Endothelin-induced modulation of neuropeptide Y and norepinephrine release from the rat mesenteric bed

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Received 12 March 2001; accepted in final form 18 June 2002

Hoang, Dan, Heather Macarthur, Alice Gardner, and Thomas C. Westfall. Endothelin-induced modulation of neuropeptide Y and norepinephrine release from the rat mesenteric bed. Am J Physiol Heart Circ Physiol 283: H1523–H1530, 2002. First published June 20, 2002; 10.1152/ajpheart.00177.2001.—The effect of three endothelin (ET) agonists [ET-1, ET-3, and sarafotoxin (STX6C)] on the nerve stimulation-induced release of norepinephrine (NE) and neuropeptide Y-immunoreactive compounds (NPY-ir) from the perfused mesenteric arterial bed of the rat as well as the effect on perfusion pressure were examined. ET-1, ET-3, and STX6C all produced a significant, concentration-dependent decrease in the evoked release of NPY-ir but had no effect on the release of NE. In contrast, all three ETs potentiated the nerve stimulation-induced increase in perfusion pressure. The inhibition of nerve stimulation-induced NPY-ir release by ET-1 was significantly blocked by the ETA/ETB antagonist PD-142893 and the ETB antagonist RES-701-1 but not by the ETA antagonist BQ-123. The potentiation of the nerve stimulation-induced increase in perfusion pressure by ET-1 was significantly blocked by PD-142893 and BQ-123 and attenuated by RES-701-1. Prior exposure of the preparation to indomethacin or meclofenamate failed to alter the attenuation of the evoked release of NPY-ir or the potentiation of the increase in perfusion pressure produced by ET-1 or ET-3. These results are consistent with the idea that sympathetic cotransmitters can be preferentially modulated by paracrine mediators at the vascular neuroeffector junction.

sympathetic neurotransmission

ENDOTHELINS (ETs) are a family of 21-amino acid peptides that consist of at least three isoforms encoded by distinct genes, ET-1, ET-2, and ET-3 (5, 44). The three ETs possess a spectrum of biological activities including both vasodilation and vasoconstriction (38, 44). There appear to be at least three major subtypes of receptors, including ETA, ETB, and ETC, with a further classification into ETA1, ETA2, ETB1, and ETB2 (5, 9, 14). The suggestion has been made that ETs can exert a modulatory effect on the release of various neurotransmitters from sympathetic neurons or other perivascular nerves. ET-1 has been suggested to attenuate sympathetic neurotransmission, whereas it enhances the contractile response to exogenous norepinephrine (NE) in a variety of isolated vascular or nonvascular smooth muscle preparations (22, 31, 32, 34, 41). A similar ET-1-induced decrease in the transmural nerve stimulation-evoked release of ACh and an enhancement of the contractile response to ACh in the guinea pig ileum has also been reported (42). Moreover, ETs have been demonstrated to enhance the contractile effect induced by nerve stimulation or by various vasoactive agents (17, 32, 33, 43).

Considerable evidence suggests that sympathetic neurons innervating the vascular smooth muscle contain at least three chemical mediators, including NE, neuropeptide Y (NPY), and ATP (21, 26). A question of major importance is what regulates the release of these cotransmitters and whether or not they can be differentially or preferentially modulated. The purpose of our study was to investigate the role of ETs on the evoked release of NE and its cotransmitter NPY as well as the postjunctional response using the perfused mesenteric arterial bed as a model of the vascular neuroeffector junction.

MATERIALS AND METHODS

All experiments were performed with male Sprague-Dawley rats weighing 300–400 g obtained from Harlan (Indianapolis, IN). Animals were kept under standard laboratory conditions with food and water ad libitum.

Tissue Preparation

Isolated and perfused mesenteric arterial bed. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and the mesenteric arterial bed was excised and perfused using a modified method as described previously (40). Briefly, the abdomen was opened, and heparin sodium (100 U/ml, 2 mg/kg) was administered via the inferior vena cava. The mesenteric arterial bed and associated intestine were removed after ligation of the descending colon proximal to the rectum and the duodenum proximal to the stomach, and the superior mesenteric artery then was cannulated with polyethylene-90 polyvinyl tubing connected to a syringe and flushed with heparinized saline. The four main branches were ligated. The mesenteric vascular bed was dissected from the intestinal wall and placed in an organ bath maintained at 37°C, perfused, and superfused with a modified...
Krebs-bicarbonate buffer using a Gilson minipump at a rate of 5 and 0.5 ml/min, respectively. The modified Krebs-bicarbonate buffer was composed of the following (in mM): 120 NaCl, 5.0 KCl, 1.2 MgSO4, 2.4 CaCl2, 11.1 dextrose, 25 NaHCO3, and 0.027 EDTA sodium. The perfusate was maintained at 37°C and aerated constantly with 95% O2-5% CO2. The tissue was allowed to equilibrate for 45–60 min with a perfusate containing 1 μM desipramine and 80 μM corticosterone to block the neuronal and extraneuronal uptake of NE, respectively.

Periarterial Nerve Stimulation

Platinum ring electrodes were placed around the artery, and, when appropriate, periarterial nerve stimulation was applied at various frequencies for 1 min (2-ms duration) of supermaximal voltage using a Grass S-88 stimulator. Perfusion effluent was collected in 1-min fractions continuously from the bottom of the organ chamber with the use of a fraction collector. Five samples were pooled into one, and aliquots were taken for analysis of NE and NPY-immunoreactive compounds (NPY-ir) as described below. Perfusion pressure was continuously monitored using a Statham Pressure Transducer and recorded on a Grass recorder. As described previously (37), periarterial nerve stimulation produced a frequency-dependent increase in both NE and NPY-ir and a concomitant increase in perfusion pressure that was abolished in the presence of tetrodotoxin (10−5M). The system was coupled to a Micron PIII computer, with which separations were performed isocratically using a C18 column and an ESA Coulochem II detector. Separations were identified and quantified by HPLC with electrochemical detection as previously described (4). The system consisted of a Varian ProStar model 220 solvent delivery system and a Varian ProStar model 410 autosampler (Varian; Walnut Creek, CA) coupled to a C18 column and an ESA Coulochem II detector. Separations were performed isocratically using a filtered and degassed mobile phase consisting of 10% methanol, 0.1 M sodium phosphate, 0.2 mM sodium octyl sulfate, and 0.1 mM EDTA, adjusted to pH 2.8 with phosphoric acid. The HPLC system was coupled to a Micron PIII computer, with which chromatograms were recorded and analyzed with Varian ProStar workstation software.

NPY-ir was determined in perfusate-effluent samples by radioimmunoassay using a specific antiserum that was raised in the rabbit against porcine NPY (6). Radioimmunoassays were performed using a 5-day disequilibrium method. Duplicated samples were incubated with NPY antiserum. Twenty-four hours later, 125I-labeled NPY was added to each tube. After a 72-h (at 4°C) incubation period, antibody-bound NPY and free NPY were separated by a second antibody method, and the radioactivity was measured in a Packard Cobra II γ-counter. Triplicates were used in standard curves with a sensitivity range of 1–100 fmol.

Table 1. Effect of repeated periarterial nerve stimulation on changes in transmitter release and perfusion pressure over time in the absence of drugs

<table>
<thead>
<tr>
<th>Perfusion Time, min</th>
<th>Increase in NPY-ir Over Basal, fmol/ml</th>
<th>Increase in NE Over Basal, ng/ml</th>
<th>Increase in Perfusion Pressure Over Basal, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.4 ± 0.5</td>
<td>0.20 ± 0.04</td>
<td>186 ± 2</td>
</tr>
<tr>
<td>15</td>
<td>7.5 ± 0.6</td>
<td>0.20 ± 0.02</td>
<td>184 ± 2</td>
</tr>
<tr>
<td>30</td>
<td>7.3 ± 0.4</td>
<td>0.24 ± 0.02</td>
<td>188 ± 3</td>
</tr>
<tr>
<td>45</td>
<td>7.7 ± 0.3</td>
<td>0.22 ± 0.03</td>
<td>184 ± 3</td>
</tr>
<tr>
<td>60</td>
<td>7.4 ± 0.4</td>
<td>0.23 ± 0.04</td>
<td>185 ± 2</td>
</tr>
<tr>
<td>75</td>
<td>7.2 ± 0.5</td>
<td>0.20 ± 0.03</td>
<td>187 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE. After an equilibration period of 45–60 min, preparations were stimulated for 1 min at 15-min intervals, and samples were collected and analyzed for neuropeptide Y-immunoreactive compounds (NPY-ir) and norepinephrine (NE) as described in MATERIALS AND METHODS. Perfusion pressure was continuously monitored.

Statistical Analysis of Data

Statistical analysis of differences in release of NPY-ir and NE as well as perfusion pressure was carried out by one-way ANOVA, followed by Newman-Keuls multiple-comparison tests. A P value of 0.05 or smaller was considered statistically significant.

RESULTS

In the first set of experiments, NPY-ir levels went from a prestimulation (basal) value of 2.1 ± 0.03 to 9.5 ± 0.7 fmol/ml after nerve stimulation. NE levels went from a prestimulation value of 0.1 ± 0.008 to 0.30 ± 0.02 ng/ml after nerve stimulation. Perfusion...
with ET-1 at various concentrations for 15 min produced a concentration-dependent attenuation of the periarterial nerve stimulation-induced release of NPY-ir but had no significant effect on the nerve stimulation-induced release of NE (Fig. 1). To determine whether perfusion with ET-1 for 15 min produced the maximal inhibition of the evoked release of NPY-ir and was long enough to see an effect on NE release, the following experiments were performed. Various concentrations of ET-1 were added to the perfusion buffer from 15 to 60 min before the periarterial nerve stimulation-induced release of NPY-ir and NE. It can be seen in Table 2 that increasing the exposure time of the mesenteric arterial bed to ET-1 did not result in further suppression of the evoked release of NPY-ir nor was an inhibitory effect on NE release revealed. Figure 2 depicts the effect of ET-3 on the periarterial nerve stimulation-induced release of NPY-ir and NE. Basal NPY-ir values were 1.7 ± 0.04 fmol/ml and increased to 8.78 ± 0.6 fmol/ml after nerve stimulation. Prestimulation NE levels were 0.06 ± 0.01 ng/ml and increased to 0.3 ± 0.02 ng/ml after stimulation. ET-3 also produced a significant and concentration-dependent attenuation in the electrically induced release of NPY-ir, whereas it had no effect on NE release (Fig. 2). We next studied the EBB-selective agonist STX6C. STX6C produced a similar response as ET-1 and ET-3. In these experiments, basal NPY-ir values went from 2.0 ± 0.04 to 9.4 ± 0.6 fmol/ml after nerve stimulation. NE went from a prestimulation level of 0.08 ± 0.004 to 0.4 ± 0.02 ng/ml after stimulation. As with ET-1 and ET-3, STX6C produced a significant decrease in the evoked release of NPY-ir, whereas it failed to alter the effect of periarterial nerve stimulation-induced release of NE (Fig. 3).

The mean basal perfusion pressure for all experiments was 32 ± 0.2 mmHg. Periarterial nerve stimulation at a frequency of 16 Hz in the absence of any drug resulted in an increase of 186 ± 2 mmHg. Time-control experiments in which the mesenteric bed was stimulated up to six times at 15-min intervals resulted in similar and reproducible increases in perfusion pressure after all six stimulation periods (Table 1). All three ET agonists resulted in a significant, concentration-dependent potentiation of the periarterial nerve stimulation-induced increase in perfusion pressure (Fig. 4). ET-1 and ET-3 were equally potent and efficacious, whereas ST6C was less potent and efficacious.

### Table 2. Effect of alterations in the time in which the perfused mesenteric arterial bed was exposed to ET-1 before periarterial nerve stimulation

<table>
<thead>
<tr>
<th>Concentration of ET-1, M</th>
<th>10^{-11}</th>
<th>10^{-10}</th>
<th>10^{-9}</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY-ir release, %control release</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of ET-1 exposure, min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>45 ± 8</td>
<td>28 ± 7</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>30</td>
<td>44 ± 10</td>
<td>27 ± 6</td>
<td>21 ± 5</td>
</tr>
<tr>
<td>45</td>
<td>44 ± 9</td>
<td>29 ± 7</td>
<td>23 ± 6</td>
</tr>
<tr>
<td>60</td>
<td>46 ± 7</td>
<td>28 ± 8</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>NE release, %control release</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of ET-1 exposure, min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>110 ± 20</td>
<td>195 ± 35</td>
<td>103 ± 8</td>
</tr>
<tr>
<td>30</td>
<td>105 ± 10</td>
<td>100 ± 7</td>
<td>104 ± 5</td>
</tr>
<tr>
<td>45</td>
<td>105 ± 6</td>
<td>110 ± 11</td>
<td>100 ± 8</td>
</tr>
<tr>
<td>60</td>
<td>111 ± 12</td>
<td>100 ± 8</td>
<td>104 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE. ET, endothelin.
before nerve stimulation to 8.60 ± 0.6 fmol/ml after nerve stimulation. Finally, in the presence of RES-701-01, basal NPY-ir levels went from 1.9 ± 0.04 fmol/ml before stimulation to 9.2 ± 0.7 fmol/ml after nerve stimulation. The effect of the three antagonists on the ET-1-induced attenuation of the periarterial nerve stimulation-induced release of NPY-ir is summarized in Table 3. It was observed that the ETA antagonist BQ-123 did not alter the inhibitory effect of ET-1 on the periarterial nerve stimulation-induced release of NPY-ir. In contrast, both the mixed ETA/ETB antagonist PD-142893 and the ETB antagonist RES-701-1 significantly blocked the ET-1-induced inhibition of the NPY-ir release. None of the three ET antagonist changed the basal or stimulation-induced increase in perfusion pressure, whereas RES-701-1 reduced the increase by 50% but did not completely block the response (data not shown).

To examine whether the inhibitory effect of the ETs was an indirect effect resulting from ET first producing a release of prostanoids, which in turn inhibited the evoked release of NPY-ir, experiments were conducted using indomethacin and meclofenamate. These agents were added to the perfusion buffer 30 min before and together with varying concentrations of ET-1 and ET-3. Treatment with both agents produced a significant increase in the basal level of NPY-ir, going from a value of 1.5 to 9.2 ± 0.5 and 8.5 ± 0.4 fmol/ml after a 30-min perfusion with indomethacin (10 μM) or meclofenamate (50 μM), respectively. In the presence of either agent, ET-1 and ET-3 still produced significant attenuation in the evoked release of NPY-ir (Fig. 5 and 6). Neither indomethacin nor meclofenamate produced any change in basal perfusion pressure, the increase seen due to periarterial nerve stimulation, or the ET-1- or ET-3-induced potentiation of the periarterial nerve stimulation-induced increase in perfusion pressure (data not shown).

DISCUSSION

With the use of the perfused mesenteric arterial bed as a model of the sympathetic neuroeffector junction, we examined the effect of several ET agonists (ET-1,
ET-3, and STX6C) for the ability to alter the high-frequency periarterial nerve stimulation-induced release of NPY-ir and NE and the accompanying increase in perfusion pressure. It was observed that all three ETs significantly attenuated the evoked release of NPY-ir without altering the release of NE. In addition, all three ETs produced a potentiation of the periarterial nerve stimulation-induced increase in perfusion pressure. These findings are consistent with numerous reports suggesting a modulatory role for ETs on sympathetic or cholinergic neurotransmission. The nature of this modulatory role is complex, however, due to the fact that ETs appear to exert both prejunctional and postjunctional actions. Regarding modulation of the NE release, there are studies suggesting that ETs decrease, increase, or have no effect on the electrically evoked release of NE from a variety of vascular or nonvascular smooth muscle preparations in vitro or in vivo (22, 31, 32, 34, 41). The present results disagree with those studies showing that ET decreases the evoked release of NE but are similar to what we reported earlier showing no effect on the evoked release of NE (16). The majority of studies reporting an inhibition in NE release utilized measurements of $^{3}$H after incubation of the tissues with $[^{3}H]NE$ as a marker for NE release. None of these studies report that they verified that the $^{3}$H was indeed $[^{3}H]NE$. In addition, there are known differences in the subcellular pools labeled by $[^{3}H]NE$ versus the endogenous amine itself. The possibility exists that ET decreases the release of $[^{3}H]NE$ from a pool that stores $[^{3}H]NE$ as opposed to the endogenous amine. A more likely explanation is that ET decreases NE release only to low-frequency nerve stimulation and not to higher frequencies. Takagi et al. (34) actually observed that ET decreased NE in the renal vasculature after renal nerve stimulation at low frequencies but not at higher frequencies. Others reported inhibition of NE release only at very high concentrations of ET-1. In those cases where inhibition of $^{3}$H was observed, the inhibition was small as opposed to the very marked inhibition of NPY-ir observed in the present study with low concentrations of ETs. In the present study, we used high-frequency nerve stimulation to simultaneously measure NPY-ir release together with NE. This is consistent with well-known observations that such a type of stimulation is necessary to evoke NPY-ir release (21). Because of the difference between our results and those reported by others, in addition to the fact that some investigators report no effect or even an increase in NE release, the question of ET-induced modulation of NE release is still an open question. In our study, however, it would appear that ETs selectively attenuate only NPY-ir and not NE when high frequencies of nerve stimulation were used as might be encountered under conditions of high sympathetic tone, stress, or disease.

Regarding the postjunctional effects of ET, although some investigators have reported inhibition of the contractile response (43), the majority of studies reported an enhancement of the postjunctional response (17, 28, 33, 43). Such a potentiation of the postjunctional response to sympathetic nerve stimulation was confirmed in the present study even when very low concentrations of ET-1, ET-3, or STX6C were used. Our results are therefore consistent with the numerous studies reporting such potentiation.

The inhibition of nerve stimulation-induced NPY-ir release by ET-1 was significantly blocked by the ET$_{A}$/ET$_{B}$ antagonist PD-142893 as well as by the selective ET$_{B}$ antagonist RES-701-1 but not by the ET$_{A}$ antagonist BQ-123. These results plus the fact that the ET$_{B}$ agonist STX6C mimicked the inhibitory effect of ET-1 provide strong evidence that the inhibitory effect of ET-1 on the evoked release of NPY-ir is due to activation of an ET$_{B}$ receptor. The fact that the ET-1-induced increase in perfusion pressure was blocked by the ET$_{A}$/ET$_{B}$ antagonist as well as the ET$_{B}$ agonist, but only attenuated by the ET$_{B}$ antagonist, suggests that the principle receptor contributing to the increase in per-

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**Fig. 5.** Effects of ET-1 alone or in the presence of indomethacin (Indo; 50 $\mu$M) or meclofenamate (Meclo; 10 $\mu$M) on the periarterial nerve stimulation-induced release of NPY-ir. Each point is the mean ± SE of 5–8 observations.

**Fig. 6.** Effects of ET-3 alone or in the presence of Indo (50 $\mu$M) or Meclo (10 $\mu$M) on the periarterial nerve stimulation-induced release of NPY-ir. Each point is the mean ± SE of 6–10 observations.
fusion pressure is an ETα receptor. However, it is not exclusively due to ETα receptors because all three ET agonists had the ability to produce this effect. It is concluded that the postjunctural response is due to activation of both an ETα and ETβ receptor, the latter most likely being ETβ2.

The fact that the ET antagonist did not affect either basal or stimulated release of NE or NPY-ir deserves mention. This could be interpreted to mean that ET does not exert a physiological relevant modulation of NE or NPY-ir release. However, an ET antagonist would only affect basal or stimulated release of NE or NPY if there were sufficient endogenous ET in the biophase of this vascular sympathetic neuroeffector junction to have an effect (presumably inhibition) that would be altered by the antagonist. ET is probably produced in modest quantities in the present experiments, and removal of the ET-dependent component alone may not lead to a significant and detectable increase in NE or NPY-ir levels. However, if there is enough ET present in the biophase, as is the case when it is applied exogenously or if it is elevated under pathophysiological conditions such as salt-dependent hypertension, stress, or increases in sympathetic tone, then we would see that ET selectively inhibits the evoked release of NPY-ir, and this in turn would be attenuated by the ETβ antagonists.

The fact that ET did not alter the basal perfusion pressure could be interpreted that the postjunctural response is also not physiologically relevant. However, the fact that ET markedly enhanced the nerve stimulation-induced increase in perfusion pressure is consistent with its ability to potentiate the contractile effect of various vasoactive agents and that this is an important response to sympathetic nerve stimulation. Several authors have concluded that the ET system does not contribute to physiological regulation of blood pressure because ET antagonists do not appear to decrease blood pressure in intact normotensive rats. However, it would appear that this point is both controversial and unresolved. For instance, the nonspecific ET antagonist bosentan has been reported to reduce blood pressure in dogs and guinea pigs (7, 37), although admittedly it was ineffective in rats. It is well known that the regulation of arterial tone is complex and involves numerous neuronal, endothelial, and autacoid interactions. It has recently been demonstrated that blockade of the renin-angiotensin system unMASKS potent vasocontractile response to ET antagonists in normotensive anesthetized rats (29). The authors conclude that this suggests that endogenous ET-1 indeed contributes to the normal vasodepressor tone and that this contribution may be evidenced after blockade of the renin-angiotensin system. We therefore maintain that numerous substances that are known or may be physiologically relevant do not increase basal perfusion pressure in the perfused mesenteric arterial bed (e.g., NPY and ET-1) but do in the in vivo situation. It is likely that the reason that the ET-1-induced inhibition of the evoked release of NPY-ir does not result in a decrease in perfusion pressure is because this decrease in NPY-ir release may be overridden by ET-induced potentiation of the increase in perfusion pressure produced by NE, whose release is not inhibited by ET-1.

Previous studies have reported that ETs induce the release of both PGI2 and nitric oxide (NO) from endothelial cells, thereby leading to vasodilatory responses (15). A great deal of evidence has been documented in support of a vasodilatory action of ET. For example, both ET-1 and ET-3 have been reported to stimulate NO release from bovine endothelial cells (39), isolated perfused rat and rabbit blood vessels (38), and other vascular tissues (3). In addition to the production and release of NO, both ET-1 and ET-3 have been shown to stimulate the release of PGI2 from a variety of species and tissues, including bovine and human vascular endothelial cells (13), isolated rat mesenteric arteries (27), and the perfused rat lung (8). The ET-induced release of PGI2 has also been demonstrated in vivo studies using rats (3) and beagle dogs (14).

Because ETs are well known to release PGI2 from endothelial cells and because we have demonstrated that activation of prostacyclin receptors inhibits NPY-ir release (19), the possibility exists that the ET-induced inhibition of NPY-ir release is an indirect effect mediated by prostacyclin rather than directly by ET itself. To answer this question, we examined the effect of ET-1 and ET-3 on the periarterial nerve stimulation-induced release of PGI2 before and after blockade of prostacyclin synthesis utilizing indomethacin and meclofenamate. We observed that both cyclooxygenase inhibitors caused an increase in basal NPY-ir levels. The most likely explanation is that both compounds are removing the endogenous prostacyclin tone that normally exerts an inhibitory effect on NPY-ir levels. In the presence of both compounds, ET-1 and ET-3 still produced a marked, concentration-dependent inhibition of the evoked release of NPY-ir. These results are interpreted to mean that ETs are acting directly to attenuate the nerve stimulation-induced release of PGI2 and not indirectly via prostacyclin. The fact that we observed the same result with two different cyclooxygenase inhibitors gives added strength to this conclusion. Further evidence for a direct effect of ET on NPY-ir release is the fact that two different prostacyclin agonists (carbaPGI2 and cica-prost) inhibited the release of both PGI2 and NE (19), whereas, as observed in the present study, the three endothelin agonists only inhibited NPY-ir release without any alteration in NE release.

Although ETs induce a release of NO as well as PGI2, we further investigated the contribution of NO because other data from our laboratory suggest that it is not likely to participate in the inhibitory effect of ET on sympathetic neurotransmission. We have previously observed that NO interacts with catecholamines (NE, dopamine, and epinephrine) to chemically deactivate them. This takes on the appearance of a NO-induced decrease in catecholamine release (23, 24). Because ET did not alter the NE release in the present study, this potential mechanism is unlikely. Moreover, we have
also observed that NO does not inhibit the evoked release of NPY-ir. In fact, we have seen that NO gradually decrease catecholamines appearing in the incubation medium of nerve growth factor-differentiated PC12 cells, whereas it increased the appearance of NPY-ir (20).

Taken together, these present results strongly suggest that there can be differential or preferential modulation of the release of the sympathetic cotransmitters at least under high-frequency nerve stimulation conditions. Our results provide strong additional evidence to that already existing in the literature. For instance, clonidine inhibited the release of NE but not ATP from the myenteric plexus of the guinea pig ileum (15). In other preparations, including the mouse vas deferens (2), rabbit ileocolic artery (1), and guinea pig (15). In other preparations, including the mouse vas deferens (2), rabbit ileocolic artery (1), and guinea pig (15). In other preparations, including the mouse vas deferens (2), rabbit ileocolic artery (1), and guinea pig.

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Taken together, these present results strongly suggest that there can be differential or preferential modulation of the release of the sympathetic cotransmitters at least under high-frequency nerve stimulation conditions. Our results provide strong additional evidence to that already existing in the literature. For instance, clonidine inhibited the release of NE but not ATP from the myenteric plexus of the guinea pig ileum (15). In other preparations, including the mouse vas deferens (2), rabbit ileocolic artery (1), and guinea pig.


