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SHORT COMMUNICATIONS

Effect of oudenone on adrenal tyrosine hydroxylase activity *in vivo* and on tissue catecholamine concentrations

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OUDENONE, a new microbial product inhibiting tyrosine hydroxylase *in vitro*, was discovered by Umezawa *et al.*[1] The structure and synthesis of oudenone will be reported in another paper. This compound was found to have a hypotensive effect.[1] Oudenone is a unique inhibitor, being competitive with the reduced pteridine cofactor and uncompetitive with tyrosine.[1] Since tyrosine hydroxylase[2] is known to be the rate-limiting step in the biosynthesis of catecholamines,[3] the effect of oudenone on the enzyme activity *in vivo* and on the endogenous levels of catecholamines is of interest. The present communication describes the inhibition of tyrosine hydroxylase in the adrenal glands *in vivo* and the reduction of tissue catecholamine levels in the adrenal glands, heart and brain.

White crystals (77–78°) of oudenone was dissolved in water and injected i.p. into Wistar rats (200 g). Rats were sacrificed at various times after the injection. The adrenal glands, brain and heart were removed. The adrenal gland of the one side was used for the assay of tyrosine hydroxylase and that of the other side for the assay of catecholamines.

Tyrosine hydroxylase inhibition *in vivo* was measured by the conversion of [^{14}C]tyrosine to [^{14}C]dihydroxyphenylalanine (DOPA) in the homogenate of the adrenal gland.[2] The adrenal gland was homogenized with 0·6 ml of 5 mM potassium phosphate buffer, pH 7·5 in a glass homogenizer by hand. Incubation mixture contained (in μmoles): acetate buffer (pH 6·0), 200; L[^{14}C-]tyrosine (0·05 μc), 0·1; $FeSO_4$, 1; mercaptoethanol, 100; 2-amino-4-hydroxy-6,7-dimethyl-tetrahydropteridine, 1; the homogenate (0·1 ml); and water to 1·0 ml. Incubation was carried out at 30° for 15 min in air under shaking. [^{14}C-]DOPA formed was isolated by an alumina column and assayed.

The concentration of catecholamines in tissues was determined by a sensitive method for the simultaneous estimation of norepinephrine and dopamine in tissue by Hogans as described previously.[4]

Estimation of the inhibition of tyrosine hydroxylase by oudenone in the adrenal medulla were shown in Fig. 1. The enzyme activity was inhibited after the injection. A maximum inhibition was observed at 8 hr after administration of oudenone. Thereafter, the enzyme activity returned to the normal level, and a slight increase was observed at 24 hr after oudenone administration.

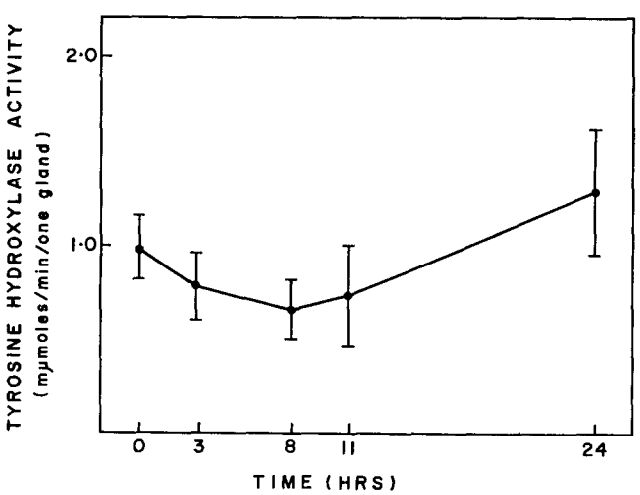

FIG. 1. Tyrosine hydroxylase activity in the adrenal gland of rats after administration of a single dose of oudenone (100 mg/kg, i.p.). Each value represents the mean value (vertical line represents ± 1 standard error) obtained from three to five rats.

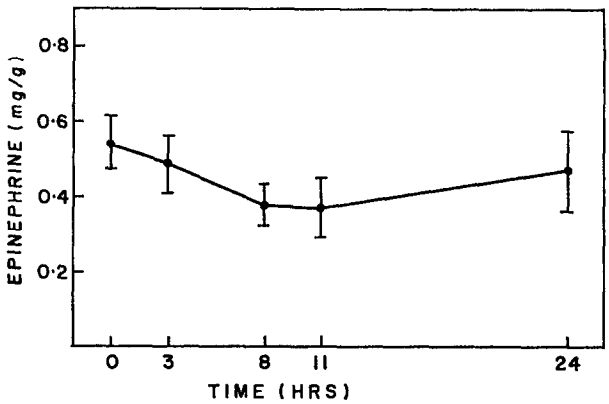

FIG. 2. Levels of epinephrine (including norepinephrine) in the adrenal gland of rats after administration of a single dose of oudenone (100 mg/kg, i.p.). Each value represents the mean value (vertical line represents ± 1 standard error) obtained from five to six rats.

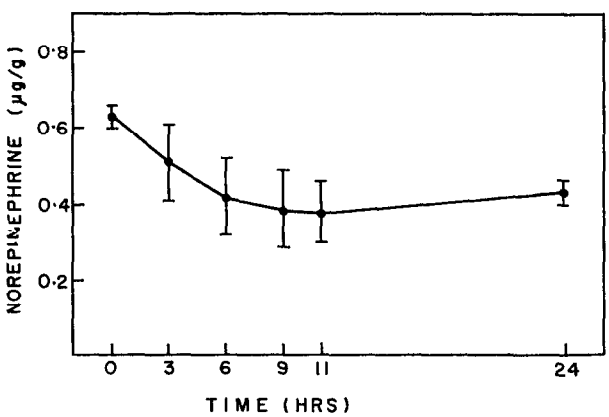

FIG. 3. Levels of norepinephrine in the heart of rats after administration of a single dose of oudenone (100 mg/kg, i.p.). Each value represents the mean value (vertical line represents ± 1 standard error) obtained from three rats.

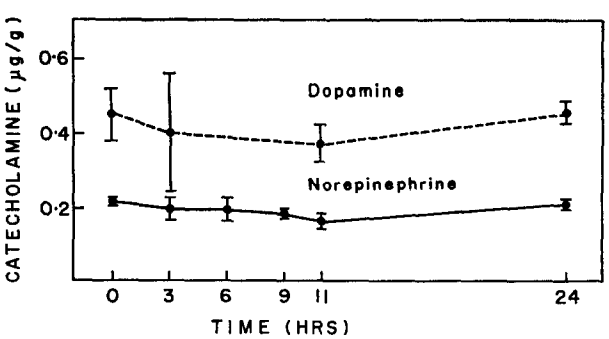

FIG. 4. Levels of dopamine and norepinephrine in the brain of rats after administration of a single dose of oudenone (100 mg/kg, i.p.). Each value represents the mean value (vertical line represents ± 1 standard error) obtained from three rats.

Catecholamines in the adrenal gland was also decreased after oudenone administration. A maximum reduction was observed between 8 and 11 hr after the administration (Fig. 2). The time course of changes of catecholamine levels in the adrenal gland was similar to that of tyrosine hydroxylase activity. Norepinephrine level in the heart was decreased after oudenone administration. A maximum reduction of norepinephrine was observed between 9 and 11 hr after the injection (Fig. 3). Dopamine and norepinephrine levels in the brain showed a slight decrease at 11 hr after oudenone administration (Fig. 4).

Since tyrosine hydroxylase activity in the adrenal gland was found to be inhibitied *in vivo* after the administration of oudenone, the decrease in the catecholamine levels may be attributed to the inhibition of catecholamine synthesis at the tyrosine hydroxylase stage. The hypotensive effect of oudenone could be attributed to the inhibition of tyrosine hydroxylase *in vivo* and the resultant reduction of tissue catecholamines.

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Department of Biochemistry,
School of Dentistry,
Aichi-Gakuin University,
Chikusa-ku, Nagoya, Japan
TOSHIHARU NAGATSU
IKUKO NAGATSU*

Institute of Microbial Chemistry,
Shinagawa-ku, Tokyo, Japan
HAMAO UMEZAWA
TOMIO TAKEUCHI

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Addendum—The structure of oudenone has been reported to be: (S)-2-[4,5-dihydro-5-propyl-2 (3H)-furylidene]-1,3-cyclopentanedione. M. OHNO, M. OKAMOTO, N. KAWABE, H. UMEZAWA, T. TAKEUCHI, H. IINUMA and S. TAKAHASHI, *J. Am. chem. Soc.* **93**, 1285 (1971).

* Present address: Department of Anatomy and Physiology, Aichi Prefectural College of Nursing Moriyama-ku, Nagoya, Japan.

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Hepatic free radical levels in ethanol-treated and carbon tetrachloride-treated rats

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THE OBSERVATION by Gallagher[1] that the antioxidants, α-tocopherol (vitamin E), sodium selenite and *N,N'*-diphenyl-*p*-phenylenediamine (DPPD), afforded rats protection against the lethal effects of carbon tetrachloride (CCl_4) and ameliorated histological damage to the liver has given impetus to a large volume of studies designed to elucidate the protective and toxic mechanisms involved. Carbon tetrachloride hepatotoxicity has been the subject of a recent comprehensive review.[2]