Inhibition of Monoamine Oxidase Type A, but Not Type B, Is an Effective Means of Inducing Anticonvulsant Activity in the Kindling Model of Epilepsy

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ABSTRACT

The anticonvulsant activity of inhibitors of monoamine oxidase (MAO) was reported early after the development of irreversible MAO inhibitors such as tranylcypromine, but was never clinically used because of the adverse effects of these compounds. The more recently developed reversible MAO inhibitors with selectivity for either the MAO-A or MAO-B isoenzyme forms have not been studied extensively in animal models of epilepsy, so it is not known which type of MAO inhibitor is particularly effective in this respect. We compared the following drugs in the kindling model of epilepsy: 1) L-deprenyl (selegiline), i.e., an irreversible inhibitor of MAO-B, which, however, also inhibits MAO-A at higher doses, 2) the novel reversible MAO-B inhibitor LU 53439 (3,4-dimethyl-7-(2-isopropyl-1,3,4-thiadiazol-5-yl)methoxy-coumarin), which is much more selective for MAO-B than L-deprenyl, 3) the novel reversible and highly selective MAO-A inhibitor LU 43839 (esuprone; 7-hydroxy-3,4-dimethyl-coumarin ethanesulfonate), and 4) the irreversible nonselective MAO inhibitor tranylcypromine. Esuprone proved to be an effective anticonvulsant in the kindling model with a similar potency as L-deprenyl. In contrast to esuprone and L-deprenyl, the selective MAO-B inhibitor LU 53439 was not effective in the kindling model; this substantiates the previous notion that the anticonvulsant activity of L-deprenyl is not related to MAO-B inhibition, but to other effects of this drug, such as inhibition of MAO-A. Drugs inhibiting both MAO-A and MAO-B to a similar extent (tranylcypromine) or combinations of selective MAO-A and MAO-B inhibitors (esuprone plus LU 53439) had no advantage over MAO-A inhibition alone, but were less well tolerated. The data thus suggest that selective MAO-A inhibitors such as esuprone may be an interesting new approach for the treatment of epilepsy.

Monoamine oxidase (MAO; EC 1.4.3.4) plays an essential role in the oxidative deamination of biogenic and food-derived amines, both in the central nervous system and in peripheral tissues (Glover and Sandler, 1986; Magyar, 1993). MAO exists in two functional isoenzyme forms, MAO-A and MAO-B, each of which shows preferential affinity for substrates and specificity toward inhibitors (Knoll, 1978; Glover and Sandler, 1986; Magyar, 1993). With respect to biogenic amines, MAO-A preferentially metabolizes serotonin, noradrenaline, and adrenaline, whereas MAO-B preferentially oxidizes phenylethylamine. Dopamine is considered as a mixed type of substrate; it is preferentially oxidized by MAO-A in the rat brain, but by MAO-B in the human brain (Magyar, 1993). MAO inhibitors can be classified according to their selectivity for the two forms of the enzyme. The first generation of MAO inhibitors with drugs such as tranylcyromine has no selective inhibitory potency toward MAO-A and MAO-B, whereas the second or new generation of inhibitors involves selective inhibitors of MAO-A, such as moclobemide, and MAO-B, such as L-deprenyl (selegiline).

The main indication for MAO-A inhibitors is depression, whereas MAO-B inhibitors such as L-deprenyl are used as an adjunct to the dopamine precursor L-dopa in therapy of Parkinson’s disease (Knoll, 1992; Murphy et al., 1995). In addition, several other brain diseases have been discussed as potential indication for MAO inhibitors (Da Prada et al., 1990; Knoll, 1992; Yu, 1994; Palomo et al., 1996). One of these is epilepsy, i.e., a common brain disorder characterized by spontaneous recurrent seizures. Early studies showed that the old MAO inhibitors exhibit anticonvulsant effects in different seizure models in rodents (cf., Przegalinski, 1985), but this effect was not clinically used because of the adverse effects of such agents. More recently, it has been shown that both selective MAO-A and MAO-B inhibitors exert anticonvulsant activity in seizure models (Sparks and Buckholtz, 1985; Mukhopadhyay et al., 1987; Dostert et al., 1991; Medvedev et al., 1992; Strolin-Bendetti et al., 1994; Lösch and Hönack, 1995; Ulugol et al., 1995; Lösch and Lehmann, 1996, 1998. Because these more recent drugs are much better

ABBREVIATIONS: ADT, afterdischarge threshold; MAO, monoamine oxidase.
tolerated than the early MAO inhibitors, it has been proposed that inhibition of MAO may be an interesting strategy for developing novel anticonvulsant agents (Löscher and Hönack, 1995; Löscher and Lehmann, 1996, 1998). The most extensively studied drug in this respect is L-deprenyl. However, the selectivity of this drug toward MAO-B is quite limited, particularly when the drug is administered chronically (Gerlach et al., 1992; Knoll, 1992; Lange et al., 1994; Gerlach et al., 1996; Olanow, 1996; Tatton and Chalmersredman, 1996; Tatton et al., 1996; Knoll, 1998). Indeed, recent experiments by our group cast doubt on whether anticonvulsant effects of L-deprenyl are mediated by irreversible MAO-B inhibition (Löscher and Hönack, 1995; Löscher and Lehmann, 1996, 1998). At higher doses (>1 mg/kg), L-deprenyl has been shown to inhibit MAO-A, too (Gerlach et al., 1992; Magyar, 1993). Furthermore, chronic administration of L-deprenyl may result in a progressive inhibition of MAO-A in both rodents and humans, even at doses that if administered acutely would be MAO-B specific (Knoll, 1978, 1986; Gerlach et al., 1992). Thus, it is not possible to judge whether the anticonvulsant activity seen with L-deprenyl at doses >1 mg/kg was related to inhibition of MAO-A, MAO-B, or both.

For clarification of the role of MAO-A and MAO-B inhibition as a means of inducing anticonvulsant effects, we directly compared selective inhibitors of MAO-A and MAO-B, nonselective MAO inhibitors, and combinations of selective MAO-A and MAO-B inhibitors in the kindling model of epilepsy. Kindling represents a model of temporal lobe epilepsy, i.e., the most common type of epilepsy in humans (Sato et al., 1990; Löscher and Schmidt, 1994). Because this type of epilepsy is often resistant to treatment with current antiepileptic drugs, there is a need to develop new principles for improved therapy of this disease. In view of the potent anticonvulsant effects of L-deprenyl in the kindling model (Löscher and Hönack, 1995), we hoped that MAO inhibitors with higher selectivity than L-deprenyl might be interesting candidates for epilepsy therapy. Two novel MAO inhibitors were used in this respect. LU 53439 (3,4-dimethyl-7-(2-isopropyl-1,3,4-thiadiazol-5-yl)-methoxy-coumarin) is a reversible MAO-B inhibitor and much more selective in this regard than L-deprenyl (IC50 of LU 53439 for MAO-B is 0.9 nM, for MAO-A, 10,000 nM; Drescher et al., 1993). LU 43839 (esuprone; 7-hydroxy-3,4-dimethylcoumarin ethanesulfonate) is a highly selective reversible inhibitor of MAO-A (IC50 for MAO-A is 7.3 nM, for MAO-B, >1,000 nM; Traut et al., 1992). Both compounds are potent and selective for MAO-A or MAO-B inhibition in vivo and were used at doses recently shown to almost completely inhibit the respective MAO isoenzyme in the rodent brain after oral administration (Traut et al., 1992; Drescher et al., 1993).

**Materials and Methods**

**Animals.** Female Wistar rats (Harlan-Winkelmann, Borchen, Germany), weighing 200 to 300 g, were used. The animals were purchased from the breeder at a body weight of 180 to 220 g. After arrival in the animal colony, the rats were kept under controlled environmental conditions (ambient temperature 24–25°C, humidity 50–60%, 12:12-h light/dark cycle, light on at 7:00 AM) for at least 1 week before being used in the experiments. Standard laboratory chow (Altromin 1324 standard diet) and tap water were allowed ad libitum. With respect to the use of females, it is important to note that we previously showed that neither seizure susceptibility nor anticonvulsant drug effects are affected by the estrous cycle in fully kindled female rats as used in the present study (Rundfeldt et al., 1999; Wahnschaffe and Löscher, 1992).

**Kindling.** A bipolar electrode was stereotaxically implanted into the basolateral nucleus of the right amygdala as described previously (Löscher and Hönack, 1995). After a postoperative period of 2 weeks, constant current stimulations (500 μA, 1 ms, monophasic square-wave pulses, 50/s for 1 s) were delivered to the amygdala at intervals of 1 day until 10 sequential fully kindled (i.e., focal and secondarily generalized clonic) seizures were elicited.

**Evaluation of Anticonvulsant Activity.** For evaluation of anticonvulsant drug effects on focal seizures, the afterdischarge threshold (ADT), i.e., the most sensitive measure of anticonvulsant activity against focal seizure activity in kindled rats, was recorded after kindling acquisition (with an interval of at least 4 days after the 10th stage 5 seizure) using an ascending stairstep procedure (Freeman and Jarvis, 1981). The initial current intensity was 10 μA, and the current intensity was increased in steps of about 20% of the previous current at intervals of 1 min until an afterdischarge at least 3 s in duration was elicited. In addition to ADT, the following parameters of kindled seizures were measured at ADT current. Seizure severity was classified according to Racine (1972): 1) immobility, eye closure, twitching of vibrissae, sniffing, facial clonus; 2) head nodding associated with more severe facial clonus; 3) clonus of one forelimb; 4) rearing, often accompanied by bilateral forelimb clonus; and 5) rearing with loss of balance and falling accompanied by generalized clonic seizures. Almost all rats showed focal (stages 1 and 2) and secondarily generalized (stages 3–5) seizures at focal seizure threshold (ADT) currents. Seizure duration was the duration of limbic seizures (stages 1 and 2) and/or motor (stages 3–5) seizures. Afterdischarge duration was the total time of spikes in the electroencephalogram recorded from the site of stimulation. After all rats showed reproducible ADTs, the effects of MAO inhibitors on ADT and severity and duration of seizures recorded at ADT were determined in groups of 6 to 10 fully kindled rats after i.p. (L-deprenyl) or oral (L-deprenyl and all other drugs) administration. All MAO inhibitors were used at doses previously shown to cause up to complete inhibition of the respective MAO isoenzyme in rodent brain after oral administration (Traut et al., 1992; Celada and Artigas, 1993; Drescher et al., 1993; Magyar, 1993). The control ADT was determined 2 to 3 days before and after each drug treatment, and the next drug experiment was only undertaken if the postdrug ADT was not significantly different from the predrug ADT. For control determinations, rats received i.p. or p.o. administration of vehicle with the same pretreatment time as in the respective drug experiment. For all drug experiments, at least 7 days were interposed between two drug injections in the same group of rats to avoid alterations in drug potency due to accumulation or tolerance. Some experiments with L-deprenyl and esuprone were repeated with the same dose and pretreatment time to examine the reproducibility of the anticonvulsant effect of these drugs. Significance of differences between seizure readings (ADT and severity and duration of seizures) in the same group of rats (e.g., the difference between control and drug trial) was calculated by the Wilcoxon signed rank test for paired replicates.

**Evaluation of Adverse Effects.** For examination of behavioral drug effects, the animals were removed from their home cages and placed singly in plastic cages. The animals were continuously observed for alterations in behavior after i.p. drug injection up to the time of amygdala stimulation. For comparative evaluation of experiments, behavioral alterations determined immediately before ADT determination were used. Control experiments with vehicle injection were done in the same way. For all observations, rigorous observational protocols described elsewhere were used (Löscher and Hönack, 1992). Hyper- or hypolocomotion, head weaving (swaying movements of the head and upper torso from side to side for at least one complete cycle; i.e., left-right-left), stereotyped sniffing, biting, licking or grooming, reciprocal forepaw treading (“piano playing”), stereotyped rearing, reduction of normal rearing, hyperexcitability (as indicated by...
increased reactions to noise or handling), tremor, abduction of hind limbs, reduction of righting reflexes, flat body posture, circling, Straub tail, and piloerection were scored using a ranked intensity scale where 0 = absent, 1 = equivocal, 2 = present, and 3 = intense. Ataxia was scored using a 6-point rating system as described previously (Hönack and Lösch, 1995). In addition to rating motor impairment by observation scores, impaired motor function was quantitated by the Rotarod test as described previously (Hönack and Lösch, 1995).

In all experiments, rectal body temperature was recorded by an electronic thermometer immediately before drug or vehicle administration as well as 2 min before ADT determination. A third body temperature recording was taken between the latter two measurements, e.g., at 30 min in experiments with a pretreatment time of 1 h. Significance of difference to predrug values in the same group of rats was determined by Student’s t test for paired data. All rats were habituated to the various manipulations before onset of the drug experiments. Vehicle injection did not induce any behavioral alterations or Rotarod failures, but sometimes significantly increased body temperature, most likely due to the stress associated with handling of the animals.

**Drugs.** LU 53439 and esuprone (LU 43839) were provided by Knoll AG, BASF Pharma (Ludwigshafen, Germany). R(-)-deprenyl (l-deprenyl) hydrochloride was obtained from RBI (Biotrend, Köln, Germany). Tranylcypromine (trans-2-phenylcyclopropylamine) hydrochloride was purchased from Sigma (Deisenhofen, Germany). For i.p. injections, l-deprenyl was freshly dissolved in distilled water before each experiment. For oral administrations, drugs were freshly suspended in an aqueous solution of 0.5% hydroxypropyl methylcellulose before each drug administration. All doses or drug concentrations refer to the free drug forms. Controls received the respective vehicle (saline i.p. or hydroxypropyl methylcellulose p.o.) administrations. Administration volumes were 2 ml/kg (i.p.) or 2.5 ml/kg (p.o.).

**Results**

**Anticonvulsant and Adverse Effects of l-Deprenyl.** As reported recently (Lösch and Hönack, 1995), i.p. administration of l-deprenyl, 10 or 20 mg/kg, significantly increased focal seizure threshold (ADT) in fully kindled rats when tested 1 h after injection (Fig. 1). When the same dose (10 mg/kg) was given in two separate experiments in kindled rats, l-deprenyl induced a significant ADT increase in both experiments (Fig. 1), demonstrating the reproducibility of this effect. Although the percent increase in ADT above predrug control was somewhat higher in the second experiment with 10 mg/kg (Fig. 1), there was no significant difference between either control ADTs or ADTs after l-deprenyl in the two experiments.

Because we wanted to test all other drugs by the oral route, we also administered l-deprenyl p.o. (Fig. 1). One hour after oral administration, l-deprenyl, 20 mg/kg, markedly increased ADT by 160% above control ($P = .0039$), but the effect was lost after 2 or 4 h, arguing against irreversible MAO-B inhibition as underlying the anticonvulsant effect. In addition to an increase of the electrical current needed to induce focal and secondarily generalized seizures, a significant decrease in seizure duration and afterdischarge duration was seen in some of the experiments with l-deprenyl, indicating that l-deprenyl not only increased focal seizure threshold but also decreased seizure spread from the focus.

Except slight (stage 1) ataxia in some rats shortly after administration, no other behavioral alterations were noted after l-deprenyl. All rats passed the Rotarod test without any problem. In some but not all experiments, a moderate but significant decrease of rectal body temperature by 0.2–0.5°C was recorded; this was not seen in vehicle control experiments (not illustrated).

**Anticonvulsant and Adverse Effects of LU 53439.** The selective and reversible MAO-B inhibitor LU 53439 did not exert any marked effects in kindled rats (Fig. 2). At 20 mg/kg p.o., this drug slightly but significantly increased ADT by 27% above control ($P = .0235$) when ADT was determined 1 h after drug administration, but no ADT increase was seen when the dose was increased to 40 mg/kg. Seizure parameters recorded at ADT were not affected by LU 53439.

No behavioral alterations were seen after administration of LU 53439, and all rats passed the Rotarod test. Body temperature was not significantly altered by this drug.

**Anticonvulsant and Adverse Effects of Esuprone (LU 43839).** In contrast to LU 53439, the selective and reversible MAO-A inhibitor esuprone was highly effective in the kindling model (Fig. 3). In a first group of kindled rats, esuprone, 20 mg/kg p.o., significantly increased ADT by 130% above control ($P = .0078$) when ADT was determined 2 h after drug administration. A repeat of this experiment in another group of kindled rats resulted in a significant, albeit less marked ADT increase. A variation in interindividual and individual ADT responses to anticonvulsant drug effects is also known for other drugs in kindled rats, but in females this is not related to different stages of the estrous cycle (Rundfeldt et al., 1990).

When ADT was determined 1 h after administration of esuprone, 20 mg/kg, an ADT increase of 120% ($P = .00293$) was obtained (Fig. 3). This anticonvulsant effect of esuprone was long-lasting, because a significant ADT increase was also seen 4 h after drug administration (Fig. 3). Increase of dosage to 40 mg/kg did not further increase the anticonvulsant effect determined 2 h after administration. There was a tendency for decreased seizure severity and duration in most experiments with esuprone, but this was insignificant in most cases. However, it should be noted in this respect that in case rats had been stimulated with a current 20% above their individual ADT, then esuprone would have completely blocked all seizure activity in most experiments.

With respect to adverse effects, no alterations in behavior and no Rotarod failures were recorded. In some but not all experiments with esuprone, a decrease in body temperature was noted. In the two experiments in which body temperature was measured 2 h after application of 20 mg/kg, body temperature decreased from 38.74 ± 0.09°C to 37.23 ± 0.14°C ($P < .0001$) and from 38.18 ± 0.08°C to 37.71 ± 0.12°C ($P = .0108$), respectively, whereas no significant decreases in body temperature were seen in the three other experiments with esuprone, including the experiment with 40 mg/kg.

**Anticonvulsant and Adverse Effects of Combinations of LU 53439 and Esuprone.** To evaluate whether combinations of a MAO-A and a MAO-B inhibitor are more effective than either drug alone, we administered a combination of 20 mg/kg of LU 53439 and 20 mg/kg esuprone and recorded ADT and seizure parameters at 1 and 2 h after oral administration (Fig. 4). The combination was more effective to increase ADT than either drug alone, but at least in the experiment with 1-h pretreatment time, the effect of the drug combination seemed to be purely additive. Because neither seizure severity nor seizure or afterdischarge duration recorded at ADT were affected by the drug combinations, they were not illustrated separately.
In contrast to single drug treatment, the combination induced ataxia (mean scores 1.3 ± 0.21 and 1.7 ± 0.15 after 1 and 2 h) and moderate hypolocomotion (mean scores 0.9 ± 0.1 and 1.0 ± 0). Furthermore, body temperature was relatively markedly decreased (from a predrug value of 38.58 ± 0.09°C to 36.75 ± 0.1°C after 1 h; *P* < .0001; and from 38.24 ± 0.1°C to 36.85 ± 0.14°C after 2 h; *P* < .0001).

**Anticonvulsant and Adverse Effects of Tranylcyprozine.** The nonselective irreversible MAO inhibitor tranylcypromine, 20 mg/kg p.o., markedly increased ADT by 450% above control (*P* = .0039) when tested 2 h after administration (Fig. 5). There was also a trend to decreased seizure severity and duration at this dose, but these trends did not become significant. When the dose was reduced to 0.5 mg/kg, no anticonvulsant effects were observed. An intermediate dose of 5 mg/kg significantly increased ADT (*P* = .0078), but other seizure parameters were not affected.

After 20 mg/kg, ataxia (mean scores 1.25 ± 0.16 and 1.38 ± 0.26 at 1 and 2 h after administration, respectively), reduced righting (0.9 ± 0.23 and 0.8 ± 0.25), piloerection (1 ± 0.4 and 1.1 ± 0.3), hypolocomotion (0.9 ± 0.13 and 0.6 ± 0.26), and pronounced salivation were observed, but all rats passed the Rotarod test and body temperature was not affected. However, seven of the eight rats of this experiment died within
the next 24 h. This was the reason to reduce the dose of tranylcypromine in all subsequent experiments. Neither 0.5 nor 5 mg/kg induced any behavioral alterations and did not cause fatalities, but at 5 mg/kg, body temperature markedly decreased (from a predrug value of 38.06 ± 0.13°C to 36.1 ± 0.35°C at 2 h after administration; \( P < 0.0001 \)).

**Discussion**

The lack of anticonvulsant efficacy of the selective MAO-B inhibitor LU 53439 but potent anticonvulsant activity of the selective MAO-A inhibitor esuprone strongly argues in favor of MAO-A but not MAO-B inhibition as an effective means of inducing anticonvulsant effects in the kindling model of epilepsy. These data also substantiate recent observations, indicating that MAO-B inhibition is not involved in the anticonvulsant activity of l-deprenyl (Löschler and Hönack, 1995; Löschler and Lehmann, 1996, 1998). Thus, although irreversible MAO-B inhibition in rodent brain occurs at doses below 1 mg/kg (Magyar, 1993), dose-dependent anticonvulsant effects were only observed at doses of above 1 mg/kg (Löschler and Hönack, 1995; Löschler and Lehmann, 1996, 1998). In doses above 1 mg/kg, l-deprenyl is a potent inhibitor of MAO-A, with almost complete inhibition in rat brain seen at about 20 mg/kg (Magyar and Tóthfalusi, 1984), i.e., the dose found in the present study to induce marked increases of focal seizure threshold (ADT) in the kindling model. Because

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**Fig. 2.** Effect of the selective MAO-B inhibitor LU 53439 on focal seizure threshold (ADT) and seizure severity, seizure duration, and afterdischarge duration recorded at ADT currents in fully kindled rats. Data are from a group of nine rats. Route of administration (p.o.), dose (in mg/kg), and time (in h) of administration before ADT determination are indicated below each column. Predrug control recordings (open columns) were performed 2 to 3 days before each drug experiment, using administration of vehicle with the same route and pretreatment time as in the respective drug experiment. Data are illustrated as means plus S.E. of nine rats per experiment. Significant differences between data of a drug experiment and its individual predrug control are indicated by asterisk (\( P < .0235 \)). The order of presentation of data from individual dose regimens corresponds to the order in which the experiments were performed.
similar ADT increases were obtained with the MAO-B inhibitor esuprone but not the MAO-A inhibitor LU 53439, one might suggest that anticonvulsant activity of L-deprenyl is due to inhibition of MAO-A rather than MAO-B, or to a combination of inhibition of both isoenzyme forms.

In vitro experiments with rat liver mitochondrial MAO-A and MAO-B have shown that L-deprenyl is an irreversible inhibitor of both enzymes ($K_i$ = 76 μM for MAO-A and $K_i$ = 0.3 μM for MAO-B; Robinson et al., 1995). Respective IC$_{50}$ values for rat brain MAO are 2 μM (MAO-A) and 0.008 μM (MAO-B; Drescher et al., 1993). The higher sensitivity of rat brain compared with rat liver MAO-A and MAO-B to L-deprenyl in in vitro experiments is also seen in in vivo studies (Magyar and Tóthfalusi, 1984). The inhibition of MAO-A and MAO-B by L-deprenyl is characterized by a first reversible inhibitory phase in which L-deprenyl forms a noncovalent complex with the enzyme. Subsequent oxidation of L-deprenyl leads to a covalent bond formation within this complex, thereby inducing irreversible "suicide inhibition" of MAO-A and MAO-B (Gerlach et al., 1992; Robinson et al., 1995). In the present and previous in vivo experiments in rats, the anticonvulsant effects of L-deprenyl were short-lived (Löscher and Hömack, 1995; Löscher and Lehmann, 1996); this seems to be not in line with irreversible MAO inhibition as a mechanism of these
In contrast with esuprone, LU 53439 does not inhibit MAO-A in doses up to 30 mg/kg, but it is a highly selective inhibitor of MAO-B (Drescher et al., 1993). In vitro, the ratio between inhibition of MAO-B and MAO-A in rat brain homogenates was >10,000 for LU 53439 compared with 250 for L-deprenyl (Drescher et al., 1993). In contrast with L-deprenyl, which affects uptake of noradrenaline and dopamine at high concentrations (Knoll, 1992), LU 53439 is devoid of such an effect (Drescher et al., 1993). In rats after single doses of 1 to 32 mg/kg, the brain concentrations of dopamine, serotonin, noradrenaline, and their metabolites were unaffected. However, after complete inhibition of MAO-A by esuprone, administration of LU 53439 induced a further decrease in the content of dopamine and serotonin metabolites in the corpus striatum of rats (Drescher et al., 1993). Similarly, LU 53439 did not increase extracellular levels of dopamine in the striatum of rats, but caused a further increase of dopamine in rats treated with esuprone (Drescher et al., 1993). As shown by the present data, however, these synergistic biochemical interactions did not lead to any marked potentiation of esuprone’s anticonvulsant efficacy by combined treatment with LU 53439.

The nonselective and irreversible MAO inhibitor tranylcypromine, given at a dose that completely inhibits both MAO-A and MAO-B (Celada and Artigas, 1993), was the most effective drug of this study to increase ADT, but proved to be highly toxic. Doses of 20 to 40 mg/kg of tranylcypromine had previously been reported to produce protection against audiogenic seizures in DBA/2J mice without mentioning any toxicity (Sparks and Buckholtz, 1985). In kindled rats, lower doses were tolerated better but were less effective compared with L-deprenyl or esuprone. These data are in line with the observations from the combination of esuprone and LU 53439 that inhibition of MAO-A and MAO-B may have advantages in terms of anticonvulsant efficacy but produces more adverse effects when compared to inhibition of MAO-A alone.

The ability of a drug to increase ADT in kindled rats indicates that the drug directly affects seizure initiation in the focus, i.e., elevates focal seizure threshold, whereas a drug’s ability to reduce seizure severity or duration recorded at ADT is thought to indicate that the drug inhibits seizure spread from the focus (Löschner and Schmidt, 1988). None of the MAO inhibitors had a significant effect on seizure severity recorded at ADT, and only L-deprenyl showed a clear effect on seizure duration, indicating that MAO inhibition primarily affects seizure threshold and not seizure spread, at least in the kindling model. This type of anticonvulsant effect is similar to that of the major antiepileptic drug phenytoin, which also affects focal seizure threshold but not seizure spread in the kindling model of temporal lobe epilepsy (Rundfeldt et al., 1990; Ebert et al., 1997).

MAO-A inhibitors are not only effective against focal seizures in the kindling model, as shown by the present data on esuprone, but also exert anticonvulsant activity against generalized seizure types. Thus, clorgyline produced anticonvulsant activity against generalized convulsions induced by hypoxia in mice (Ulugol et al., 1995). The selective and reversible MAO-A inhibitor pirlindole was shown to pro-

**Fig. 4.** Effect of the selective MAO-A inhibitor esuprone and the selective MAO-B inhibitor LU 53439 alone or in combination on focal seizure threshold (ADT) in fully kindled rats. Data are from two groups of 9 and 10 rats. Route of administration (p.o.), dose (in mg/kg), and time (in h) of administration before ADT determination are indicated below each column. Predrug control recordings (open columns) were performed 2 to 3 days before each drug experiment, using administration of vehicle with the same route and pretreatment time as in the respective drug experiment. Data are illustrated as means plus S.E. of 9 to 10 rats per experiment. Significant differences between data of a drug experiment and its individual predrug control are indicated by asterisks (* at least .025). Seizure severity, seizure duration, and afterdischarge duration recorded at ADT currents were not significantly altered by the treatments (not illustrated).

effects. However, it is conceivable that the combination of reversible and irreversible inhibition of brain MAO-A and MAO-B shortly after acute administration of L-deprenyl leads to a marked MAO inhibition that is reduced when the reversible inhibitory effect vanishes. An alternative explanation for the short-lived anticonvulsant effect of L-deprenyl after single dose administration is that this effect is not related to MAO inhibition, but to the various other actions of this drug (Gerlach et al., 1992; Knoll, 1992; Lange et al., 1994; Gerlach et al., 1996; Olanow, 1996; Tatton and Chalmers, 1996; Tatton et al., 1996; Knoll, 1998).

Esuprone is a novel reversible and highly selective MAO-A inhibitor that is about 3 times more potent than moclobemide (Traut et al., 1992). About 60% MAO-A inhibition in rat brain is seen after oral administration of 0.3 mg/kg; even at 10 mg/kg, at which MAO-A is almost completely inhibited, MAO-B is not inhibited by more than 10%. The MAO-A inhibiting effect of esuprone (10 mg/kg) appears within 1 h of oral dosing and persists at a high level for 2 to 6 h. The half-life of MAO-inhibiting activity is about 12 to 14 h, and dopamine, noradrenaline, and serotonin are significantly increased in rat brain after administration of esuprone (Traut et al., 1992). General pharmacology studies did not reveal any activity not related to inhibition of MAO-A.
long the onset and to decrease the intensity of seizures in audiogenic seizure susceptible rats (Medvedev et al., 1992). These observations and the present data indicate that selective MAO-A inhibitors are effective against diverse seizure types and thus might be interesting candidates for the treatment of epilepsy.

Why are MAO-A inhibitors effective anticonvulsants when the present data indicate that MAO-B inhibitors are less effective in this regard? In contrast to the human brain, MAO-A is predominant in the brain of mice and rats and mainly responsible for oxidative deamination of monoamines, including dopamine (Magyar, 1993). As shown for the selective MAO-A inhibitor esuprone, MAO-A inhibition therefore increases brain concentrations of noradrenaline, serotonin, and dopamine in rats (Traut et al., 1992), which explains the anticonvulsant activity associated with this enzyme inhibition. Indeed, drugs selectively enhancing dopaminergic, noradrenergic, or serotonergic activity in the rodent brain have been shown to increase seizure thresholds, although with different potency in different seizure models (Kilian and Frey, 1973; Przegalinski, 1985). In contrast, selective MAO-B inhibitors such as LU 53439 do not increase dopamine, noradrenaline, or serotonin in brain regions or in extracellular fluid obtained by microdialysis from brain re-
gions in rats (Drescher et al., 1993); this explains the lack of anticonvulsant activity, at least in the kindling model, in rodents. This situation may be different in humans because, in contrast to rodents, dopamine is deaminated predominantly by MAO-B in the human brain (Magyar, 1993). However, there is evidence that increase of noradrenaline rather than increase of dopamine is an effective means of inducing anticonvulsant effects in different seizure models, including kindled rats (Kilian and Frey, 1973; Peterson and Albertson, 1982; Przegalinski, 1985; Corcoran and Weiss, 1990), so that from this point of view MAO-A inhibition may be more efficacious than MAO-B inhibition to yield anticonvulsant effects in humans, too.

In conclusion, the novel highly selective and reversible MAO-A inhibitor esuprone proved to be an effective anticonvulsant in the kindling model of temporal lobe epilepsy. With its potent effect on focal seizure threshold (ADT), it resembles antiepileptic drugs such as phenytoin or carbamazepine, i.e., major drugs for treatment of temporal lobe epilepsy (Löschner and Schmidt, 1988). In contrast to esuprone, the selective MAO-B inhibitor LU 53439 was not effective in the kindling model, which substantiates the previous notion that the anticonvulsant activity of L-depranol is not related to MAO-B inhibition, but to other effects of this drug, such as inhibition of MAO-A (Löschner and Hönnack, 1995; Löschner and Lehmann, 1996, 1998). Drugs inhibiting both MAO-A and MAO-B to a similar extent (tryptophanpyrimidine) or combinations of selective MAO-A and MAO-B inhibitors had no advantage over MAO-A inhibition alone, but were less well tolerated. The data thus suggest that long-acting MAO-A inhibitors such as esuprone may be an interesting new approach for treatment of epilepsy.

References