J. Pharm. Pharmacol. 1994, 46: 814–819
Received September 15, 1993
Accepted January 27, 1994

The Anticonvulsant FCE 26743 is a Selective and Short-acting MAO-B Inhibitor Devoid of Inducing Properties towards Cytochrome P450-dependent Testosterone Hydroxylation in Mice and Rats

M. STROLIN BENEDETTI, P. MARRARI, M. COLOMBO, M. G. CASTELLI, M. ARAND*, F. OESCH* AND P. DOSTERT

*Pharmacia-Farmitalia Carlo Erba, Research and Development, Via Carlo Imbonati 24, I-20159 Milan, Italy and *Institute of Toxicology, University of Mainz, Obere Zahlbacher Str. 67, D-6500 Mainz, Germany*

Abstract—The effects of the potent anticonvulsant FCE 26743 ((*S*)-2-(4-(3-fluorobenzyloxy)benzylamino)propionamide) on monoamine oxidase (MAO) activity were measured in-vitro and ex-vivo using rat tissue homogenates. In-vitro, FCE 26743 showed potent and selective inhibitory properties towards liver MAO-B, with IC50 values about 10^{-7} M for MAO-B and higher than 10^{-5} M for MAO-A. When determined ex-vivo in brain, the ED50 value for the inhibition of MAO-B was 1·1 mg kg^{-1} (p.o.) 1 h post-dosing, whereas MAO-A remained virtually unaffected after administration of 60 mg kg^{-1}. Similar effects were seen in liver. Following oral administration of 5 mg kg^{-1} FCE 26743 to rats, brain MAO-B inhibition was 79% after 1 h and 13% after 24 h, indicating that FCE 26743 behaves as a short-acting MAO-B inhibitor. The ability of FCE 26743 to act as a MAO substrate was assessed in mice by measuring the urinary excretion of alaninamide, a potential metabolite of FCE 26743 which would result from the action of MAO. No alaninamide was detectable in the 0–8 h urines after administration of a 119 mg kg^{-1} dose, suggesting that FCE 26743 is not, or only to a small degree, a substrate of MAO. The effects of FCE 26743 on cytochrome P450 enzymes involved in testosterone hydroxylation were determined in rats after repeated administration. No induction of the cytochrome P450 system was noted.

The administration of the glycine derivative milacemide (2-(*n*-pentylamino)acetamide) (Fig. 1), an atypical antiepileptic agent, to rats has been shown to increase brain glycine concentrations (Christophe et al 1983). The delivery of glycine from milacemide was later found to result from the action of monoamine oxidase (EC 1·4·3·4, MAO) type B (Janssens de Varebeke et al 1988). We reported that 2-(benzylamino)acetamide (Fig. 1), another glycine derivative, is less readily de-aminated by MAO-B than milacemide and displays lower anticonvulsant activity (Dostert et al 1991). In contrast, FCE 26743, (*S*)-2-(4-(3-fluorobenzyloxy)benzylamino)propionamide (Fig. 1), showed potent protective properties in a number of models of epilepsy in rodents, such as bicuculline-induced convulsions and lethality, maximal electroshock seizures and kainic acid-induced seizures, and status epilepticus (Dostert et al 1991; Maj et al 1993).

Although milacemide and 2-(benzylamino)acetamide are derivatives of the amino acid glycine, they can also be considered as acetamide derivatives of pentylamine and benzylamine, respectively. Pentylamine is an especially good MAO-B substrate (Strolin Benedetti et al 1981), while milacemide (Janssens de Varebeke et al 1988) and benzylamine (Mitra & Guha 1980) are de-aminated more slowly, but to an approximately similar rate to each other. It is thus not surprising that 2-(benzylamino)acetamide is less readily metabolized by MAO-B as compared with milacemide (Dostert et al 1991). Indeed, in 2-(benzylamino)-acetamide are combined both structural elements, the acetamido moiety and the aromatic residue, that separately lead to diminished de-amination of milacemide and benzylamine as compared with pentylamine. The potent anticonvulsant FCE 26743 differs from milacemide and 2-(benzylamino)acetamide in that an alaninamide residue replaces the glycinamide moiety and in the presence of a phenyl ring substituted in the para-position by a 3-fluorobenzyloxy group. Introduction of a benzyloxy group in para of a phenyl ring in MAO substrates and inhibitors has been shown to transform the substrates into inhibitors (Sullivan & Tipton 1990), and to increase the inhibitory potency of the inhibitors (Dostert et al 1989; Mazouz et al 1993).

In this study, the behaviour of FCE 26743 as a MAO inhibitor and substrate was examined. The inhibitory properties of FCE 26743 were assayed in-vitro and ex-vivo. The ability of FCE 26743 to act as a MAO substrate was assessed in-vivo by measuring the urinary excretion of alaninamide in mice. In a previous study, no changes in the urinary excretion of glycinamide were noted in mice after administration of the MAO-resistant α-methyl milacemide (Fig. 1), whereas there was a substantial increase in urinary glycinamide after the MAO-B substrate milacemide (O'Brien et al 1991). Since most of the drugs currently used in anticonvulsant therapy, such as phenobarbitone and carbamazepine (Conney 1986; Eichelbaum et al 1985), are strong inducers of the microsomal cytochrome P450-dependent mono-oxygenase system, the effects of FCE 26743 on the regio- and stereoselective hydroxylation of testosterone in rat liver was studied in comparison with both standard anticonvulsants.

Correspondence: P. Dostert, Pharmacia-Farmitalia Carlo Erba, Research and Development, Via C. Imbonati 24, I-20159 Milan, Italy.

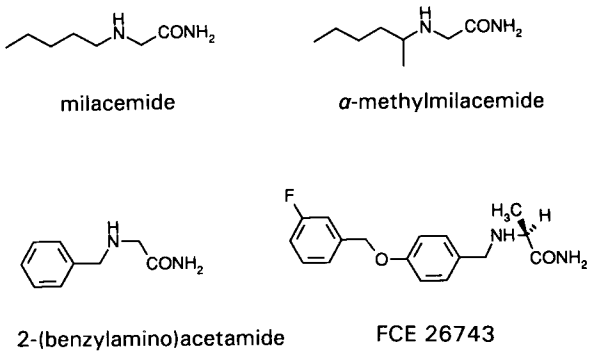

FIG. 1. Structures of milacemide, α-methyl milacemide, 2-(benzylamino)acetamide and FCE 26743.

Materials and Methods

Chemicals

FCE 26743 was synthesized in the General Medicinal Chemistry Group of Farmitalia Carlo Erba, both as the hydrochloride and the methanesulphonate salt. L-Alaninamide hydrochloride, 5-hydroxytryptamine (5-HT) creatinine sulphate and phenyl-ethylamine (PEA) hydrochloride were from Sigma, St Louis, USA. The radiolabelled substrates 5-hydroxy [side chain-2-^{14}C] tryptamine creatinine sulphate ([^{14}C]5-HT, sp. act., 57 mCi mmol^{-1}) and [β-ethyl-1-^{14}C] phenylethylamine hydrochloride ([^{14}C]PEA, sp. act., 55 mCi mmol^{-1}) were purchased from Amersham, UK. Phenobarbitone and carbamazepine were from Compagnia Farmaceutica Milanese (Milan, Italy) and Sigma, respectively, while bovine serum albumin and steroid standards were purchased from Sigma (Deisenhofen, Germany).

Determination of alaninamide in mouse urine

Male mice (CD-1; Charles River, Italy), 20–25 g, were used. The animals, fasted overnight, were housed in separate stainless steel metabolism cages in groups of five. Each group was allowed free access to drinking water.

Twenty mice (four groups of five) received orally 0·38 mmol kg^{-1} FCE 26743 methanesulphonate (114 mg kg^{-1}, expressed in terms of free base) and a further twenty (four groups of five) were administered water as vehicle control. The dose administered was about four times the ED50 in the antibicuculline test (Dostert et al 1991). The 0–8 h urines from each group were collected in dry ice-cooled receivers for the determination of alaninamide.

The determination of alaninamide in urine was performed using high-performance liquid chromatography (HPLC) essentially as described for glycinamide (O'Brien et al 1991). Samples (100 μL) of urine from each group were diluted to 1 mL with water. Samples (20 μL) of the diluted urine were reacted with 20 μL *o*-phthaldialdehyde solution (Pierce, Rockford, IL, USA) using a LABNET modular Spectra-Physics liquid chromatography system equipped with an automatic system for pre-column primary amino acid derivatization. A 10 μL sample of the reaction mixture was automatically injected into the analytical column. The HPLC system consisted of a Spectra-Physics 8800 pump, a Spectra-Physics 8880 autosampler and a Spectra-Physics 4270 computing integrator equipped with a LABNET data-capture module. A Perkin Elmer LS-5 fluorescence detector, set at excitation and emission wavelengths of 230 and 418 nm, respectively, was used to analyse the eluted samples. Chromatography was performed with gradient elution on a 5 μm particle-size Hypersil ODS column (250 × 4.6 mm, i.d.) at a flow rate of 1·5 mL min^{-1}. For gradient elution chromatography solvent A was 0·1 M sodium acetate pH 7·2/methanol/tetrahydrofuran (90/9·5/0·5) and solvent B was methanol. The gradient consisted of solvent A 100 to 95% in 2 min, 95 to 85% in 10 min, 85 to 75% in 12 min, 75 to 70% in 11 min, 70 to 65% in 5 min, and finally 65 to 50% in 5 min. The limit of quantitation was 47·5 pmol alaninamide injected, equivalent to 95 nmol mL^{-1} urine. The retention time of alaninamide was about 42 min.

MAO inhibition

Inhibition of MAO-A and MAO-B was determined in-vitro using rat liver homogenates and ex-vivo using rat brain and liver homogenates, essentially as described by Cao Dahn et al (1984). 5-HT and PEA were used as substrates for MAO-A and MAO-B, respectively.

In-vitro assay

Male Sprague-Dawley rats (Charles River, Italy), 150–175 g, fasted for 16 h were killed by decapitation. The livers were rapidly removed, rinsed in saline, blotted on filter paper, weighed, frozen in liquid nitrogen and stored at −20°C until use. After thawing, the tissues were homogenized in phosphate buffer 0·1 M, pH 7·4, using an Ultra-Turrax (1 g tissue/16 mL buffer). Aliquots (0·1 mL) of tissue homogenates were taken for the determination of MAO activity in a final volume of 0·5 mL. Increasing concentrations of FCE 26743 (range 10^{-9} – 10^{-4} M) in distilled water were added to the samples. The tubes were then removed from the ice-bath and preincubated at 37°C in normal air for 2, 60, or 120 min. The enzymatic reaction conducted at 37°C was started by adding 0·1 mL [^{14}C]PEA (final concentration 4 μM) or [^{14}C]5-HT (final concentration 160 μM). The incubation times were 2 and 5 min for PEA and 5-HT, respectively. The reaction was stopped by cooling the tubes on ice and acidifying with 0·2 mL 4 M HCl. After addition of 7 mL toluene-ethylacetate (1/1, v/v), the organic phase obtained after centrifugation (400 *g*, 10 min) was counted in 10 mL Insta-Fluor II scintillation fluid (Canberra-Packard, USA) using a β-counter (TRI-CARB 1900 CA). The results were expressed as percentage inhibition with respect to the appropriate control. The inhibition curves were obtained by computer-assisted analysis using the ALLFIT program kindly provided by Dr P. Munson, National Institute of Health, Bethesda, USA.

Ex-vivo assay

Groups of five male Sprague-Dawley rats (Charles-River, Italy), 180–200 g, fasted for 16 h, were treated orally with different doses (1, 2, 5, 10 mg kg^{-1} or 10, 20, 40, 60 mg kg^{-1}, as free base) of FCE 26743 in aqueous solution. Control rats were given water. Rats were killed by decapitation 1 h after dosing. In the time-course experiments, the animals were treated orally with FCE 26743 (5 or 60 mg kg^{-1}) and killed at different times after dosing. Control rats received water and were killed after 1 h.

Brain and liver were rapidly removed, rinsed in saline, blotted on filter paper, weighed, frozen in liquid nitrogen and stored at −20°C pending analysis. The homogenization of brain and liver, the enzymatic reaction (without preincubation), the extraction and the sample counting, were carried out as described for the in-vitro assay. The results were expressed as percentage inhibition of MAO activity vs controls.

Regio- and stereoselective hydroxylation of testosterone

Male Sprague-Dawley rats (Charles-River, Italy; initial weight 200–250 g) were assigned to groups of 3–5 animals and housed with free access to standard rat chow and water. FCE 26743 and phenobarbitone were administered orally (gavage) as an aqueous solution for four consecutive days. Carbamazepine was administered as an aqueous suspension in 0·5% (w/v) methocel for the same period of time. The three compounds were administered at a dose of 300 μmol kg^{-1} per day, which for FCE 26743 is equivalent to 91 mg kg^{-1} as free base. One control group (control I) received 0·5% (w/v) methocel in water and another control group (control II) was given water only. The treatment volume was 4 mL kg^{-1} for each administration. The body weight of the animals was recorded on each day from the beginning of the treatment.

All animals were killed after a 24-h fasting period (16 h after the last drug administration) by cervical dislocation. The livers were taken out within 3 min, washed with saline solution (0·9% NaCl), dried briefly on blotting-paper, weighed, immediately frozen in liquid nitrogen, and stored at −80°C until preparation of subcellular fractions.

The livers were separately homogenized in sucrose solution (250 mM containing 10 mM Tris/HCl, pH 7·4) with an Ultra-Turrax homogenizer to give 25% (w/v) homogenates that were subsequently centrifuged for 20 min at 10 000 *g* and 4°C. The resulting supernatant was subjected to a second centrifugation for 60 min at 100 000 *g* and 4°C. The obtained microsomal pellet was resuspended in a volume of homogenization buffer equivalent to the initial liver weight, dispensed into vials, snap-frozen in liquid nitrogen and stored at −80°C until assayed. Protein concentrations were determined according to the method of Bradford (1976) using bovine serum albumin as standard.

The assay for the regio- and stereoselective testosterone hydroxylations indicative for the activities of individual cytochrome P450 isozymes, was performed as described by Oesch et al (1992). The different metabolites were identified by their respective retention times as compared with external standards. Data are presented as the average of the respective groups ± s.d. Significance of the influence of the treatment on the recorded parameters (*t*-test) was analysed using the procedure of Dunnett for multiple comparisons with a control (Dunnett 1964).

Results

The concentration of alaninamide in urine was below the limit of quantitation (95 nmol mL^{-1}) in the treated animals and in the control group of mice.

In-vitro, FCE 26743 was found to inhibit liver MAO-B with IC50 values (mean ± s.d.; n = 5) of 0·25 ± 0·10, 0·14 ± 0·06 and 0·13 ± 0·04 μM after 2, 60 and 120 min of preincubation, respectively. The difference between the IC50 values after 2 and 60 min preincubation was statistically significant ($P < 0.01$, two-way analysis of variance followed by multiple comparison). MAO-A activity was not affected (IC50 > 10 μM) under any of the preincubation conditions.

Table 1. Ex-vivo inhibition of rat brain MAO-A and MAO-B 1 h after oral administration of FCE 26743. Results are given as percent inhibition of MAO.

MAO type	Dose of FCE 26743 (mg kg^{-1})			
	1	2	5	10
MAO-B	37·9 ± 9·2	61·9 ± 4·9	85·9 ± 3·4	88·4 ± 1·6
MAO-A	−1·5 ± 2·8	−1·1 ± 3·5	0·3 ± 4·8	0·9 ± 8·9

MAO-A and MAO-B were assayed using 5-HT (160 μM) and PEA (4 μM) as substrates, respectively. Each value is the mean ± s.d. from five animals.

When rats were given oral graded doses of FCE 26743 and killed 1 h after dosing, brain MAO-B activity was substantially inhibited from the 2 mg kg^{-1} dose, whereas no inhibition of MAO-A activity was found up to the 10 mg kg^{-1} dose (Table 1). Under these experimental conditions, the ED50 for the inhibition of rat brain MAO-B was 1·1 mg kg^{-1} (0·9–1·2, 95% confidence limits). When inhibition of both forms of MAO was determined in brain and liver 1 h after oral administration of FCE 26743 (Table 2), brain MAO-B activity was inhibited by more than 90% from the 10 mg kg^{-1} dose and liver MAO-B from the 20 mg kg^{-1} dose, whereas MAO-A activity remained virtually unaffected in both tissues even at the 60 mg kg^{-1} dose.

The time-course of the inhibition of brain MAO-B in rats given 5 mg kg^{-1} FCE 26743 is shown in Fig. 2. After 1 h the percentage of inhibition of MAO-B was 79 ± 4 (mean ± s.d.) and decreased thereafter. The activity of brain MAO-B returned to almost normal values 24 and 48 h after dosing. When rats were administered an oral dose of 60 mg kg^{-1}

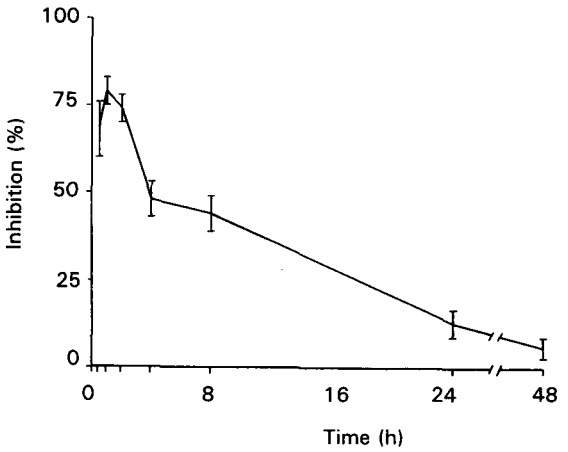

FIG. 2. Time-course of the ex-vivo inhibition of brain MAO-B after the oral administration of 5 mg kg^{-1} FCE 26743 to rats (mean values ± s.d., n = 5).

Table 2. Ex-vivo inhibition of MAO-A and MAO-B in rat brain and liver 1 h after oral administration of FCE 26743. Results are given as percent inhibition of MAO.

MAO type	Tissue	Dose of FCE 26743 (mg kg^{-1})			
		10	20	40	60
MAO-B	Brain	91·3 ± 1·5	94·3 ± 0·9	94·0 ± 0·4	93·6 ± 1·1
	Liver	85·1 ± 1·5	91·0 ± 1·6	94·7 ± 0·7	95·2 ± 0·4
MAO-A	Brain	18·2 ± 4·2	16·7 ± 6·3	14·1 ± 7·9	6·6 ± 6·6
	Liver	−5·9 ± 6·7	0·6 ± 7·4	0·3 ± 7·8	1·0 ± 5·9

MAO-A and MAO-B were assayed using 5-HT (160 μM) and PEA (4 μM) as substrates, respectively. Each value is the mean ± s.d. from five animals.

Table 3. Effect of carbamazepine, phenobarbitone and FCE 26743 on the regio- and stereoselective testosterone hydroxylation in rat liver microsomes.

Treatment (300 μmol kg^{-1}/day, 4 days)	Testosterone hydroxylation (pmol (mg protein)$^{-1}$ min^{-1})								
	2α	2β	6α	6β	7α	15β	16α	16β	Androstenedione
Control	1661 ±672	108 ±45	208 ±15	1361 ±352	607 ±88	38 ±16	2480 ±1004	18 ±8	1496 ±518
Carbamazepine	778 ±312*	227 ±53**	237 ±16*	1780 ±529	487 ±26	92 ±19**	2146 ±722	621 ±253**	1874 ±561
Control	1370 ±363	88 ±21	218 ±23	1198 ±254	677 ±180	26 ±6	1969 ±512	16 ±5	1324 ±328
Phenobarbitone	618 ±122	606 ±178**	258 ±29	2952 ±836**	885 ±128	292 ±91**	2504 ±521	960 ±330**	2137 ±558*
FCE 26743	1331 ±542	55 ±28	209 ±12	899 ±259	481 ±92	20 ±11	2163 ±901	25 ±6	1228 ±391

*$P < 0.05$, **$P < 0.01$ with respect to the corresponding control group (Dunnett's test). Values are mean ± s.d., n = 3–5.

FCE 26743 and inhibition of both forms of MAO was determined 0·5, 1, 2 and 6 h after dosing, about 92% inhibition of MAO-B activity was found at all times in brain, whereas inhibition of MAO-A never exceeded 10%. Similar results (data not shown) were found in liver, where, however, the inhibition of MAO-B was slightly lower (range 79–87%) than that measured in brain.

In the induction study, no signs of toxicity were observed in rats of any of the investigated dose groups throughout the treatment period. The liver weight appeared to be moderately enhanced after carbamazepine (116% of control, $P < 0.05$) and phenobarbitone (135% of control, $P < 0.01$) treatment. For FCE 26743, no significant treatment-related effect was observed concerning liver weight. No significant changes in the microsomal protein content were observed in any of the treatment groups.

The effects of FCE 26743, carbamazepine and phenobarbitone on testosterone metabolism are reported in Table 3. No changes in the various regio- and stereoselective hydroxylations of testosterone were observed in the rats treated with FCE 26743.

Discussion

FCE 26743 was found to be a potent and highly selective inhibitor of MAO type B. Thus, 1 h after the oral administration of 5 mg kg^{-1} FCE 26743, brain MAO-B was inhibited by about 86%, whereas MAO-A activity remained virtually unaffected after a 60 mg kg^{-1} dose. When determined in-vitro, the selectivity of FCE 26743 toward MAO-B as measured by the ratio IC50 MAO-A/IC50 MAO-B was greater than 400 and 700 after 2 and 60 min incubation, respectively. The short-lasting MAO-B inhibitory activity of FCE 26743 was established in the experiment reported in Fig. 2, where the inhibition of brain MAO-B activity was about 80% 1 h after the oral administration of a 5 mg kg^{-1} dose to rats and almost negligible (13%) 24 h post-dosing.

Although the determination of urinary alaninamide does not allow a sensitive and direct evaluation of the ability of FCE 26743 to behave as a MAO substrate, the absence of detectable alaninamide in urine suggests that FCE 26743 is, at least to a large extent, resistant to the action of MAO. For comparison, when the MAO-B substrate milacemide was given to mice 15% of the dose was recovered in the 0–8 h urine as glycinamide (O'Brien et al 1991). In the present study, 0·38 mmol kg^{-1} FCE 26743 was given to each mouse and an average volume of 5 mL urine was collected per group of five animals. Assuming that all administered FCE 26743 had been metabolized to, and excreted in urine as alaninamide, a concentration of 8·4 μmol mL^{-1} urine would have been found. Provided that 15% of the dose had been excreted as unchanged alaninamide, as was the case for the excretion of glycinamide from milacemide, a concentration of 1260 nmol mL^{-1} alaninamide would be expected. Therefore, according to the limit of quantitation of alaninamide in urine, a rough estimate of the ability of FCE 26743 to act as a MAO-B substrate indicates that FCE 26743 is metabolized at a rate less than one-thirteenth that of milacemide, assuming that glycinamide and alaninamide have a similar metabolic fate in the mouse. In-vitro, the potency of FCE 26743 as a MAO-B inhibitor was significantly higher after 60 min compared with 2 min preincubation. Although a time-dependent increase in MAO-B inhibitory potency

was also shown for the MAO-B substrate milacemide (Janssens de Varebeke et al 1989), the effect observed with FCE 26743 does not necessarily imply that this compound also behaves as a MAO-B substrate. Additional experiments are needed to establish whether FCE 26743 acts as a slow-binding inhibitor, or is metabolized to some degree by MAO-B to form an adduct with the enzyme, which would dissociate under in-vivo conditions, as has already been shown to occur with other short-acting MAO-B inhibitors (Dostert et al 1983; Tipton et al 1983).

Both carbamazepine and phenobarbitone treatment strongly enhanced the hydroxylation of testosterone in the 16β-position, which is indicative for the induction of isoenzymes of the cytochrome P4502B subfamily. In addition, phenobarbitone and, to a smaller extent carbamazepine, increased the formation of 2β- and 15β-hydroxytestosterone that are the specific metabolites of cytochrome P4503A isoenzymes. The observed increase in testosterone 6β-hydroxylation by phenobarbitone can also be explained by a cytochrome P4503A induction. As this metabolite occurs as the product of a variety of different cytochromes P450 the increase in its formation is less prominent than that observed with the more specific metabolites. In contrast to the findings with phenobarbitone and carbamazepine, the administration of FCE 26743 to rats for four days at a daily dose about eight times the ED50 value in the maximal electroshock test resulted in no apparent effect on the pattern of testosterone metabolites formed by cytochrome P450 enzymes. If confirmed by further work, this characteristic of the anticonvulsant FCE 26743 would be of great interest as a number of cytochrome P450 enzymes, such as the 3A subfamily, have been shown to be involved in the metabolism of many drugs (for review see Gonzalez 1992). Moreover, epileptic patients are treated for all their lifespan and are generally polymedicated.

Although the presence of both anticonvulsant and MAO inhibitory properties in the same molecule is common, none of the currently used anti-epileptics, have been shown to be potent inhibitors of MAO, with the exception of milacemide whose clinical evaluation as an anti-epileptic was discontinued. The potential interest of combining both MAO-B inhibition and anti-epileptic activity in the same molecule remains to be established.

The pathogenesis of epilepsy is poorly understood. There is evidence that brain lipid peroxidation is increased as a consequence of oxidative stress in iron-induced and also in transition metal-independent animal models of epilepsy (Triggs & Willmore 1984; Sobaniec et al 1989; Singh & Pathak 1990). This effect is antagonized by administration of vitamin E (Willmore et al 1986; Sobaniec et al 1989) but is not affected by phenytoin treatment (Willmore & Triggs 1984). Moreover, it has been reported that the catalytic properties of brain mitochondrial MAO are modified upon incubation with lipid peroxidation inducers (Medvedev et al 1992), and in a strain of rats with inherited audiogenic epilepsy (Rajgorodskaya et al 1991). In both cases, γ-hydroxybutyric acid was found to be metabolized by the modified MAO.

The involvement of neurotransmitter systems in the cellular mechanism of epilepsy has been suggested (for review see Sherwin & van Gelder 1986). Higher brain monoamine levels have been shown to reduce seizure susceptibility (Prockop et al 1959; Mishra et al 1993). It has also been reported that the synthesis and release of monoamines, of dopamine in particular, is increased in seizure foci compared with nonfocus regions in the human brain (Goldstein et al 1988; Pintor et al 1990), maybe as a defence response to seizure susceptibility. Administration of the MAO-B inhibitor selegiline to Parkinson's disease patients was shown to enhance brain dopamine concentrations (Riederer & Youdim 1986). Moreover, binding of selegiline was shown to be significantly higher in the hippocampus from patients with intractable partial complex epilepsy as compared with nonepileptic controls (Kumlien et al 1992). Therefore, one can ask whether the MAO-B inhibitory property of FCE 26743 might contribute to its anti-epileptic activity by increasing brain dopamine levels during long-term treatment.

The inhibition of MAO-B by FCE 26743 is likely to result in a decreased formation of H_2O_2 in brain. Whether this effect might induce a lower level of oxidative stress in epileptic patients can hardly be anticipated. The increase in oxidative stress in striatum, as measured by oxidized glutathione levels, caused by haloperidol-induced dopamine turnover was largely suppressed by pretreatment of mice with selegiline (Cohen 1990). This result suggests that a greater risk of peroxidative damage is associated with increased dopamine availability, and can be prevented by inhibition of MAO-B. However, it has recently been reported that the protective effects of selegiline against oxidative damage caused by dopamine could result from a radical scavenging property of the molecule rather than from the prevention of H_2O_2 formation by inhibition of MAO activity (Chiueh et al 1994). It is not known whether FCE 26743 possesses the same radical scavenging ability as selegiline. If not, it would, however, remain to be determined whether oxidative stress associated with epilepsy might be attenuated appreciably by FCE 26743 treatment as a consequence of the sole decrease in H_2O_2 formation.

The cytochrome P450 system-inducer phenobarbitone has been shown to increase lipid peroxidation and to decrease vitamin E levels in rat liver (Ono et al 1986). This effect is likely to result from an increased formation of superoxide radical by phenobarbitone-induced cytochrome P450 isoforms (Paolini et al 1992) and should not occur with FCE 26743.

In conclusion, a molecule like FCE 26743 that, in addition to its potent anticonvulsant properties, might decrease oxidative stress and contribute to enhancement of brain dopamine levels by inhibiting MAO-B activity, and should not affect the levels of the natural radical scavenger vitamin E as a result of its lack of effect on the phenobarbitone inducible cytochromes P450, might be particularly well equipped to compete advantageously with the currently used anti-epileptics.

Acknowledgement

The authors wish to thank Dr P. Tocchetti and Mrs H. Wagner for their contribution to this study, and Ms L. Magné for the preparation of the manuscript.

References

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem. 72: 248–254

Cao Dahn, H., Strolin Benedetti, M., Dostert, P. (1984) Differential changes in monoamine oxidase A and B activity in aging rat tissues. In: Tipton, K. F., Dostert, P., Strolin Benedetti, M. (eds) Monoamine Oxidase and Disease. Prospects for Therapy with Reversible Inhibitors. Academic Press, London, pp 301–317

Christophe, J., Kutzner, R., Nguyen-Bui, N.D., Damien, C., Chatelain, P., Gillet, L. (1983) Conversion of orally administered 2-n pentylaminoacetamide into glycinamide and glycine in the rat brain. Life Sci. 33: 533–541

Chiueh, C. C., Huang, S.-J., Murphy, D. L. (1994) Suppression of hydroxyl radical formation by MAO inhibitors: a novel possible neuroprotective mechanism in dopaminergic neurotoxicity. J. Neural Transm. In press

Cohen, G. (1990) Monoamine oxidase and oxidative stress at dopaminergic synapses. J. Neural Transm. 32 (Suppl.): 229–238

Conney, A. H. (1986) Induction of microsomal cytochrome P-450 enzymes: the first Bernard B. Brodie lecture at Pennsylvania State University. Life Sci. 39: 2493–2518

Dostert, P., Strolin Benedetti, M., Guffroy, C. (1983) Different stereoselective inhibition of monoamine oxidase-B by the *R*- and *S*-enantiomers of MD 780236. J. Pharm. Pharmacol. 35: 161–165

Dostert, P., Strolin Benedetti, M., Tipton, K. F. (1989) Interactions of monoamine oxidase with substrates and inhibitors. Med. Res. Rev. 9: 45–89

Dostert, P., Strolin Benedetti, M., Tipton, K. F. (1991) New anticonvulsants with selective MAO-B inhibitory activity. Eur. J. Neuropsychopharmacol. 1: 317–319

Dunnett, C. W. (1964) New tables for multiple comparisons with a control. Biometrics 20: 482–491

Eichelbaum, M., Tomson, Y., Tybring, G., Bertilsson, L. (1985) Carbamazepine metabolism in man. Induction and pharmacogenetic aspects. Clin. Pharmacokinet. 10: 80–90

Goldstein, D. S., Nadi, N. S., Stull, R., Wyller, A. R., Porter, R. J. (1988) Levels of catechols in epileptogenic and nonepileptogenic regions of the human brain. J. Neurochem. 50: 225–229

Gonzalez, F. J. (1992) Human cytochromes P-450: problems and prospects. Trends Pharmacol. Sci. 13: 346–352

Janssens de Varebeke, P., Cavalier, R., David-Remacle, M., Youdim, M. B. H. (1988) Formation of the neurotransmitter glycine from the anticonvulsant milacemide is mediated by brain monoamine oxidase B. J. Neurochem. 50: 1011–1016

Janssens de Varebeke, P., Pauwels, G., Buyse, C., David-Remacle, M., De Mey, J., Roba, J., Youdim, M. B. H. (1989) The novel neuropsychotropic agent milacemide is a specific enzyme-activated inhibitor of brain monoamine oxidase B. J. Neurochem. 53: 1109–1116

Kumlien, E., Hilton-Brown, P., Spännare, B., Gillberg, P.-G. (1992) In vitro quantitative autoradiography of [^{3}H]-l-deprenyl and [^{3}H]-PK 11195 binding sites in human epileptic hippocampus. Epilepsia 33: 610–617

Maj, R., Antongiovanni, V., Bonsignori, A., Breda, M., Dostert, P., Fariello, R. G., McArthur, R. A., Varasi, M., Bianchetti, A. (1993) Anticonvulsant profile of benzylaminopropanamide derivatives in mice and rats. Abstract presented at the Focus on Epilepsy II, International Conference, Whistler (Canada), September 11–14

Mazouz, F., Gueddari, S., Burstein, C., Manusy, D., Milcent, R. (1993) 5-[4-(Benzyloxy)phenyl]-1,3,4-oxadiazol-2(3H)-one derivatives and related analogues: new reversible, highly potent, and selective monoamine oxidase type B inhibitors. J. Med. Chem. 36: 1157–1167

Medvedev, A. E., Rajgorodskaya, D. I., Gorkin, V. Z., Fedotova, I. B., Semiokhina, A. F. (1992) The role of lipid peroxidation in the possible involvement of membrane-bound monoamine oxidases in gamma-aminobutyric acid and glucosamine deamination in rat brain. Mol. Chem. Neuropathol. 16: 187–201

Mishra, P. K., Kahle, E. H., Bettendorf, A. F., Dailey, J. W., Jobe, P. C. (1993) Anticonvulsant effects of intracerebroventricularly administered norepinephrine are potentiated in the presence of monoamine oxidase inhibition in severe seizure genetically epilepsy-prone rats (GEPR-9s). Life Sci. 52: 1435–1441

Mitra, C., Guha, S. R. (1980) Serotonin oxidation by type B MAO of rat brain. Biochem. Pharmacol. 29: 1213–1216

O'Brien, E., Tipton, K. F., Strolin Benedetti, M., Bonsignori, A., Merrari, P., Dostert, P. (1991) Is the oxidation of milacemide by monoamine oxidase a major factor in its anticonvulsant actions? Biochem. Pharmacol. 41: 1731–1737

Oesch, F., Wagner, H., Platt, K. L., Arand, M. (1992) Improved sample preparation for the testosterone hydroxylation assay using disposable extraction columns. J. Chromatogr. 582: 232–235

Ono, J., Mimaki, T., Yabuuchi, H. (1986) Effects of phenobarbital on lipid peroxidation in vitamin E-deficients rats. Pediatr. Pharmacol. 5: 223–227

Paolini, M., Pedulli, G. F., Borzatta, V., Pozzetti, L., Cipollone, M., Cantelli-Forti, G. (1992) Superoxide production by IA1, IA2, IIB1, IIIA, IVA, IIE1-inducible cytochrome P450 isoforms in biological systems. An EPR investigation. Proceedings of the Fourth European ISSX Meeting, Bologna, Italy, July 3–6, p. 90

Pintor, M., Mefford, I. N., Huttler, I., Pocotte, S. L., Wyler, A. R., Nadi, N. S. (1990) Levels of biogenic amines, their metabolites, and tyrosine hydroxylase activity in the human epileptic temporal cortex. Synapse 5: 152–156

Prockop, D. J., Shore, P. A., Brodie, B. B. (1959) An anticonvulsant effect of monoamine oxidase inhibitors. Experientia 15: 145–147

Rajgorodskaya, D. I., Medvedev, A. E., Gorkin, V. Z., Fedotova, J. B., Semiokhina, A. F. (1991) On the alteration of catalytic properties of mitochondrial monoamine oxidases in experimental audiogenic epilepsia. Vopr. Med. Khim. 37: 46–48

Riederer, P., Youdim, M. B. H. (1986) Monoamine oxidase activity and monoamine metabolism in brains of parkinsonian patients treated with L-deprenyl. J. Neurochem. 46: 1359–1365

Sherwin, A. L., van Gelder, N. M. (1986) Amino acid and catecholamine markers of metabolic abnormalities in human focal epilepsy. Adv. Neurol. 4: 1011–1032

Singh, R., Pathak, D. N. (1990) Lipid peroxidation and glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, and glucose-6-phosphate dehydrogenase activities in Fe Cl_3-induced epileptogenic foci in the rat brain. Epilepsia 31: 15–26

Sobaniec, W., Rudzinski, P., Jankowicz, E., Sobaniec-Lotowska, M., Kulak, W. (1989) Cardiazol-induced seizures and the concentration of lipid peroxides in the brain of rats under the influence of valproic acid and vitamin E. Neuropath. Pol. 27: 129–136

Strolin Benedetti, M., Sontag, N., Boucher, T., Kan, J. P. (1981) Aliphatic amines as MAO substrates in the rat: the effect of selective inhibitors on the deamination of n-pentylamine. In: Usdin, E., Weiner, N., Youdim, M. B. H. (eds) Function and Regulation of Monoamine Enzymes. Macmillan, London, pp 527–538

Sullivan, J. P., Tipton, K. F. (1990) The interactions of monoamine oxidase with some derivatives of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). J. Neural Transm. 29(Suppl.): 269–277

Tipton, K. F., Fowler, C. J., McCrodden, J. M., Strolin Benedetti, M. (1983) The enzyme-activated irreversible inhibition of type-B monoamine oxidase by 3-(4-[(3-chlorophenyl)methoxy]phenyl)-5-[(methylamino)methyl]-2-oxazolidinone methanesulphonate (compound MD780236) and the enzyme-catalysed oxidation of this compound are competing reactions. Biochem. J. 209: 235–242

Triggs, W. J., Willmore, L. J. (1984) In vivo lipid peroxidation in rat brain following intracortical Fe^{2+} injection. J. Neurochem. 42: 976–980

Willmore, L. J., Triggs, W. J. (1984) Effect of phenytoin and corticosteroids on seizures and lipid peroxidation in experimental posttraumatic epilepsy. J. Neurosurg. 60: 467–472

Willmore, L. J., Triggs, W. J., Gray, J. D. (1986) The role of iron-induced hippocampal peroxidation in acute epileptogenesis. Brain Res. 382: 422–426