Differential effects of petit mal anticonvulsants and convulsants on thalamic neurones: GABA current blockade

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1 Currents evoked by applications of γ-aminobutyric acid (GABA) to acutely dissociated thalamic neurones were analysed by voltage-clamp techniques, and the effects of the anticonvulsant succinimides ethosuximide (ES) and α-methyl-α-phenylsuccinimide (MPS) and the convulsants tetramethylsuccinimide (TMS), picrotoxin, pentylenetetrazol (PTZ), and bicuculline methiodide were assessed.

2 TMS (1 μM–10 mM) reduced responses to iontophoretically applied GABA, as did picrotoxin (0.1–100 μM), PTZ (1–100 mM) and bicuculline (1–100 μM).

3 ES, in high concentrations (1–10 mM), reduced GABA responses to a lesser extent, and also occluded the reductions in GABA-evoked currents produced by TMS, picrotoxin, and PTZ. ES did not occlude the effects of bicuculline on GABA responses. Therefore, we propose that ES acts as a partial agonist at the picrotoxin GABA-blocking receptor.

4 MPS had no effect on GABA responses (at a concentration of 1 mM), and, like ES, occluded the GABA-blocking actions of TMS, apparently acting as a full antagonist.

5 The anticonvulsant actions of ES and MPS against TMS and PTZ-induced seizures may thus involve two independent mechanisms: (1) the occlusion of TMS and PTZ GABA-blocking effects; and (2) the previously described specific effect of ES and MPS on low-threshold calcium current of thalamic neurones. The latter cellular mechanism may be more closely related to petit mal anticonvulsant activity.

Introduction

We have previously shown that clinically relevant concentration ranges of petit mal anticonvulsant succinimides such as ethosuximide (ES, Zarontin, Parke Davis), α-methyl-α-phenylsuccinimide (MPS, the active metabolite of Celontin, Parke Davis), and the structurally similar oxazolidinedione dimethadione (DMD) reduce low-threshold calcium current (LTCC) in thalamic neurones (Coulter et al., 1989a,b; 1990). Inactive (succinimide; Ferrendelli & Kupferberg, 1980) and convulsant (tetramethylsuccinimide, TMS; Klunk et al., 1982a,c) succinimides were ineffective in reducing LTCC (Coulter et al., 1989a; 1990). When considered in the context of the hypothesized role of the LTCC in the oscillatory thalamocortical activity underlying petit mal, the LTCC-reducing action of these anticonvulsant agents is consistent with their clinical utility (see Coulter et al., 1989b for a more complete discussion on the role of LTCC in petit mal). TMS reduces γ-aminobutyric acid (GABA) responses in cultured cortical neurones (Barnes & Dichter, 1984), an effect which has been proposed to be at least partially responsible for its convulsant actions. The cellular actions of TMS on GABA responses are similar to those of pentylenetetrazol (PTZ) (Nicoll & Padjen, 1976; Macdonald & Barker, 1977; De Deyn & Macdonald, 1989), and both agents are thought to bind to the picrotoxin receptor in the GABA receptor/ionophore complex (Klunk et al., 1982a,c; 1983; Ramanjaneyulu & Ticku, 1984).

In order to understand better the role of modulation of GABA responses in the generation and control of epilepsy by succinimides and structurally similar compounds, we examined the actions of ES, MPS, TMS, picrotoxin, PTZ and bicuculline methiodide on GABA responses of acutely isolated thalamic neurones under voltage-clamp recording conditions. We found that TMS, picrotoxin, PTZ and bicuculline all reduced GABA responses in a concentration-dependent manner. ES also reduced GABA responses to a lesser extent, while MPS had no apparent effect on GABA responses. ES significantly occluded the GABA-blocking actions of TMS, picrotoxin and PTZ, but not of bicuculline, and therefore

appeared to act as a partial agonist at the picrotoxin GABA-blocking receptor. We suggest that this occluding action of ES may be responsible for the anticonvulsant activity of this agent in some chemically-induced seizures, but that this action is unrelated to the LTCC-blocking effects of ES, which may underlie its ability to suppress the spike wave discharges of petit mal epilepsy.

Methods

Dissociation

All experiments were performed on thalamic neurones isolated from the ventrobasal nucleus of young rats (age 1–15 days) by methods described in the preceding paper (Coulter et al., 1990).

Voltage-clamp recording

Whole-cell voltage-clamp recordings were made by methods described in the preceding paper (Coulter et al., 1990). The intracellular (pipette) solution contained (in mM): Trizma phosphate (dibasic) 110, Trizma base 28, ethylene glycol bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid 11, $MgCl_2$ 2, $CaCl_2$ 0.5 and Na-ATP 4, pH 7.35. The pipette solution also contained an intracellular adenosine 5'-triphosphate (ATP) reconstitution system consisting of creatine phosphokinase, 50 u ml^{-1}, and phosphocreatine, 22 mM (Forscher & Oxford, 1985; Mody et al., 1988). GABA currents have been shown to run down or fade (Huguenard & Alger, 1986), a process apparently triggered by lack of high energy phosphates or inadequate calcium buffering being present in the intracellular medium (Stelzer et al., 1988). By use of the calcium-buffered intracellular solution, with ATP and an ATP reconstitution system as described above, stable GABA responses could routinely be recorded for 2–3 h. The external solution contained (in mM): NaCl 155, KCl 3, $MgCl_2$ 1, $CaCl_2$ 3, HEPES-Na$^+$ 10 and tetrodotoxin 0.0005; pH 7.4. All recordings were conducted at room temperature (20–22°C).

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Drug concentrations and method of application

All drug solutions were freshly prepared daily. In order to explore the full concentration-dependence of the effects on GABA, concentration ranges of MPS (Sigma, St. Louis, MO, U.S.A.) and ES (a gift of Parke-Davis, Ann Arbor, MI, U.S.A.) were chosen to overlap and extend those which are achieved as free serum concentrations in patients medicated with the anticonvulsant: 50–250 μM for MPS (Strong *et al.*, 1974; Porter *et al.*, 1979; Browne *et al.*, 1983), and 250–750 μM for ES (Browne *et al.*, 1975). Free serum concentrations of the convulsant TMS (ICN Pharmaceuticals, Plainview, NY, U.S.A.) have not been studied. Since previous work has shown that 0.5–1 mM TMS reduces GABA responses in cultured cortical neurones by 60–80% (Barnes & Dichter, 1984), we applied this agent at 1 μM–10 mM. Other convulsants were applied in the following concentrations: picrotoxin (Sigma) 0.1–100 μM, PTZ (Sigma) 100 μM–100 mM and bicuculline (Research Biochemicals Inc., Natick, MA, U.S.A.) 0.1 μM–1 mM. All drugs were bath applied, except for γ-aminobutyric acid (GABA), which was applied iontophoretically. ES, MPS, TMS, picrotoxin, PTZ and bicuculline were soluble directly in the perfusion medium. MPS often required sonication to speed solubilization.

GABA iontophoresis

GABA (1 M, pH 4.5, dissolved in normal bath solution) was iontophoresed from either one- or two-barrelled electrodes. When a two-barrelled electrode was used, the return barrel contained bath solution and the control unit (WPI, Worcester, MA, U.S.A.) automatically compensated the iontophoretic current ejected through one barrel via the return barrel. The iontophoretic electrode was placed within 20 μm of the neurone, and GABA was applied to cells held at −60 mV, near the reversal potential for the GABA response (Figure 1). The conductance increase elicited by GABA application was

assessed with +20 mV, 50 ms voltage commands, delivered at 10 Hz. No active conductances were elicited by the voltage steps from −60 to −40 mV, since the LTCC was steady-state inactivated at −60 mV, −40 mV is subthreshold for eliciting the HTCC, and Na$^+$ and K$^+$ currents were blocked by the internal and external solutions (Coulter *et al.*, 1989c). GABA effects were quantitated either as peak conductance (minus leak conductance) elicited following GABA application, or peak outward current elicited at a holding potential of −30 mV to −40 mV. Drug effects are expressed as percentage reduction in GABA-evoked outward current.

Results

GABA responses

Iontophoretic application of GABA to thalamic neurones elicted a current with a reversal potential (E_{GABA}) of −67.1 ± 2.1 mV (mean ± s.e., n = 12) accompanied by an increase in conductance (Figure 1a,b). The reversal potential was close to that for a chloride current derived from the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin & Katz, 1949). Under the ionic conditions of this study, assuming a phosphate to chloride permeability ratio of 0.025 (Bormann *et al.*, 1987), and an activity coefficient of 0.75 for the 166 mM external chloride solution, the calculated reversal potential was −70 mV. Our measured E_{GABA} was 2.9 mV depolarized from this theoretical value. The discrepancy might be due to an elevation of internal chloride concentration over that present in the recording pipette because of chloride entry into the cell by either leak, or GABA-activated chloride conductance. This type of shift of E_{GABA} at low internal chloride concentrations has also been seen in hippocampal neurones recorded under similar conditions (Huguenard & Alger, 1986). E_{GABA} was dependent on external chloride concentration. Application of GABA to a cell perfused with a solution con-
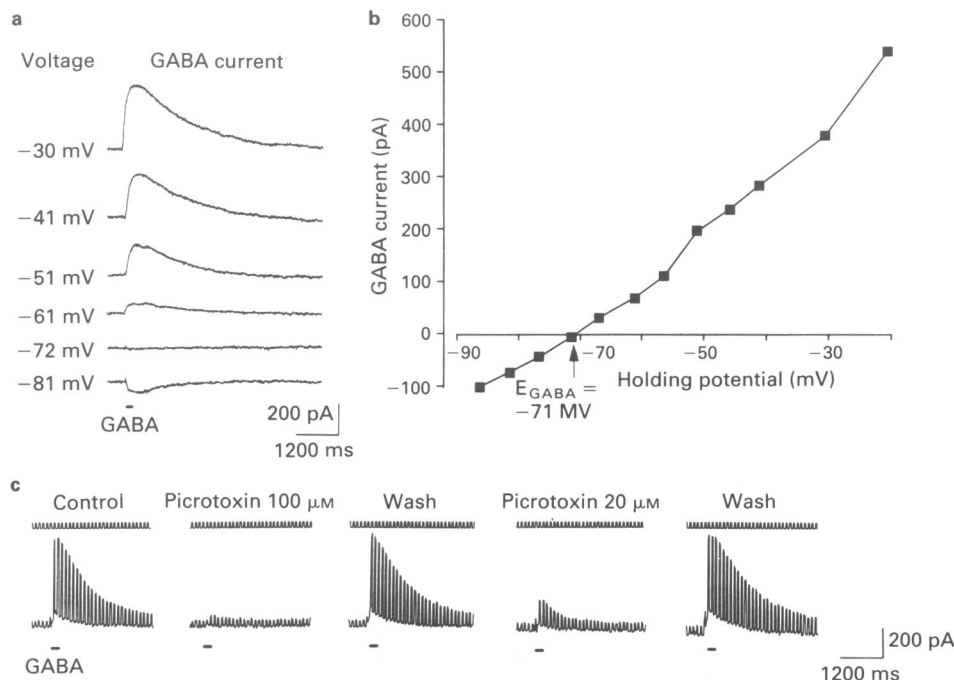

Figure 1 Characterization of the cellular response to iontophoretically applied γ-aminobutyric acid (GABA). (a) Traces illustrating the voltage-dependence of the GABA response. The holding potential appears to the left of the elicited GABA current, and the period of GABA application is indicated below the traces. (b) Plot of holding potential vs amplitude of the GABA current elicited for that holding potential (same cell as in (a)). The reversal potential of the GABA current (E_{GABA}, indicated by arrow) was −71 mV for this cell. (c) Blockade of the GABA response by picrotoxin in another neurone held at −60 mV. Control: response to GABA in normal bath solution, with 20 mV conductance pulses (voltage commands above each trace in c) illustrating the marked increase in conductance accompanying the GABA current. This response was virtually abolished by application of 100 μM picrotoxin, in a reversible manner. Picrotoxin 20 μM reduced the GABA response by 80–90%, and this effect was also completely reversible.

taining reduced chloride concentration (88 mM, NaCl replaced with Na isethionate) resulted in a shift of E$_{GABA}$ to -55.8 ± 2.8 mV ($n = 5$), and a reduction in amplitude of the peak GABA-induced current. The expected reversal potential, calculated as above with the Goldman-Hodgkin-Katz equation, was -54 mV. In the same 5 cells, E$_{GABA}$ in normal (166 mM) extracellular chloride concentration was -67.6 ± 4.5 mV. The GABA-induced chloride current showed pronounced outward rectification, as has been previously described in other neurones (Segal & Barker, 1984; Barker & Harrison, 1988). This current and the accompanying conductance increase elicited by GABA were both reduced or blocked by bath application of picrotoxin (0.1–100 μM, $n = 11$; Figure 1c), or bicuculline (0.1–100 μM, $n = 9$, not shown). No conductance change was noted during application of equivalent iontophoretic current without GABA in the ejecting pipette (not shown), and a broken iontophoretic pipette could elicit a similar conductance without application of iontophoretic current. These data are consistent with activation of GABA$_A$ receptors, and opening of GABA-gated chloride channels by the applied GABA.

Drug effects on GABA responses

We examined the actions of the anticonvulsant succinimides ES and MPS, and the convulsants TMS, picrotoxin, PTZ and bicuculline on GABA-induced currents in a total of 68 neurones. At concentrations of 10 μM or greater, TMS depressed GABA responses in a reversible manner in all of the 28 cells tested. Reductions of $22.7 \pm 5.4\%$ ($n = 8$, mean $\pm$ s.e.) and $40.2 \pm 2.5\%$ ($n = 10$) were produced by TMS concentrations of 100 μM and 1 mM, respectively. We also tested ES effects on GABA responses in 21 neurones (Figure 2). At 200 μM (the IC$_{50}$ for ES reduction of LTCC (Coulter et al., 1989b)) and 1 mM, ES reversibly reduced GABA responses by $5.5 \pm 2.4\%$ ($n = 9$) and $18.8 \pm 3.8\%$ ($n = 10$), respectively. The concentration-dependence of ES- and TMS-induced GABA response reductions is plotted in Figure 2. TMS was >10 fold more effective in reducing GABA responses than ES. These findings could be explained by differences in potency and/or efficacy of the two succinimides at the same receptor, or by the two drugs acting at different receptors. Reduced efficacy of ES at a common receptor might be associated with expression of partial agonist activity by ES. Under these circumstances, if both drugs were applied simultaneously, ES would be
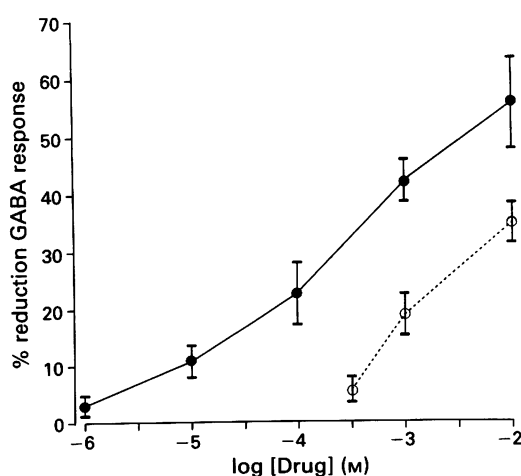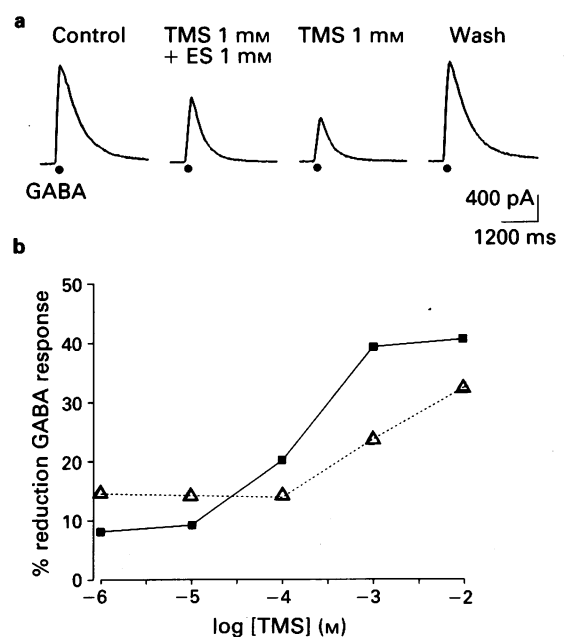

Figure 3 Ethosuximide (ES) occlusion of the γ-aminobutyric acid (GABA)-blocking effect of tetramethylsuccinimide (TMS) in 2 neurones. (a) GABA-elicted outward currents (GABA applied iontophoretically at ●) in normal bath solution, solution containing TMS 1 mM and ES 1 mM, TMS 1 mM alone and wash conditions. ES occluded the GABA-blocking action of TMS (1 mM) by 39% in this cell (cf. 2nd and 3rd traces with control). (b) Concentration-response curve for TMS block of GABA current in another neurone for TMS applied alone (■) and in the presence of 1 mM ES (△). At all TMS concentrations >30 μM, ES occluded the GABA-blocking actions of TMS.

expected to occlude TMS block of GABA responses in certain concentration ranges. If the two drugs were acting at different receptors, an independent additive interaction between the two effects would be expected. We tested these possibilities by analysing the effects of concurrent ES and TMS application. In 18 cells, ES (200 μM–3 mM) decreased the TMS-induced block of GABA responses (Figure 3).

Concentrations of 400 μM and 1 mM ES occluded the GABA blocking effect of 1 mM TMS by $17.1 \pm 4.3\%$ ($n = 3$) and $31.6 \pm 8.3\%$ ($n = 4$), respectively. Figure 3a illustrates this action in one cell to which TMS 1 mM, and TMS 1 mM plus ES 1 mM were applied. This concentration of TMS reduced the GABA current by 56% when applied alone. When TMS and ES were bath applied to the same cell, only a 34% reduction in GABA response was seen. Thus, concurrent ES application caused a 39% occlusion of the GABA-blocking effect of TMS. In another neurone, various concentrations of TMS were applied alone and in the presence of 1 mM ES, and the resulting GABA-blocking effects plotted as log[TMS] vs % reduction in GABA response (Figure 3b). ES had a small GABA-blocking effect of its own, seen in Figure 3b as an increased block of GABA responses by TMS + ES compared to low concentrations of TMS alone (when TMS concentrations <50 μM were applied). ES (1 mM) occluded the GABA-blocking action of TMS, when the latter was applied in concentrations of 50 μM–10 mM, an effect that is analogous to partial agonist effects on a full agonist (e.g. Kenakin, 1987). Another petit mal anticonvulsant, MPS, had no effect on GABA responses when applied at 1 mM (n = 7, not shown), although it caused a $64.6 \pm 7.0\%$ occlusion of the GABA-blocking actions of 1 mM TMS when applied concurrently (n = 5, not shown).

We did further experiments to determine which receptor in the GABA receptor/ionophore complex might be the site mediating the ES-induced occlusion of convulsant GABA-blocking effects. The GABA receptor/ionophore complex contains binding sites for GABA, and at least 3 binding sites

Figure 2 Concentration-response curve for block of γ-aminobutyric acid (GABA) current by the convulsant tetramethylsuccinimide (TMS), and the anticonvulsant ethosuximide (ES). TMS (●——●) was >10 fold more potent in blocking GABA current than ES (○– – –○), which blocked GABA current by a maximum of 10–15% in clinically relevant anticonvulsant concentrations (250–270 μM). V$_{hold}$ = -30 mV. GABA current reduction is expressed as % of control, in this and subsequent figures.

mediating allosteric modulation of GABA responses, including those for picrotoxin and picrotoxinin analogues, barbiturates, and benzodiazepines (Olsen, 1982; Maksey & Ticku, 1985). Bicuculline and picrotoxin antagonize GABA responses by binding to different receptors. Bicuculline is thought to compete with GABA for the GABA receptor, and competitively antagonize GABA responses (Simmonds 1982a,b), while picrotoxin is thought to bind to its receptor and block the chloride channel, and therefore block GABA responses allosterically, in a non-competitive manner (Simmonds, 1982b). The convulsant PTZ has also been shown to block GABA responses (Nicoll & Padjen, 1976; Macdonald & Barker, 1977; De Deyn & Macdonald, 1989), and it has been suggested that this block is due to PTZ binding to the picrotoxin receptor on the GABA receptor/ionophore complex (Ramanjaneyulu & Ticku, 1984; Maksey & Ticku, 1985). Therefore, we examined the effects of picrotoxin on GABA responses in 11 cells. A picrotoxin concentration of 100 μM blocked the GABA response entirely while a concentration of 20 μM blocked GABA responses by 80–90% (Figure 1c). Figure 4 shows the concentration-dependence of picrotoxin block of GABA responses. The curve is generated by an equation assuming a bimolecular interaction between drug and receptor, a 100% maximal effect, and an IC_{50} of 3 μM. Since this concentration-response curve was produced by application of picrotoxin, which is an equal mixture of picrotoxinin (the active GABA blocker) and picrotin (inactive as a GABA blocker), this IC_{50} of 3 μM for picrotoxin would correspond to an IC_{50} of 1.5 μM for picrotoxinin. Figure 4 also shows the concentration-dependence of PTZ block of GABA responses for 12 cells. The curve was generated by an equation assuming a bimolecular interaction between drug and receptor, a 60% maximal effect, and an IC_{50} of 1 mM. This is in reasonable agreement with the results of De Deyn & Macdonald (1989) in current-clamped cultured spinal neurones. These authors obtained an IC_{50} of 1.1 mM, and full blockade of GABA responses by 10 mM PTZ. Thus, in thalamic neurones, PTZ is approximately 1000 times less potent, and 40% lower in efficacy, than picrotoxin in blocking GABA responses. In 8 cells, ES, in varying concentrations, occluded the GABA-blocking effect of picrotoxin (Figure 5a). At a concentration of 1 mM, ES decreased the GABA-blocking action of 10 μM picrotoxin by 13.1 $\pm$ 3.0% (*n* = 4). Figure 5a illustrates this effect in one cell where ES (1 mM) occluded the GABA-blocking effect of 10 μM picrotoxin by 33%. Figure 5b shows the concentration-
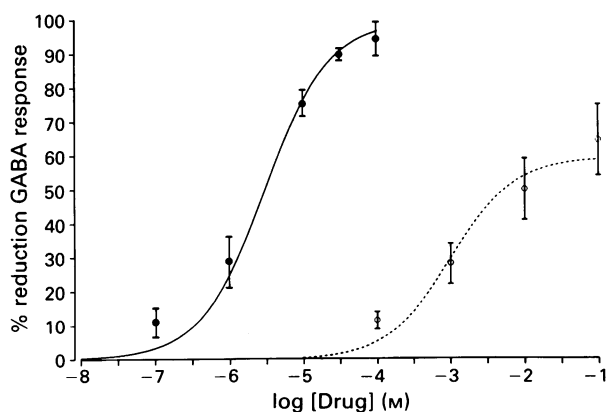

Figure 5 Ethosuximide (ES) occlusion of the γ-aminobutyric acid (GABA)-blocking effect of picrotoxin in 2 neurones. (a) GABA-elicted outward currents in 1 cell under control, picrotoxin 10 μM, picrotoxin 10 μM and ES 1 mM, and wash conditions. ES occluded the GABA-blocking action of picrotoxin (10 μM) by 33% in this cell. (b) Concentration-response curve for picrotoxin block of GABA current in another neurone in the absence (■) and presence (△) of 1 mM ES. ES occluded the GABA-blocking actions of picrotoxin at all picrotoxin concentrations >3 μM.

dependence of this effect. ES, which had a small GABA-blocking effect of its own (not shown), occluded the effects of 30–100 μM picrotoxin on GABA responses, and produced an added reduction in responses at picrotoxin concentrations less than 30 μM.

In 9 cells, we examined the effect of 400 μM–1 mM ES on the GABA-blocking actions of PTZ. ES (1 mM) occluded the effects of 10 and 100 mM PTZ by 30.1 $\pm$ 12.5% (*n* = 5) and 21.8 $\pm$ 4.6% (*n* = 4), respectively. Figure 6a illustrates this effect in one exceptional cell in which ES (1 mM) occluded the GABA-blocking action of 10 mM PTZ by 60%. Figure 6b shows the concentration-dependence of this effect. ES (1 mM) occluded the GABA-blocking actions of PTZ at all PTZ concentrations > 1 mM, and was additive at lower concentrations. To determine whether this effect of ES on convulsant actions is specific for agents acting at the picrotoxin receptor, we examined the GABA-blocking effects of bicuculline alone and with 1 mM ES. Bicuculline is thought to bind to the GABA receptor itself, and act as a competitive inhibitor of GABA responses, in contrast to the allosteric inhibitory action of picrotoxin (Simmonds, 1982b). In all 7 cells tested, ES elicited clear independent and additive effects on the GABA-blocking actions of bicuculline, at all concentrations. Figure 7a illustrates this effect in one cell. Superimposed traces of bicuculline alone and with 1 mM ES demonstrate an additive effect of bicuculline and ES at bicuculline concentrations ranging from 1 to 100 μM. Figure 7b shows the concentration-dependent reductions produced by bicuculline alone and with ES (1 mM). The data from bicuculline alone could be fitted by a curve which assumed a bimolecular interaction between drug and receptor, an IC_{50} of 40 μM, and a 95% maximal effect. Data from concurrent application of 1 mM ES and the same concentrations of bicuculline in the same cell also showed a concentration-dependent reduction of GABA responses. These data were fitted by a curve developed with the same assumptions that were employed for the bicuculline alone data, except that an additional (presumably ES-elicited) reduction of 28% of the remaining current was added at each point to

Figure 4 Plot of log drug concentrations vs block of γ-aminobutyric acid (GABA) current for the convulsants picrotoxin and pentylenetetrazol (PTZ). Picrotoxin (●——●) was approximately 100 fold more potent, and greater in efficacy in blocking GABA current than PTZ (○ — — ○). The curve fitted to the picrotoxin data was generated by an equation in which a bimolecular interaction between drug and receptor, an IC_{50} of 3 μM and a 100% maximal effect were assumed. The curve fitted to the PTZ data was generated by an equation in which a bimolecular interaction between drug and receptor, an IC_{50} of 1 mM and a 60% maximal effect were assumed. Curves were fitted by eye.
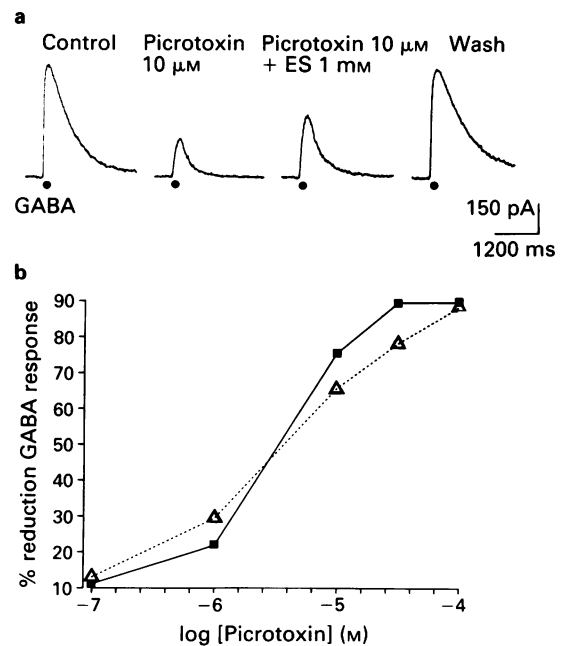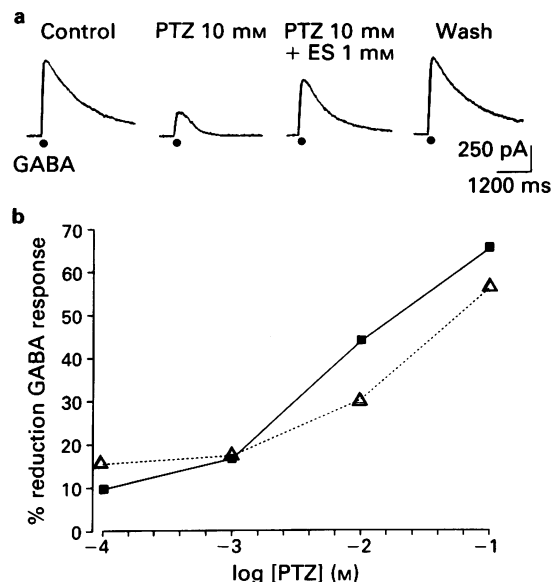
Figure 6 Ethosuximide (ES) occlusion of the γ-aminobutyric acid (GABA)-blocking effect of pentylenetetrazol (PTZ) in 2 neurones. (a) GABA-elicited outward currents under control, PTZ (10 mM), PTZ (10 μM) and ES (1 mM), and wash conditions. ES occluded the GABA-blocking action of PTZ (10 mM) by 60% in this cell. (b) Concentration-response curve for PTZ block of GABA current in another neurone in the absence (■) and presence (△) of 1 mM ES. ES occluded the GABA-blocking effect of PTZ at all PTZ concentrations > 1 mM.

the effect of bicuculline alone. Thus, ES acts on GABA responses by a mechanism entirely independent of the action of bicuculline, presumably by binding to a physiologically distinct receptor. This is in marked contrast to the occlusive ES interactions with convulsants acting at the picrotoxinin receptor.
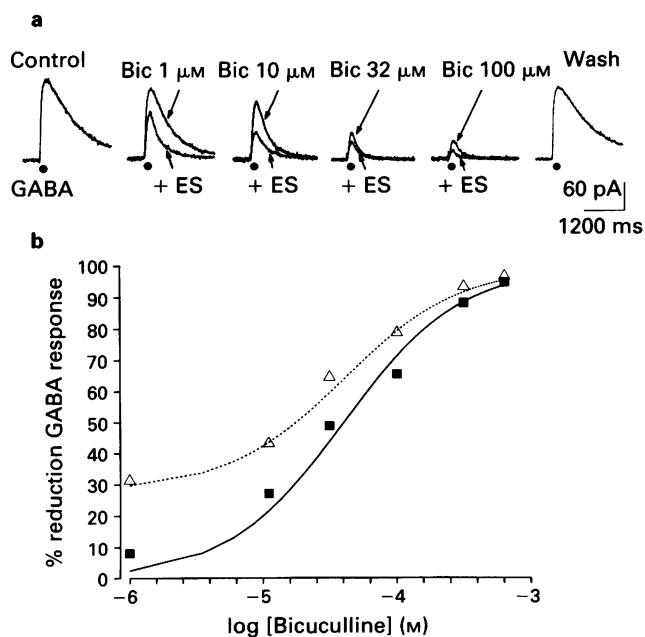

Figure 7 Ethosuximide (ES) and bicuculline methiodide (Bic) exerted an independent and additive reduction in γ-aminobutyric acid (GABA)-current when applied concurrently. Recordings are from 2 neurones (a and b). (a) GABA-elicited outward currents under control, bicuculline 1 μM, 10 μM, 32 μM and 100 μM alone and in the presence of ES 1 mM and wash conditions. Bicuculline and ES, when applied concurrently, exert an independent and additive effect in blocking GABA currents. (b) Concentration-response curve for bicuculline block of GABA current for bicuculline alone (■) and in the presence of 1 mM ES (□). The fitted curves are described in the text.

Discussion

It appears that the reductions in GABA responsiveness and the decreases in LTCC elicited by succinimides are mediated by different receptors. This conclusion is supported by the differential effects of TMS versus MPS and ES on the conductances in question. TMS effects are consistent with it binding much more readily to the GABA-blocking receptor than to the LTCC-blocking receptor, as judged by the reductions in GABA conductance that can be produced by concentrations of this agent that have no effects on LTCC, and the inability of TMS to occlude MPS effects on LTCC (Coulter et al., 1990). By contrast, MPS and ES are more effective at the LTCC-blocking receptor (Coulter et al., 1989b; 1990). Also, the order of potency of the various succinimides and convulsants in reducing LTCC is ES > MPS ≫ TMS > PTZ (Coulter et al., 1990), while the potency of these same compounds in reducing GABA responsiveness is TMS > PTZ ≫ ES > MPS.

We have previously suggested (Coulter et al., 1989a,b) that one action responsible for the anticonvulsant effects of the succinimides is their ability to reduce the LTCC of thalamic neurones. The receptor mediating this action appears to be distinct from the picrotoxin receptor, which is responsible for the convulsant actions of TMS (Ramanjaneyulu & Ticku, 1984). Ferrendelli and colleagues (Klunk et al., 1982a,b; 1983) have developed a model for the structure-activity relationship of alkyl substituted succinimides and γ-butyrolactones, and have suggested that α-substitution alone is linked to anticonvulsant activity against PTZ-induced seizures, while β- or α- and β-substitution confers convulsant activity on a molecule binding to the picrotoxin receptor. These authors hypothesized that β-substitution by an alkyl group on the heterocyclic 5-membered ring directly blocks the chloride channel associated with the GABA receptor. Ticku & Maksey (1983) suggested that β-substitution by such an alkyl group may interact with an allosteric hydrophobic regulatory site, which in turn may block or close the ligand-gated chloride channel. Our results are in reasonable agreement with this Ferrendelli/Ticku hypothesis. Picrotoxin, TMS, and PTZ, all of which block GABA responses (Figures 2 and 4), are convulsants. All three of these drugs have areas within their chemical structures which correspond to β-alkyl substitution on the lactone ring (Klunk et al., 1983; Ticku & Maksey, 1983). The α-substituted anticonvulsant succinimides ES and MPS exhibit low efficacy as GABA-blockers, but do occlude the effect of full (or higher efficacy) antagonists in an apparently competitive manner (Figures 3, 5 and 6).

Our results agree in part with those of Barnes & Dichter (1984), who showed that both ES and TMS reduce GABA responses in cortical neurones. However, these authors also demonstrated that ES did not occlude the reductions in GABA responses produced by TMS or picrotoxin, when applied concurrently at concentrations of 500 μM to 1 mM. We were able to demonstrate such occlusive effects of ES on the GABA-blocking actions of TMS, picrotoxin and PTZ (Figures 3, 5 and 6), but not of bicuculline (Figure 7). This action of ES may therefore be a mechanism by which it acts to depress chemically-induced seizures. The finding that a second succinimide petit mal anticonvulsant, MPS, was also effective in occluding the GABA blocking actions of TMS further supports this conclusion.

It is possible that the discrepancy between our data and those of Barnes & Dichter (1984) is due to the different techniques employed in the two sets of experiments. Pressure-puff application (Barnes & Dichter, 1984) may not allow all drugs to come to an equilibrium concentration before a GABA response is assessed, which makes interpretation of the data more difficult. Also, assessment of partial agonist activity requires quantitative data analysis, which is facilitated by the voltage-clamp techniques used in the present experiments. De Deyn & Macdonald (1989) recently showed that PTZ reduces

GABA responses in a concentration-dependent manner, but also found that a single concentration of ES ($600\,\mu M$) did not occlude the GABA-reducing action of 1 mM PTZ. The ES occlusion of the PTZ- (or TMS- or picrotoxin-) GABA-blocking actions demonstrated in our experiments was concentration-dependent, and would have been missed if the interaction of the two drugs had not been examined over the entire concentration range of full agonist response. For example, although concurrent application of ES (1 mM) with 1 mM PTZ had no effect on the GABA-blocking actions of PTZ (Figure 6), at higher PTZ concentrations (e.g. 10 mM), ES could occlude these PTZ actions by >30%. These types of interactions are characteristic of partial agonist actions at a receptor (Kenakin, 1987).

The specificity of the ES occluding actions for agents binding to the picrotoxinin receptor (PTZ, picrotoxin, and TMS), but not the GABA/bicuculline receptor, suggests that ES is interacting at this site to exert its effects. However, binding data show that ES exhibits very low potency in displacing [^{35}S]-t-butylbicyclophosphorothionate (TBPS), a high affinity picrotoxin receptor ligand, from its receptor (Squires *et al.*, 1983). This finding is consistent with our data which indicate that 1 mM ES is only moderately effective in reducing the GABA-blocking actions of picrotoxin (13% occlusion of $10\,\mu M$ picrotoxin GABA-blocking actions, Figure 5). ES produced a larger reduction of the actions of TMS and PTZ (Figure 3 and 6), agents which bind with lower affinity to the picrotoxin receptor (Ramanjaneyulu & Ticku, 1984), and reduce GABA responses with lower potency than picrotoxin (Figures 2 and 4).

There is a discrepancy betwen the low ES binding affinity at the picrotoxin/TBPS receptor, and its physiological occlusive effects against agents binding at this receptor. One possible explanation is that only a low level of ES binding to the picrotoxin receptor is necessary to exert its action. A second possibility is that there is a chemical interaction between ES

and picrotoxin receptor ligands that does not involve ES binding to the picrotoxin receptor at all. Finally, ES may bind to another receptor, allosterically linked to the picrotoxin receptor. For example, barbiturates, which bind to a receptor distinct from the picrotoxin/TBPS receptor (Trifiletti *et al.*, 1985), can inhibit picrotoxin and TBPS binding, suggesting a close (negative) coupling between the barbiturate and picrotoxin receptors (Wong *et al.*, 1984; Maksey & Ticku, 1985; Trifiletti *et al.*, 1985). ES is structurally similar to barbiturates, making this type of allosteric interaction with negative effects on picrotoxin/TBPS ligand binding a reasonable possibility that could be tested in future experiments.

The ability of succinimide petit mal anticonvulsants to occlude GABA-blocking actions of drugs acting at the picrotoxin receptor may partially explain their actions against PTZ- and TMS-induced convulsive seizures. However, these seizures are behaviourally dissimilar from the non-convulsive attacks of petit mal, and not all agents which block PTZ seizures are useful against petit mal. For example, primidone and phenobarbital are quite effective in controlling PTZ-induced seizures, but are ineffective or may even exacerbate petit mal (Löscher & Schmidt, 1988). The anticonvulsant actions of ES and other α-substituted succinimides against TMS and PTZ-induced seizures may thus involve two independent mechanisms: (1) the occlusion of TMS and PTZ GABA-blocking effects; and (2) the specific effect of ES and MPS on low-threshold calcium current of thalamic neurones (Coulter *et al.*, 1989a,b; 1990). The latter cellular action of anticonvulsant succinimides may be more closely related to their effectiveness in petit mal.

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