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THE COMBINED USE OF α-METHYLTYROSINE AND *THREO*-DIHYDROXYPHENYLSERINE— SELECTIVE REDUCTION OF DOPAMINE LEVELS IN THE CENTRAL NERVOUS SYSTEM

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Abstract—Dihydroxyphenylserine-α-^{14}C (DOPS-α-^{14}) is taken up into mouse brain and decarboxylated to form norepinephrine-^{14}C. The combined use of nontracer doses of this amino acid (*threo-* or *erythro*-DOPS) and the tyrosine hydroxylase inhibitor, α-methyltyrosine ethyl ester, causes a 50 per cent depletion of dopamine in the central nervous system in mice while leaving norepinephrine levels unchanged. Without *threo*-DOPS, α-methyltyrosine depletes both norepinephrine and dopamine levels to less than one-half their normal values. DL-*Threo*-DOPS rapidly replenishes norepinephrine levels in reserpine-treated mice, but does not cause the awakening effect shown by L-DOPA under the same conditions. It is suggested that the reversal of the reserpine syndrome by L-DOPA is due to formation of dopamine rather than norepinephrine in the central nervous system.

THE STUDY of drug interactions with biogenic amines in the central nervous system is complicated by the difficulty in altering the level of one amine, such as dopamine, without simultaneously affecting the stores of other amines such as norepinephrine and serotonin. Pharmacological agents, e.g. reserpine, cause profound decreases in dopamine, norepinephrine, and serotonin levels in the central nervous system, and it is difficult to correlate the concomitant sedation with the depletion of a particular amine. Selective agents, such as the tyrosine hydroxylase inhibitor, α-methyltyrosine, have provided a means of depleting central stores of norepinephrine and dopamine without affecting serotonin levels.[1] Mice treated in this way are mildly sedated. When serotonin levels are reduced by the administration of the tryptophan-5-hydroxylase inhibitor, *p*-chlorophenylalanine,[2] no sedation is observed. *p*-Chlorophenylalanine also inhibits phenylalanine hydroxylase and causes a chemically induced phenylketonuria.[3] Another method of selectively depleting brain serotonin is through use of tryptophan-free diets.[4] The results of these studies seem to indicate that the effects of reserpine are due to depletion of catecholamines rather than serotonin.

The now recognized importance of dopamine as a neurohormone[5] makes it desirable to study separately the physiological effects of depletion of either norepinephrine or dopamine in the central nervous system.

Selective depletion of central norepinephrine has been achieved by the catecholamine-releasing agent, α-methyl-*m*-tyrosine. Dopamine levels return to normal within 24 hr after depletion with this agent, while norepinephrine remains at a low level for several days.[6] The motor excitation that follows treatment of mice with reserpine or

α-methyl-*m*-tyrosine in connection with a monoamine oxidase inhibitor appeared to depend selectively upon dopamine levels.[7] Alternatively, norepinephrine has been selectively depleted by inhibition of dopamine-β-hydroxylase.[8]

So far no method has been available for the selective depletion of dopamine, although such a system would permit insights into the role of dopamine in the central nervous system. In this report a method is described which employs the tyrosine hydroxylase inhibitor, α-methyltyrosine ethyl ester, in conjunction with *threo*-dihydroxyphenyl serine (*threo*-DOPS). By this procedure dopamine can be selectively depleted while the normal brain level of norepinephrine is maintained (Fig. 1).

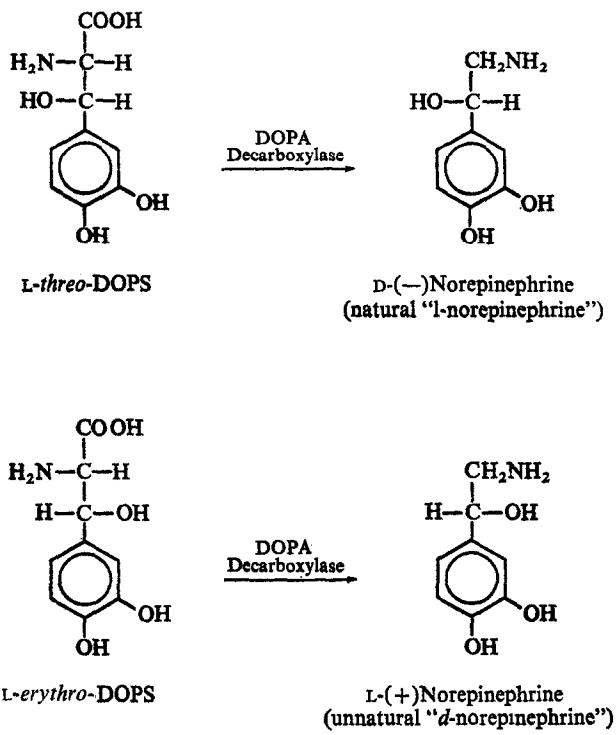

FIG. 1.

MATERIALS AND METHODS

DL-*Threo*-DOPS and DL-*erythro*-DOPS were synthesized by the method of Bolhofer,[9] A mixture of DL-*threo*-DOPS-α-^{14}C (3 parts) and DL-*erythro*-DOPS-α-^{14}C (1 part) was synthesized by the same procedure, starting with 9 mg of glycine-2-^{14}C (New England Nuclear Corp., 4·2 mc/m-mole) and 88 mg of 3,4-dibenzyloxybenzaldehyde. The purity and ratio of *threo*- and *erythro*-DOPS-α-^{14}C in the final product was ascertained by paper chromatography (Whatman No. 1) in isopropanol–acetic acid–water (70:5:25). α-Methyltyrosine ethyl ester was prepared from α-methyltyrosine by esterification in ethanol with hydrogen chloride. Other compounds were obtained from commercial sources. The animals used in this study were male, general purpose, albino mice weighing 14–20 g.

Animals were sacrificed by cervical fracture; the tissues were dissected, frozen on a block of dry ice, weighed, and homogenized in cold 0·4 N perchloric acid. The catecholamines of heart and brain tissues were isolated by adsorption on alumina according to the procedure of Crout *et al.*[10] Norepinephrine was assayed by the trihydroxyindole method of von Euler and Floding[11] and dopamine by the method of Carlsson and Waldeck.[12] When it was necessary to separate DOPS from other catechol compounds, the eluate from the alumina column containing the isolated catechols was passed through an IRC-50 Na^+ column buffered at pH 6·5, according to the procedure described by Lovenberg *et al.*[13] for the separation of DOPA and dopamine. The isolated DOPS was assayed by the trihydroxyindole method. The noncatechol metabolites in the effluent of the alumina column were separated into phenolic amines (normetanephrine) and phenols (3-methoxy-4-hydroxymandelic acid, 3-methoxy-4-hydroxyphenylglycol) by ion-exchange chromatography as described by Pisano.[14] Radioactivity was determined by liquid scintillation spectrophotometry in Bray's phosphor solution.[15]

RESULTS

Metabolism of DL-*DOPS-α-*^{14}*C in mouse brain.* The organ distribution of radioactivity after a single, i.v. injection (tail vein) of DL-DOPS-^{14}C (0·5 μm; sp. act., 4 mc/m-mole) was followed over a period of 5 hr. Little organ specificity was observed, except for a reduced amount of radioactivity in brain and the characteristic elevation of radioactivity in kidney. Approximately 3 per cent of the administered radioactivity was present in brain after 30 min, with measureable quantities present after 5 hr. The half-life of the DL-DOPS-^{14}C in the whole animal was 17 min.

The distribution of labelled DOPS and its metabolic products was determined in brain over a period of 3 hr, as shown in Table 1. The amino acid disappeared rapidly

TABLE 1. METABOLISM OF DOPS-^{14}C (4 MC/M-MOLE) IN MOUSE BRAIN AFTER THE INTRAVENOUS INJECTION OF 0·5 μMOLE OF *threo-erythro*-DOPS PER MOUSE*

Time (min)	Radioactivity (cpm/g brain)				
	Total	Dihydroxyphenylserine	Norepinephrine	Amine metabolites (NMN)	Non-amine metabolites (VMA)
30	9600	2070	970	0	5660
90	7000	240	960	0	4940
180	5000	0	760	210	3950

* Animals (3) were sacrificed at 30, 90 and 180 min. Brain levels of DOPS-^{14}C, norepinephrine, and its metabolites were determined as described under Methods.

from brain with only a trace detectable after 90 min. The level of labelled norepinephrine decreased more slowly with detectable amounts present after 3 hr. Between 30 and 40 per cent of the activity in brain was present in the fraction containing non-amine metabolites of norepinephrine, whereas only a trace of the amine metabolite, normetanephrine, could be detected.

Selective replenishment of norepinephrine in mouse brain. Brain levels of both norepinephrine and dopamine are reduced to approximately 50 per cent of their normal

values 4·5 hr after i.p. administration of α-methyltyrosine ethyl ester. The freely soluble ethyl ester was used because of the limited solubility of the free amino acid. The ethyl ester is rapidly hydrolyzed *in vivo* to α-methyltyrosine.[16] As shown in Table 2, administration of either *erythro-* or *threo*-DOPS restores norepinephrine levels to normal, but has no effect on the dopamine level. L-DOPA, on the other hand, restores the level of both catecholamines. Administration of these precursor amino acids to normal mice has virtually no effect on central catecholamine levels. It should also be noted that *erythro-* and *threo*-DOPS appear to refill peripheral norepinephrine stores (heart).

TABLE 2. THE EFFECT OF L-DOPA, DL-*threo*-DOPS AND DL-*erythro*-DOPS ON THE α-METHYLTYROSINE ETHYL ESTER-INDUCED DEPLETION OF CATECHOLAMINE*

	Norepinephrine (μg/g)		Dopamine (μg/g)
	Heart	Brain	Brain
Control	1·06 ± 0·11	0·39 ± 0·02	0·47 ± 0·03
DL-*threo*-DOPS	1·32 ± 0·10	0·36 ± 0·01	0·40 ± 0·03
DL-*erythro*-DOPS	1·16 ± 0·05	0·35 ± 0·03	0·39 ± 0·03
L-DOPA	1·20 ± 0·15	0·39 ± 0·01	0·46 ± 0·03
α-Methyltyrosine ethyl ester	0·61 ± 0·04	0·19 ± 0·01	0·20 ± 0·02
α-Methyltyrosine plus DL-*threo*-DOPS	1·26 ± 0·18	0·45 ± 0·02	0·26 ± 0·04
α-Methyltyrosine plus DL-*erythro*-DOPS	1·24 ± 0·20	0·40 ± 0·01	0·28 ± 0·04
α-Methyltyrosine plus L-DOPA	0·98 ± 0·03	0·32 ± 0·01	0·53 ± 0·03

* α-Methyltyrosine ethyl ester (250 mg/kg; 2 x, i.p.) was administered 11 and 4·5 hr and the amino acids (125 mg/kg, i.p.) 3 hr before the animals were sacrificed. Brains and hearts were removed and homogenized (1:3, w/v) in 0·4% perchloric acid, and norepinephrine and dopamine were isolated and assayed as described under Methods. Values ± S.D. represent the means obtained from 4 groups of 5–8 mice.

Effect of threo-*DOPS on reserpinized mice.* The following procedure was used to reserpinize mice. Subcutaneous injections of reserpine were given daily for three days: 2 mg/kg on the first day, 1 mg/kg on the second, and 0·5 mg/kg on the third day. At this time the levels of norepinephrine and dopamine in brain were reduced to 0·12 ± 0·06 and 0·17 ± 0·03 μg/g respectively. The mice appeared asleep, with a typical hunch-backed posture and with eyelids closed (Fig. 2). They responded to touch with a peculiar mincing gait and trembling. Amino acids were administered i.v. into the tail vein as saturated solutions in normal saline to groups of 5 mice. When L-DOPA was given (130 mg/kg) the "awakening effect" described previously[17,18] ensued within 1 min; the excited motor activity persisted for 2–3 hr, gradually decreasing until the animals returned to the reserpinized state in 4–8 hr. When DL-*threo*-DOPS was given (130, 260, 390, 540, and 600 mg/kg), the only change observed was an occasional opening of the eyes; otherwise, the mice were indistinguishable from reserpinized control animals which received only saline. As shown in Fig. 3, the brain level of norepinephrine was restored to normal for approximately 90 min after the *threo*-DOPS administration. Comparable injections of *erythro*-DOPS, *threo-m*-hydroxyphenylserine α-methylDOPA, 5-hydroxyDOPA, and 5-hydroxytryptophan did not induce the awakening response.

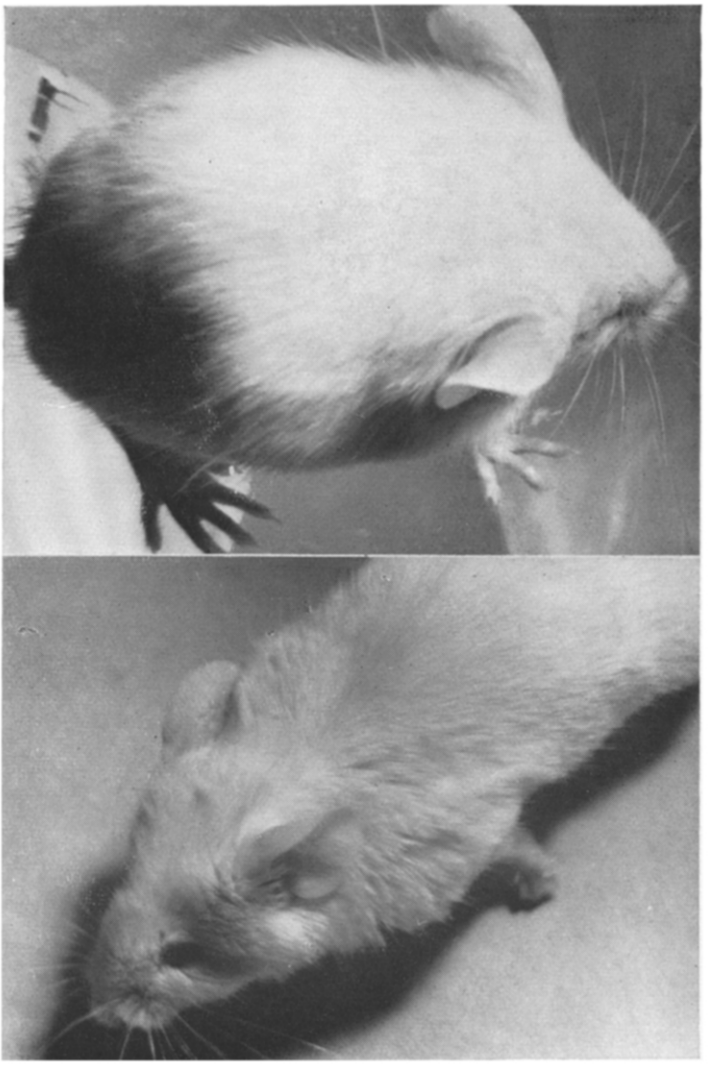

FIG. 2. Effect of *threo*-DOPS and L-DOPA on reserpinized mice. Top: reserpinized mouse 10 min after i.v. (tail vein) injection of *threo*-DOPS (540 mg/kg). No change from the typical appearance of reserpinized control mice was observed. Bottom: reserpinized mouse 10 min after the injection of L-DOPA (130 mg/kg), which resulted in a marked "awakening" response.

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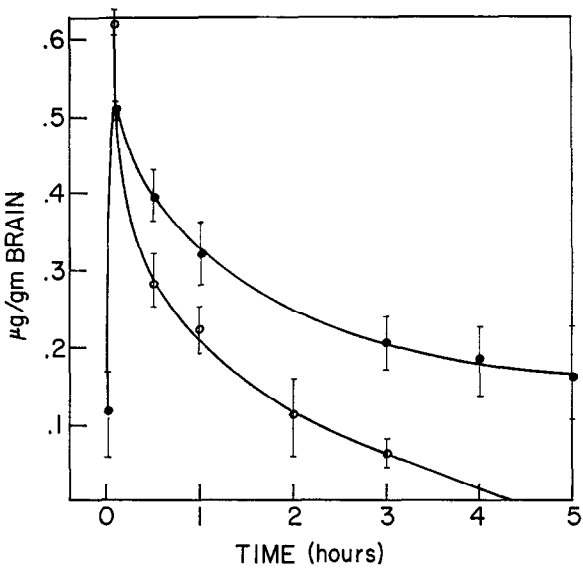

FIG. 3. Replenishment of brain norepinephrine after administration of DL-*threo*-DOPS to reserpinized mice. DL-*threo*-DOPS (540 mg/kg) was injected i.v. (tail vein) 24 hr after a final dose of reserpine. Brain levels of DOPS (○———○) and norepinephrine (●———●) were measured as described under Methods in 3 groups of 5 mice at the times indicated. The level of norepinephrine in normal mouse brain was 0·39±0·02.

DISCUSSION

Both *threo*- and *erythro*-DOPS are substrates for mammalian aromatic L-amino acid decarboxylase, giving rise to *l*- and *d*-norepinephrine respectively.[19,20] The possibility that DOPS might be a naturally occurring precursor for the biosynthesis of norepinephrine has been considered several times in the past 50 yr,[21] but no conclusive evidence for the formation or occurrence of DOPS has been found. DOPS, however, is a valuable pharmacological tool to introduce an immediate precursor of norepinephrine into the central nervous system.

DOPS is rapidly taken up into the brain and decarboxylated to form norepinephrine (Tables 1 and 2), which then is metabolized by catechol-*O*-methyltransferase and monoamine oxidase. If the normal biosynthesis of catecholamines is interrupted by inhibition of tyrosine hydroxylase through the administration of α-methyltyrosine ethyl ester, the levels of both dopamine and norepinephrine are greatly reduced in brain.[1] Under these conditions the administration of either *threo*- or *erythro*-DOPS can restore central norepinephrine stores as effectively as L-DOPA (Table 2). The administration of L-DOPA also restores dopamine levels, whereas the administration of *threo*- or *erythro*-DOPS has no effect on dopamine levels, which remain at approximately one-half of their normal value.

Erythro-DOPS will replenish the depleted stores of norepinephrine with the unphysiological *d*-isomer of norepinephrine. The effects of such a "false transmitter" in the central nervous system would be of interest. The use of *threo*-DOPS-α-^{14}C of higher spec. act. than that reported here appears to be a feasible alternate to the use

of DOPA-H^3 or intracisternal injection of norepinephrine-H^3 for isotopic labelling of central norepinephrine stores.

In reserpinized animals the central stores of norepinephrine are rapidly restored to normal after the i.v. administration of *threo*-DOPS (Fig. 3) and then return to the depleted level after 1–2 hr. During the period in which the norepinephrine levels are normal, the animals remain sedated and cannot be distinguished from the reserpinized controls (Fig. 3). The awakening effect that immediately follows the intravenous administration of L-DOPA (130 mg/kg, Fig. 2) and then diminishes over a period of 2–4 hr was absent, even at doses of *threo*-DOPS up to 600 mg/kg. Blaschko and Chrusciel[18] commented on these effects, but did not demonstrate the replenishment of central norepinephrine stores. The present results demonstrate that *threo*-DOPS can effectively restore central norepinephrine levels in reserpinized mice and that this replenishment fails to cause any perceivable awakening effect. It is suggested, therefore, that both the "awakening" of reserpinized mice and the temporary amelioration of parkinsonism in man[22,23] by the administration of L-DOPA result from the formation of dopamine in the central nervous system.

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