Attenuation of Long-Lasting Effects of Sympathetic Stimulation After Repeated Stimulation

G.T. Hall, T.D. Gardner, and Erica K. Potter

Neuropeptide Y (NPY) is stored with norepinephrine in sympathetic nerves throughout the cardiovascular system and is released during activation of the sympathetic nervous system in humans and other animals. After stimulation of the cardiac sympathetic nerves in anesthetized dogs, the action of the vagus nerve on heart rate is attenuated for a prolonged period. This attenuation of cardiac vagal action is also seen after injection of NPY. Both sympathetic stimulation and exogenous NPY inhibit cardiac vagal effects by acting on postganglionic vagal nerves. Because the supply of neuropeptides to nerve terminals is by axonal transport, it might be expected that repeated stimulation of cardiac sympathetic nerves would deplete the sympathetic neural factor, proposed to be NPY. In all 11 dogs of this study, repeated episodes of stimulating the cardiac sympathetic nerve (16 Hz for 1 minute each) had a diminishing effect in attenuating cardiac vagal action. However, the episodes of sympathetic stimulation did not show diminishing effectiveness in increasing heart rate. Exogenous NPY had similar inhibitory effects on vagal action whether given at the beginning or the end of the episodes of sympathetic stimulation. Transmural stimulation of sympathetic nerves around rabbit ear arteries produced effects that are also mimicked by NPY. These are prolonged potentiation of contractions evoked by injection of norepinephrine or by brief bursts of transmural stimulation. Repeated stimulations in this case also had diminishing abilities to evoke such potentiations. Both sets of observations are consistent with repeated stimulation of sympathetic nerves causing depletion of a nonadrenergic transmitter, possibly NPY. (Circulation Research 1990;67:193–198)

Neuropeptide “cotransmitters” have been described as localized and released with classical transmitters in many central and peripheral nerves. In a number of tissues, administration of classical transmitter and neuropeptide cotransmitter together, but not classical transmitter alone, mimics the response seen after electrical stimulation. Generally, the component of the response characterized by slow onset and long duration can be attributed to the neuropeptide(s), whereas the classical transmitter causes rapid and short-lasting effects. This property of long-lasting action may also allow neuropeptides to act as neuromodulators.

One notable difference between peptide and classical transmitters is their method of replenishment to nerve endings. Unlike the classical transmitters, peptide transmitters are not replenished in the nerve endings by reuptake or resynthesis. Rather, replenishment of released peptide is dependent on synthesis in the cell body and axonal transport to the nerve ending. This is a comparatively slow mechanism. One can predict, therefore, that repeated stimulation of cut nerves should exhaust the nerve endings of peptide, while the primary transmitter may remain in good supply because of reuptake or resynthesis in the nerve terminal. The present study tested this prediction. The principal element of the study was to observe the effects on cardiac vagal action of repeated trials of high-frequency electrical stimulation of the cardiac sympathetic nerve. After such stimulation, the action of the vagus nerve on the heart is known to be attenuated for a prolonged period, an action proposed to be due to a neuropeptide, probably neuropeptide Y (NPY), released during stimulation of the sympathetic nerve. Injected NPY is known to attenuate cardiac vagal action in a
manner similar to sympathetic nerve stimulation.7-10 Furthermore, it is known that an NPY-like immunoreactive material appears in coronary sinus blood after sympathetic stimulation.11

Additional experiments addressing the same principle were also conducted and carried out on isolated rabbit ear arteries, in which the sympathetic nerves were stimulated transmurally by field stimulation. Such stimulation is known to be followed by a prolonged potentiation of the vasoconstrictive effects of brief periods of nerve stimulation and of exogenous norepinephrine on the artery.12 This effect also has been ascribed to a neuropeptide (again, probably NPY) released during the sympathetic stimulation.

**Materials and Methods**

**Dogs**

Experiments were conducted on 11 adult mongrel dogs (4–11 kg). Each animal was anesthetized with 60–100 mg/kg α-chloralose (Sigma Chemical Co., St. Louis) after induction with 15 mg/kg thiopentone (Pentothal, Abbott Laboratories, Sydney, Australia). The trachea was cannulated, and the dog was artificially ventilated. A femoral artery was cannulated for measuring arterial blood pressure and a femoral vein for administering drugs or further doses of anesthetic. Blood pressure was recorded on one channel of a Grass polygraph (model 79D, Quincy, Mass.). Pulse interval (PI, the interval between successive beats of the heart) was also measured, triggered beat by beat from the electrocardiogram, which was monitored on an oscilloscope to confirm the absence of atrioventricular blockade during stimulation of the vagus nerve. Body temperature was maintained at 38±0.5°C.

Both vagus nerves were cut high in the neck, and the right vagus was used for stimulation with platinum electrodes. The vagus nerve was stimulated supramaximally (approximately 35 V, 1 msec) for 6 seconds every minute at approximately 2 Hz. The precise stimulus frequency was adjusted to give a submaximal increase in PI of the order of 400 msec.

The stellate ganglion on the right was exposed, and all its branches were cut except the cardiac sympathetic nerve that had been first identified by acceleration of the heart when it was stimulated electrically.7-9 The portion of the cardiac sympathetic nerve that ran between the stellate ganglion and the heart was stimulated supramaximally at approximately 20 V, 1 msec 16 Hz (20 Hz in some trials) for 1 minute in each trial. Only some of the cell bodies of postganglionic neurons that run to the heart lie in the stellate ganglion. A large number of preganglionic fibers traverse the stellate ganglion to synapse nearer to the heart.13-15 Thus, stimuli can be assumed to have been delivered to some preganglionic and to some postganglionic fibers in the experiments described here.

Each trial consisted of repeating intermittent vagal stimulation over a period during which a reproducible increase in PI (Δ PI) was first established (usually a PI change of approximately 400 msec), followed by a 1-minute period of sympathetic stimulation. This sympathetic stimulation caused an immediate, short-lasting cardioacceleration, followed by a period of vagal attenuation lasting up to 45 minutes.8 This period of attenuation was allowed to wear off, and vagal action on PI was allowed to return to control levels before proceeding with the next trial. Because the return of the depressed vagal action on PI to control levels is linear with time,13 full recovery for each trial took twice the half-times recorded in Table 1. Usually, three or four stimuli were given at the regained control level before a new trial was begun. This whole procedure was repeated for many trials (range, 14–37 trials; average, 22 trials). The duration of each experiment was long (range, 4.5–14.5 hours; average, 6.5 hours). Two variables were used to measure the extent of attenuation of vagal action after sympathetic stimulation: the half-time to recovery measured in minutes,16 and the maximum percent inhibition of the change in PI evoked by vagal stimulation.

### Table 1. Diminished Attenuation of Vagal Inhibitory Effects After Repeated Trials of Sympathetic Stimulation

<table>
<thead>
<tr>
<th>Dog</th>
<th>No. of trials</th>
<th>Maximum percent inhibition at trial 1</th>
<th>Half-time to recovery at trial 1 (min)</th>
<th>Maximum percent inhibition at final trial</th>
<th>Half-time to recovery at final trial (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>48</td>
<td>16</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>60</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>65</td>
<td>24</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>75</td>
<td>18</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>70</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>55</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>70</td>
<td>11</td>
<td>20</td>
<td>4</td>
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<td>8</td>
<td>27</td>
<td>60</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
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<td>44</td>
<td>10</td>
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<tr>
<td>10</td>
<td>15</td>
<td>49</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>50</td>
<td>12</td>
<td>15</td>
<td>3</td>
</tr>
</tbody>
</table>
Max percent inhibition =
\[
\frac{\Delta \text{PI control} - \Delta \text{PI when maximally inhibited}}{\Delta \text{PI control}} \times 100
\]

In four of the dogs, a test injection of NPY (50 \(\mu\)g/kg i.v. bolus, porcine NPY, Peninsula Lab, Belmont, Calif.) was given early in the series of trials (usually after trial 3) and another at the end of the series of trials when the attenuation of vagal action had been reduced.

**Rabbit Ear Arteries**

A complementary series of experiments was conducted in isolated, perfused rabbit ear arteries. Isolated segments of artery were cannulated and perfused at constant flow with Krebs’ bicarbonate solution of the following millimolar composition: NaCl 118, NaHCO3 25, NaH2PO4 1.33, KCl 4.7, CaCl2 2.7, MgCl2 1.44, aerated with 5% CO2 in O2 and maintained at 37° C. Perfusion pressure was measured using a Statham P23AC transducer (Gould Instruments, Cleveland) and recorded on a polygraph (Grass model 79). Contractions of the perfused arterial segment were evoked in two ways: 1) by injecting norepinephrine (0.5–3 ng in 0.05–0.2 ml) through a rubber connection close to the cannula, or 2) by transmural nerve stimulation\(^{17}\) (8–20 Hz, 0.3 msec, 80–100 V for 5 seconds) using a Grass SD 9 square-wave stimulator. Potentiation of both kinds of contraction is seen after high-frequency transmural nerve stimulation (15 Hz for 3 minutes), and this has been attributed to the effects of NPY released from the nerve terminals during the high-frequency stimulation.\(^{10}\) Repeated trials of high-frequency stimulation were given, and the potentiating effects on contraction of the arterial segment were recorded. Arteries from both ears were used, and one was always used as a control because the perfusion was stopped during the high-frequency transmural nerve stimulation.

**Results**

**Dogs**

In all 11 dogs tested, there was marked attenuation of vagal action after sympathetic stimulation. This attenuation was reduced after repeated trials of such stimulation in all dogs (Table 1). The maximum percent inhibition at trial 1 ranged from 44% to 75%, and this was reduced to a range of 0–30% (\(p<0.001\), paired \(t\) test). In six dogs, it was reduced to zero; that is, in the final trial there was no attenuation of vagal action after sympathetic stimulation. In the other five dogs, this attenuation of vagal action after sympathetic stimulation was reduced to 10–30%. The half-time for recovery ranged from 10 to 35 minutes, and this was reduced to 0–7 minutes (\(p<0.001\), paired \(t\) test). The number of trials for each dog ranged from 14 to 37 trials. In every dog, the immediate decrease in PI seen during the 1-minute sympathetic stimulation remained within ±10% of control level, and there was no systematic or significant change with progressive trials.

Figure 1 shows an example of the effects of repeated trials. Each point represents one trial, that is, a period of vagal attenuation after sympathetic stimulation. In the example illustrated (dog 6 in Table 1), there was an initial inhibition in trial 1 of 55% (upper panel) and the half-time to recovery was 35 minutes (middle panel). By the final trial, there was no inhibition of cardiac vagal action after sympathetic stimulation. The lower panel shows that the decrease in PI evoked by sympathetic stimulation showed no significant or systematic change over the 37 trials shown in this figure. (Because of the initially
TABLE 2. Diminished Potentiation of Arterial Contractions Induced by Norepinephrine or Brief Electrical Nerve Stimulation After Repeated Trials of Prolonged Transmural Sympathetic Nerve Stimulation

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>No. of trials</th>
<th>Potentiation at trial 1</th>
<th>Potentiation at last trial</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NE</td>
<td>ES</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
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<td>4.7</td>
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<td>5</td>
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<td>2.5</td>
<td>1.6</td>
</tr>
<tr>
<td>6</td>
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<td>2.4</td>
<td>2.5</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
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<td>8</td>
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<td>1.4</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>31</td>
<td>4.9</td>
<td>1.7</td>
</tr>
</tbody>
</table>

NE, norepinephrine; ES, electrical nerve stimulation.

long recovery times in such experiments, whole experiments of this type take many hours to complete; for example, the data in Figure 1 were collected over 14 hours from the first trial of sympathetic stimulation.)

In four dogs, an intravenous injection of NPY (50 μg/kg) was given early in the series of trials, usually after trial 3, and at the end of the series when little or no attenuation of vagal action after sympathetic stimulation was seen. The amount or duration of attenuation seen with NPY injections was not diminished in any of these animals after the series of progressive trials of sympathetic stimulation. This result is also illustrated in Figure 1.

**Rabbit Ear Arteries**

Pairs of arteries from nine rabbits were used to measure the effect of repeated trials of prolonged high-frequency transmural nerve stimulation on contractions evoked by brief transmural nerve stimulation or by injection of norepinephrine. Perfusion was stopped during prolonged stimulation to allow accumulation of any neuropeptide released. Stopping the perfusion in itself, however, had no effect on the contractions subsequently evoked by transmural nerve stimulation or injection of norepinephrine. In the initial trials, such contractions were potentiated in all nine pairs of arteries. Potentiation in this context is measured as a ratio greater than one, of the first contraction (evoked either by norepinephrine injection or transmural electrical stimulation) after continuous prolonged transmural stimulation (15 Hz for 3 minutes) to the contraction immediately before the prolonged stimulation. This confirms the findings of Glover. Periods of prolonged transmural nerve stimulation were repeated until the potentiation seen after prolonged transmural nerve stimulation was abolished or greatly attenuated. The number of such trials necessary to achieve this varied from six to 31 for the nine rabbits (Table 2). The ratios for potentiation of contraction evoked by norepinephrine injection ranged from 1.4 to 5.1 initially, and in the final trial from 1 to 1.3 (p<0.01, paired t test). The ratios for potentiation for contraction evoked by electrical stimulation ranged from 1.3 to 4.7 initially, and in the final trial from 0 to 2.5 (p<0.001, paired t test). An example from one pair of arteries is seen in Figure 2 (rabbit 1 from Table 2) in which it took 15 trials to abolish the potentiation seen after transmural sympathetic stimulation. Only the first two and the last two trials are shown. Contractions caused by nerve stimulation and by injected norepinephrine are potentiated after electrical stimulation in the early but not in the later trials in the series.

**Discussion**

In the present study we have demonstrated that repeated stimulation of the cut, cardiac sympathetic nerve reduces the ability of such stimulation to attenuate vagal action at the heart. This marked decline in the effect of sympathetic nerve stimulation in attenuating cardiac vagal action cannot be
ascribed to a failure of the electrical stimulus to be reliably transmitted to the nerve terminals because in every dog, the effect of sympathetic nerve stimulation increasing heart rate (presumably, the immediate action of released norepinephrine) did not change significantly during the course of the experiments. Nor can the findings be attributed to a general desensitization of tissues to released NPY because the effect of exogenous NPY on cardiac vagal action was similar whether injected at the beginning or end of the decline. Thus, the observed decline in the effect of sympathetic nerve stimulation in attenuating vagal action, while leaving the effect on increasing heart rate unchanged, suggests that the inhibition of the vagus nerve is mediated, at least in part, by a depletatable transmitter, conforming in this respect to the properties of a peptide transmitter. Such a peptide could be NPY because NPY is known to be colocalized with norepinephrine in many or most nerves going to the heart and blood vessels and because exogenous NPY is known to cause prolonged attenuation of cardiac vagal action.7–10

These findings help reveal the probable participation of a second transmitter in the range of effects of sympathetic nerves. Its depletion was demonstrated here in an experimental arrangement that involved disconnecting the stellate ganglion from all its branches except the nerves that run from it to the heart, and then stimulating those cardiac sympathetic nerves electrically between the ganglion and the heart. Some, but not all, cell bodies of cardiac sympathetic nerves in the dog lie in the stellate ganglion; some preganglionic fibers extend through the ganglion to synapse closer to the heart.13–15 For those cardiac sympathetic fibers that were postganglionic, this arrangement was well suited to such demonstration because replenishment from the cell bodies in that ganglion was thereby prevented. However, it cannot be assumed that, in physiological circumstances, such depletion would be a significant factor in neural control of the heart; replenishment from cell bodies along intact nerves may keep pace with release.

In the rabbit ear artery, the long-lasting potentiation of contractions evoked either by norepinephrine or by transmural nerve stimulation after high-frequency nerve stimulation has been ascribed to an action of NPY released from the sympathetic nerves during stimulation.12 NPY is colocalized with norepinephrine in perivascular nerves, as mentioned above, and injection of NPY into the perfusion fluid causes a similar long-lasting potentiation of evoked contraction.12 Despite abolition of the constrictor potentiating effect of prolonged stimulation, the artery remained able to contract in response to either exogenous norepinephrine or brief nerve stimulation. We therefore attribute the decline in this potentiating response to the depletion of a transmitter, again possibly NPY, after repeated trials of high-frequency transmural nerve stimulation. One further observation is consistent with the depletatable, potentiating factor being NPY. In a recent study of rabbit ear arteries,20 Daly et al used an anti-NPY antibody and showed a decrease in the constrictor responses to brief transmural stimuli similar to the brief test stimuli used in the current study. They concluded that NPY participates in these vasoconstrictor responses. It was noted in the present study that the electrically evoked constrictions declined after repeated, prolonged periods of transmural stimulation. This is shown in Figure 2, for example, and was seen in all experiments. If NPY is partially responsible for the constriction evoked by brief nerve stimulation, then its depletion with repeated prolonged stimulations could account for the smaller test responses.

The experimental preparation in the current study involved field stimulation of postganglionic nerve endings, disconnected from their cell bodies, and was particularly suited to demonstrating depletion of the potentiating factor. It cannot be assumed that such depletion from intact sympathetic nerves would be a physiologically significant factor in neural vasmotor control because normally, replenishment from remote cell bodies might keep pace with release.

There are several examples in the literature of attenuation of a physiological response after repeated nerve stimulation. Stimulation of the parasymathetic nerve to the salivary gland at 40 Hz for 20–80 minutes reduces the atropine-resistant secretion of fluid and proteins.21 Vasoactive intestinal polypeptide and substance P nerves both innervate the parotid gland and are thought to be responsible for the nonadrenergic noncholinergic response. The likely explanation for the decrease in secretion after prolonged nerve stimulation was the neuronal depletion of nonadrenergic noncholinergic transmitters. Furthermore, depletion of NPY from the sympathetic nerves to the heart and blood vessels of skeletal muscle has been measured after reserpine treatment.22 An earlier but more physiological method of peptide depletion has also been described in the central nervous system. Vasopressin can be depleted from the hypothalmo-neurohypophysial system by dehydration.23

It is suggested that the responses described in the two series of experiments reported here are two further examples of depletion of a peptide leading to a functional change. It is likely that the peptide concerned is NPY.

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References


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