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Pharmacological profile of an essential oil derived from *Melissa officinalis* with anti-agitation properties: focus on ligand-gated channels

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Abstract

A dual radioligand binding and electrophysiological study, focusing on a range of ligand-gated ion channels, was performed with a chemically-validated essential oil derived from *Melissa officinalis (*MO), which has shown clinical benefit in treating agitation. MO inhibited binding of [^{35}S] *t*-butylbicyclophosphorothionate (TBPS) to the rat forebrain gamma-aminobutyric acid $(GABA)_A$ receptor channel (apparent IC50 0.040 ± 0.001 mg mL^{-1}), but had no effect on *N*-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropianate (AMPA) or nicotinic acetylcholine receptors. Electrophysiological analyses with primary cultures of rat cortical neurons demonstrated that MO reversibly inhibited GABA-induced currents in a concentration-dependent manner (0.01–1 mg mL^{-1}), whereas no inhibition of NMDA- or AMPA-induced currents was noted. Interestingly, MO elicited a significant dose-dependent reduction in *both* inhibitory and excitatory transmission, with a net depressant effect on neurotransmission (in contrast to the classical $GABA_A$ antagonist picrotoxinin which evoked profound epileptiform burst firing in these cells). The anti-agitation effects in patients and the depressant effects of MO in in-vitro we report in neural membranes are unlikely to reflect a sedative interaction with any of the ionotropic receptors examined here.

Introduction

Agitation is the most frequent (> 75%) and most persistent of the behaviour and psychological symptoms in dementia (BPSD) syndrome in patients with severe dementia, usually characterized by a combination of aggression (verbal and/or physical), restlessness and shouting in the context of subjective anxiety (Akhondzadeh et al 2003). These symptoms can be very distressing and have a major impact on the quality of interaction with staff and other residents in care settings. Pharmacological treatment with neuroleptic agents is often the first-line treatment for BPSD, despite evidence of only modest efficacy (Ballard et al 2002). Over-prescribing has however become a major problem, especially in residential and nursing care environments, where more than 40% of people with dementia are taking neuroleptic medication, often inappropriately and usually with little subsequent monitoring (Beabrun & Gray 2000). In addition, these drugs have considerable adverse effects. Most classes of anti-psychotic agents are associated with an increased risk of falls and drowsiness; additional problems with the neuroleptics include parkinsonism, akathisia, tardive dyskinesia, reduced well-being, risk of cardiac arrhythmias, social withdrawal, severe neuroleptic sensitivity reactions and possibly stroke (Brodaty et al 2003). Neuroleptic agents may also substantially accelerate cognitive decline and neuronal loss (specifically in the substantia nigra) (Brodaty et al 2003). In light of the potentially harmful side-effects of these agents, the Chief Medical Officer in the UK has recommended particular caution when prescribing neuroleptics to people with dementia, and in the USA legislation has been introduced to regulate the prescription of neuroleptics to nursing home residents (Carson et al 2006).

Neuronal systems implicated in agitation or anxiety associated with neuropsychiatric symptoms include gamma-aminobutyric acid (GABA), glutamate and cholinergic systems (Hardy et al 1987; Herrmann & Lanctot, 1997; Garcia-Alloza et al 2005). The application of plant essential oils in aromatherapy has been recorded for thousands of years. Knowledge of the distillation of the essential oils and application of individual species to improve specific health problems and well-being was introduced into Europe in the tenth century. There is a need to evaluate the efficacy of specific essential oils in placebo-controlled trials and to set this in the context of mechanistic pharmacological studies. Several plant species are used in medical herbalism for their effects on anxiety, restlessness, excitability and depression. These include lemon balm (*Melissa officinalis*), lavender (*Lavandula augustifolia*), chamomile, bergamot, neroli and valerian (e.g. Viola et al 1995; Brum et al 2001; Perry & Perry 2006). Several recent clinical trials have attested to the value of aromatherapy in people with dementia (Lee 2005; Perry & Perry 2006; Lin et al 2007). Despite this, however, the central mechanisms by which the essential oils exert their effects are largely unknown. The safety of these essential oils has also been well established in clinical populations.

M. officinalis is of particular interest on account of the known sedative, cognitive-enhancing and relevant physiological actions (Viola et al 1995; Ballard et al 2002; Akhondzadeh et al 2003; Perry & Perry 2006; Kennedy et al 2002, 2003, 2004, 2006). In order to maximize clinical benefit and develop new and more effective treatments it is important to select the most appropriate plant essential oil(s) and clarify the mechanism of action. Limited pharmacological data are available to explain the clinical effects of these oils (Perry & Perry 2006). We have performed a detailed pharmacological study of the essential oils derived from *M. officinalis* (MO). Four batches of these oils, obtained from reputable European suppliers, were used in this study. The quality of these essentials oils was validated in a previous detailed chemical gas chromatography mass spectroscopy (GC-MS) analysis (Elliott et al 2007). A specific batch for the current study was selected on the basis of the GC-MS analysis and preliminary pharmacological study. MO sourced from Baldwin's and Fytosan gave qualitatively similar results (Elliott et al 2007).

Materials and Methods

Chemicals and herb samples

Samples of MO were sourced from four separate authenticated suppliers: Baldwins (London, UK), Pranarom (Lille, France), Quinessence (Coalville, UK) and Fytosan (Die, France). An analysis of the terpene constituents based on GC-MS was carried out at the Royal Botanic Garden at Kew (London), using a Perkin-Elmer (Waltham, MA, USA) autosystem XL GC coupled to a Perkin-Elmer TurboMass (quadrupole) MS (DB-5MS column (30 m × 0.25 mm; film thickness, 0.25 μm; helium as carrier gas and temperature programming from 40°C to 300°C at 3°C min^{-1}; injection temperature 220°C). Substances were identified by comparing retention indices with literature values, and MS data with data in the NIST/EPA/MSDC MASS Spectral Database (Elliot et al 2007).

The principal monoterpenes detected in all MO samples were geranial and neral (citral). The mean percentage composition of citral in the samples was 54.9%. The principal sesquiterpene detected in all oils was (E)-caryophillene (mean 12.2%). These compounds are reported to be the major components of MO essential oil (Adams et al 2001). We selected a Baldwin's batch of MO to perform the following study. Further details of the compositions of the essential oils from the four suppliers have been published previously (Elliott et al 2007). Importantly, the GC-MS profile displayed no major changes in composition following long-term storage (18 months) at 4°C in the dark (not shown, Royal Botanic Garden Kew). Dilutions of essential oil stock were performed fresh on the day of the assay.

[^{3}H] Flunitrazepam (specific activity 91.0 Ci mmol^{-1}) and [^{3}H] nicotine (specific activity 77 Ci mmol^{-1}) were obtained from Amersham Biotech (Amersham UK). [^{35}S]-*t*-butylbicyclophosphorothionate (TBPS; specific activity 80 Ci mmol^{-1}) was from Perkin Elmer Life Science (Waltham, MA, USA); [^{3}H] MK-801 (specific activity 25 Ci mmol^{-1}) and [^{3}H] muscimol (specific activity 36.5 Ci mmol^{-1}) were obtained from ARC (Melville, NY, USA); [^{3}H] amino-3-hydroxy-5-methyl-4-isoxazolepropianate (AMPA; specific activity 40.8 Ci mmol^{-1}) was obtained from Perkin Elmer. Picrotoxinin, diazepam, GABA, glutamate and ketamine were all obtained from Sigma (Poole, UK). All other chemicals were purchased from Sigma (Poole, UK) unless stated otherwise.

GABA and ketamine stock solutions (10^{-2} M) were prepared in the appropriate assay buffers. Diazepam stock solutions (10^{-2} M) were prepared in absolute ethanol. Picrotoxinin stock solutions (10^{-2} M) were prepared in DMSO. The solvents had no effect on radioligand binding assays at concentrations below 0.1% (v/v) ethanol or DMSO (data not shown; Abuhamdah et al 2005)

Tissue preparation

Adult male Wistar rats (200–300g) were maintained under a 12 h light:dark cycle at 23°C and 65% humidity, with water and standard laboratory food available ad libidum. Animal treatment and husbandry were in accordance with approved use of animals in scientific procedures regulated by the Animals (Scientific Procedures) Act 1986, UK (PPL 60/437).

Animals were killed humanely using a Schedule 1 procedure. The brains were removed rapidly, and the required tissue (forebrain) dissected immediately and kept cool on ice. The tissue was then homogenized in ice-cold homogenization buffer (50 mM Tris-HCl, pH 7.4, containing 5 mM EDTA, 5 mM EGTA and 320 mM sucrose) using a Dounce glass/glass homogenizer. The homogenate was centrifuged at 1000*g* for 10 min at 4°C. The supernatant was stored in ice, and the pellets re-homogenized in ice-cold buffer, centrifuged at 1000*g* fror 10 min at 4°C. The supernatants from the first and second centrifugation steps were pooled

and centrifuged at 12 000*g* for 30 minutes at 4°C. The supernatant was discarded and the pellet re-suspended in 50 mM Tris containing 5 mM EDTA and 5 mM EGTA (5 mL g^{-1} original tissue), processed using a five-step freeze–thaw protocol (Abuhamdah et al 2005), frozen and stored at −20°C.

Determination of protein concentration

The protein concentration was determined using the Lowry assay protocol (Lowry et al 1951) using bovine serum albumin as the standard protein.

Radioligand competition binding assays

A series of dose–response competition binding experiments were performed using [^{35}S] TBPS, [^{3}H] muscimol, [^{3}H] flunitrazepam, [^{3}H] AMPA, [^{3}H] MK-801 and [^{3}H] nicotine, using well-washed adult rat forebrain membranes.

$GABA_A$ receptors

[^{35}S] TBPS, [^{3}H] muscimol and [^{3}H] Flunitrazepam binding were determined as described by Abuhamdah et al (2005) using well-washed rat forebrain membranes. To measure [^{35}S] TBPS binding, membranes were incubated in 50 mM Tris buffer containing 0.2 M NaCl, pH 7.4, using approximately 20 nM [^{35}S] TBPS for 90 min at 25°C with a range of test concentrations of MO (0.001–1 mg mL^{-1}). Non-specific binding was defined in the presence of 100 μM picrotoxinin.

To measure [^{3}H] muscimol binding membranes were incubated in 50 mM Tris buffer pH 7.4 using approximately 10 nM [^{3}H] muscimol for 1 h at 4°C with a range of test concentrations of MO (0.001–1 mg mL^{-1}). Non-specific binding was defined in the presence of 100 μM GABA.

To measure [^{3}H] flunitrazepam binding membranes were incubated in 50 mM Tris buffer containing 5 mM EDTA and 5 mM EGTA using approx 1 nM [^{3}H] flunitrazepam for 1 h at 4°C with a range of test concentrations of MO (0.001–1 mg mL^{-1}). Non-specific binding was defined in the presence of 100 μM diazepam.

Glutamate receptors

[^{3}H] AMPA binding assays were performed essentially as described by Honoré et al (1985). The assay was performed using 5–10 nM [^{3}H] AMPA in 30 mM Tris-HCl, 2.5 mM $CaCl_2$, 100 mM KSCN, pH 7.1 at 4°C for 1 h. Non-specific binding was determined using 1 μM glutamate.

[^{3}H] MK-801 binding assays were performed as described by Chazot et al (1993). Briefly, well-washed rat forebrain membranes were incubated in 25 mM sodium phosphate buffer with 10 μM glutamate, pH 7.4, using approximately 1 nM [^{3}H] MK-801 for 2 h at 22°C with a range of test concentrations of MO ($10^{-11} - 10^{-4}$ M). Non-specific binding was defined in the presence of 10 mM ketamine.

[^{3}H] nicotine binding assay

[^{3}H] nicotine binding was measured as described by Court et al (1997). The assay was performed in 50 mM Tris-HCl pH 7.8 containing 8 mM $CaCl_2$, using approximately 10 nM [^{3}H] nicotine at room temperature for 1 h. Non-specific binding was determined using 100 μM nicotine.

All six binding assays were terminated by rapid filtration through Whatman GF/B filters pre-soaked in phosphate buffer, which were then washed three times using 3 mL ice-cold 10 mM sodium phosphate buffer (pH 7.4), using a Brandel cell harvester. Filters were transferred into scintillation vials, liquid scintillation fluid added and incubated for 16–24 h at room temperature. The bound radioactivity was quantified using a Beckman LS 500 CE scintillation spectrophotometer (High Wycombe, UK) with a counting time of 4 min per vial.

Primary cell culture

Neuronal cultures were prepared from 16–18-day-old rat embryos (Sprague–Dawley strain). Cortical cells were removed by mechanical trituration without trypsin and plated onto coverslips coated with poly-D-lysine (100 000 cells mL^{-1}) in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 100 units (μg mL^{-1}) penicillin/streptomycin; cells were maintained at 37°C under 5% CO_2/95% O_2. After 24 h, the plating medium was replaced by a maintenance medium comprising neurobasal medium, with 2% B27 supplement, 1% glutamax (Invitrogen, Paisley, Scotland, UK) and 100 units (μg mL^{-1}) penicillin/streptomycin. Cells were used in experiments after 14–28 days in-vitro (Lees et al 1993, 2000).

Recombinant $GABA_A$ receptors expressed in HEK 293 cells

HEK cells stably expressing $GABA_A$ $\alpha1\beta2\gamma2$L receptors (a kind gift from Dr David Graham, Synthélabo, Paris, France) were grown and maintained as described by Besnard et al (1997). Cells were harvested at 80% confluence and homogenates subjected to radioligand binding assays as described above.

Electrophysiology

Coverslips containing cultured neurons were placed in a 5 mm Perspex trench with continuous flow of buffer on the stage of an inverted microscope. The basal extracellular buffered saline contained 142 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES and 30 mM D-glucose, pH 7.4 (NaOH). Intracellular buffered saline consisted of 142 mM K-gluconate, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES and 11 mM EGTA, pH 7.4 (KOH).

Electrodes were pulled from GC150T-10 borosilicate glass (Harvard Apparatus Ltd, Edinbridge, Kent, UK) with resistance of 3–5 MΩ. All potentials cited are based on the preamplifier null potential and take no account of the liquid junction offset inherent in the use of the asymmetrical solutions. Phase bright pyramidal cells were identified and selected under ultra-long working distance phase-contrast optics (Nikon Diaphot, Segensworth, UK). At a holding potential of −40mV, GABA (10 μm 500 ms) was rapidly and quantitatively delivered to cultured cells using the Y-tube technique (Murase et al 1989). Whole-cell currents were filtered at 3 KHz before digitization at 6 kHz on a CED

1401 plus A/D D/A converter (Isis Electronics Ltd, Cheltenham, UK). The herbal oils were initially dissolved in DMSO and then diluted 1:1000 into test buffered salines. The same concentration of DMSO (0.1%) was added routinely to drug-free buffered salines and had no effect on any of the parameters reported (Lees et al 1993; 2000).

Statistical analysis

Data are presented as mean ± s.e.m. Repeated measures one-way analysis of variance and Student's *t*-tests were used as appropriate using Prism 4 software (Graphpad, CA, USA). *P* values below 0.05 were considered significant.

Results

Previous clinical evidence suggests that MO elicits an anti-agitation or calming effect, which indicates a sedative neurosteroid-, benzodiazepine- or barbiturate-like activity at the $GABA_A$ receptor. To test this hypothesis, the effects of MO essential oils were investigated using a range of radioligands targeting the major binding sites of the $GABA_A$ receptor (Abuhamdah et al 2005).

Radioligand binding at the benzodiazepine site of the $GABA_A$ receptor

The effect of MO on radioligand binding to the benzodiapepine binding site of the $GABA_A$ receptor was studied using the [^{3}H] flunitrazepam binding assay. Specific [^{3}H] flunitrazepam binding was defined using diazepam (100 μM). MO alone did not alter the equilibrium binding of [^{3}H] flunitrazepam to $GABA_A$ receptors in adult rat forebrain membranes (Figure 1).

Agonist binding site at the $GABA_A$ receptor

The effect of MO on radioligand binding to the agonist binding site of $GABA_AR$ was studied using [^{3}H] muscimol binding assay; specific binding was defined using 100 μM GABA. MO enhanced the specific binding of [^{3}H] muscimol to well-washed adult rat forebrain membranes in a concentration-dependent manner, with a maximum enhancement at a concentration of 1 mg mL^{-1}, and apparent EC50 of 0.099 ± 0.001 mg mL^{-1} (Figure 2).

Effects at the channel binding site of the $GABA_A$ receptor

To investigate the effect of MO on the ion channel binding site of $GABA_A$ receptors, [^{35}S] TBPS binding activity was determined in well-washed adult rat forebrain membranes; specific binding was defined using 100 μM picrotoxinin (Figure 3). A dose-dependent complete inhibition of [^{35}S] TBPS binding was observed (IC50 0.040 ± 0.001 mg mL^{-1}).

Activity at other ligand-gated ion channels

In order to assess the selective pharmacology of MO in more detail, the effects of the oil were determined on a number of other common neuronal ligand-gated ion channels, including the excitatory ion channels gated by N-methyl-D-aspartate (NMDA) using [^{3}H] MK-801 binding assay, and by

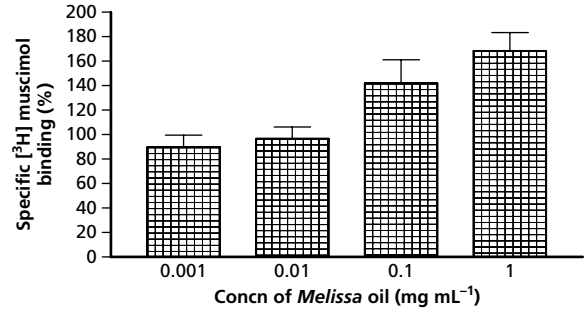

Figure 2 Effect of *Melissa officinalis* essential oil on [^{3}H] muscimol binding to well-washed rat forebrain membranes. Data are mean ± s.d. from at least three separate experiments.

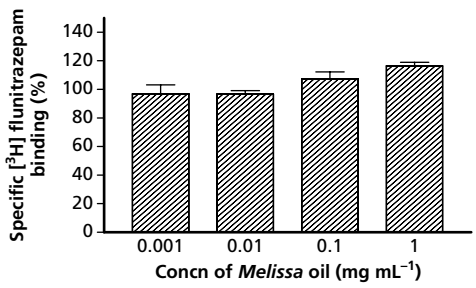

Figure 1 Effect of *Melissa officinalis* essential oil on [^{3}H] flunitrazepam binding to well-washed rat forebrain membranes. Data are mean ± s.d. from at least three separate experiments.

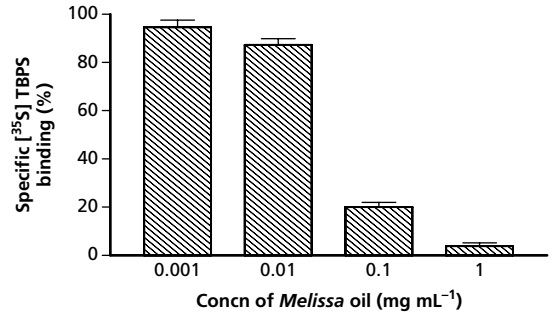

Figure 3 Effect of *Melissa officinalis* essential oil on TBPS binding to well-washed rat forebrain membranes. Data are mean ± s.d. from at least three separate experiments. The IC50 for [35S] TBPS is 0.040 ± 0.001 mg mL^{-1} for at least three independent experiments.

AMPA using [^{3}H] AMPA. In addition, the effect of MO on neuronal nicotinic receptors ($\alpha4\beta2$, $\alpha7$ nAChRs) was determined using a [^{3}H] nicotine binding assay (Court et al 1997). MO had no effect (positive or negative) on [^{3}H] MK-801, [^{3}H] AMPA or [^{3}H] nicotine binding, up to a concentration of 1 mg mL^{-1}.

Effects of MO on the GABA$_A$ receptor complex in HEK293 cells

The effects of MO on the three binding sites on the GABA$_A$ receptor labelled by [^{35}S] TBPS, [^{3}H] muscimol and [^{3}H] flunitrazepam in native membranes were also examined using a HEK293 cell line stably expressing $\alpha1\beta2\gamma2$L (the most abundant GABA$_A$ receptor subunit combination in the brain). Similar effects were observed to the native preparation (data not shown).

Electrophysiology

The apparent inhibitory effects of MO on [^{35}S] TBPS binding predict a pro-convulsant effect, which is obviously not apparent in the clinical use of this essential oil, and may not be its primary effect. We therefore investigated the electrophysiological effects of MO. Patch clamp experiments on primary cultures of rat cortical neurons demonstrated that MO reversibly reduced currents through the GABA$_A$ channel in a concentration-dependent (0.01–1 mg mL^{-1}) manner, consistent with the binding profile (Figure 4). The data fitted well to the four-term/logistic Hill equation. (With the minimum and maximum constrained to 0 and 100%, respectively, the IC50 was 0.071 mg mL^{-1}.) The blocking action at both 0.01 and 0.1 mg mL^{-1} was statistically significant compared with control currents (data not shown). Note that at 0.1 mg mL^{-1} spontaneous inhibitory post-synaptic currents (IPSCs) were almost completely blocked by MO and recovered relatively quickly compared with the partial blocking effect noted

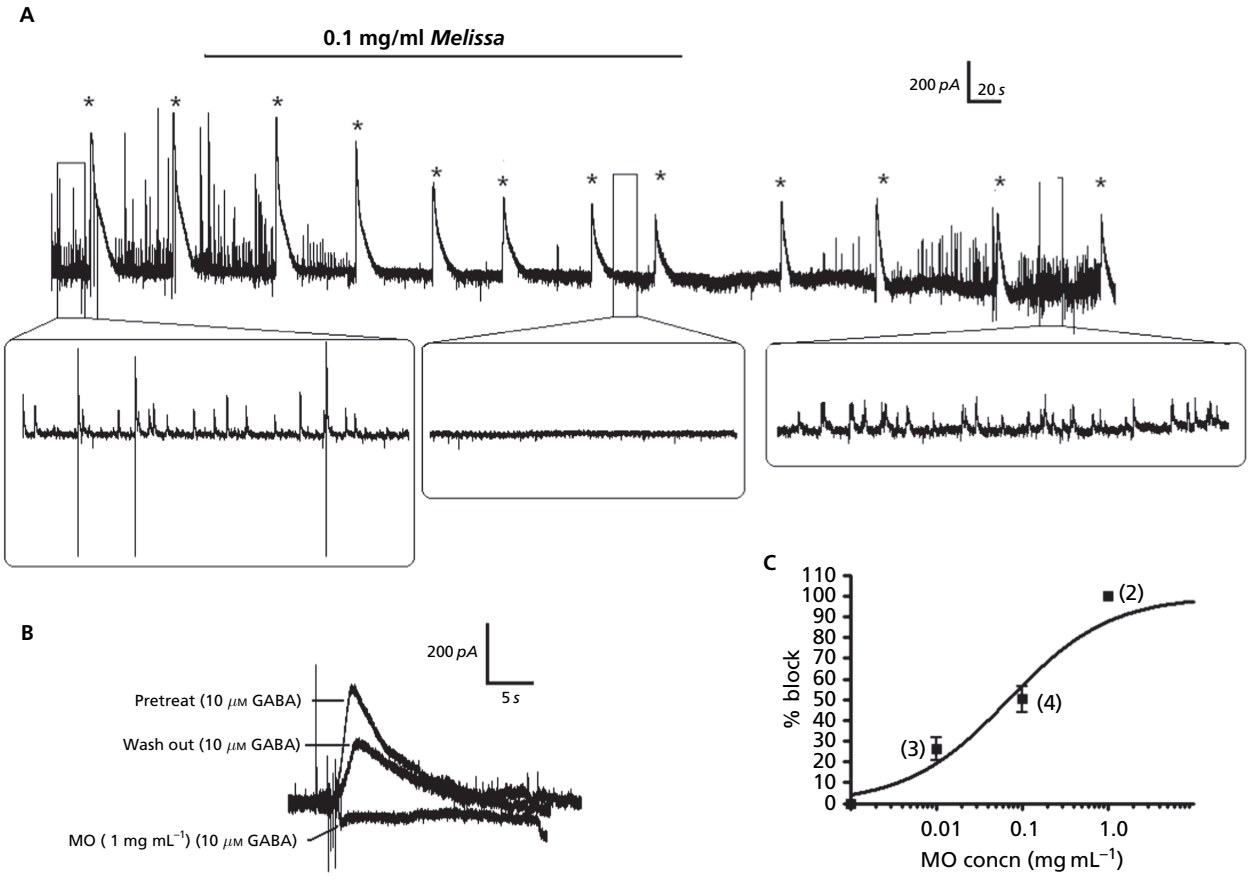

Figure 4 Effect of*Melissa officinalis* essential oil (MO) on GABA-mediated currents in primary cortical neurons. (A) MO 0.1 mg mL^{-1} produced a rapid and partially reversible block of spontaneous inhibitory post-synaptic currents (fast upward deflections in the trace: shown in detail in the expanded insets below the compressed trace). The asterisks indicate application of 500 ms pulses of exogenous GABA (10 μM). Note that these events are blocked to a similar extent as the multi-quantal synaptic inhibitory events and that the excitatory input to the cell (downward deflections indicate excitatory post-synaptic currents and spontaneous action currents) is also reduced during exposure to MO. (B) Expanded traces from a representative experiment showing that the higher concentration of 1 mg mL^{-1} MO completely blocked the evoked GABA currents (again partially reversible). (C) Concentration–response plot showing the compounded data on the effects of MO on evoked responses to exogenous GABA application. The IC50 is 0.071 mg mL^{-1}. Data are mean ± s.e.m. (n values are given in parentheses on the graph).

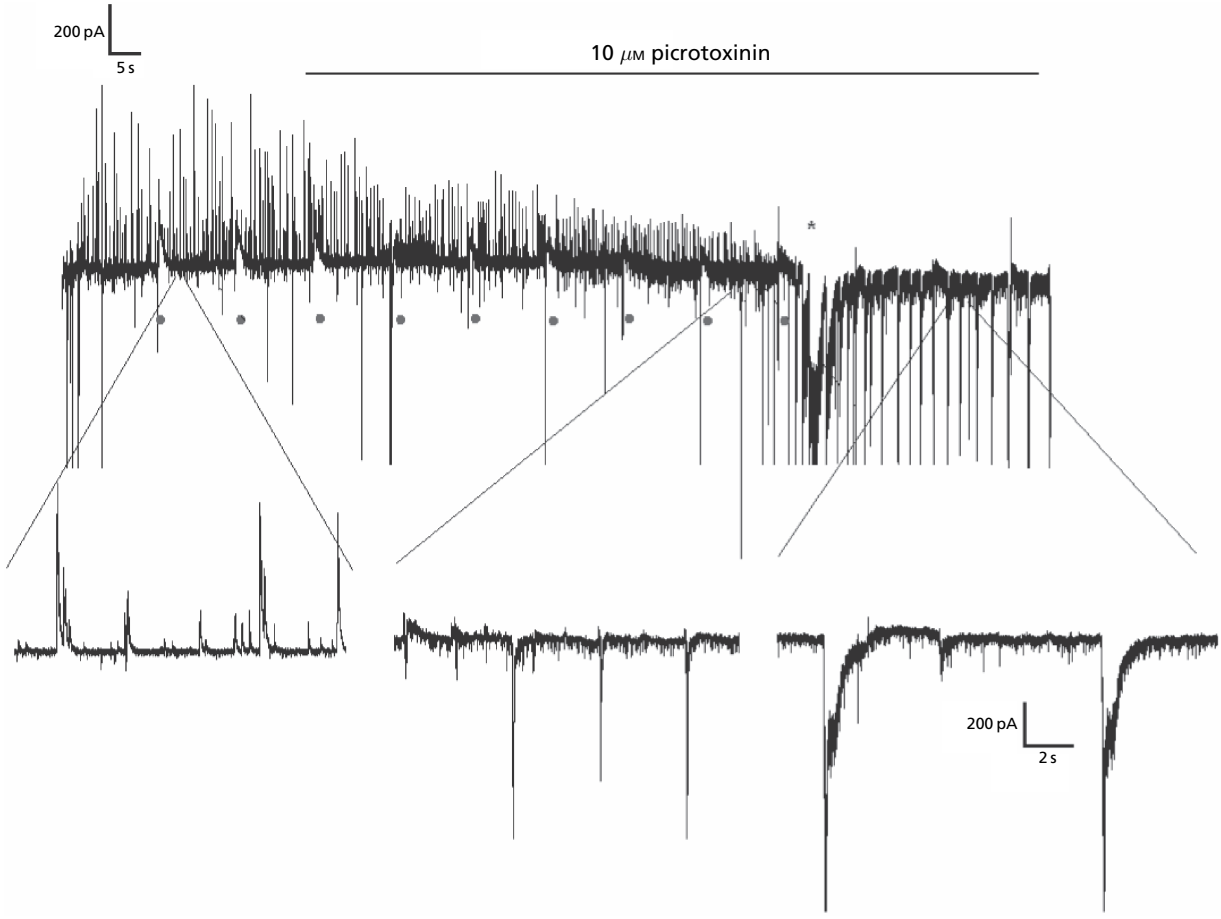

Figure 5 Effect of the selective $GABA_A$ antagonist, picrotoxinin. Note the complete erosion of spontaneous synaptic inhibition (fast upward events) and the responses to exogenous GABA (indicated by dots below the trace). The typical result of this disinhibition (all of the four cells tested) is illustrated at the end of the treatment period. Note the marked enhancement in excitatory (downward events) and the evolution of protracted paroxysmal shifts (asterisk) with burst firing profiles, which are typical of epileptiform firing in brain cells. The expanded traces show the control activity (left), the enhanced synaptic excitation during the onset of GABA blockade (centre) then paroxysmal shifts with superimposed bursts of action currents (right).

on the evoked GABA responses (Figure 4A). MO concurrently reduced the incidence of both excitatory and inhibitory spontaneous synaptic traffic in the cultured networks (see inset traces in Figure 4B) which is not a feature of selective $GABA_A$ antagonists in these cells. Figure 5 shows the selective blocking effect of the non-competitive and selective $GABA_A$ antagonist picrotoxinin, which blocked both evoked outward currents and spontaneous IPSCs. This disinhibition resulted in paroxysmal depolarizing shifts and epileptiform burst firing in all four of the picrotoxinin-treated pyramidal cells.

Discussion

BPSD have a negative impact on patients' activities of daily living and on caregivers' quality of life. Aggression and agitation are particularly serious and problematic symptoms for family caregivers, and these symptoms are often the primary cause of hospital admission or institutional care. Aggression and agitation occur in 20–80% of patients with Alzheimer's disease. Imbalances of different neurotransmitters (acetylcholine, dopamine, noradrenaline, serotonin and GABA) have been proposed as neurobiological causes of BPSD (Hardy et al 1987; Herrmann & Lanctot 1997; Garcia-Alloza et al 2005). Although non-pharmacological interventions, such as the verbal environmental intervention, should be first-line treatments for milder BPSD, many psychotropic agents (e.g. conventional antipsychotics, benzodiazepines, antidepressants, anticonvulsants and beta-blockers) have been used in attempts to manage aggressive behaviour. However, their efficacy is insufficient and their use has been limited because of adverse effects such as orthostatic hypotension, arrhythmia, extra-pyramidal symptoms, urinary retention, constipation, sedation and delirium (Carson et al 2006).

Pharmacological targets for the reduction of agitation and accompanying or underlying aggression or anxiety include the neurotransmitter systems serotonin, dopamine, acetylcholine (via nicotinic and muscarinic receptors) and GABA.

In this work, we have characterized the effect of MO on the three major binding sites of the $GABA_A$ receptor: the benzodiazepine site, the GABA site and the ion channel site, to detect any $GABA_A$ modulatory activity. To confirm selectivity, interactions with other common ligand-gated ion channel receptors (NMDA and AMPA) and neuronal nicotinic receptor were also investigated.

We have shown that MO inhibited [^{35}S] TBPS binding in a concentration-dependent manner in native and recombinant $GABA_A$ receptors. Interestingly, the oil alone showed stimulatory effects on agonist binding, but no effect on the benzodiazepine site. Some degree of specificity towards the $GABA_A$ receptor was evident, with no effects on other major ligand-gated ion channels expressed in the central nervous system.

To confirm our radioligand binding findings, an electrophysiological study was performed using cultured rat cortical neurons. We found that 0.1–1 mg mL^{-1} MO significantly reduced GABA-evoked current in cultured neurons and, surprisingly, silenced both inhibitory and excitatory traffic in neuronal networks. Our comparative experiment with the selective $GABA_A$ receptor antagonist picrotoxinin confirms that selective block of the $GABA_A$ channel enhances synaptic drive, elicits full-blown action potentials and evokes epileptiform activity in the form of paroxysmal depolarizing shifts and concomitant high-frequency firing. At 0.1 mg mL^{-1} MO profoundly inhibited GABA-induced current, and both excitatory and inhibitory synaptic activity was almost completely blocked in neuronal networks at this concentration. (Note that full block of post-synaptic currents evoked by exogenous GABA was achieved at 1 mg mL^{-1}.) These net 'depressant' effects are possibly mediated by a relatively high-affinity pre-synaptic interaction. Similar depressant actions are seen with clinically useful drugs that target voltage-gated ion channels and depress membrane excitability via a pre-synaptic interaction (Lees & Leach 1993).

Conclusion

The pharmacological activities of MO reflect its uses in traditional medicine as a sedative and/or anxiolytic agent (Perry & Perry 2006). We conclude that MO does exert depressant effects on neural activity, but that this is not a reflection of its interaction with the $GABA_A$ complex. At least one component in the oil does appear to bind to the GABA receptor but functionally this reduces inhibitory synaptic currents and responses to exogenous GABA application. We are currently attempting to delineate the active elements in this oil using functional group-based fractionation, to identify the depressant component(s), and to assess the behavioural effects of MO.

References

Abuhamdah, S., Fuerstner, A., Lees, G., Chazot, P. L. (2005) Pharmacological binding studies of caloporoside and novel congeners with contrasting effects upon [^{35}S] TBPS binding to mammalian $GABA_A$ receptor. *Biochem. Pharmacol*. **70**: 1382–1388

Adams, R. P. (2001) *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. USA: Allured Publishing Corporation

Akhondzadeh, S., Noroozian, M., Mohammadi, M., Ohadinia, S., Jamshidi, A. H., Khani, M. (2003) Melissa officinalis extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomised, placebo controlled trial. *J. Neurol. Neurosurg. Psychiatry* **74**: 863–866

Ballard, C. G., O'Brien, J. T., Reichelt, K., Perry, E. K. (2002) Aromatherapy as a safe and effective treatment for the management of agitation in severe dementia: the results of a double-blind, placebo-controlled trial with Melissa. *J. Clin. Psychiatry* **63**: 553–558

Beabrun, G., Gray, G. E. (2000) A review of herbal medicines for psychiatric disorders. *Psychiatr. Serv*. **51**: 1130–1134

Besnard, F., Even, Y., Itier, V., Granger, P., Partiséti, M., Avenet, P., Depoortere, H., Graham, D. (1997) Development of stable cell lines expressing different subtypes of $GABA_A$ receptors. *J. Recept Signal Transduct Res*. **17**: 99–113

Brodaty, H., Ames, D., Snowdon, J., Woodward, M., Kirwin, J., Clarnette, R., Lee, E., Lyons, B., Grossman, F. (2003) A randomized placebo-controlled trial of risperidone for the treatment of aggression, agitation, and psychosis of dementia. *J. Clin. Psychiatry* **64**: 134–143

Brum, L. F., Elisabetsky, E., Souza, D. (2001) Effects of linalool on [(3)H] MK801 and [(3)H] muscimol binding in mouse cortical membranes. *Phytother. Res*. **15**: 422–425

Carson, S., Mcdonagh, M. S., Peterson, K. (2006) A systematic review of the efficacy and safety of atypical antipsychotics in patients with psychological and behavioral symptoms of dementia. *J. Am. Geriatr. Soc*. **54**: 354–361

Chazot, P. L., Fotherby, A., Stephenson, F. A. (1993) Evidence for the involvement of a carboxyl group in the vicinity of the MK801 and magnesium ion binding site of the N-methyl-D-aspartate receptor. *Biochem. Pharmacol*. **45**: 605–610

Court, J. A., Lloyd, S., Johnson, M., Griffiths, M., Birdsall, N. J. M., Piggott, M. A., Oakley, A. E., Ince, P. G., Perry, E. K., Perry, R. H. (1997) Nicotinic and muscarinic cholinergic receptor binding in the human hippocampal formation during development and aging. *Dev. Brain Res*. **101**: 93–105

Elliott, M. S. G., Abuhamdah, S., Howes, M. J. R, Lees, G., Ballard, C. G., Burns, A., Chazot, P. L., Perry, E. K., Francis, P. T. (2007) The essential oils from *Melissa officinalis* L. and *Lavandula angustifolia* Mill. as potential treatment for agitation in people with severe dementia. *Int. J. Essential Oil Ther. In press*

Garcia-Alloza, M., Gil-Bea, F. J., Diez-Ariza, M., Chen, C. P., Francis, P. T., Lasheras, B., Ramirez, M. J. (2005) Cholinergic-serotonergic imbalance contributes to cognitive and behavioral symptoms in Alzheimer's disease. *Neuropsychologia* **43**: 442–449

Hardy, J., Cowburn, R., Barton, A., Reynolds, G., Dodd, P., Wester, P., O'Carroll, A. M., Lofdahl, E., Winblad, B. (1987) A disorder of cortical GABAergic innervation in Alzheimer's disease. *Neurosci. Lett*. **73**: 192–196

Herrmann, N., Lanctot, K. L. (1997) From transmitters to treatment: the pharmacotherapy of behavioural disturbances in dementia. *Can. J. Psychiatry* **42** (Suppl 1): 51S–64S

Honoré, T., Nielsen, M. (1985) Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosci. Lett*. **54**: 27–32

Kennedy, D. O., Scholey, A. B., Tildesley, N. T., Perry, E. K., Wesnes, K. A. (2002) Modulation of mood and cognitive performance following acute administration of Melissa officinalis (lemon balm). *Pharmacol. Biochem. Behav*. **72**: 953–964

Kennedy, D. O., Wake, G., Savelev, S., Tildesley, N. T., Perry, E. K., Wesnes, K. A., Scholey, A. B. (2003) Modulation of mood and cognitive performance following acute administration of single doses of Melissa officinalis (lemon balm) with human CNS nicotinic and muscarinic receptor-binding properties. *Neuropsychopharmacology* **28**: 1871–1881

Kennedy, D. O., Little, W., Scholey, A. B. (2004) Attenuation of laboratory-induced stress in humans after acute administration of Melissa officinalis (lemon balm). *Psychosom. Med*. **66**: 607–613

Kennedy, D. O., Little, W., Haskell, C. F., Scholey, A. B. (2006) Anxiolytic effects of a combination of Melissa officinalis and Valeriana officinalis during laboratory induced stress. *Phytother. Res*. **20**: 96–102

Lee, S. Y. (2005) The effect of lavender aromatherapy on cognitive function, emotion, and aggressive behavior of elderly with dementia. *Taehan Kanho Hakhoe Chi* **35**: 303–312

Lees, G., Leach, M. J. (1993) Studies on the mechanism of action of the novel anticonvulsant lamotrigine (Lamictal) using primary neuroglial cultures from rat cortex. *Brain Res*. **612**: 190–199

Lees, G., Chazot, P. L., Vankayalapati, H., Singh, G. (2000) A simple polar deacetylated caloporoside derivative is a positive modulator of the GABA(A) chloride channel complex in cortical mammalian neurones. *Bioorg. Med. Chem. Lett*. **10**: 1759–1761

Lin, P. W., Chan, W. C., Ng, B. F., Lam, L. C. (2007) Efficacy of aromatherapy (Lavandula angustifolia) as an intervention for agitated behaviours in Chinese older persons with dementia: a cross-over randomized trial. *Int. J. Geriatr. Psychiatry* **22**: 405–410

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem*. **193**: 265–275

Murase, K., Ryu, P. D., Randic, M. (1989) Excitatory and inhibitory amino acids and peptide-induced responses in acutely dissociated dorsal horn neurons. *Neurosci. Lett*. **103**: 56–63

Perry, N., Perry, E. (2006) Aromatherapy in the management of psychiatric disorders: clinical and neuropharmacological perspectives. *CNS Drugs* **20**: 257–280

Viola, H., Wasowski, C., Levi de Stein, M., Wolfman, C., Silveira, R., Dajas, F., Medina, J. H., Paladini, A. C. (1995) Apigenin, a component of Matricaria recutita flowers, is a central benzodiazepine receptor-ligand with anxiolytic effects. *Planta Med*. **61**: 213–216