Short communications

Monoamine oxidase and aldehyde dehydrogenase activity in the striatum of rats after 6-hydroxydopamine lesion of the nigrostriatal pathway

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Monoamine oxidase and aldehyde dehydrogenase activity were significantly reduced in the striatum of rats 8 days after lesion of the nigrostriatal nerve pathway caused by 6-hydroxydopamine. Evidence is presented for the existence of an intraneuronal 'dopamine monoamine oxidase' and intraneuronal aldehyde dehydrogenase. Dopamine concentrations were reduced to 10% of their control values.

The solubilization of mitochondrial monoamine oxidase (MAO) and its subsequent electrophoretic separation into distinct multiple forms has been observed in a number of tissues (Youdim, 1972; Sandler & Youdim, 1972). Preliminary investigations suggested that these multiple forms differ in their physiochemical properties. For example, they differ in their substrate specificity and sensitivity to different inhibitors of the classical mitochondrial monoamine oxidase originally described by Youdim & Sandler (1967). Thus, after electrophoresis, the cathodally migrating band of monoamine oxidase from human and rat brain (Youdim, 1972; Sandler & Youdim, 1972) and beef adrenal gland MAO (Tipton, Youdim & Spires, 1972) has a substance specificity pattern which differs greatly from those of the other forms of the enzyme. The 'cathodic' enzyme has a much greater affinity for dopamine as substrate when compared with the other forms (Youdim, 1972). It is also resistant to inhibition by certain inhibitors of MAO activity (Sandler & Youdim, 1972; Tipton *et al.*, 1972). It has been tentatively called a 'dopamine monoamine oxidase' (Youdim, 1972).

Biochemical (Ungerstedt, 1968; Uretsky & Iversen, 1969) and microscopical studies (Malmfors & Sachs, 1968; Thoenen & Tranzer, 1968) have provided strong evidence that 6-hydroxydopamine, when injected directly into the brain, is able to cause degeneration of central noradrenergic and dopaminergic neurones while 5-hydroxytryptaminergic neurones are little affected (Ungerstedt, 1968). Since the activity of 'dopamine monoamine oxidase' in the striatum is almost seventy times greater than that found in the cerebral cortex (Collins, Sandler, Williams & Youdim, 1970) this form of the enzyme may be localized within the dopamine containing neurones. It was decided to inject 6-hydroxydopamine directly into the substantia nigra to bring about a degeneration of dopaminergic neurones running to the striatum. We have investigated the action of such degeneration on the activity of striatal MAO and aldehyde dehydrogenase (AldDH).

Methods.—Male Charles Rivers rats (250–300 g body weight) were given an intracerebral injection of 6-hydroxydopamine hydrochloride (Hässle, Sweden) into the right substantia nigra. The drug (2 μg base/μl) was dissolved in Merles solution of the following composition: NaCl, 8·98 g; KCl, 0·25 g; $CaCl_2$, 0·14 g; $MgCl_2$, 0·11 g; NaH_2PO_4, 0·07 g; urea, 0·13 g; glucose, 0·16 g; distilled water to 1 litre. Ascorbic acid (0·5 μg/μl) was added to prevent the autooxidation of 6-hydroxydopamine. The drug solution was injected stereotaxically (4 μl or 10 μl at the rate of 1 μl/min) into the rostral part of the dopamine containing cell group of the zona compacta. Control animals received the same amount of the Merles-ascorbic acid solution containing no 6-hydroxydopamine. Eight to twelve days after the injection of the drug the animals were decapitated and the striatum from each half of the brain was removed and chilled rapidly. Each striatum was then homogenized in 2·0 ml of water. One ml was removed to measure MAO and AldDH activity. MAO was assayed with ^{14}C-dopamine (Robinson, Lovenberg, Keiser & Sjoerdsma, 1968) and kynuramine (Kraml, 1965) as substrates. Activity of AldDH was determined by the method of Deitrich (1966) with 3-indoleacetalde-

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hyde bisulphate as the substrate. All results are expressed per mg protein (Lowry, Rosebrough, Farr & Randall, 1951). To the rest of the homogenate 4·6 ml of ethanol (95%) was added to precipitate the proteins and dopamine was determined by the method of Javoy, Hamon & Glowinski, 1970).

Results.—The dopamine concentration in the right striatum fell to about 10% of that of the contralateral side (Table 1) providing evidence for the degeneration of the nigrostriatal dopamine pathway ipsilateral to the lesion. A small (15–25%) but significant decrease in the total MAO activity was observed in the groups of rats pretreated with 8 μg of 6-hydroxydopamine when ^{14}C-dopamine was used as substrate. With 20 μg 6-hydroxydopamine injection, MAO activity was reduced even more, the reduction being 30–35%. This larger dose of 6-hydroxydopamine caused an almost complete destruction of the dopaminergic neurone pathway as seen by the near total disappearance of dopamine in the striatum ipsilateral to the lesion (Table 1). The MAO activity in the striatum of control animals and of the contralateral side in treated animals were almost identical. However, when kynuramine was used as the substrate no differences could be observed after 6-hydroxydopamine treatment. With indoleacetaldehyde as substrate AldDH activity was greatly reduced. In some experiments the reduction was up to 67%.

Discussion.—The reduction in MAO activity observed in these studies, using ^{14}C-dopamine as substrate is in contrast to that reported by others (Goldstein, Anagnoste, Battista, Owen & Nakatani, 1969; Uretsky & Iversen, 1969). These investigators used either tryptamine or tyramine as substrate to measure MAO activity. It was concluded that the major part of MAO activity in the striatum was not associated with dopaminergic neurones. For example glial cells appear to contain highly active MAO (unpublished results). Therefore the reduction in intraneuronal MAO activity may, to a large extent, be masked. The 15–25% reduction in MAO activity with ^{14}C-dopamine as substrate could represent the loss of intraneuronal MAO associated with the dopaminergic nerve terminals.

The body of evidence for the existence of multiple molecular forms of MAO associated with the mitochondria, as well as the presence of different forms of the enzyme in a number of tissues within the same species has been accumulating (Sandler & Youdim, 1972). One important feature of these reports has been the difference observed in the substrate specificities of these forms of MAO including that of intra- and extraneuronal enzymes (Almgren, Anden, Jonason, Norberg &

TABLE 1. *The effect of 6-hydroxydopamine (6-OHDA) induced degeneration of the right nigrostriatal dopaminergic system on dopamine (DA) concentration and on monoamine oxidase (MAO) and aldehyde dehydrogenase (AldDH) activities*

μg of 6-OHDA injected in the right substantia nigra		Left side	Right side	% Fall	No. of observations
Control	DA	9·6±0·1*	9·4±0·1		(8)
	MAO	48·4±3·1†	50·1±2·6	(+4)	(8)
	AldDH	54·0±2·5‡	52·5±3·4	(−3)	(8)
8	DA	9·8±0·2	1·3±0·5 $P<0·001$	(−87)	(8)
	MAO	40·3±2·8	29·4±4·4 $P<0·05$	(−25)	(8)
	AldDH	59·8±4·1	30·0±3·6	(−50)	(8)
20	DA	10·3±0·4	0·4±0·1 $P<0·001$	(−95)	(8)
	MAO	40·1±2·7	28·8±4·7 $P<0·01$	(−35)	(8)
	AldDH	53·4±3·5	17·7±4·3 $P<0·001$	(−67)	(8)

* μg/g of fresh tissue. This is the value obtained in one experiment (8 animals). The fall of dopamine levels in the other experiments were always 85–90% in the right lesioned side as compared with contralateral side. † (nmol deaminated product formed/20 min)/mg protein. ‡ (nmol indoleacetic acid formed/h)/mg protein.

Olson, 1966; Jarrott, 1971; Jarrott & Iversen, 1971. The present study indicates indirectly that the dopaminergic neurones contain a significant amount of MAO and AldDH, and destruction of these neurones by chemical lesion, reduces their activity. Denervation (Jarrott, 1971) has already been shown to achieve such results with peripheral adrenergic nerves. Differences in the fall of MAO activity with a number of substrates have been reported after the sympathetic denervation of salivary gland (Almgren *et al.*, 1966) and vas deferens (Jarrott & Iversen, 1971). The present results suggest that in the rat striatum there may also be similar differences in the properties of intra- and extraneuronal MAO.

The evidence for the presence of an MAO which had tentatively been called 'dopamine monoamine oxidase' (Youdim, 1972) has been strengthened by the recent observation of Kroon & Veldestra (1972). They reported that rat brain mitochondria can be separated into different forms by sucrose density gradient. One interesting feature of their studies was to show that the different populations of mitochondria have different MAO activities with at least four substrates, one of which was dopamine. These authors have presented evidence for the existence of 'dopamine monoamine oxidase' associated with the synaptosomes.

The role of AldDH in the neurone is not fully understood. It is known that the enzyme is distributed between the soluble and particulate fraction of brain homogenate (Deitrich, 1966). During preparation of this communication Duncan, Sourkes, Boucher, Poirier & Roberge 1972) reported the reduction of AldDH activity in the striatum of cat after electrolytic lesion of nigrostriatal tract. In our studies we show a similar result in the rat after a chemical lesion. It should be noted that the fall in AldDH activity observed does not necessarily prove the presence of the enzyme in the neurone. Although Duncan *et al.* (1972) suggest that most of striatal AldDH is localized in the presynaptic innervation of the striatum, it may not be restricted to dopamine containing nerve terminals. Additional evidence is required to elucidate further the distribution and physiological role of AldDH in the C.N.S.

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