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The anticonvulsant actions of σ receptor ligands in the Mg^{2+}-free model of epileptiform activity in rat hippocampal slices

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1 The anticonvulsant potency of a series of structurally-dissimilar compounds which possess nanomolar affinities for high-affinity σ binding sites was examined in the Mg^{2+}-free model of epileptiform activity in rat hippocampal slices. Extracellular field potential recordings in the CA1 region were employed to examine the effects of test compounds on spontaneous epileptiform activity and multiple population spikes evoked by stimulation of the Schaffer collateral-commissural pathway.

2 Applied at σ site-selective (i.e. nanomolar) concentrations, dextromethorphan, ditolylguanidine, caramiphen and opipramol failed to modify Mg^{2+}-free epileptiform activity; neither pro- nor anticonvulsant effects were observed. However, applied at micromolar concentrations, these and additional test compounds reversibly inhibited orthodromically-evoked epileptiform field potentials with a rank order potency (IC_{50} values in μM): dextrorphan (1.5) > ifenprodil (6.3) > dextromethorphan (10) > ditolylguanidine (15) > loperamide (28) > carbetapentane (38) > caramiphen (46) > opipramol (52). Micromolar concentrations of the same compounds also inhibited spontaneous epileptiform bursts recorded during perfusion with Mg^{2+}-free medium.

3 Co-application of ropizine (10 μM), an allosteric modulator of dextromethorphan binding to high-affinity σ receptors, failed to endow dextromethorphan 10 nM with anticonvulsant properties and did not modify the anticonvulsant potency of 10 μM dextromethorphan.

4 The effects of dextrorphan (10 μM), ifenprodil (20 μM), loperamide (50 μM) and caramiphen (100 μM) were examined in the presence of external Mg^{2+} on field potential input/output (I/O) relationships and paired-pulse facilitation (PPF) of field excitatory postsynaptic potentials. Only caramiphen elicited effects on these parameters, affecting synaptic transmission at the point of synaptic transfer and depressing PPF ratios to below baseline values. The effects of caramiphen on I/O relationships mimicked those of the established anticonvulsant adenosine; in contrast, adenosine evoked an increase in PPF ratios.

5 Because anticonvulsant activity was observed only at micromolar concentrations of the σ ligands tested, the results indicate that their anticonvulsant actions should not be ascribed to their occupancy, observed at nanomolar concentrations, of high-affinity σ binding sites. Rather, anticonvulsant activity more likely reflects functional NMDA receptor antagonism and/or blockade of high voltage-activated Ca^{2+} channels, effects which are associated with micromolar concentrations of the test compounds. Modulation of GABAergic inhibitory mechanisms may also contribute to the anticonvulsant properties of caramiphen.

Keywords: Anticonvulsant activity; σ receptor; *N*-methyl-D-aspartate (NMDA) receptor; voltage-activated Ca^{2+} channel; hippocampal slice

Introduction

Much interest has centered on the mechanism(s) which subserve the anticonvulsant properties of dextromethorphan (DXM). Because the relatively weak potency of DXM as an *N*-methyl-D-aspartate (NMDA) antagonist *in vivo* appears unable to account for all of its therapeutically-useful actions (Leander *et al*., 1988; Church *et al*., 1989; Tortella *et al*., 1989; Church & Lodge, 1990), the suggestion has been made that high affinity σ DXM binding sites, which may (Hayashi *et al*., 1995; Yamamoto *et al*., 1995) or may not (Fletcher *et al*., 1995; Whittemore *et al*., 1997) be associated with the NMDA receptor-channel complex, represent the functional receptors responsible for the anticonvulsant properties of DXM and other σ receptor ligands (reviewed by Tortella *et al*., 1989; Walker *et al*., 1990; also see Musacchio *et al*., 1988; Klein & Musacchio, 1989; Leander, 1989; Apland & Braitman, 1990; Pontecorvo *et al*., 1991). Other non-opioid antitussives with anticonvulsant activity (e.g. caramiphen, carbetapentane; Tortella & Musacchio, 1986; Tortella *et al*., 1988; Aram *et al*., 1989; Leander, 1989; Apland & Braitman, 1990) also bind to one or more of the high affinity σ sites and high affinity ligand binding to these site(s) can be allosterically modulated by the anticonvulsant agents phenytoin and ropizine (Musacchio *et al*., 1988; Klein & Musacchio, 1989; 1992). In turn, caramiphen, carbetapentane and DXM potentiate the anticonvulsant efficacy of phenytoin against maximal electroshock-induced convulsions (Tortella & Musacchio, 1986; Tortella *et al*., 1988).

Although the anticonvulsant effects of σ site ligands have been ascribed to the (ill-defined) consequences of σ receptor occupancy, which is observed at nanomolar concentrations, it appears from the limited number of agents tested that anticonvulsant activity is associated with micromolar concentrations of the compounds (Aram *et al*., 1989; Apland & Braitman, 1990). This raises the possibility that, at these relatively high concentrations, σ ligands may be exerting their anticonvulsant actions via mechanism(s) other than the occupancy of high affinity σ binding sites. In particular,

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micromolar concentrations of σ ligands exert both NMDA antagonist (Fletcher *et al*., 1995; Whittemore *et al*., 1997) and voltage-activated Ca^{2+} channel blocking (Netzer *et al*., 1993; Church *et al*., 1994b; Church & Fletcher, 1995) actions. Given the established involvement of NMDA receptors and voltage-activated Ca^{2+} channels to the pathogenesis of seizure activity and the known anticonvulsant properties of both NMDA receptor antagonists and blockers of some subtypes of high voltage-activated (HVA) Ca^{2+} channel (reviewed by Rogawski & Porter, 1990; also see Dingledine *et al*., 1986; Leander *et al*., 1988; Bingmann & Speckmann, 1989; DeSarro *et al*., 1990; Parsons *et al*., 1995), the anticonvulsant effects of σ ligands may reflect their ability to modulate either or both of these mechanisms.

In the present study we have examined the anticonvulsant activities of a series of σ site ligands the inhibitory potencies of which at both NMDA receptors and at HVA Ca^{2+} channels have been established (Church & Fletcher, 1995; Fletcher *et al*., 1995). The Mg^{2+}-free model of epileptiform activity was employed, given the known sensitivity of this model to both NMDA antagonists and inhibitors of multiple subtypes of HVA Ca^{2+} channel (e.g. Mody *et al*., 1987; Aram *et al*., 1989; Pohl *et al*., 1992). In addition, inhibitory mechanisms are preserved in this model and act to limit the amount of synchronous firing associated with epileptiform discharges (Mody *et al*., 1987; Tancredi *et al*., 1990; Westerhoff *et al*., 1995). The possibility exists that compounds with antagonist activity at multiple subtypes of HVA Ca^{2+} channel, such as those examined here, may interrupt inhibitory as well as excitatory synaptic transmission and thus potentiate rather than suppress epileptiform activity (see Horne & Kemp, 1991; Potier *et al*., 1993). Since many of the compounds tested possess inhibitory activity at both NMDA receptors and HVA Ca^{2+} channels, the experiments also provided an opportunity to assess whether such dual antagonism results in an additive or synergistic anticonvulsant effect greater than that which might result from NMDA antagonism or blockade of HVA Ca^{2+} channels alone.

Methods

Slice preparation

Adult male Wistar rats were anaesthetized with 3% halothane in air, decapitated and transverse hippocampal slices (400 μm) prepared as described by Church & McLennan (1989). Slices were placed at the interface between an humidified atmosphere (95% O_2; 5% CO_2) and artificial cerebrospinal fluid (aCSF) containing (mM): NaCl 125, KCl 3, $NaHCO_3$ 24, NaH_2PO_4 1.5, $MgSO_4$ 1.5, D-glucose 10 and $CaCl_2$ 2. The aCSF was equilibrated with 95% O_2; 5% CO_2, giving a pH in the recording chamber of 7.4, and perfused at a rate of 2 ml min^{-1}. Magnesium-free aCSF was prepared by omitting $MgSO_4$. Experiments were performed at 35 ± 1°C.

Recording and stimulating techniques

Extracellular field potentials were recorded using glass micropipettes (8–20 MΩ) filled with 4 M NaCl or aCSF. For assessment of anticonvulsant activity, the recording electrode was positioned in the CA1 stratum pyramidale at the depth at which maximal evoked potentials for a given stimulus intensity were elicited. In experiments in which input/output (I/O) functions or paired-pulse facilitation (PPF) were examined, a second recording electrode was placed in the CA1 stratum radiatum. Single or paired orthodromic stimuli were delivered via a bipolar electrode to the Schaffer collateral-commissural (SC) pathway (square-wave pulses, 0.05 ms duration, 1–100 V intensity) at a maximum frequency of 0.05 Hz.

Orthodromically-evoked field responses and spontaneous epileptiform burst waveforms were amplified (Axoclamp 2, Axon Instruments Inc., Foster City, CA) and displayed on an oscilloscope. Voltage traces evoked by orthodromic stimulation were filtered (d.c. - 10 kHz), digitized on-line (TL-1, Axon Instruments Inc.; sample rate = 50 kHz) and stored on a personal computer using pClamp software (v.6.0.3; Axon Instruments Inc.) for subsequent analysis. Spontaneous epileptiform activity (a.c. coupled; 1 Hz - 10 kHz) was displayed continuously on a chart recorder (Gould 2200S, Gould Inc., Cleveland, OH) and individual spontaneous burst waveforms were captured and stored for later analysis.

Experimental protocols

Assessment of anticonvulsant activity Slices were exposed sequentially to: (a) Mg^{2+}-containing aCSF for 60 min to allow recovery following preparation. At the end of this period, single orthodromic stimuli were applied and a stimulus intensity which produced a single population spike of half-maximal amplitude was identified and employed for the remaining phases of the experiment. Paired stimuli (50 ms interstimulus interval (ISI)) were employed for the remainder of the experiment. Slices exhibiting multiple population spikes in response to single or paired orthodromic stimuli were rejected; (b) Mg^{2+}-free aCSF for a period (30–100 min) until a stable level of epileptiform activity, as assessed by field potential responses to paired stimuli at the previously chosen stimulus intensity, had been reached. Spontaneous burst activity and evoked epileptiform responses recorded at the end of this period served as control responses for the effects of a test compound. Slices in which paired stimuli failed to evoke less than a total of 6 population spikes of at least 0.5 mV in amplitude were excluded from further experimentation; (c) Mg^{2+}-free aCSF containing test compound for 60 min; (d) Mg^{2+}-free aCSF in the absence of test drug, for the time required for epileptiform activity to recover to the level observed in period (b) or 4 h, whichever was shorter. During this period, spontaneous epileptiform bursts and evoked field potential responses were recorded at 60 min intervals; and (e) Mg^{2+}-containing aCSF for 30 min. The final period of reperfusion with Mg^{2+}-containing aCSF was conducted to assess any long term effects of exposure to a test compound and to ensure that the recovery of epileptiform activity following exposure to a test compound was not the result of a deterioration in slice viability.

The anticonvulsant activity of the test compounds was quantified by comparing epileptiform responses evoked in Mg^{2+}-free aCSF immediately before the application of a test compound with responses evoked at the end of a 60 min period of perfusion with drug-containing, Mg^{2+}-free medium. Responses were analysed using a Visual Basic macro which measured the total length of the line representing the evoked waveform (i.e. a coastline measurement; see Dingledine *et al*., 1986; Apland & Cann, 1995). Measurements of the first and second field potential responses (FP1 and FP2, respectively) were performed independently, in order to avoid contamination of the measurements of the lengths of the lines by stimulus artifacts. For both FP1 and FP2, measurement of waveform length commenced at the end of the primary evoked population spike. For FP1, length measurement terminated at the data acquisition point immediately before the second

stimulus artifact; for FP2, the end of the coastline was the point at which the epileptiform potential returned to the baseline observed before the first stimulus. Lengths of corresponding traces obtained without stimulation were subtracted from the length of each of the waveforms and, finally, the 'background-subtracted' coastline measurements for FP1 and FP2 were added together to produce a value which represented the total length of the response to the paired stimuli. In any given experiment, the mean of 3 coastline measurements under each experimental condition was taken and inhibition of epileptiform activity by each concentration of each test compound was then calculated using the equation: % inhibition $= 100 - [(FP1_d + FP2_d)/(FP1_c + FP2_c)] \times 100$, where $FP1_d$ and $FP2_d$ denote the length of the field potential waveforms in drug-containing aCSF, and $FP1_c$ and $FP2_c$ represent the length of the (control) field potential waveforms in drug-free aCSF. % inhibition values are presented as means $\pm$ s.e.mean. The effect of each concentration of each test compound was examined on a minimum of four (and usually >6) different slices. To derive IC_{50} values (the concentration of test compound resulting in 50% inhibition of the control response), data points were fit to the logistic equation: $R = R_{max}[\text{concentration}^{n_H}/(\text{concentration}^{n_H} + IC_{50}{}^{n_H})]$, where R is the observed change at the test concentration, R_{max} is the maximum observed change, concentration refers to the concentration of test drug and n_H is the Hill coefficient. In experiments used for the calculation of IC_{50} values, only one concentration of test compound was examined per slice; experiments in which more than one concentration of a given test compound was applied were used for illustrative purposes only (Figures 1 and 4).

I/O functions and paired-pulse facilitation Hippocampal slices were perfused for 60 min with Mg^{2+}-containing aCSF, followed by a 60 min period of perfusion with Mg^{2+}-containing aCSF in the presence of test compound and, finally, a period of reperfusion ($\leqslant$4 h) with drug-free medium. At the end of each 60 min period, field potential waveforms were recorded simultaneously from the CA1 pyramidal cell body and dendritic layers in response to single (I/O functions; intensity range 10–60 V) or paired (PPF experiments; ISIs of 20, 30 and 40 ms) orthodromic stimuli. The variables measured to construct I/O curves were the amplitude of the presynaptic fibre volley, the maximum rate of change (i.e. slope) of the field excitatory postsynaptic potential (f-e.p.s.p.) and the amplitude of the population spike. Three consecutive responses were collected and averaged at each stimulus intensity, and four experiments were performed for each test compound at a concentration above its previously established anticonvulsant IC_{50} value. In studies of PPF, stimulus intensity was adjusted during the course of drug perfusion to elicit a conditioning f-e.p.s.p. of approximately constant amplitude (corresponding to half maximal amplitude before drug administration; see Harris & Cotman, 1983; Dunwiddie & Haas, 1985; Kahle & Cotman, 1993).

I/O functions were analysed by comparing responses evoked in Mg^{2+}-containing aCSF immediately before the application of a test compound with responses evoked at the end of a 60 min period of perfusion with drug-containing, Mg^{2+}-containing aCSF. The relationships between the following pairs of variables were examined (see Aitken, 1985): (a) stimulus intensity *vs* population spike amplitude, a measure of the effectiveness of overall synaptic transmission; (b) stimulus intensity *vs* presynaptic fibre volley ('prevolley') amplitude, an indication of presynaptic fibre excitability; (c) prevolley amplitude *vs* f-e.p.s.p. slope, to indicate the efficiency of synaptic transfer; and (d) f-e.p.s.p. slope *vs* population spike amplitude, to assess postsynaptic CA1 pyramidal cell excitability. In studies of PPF, the effects of a test compound, at a concentration above the previously determined anticonvulsant IC_{50} value, were examined in a minimum of 3 slices. A PPF ratio (i.e. the ratio of the amplitude of the test f-e.p.s.p. to the conditioning f-e.p.s.p. amplitude) was calculated for each ISI tested using the means of three consecutively-evoked paired responses.

Preparation and sources of compounds

Stock solutions of test compounds, prepared fresh immediately before each experiment, were made up in distilled water, with the exceptions of ifenprodil, 1,3-di(2-tolyl)guanidine (DTG) and loperamide, which were dissolved in ethanol, methanol and dimethylsulphoxide, respectively. Final working solutions contained $<0.1\%$ solvent which, in control experiments, had no effect on responses (data not shown). Drugs were obtained from Sigma Chemical Co. (St. Louis, MO), with the exceptions

Table 1 Potency of test compounds as anticonvulsant agents (this study) and as inhibitors of NMDA-evoked currents and barium currents (I_{Ba}) in voltage-clamped neurones

	IC_{50}-anticonvulsant activity (μM)	*IC_{50}-NMDA inhibition* (μM)	*IC_{50}-I_{Ba} inhibition* (μM)
Loperamide	28 $\pm$ 2	73 $\pm$ 7	2.5 $\pm$ 0.4
Caramiphen	46 $\pm$ 7	110 $\pm$ 12	47 $\pm$ 3
Carbetapentane	38 $\pm$ 5	112 $\pm$ 13	40 $\pm$ 3
Opipramol	52 $\pm$ 5	96 $\pm$ 9	32 $\pm$ 4
Dextromethorphan	10 $\pm$ 3	1.8 $\pm$ 0.2	73 $\pm$ 10
Ifenprodil	6.3 $\pm$ 0.6	0.8 $\pm$ 0.2	18 $\pm$ 2
Ditolyguanidine	15 $\pm$ 1	37 $\pm$ 5	200 $\pm$ 20
Dextrorphan	1.5 $\pm$ 0.3	0.07 $\pm$ 0.02	334 $\pm$ 36

Data are means $\pm$ s.e.mean of the IC_{50} values (μM) of the compounds, indicated to the left, for inhibition of epileptiform activity in hippocampal slices (first column; this study), inhibition of NMDA-induced currents in voltage-clamped hippocampal neurones (second column) and for antagonism of whole-cell I_{Ba} in hippocampal neurones (third column). Data in the second and third columns are from Fletcher *et al.* (1995) and Church & Fletcher (1995), respectively, except for loperamide (Church *et al.*, 1994a) and dextrorphan (Trube & Netzer, 1994). The data of Trube & Netzer (1994) were obtained in rat cortical neurones. For purposes of comparison to the other values shown, which were obtained in hippocampal neurones, IC_{50} values for inhibition of NMDA-induced currents and I_{Ba} for DXM in cortical neurones were 0.55 $\pm$ 0.07 μM and 77 $\pm$ 9 μM (means $\pm$ s.d.), respectively (Trube & Netzer, 1994). Using extracellular recording techniques in hippocampal slices, Cole *et al.* (1989) estimated an EC_{50} value of 0.65 μM for dextrorphan inhibition of NMDA-induced depolarization in rat hippocampal slices, whereas Parsons *et al.* (1995) obtained an IC_{50} value of 1.3 $\pm$ 0.3 μM for dextrorphan inhibition of NMDA-induced currents in rat superior collicular neurones under whole-cell voltage clamp.

of adenosine, dextrorphan, DTG and ifenprodil (Research Biochemicals International, Natick, MA); opipramol (a gift from CIBA-GEIGY Corp., Summit, NJ); and ropizine (a gift from G.D. Searle & Co., Skokie, IL).

Results

Mg^{2+}-free epileptiform activity

In the presence of external Mg^{2+}, paired stimulations of the SC pathway evoked single population spikes recorded in the CA1 stratum pyramidale following each stimulus (e.g. Figures 3b and 5b). During perfusion with Mg^{2+}-free aCSF, paired stimuli evoked multiple populations spikes. Spontaneous epileptiform bursts were observed during perfusion with Mg^{2+}-free medium in 121/167 slices examined; the amplitude and frequency of bursts were relatively constant in an individual slice but varied considerably between slices (amplitudes varying from 0.5–4 mV and frequency ranging from 2–24 bursts min^{-1}, Mody *et al*., 1987; Tancredi *et al*., 1990; Lücke *et al*., 1996).

Anticonvulsant effects of test compounds

All compounds tested inhibited both evoked and spontaneous epileptiform activity when applied at micromolar concentrations. IC_{50} values for inhibition of orthodromically-evoked epileptiform field potentials are presented in Table 1.

Loperamide Loperamide blocks both dihydropyridine-sensitive (L-type) and dihydropyridine-resistant, ω-conotoxin GVIA-sensitive (N-type) HVA Ca^{2+} channels in hippocampal neurones with an IC_{50} value of 2.5 μM (Table 1). However, it is only a weak NMDA antagonist (IC_{50} value for block of NMDA-evoked whole-cell currents in hippocampal neurones = 73 μM). Although the affinity of loperamide for σ binding sites has not been studied, loperamide was used as a control compound representative of a class of agents which block multiple subtypes of HVA Ca^{2+} channel whilst having relatively weak NMDA antagonist activity.

Loperamide blocked evoked and spontaneous epileptiform activity in a concentration-dependent manner; the IC_{50} value for inhibition of evoked epileptiform activity was 28 μM (Figure 1). The anticonvulsant action of loperamide was reversible, full recovery of epileptiform activity occurring within 120 min of the start of washout of ⩽50 μM of the compound. Loperamide appeared to exert a more pronounced

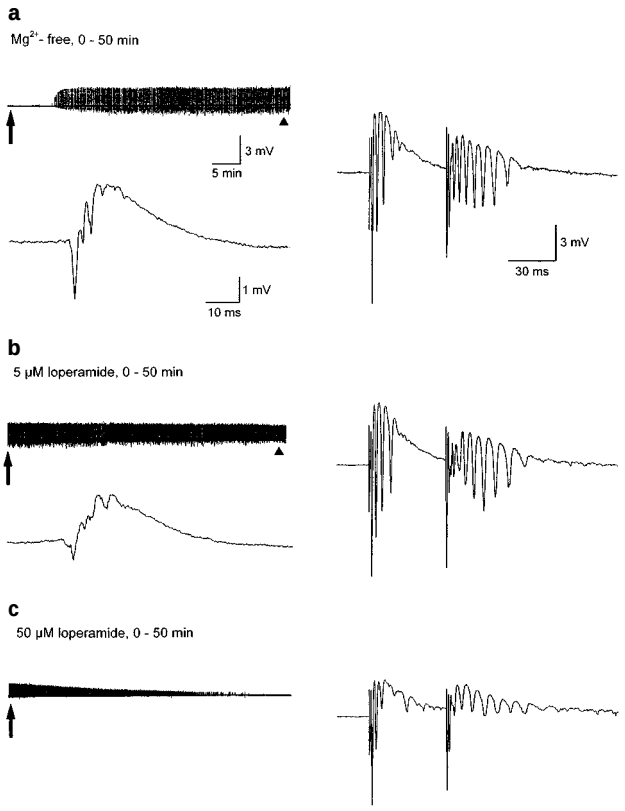

Figure 1 Loperamide attenuated spontaneous burst activity and evoked epileptiform field potentials in a concentration-dependent manner. To the left, from top to bottom, are shown chart records of: (a) the development of spontaneous burst activity upon exposure to Mg^{2+}-free medium; (b) spontaneous burst activity following the start of perfusion with loperamide 5 μM; and (c) spontaneous burst activity following the start of perfusion with loperamide 50 μM. The arrow beneath each chart record indicates the start of perfusion with the medium shown on the figure. The traces beneath the chart records in (a) and (b) show single spontaneous bursts captured at the time indicated by the filled triangle shown beneath the chart record. The absence of a representative single spontaneous burst in (c) indicates that spontaneous activity was abolished, as can be seen in the accompanying chart record. Scale bars for the chart record and the spontaneous burst shown in (a) also apply to corresponding records in (b) and (c). To the right, from top to bottom, are shown extracellular field potentials recorded in CA1 stratum pyramidale in response to paired stimulations of the SC pathway (50 ms ISI). Each record was obtained 60 min after the start of perfusion with the medium indicated at the left of the figure (i.e. ~10 min following the end of the chart record shown to the left). Scale bars shown in (a) also apply to (b) and (c). Recovery of both spontaneous and evoked epileptiform potentials from the effects of loperamide 50 μM was observed 120 min after the start of reperfusion with drug-free, Mg^{2+}-free medium (not shown). All records were obtained from the same hippocampal slice.

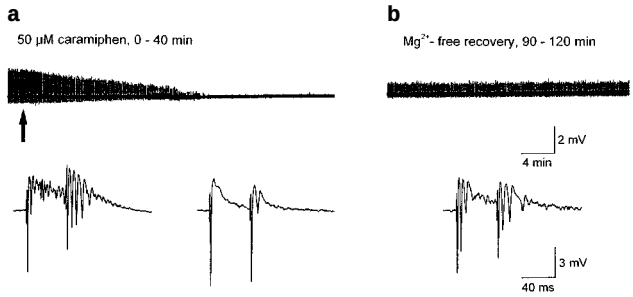

Figure 2 Caramiphen inhibited spontaneous and evoked epileptiform activity. To the left, in (a), is shown a chart record of the effect of caramiphen 50 μM on Mg^{2+}-free spontaneous burst activity. The start of perfusion with caramiphen is indicated by the arrow beneath the chart record. Beneath the chart record are extracellular field potentials recorded in the CA1 pyramidal cell body layer in response to paired stimulations of the SC pathway (50 ms ISI). The left-hand record was obtained 60 min after the start of perfusion with Mg^{2+}-free medium (i.e. just before the start of the chart record shown immediately above); the right-hand record was obtained 60 min following the start of perfusion with Mg^{2+}-free medium containing 50 μM caramiphen (i.e. ~20 min following the end of the chart record shown immediately above). To the right, in (b), is shown a chart record of the recovery of spontaneous burst activity 90–120 min following the start of washout of 50 μM caramiphen. Beneath the chart record is shown the epileptiform field potential evoked 2 h following the return to drug-free, Mg^{2+}-free medium (i.e. at a time just after the end of the chart record shown immediately above). Spontaneous and evoked epileptiform activity was abolished within 12 min of the return to Mg^{2+}-containing medium (not shown). Scale bars shown in (b) also apply to the appropriate records in (a). All records were obtained from the same hippocampal slice.

effect on spontaneous than an evoked epileptiform activity; at a concentration of 50 μM, for example, loperamide failed to inhibit completely evoked epileptiform activity (a $74\pm6\%$ reduction, $n=4$), whereas spontaneous epileptiform activity was abolished in 4/4 slices tested (see Figure 1). Although loperamide has affinity for opioid receptors, its Ca^{2+} channel blocking and NMDA antagonist actions are insensitive to naloxone (Church *et al*., 1994a). Pretreatment with 1 μM naloxone for 45 min failed to affect the anticonvulsant potency of 20 μM loperamide. Applied alone, 20 μM loperamide produced a $37\pm3\%$ ($n=5$) reduction in the evoked epileptiform response; in the presence of 1 μM naloxone, the corresponding reduction was $34\pm5\%$ ($n=3$).

Caramiphen, carbetapentane and opipramol Caramiphen (CM), carbetapentane (CBT) and opipramol possess nanomolar affinities for σ binding sites and, at micromolar concentrations, exhibit HVA Ca^{2+} channel blocking properties and, to a lesser degree, NMDA antagonist activity (Table 1). As such, CM, CBT and opipramol were employed as compounds representative of mixed NMDA/Ca^{2+} channel blockers which exhibit a more potent inhibitory action at HVA Ca^{2+} channels than at NMDA receptors.

Applied at 10 and 100 nM ($n=3$ in each case), CM and opipramol failed to modify Mg^{2+}-free epileptiform activity; neither pro- nor anticonvulsant effects were observed (not illustrated). The anticonvulsant effects of nanomolar concentrations of CBT were not examined. However, tested in the concentration range 10–200 μM, CM, CBT and opipramol blocked evoked epileptiform activity in a concentration-dependent and reversible manner. At a concentration of 50 μM ($n\geqslant6$ in each case), CM, CBT and opipramol reduced evoked epileptiform responses by $46\pm3\%$, $56\pm5\%$ and $48\pm8\%$, respectively; the respective IC_{50} values were 46 μM, 38 μM and 52 μM. Examples of the anticonvulsant actions of CM and opipramol are presented in Figures 2 and 3, respectively. Despite their net anticonvulsant actions, application of CM or opipramol at 20 or 50 μM increased the amplitude of the first and sometimes subsequent population spikes in, respectively, 11/19 and 9/15 slices examined. A similar 'low-dose enhance-

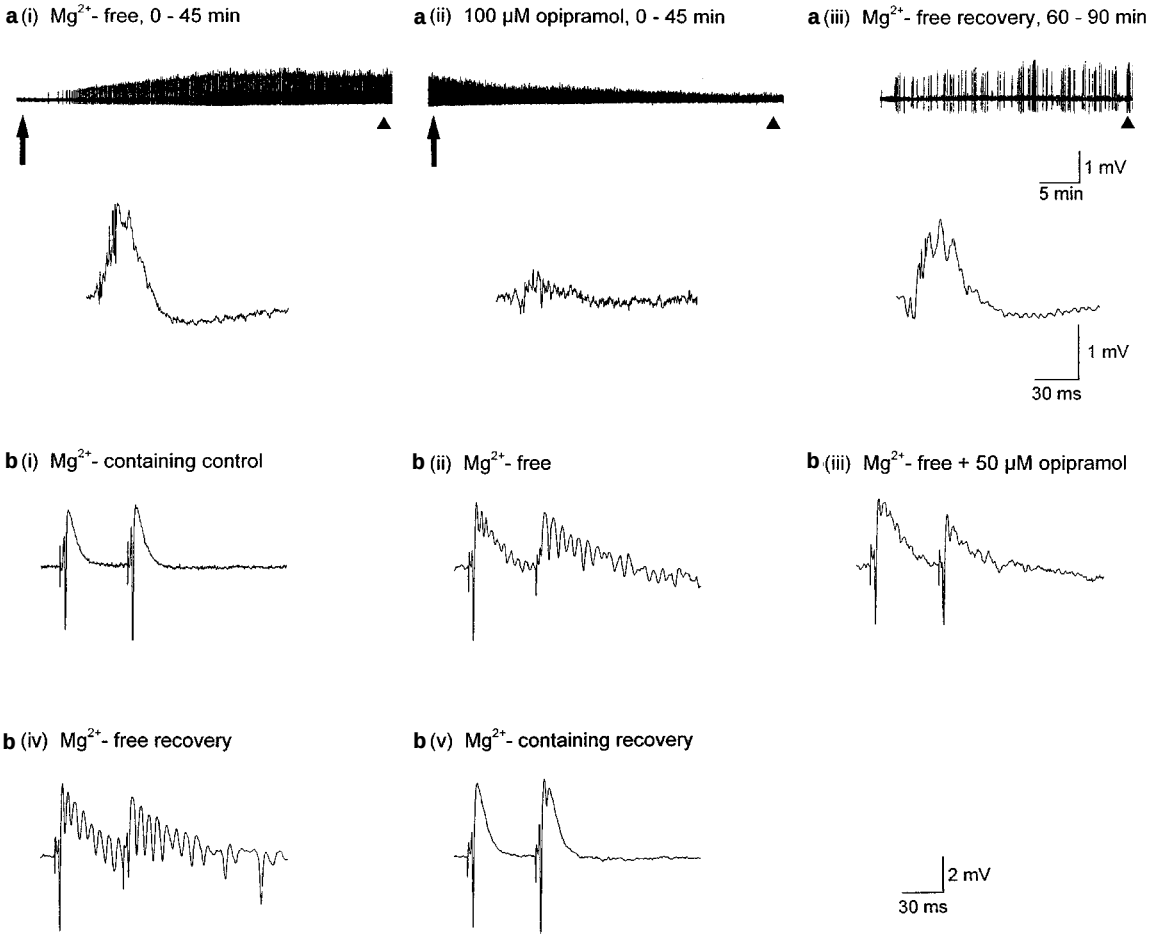

Figure 3 Opipramol inhibited epileptiform activity in a reversible manner. (a) From left to right are shown chart records of: (i) the development of spontaneous burst activity upon exposure to Mg^{2+}-free medium; (ii) the reduction in spontaneous burst amplitude following the start of perfusion with opipramol 100 μM; and (iii) spontaneous burst activity 60–90 min following the start of washout of 100 μM opipramol. The arrow beneath each chart record indicates the start of perfusion with medium indicated on the figure. The traces below the chart records show single spontaneous bursts captured at the time indicated by the solid triangle shown beneath the corresponding chart record. Scale bars for the chart record and the spontaneous burst shown in (iii) also apply to the corresponding traces in (i) and (ii). (b) In a different hippocampal slice, opipramol 50 μM inhibited evoked epileptiform activity. In the top row, from left to right, are shown field potentials recorded in the CA1 pyramidal cell body layer in response to orthodromic stimuli (50 ms ISI): (i) during perfusion with Mg^{2+}-containing control medium; (ii) 60 min after the start of perfusion with Mg^{2+}-free medium; and (iii) 60 min after the start of exposure to opipramol 50 μM. In the lower row, from left to right, are shown: (iv) recovery of epileptiform activity 60 min following the start of reperfusion with drug-free, Mg^{2+}-free medium; and (v) responses evoked 30 min following the return to Mg^{2+}-containing medium. Scale bars shown apply to all traces.

ment' of epileptiform activity has been observed previously with a variety of NMDA antagonists (Dingledine *et al.*, 1986; Cole *et al.*, 1989) and, in the present study, DTG and dextrorphan elicited similar effects (see below). At concentrations $\geqslant 100$ μM, CM and CBT elicited a marked broadening of the population spikes evoked by paired stimuli in 10/16 slices tested, possibly reflecting blockade of voltage-activated K^+ channels (see Fletcher *et al.*, 1989).

Spontaneous epileptiform activity was abolished by even the lowest concentration (20 μM) of CM ($n=6$; see Figure 2) and CBT ($n=4$; not illustrated) tested (see Apland & Braitman, 1990). An example of the effect of opipramol on spontaneous epileptiform activity is shown in Figure 3a.

Ifenprodil, DTG and DXM Ifenprodil, DTG and DXM possess nanomolar affinities for high-affinity σ binding sites. Applied at micromolar concentrations, they are NMDA antagonists which also possess weak HVA Ca^{2+} channel blocking activity (cf CM, CBT and opipramol, which are more potent HVA Ca^{2+} channel blockers than NMDA antagonists; Table 1).

In the majority of slices examined (25/29), evoked epileptiform activity was reduced by ifenprodil (1 – 50 μM) in a reversible and concentration-dependent manner, with an IC_{50} value of 6.3 μM (Figure 4a). However, in the remaining four slices, in which epileptiform activity before drug administration was not well-developed, 10 or 20 μM ifenprodil ($n=2$ in each case) elicited a fully-reversible enhancement of epileptiform activity (Figure 4b). In an additional four slices, ifenprodil 20 or 50 μM ($n=2$ in each case) had little effect on Mg^{2+}-free epileptiform activity but, following the start of reperfusion with drug-free, Mg^{2+}-free medium, a profound inhibition of evoked activity was observed which slowly recovered during prolonged (4 h) washing with drug-free, Mg^{2+}-free aCSF (not illustrated). This depression was reminiscent of the effect of bath-applied NMDA on orthodromically-evoked field potentials recorded in the CA1 region of rat hippocampal slices, an action which is a secondary consequence of the depolarization of neurones in the recording region (see Anderson *et al.*, 1987; Apland & Braitman, 1990; Apland & Cann, 1995). Spontaneous epileptiform activity was also inhibited by ifenprodil in a concentra-

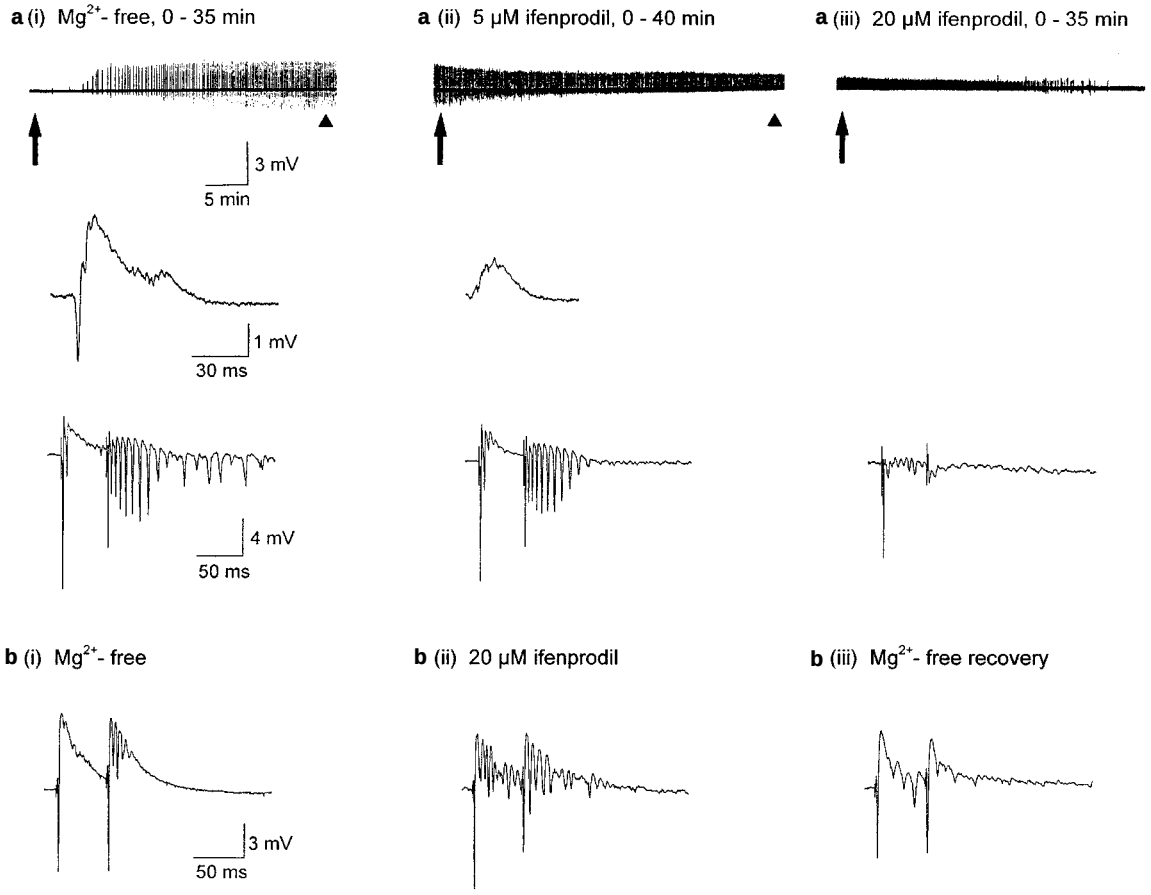

Figure 4 Anti- and proconvulsant effects of ifenprodil. (a) Spontaneous and evoked epileptiform activity was attenuated by ifenprodil in a concentration-dependent manner. In (i), (ii) and (iii), arrows beneath the chart records indicate the start of perfusion with the medium indicated on the figure. The traces immediately beneath the chart recordings are single spontaneous bursts captured at the time indicated by the solid triangle in the corresponding chart recorder trace. The absence of a representative burst in (iii) indicates that spontaneous activity was abolished, as can be seen in the accompanying chart record. The lowermost records in (i), (ii) and (iii) are evoked epileptiform potentials recorded in the CA1 pyramidal cell layer: (i) 60 min following the start of perfusion with Mg^{2+}-free medium; (ii) 60 min after the start of perfusion with ifenprodil 5 μM; and (iii) 60 min after the start of perfusion with ifenprodil 20 μM. Recovery of both spontaneous and evoked epileptiform potentials was observed 120 min after the start of reperfusion with drug-free, Mg^{2+}-free medium (not shown). All records were obtained from the same hippocampal slice. Scale bars shown in (i) also apply to the corresponding records in (ii) and (iii). (b) In a different hippocampal slice, ifenprodil 20 μM exerted a proconvulsant effect. (i) The record shows evoked epileptiform potentials 60 min after the start of perfusion with Mg^{2+}-free medium. (ii) Sixty minutes of perfusion with 20 μM ifenprodil elicited a proconvulsant effect. (iii) One hundred and twenty minutes after the start of washout of ifenprodil, evoked epileptiform potentials returned towards pre-drug exposure levels. Scale bars in (i) also apply to (ii) and (iii).

tion-dependent manner, with 20 or 50 μM ifenprodil abolishing spontaneous activity in 15/18 slices examined (Figure 4a). In the three remaining slices, in which spontaneous activity before drug application was not well-developed, block of spontaneous activity by 20 μM ifenprodil was preceded by a marked increase in the frequency of spontaneous bursts from 9 ± 4 bursts min^{-1} (mean $\pm$ s.d.) before drug application to 24 ± 1 bursts min^{-1} measured at 20–30 min after the start of perfusion with ifenprodil ($P < 0.05$, paired Student's t test). A qualitatively similar phenomenon has been described following

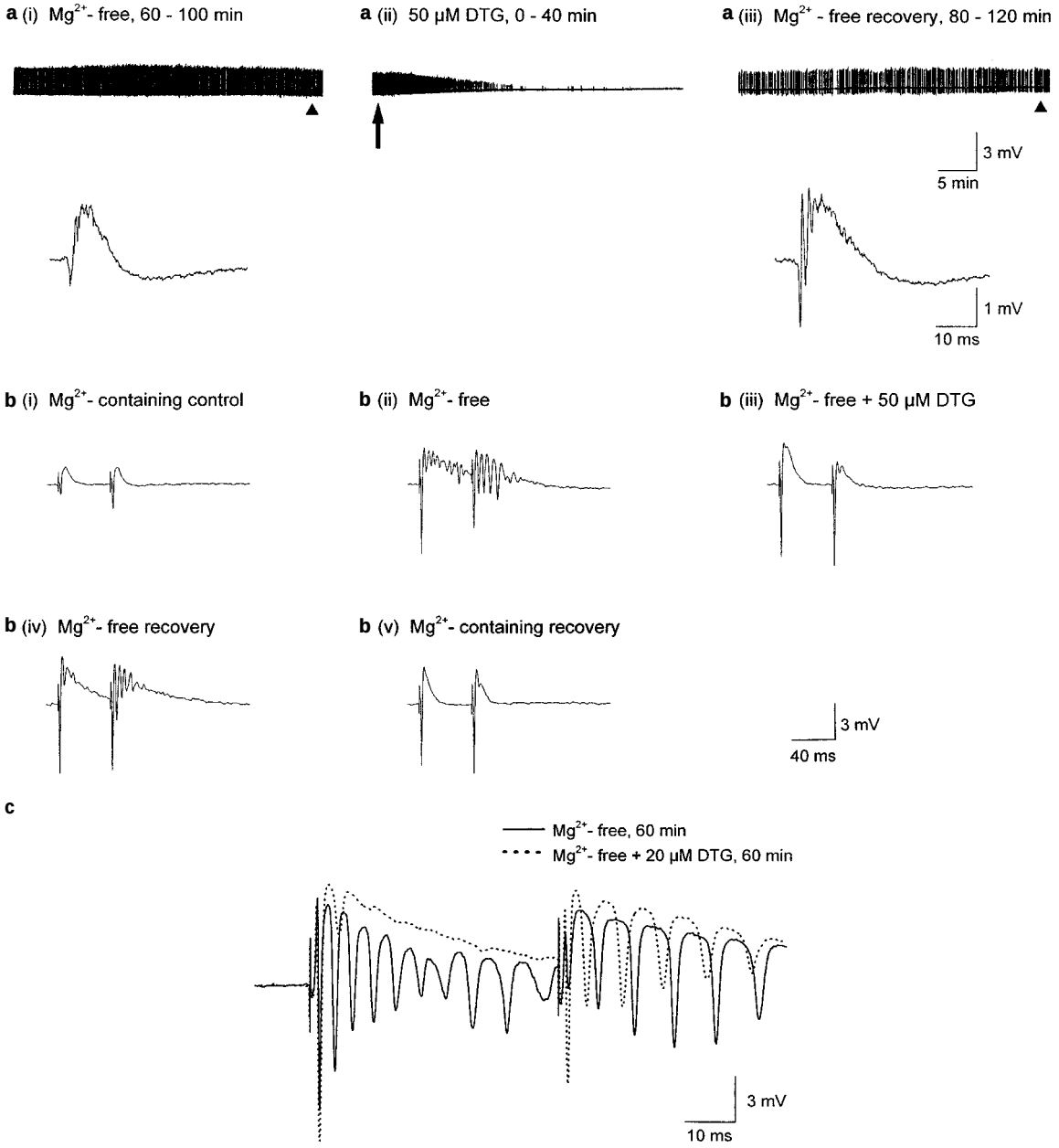

Figure 5 The anticonvulsant actions of ditolylguanidine. (a) From left to right are shown chart records of: (i) spontaneous burst activity 60–100 min following the start of perfusion with Mg^{2+}-free medium; (ii) the effect of DTG 50 μM on spontaneous burst activity (DTG exposure commenced at the arrow shown beneath the chart record); and (iii) recovery of spontaneous burst activity 80–120 min following the start of washout of DTG. Beneath the chart records in (i) and (iii) are shown single spontaneous bursts captured at the time indicated by the solid triangle shown beneath the accompanying chart record. The absence of a representative single spontaneous burst in (ii) indicates that spontaneous activity was abolished, as can be seen in the accompanying chart record. Scale bars for the chart record and the spontaneous burst shown in (iii) also apply to corresponding traces in (i) and (ii). All records were obtained from the same hippocampal slice. (b) In the upper row, from left to right, are shown field potentials recorded in the CA1 pyramidal cell body layer: (i) during perfusion with Mg^{2+}-containing control medium; (ii) 60 min after the start of perfusion with Mg^{2+}-free medium; and (iii) 60 min after the start of exposure to DTG 50 μM. In the lower row, from left to right, are shown: (iv) recovery of evoked epileptiform activity 120 min following the start of reperfusion with drug-free, Mg^{2+}-free medium; and (v) responses evoked 30 min following the return to Mg^{2+}-containing medium. Scale bars apply to all traces. All records were obtained from the same hippocampal slice (a different slice to that used in (a)). (c) Low concentrations of DTG, although anticonvulsant in their overall effect, occasionally caused an increase in the amplitude of the first (and sometimes subsequent) population spikes. The solid and dashed lines represent responses evoked by paired orthodromic stimuli in Mg^{2+}-free aCSF immediately before and 60 min following the addition of 20 μM DTG, respectively. Note the increased amplitude of the first population spike following both the first and second stimuli, accompanied by a decrease in the amplitude and/or number of subsequent population spikes in the presence of DTG. Records are from a different hippocampal slice to those used in (a) and (b). Scale bars apply to both traces.

the focal application of NMDA to the CA3 region during perfusion with Mg^{2+}-free medium (Lücke *et al.*, 1996). Although the mechanism(s) underlying the atypical (proconvulsant) responses to ifenprodil were not examined, they may reflect the ability of relatively low concentrations of the drug to potentiate NMDA-induced responses under Mg^{2+}-free conditions (see Kew *et al.*, 1996).

Applied at 10, 50 and 100 nM ($n=3$ in each case), DTG failed to modify Mg^{2+}-free epileptiform activity; neither pro- nor anticonvulsant effects were observed (not illustrated). However, at micromolar concentrations, DTG exhibited reversible and concentration-dependent anticonvulsant activity against evoked epileptiform bursts ($IC_{50}=15$ μM; Figure 5b). Despite this net anticonvulsant effect, DTG 10, 20 or 50 μM increased the amplitude of the first and sometimes subsequent population spikes in 12/24 slices examined (Figure 5c). Spontaneous epileptiform bursting was inhibited by DTG in a concentration-

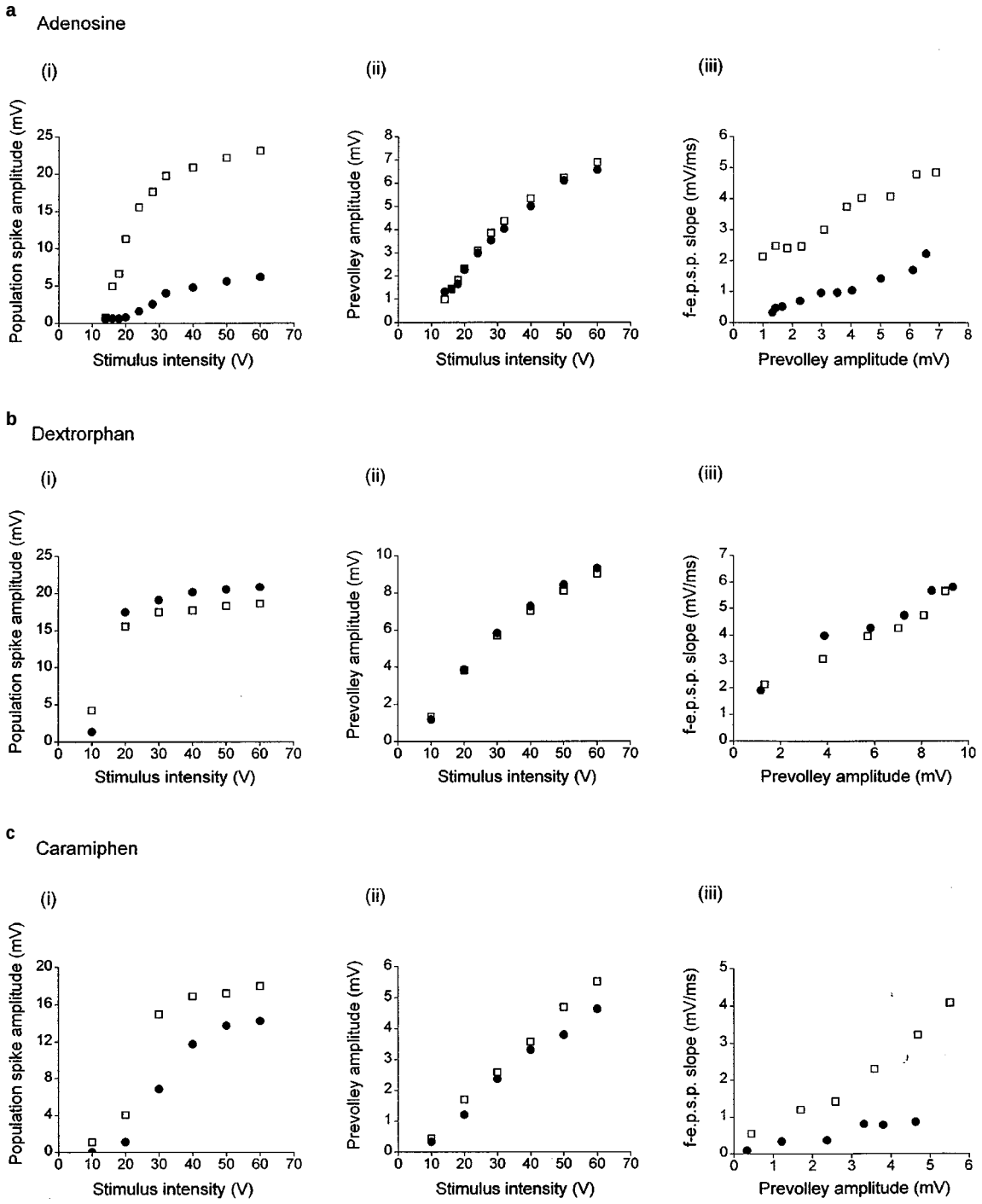

Figure 6 The effects of adenosine, dextrorphan and caramiphen on input/output functions. Results of typical experiments showing the effects of (a) adenosine 300 μM, (b) dextrorphan 10 μM and (c) caramiphen 100 μM on: (i) the overall strength-response function; (ii) the stimulus intensity *vs* prevolley amplitude relationship; and (iii) the prevolley amplitude *vs* f-e.p.s.p. slope relationship. In all cases, open squares represent data obtained immediately before drug exposure and solid circles represent data obtained 60 min after the start of perfusion with test compound. Each data point represents the average of three consecutively-evoked responses. Washout of the test compounds resulted in recovery of the I/O relationships towards pre-drug controls (not shown). For each compound, the relationships presented are representative of the consistent effects observed in four experiments.

dependent and reversible manner; 20 μM DTG, for example, abolished spontaneous activity in 7/18 slices tested whereas 50 μM DTG abolished spontaneous activity in 7/7 slices tested (Figure 5a).

DXM consistently attenuated evoked epileptiform activity ($IC_{50}=10$ μM; not illustrated). Full recovery from the anticonvulsant effects of DXM required prolonged (up to 4 h) washing with Mg^{2+}-free aCSF. DXM also decreased the

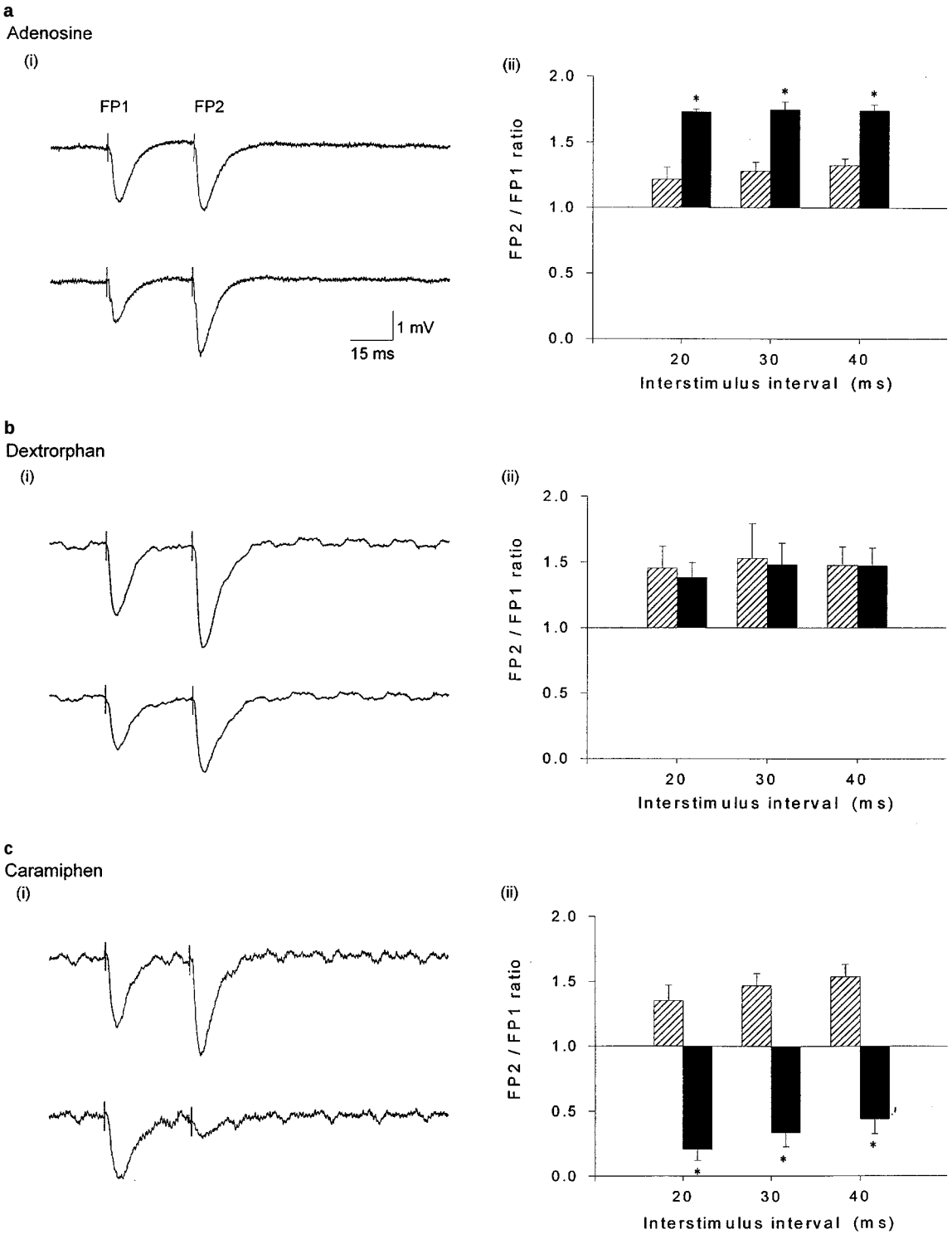

Figure 7 The effects of adenosine, dextrorphan and caramiphen on paired-pulse facilitation. To the left in (a,i), (b,i) and (c,i) are shown sample records of f-e.p.s.p.s evoked by paired stimuli (30 ms ISI) under control conditions (upper traces) and following 60 min of perfusion with, respectively, adenosine 300 μM, dextrorphan 10 μM and caramiphen 100 μM (lower traces). To the right in (a,ii), (b,ii) and (c,ii) are histograms of pooled data ($n=3$ for each compound at each ISI) showing the ratio between the amplitude of FP2 to that of FP1 at the interstimulus intervals indicated. Error bars represent s.e.mean. The first response (FP1) was normalized and the relative magnitude of the second response was then plotted. Adenosine 300 μM (a,ii, solid columns) significantly ($^*P<0.05$, paired Student's t test) increased the ratio between FP2 and FP1 when compared to control responses (hatched columns). Dextrorphan 10 μM (b,ii, solid columns) had no significant effect on the ratio between FP2 and FP1 when compared to control responses (hatched columns). Caramiphen 100 μM (c,ii, solid solumns) significantly ($^*P<0.05$, paired Student's t test) altered the FP2/FP1 ratio seen in control (hatched columns), transforming paired-pulse facilitation to paired-pulse depression. The effects of all compounds tested on PPF ratios were fully reversible (not shown).

amplitude and frequency of spontaneous epileptiform bursts; at the highest concentrations examined (50 and 100 μM, $n=5$ and 6, respectively), spontaneous epileptiform activity was abolished. Prolonged periods of reperfusion with Mg^{2+}-free aCSF were also required to reverse the effects of DXM on spontaneous activity. The anticonvulsant agent ropizine (10 μM) allosterically modulates the binding of DXM to high-affinity σ binding sites (Musacchio *et al*., 1988; Klein & Musacchio, 1992). Applied alone, ropizine 100 μM attenuated both evoked and spontaneous epileptiform activity (not illustrated). However, exposure of slices to a low concentration of ropizine (10 μM) which, by itself, exerted no measurable effect on evoked or spontaneous epileptiform activity, failed to endow DXM 10 nM with anticonvulsant properties and did not affect the anticonvulsant potency of DXM 10 μM ($n=4$ in each case). Thus, applied alone, DXM 10 μM reduced epileptiform activity by $46\pm9\%$ ($n=6$); the corresponding reduction in the presence of ropizine 10 μM was $48\pm6\%$ ($n=4$).

Dextrorphan Dextrorphan was employed as a control compound representative of agents which are potent NMDA antagonists with weak Ca^{2+} channel blocking actions (Table 1). Dextrorphan exhibits poor affinity for high-affinity σ binding sites (Roth *et al*., 1996). Evoked epileptiform activity was inhibited by dextrorphan in a concentration-dependent manner ($IC_{50}=1.5$ μM). As previously observed (Aryanpur *et al*., 1990), and in a manner similar to that found with CM, opipramol and DTG (see above), low concentrations of dextrorphan (1 – 5 μM) increased the amplitude of the first and sometimes subsequent population spikes in 11/27 slices examined. The reversible anticonvulsant effect of dextrorphan on spontaneous epileptiform bursting was also concentration-dependent and was accompanied by a reduction in both the amplitude and frequency of spontaneous bursts at concentrations $\leqslant 5$ μM and abolition of burst activity at concentrations $\geqslant 10$ μM. Recovery from the anticonvulsant effects of dextrorphan required prolonged ($\geqslant 4$ h) washing with Mg^{2+}-free aCSF.

I/O functions and paired-pulse facilitation

Adenosine was examined initially as a reference compound. Adenosine 300 μM ($n=4$) elicited a reversible rightward shift in the stimulus intensity *vs* population spike relationship, with no effect on the stimulus intensity *vs* presynaptic volley amplitude curve, indicating that it reduced the effectiveness of overall synaptic transmission (Figure 6a,i). This effect was due to a reduction in synaptic transfer because, in the presence of adenosine, a given prevolley amplitude evoked a smaller f-e.p.s.p. slope (Figure 6a,iii). It did not reflect changes in postsynaptic excitability because the amplitude of the population spike evoked by a given magnitude of f-e.p.s.p. did not change (not illustrated). In the PPF experiments ($n=3$; Figure 7a), 300 μM adenosine produced an increase in the ratios of the amplitudes between the second (test) and first (conditioning) f-e.p.s.p.s (see Dunwiddie & Haas, 1985; Kahle & Cotman, 1993).

Loperamide, CM, ifenprodil and dextrorphan were examined as agents representative of each of the four groups of compounds tested for anticonvulsant activity. Field potential I/O relationships and PPF ratios ($n=4$ in each case) were not affected by dextrorphan (10 μM; Figures 6b and 7b) or ifenprodil (20 μM; not illustrated), as shown for NMDA antagonists tested in the presence of external Mg^{2+} (see Harris & Cotman, 1983; Dingledine *et al*., 1986; Albertson *et al*., 1992). Loperamide (50 μM; $n=4$) was similarly without effect on non-epileptiform synaptic activity, as previously found for other organic Ca^{2+} channel blockers (Bingmann & Speckmann, 1989; Pohl *et al*., 1992; Wang *et al*., 1997). In contrast, caramiphen (100 μM; $n=4$), like adenosine, depressed both the stimulus intensity *vs* population spike relationship (Figure 6c,i) and the presynaptic volley *vs* f-e.p.s.p. relationship (Figure 6c,iii). However, in direct contrast to the effects of adenosine, CM 100 μM evoked a significant depression of PPF ratios to below baseline values, with the amplitude of the second f-e.p.s.p. diminished markedly relative to the first ($n=4$; Figure 7c).

Discussion

The compounds tested exerted concentration-dependent anticonvulsant actions against Mg^{2+}-free epileptiform activity. The absolute anticonvulsant potency values established in the present study (Table 1) agree well with those previously established for CM, CBT, DXM and DTG against Mg^{2+}-free epileptiform activity in neocortical (Aram *et al*., 1989) and/or hippocampal (Apland & Braitman, 1990) slices. The anticonvulsant activities of loperamide and opipramol, together with the *in vitro* anticonvulsant properties of ifenprodil, have not been previously studied.

Although structurally dissimilar, all the compounds (apart from dextrorphan and with the possible exception of loperamide, which has not been examined) possess nanomolar affinities for high-affinity σ binding site(s). Although it has been suggested that these sites mediate the anticonvulsant actions of σ ligands (Musacchio *et al*., 1988; Klein & Musacchio, 1989; Leander, 1989; Tortella *et al*., 1989; Apland & Braitman, 1990; Pontecorvo *et al*., 1991), the present results indicate that this is unlikely to be the case. First, applied at nanomolar (i.e. σ site-selective) concentrations, selected compounds failed to modulate Mg^{2+}-free epileptiform activity. Second, for all drugs tested, anticonvulsant effects were observed only at the micromolar concentrations associated with NMDA antagonist and/or HVA Ca^{2+} channel blocking actions (see below). Third, ropizine, which modulates DXM binding to σ binding sites (Klein & Musacchio, 1992), failed to affect the anticonvulsant potency of DXM. Fourth, preliminary observations indicate that the high-affinity σ receptor ligand L-687,384 does not possess anticonvulsant activity at nanomolar concentrations; weak inhibitory activity is observed with L-687,384 $\geqslant 50$ μM but, at these concentrations, the compound acts as a mixed NMDA antagonist/Ca^{2+} channel blocker in a manner similar to many of the compounds tested here (McLarnon *et al*., 1994). We therefore conclude that the anticonvulsant properties of the σ site ligands examined in the present study are not mediated by occupation of high-affinity σ binding site(s). Similarly, the increase in the amplitude of the primary, and sometimes subsequent, population spike(s) by low micromolar concentrations of CM, opipramol, DTG and dextrorphan (which, in the case of dextrorphan, has been suggested to be mediated by high-affinity σ site(s); Aryanpur *et al*., 1990) is also unlikely to reflect σ site occupancy. Thus, no potentiation was observed with nanomolar concentrations of DTG or during the application of low micromolar concentrations of CBT, ifenprodil or DXM. Furthermore, dextrorphan elicited potentiation despite having poor affinity for σ binding sites.

If high-affinity σ binding sites do not mediate the anticonvulsant properties of micromolar concentrations of σ ligands, what mechanism(s) might be involved? A variety of

high-affinity σ ligands (including some of those examined in the present study) inhibit K^+-stimulated efflux of ^{86}Rb from cortical synaptosomes at micromolar concentrations (Fletcher *et al*., 1989). However, K^+ channel block leads to the development of epileptiform activity and K^+ channel openers have been shown to possess anticonvulsant activity (Gandolfo *et al*., 1989). Rather, the anticonvulsant properties of micromolar concentrations of σ site ligands might reflect their ability to inhibit NMDA receptor-mediated events and/or HVA Ca^{2+} channels, given that these actions are observed at concentrations similar to those established in the present study for anticonvulsant activity (Table 1) and that the anticonvulsant activities of NMDA antagonists and blockers of some subtypes of HVA Ca^{2+} channel have been well-documented (see Introduction).

When applied at micromolar concentrations, many high affinity σ receptor ligands (including those examined in the present study) exhibit NMDA antagonist actions (e.g. Fletcher *et al*., 1995; Whittemore *et al*., 1997) and it seems clear that functional NMDA antagonism is in large part responsible for the anticonvulsant properties of many of the compounds tested. Thus, the absolute anticonvulsant potencies of the test compounds correspond reasonably well with their potencies as NMDA antagonists in hippocampal neurones (Table 1). In addition, the rank order potency for anticonvulsant activity is similar to the rank order potency for NMDA antagonism (Spearman rank order correlation coefficient = 0.95; $P<0.05$). Despite the good overall rank order correlation, it is apparent that the anticonvulsant potencies of CM, CBT and opipramol (together with loperamide) exceed those which might be expected if their anticonvulsant actions were simply a reflection of NMDA antagonism, suggesting in turn that additional mechanism(s) may contribute to the anticonvulsant properties of these compounds.

Blockade of multiple subtypes of HVA Ca^{2+} channel is a third feature of the spectrum of activity of micromolar concentrations of most of the compounds examined in the present study. Several lines of evidence support the possibility that compounds which act to block multiple subtypes of HVA Ca^{2+} channel, and thereby affect neurotransmitter release as well as postsynaptic Ca^{2+} influx, might demonstrate a more favourable profile of anticonvulsant activity than agents which act selectively at dihydropyridine-sensitive Ca^{2+} channels. First, anticonvulsant agents such as phenytoin include blockade of dihydropyridine-resistant HVA Ca^{2+} channels and inhibition of neurotransmitter release as part of their spectrum of activity (Rogawski & Porter, 1990). Second, selective L-type Ca^{2+} channel blockers, which are ineffective at blocking excitatory neurotransmitter release, possess equivocal anticonvulsant efficacy (e.g. Morón *et al*., 1990; Horne & Kemp, 1991; Mangano *et al*., 1991). In contrast, anticonvulsant activity is associated with selective blockers of P- and Q-type Ca^{2+} channels which reduce the release of excitatory neurotransmitters but fail to affect inhibitory postsynaptic potentials (i.p.s.p.s; Robichaud *et al*., 1994; Poncer *et al*., 1997; Wang *et al*., 1997). Third, Ca^{2+} channel blockers such as verapamil and flunarizine, which have activity against multiple subtypes of HVA Ca^{2+} channel and which inhibit dihydropyridine-resistant Ca^{2+}-dependent release of glutamate (Mangano *et al*., 1991; Dickie & Davies, 1992; Cousin *et al*., 1993; Ishibashi *et al*., 1995), possess anticonvulsant properties, including activity against seizures which are resistant to dihydropyridines (Bingmann & Speckmann, 1989; DeSarro *et al*., 1990; Pohl *et al*., 1992).

Although there was a poor correlation between rank order anticonvulsant potency and rank order potency for inhibition of HVA Ca^{2+} channels (Spearman rank order correlation coefficient = -0.36), it remains possible that blockade of HVA Ca^{2+} channels by loperamide, CM, CBT and opipramol (all of which are more potent Ca^{2+} channel blockers than NMDA antagonists and whose anticonvulsant potencies exceed their NMDA antagonist potencies) may contribute to their anticonvulsant properties. Involvement of Ca^{2+} channel blockade in the anticonvulsant actions of CM and CBT is supported by findings that both drugs inhibit K^+-stimulated release of endogenous glutamate (but not γ-aminobutyric acid GABA) from hippocampal slices at low micromolar concentrations (Annels *et al*., 1991; Mangano *et al*., 1991). Furthermore, whilst CM and CBT are effective against epileptiform activity induced by Mg^{2+}-free aCSF, they have little activity against seizures induced by NMDA (Leander, 1989; Apland & Braitman, 1990). In the case of loperamide, this blocker of multiple subtypes of HVA Ca^{2+} channel appeared more efficacious against spontaneous epileptiform events than against field bursts evoked by stimulation of the SC pathway. This may reflect the fact that interictal bursts recorded in CA1 are typically generated by CA3 pyramidal cells which are endowed with particularly robust voltage-activated Ca^{2+} currents (Mody *et al*., 1987; Tancredi *et al*., 1990). On the other hand, the present results do not support an involvement of Ca^{2+} channel blockade in the anticonvulsant properties of DXM (Church & Fletcher, 1995). Thus the *in vitro* anticonvulsant potency of DXM greatly exceeds its inhibitory activity at HVA Ca^{2+} channels (Table 1) and its potency for inhibition of K^+-stimulated glutamate or [^{3}H]-D-aspartate release from hippocampal slices (IC_{50} = 130 μM and 100 μM, respectively; Annels *et al*., 1991; Mangano *et al*., 1991).

Despite the possible involvement of Ca^{2+} channel blockade in the anticonvulsant actions of some of the compounds tested, taken as a whole the present study failed to demonstrate clear-cut synergistic anticonvulsant effects for compounds which possess NMDA antagonist and broad-spectrum HVA Ca^{2+} channel blocking activities in combination. The most parsimonious explanation for this finding is that the compounds examined do not possess a useful combination of inhibitory activities at multiple subtypes of HVA Ca^{2+} channel and may reduce not only glutamate (see above) but also GABA release. The latter effect would result in at least a partial attenuation of any anticonvulsant action consequent upon NMDA antagonism, inhibition of postsynaptic HVA Ca^{2+} channels and/or reduction of glutamate release. Indeed, the compounds tested (with the exception of dextrorphan) block N- as well as L-type Ca^{2+} channels (Church *et al*., 1994a, b; Church & Fletcher, 1995). Selective N-type Ca^{2+} channel blockers have minimal effects on evoked excitatory transmitter release, depress i.p.s.p.s to a greater extent than e.p.s.p.s, and exhibit proconvulsant activity (Horne & Kemp, 1991; Mangano *et al*., 1991; Potier *et al*., 1993), findings which may reflect the fact that this subtype of HVA Ca^{2+} channel mediates GABA release from interneurones in the stratum radiatum (Poncer *et al*., 1997). In contrast to N-type Ca^{2+} channel blockers, P-type Ca^{2+} channel blockers suppress excitatory synaptic transmission in the hippocampus by depressing glutamate release, with no evidence of either a decrease in i.p.s.p.s or proconvulsant activity (see Poncer *et al*., 1997). However, with the exception of ifenprodil, which inhibits P- as well as N-type HVA Ca^{2+} channels (IC_{50} for inhibition of P-type channels = $\sim$60 μM; Bath *et al*., 1996) and which reduces K^+-stimulated release of glutamate (IC_{50} = 35 μM; Ellis & Davies, 1994; also see Mangano *et al*., 1991), the specific inhibitory activities of the test compounds at P-, Q- (or R-) type HVA Ca^{2+} channels remain unknown.

Although anticonvulsant activity was evident at the micromolar concentrations associated with functional NMDA antagonism and/or blockade of multiple subtypes of HVA Ca^{2+} channel, other mechanisms may contribute to the anticonvulsant activities of at least some of the compounds tested. Most notably, although adenosine and CM had similar effects on I/O functions, they elicited opposite actions on PPF in that CM markedly depressed the facilitation of test responses to the extent that paired-pulse depression (PPD) emerged. The ability of CM to elicit PPD appears unlikely to reflect its ability to reduce excitatory transmitter release (see above) because this would have the effect, as in the case of adenosine, to enhance PPF (Dunwiddie & Haas, 1985; Debanne *et al*., 1996). Rather, the depressant effect of CM on PPF observed at short conditioning-testing intervals may reflect an augmentation of an underlying (feed-forward) inhibitory conductance activated by the conditioning stimulus (Alger & Nicoll, 1982; Turner, 1990). This would have the effect of shunting the response to the test stimulus, as previously observed for anticonvulsant compounds which potentiate $GABA_A$ receptor-mediated inhibition (e.g. Albertson *et al*., 1992). Surprisingly, there are no published results concerning the effects of CM on γ-aminobutyric acid (GABA) release, GABA uptake, $GABA_A$ receptor binding or $GABA_A$ receptor-mediated responses. The present results suggest that an evaluation of the possibility that the anticonvulsant properties of CM may involve the modulation of GABAergic inhibitory mechanisms would appear to be worthwhile.

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