Histochemical Demonstration in Rat of Monoamine Oxidase Inhibition by β-Phenyl-Isopropyl Hydrazine

Abstract. A histochemical method for demonstration of monoamine oxidase activity was found to be almost as sensitive as a biochemical method when used for revealing total inhibition of monoamine oxidase in rat tissues after administration of β-phenyl-isopropyl hydrazine. The histochemical method is of special value in the study of monoamine oxidase inhibition in the complex structures of the brain.

β-Phenyl-isopropyl hydrazine is a very potent monoamine oxidase inhibitor. In the rat, Horita (*1*) demonstrated biochemically total inhibition of monoamine oxidase in pooled brain samples after a dose of $5 \times 10^{-6} M$ β-phenyl-isopropyl hydrazine per kilogram of body weight. To our knowledge, no attempts have been made to locate monoamine oxidase inhibition in rat tissues. The histochemical tetrazolium method of Glenner *et al.* (*2*) was found to be sensitive enough for this purpose.

Female albino rats of the Wistar strain, weighing 160 to 200 g, were used in the experiments. β-Phenyl-isopropyl hydrazine (*3*) was dissolved in water to different concentrations so that 0.1-ml solutions injected intraperitoneally contained doses ranging from 1×10^{-3} to $5 \times 10^{-7} M$/kg body weight. After 6 hours, the rats were killed by decapitation. Brain, liver, and kidney were rapidly removed and frozen with carbon dioxide snow on metal blocks together with a corresponding control specimen. Untreated litter mates, and in some experiments also rats treated with equimolar doses of iproniazid and nialamide, served as controls. In a cryostat 10-μ sections were cut simultaneously, picked up on coverslips, and incubated at 37°C for 45 minutes with Nitro-Blue tetrazolium as electron acceptor and tryptamine as substrate (*2*).

In the brain of the control rats, the distribution of monoamine oxidase corresponded well with the observations of Shimizu *et al.* (*4*). In the rats treated with β-phenyl-isopropyl hydrazine, the monoamine oxidase of brain was totally inhibited even by a concentration of $5 \times 10^{-6} M$/kg (Fig. 1, top). The locus coeruleus, which is the most strongly active nucleus in the rat brain (*4*), however, retained a moderate activity.

In the liver and kidney, monoamine oxidase inhibition was total after administration of β-phenyl-isopropyl hydrazine in a dose of $4 \times 10^{-5} M$/kg (Fig. 1, bottom). At a concentration of $2 \times 10^{-5} M$/kg, the periportal areas of the liver lobules retained traces of activity, the central areas being inactive. At this concentration, the straight terminal portions of the proximal convoluted kidney tubules exhibited a faint staining.

In the demonstration of total monoamine oxidase inhibition, the histo-chemical tetrazolium method appears to be almost as sensitive as the spectrophotometric method used by Horita (*1*). In the brain, both methods revealed complete inhibition at the same dosage of β-phenyl-isopropyl hydrazine, whereas in the liver there was a slight difference in favor of the biochemical method.

Consequently, the histochemical method offers an excellent tool for the localization of monoamine oxidase inhibition in the complex structures of the brain. It is known that the various substrates of monoamine oxidase are concentrated in different parts of the brain (*5*). Moreover, monoamine oxidase inhibitors are selective in their action, causing differences in the amounts of individual monoamines accumulated in pooled brains (*6*). This may be because of differences in the distribution of the monoamine oxidase inhibitors in the brain, which, in turn, could be a consequence of their dissimilar chemical structure (*7*).

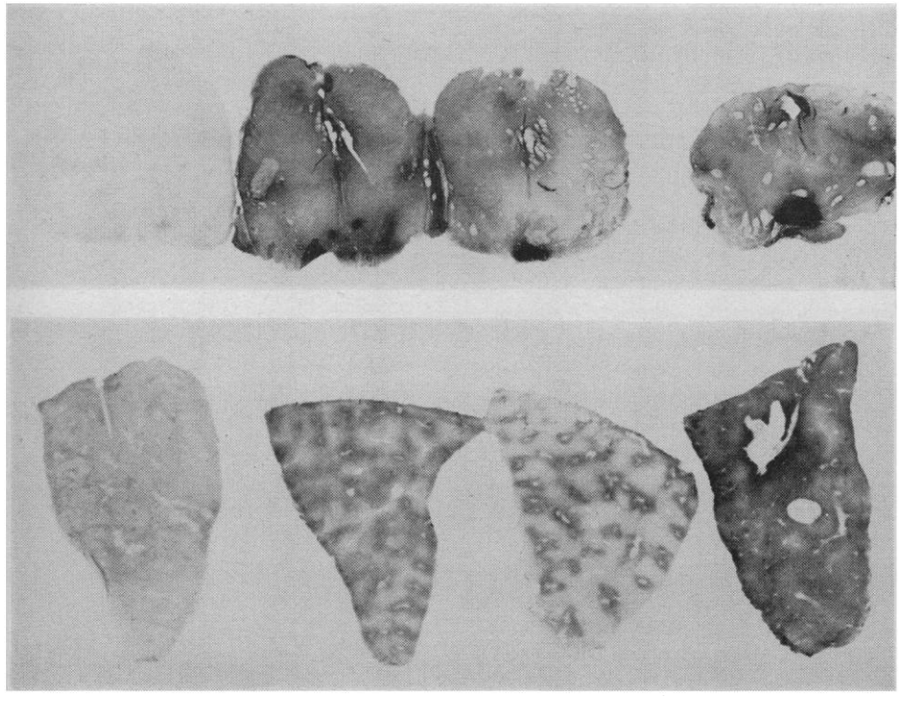

Fig. 1. Top, Vertical cross sections of the rat pons. From left to right: untreated control, and animals 6 hours after administration of iproniazid, nialamide, and β-phenyl-isopropyl hydrazine in a dose of $5 \times 10^{-6} M$/kg, respectively. Note the total inhibition after administration of β-phenyl-isopropyl hydrazine. Bottom, Liver of rat. A similar arrangement, but a higher dose of $2 \times 10^{-5} M$/kg. Note the subtotal inhibition caused by β-phenyl-isopropyl hydrazine and the centrolobular inhibition caused by iproniazid.

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References and Notes

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3. A generous supply of β-phenyl-isopropyl hydrazine (Catron) was obtained from the Lakeside Labs through the courtesy of Messrs. Astra, Sweden.
4. N. Shimizu, N. Morikawa, M. Okada, *Z. Zellforsch. u. mikroskop. Anat.* **49**, 389 (1959).
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6. A. Carlson, M. Lindquist, T. Magnusson, *Intern. Symp. on Nialamide* (1959), p. 96.
7. Further studies along these lines are in progress. This work was partially aided by a grant to the department from the Sigrid Juselius Foundation, Helsinki, Finland.

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