Electropharmacograms of Rasagiline, Its Metabolite Aminoindan and Selegiline in the Freely Moving Rat

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\textbf{Abstract}

\textbf{Background:} Rasagiline and selegiline are classified as monoamine oxidase B (MAO-B) inhibitors. The present investigation deals with time-dependent electrical frequency changes (electropharmacograms) induced by these, as well as by aminoindan, the major metabolite of rasagiline. \textbf{Method:} Adult rats (day-night converted, >5 months old) were fitted with 4 bipolar concentric steel electrodes connected to a small base plate, which was positioned stereotactically for insertion of the electrodes into the frontal cortex, hippocampus, striatum and reticular formation. The plate carried a small plug to receive a telemetric device during the experimental session. Changes in field potentials were recorded during a pre-drug reference period of 45 min, followed by intraperitoneal administration and 5 h of recording thereafter. Data were transmitted wirelessly for frequency analysis. Data from 10 animals treated within a crossover design were averaged. \textbf{Results:} A dose of 0.25 mg/kg i.p. rasagiline produced statistically significant decreases in spectral alpha2 and beta1 power. Higher dosages showed a linear enhancement of this effect. A similar pattern was obtained after administration of aminoindan (2–10 mg/kg), but of shorter duration. Selegiline produced a similar pattern only for the first 1–2 h. After this, statistically significant increases in delta and theta power were observed. \textbf{Conclusion:} Despite the feature of MAO-B inhibition in both drugs and its reflection in the initial changes of the frequency pattern during the first hour, the pharmacological action of selegiline during the following hours differs profoundly from that of rasagiline, presumably due to the toxicity of its major metabolites methamphetamine and amphetamine.

\textbf{Key Words}

Electropharmacogram · Monoamine oxidase B inhibitors · Rat · Telemetry · Rasagiline · Selegiline

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\textbf{Introduction}

Rasagiline has been successfully administered to Parkinson patients as adjunctive therapy [1] and also as monotherapy [2]. Its best-known aspect of the mechanism of action consists of a highly specific irreversible inhibition of monoamine oxidase (MAO) B in comparison to MAO-A [3]. In comparison to selegiline, an older MAO-B inhibitor, rasagiline has 3–15 times greater activity at this enzyme [4] and is metabolized differently, resulting in the production of aminoindan, whereas selegi-
line is metabolized into L-methamphetamine and L-amphetamine [5]. The current investigation aimed to further characterize these 2 compounds with respect to changes in spectral field power (electropharmacogram) in freely moving rats in order to learn more about possible contributions of aminoidan to the therapeutic effects of rasagiline. Since pharmaco-EEG changes for selegiline have been reported at a dose of 5 mg/kg [6] and preclinical results for rasagiline have been reported to be in the range of 1 mg/kg [7], this dose range was also tested in the present investigation.

Material and Methods

Ten adult Fisher 344 rats (8 months of age and day-night converted, weight approx. 400 g) were implanted with 4 bipolar concentric steel electrodes within a stereotactic surgical procedure. All 4 electrodes were placed 3 mm laterally within the left hemisphere. Anterior coordinates were 12.2, 5.7, 9.7 and 3.7 mm for the frontal cortex, hippocampus, striatum and reticular formation (according to the rat brain atlas [8]). A base plate carrying 4 bipolar stainless steel semi-micro electrodes (neurological electrodes ‘SNF 100’, Rhodes Medical Instruments, Summerland, Calif., USA) and a 5-pin-plug was fixed to the skull by dental cement in spectral field power (electropharmacogram) in freely moving rats in order to learn more about possible contributions of aminoindan to the therapeutic effects of rasagiline. Since pharmaco-EEG changes for selegiline have been reported at a dose of 5 mg/kg [6] and preclinical results for rasagiline have been reported to be in the range of 1 mg/kg [7], this dose range was also tested in the present investigation.

The electropharmacogram was recorded continuously for 5 h after drug administration. Changes in spectral power within the 4 brain regions following saline administration could not be detected. The pattern remained stable. Statistically significant electropharmacograms of rasagiline were obtained by intraperitoneal administration of 3 doses (0.25, 0.50 and 1 mg/kg). Consistent changes in comparison to pre-drug values were already observed during the first hour after administration (fig. 1). The main effects were seen in the frontal cortex (statistically significant decreases in alpha2 and beta1 frequencies) and hippocampus. Changes remained visible for 5 h (fig. 1), which mainly consisted of changes in the alpha2 and beta1 frequencies in all brain areas. Even during the last hour, decreases in alpha2 and beta1 in the hippocampus were statistically highly significant. No changes in motion were observed (fig. 2).

Administration of 2 mg/kg aminoindan was nearly devoid of any effect on field potentials (fig. 3). A stable electropharmacogram of aminoindan could not be obtained before administration at a dose of 5 mg/kg (fig. 3). Under this experimental condition, a pattern of changes emerged which was very reminiscent to that observed after administration of rasagiline during the first hour of...
Fig. 1. Time dependence of changes in spectral power (%) of the 45 min recordings in brain regions of the freely moving rat after intraperitoneal administration of rasagiline, selegiline and aminoindan. Frequency ranges are depicted as bar graphs representing delta (δ), theta (θ), alpha1 (α1), alpha2 (α2), beta1 (β1) and beta2 (β2) spectral power. *p<0.10, **p<0.05, ***p<0.01.
recording. Major statistically significant effects were likewise observed within the frontal cortex and hippocampus consisting of decreases in spectral power in alpha2 and beta1 frequencies; this was somewhat less but still also statistically significant for alpha1 frequencies during the first hour after administration (fig. 1). During the second hour, only deeper brain areas showed a statistically significant decrease in alpha2 and beta1 power. As observed in the presence of rasagiline, changes in the frequency pattern were still visible 5 h after administration. A statistically significant pattern of changes could be observed after increasing the dosage to 10 mg/kg, but with even stronger decreases in alpha and beta1 power (fig. 3). Motion was somewhat increased during the first hour after the higher dosage (fig. 2).

The administration of selegiline led to a more complicated pattern of changes in comparison to rasagiline or aminoindan. During the first 2 h, a rather similar pattern in comparison to rasagiline evolved, but then the pattern of spectral frequencies changed completely. At the dosage of 1.5 mg/kg, an increase in delta and theta power – mainly in the frontal cortex and hippocampus – could be observed beginning from the third hour after administration (fig. 1). At the higher doses of 3 and 6 mg/kg, the start of this late pattern of delta and theta increases in the frontal cortex and hippocampus was shifted to the fourth hour after administration, and in addition was more clearly visible in the striatum and reticular formation (not shown). This peculiar pattern of delta and theta increases was statistically significant (fig. 1). The time-dependent pattern of changes, therefore, was basically completely different from that observed after the administration of rasagiline. Motility in general was within the scatter of normality showing a significant increase only during the first hour (fig. 2).

**Discussion**

Rasagiline produced statistically significant effects on spectral field power in all 4 regions of the brain within a dose range of 0.25–1 mg/kg. Similar effects were observed with higher doses of 3 and 6 mg/kg, but which lasted longer (not shown). The strongest effects were documented within the alpha2 and beta1 spectral power, observed as decreases in spectral power which were statistically different from saline. Alpha2 frequencies have repeatedly been shown to change with the activity of dopaminergic transmission [12, 13]. Thus, a decrease in electrical power at this frequency band represents enhanced dopaminergic activity consistent with the idea of the proposed mechanism of action in Parkinson patients. Beta1 frequencies seem to be under the control of glutamatergic transmission, as has for example been shown with memantine, an antidementive drug that enhances AMPA-receptor-dependent electrical activity in the hippocampus and decreases beta1 power in this model [12]. Alpha1 power changes in relation to serotonergic transmission, as exemplified with 5HT-2 receptor active compounds [9] as well as with 5HT-1A active compounds [Dimpfel, unpubl. work]. Decreases in spectral alpha1 power signalize enhancement of serotonergic transmission, as observed for example in the presence of antidepressive drugs [10]. An alpha1 decrease, in addition to the decrease in alpha2 and beta1 spectral power, was observed in the presence of aminoindan.

Comparison of the action of rasagiline with other drugs tested under identical conditions suggests a relationship to neuroprotective drugs like donepezil (fig. 4), which means that MAO-B inhibition is probably not the only mechanism of action within the brain. According to Youdim [14], the propargyl moiety exhibits intrinsic neuroprotective pharmacological activity that requires identification. Both rasagiline and aminoindan share this moiety. Meanwhile, the neuroprotective mechanism of 1-(R)-aminoindan has been analyzed and reported [15]. Another reason to assume that we are not tracing the enzyme inhibitory effects is the fact that rasagiline is 3–15
times more potent in this respect in comparison to selegiline, but changes in the electropharmacogram are quantitatively similar during the first 2 h after administration. Neuroprotective effects of rasagiline have been reported at a dose of 1 mg/kg in the mouse [16]. Thus, rasagiline potentially has a dual action, which fits well to reports in the literature reporting neuroprotective features [17, 18]. The other MAO-B inhibitor, selegiline, seemed to be equally effective in MPTP-induced neurotoxicity in marmosets [19], but a more detailed analysis of both compounds on glutamate-receptor-induced physiological responses revealed profound differences [20].

Selegiline and rasagiline are metabolized in a different manner. Whereas selegiline is metabolized to methamphetamine and amphetamine (which are known for their negative effects [21]), rasagiline is metabolized to aminoindan, which had not been characterized in depth up to now. The present investigation resulted in the detection of a pharmacodynamic potential of aminoindan of its own. This finding is corroborated by results from a study performed in the hippocampus slice model in vitro. Here, aminoindan behaved like neuroprotective drugs or antidepressive drugs [10]. The extent of the changes seen after 5 mg/kg aminoindan during the first hour is similar
to that observed after 1 mg/kg rasagiline. Therefore, the presence of the active metabolite aminoindan might add a considerable benefit to Parkinson patients due to the longer therapeutic action. Since the combined action of rasagiline and aminoindan cumulates into time-dependent stronger action, with a profile resembling antidepressive drugs, further benefits for the patients can be assumed.

There is a profound difference with respect to the time course of action between rasagiline and selegiline. At nearly equal dosages, rasagiline shows time-dependent attenuation of frequencies, whereas in the presence of selegiline the pattern reverses with respect to delta and theta powers. These 2 frequencies have been shown to change with the activity of the cholinergic [22] and noradrenergic systems [23], respectively. Attenuation of cholinergic and noradrenergic transmission according to increase in delta and theta frequencies could have serious consequences in terms of cognitive impairment and tiredness. This kind of a reversal is shifted with higher dosages as has been observed with the administration of low dosages of L-DOPA and dopamine D₂ agonists [13]. There is circumstantial evidence for the involvement of dopamine D₂ receptors. In the case of L-DOPA, cocaine and D₂ agonists, this seems to be a presynaptic action at the dopamine D₂ receptor.

This kind of delta and theta enhancement of spectral power could also indirectly derive from stimulation of dopamine D₂ autoreceptors on GABAergic neurons leading to depression of GABAergic transmission as proposed by Centonze [24]. The late effects of selegiline might therefore be interpreted as stemming from the action of methamphetamine or amphetamine, the main metabolites of selegiline. Amphetamine is known to raise extracellular dopamine levels. At low dosages, this presumably leads to stimulation of presynaptic dopamine D₂ autoreceptors. Previous studies indicated that amphetamine can also decrease stimulation-dependent vesicular dopamine release. Using a dopamine D₂ blocker (sulpiride) and mice lacking this receptor, it could be shown by Schmitz et al. [25] that this feature was due to D₂ receptor activation. A further possibility is that heterosynaptic dopamine neurotransmission might play a role as shown by Bamford et al. [26]. Since scopolamine, a cholinergic muscarinic antagonist, and biperiden provide a similar type of pattern, involvement of the cholinergic system must also be discussed as dopaminergic and cholinergic transmission influence each other [22]. Thus, there is a
high probability that the late increases in delta and theta power observed after administration of selegiline reflect the activity of its main metabolites. Since increases in these frequencies are observed in cognitive impairment in humans and during enhanced tiredness, this feature must be regarded as a potential risk in the treatment of patients. In addition, the psychostimulant methamphetamine is a highly addictive drug with neurotoxic actions leading to persistent forms of cognitive impairment including deficits in attention [27], which is in line with our interpretation.

Since field potentials (in the presence of drugs, called electropharmacograms) are based on the activity of ionic currents [28], speculation on the involvement of ion channels is allowed. From the pattern of changes on the electropharmacograms in the presence of drugs acting on L-type calcium channels, we can speculate on a common mechanism of action. Agonists targeting at this type of channel, like BAY K8644, induce a pattern of spectral frequency changes very reminiscent of the action of acetylcholinesterase inhibitors like donepezil. In addition, antagonists at this channel, like gabapentin, induce a pattern very reminiscent of the low-dose phenomenon or action of dopamine antagonists. It is therefore very tempting to assume that this increase in delta activity is based on attenuation of cholinergic transmission possibly induced by activation of presynaptic dopamine D2 auto- or heteroreceptors eventually linked to L-type calcium channels. Alternatively, the dopamine D2-receptor-dependent second signaling pathway involving β-arrestin-2 [for a review, see 29], which has a comparable time course as our data with maxima in the second hour, could likewise be responsible for the late effects observed in our study.

A last point has to be discussed dealing with the different clinical dosages used for rasagiline and selegiline. In rats, the pharmacologic effect during the first hour was comparable for both drugs. From this, no higher dosage would be required for selegiline. This also speaks against the view that MAO-B inhibition is the only mechanism of action. However, selegiline has a much shorter time-limited action due to the late presynaptic effects of its metabolites antagonizing its antiparkinsonian effects and leading to sleep attacks in patients.

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