The Effect of Pargyline Pretreatment on the Enhancement of the Exocytotic Release of Norepinephrine during Nerve Stimulation which is Induced by a Benzoquinolizine Compound with Reserpine-Like Properties

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ABSTRACT


Pretreatment of guinea pigs with paragline (100 mg/kg i.p., 18 hr before sacrifice) resulted in a significant depression in the overflow of endogenous norepinephrine (NE), total [3H], [1H]-NE and dopamine β-hydroxylase associated with stimulation of the sympathetic nerves to the isolated heart. The depression was pronounced at 5 Hz. At 10 Hz, pargyline pretreatment was without effect. The effect on dopamine β-hydroxylase output was not as great as that on total [3H]NE or endogenous NE release, suggesting the possibility that more than one mechanism is responsible for the depressant effect of monoamine oxidase (MAO) inhibition on sympathetic neurotransmission. A benzoquinolizine compound with reserpine-like properties, 2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b-H-benzo[e]quinolizine (RO 4-1284), increased the nerve stimulation-mediated overflow of all the measured indices of neurotransmitter release, in addition to producing a significant increase in the spontaneous overflow of [3H] from hearts of untreated guinea pigs. The augmentation of nerve-stimulated NE release by RO 4-1284 was even greater in hearts from pargyline pretreated guinea pigs. These results would tend to eliminate a causal role for a deaminated metabolite of NE in the augmenting effect on release seen with RO 4-1284. Conversely, the inhibition of neurotransmitter release associated with MAO inhibition is not mediated by the blockade in the formation of deaminated catecholamine metabolites. Enhancement in the negative feedback mechanism on release and the accumulation of false transmitter probably account for the local effects of MAO inhibitors on neurally mediated norepinephrine release.

The mechanism by which monoamine oxidase (MAO) inhibitors decrease peripheral sympathetic nervous system function remains controversial. Among the explanations for the antidepressant effects of MAO inhibitors are: 1) depression of ganglionic transmission (Gertner, 1961); 2) accumulation and release of false transmitter with low intrinsic activity (Kopin et al., 1965); 3) exaggerated activation of the presynaptic alpha adrenergic negative feedback mechanism (DePotter et al., 1971; Cubeddu et al., 1974; Langer et al., 1975); 4) bretylium-like blockade of the adrenergic neuron (Antonaccio and Smith, 1969); 5) decrease in the synthesis of norepinephrine due to feedback inhibition of tyrosine hydroxylase (Berkowitz et al., 1974); and 6) a central effect which results in reduced sympathetic nervous system outflow (Fuentes et al., 1979).

Dopamine-β-hydroxylase (DBH) is stored in synaptic vesicles and released with norepinephrine (NE) during nerve stimulation (DePotter et al., 1971; Geffen et al., 1969; Gewirtz and Kopin, 1970; Weinshilboum et al., 1971; Smith, 1973). The determination of DBH activity has been used as a measure of exocytotic release of neurotransmitter and its modulation by such diverse agents as alpha adrenergic agonists and antagonists (Johnson et al., 1971; Cubeddu et al., 1974, 1975a), muscarinic cholinergic agonists (Langley and Gardier, 1977) and beta adrenergic agonists and antagonists (Dixon et al., 1979). Roizen et al. (1975) have shown that the anesthetic agent halothane can inhibit the release of NE induced by nerve stimulation without altering DBH outflow.

Measuring DBH release consequent to nerve stimulation should help to clarify the mechanism by which MAO inhibitors depress sympathetic nerve function. If, for instance, the mechanism exclusively involves the formation of false transmitters and their subsequent storage in the vesicles in place of NE, nerve stimulation should produce a decrease in NE outflow but no reduction in DBH release. Conversely, any mechanism that
NE and DBH metabolites of NE in the mechanism of the antiadrenergic nialamide with RO 4-1284, then it should be possible to prevent the formation of deaminated metabolites. MAO inhibition with metabolite(s) is ethylene glycol. If the formation of one or more deaminated significant spleen in the presence of 0.9 M NE metabolite was isolated, should produce a parallel decrease in the release of total 3H and [3H]NE (20 vs. 30% decrease exocytosis through an action on the vesicle membrane. Stimulation of the isolated spleen in the presence of 0.9 µM RO 4-1284 resulted in a significant increase in the outflow of [3H]dihydroxyphenylethylene glycol. If the formation of one or more deaminated metabolite(s) is responsible for the increased exocytosis seen with RO 4-1284, then it should be possible to prevent the augmentation of exocytosis due to this compound by inhibiting the formation of deaminated metabolites. MAO inhibition with halamidene has been shown to prevent reserpine-induced depletion of NE in the rat salivary gland (Jonason, 1970). Inhibition of MAO by pargyline pretreatment was carried out in order to: 1) assess the possible role of reduced production of deaminated metabolites of NE in the mechanism of the antiadrenergic action of MAO inhibitors; and 2) determine whether or not a deaminated metabolite of NE is involved in the increased release of NE and DBH seen with RO 4-1284.

**Methods**

Male Hartley guinea pigs (450-550 g) were pretreated with pargyline HCl (100 mg/kg i.p.) 18 hr before sacrifice. Control animals were pretreated with isonic saline. The hearts were isolated, perfused and labeled as previously described (Langley and Weiner, 1978). In the experiments in which the effect of pargyline pretreatment alone was examined, the left and right postganglionic sympathetic nerve bundles were stimulated simultaneously with a total train of 300 pulses at a frequency of 5, 7, or 10 Hz and 1-msec duration. Only one frequency was used in a single experiment.

All experiments involving the effect of RO 4-1284 alone and after pargyline pretreatment were performed at a frequency of 5 Hz with a total train of 300 impulses (1 min). Six stimulations were carried out in each experiment and 15 min separated the stimulation periods, except for the interval between stimulations 2 and 3 which was of 20 minutes duration. In experiments in which RO 4-1284 was employed, the compound was perfused through the heart immediately after stimulation 2 and continued through stimulation 6.

**NE determination.** One milliliter of the perfusate was counted by liquid scintillation spectrometry for total [3H]outflow. Three milliliters of perfusate were frozen for the assay of DBH activity.

The remainder of the perfusate was used for the separation of [3H]NE from its [3H]metabolites and for the isolation of endogenous NE. The pH of the perfusate was adjusted to 2.5 with 1 N HCl and applied to a Dowex-50 × 4, H+ (200-400 mesh) ion exchange column (5 cm × 0.5 cm). The column was washed with 15 ml of double distilled water, and 2 ml of the combined effluent and H2O wash were counted to determine deaminated metabolites. NE and its O-methylated metabolite were eluted from the column with 8 ml of 2.0 N HCl and 0.5 ml was counted by liquid scintillation spectrometry. One-half milliliter of 10-3 M EDTA and 1 ml of 2 M Na acetate, pH 8.6, were added to the remainder of the eluate and the pH was adjusted to 8.5. The adjusted eluate was applied to an alumina oxide column (1 cm × 0.5 cm, Woelm). The column was washed with 6 ml of double distilled H2O and 1 ml of the combined effluent and the H2O wash was counted for normetanephrine determination. NE was eluted from the column with 3.0 ml of 0.2 N HCl and 0.5 ml of the eluate was counted by liquid scintillation spectrometry for [3H]NE determination.

NE was measured fluorometrically by using the trihydroxyindole method (Chang, 1964). Recovery of NE was 75% (±1.4) and normetanephrine was 86% (± 1.8). Results are corrected for recoveries.

**DBH assay.** DBH activity was determined by using a modification of the two-step enzymatic method of Molinoff et al. (1971). One hundred microliters of perfusate was incubated for 1 hr at 37°C in a reaction mixture containing the following components: 12.5 µl of 30 mM tyramine HCl, 10 µl of 6 mM pargyline HCl (Abbott Laboratories, North Chicago, IL); 6 µl of 5 mM Tris-HCl, pH 7.4, containing 100 µg catalase (Boehringer-Mannheim Corp., New York, NY) and 5.0 µl of 1.0 M sodium acetate buffer, pH 5.0. In order to eliminate the effects of endogenous DBH inhibitors present in the perfusate and in the heart homogenate, a range of copper concentrations was employed (10 to 80 µM for the perfusate and 40 to 160 µM for the heart homogenate).

After 1 hr of incubation, the pH was changed to 8.6 by the addition of 50 µl of a mixture of the following components: 43 µl of 1.0 M Tris-HCl, pH 8.6; 5 µl of S-[methyl-C14]adenosyl-L-methionine (25 nCi; specific activity (S.A.) 55 mCi/mmol); 1 µl of 1.6 × 10-3 M diithiothreitol (DTT); and 1 µl of partially purified phenylethanolamine-N-methyltransferase (Nelson and Molinoff, 1976). After an additional 30 min of incubation, the reaction was stopped with 0.3 ml of 0.5 M borate buffer, pH 10. The [14C]-N-methyloctopamine formed in the coupled reaction was extracted into ethyl acetate and counted by liquid scintillation spectrometry. Blanks consisted of 100 µl of perfusate heated at 90°C for 5 min and carried through the two-step reaction. Internal standards were prepared by adding 20 or 40 ng of octopamine to 100 µl of heated perfusate and the complete reaction mixture. DBH activity was calculated as total nanomoles of octopamine formed per hour for the 3.5-min collection period.

At the termination of the experiment, the heart was blotted, weighed and homogenized in 50 volumes of Tris (5 mM, pH 7.4)-Triton X-100 (0.1%). The homogenate, which was about 100 to 150 ml in volume, was centrifuged at 10,000 × g for 10 min and a 5-ml aliquot was frozen for tissue DBH determination. The pellet, which contained only a small fraction of the total tissue [3H], was acidified with 4 ml of 1 N HCl and centrifuged at 10,000 × g for 10 min. Both supernatants were combined and 1 ml of the acidified supernatant was counted for total [3H]. Twenty-five milliliters of the remaining supernatant were carried through the double column chromatography procedure (described above for the perfusate) for the isolation and assay of [3H]-metabolites, [3H]NE and endogenous NE. Total homogenate MAO activity was assayed according to the method of Wurtman and Axelrod (1963).

**Results**

Effect of pargyline pretreatment on neurotransmitter release induced by nerve stimulation at different frequencies. The effect of pargyline, 100 mg/kg i.p., administered 18 hr before the experiment, on release of total [3H]NE, NE and DBH from the isolated perfused heart was assessed during stimulation at 5, 7 and 10 Hz. Pargyline treatment was associated with a significant reduction in the outflow of total [3H]NE and NE during each of 3 periods of stimulation at 5 Hz (fig. 1). A significant decrease in DBH output occurred with stimulations 1 and 2 (fig. 1). The inhibitory effect of pargyline pretreatment on DBH release was less pronounced than the reduction in the release of total [3H] and [3H]NE (20 vs. 30% decrease) or endogenous NE (20 vs. 39%).

Stimulation at 7 Hz resulted in a decreased outflow of total [3H] and [3H]NE, but no significant change in endogenous NE or DBH release from hearts of pargyline-pretreated animals (fig. 2). Stimulation at 10 Hz produced no significant change in the outflow of any of the measured indices of release (fig. 3).

Effect of RO 4-1284. All measured indices of neurotransmitter release were significantly (P < .001) increased in the presence of 0.9 µM RO 4-1284 (figs. 4 and 5). The pattern of metabolism of the neurally released [3H]NE did not differ.
Fig. 1. Left panel, the effect of pargyline on the release of total $^3$H and $[^3$H]NE in response to nerve stimulation at 5 Hz. Data are presented as percentage of tissue radioactivity: ●, total $^3$H control; ■, total $^3$H after pargyline pretreatment (100 mg/kg); ○, $[^3$H]NE control; □, $[^3$H]NE after pargyline pretreatment. Right panel, the effect of pargyline on the release of NE (total nanograms) and DBH activity (total nanomoles per hour) in response to nerve stimulation at 5 Hz: ○, NE control; △, NE after pargyline pretreatment; ●, DBH control; △, DBH after pargyline.

* $P < .05$; ** $P < .01$; *** $P < .001$ (Student’s t test for two population means; control experiments, $n = 10$, pargyline experiments, $n = 8$).

Fig. 2. Release of total $^3$H, $[^3$H]NE, NE and DBH in response to nerve stimulation at 7 Hz. See legend to figure 1 for details. * $P < .05$; control experiments, $n = 5$; pargyline experiments, $n = 6$.

Fig. 3. Release of total $^3$H, $[^3$H]NE, NE and DBH in response to sympathetic nerve stimulation at 10 Hz. See legend to figure 1 for details. Control experiments, $n = 5$; pargyline experiments, $n = 6$.

Fig. 4. The ratio of the outflow of the total $^3$H and $[^3$H]NE during stimulations 3 through 6 to the outflow during stimulation 2 within each experiment (Sn = S3, S4, S5 and S6). ○, control (n = 10); □, pargyline pretreated (100 mg/kg, n = 12); ●, RO 4-1284 (0.9 μM, n = 5); ■, RO 4-1284 after pargyline pretreatment (100 mg/kg, n = 5).

Fig. 5. The ratio of the outflow of NE and DBH during stimulations 3 through 6 to the outflow during stimulation 2 within each experiment (Sn = S3, S4, S5 and S6). ○, control (n = 10); △, pargyline pretreated (100 mg/kg, n = 12); ●, RO 4-1284 (n = 5); △, RO 4-1284 after pargyline pretreatment (n = 5). * $P < .05$.

significantly from that of control stimulations. Deaminated metabolites represented 18.1% (±1.9) of the total $^3$H recovered in stimulation samples from control hearts and 16.0% (±4.2) of the total $^3$H recovered during nerve stimulation in the presence of RO 4-1284. Normetanephrine accounted for 14.3% (±1.8) of the total $^3$H in the perfusate after stimulation of sympathetic nerves to control hearts and 18.9% (±2.2) of the total $^3$H in the perfusate after neural stimulation of hearts perfused with RO 4-1284. Tissue MAO activity was essentially the same in both control hearts (2.78 ± 0.34 nmol [14C]indole acetic acid per mg of tissue per 15 min) and hearts perfused with RO 4-1284 (3.01 ± 0.17 nmol [14C]indole acetic acid per mg of tissue per 15 min).

**RO 4-1284 after pargyline pretreatment.** Pretreatment of guinea pigs with pargyline (100 mg/kg i.p., 18 hr before the experiment) failed to prevent the enhancing effect of RO 4-1284 on exocytosis (figs. 4 and 5). In fact, endogenous NE outflow was significantly elevated in the presence of RO 4-1284 during stimulations 4, 5 and 6 after pargyline treatment compared with NE outflow from hearts of untreated guinea pigs in the presence of RO 4-1284. There was a slight but nonsignificant elevation in the outflow of DBH after RO 4-1284 in hearts from the pargyline-pretreated guinea pigs (fig. 5). Deaminated metabolites were significantly lower in the perfusate from hearts of pargyline-pretreated guinea pigs ($6.2 ± 0.7\%$ of the total $^3$H released). Tissue MAO activity was below the sensitivity of the method in most of the hearts from pargyline-pretreated animals (less
TABLE 1
Tissue levels of total $^3$H, $[^3]$HNE, NE and total DBH activity
At the termination of the experiments, hearts were homogenized and assayed for total $^3$H, $[^3]$HNE, NE and DBH activity as described in "Methods." Values are means ± S.E.M. of the number of experiments shown in parentheses.

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<tr>
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<th>$^3$H</th>
<th>$[^3]$HNE</th>
<th>NE</th>
<th>DBH</th>
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<tr>
<td></td>
<td>µCi/g</td>
<td>µCi/g</td>
<td>µg/g</td>
<td>µmol/h/g/g</td>
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<tr>
<td>Control (20)</td>
<td>1.37 ± 0.26</td>
<td>1.19 ± 0.25</td>
<td>0.92 ± 0.23</td>
<td>172 ± 30</td>
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<tr>
<td>Pargyline (20)</td>
<td>1.54 ± 0.33</td>
<td>1.38 ± 0.26*</td>
<td>1.24 ± 0.28***</td>
<td>181 ± 40</td>
</tr>
<tr>
<td>RO 4-1284 (5)</td>
<td>1.09 ± 0.11*</td>
<td>0.91 ± 0.10*</td>
<td>0.66 ± 0.09**</td>
<td>186 ± 34</td>
</tr>
<tr>
<td>RO 4-1284 after pargyline pretreatment (5)</td>
<td>1.65 ± 0.21*</td>
<td>1.50 ± 0.21*</td>
<td>1.17 ± 0.52</td>
<td>141 ± 29*</td>
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* P < .05; ** P < .01; *** P < .001. Significantly different from control (Student's t test).

TABLE 2
Prestimulation outflow of total $^3$H
The total radioactivity (nanocurie) released spontaneously during the 1-min period immediately before nerve stimulation. Within each treatment group, prestimulation $^3$H did not vary significantly and the data have been pooled. Number of experiments are in parentheses.

<table>
<thead>
<tr>
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<th>Total $^3$H outflow (nCi)</th>
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<tbody>
<tr>
<td>Control (20)</td>
<td>4.36 ± 1.53</td>
</tr>
<tr>
<td>Pargyline Treatment (20)</td>
<td>2.33 ± 0.36***</td>
</tr>
<tr>
<td>RO 4-1284 (5)</td>
<td>6.38 ± 2.45***</td>
</tr>
<tr>
<td>Pargyline Treatment and RO 4-1284 (5)</td>
<td>2.60 ± 0.48***</td>
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*** P < .001.

Discussion

The depression of sympathetic nervous system function with MAO inhibitors may not be explicable by a single mechanism. MAO inhibitors have been found to depress the response of some tissues to nerve stimulation (Smith, 1966; Antonaccio and Smith, 1969) and decrease the outflow of NE in response to nerve stimulation (Axelrod et al., 1961; Davey et al., 1963). The present investigation supports the earlier observation that MAO inhibitors like pargyline decrease the release of sympathethic neurotransmitter. This inhibition of release is more pronounced at 5 than 7 Hz and is not apparent at 10 Hz. Several other modulators of the release of NE during sympathetic nerve stimulation have been reported to be most effective at low frequencies; e.g., prostaglandins (Junstad and Wennmalm, 1973), alpha adrenoceptor agonists (DePotter et al., 1971; Cubeddu et al., 1974; Langer et al., 1975) and beta adrenoceptor agonists (Adler-Graschinsky and Langer, 1975; Stjärne and Brundin, 1975).

The fact that the release of amine is depressed to a greater extent than the release of DBH suggests that more than one mechanism may be functioning to produce this inhibitory effect (fig. 1). The decrease in both NE and DBH tends to support the conclusion that pargyline pretreatment causes a decrease in exocytosis; i.e., either a bretylium-like neuronal blockade (Antonaccio and Smith, 1969) or enhancement of the presynaptic alpha adrenoceptor negative feedback mechanism (DePotter et al., 1971; Cubeddu et al., 1974; Langer et al., 1975). In addition, the disproportionately greater fall in NE output with stimulation suggests that the synaptic vesicles contain a lower NE-DBH ratio and lends support to the false transmitter concept which may be important during MAO inhibition (Kopin et al., 1965).

A recent proposal by Rand et al. (1975) may also help to account for the decrease in sympathetic nerve function with pargyline. Inhibition of MAO increases not only the concentration of NE which is formed within the synaptic vesicle but also that of dopamine which is formed extravesicularly and is more likely to come into contact with MAO under normal circumstances (Cooper et al., 1974). Evidence has accumulated supporting the presence of specific presynaptic dopamine receptors at the sympathetic nerve terminal which can act to decrease the release of NE during sympathetic nerve activity (Langer, 1973; McCulloch et al., 1973, Ilhan and Long, 1975). These findings have led Rand et al. (1975) to suggest that the antihypertensive activity of MAO inhibitors may result from their preservation of neuronal dopamine, which, when released, can stimulate the inhibitory presynaptic dopamine receptors as well as behave as a false transmitter with relatively low potency on...
postsynaptic adreceptors. Such a mechanism can explain reduced exocytosis but it cannot account for the greater inhibitory effect of pargyline on NE release than on DBH release.

Inhibition of MAO with pargyline failed to prevent the augmenting effect of RO 4-1284 on the stimulated release of total $^{3}$H-NE, NE and DBH. After MAO inhibition, RO 4-1284 actually produced a significant increase in NE outflow above that produced by RO 4-1284 alone. These results suggest that a pool of NE whose existence is favored by RO 4-1284 is preserved by pargyline pretreatment and is released by nerve stimulation. Since DBH outflow is not significantly increased by RO 4-1284 after pargyline pretreatment, the NE may be from a nonvesicular pool. However, tissue levels of DBH in RO 4-1284 perfused hearts from pargyline-pretreated guinea pigs are significantly less than controls (table 1). An explanation for the lower DBH activity in these experiments is lacking but may account for the significant elevation of NE release without a similar increase in DBH outflow (fig. 5).

The amount of deaminated metabolite released during stimulation of hearts from pargyline-pretreated guinea pigs was unaffected by perfusion with RO 4-1284. Prestimulation outflow in the experiments in which RO 4-1284 was perfused into hearts from pargyline-pretreated guinea pigs is also essentially the same as that from hearts in which the effect of pargyline alone was tested. These results tend to eliminate an active deaminated metabolite as being responsible for the enhanced exocytosis seen with RO 4-1284.

References


