PHARMACOLOGICAL REVIEWS
Vol. 18, No. 1, Part 1

Printed in U.S.A.

N. SUMMARY OF DISCUSSION AND COMMENTARY

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Unfortunately, the discussants did not concentrate on specific enzyme problems, although enzymology was the major topic of this session, and although the papers presented could have served as an excellent starting point for discussions going along these lines.

A few points were added to the enzymology of monoamine oxidase (MAO), introduced by Gorkin. Witkop mentioned that the introduction of an *ortho*-hydroxyl into catecholamines markedly reduced the rate of degradation catalyzed by MAO (J. W. Daly *et al.*, Biochemistry, in press). Similarly, it had been found (E. A. Zeller, Biochem. Z. **339:** 13, 1963), that *ortho*-substitution partially or totally abolishes the ability of benzylamine to serve as a substrate of MAO. Nagatsu made an heroic effort to purify beef brain MAO with the help of sonication and detergents. Although only a small increase of specific activity was accomplished, a better understanding of the specificity pattern of brain MAO was obtained with this new preparation, and serotonin turned out to be a better substrate than the classical tyramine. Zeller presented some data concerning the marked effect of sex hormones on the activity of liver MAO. They resulted from the cooperative efforts of F. Chordikian, J. S. Schweppe and G. Stanick. Adult female mice display twice as high MAO activity in the liver as males. Upon castration, the difference disappears. The high MAO level found in adult females and in castrated males and females can be reduced by testosterone, nortestosterone, estradiol, and progesterone with decreasing power. Brain MAO proved to be much more resistant to the action of these steroids than the liver enzyme. Preliminary studies carried out with iproniazid- and puromycin-treated animals seem to indicate that the hormones exert their influence by affecting the synthesis of this enzyme. The activity determinations were made with the help of a new photometric procedure based on the degradation of *m*-iodobenzylamine, which requires much less material and time than the classical methods. (V. Zeller *et al.*, J. Medicin. Chem. **8:** 440, 1965). Puromycin was also used to analyze the effect of ecdysone on the dopa-decarboxylase of the Calliphora larva, as C. E. Sekeris mentioned in answer to a question by S. Udenfriend: puromycin abolished the appearance of the decarboxylase.

The paper on MAO inhibitors by Pletscher found considerable attention. Again the basic principles were neglected entirely in the discussion in favor of the application of the MAO inhibitors as tools in physiological and pharmacological research. Furchgott had previously reported that a short incubation of reserpinized guinea pig atria with a moderate concentration of norepinephrine (NE) largely restored the positive inotropic response to tyramine. Pretreatment of this preparation with iproniazid greatly facilitated the ability of NE to restore the indirect sympathomimetic effect. It was previously assumed that this effect

was due to a protection of tyramine against MAO. Recent detailed analysis, however, revealed that the inhibitor protected the NE taken up by the nerve terminals from inactivation by MAO and thus allowed effective quantities to be stored. Some of the effects of bretylium in this system may also be explained by its reversible inhibition of MAO. Since the effects are relatively small *in vitro*, it is assumed—as so often in similar circumstances—that bretylium is actively taken up and concentrated to a sufficient extent to produce substantial inhibition. This problem should be tackled experimentally, *e.g.*, with the help of histoenzymological methods. Pletscher had suggested that dopamine may serve as a transmitter for NE during treatment with MAO inhibitors, and this may explain the antihypertensive effect observed with some of these compounds. Rand reported experiments carried out by J. Farmer: the responses of the cat nictitating membrane to sympathetic stimulation are diminished after infusions of dopamine. In cats pretreated with a MAO inhibitor dopamine did not affect sympathetic responses. These observations are in good accord with Pletscher's suggestions. Iversen agreed with this author's statement that MAO inhibitors may have an action other than inhibiting MAO. Several MAO inhibitors, such as tranylcypromine, phenelzine, and harmaline, also prevent NE uptake. Obviously, this phenomenon could be quite independent of MAO. On the other hand, one still has to explain why compounds of such different structures elicit the same responses. One could also consider the suggestion, made some years ago (E. A. Zeller and J. R. Fouts, Ann. Rev. Pharmacol., **3**: 9–32, 1963), that the degradation products of MAO action, the aldehydes, may pass more easily through the lipid layer of the cell membrane or similar structural units than the original biogenic amines in their ionized form. One may find some support of this working hypothesis in the data presented by LaBrosse. After administration of reserpine to the isolated rat heart treated with H^3-NE, it was not the H^3-NE which left the heart; but tritium-labeled deaminated catechol metabolites, such as H^3-3,4-dihydroxymandelic acid. From these data the author concluded that reserpine depletes NE by enhancing the action of MAO on stored NE rather than by releasing intact catecholamines. Again, direct experimental proof of this reserpine effect is needed.

Everett expressed some doubts about the role of MAO in the hypotensive action of MAO-inhibitors. While pargyline is a most effective hypotensive drug, MO 1255 has no effect on blood pressure whatsoever. Since both drugs are powerful MAO inhibitors one has to seek other properties to explain this difference. MO 1255, however, has to be converted *in vivo* into the active inhibiting compound. Thus it remains to be seen whether the necessary transformation takes place at the site of the hypotensive action, before MAO is to be excluded. A most remarkably long lasting effect of MAO inhibitors on catechol amine metabolism was reported by Pekkarinen and his co-workers (Acta pharmacol. toxicol., in press). After treating patients with nialamide for 35 days, their excretion of vanilylmandelic acid (VMA) was significantly reduced for at least 177 days after cessation of drug administration. These and many other contributions to this symposium, impressively demonstrated the versatility and

wide range of applications of the MAO inhibitors, which came into being only in 1952.

From Axelrod's presentation on methylation processes, which included many substances other than catecholamines, several points were brought up in the discussion. Axelrod had concluded that the different metabolic fate observed for NE released by tyramine and by reserpine respectively might be due to the fact that tyramine produces a faster depletion than reserpine. Costa referred to two publications (in press) from Brodie's laboratory in which it was shown that the maximal rate of decline of endogenous NE elicited by tyramine is smaller than that caused by reserpine. These results do not support the existence of a direct relationship between the rate of decline and the type of NE metabolism, as suggested by Axelrod.

deSchaepdryver reported that metanephrine-7-H^3 can be O- and N-demethylated not only *in vitro*, as previously reported by Axelrod, but also in the intact rabbit (W. DePotter *et al.* Biochem. Pharmacol. **12:** 661, 1963).

Belleau's attempts to interpret the structure of adrenergic receptors with the help of some concepts of modern physical organic chemistry was followed by a half-hearted discussion only. This outcome is deplorable because in this community of investigators an unrivaled body of precise data were available which either agree or disagree with the proposed models. According to Bueding the exposure of intestinal smooth muscle (taenia coli of the guinea pig) to threshold concentrations of epinephrine (E) increased the turnover of P^{32} in ATP, a finding which is in support of Belleau's idea that there is an interaction between ATP and the adrenergic receptor. On the other hand, Schild mentioned that for the inhibitory action of isoproterenol on the depolarized rat uterus magnesium is not required; this metal, therefore, appears not to be involved in the chelation of the catechol grouping. However, protein-bound metals, even magnesium, could still serve as an anchoring point. Finally, Dornhorst pointed out that the catechol configuration is not an absolute necessity for "*beta*-activity," because the 3,5-dihydroxy analog of isoproterenol shows typical *beta*-activity with a potency about one fifth of that of the parent substance.