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Electrophysiological effects of monoamine oxidase inhibition on rat midbrain dopaminergic neurones: an *in vitro* study

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1 The effects of the inhibition of monoamine oxidase (MAO) type A and B have been evaluated on the spontaneous firing activity of the dopaminergic (principal) neurones of the rat midbrain intracellularly recorded from a slice preparation.

2 The non-specific MAO inhibitor, pargyline, superfused at a concentration of 10–100 μM, decreased or abolished the spontaneous firing discharge of the principal neurones in the subtantia nigra pars compacta and ventral tegmental area. This effect had a slow onset and appeared to be sustained.

3 The administration of the dopamine $D_{2/3}$ receptor antagonist, sulpiride (100–300 nM), antagonized the pargyline-induced effect, while the superfusion of the dopamine D_1 receptor antagonist, SCH 23390 (1–3 μM) did not counteract the induced inhibition of the firing rate.

4 The inhibitor for the MAO A, clorgyline (30–100 μM), reduced the firing rate of the dopaminergic neurones. A similar depressant effect was also observed when a MAO B inhibitor, deprenyl (30–100 μM), was applied. Lower concentrations of both drugs (300 nM–10 μM) did not produce consistent effects on neuronal discharge.

5 Our data suggest that only the blockade of both types of MAO enzymes favours the inhibitory action of endogenous dopamine on somato-dendritic $D_{2/3}$ autoreceptors.

Keywords: Ventral tegmental area; substantia nigra; dopamine; spontaneous firing; pargyline; clorgyline; deprenyl; -sulpiride, SCH 23390

Introduction

Monoamine oxidases (MAO) are mitochondrial enzymes which participate in the degradation of dopamine (Yang & Neff, 1974; Green *et al.*, 1977; Weiner & Molinoff, 1989; Juorio *et al.*, 1994). Two forms of MAO (A and B) (Johnston, 1968; Yang & Neff, 1974; Schoepp & Azzaro, 1982) have been described so far with a different distribution in the brain: (a) the MAO A are mainly located intraneuronally in the dopaminergic cells (Roffler-Tarlov *et al.*, 1971; Marsden *et al.*, 1972; Demarest *et al.*, 1980; Commissioning, 1985). (b) the MAO B are either primarily present in the 5-hydroxytryptaminergic terminals or in glial cells (Levitt *et al.*, 1982; Francis *et al.*, 1985; Westlund *et al.*, 1985).

An inhibition of both types of monoamine oxidase can be readily obtained by the non-specific inhibitor pargyline (Butcher *et al.*, 1990). Furthermore, there is evidence that a rather selective inhibition of MAO A can be obtained by a low concentration (nM) of clorgyline while a quite selective MAO B inhibition is caused by (−)-deprenyl treatment (nanomolar range) (Johnston, 1968; Knoll & Magyar, 1972; Harsing & Vizi, 1984). Although it has been demonstrated that the inhibition of MAO with pargyline prevents the formation of 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and increases the efflux of dopamine and 3-methoxytyramine (3-MT) from the striatum of animals pretreated with this drug (Harsing & Vizi, 1984; Imperato & DiChiara, 1984; Kato *et al.*, 1986; Wood *et al.*, 1987; Butcher *et al.*, 1990; Juorio *et al.*, 1994), there are still controversial results about the relative effects of MAO A and B inhibition on dopamine metabolism (Houslay *et al.*, 1976; Green *et al.*, 1977; Schoepp & Azzaro, 1983; Harsing & Vizi, 1984; Juorio *et al.*, 1994).

In addition, in spite of the large amount of evidence showing a pattern of different distribution of MAO and the use of rather selective MAO inhibitors to modify the levels of dopamine and dopamine catabolites in the brain, there are still few results showing the functional effects of MAO inhibition at neuronal level (Engberg *et al.*, 1991).

The aim of the present work was to show the acute effects of MAO inhibitors on the firing rate of the dopaminergic neurones intracellularly recorded *in vitro* in the substantia nigra and ventral tegmental area of the rat mesencephalon.

Methods

Preparation of the tissue

A detailed description of the methods has been published elsewhere (Lacey *et al.*, 1989; Mercuri *et al.*, 1994; 1995). Albino Wistar rats (150–300 g) (Morini, Reggio Emilia) were anaesthetized with halothane (Aldrich) and killed by a blow to the chest. The brain was removed and horizontal slices (thickness 300 μm) were cut by a vibratome starting from the ventral surface of the midbrain. A single slice containing the substantia nigra and the ventral tegmental area was then transferred into a recording chamber and completely submerged in an artificial cerebrospinal fluid containing (mM): NaCl 126, KCl 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.4, glucose 11, $NaHCO_3$ 25, warmed at 35°C and gassed with 95% O_2 and 5% CO_2. The ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) were identified with a dissecting microscope as the regions medial (VTA), rostral, lateral and caudal (SNc) to the medial terminal nucleus of the accessory optic tract.

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Recordings

The recording electrodes (Clark, 1–1.5 mm, thick wall), pulled by Narishige vertical and horizontal pullers, were filled with 2 M KCl or 1 M K^+-acetate and 2 M K^+-citrate and had a tip resistance of 40–120 MΩ. The signals were obtained by an amplifier (Axoclamp-2A, Axon Instruments) and were displayed on a pen recorder (Gould 2400 S) and on a digital oscilloscope (Tektronix) or saved in a tape recorder (Biologic) for off-line analysis. Data were presented as a mean ± s.e.mean.

Application of drugs

The drugs were bath-applied via a three-way tap system. Complete exchange of the bath solution occurred in about 1 min. The following drugs were used: dopamine hydrochloride, pargyline (Sigma), L-deprenyl and clorgyline (RBI), SCH 23390 (R(+)-7 chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride; Shering), (−)-sulpiride (Ravizza).

Results

Electrophysiological and pharmacological characterization of the cells

Intracellular recordings were obtained from 56 'principal' neurones of the substantia nigra pars compacta and ventral tegmental area which fired spontaneously at a rate of 1.5 ± 0.1 Hz ($n=35$). The physiological and pharmacological properties of these cells have been previously described for dopamine containing neurones of the midbrain *in vitro* (Llinas *et al.*, 1984; Kita *et al.*, 1986; Grace & Onn, 1989; Lacey *et al.*, 1989; Yung *et al.*, 1991; Johnson & North, 1992; Hajos & Greenfield, 1993; Mercuri *et al.*, 1995). Because no clear differences were observed between the effects of MAO inhibitors on neurones in the SNc and VTA the data were pooled.

Effects of pargyline

Bath-application of the non-specific MAO inhibitor, pargyline, reduced or blocked the spontaneous firing discharge of the dopaminergic cells. This effect was associated with a hyperpolarization of the membrane. (Figure 1). Although, there was a great variability in the degree of the inhibition, all the neurones exposed to 100 μM pargyline ($n=6$) were clearly inhibited by this MAO inhibitor (Figures 1 and 2). On the other hand, 30, 10 and 3 μM pargyline inhibited only 5 out of 7, 4 out of 8 and 2 out of 6 neurones, respectively. Analysis of the effects of pargyline showed that the inhibition of firing was time-dependent. In fact, it occurred within 8–10 min and reached a maximum in about 40 min (Figure 2). This inhibition was sustained and did not recover even after 2 h of wash out. Thus the effect of pargyline appeared to be permanent.

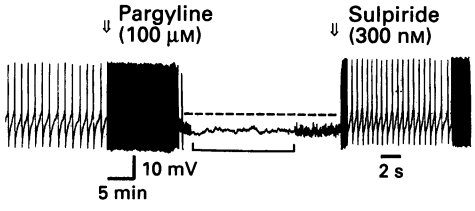

Figure 1 Effects of pargyline on midbrain dopaminergic neurones and antagonism caused by (−)-sulpiride: the superfusion of pargyline, initiated at the time indicated by the arrow, hyperpolarized the cell and blocked the spontaneous firing activity. Note that: (a) the inhibition of the cell occurred for several minutes during application of pargyline; (b) (−)-sulpiride (superfused at the point indicated) antagonized the effects of pargyline. In order to show, individual action potentials the speed of the chart was changed (see time bar below), before, during (full line underneath) the application of pargyline and during the superfusion of (−)-sulpiride. Full amplitude of the action potential was not reproduced due to the limited frequency of the pen recorder. The dashed line indicates −48 mV.

Effects of dopamine antagonists

The administration of the $D_{2/3}$ dopamine antagonist (−)-sulpiride (100–300 nM) blocked the inhibition caused by pargyline ($n=10$) (Figures 1 and 3). When this antagonist was applied to cells that had been pretreated with pargyline ($n=5$), it produced a reversible increase of the spontaneous firing (Figure 4). Occasionally, ($n=4$) when (−)-sulpiride was perfused after pargyline inhibition, it augmented the firing rate above the control value.

On the other hand, the D_1 antagonist, SCH 23390 (1–3 μM), had no effect on the pargyline inhibition ($n=3$) nor when coadministered with (−)-sulpiride did it produce any further increase in the spontaneous firing activity ($n=2$) (not shown).

Effects of clorgyline and L-deprenyl The MAO A inhibitor, clorgyline, was bath-applied at a concentration of 1–100 μM on the 'principal' neurones for a period ranging between 10 and 60 min. At concentrations of 1–10 μM it did not produce clear effects on all the tested cells ($n=7$) while at 30–100 μM it decreased the spontaneous firing activity of 3 out of 7 neurones (61% of control at 30 min with 100 μM) (Figure 5).

The superfusion of the neurones with the MAO B inhibitor, L-deprenyl (300 nM–10 μM) for more than 50 min did not produce any clear change of the firing discharge in 8 out 10 cells. In two cells a slight depression (15% of control) was observed with 10 μM. Only in 4 out 7 neurones 30–100 μM deprenyl caused an inhibition of the spontaneous activity (79 ± 7.5% of control at 40 min with 100 μM). This effect was antagonized by (−)-sulpiride (Figure 6).

Discussion

We have already shown (Mercuri *et al.*, 1989; 1992) that electrophysiological recordings from dopaminergic neurones of the ventral mesencephalon *in vitro* can be used as a valid model to study interesting aspects of dopaminergic neurotransmission. In line with those previous observations, the present results help to understand part of the acute functional events that occur in the central nervous system after MAO inhibition. The inhibition of both types (A and B) of MAO obtained with pargyline reduces the spontaneous firing discharge of the dopaminergic cells by hyperpolarizing the membrane potential. The relative prolonged period of application of the drug which is necessary to observe and to reach a steady-state response supports the contention that an inhibition of dopamine metabolism causes an increased level of dopamine in the extracellular space. The blockade of the long lasting pargyline-induced inhibitory effects by the selective $D_{2/3}$-receptor antagonist, (−)-sulpiride and the lack of additional actions of the D_1 antagonist, SCH 23390 suggest that the pargyline-induced inhibition is mainly the consequence of activation of $D_{2/3}$ dopamine autoreceptors by endogenous non-degraded dopamine. Thus, the indirect activation of these somatodendritic autoreceptors inhibits the principal neurones by hyperpolarizing the membrane via an increase of potassium conductance (Lacey *et al.*, 1987; 1988). Since we had to use high and unspecific concentrations of both clorgyline and L-deprenyl to observe consistent acute effects on firing discharge, it can be argued that a simple inhibition of MAO A or B activity does not produce a significant elevation of extracellular dopamine to activate the dopaminergic autoreceptors. Thus, only a mixed inhibition of type A and type B MAO obtained with pargyline and high concentrations of clorgyline and deprenyl, is able to affect clearly the functioning of the

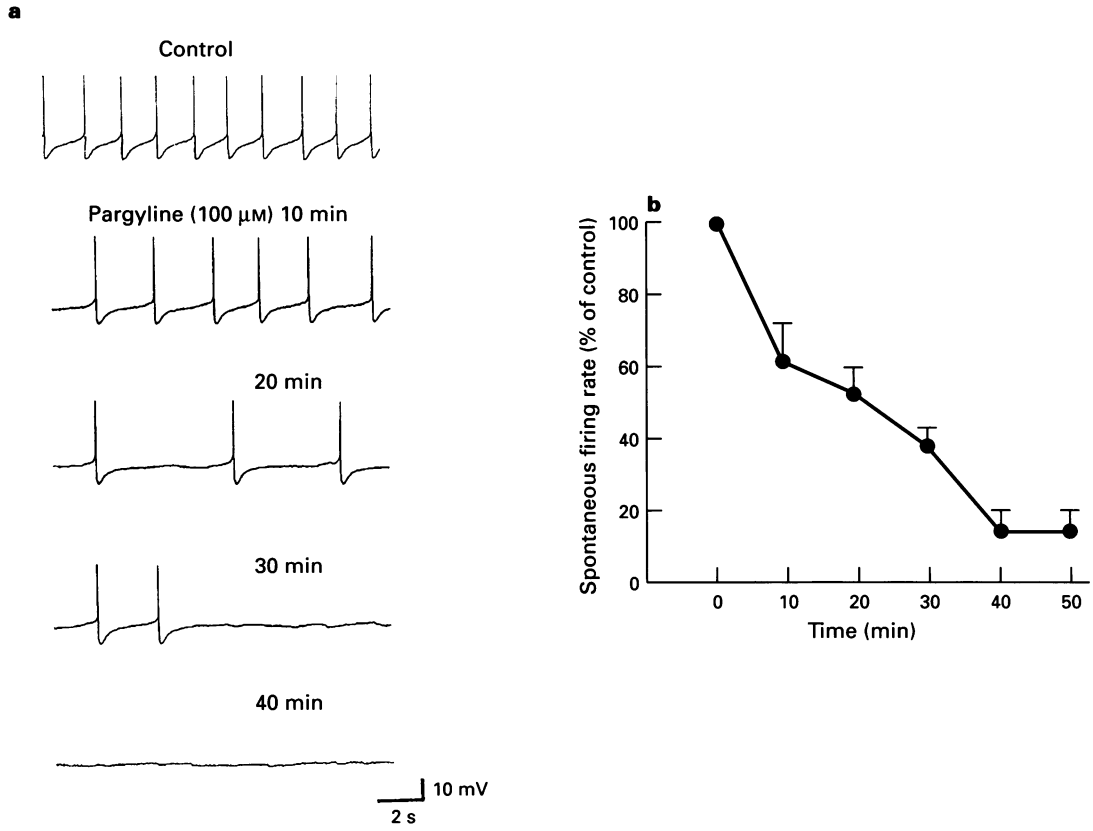

Figure 2 The spontaneous firing activity of the dopaminergic cells is inhibited by pargyline: (a) the traces are voltage recordings. Pargyline (100 μM) caused a progressive inhibition of this principal cell. (b) The graph shows the time course of effect of pargyline (100 μM). The firing is expressed as a percentage of control. Pargyline application started at time 0. Each point represent the mean ± s.e.mean of 5–6 determination (about 1 min) firing at 0, 10, 20, 30, 40, 50 min from 6 cells.

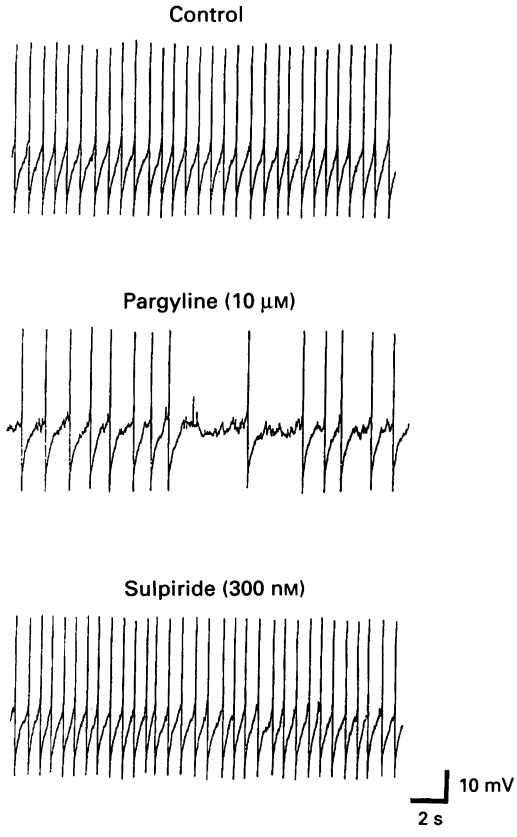

Figure 3 Sulpiride antagonizes effects of pargyline: the inhibition of the spontaneous firing induced by pargyline (10 μM for 30 min) was antagonized by superfusion with (−)-sulpiride (300 nM).

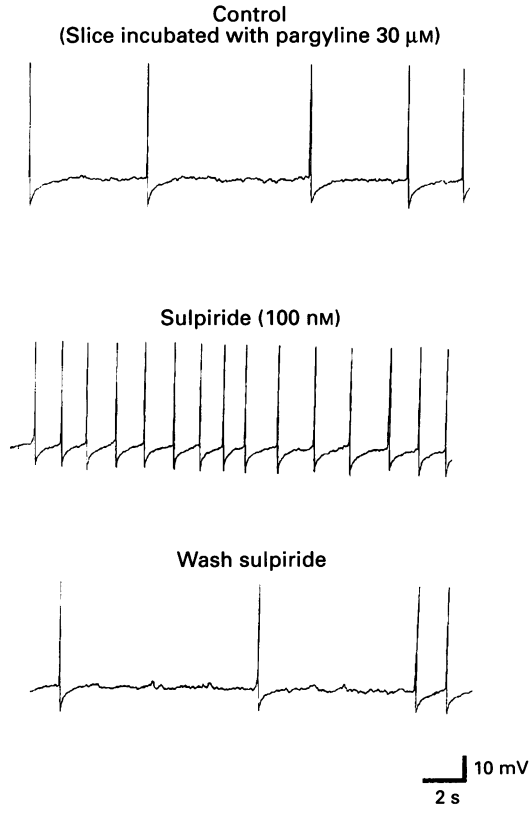

Figure 4 Pargyline induces long-lasting effects on the dopaminergic cells: reversible antagonism by (−)-sulpiride (100 nM) of the pargyline-induced reduction of firing. This slice was pretreated with pargyline 30 μM for 40 min, 3 h beforehand.

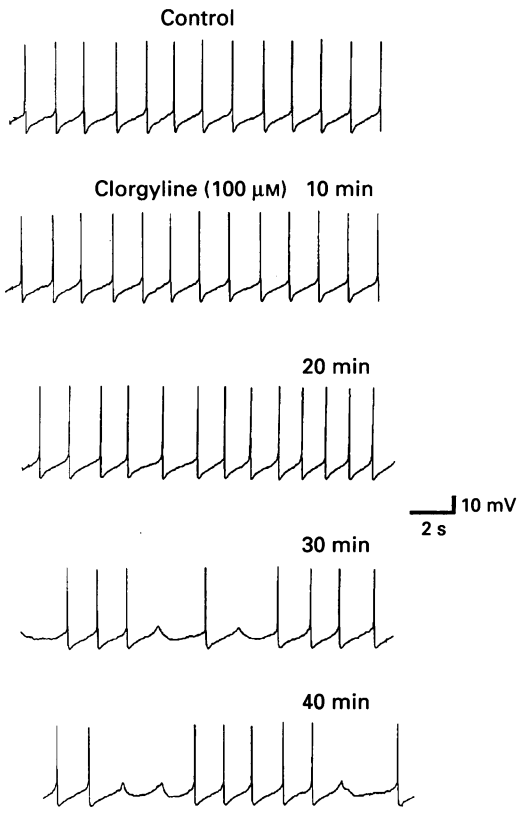

Figure 5 Effects of clorgyline: the spontaneous firing of this principal neurone was reduced by the perfusion with clorgyline (100 μM) for 40 min.

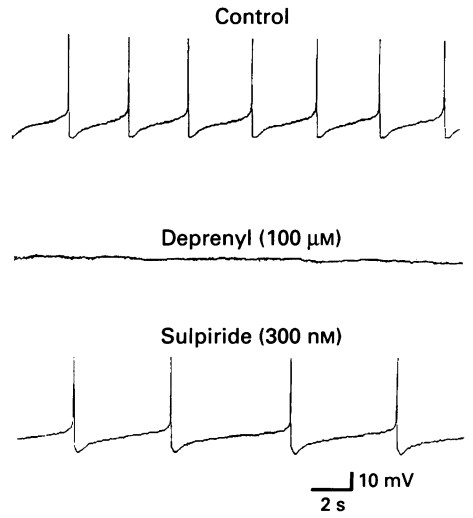

Figure 6 Effects of deprenyl: the spontaneous firing of this principal neurone was depressed by perfusion with 100 μM deprenyl. This inhibitory effect was antagonized by (−)-sulpiride (300 nM).

dopaminergic neurones of the rat mesencephalon. It has already been proposed that a complete inhibition of both forms of MAO is required to elevate maximally extracellular dopamine (Butcher *et al.*, 1990; Juorio *et al.*, 1994) and to induce dopamine-related behavioural activation (Green *et al.*, 1977). In order to explain the lack of effects of low concentrations of clorgyline on firing discharge, it is conceivable that the preferential blockade of MAO A by this drug might switch the metabolism of dopamine to extraneuronal non-dopaminergic sites where MAO B still plays an important role (Schoepp & Azzaro, 1982; 1983; Butcher *et al.*, 1990). Furthermore, since the blockade of MAO A should not only produce an increase of the dopaminergic but also of the 5-hydroxytryptaminergic tone (Yang & Neff, 1974; Suzuki *et al.*, 1982), a possible inhibition caused by an enhanced extracellular level of dopamine could be masked by a concomitant disinhibition which might result from increased 5-hydroxytryptaminergic transmission (Johnson *et al.*, 1992). An augmentation of the 5-hydroxytryptaminergic tone during MAO inhibition could also explain our experimental observation that (−)-sulpiride not only restored the initial firing activity after pargyline treatment but in some cases augmented it above control level.

The lack of effect of low concentrations of deprenyl on the spontaneous firing is in accordance with a recent *in vivo* study (Engberg *et al.*, 1991) which showed that the inhibition of MAO B by deprenyl did not clearly change the firing activity of the principal neurones of the rat substantia nigra as well as with biochemical studies which have recently demonstrated that the extracellular concentration of dopamine is little affected by the acute administration of deprenyl (Kato *et al.*, 1986; Kito *et al.*, 1986; Wood *et al.*, 1987; Butcher *et al.*, 1990; Juorio *et al.*, 1994).

In conclusion, our results suggest that only a combined inhibition of monoamine oxidase A and B is able to decrease the firing activity of the dopaminergic cells of the rat mesencephalon.

This supports the concept that both types of monoamine oxidase should be blocked in order to arrest the degradation of dopamine and to obtain a substantial enhancement of dopaminergic transmission either in control conditions or in dopamine deficient pathological states such as Parkinson's disease and depressive disorders.

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