Daily transdermal administration of selegiline to guinea-pigs preferentially inhibits monoamine oxidase activity in brain when compared with intestinal and hepatic tissues

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Abstract

Selegiline has been formulated in an acrylic polymer adhesive mixture to be employed as a constant release topical patch for daily transdermal administration. Application of this selegiline transdermal system (STS) to guinea-pigs resulted in an average delivery of 1.185 mg selegiline/cm^2 patch/24 h. STS dose–response curves were generated by altering patch size (cm^2). A transdermal dose range was identified which inhibited guinea-pig brain monoamine oxidase-B (MAO-B) by greater than 95% yet provided for a dose-dependent inhibition of monoamine oxidase-A (MAO-A) activity. The ID50 for inhibition of MAO-A activity in response to a 21-day daily regimen with transdermal selegiline was approximately 7.5-fold lower for cortical and striatal brain regions compared with that obtained for duodenum; hepatic MAO-A was unaffected following the same dosing regimen. By contrast, orally administered selegiline inhibited brain and duodenal MAO-A to the same extent, and generated a shallower dose–inhibition curve for brain MAO-A inhibition. In addition, transdermal delivery was approximately 6–8-times more potent than oral selegiline for the inhibition of brain MAO-A activity. It is concluded that daily transdermal selegiline administration may provide therapeutic advantages over oral treatment, based on its preferential, dose-dependent inhibition of brain vs peripheral MAO-A activity.

Introduction

Monoamine oxidase (MAO) is a ubiquitous enzyme present in mammalian tissues as two genetically distinct isozymes referred to as MAO-A and MAO-B (Shih et al 1999). Selegiline (R-($-$)-N-2-dimethyl-N-2-propynyl-phenethylamine; ($-$)-deprenyl) is a preferential MAO-B inhibitor (Schoepp & Azzaro 1981; Knoll 1986; Chrisp et al 1991) that is currently used as adjunctive therapy for the treatment of late stage Parkinson's disease (Golbe 1988; Heinonen & Rinne 1989). Clinical studies have demonstrated the potential use of oral selegiline as antidepressant therapy, however at doses that are three to six times greater than approved for the treatment of Parkinson's disease (Mann et al 1989; McGrath et al 1989; Sunderland et al 1994). While these oral doses of selegiline appear to be efficacious in the treatment of depression, they no longer maintain selectivity for MAO-B inhibition (Sunderland et al 1985; Prasad et al 1988) and carry the risk of acute hypertensive episodes associated with the ingestion of tyramine containing foods or sympathomimetic decongestants (Blackwell 1963, 1991; Blackwell et al 1967). This acute hypertensive syndrome following dietary tyramine has been referred to as the ''cheese effect'' and appears to be due to clinically significant inhibition of MAO-A at the intestinal barrier to systemic tyramine exposure (Elsworth et al 1978; Da Prada et al 1988; Hasan et al 1988; Anderson et al 1993).

It has been suggested that there is a need for significant MAO-A inhibition in brain tissue for antidepressant efficacy of MAO inhibitor drugs (Lipper et al 1979). The need for MAO-A inhibition in brain tissue is based upon the preferential metabolism by MAO-A of brain noradrenaline (norepinephrine) and serotonin (5-hydroxytryptamine) (Cesura & Pletscher 1992), two neurotransmitters that have putative involvement in the

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genesis of a depressive syndrome and the actions of anti-depressant agents (Youdim et al 1988). However, currently available MAO inhibitor antidepressant agents, such as phenelzine or tranylcypromine, that are highly effective inhibitors of MAO-A throughout the body, may only be administered to patients who refrain from tyramine-rich foods or beverages and sympathomimetic decongestants.

More recently, a selegiline transdermal patch (the Selegiline Transdermal System; Somerset Pharmaceutical, Inc., Tampa, FL) has been developed that produces sustained, high plasma levels of selegiline but lowers the exposure to selegiline metabolites when compared with equivalent oral selegiline doses (Barrett et al 1996; Rohatagi et al 1997). It was hypothesized that transdermal administration might provide selegiline plasma levels sufficient to inhibit MAO-A in the CNS while avoiding this interaction in the gastrointestinal tract and other peripheral tissues of critical importance to the production of the "cheese effect". This hypothesis was based upon the preferential pharmacological effects of selegiline on brain vs hepatic MAO-B activity when administered via subcutaneous injection in sufficiently low concentrations to achieve selective MAO-B inhibition (Ekstedt et al 1979; Felner & Waldmeier 1979).

This study was conducted as a "proof of concept" comparing oral vs transdermal administration of selegiline on the ability to inhibit MAO activities in brain vs the intestinal epithelium and liver. The guinea-pig was used as the model system since this animal species demonstrates ratios of MAO-B/MAO-A activity and apparent affinity constants for dopamine that are similar to those observed in human brain tissue (Azzaro et al 1985). In addition, this animal was found to be amenable to chronic dosing with transdermal patches. The hypothesis was tested by generating complete dose–response curves for MAO-A inhibition at selegiline doses that exceeded complete inhibition of brain MAO-B activity. This exercise was conducted following repeated dosing of animals with oral or transdermal selegiline administration. Validation of selegiline absorption following transdermal dosing was supported via residual patch analysis.

Materials and Methods

Animals

Young adult male guinea-pigs (450–550 g) were purchased from Hilltop Lab Animals (Scottdale, PA) and housed in the university animal quarters for a minimum of five to seven days before use. All procedures performed with guinea-pigs adhered to the NIH Guide for the Care and Use of Laboratory Animals (West Virginia University ACUC Protocol #9606-04).

Transdermal dosing

Placebo and selegiline-containing 10-cm^2 patches (1.83 mg cm^{-2}, selegiline base) were provided by Somerset Pharmaceuticals (Tampa, FL). Minipatches (0.041–

10.0 cm^2) were prepared from the 10-cm^2 circular patches by cutting rectangular segments with a MacIlwain Tissue Chopper (Brinkman Instruments for the Mickle Laboratory Engineering Co, Surrey, UK). Minipatches were cut with the original release linear in place. Choice of 0.041 cm^2 as the lower limit was based on the estimated 24-h selegiline delivery of 0.1 mg kg^{-1}. Felner & Waldmeier (1979) showed that this dose caused complete inhibition of brain MAO-B activity with no effect on MAO-A activity following 14 days of subcutaneous selegiline administration to rats.

One day in advance of patch application, the dorsal hair of the guinea-pigs was clipped and the remaining hair stubble was removed by close shaving with an electric razor. Care was taken to minimize skin trauma. Placebo or selegiline-containing minipatches were removed from their respective release liners and placed on the shaved (electric razor) dorsal skin using a fine forceps. Minipatches were covered by an approximate 3 cm^2 piece of Dental Dam (Hygenics Pharmaceuticals, Laguna Hills, CA), which in turn was held in place with an overlapping Elastikon wrap around the trunk of the animal. The wrap and patch were removed 24 h later. With chronic dosing, fresh placebo or drug-containing minipatches were applied daily. Every third day the skin area was re-shaved to remove new hair growth.

Minipatch samples were analysed for initial total selegiline content, uniformity between patch preparations, and for residual selegiline following a 24-h application period. New minipatches (0.041 and 0.41 cm^2) were prepared and removed from the release liner with a fine forceps and immediately placed on a clean release liner and stored at $-20°C$. Minipatches applied to the skin for 24 h were likewise stored on a clean release linear at $-20°C$. The difference between the mean of the total amount in new unapplied minipatches and post 24-h minipatch residual per animal was considered to represent the mg of drug delivered per 24 h.

Selegiline contents in the minipatches were analysed post-extraction by high-pressure liquid chromatography at Wisconsin Analytical and Research Services LTC (Madison, WI). The average selegiline delivery from a representative sampling (n = 9–10) of the two minipatches was 1.185 mg cm^{-2}/24 h. In this study, doses of transdermal selegiline have been presented as mg delivered kg^{-1}/24 h (based on 1.185 mg cm^{-2}/24 h) and as cm^2 minipatch applied per 24 h.

Oral dosing

Selegiline HCl (Somerset Pharmaceuticals, Tampa, FL) solutions were prepared fresh daily in H$_2$O and administered as a gavage (0.25 mL) using a round tip gavage needle.

MAO assays

All tissues (cortex, striatum, duodenum, ileum and liver) were homogenized (10% w/v) in 0.05 M sodium/potassium phosphate buffer (pH = 7.4) at 0–4°C using a hand driven

Teflon-smooth glass homogenizer. Whole duodenum or ileal muscle was chopped twice (right angles, 0.5 mm^2) before homogenization. Homogenates of cortex or striatum were used undiluted in the assays while other homogenates were further diluted as follows: liver 1:25 for MAO-B and 1:10 for MAO-A; intact duodenum or ileal epithelium 1:25; ileal muscle 1:10. The reaction mixture (600 μL) consisted of 540 μL 0.05 M sodium/potassium phosphate buffer (pH = 7.4), 40 μL tissue homogenate and 20 μL radioactive substrate stock preparation (to yield final concentrations of 0.1 mM [^{14}C]5-hydroxytryptamine (serotonin) for MAO-A and 10 μM [^{14}C]phenylethylamine for MAO-B). [^{14}C]Phenylethylamine (50–60 mCi mmol^{-1}) was obtained from Amersham-Pharmacia (Piscataway, NJ) and [^{14}C]5-hydroxytryptamine (40–60 mCi mmol^{-1}) was purchased from DuPont/NEN (Boston, MA). The duration of the MAO-B assays was 4 min while MAO-A was assayed at 15 min with the exception of ileal muscle (10 min). Reactions were terminated with sequential additions of 100 μL 2.0 M citric acid and 40 μL 20.0 mg mL^{-1} ascorbic acid in 1.5% EDTA, and then extracted with either 3.0 mL ethylacetate (MAO-A) or octane (MAO-B). The organic phase (2.0 mL) was then mixed with 4.0 mL scintillation fluid (Scinti Safe Econo 2; Fisher Scientific Corp, Pittsburgh, PA) and assessed for radioactivity using a scintillation spectrometer (Tri-Carb #1900CA, Packard Instrument Corp, Downer's Grove, IL). Enzymatic activities were expressed per unit of Lowry protein and per unit of time (15 min for MAO-A; 4 min for MAO-B).

[^{14}C]tyramine oxidation

Initial experiments focused on establishing valid assay conditions for [^{14}C]tyramine oxidation by whole duodenum, ileal epithelium and muscle. The ileum was removed, trimmed of surface fat and adventitia, slit longitudinally and the luminal contents rinsed free in saline. Segments of non-separated or whole ileum were frozen with dry ice–acetone and the remainder was separated into epithelium and muscle tissues using a blunt spatula and frozen with dry ice–acetone. All samples were then stored at -80°C until the time of assay. Histological analyses of intact ileum vs the surgically-separated ileal epithelium and muscle revealed pure separated tissue types harvested with a consistent plane of cleavage between these tissues (not shown). The tyramine oxidation assays were conducted without major modification of the procedures outlined above under "MAO assays", but were performed using a final concentration of 400 μM tyramine containing 0.06 μCi [^{14}C]tyramine (50 Ci mmol^{-1}, New England Nuclear NEC-325) in a final volume of 1.0 mL. Linear reaction rates (stable positive slope for product formation per unit time) for [^{14}C]tyramine oxidation were established for whole duodenum, ileal epithelium and muscle. Likewise, linear relationships were established between tissue homogenate concentration (mg tissue equivalents per assay) and tyramine oxidation in whole duodenum, ileal epithelium and muscle. Based on these results, the routine assays of [^{14}C]tyramine oxidation employed for whole duodenum,

0.16 mg tissue equivalent and 15-min reaction time; for ileal epithelium, 0.16 mg tissue equivalent and 15-min reaction time; and for ileal muscle 0.4 mg tissue equivalent and a 10-min reaction time. Reactions were terminated with citric acid, and [^{14}C]tyramine oxidation products were extracted with ethyl acetate and quantified as described above.

Statistics

Comparisons were made between ID50 values using a Z statistic: Z = (ID50-#1)−(ID50-#2)/s.e. (s.e. is the standard error of the estimate of the difference). Analyses were performed by Dr Gerry Hobbs, WVU Dept. Community Medicine. The t-test was used where appropriate to establish statistical significance between individual sample means.

Results

Oral and transdermal administration of selegiline on MAO-A inhibition

Preliminary experiments were conducted to determine the time period required for optimal dosing with transdermal selegiline. A maximal-effective and selective MAO-B inhibitory dose of 0.1 mg kg^{-1} was chosen for this exercise based upon the subcutaneous data obtained by Felner & Waldmeier (1979). These experiments revealed a cumulative inhibition on brain MAO-B activity following the initial 24-h dose of transdermal selegiline that stabilized during the 7–21-day treatment period. Specifically, after a single 24-h patch application MAO-B activity in cortex and striatum was inhibited 32% and 41%, respectively; however, after 7 or 21 days of daily patch applications, the MAO-B in cortex and striatum was inhibited >95%. Based upon these data, complete dose–inhibition curves to both oral and transdermal selegiline were generated following 21 days of treatment to test for dose-dependent,

Table 1 ID50 (mg kg^{-1}) for MAO-A inhibition[a].

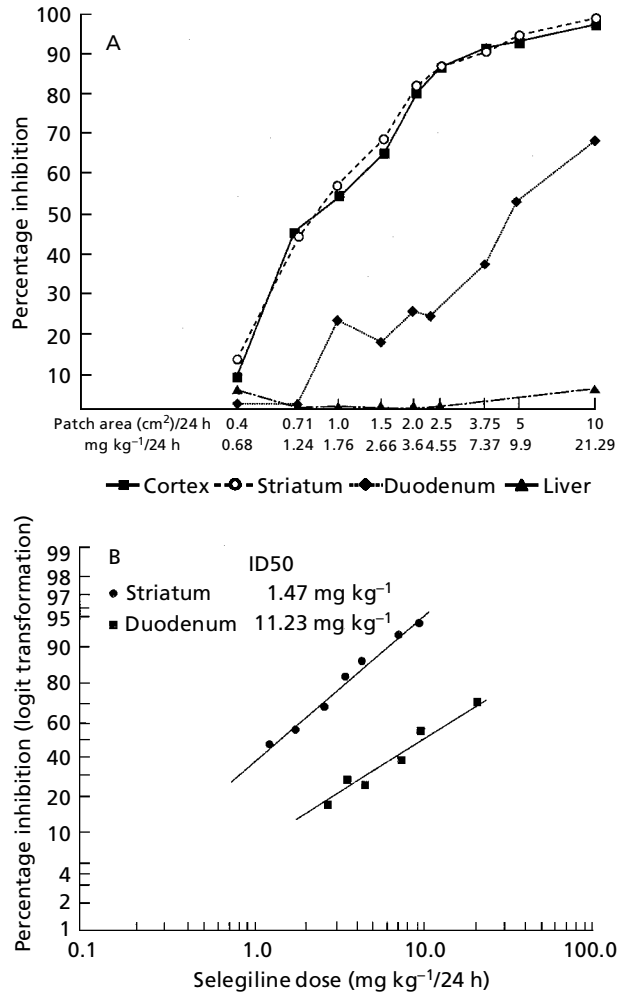| Tissue	Oral selegiline	Transdermal selegiline
Cortex	12.4 (7.03–24.01)	1.63[b] (1.06–2.34)
Striatum	9.31 (5.67–15.59)	1.47[b] (1.18–2.11)
Duodenum	7.03 (3.42–14.49)	11.23 (7.72–19.9)
Liver	91.2[c]	> 21.3[c]

[a]Drug treatment was daily for 21 days (values represent the mean of four to six separate experiments; numbers in parentheses represent 95% confidence intervals). [b]$P \leqslant 0.05$, statistically different relative to duodenum from oral and transdermal groups and relative to cortex and striatum from oral group. Comparisons were made between ID50 values using a Z statistic. [c]Values were estimated based on extrapolations from log-logit plots.
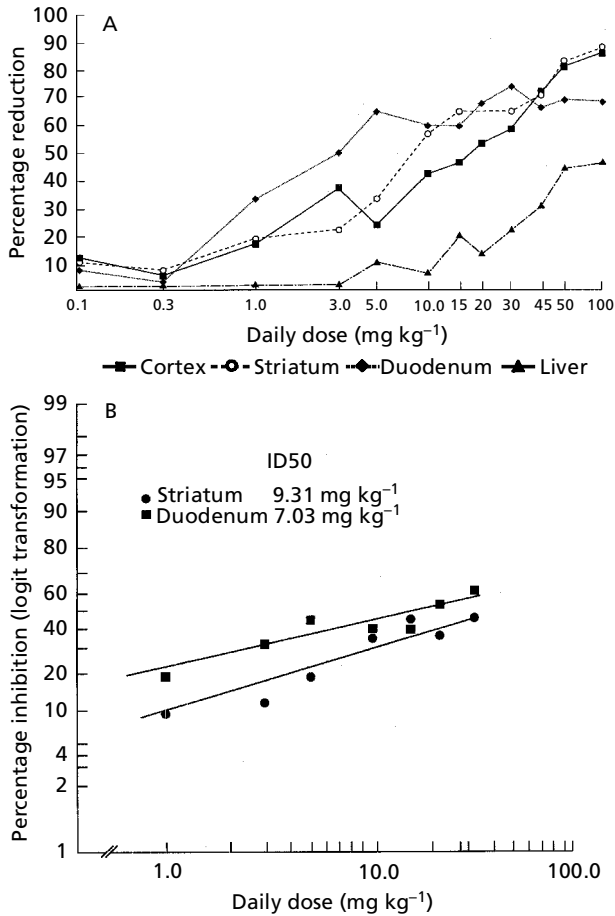
Figure 1 A. The effect of oral selegiline treatment on MAO-A inhibition. Animals (550–700 g) were treated daily for 21 days with selegiline gavage in doses ranging from 0.1 mg kg^{-1} to 100 mg kg^{-1}. Cortex, striatum, liver and duodenum control values were 21.0 ± 1.5, 13.25 ± 0.8, 146.3 ± 10.0 and 396.4 ± 29.1 pmol (μg protein)$^{-1}$/15 min, respectively. All values for control and treatment groups represent the mean$\pm$s.e.m. of 4–6 separate experiments. The values given on the x-axis are on a log scale. B. Log-logit analysis of the effects of oral selegiline treatment on MAO-A inhibition. Results depicted in A were transformed (linearized) using a log-logit analysis to interpolate ID50 values for MAO-A inhibition. The plot for cortex and liver (not shown) revealed respective ID50 values of 12.4 and 91.2 mg kg^{-1}. Table 1 provides a summary of the ID50 values for chronic selegiline responses.

Figure 2 A. The effect of transdermal selegiline treatment on MAO-A inhibition. Animals (550–700 g) were treated daily for 21 days with fresh selegiline patches ranging in doses from 0.68 mg kg^{-1} (0.4 cm^2) to 21.29 mg kg^{-1} (10 cm^2). Respective control values for MAO-A activity in cortex, striatum, liver and duodenum were 21.2 ± 1.1, 10.6 ± 0.7, 120.4 ± 48, 405.7 ± 17.4 pmol (μg protein)$^{-1}$/15 min. All values for control and treatment groups represent the mean$\pm$s.e.m. of 4–6 separate experiments. Values on the x-axis are on a log scale. B. Log-logit analysis of the effects of transdermal selegiline treatment on MAO-A inhibition. Results depicted in A were linearized using log-logit analysis to interpolate ID50 values for MAO-A inhibition. The plot for cortex (not shown) revealed an ID50 of 1.63 mg kg^{-1}. Table 1 provides a summary of the ID50 values for chronic selegiline responses.

tissue-selective MAO-A inhibition. Over the entire dose range used in these 21-day studies, MAO-B in cortex and striatum was inhibited by more than 95%.

Daily gavage dosing of selegiline inhibited duodenal and brain (cortex or striatum) MAO-A to approximately the same extent (Table 1, Figure 1A, B). By contrast, transdermally-administered selegiline resulted in a marked preferential inhibition of brain MAO-A (Table 1, Figure 2A, B). Following transdermal administration of selegiline, the ID50 value for brain MAO-A was approximately 7.5-times lower than that for the duodenum. It was also apparent that a more precise and steeper dose–inhibition curve was generated following the transdermal route of administration (Figure 1A, B vs Figure 2A, B). Differences in precision between the two routes of administration were reflected in a comparison of the 95% confidence limits for the brain and duodenal ID50 values (Table 1). In addition, the potency of transdermal selegiline as an inhibitor of brain MAO-A activity was 6–8-times greater than that seen during gavage treatment (Table 1).

At all doses tested (0.68–21.29 mg kg^{-1}/day), hepatic MAO-A was virtually resistant to inhibition by transdermal administration of selegiline (Figure 2A, Table 1). Following oral treatment, the liver demonstrated reduced sensitivity

to selegiline with a shift in the dose–inhibition curve to the right of that obtained in intestine and brain tissues (Figure 1A). The extrapolated ID50 value for liver after oral selegiline treatment was approximately an order of magnitude greater than that demonstrated in brain or intestine (Table 1).

Tyramine vs serotonin metabolism in intestinal tissue

Oral therapy with a traditional non-selective MAO inhibitor directly exposes the intestinal mucosa to relatively high inhibitor concentrations of drug. With insufficient mucosal MAO-A activity, dietary tyramine can gain entry into the systemic circulation and produce undesirable pressor effects (see Introduction). In the experiments described above, MAO-A activity was assayed using serotonin as the substrate. This is a common assay procedure for the determination of MAO-A activity in mammalian tissues since serotonin is regarded as a specific substrate for MAO-A isozyme (see Cesura & Pletscher 1992). However, tyramine is a substrate for either isoform of MAO (Strolin Benedetti et al 1983; Strolin Benedetti & Dostert 1985; Hasan et al 1988), and to ensure that the inhibition of serotonin metabolism by selegiline was an accurate reflection of what might occur for tyramine, an additional dose–inhibition curve for tyramine metabolism was obtained in segments of the guinea-pig intestine after transdermal selegiline administration.

Comparisons of the metabolism of tyramine and serotonin in whole duodenal homogenates obtained following repeated dosing with transdermal selegiline revealed a strong correlation between the metabolism of these two substrates (Figure 3). Resolution of the ileum into mucosa and smooth muscle for enzymatic analysis demonstrated

Table 2 Dose related inhibition of [^{14}C]tyramine oxidation in ileal epithelium and ileal muscle after 21-days of transdermal selegiline treatment[a].

Patch size (cm^2)/dose (mg kg^{-1})	Ileal epithelium: percentage inhibition	Ileal muscle: percentage inhibition
Control	–	–
0.4 cm^2/0.68 mg kg^{-1}	11.7±1.1	12.8±6.5
0.71 cm^2/1.24 mg kg^{-1}	19.3±6.8	12.8±2.6
1.5 cm^2/2.66 mg kg^{-1}	25.3±1.9	24.0±6.2
2 cm^2/3.6 mg kg^{-1}	30.4±5.2	43.8±7.8
2.5 cm^2/4.55 mg kg^{-1}	37.9±7.7	50.7±10.0
3.75 cm^2/7.37 mg kg^{-1}	48.8±2.8	61.7±6.0
5 cm^2/9.9 mg kg^{-1}	49.5±4.5	77.0±2.2[b]
10 cm^2/21.29 mg kg^{-1}	77.6±1.6	86.2±1.0[b]

[a]All data for treatment groups are expressed as percentage inhibition from control and represent the mean±s.e.m. of three to four separate experiments. The control value for ileal epithelium MAO-A activity was 1060.7±31.6 nmol tyramine oxidized (mg protein)$^{-1}$/15 min. The control value for ileal muscle MAO-A activity was 100.5±6.2 nmol tyramine oxidized (mg protein)$^{-1}$/15 min. Both control values represent the mean±s.e.m. of three to four separate experiments. [b]Significantly different ($P \leqslant 0.05$) from respective epithelium based on an analysis of variance matched with the Neuman-Keuls test.

that the mucosa contained 10-fold greater MAO-A activity compared with the muscle, but the percentage inhibition in response to transdermal treatment was slightly greater in the muscle sub-fraction at mini-patch sizes $\geqslant 2$ cm^2 (Table 2).

Resistance of hepatic MAO-A to inhibition by transdermal selegiline

Since hepatic MAO-A activity appeared to be resistant to inhibition by repeated daily dosing with transdermal selegiline (Figure 2A), it raised the possibility that serotonin (the substrate employed for assay of MAO-A activity) may be metabolized in this tissue by way of an additional amine oxidase pathway, such as semicarbazide sensitive amine oxidase (SSAO). SSAO was considered as a possibility since it is capable of metabolizing amine substrates but is resistant to inhibition by acetylenic MAO inhibitors such as clorgyline or selegiline (Callingham & Barrand 1987).

Selective inhibitors of MAO-A, MAO-B and SSAO were employed to pharmacologically test for the presence of amine metabolism by these amine-oxidizing enzymes in hepatic tissue. In this regard, the in-vitro metabolism of phenylethylamine (MAO-B substrate) and serotonin (MAO-A substrate) was examined in hepatic tissue before and after in-vitro treatment with selegiline (a selective MAO-B inhibitor), clorgyline (a selective MAO-A inhibitor (Johnston 1968)), or semicarbazide (specific inhibitor of SSAO). Phenelzine (a nonselective MAOA/B inhibitor) was employed also as a means of examining amine metab-
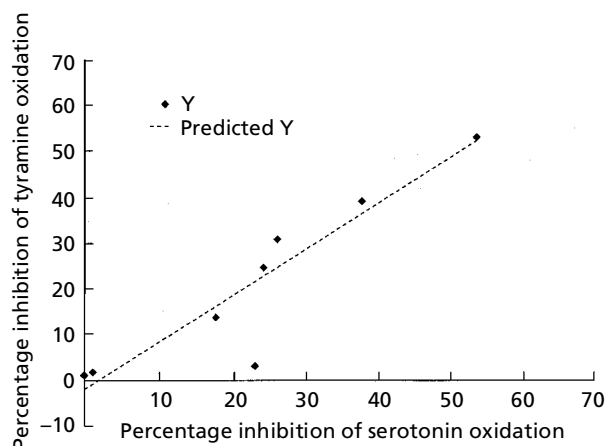
Figure 3 Correlation between selegiline inhibition of serotonin and tyramine oxidation by duodenum ($r^2 = 0.91$; slope = 1.03). Duodenal samples were assayed from the chronic (daily for 21 days) transdermal selegiline dose–response study depicted in Figure 2A. The dashed line depicts the calculated linear regression.

Table 3   Absence of semicarbazide-sensitive amine oxidase activity in guinea-pig liver. Normal guinea-pig liver crude homogenates were assayed for MAO-A and MAO-B activity as described in Materials and Methods using the substrates serotonin and phenylethylamine, respectively. The presence of hepatic semicarbazide-sensitive amine oxidase activity was based upon its sensitivity to inhibition by semicarbazide and its resistance to inhibition by clorgyline and selegiline.

Group	MAO-A activity (pmol (mg protein)$^{-1}$/15 min)	
		Percentage reduction
Control for semicarbazide	110000 ± 4643[a]	
Semicarbazide 0.1 mM	109030 ± 1770	0.9%
Control for other drugs	97264 ± 8790	
Selegiline 30 nM	102863 ± 8508	0%
Clorgyline 10 nM	10213 ± 1531	89.5%
Phenelzine 0.01 μM	71828 ± 2088	26.1%
Phenelzine 100 μM	234 ± 100	99.8%
	MAO-B activity (pmol (mg protein)$^{-1}$/4 min)	
Control for semicarbazide	15401 ± 876	
Semicarbazide 0.1 mM	14073 ± 357	9%
Control for other drugs	14619 ± 448	
Selegiline 30 nM	664 ± 127	99.5%
Clorgyline 10 nM	13937 ± 437	4.6%
Phenelzine 0.01 μM	10558 ± 370	27.7%
Phenelzine 100 μM	108 ± 65	99.26%

[a]Denotes mean$\pm$s.e.m. for three separate experiments.

olism in the absence of MAO activity. The results of these experiments are summarized in Table 3.

A high concentration of semicarbazide (0.1 mM) was unable to alter the metabolism of serotonin or phenyl-ethylamine in guinea-pig liver homogenates. By contrast, clorgyline and selegiline produced a highly selective and near complete inhibition of serotonin and phenylethyl-amine metabolism, respectively, and phenelzine inhibited the metabolism of both substrates in a concentration-dependent and non-selective fashion. Thus it may be concluded that neither phenylethylamine nor serotonin metabolism was associated with SSAO in guinea-pig liver, and that MAO-B and MAO-A accounted for their respect-ive metabolic degradation in this tissue. Moreover, the observation that serotonin metabolism in hepatic tissue is not affected by selegiline can be attributed to its trans-dermal delivery and not a unique enzymatic property of this guinea-pig tissue.

Discussion

This study has demonstrated a tissue-selective effect of selegiline as a MAO inhibitor when delivered via a trans-dermal formulation. Transdermal selegiline preferentially inhibited brain MAO-A activity when compared with duodenal and hepatic tissues. This was in direct contrast to the results of oral selegiline treatment where brain and duodenal tissues demonstrated similar ID50 values for inhibition of MAO-A activity by selegiline. The preferential effects of transdermally administered selegiline on brain MAO-A activity were consistent with the findings of Felner

& Waldmeier (1979) and Eckstedt et al (1979), who demonstrated that repeated subcutaneous dosing of rats with selegiline was more effective in inhibiting MAO ac-tivity in the brain than in hepatic tissue. These investigators demonstrated also that 7–21 days of subcutaneous dosing with selegiline lowered the ID50 value relative to a single administration and led to a loss of selectivity for MAO-B inhibition that was more pronounced in brain than liver tissue. These latter results were also consistent with our findings in guinea-pigs administered selegiline via a trans-dermal patch.

The importance of the tissue selectivity observed after treatment with the transdermal selegiline relates to the ability to deliver antidepressant doses of selegiline without the risk of acute hypertensive reactions associated with dietary ingestion of tyramine (Blackwell 1963; Blackwell et al 1967). Normally, dietary tyramine is prevented from entering the systemic circulation because of a series of enzymatic barriers, including MAO-A, MAO-B, and phenolsulfotransferase (Da Prada et al 1988). However, it is MAO-A that assumes the major role in the metabolic destruction of this pressor amine as it passes through the intestinal mucosa (Elsworth et al 1978; Da Prada et al 1988; Hasan et al 1988; Anderson et al 1993). It was for this reason that experiments in this study were focused upon the interactions between selegiline delivered via a transdermal patch and MAO-A inhibition in intestinal tissues. The results demonstrated a sparing of intestinal tissue MAO-A at concentrations of transdermal selegiline that produced substantial inhibition of MAO-A activity in the brain. Moreover, an additional selegiline dose–response curve obtained in intestinal tissue using tyramine as the

assay substrate yielded results identical to those obtained with serotonin (as demonstrated by highly significant correlation coefficient), suggesting that indeed the results obtained with serotonin would apply to the metabolism of tyramine as well. Thus it would appear that selegiline administered by transdermal patch would effectively inhibit brain MAO-B and MAO-A activity while avoiding significant inhibition of intestinal MAO-A and the potential for acute hypertension associated with dietary tyramine.

Ex-vivo assays conducted with liver tissue demonstrated that liver MAO-A activity was unaffected by selegiline delivered via transdermal patch. This level of hepatic resistance was not found after oral selegiline dosing; however, the inhibition curve to oral selegiline in hepatic tissue was shifted to the right of those obtained from brain and intestinal tissues. While the basis for the hepatic MAO resistance to inhibition by transdermal selegiline is currently unknown, the presence of a selegiline-resistant SSAO does not appear offer a solution. In this regard, it was theorized that the presence of SSAO could mask an inhibitory effect of selegiline on MAO-A by oxidizing the assay substrate, serotonin. However, assays conducted invitro in the presence of semicarbazide demonstrated that metabolism was not occurring by way of an alternate pathway through the enzyme SSAO. In these experiments, semicarbazide was totally without effect on either serotonin or phenylethylamine metabolism. By contrast, in-vitro incubation of hepatic tissue with selegiline or clorgyline demonstrated the selective properties appropriate for these agents regarding inhibition of phenylethylamine and serotonin metabolism, respectively.

It is conceivable that the hepatic resistance to MAO inhibition by transdermal selegiline is related in part to the rapid turnover of hepatic MAO isozymes. Turnover rates for MAO-A and MAO-B activity are highly tissue and species specific (see Strolin Benedetti & Dostert 1992). In general, MAO turnover rates are approximately three times faster in hepatic tissue as compared with brain (Planz et al 1972). The inhibition of MAO activity by selegiline is irreversible in nature, and so the rate of enzyme turnover would determine the degree of steady-state enzyme inhibition at a given tissue concentration of selegiline, provided that the selegiline concentration remained below the amount necessary for complete enzyme inhibition (Felner & Waldmeier 1979). However, it is also conceivable that selegiline concentrations are much lower in hepatic tissue compared with other tissue sites because of the high level of cytochrome P450 activity available to metabolize selegiline in hepatic cells (Taavitsainen et al 2000). If the latter case were indeed true, a lower level of MAO inhibition would be observed compared with other tissue examined.

Selegiline is a highly lipid soluble substance (as demonstrated by a hexane/water ratio of 82/18) that allows for its rapid penetration of biological membranes and entry into tissues (Magyar & Tothfalusi 1984). Thus its distribution among the different tissues of the body would occur based primarily upon blood flow to those tissues (Goldstein et al 1974). Due to the chemical properties and distribution characteristics of selegiline, the preferential inhibition of

brain vs intestinal MAO-A activity observed after transdermal selegiline administration may relate to differences in the distribution of selegiline to brain and intestinal tissues. Support for this suggestion comes from a recent study by Gaal et al (2000) who demonstrated that guinea-pigs administered selegiline by transdermal patch exhibited much higher concentrations of selegiline within brain tissue as compared with intestine. These authors, however, did not evaluate the pharmacodynamic effects of selegiline on MAO activity in those same tissues and therefore a direct correlation to the findings presented here cannot be made.

Conclusions

We have demonstrated a tissue-selective effect of selegiline on MAO inhibition when administered by transdermal patch but not by oral gavage. The basis for this finding is currently unknown, but may reflect a difference in the pharmacokinetic profile and/or tissue distribution of selegiline when administered by these two routes of administration. The ability to inhibit brain MAO activity while sparing intestinal and hepatic enzymes has therapeutic value for the safe delivery of antidepressant concentrations of selegiline without the risk of acute hypertension following concomitant consumption of dietary tyramine or over-the-counter sympathomimetic amine decongestants. Recent studies conducted in volunteers have confirmed this safety factor for transdermal selegiline administered with oral, encapsulated tyramine or over-the-counter sympathomimetic decongestants (Azzaro et al 2000).

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