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EFFECT OF DIPHENYLHYDANTOIN ON THE UPTAKE AND CATABOLISM OF L-[^{3}H]NOREPINEPHRINE *IN VITRO* IN RAT CEREBRAL CORTEX TISSUE*

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Abstract—The effect of diphenylhydantoin on the accumulation of [^{3}H]norepinephrine *in vitro* was examined in brain slices prepared from rat cerebral cortex. High concentrations of diphenylhydantoin (10^{-3} M) caused a significant reduction in the 5-min accumulation of [^{3}H]norepinephrine. On the other hand, 10^{-5}–10^{-4} M diphenylhydantoin facilitated the 20-min accumulation of [^{3}H]norepinephrine. This facilitative action of diphenylhydantoin was (1) associated with a reduction in oxidative catabolism of [^{3}H]norepinephrine and (2) abolished by the 2-hr pretreatment of rats with 100 mg/kg of nialamide (i.p.). The inhibitory action of diphenylhydantoin on the oxidative catabolism of [^{3}H]norepinephrine was observed in both whole and lyzed crude synaptosomal preparations. When diphenylhydantoin and pargyline were compared, it was found that pargyline ($ID_{50} = 1{\cdot}5 \times 10^{-6}$ M) was 37 times more effective than diphenylhydantoin ($ID_{50} = 5{\cdot}5 \times 10^{-5}$ M) in inhibiting the oxidative deamination of [^{3}H]norepinephrine. These results suggest that diphenylhydantoin alters norepinephrine metabolism in cerebral cortex slices by an inhibitory action on (1) monoamine oxidase activity and (2) the neuronal uptake system.

SINCE ITS DISCOVERY by Merritt and Putnam[1] in 1938, diphenylhydantoin (DPH) has become one of the most widely used drugs in the treatment of convulsive disorders. In spite of the universal acceptance of DPH as an effective anticonvulsant agent, its exact mechanism of action for suppressing seizures remains obscure.

There are data to suggest that the anticonvulsant properties of DPH are mediated through the interaction of this compound with neurons which contain such putative neurotransmitters as the catecholamines (norepinephrine and dopamine) and/or 5-hydroxytryptamine. Chen *et al.*[2] reported in 1954 that reserpine will abolish the anticonvulsant properties of DPH as well as lower the electroshock seizure threshold in rodents. This latter effect of reserpine appears to be related to the catecholamine and/or 5-hydroxytryptamine depleting action of this agent.[3] Other studies have shown that DPH in doses that protect rats against maximal electroshock will cause a significant rise in brain 5-hydroxytryptamine levels.[4] And finally, recently it has been shown that DPH will block the accumulation of [^{3}H]-dopamine into slices prepared from rat corpus striatum.[5]

The present studies were performed in an attempt to examine the effects of DPH on a third brain amine, norepinephrine (NE). Our studies were performed in rat cerebral

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cortex since (1) DPH inhibits the spread of electrical activity associated with seizures in the cerebral cortex,[6] and (2) the catecholamine neuronal system in this brain region contains NE as the neurotransmitter substance.[7] The results of these studies would suggest that DPH alters both the neuronal uptake and catabolism of [^{3}H]NE in brain slices prepared from rat cerebral cortex.

MATERIALS AND METHODS

Animals. Normal male, adult (250–300 g) Sprague–Dawley rats were housed in the University animal quarters for at least 3 days prior to sacrifice and given food and water *ad lib.*

Drugs. All concentrations of diphenylhydantoin Na^+ (Park, Davis & Company) were prepared by dissolving in 0·001 N NaOH. Pargyline HCl (Abbott Laboratories) was dissolved in Krebs–Henseleit bicarbonate solution (see below). Nialamide (Pfizer, Inc.) was prepared by dissolving nialamide in 1×10^{-3} N HCl. DL-Norepinephrine was obtained from Winthrop Laboratories and normetanephrine from CalBiochem Company.

Radioisotopes. L-[7-^{3}H]norepinephrine (6·6 Ci/m-mole), Amersham/Searle Corp., was used in all of the studies.

Accumulation and metabolism of [^{3}H]NE in cerebral cortex slices. Two rats were decapitated, their brains removed and the cerebral cortex (with corpus striatum and white matter removed) was dissected and placed in an ice-cold Krebs–Henseleit bicarbonate solution[8] (m-mole/l: NaCl, 118·00; KCl, 4·69; $CaCl_2$-$2H_2O$, 2·51; $MgSO_4$, 0·71; Na_2EDTA-$2H_2O$, 0·02; KH_2PO_4, 0·75; $NaHCO_3$, 14·74; glucose, 11·10). The cortex was weighed and sliced with a McIlwain tissue chopper at 0·3 mm intervals, turned 90° and resliced as before.[9] Sliced cortex (1·5 g) was added to 2 ml of Krebs–Henseleit solution vortexed and centrifuged (4°) at 1000 *g* for 5 min. The supernatant was discarded and the cortical slices were resuspended in 4 ml of ice-cold Krebs–Henseleit solution. An 0·2-ml aliquot of the suspension (6–7 mg of protein) was added to each of 18 centrifuge tubes containing 1·5 ml of Krebs–Henseleit solution 0·1 ml of 0·2 per cent ascorbic acid, 0·1 ml of DPH (10^{-6}–10^{-3} M, final concn in tube) and incubated for 10 min at 37° (95 per cent O_2–5 per cent CO_2). Control sample tubes contained 0·1 ml of 0·001 N NaOH (solvent medium for DPH) instead of the DPH solution. After the 10-min preincubation period, 2×10^{-6} M [^{3}H]NE (1·3 μCi), in a volume of 0·1 ml, was added to each tube to give a final concentration of 1×10^{-7}M. Samples were allowed to incubate for an additional 5 or 20 min (95 per cent O_2–5 per cent CO_2; 37°). The incubation was terminated by chilling the tubes in ice, immediately followed by centrifugation at 10,000 *g* for 2 min (4°). The supernatant was poured off and the pellet washed with 1 ml of ice-cold Krebs–Henseleit solution, vortexed and recentrifuged at 10,000 *g* for 10 min (4°). The supernatant of each sample was combined with supernatants from the initial centrifugation (medium fraction) and saved for analysis of [^{3}H]NE, [^{3}H]normetanephrine ([^{3}H]NM) and [^{3}H]-deaminated metabolites. Medium fractions were acidified with 2 ml of 2 N HCl and 100 μg of both NE and NM were added for recovery determination. The final pellet was acidified with 2 ml of 2 N HCl, 100 μg of both NE and NM added to each sample and homogenized. Homogenates were centrifuged at 10,000 *g* for 10 min (4°) after which the supernatants (tissue extracts) were decanted and assayed for [^{3}H]NE, [^{3}H]NM and [^{3}H]-deaminated metabolites. The amount of protein in each sample was

determined by the biuret method as described by Layne.[10] The amount of protein in each sample was summed and compared to the total tissue wet weight so that each protein value could be converted into wet weights.

Separation and determination of [^{3}*H*]*NE,* [^{3}*H*]*NM and total* [^{3}*H*]*-deaminated catabolites.* Acidified tissue extracts and medium from each sample were adjusted to pH = 6·0 with NaOH and passed over a strong cation exchange resin[11,12] (Baker grade CGC-241; x—8, 200–400 mesh). The ion-exchange column dimensions were 40 × 5 mm at pH = 6·0. Prior to addition of the sample, the column was prepared by passing 20 ml of 0·1 M sodium phosphate buffer (pH = 6·5) followed by 3 ml of glass distilled water (GDW). Column effluents plus 5 ml of a GDW wash were collected and saved for the determination of total [^{3}H]-deaminated catabolites. After a 35-ml GDW wash, [^{3}H]NE and [^{3}H]NM were eluted from the column with 2 N HCl. Recovery values as determined fluorometrically (280/335) were consistently 85 and 75 per cent respectively.

Aliquots of the [^{3}H]NE, [^{3}H]NM and [^{3}H]-deaminated catabolite fractions were assayed with a Packard Tri-Carb liquid scintillation spectrometer equipped with automatic standardization. The liquid scintillation mixture consisted of 5 g of 2,5-diphenyloxazole, 0·2 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene, 500 ml of toluene and 500 ml of Triton X-100.

Oxidative deamination of [^{3}*H*]*NE in intact and lyzed crude synaptosomal fractions.* A crude synaptosomal fraction of rat cerebral cortex was prepared as described by Gray and Whittaker.[13] Briefly, a 10% (w/v) homogenate of rat cerebral cortex (2·8 g wet weight; corpus striatum and white matter were removed) was prepared, in 0·32 M sucrose (4°), with a Teflon-glass, loose fitting tissue grinder (12 strokes). The homogenate was centrifuged at 1000 *g* for 10 min at 4°. The 1000 *g* supernatant was recentrifuged at 10,000 *g* for 20 min (4°) and the pellet was resuspended in 3 ml of 0·32 M sucrose (crude synaptosomal suspension). In some experiments the 10,000 *g* pellet was osmotically lyzed in GDW (7 ml/g of original tissue), recentrifuged at 10,000 *g* for 20 min (4°) and resuspended in 0·32 M sucrose (lyzed crude synaptosomal suspension).

Oxidative metabolism of [^{3}H]NE in both synaptosomal fractions (see above) was determined by adding 0·5 ml (7–9 mg of protein) of either synaptosomal suspension to centrifuge tubes containing 1·2 ml of Krebs–Henseleit solution, 0·1 ml of 0·2% ascorbic acid and 0·1 ml of DPH (10^{-6} to 10^{-3} M, final concn in tube) or pargyline hydrochloride (10^{-6}–10^{-4} M, final concn in tube). The centrifuge tubes were preincubated for 5 min at 37° (95% O_2–5% CO_2) after which 0·1 ml of [^{3}H]NE (1·3 μCi) was added to each tube to give a final concentration of 1×10^{-7} M. The tissue was incubated for 20 min at 37° (95% O_2–5% CO_2). The reaction was stopped by addition of 2 ml of 2 N HCl. Acidified controls were determined by addition of 2 ml of 2 N HCl prior to incubation. All samples were centrifuged at 10,000 *g* for 30 min and the supernatant was assayed for [^{3}H]-deaminated catabolites. Protein was determined as above.

[^{3}H]-deaminated catabolites were separated from [^{3}H]NE and [^{3}H]NM by ion-exchange chromatography (Dowex 50 W, x-4, 200–400 mesh; 30 × 5 mm, pH = 6·0) and assayed by liquid scintillation spectrometry as described above.

Electromicroscopy examination of intact and osmotically-lyzed crude synaptosomal fractions. The intact and osmotically lyzed crude synaptosomal pellets were resuspended in 3 ml of 0·32 M sucrose, and 0·02 ml was centrifuged at 10,000 *g* through 12 ml of

4 per cent glutaraldehyde in 0·078 N cacodylate buffer (pH 7·4) at 4° for 10 min. Half of the supernatant was decanted and the pellet (about 3 mm dia.) was loosened from the centrifuge tube. The remainder of the aldehyde fixative was exchanged for a solution of osmium tetroxide (2 per cent osmium tetroxide in 0·01 M phosphate buffer, pH 7·2, with 0·32 M sucrose, at 4°) by the continuous dropwise addition of the latter while the mixture was being agitated and slowly decanted. The exchange was completed in approximately 3 min and fixation was continued for 2 hr at 4°. The fixed pellet was rinsed in saline, dehydrated through a graded series of ethyl alcohols (30–100 per cent) and embedded in an epoxy resin [Epon 812, 35 parts; phenylglycidyl ether, 22 parts; nadic methylanhydride, 42 parts and epoxy S-1 (dimethylaminoethanol), 1·5 parts].

Ultra thin sections (60–90 nm) were cut at various levels through the pellet using an LKB III ultratome. The sections were mounted on copper grids and stained with uranyl acetate and lead citrate. The grids were viewed in an RCA EMU 3G electron-microscope at an accelerating voltage of 100 kV. Micrographs were printed at a final magnification of 32,000 ×.

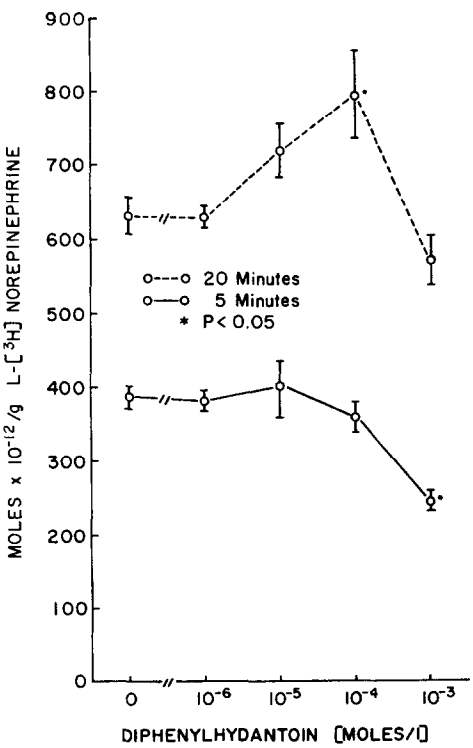

FIG. 1. Effect of diphenylhydantoin on [^{3}H]norepinephrine accumulation. Slices of rat cerebral cortex were allowed to accumulate [^{3}H]norepinephrine for 5 or 20 min at 37° (95% O_2–5% CO_2) in the presence of varying concentrations of diphenylhydantoin. Tissue [^{3}H]norepinephrine was separated from its [^{3}H]-catabolites by ion-exchange chromatography. The ordinate represents the number of moles × 10^{-12}/g of wet weight of [^{3}H]norepinephrine accumulated. The final concentration of [^{3}H]norepinephrine in the incubation media was 1 × 10^{-7} M (1·3 μCi). Each value represents mean ±S.E.M. of 4–15 experiments.

RESULTS

Effect of DPH on the accumulation and metabolism of [^{3}H]NE in cerebral cortex slices. The effect of DPH on the accumulation of [^{3}H]NE into cerebral cortex slices was studied (Fig. 1). The values expressed in Fig. 1 represent the concentration of [^{3}H]NE found in the tissue after a 5- or 20-min incubation period. The effect of DPH on accumulation was highly dependent upon the time of incubation. After 5-min incubations, an inhibition ($P < 0.01$) of the accumulation of [^{3}H]NE was seen with high doses of DPH (10^{-3} M). When cerebral cortex slices were incubated for 20 min with [^{3}H]NE, a stimulation of [^{3}H]NE accumulation was seen at 10^{-5} and 10^{-4} M DPH ($P < 0.05$); at 10^{-3} M DPH, accumulation of [^{3}H]NE returned to control values.

Tissue/medium (T/M) values of [^{3}H]NE accumulation after 5- or 20-min incubation periods are expressed in Table 1. A reduction in the T/M value was only seen when 10^{-3} M DPH was studied. This effect of 10^{-3} M DPH was seen after both 5- and 20-min incubation periods.

TABLE 1. EFFECT OF DIPHENYLHYDANTOIN (DPH) ON THE TISSUE/MEDIUM VALUES OF L-[^{3}H]NOREPINEPHRINE IN CEREBRAL CORTEX SLICES*, †

Concn of DPH (M)	Incubation (5 min)	Incubation (20 min)
Control	6·2 ± 0·4 (4)	21·0 ± 1·5 (22)
DPH, 10^{-6}	6·1 ± 0·4 (4)	19·3 ± 1·4 (8)
DPH, 10^{-5}	6·4 ± 0·7 (4)	21·0 ± 1·9 (9)
DPH, 10^{-4}	5·2 ± 0·2 (3)	20·5 ± 1·3 (9)
DPH, 10^{-3}	2·8 ± 0·3‡ (4)	8·9 ± 0·9‡ (9)

* Rat cerebral cortex slices were allowed to accumulate L-[^{3}H]norepinephrine (10^{-7} M) for 5 or 20 min at 37° (95% O_2–5% CO_2). Tissue and medium were separated by centrifugation and L-[^{3}H]norepinephrine was measured in each fraction by liquid scintillation spectrometry. Values in parentheses represent the number of experiments performed.

† moles × 10^{-12}/g/moles × 10^{-12}/ml = tissue/medium values.

‡ $P < 0.001$ as compared to control values.

The effect of DPH on the formation of [^{3}H]-deaminated and [^{3}H]*O*-methylated catabolites of [^{3}H]NE during the 20-min accumulation study was also examined (Fig. 2). The values for [^{3}H]NM and [^{3}H]-deaminated catabolites expressed in Fig. 2 represent the total amount of each of these catabolites found in both the tissue and medium fractions. At all concentrations studied, DPH had no effect on the formation of [^{3}H]NM, the *O*-methylated catabolite of NE. However, the DPH (10^{-5} to 10^{-4} M)-induced increase in tissue accumulation of [^{3}H]NE was accompanied by a corresponding decrease in the formation of total [^{3}H]-deaminated catabolites.

Effect of DPH on the accumulation of [^{3}H]*NE into cerebral cortex slices after treatment with nialamide.* Rats were treated with 100 mg/kg (i.p.) of nialamide, an inhibitor of monoamine oxidase (EC 1.4.3.4) (MAO),[14] 2 hr prior to sacrifice. This dose of nialamide was necessary to completely inhibit the formation of [^{3}H]-deaminated catabolites of [^{3}H]NE *in vitro*. In the absence of DPH, nialamide produced a significant ($P < 0{\cdot}05$) increase in the 20-min accumulation of [^{3}H]NE (compare Fig. 1 with Fig. 3). In nialamide-treated rats, DPH did not cause an increase in the 20-min accumulation of [^{3}H]NE (Fig. 3) as was seen in the absence of nialamide (Fig. 1); however, large concentrations of DPH (10^{-3} M) caused a reduction ($P < 0{\cdot}05$) in the 20-min accumulation of [^{3}H]NE. This effect of DPH in nialamide-treated rats was similar to that seen after 5-min accumulation studies in the absence of nialamide (compare Fig. 3 with Fig. 1).

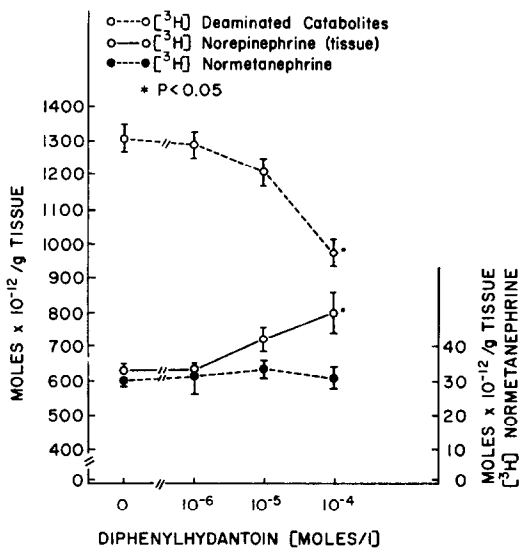

FIG. 2. Effect of diphenylhydantoin on the accumulation and catabolism of [^{3}H]norepinephrine. Slices of rat cerebral cortex were allowed to accumulate L-[^{3}H]norepinephrine (10^{-7} M; 1·3 μCi) for 20 min at 37° (95% O_2–5% CO_2) in the presence of varying concentrations of diphenylhydantoin. Tissue and medium [^{3}H]normetanephrine and [^{3}H]-deaminated catabolites of [^{3}H]norepinephrine were separated by ion-exchange chromatography and determined by liquid scintillation spectrometry. The [^{3}H]norepinephrine presented represents only the [^{3}H]norepinephrine accumulated in the tissue (i.e. not tissue and media). The left ordinate represents values corresponding to [^{3}H]norepinephrine and [^{3}H]-deaminated catabolites of [^{3}H]norepinephrine. The right ordinate represents the values corresponding to [^{3}H]normetanephrine. Each value represents the mean ±S.E.M. of 4–15 experiments.

Effect of DPH on the oxidative metabolism of [^{3}H]*NE in intact and lyzed crude synaptosomal fractions.* Additional catabolism studies were carried out in whole and osmotically lyzed crude synaptosomal preparations of rat cerebral cortex (see Methods). The results of these experiments are summarized in Tables 2 and 3. Electron micrographs of the intact (Fig. 4a), synaptosomal preparation revealed well-preserved synaptosomes (pinched-off nerve terminals) while the lyzed (Fig. 4b) synaptosomal preparation was devoid of synaptosomes and contained only swollen mitochondria and membrane fragments.

TABLE 2. EFFECT OF DIPHENYLHYDANTOIN (DPH) ON THE OXIDATIVE CATABOLISM OF L-[^{3}H]NOREPINEPHRINE IN CRUDE SYNAPTOSOMAL PREPARATIONS OF RAT CEREBRAL CORTEX*

Compound	N†	[^{3}H]-deaminated catabolites‡	Inhibition§ (%)
Control	8	5·33 ± 0·20	
DPH, 10^{-6} M	7	5·53 ± 0·24	0
DPH, 10^{-5} M	9	4·62 ± 0·26‖	13
DPH, 10^{-4} M	9	3·06 ± 0·30‖	42
Cocaine, 10 μg/ml	4	3·02 ± 0·26‖	41

* Crude synaptosomal preparations of rat cerebral cortex were incubated with 10^{-7} M L-[^{3}H]norepinephrine (1·3 μCi) for 20 min at 37° (95% O_2–5% CO_2). [^{3}H]-deaminated catabolites were isolated by ion-exchange chromatography and measured by liquid scintillation spectrometry. All drugs were added *in vitro*.

† Number of experiments performed.

‡ Moles × 10^{-12}/mg of protein (with acid control subtracted). Each value represents the mean ± S.E.M. Acidified control = 2·84 ± 0·12 moles × 10^{-13}/mg of protein.

§ (Control)–(Experimental)/(Control) × 100 = per cent inhibition.

‖ $P < 0.05$.

TABLE 3. EFFECT OF DIPHENYLHYDANTOIN (DPH) ON THE OXIDATIVE CATABOLISM OF L-[^{3}H]NOREPINEPHRINE IN OSMOTICALLY LYZED CRUDE SYNAPTOSOMAL PREPARATIONS OF CEREBRAL CORTEX*

Compound	N†	[^{3}H]-deaminated catabolites‡	Inhibition§ (%)
Control	9	9·88 ± 0·21	
DPH, 10^{-6} M	6	9·36 ± 0·36	5
DPH, 10^{-5} M	5	7·64 ± 0·30‖	23
DPH, 10^{-4} M	5	2·82 ± 0·21‖	71
Cocaine, 10 μg/ml	4	10·22 ± 0·28	0

* Osmotically lyzed (with glass distilled water) crude synaptosomal preparations of rat cerebral cortex were incubated with 10^{-7} M L-[^{3}H]norepinephrine (1·3 μCi) for 20 min at 37° (95% O_2–5% CO_2). [^{3}H]-deaminated catabolites were isolated by ion-exchange chromatography and measured by liquid scintillation spectrometry. All drugs were added *in vitro*.

† Number of experiments performed.

‡ Moles × 10^{-12}/mg of protein (with acid control subtracted). Each value represents the mean ±S.E.M. Acidified control = 5·10 ± 0·74 moles × 10^{-13}/mg of protein.

§ (Control)–(Experimental)/(Control) × 100 = per cent inhibition.

‖ $P < 0.05$.

In the crude synaptosomal preparation (Table 2), 10^{-5} and 10^{-4} M DPH produced a significant ($P < 0.05$) reduction in the oxidative catabolism of [^{3}H]NE. Cocaine (10 μg/ml), an inhibitor of the NE neuronal uptake, produced the same amount of inhibition of [^{3}H]NE catabolism as 10^{-4} M DPH. This high concentration of cocaine has been shown to produce virtually complete inhibition of the neuronal uptake process in brain tissue.[15]

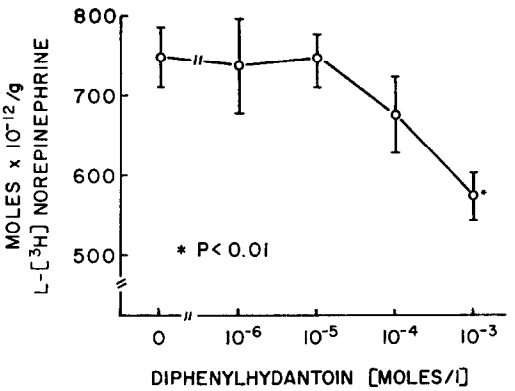

FIG. 3. Effect of nialamide on the facilitative action of diphenylhydantoin on [^{3}H]norepinephrine accumulation. Rats were pretreated with 100 mg/kg (i.p.) of nialamide 2 hr prior to sacrifice. The 20-min accumulation of [^{3}H]norepinephrine *in vitro* in the presence of varying concentrations of diphenylhydantoin was examined as described in Fig. 1. Each value represents the mean ±S.E.M. of 9–17 experiments.

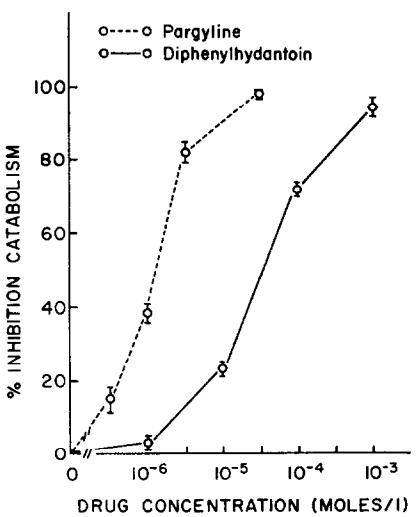

FIG. 5. Effect of pargyline and diphenylhydantoin on the oxidative catabolism of L-[^{3}H]norepinephrine in lyzed crude synaptosomal preparations. A crude synaptosomal preparation of rat cerebral cortex (see Methods) was osmotically lyzed with glass distilled water and incubated for 20 min at 37° (95% O_2–5% CO_2) with [^{3}H]norepinephrine (10^{-7} M; 1·3 μCi) in the presence of either pargyline or diphenylhydantoin. The incubation was terminated by addition of 2 ml of 2 N HCl. [^{3}H]-deaminated catabolites of [^{3}H]norepinephrine were isolated by ion-exchange chromatography and measured by liquid scintilation spectrometry. Per cent inhibition of oxidative catabolism was calculated as follows: [Control] − [Experimental]/[Control] × 100. Each value represents the mean ±S.E.M. of four to six experiments.

In lyzed crude synaptosomal preparations (Table 3), DPH also produced a concentration-dependent reduction in oxidative catabolism of [^{3}H]NE. This effect was again significant ($P < 0.05$) at 10^{-5} and 10^{-4} M DPH, but appeared greater than in the unlyzed preparation. With the neuronal membranes lyzed, cocaine (10 μg/ml) had no effect on the oxidative catabolism of [^{3}H]NE.

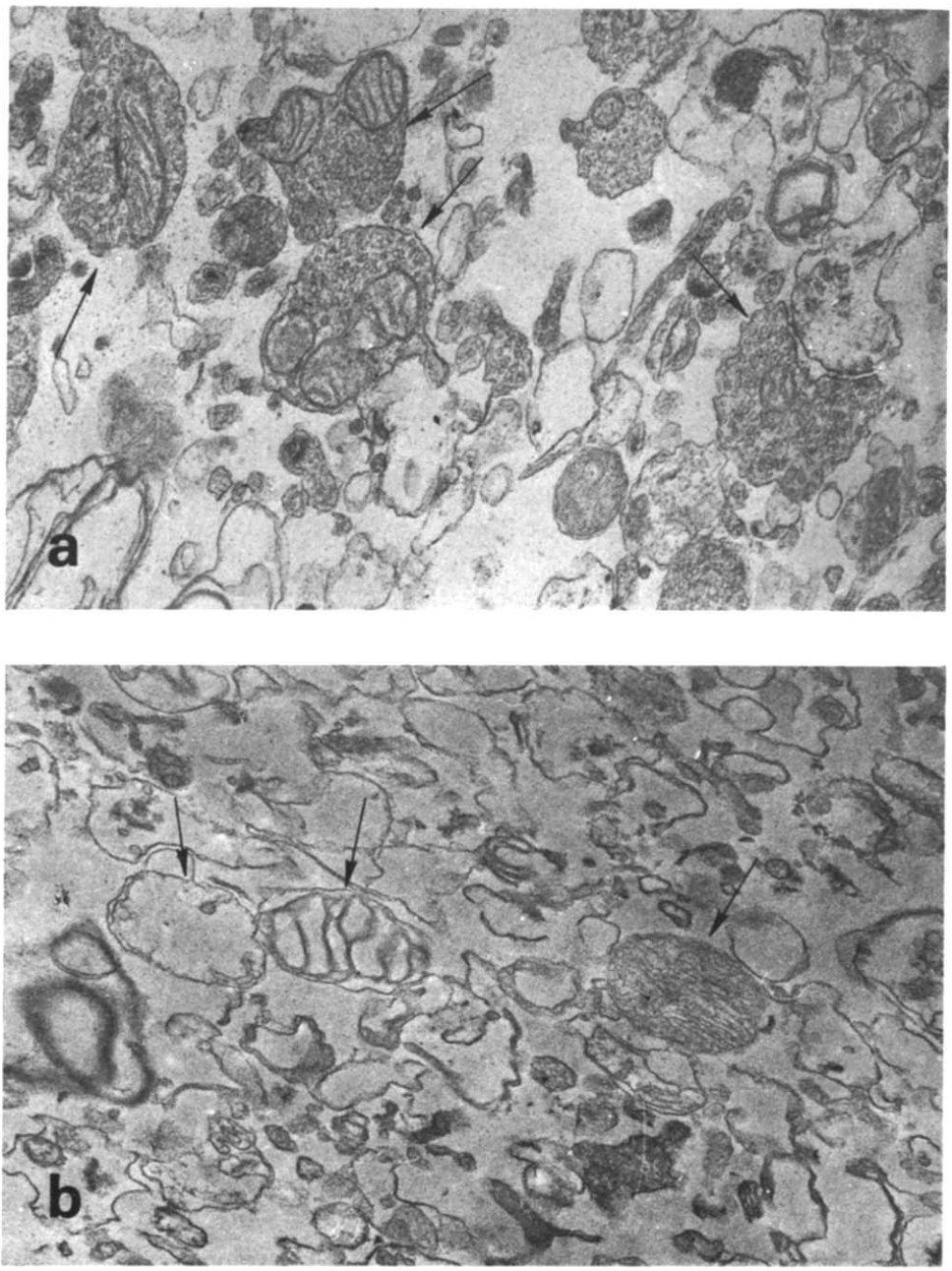

FIG. 4. (a) A representative electronmicrograph taken of the crude synaptosomal fraction of the rat cerebral cortex (32,000 ×). Intact synaptosomes (↓) are shown. (b) A representative electronmicrograph taken of the osmotically lyzed crude synaptosomal preparation (32,000 ×). Pellet of the lyzed preparation consisted primarily of broken membrane fragments while intact synaptosomes were absent. Swollen mitochondria (↓) are shown in various stages of preservation.

B.P.—*facing page* 2726

The effects of pargyline hydrochloride, an inhibitor of oxidative catabolism of NE in brain tissue,[14,16] were compared to the action of DPH on the oxidative catabolism of [^{3}H]NE in lyzed crude synaptosomal preparations (Fig. 5). ID_{50} values for DPH and pargyline were $5{\cdot}5 \times 10^{-5}$ M and $1{\cdot}5 \times 10^{-6}$ M respectively. Thus, pargyline was 37 times more potent in inhibiting the oxidative catabolism of [^{3}H]NE in cerebral cortex tissue.

DISCUSSION

The accumulation of catecholamines *in vitro* by brain slices represents at least three metabolic events within the catecholamine nerve terminal. Catecholamines are initially transported across the neuronal membrane by a high affinity uptake system which is sodium- and energy-dependent.[17] Once inside the neuron, the catecholamine is either taken-up and stored in intraneuronal storage vesicles[18–20] or catabolized by mitochondrial MAO (EC 1.4.3.4).[12] Drugs which alter one or more of these neuronal events have profound effects on the accumulation of catecholamines. For example, reserpine, a drug which alters storage of catecholamines,[21,22] will severely depress the accumulation of catecholamines.[23] Similar results can be obtained with neuronal uptake inhibitors such as desmethylimipramine.[23] On the other hand, pretreatment with inhibitors of MAO leads to an increase of the catecholamine accumulation by brain slices, since these agents inhibit the oxidative catabolism of catecholamines that have been transported across the neuronal membrane (see Figs. 1 and 3).

The present studies were carried out in an attempt to examine the effects of DPH on neuronal uptake, storage and catabolism of [^{3}H]NE in brain tissue by studying the effects of DPH on [^{3}H]NE accumulation. Our studies indicate that DPH has at least two effects on [^{3}H]NE accumulation in cerebral cortex tissue which is dependent on the concentration of DPH and the time allowed for [^{3}H]NE accumulation to occur. Five-min accumulation studies would indicate that DPH had no effect on [^{3}H]NE accumulation until high concentrations of DPH (10^{-3} M) were tested. At this concentration of DPH (10^{-3} M), a severe reduction in the 5-min accumulation of [^{3}H]NE was seen. On the other hand, 10^{-5}–10^{-4} M DPH facilitated the 20-min accumulation of [^{3}H]NE. This action of DPH was not seen when 10^{-3} M DPH was allowed to interact with the tissue. Manipulation of the incubation time can be a useful tool in determining if a drug that alters accumulation of [^{3}H]NE does so through an action on the neuronal uptake system or the intraneuronal storage processes. Snyder *et al.*[23] have shown that the accumulation of [^{3}H]NE seen after short incubation periods (5 min or less) occurs primarily through the neuronal uptake system while the accumulation of [^{3}H]NE seen after longer incubation periods is due to neuronal uptake and storage of [^{3}H]NE. Oxidative catabolism of [^{3}H]NE would also play a role in the amount of [^{3}H]NE accumulated in a period of time; however, it would seem that the effect of catabolism would be of greater importance during long incubation periods. Accordingly, our data would suggest that high concentrations of DPH (10^{-3} M) inhibit the neuronal uptake of [^{3}H]NE. On the other hand, since the facilitative action of DPH on the accumulation of [^{3}H]NE was seen only after 20-min incubation periods (and not 5-min periods), it would suggest an additional effect of DPH on neuronal storage or oxidative catabolism of [^{3}H]NE.

That the facilitative action of DPH on the 20-min accumulation of [^{3}H]NE was due to an inhibitory effect of DPH on the oxidative catabolism of [^{3}H]NE was demon-

strated in studies where we simultaneously measured the quantity of [^{3}H]-deaminated catabolites of [^{3}H]NE formed during the accumulation period. In these studies, as the concentration of [^{3}H]NE increased in cerebral cortex tissue, the concentration of total [^{3}H]-deaminated catabolites decreased in a concentration-related fashion. In addition, treatment of rats with the MAO inhibitor, nialamide, completely abolished the facilitative action of DPH. If the facilitative action of DPH were due to an effect of DPH on neuronal storage rather than catabolism of [^{3}H]NE, a summation of the effects would be expected after nialamide. In these latter experiments, only the inhibitory action seen after large concentrations of DPH (10^{-3} M) was observed. It has already been suggested above that the effect of high concentrations of DPH is due to an inhibitory action of DPH on neuronal uptake of [^{3}H]NE.

The medium concentration of [^{3}H]NE is ultimately related to its tissue uptake, storage and catabolism. It is of interest that although 10^{-5} and 10^{-4} M DPH facilitated the tissue accumulation of [^{3}H]NE, T/M values of [^{3}H]NE remained unchanged with respect to control values. Thus as the [^{3}H]NE concentration increased in cerebral cortex, medium concentrations of [^{3}H]NE failed to decline as in the absence of DPH. This consistent finding most likely reflects the inhibitory action of DPH on tissue oxidative catabolism of [^{3}H]NE.

Although the 20-min accumulation of [^{3}H]NE seen after 10^{-3} M DPH was the same as control values (i.e. neither stimulated nor depressed), this value probably represents a mixed effect of the two actions of DPH. This latter point is exemplified by analysis of the T/M values of [^{3}H]NE. It can be seen that although the tissue value of [^{3}H]NE was not depressed, as compared to control values, the T/M value was severely depressed. This latter effect on the T/M value was due to the failure of the medium content of [^{3}H]NE to decline as in control preparations. Therefore, it would suggest that although a reduced amount of [^{3}H]NE was transported across the neuronal membrane (because of inhibition of neuronal uptake), less of what was transported was catabolized and more was stored.

Agents which inhibit the neuronal uptake of NE also inhibit oxidative catabolism of NE by limiting the access of the substrate (NE) to intraneuronal MAO.[16] To be sure that the inhibition of oxidative catabolism of [^{3}H]NE seen after DPH was not due to an effect of this agent on the neuronal uptake of [^{3}H]NE, it was felt that additional catabolism studies should be carried out in broken cell preparation (lyzed synaptosomal preparations). In these experiments, the oxidative catabolism of [^{3}H]NE was inhibited in a concentration-related fashion similar to that seen in whole synaptosomes. Thus, it is suggested that the reduction in the total [^{3}H]-deaminated catabolites formed from [^{3}H]NE in the 20-min accumulation studies was due to a direct inhibitory effect of DPH on intraneuronal MAO activity and not a result of the effect of DPH on the NE neuronal uptake system.

The mechanisms involved in the anticonvulsant actions of DPH are unknown. It has been suggested that the anticonvulsant actions of DPH may be related to alterations in electrolyte (Na^+) concentrations seen after DPH.[24] More recently, it has been suggested that DPH may act by altering the neuronal uptake of brain catecholamines.[5] This latter suggestion is particularly intriguing since there is now extensive evidence to suggest that brain NE and dopamine function as neurotransmitter substances. Our results would confirm the action of DPH on the neuronal uptake of catecholamines but only at very high concentrations of DPH. Of greater interest is the action of DPH

on the oxidative catabolism of [^{3}H]NE. This effect of DPH was seen with 10- to 100-fold less DPH than was required to inhibit the NE neuronal uptake system. It is of interest that Anderson *et al.*[4] demonstrated that anticonvulsant doses of DPH caused an elevation in the endogenous concentrations of 5-hydroxytryptamine (an additional putative neurotransmitter). Since both NE and 5-hydroxytryptamine are oxidatively deaminated by MAO[12,14,25] and since inhibitors of MAO cause elevations of the endogenous concentrations of both amines,[26] it is possible that the elevation of brain 5-hydroxytryptamine reported by Anderson *et al.*[4] may be due to the inhibitory effect of DPH on intraneuronal MAO activity, as was seen in the studies *in vitro* presented in this manuscript.

It has been previously shown that other inhibitors of MAO have anticonvulsant properties.[27] Whether this action of MAO inhibitors is due to an effect on MAO or some other unknown action is uncertain. However, with the finding that DPH is also a weak inhibitor of oxidative catabolism of [^{3}H]NE, additional studies should be carried out to explore the relationship which may exist between anticonvulsant properties of drugs and brain amine metabolism.

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