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Effects of anticonvulsants on soman-induced epileptiform activity in the guinea-pig in vitro hippocampus

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Abstract

Seizures arising from acetylcholinesterase inhibition are a feature of organophosphate anticholinesterase intoxication. Although benzodiazepines are effective against these seizures, alternative anticonvulsant drugs may possess greater efficacy and fewer side-effects. We have investigated in the guinea-pig hippocampal slice preparation the ability of a series of anticonvulsants to suppress epileptiform bursting induced by the irreversible organophosphate anticholinesterase, soman (100 nM). Carbamazepine (300 μM), phenytoin (100 μM), topiramate (100–300 μM) and retigabine (1–30 μM) reduced the frequency of bursting but only carbamazepine and phenytoin induced a concurrent reduction in burst duration. Felbamate (100–500 μM) and clomethiazole (100–300 μM) had no effect on burst frequency but decreased burst duration. Clozapine (3–30 μM) reduced the frequency but did not influence burst duration. Levetiracetam (100–300 μM) and gabapentin (100–300 μM) were without effect. These data suggest that several compounds, in particular clomethiazole, clozapine, felbamate, topiramate and retigabine, merit further evaluation as possible treatments for organophosphate poisoning.

Keywords: Acetylcholinesterase; Anticonvulsant; Epileptiform activity; Hippocampal slice; Organophosphate; Soman

1. Introduction

Organophosphate anticholinesterases exert their primary toxic effects by preventing the hydrolysis of acetylcholine by acetylcholinesterase (Taylor, 1996). The subsequent accumulation of acetylcholine at peripheral cholinergic sites produces the classical muscarinic and nicotinic receptor-mediated signs of anticholinesterase intoxication including salivation, lacrimation, urination, miosis or mydriasis, diarrhoea, bronchospasm, bronchorrhoea, vomiting, muscle fasciculation, sweating, hypertension and tachycardia or bradycardia (Taylor, 1996; Verhulst et al., 2002). Signs of central nervous system involvement are commonly observed in severe organophosphate poisoning: in a retrospective case note review of 5541 children intoxicated with organophosphate anticholinesterase insecticides, Verhulst et al. (2002) documented signs of seizures (tonic-clonic convulsions) in 30% of the cohort, depressed levels of consciousness in 31% and respiratory failure requiring mechanical ventilation in 35%.

First-line treatment for organophosphate anticholinesterase intoxication consists of a muscarinic receptor antagonist (usually atropine) combined with an oxime reactivator of organophosphate-inhibited cholinesterase (e.g. pralidoxime or obidoxime—Karalliedde and Senanayake, 1989; Marrs, 2003). A benzodiazepine anticonvulsant (usually diazepam) is the treatment currently indicated for control of seizures (Karalliedde and Senanayake, 1989; Marrs, 2003).

Although the efficacy of diazepam against organophosphate-induced seizures is apparent both clinically (Marrs, 2003) and in animal studies (McDonough et al., 2000), the well-established depressant actions of benzodiazepines on central respiratory drive (Forster et al., 1980), in combination with the deleterious consequences of cholinergic excess on airway and respiratory muscle function, are a potential cause for concern. An alternative anticonvulsant,

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combining the anti-seizure efficacy of benzodiazepines but without any impairment of respiration, would constitute a significant therapeutic advance. Moreover, some animal studies have suggested that organophosphate-induced seizures can recur following apparently successful initial treatment with benzodiazepines, thereby increasing the risk of neuropathological sequelae (Anderson et al., 1997).

Organophosphate-induced seizures cannot easily be reproduced in naïve animals due to the steep dose-toxicity profile shown by irreversible organophosphate anticholinesterase. Instead, studies employ adjuncts (e.g. atropine and oxime) in order to produce seizure activity but prevent immediate mortality (McDonough et al., 2000). However, these adjuncts, together with the inherent complexities of an in vivo test system, can confound interpretation of anticonvulsant efficacy. Thus, studying the effects of potential anticonvulsants against epileptiform activity induced by organophosphate anticholinesterases in vitro may prove advantageous. With this in mind, we have developed a guinea-pig hippocampal slice preparation in which organophosphate anticholinesterase-induced epileptiform activity can be reliably induced (Harrison et al., 2004).

In the present study, we have evaluated a series of established, emergent and experimental anticonvulsant drugs for their ability to reverse epileptiform activity induced by the irreversible organophosphate anticholinesterase, soman, in the CA1 cell body layer (an area which develops and maintains epileptiform bursting following exposure to organophosphate anticholinesterases (Endres et al., 1989). Some of this work has appeared in abstract form (Harrison et al., 2001, 2002).

2. Methods

2.1. Slice preparation and recording

Male Dunkin–Hartley guinea-pigs (250–500 g) were anaesthetised with halothane and killed by decapitation. The brain was then removed and the left and right hippocampi removed and bisected transversely into temporal and septal tissue blocks. Five to seven transverse slices (500 μm thick) from the temporal section were prepared using a Vibratome (Vibratome Company, Missouri, USA). The slices were stored at room temperature in an artificial cerebral spinal fluid (CSF) of the following composition (in mM): NaCl 118, KCl 3, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgCl_2$ 1, $CaCl_2$ 1.5, D-glucose 10 (gassed with 95% O_2/5% CO_2). Slices were allowed to recover for at least 1 h before transfer to a recording chamber (volume ≈ 1 ml) where they were submerged and continuously superfused (at 10–15 ml/min) with gassed artificial CSF at 31 °C.

2.2. Extracellular recording

After a further 30 min equilibration, an extracellular recording electrode (filled with 2 M NaCl; 1–5 MΩ resistance) was placed in CA1 *stratum pyramidale* in order to monitor neuronal activity. The Schaffer collateral/commissural pathway was then stimulated every 30 s via a bipolar stimulating electrode (MCE 100, Harvard apparatus; stimulation pulse width ≈ 150 μs, amplitude ≈ 250 μA) and the recording electrode gradually lowered into the CA1 cell layer until the population spike amplitude was maximal. Stimulation was then terminated. Baseline activity was monitored for at least 30 min before application of soman; any slices showing spontaneous epileptiform activity during this period were discarded. Soman was applied for up to 60 min—if epileptiform activity had not appeared by this time the slice was classified as 'unresponsive', but only after slice viability had been confirmed by the appearance of epileptiform activity following exposure to the K^+channel blocker, 4-aminopyridine (30 μM). In slices in which epileptiform activity appeared, the activity was allowed to continue for 15 min before addition of the test compound. All compounds were applied by addition of appropriate volumes of concentrated stock solution directly into the perfusion reservoir (exchange and equilibration of added compounds with the recording chamber occurred within 3 min). In a series of separate vehicle control experiments, dimethylsulphoxide (DMSO), at the highest concentration used (0.3% v/v), exerted no effect on either baseline or soman-induced epileptiform activity (data not shown).

2.3. Data analysis

Data were captured via a CED 1401 interface (2 kHz sampling rate) controlled by Spike 2 software (Cambridge Electronic Design, UK). At the end of each experiment the digitised trace was manually analysed for burst characteristics (amplitude, duration and rate) using on-screen cursors. A burst was defined as a deflection from baseline noise lasting >10 ms, with a magnitude of >0.1 mV above baseline and separated from neighbouring "bursts" by a quiescent period of >2 s. To determine the effect of the various treatments on epileptiform activity, the average burst rate in the 10 min epoch immediately before anticonvulsant application was compared with the burst rate 30–40 min after treatment application (throughout this paper the terms bursting and epileptiform activity are used interchangeably). To determine the effect of the various treatments on burst duration, the burst immediately before drug application was compared to the last burst after 30–40 min of anticonvulsant exposure. Due to the good antiepileptiform efficacy of some of the treatments, bursting was not always observed during the 30–40 min epoch. In these cases the number of slices from which the burst duration was calculated is provided in the figure legend.

Unless otherwise specified, summarised data are presented as mean ± S.E.M., with a significant difference determined using one-way analysis of variance (ANOVA) followed by Dunnett's *post-hoc* test.

2.4. Drugs

All chemicals were obtained from either Sigma-Aldrich Co Ltd (Poole, UK) or Tocris Cookson Ltd (Langford, UK) with the following exceptions: soman (pinacolyl methylphosphonofluoridate; purity >95%; approximately 5 mg/ml in isopropyl alcohol) was synthesised by the Department of Chemistry, Dstl Porton Down; levetiracetam was a kind gift from UCB Pharma (Belgium); topiramate was a kind gift from The R.W. Johnson Pharmaceutical Research Institute (PA, USA); retigabine was a kind gift from ASTA Medica (Frankfurt, Germany—now licensed by Xcel Pharmaceuticals Inc., San Diego, USA).

Soman was diluted daily from a ≈5 mg/ml stock (isopropyl alcohol) using artificial CSF to a final concentration of 100 nM. Candidate compounds were made up into a stock solution and frozen into aliquots. A fresh aliquot was diluted as necessary and used for each experiment (maximum vehicle concentration 0.3% v/v). Compounds (generic name; chemical name; solvent) were dissolved as follows: baclofen (4-amino-3-(4-chlorophenyl) butanoic acid; water), carbamazepine (5H-dibenz(*b*,*f*)azepine-5-carboxamide; DMSO), clomethiazole (5-(2-chloroethyl)-4-methyl-thiazole hydrochloride; water), clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[*b*,*e*][1,4]diazepine; DMSO), felbamate (2-phenyl-1,3-propanediol dicarbamate; DMSO), gabapentin (1-(aminomethyl)-cyclohexaneacetic acid; water), galanin (galanin (1–30) (human); water), L733,060 (3-[[3,5-*bis*(trifluoromethyl) phenyl]methoxy]-2-phenyl-piperidine; DMSO), levetiracetam ((*S*)-(-)-α-ethyl-2-oxo-1-pyrrolidine acetamide; water), MPEP (2-methyl-6-(phenylethynyl)pyridine hydrochloride; water), phenytoin (5,5-diphenyl-2,4-imidazolidinedione; DMSO), retigabine (*N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester; DMSO) and topiramate (2,3:4,5-*bis*-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate; water).

3. Results

3.1. Soman-induced epileptiform activity

In agreement with previous work (Harrison et al., 2004), addition of 100 nM soman to the circulating artificial CSF induced epileptiform bursting within the CA1 *stratum pyramidale* in ≈75% of the hippocampal slices studied. In the 126 slices in which epileptiform activity was induced by soman (100 nM) in the present study, the mean latency to onset of bursting was 20.0±1.3 min, discharge frequency 5–15 min after initiation of bursting activity was 2.1±0.1 bursts/min, peak-to-peak amplitude was 2.0±0.1 mV and burst duration was 625±19 ms. In the absence of drug intervention in control slices, bursting continued in all slices for up to 4.5 h (the longest period recorded).

3.2. Effect of drug application on soman-induced bursting

A range of drugs was applied with a diverse spectrum of actions in order to characterise the soman-induced epileptiform activity and to establish which of the compounds would be worth investigating further in vivo. A summary of these compounds is provided in Table 1 along with their known (or proposed) mechanisms of action and, where applicable, their approximate therapeutic plasma concentration in humans.

3.2.1. Carbamazepine and phenytoin

Application of carbamazepine (30, 100 and 300 μM) abolished soman-induced bursting at the highest concentration tested, and produced a significant reduction in burst duration at 30 and 100 μM ($P<0.01$; see Figs. 1 and 2). Phenytoin (10, 30 and 100 μM) abolished soman-induced bursting at 100 μM and a significant reduction in the duration of bursts was seen at 30 μM ($P<0.05$; see Figs. 1 and 2). The concentrations at which carbamazepine and phenytoin exerted these effects are comparable to those reported for their effects on penicillin-induced epileptiform activity in guinea-pig hippocampus (Oliver et al., 1977; Schneiderman and Evans, 1986) but higher than those reported to block epileptiform activity induced by removal of Ca^{2+} from the artificial CSF (Ashton et al., 1988a).

3.2.2. Clomethiazole and felbamate

Application of clomethiazole (100 and 300 μM) failed to inhibit soman-induced bursting ($P>0.05$). However, a significant reduction in the duration of soman-induced bursts was seen at both concentrations tested ($P<0.01$; see Figs. 3 and 4). Application of felbamate (100 and 500 μM) also failed to inhibit soman-induced bursting ($P>0.05$), although a significant reduction in the duration of soman-induced bursts was seen ($P<0.05$ at 100 μM and

Table 1
Summary of the principal drugs evaluated with their mechanisms of action and therapeutic concentrations

Drug	Mechanism of action	Concentration (μM)	References
Carbamazepine	Na^+ channel block	≈40	MacDonald and Kelly, 1994; McNamara, 1996
Phenytoin	Na^+ channel block	≈30	MacDonald and Kelly, 1994; McNamara, 1996
Clomethiazole	Modulation of $GABA_A$ receptor Potentiation of glycine responses	≈70	Reviewed by Ogren, 1986; Green, 1998; Kong et al., 2002
Felbamate	NMDA subunit selective inhibitor Na^+ channel block Potentiation of GABA responses	≈50–600	Wilson and Brodie, 1996; Kleckner et al., 1999; Harden et al., 1996
Retigabine	Activation of K^+ channels	≈1	Rundfeldt and Netzer, 2000; Bialer et al., 1999
Topiramate	Inhibition of Na^+ or Ca^{2+} channels Enhancement of GABA currents Inhibition of kainate currents Inhibition of carbonic anhydase	≈20	Reviewed by Shank et al., 2000
Clozapine	Dopamine receptor antagonist Muscarinic receptor antagonist Many other interactions	≈1	Ashby and Wang, 1996; Goossen et al., 2003
Levetiracetam	Modulation of Ca^{2+} channels Modulation of GABA and glycine currents Reduction of Ca^{2+} release	≈5–10	Klitgaard et al., 2003; Lukyanetz et al., 2002; Rigo et al., 2002; Angehagen et al., 2003; Perucca et al., 2003
Gabapentin	Ca^{2+} channel block Increased GABA synthesis	≈30	Taylor et al., 1998; Gatti et al., 2003

The table above provides a brief summary of some of the clinical drugs employed in this study, along with proposed mechanisms of action, human therapeutic plasma concentrations and appropriate references. This is intended as a broad overview and not as a definitive guide.

$P<0.01$ at 500 μM; see Figs. 3 and 4). The concentrations of these compounds are similar to those shown to have significant effects on epileptiform activity induced by removal of Mg^{2+} from the circulating artificial CSF, with slightly higher concentrations required to block bursting (Empson et al., 2000; Libri et al., 1996).

3.2.3. Retigabine

Application of retigabine (1, 3, 10 and 30 μM) produced significant inhibition of bursting activity at all concentrations tested ($P<0.05$ at 1 μM; $P<0.01$ at 3 μM), with bursting abolished at 10 and 30 μM (see Fig. 5). However, retigabine produced no significant effect on the duration of bursts ($P>0.05$; see Fig. 5). The potency of retigabine was comparable to that reported elsewhere against low Ca^{2+}- and low Mg^{2+}-induced epileptiform activity in the rat hippocampal slice (Dost and Rundfeldt, 2000).

3.2.4. Topiramate

Application of topiramate (30, 100 and 300 μM) reduced the burst rate with a significant effects observed at concentrations ≥100 μM ($P<0.05$; Fig. 6). Unexpectedly, topiramate (300 μM) induced a significant increase in the duration of soman-induced bursts ($P<0.05$; Fig. 6). The concentrations employed (and the corresponding degree of inhibition of bursting) are in accord with those previously reported for electrical stimulation-induced epileptiform activity in rat cortex/hippocampal slices (Jahromi et al., 2000).

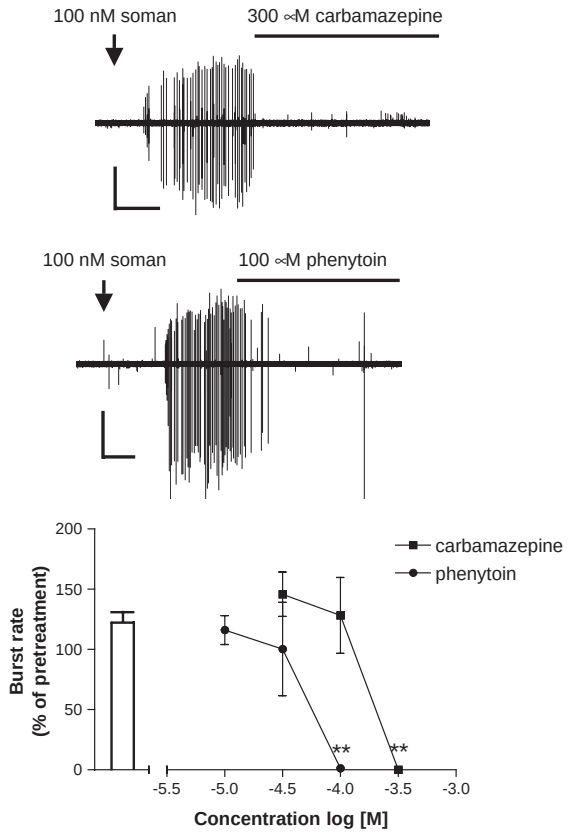

Fig. 1. The effect of carbamazepine and phenytoin on the rate of soman-induced bursting. An example of the effect of carbamazepine on bursting (top; horizontal bar: 10 min, vertical bar: 1 mV) and phenytoin (middle horizontal bar: 10 min, vertical bar: 1 mV) on bursting is shown above. A summary of these effects 30–40 min after intervention is shown bottom (abscissa: log [*M*]; ordinate: burst rate as a percentage of pretreatment). Each point represents the mean of 4–5 individual experiments ± S.E.M. **, $P=<0.01$ and denotes statistical significance from control (as illustrated by the column).

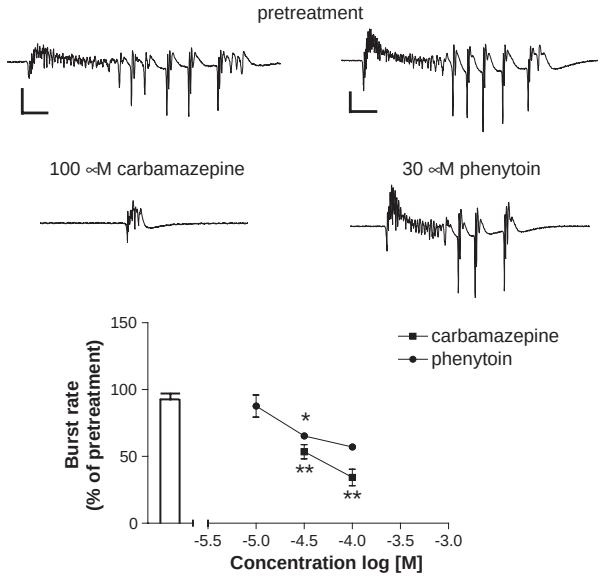

Fig. 2. The effect of carbamazepine and phenytoin on the duration of soman-induced bursts. An example of the effect of carbamazepine (top left; horizontal bar: 100 ms, vertical bar: 1 mV) and phenytoin (top right; horizontal bar: 100 ms, vertical bar: 1 mV) on burst duration is shown above. A summary of these effects 30–40 min after intervention is shown bottom (abscissa: log [*M*]; ordinate: burst duration as a percentage of pretreatment). Each point represents the mean of 3–5 individual experiments ± S.E.M. *, $P=<0.05$; **, $P=<0.01$ and denotes statistical significance from control (as illustrated by the column). Only 1/4 slices showed bursting 30–40 min after intervention with 100 μM phenytoin.

3.2.5. Clozapine

Application of clozapine (3, 10 and 30 μM, $n=3$–4 slices) produced a significant inhibition of bursting activity ($P<0.01$) at all concentrations tested (Fig. 7). However, no significant reduction in the duration of soman-induced bursts was seen at any concentration tested ($P>0.05$). Although classed as an antipsychotic agent (thought to act via blockade of dopamine and 5-hydroxytryptamine receptor subtypes), clozapine has marked affinity for muscarinic receptors (Bolden et al., 1992). Although the mechanism by which clozapine inhibited soman-induced bursting is unknown, the concentration range over which this compound inhibited bursting is comparable to that at which antimuscarinics inhibit soman-induced bursting relative to their affinity for muscarinic receptors (Harrison et al., 2004; Bolden et al., 1992).

3.2.6. Levetiracetam

Levetiracetam (10, 30, 100 and 300 μM, $n=4$ slices) failed to reduce either the burst rate or the burst duration ($P>0.05$; Fig. 8). The concentrations used here cover those shown to have significant effects in other in vitro models of seizure activity (Birnstiel et al., 1997; Margineanu and Klitgaard, 2000).

3.2.7. Gabapentin and miscellaneous others

Application of gabapentin (100 and 300 μM, $n=3$-4 slices) failed to reduce burst rate or burst duration ($P>0.05$). These concentrations are known to have significant effects in vitro (Lucke et al.,

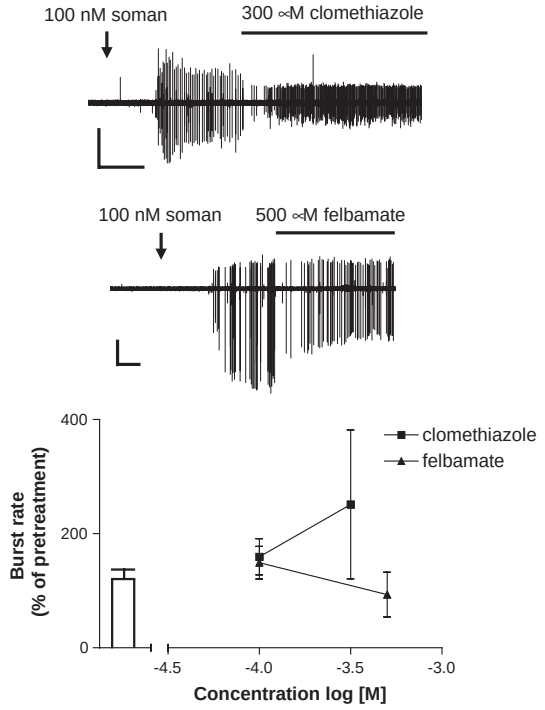

Fig. 3. The effect of clomethiazole and felbamate on the rate of soman-induced bursting. An example of the effect of clomethiazole (top; horizontal bar: 10 min, vertical bar: 1 mV) and felbamate (middle horizontal bar: 10 min, vertical bar: 1 mV) on bursting is shown above. A summary of these effects 30–40 min after intervention is shown bottom (abscissa: log [*M*]; ordinate: burst rate as a percentage of pretreatment). Each point represents the mean of 4–5 individual experiments±S.E.M. No statistical difference from control (as illustrated by the column) values was observed ($P>0.05$).

1998; Taylor et al., 1998). As this compound has been proposed as a subtype-selective $GABA_B$ (gamma-aminobutyric acid) receptor agonist (Ng et al., 2001), we were interested to determine the effect of the non-selective $GABA_B$ receptor agonist, baclofen. Application of 1 μM baclofen ($n=3$ slices) completely abolished bursting in all slices tested. A similarly potent effect on epileptiform activity has been reported previously (Ault et al., 1986).

In addition, a number of other compounds that have also been reported to have potential anticonvulsant/antiseizure properties were also assessed in a few slices (Chapman et al., 2000; Wasterlain et al., 2000; Mazarati et al., 1998). The metabotropic glutamate receptor antagonist, MPEP (30 μM, $n=4$), the novel tachykinin NK1 receptor antagonist, L733,060 (10 and 30 μM, $n=4$ and 1, respectively) and the neuropeptide galanin (300 nM, $n=4$) all failed to induce a reduction in either the rate or burst duration of soman-induced epileptiform activity ($P>0.05$, unpaired *t*-test).

4. Discussion

One potential result of organophosphate intoxication, even with medical intervention, is the development of seizures. Rapid control of these seizures is essential in order to minimise lethality, incapacitation, neuropathology and other undesirable effects. Currently, the benzodiazepine anticonvulsant, diazepam, is indicated for control of organophosphate-induced seizures. However, some animal models have suggested that despite initial success with diazepam treatment, organophosphate-induced seizures can recur. Moreover, the side-effects of benzodiazepines (e.g., respiratory depression and sedation) are undesirable. Thus, there is continuing need to evaluate new anticonvulsant compounds as possible replacements for benzodiazepines in the treatment organophosphate-induced seizures.

We therefore wished to assess a range of compounds to determine whether they show anticonvulsant properties, with the hope of identifying compounds meriting more extensive evaluation in the treatment of organophosphate intoxication. Moreover, the data obtained allow us to compare our slice model with other in vitro and in vivo models of epileptiform/seizure activity.

Application of candidate compounds produced a variety of effects, from complete abolition of epileptiform activity to no noticeable effect. Both of the classical voltage sensitive Na^+ channel blockers, carbamazepine and phenytoin, were capable of abolishing epileptiform activity. Interestingly, both also produced a reduction in the duration of soman-induced bursts at concentrations which had no significant effect on the rate of bursting. The reduction in the duration of epileptiform events was not limited to use dependent Na^+ channel blockers, as both clomethiazole and felbamate also produced a similar effect. However, a reduction in the burst duration was not seen with all compounds, e.g. retigabine and clozapine were capable of

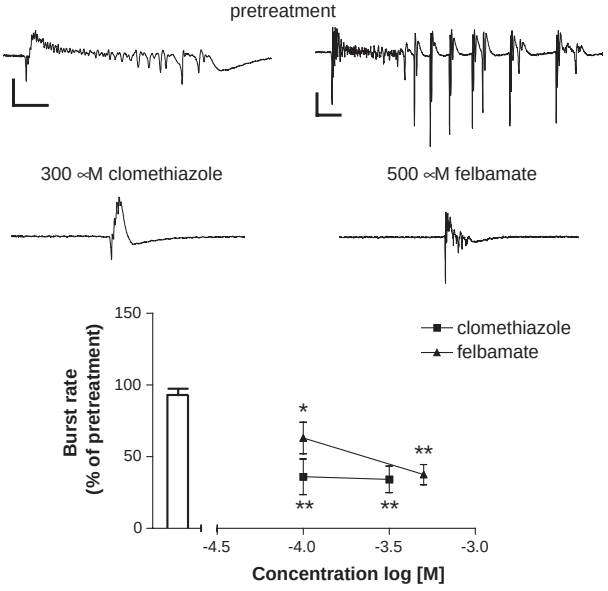

Fig. 4. The effect of clomethiazole and felbamate on the duration of soman-induced bursts. An example of the effect of clomethiazole (top left; horizontal bar: 100 ms, vertical bar: 1 mV) and felbamate (top right; horizontal bar: 100 ms, vertical bar: 1 mV) on burst duration is shown above. A summary of these effects 30–40 min after intervention is shown bottom (abscissa: log [*M*]; ordinate: burst duration as a percentage of pretreatment). Each point represents the mean of 3–5 individual experiments±S.E.M. *, $P=<0.05$; **, $P=<0.01$ and denotes statistical significance from control (as illustrated by the column).

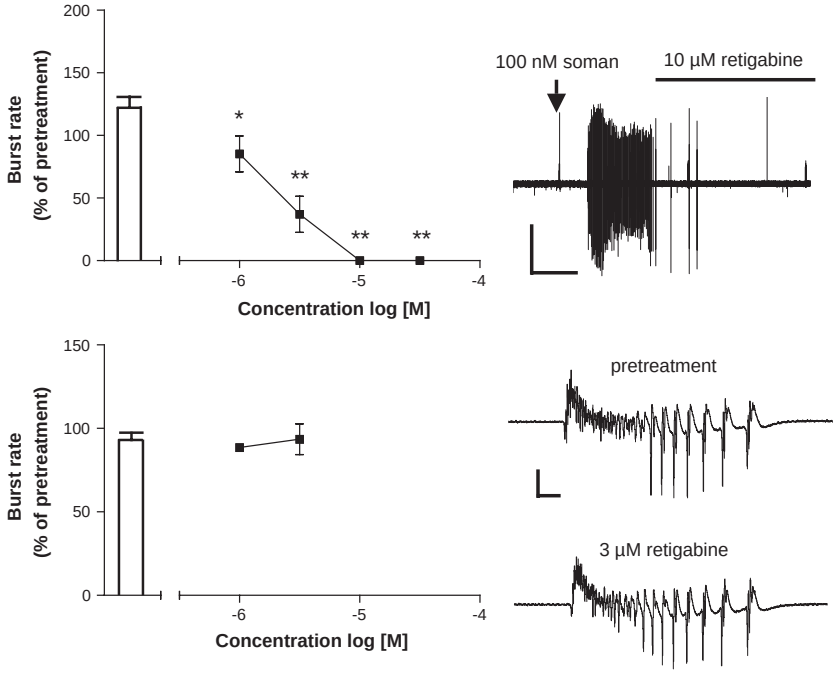

Fig. 5. The effect of retigabine on soman-induced bursting. An example of the effect of retigabine on the rate of soman-induced bursting is shown (top right; horizontal bar: 10 min, vertical bar: 1 mV) with a summary of these effects 30–40 min after intervention to the left (abscissa: log [*M*]; ordinate: burst rate as a percentage of pretreatment). An example of the effect of retigabine on the duration of soman-induced bursts is shown (bottom right; horizontal bar: 100 ms, vertical bar: 1 mV) with a summary of these effects 30–40 min after intervention to the left (abscissa: log [*M*]; ordinate: burst duration as a percentage of pretreatment. Each point represents the mean of 4–5 individual experiments±S.E.M. *, $P=<0.05$; **, $P=<0.01$ and denotes statistical significance from control (as illustrated by the column).

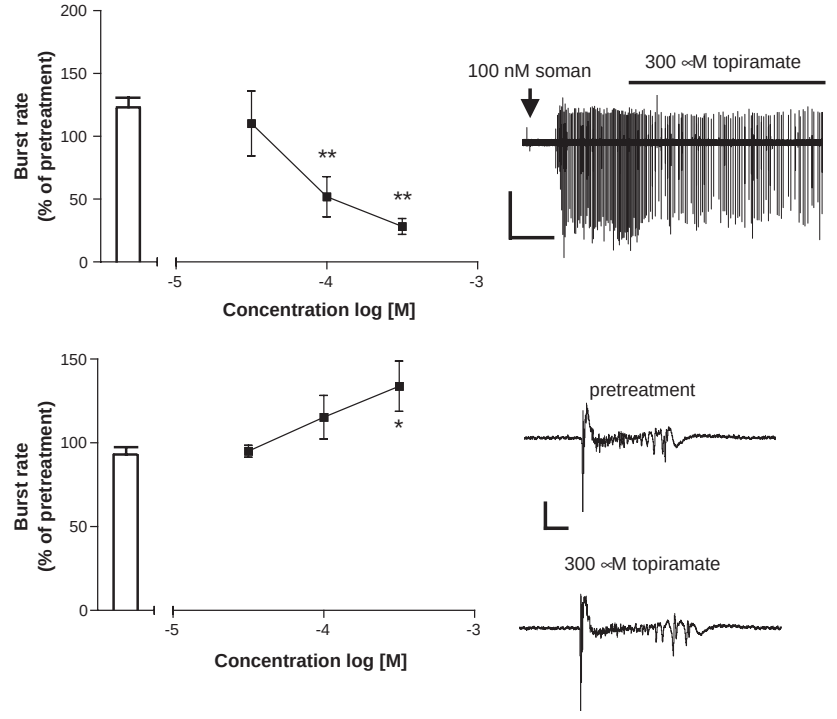

Fig. 6. The effect of topiramate on soman-induced bursting. An example of the effect of topiramate on the rate of soman-induced bursting is shown (top right; horizontal bar: 10 min, vertical bar: 1 mV) with a summary of these effects 30–40 min after intervention to the left (abscissa: log [*M*]; ordinate: burst rate as a percentage of pretreatment). An example of the effect of topiramate on the duration of soman-induced bursts is shown (bottom right; horizontal bar: 100 ms, vertical bar: 1 mV) with a summary of these effects 30–40 min after intervention to the left (abscissa: log [*M*]; ordinate: burst duration as a percentage of pretreatment. Each point represents the mean of 3–5 individual experiments±S.E.M. *, $P=<0.05$; **, $P=<0.01$ and denotes statistical significance from control (as illustrated by the column).

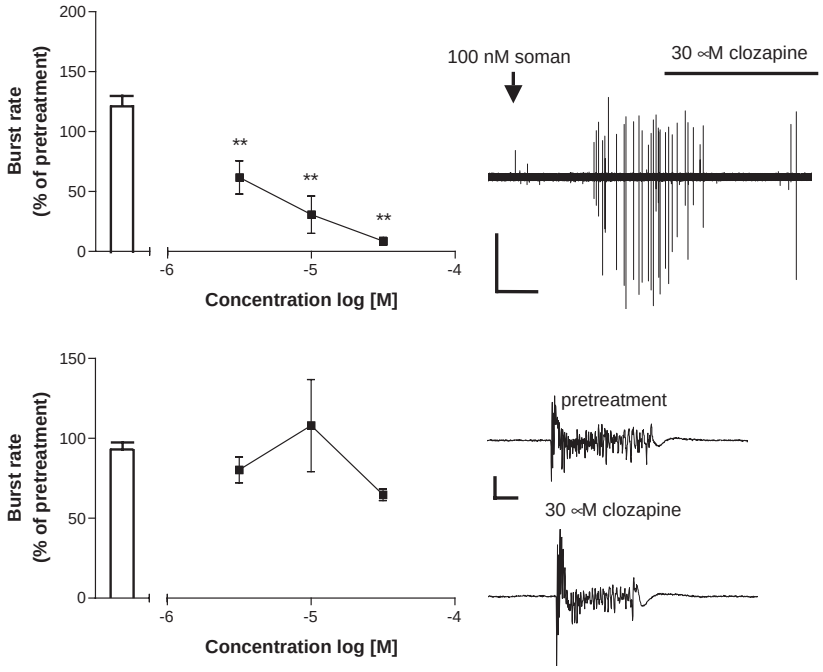

Fig. 7. The effect of clozapine on soman-induced bursting. An example of the effect of clozapine on the rate of soman-induced bursting is shown (top right; horizontal bar: 10 min, vertical bar: 1 mV) with a summary of these effects 30–40 min after intervention to the left (abscissa: log [*M*]; ordinate: burst rate as a percentage of pretreatment). An example of the effect of clozapine on the duration of soman-induced bursts is shown (bottom right; horizontal bar: 100 ms, vertical bar: 1 mV) with a summary of these effects 30–40 min after intervention to the left (abscissa: log [*M*]; ordinate: burst duration as a percentage of pretreatment. Each point represents the mean of 3–5 individual experiments ± S.E.M. **, $P = < 0.01$ and denotes statistical significance from control (as illustrated by the column). Only 3/4 slices showed bursting 30–40 min after intervention with 30 μM clozapine.

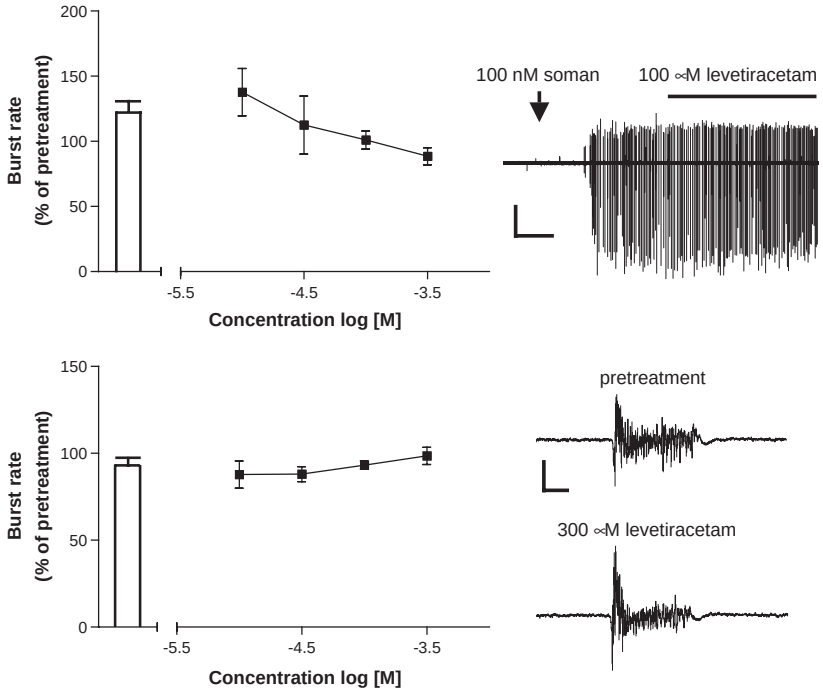

Fig. 8. The effect of levetiracetam on soman-induced bursting. An example of the effect of levetiracetam on the rate of soman-induced bursting is shown (top right; horizontal bar: 10 min, vertical bar: 1 mV) with a summary of these effects 30–40 min after intervention to the left (abscissa: log [*M*]; ordinate: burst rate as a percentage of pretreatment). An example of the effect of levetiracetam on the duration of soman-induced bursts is shown (bottom right; horizontal bar: 100 ms, vertical bar: 1 mV) with a summary of these effects 30–40 min after intervention to the left (abscissa: log [*M*]; ordinate: burst duration as a percentage of pretreatment. Each point represents the mean of 4–5 individual experiments ± S.E.M. No statistical difference from control (as illustrated by the column) values was observed ($P > 0.05$).

inhibiting the rate of soman-induced discharges without affecting the duration. An explanation for this difference may lie in the cause of the epileptiform activity and the mechanism of action for each drug. The primary stimulus for the epileptiform activity is the overstimulation of cholinergic receptors. Thus activation of muscarinic receptors by elevated acetylcholine levels causes the block of the resting K^+ current (I_m) and thus excitation of the *stratum pyramidale* cell layer (Cole and Nicoll, 1984). Consequently both clozapine (by competitive inhibition of the muscarinic receptor) and retigabine (by activation of the resting K^+ current) antagonise the stimulus for epileptiform discharges. In contrast, carbamazepine, phenytoin, clomethiazole and felbamate act further "downstream" to antagonise or dampen the spread of excitatory transmission. Topiramate was an exception in that an actual increase in burst duration was observed in conjunction with a reduction in burst rate. This compound has been reported to have a wide spectrum of actions, including the antagonism of muscarinic responses (Shank et al., 2000; Palmieri et al., 2000). It seems probable that it is one of these diverse mechanisms of action, not present in other compounds, that is responsible for the lengthening of duration.

Overall, the effects of the in vitro action of the tested compounds on soman-induced epileptiform activity are comparable with other slice models. Thus it has been reported that phenytoin at concentrations similar to those used here can block epileptiform discharges in the guinea-pig hippocampus induced by penicillin (via blockade of the post-synaptic $GABA_A$ receptor-channel complex) as well as reduce the duration of individual events (Oliver et al., 1977; Schneiderman and Evans, 1986). In contrast the concentrations of carbamazepine and phenytoin here are much higher than those required to block epileptiform activity induced by removal of Ca^{2+} in the guinea-pig hippocampus where IC_{50}s of 5 and 1 μM were reported for carbamazepine and phenytoin, respectively (Ashton et al., 1988a). This indicates that the soman-induced bursting is more akin to models where epileptiform activity is induced by "synaptic" methods, e.g. $GABA_A$ blockers, low Mg^{2+}, than by those of "non-synaptic" methods, e.g. low Ca^{2+} (Ashton et al., 1988b).

In comparison with the in vivo situation the concentrations of compounds employed here to abolish epileptiform activity are typically higher than those employed clinically, see Table 1. This discrepancy may in part be explained by the partitioning of drugs into CNS tissue in vivo, by different species sensitivities and different brain region sensitivity, and by problems in drug equilibration across the hippocampal slice (Heinemann et al., 1994). Despite these potential variables many drugs did show statistically significant effects on epileptiform activity at clinically relevant concentrations, supporting the view that similar actions would also occur in vivo.

The data here have demonstrated that several compounds show efficacy against soman-induced epileptiform activity in vitro; however, the situation in vivo is more complicated. Of those drugs tested in our slice model, phenytoin (and its pro-drug fosphenytoin) has been assessed the most comprehensively in vivo, where it has generally been found to be ineffective in controlling soman-induced seizures, although some protective effects were observed (Shih et al., 1991; Shih et al., 1999; McDonough et al., 2004). However, interpretation of in vivo data is complicated by the requirement of adjunctive therapy (typically atropine and oxime) due to the steep dose-lethality curve of organophosphorous agents such as soman. It seems reasonable to suggest, therefore, that this model as with all in vitro models, will not be 100% predictive and that some of the compounds effective here will not be so in vivo. However, initial work done here indicates that the converse may not be true, notably drugs which fail to attenuate seizures in vitro and fail in vivo.

Overall, this study has demonstrated that several compounds (which are known to have beneficial effects in seizure activity) possess the ability to inhibit soman-induced epileptiform activity in the hippocampal slice. Of particular interest are the compounds clomethiazole, felbamate, retigabine, topiramate and clozapine. The reported neuroprotective ability of clomethiazole, felbamate and topiramate indicate that these compounds either alone or as an adjunct are attractive candidates for enhancing current anticonvulsant therapy. Whilst the ability of retigabine to physiologically antagonise one of the primary excitatory actions of acetylcholine mean that this compound is worth further investigation in vivo. The ability of clozapine to abolish seizures is an interesting finding as generally antimuscarinic compounds are only partially effective in this model (Harrison et al., 2004). It has been suggested dopamine antagonists may have protective effects against soman-induced seizures (Bourne et al., 2001) and that dopaminergic and cholinergic pathways interact (Gainetdinov and Caron, 1999), thus application of a drug which antagonises both of these pathways (such as those developed for schizophrenia) may prove more efficacious.

In summary, this investigation has demonstrated that soman-induced epileptiform activity can be antagonised by a range of diverse compounds but is resistant to others. With the caveat that in vitro models are not fully predictive for in vivo models this work suggests that several compounds are worth further investigation for the treatment of organophosphate induced seizures.

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