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Effect of lamotrigine on the activities of monoamine oxidases A and B in vitro and on monoamine disposition in vivo

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Abstract

Recent clinical evidence indicates that the broad spectrum anticonvulsant drug lamotrigine is effective against the depressive phase of bipolar illness and the difficult to treat rapid cycling form of the disorder. However, the molecular mechanism underlying this therapeutic action remains uncertain. Given that inhibition of the A-type of monoamine oxidase (MAO) is a proven antidepressant mechanism, we investigated the effects of lamotrigine on MAO activities in vitro and on monoamine disposition in vivo.

In vitro, lamotrigine inhibited rat brain MAO activities with K_i values (MAO-A, 15 μM; MAO-B, 18 μM) potentially within the therapeutic range for this drug. The effects of lamotrigine on the MAO-A activities of rat brain and human liver preparations were almost identical suggesting minimal species or tissue variation. In contrast, there was no (MAO-A) or minimal (MAO-B) reduction in brain MAO activities when assayed ex vivo following the administration of lamotrigine to rats. In vivo brain microdialysis failed to detect meaningful alterations in extracellular hippocampal or frontal cortex monoamine concentrations. Furthermore, lamotrigine did not modulate oral tyramine-induced hypertension in rats or 5-hydroxytryptophan-induced head shaking in mice, providing strong evidence that the drug does not perturb monoamine metabolism in vivo. The absence of observable effects of lamotrigine on monoamine disposition in vivo may be explained by the competitive and highly reversible nature of the interaction of lamotrigine with MAO isoforms. Thus, altered monoamine metabolism in vivo is unlikely to account for the antidepressant action of the drug in bipolar depression.

Keywords: Lamotrigine; Bipolar; Monoamine oxidase inhibition; Moclobemide; 5-hydroxytryptophan; Tyramine

1. Introduction

Lamotrigine (3,5-diamino-6-(2,3-dichlorphenyl)-1,2,4-triazine; Lamictal) is an anticonvulsant drug with a broad spectrum of activity (Goa et al., 1993). In common with a number of other anticonvulsants, including sodium valproate and carbamazepine, lamotrigine exhibits mood-stabilising properties and has, therefore, become a useful treatment for bipolar disorder (Bowden, 1998; Post et al., 1998). In recent years, clinical evidence indicates that lamotrigine is also effective against the depressive phase of bipolar illness (Bowden et al., 1999a,b; Calabrese et al., 1999a,b; Kotler and Matar, 1998; Suppes et al., 1999) and the difficult to treat rapid cycling form of the disorder (Bowden et al., 1999a; Calabrese et al., 2000, 2001; Suppes et al., 1999; Walden et al., 2000) with a low potential for inducing the switch to mania thus minimising

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the risk of accelerated cycling that is often associated with the use of conventional antidepressants (Calabrese et al., 1999c; Calabrese et al., 2001). Interestingly, a recent placebo-controlled, double blind trial suggested that lamotrigine may also augment antidepressant treatment for unipolar depression (Normann et al., 2002).

The best characterised action of lamotrigine is the use- and voltage-dependent inhibition of voltage gated sodium channels (Xie et al., 1995). This mechanism is believed to mediate the seizure suppressing activity of lamotrigine and, via the consequent reduction in excessive neurotransmitter release in basal forebrain regions, may account for its anti-mania properties (Berk, 1999; Xie and Hagan, 1998). It is not entirely clear, however, how sodium channel blockade might provide the observed therapeutic benefits of lamotrigine in bipolar depression (Xie and Hagan, 1998). Consequently, a series of pre-clinical investigations were instigated to explore possible alternative mechanisms. Since inhibitors of monoamine uptake and metabolism are well established antidepressant mechanisms in bipolar depression (Thase and Sachs, 2000), our studies have centred on the effects of lamotrigine on monoamine disposition. Initially we focussed on transport and found that monoamine uptake by human platelets (5-hydroxy-tryptamine, 5-HT) and rat brain synaptosomes (5-HT, dopamine, noradrenaline) was inhibited by lamotrigine but with a potency that suggested that this mechanism was unlikely to be of therapeutic significance (Southam et al., 1998). We have now examined the effects of lamotrigine on monoamine metabolism by monoamine oxidase (MAO) and describe a series of investigations designed to determine the effects of lamotrigine on MAO type A and B activities in vitro and in vivo. In addition, we have tested lamotrigine in two rodent models believed to represent serious adverse events associated with the use of irreversible MAO inhibitors that have severely restricted their use in bipolar depression (Thase and Sachs, 2000).

2. Materials and methods

2.1. Preparation of rat brain homogenate

Forebrains from adult male Han–Wistar rats (Charles River, U. K., $n=4$) were quickly removed and placed in ice-cold 0.154 M phosphate buffer (pH 7.8). After scissor-mincing in 10 ml 1 M phosphate buffer (ice-cold, pH 7.8), tissue was homogenised using a mechanical homogenisor (Ystral, Germany) and then centrifuged (2000 ×*g*, 10 min, 4 °C). The protein concentration of the supernatant was measured according to the method of Bradford (Bradford, 1976) before snap freezing in liquid nitrogen and storing at −70 °C until use.

2.2. Preparation of human liver microsomes

Frozen human liver was thawed in 4 volumes of ice cold 10 mM phosphate buffer containing 10 mM EDTA and 1.154 M KCl, pH 7.4. After the removal of connective tissue and fat, liver was scissor-minced and homogenised in a potter-type homogeniser and centrifuged at 9000 ×*g* for 20 min at 4 °C. The supernatant was further centrifuged (10^5 ×*g*, 1 h, 4 °C) and the microsomal pellet resuspended in the original volume of 50 mM phosphate buffer containing 0.1 mM EDTA and 20% glycerol. At −80 °C, 5 μl aliquots were stored until use. MAO-A activity assays of the microsomal preparation was performed as described for rat brain homogenate.

2.3. MAO activity assays

Assays were performed in triplicate in a final volume of 100 μl containing 0.15–0.5 mg protein (rat brain or human liver microsome homogenate) and incubated at 37 °C for 10 min. For MAO-A assay, the substrate was 5 μM [^{3}H]5-HT and for MAO-B was 13.5 μM [^{14}C]benzylamine. Activities of the A and B isoforms were isolated pharmacologically by incorporating 1 μM deprenyl (selective inhibitor of MAO-B) or 1 μM clorgyline (selective MAO-A inhibitor) into the reaction mix. Assays were terminated by the addition of 50 μl 2 M HCl and deaminated reaction products extracted by mixing well with 600 μl 1 : 1 ethyl acetate / toluene. In duplicate, 200 μl of extract was added to 3 ml scintillation fluid and radioactivity assessed by scintillation counting. Non-specific activity was determined by adding HCl before the homogenate. Total activity was determined by counting label alone. Experiments designed to test the reversibility of enzyme inhibition were analysed statistically using analysis of variance (ANOVA) with post-hoc contrast test.

2.4. Ex vivo rat brain MAO activity assays

Adult male Han–Wistar rats (4–6 per group, Charles River, U. K.) were administered lamotrigine (30 mg/kg, prepared as a suspension in 0.25% methyl cellulose), tranylcypromine (3 mg/kg in saline), moclobemide (30 mg/kg, in saline), clorgyline (10 mg/kg, in saline) or vehicle, intraperitoneally. Animals were killed by decapitation and the forebrains removed, homogenised, and the soluble fraction assayed for MAO-A and MAO-B activities (see above) 2 h after the administration of drugs except in those animals treated with both lamotrigine and clorgyline when the former was administered 2 h and the latter 1 h before forebrains were taken and homogenates prepared. Ex vivo MAO activities of lamotrigine and standards were compared with their vehicle controls using Student's *t*-test.

2.5. Preparation and in vitro maintenance of slices of rat cerebellum

Adult male Han–Wistar rat (Charles River, U.K.) cerebellum was excised and the hemispheres removed by razor cuts. One of the cut surfaces of the vermis was glued (with cyanoacrylate) onto the stage of a vibroslice (Campden Instruments, U.K.) and then immersed into cool (10–12 °C) Krebs–Henseleit solution containing (mM) NaCl (120), KCl (2.0), $MgSO_{4.7H_20}$ (1.19), KH_2PO_4 (1.18), $NaHCO_3$ (26.0), $CaCl_2$ (2.0), and glucose (11.1), gassed with 5% CO_2 in O_2, pH 7.4. Parasaggital slices were cut at 400 μm intervals and transferred to Krebs–Henseleit solution maintained at 37 °C in a shaking water bath where they were allowed to recover for 1–2 h before use. Lamotrigine (30

μM) or vehicle was added to the medium and incubation continued for a further 15 min before slices (four slices per treatment from two rats) were plunged into ice-cold 1 mM phosphate buffer, homogenised using a mechanical homogenisor (Ystral, Germany) and MAO-A activity determined as described above.

2.6. In vivo brain microdialysis

Male Lister hooded rats (250–300 g, University of Nottingham) were anaesthetised with a halothane N_2O/O_2 mixture and placed in a stereotaxic frame. Microdialysis probes (Hospal, U.K., 4 mm membranes, artificial cerebral spinal fluid perfusion) were implanted into the frontal cortex (stereotaxic coordinates relative to bregma: 3.2 mm anterior, 3.0 mm lateral, and 4.0 mm ventral) or ventral hippocampus (stereotaxic coordinates relative to bregma: 4.8 mm posterior, 4.8 mm lateral, and 8.4 mm ventral). Rats were allowed to recover for 24 h before the commencement of drug treatments. To determine acute effects extracellular neurotransmitter concentrations, animals received either lamotrigine (20 mg/kg), paroxetine (10 mg/kg), sodium valproate (250 mg/kg) or 0.25% methyl cellulose vehicle via a single intraperitoneal injection. For chronic studies, animals were dosed orally every day (at 1100 hours) for 21 days with lamotrigine (20 mg/kg) or 0.25% methyl cellulose vehicle. Twenty minute dialysate fractions (flow rate 1.2 μl/min) were collected into 5 μl PCA for 2 h prior to and for 2.5 h post drug administration (acute studies) or for a total of 4.5 h commencing 24 h after the last dose of drug or vehicle (chronic studies) and snap frozen for subsequent analysis by HPLC using electrochemical detection. On completion of dialysate collection, rats were killed and brains removed for histological verification of microdialysis probe position. Statistical analysis was performed using ANOVA with post-hoc Dunnett's test.

2.7. Tyramine pressor effects in conscious rats

Adult male Random Hooded rats (GlaxoSmithKline Animal Breeding Unit) were fasted overnight before the implantation of a PVC cannula (0.58 mm internal diameter, 0.96 mm outside diameter, Critchley Electronics, Australia) into the right carotid artery and exteriorised through a tether at the back of the neck under 3% isoflurane in O_2 (1.4 l/min) anaesthesia. The patency of the cannula, which was passed through a swivel system designed to allow animals' free movement, was maintained by the infusion of sterile saline containing 50 U/ml heparin at 0.3 ml/h. Blood pressure and integrated heart rate were monitored continuously and sampled at 2 min intervals using the MI^2 Bioreport. Animals were allowed to recover until basal cardiovascular and behavioural parameters were regained (at least 2 h) before the oral administration of drugs (lamotrigine, 60 mg/kg; phenelzine, 30 mg/kg; moclobemide, 30 mg/kg; or saline vehicle) followed 2 h later by oral tyramine (10 mg/kg) or saline vehicle and heart rate and blood pressure subsequently monitored for 60 min.

2.8. 5-hydroxytryptophan-induced head shaking in mice

Adult male CD-1 mice (Charles River, U.K.) were dosed intraperitoneally with test compounds or vehicle (0.25% methyl cellulose) followed 60 min later by 100 mg/kg 5-hydroxytryptophan by the same route. The number of episodes of head shaking was recorded for each animal during the period 10–30 min post 5-hydroxytryptophan dosing. Statistical comparisons were made using an unpaired non-parametric two sided Mann–Whitney U test.

2.9. Drugs

Lamotrigine free base (5-hydroxytryptophan induced head shaking and microdialysis studies), lamotrigine isothionate (all other studies), and paroxetine were synthesized by GlaxoSmithKline. Moclobemide was used in the form of ground Manerix tablets (active ingredient 37%, Roche Products Ltd.). Tyramine HCl, phenelzine sulphate, tranylcypromine hemisulphate, 5-hydroxytryptophan, clorgyline HCl, deprenyl HCl and sodium valproate were obtained from Sigma-Aldrich, U.K; Fluoxetine HCl from Tocris, U.K.; and [^{3}H]5-HT creatine sulphate (370 GBq/mmol) and [^{14}C]benzylamine HCl (1.85 GBq/mmol) from Amersham Biosciences U.K. Ltd.

2.10. Use of animals

All animal studies were performed in accordance with UK Home Office Regulations and company policies on the use and welfare of animals.

3. Results

3.1. Effect of lamotrigine on the MAO-A and MAO-B activities of rat brain homogenate

On the basis of preliminary investigations, in which it was established that 10, 20, or 30 min preincubation of lamotrigine (50 μM) with rat brain homogenate did not enhance the degree of inhibition of MAO-A activity exerted by the drug, lamotrigine and substrate were added simultaneously to the enzyme preparation. Lamotrigine was found to inhibit both MAO-A and MAO-B activities of rat brain homogenate in a concentration-dependent manner (Fig. 1A, B). When substrate concentrations were set at 20 μM, IC_{50} values for A and B isoforms were determined as 10 and 19 μM, respectively. Double reciprocal plots of reaction rate (1/v) as a function of substrate concentration (1/[s]) suggested k_m values of 60 μM 5-HT for MAO-A and 160 μM benzylamine for MAO-B. Increasing concentrations of lamotrigine resulted in increasing slopes of plots which converged on the ordinate indicating that lamotrigine inhibited both isoforms competitively with K_i values determined as 15 μM (MAO-A) and 18 μM (MAO-B) (Fig. 1C, D).

3.2. Comparison of the in vitro effects of lamotrigine on rat brain and human liver MAO-A activities

When the inhibitory effect of lamotrigine on the MAO-A activity of a preparation of human liver was compared in the same experiment with that of rat brain homogenate, the resulting concentration inhibition curves suggested that there was very little species difference between rats and humans (Fig. 2). In the

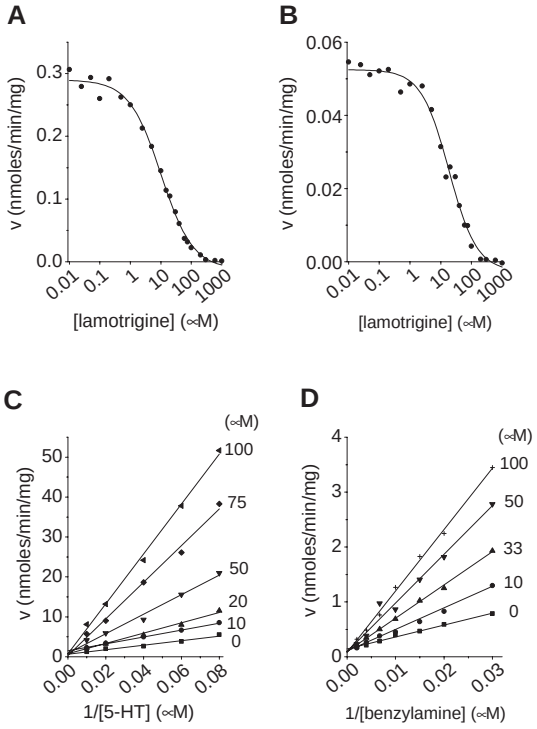

Fig. 1. Inhibition of rat brain MAO isoform activities by lamotrigine. Concentration inhibition curves describing the effect of lamotrigine on the MAO-A (A) and MAO-B (B) activities of rat brain homogenate in the presence of 20 μM 5-HT and 20 μM benzylamine, respectively. Double reciprocal plots of the activities of rat brain homogenate MAO-A (C) MAO-B (D) as a function of substrate concentration in the absence and presence of increasing concentrations of lamotrigine. K_i values were determined as 15 and 18 μM for MAO-A and MAO-B, respectively. Each data point represents the mean of three separate determinations.

presence of 20 μM 5-HT, IC_{50} values were determined as 12 μM and 22 μM for preparations derived from rat and human tissues, respectively.

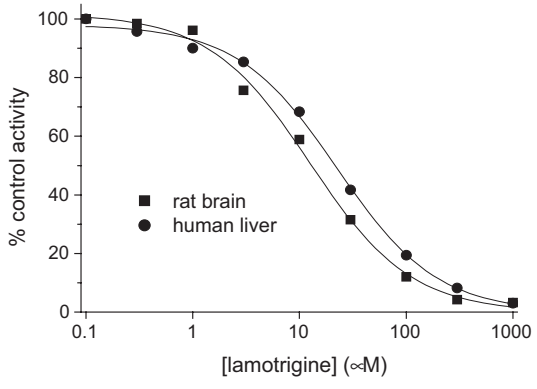

Fig. 2. Lamotrigine inhibited human liver and rat brain MAO-A activities with similar potencies. IC_{50} values for the concentration-dependent inhibition of rat brain homogenate and lysed human liver microsome MAO-A activities were 12 and 22 μM, respectively. In both cases, the substrate (5-HT) concentration was 20 μM. Data points represent the mean of three separate determinations.

3.3. Reversibility of the in vitro inhibition of rat brain MAO-A activity by lamotrigine

The concentration–inhibition curve describing the effect of lamotrigine on the MAO-A activity of rat brain homogenate (Fig. 1A) established that enzyme activity was markedly inhibited in the presence of 30 μM lamotrigine but was unaffected by 1 μM. To assess the reversibility of the inhibition, an assay mix containing homogenate and 30 μM lamotrigine was diluted 30-fold (i.e., 30 to 1 μM) and the resultant activity compared with that of a mix diluted 30-fold with 30 μM lamotrigine (i.e., 30 to 30 μM) and a mix of homogenate containing 1 μM lamotrigine diluted 30-fold in 1 μM lamotrigine (i.e., 1 to 1 μM). The 55% reduction in rat brain MAO-A activity caused by 30 μM lamotrigine was immediately lost following dilution suggesting that the inhibitory action of the drug was highly reversible (Table 1).

3.4. MAO-A activity of homogenate prepared from slices of rat cerebellum incubated in the presence of lamotrigine

The MAO-A activity of homogenate prepared from 400 μm thick slices of rat cerebellum which had been incubated in vitro (0.086±0.024 nmoles/mg/min, n=4), although lower than that prepared directly from rat brain, was not affected by the addition of 30 μM lamotrigine (15 min) to the incubation medium (0.085±0.015 nmoles/mg/min, n=4).

3.5. Effect of lamotrigine administration on ex vivo rat brain MAO-A and MAO-B activities

Despite the high dose, the MAO activity of rat brain homogenate prepared 2 h after the intraperitoneal administration of 30 mg/kg lamotrigine was either unchanged (MAO-A, Fig. 3A) or slightly reduced (MAO-B, to 79% of controls, Fig. 3B) compared with that of tissue obtained from animals administered vehicle. In contrast, the irreversible non-selective MAO inhibitor tranylcypromine (3 mg/kg), the MAO-A selective irreversible inhibitor clorgyline (10 mg/kg) and the MAO-A selective reversible inhibitor moclobemide (30 mg/kg) all inhibited ex vivo activities of the A isoform by 80–90% (Fig. 3A) 2 h after dosing. Tranylcypromine also inhibited MAO-B activity ex vivo by a similar extent (Fig. 3B).

In a separate experiment designed to examine the capacity of lamotrigine to protect against subsequent irreversible inhibition, it

Table 1
Reversal of the inhibition of rat brain MAO-A activity by lamotrigine

Initial lamotrigine concentration (μM)	Final lamotrigine concentration (μM)	MAO-A activity (nmoles/min/mg)
0	0	0.561±0.024
1	1	0.572±0.048
30	30	0.260±0.011[a]
30	1	0.568±0.093

Rat brain homogenate incubated with 30 μM lamotrigine was diluted to produce a final lamotrigine concentration of 1 μM immediately before assay. For comparison, homogenate incubated with 0, 1 or 30 μM lamotrigine was diluted in the same concentrations of lamotrigine. Assays were performed in triplicate, [a]P<0.001 versus 1 to 1 μM and 30 to 1 μM.

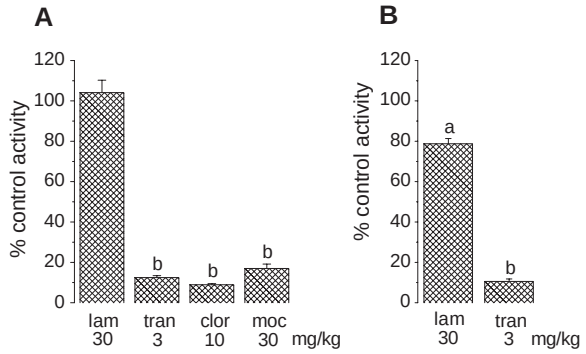

Fig. 3. Effect of administering lamotrigine to rats on ex vivo brain homogenate MAO activities. (A) MAO-A activities of brain homogenates prepared from rats administered lamotrigine (lam), tranylcypromine (tran), clorgyline (clor), or moclobemide (moc). (B) MAO-B activities of brain homogenates prepared from rats administered lamotrigine or tranylcypromine. Each column represents the mean±S.E.M. MAO activity determinations of homogenates prepared from 4–6 rats, each assay performed in triplicate. [a]$P<0.05$, [b]$P<0.001$.

was confirmed that ex vivo rat brain MAO-A activity 2 h after the administration of 30 mg/kg lamotrigine (0.377±0.009 nmoles/mg/min) was no different from that of vehicle treated rats (0.358±0.024 nmoles/mg/min), that clorygyline administration reduced ex vivo rat brain MAO-A activity by about 90% (0.032 ±0.002 nmoles/mg/min, 1 h post-dose), and that the inhibitory effect of clorgyline (1 h post-dose) was not diminished by the administration of lamotrigine 1 h earlier (MAO-A activity=0.021 ±0.010 nmoles/mg/kg).

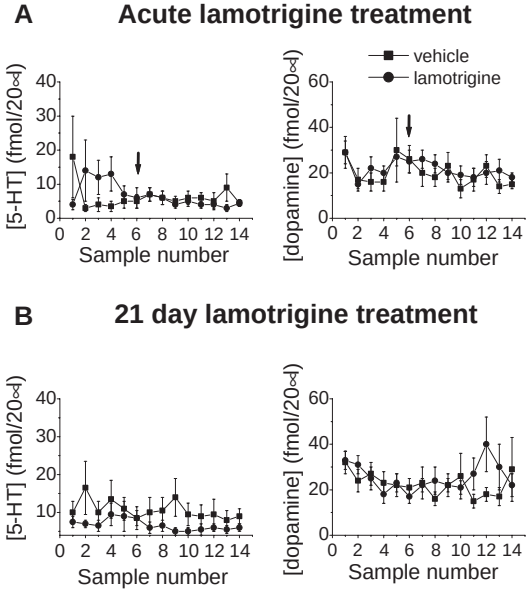

Fig. 4. Effect of lamotrigine on extracellular 5-HT or dopamine concentrations in the frontal cortex of conscious rats. Lamotrigine was administered (A) as a single intraperitoneal dose (20 mg/kg, after 2 h basal sampling indicated by downward arrow) or (B) daily oral dosing (20 mg/kg) for 21 days prior to sampling. Samples represent mean±S.E.M. 5-HT or dopamine concentrations in 20 min microdialysis fractions ($n=6$–10).

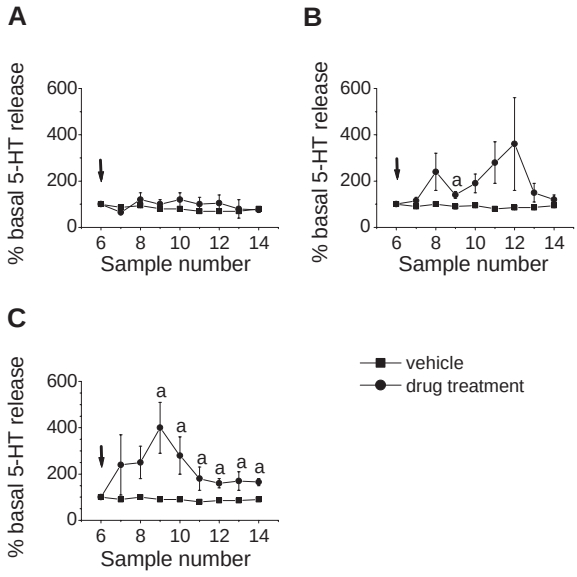

Fig. 5. Paroxetine and sodium valproate but not lamotrigine raised extracellular 5-HT concentrations in the ventral hippocampus of conscious rats. (A) Lamotrigine (20 mg/kg), (B) paroxetine (10 mg/kg), or (C) sodium valproate (250 mg/kg) were administered as a single intraperitoneal (indicated by downward arrow) dose after 2 h basal sampling. Samples represent mean±S.E.M. 5-HT concentrations in 20 min microdialysis fractions ($n=6$–10) and data normalised to basal values (average of the first six samples, not shown). [a]$P<0.05$.

3.6. Effect of lamotrigine on brain extracellular 5-HT and dopamine concentrations in conscious rats

In vivo microdialysis did not detect any modulation of extracellular 5-HT, dopamine (Fig. 4A), or 5-hydroxyindoleacetic acid (5-HIAA, not shown) concentrations in the frontal cortex of conscious rats following a single systemic (intraperitoneal) dose of lamotrigine (20 mg/kg). Similarly, chronic dosing with lamotrigine (20 mg/kg daily), also failed to produce any consistent effect on

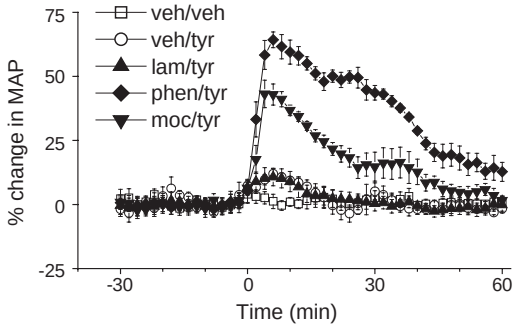

Fig. 6. Effect of lamotrigine on oral tyramine-induced increase in mean arterial pressure in conscious rats. Rats were orally administered tyramine (tyr, 10 mg/kg) or vehicle (veh) at time 0. Lamotrigine (lam, 60 mg/kg), phenelzine (phen, 30 mg/kg), moclobemide (moc, 30 mg/kg), or vehicle (veh) were administered orally 60 min before tyramine. Mean arterial pressure was recorded at 2 min intervals and results normalised to the average of recordings made during the 30 min before tyramine dosing. Data points represent mean±S.E.M. values obtained from groups of 6 animals.

frontal cortex 5-HT, dopamine (Fig. 4B), or 5-HIAA (not shown) concentrations.

In the ventral hippocampus, acute treatment with lamotrigine again failed to modulate 5-HT concentrations (Fig. 5A). In contrast, both the selective serotonin reuptake inhibitor paroxetine (10 mg/kg, Fig. 5B) and the anticonvulsant sodium valproate (250 mg/kg, Fig. 5C) significantly elevated 5-HT levels in the ventral hippocampus of conscious rats following acute administration.

3.7. Effect of lamotrigine on tyramine pressor effects in conscious rats

Oral dosing with saline vehicle produced no consistent effect on mean arterial pressure (Fig. 6). Oral tyramine (10 mg/kg), administered 2 h after saline pretreatment, produced a modest 12% (15–20 mm Hg) increase in mean arterial pressure which peaked by 10 min after dosing and returned to normal values within 20 min. Oral pretreatment with a high dose of lamotrigine (60 mg/kg) had no effect on the extent or duration of the tyramine response (Fig. 6). In contrast, oral phenelzine (30 mg/kg) and moclobemide (30 mg/kg) pretreatment magnified the peak tyramine pressor effect 5.5-fold (70 mm Hg) and 3.5-fold (45 mm Hg), respectively, and extended its duration up to 60 min (moclobemide) or longer (phenelzine).

3.8. Effect of lamotrigine on 5-hydroxytryptophan-induced head shaking in mice

Intraperitoneal injection of the 5-HT precursor 5-hydroxytryptophan (100 mg/kg) induced a serotonin behavioural syndrome characterised by repeated head shaking. Inhibition of 5-HT uptake by pretreating animals with fluoxetine (30 mg/kg, i.p., 1 h before 5-hydroxytryptophan) caused a marked (5-fold) increase in the rate of head shaking (Table 2). Similar increases in head shaking resulted when animals were pretreated with the irreversible non-selective MAO inhibitor tranylcypromine (3 mg/kg, i.p.) or the reversible MAO-A-selective inhibitor moclobemide (5 mg/kg, i.p.) (Table 2). In contrast, the administration of lamotrigine (10 or 30 mg/kg, i.p.) did not alter the rate of head-shaking induced by 5-hydroxytryptophan administered 1 h later (Table 2).

Table 2
Effect of lamotrigine on 5-hydroxytryptophan-induced head shaking in mice

Drug (mg/kg)	Head shakes per 20 min
Vehicle	2.3±0.8
Lamotrigine (10)	1.2±0.3
Lamotrigine (30)	1.5±0.8
Tranylcypromine (3)	9.2±2.7[a]
Fluoxetine (30)	9.8±1.7[b]
Moclobemide (5)	12.7±4.2[a]

Mice were dosed intraperitoneally with lamotrigine (10 or 30 mg/kg), fluoxetine (30 mg/kg), tranylcypromine (3 mg/kg), or moclobemide (5 mg/kg) 60 min before 100 mg/kg 5-hydroxytryptophan. Data represent mean (±S.E.M., $n=6$) number of episodes of head shaking recorded between 10 and 30 min post-5-hydroxytryptophan. [a]$P<0.05$ and [b]$P<0.01$ versus vehicle controls.

4. Discussion

We have examined the effects of lamotrigine on monoamine metabolism and present data demonstrating the competitive and reversible inhibition of both A and B isoforms of MAO in vitro with a marked increase in potency compared with the inhibition of monoamine uptake (Southam et al., 1998). There was little selectivity between the A and B isoforms and, at least in the case of the former, there appeared to be minimal species difference, with concentration–inhibition curves for rat brain and human liver preparations almost identical. The K_i values determined for the inhibition of rat brain MAO-A and MAO-B by lamotrigine (15 and 18 μM, respectively) are within the concentration range resulting in cerebral spinal fluid and brain extracellular space from the administration of an anticonvulsant dose of the drug. Following a single intraperitoneal injection of 20 mg/kg, for example, concentrations of lamotrigine in rat cerebrospinal fluid are reported to exceed 20 μM (Walker et al., 2000). They are also close to the estimated affinity (12 μM) for lamotrigine at sodium channels in their inactivated state (Xie et al., 1995).

In the light of the above observations, we set out to investigate the action of lamotrigine on MAO acitivities and monoamine disposition ex vivo, in intact tissues, and in vivo. Intitially we investigated whether inhibition of MAO by lamotrigine could be observed ex vivo after administration of a high anticonvulsant dose (30 mg/kg) of the compound and found rat brain MAO-A and MAO-B activities to be either unaltered or only marginally inhibited. Similarly, the clearly demonstrable inhibition of homogenate MAO activities by lamotrigine was not evident when in vitro preparations of intact tissue (rat cerebellar slices) were incubated with the drug prior to its homogenisation and determination of MAO activities. Furthermore, prior dosing of rats with lamotrigine did not modulate the capacity of the irreversible inhibitor clorgyline to block ex vivo 5-HT metabolism by MAO-A. Although a shorter acting irreversible inhibitor than clorgyline may provide a more accurate estimate of the level of reversible inhibition in this protection assay (Green and El Hait, 1980), this data does suggest that lamotrigine was not able to compete with clorgyline for its binding site on the enzyme.

We then employed microdialysis to determine the consequences of lamotrigine administration on rat brain extracellular monoamine concentrations in vivo. Neither acute (frontal cortex and ventral hippocampus) nor chronic (frontal cortex) administration of lamotrigine appeared to produce meaningful alterations in extracellular concentrations of 5-HT or dopamine. Whilst systemic administration of the reversible MAO-A inhibitor brofaromine is reported to increase extracellular 5-HT concentrations in the frontal cortex (Bel and Artigas, 1995), the failure of clorgyline and the selective MAO-B inhibitor deprenyl to do likewise (Celeda and Artigas, 1993) suggests that the modulation of extracellular monoamine concentrations by selective MAO

inhibitors is not a particularly robust effect. However, the non-selective MAO inhibitor tranylcypromine (both acute and chronically) (Celeda and Artigas, 1993; Ferrer and Artigas, 1994) and the concurrent administration of MAO-A and -B inhibitors (Celeda and Artigas, 1993; Bel and Artigas, 1995) are reported to increase extracellular 5-HT concentrations in the frontal cortex. Thus, if the relatively non-selective inhibition of rat brain MAO by lamotrigine observed in vitro was translated to meaningful MAO-A and -B inhibition in vivo, evidence of altered extracellular monoamine concentrations might have been anticipated. In fact, a lamotrigine-induced decrease in hippocampal extracellular monoamine concentrations has recently been reported (Ahmad et al., 2004) which, although partially discrepant with our own observations, does confirm that lamotrigine challenge does not increase extracellular monoamine concentrations in rat brain. In contrast and in accordance with earlier reports (Biggs et al., 1992; Murakami et al., 2001; Ramaiya et al., 1997), we were able to demonstrate elevated extracellular 5-HT concentrations in the hippocampus arm of our experiments following administration of the serotonin reuptake inhibitor paroxetine and the anticonvulsant sodium valproate.

To address further the effect of lamotrigine on MAO activities in vivo, we tested the drug in two animal models considered to reflect monoamine disposition in vivo and also to represent side-effects associated with the clinical use of MAO inhibitors. The first of these was tyramine-induced hypertension in the rat (Fankhauser et al., 1994). Ingestion of this vasoactive amine, which is found in high concentrations in certain foodstuffs including cheese and red wine, can cause an elevation in blood pressure, an effect which may be exacerbated when the metabolism of tyramine is blocked by MAO-A inhibitors resulting in a hypertensive crisis known as the 'cheese effect' (Livingston and Livingston, 1996). In contrast to phenelzine and, to a lesser extent, molcobemide, we found no potentiation of tyramine-induced hypertension following lamotrigine administration. Thus, administration of lamotrigine appeared not to compromise tyramine metabolism by inhibiting MAO activity in vivo. The second model to be tested was 5-hydroxytryptophan-induced head shaking in mice which is one of several stereotypical behaviours, known collectively as the serotonin syndrome, that can arise (via 5-HT_{2A} receptor activation) as a consequence of 5-HT build up in the extracellular space following administration of the 5-HT precursor 5-hydroxytryptophan and/or inhibitors of 5-HT uptake or metabolism (Ortmann et al., 1980; Schreiber et al., 1995). The failure of lamotrigine to increase the frequency of head shaking strongly suggests that lamotrigine does not alter 5-HT disposition in the rodent brain in vivo.

Why does the capacity of lamotrigine to inhibit MAO-A and -B isoforms in vitro not result in altered monoamine disposition in vivo? One possibility is that, unlike membrane-associated voltage gated sodium channels, MAOs are located intracellularly and that inadequate access to this compartment may account for the lack of effect of lamotrigine on MAO activities in intact tissues. Another, and in the light of the oral bioavailability of the drug, perhaps more likely explanation may be found by examining the kinetics of MAO inhibition by lamotrigine. Lamotrigine inhibited both A and B isoforms of MAO competitively. Thus, increasing concentrations of substrates, including 5-HT in the brain or tyramine in the periphery, would displace lamotrigine from the enzyme and thereby minimize its impact. The inhibition was also highly reversible, as illustrated by the immediate and complete loss of the inhibitory effect of lamotrigine on MAO-A activity following dilution of the drug to sub-threshold levels. This would also be expected to minimise the effects of the inhibition in vivo.

The significance of such inhibition kinetics is highlighted by comparison with those of standard MAO inhibitors used in the clinic to treat depression. First generation inhibitors bind irreversibly and non-selectively to MAOs. The efficacy of such drugs in bipolar depression is beyond doubt but potentially serious side effects have restricted their use (Thase and Sachs, 2000). The propensity for irreversible MAO inhibitors to trigger the 'cheese effect' was illustrated by the five-fold potentiation of the tyramine-induced increase in mean arterial pressure in rats by phenelzine. Similarly, the marked increase in 5-hydroxytryptophan-induced head shaking in mice caused by tranylcypromine is indicative of the potential of this drug to induce serotonin syndrome. Attempts to reduce such complications led to the introduction of reversible and MAO-A selective inhibitors typified by moclobemide (Wouters, 1998). Interestingly, although a reversible inhibitor of MAO-A and with a vastly improved side-effect profile in the clinic compared with irreversible inhibitors, MAO-A activity is inhibited by moclobemide when assayed ex vivo (present data, Da Prada et al., 1989) and significant potentiation of tyramine pressor effects and 5-hydroxytryptophan stereotypies can be detected in vivo (present data; Burkard et al., 1989). Again, the explanation for this paradox may be found in the kinetics of the inhibitory activity of moclobemide. Unlike lamotrigine, moclobemide required preincubation with MAO-A to achieve maximal inhibitory effect and, when assayed ex vivo following 2 h pretreatment, rat brain homogenate MAO-A activity was inhibited in a non-competitive fashion (Da Prada et al., 1989). Furthermore, whereas the recovery of enzyme activity upon dilution of lamotrigine was immediate, dilution and dialysis experiments indicate that several hours are required for complete reversal of MAO-A activity by moclobemide (Da Prada et al., 1989). It is likely that the slow reversibility of MAO-A inhibition by moclobemide has resulted in a drug in which antidepressant activity is maintained but with reduced liability to cause the adverse events associated with the use of irreversible inhibitors (Norman and Burrows, 1995). However, the present results suggest that a competitive and highly reversible MAO inhibitor in vitro, such as lamotrigine, is unlikely to exert

meaningful effects on monoamine disposition in vivo and, therefore, the action of lamotrigine in combating the depression phase of bipolar disorder appears unlikely to be mediated via this mechanism. By the same argument, adverse events associated with the altered monoamine disposition would not be anticipated. In this respect, it is noteworthy that lamotrigine has not been observed to trigger tyramine-driven cardiovascular adverse events or serotonin syndrome in the clinic (Messenheimer et al., 1998, 2000).

In the light of the failure to demonstrate any effects of lamotrigine on monoamine disposition in vivo, the mechanism underlying the therapeutic activity of lamotrigine in bipolar depression remains unknown. However, emerging data, which includes the demonstration of specific effects on gene expression (Wang et al., 2002), protection against glycogen synthase kinase-3β-induced apoptosis (Li et al., 2002), and the modulation of non-specific cationic conductances (Poolos et al., 2002) and certain potassium (Zona et al., 2002) and calcium (Xiong et al., 2001) currents, continues to suggest alternative possibilities.

In conclusion, we demonstrate the inhibition of MAO activity in both rat and human tissues in vitro at concentrations within the anticonvulsant therapeutic range. However, lamotrigine did not alter extracellular monoamine concentrations in the rat brain or potentiate 5-hydroxytryptophan-induced head shaking in mice or tyramine-induced hypertension in rats suggesting that monoamine disposition is not altered by lamotrigine in vivo. Competitive kinetics and the ready reversibility of the inhibition may explain the failure of lamotrigine to exert meaningful effects on MAO activity in vivo and suggest that this mechanism is unlikely to account for the efficacy of lamotrigine in bipolar depression.

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