Dopamine-β-hydroxylase activity and noradrenaline content in pial arteries of cat and goat

JESÚS MARÍN*, MERCEDES SALAICES, ANTONIO G. GARCÍA *Departamento de Farmacologia y Terapéutica, Facultad de Medicina, Universidad Autónoma, C/Arzobispo Morcillo,* s/n *Madrid-34-Spain*

The main origin of the sympathetic innervation of cerebral vessels arises from the superior cervical ganglia, since gangliectomy produces: (i) a decrease of perivascular nerve fibres (Nielsen & Owman 1967; Falck et al 1968); (ii) an increase of cerebral blood flow (Alborch et al 1977); (iii) a decrease in the vasoconstriction induced by tyramine (Lluch et al 1975; Alborch et al 1977), and (iv) a reduction in the content of pial artery noradrenaline (NA) (Edvinsson et al 1972; Conde et al 1978).

Little is known about the activity of the dopamine-β-hydroxylase (DBH), the enzyme responsible for conversion of dopamine to NA (Kaufman & Friedman 1965), in brain arteries. Only recently has the presence of this enzyme been reported in rat brain microvessels (Spector et al 1977) and in dog cerebral arteries (Shibata 1977).

We have assayed DBH activity and NA content in the pial arteries of the cat and goat and investigated their variations after denervation procedures.

Goats (25–40 kg) were killed by 30 ml of KCl saturated solution given i.v. and cats (1.5–4 kg) by bleeding under anaesthesia (35 mg kg^{-1} of sodium pentobarbitone i.p.). The brains were removed and the pial arteries of the circle of Willis with their branches were dissected in a Petri dish containing Krebs-Henseleit solution at 4 °C previously oxygenated with 5% CO_2 in oxygen mixture, after which the arteries were washed free of blood.

Three goats, were anaesthetized with 2% sodium thiopentone i.v. and both superior cervical ganglia were removed under sterile conditions and also those of six cats previously injected with 0·5 mg kg^{-1} of atropine (i.p.) and anaesthetized with 35 mg kg^{-1} of sodium pentobarbitone (i.p.). Another six cats were treated with 6-hydroxydopamine (6-OHDA, 5 mg intracisternally) dissolved in 1 ml of 0·9% NaCl (saline) containing 0·01 % (w/v) ascorbic acid. Experiments were performed 15 days after surgery or 6-OHDA treatment.

To assay DBH activity, arteries from each animal were blotted, weighed and homogenized in 100 volumes of 5 mM-Tris buffer, pH 6·8, containing 0·2% Triton X-100 and 0·25% of bovine serum albumin solution. The homogenates were centrifuged at 12 000 *g* for 15 min and DBH activity in the supernatants was assayed on the same day according to Molinoff et al (1971). The results were expressed as n mol octopamine formed h^{-1} g^{-1} of tissue.

NA was assayed in the supernatant of arteries homogenized in 1 ml of 0·1 N HCl and centrifuged at 12 000 *g* for 15 min, according to Henry et al (1975). The results were expressed as μg g^{-1} of wet tissue.

The result are means ± s.e. of the means. The statistical significance was evaluated by means of Student's *t*-test; a probability value of less than 5% was considered significant.

The arteries of both species had a high DBH activity. That in goat vessels (189 ± 30 nmol h^{-1} g^{-1} n = 4) was twice that in the cat (96 ± 15 nmol h^{-1} g^{-1} n = 6). Gangliectomy caused a marked decline in DBH activity in arteries from both species (goat 48 ± 12 and cat 20 ± 10 nmol h^{-1} g^{-1}) ($P < 0·001$; n = 3). A similar reduction in DBH activity was observed in the cats (12 ± 10 nmol h^{-1} g^{-1}) pretreated with 6-OHDA ($P < 0·001$; n = 3).

The arteries also possessed an elevated NA content which was also higher in the goat (3·2 ± 0·8 μg g^{-1}) than in the cat (1·3 ± 0·2 μg g^{-1}) (Table 1). Gangliectomy and 6-OHDA pretreatment produced a large decrease in NA content ($P < 0·001$, Table 1).

The finding of high DBH activity indicates that the brain vessels have the ability to synthesize their own NA. The 2:1 ratio of activity for both DBH and NA in goats compared with cats indicates that the adrenergic innervation of the pial arteries of the goat is more dense than in the cat. The effect of gangliectomy and of 6-OHDA treatment which specifically destroys the adrenergic nerve endings, showed that the adrenergic innervation of pial arteries essentially originates from the superior cervical ganglia. These findings agree with those reported by others using different methods (Nielsen & Owman 1967; Falck et al 1968; Iwayama 1970; Lluch et al 1975; Alborch et al 1977).

Table 1. Noradrenaline (NA) content in pial arteries of the cat and goat.

	NA content (μg g^{-1})	
	Cat	Goat
Control	1·3 ± 0·2 (5)	3·2 ± 0·8 (6)
Gangliectomy	0·2 ± 0·1* (4)	0·5 ± 0·2* (4)
6-OHDA	0 (3)	—

Number of experiments are shown in parentheses. Values are means ± s.e. of mean. * $P < 0·001$.

* Correspondence.

The authors acknowledge the technical assistance of M. Carmen Molinos. This work was supported by Comisión Asesora de Investigación Científica y Técnica de la Presidencia del Gobierno and Instituto Nacional de Previsión.

June 11, 1979

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Exposure of mitochondrial outer membranes to neuraminidase selectively destroys monoamine oxidase A activity

MILES D. HOUSLAY*, ROBERT J. MARCHMONT, *Department of Biochemistry, University of Manchester Institute of Science and Technology, Sackville Street, Manchester M60 1QD U.K.*

The enzyme monoamine oxidase is involved in the catabolism of biogenic amines, and is the site of action of a number of antidepressant drugs (see Blaschko 1972; Knoll 1976).

The monoamine oxidase activity of rat liver mitochondrial outer membranes can be divided into two categories, MAO-A and MAO-B based upon their substrate specificity and sensitivity to irreversible and reversible inhibition (Houslay & Tipton 1974). The binding sites for the selective inhibitors, clorgyline and pargyline, appear to have a different localization in the membrane (Houslay 1977) and it has been suggested that the membrane environment may give rise to some of the features of enzyme multiplicity (Houslay & Tipton 1973a).

Recently Russell et al (1979a, b) have demonstrated that the active site of MAO-A faces the mitochondrial inner membrane and the active site of MAO-B faces the cytosol. The possibility exists that membrane components that are asymmetrically disposed could modulate the activity of MAO-A and MAO-B.

It is envisaged for biosynthetic purposes that the external surface of the mitochondrial outer membrane should correspond to the external surface of the endoplasmic reticulum (see Rothman & Lenard 1977). This may imply that carbohydrate residues attached to proteins exposed at the lumenal side of the endoplasmic reticulum should be found associated with the inner face of the mitochondrial outer membrane. We thus decided to investigate the effect of neuraminidase treatment on isolated mitochondrial outer membranes. This enzyme is known to remove substantial amounts of sialic acid from mitochondrial outer membranes (see Hughes 1976).

Mitochondrial outer membranes from male, Sprague Dawley rats were prepared as described before (Houslay & Tipton 1973b), except that K phosphate buffers were used instead of Tris buffers which have a selective inhibitory effect on the two enzymes (Fowler et al 1977). Also the final mitochondrial outer membrane fraction was obtained by diluting the fraction obtained from the sucrose gradient, tenfold with 10 mM K phosphate pH 7·2 and centrifuging it for 1 h at 100 000*g*. The pellet was resuspended at 3–4 mg ml^{-1} protein in the same buffer.

These membranes were treated with purified neuraminidase preparations from *Clostridium perfringens* obtained from either Boerhinger Corp. (U.K.), East Sussex or type VIII from Sigma (U.K.) Ltd., Kingston-Upon-Thames with similar results. Final concentrations of 1·3 mg ml^{-1} mitochondrial outer membranes, 1·1 mg ml^{-1} neuraminidase in 67 mM K phosphate buffer pH 6·0 were incubated for periods of up to 30 min at 30 °C. Any residual phospholipase activity of these enzyme preparations would be effectively inhibited by the absence of high [Ca^{2+}] and the presence of high phosphate concentrations in our incubations. At appropriate time intervals aliquots were taken for assay of monoamine oxidase activity. [1-^{14}C]β-phenethylamine (from NEN, Germany) and [^{3}H]-5-hydroxytryptamine (Amersham) were used as substrates, assayed as described by Callingham & Laverty (1973). Incubation of mitochondrial outer membranes for a

* Correspondence.