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SHORT COMMUNICATIONS

Difference in oral effectiveness of two tyrosine hydroxylase inhibitors

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THE ISOLATION and purification of the enzyme tyrosine hydroxylase from bovine adrenals[1] has stimulated an intensified study of the role of this enzyme in the biosynthesis of dopamine and norepinephrine. Several derivatives of tyrosine have since been demonstrated to inhibit competitively the hydroxylation of tyrosine by this enzyme *in vitro*, including α-methyltyrosine (α-MT), and 3-iodotyrosine.[1–3] Inhibition *in vivo* of tyrosine hydroxylase by α-MT has been reported in guinea pigs,[4,5] and rats.[6] In several experiments the effects of this inhibitor upon endogenous tissue catecholamine levels[4,5,7,8] and behavior[6,8] have been determined after repeated parenteral injections. Inhibition *in vivo* of catecholamine biosynthesis in rats and guinea pigs after both acute and chronic i.p. administration of 3-iodotyrosine was recently described.[9,10]

Tyrosine is principally metabolized by transamination to *p*-hydroxyphenylpyruvate. A structural comparison of 3-iodotyrosine with tyrosine suggests for it a similar metabolic fate. Indeed, these compounds are equally active as substrates for a purified rat liver transaminase.[11] However, α-MT lacks an αH necessary for this reaction and is impervious to transamination. Both α-MT and 3-iodotyrosine are very active inhibitors *in vitro*[2] and, in separate experiments with relatively large parenteral doses, have decreased brain catecholamines in a manner consistent with inhibition *in vivo* of tyrosine hydroxylase[4,5,7–10] The purpose of this parallel study with the two inhibitors was to determine whether the noted structural variations would affect their inhibition of mouse brain catecholamine biosynthesis. The inhibitors were administered admixed with diet. Since mice eat throughout the day, incorporation of test compounds in their diet provides relatively continuous exposure to the drug without problems inherent in repetitive manual dosing techniques.

Groups of eight Carworth Farm male mice were weighed, housed as a group, and fed a stock ground diet (Upjohn B.A.). In addition to two control groups, a single group was placed on a diet containing one of the following: L-α-methyltyrosine, 0·3% and 1·0%; 3-iodotyrosine (Aldrich Chemical Co.), 0·3% and 1·0%; *d*-amphetamine sulfate, 0·03%; reserpine, 0·01%. The feeding program was continued for 48 hr. After each 24-hr period, food consumption and body weight changes were recorded. At the conclusion of the feeding trial, locomotor activity was measured in two sets of three mice from each of the groups. Motor activity was measured for a 10-min period after an initial 10-min acclimation period in six circular actophotometers (Woodard Research Corp.), each equipped with six light beams and six photoelectric cells connected to a digital counter.

Within 90 min of being removed from the diet, mice were sacrificed by decapitation, and whole brains were removed and placed on dry ice. Two brains were pooled, homogenized in three volumes of 0·01 N HCl, and extracted.[12] Aliquots of the aqueous extract were assayed for norepinephrine,[13] dopamine,[14] and serotonin.[15] Amine content was determined by comparison with the fluorescence of duplicate tissue-containing internal standards. These results were not corrected for recovery in the extraction procedure.

Data in Table 1 clearly indicate the markedly different effects of the two tyrosine hydroxylase inhibitors upon the parameters measured. 3-Iodotyrosine had no effect upon food intake at either concentration and produced an increase in body weight exceeding that shown by the control animals. α-MT at the lower level (0·3%) produced little alteration of food intake or weight change. At the 1% level, consumption of food was greatly diminished (34 per cent of control intake), and the change in body weight exceeded that induced by *d*-amphetamine and reserpine. The decrease in locomotor

activity corresponded with the depression observed in this group after 16 hr; this response was paralleled only by the reserpine-fed mice. The increase in motor activity of the mice fed the higher level of 3-iodotyrosine was approximately half that observed in mice fed *d*-amphetamine.

TABLE 1. FOOD AND DRUG INTAKE, BODY WEIGHT CHANGE, AND MOTOR ACTIVITY MEASUREMENTS AFTER A 48-HOUR FEEDING EXPERIMENT

Additive	(%)	Food intake (g)	Change in body wt/group (g)	Drug intake (mg/kg/48 hr)	Motor activity (% of control)*
Control†		56	+2		100
α-Methyltyrosine	0·3	50	−1	891	52§
	1·0	19‡§	−35‡§	1245‡	7§
3-Iodotyrosine	0·3	56	+6	960	92
	1·0	54	+3	3127	126
d-Amphetamine	0·03	40	−17§	76	146§
Reserpine	0·01	23§	−29§	14	14§

* These data are the average counts of two runs in each of the drug groups relative to the average count of four runs in the control groups.

† Food intake and body weight change are the average of two control groups.

‡ These values are adjusted for the death of one mouse in this group noted at 42 hr.

§ These values are significantly different ($P < 0·05$) from control groups.

Table 2 summarizes the varied effects of the two inhibitors upon mouse brain amines as compared with reserpine and *d*-amphetamine similarly administered. Although the intake of 3-iodotyrosine exceeded 3 g/kg for the 48-hr period (equivalent to 65 mg/kg/hr), it did not affect brain amine levels. The decrease of endogenous norepinephrine and dopamine by α-MT was consistent with the drug

TABLE 2. EFFECT OF SEVERAL COMPOUNDS ADDED TO DIET UPON MOUSE BRAIN AMINE CONTENT*

Additive	(%)	Norepinephrine	Dopamine	Serotonin
Control		0·53 ± 0·03 (3)†	0·78 ± 0·01 (3)	0·78 ± 0·03 (3)
α-Methyltyrosine	0·3	0·32 ± 0·04 (3)	0·38 ± 0·05 (2)	0·71 ± 0·01 (3)
	1·0	0·20 ± 0·01 (3)	0·23 ± 0·04 (3)	0·81 ± 0·06 (3)
3-Iodotyrosine	0·3	0·52 ± 0·01 (3)	0·80 ± 0·05 (3)	0·68 ± 0·03 (3)
	1·0	0·51 ± 0·04 (3)	0·75 ± 0·03 (3)	0·73 ± 0·05 (3)
d-Amphetamine	0·03	0·46 ± 0·06 (2)	0·80 ± 0·04 (2)	0·70 ± 0·06 (3)
Reserpine	0·01	0·18 (2)	0·10 ± 0·05 (2)	0·39 ± 0·01 (2)

* All values are expressed as μg amine/g wet weight of brain tissue.

† ±S.D.; ( ) indicates the number of determinations.

intake at both dietary levels. Depletion of norepinephrine by 1% α-MT was equivalent to that produced by reserpine. Dopamine levels, when altered, were affected to a greater extent than were the corresponding norepinephrine levels. *d*-Amphetamine did not affect amine levels. Only reserpine altered brain serotonin content.

Data presented here indicate that under the conditions of this experiment 3-iodotyrosine given orally, in contrast to α-MT, does not block the biosynthesis of catecholamines in mouse brain. The results of this investigation agree with a similar investigation of 7 days' duration in which mouse

brain norepinephrine levels were reduced only 15 per cent with an average intake of 3-iodotyrosine of 450 mg/kg/day. A probable explanation for the failure of 3-iodotyrosine to lower mouse brain norepinephrine and dopamine is that this inhibitor is effectively metabolized by transamination.

This theory is partially substantiated by the effect of D-cycloserine, a transaminase inhibitor,[16,17] upon the inhibitory action of 3-iodotyrosine. Although 3-iodotyrosine at 200 mg/kg, i.p., had no effect upon brain norepinephrine, a single i.p. dose of 3-iodotyrosine (300 mg/kg) reduced brain norepinephrine 30 per cent after 2 hr. When D-cycloserine (1000 mg/kg, i.p.) was given 30 min before 3-iodotyrosine (300 mg/kg, i.p.), brain norepinephrine was further decreased to 50 per cent of control levels in 2 hr. No significant change in brain amine levels was noted with D-cycloserine (1000 mg/kg i.p.) alone. Depletion of norepinephrine produced by the combined treatment of D-cycloserine and 3-iodotyrosine (300 mg/kg, i.p.) approximated that produced by 3-iodotyrosine (500 mg/kg, i.p.) It should be noted that D-cycloserine did not alter the depletion of brain norepinephrine by α-MT (200 mg/kg, i.p.) at 2 hr.

The widespread distribution of high quantities of α-MT in rat tissues 30–48 hr after a single i.p. injection of 200–300 mg/kg,[18] in contrast to the extremely low tissue concentrations of 3-iodotyrosine found shortly after its administration,[10] also substantiates this explanation.

3-Iodotyrosine is readily absorbed from the peritoneal cavity.[10] Although the efficiency of its absorption after oral administration in mice was not determined, the relative decreases in brain norepinephrine and dopamine after high doses of 3-iodotyrosine (500 mg/kg) given orally or parenterally indicate that, although absorption of 3-iodotyrosine after oral administration may be a factor in its slightly lower activity *in vivo*, 3-iodotyrosine does inhibit catecholamine biosynthesis in mice at very high doses by either route.

The chronic feeding procedure successfully overcomes the problems of multiple dosing as a means of producing enzyme inhibition *in vivo* by α-MT. With this method the amount of drug to be ingested can be regulated by the dietary level employed.

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Pharmacology Research Laboratories,
The Upjohn Company,
Kalamazoo, Mich., U.S.A.

GARLAND A. JOHNSON
ELIZABETH G. KIM
WILLIAM VELDKAMP
ROLAND RUSSELL

REFERENCES

1. T. NAGATSU, M. LEVITT and S. UDENFRIEND, *J. biol. Chem.* **239**, 2910 (1964).
2. S. UDENFRIEND, P. ZALTZMAN-NIRENBERG and T. NAGATSU, *Biochem. Pharmac.* **14**, 837 (1965).
3. M. GOLDSTEIN and Z. WEISS, *Life Sciences* **4**, 261 (1965).
4. S. SPECTOR, A. SJOERDSMA and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **147**, 86 (1965).
5. K. E. MOORE, *Life Sciences* **5**, 55 (1966).
6. A. WEISSMAN and B. K. KOE, *Life Sciences* **4**, 1037 (1965).
7. K. E. MOORE, *Pharmacologist* **7**, 170 (1965).
8. B. P. H. POESCHEL and F. W. NINTEMAN, *Life Sciences* **5**, 11 (1966).
9. M. GOLDSTEIN, B. ANAGNOSTE and K. NAKAJIMA, *Biochem. Pharmac.* **14**, 1914 (1965).
10. S. SPECTOR, R. O. MATA, A. SJOERDSMA and S. UDENFRIEND, *Life Sciences* **4**, 1307 (1965).
11. G. A. JACOBY and B. N. LADU, *J. biol. Chem.* **239**, 419 (1964).
12. P. A. SHORE and J. S. OLIN, *J. Pharmac. exp. Ther.* **122**, 295 (1958).
13. U. S. VON EULER and I. FLODING, *Acta physiol. scand.* **33**, Suppl. 118, 45 (1955).
14. A. CARLSSON and B. WALDECK, *Acta physiol. scand.* **44**, 293 (1958).
15. S. SNYDER, J. AXELROD and M. ZWEIG, *Biochem. Pharmac.* **14**, 831 (1965).
16. T. MATSUMOTO, *Kekkaku* **33**, 338 (1958), cited in *Chem. Abstr.* **52**, 20656i (1958).
17. R. M. AZARKH, A. E. BROUNSHTEIN, T. S. PASHKINA and T.-S. HSU, *Biokhimiya* **25**, 954 (1960).
18. K. E. MOORE, *Fedn Proc.* **25**, 688 (1966).