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In vitro activation of monoamine oxidase in rat tissue homogenates by 4 (or 5)-diazoimidazole-5(or 4)-carboxamide

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IZUMI *et al.*[1] first noted an increase of monoamine oxidase (MAO) activity in a guinea pig heart homogenate and in mitochondria after reserpine administration. Youdim and Sandler[2] showed that reserpine activated rat microsomal MAO more than the mitochondrial enzyme and suggested that oxidative deamination of reserpine-released catecholamine might take place within the microsome-like amine storage granule.

Iwata and Yamamoto[3] reported that 4(or 5)-diazoimidazole-5(or 4)-carboxamide (Diazo-ICA) which has pharmacological properties like those of serotonin in laboratory animals, released serotonin from rabbit platelets *in vivo* and *in vitro*.

This diazonium compound has recently been demonstrated to have positive ino- and chronotropic actions on isolated guinea pig atria, and it was found that these were in part due to liberation of catecholamines from their cardiac depots.[4]

Iwata and Yamamoto[5] noted that administration of Diazo-ICA to rabbits caused increased urinary excretion of 5-hydroxyindole acetic acid (5-HIAA), a metabolic product of serotonin.

These observations prompted us to study the effect of Diazo-ICA on monoamine metabolism. This communication reports the activation of MAO in rat tissue homogenates by Diazo-ICA *in vitro*.

Male Sprague–Dawley rats, weighing 250–300 g, were decapitated and tissues were removed immediately, blotted to remove blood and kept on ice. Tissues were homogenized in two volumes of ice-cold distilled water in a glass homogenizer. Duplicate samples for incubation each contained, in a final volume of 3 ml: 0·5 M phosphate buffer (pH 7·0) 0·3 ml, whole homogenate 1 ml, substrate (2–4 μmoles/3 ml in 0·01 N hydrochloride) 1 ml, and water or drug solution 0·7 ml. Monoamine oxidase activity was determined by measuring substrate disappearance by the method of Sjoerdsma *et al.*[6] MAO activity was estimated by assay of serotonin by the method of Udenfriend *et al.*[7] or of tyramine by the method of Udenfriend and Cooper.[8]

Diazo-ICA activated MAO in homogenates of all tissues tested *in vitro*. The activatory effect of the diazonium salt *in vitro* developed rapidly and preincubation with the compound did not increase the activation. As shown in Fig. 1, the strongest activation with serotonin as substrate was obtained with the heart preparation where there was about 250 per cent activation. MAO in the liver preparation was also strongly activated by this diazonium salt, MAO activity increasing to 200 per cent of the

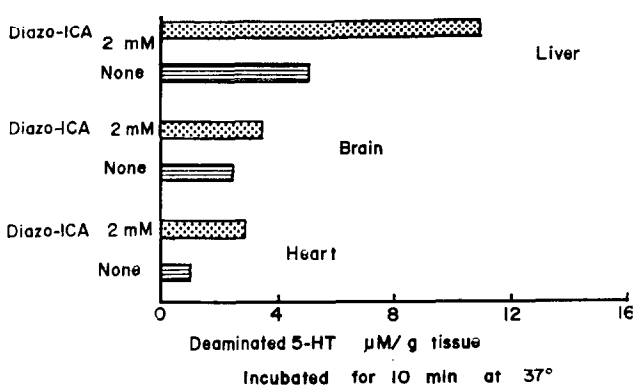

FIG. 1. Activation of MAO in rat tissue homogenates by Diazo-ICA.

control value when the compound was present at a final concentration of 2×10^{-3} M. Activation of MAO by Diazo-ICA was also observed with tyramine as substrate.

With liver preparations, no activation of serotonin deamination occurred in the presence of compounds related to Diazo-ICA, namely, 4(or 5)-aminoimidazole-5(or 4)-carboxamide (AICA) and 4(or 5)-(dimethyltriazeno)imidazole-5(or 4)-carboxamide (Dimethyl-TICA) at concentrations of less than 2×10^{-3} M. A high concentration of Dimethyl-TICA (5×10^{-3} M) in the test solution inhibited serotonin deamination. Similar inhibition was observed using Diazo-ICA at a final concentration of 5×10^{-3} M or more (Fig. 2).

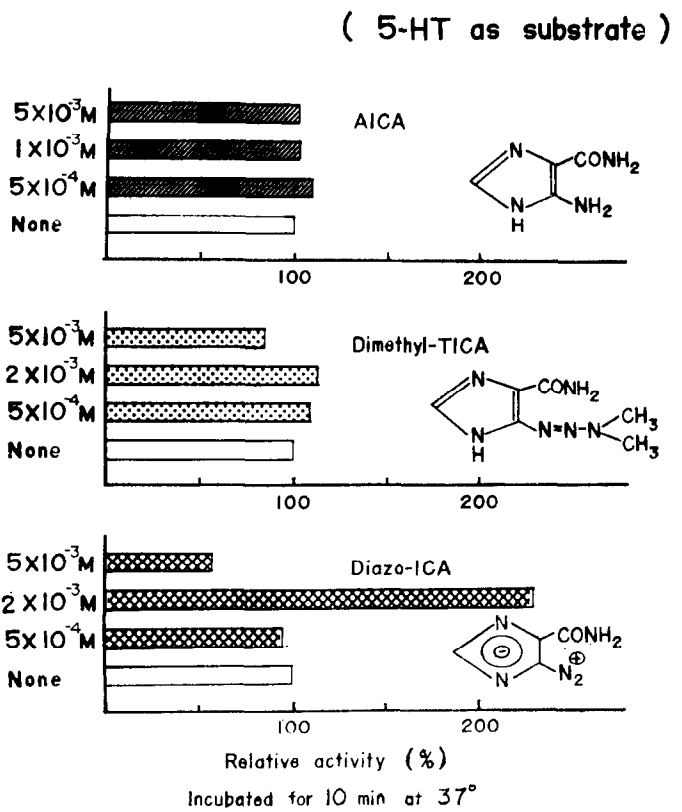

FIG. 2. Effects of AICA derivatives on MAO activity in rat liver homogenates. Diazo-ICA: 4(or 5)-diazoimidazole-5(or 4)-carboxamide AICA: 4(or 5)-aminoimidazole-5(or 4)-carboxamide Dimethyl-TICA: 4(or 5)-(dimethyltriazeno)imidazole-5(or 4)-carboxamide.

Activation of MAO induced by Diazo-ICA could be completely inhibited by addition of cysteine or other mercaptoamino compounds which themselves slightly inhibit MAO activity.

To investigate whether the observed activation by Diazo-ICA was due to a chemical interaction between the compound and substrate, substrate was incubated without enzyme preparation at pH 7·0 in the absence and presence of Diazo-ICA and the serotonin estimation were carried out by the method of Sjoerdsma *et al.*[6] The pH of the incubation medium did not change during this incubation period and serotonin assay was not influenced. In another experiment, activation of MAO activity was also observed when tyramine as well as serotonin was used as substrate. Thus these results suggested that there might be no interaction between the substrate and Diazo-ICA.

The action of reserpine on MAO was studied *in vivo* by Izumi *et al.*[1] Activation of MAO by reserpine

was also described by Youdim and Sandler,[2] who found that the increase was greater in heart than in brain or liver. Similar results were obtained with Diazo-ICA.

An increase in membrane permeability was proposed by Izumi *et al.*[1] to explain the activation of MAO in mitochondria after reserpine administration, although a more direct chemical action of reserpine on the enzyme itself may also be possible. It appears that a permeability change may be involved to some extent in activation of a tissue homogenate by Diazo-ICA, because the increase in activity caused by Diazo-ICA was somewhat less after sonication of the tissue homogenates.

In the presence of calcium, activation was complete and EDTA reversed only calcium-potentiated activity, although calcium alone had no effect on the MAO activity of a whole homogenate of liver.

Previous studies showed that Diazo-ICA caused chemical modification of the sulfhydryl groups of mercapto compounds and biological sulfhydryl groups.[4] Mercaptoamino compounds such as cysteine or cysteamine were found to prevent activation of the enzyme activity by Diazo-ICA completely and preliminary treatment of MAO with iproniazid also prevented activation of the MAO by Diazo-ICA.

Gokin and Krivchenkova[9] found that various mercaptoamino compounds inhibit rat liver mitochondrial MAO activity and that preliminary treatment of the enzyme with cysteamine prevented the irreversible inhibition of the enzyme activity induced by iproniazid to a significant extent.

These findings indicate that Diazo-ICA may interact with the active center of MAO attacked by iproniazid or mercaptoamino compounds.

Department of Pharmacology,
Faculty of Pharmaceutical Sciences,
Osaka University,
Toneyama 6-5, *Toyonaka,*
Osaka-fu,
Japan

ITARU YAMAMOTO
MAKOTO OKA
HEITAROH IWATA

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Human metabolism of orally administered pentazocine

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AN EARLIER communication[1] has described the metabolism *in vitro* and *in vivo* of pentazocine in the monkey and the metabolism *in vitro* of this drug in the mouse and rat. It was found that either of the methyl groups of the dimethylallyl side-chain of pentazocine could be hydroxylated and that one of