

THE EFFECTS OF ANTIDEPRESSANTS ON THE RETENTION AND METABOLISM OF [³H]-NOREPINEPHRINE IN RAT BRAIN SLICES

F. T. CREWS* and C. B. SMITH

Department of Pharmacology, The University of Michigan Medical School,
Ann Arbor, MI 48109, U.S.A.

(Accepted 20 September 1980)

Summary—Tricyclic antidepressants acutely decrease the neuronal retention of [³H]-norepinephrine ([³H]-NE) by blocking neuronal membrane uptake and/or vesicular uptake and binding. To distinguish between effects upon the plasma membrane and upon the vesicular membrane, the retention, deamination, and *O*-methylation of [³H]-NE by rat brain slices were investigated in the presence of several antidepressant agents. The effects of antidepressants were compared to those of the prototype inhibitors, cocaine and reserpine, using slices of hypothalamus, brainstem, parietal cortex and caudate nucleus. Cocaine, which inhibits neuronal membrane uptake, *decreased* both the deamination and retention of [³H]-NE, while *O*-methylation was increased. Reserpine, which inhibits vesicular transport and binding, *increased* deamination, while it reduced retention without affecting the *O*-methylation of [³H]-NE. The effects of desipramine, a prototype tricyclic antidepressant, were found to depend on the concentration. At low concentrations (10^{-9} – 10^{-8} M), desipramine inhibited the retention and deamination of [³H]-NE in each brain region except the caudate. At higher concentrations (10^{-7} – 10^{-4} M), the retention of [³H]-NE was reduced further. However, deamination was increased in the caudate and, in the other three regions, deamination did not decrease further. Nortriptyline and protriptyline had actions similar to desipramine, whereas, iprindole did not affect [³H]-NE retention. These results suggest that tricyclic antidepressants are not specific selective inhibitors of neuronal membrane transport.

The ability of tricyclic antidepressants to inhibit the uptake and retention of norepinephrine has been attributed to actions upon the neuronal membrane (Maxwell, Ferris and Burcsu, 1976; De Paulis, Kelder and Ross, 1978). However, studies using isolated synaptic vesicles have found that tricyclic antidepressants are potent inhibitors of the vesicular transport and binding of norepinephrine (Philippu, 1976). Studies solely on the retention of [³H]-norepinephrine cannot distinguish between inhibition at neuronal membrane or vesicular sites. The purpose of the present study was to investigate the sites of action at which tricyclic antidepressants inhibit the retention of [³H]-norepinephrine in intact noradrenergic nerve endings.

To distinguish between actions at the neuronal membrane or the synaptic vesicle, both the retention and metabolism of [³H]-norepinephrine were studied using rat brain slices. The effects of desipramine (DMI), protriptyline, nortriptyline and iprindole upon the uptake, metabolism and retention of [³H]-norepinephrine ([³H]-NE) were determined by incubating slices from various areas of the rat brain with [³H]-NE. When taken up by adrenergic neurons, [³H]-NE can be deaminated to [³H]-3,4-dihydroxymandelic acid ([³H]-DOMA) or [³H]-3,4-dihydroxyphenylglycol ([³H]-DOPG) by monoamine oxidase

(Kopin, 1972), or it can be transported into synaptic vesicles and stored (Trendelenburg, 1972; Von Euler, 1972). Cocaine and reserpine were used as prototype inhibitors of norepinephrine uptake and retention at either the neuronal or vesicular sites.

The results of the present study indicate that both sites of norepinephrine uptake and retention can be affected by tricyclic antidepressants. Low concentrations of desipramine decreased both the retention and deamination of [³H]-NE in the brainstem, hypothalamus and cortex slices. In these brain regions, higher concentrations of DMI continued to decrease the retention of [³H]-NE whereas deamination was not decreased further. In the caudate, low concentrations of DMI were ineffective whereas higher concentrations increased the deamination and decreased the retention of [³H]-NE. Protriptyline and nortriptyline acted in a similar manner to DMI, whereas iprindole had no major effect upon the uptake and the retention of [³H]-NE in slices from any brain region studied.

METHODS

Adult, male, Sprague–Dawley rats (200–300 g) were decapitated, their brains rapidly removed and dissected according to a modification of the method described by Glowinski and Iversen (1966). Slices (about 0.5 mm thick) of uniform size and shape were made from the hypothalamus, brainstem, caudate nucleus, and parietal cortex as described by Steinberg and

*Present address: Department of Pharmacology, The University of Florida Medical School, Gainesville, FL 32610, U.S.A.

Smith (1970). Four slices from each area were weighed and placed in 5 ml of ice-cold Krebs-Henseleit solution (pH 7.4) containing: NaCl, 119.4 mM; KCl, 4.7 mM; CaCl₂ 2H₂O, 2.6 mM; MgSO₄ 7H₂O, 1.2 mM; KH₂PO₄, 1.9 mM; NaHCO₃, 22.1 mM and anhydrous dextrose, 20.3 mM. The combined weights (mg wet wt \pm SEM) of the 4 slices from each area were: hypothalamus, 29.8 \pm 1.7; brainstem, 36.1 \pm 1.8; caudate, 29.0 \pm 1.5 and parietal cortex, 48.2 \pm 2.6.

The slices were equilibrated in an atmosphere of 95% O₂-5% CO₂ for 15 min in a Dubnoff metabolic shaking incubator at 37°C. At the end of this preincubation, [³H]-norepinephrine (2.9 μ Ci/sample) was added to a concentration of 1.1×10^{-7} M. Previous studies have shown that rates of uptake and retention of [³H]-norepinephrine are linear for incubations up to 30 min duration. Therefore, [³H]-norepinephrine was incubated with the tissue slices for 10 min. At the end of the 10 min incubation, the bathing solution was removed and the slices were rinsed with an additional 5 ml of 37°C Krebs-Henseleit solution (K-H solution). The slices were then washed with a third 5 ml of fresh 37°C K-H solution for 6 min. At the end of this 6 min wash, the slices were rinsed with a final 5 ml of 37°C K-H solution. Preliminary studies indicated that 93.4 \pm 1.3% (10 determinations) of norepinephrine catabolites were in the incubation bath and first rinse, 4.2 \pm 0.9% (10 determinations) of the norepinephrine catabolites were in the six minute wash and final rinse, while only 2.0 \pm 0.6% of the catabolites remained in the slices. Therefore, the catabolites of norepinephrine were assayed in the bath, wash and rinse solutions, while the slices were separated and assayed for the unchanged norepinephrine which was retained.

Conjugates of the catabolites were estimated by use of the method of Kopin, Axelrod and Gordon (1961). Total catabolite values, free and conjugated, were identical to free catabolite values which indicated that little or no conjugation occurred in these preparations. Therefore, in subsequent experiments only free catabolites were determined.

Determination of [³H]-norepinephrine retained

Slices from the 4 areas of the rat brain were homogenized in 7.5 ml of ice-cold 5% trichloroacetic acid. The homogenates were then centrifuged for 20 min at 12,000 *g*. The supernatant was poured off and saved. The pellet was suspended in 2.5 ml of 5% trichloroacetic acid and then centrifuged a second time. The two supernatants were combined. [³H]-norepinephrine was extracted by absorption on alumina as described by Crout (1961). The eluates were collected in glass scintillation vials. To each eluate 15 ml of scintillation fluid (200 mg 2-p-phenylenebis-(5-phenyloxazole), 24 g 2,5-diphenyloxazole, 200 g naphthalene dissolved in 3.8 l p-dioxane) was added. The samples were counted in a Packard Tri-Carb liquid scintilla-

tion counter for a period of time sufficient to give a standard error of counting less than 2%.

Determination of deaminated catabolites

The bath and wash solutions were each combined with 2 ml of 1 M phosphate buffer (pH, 5.5) which contained 75 μ g of each of the following carriers: 3,4-dihydroxymandelic acid (DOMA), 3,4-dihydroxyphenylglycol (DOPG), 3-methoxy-4-hydroxymandelic acid (VMA), 3-methoxy-4-hydroxyphenylglycol (MOPEG) and normethanephrine (MNM). The carriers were used for the determination of recoveries. The bath and wash solutions were then passed over Dowex 50-Na⁺ columns (5 \times 35 mm). The bath and wash effluents as well as a 5 ml glass-distilled water rinse of the column were collected in 50 ml pear-shaped flasks. The effluents were brought to a pH of 2.8 with 0.3 ml of 6 N HCl. They were then frozen in a dry-ice bath and lyophilized to dryness. The residue was dissolved in 1 ml of glass-distilled water. The final pH of this solution was 2.0. The deaminated metabolites were then extracted into 40 ml of a 5% ether-ethyl acetate (v/v) mixture. A 15 ml aliquot of the ether-ethyl acetate extract was blown down under nitrogen to 0.1 ml. This was spotted on an Eastman chromatogram sheet 6060 (Eastman Kodak Company, Rochester, NY) composed of silica gel with a fluorescent indicator. The chromatograms were developed by ascending chromatography in a solvent system composed of benzene, acetic acid, and water (53.5:45:1.5). Ultraviolet light revealed four spots with *R_f* values identical with those of DOPG, DOMA, VMA and MOPEG. The spots were cut out and eluted into 3 ml of glass-distilled water. The silica particles were removed by centrifugation. The 3 ml sample was then divided into a 2 ml aliquot which was counted for radioactivity as described above and a 0.5 ml aliquot which was used to determine recoveries. Recoveries were done on all deaminated catabolite samples. All deaminated catabolite values are corrected for recovery.

Determination of deaminated ³H-catabolite recovery

The recoveries of deaminated catabolites were determined by a modification of the phenol reaction described by Barnes, Mellman, Tedesco, Young and Nocho (1963). The 0.5 ml recovery aliquot was diluted to 1 ml with glass-distilled water. To this was added 0.1 ml of 1 N phenol reagent which was followed by 0.3 ml of 20% sodium carbonate. The samples were then heated in a 95°C water bath for 1 min. The absorption of the solutions was then determined with a Gilford 300 Spectrophotometer at 725 nm. The mean recoveries \pm SEM were as follows: DOMA, 73.6 \pm 0.8%; DOPG, 51.4 \pm 1.2%; VMA, 91.8 \pm 1.1%; MOPEG, 60.5 \pm 1.5%.

Determination of [³H]-normetanephrines

The bath, wash, and rinse solutions were passed over Dowex 50-Na⁺ columns (5 \times 35 mm). The

amines, $[^3\text{H}]$ -normetanephrine and $[^3\text{H}]$ -norepinephrine were absorbed to the resin and were thus separated from the deaminated catabolites which passed through and were assayed in the effluent. Following a 5 ml glass-distilled water rinse, 6 ml of 1 N HCl was passed over the column to remove some $[^3\text{H}]$ -norepinephrine. $[^3\text{H}]$ -normetanephrine as well as the remaining $[^3\text{H}]$ -norepinephrine was then eluted with 15 ml of 3 N NH_4OH . To this eluate 1.5 ml of 6N HCl and 1 ml of Tris-(hydroxymethyl) aminomethane (pH 8.3) was added. The pH of each sample was brought to 8.3, and the $[^3\text{H}]$ -norepinephrine was absorbed onto alumina by the method of Crout (1961). The effluent of the alumina columns contained the $[^3\text{H}]$ -normetanephrine, and this was collected. The effluent was brought to a pH of 5.5 with 2 ml of 4M NaAc (pH 5.5) and passed over a second Dowex 50- Na^+ column. The column was washed with 10 ml of glass-distilled water and the $[^3\text{H}]$ -normetanephrine eluted with 15 ml 3N NH_4OH . The eluates were evaporated to dryness. The residue was then washed with 0.5 ml of isoamyl alcohol. A 0.1 ml aliquot was spotted on an Eastman Chromatogram sheet 6065 (Eastman Kodak Company, Rochester, NY) composed of cellulose with a fluorescent indicator. The chromatograms were developed by ascending chromatography in a solvent system composed of butanol, acetic acid and water (60:15:25). $[^3\text{H}]$ -normetanephrine was visualized by spraying the chromatogram first with 1 N phenol reagent and then with 20% sodium carbonate. The spots were cut out and counted by liquid scintillation spectrometry as described above. Recovery of $[^3\text{H}]$ -normetanephrine was $61.8 \pm 2.8\%$ (20 determinations).

Drugs used

The following drugs and reagents were used: levo- $[^3\text{H}(\text{N})]$ norepinephrine (specific activity 2.7 Ci/mmol, radiochemical purity > 98.5%, New England Nuclear Corp., Boston, MA), *l*-norepinephrine bitartrate and 3,4-dihydroxymandelic acid (Calbiochem, San Diego, CA), cocaine hydrochloride (Merck Chemicals, Rahway, NJ), 3-methoxy-4-hydroxymandelic acid, 3,4-dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol (Sigma Chemical Company, St Louis, MO), desipramine hydrochloride (USV Pharmaceuticals, Scarsdale, NY), and normetanephrine (Winthrop Labs., New York, NY).

All drugs were dissolved in saline except reserpine. Reserpine was dissolved in 20% ascorbic acid and diluted to the appropriate concentrations with glass-distilled water. Cocaine, desipramine and the other tricyclic antidepressants were added to the brain slice incubation baths 15 min before the addition of $[^3\text{H}]$ -norepinephrine.

Results are expressed as the mean value \pm SEM. The significance of differences was assessed by Student's *t*-test (Fisher, 1950), and *P* values are given in parentheses.

RESULTS

Control experiments

Control slices from different brain regions varied in their ability to retain and deaminate $[^3\text{H}]$ -NE (Fig. 1). The caudate slices which contain dopaminergic nerve terminals (Hornykiewicz, 1973) retained more than double the $[^3\text{H}]$ -NE retained by the slices of hypothalamus, cortex and brainstem where the adrenergic terminals are primarily noradrenergic (Holzbauer and Sharman, 1972). In contrast, the hypothalamus, cortex

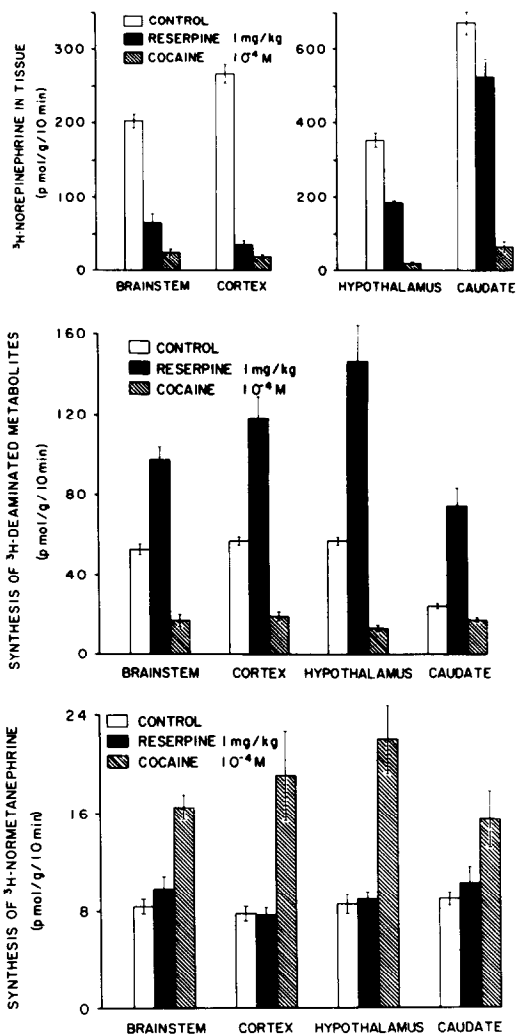


Fig. 1. Effects of reserpine and cocaine on the retention and metabolism of $[^3\text{H}]$ -norepinephrine in slices from various areas of the rat brain. (Upper graph) $[^3\text{H}]$ -norepinephrine retained by slices, pmol/g tissue wet wt. (Middle graph) $[^3\text{H}]$ -deaminated metabolites synthesized by slices, pmol/g tissue wet wt. (Lower graph) $[^3\text{H}]$ -normetanephrine synthesized by slices, pmol/g tissue wet wt. All values are for a 10 min incubation with $[^3\text{H}]$ -NE (10^{-7} M) at 37°C . Open bar, control slices; solid bar, reserpine (1 mg/kg, i.p., 18 hr before sacrifice); cross-hatched bars, cocaine, 10^{-4} M, added to the incubation flask 15 min before the addition of $[^3\text{H}]$ -NE. Each value represents the mean of from 4 to 6 determinations. Vertical lines represent SE.

Table 1. Effects of reserpine and cocaine on the synthesis of [³H]-norepinephrine in slices from various areas of the rat brain

	Brainstem	Hypothalamus	Caudate	Cortex
<i>Control</i>				
DOMA†	9.27* ± 0.62†	8.90 ± 0.41	4.40 ± 0.43	9.65 ± 0.51
DOPG§	26.26 ± 1.33	28.90 ± 1.06	11.86 ± 0.90	29.75 ± 1.32
VMA¶	4.94 ± 0.65	5.06 ± 0.51	2.28 ± 0.28	4.51 ± 0.40
MOPEG	10.90 ± 0.91	14.15 ± 1.24	5.32 ± 0.63	13.02 ± 1.46
TOTALS	52.42 ± 2.49	56.91 ± 1.91	23.86 ± 1.18	56.80 ± 2.05
<i>Reserpine 1 mg/kg</i>				
DOMA	18.52 ± 1.83	25.70 ± 3.88	15.24 ± 1.82	15.69 ± 0.84
DOPG	44.56 ± 3.20	74.10 ± 8.54	32.47 ± 3.76	65.05 ± 5.55
VMA	7.76 ± 0.57	10.43 ± 1.12	5.42 ± 0.93	11.56 ± 0.93
MOPEG	26.56 ± 1.30	35.62 ± 4.67	20.60 ± 3.72	25.92 ± 3.20
Totals	97.39 ± 6.44	145.85 ± 18.21	73.74 ± 9.14	118.22 ± 10.50
<i>Cocaine 10⁻⁴ M</i>				
DOMA	2.96 ± 0.81	1.72 ± 1.02	2.59 ± 1.29	3.07 ± 0.36
DOPG	5.03 ± 1.44	4.26 ± 1.40	5.79 ± 0.22	6.95 ± 1.02
VMA	1.85 ± 0.25	1.43 ± 0.58	2.48 ± 0.36	1.56 ± 0.42
MOPEG	6.96 ± 0.94	5.38 ± 0.76	6.06 ± 0.49	7.34 ± 1.26
Total deaminated	16.81 ± 2.96	12.79 ± 1.58	16.92 ± 0.89	18.91 ± 1.96

* pmol/g/10 min. Each value represents the mean of at least 3 determinations.

† SEM.

‡ DOMA, 3,4-dihydroxymandelic acid.

§ DOPG, 3,4-dihydroxyphenylglycol.

¶ VMA, 3-methoxy-4-hydroxyphenylglycol.

|| MOPEG, 3-methoxy-4-hydroxyphenylglycol.

and brainstem slices all deaminated very similar amounts of [³H]-NE while the caudate slices deaminated much smaller amounts.

Although the amount of [³H]-NE deaminated varied among brain regions, the distribution of the four deaminated catabolites within that total was very similar among the regions (Table 1). In all four brain regions [³H]-DOPG was the major catabolite: [³H]-DOPG averaged 51%, [³H]-DOMA, 17%, [³H]-MOPEG 23%; and [³H]-VMA, 9% of the total deaminated catabolites forms.

Reserpine and cocaine

Both cocaine, added *in vitro* to the incubates at a concentration of 10⁻⁴ M and reserpine, 18 hr pretreatment with 1 mg/kg (i.p.), decreased the retention of [³H]-norepinephrine. Cocaine decreased the retention of [³H]-NE to less than 12% ($P < 0.001$) of control values in all 4 brain regions (Fig. 1). Reserpine reduced the retention of [³H]-NE in the brainstem and cortex to 24% ($P < 0.001$) and 19% ($P < 0.001$) of control values, respectively. In the hypothalamus [³H]-NE retention was reduced to 52% ($P < 0.001$) of control values while caudate retention was only reduced to 78% ($P < 0.025$) of control values by reserpine.

Cocaine decreased the synthesis of [³H]-DOPG in a manner similar to [³H]-NE retention (Table 1). In direct contrast to cocaine, reserpine pretreatment, 1 mg/kg (i.p.), 18 hr, approximately doubled the synthesis of the deaminated metabolites in all 4 brain regions (Table 1). These increases indicate that the

inhibition of vesicular transport and binding markedly increases the deamination of [³H]-NE.

Reserpine and cocaine also had differing effects on the *O*-methylation of [³H]-NE. [³H]-normetanephrine formation was not significantly altered by reserpine. However, cocaine almost doubled the formation of normetanephrine in all 4 brain regions ($P < 0.005$, Fig. 1).

Desipramine concentration response curves

When added directly to the incubates, DMI (10⁻⁹-10⁻⁴ M) decreased the retention of [³H]-NE in all 4 brain regions (Fig. 2). The retention of [³H]-NE in the hypothalamus, brainstem and cortex was decreased in an almost identical concentration-dependent manner starting at 10⁻⁹ M DMI and continuing to the highest concentration of 10⁻⁴ M DMI. In these three brain regions the deamination of [³H]-NE was also decreased. The deaminated metabolites were decreased most dramatically at the lower concentrations of DMI (10⁻⁹-10⁻⁸ M). As the concentrations of DMI were increased above 10⁻⁷ M, the magnitude of the decreases in the synthesis of [³H]-DOPG (Fig. 3) and the other deaminated metabolites was small if present at all (data not shown). This is in contrast to the retention of [³H]-NE which continued to decrease throughout the concentration range tested (10⁻⁹-10⁻⁴ M).

The effects of DMI in the caudate are strikingly different from those in the other brain regions. In the caudate, neither the retention nor the deamination of [³H]-NE was significantly affected by concentrations

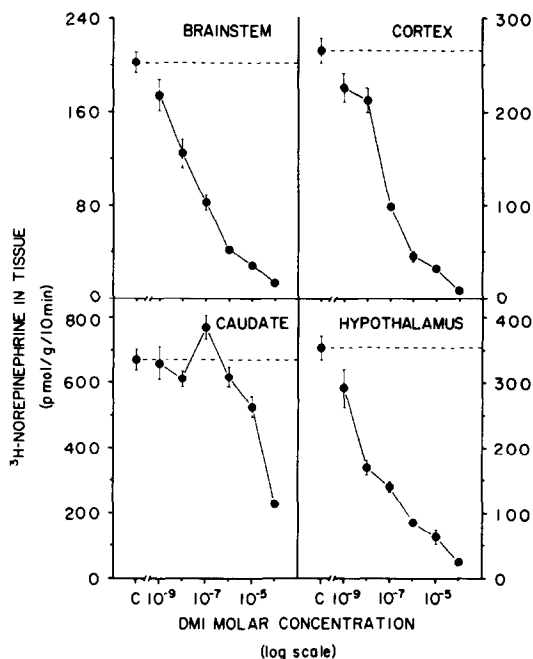


Fig. 2. Effects of DMI added to incubates on the retention of $[^3\text{H}]$ -norepinephrine in slices from various areas of the rat brain; DMI (10^{-9} – 10^{-4} M) was added 15 min before the addition of $[^3\text{H}]$ -norepinephrine (10^{-7} M). The slices were incubated with $[^3\text{H}]$ -norepinephrine for 10 min at 37°C . Each value represents the mean of at least 3 determinations. The control value is on the left of each graph. Vertical lines represent SE.

of DMI as high as 10^{-6} M. At the high concentrations of DMI (10^{-5} – 10^{-4} M), the retention of $[^3\text{H}]$ -NE was reduced in the caudate (Fig. 2) while synthesis of $[^3\text{H}]$ -DOPG (Fig. 3) was more than doubled. Thus, in the caudate, high concentrations of DMI decreased the retention while increasing the deamination of $[^3\text{H}]$ -NE.

In all 4 brain regions $[^3\text{H}]$ -normetanephrine synthesis was increased by DMI. The lower concentrations of DMI (10^{-9} – 10^{-7} M) only slightly increased the synthesis of $[^3\text{H}]$ -normetanephrine while concentrations of DMI above 10^{-6} M, significantly increased the *O*-methylation of $[^3\text{H}]$ -NE in all 4 brain regions ($P < 0.05$, Fig. 4).

Nortriptyline, protriptyline, and iprindole

In slices of the hypothalamus, brainstem, and cortex, nortriptyline (10^{-7} M) significantly ($P < 0.005$) reduced both the retention and deamination of $[^3\text{H}]$ -NE in a manner similar to DMI (Fig. 5). Nortriptyline did not affect the retention of $[^3\text{H}]$ -NE in the caudate. However, it decreased the synthesis of the deaminated metabolites in the caudate to 59% ($P < 0.005$) of control values, unlike DMI, which either did not change or increased deamination in the caudate.

Protriptyline (10^{-7} M) also affected the retention and metabolism of $[^3\text{H}]$ -NE in a manner similar to DMI, except in the caudate (Fig. 5). While an equal-

ent concentration of DMI did not affect either the retention or deamination of $[^3\text{H}]$ -NE in the caudate, protriptyline decreased the retention of $[^3\text{H}]$ -NE to 82% ($P < 0.025$) of control values and slightly reduced the deamination of $[^3\text{H}]$ -NE to 92% of controls. $[^3\text{H}]$ -normetanephrine synthesis was increased in all 4 brain regions by protriptyline.

Iprindole, although a clinically effective tricyclic antidepressant (El-Deiry, Forrest and Littman, 1967; McClatchey, Moffat and Irvine, 1967), differs from the tricyclic prototypes both structurally and through its effects on $[^3\text{H}]$ -NE retention and metabolism (Fig. 5). Iprindole, an indole with an eight-member saturated ring, when added *in vitro* to the incubates at a concentration as high as 10^{-6} M, did not affect the retention of $[^3\text{H}]$ -NE in any of the 4 brain regions studied. Furthermore, the synthesis of the deaminated metabolites was not significantly affected in the caudate and cortex. Deamination was decreased to 73% ($P < 0.05$) of control values in the brainstem and to 78% ($P < 0.05$) of controls in the hypothalamus. The synthesis of $[^3\text{H}]$ -normetanephrine was not affected in any of the 4 brain regions studied. Thus, in contrast to the other 3 antidepressants studied, iprindole did not decrease the retention of $[^3\text{H}]$ -NE in any brain region, slightly decreased deamination only in the brainstem and hypothalamus, and did not affect *O*-methylation.

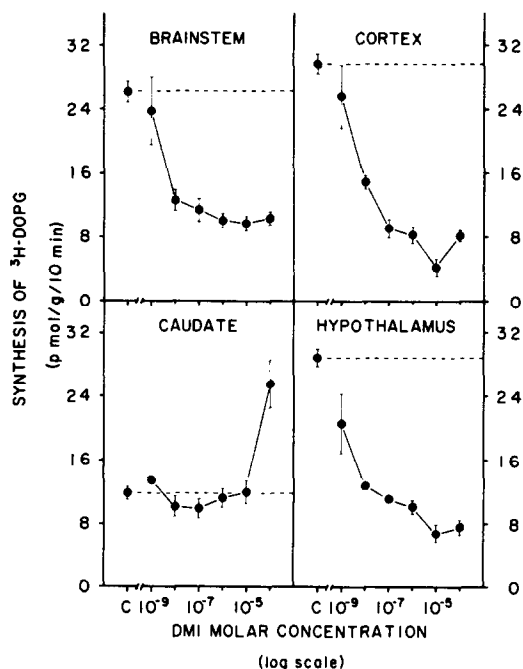


Fig. 3. Effects of DMI added to incubates on the synthesis of $[^3\text{H}]$ -3,4-dihydroxyphenylglycol ($[^3\text{H}]$ -DOPG) in slices from various areas of the rat brain; DMI (10^{-9} – 10^{-4} M) was added 15 min before the addition of $[^3\text{H}]$ -norepinephrine (10^{-7} M). The slices were incubated with $[^3\text{H}]$ -norepinephrine for 10 min at 37°C . Each value represents the mean of at least 3 determinations. The control value is on the left of the graph in each case. Vertical lines represent SE.

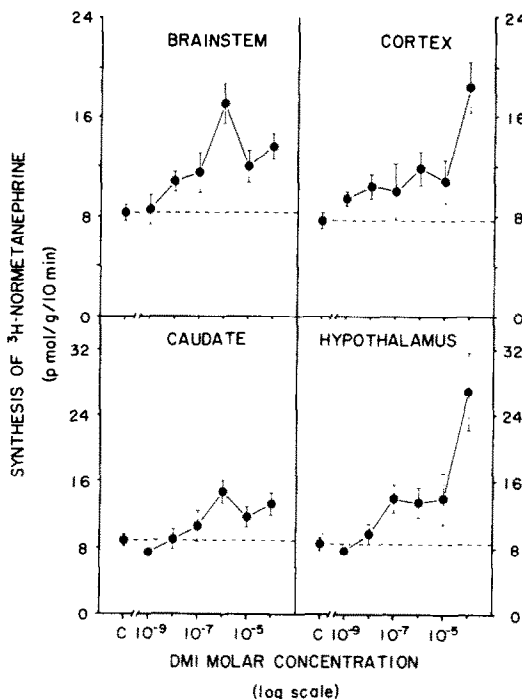


Fig. 4. Effects of DMI added to incubates on the synthesis of [^3H]-normetanephrine in slices from various areas of the rat brain; DMI (10^{-9} – 10^{-4} M) was added 15 min before the addition of [^3H]-norepinephrine (10^{-7} M). The slices were incubated with [^3H]-norepinephrine for 10 min at 37°C . Each value represents the mean of at least 3 determinations. The control value is on the left of the graph in each case. Vertical lines represent SE.

DISCUSSION

The differences between the effects of reserpine and those of cocaine on the metabolism of [^3H]-NE may be used to distinguish between the site or sites at which drugs act to inhibit the uptake and retention of norepinephrine. In this preparation, cocaine-like drugs, which block neuronal membrane transport, are characterized by decreases in both the retention and deamination of [^3H]-NE, while the *O*-methylation of [^3H]-NE increases. Drugs like reserpine, which block vesicular transport, decrease the retention of [^3H]-NE whereas they increase deamination of [^3H]-NE and do not affect its *O*-methylation. Therefore, this preparation was used to investigate the sites of the acute and chronic actions of tricyclic anti-depressants.

Complete concentration-response curves for DMI were determined in all 4 brain regions. Both the retention and deamination of [^3H]-NE in the brainstem, hypothalamus and cortex were decreased by DMI (Figs 2 and 3). In these 3 brain regions, low concentrations of DMI (10^{-9} – 10^{-8} M) decreased [^3H]-NE retention and [^3H]-DOPG synthesis to the same extent, which suggests a blockade of neuronal membrane transport. With increasing concentrations, greater than 10^{-7} M DMI, [^3H]-NE retention continued to decrease while the synthesis of [^3H]-DOPG

remained relatively constant. The continuing decreases in [^3H]-NE retention, without concomitant decreases in deamination, suggest that DMI also has reserpine-like effects at these concentrations. Thus, in the brainstem, hypothalamus and cortex, concentrations of DMI below 10^{-7} M seem to block neuronal membrane transport while higher concentrations affect both neuronal membrane and vesicular sites.

In contrast to the other areas of the brain, the caudate slices showed no change in either [^3H]-NE retention or deamination at low concentrations of DMI (10^{-9} – 10^{-6} M). Higher concentrations of DMI decreased the amount of [^3H]-NE retained by caudate slices while increasing the deamination of [^3H]-NE. These effects are identical to those produced by reserpine and, therefore, imply that DMI is interfering with the transport and/or binding of [^3H]-NE by the synaptic vesicle. Caudate neuronal membrane transport is not affected by low concentrations of DMI. This allows the reserpine-like

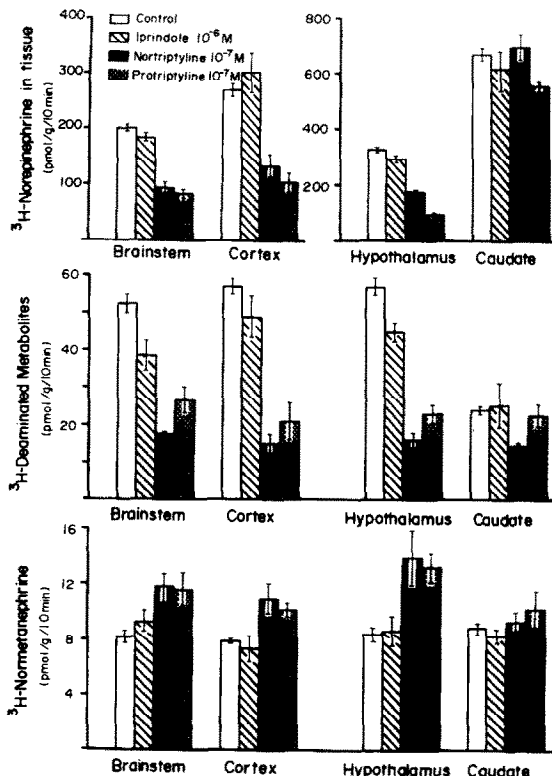


Fig. 5. Effects of iprindole, nortriptyline, and protoipryline on the retention and metabolism of [^3H]-norepinephrine in slices from various areas of the rat brain. (Upper graph) [^3H]-norepinephrine retained by slices, pmol/g tissue wet wt. (Middle graph) [^3H]-deaminated metabolites synthesized by slices, pmol/g tissue wet wt. (Lower graph) [^3H]-normetanephrine synthesized by slices, pmol/g tissue wet wt. All values are for a 10 min incubation with [^3H]-NE (10^{-7} M) at 37°C . Open bar, control slices; hatched bars, iprindole, 10^{-6} M; solid bars, nortriptyline, 10^{-7} M; cross hatched bars, protoipryline, 10^{-7} M. All drugs were added to the incubation flask 15 min before the addition of [^3H]-NE. Each value represents the mean of from 4 to 6 determinations. Vertical lines represent SE.

vesicular effects of higher concentrations of DMI to predominate in caudate slices.

Nortriptyline and protriptyline affected the retention and metabolism of [³H]-NE in a manner similar to DMI. These cocaine-like effects suggest a blockade of neuronal membrane transport in the noradrenergic neurons of these brain regions. At these same concentrations there were only slight effects upon the caudate retention and deamination of [³H]-NE by caudate slices.

Although most investigators have only determined the retention of [³H]-norepinephrine, they attribute the inhibition of norepinephrine retention by tricyclic antidepressants to an action solely at the neuronal membrane. The results of the present study indicate that the sites of action of tricyclic antidepressants depend on the antidepressant, the brain region studied and the concentration used. Very low concentrations of desipramine (10^{-9} – 10^{-8} M) appear to inhibit primarily neuronal membrane transport in the hypothalamus, brainstem and cortex. However, at concentrations above 10^{-7} M, a concentration frequently reached *in vivo* (Bickel, 1975; Vetulani, Stawarz, Dingell and Sulser, 1976), both the neuronal membrane and synaptic vesicle transport are affected. In contrast, iprindole does not appear to affect either neuronal membrane or vesicular transport sites. These results indicate that no generalization can be made concerning the sites of action of tricyclic antidepressants on norepinephrine uptake and retention.

Acknowledgements—We thank Drs Irwin J. Kopin, Martin Zatz and John F. Tallman for their careful reading and comments during the preparation of this manuscript.

REFERENCES

- Barness, L. L., Mellman, W. J., Tedesco, T., Young, D. G. and Nocho, R. (1963). A quantitative method for determining urinary phenols. *Clin. Chem.* **9**: 600–607.
- Bickel, M. H. (1975). Poisoning by tricyclic antidepressant drugs. *Int. J. clin. Pharmac. Ther. Toxic.* **11**: 145–176.
- Crout, J. R. (1961). Catecholamines in urine. In: *Standard Methods in Clinical Chemistry* (Seligson, D., Ed.), Vol. 3, pp. 62–80. Academic Press, New York.
- De Paulis, T., Kelder, D. and Ross, S. B. (1978). On the topology of the norepinephrine transport carrier in rat hypothalamus. The site of action of tricyclic uptake inhibitors. *Mol. Pharmac.* **14**: 596–606.
- El Deiry, N. K., Forrest, A. D. and Littman, S. K. (1967). Clinical trial of a new antidepressant (Wy-3262). *Br. J. Psychiat.* **113**: 99–1004.
- Fisher, R. A. (1950). *The Design of Experiments*, 11th Edition. Oliver & Boyd, Edinburgh.
- Glowinski, J. and Iversen, L. L. (1966). Regional studies of catecholamines in the rat brain. I. The disposition of [³H]-norepinephrine, [³H]-dopamine, and [³H]-DOPA in various regions of the brain. *J. Neurochem.* **13**: 655–669.
- Holzbauer, M. and Sharman, D. F. (1972). The distribution of catecholamines in vertebrates. In: *Catecholamines* (Blaschko, H. and Muscholl, E., Eds), pp. 110–185. Springer-Verlag, Berlin.
- Hornykiewicz, O. (1973). Dopamine in the basal ganglia. *Br. Med. Bull.* **29**: 172–172.
- Kopin, I. J. (1972). Metabolic degradation of catecholamines. The relative importance of different pathways under physiological conditions and after administration of drugs. In: *Catecholamines* (Blaschko, H. and Muscholl, E., Eds), pp. 270–282. Springer-Verlag, Berlin.
- Kopin, I. J., Axelrod, J. and Gordon, E. K. (1961). The metabolic fate of ³H-epinephrine and ¹⁴C-metanephrine in the rat. *J. biol. Chem.* **236**: 2109–2113.
- Maxwell, R. A., Ferris, R. M. and Burcsu, J. E. (1976). Structural requirements for inhibition of norepinephrine uptake by phenethylamine derivatives, desipramine, cocaine and other compounds. In: *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines* (Paton, D. M., Ed.), pp. 95–147. Raven Press, New York.
- McClatchey, W. T., Moffat, J. and Irvine, G. M. (1967). A double-blind study of WY-3263, imipramine and placebo. *J. Ther. Clin. Res.* **5**: 13–15.
- Philippu, A. (1976). Transport in intraneuronal storage vesicles. In: *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines* (Paton, D. M., Ed.), pp. 215–240. Raven Press, New York.
- Steinberg, M. I. and Smith, C. B. (1970). Effects of desmethyl-imipramine and cocaine on the uptake, retention, and metabolism of [³H]-tyramine in rat brain slices. *J. Pharmac. exp. Ther.* **173**: 176–192.
- Trendelenburg, U. (1972). Factors influencing the concentration of catecholamines at receptors. In: *Catecholamines* (Blaschko, H. and Muscholl, E., Eds), pp. 726–761. Springer-Verlag, Berlin.
- Vetulani, J., Stawarz, R. J., Dingell, J. V. and Sulser, F. (1976). A possible common mechanism of action of antidepressant treatments. *Naunyn-Schmiedeberg's Arch. Pharmac.* **293**: 109–114.
- Von Euler, W. S. (1972). Synthesis, uptake and storage of catecholamines in adrenergic nerves. The effect of drugs. In: *Catecholamines* (Blaschko, H. and Muscholl, E., Eds), pp. 186–230. Springer-Verlag, Berlin.