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ROLE OF TYPE A AND TYPE B MONOAMINE OXIDASE IN THE METABOLISM OF RELEASED [^{3}H]DOPAMINE FROM RAT STRIATAL SLICES*

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Abstract—The effects of selective inhibition of multiple forms of monoamine oxidase (MAO) on the *in vitro* release and metabolism of newly-synthesized [^{3}H]dopamine (DA) were examined using rat brain slices. Striatal slices were preincubated in the presence of [^{3}H]L-tyrosine (20 μM) followed by a short incubation period in the presence of the selective irreversible MAO-inhibitor agents clorgyline (type A) and deprenyl (type B). Tissue pretreated in this manner was then subjected to a release incubation, and DA release and metabolism were determined under spontaneous and depolarizing conditions. Pretreatment with clorgyline (10^{-7} M) significantly reduced the spontaneous, as well as K^+-evoked, formation of both 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Deprenyl (10^{-7} M) pretreatment did not significantly affect these variables, but clorgyline + deprenyl pretreatment resulted in a reduction of both DOPAC and HVA that was greater than that produced by clorgyline alone. By contrast, deprenyl pretreatment significantly decreased both DOPAC and HVA under depolarizing conditions, but only in the presence of the DA uptake inhibitor nomifensine (10^{-5} M). In the absence of MAO inhibition, nomifensine increased K^+-evoked formation of DOPAC and HVA, while spontaneous formation was not affected. The results suggest that released DA is deaminated primarily by the type A form of MAO; however, in the absence of the type A MAO, or under conditions that promote exclusive postsynaptic deamination, minor but significant metabolism occurs via the type B enzyme. Data obtained are further discussed in relation to the mechanism of MAO-inhibitor drug action and pre- versus postsynaptic formation of DOPAC and HVA.

The enzyme monoamine oxidase (MAO; monoamine:O_2 oxidoreductase; EC 1.4.3.4) has been shown to be present in a variety of tissues, including rat brain, in at least two forms [1, 2]. These isozymes exhibit different substrate and inhibitor characteristics and have been termed type A and type B MAO [3]. The A form of MAO is more sensitive to inhibition by clorgyline [3] and preferentially deaminates serotonin [4]. The type B isozyme is more sensitive to inhibition by deprenyl [5, 6] and preferentially deaminates β-phenylethylamine [7]. Dopamine (DA) is readily deaminated by both the clorgyline and deprenyl sensitive forms of MAO and is therefore considered a common substrate [6, 8].

The role of endogenous DA as a neurotransmitter in striatal tissue is well established [9]. Moreover, MAO is considered to play an important role in the metabolism of synaptic DA. In this regard, intracellular MAO functions to deaminate neuronally released DA, subsequent to recapture by carrier-mediated uptake mechanisms within the synaptic region [10–12].

In rat brain, evidence exists for the exclusive presence of the type A form of MAO within striatal DA neurons [13–15]. Thus, the deamination of released DA, recaptured by DA nerve terminals, would occur through an interaction with type A MAO. However, since the high-affinity reuptake mechanism associated with DA neurons is inhibited under depolarizing ionic conditions [16, 17], it appears that postsynaptic deamination of DA may be considerable under conditions of elevated impulse flow [18]. Yet in sites external to DA neurons, where the majority of striatal MAO activity is found [15, 19, 20], no information is available on the relative degree of DA deamination occurring through multiple forms of MAO.

Accordingly, we have examined the functional role of postsynaptic type A and type B MAO in the deamination of released DA in rat striatal tissue. Tissue slice preparations were employed in these experiments to maintain the structural integrity of the DA synapse with regard to all cell-types which may contain the multiple forms of MAO. The relative contributions of types A and B MAO in DA deamination were determined utilizing the selective irreversible MAO inhibitors, clorgyline and deprenyl. In addition, postsynaptic deamination was determined in the presence of the DA neuronal uptake inhibitor, nomifensine.

METHODS

Drugs and chemicals. Nialamide, L-tyrosine, 3-hydroxytyramine (dopamine), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid), and

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3,4-dihydroxyphenylacetic acid were all obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Clorgyline HCl and (−)deprenyl HCl were supplied by May & Baker Ltd. (Dagenham, England) and Dr. J. Knoll (Semmelweiss University, Budapest) respectively. Nomifensine (8-amino-2-methyl - 4 - phenyl - 1,2,3,4 - tetrahydroisoquinoline hydrogen maleate) was obtained from Hoechst–Roussel Pharmaceuticals Inc. (Sommerville, NJ, U.S.A.). [^{3}H]L-Tyrosine was purchased from the Amersham Corp. (Arlington Heights, IL, U.S.A.) and is described as follows: L-[2,6-^{3}H]tyrosine (35 Ci/mmole). [^{3}H]Dopamine (3,4-[7-^{3}H]dihydroxyphenylethylamine, 33.4 Ci/mmole) was purchased from the New England Nuclear Corp. (Boston, MA, U.S.A.).

[^{3}H]Tyrosine was purified before use by the method of Weiner and Selvaratnam [21], and [^{3}H]DA was purified by adsorption to alumina and elution with 0.2 N acetic acid.

Tissue preparation. Male Sprague–Dawley rats (225–300 g) were decapitated, and the brains were immediately placed in ice-cold, oxygenated (95% O_2–5% CO_2) physiological buffer containing (mmole/liter): NaCl, 126.45; KCl, 2.40; $CaCl_2 \cdot 2H_2O$, 1.10; $MgCl_2 \cdot 6H_2O$, 0.83; $Na_2SO_4 \cdot 10H_2O$, 0.50; KH_2PO_4, 0.50; $NaHCO_3$, 27.62; and glucose, 5.88 [22]. Additionally, ascorbic acid (1.1 mM) was added as an anti-oxidant resulting in a final pH of 7.35. Striata from four to seven rats were dissected on ice as described by Knapp *et al.* [23]. Striata were pooled, blotted dry, weighed (about 50 mg each), and chopped once in perpendicular directions using a McIlwain tissue chopper (0.3 mm intervals). Resultant tissue slices were suspended in fresh cold buffer (10 ml/g) and used in release and accumulation experiments as described below.

Release and metabolism of newly-synthesized [^{3}H]*DA.* Release of newly-synthesized [^{3}H]DA was performed using a modification of the procedure of Azzaro and Rutledge [24]. Striatal slices (see above) were transferred to a plastic beaker in a shaking water bath and incubated in the presence of [^{3}H]tyrosine (10 μCi/ml, final concentration 20 μM) for 30 min. All incubations were performed at 37° under an atmosphere of 95% O_2–5% CO_2. Following incubation, the beaker contents were centrifuged [1500 *g* for 3 min at room temperature (RT)] and resuspended in fresh buffer (5 ml/g original tissue). Tissue aliquots (0.5 ml) were incubated in the presence of the selective MAO inhibitors, clorgyline (10^{-7} M) and/or deprenyl (10^{-7} M), for 10 min (incubation volume 2.0 ml). We have demonstrated previously that, under these assay conditions, this concentration of clorgyline or deprenyl results in selective loss of greater than 90% of type A or type B MAO activity, respectively, in striatal slices without significant effect on the corresponding insensitive form of MAO [25]. Controls were performed in the absence of MAO-inhibitor drugs. The tissue was then washed twice, resuspended in 1.0 ml of fresh buffer, and transferred to plastic beakers. A release incubation was performed for 20 min in the presence of buffer to which KCl was added to increase the total concentration of K^+ (final volume 2.0 ml). In some experiments, the buffer contained nomifensine (10^{-5} M). Following the release incubation, the medium was separated from the tissue by centrifugation (1500 *g* for 3 min, RT) and acidified by addition of a 2 N acetic acid solution containing 0.1% ascorbic acid. The tissue was then homogenized in 2.0 ml of 2 N acetic acid solution using a ground-glass homogenizer. Prior to homogenization, 500 μg of homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) and 25 μg of DA were added to both tissue and medium as carrier substances for determination of recoveries. Protein in each sample was removed by centrifugation (10,000 *g* for 10 min, 4°), and the amounts of [^{3}H]DA, [^{3}H]HVA, and [^{3}H]DOPAC in the supernatant fractions were determined as described below.

To correct for the amount of [^{3}H]HVA and [^{3}H]DOPAC present in the tissue prior to the 20-min release incubation, an appropriately pretreated tissue sample (i.e. control, deprenyl, clorgyline, or deprenyl + clorgyline) was immediately homogenized rather than exposed to the release incubation. The amount of [^{3}H]HVA and [^{3}H]DOPAC formed in this sample served as the "tissue blank" for that particular treatment. In each experiment, this "tissue blank" was substracted so that the values presented reflect only the metabolites of DA formed during the release incubation. The mean ± S.E.M. of values for the "tissue blanks" (dpm/mg protein) in each pretreatment group were as follows: control pretreatment, HVA 5,972 ± 294, DOPAC 14,357 ± 897; deprenyl, HVA 5,439 ± 280, DOPAC 14,971 ± 1,161; clorgyline, HVA 4,510 ± 312, DOPAC 4,209 ± 462; deprenyl + clorgyline, HVA 5,213 ± 430, DOPAC 5,357 ± 443. [^{3}H]DA present in tissue blanks was also measured and compared among the different MAO-inhibitor pretreatments to determine possible effects on [^{3}H]DA formation and, therefore, the amount of [^{3}H]DA available for release and metabolism. No significant differences were detected. In control pretreated tissue, this value was 284 ± 25 dpm/mg protein × 10^3 (mean ± S.E.M.)

Accumulation and metabolism of [^{3}H]*DA.* Accumulation and metabolism of [^{3}H]DA in rat striatal slices were generated using a modification of methods previously described [26, 27]. Briefly, aliquots of tissue slices (0.5 ml) were pretreated *in vitro* with selective MAO-inhibitors as described above. The tissue was washed twice, resuspended in 1.0 ml of fresh buffer, and then transferred to plastic beakers containing 0.96 ml buffer and 20 μl of nomifensine vehicle (0.01 N HCl) or nomifensine solution. Samples were preincubated for 5 min, then 20 μl of [^{3}H]DA solution (2 μCi, final concentration 0.1 μM) was added, and the incubation was continued for an additional 5 or 10 min. Following incubation, the tissue and medium were separated by centrifugation. Medium samples were acidified, and tissue samples were homogenized as described above.

Tissue blanks, to correct for non-enzymatic formation of [^{3}H]HVA and [^{3}H]DOPAC, were prepared using tissue slices denatured by heating (100° for 20 min). These values represented less than 10% of control pretreatment values for both [^{3}H]HVA and [^{3}H]DOPAC and were routinely subtracted from all samples.

In some experiments, rats were administered nialamide (100 mg/kg, i.p.) for 120 min and reserpine (2.5 mg/kg, i.p.) for 90 min before being killed. Animals were pretreated in this manner in order to inhibit metabolism and vesicular storage of accumulated amine [28].

Wet tissue weights used in expressing accumulation data and calculation of percentage inhibition of accumulation were determined according to procedures described by Ziance and Rutledge [26].

Chromatographic separation of DA and DA metabolites. [^{3}H]DOPAC, [^{3}H]HVA, and [^{3}H]DA were separated by a modification of the procedure of Taylor and Laverty [29]. Tissue and medium samples obtained above (pH 3.0) were passed over an AG50W-X4 cation-exchange resin column (60 × 5 mm, H^+ form). Column effluents plus a water wash (4 ml) were collected and saved for analysis of [^{3}H]DOPAC and [^{3}H]HVA. The column was treated with 20 ml of 0.1 M phosphate buffer at pH 6.5 ([^{3}H]tyrosine fraction), 16 ml of 1 N HCl, and [^{3}H]DA was then eluted in the next 10 ml of 2 N HCl. Aliquots of [^{3}H]DA (2.0 ml) were added to minivials containing 4 ml Insta-Gel (Packard Instruments) and assayed in a Packard Tricarb liquid scintillation spectrometer equipped with automatic standardization (counting efficiency 22–24%). Average recovery for [^{3}H]DA using fluorometric analysis (280/330 nm) was 82%, and all samples were corrected.

The sample effluents plus water wash obtained above were combined and adjusted to pH 5.5 with NaOH. [^{3}H]DOPAC and [^{3}H]HVA in the samples were then adsorbed on a Dowex 1-X2 anion exchange resin column (40 × 5 mm, acetate form). Following a water rinse (10 ml), the column was treated with 4 ml of 0.2 N HCl and [^{3}H]DOPAC and [^{3}H]HVA were eluted in the next 10 ml of 0.2 N HCl. [^{3}H]DOPAC and [^{3}H]HVA were then separated by alumina chromatography using a modification of the procedure described by Westfall *et al.* [30]. An aliquot of the [^{3}H]HVA + [^{3}H]DOPAC eluate was adjusted to pH 8.3 using 1 M Tris base and, immediately, passed over alumina columns (200 mg of alumina; 0.5 cm in diameter). The effluent plus a water rinse containing [^{3}H]HVA was collected: [^{3}H]DOPAC was eluted with 2 × 1 ml of 1 N HCl. Aliquots of these fractions were assayed for tritium using liquid spectrometry as described above for [^{3}H]DA. Average recoveries for [^{3}H]HVA and [^{3}H]DOPAC were 65 and 77%, respectively, and all samples were corrected.

Statistics. Data were analyzed statistically using a two-way analysis of variance followed by a Duncan's New Multiple Range test [31] for comparison of mean values. A $P < 0.05$ was considered significant.

Protein determination. The amount of protein in all samples was determined by the biuret method as described by Layne [32], using bovine serum albumin as the standard.

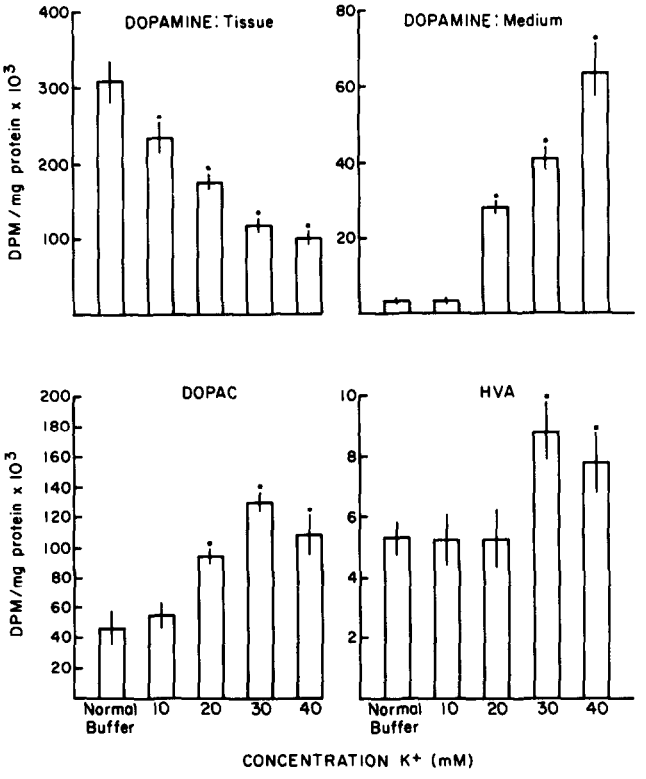

Fig. 1. K^+-evoked release and metabolism of newly-synthesized [^{3}H]DA in rat striatal slices. Striatal slices were preincubated with [^{3}H]tyrosine (20 μM) for 30 min to allow formation of newly-synthesized [^{3}H]DA. The tissue was washed in fresh buffer and then a release incubation was performed for 20 min in the presence of increasing concentrations of K^+. The amounts of [^{3}H]DA in the tissue, [^{3}H]DA in the medium, and total (tissue + medium) [^{3}H]HVA and [^{3}H]DOPAC formed were determined using column chromatographic separation (see Methods). Data represent mean ± S.E.M. of five experiments.

Key: (*) significantly different when compared to corresponding normal buffer ($P < 0.05$).

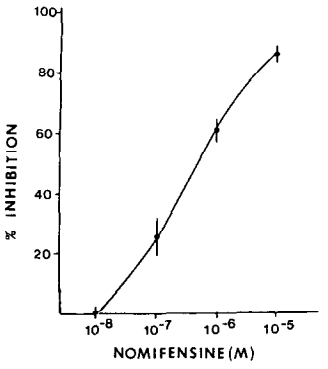

Fig. 2. Inhibition of [^{3}H]DA accumulation in rat striatal slices by nomifensine. Rats were administered nialamide (100 mg/kg, i.p.; 120 min) and reserpine (2.5 mg/kg, i.p.; 90 min), and striatal slices were prepared as described in Methods. Five-minute uptake of [^{3}H]DA (0.1 μM) was examined in the presence of nomifensine. Controls were performed using aliquots of tissue incubated in the absence of nomifensine. Percent inhibition was calculated according to the formula $((Rc - Ri)/(Rc - 1))100$ where *Rc* is the ratio of [^{3}H]DA in the tissue to [^{3}H]DA in the medium, expressed as (pmoles/g wet wt)/(pmoles/ml) for control samples, and *Ri* is the ratio in the presence of the inhibitor drug nomifensine. Data represent mean ± S.E.M. of three experiments performed in duplicate. Mean ± S.E.M. of control values (*Rc*) is 26.5 ± 4.2.

RESULTS

Potassium-evoked release and metabolism of newly-synthesized [^{3}H]DA in rat striatal slices. The potassium-induced depolarization of striatal slices produced a concentration-dependent release of newly-synthesized [^{3}H]DA (Fig. 1). Increases in the potassium-ion (K^+) concentration above that found in normal buffer (2.9 mM) caused a decrease in tissue [^{3}H]DA, an increase in medium [^{3}H]DA, and an increase in total [^{3}H]DOPAC and [^{3}H]HVA. The formation of both [^{3}H]deaminated metabolites of DA was maximal at 30 mM K^+. At this concentration, [^{3}H]DOPAC and [^{3}H]HVA were increased significantly by 290 and 160% respectively, when compared to normal buffer. Based on these results, the remaining experiments were designed to examine the role of multiple forms of MAO on the deamination of released [^{3}H]DA using either normal buffer (spontaneous release) or buffer containing 30 mM K^+ (K^+-evoked release).

Dose–response inhibition of [^{3}H]DA accumulation by nomifensine in rat striatal slices. Initially, experiments were performed to determine the concentration of nomifensine required to maximally inhibit [^{3}H]DA reuptake. Rat striatal slices, obtained following *in vivo* nialamide and reserpine pretreatment (see Methods), were allowed to accumulate [^{3}H]DA for 5 min in the presence of differing concentrations of nomifensine. As shown in Fig. 2, a concentration of 10^{-5} M was required to produce greater than 80% inhibition. Consequently, this concentration (10^{-5} M) was used in subsequent release experiments to inhibit DA neuronal reuptake of released [^{3}H]DA. Previous studies [33–35] have reported that concentrations of nomifensine as high as 10^{-5} M do not elicit dopamine release or inhibit MAO activity [34].

Effects of selective type A and type B MAO-inhibitor pretreatment and nomifensine on release and metabolism of newly-synthesized [^{3}H]DA in rat striatal slices. The effects of selective type A (clorgyline) and type B (deprenyl) MAO-inhibitor pretreatment on spontaneous release and metabolism of newly-synthesized [^{3}H]DA are shown in Fig. 3 (see normal buffer). While deprenyl produced no significant changes in any of the variables measured, clorgyline increased both tissue and medium [^{3}H]DA; however, the observed elevation in tissue [^{3}H]DA was not significant. Furthermore, clorgyline resulted in significant reductions of both [^{3}H]DOPAC and [^{3}H]HVA to 13 and 39% of control respectively. Inhibition of both type A and type B MAO (clorgyline plus deprenyl pretreatment group) also caused

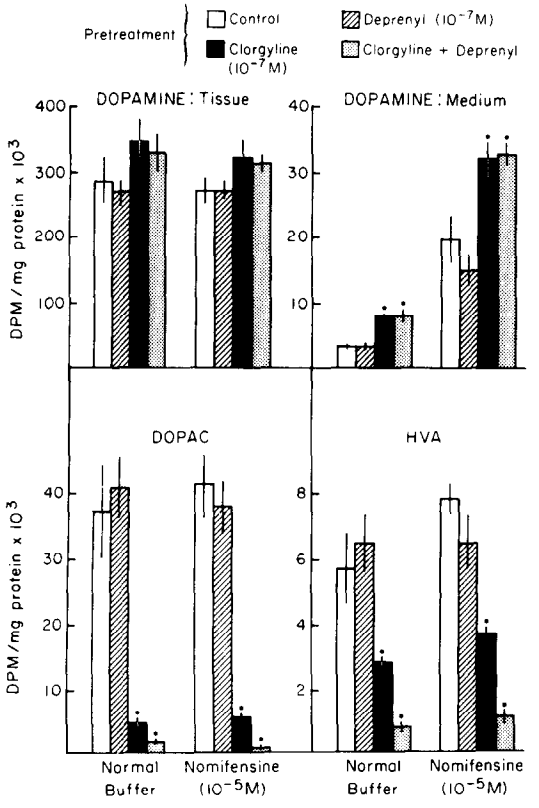

Fig. 3. Effect of selective MAO-inhibitor pretreatment and nomifensine on spontaneous release and metabolism of newly-synthesized [^{3}H]DA in rat striatal slices. Tissue slices were preincubated with [^{3}H]L-tyrosine (20 μM) for 30 min and then were incubated for 10 min in fresh buffer containing the selective irreversible MAO inhibitors, clorgyline and/or deprenyl (10^{-7} M). Controls were incubated in the absence of MAO-inhibitor drugs. Tissue slices were washed, and release and subsequent metabolism of [^{3}H]DA were examined for 20 min. The amounts of [^{3}H]DA in the tissue, [^{3}H]DA in the medium, and total (tissue + medium) [^{3}H]HVA and [^{3}H]DOPAC formed were determined using column chromatographic separation (see Methods). Data represent mean ± S.E.M. of five to eight experiments. Key: (*) significantly different when compared to corresponding control pretreatment ($P < 0.05$).

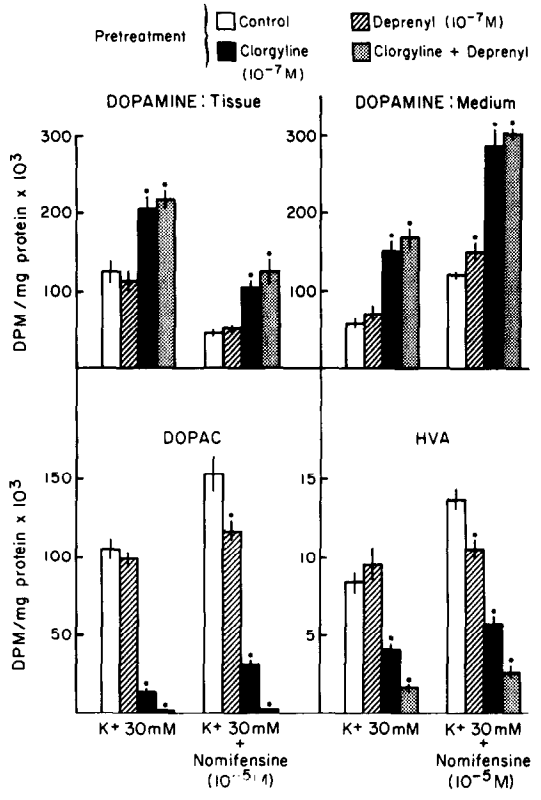

Fig. 4. Effect of selective MAO-inhibitor pretreatment and nomifensine on K^+-evoked (30 mM) release and metabolism of newly-synthesized [^{3}H]DA in rat striatal slices. For details see legend for Fig. 3. Data represent mean ± S.E.M. of five to eight experiments. Key: (*) significantly different when compared to corresponding control pretreatment ($P < 0.05$).

a significant elevation above control in medium [^{3}H]DA. While this increase in medium [^{3}H]DA was not significantly different from the clorgyline pretreated tissue, clorgyline + deprenyl did further significantly reduce the formation of [^{3}H]DOPAC and [^{3}H]HVA ($P < 0.05$). These reductions were 4 and 14% of control values for [^{3}H]DOPAC and [^{3}H]HVA respectively.

When measuring release and metabolism of newly-synthesized [^{3}H]DA under spontaneous conditions, the presence of nomifensine (10^{-5} M) significantly elevated medium [^{3}H]DA in all pretreatment groups (see Fig. 3, nomifensine (10^{-5} M)). These increases were most pronounced after clorgyline or clorgyline + deprenyl. However, nomifensine did not produce any detectable changes in tissue [^{3}H]DA or [^{3}H]DOPAC and [^{3}H]HVA formed when compared to the corresponding normal buffer.

When K^+-evoked (30 mM) release and metabolism of newly-synthesized [^{3}H]DA was examined, again deprenyl produced no significant changes in any of the variables measured (Fig. 4, see K^+ 30 mM). In contrast, clorgyline resulted in significant increases of tissue and media [^{3}H]DA to 166 and 263% of control values respectively. This elevation was more pronounced in clorgyline + deprenyl pretreated tissues, but not significantly when compared to clorgyline alone. Nevertheless, clorgyline + deprenyl produced increases of 175 and 294% in tissue and medium [^{3}H]DA, respectively, when compared to control. When [^{3}H]DOPAC and [^{3}H]HVA formation was examined (Fig. 4, see K^+ 30 mM), clorgyline significantly reduced [^{3}H]DOPAC to 12% and [^{3}H]HVA to 48% of control values. Again, a further significant reduction of both deaminated metabolites resulted from clorgyline + deprenyl pretreatment (i.e. 2% of control for [^{3}H]DOPAC and 19% of control for [^{3}H]HVA).

In the presence of nomifensine (10^{-5} M), 30 mM K^+ caused a significant decrease in tissue [^{3}H]DA and a significant increase in medium [^{3}H]DA in all pretreatment groups when compared to the corresponding 30 mM K^+ alone (see Fig. 4). Additionally, [^{3}H]DOPAC and [^{3}H]HVA formed in the control

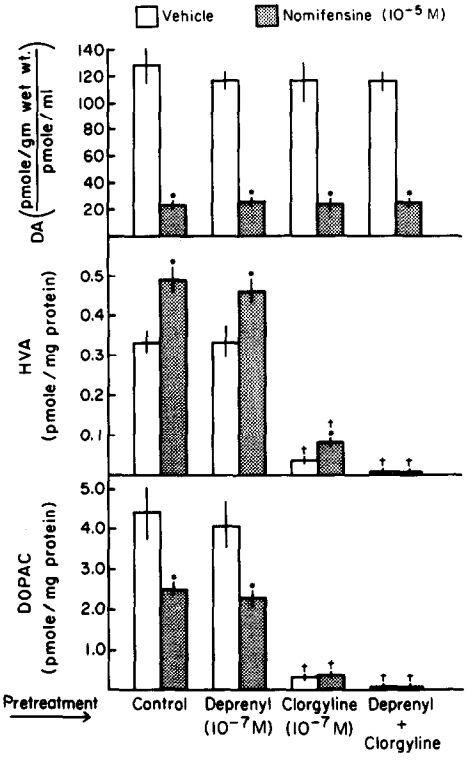

Fig. 5. Effect of selective MAO-inhibitor pretreatment and nomifensine on accumulation and metabolism of [^{3}H]DA in rat striatal slices. Tissue slices were pretreated *in vitro* with the selective, irreversible MAO inhibitors, clorgyline and/or deprenyl (10^{-7} M). Controls were incubated in the absence of MAO-inhibitor drugs. Resultant tissue slices were washed and preincubated with nomifensine (10^{-5} M) or nomifensine vehicle for 5 min. [^{3}H]DA (0.1 μM) accumulation and metabolism were examined for 10 min (37°). The amounts of [^{3}H]DA in the tissue, [^{3}H]DA in the medium, and total (tissue + medium) [^{3}H]HVA and [^{3}H]DOPAC formed were determined using column chromatographic separation (see Methods). [^{3}H]DA accumulation is expressed as a tissue to medium ratio (pmoles [^{3}H]DA per g wet wt tissue/pmoles [^{3}H]DA per ml). Data represent mean ± S.E.M. of five to seven experiments. Key: (*) significantly different from corresponding nomifensine vehicle ($P < 0.05$); and (†) significantly different when compared to corresponding control pretreatment ($P < 0.05$).

groups were increased significantly when compared to 30 mM K^+ alone (Fig. 4).

In contrast to what was observed under spontaneous conditions (compare Figs. 3 and 4), 30 mM K^+ in the presence of nomifensine significantly increased medium [^{3}H]DA in deprenyl pretreated tissue above that observed in the control group. Moreover, deprenyl significantly decreased both [^{3}H]DOPAC and [^{3}H]HVA formation to 77 and 75% of control values. Further significant reductions were observed following clorgyline (DOPAC 20% and HVA 41% of control) and the greatest reductions were observed following clorgyline + deprenyl (DOPAC 2% and HVA 20% of control).

Effects of selective MAO-inhibitor pretreatment and nomifensine on accumulation and metabolism of [^{3}H]*DA in striatal slices.* To further examine pre- versus postsynpatic formation of [^{3}H]DOPAC and [^{3}H]HVA, the effects of selective MAO-inhibitor pretreatment and nomifensine on accumulation and metabolism of [^{3}H]DA (0.1 μM) were determined. As shown in Fig. 5 (see vehicle, upper graph) deprenyl, clorgyline, or deprenyl + clorgyline did not significantly alter DA accumulation when compared to control. Furthermore, deprenyl did not alter the formation of [^{3}H]HVA or [^{3}H]DOPAC when compared to control tissue (Fig. 5; see vehicle, middle and lower graphs). However, clorgyline significantly decreased both [^{3}H]HVA (11% of control) and [^{3}H]DOPAC (7% of control), and clorgyline + deprenyl resulted in further reductions of both metabolites (HVA, 2% of control; DOPAC, 2% of control).

The addition of nomifensine (10^{-5} M) significantly inhibited [^{3}H]DA accumulation in all pretreatment groups to approximately 20% of vehicle controls (Fig. 5). However, in control, deprenyl, or clorgyline groups, nomifensine (10^{-5} M) significantly increased [^{3}H]HVA formation by 147, 136, and 228%, respectively, when compared to the corresponding vehicle control. In contrast, the addition of nomifensine significantly decreased [^{3}H]DOPAC formation in control and deprenyl pretreated tissue to 56 and 55% of vehicle controls respectively (Fig. 5, lower graph).

DISCUSSION

The present experiments were designed to gain insight into the role of multiple forms of MAO in the deamination of striatal DA in the synaptic region. These *in vitro* experiments were performed in physiological buffer containing a normal ionic composition (normal buffer) or depolarizing concentration of K^+ (30 mM). This design was used in order to further evaluate synaptic DA deamination in a resting as well as an active, state of transmitter release. Incremental increases in K^+ concentration resulted in the release of newly-synthesized [^{3}H]DA, which was associated with an increase in the formation of [^{3}H]DOPAC and [^{3}H]HVA, the major deaminated products of DA metabolism ([36], see Fig. 1). The formation of other minor deaminated DA metabolites, such as dihydroxyphenylethanol and 3-methoxy-4-hydroxyphenylethanol, could not be detected. Moreover, sulfate conjugation of [^{3}H]DOPAC and [^{3}H]HVA [37] could not be demonstrated under these *in vitro* conditions.

To evaluate the role of the type A and B forms of MAO in the deamination of [^{3}H]DA under conditions of resting and active transmitter release, striatal slices were treated with the selective type A and/or type B MAO inhibitors (clorgyline and/or deprenyl). In these experiments, it was necessary to allow the slices to form [^{3}H]DA prior to exposure to the MAO inhibitor drugs. Previous experiments had shown that selective type A MAO inhibition would decrease the formation of [^{3}H]DA from [^{3}H]tryosine [25]. Furthermore, since clorgyline and deprenyl produce irreversible MAO inhibition under these experimental conditions [25], the tissue was washed prior to the release incubation in order to remove excess drug that might have additional effects on the release process *per se*. Thus, under these conditions, any changes in metabolism induced by the MAO-inhibitor treatments most likely reflect only the presence or absence of the type A and/or type B MAO activity.

The results of these experiments showed that pretreatment with the type B MAO inhibitor, deprenyl, was ineffective in altering the formation of ^{3}H-deaminated metabolites from [^{3}H]DA, whether, the experiments were conducted in normal buffer (Fig. 3) or under depolarization conditions (K^+ 30 mM, Fig. 4). By contrast, selective type A MAO inhibition (clorgyline pretreatment) caused a substantial reduction in both [^{3}H]DOPAC and [^{3}H]HVA. However, this reduction of both metabolites was enhanced when both type A and type B MAO were inhibited (Figs. 3 and 4; clorgyline + deprenyl pretreatment). These results suggest that released DA is metabolized almost exclusively by the type A form of MAO. Nevertheless, in the absence of the type A MAO, a small but significant amount of DA deamination occurred through type B MAO. This would suggest that in the presence of both enzyme forms, the type A form possesses some characteristic which results in preferential type A metabolism of released DA. In this regard, we have demonstrated previously in rat striatum that the type A MAO exhibits a 3-fold greater affinity for DA than the type B enzyme [38].

While some investigators [13, 39] have suggested that the *in vivo* deamination of DA, in rat brain, occurs only through an interaction with type A MAO, the results of our *in vitro* experiments are more consistent with the conclusions of Green *et al.* [40]. These investigators, using biochemical and behavioral data, reasoned that *in vivo* DA deamination occurs predominantly through type A MAO. However, a small type B MAO component becomes evident in the absence of type A MAO, and only inhibition of both enzyme forms will allow for the full expression of the behavioral effects of MAO-inhibitor drugs.

To evaluate the effect of compartmentalization on the role of multiple MAOs in DA deamination within the synaptic region, studies were performed in the presence of the DA neuronal reuptake inhibitor, nomifensine. With this agent it was possible to determine the extent of DA deamination occurring external to the DA neuron (postsynaptic) under

conditions of active transmitter release. These experiments showed that, under depolarizing conditions (30 mM K^+), the presence of nomifensine increased the formation of ^{3}H-deaminated metabolites (Fig. 4). Thus, it is suggested that during active transmitter release, DA deamination occurs largely at sites external to the DA neuron. The findings of Bruinvels [16] and Holz and Coyle [17] would support such an hypothesis since their studies showed that the high-affinity DA uptake system, associated with DA neurons, is inhibited under depolarizing conditions. If this is indeed the case, during periods of increased impulse flow, released DA would remain for a longer period of time in the synapse, a situation that would favor deamination within postsynaptic sites.

When nomifensine was added to tissues treated with selective inhibitors of MAO, it was observed that, in the presence of 30 mM K^+, [^{3}H]DOPAC and [^{3}H]HVA were reduced after either type A (clorgyline) or type B (deprenyl) MAO inhibition. This result is in contrast to that seen under resting conditions or in the absence of nomifensine. These data suggest that, under conditions which favor and promote postsynaptic (i.e. outside of DA neurons) DA metabolism, significant deamination can occur through both the type A or type B form of MAO. The fact that the type B component is only detectable under depolarizing conditions in the presence of nomifensine may merely reflect the small contribution of the type B MAO in the total deamination of synaptic DA. Nevertheless, in the presence of an active DA reuptake system, DA deamination was reduced by clorgyline, but not deprenyl. This suggests that the deamination of DA occurring within DA neurons is only through a type A form of MAO. These results are consistent with our previous findings [15, 25] and the findings of other [13, 14] with regard to the exclusive presence of the type A form of MAO within striatal DA neurons.

Under spontaneous release conditions (normal buffer), [^{3}H]DOPAC and [^{3}H]HVA formation were unchanged following DA reuptake inhibition with nomifensine. Thus, it was not possible to determine the site(s) of metabolite formation from this experiment. It is conceivable that a decrease in presynaptic DA metabolism, following reuptake inhibition, was compensated by an increased postsynaptic metabolism resulting from an increase in synaptic (media) DA. However, in metabolism studies, under conditions that would favor accumulation of [^{3}H]DA by striatal DA nerve terminals (Fig. 5), nomifensine produced an 80% inhibition of [^{3}H]DA accumulation, but only decreased [^{3}H]DOPAC formation by 50%. Thus, it would appear that DOPAC formation occurs both within and outside of DA neurons. However, since nomifensine did not reduce [^{3}H]HVA, but actually enhanced its formation, it is suggested that HVA formation only occurs at sites external to the DA neuron. This latter result is supportive of the conclusion of Roffler-Tarlov *et al.* [41] who also suggest that HVA may represent a postsynaptic DA metabolite.

Our DA release experiments demonstrated that decreases in metabolite formation, following MAO-inhibitor pretreatment, were consistently associated with an increase in medium DA (see Figs. 3 and 4). Since experimental pretreatment with these drugs did not affect DA accumulation (see Fig. 5), the enhanced medium DA appears to have been an exclusive result of inhibited deamination. Presumably, such DA would be available for interaction at postsynaptic DA receptors. Therefore, it is suggested that inhibition of metabolism *per se* may represent a mechanism by which MAO-inhibitor drugs enhance synaptic DA and could account, in part, for their therapeutic actions. However, it is difficult to know if tissue slice preparations truly reflect the actions of drugs under *in vivo* conditions. At higher concentrations, MAO-inhibitor drugs also possess inhibitory effects on the DA neuronal reuptake system [42–44]. Therefore, since nomifensine was able to further increase medium DA in MAO inhibited tissue, it suggests that an effect of MAO inhibitors on the DA reuptake system would serve to further enhance synaptic DA and presumably DA receptor interaction.

Since the present experiments suggest that both type A and type B MAO are involved in the deamination of DA occurring outside of the DA neuron, it raises the question of cell-type localization. A number of non-DAergic cell-types have been shown to both accumulate DA and possess MAO activity. These include serotonergic neurons [45], glial cells [46–48] and brain capillary pericytes [49, 50]. While the importance of each of these sites must await future studies, it is conceivable that uptake and metabolism of neuronally released DA by these cell-types may operate analogously to the uptake processes previously shown to play a role in the postsynaptic metabolism of peripheral and central norepinephrine [51, 52].

In conclusion, the present study demonstrates that released DA is primarily deaminated by the type A form of MAO in rat striatal tissue. However, in the absence of type A MAO or under conditions which promote exclusive postsynaptic deamination, type B MAO plays a minor but significant role in striatal DA metabolism.

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