

## Halothane Decreases the Release of Neuropeptide Y and 3,4-Dihydroxyphenylglycol from Superfused Segments of Dog Pulmonary Artery

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Neuropeptide Y (NPY), norepinephrine (NE), and 3,4-dihydroxyphenylglycol (DOPEG), the metabolite of NE that arises intraneuronally, were measured in superfusates before, during, and after nerve stimulation and in extracts of dog pulmonary artery after superfusion and electrical stimulation (ES) at 12, 6, and 1 Hz. NE and DOPEG were quantified by high-pressure liquid chromatography with electrochemical detection; peptides were quantified by radioimmunoassay. The rate of overflow of NPY, NE, and DOPEG into superfusate was measured over time. The overflow of DOPEG into superfusate during basal conditions was 3.0 times that of NE. Efflux of DOPEG and NPY increased during ES; peak effluxes were not reached, however, until after cessation of stimulation. NE efflux peaked during ES. Effluxes of NE, NPY, and DOPEG were frequency-dependent at 12 and 6 Hz; at 1 Hz efflux of only NE was greater than basal. Halothane decreased significantly the rates of NPY and DOPEG efflux during and after 12 Hz ES; DOPEG efflux evoked by 6 Hz stimulation was also decreased by halothane. The percentage of the total tissue content of NPY that overflowed was decreased by halothane. Halothane did not affect the molar ratios of NE:DOPEG or NE:NPY during basal conditions or ES. These studies provide evidence that halothane slows efflux of NPY that is released along with NE from dog pulmonary artery during high frequencies of stimulation. Halothane also reduces the metabolism of NE to DOPEG. (Key words: Anesthetics, volatile: halothane. Peptides: co-transmitter peptides, neuropeptide Y. Blood vessels: dog pulmonary artery. Sympathetic nervous system, catecholamines: norepinephrine, 3,4-dihydroxyphenylglycol. Enzyme: monoamine oxidase.)

IN RECENT YEARS peptides capable of enhancing contraction or relaxation have been found in blood vessels and airways.<sup>1-3</sup> These peptides, which co-exist and are co-released with the classical neurotransmitter, increasingly appear to modulate the release or action of the classical neurotransmitter.<sup>4-6</sup> Neuropeptide Y (NPY) is a 36-amino acid peptide present in blood vessels having a sympathetic innervation.<sup>4</sup> NPY appears to be stored, along with norepinephrine (NE), in the large dense core granules in postganglionic sympathetic nerve endings inner-

vating a variety of structures, including blood vessels.<sup>4,7,8</sup> NPY and NE are co-released, under certain conditions, into the synaptic cleft.<sup>5,6,9,10</sup> Postsynaptically, NPY evokes a direct vasoconstrictor response in high concentrations in some but not all blood vessels.<sup>5,10-12</sup> NPY also potentiates NE-induced contractions, most likely by an action at postsynaptic sites.<sup>4,11</sup> The potentiation of NE-induced contractions by NPY is manifested at nanomolar concentrations—far below that required by NPY to evoke a direct vasoconstrictor response.<sup>11</sup> Electrical stimulation (ES) of efferent nerves to the spleen,<sup>6</sup> or field stimulation of isolated blood vessels,<sup>13</sup> causes release of NPY along with NE. The release of NPY has been reported to occur predominantly at higher frequencies of ES,<sup>6</sup> in contrast to the release of NE, which occurs at low and high frequencies.<sup>14</sup> Thus, it appears that strong sympathetic activation results in the release of NPY as well as of NE.

Halothane suppresses the response of the sympathetic nervous system to surgery.<sup>15</sup> Most studies of this effect of halothane have focused on NE. Studies of levels of NE in plasma during halothane anesthesia have produced conflicting results: no change, decreased, or increased levels of NE in plasma during halothane have been reported.<sup>15</sup> *In vitro* studies using blood vessels have shown halothane to impair the release of NE induced by 2-Hz ES.<sup>16,17</sup> Since only a small amount of the NE released at peripheral sympathetic nerve endings (*e.g.*, blood vessel walls) escapes both neuronal and extraneuronal uptakes and overflows from the synaptic cleft to ultimately enter the bloodstream,<sup>18</sup> this apparent conflict in results of studies may relate to the inadequacy of plasma NE level as an indicator of NE release at sympathetic nerve endings. NPY is co-stored with NE in sympathetic nerve endings and is a potent potentiator of the pressor effects of NE.<sup>4,11</sup> The current studies were undertaken to determine if the release of NPY was altered by halothane; whether any altered release of NPY caused by halothane corresponded with halothane-induced alterations in NE release; and lastly, whether halothane affected the intraneuronal disposition of NE. Formation of 3,4-dihydroxyphenylglycol (DOPEG), the metabolite of NE that arises intraneuronally through the action of monoamine oxidase (MAO) and aldehyde reductase on NE, was used as the indicator of halothane-induced alterations in the intraneuronal disposition of NE.

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## Materials and Methods

### VESSEL COLLECTION AND PREPARATION FOR STUDY

The studies described in this report were approved by the Institutional Animal Care and Use Committee. Adult mongrel dogs of either sex were fasted for 1 day and then anesthetized with pentobarbital sodium (30 mg/kg iv). After exsanguination, the thorax was opened and the lungs removed by a cut through each hilus. The lungs were placed in preoxygenated Krebs'-Ringer's solution.<sup>19</sup> Lung parenchyma was dissected away from small pulmonary artery selected randomly throughout the lower lobes. Helical strips were prepared from arterial segments between 3 and 1 mm (outside diameter) by cutting transverse to the long axis of the vessel.<sup>20</sup>

### SUPERFUSION OF PULMONARY ARTERIES

The strips of vessel were suspended vertically between and in contact with platinum wire electrodes in a superfusion apparatus maintained at 37° C.<sup>19</sup> Initial force was set at 2 g. The electrodes were attached to a stimulator (Grass model S-44) that delivered impulses of rectangular waves (10 V; 0.2 ms; 1, 6, or 12 Hz). Krebs'-Ringer's solution served as the superfusate. The superfusate was prewarmed and preaerated with 95% O<sub>2</sub>—5% CO<sub>2</sub> in a reservoir tower before being pumped to the upper end of the strip and allowed to flow down over the tissue at 2 ml/min. The superfusate was allowed to accumulate to a volume of approximately 200 μl in the bottom of the superfusion chamber before being pulled (at the same rate of superfusion) by a second pump through a Sep-Pak C-18 cartridge attached directly to the bottom of the superfusion chamber.

Samples of superfusate were collected at 10-min intervals throughout the 100 min of each experiment (samples 1–10). ES was applied to the tissue only during collection of the second sample of superfusate (10–20 min). Eight poststimulation samples were collected subsequently. After the last sample of superfusate was collected, the tissues were removed from the superfusion apparatus, blotted dry, and weighed, and NE and NPY were extracted as described previously.<sup>13</sup> The procedure used for separating NPY and NE from superfusate and from tissue has been described previously.<sup>13</sup>

### QUANTITATION OF NE AND PEPTIDES

For NE and DOPEG a reversed-phase high-pressure liquid chromatography (HPLC) system was used.<sup>13,21</sup> Peptides were quantitated by radioimmunoassay.<sup>22</sup> Recoveries (mean ± SEM) in superfusates were 87.7 ± 1.6% (n = 38) for NE, 62.0 ± 1.2% (n = 36) for DOPEG, and

96.3 ± 2.5% (n = 36) for NPY. The authenticity of the NPY released into superfusate and measured in tissues has been established previously<sup>13</sup> by comparing the elution profile against the profile of authentic NPY when separated by the HPLC system described by Yaksh *et al.*<sup>23</sup>

### HALOTHANE DELIVERY TO SUPERFUSATE

In some of the experiments halothane was added to the gas aerating the Krebs'-Ringer's solution. When halothane was used, the gas used to aerate the Krebs'-Ringer's solution flowed into a copper kettle containing halothane. From there it flowed into a 1-l rubber reservoir bag. A variable speed rotary pump forced the gas from the reservoir bag through a fritted glass disc in the bottom of the reservoir tower where the gas mixture bubbled through the Krebs'-Ringer's solution. The rate of flow of the gas mixture containing halothane into the reservoir tower was approximately 150 ml/min. In addition, this gas mixture flowed through the superfusion chamber, which was enclosed except for a 2-mm opening at the top through which the string used to suspend the vessel strip passed. Thus, little, if any, anesthetic gradient existed between the superfusate saturated with halothane and the air filling the superfusion chamber. The concentration of volatile anesthetic in the gas mixture in transit between the rotary pump and reservoir tower and superfusion chamber was monitored continuously by a Beckman LB II gas analyzer just prior to the point at which the gas entered the reservoir tower and superfusion chamber. Before each experiment, the gas analyzer was calibrated with 1.0 and 2.77% halothane. The concentration of halothane was also measured in samples of superfusate using gas chromatography.<sup>24</sup> When 1.5% halothane was measured by the Beckman gas analyzer in the aerating gas, 1.38 ± 0.02% was measured by gas chromatography in the Krebs'-Ringer's solution used as superfusate.

*Analysis of data* All data are expressed per 100 mg of tissue in order to correct for small variations in weight among the vessel strips. The mean weights were 93.8 ± 1.9 mg (mean ± SE). The data are expressed as means ± SE. Statistical analyses were performed with two-way analysis of variance (ANOVA) followed by Student's *t* tests. A *P* < 0.05 was considered significant.

## Results

### OVERFLOW OF NE, DOPEG, AND NPY DURING BASAL CONDITIONS BEFORE STIMULATION

During basal conditions preceding ES, only small amounts of NE were measured in the superfusate (fig. 1). The rate of overflow of NE into superfusate of vessel strips exposed to halothane (0.06 ± 0.01 pmol/min) was not significantly different from the rate of overflow of

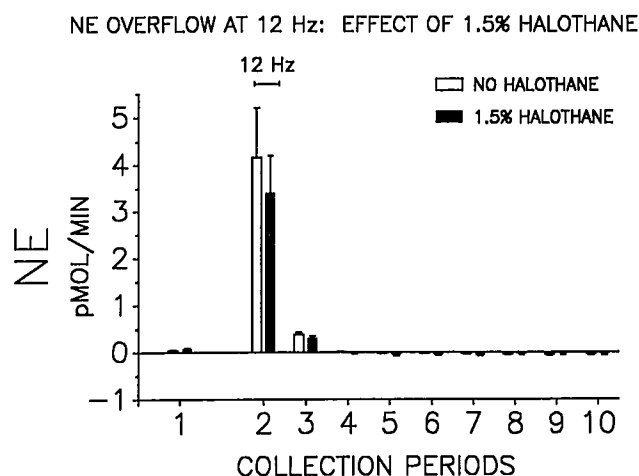


FIG. 1. Norepinephrine (NE) overflow into superfusate from isolated dog pulmonary artery strips during basal conditions (collection period 1) and the increase in overflow of NE above basal during and after continuous ES (12 Hz). ES was delivered only during the second collection period. All collection periods were 10 min. Shaded bars represent overflow from tissues studied in the presence of 1.5% halothane. Data are mean  $\pm$  SE,  $n = 7$  tissues from seven dogs.

NE measured in control strips ( $0.05 \pm 0.006$  pmol/min). In control vessels the rate of DOPEG overflow into superfusate during basal conditions ( $0.14 \pm 0.02$  pmol/min) (fig. 2) was approximately 3.0 times greater than the rate

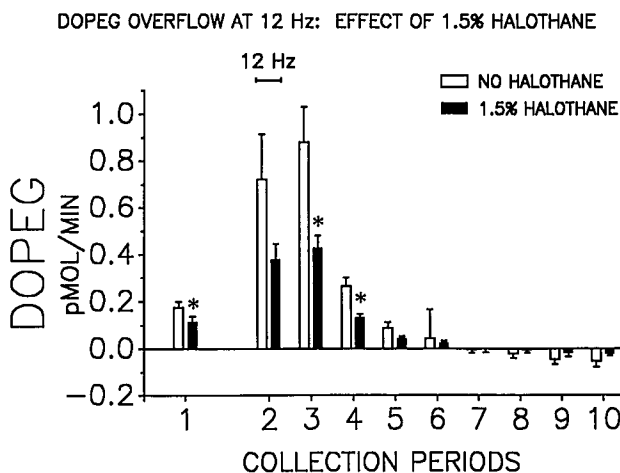


FIG. 2. 3,4-Dihydroxyphenylglycol (DOPEG) overflow into superfusate from isolated dog pulmonary artery strips during basal conditions (collection period 1) and the increase in overflow of DOPEG above basal during and after continuous ES (12 Hz). ES was delivered only during the second collection period. All collection intervals were 10 min. Shaded bars represent overflow from tissues studied in the presence of 1.5% halothane. Data are mean  $\pm$  SE,  $n = 7$  tissues from seven dogs.  $P < 0.01$  for control versus halothane during basal, during ES, and after ES (ANOVA).  $*P < 0.05$  for DOPEG overflow in control versus halothane tissues during collection periods 3 and 4 (unpaired Student's  $t$  test).

of NE overflow ( $0.05 \pm 0.006$  pmol/min) under basal conditions (fig. 1). Exposure of vessel strips to halothane significantly decreased the rate of DOPEG overflow into the superfusate (to  $0.09 \pm 0.008$  from  $0.14 \pm 0.02$  pmol/min) during basal conditions (fig. 2). During basal conditions the rate of NPY overflow into superfusate of vessel strips exposed to halothane ( $0.56 \pm 0.12$  fmol/min) was not significantly different from the rate of NPY overflow into superfusate in control vessels ( $0.76 \pm 0.13$  fmol/min) (fig. 3).

#### OVERFLOW OF NE, DOPEG, AND NPY DURING AND AFTER ELECTRICAL STIMULATION AT 12 Hz

The rate of overflow of NE into superfusate increased markedly during ES; the peak rate of NE overflow occurred during the 10 min of ES (fig. 1). The increase in rate of overflow of NE had returned to a rate of overflow at or below baseline overflow rate by the end of collection of the first 10-min sample of superfusate after cessation of stimulation. The rate of overflow of NE during ES at 12 Hz and during the interval of collection of the first poststimulation sample of superfusate was not decreased significantly by halothane. In contrast to NE, the time of peak overflow of DOPEG or NPY did not coincide with ES; the peak rate of DOPEG overflow was measured in the first poststimulation sample (fig. 2), and for NPY the

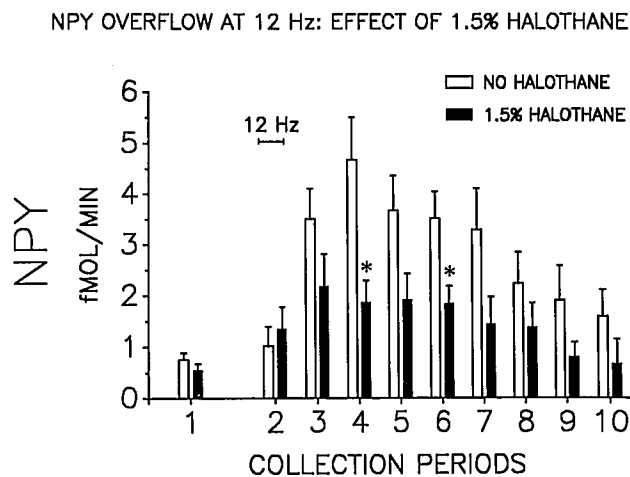


FIG. 3. Neuropeptide Y (NPY) overflow into superfusate from isolated superfused dog pulmonary artery strips during basal conditions (collection period 1) and the increase in overflow of NPY above basal during and after continuous ES (12 Hz). ES was delivered only during the second collection period. All collection intervals were 10 min. Shaded bars represent overflow from tissues studied in the presence of 1.5% halothane. Data are mean  $\pm$  SE,  $n = 7$  tissues from seven dogs.  $P < 0.01$  overflow of NPY in control versus halothane strips (ANOVA).  $P < 0.05$  for NPY overflow in control versus halothane during collection periods 4 and 6 (unpaired Student's  $t$  test).

peak rate of overflow resulting from ES was measured during collection of the second sample of superfusate after cessation of stimulation (fig. 3). In addition, the increase above basal in rate of overflow of DOPEG and NPY persisted for a longer time than did the increase in rate of overflow of NE. The increase in rate of overflow of NPY above basal was still evident 70 min after cessation of ES.

Halothane significantly decreased (ANOVA,  $P < 0.01$ ) the rates of DOPEG and NPY overflow both during and after ES (figs. 2 and 3). Student's *t* test indicated that during collection intervals 3 and 4 for DOPEG and during collection intervals 4 and 6 for NPY, halothane significantly decreased the rate of overflows compared to controls.

#### OVERFLOW OF NE, DOPEG, AND NPY DURING AND AFTER ELECTRICAL STIMULATION AT 6 HZ

The rate of overflows of NE, DOPEG, and NPY increased substantially above basal rates during ES (figs. 4–6); however, the peak rates reached were always less than those measured during stimulation at 12 Hz (figs. 1–3). The collection intervals in which peak overflows of NE, DOPEG, and NPY were reached were similar to those in which peak overflows were measured during and after ES at 12 Hz; *i.e.*, the peak rate of NE overflow occurred during ES, whereas that of DOPEG and NPY occurred after ES had been discontinued.

Halothane significantly decreased (ANOVA,  $P < 0.05$ )

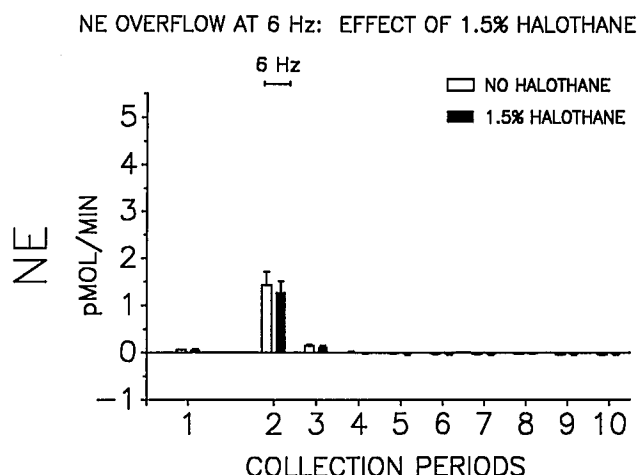


FIG. 4. Norepinephrine (NE) overflow into superfusate from isolated dog pulmonary artery strips during basal conditions (collection period 1) and the increase in overflow of NE above basal overflow during and after continuous ES (6 Hz). ES was delivered only during the second collection period. All collection periods were 10 min. Shaded bars represent overflow from tissues studied in the presence of 1.5% halothane. Data are means  $\pm$  SE,  $n = 7$  tissues from seven dogs.

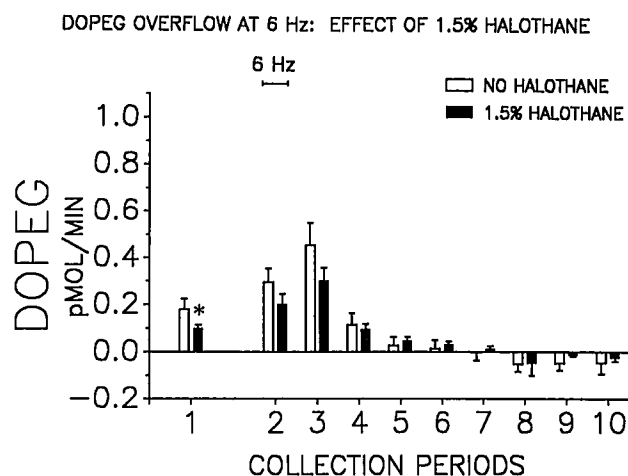


FIG. 5. 3,4-Dihydroxyphenylglycol (DOPEG) overflow into superfusate from isolated dog pulmonary artery strips during basal conditions (collection period 1) and the increase in overflow of DOPEG above basal during and after continuous ES (6 Hz). ES was delivered only during the second collection period. All collection periods were 10 min. Shaded bars represent overflow from tissues studied in the presence of 1.5% halothane. Data are mean  $\pm$  SE,  $n = 7$  tissues from seven dogs.  $P < 0.05$  for control *versus* halothane (ANOVA).  $*P < 0.05$  for DOPEG overflow under basal conditions in halothane-treated tissues (unpaired Student's *t* test).

the rate of DOPEG overflow resulting from ES (fig. 5). The decreases in rate of NE and NPY overflows in the presence of halothane did not reach statistical significance (figs. 4 and 6).

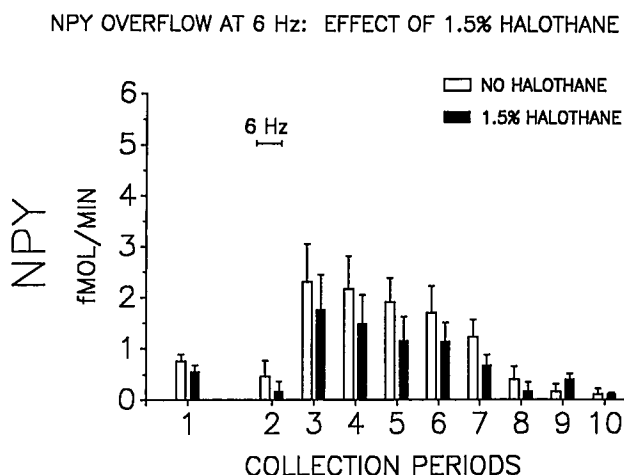


FIG. 6. Neuropeptide Y (NPY) overflow into superfusate from isolated superfused dog pulmonary artery strips during basal conditions (collection period 1) and the increase in NPY overflow above basal during and after continuous ES (6 Hz). ES was delivered only during the second collection period. All collection periods were 10 min. Shaded bars represent group studied in the presence of 1.5% halothane. Data are mean  $\pm$  SE,  $n = 7$  tissues from seven dogs.

### OVERFLOW OF NE, DOPEG, AND NPY DURING ES AT 1 HZ AND AFTER ES

The rate of overflow of NE above basal was increased by ES; however, ES did not increase the rates of overflow of DOPEG or NPY above basal levels (data not shown). Halothane did not affect the evoked overflows of NE, DOPEG, or NPY.

### TISSUE CONTENT OF NE, DOPEG, AND NPY; PERCENTAGE OVERFLOWING BEFORE, DURING, AND AFTER ES AT 12 OR 6 HZ

The tissue contents of NE, DOPEG, and NPY were not significantly different in control vessels after stimulation at 12 *versus* 6 Hz (tables 1 and 2). Stimulation at 12 Hz resulted in loss of a significantly greater percentage of the tissue content of NE and NPY than did stimulation at 6 Hz. By contrast, most DOPEG that was formed left the tissue after its formation regardless of whether the tissue was stimulated at 6 or 12 Hz. Halothane significantly decreased the percentage of the total tissue content of NPY that overflowed at 12 Hz but not at 6 Hz (table 1).

### NE:DOPEG RATIOS IN SUPERFUSATE BEFORE, DURING, AND AFTER ES AT 12 OR 6 HZ; THE EFFECT OF 1.5% HALOTHANE

Under basal conditions in control vessels the concentration of NE in superfusates was approximately one third that of DOPEG (table 3). During the interval of ES there was reversal of the NE:DOPEG molar ratios evident under basal conditions, and NE became more abundant than DOPEG. The magnitude of the reversal of the ratio was greater in vessels stimulated at 12 Hz than in vessels stim-

TABLE 1. Content of NE, DOPEG, and NPY and Tissue Contents Lost Before, During, and After Electrical Stimulation at 12 Hz in the Absence and Presence of Halothane

Analyte	Tissue Content	Overflow (% of Tissue Content)
NE (pmol/0.1 g)		
Control	339.2 ± 94.7	15.7 ± 3.8†
Halothane (1.5%)	330.0 ± 40.4	12.5 ± 4.0†
DOPEG (pmol/0.1 g)		
Control	0.89 ± 0.62	97.7 ± 1.7
Halothane (1.5%)	0.54 ± 0.54	98.4 ± 1.6
NPY (pmol/0.1 g)		
Control	2.8 ± 1.1	12.1 ± 4.4†
Halothane (1.5%)	4.3 ± 1.6	5.4 ± 1.2*†

Values are mean ± SE, n = 6. Percentage overflow = basal overflow + ES-induced overflow + post-ES overflow ÷ tissue content.

\*  $P < 0.05$  from control vessels.

† Significantly different from the percentage of tissue content that overflowed during 6-Hz ES.

TABLE 2. Content of NE, DOPEG, and NPY and Tissue Contents Lost into Superfusate Before, During, and After Electrical Stimulation at 6 Hz in the Absence and Presence of Halothane

Analyte	Tissue Content	Overflow (% of Tissue Content)
NE (pmol/0.1 g)		
Control	338.6 ± 52.7	5.5 ± 1.2
Halothane (1.5%)	471.9 ± 89.2	3.8 ± 1.1
DOPEG (pmol/0.1 g)		
Control	0.3 ± 0.3	98.8 ± 1.2
Halothane (1.5%)	0.2 ± 0.2	98.3 ± 1.7
NPY (pmol/0.1 g)		
Control	5.7 ± 1.1	2.5 ± 0.4
Halothane (1.5%)	5.9 ± 1.2	2.3 ± 0.3

Values are mean ± SE, n = 6. Percent overflows = basal overflow + ES-induced overflow + post-ES overflow ÷ tissue content.

ulated at 6 Hz. Compared to controls, halothane did not affect the molar ratio of NE:DOPEG either under basal conditions or during ES. Similarly, halothane did not affect the ratio of NE:DOPEG in the samples of superfusate collected immediately after stimulation ended; ratios of NE:DOPEG in subsequent poststimulation samples are variable but are not significantly different in control and halothane groups.

### NE:NPY RATIOS IN SUPERFUSATE BEFORE, DURING, AND AFTER ES AT 12 OR 6 HZ; THE EFFECT OF 1.5% HALOTHANE

Under basal conditions the ratios of NE:NPY were similar and were not affected significantly by halothane in the strips of vessel stimulated at 6 Hz or at 12 Hz (table 4). During ES the ratio of NE:NPY increased compared to controls in all tissues; however, the increases were not significantly different at 6 and 12 Hz (table 4). The increase in the ratio of NE:NPY with ES was of a similar magnitude in control tissues and tissues exposed to halothane. The presence of halothane did not affect the rate of decline in ratios of NE:NPY immediately after cessation of ES. Similar rates of decline in NE:NPY ratios were evident in tissues stimulated at 6 and 12 Hz.

### Discussion

These studies provide evidence that the concept of a single transmitter substance released at neuroeffector junctions in pulmonary artery is no longer valid. In addition to NE, NPY is released. The NPY released can be measured in superfusate of an isolated segment of tissue under basal conditions, and during ES its release is increased coincidentally with increased NE release. The amount of NPY released is greater at higher than at lower frequencies of ES, as has been suggested previously in other tissues.<sup>6</sup> The implications of these studies are that

TABLE 3. Ratio of NE:DOPEG in Superfusate of Dog Pulmonary Artery under Basal Conditions During and After Electrical Stimulation in the Absence and Presence of Halothane

	Basal	ES	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8
Control, 12 Hz Halothane (1.5%), 12 Hz	0.29 ± 0.04	4.63 ± 0.49	0.43 ± 0.04	0.12 ± 0.03	0.09 ± 0.03	0.12 ± 0.05	0.08 ± 0.03	0.08 ± 0.04	0.05 ± 0.05	0.14 ± 0.14
Control, 6 Hz Halothane (1.5%), 6 Hz	0.69 ± 0.22	6.25 ± 0.87	0.63 ± 0.06	0.15 ± 0.04	0.10 ± 0.04	0.13 ± 0.05	0.09 ± 0.05	0.07 ± 0.07	0.18 ± 0.18	0.13 ± 0.13
	0.33 ± 0.06	2.86 ± 0.65*	0.28 ± 0.05*	0.40 ± 0.05*	0.17 ± 0.008*	0.14 ± 0.08	0.11 ± 0.05	0.31 ± 0.31	—	—
	0.41 ± 0.13	3.22 ± 0.83*	0.35 ± 0.07*	0.20 ± 0.04	0.20 ± 0.04	0.19 ± 0.07	0.20 ± 0.10	—	—	—

Data are means ± SE, n = 8. PS = Poststimulation interval. Each interval of collection was 10 min. Dash = no samples were collected during this time interval. \* Significantly different from equivalent group stimulated at 12 Hz.

TABLE 4. Ratio of NE:NPY in Superfusate of Dog Pulmonary Artery under Basal Conditions During and After Electrical Stimulation in the Absence and Presence of Halothane

	Basal	ES	PS1	PS2	PS3	PS4	PS5	PS6	PS7	PS8
Control, 12 Hz Halothane (1.5%), 12 Hz	71.9 ± 32.0	1323.7 ± 218.2	82.1 ± 16.8	8.4 ± 2.7	5.4 ± 2.4	5.5 ± 2.2	4.6 ± 2.1	4.5 ± 3.3	6.2 ± 6.2	16.3 ± 16.3
Control, 6 Hz Halothane (1.5%), 6 Hz	74.2 ± 18.8	1803.5 ± 615.7	121.1 ± 28.2	12.6 ± 5.6	6.2 ± 2.9	5.7 ± 2.3	5.5 ± 2.9	6.4 ± 6.4	13.6 ± 13.6	10.3 ± 10.3
	128.8 ± 42.3	2223.1 ± 445.3	130.7 ± 66.5	39.4 ± 22.6	31.9 ± 19.6	29.8 ± 24.6	36.9 ± 32.3	22.7 ± 22.7	—	—
	78.9 ± 19.0	1356.2 ± 228.4	122.4 ± 65.6	53.4 ± 35.3	32.2 ± 29.4	60.4 ± 46.6	17.5 ± 14.8	7.8 ± 7.8	—	—

Data are means ± SE, n = 7. PS = Poststimulation interval. Each interval of collection was 10 min. Dash = no sample was collected during this time interval.

NPY is released in addition to NE during major or maximum activation of the sympathetic nervous system. The rather slow onset of overflow of NPY into superfusate and its continued overflow after cessation of ES (as compared to NE [figs. 1 and 2]) may be of importance in its functional role. The large molecular weight of NPY (4272 d) may be in part responsible for the slow diffusion of NPY from the synaptic cleft. The prolonged presence of NPY in the superfusate after ES also is consistent with the absence of a high-capacity NPY reuptake system comparable to the neuronal uptake system for NE. If NPY serves to prolong the action of the sympathetic response to the benefit of the organism, then a high-capacity uptake system for removing NPY released into the synapse would not seem to serve the organism best. An antagonist to NPY would be of help in identifying the physiologic role of NPY but is not currently available.

Halothane (1.5%) significantly reduced the overflow of NPY into the superfusate. This suggests that the magnitude and duration of activation of the sympathetic nervous system during anesthesia with halothane might have been blunted. The absence of a significant halothane-induced alteration in the NE:NPY ratio suggests that NE release must have been decreased correspondingly even though the decrease in NE overflow when measured directly did not reach statistical significance.

The absence of a decrease in the ES-induced release of NE in the presence of halothane appears initially to be at variance with our previous work<sup>16,17</sup> and that of others.<sup>25</sup> The previous studies, however, were in different tissues and used 2 Hz ES, a considerably lower frequency than was used to obtain the data presented here. Also, it is important to note that the NE measured in these studies represents "overflow" and not "release" of NE (*i.e.*, only the NE that escapes both neuronal and extraneuronal uptake overflows from the cleft into the superfusate). In a previous study using dog saphenous vein, we were not able to measure a halothane-induced decrease in NE overflow; however, when inhibitors of neuronal and extraneuronal uptakes were used, halothane significantly reduced the ES-induced release of NE.<sup>17</sup>

The current studies also help to resolve a question unanswered by previous work. A previous study of the metabolism of NE in the presence of halothane indicated that DOPEG formation was decreased in the presence of halothane.<sup>16</sup> A decrease in DOPEG formation does not necessarily mean inhibition of intraneuronal catabolic enzymes, since DOPEG is formed through two processes.<sup>25-28</sup> Under basal conditions DOPEG is formed independent of neuronal uptake of NE; presumably NE is leaked from intraneuronal storage vesicles into the cytoplasm, where it is converted to DOPEG. In contrast, the remaining component of DOPEG is formed after

reuptake of NE into nerve endings; hence this formation is eliminated by drugs that inhibit neuronal uptake of NE. A decrease in DOPEG formation during ES could thus result from halothane-induced impaired neuronal uptake of NE. In a recent study, however, we could not demonstrate evidence of halothane-induced impaired neuronal (or extraneuronal) uptake of NE.<sup>29</sup> The studies in the current report, which directly measured the formation of DOPEG during basal conditions and during ES, clearly demonstrate a decrease in DOPEG formation in the presence of halothane. These data, when considered together with those from previous studies, are strong evidence that halothane inhibits intraneuronal MAO or aldehyde reductase. The apparent enzyme inhibition is not complete: some DOPEG was still measurable in this as well as in our previous study.<sup>16</sup> In our previous study<sup>16</sup> less DOPEG was formed with 3% than with 1.5% halothane. Thus, the effect of halothane on intraneuronal metabolism of NE may be dose-dependent. Impaired intraneuronal metabolism of NE and decreased release of NE in the presence of halothane, if of sufficient magnitude, could have been expected to increase the tissue content of NE but did not. That increased tissue content of NE was not measurable in the tissue may reflect a relatively small increase in NE content relative to the total NE content. Only 5-15% of the tissue content of NE was released throughout the experiments. It is also unclear how this effect of halothane would be manifested *in vivo* during halothane anesthesia. The high percentage of the DOPEG formed ( $\pm 98\%$ ) that diffused from the synaptic cleft into the superfusate indicates the ease with which DOPEG leaves the nerve ending once it is formed. NPY, in contrast, has a larger molecular weight, and thus it diffuses from the synaptic cleft into the superfusate much more slowly. As a result, the peak overflow of NPY is reached after stimulation has been stopped and remains elevated for the duration of the studies.

The NE:DOPEG ratios indicate that under basal conditions preceding ES, approximately three times as much DOPEG as NE was present. ES sharply increased the release of NE so that NE overflow exceeded DOPEG overflow during ES. The significantly greater reversal of the NE:DOPEG ratio with 12 as compared to 6 Hz reflects the increased release of NE occurring with 12 Hz. It is noteworthy that halothane did not significantly alter the NE:DOPEG ratio even though DOPEG formation was decreased. This likely reflects a decrease in NE release as well as a reduction in DOPEG formation.

The halothane concentration used (1.5%) represents the amount that was measured continuously in the gas aerating the Krebs'-Ringer's superfusate and that filled the superfusion chamber. The halothane concentration in the superfusate as determined by gas chromatography

was  $1.38 \pm 0.02\%$  (mean  $\pm$  SE). It was assumed that the partial pressure of halothane in the tissue and in the bath were in equilibrium.

The current studies show that NPY is present in dog pulmonary artery; is released with NE in response to ES; is released in greater amounts at 12 than at 6 or 1 Hz; and when in response to 12 Hz ES, is decreased by 1.5% halothane. Once NPY has been released, its clearance from the synaptic cleft is prolonged compared to that of NE. In addition, halothane has been found to decrease formation of DOPEG, the metabolite of NE produced intraneuronally by the action of MAO and aldehyde reductase on NE.

From a clinical perspective, it is noteworthy that halothane modifies the disposition of NPY. The peptide is widely distributed within the cardiovascular system. NPY has been found to have no direct inotropic or chronotropic effects;<sup>30</sup> however, it is a potent potentiator of the actions of several vasoconstrictive agents, including NE, epinephrine, prostaglandin  $F_{2\alpha}$ , angiotensin II, and histamine.<sup>31-33</sup> Moreover, NPY may contribute to blood pressure regulation by suppressing renin release<sup>34</sup> or by increasing the release of atrial natriuretic factor.<sup>35</sup> Plasma NPY and NE concentrations are increased by hemorrhagic stress<sup>36</sup> and by surgery.<sup>12</sup> Importantly, the main source of these increased concentrations appears to be peripheral sympathetic nerve endings.<sup>36</sup> The concentrations of NPY and NE do not increase proportionately, however, and as seen in the current *in vitro* study, NPY concentrations fall more slowly than NE levels. Increased levels of NPY in the synaptic cleft actually serve to decrease NE release *via* specific presynaptic NPY receptors, while at the same time sensitizing the smooth muscle cells to the vasoconstrictor effects of NE.<sup>2,37,38</sup> Conversely, NE in the cleft inhibits NPY release *via*  $\alpha_2$  receptors.<sup>2,38</sup> The net effect appears to be improved regulation of vascular homeostasis. Within this context, the finding that halothane reduces NPY release may be clinically relevant, since halothane decreases the response of the sympathetic nervous system to surgery. This blunting effect seems not to be related to neuronal or extraneuronal amine uptake<sup>29</sup> or to the rate of NE synthesis.<sup>39</sup> It seems possible that this decreased sympathetic response to halothane may be due, at least in part, to reduced NPY release. Further studies are required to evaluate this possibility.

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