

Effect of mao-A and mao-B inhibitors in ganglia and nerve endings

*Efecto de los inhibidores de mao-A y B en el ganglio
y en los terminales nerviosos*

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RESUMEN

Se estudio el efecto de clorgilina y deprenil sobre la liberación y el metabolismo de ^3H -noradrenalina (^3H -NA) en el ganglio superior (GCS) y en la membrana nictitante (MN) de gato. Nuestros resultados muestran que en el GCS, clorgilina y deprenil redujeron del mismo modo la formación de 3,4-dihidroxi-fenilglicol (DOPEG) mientras que en la MN la disminución del DOPEG fue mayor con clorgilina que con deprenil. En el GCS la monoaminooxidasa (MAO) A y B cataliza la deaminación de NA a ^3H -DOPEG, a ^3H -3,4-acido-dihidroxi-mandílico (^3H -DOMA) o a los metabolitos ^3H -O-metilados deaminados (^3H -OMDA). En la MN la MAO-A presináptica cataliza la deaminación de NA principalmente a ^3H -DOPEG, la MAO-B postsináptica a ^3H -NMN y; la MAO-A y B a ^3H -DOMA y ^3H -OMDA.
PALABRAS CLAVES: clorgilina, deprenil, MAO-A y MAO-B

ABSTRACT

The effect of clorgyline and deprenyl on the release and metabolism of ^3H -noradrenaline (^3H -NA) was studied in the superior cervical ganglion (SCG) and nictitating membrane (NM) of the cat. Our results show that in SCG, clorgyline and deprenyl reduced to the same degree the formation of 3,4-dihydroxy-phenylglycol (DOPEG) whereas in NM the decrease of DOPEG was more pronounced in presence of clorgyline than of deprenyl. In SCG the deamination of NA to either ^3H -DOPEG, ^3H -3,4-dihydroxymandelic acid (^3H -DOMA) or ^3H -O-methylated deaminated metabolites (^3H -OMDA) is catalized by monoamineoxydase (MAO) A and B. In NM the deamination of NA to ^3H -DOPEG is mainly catalized by MAO-A presynaptic, ^3H -NMN by MAO-B postsynaptic, and ^3H -DOMA and ^3H -OMDA by MAO-A and B.

KEY WORDS: clorgyline, deprenyl, MAO-A and MAO-B

INTRODUCTION

The oxydative deamination of neurotransmitter amines and other monoamines is shown to be accomplished by at least two functionally different forms of monoamine oxidase (MAO), type A and type B (Jaim 1977; Tipton et.al 1982). These two forms of MAO can be distinguished on the basis of their differing relative sensitivities to the irreversible MAO inhibitors clorgyline and deprenyl (Johnston 1968; Knoll and Magyar 1972).

Studies in canine saphenous vein demonstrated that MAO-A is involved in the metabolism

of ^3H -noradrenaline (^3H -NA) released from adrenergic nerve endings (Caramona and Osswald 1985; Verbeuren and Vanhoutte 1982). In a previous study carried out in our laboratory, it was shown that both MAO-A and MAO-B were present in the SCG and in the NM of the cat, although some differences between both structures were found (Filinger and Stefano 1981b).

However little information is available, concerning the forms of MAO that are responsible of the deamination of ^3H -NA in cat's superior

cervical ganglion (SCG) and nictitating membrane (NM).

This study was designed to compare the effect of specific inhibitors of MAO-A (clorgyline) and

MAO-B (deprenyl) on the release and the metabolism of ^3H -noradrenaline (^3H -NA) in the SCG and in the NM of the cat, as models of cell body and nerve endings respectively.

MATERIALS AND METHODS

1. Isolated Superior Cervical Ganglion and Nictitating Membrane of the Cat

Adult cats of 2.0-4.0 kg body weight and of either sex, were anaesthetized with sodium pentobarbital (35 mg/kg i.p.). The ganglia were dissected free of postganglionic sympathetic fibres and decapsulated under a binocular dissecting microscope. The medial muscle of the nictitating membrane was also dissected for further use. The tissues were placed in a Petri dish with modified Krebs' solution previously bubbled with 95% O_2 and 5% CO_2 . The composition of the Krebs' solution was as follows (millimolar concentrations): NaCl, 118.0; KCl, 4.7; CaCl_2 , 2.6; MgCl_2 , 1.2; NaH_2PO_4 , 1.0; NaHCO_3 , 25.0; glucose, 11.1; ethylenediamine tetraacetic acid (EDTA), 0.004 and ascorbic acid, 0.11.

The tissues were incubated at 37°C for 30 min with 5 $\mu\text{Ci/ml}$ (0.9 μM) of (-)-([7- ^3H]) noradrenaline ([^3H]NA) (New England Nuclear, Boston, MA, USA sp. act. 5.71 Ci/mol). At the end of the incubation period the tissues were submitted to 5 consecutive 1 min washes and then to consecutive 5 min washes with Krebs' solution.

Clorgyline 10^{-7}M and Deprenil 10^{-6}M were added to the medium during 30 min after six 5 min washes subsequent to the labelling of the tissue with (^3H)-NA. Aliquots of 0.5 ml of the bathing solution were used to determine total radioactivity.

At the end of the experiments, the tissues were blotted dry, weighed and homogenized in 5 ml of cold 0.4 N perchloric acid containing 1 mg/ml of EDTA and 1.25 mg/ml of Na_2SO_3 . Aliquots of tissue extracts were analyzed for labelled compounds.

2. Chemical Methods

The analysis of ^3H -NA and its metabolites, carried out 30 min after the incubation with clorgyline 10^{-7}M and deprenil 10^{-6}M , was performed according to the method described by Graefe et al (1973). Five fractions were isolated NA, 3,4-dihydroxyphenylglycol (DOPEG), 3,4-dihydroxymandelic acid (DOMA), normetanephrine (NMN) and the O-methylated deaminated fraction (OMDA) which represents 4-hydroxy-3-methoxy-phenylglycol (MHPG) plus 4-hydroxy-3-methoxymandelic acid (VMA). Each fraction was corrected for recoveries and cross-contamination. Statistical calculations were performed according to conventional procedures (Snedecor and Cochran 1967).

Clorgyline N-methyl-N-propargyl-3 (2,4-dichlorophenoxy)-propylamine hydrochloride, M&B 9302 was obtained from May and Baker Ltd, Dagenham, U.K. Deprenyl (phenylisopropylmethylpropionylamine hydrochloride) was kindly provided by Professor J. Knoll, Semmelweis, University of Medicine, Budapest, Hungary.

RESULTS

1. Effect of Clorgyline and Deprenyl on Total Spontaneous ^3H -efflux in Superior Cervical Ganglion (SCG) and Nictitating Membrane (NM) of the Cat

To determine if the MAO inhibitors, clorgyline and deprenyl, affected the spontaneous efflux of total radioactivity in the SCG and NM of the cat, the total activity in the samples collected

prior to the exposure to clorgyline 10^{-7}M or deprenyl 10^{-6}M (basal efflux 1) was compared to the total activity in samples collected 15 min after exposure of MAO inhibitors (basal efflux 2).

As shown in Table 1 and 2, clorgyline 10^{-7}M and deprenyl 10^{-6}M slightly decreased the basal efflux of total radioactivity in cat ganglia as well as in NM.

TABLE I. Effect of Clorgyline and Deprenyl on Total Spontaneous ³H-efflux in SCG of the cat

| | n | Basal efflux 1 nCi/100 mg | Basal efflux 2 nCi/100 mg | Basal efflux as % of basal efflux 1 |
|----------------------------------|---|------------------------------|------------------------------|--|
| Control | 5 | 28.9 ± 3.8 | 22.9 ± 3.0 | 79.2 ± 2.7 |
| Clorgyline 10 ⁻⁷ M | 5 | 26.0 ± 3.5 | 18.0 ± 2.2 | 69.2 ± 2.4 * |
| Deprenyl 10 ⁻⁶ M | 5 | 25.5 ± 4.7 | 17.0 ± 2.8 | 66.7 ± 1.9 * |

Notes: 'Spontaneous efflux' or 'basal efflux' is the efflux of tritiated compounds observed in the absence of any stimulus. Basal efflux 1: control values. Basal efflux 2: Values 15 min after exposure to clorgyline or deprenyl. *p<0.05; when compared against the corresponding controls. Shown are mean values ± SEM and expressed as nCi/100 mg tissue. n: number of experiments.

TABLE II. Effect of Clorgyline and Deprenyl on Total Spontaneous ³H-efflux in NM of the cat.

| | n | Basal efflux 1 nCi/100 mg | Basal efflux 2 nCi/100 mg | Basal efflux as % of basal efflux 1 |
|----------------------------------|---|------------------------------|------------------------------|--|
| Control | 4 | 41.3 ± 3.9 | 37.1 ± 2.9 | 89.8 ± 1.8 |
| Clorgyline 10 ⁻⁷ M | 3 | 37.5 ± 2.7 | 29.4 ± 2.2 | 78.4 ± 2.7 * |
| Deprenyl 10 ⁻⁶ M | 3 | 34.0 ± 2.8 | 28.6 ± 1.7 | 84.1 ± 1.3 * |

Notes: 'Spontaneous efflux' or 'basal efflux' is the efflux of tritiated compounds observed in the absence of any stimulus. Basal efflux 1: control values. Basal efflux 2: Values 15 min after exposure to clorgyline or deprenyl. *p<0.05; when compared against the corresponding controls. Shown are mean values ± SEM and expressed as nCi/100 mg tissue. n: number of experiments.

2. Metabolism of ³H-NA Released by Clorgyline and Deprenyl in Superior Cervical Ganglion (SCG) of the Cat

In the SCG unmetabolized ³H-NA corres-

ponded to 24.1 ± 3.9 % of the total tritium efflux (Fig 1). The ³H-DOPEG represented 33.4 ± 2.6 % of the total, while the ³H-OMDA accounted for 25.9 ± 3.1 % of the total radioactivity in spontaneous outflow.

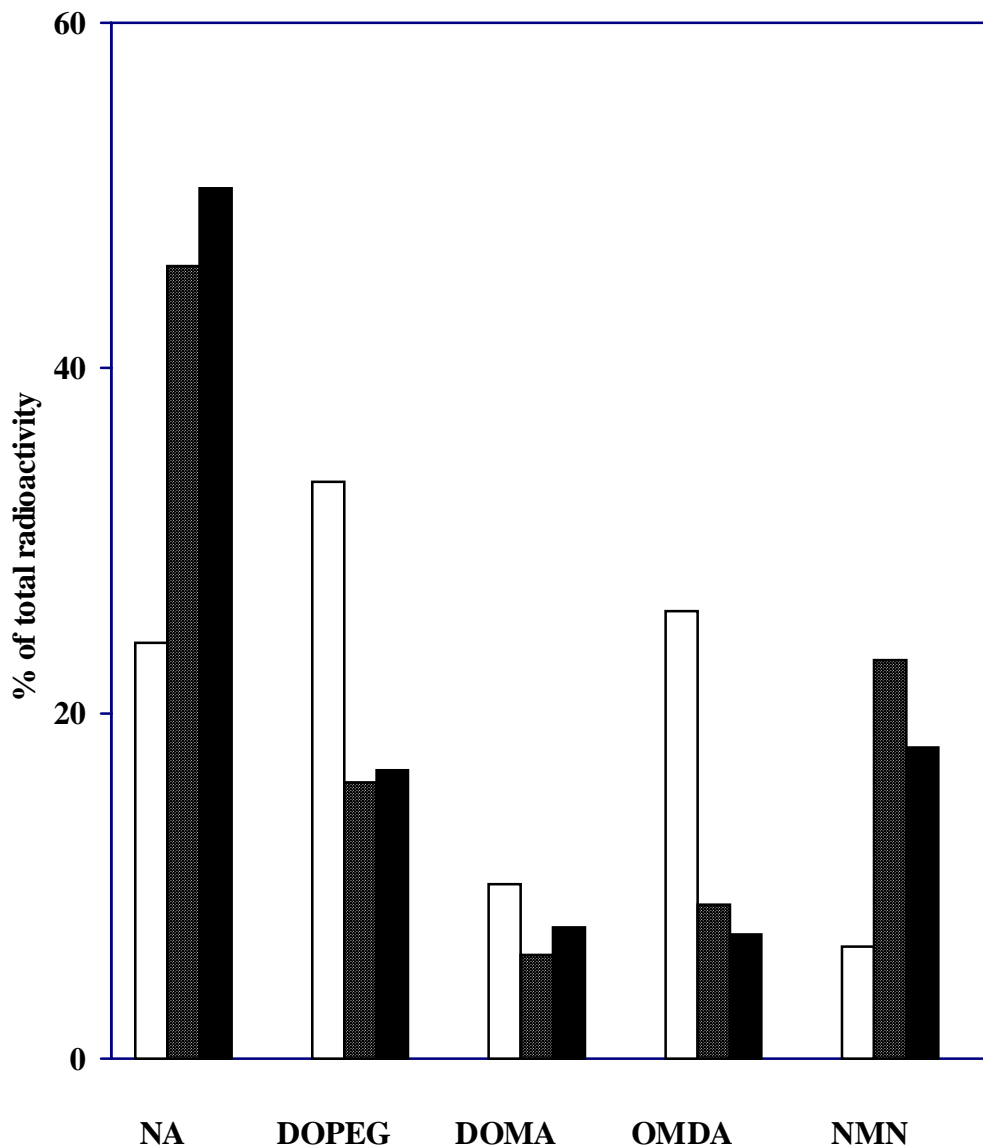


Fig 1. Metabolism of ^3H -NA released by clorgyline 10^{-7} M and deprenyl 10^{-6} M from cat SCG. Ordinate: percentage of total radioactivity. Spontaneous efflux of radioactivity in samples collected 90 min after the incubation with ^3H -NA. □ control ($n=5$); ▨ clorgyline 10^{-7} M ($n=5$); ■ deprenyl 10^{-6} M ($n=5$). NA, ^3H -noradrenaline; DOPEG ^3H -3,4-dihydroxyphenylglycol; OMDA, ^3H -O-methylated deaminated metabolites; DOMA, ^3H -3,4-dihydroxymandelic acid; NMN, ^3H -normetanephrine. Shown are mean values \pm SEM. * $p < 0.001$; ** $p < 0.005$; *** $p < 0.010$; **** $p < 0.025$ (when compared with the control)

The metabolism of ^3H -NA released by clorgyline 10^{-7} M and deprenyl 10^{-6} M in the SCG was determined (Fig 1). In this tissue, significant decreases in the proportions of ^3H -DOPEG, ^3H -OMDA and ^3H -DOMA were observed in the presence of clorgyline 10^{-7} M and deprenyl 10^{-6} M. As can be seen in Fig 1, both MAO inhibitors increase the overflow of ^3H -NMN.

3. Metabolism of ^3H -NA Released by Clorgyline and Deprenyl in Nictitating Membrane (NM) of the Cat

The metabolic fate of ^3H -NA released spontaneously from the NM differed somewhat from that observed in SCG (Fig 2)

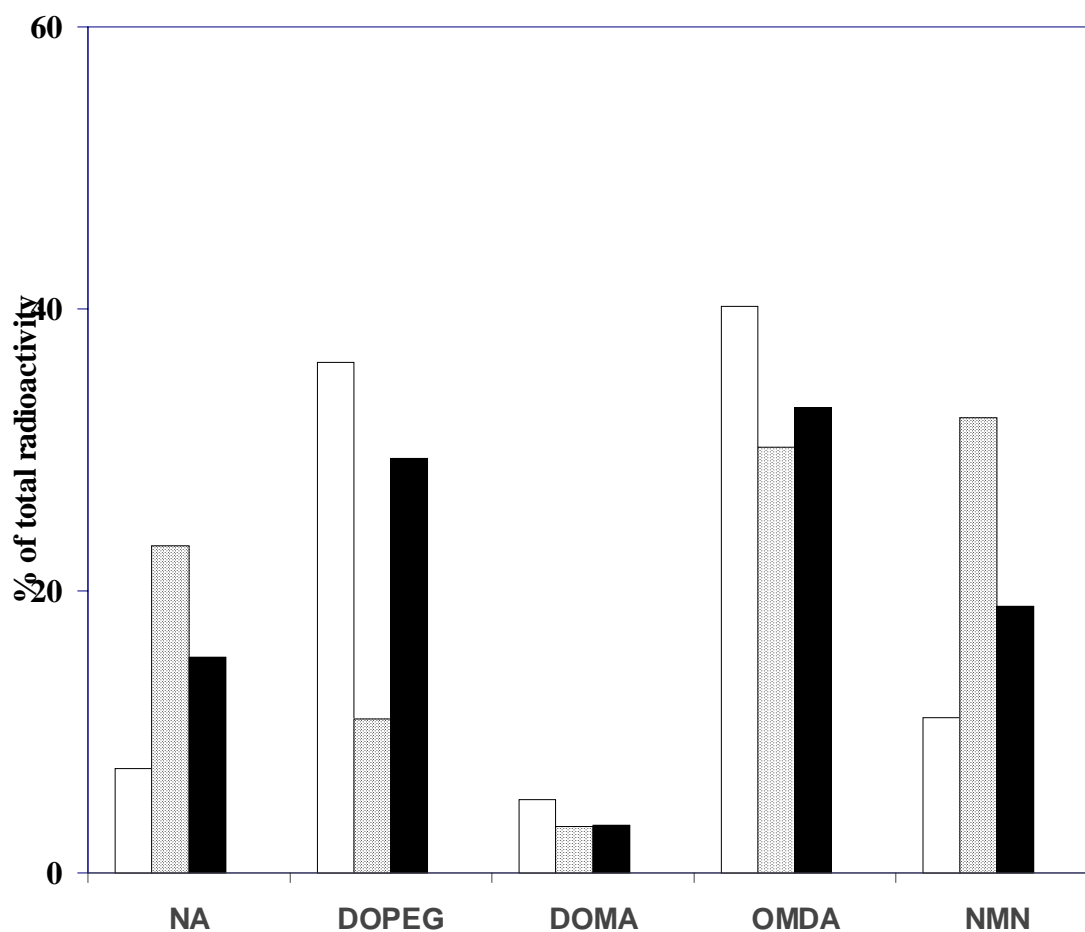


Fig 2. Metabolism of ^3H -NA released by clorgyline 10^{-7} M and deprenyl 10^{-6} M from cat NM. Ordinate: percentage of total radioactivity. Spontaneous efflux of radioactivity in samples collected 90 min after the incubation with ^3H -NA. □ control ($n=4$); ▨ clorgyline 10^{-7} M ($n=3$); ■ deprenyl 10^{-6} M ($n=3$). NA, ^3H -noradrenaline; DOPEG ^3H -3,4-dihydroxyphenylglycol; OMDA, ^3H -O-methylated deaminated metabolites; DOMA, ^3H -3,4-dihydroxymandelic acid; NMN, ^3H -normetanephrine. Shown are mean values \pm SEM. * $p < 0.001$; ** $p < 0.005$; *** $p < 0.010$; **** $p < 0.025$ (when compared with the control)

In the NM the $^3\text{H-NA}$ represented 7.4 ± 0.6 % of the total radioactivity in spontaneous outflow. The $^3\text{H-OMDA}$ and $^3\text{H-DOPEG}$ accounted for 40.2 ± 1.0 % and 36.2 ± 2.3 % of the total tritium efflux respectively.

When clorgyline 10^{-7} M or deprenyl 10^{-6} M was added to the medium, the percentage contribution of NA and its metabolites to the

amount of ^3H -released varied markedly (Fig 2). The percentage of $^3\text{H-DOPEG}$ and $^3\text{H-OMDA}$ decrease significant in presence of both MAO inhibitors. It is, to note, that the diminution in the total radioactivity of the $^3\text{H-DOPEG}$ was more pronounced in presence of clorgyline 10^{-7} M than of deprenyl 10^{-6} M.

DISCUSSION

The present study shows that clorgyline and deprenyl modify the metabolism of $^3\text{H-NA}$ in the SCG as well as in the NM of the cat. It was also observed that the MAO inhibitors slightly decrease the spontaneous efflux of total radioactivity in both tissues.

The choice of clorgyline and deprenyl as specific inhibitors of MAO-A and MAO-B respectively is based on generally accepted evidence (Fowler and Tipton 1983; Johnston 1968). However their specificity is only relative because high concentrations of MAO-inhibitors may inhibit the type of MAO for which they are not specific (Knoll and Magyar 1972).

Filinger and Stefano (1981b) characterized the type A and type B MAO in the cell body and in the nerve terminals and found that the potency of clorgyline, to inhibit the A form of MAO, using tyramine as substrate, was lower in ganglia than that observed in the NM. It has been also demonstrated that in order to evoke a similar overflow of NA from noradrenergic structures in the cat SCG and from nerve terminals in the cat NM, higher concentrations of amphetamine (Filinger and Stefano 1981a), tyramine (Filinger 1994a) and Ro 4-1284 (Filinger 1994b) are required in the isolated ganglia than in the nerve endings.

Our experiments show that in the SCG, clorgyline and deprenyl significantly reduced the ^3H -efflux of DOPEG and DOMA to 50 % of the control values. The diminution of the $^3\text{H-OMDA}$ fraction was more pronounced. A marked increase in the fraction of unmetabolized $^3\text{H-NA}$ and $^3\text{H-NMN}$ was observed in presence of the MAO-inhibitors. Similar effects were seen with the non-specific inhibitor pargyline in canine saphenous vein (Verbeuren and Vanhoutte 1982) and in cat ganglia (Filinger 1986).

In the NM, clorgyline decrease the efflux of DOPEG more than that of the other deaminated

metabolites. Deprenyl reduced the proportion of DOPEG in a lesser extent than clorgyline. With both MAO inhibitors, a compensatory increase in the overflow of $^3\text{H-NA}$ and 3,4-normetanephine ($^3\text{H-NMN}$) was observed.

These results indicate that in the SCG the contribution of the of MAO A and B to the deamination of NA is similar, whereas in the NM a higher contribution of MAO-A is evident.

Biochemical and histochemical results indicate that the use of the irreversible inhibitors, clorgyline and deprenyl, together with several monoamines as substrate reveals that the nature and proportions of the two forms of MAO may vary between animal species as well as in different tissues of the same animals (Fowler and Tipton 1983; Uchida and Koelle 1984).

It is also considered that the specific localization of the two forms of MAO within different body tissues is of biological and clinical significance (Kamijo et.al 1982). Marked differences in the abundance and patterns of distribution of MAOs, particularly MAO-B, were shown in human and rodent peripheral tissues (Saura et.al 1996). MAO B inhibition could also be involved in understanding and treating tobacco smoke addiction (Fowler et.al 1998).

We can conclude that: (1) In the SCG, the deamination of NA to either $^3\text{H-DOPEG}$, $^3\text{H-DOMA}$ or $^3\text{H-OMDA}$ is catalyzed by MAO-A and MAO-B. (2) In the NM, the deamination of NA to $^3\text{H-DOPEG}$ is catalyzed by MAO-A presynaptic. Since the overflow of $^3\text{H-DOMA}$ and $^3\text{H-OMDA}$ are slightly affected by both inhibitors of MAO, its deamination must be catalyzed by MAO-A and MAO-B, and $^3\text{H-NMN}$ is deaminated preferentially by MAO-B.

It is to note that in a previous study sympathetic denervation also reduced MAO-A than MAO-B in the NM, to a greater extent, with

predominance of the former in adrenergic nerve endings and of the latter at the extraneuronal sites (Filinger and Stefano 1981b).

Finally, our results support that under conditions in which transport mechanisms govern

the access to the enzymatic sites, the cell body is endowed with MAO-A and MAO-B activity, whereas the nerve endings are related with MAO-A presynaptic and MAO-B postsynaptic.

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