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POSTNATAL DEVELOPMENT OF DOPAMINE DEAMINATION IN THE STRIATUM OF THE RAT

A.J. DAVIS, MARGARETHE HOLZBAUER & D.F. SHARMAN

Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

M.B.H. YOUDIM

Medical Research Council Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford

In the striata of litter-mate rats the development of the monoamine oxidase (MAO) activity towards dopamine *in vitro* followed a similar time course with age as the tissue concentrations of homovanillic acid and dihydroxyphenylacetic acid, the two acid metabolites of dopamine formed by the action of MAO.

Introduction During a study of the development of the enzyme monoamine oxidase (E.C. 1.4.3.4.; MAO) it has been observed that the ability of homogenates of the striata of 5 and 10 day old rats to deaminate dopamine was very low or not detectable. Depending on the strain of rats, this was followed by a more or less steep rise in dopamine deamination and a maximum was reached around days 30 – 40. In contrast to dopamine, considerable quantities of tyramine were metabolized by the same striatal homogenates of the 5 day old rats. Deamination of tyramine also increased up to day 40, but the rate of increase with age was slower than that for dopamine (Blatchford, Holzbauer & Youdim, 1975; Youdim & Holzbauer, 1975).

Our observations were made on tissue homogenates *in vitro*. The question arose whether the *in vitro* observations reflected the rates at which dopamine is being deaminated *in vivo*. As an index of the *in vivo* deamination the concentrations in the striatum of the two naturally occurring acidic metabolites of dopamine, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) can be used.

That dopamine deamination in the striatum is low in the young animal has been indicated in previous experiments. Thus in the sheep the concentration of HVA in the caudate nucleus was found to increase between foetal and adult life (Sharman, 1963). Keller, Bartholini & Pletscher (1973) have measured striatal concentrations of dopamine and HVA in Wistar rats of the Füllinsdorf colony aged between 4 and 60 days. In these rats only small quantities of HVA were detected at day 4. The value obtained on day 12 was no longer significantly different from the highest value, which was found on day 18. Because of the strain differences observed in our previous experiments a direct comparison between these *in vivo* studies and our *in vitro* studies was not possible. In the present work we have, therefore, used litter-mate rats to compare directly the development of MAO activity towards dopamine in the striatum *in vitro*, with the striatal concentrations of both acidic dopamine metabolites, HVA and DOPAC.

Methods Rats from 14 litters born between March 9th and 26th 1975 of mothers belonging to the same Wistar colony were used. The tissues from these rats were distributed between the samples to be analysed for enzyme activity and phenolic acid so that each age group included the striata from at least one rat from each litter. Each sample contained either male or female tissue. No significant sex differences were seen in the phenolic acid concentration or MAO activity. The rats were weaned at day 21. They were killed by rapid decapitation. MAO activity was measured in the striata from one rat. For the measurement of HVA and DOPAC at days 5, 8 and 10 the striata of 5 rats were pooled for each sample. At day 20, three rats were used per sample and, at day 30, two rats. The acids were also measured in individual striata of 6 of the mother rats.

For monoamine oxidase assay the tissues (which were kept up to 14 days at -18° C between dissection and assay) were homogenized in 1 ml 0.1 M sodium phosphate buffer pH 7.4. MAO activity was assayed by a radiochemical method similar to that of Robinson, Lovenberg, Keiser & Sjoerdsma (1968). Each assay sample consisted of 0.4 ml buffer to which 0.1 ml tissue homogenate was added and, after a preincubation period of 5 min at 37° C, 0.1 ml of substrate solution. The substrate solution contained 100 nmol of cold dopamine or tyramine per 0.1 ml plus approx. 50,000 d/min of ^{14}C-labelled amine. The samples were incubated for 30 min at 37° C and then passed through a column (approx. 0.5 x 2.5 cm) of Amberlite CG50 (mesh 200-400) to separate the amines, which were retained on the column, from

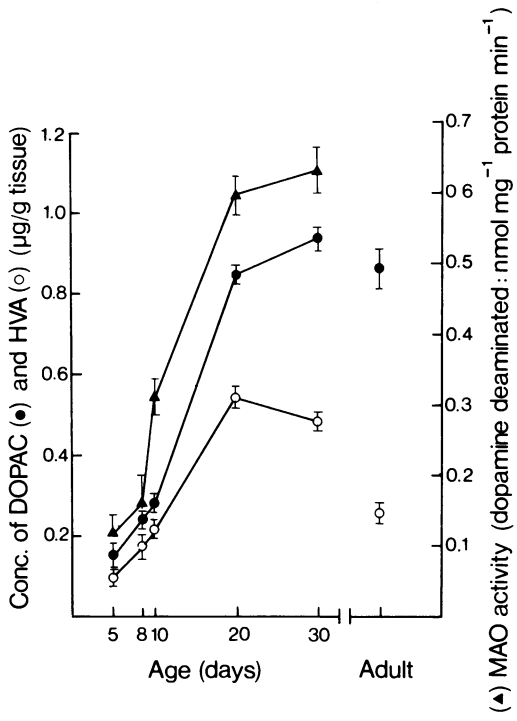

Figure 1 Postnatal development of dopamine deamination in rat striatum. Mean values are given; vertical lines show s.e. mean. (▲) Monoamine oxidase (MAO) activity towards dopamine; (•) dihydroxyphenylacetic acid (DOPAC); (○) homovanillic acid (HVA). Number of samples for each mean value: MAO: day 5: 12; day 8: 5; day 10: 12; day 20: 7; day 30: 10. HVA and DOPAC: day 5: 8; day 8: 4; day 10: 7; day 20: 8; day 30: 8; adults: 6.

their metabolites. The radioactivity of the metabolites was counted in a liquid scintillation spectrometer using Unisolve scintillation fluid. The protein content of the tissue homogenates was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951). The results were expressed in nmol amine deaminated/mg protein per minute.

For the assay of HVA and DOPAC the striatial tissue immediately after removal from the brain was frozen on a sheet of aluminium foil placed over liquid N_2 and then stored for up to 10 days, wrapped in aluminium foil, immersed in liquid N_2. The phenolic acids were estimated by a fluorimetric method based on that of Murphy, Robinson & Sharman (1969). The mean recovery for DOPAC was 54% and for HVA 69%. All values were corrected for losses.

Results Figure 1 shows the simultaneous rise in the concentrations of HVA and DOPAC in the rat striatum with increasing age and the ability of the tissue homogenates to deaminate dopamine *in vitro*. The estimates for the phenolic acids and for the MAO activity obtained on day 5 were at the lower limits of the estimation methods. The increase in MAO activity towards dopamine which occurred with age were similar to the increases in the concentrations of HVA and DOPAC in the striatum. Between days 8 and 20 dopamine deamination increased 3.8 fold; during the same period the rise in the striatal concentration of HVA was 3.2 fold and that of DOPAC 3.5 fold. The ratio of DOPAC to HVA lay between 1.3 and 1.7 until day 20 when the HVA concentrations reached a maximum. On day 30 the DOPAC concentration was about 10% higher than on day 20 and the ratio DOPAC to HVA was 1.9. In the adult rats the concentrations of both acids were lower than in their 30 day old offspring and the ratio DOPAC/HVA was 3.4.

The rise in MAO activity towards tyramine with age was slower than that towards dopamine. On day 5, four times more tyramine than dopamine was deaminated by the same homogenates whereas on day 30 only twice as much tyramine as dopamine was deaminated. There was a twofold rise in the deamination of 5-hydroxytryptamine (5-HT) between the 5 and 10 day old rats. At both ages the rates of deamination of 5-HT by striatal homogenates were similar to those of tyramine.

Discussion When measuring *in vitro* activities of enzymes in tissue homogenates the question arises whether such measurements can be used as an indication for the events occurring *in vivo*. From the present findings it appears that the increase in the MAO activity towards dopamine during the first 4 weeks of life parallels the development of the *in vivo* deamination of dopamine in the striatum of the rat. The validity of this interpretation depends on the premise that the disposition of the phenolic acids does not change with age.

The development of the striatal catabolism of dopamine closely follows the anatomical development of the dopaminergic neurones. In a detailed fluorescence-histochemical study Loizou (1972) showed that during the first week after birth the presumed dopamine containing varicosities are sparse. The rate of development thereafter was strain dependent. However, after the third week the varicosities became individually non-distinct as in the adult rat, indicating the proliferation of axon terminals. Thus the *in vivo* and *in vitro* deamination of dopamine seems closely related to the development of the dopamine containing neurones. However, this may be a purely fortuitous occurrence as the development of dopamine deamination *in vitro*

follows a similar time course with age in other brain regions (e.g. the cerebellum) in which no extensive dopamine containing systems have so far been described. This is also true for the adrenal glands and the heart, but not for the liver (Youdim & Holzbauer, 1975).

In confirmation of previous findings (Blatchford *et al.*, 1975; Youdim & Holzbauer, 1975), there was a difference in the increase with age between the rates at which dopamine and tyramine were deaminated by homogenates of rat brain striata.

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