Biochemical Pharmacology, Vol. 23, pp. 1906–1910. Pergamon Press, 1974. Printed in Great Britain.

Computer reconstruction of tyramine breakdown in brain

(*Received* 28 *September* 1973; *accepted* 28 *November* 1973)

THE END products of the metabolism of a number of biogenic amines in the central nervous system are the corresponding alcohols and acids which may be excreted as such or after conjugation.[1–3] The amines are oxidised by monoamine oxidase to form the aldehydes which may then either be oxidised by aldehyde dehydrogenase to form the acid or reduced by aldehyde reductase to form the alcohol. In the case of the catecholamines *O*-methylation can also occur.[4,5] The predominant pathway followed by a specific amine appears to depend upon its structure; phenethylamines (e.g. dopamine and tyramine) and indolethylamines are converted mainly to the carboxylic acids in the brain whereas the β-hydroxylated derivatives of the phenethylamines (e.g. noradrenaline and octopamine) are mainly converted to the glycols.[1,2,6,7] We have suggested that this effect may be due to the specificities of the enzymes involved since β-hydroxylation results in a pronounced fall in the K_m values of the aldehyde reductases whereas aldehyde dehydrogenase does not appear to be much affected.[8] This difference in metabolic fate has allowed the measurement of 5-hydroxyindoleacetic acid, homovanillic acid and 3-methoxy-4-hydroxyphenethylene glycol to be used as indices of serotonin dopamine and noradrenaline metabolism respectively[4,5] and such studies have been carried out with subjects in a number of different mental states.[9,10]

The pathway of metabolism followed by a specific amine, is, however, also affected by a number of drugs. The administration of ethanol, the anti-alcoholism drug disulfiram or the tranquillizer reserpine can cause an increase in the production of the alcoholic metabolites.[11–13] The effect of disulfiram may be due simply to its action as an inhibitor of aldehyde dehydrogenase and this explanation is supported by the observation that although this compound is a powerful inhibitor of aldehyde dehydrogenase,[14] it does not affect the activity of the aldehyde reductases.[8,15]

The action of ethanol is probably mediated by its metabolite acetaldehyde[16] in a similar fashion since acetaldehyde, which can reach a concentration of up to 75 μM in rat brain following ethanol administration,[17] is a good substrate for aldehyde dehydrogenase[18] and will thus act as a competitive inhibitor towards the oxidation of other aldehydes.[19] In contrast acetaldehyde is very poor both as a substrate and an inhibitor of aldehyde reductases[8,15,20] and thus might be expected to have little direct effect on the reductive pathway. Reserpine is known to cause the release of bound amines in nerve endings thereby exposing them to the action of monoamine oxidase.[21] The action of this enzyme would result in an increased local concentration of the aldehydes and it has been suggested that this may cause the activity of aldehyde dehydrogenase to approach saturation, whereas the aldehyde reductases, which have higher K_m values, would continue to increase in velocity.[15]

These explanations which depend solely on the kinetic properties of the enzymes involved predict that these drugs would cause changes in the steady-state levels of aldehydes in the brain and since these compounds are known to possess pharmacological activity themselves[22–24] such changes cannot be ignored. In order to gain an insight into the extent of these variations of aldehyde levels and to test the validity of this simple approach to the function of the amine breakdown pathway we have attempted to reconstruct this pathway in terms of a computer simulation based on kinetic data obtained from the enzymes purified from a single source, pig brain. The kinetic parameters of monoamine oxidase, aldehyde dehydrogenase and the two aldehyde reductases (the "low K_m" and "high K_m" enzymes[15]) were taken from our previously published results[8,15,18,25,26] and the computer simulation was carried out using the program of Illingworth[27] with the Cambridge University Titan computer.

Since no complete information is available on the kinetics and specificity of pig brain catechol-*O*-methyltransferase we chose to investigate the computer simulation approach by taking amines which did not give rise to significant amounts of *O*-methylated metabolites *in vivo*. Figure 1 shows the reaction scheme used for this simulation. In the case of monoamine oxidase it was assumed that the oxygen concentration in the cell was close to that of air saturated water and the K_m value for tyramine is that determined under these conditions.[25] The concentrations of the nicotinamide coenzymes in the brain have been estimated by Jacobson and Kaplan[28] and from these values it can be concluded the the reductases will be saturated with NADPH whereas, since the K_i value for $NADP^+$ (22 μM) is some 10 fold higher than the K_m value for NADPH (2·0 μM) and the $NADP^+$ and NADPH levels are 13 μM and 43 μM respectively, inhibition by the oxidized coenzyme will be unimportant. The levels of NAD^+ (215 μM) will be sufficient to virtually saturate the dehydrogenase but in this case the K_i value for NADH (7·2 μM) is sufficiently close to the K_m value for NAD^+ (7·5 μM) for the inhibition by the NADH present (75 μM) to be important. The inhibition of aldehyde dehydrogenase by NADH is mixed with respect to the amine and the K_m and K_i values[18] were used to provide correction factors to the K_m and V_{max} values shown in Fig. 1. Inhibition by the acid

and alcohol products may be neglected since the enzymes have been shown to catalyse reactions which are essentially irreversible at physiological pH values.[15,18] Although two NAD^+-dependent alcohol dehydrogenases, one of which is inhibited by pyrazole, have been detected in brain,[29,30] the available evidence indicates that they do not play an important role in biogenic amine metabolism since pyrazole has no significant effect on this system[31] and the pyrazole-insensitive enzyme appears to be unable to reduce the biogenic aldehydes.[30]

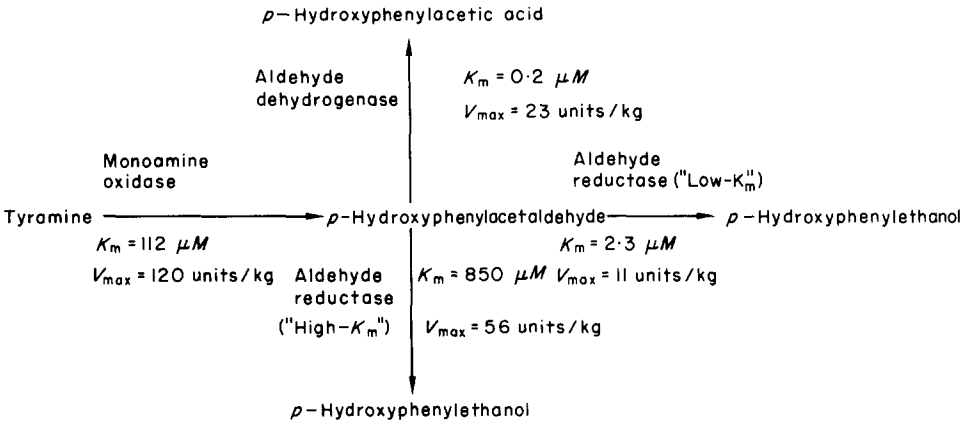

FIG. 1. The pathway of tyramine breakdown used for the simulation study. The kinetic constants were all determined at pH 7·2 and at 30°. One unit of activity is defined as the amount that catalyses the conversion of 1 μmole of substrate/min.

The levels of aldehydes available for metabolism will depend to a large extent upon the steady-state levels of amines available to monoamine oxidase. Although the concentration of vesicle bound amines has been estimated, it is more difficult to evaluate the concentration of the free cytoplasmic amines—an average value of less than 11 μM has been suggested[32] whereas it has been argued that the free amine concentration within the nerve endings may reach considerably higher levels.[33] The distribution of amines throughout the central nervous system is not uniform[34] and localization of the various enzymes involved in amine catabolism in discrete brain areas or cell types could result in the metabolism of amines by preferred routes in specific areas. The presently available evidence suggests, however, that aldehyde oxidizing and reducing enzymes are fairly uniformly distributed in brain tissue.[20,35] As a first approximation for this simulation study it was assumed that there were no important permeability barriers affecting this system and that the whole brain concentrations of the enzymes would provide the best approximation to the published results obtained *in vivo* or with whole brain slices or homogenates.

Figure 2 shows the simulated curves for the rates of aldehyde, acid and alcohol formed by this system at a series of tyramine concentrations. The velocities of the aldehyde utilizing enzymes will, of course, give a direct measure of the relative proportions of acid and alcohol metabolites formed. The rate of formation of the alcohol metabolite is almost completely due to the "low K_m" aldehyde reductase even at amine concentrations as high as 35 μM. Thus it would appear that the "high K_m" enzyme is unlikely to be important in the metabolism of amines that lack the β-OH group (similar results having been obtained with other amines lacking this group). The rate of alcohol production increases over the amine concentration range tested whereas the rate of acid production approaches saturation as the amine level rises. At a tyramine concentration of 10 μM the ratio of acid:alcohol production is about 10:1. Breese *et al.*[2] using rat brain slices obtained a ratio of acid to alcohol products of about 9:1 after a 30 min incubation with 0·4 μM tyramine. It is however, not possible to evaluate the total amine concentration present in that preparation. At amine concentrations of 20 μM the approaching saturation of aldehyde dehydrogenase results in the ratio of acid:alcohol production falling to about 4:1 indicating that an increase in amine levels as, for example, might be expected initially after reserpine treatment, results in an increase in the relative amount of alcohol produced. This change is similar to that observed *in vivo* by Sandler and Youdim[13] where reserpine treatment causes an increase in the total metabolite formation with the ratio of alcohol:acid metabolites being approximately doubled 12 hr after administration.

Preliminary simulation results obtained with β-hydroxylated aldehydes indicate that the lower K_m values of the "high K_m" reductase for such aldehydes results in this enzyme playing a larger role in the metabolism of these aldehydes.

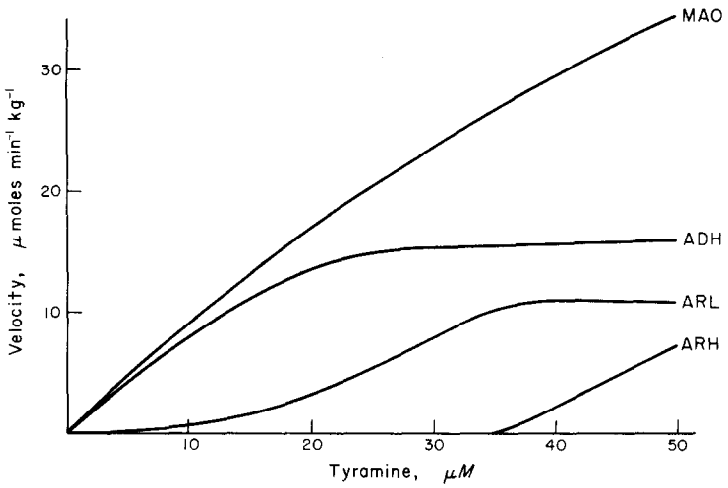

FIG. 2. Simulation of tyramine metabolism using the data given in Fig. 1 and the computer program of Illingworth.[27] The curves for the activities of monoamine oxidase, aldehyde dehydrogenase and the "low- and high-K_m" aldehyde reductases are labelled MAO, ADH, ARL and ARH respectively.

The possible effects of acetaldehyde on this system were appraised by including the competitive inhibition of aldehyde dehydrogenase in the simulation program, the K_i value for this inhibition was taken to be 2·6 μM.[19] Figure 3 shows the effects of increasing acetaldehyde concentrations at two fixed amine levels. As the acetaldehyde concentration rises an increasing proportion of the aldehyde dehydrogenase becomes involved with its oxidation and the rate of *p*-hydroxyphenylacetic acid production falls dramatically. This is compensated for by an increase in production of the alcohol as the steady-state aldehyde concentration rises (Fig. 4). These simulated results are similar to those observed in a variety of species

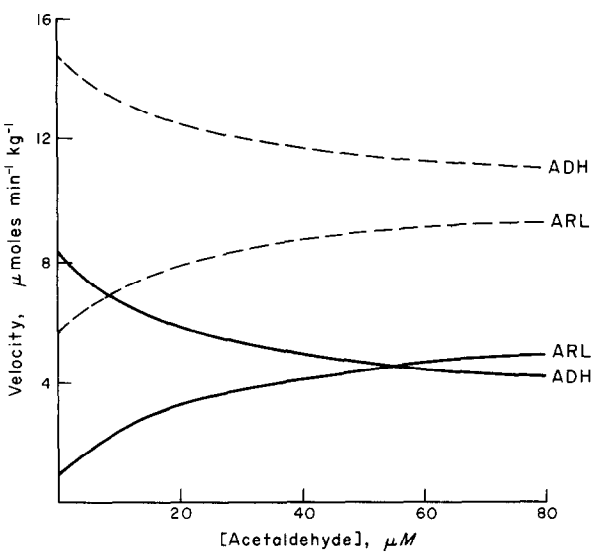

FIG. 3. Simulation of the effects of acetaldehyde on the metabolism of tyramine. Solid lines were determined at a tyramine concentration of 10 μM and broken lines at a tyramine concentration of 25 μM. Curves for the activities of aldehyde dehydrogenase are labelled ADH and those for the "low K_m" aldehyde reductase are labelled ARL. The curves for the "high K_m" aldehyde reductase are not shown since in no case did the activity of this enzyme reach 10 per cent of that of the "low K_m" enzyme.

(see 36 for review) and suggest that simple inhibition of this type may be sufficient to explain this phenomenon without the need to postulate changes in the NAD^+:NADH ratio, although a relative increase in the NADH levels following ethanol oxidation would depress acid formation even further.

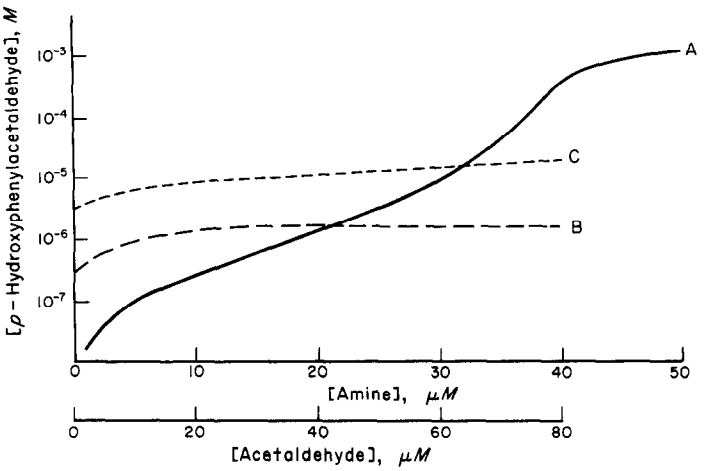

FIG. 4. Changes in the steady-state levels of *p*-hydroxyphenylacetaldehyde under the conditions represented in Figs. 2 and 3. The line labelled A represents the variation as the amine level is increased. Lines B and C represent the changes that occur when the concentration of acetaldehyde is increased at an amine level of 10 μM and 25 μM respectively.

The predicted changes in the steady-state levels of the aldehyde under the conditions discussed above are shown in Fig. 4. The three aldehyde metabolising enzymes tend to buffer changes in the free aldehyde concentrations but outside their buffering ranges large changes in the levels can occur and such changes should be taken into account when considering the behavioural effects of drugs which affect this system.

The agreement between these simulated results and measurements which have been made *in vivo* and *in vitro* is remarkably good considering the approximations that have been made and the fact that the latter results have been obtained from different preparations from several species. These results would suggest that it may indeed by possible to understand the functioning of this pathway in terms of the properties of the individual enzymes alone and that there are no permeability barriers that cause rate-determining steps in the system. An approach such as this may be of use in the evaluation of the effects of drugs on this pathway.

Acknowledgement—A.J.T. was supported by a grant from the Medical Research Council.

Department of Biochemistry,
Tennis Court Road,
Cambridge CB2 1QW, England

K. F. TIPTON
A. J. TURNER*

REFERENCES

1. C. O. RUTLEDGE and J. JONASON, *J. Pharmac. exp. Ther.* **157,** 493 (1967).
2. G. R. BREESE, T. N. CHASE and I. J. KOPIN, *J. Pharmac. exp. Ther.* **165,** 9 (1969).
3. D. ECCLESTON, A. T. B. MOIR, H. W. READING and I. M. RITCHIE, *Br. J. Pharmac. Chemother.* **28,** 367 (1966).
4. N. E. ANDÉN, B. E. ROOS and B. WERDINIUS, *Experientia* **19,** 359 (1963).
5. S. WILKS and R. MONES, *J. Neurochem.* **18,** 1771 (1971).
6. J. JONASON and C. O. RUTLEDGE, *Acta Physiol. Scand.* **73,** 411 (1968).
7. D. ECCLESTON, H. W. READING and I. M. RITCHIE, *J. Neurochem.* **16,** 274 (1969).
8. A. J. TURNER and K. F. TIPTON, *Eur. J. Biochem.* **30,** 361 (1972).
9. S. WILKS, B. SHOPSIN, S. GERSHON and M. SUHL, *Nature, Lond.* **235,** 440 (1972).

* Present address: Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds LS2 9LS.

10. S. J. DENKER, U. MALM, B. E. ROOS and B. WERDINIUS, *J. Neurochem.* **13,** 1545 (1962).
11. M. TACKER, P. J. CREAVEN and W. M. MCISAAC, *Biochem. Pharmac.* **19,** 604 (1970).
12. A. A. SMITH and S. B. WORTIS, *Biochem. Pharmac.* **3,** 333 (1960).
13. M. SANDLER and M. B. H. YOUDIM, *Nature, Lond.* **217,** 771 (1968).
14. R. A. DEITRICH and V. G. ERWIN, *Mol. Pharmac.* **7,** 301 (1971).
15. A. J. TURNER and K. F. TIPTON, *Biochem. J.* **130,** 765 (1972).
16. E. B. TRUITT and M. J. WALSH, in *The Biology of Alcoholism* (Eds. B. KISSIN and H. BEGLEITER), Vol. 1, p. 161. Plenum Press, New York (1971).
17. J. W. RIDGE, *Biochem. J.* **88,** 95 (1963).
18. R. J. S. DUNCAN and K. F. TIPTON, *Eur. J. Biochem.* **22,** 538 (1971).
19. R. A. LAHTI and E. MAJCHROWICZ, *Biochem. Pharmac.* **18,** 535 (1969).
20. B. TABAKOFF and V. G. ERWIN, *J. biol. Chem.* **245,** 3262 (1970).
21. N. WEINER and C. O. RUTLEDGE, in *Mechanisms of Release of Biogenic Amines* (Eds. U. S. VON EULER, S. ROSELL and B. URNÄS), p. 307. Pergamon Press, Oxford (1966).
22. M. JOUVET, *Science, N.Y.* **163,** 32 (1969).
23. H. C. SABELLI, W. J. GIARDINA, S. G. A. ALIVISATOS, P. K. SETH and F. UNGAR, *Nature, Lond.* **223,** 73 (1969).
24. H. C. SABELLI and W. J. GIARDINA, *Biol. Psychiatry* **2,** 119 (1970).
25. K. F. TIPTON, *Eur. J. Biochem.* **4,** 103 (1968).
26. K. F. TIPTON and A. P. DAWSON, *Biochem. J.* **108,** 95 (1968).
27. J. A. ILLINGWORTH, in *Analysis and Simulation of Biochemical Systems* (Eds. H. C. HEMKER and B. HESS), *FEBS Symp.* **25,** p. 345. North Holland, Amsterdam (1972).
28. K. B. JACOBSON and N. O. KAPLAN, *J. biol. Chem.* **226,** 603 (1957).
29. N. H. RASKIN and L. SOKOLOFF, *J. Neurochem.* **17,** 1677 (1970).
30. V. G. ERWIN, W. D. W. HESTON and B. TABAKOFF, *J. Neurochem.* **19,** 2269 (1972).
31. R. A. DEITRICH, A. C. COLLINS and V. G. ERWIN, *Biochem. Pharmac.* **20,** 2663 (1971).
32. A. L. GREEN, *Biochem. J.* **121,** 32P (1971).
33. K. F. TIPTON, *Br. Med. Bull.* **29,** 116 (1972).
34. S. S. KETY and F. G. SAMSON, *Neurosciences Res. Symp. Summaries* **2,** 494 (1967).
35. V. G. ERWIN and R. A. DEITRICH, *J. biol. Chem.* **241,** 3533 (1966).
36. A. FELDSTEIN, in *The Biology of Alcoholism* (Eds. B. KISSIN and H. BEGLEITER), Vol. 1, p. 127. Plenum Press, New York (1971).

Biochemical Pharmacology, Vol. 23, pp. 1910–1913. Pergamon Press, 1974. Printed in Great Britain.

Role of adrenochrome in aryl hydrocarbon hydroxylase induction by epinephrine in rat liver cell culture

(*Received* 25 *October* 1973; *accepted* 24 *November* 1973)

THE MICROSOMAL "mixed function oxidases" of rat liver are centred on cytochrome P-450. This family of enzymes metabolise drugs such as phenobarbitone, polycyclic hydrocarbon carcinogens, e.g. 3,4-benzo(α) pyrene (BP) and 1,2-benzo(α)anthracene (BA) in addition to many normal body constituents. Aryl hydrocarbon hydroxylase (AHH) is a member of this group of enzymes and prior treatment of the rat with BP induces hepatic AHH to higher levels of activity.

Similarly AHH activity can be found and induced to higher activities by BA in cell-cultures derived from hamster, rat, mouse and chick embryonic tissues, in addition to a few established cell-lines.[2] In cultures of foetal rat hepatocytes Geilen and Nebert[3] observed that very high, unphysiological, concentrations of catecholamines such as epinephrine increased AHH activity by a process similar to BA induction. On repeating these experiments in the course of other observations, we noticed that cell-growth medium containing high epinephrine concentrations became dark brown during the induction period.

Abbreviations: AHH—Aryl hydrocarbon hydroxylase; BP—3,4-Benzo(α)pyrene; BA—1,2-Benzo(α)anthracene; RL—Rat-liver epithelial cell line; DMSO—Dimethylsulphoxide.