guinea pig hearts,16 in isolated guinea pig atria,17 and in isolated human submandibular arteries.20 However, these effects of NPY on sympathetic neurotransmission were either produced by exogenous neuropeptide Y or elicited in blood vessels in vitro. Therefore, whether sufficient quantities of NPY are released from the sympathetic nerve endings to influence sympathetic neurotransmission appreciably under more physiological conditions is still uncertain.

The present experiments were designed to determine whether an antecedent period of intense sympathetic stimulation, presumably by releasing NPY, inhibits cardiac sympathetic neurotransmission in vivo. We compared the observed effects on sympathetic neurotransmission with those on cardiac vagal neurotransmission, because substantial evidence has already been accrued to show that such intense sympathetic stimulations, probably by releasing NPY, do inhibit vagal neurotransmission substantially and persistently.7,14–18 Hence, the effects on vagal neurotransmission serve as an index of the neuronal release of NPY.

Materials and Methods

Surgical Preparations and Recording Techniques

Thirty mongrel dogs (8–24 kg) were premedicated with morphine sulfate (2 mg/kg i.m.) and anesthetized with a-chloralose (100 mg/kg i.v.). A tracheal cannula was inserted through a midline cervical incision. The
A. Series 1 and 2

![Diagram of series 1 and 2 with labels and timing details]

B. Series 3

![Diagram of series 3 with labels and timing details]

FIGURE 1. Panel A: Time course of the vagal (V1–V5) and sympathetic (S1–S8) test stimuli and of the sympathetic release stimulation in the experiments in series 1 and 2. Panel B: Time course of the vagal test stimuli (V1–V4), the isoproterenol infusions (ISO1–ISO3), and the sympathetic release stimulation in the experiments in series 3.

chest was opened transversely at the fourth intercostal space, and intermittent positive-pressure ventilation was instituted via the tracheal cannula. Arterial blood pressure was recorded from a femoral artery by means of a Statham transducer (model P23BB).

Both cervical vagi were crushed with ligatures to interrupt conduction. A pair of stainless-steel plunge electrodes (0.2 mm, insulated to within 1 mm of the tip) was inserted into the right vagus nerve caudal to the ligature. The electrodes were connected to an electronic stimulator (model S-4, with isolation unit, Grass Instrument Co., Quincy, Mass.). Vagal stimulation by this technique evokes stable responses over many hours.

Both stellate ganglia were isolated, and their upper poles were crushed by tight ligatures to interrupt the sympathetic neural impulses to the heart. Shielded iridium electrodes (Harvard Apparatus, South Natick, Mass.) were applied to the right and left ansae subclaviae. The electrodes were connected in parallel to a second Grass stimulator (model S-4, with isolation unit).

The atrial electrogram (A wave) was recorded from a bipolar electrode catheter that was introduced into the right atrial appendage. The cardiac cycle length was computed by inputting the atrial electrogram signal to a parallel-logic analog computer (model EAI 580). The cardiac cycle length and the arterial blood pressure were recorded on an eight-channel oscillograph (Gould ES 1000).

Experimental Protocols

Three series of experiments were conducted. In series 1, we determined whether a period of intense sympathetic stimulation inhibits the subsequent chronotropic responses to weaker vagal and sympathetic test stimulations. In series 2, we determined the effects of the frequency and duration of the intense sympathetic stimulations and the effects of an α-adrenergic blocking agent (phenolamine) on the chronotropic responses to vagal and sympathetic test stimulations. In series 3, we compared the effects of intense sympathetic stimulation on the chronotropic responses to vagal test stimulation with its effects on the chronotropic responses to a β-adrenergic agonist (isoproterenol [ISO]). No animal was used in more than one of these series.

Series 1: Comparative effects on sympathetic and parasympathetic neurotransmission. We used the stimulation regimen diagrammed in Figure 1A to assess the effects of an intense bilateral sympathetic stimulation, designed to release substantial amounts of NPY, on the chronotropic responses to sympathetic and vagal test stimulations. During an initial control period in one subgroup of animals (n=13), we evoked three chronotropic responses to vagal (V1–V3 in Figure 1A) and sympathetic (S1–S3) test stimulations to obtain control indexes of vagal and sympathetic neurotransmission. The vagal test stimulations consisted of 10-second trains of electrical pulses, each 1 msec in duration and supramaximal in voltage (10–15 V). The frequency of these stimuli was adjusted initially to double the cardiac cycle length; the stimulation characteristics were not altered thereafter. The mean±SD frequency was 5.2±4.1 Hz. The time between successive vagal test stimulations was 2.5 minutes (Figure 1A).

Twenty seconds after the cessation of each vagal test stimulation, we delivered a sympathetic test stimulation.
These stimulations consisted of 30-second trains of electrical pulses, each 1 msec in duration, supramaximal in voltage, and delivered at a frequency of 3 Hz. We used the chronotropic responses (i.e., the changes in cardiac cycle lengths) to the vagal and sympathetic test stimuli as indexes of parasympathetic and sympathetic neurotransmission.

After we had recorded the control responses to the test stimulations, we delivered a single 5-minute train of intense bilateral sympathetic stimulation (15 V, 30 Hz, 1-msec pulse width) to release appreciable quantities of NPY from the sympathetic nerve endings in the heart (Figure 1A). We will refer to this intense stimulation as a sympathetic “release” stimulation. To assess the effects of the release stimulation on subsequent vagal and sympathetic neurotransmission, we again measured the chronotropic responses to periodic vagal (V1, V2, . . .) and sympathetic (S1, S2, . . .) test stimulations until the responses returned to their respective control levels.

In a second subgroup of animals (n = 6), we implemented an abridged protocol similar to that shown in Figure 1A. The principal difference was that we introduced a sham, rather than an actual, sympathetic release stimulation. The purpose of this sham stimulation experiment was to determine whether the sympathetic test stimulations that were given before the release stimulation exerted any detectable effects on the responses to the sympathetic test stimulations that followed the release stimulation.

Series 2: Effects of the frequency and duration of the sympathetic release stimulation and of a-adrenergic receptor blockade. Animals were assigned randomly either to a control group (n = 6) or to a group that received phentolamine (n = 5); no animal was assigned to both groups. Each experiment, regardless of the group, was divided into two observation periods. Each period included four stimulation regimens (each similar to that shown in Figure 1A). In two of the regimens, the sympathetic release stimulations were 1 minute in duration (D1), and in the other two, they were 5 minutes in duration (D2). For each stimulation duration, the frequencies were 5 Hz (F5) and 10 Hz (F10). Thus, four combinations of durations and frequencies (D1F5, D2F5, D1F10, and D2F10) were applied in a random sequence within each observation period. For each stimulation regimen, the prerelease and postrelease test stimulations were delivered as shown in Figure 1A. For each stimulation regimen, we waited for the test stimulation responses after the release stimulation to recover to at least 95% of the control (prerelease) responses before we began the next stimulation regimen.

During period 1, the test stimulation regimens were identical, regardless of the group. In the phentolamine group, a bolus of phentolamine (2 mg/kg) was given intravenously at the beginning of period 2, and a supplemental infusion of 1 mg/kg per hour was given throughout that period. In this group, period 1 served as an internal control for the effects of phentolamine. The control group did not receive phentolamine, but these animals were given an equivalent volume of saline. In this group, we compared the responses in periods 1 and 2 to assess the changes in the cardiac responses over the elapsed time between periods.

Series 3: Effects on the chronotropic responses to isoproterenol infusions. This series of experiments was designed to determine whether intense sympathetic stimulation, presumably by releasing NPY, affects sympathetic neurotransmission by inhibiting the responsiveness of the cardiac effector cells to β-adrenergic agonists. Animals were assigned randomly to a sympathetic release stimulation group (n = 6) or to a sham stimulation group (n = 3). During a control period in the release stimulation group, we measured the chronotropic responses to two vagal test stimulations and to two ISO infusions (Figure 1B). The vagal test stimulations and the sympathetic release stimulations were identical to those described in series 1. Beginning 10 seconds after cessation of the first vagal test stimulation (V1), we began to infuse ISO (ISO1, 0.1 μg/kg) intravenously at a constant rate for 1 minute. The second vagal test stimulation (V2) was delivered 10 minutes after the first vagal test stimulation, and V3 was delivered immediately by a second ISO infusion (ISO2). Ten minutes after the cessation of ISO2, we delivered the sympathetic release stimulation (15 V, 30 Hz, 1-msec pulse width, for 5 minutes). In the sham group, we did not stimulate the sympathetic nerves, but we allowed the same time to elapse as in the release stimulation group before we tested the subsequent responses to vagal test stimuli and to ISO infusions.

After the sympathetic release or the sham stimulation, we again measured the chronotropic responses to two vagal test stimulations (V1 and V2) at 5 and 15 minutes after cessation of the release stimulation and to one ISO infusion (ISO1) that was begun 10 seconds after cessation of V1 (Figure 1B). The responses to the ISO infusions required 10 minutes or more to recover; therefore, we determined the response to only one ISO infusion after the sympathetic release stimulation. By 15 minutes after cessation of the release stimulation, its inhibitory effects on sympathetic neurotransmission would have diminished by over 50% (as shown in Figure 4).

Results

Representative Experiment

Figure 2 shows the changes in cardiac cycle length (AA interval) evoked by the vagal and sympathetic test stimulations in the first series of experiments. Before the sympathetic release stimulation (Figure 2A), the basic cycle length was 610 msec. The vagal test stimulation increased the AA interval by 590 msec, and the sympathetic test stimulation decreased the AA interval by 180 msec.

The ansae subclaviae were then stimulated at 15 V and 30 Hz for 5 minutes to release NPY. The first vagal test stimulation, delivered 2.5 minutes after cessation of the release stimulation, increased the AA interval by only 230 msec, and the first sympathetic test stimulation decreased the AA interval by only 130 msec (Figure 2B). We will call the chronotropic response to the first postrelease test stimulation the “initial response” (Ri). We used the formula 100(Ri−Rc)/Rc to express R as a percentage of the corresponding mean control response (Rc). The initial vagal and sympathetic responses shown in Figure 2 were 39% and 72% of their respective control values.

After the release stimulation, the vagal and sympathetic test stimuli were applied every 2.5 minutes (Figure 1A) until the chronotropic responses returned to

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their control values. Figure 3 shows the time courses of the recovery of the chronotropic responses after cessation of the release stimulation (at t=0) in the same animal from which Figure 2 had been recorded. Note that the responses to the vagal and sympathetic test stimuli were maximally inhibited initially, and then they recovered gradually toward their respective control responses (i.e., toward 100%). By 35 minutes after cessation of the release stimulation, the responses to the sympathetic and vagal test stimulations were 5% and 17% below their respective control responses. In this experiment, full recovery of the responses to the sympathetic and vagal test stimulations required 45 and 55 minutes, respectively.

We refer to the area between a recovery curve and the horizontal line that represents the mean control response (100%) as the “summated response.” The summated response represents the effect of the sympathetic release stimulation on the chronotropic responses to vagal or sympathetic test stimulations, integrated over the entire time course of that effect. Thus, the summated response conveys information not only about the magnitude but also about the duration of the response. The shaded area in Figure 3 represents the first 35 minutes of the summated response to the sympathetic test stimulations.

Composite Data

Series 1: Sympathetic and parasympathetic neurotransmission. The mean effects of sympathetic release stimulation on the chronotropic responses to the sympathetic and vagal test stimulations in this subgroup of 13 animals (Figure 4) resembled those evoked in the representative experiment (Figure 3). The mean±SEM baseline AA interval for this subgroup was 443±13 msec. The initial vagal responses (37% of the control response) elicited after the release stimulation were more severely attenuated (p=0.001 by analysis of variance) than were the initial sympathetic responses (55% of the control response). The subsequent responses gradually returned toward their respective control values (Figure 4). The mean±SEM recovery times for the vagal and sympathetic responses were 52±1.3 and 63±2.9 minutes, respectively. The mean summated response to the vagal test stimulations exceeded that to the sympathetic test stimulations (p=0.005).

In the subgroup of six animals in which a sham release stimulation was carried out, the responses to the sympathetic test stimulations immediately after the sham release stimulation were not detectably different.
from the responses before the sham release stimulation. The mean±SEM difference was 0.0±2.3 msec. Hence, the initial test stimulations had no appreciable effect on the responses to the postrelease stimulations.

Series 2: Effects of release stimulation characteristics and of α-adrenergic receptor blockade. The mean effects of the frequency and duration of the sympathetic release stimulation on the initial response ($R_i$) to sympathetic and vagal test stimulations are summarized in Figure 5. The initial inhibitions (100–$R_i$) of the responses to the sympathetic test stimulations (Figures 5A and 5B) varied directly with the frequency ($p=0.05$) and duration ($p=0.003$) of the sympathetic release stimulation (analysis of variance). Similarly, the initial inhibitions of the chronotropic responses to the vagal test stimulations (Figures 5C and 5D) varied directly with the frequency ($p=0.005$) and duration ($p=0.003$) of the sympathetic release stimulation.

In the control group (no phentolamine), the initial inhibitions of the chronotropic responses to sympathetic and vagal test stimulations in period 1 were not significantly different from those obtained in period 2 (Figures 5A and 5C). Hence, the responses did not change as a function of the time that had elapsed between the two periods. Furthermore, in the group that received phentolamine during period 2, the initial inhibitions of the chronotropic responses to sympathetic ($p=0.001$) and vagal ($p=0.02$) test stimulations during period 2 were significantly greater than they were during period 1, before phentolamine had been given (Figures 5B and 5D). Thus, phentolamine significantly augmented the inhibitory effects of the sympathetic release stimulations on the responses to the sympathetic and vagal test stimulations.

The effects of the frequency and duration of sympathetic release stimulation on the summated inhibitions of the responses to the sympathetic and vagal test stimulations are shown in Figure 6. The summated inhibitions of the responses to sympathetic and vagal test stimulations were greater ($p=0.01$ and $p=0.005$, respectively) when the mean frequency of sympathetic release stimulation was 10 Hz than when it was 5 Hz. The summated inhibitions of the responses to the sympathetic and vagal test stimulations were also greater ($p=0.01$ and $p=0.001$, respectively) when the mean duration of sympathetic stimulation was 5 minutes than when it was only 1 minute. Furthermore, in the control group, the summated inhibitions of the responses obtained in period 1 were not significantly different from those obtained in period 2 (Figures 6A and 6C). Except for the responses after the 1-minute release stimulations at 5 Hz, the summated inhibitions of the responses to the vagal and sympathetic test stimulations (Figures 6B and 6D) were augmented by phentolamine ($p=0.001$ and $p=0.01$, respectively).

Series 3: Responses to isoproterenol. The mean effects of sympathetic release stimulation on the chronotropic responses to ISO infusions and vagal test stimulations are summarized in Figure 7. In the release stimulation group, the mean±SEM control AA interval was 614±17 msec. Before the release stimulation, ISO infusions decreased
the AA intervals by 190±50 msec, and the vagal test stimulations increased the AA intervals by 649±69 msec (Figures 7B and 7D). After the release stimulations, the chronotropic responses to the ISO infusions (187±52 msec) did not differ significantly from the control responses. However, the responses to the vagal test stimulations (302±93 msec) were substantially less than the control responses (p<0.001).

Before the sham stimulations (Figures 7A and 7C), the ISO infusions decreased the AA intervals by 169±58 msec, and the vagal test stimulations increased the AA intervals by 680±76 msec. The chronotropic responses to these interventions after the sham stimulations did not differ significantly from those elicited before the sham stimulations.

**Discussion**

Potter\textsuperscript{14,15} was the first investigator to characterize the sustained inhibitory effects of intense sympathetic stimulation on the cardiac chronotropic responses to subsequent vagal test stimulations. The inhibitory effects of sympathetic release stimulation on the responses to subsequent vagal test stimulations in our present experiments confirm Potter's observations\textsuperscript{14,15} and also confirm previous observations from our laboratory.\textsuperscript{7,16,17}

These enduring inhibitory effects of sympathetic release stimulation on vagal neurotransmission\textsuperscript{14–17} cannot be ascribed to the NE released during the antecedent sympathetic release stimulation. The administration of exogenous NE does not persistently inhibit the chronotropic responses to vagal test stimulations.\textsuperscript{14}

Conversely, an intravenous injection of NPY does inhibit vagal neurotransmission persistently.\textsuperscript{14,15,17} NPY is known to be released along with NE from the sympathetic nerve endings.\textsuperscript{4–10} These findings suggest, therefore, that the prolonged attenuation of the vagal responses may be mediated by neurally released NPY. Potter's experiments\textsuperscript{14,15} showed that exogenous NPY did not alter the chronotropic responses to a muscarinic agonist,\textsuperscript{12} and she concluded that NPY, released during intense sympathetic stimulation, persistently depresses vagal neurotransmission by inhibiting the release of acetylcholine from the vagal nerve endings.\textsuperscript{14} Although her conclusions and those of subsequent investigators are based on substantial physiological and pharmacological evidence, they cannot yet be considered established, because a potent, highly specific antagonist to NPY has not been available.

In the present study, we used an experimental protocol similar to that designed by Potter\textsuperscript{14,15} to determine whether cardiac sympathetic neurotransmission is also inhibited by intense sympathetic stimulation. Abundant evidence already indicates that NPY may interact with NPY receptors on the sympathetic nerve endings to suppress the release of NE from those nerve endings. Exogenous NPY decreases the stimulation-evoked release of NE in the perfused guinea pig heart,\textsuperscript{19} in guinea pig atria,\textsuperscript{11} and in isolated human submandibular arteries.\textsuperscript{20}

These findings suggest that NPY may inhibit sympathetic neurotransmission, at least in part, by suppressing the release of NE from the sympathetic nerve endings. However, these previous experiments involved isolated tissue preparations or the administration of exogenous NPY. Therefore, it had not yet been established whether sufficient NPY was released from the cardiac sympathetic nerves in situ to affect cardiac sympathetic neurotransmission.

In the present study, we determined the effects of neurally released NPY on sympathetic neurotransmission in a much more intact preparation than had been used previously. We observed that intense sympathetic
stimulation persistently inhibited the chronotropic responses to sympathetic as well as to vagal test stimulations (Figures 2–4). However, the effects on the sympathetic responses were less pronounced than were those on the vagal responses (Figures 2–4). The attenuation varied directly with the frequency and duration of the sympathetic release stimulation (Figure 5). The initial and summated responses to sympathetic test stimulations were inhibited significantly, even when the sympathetic release stimulations were delivered for only 1 minute at 5 Hz (Figures 5 and 6).

The attenuation of sympathetic neurotransmission in our experiments might have been produced, at least partly, by a depletion of the releasable pool of NE in the sympathetic nerve terminals during the antecedent period of intense sympathetic stimulation or by direct damage to the nerve fibers. We would anticipate, however, that such processes would be minimal and transient. The sympathetic nerve terminals are able to synthesize NE, to adjust the rate of synthesis to the rate of neural activity, and to take up much of the released NE.23,24 Hence, the sympathetic nerves can release substantial quantities of NE for long periods of time; the releasable pools of NE in the sympathetic nerve endings resist depletion very effectively.23,24

Hall et al25 modified Potter’s stimulation regimen14,15 to determine the effects of a series of intense sympathetic stimulations, each for 1 minute, on cardiac vagal neurotransmission in anesthetized dogs. They observed that the intense sympathetic release stimulations inhibited cardiac vagal neurotransmission, in confirmation of previous studies.7,14–17 However, the inhibitory influence exerted by each successive release stimulation diminished progressively, until the inhibitory effect became negligible after an average of about 20 such stimulations.25 Hall et al also observed that the cardiac chronotropic responses to the repetitive sympathetic release stimulations remained remarkably constant.

Hall et al25 concluded, therefore, that the successive sympathetic release stimulations did not deplete the releasable pool of NE in the sympathetic nerve terminals; they postulated instead that the releasable pool of NPY in the sympathetic nerve terminals was progressively depleted on successive release stimulations. They invoked such a depletion of NPY to explain the progressive attenuation of the inhibitory influence of successive release stimulations on vagal neurotransmission. Their hypothesis is consistent with 1) the recognized tendencies for the releasable pools of NE in the sympathetic nerve terminals to resist depletion23,24,26 and 2) the recognized tendencies for neuropeptides in general, and NPY in particular, to be vulnerable to depletion.24–27 The neuropeptides are synthesized in the neuronal cell bodies, and the slow process of axonal transport is required to convey them to the nerve terminals, from which they are ultimately released.23,25,27

For the reasons described above, the sympathetic release stimulations probably did not deplete the releasable stores of NE appreciably in our experiments. Therefore, the attenuation of sympathetic neurotransmission in our experiments was probably induced by the same process that attenuated vagal neurotransmission. This hypothesis is supported by our observation (Figure 4) that the attenuations of sympathetic and vagal neurotransmission follow a very similar time course. Furthermore, phentolamine exerts similar influences on sympathetic and vagal neurotransmission (Figures 5 and 6). As stated above, substantial, but not incontrovertible, evidence indicates that NPY, released during intense sympathetic stimulation, is responsible for the attenuation of vagal neurotransmission. It is likely, therefore, that neurally released NPY is also responsible for the attenuation of sympathetic neurotransmission in our experiments.

The accentuated inhibition of the chronotropic responses to the sympathetic test stimulations by phentolamine (Figures 5 and 6) parallel our previous observations16,17 that phentolamine significantly augmented the inhibitory effects of intense sympathetic stimulation on vagal neurotransmission. Activation of α-adrenergic receptors on the sympathetic nerve endings inhibits the release of NPY and of NE from those nerve endings, and α-adrenergic receptor blockade enhances the release of these mediators.5,26,29

Potter15 attempted to determine whether the attenuation of the vagal effects on heart rate induced by intense sympathetic stimulation was mediated prejunctionally (by a reduction in acetylcholine release) or postjunctionally (by a reduction in the responsiveness of the sinoatrial node cells). She found that, in anesthetized dogs, neither intense sympathetic stimulation (which releases NPY) nor exogenous NPY altered the chronotropic responses to a muscarinic agonist.15 Thus, the effect of intense sympathetic stimulation on parasympathetic neurotransmission appeared to be prejunctional.

We used a similar strategy in the present experiments to assess whether sympathetic release stimulation affected sympathetic neurotransmission prejunctionally or postjunctionally. We tried to distinguish whether the attenuation of the responses to sympathetic test stimulations (Figures 2–4) evoked by sympathetic release stimulation was induced 1) by a diminished neuronal release of NE during the postrelease sympathetic test stimulations or 2) by a diminished responsiveness of the cardiac pacemaker cells to β-adrenergic agonists.

In our experiments in series 3, the responses to ISO infusions were not affected by the antecedent sympathetic release stimulation (Figure 7B). Hence, either the NPY released by the intense sympathetic stimulation did not alter the sensitivity of the cardiac pacemaker cells to β-adrenergic agonists, or our intense sympathetic stimulations did not release appreciable quantities of NPY in this series of experiments. With regard to the first of these alternatives, Franco-Cereceda et al11 observed responses that paralleled our results; they found that exogenous NPY did not affect the response of the guinea pig atrium to NE.

With regard to the second alternative proposed above, we used the cardiac responses to the vagal test stimulations as an index of the quantity of NPY released by our sympathetic stimulations. A recent study7 from our laboratory demonstrated that the overflow of NPY into the coronary sinus blood in response to stimulation of the cardiac sympathetic nerves correlated well with the subsequent attenuation of vagal neurotransmission elicited by that sympathetic stimulation. The results of our present experiments showed that the chronotropic responses to the vagal test stimulations were significantly attenuated after sympathetic release stimulation (Figure 7D). The sustained attenuation of the vagal
responses suggests that NPY was indeed released by the intense sympathetic stimulation. Our present experiments also showed that the chronotropic responses to ISO infusions were not appreciably affected by intense sympathetic release stimulation (Figure 7D). The most likely conclusion, therefore, is that the NPY released by intense sympathetic stimulation acts prejunctionally to suppress the subsequent release of NE from the sympathetic nerve endings, just as it acts prejunctionally to suppress the release of acetylcholine from the parasympathetic nerve endings. However, the conclusion that these phenomena are mediated by NPY will probably not be established unequivocally until a specific and potent NPY antagonist becomes available.

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References

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