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Felbamate neuroprotection against CA1 traumatic neuronal injury

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Abstract

Traumatic brain injury is a leading cause of disability and death. Since the anticonvulsant felbamate provides hypoxic neuroprotection, we investigated whether felbamate would provide protection against traumatic neuronal injury as well. Traumatic injury to CA1 neurons in hippocampal slices was induced by fluid percussion, and CA1 evoked response was monitored. Pre-treatment with felbamate was strongly protective against neuronal injury, and permitted CA1 antidromic population spike recovery to a mean 94 ± 1% (S.E.M.) of initial amplitude, compared to unmedicated slices which regained only 15 ± 6%. The felbamate EC_{50} for this protection was 136 mg/l, and significant protection was found at felbamate concentrations similar to those reported in felbamate monotherapy for seizures. Significant protection was also detected when felbamate was initiated 15 min after trauma. Slices given brief post-trauma felbamate treatment could demonstrate long-term potentiation when assessed 8 h after trauma. These studies indicate that felbamate is neuroprotective against CA1 traumatic neuronal injury.

Keywords: Traumatic brain injury; Felbamate; Long-term potentiation; CA1; Hippocampus

1. Introduction

Felbamate (2-phenyl-1,3-propanediol dicarbamate) is an anticonvulsant (Dodson, 1993; Faught et al., 1993; Jenson, 1993) which has also been shown to be neuroprotective. This protection was initially established in vitro against hypoxia using the hippocampal slice (Wallis et al., 1992) and later confirmed with in vivo studies of ischemia (Wasterlain et al., 1992). Because drugs protecting against hypoxia-ischemic injury have often been found to be protective against other neuronal insults as well (Bullock and Fujisawa, 1982; Papagapiou and Auer, 1990; Tasker et al., 1992; Nellgard and Wieloch, 1992) we investigated the effect of felbamate upon CA1 traumatic neuronal injury. For our study we used the hippocampal slice, and we examined the effect of CA1 trauma induced by fluid percussion. This in vitro preparation permits electrophysiological monitoring of mature CA1 pyramidal cells, known to be exquisitely vulnerable to traumatic brain injury (Kotapka et al., 1992) while affording relatively precise control of the neuronal environment. Additionally, this preparation preserves neuronal-glial relationships which play an important role in excitotoxicity (Sugiyama et al., 1989), a pathological process shown to be active in the development of traumatic neuronal injury (Faden et al., 1989).

2. Materials and methods

Adult male Sprague-Dawley rats, 175–425 g, were briefly anesthetized with halothane and decapitated, as previously described (Wallis et al., 1992). The brain was removed within 1 min and placed in cold artificial cerebrospinal fluid for an additional minute. Artificial cerebral spinal fluid was composed of (in Mm) NaCl, 126; KCl, 4.0; KH_2PO_4, 1.4; $MgSO_4$, 1.3; $CaCl_2$, 2.4; $NaHCO_3$, 26; and glucose, 4.0, with a pH of 7.4 and saturated with 95% O_2/5% CO_2. Hippocampal slices were dissected and placed in a submerged-type recording-perfusion chamber of 4 ml volume, which was perfused at a rate of 2 ml/min with artificial cerebral spinal fluid and maintained at 34 ± 0.5°C.

One hour after slice placement, the CA1 orthodromic population spike of each slice was assessed.

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This potential is an indicator of neuronal cell body function, and was elicited by stimulation with a twisted, bipolar electrode placed over the CA3 Schaffer collaterals. Responses were recorded in the CA1 pyramidal layer using a tungsten electrode. Strengths of stimulating currents and recording electrode depth were adjusted to obtain maximal population spike amplitude. Stimulating currents were not changed thereafter. Orthodromic stimulation produced an evoked response with wave components which include the CA3 fiber volley which reflects CA3 axonal response, the CA1 excitatory post-synaptic potential (e.p.s.p.) reflective of CA1 dendritic response and the CA1 orthodromic population spike indicative of CA1 pyramidal cell response (Schurr et al., 1984). Orthodromic stimulation was given every 30 s; CA3 fiber volley amplitude, CA1 e.p.s.p. slope and CA1 orthodromic population spike amplitude were measured (Schurr et al., 1984). Only slices having an orthodromic CA1 population spike of 3 mV or greater were used for testing. CA1 antidromic population spike response was elicited with stimulation over the alveus, while responses were monitored in the CA1 pyramidal layer. Because CA1 antidromic population spike response does not require synaptic activation, this evoked response was used to evaluate CA1 neuronal cell body response, apart from effects of possible synaptic depression. CA1 antidromic population spike was assessed at the beginning and end of each experiment.

To induce trauma, slices were transferred to a 7 ml fluid percussion chamber filled with artificial cerebral spinal fluid and sealed with a rubber piston. A 1 kg weight was then dropped upon the piston from a height of 61 cm through a guide tube, producing percussion of the fluid surrounding the slice. Within 1 min after trauma, each slice was returned to the recording chamber and electrodes were positioned to allow monitoring of CA1 orthodromic evoked response.

Felbamate was introduced directly into the perfused artificial cerebral spinal fluid at concentrations of 45, 90, 200, 300 and 400 mg/l (0.19, 0.38, 0.84, 1.30 and 1.70 mM, respectively). In felbamate pre-treatment experiments, felbamate exposure was begun 30 min before trauma, and continued through the first 15 min of recovery with final recoveries assessed 60 min after trauma. In felbamate post-trauma treatment studies, felbamate exposure was begun at 1, 5, 15 or 30 min following trauma and continued thereafter for 35 min. Final recoveries were assessed in these cases, 60 min after cessation of felbamate treatment. Extended electrophysiological monitoring for 8 h was performed in a small number for felbamate-treated traumatized slices, unmedicated traumatized slices and unmedicated non-traumatized slices. After 8 h of recovery, tetanic stimulation with a high frequency train of 100 Hz for 1 s was given to slices via the orthodromic stimulating electrode to assess the ability to induce long-term potentiation. The strengths of the stimulating currents were not changed from those used on initial testing to achieve a maximal population spike amplitude. In all cases, percent recovery of evoked responses was calculated by dividing final value by the initial value, multiplied by 100. In neuroprotection studies, CA1 response prior to trauma was used as the initial value. In studies of long-term potentiation, CA1 response prior to tetanic stimulation was used as initial value. In all cases, n equalled at least three paired trials or more. Initial electrophysiological responses of felbamate-treated and unmedicated slices were compared by one-way analysis of variance. Final recoveries were compared using Student's correlated t-test. Wilcoxon rank-sums test was used for other comparisons.

3. Results

The findings of these experiments indicate that felbamate provides robust protection against CA1 traumatic neuronal injury in the hippocampal slice. This neuroprotection was demonstrated with both pre-trauma and post-trauma felbamate treatment protocols. With administration of 400 mg/l felbamate for 30 min before trauma, protection of CA1 evoked response was nearly complete (Fig. 1). With that treatment, mean CA1 orthodromic population spike recovered after trauma to 98 ± 5% (S.E.M.) of initial amplitude,

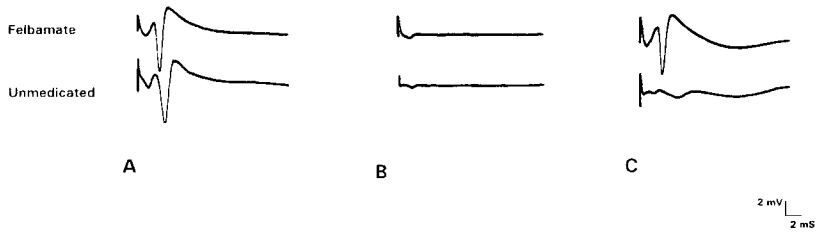

Fig. 1. Treatment with felbamate after trauma protects against the development of CA1 traumatic neuronal injury. Panel A: Initial CA1 orthodromic evoked response in two paired hippocampal slices taken from the same dissection. Panel B: Loss of evoked responses is seen for both slices at 1 min following fluid percussion trauma. Slice in upper tracing received exposure to felbamate 400 mg/l for 30 min prior to trauma. Felbamate treatment was continued for the first 15 min of recovery after trauma. Panel C: Final responses of both slices are 60 min after trauma. The felbamate-treated slice shows nearly full recovery of CA1 population spike amplitude after trauma, while poor recovery is seen in the paired, unmedicated slice.

and CA1 antidromic population spike recovered to 94 ± 1% (Table 1). In contrast, paired unmedicated slices given trauma alone regained only 2 ± 2% recovery of CA1 orthodromic population spike amplitude. Similarly, these unmedicated slices recovered a mean CA1 antidromic population spike amplitude of only 15 ± 6% ($P < 0.05$), signifying the occurrence of severe neuronal cell body dysfunction.

The electrophysiological responses reflective of post-synaptic dendritic and cell body function were most affected by slice trauma, a pattern of dysfunction consistent with excitotoxicity. In the 30 slices subjected to trauma alone, CA1 orthodromic population spike amplitude recovered to a mean of only 17 ± 2% of initial amplitude, while CA1 e.p.s.p. slope showed no recovery at all. Similarly, CA1 antidromic population spike regained a mean of only 17 ± 1% of initial amplitude, confirming that the loss of CA1 orthodromic population spike was not merely a result of synaptic depression. Axonal function showed significantly less injury, and was somewhat more variable in response to trauma. In 30 slices subjected to trauma alone, CA3 fiber volley recovered to an overall mean of 42 ± 6% of initial amplitude.

Electrophysiological dysfunction produced by trauma occurred rapidly. At 1 min after trauma, both CA1 e.p.s.p. and orthodromic population spike showed no response. Felbamate 400 mg/l pre-treatment did not prevent this rapid loss of evoked response. After 15 min, however, a small degree of recovery was seen in slices pre-treated with felbamate. At this time, recovery of CA1 orthodromic population spike in slices treated with felbamate, reached 18 ± 4%, a significant difference from untreated slices which showed no recovery.

The protection afforded by felbamate against CA1 traumatic neuronal injury was concentration-dependent (Fig. 2). The EC_{50} for orthodromic population spike protection with felbamate pre-treatment was 138 mg/l, and the EC_{50} for CA1 antidromic population spike protection was 136 mg/l. Significant protection of CA1 population spike amplitude was seen with pre-trauma felbamate exposures of 90 mg/l and above. With 90 mg/l felbamate treatment, CA1 orthodromic population spike amplitude recovered to a mean 41 ± 4%, and CA1 antidromic population spike recovery to 41 ± 2%, while recovery of CA1 orthodromic and antidromic population spike amplitude in unmedicated slices was only 15 ± 1% and 16 ± 3% ($P < 0.05$). Protection against trauma with felbamate also extended to CA1 e.p.s.p. response (Table 1). With felbamate pre-treatment, CA1 e.p.s.p. recovered after trauma to 87 ± 13% of initial slope. In contrast, paired unmedicated slices showed no recovery of e.p.s.p. slope. Consistent with the greater susceptibility to trauma seen with CA1 e.p.s.p. response as compared to CA1 population spike response, the felbamate EC_{50} for CA1 e.p.s.p. protection was 200 mg/l, a concentration somewhat higher than the felbamate EC_{50} for CA1 orthodromic population spike recovery.

Post-trauma treatment with 300 mg/l felbamate was also strongly protective against traumatic neuronal injury (Fig. 4). When this felbamate concentration was initiated within 1 min after trauma, CA1 orthodromic population spike eventually regained a mean recovery of 77 ± 11% of original amplitude (Table 2). This fel-

Table 1
Pre-treatment with 400 mg/l felbamate protects against CA1 traumatic neuronal injury

	Initial potential	% Response present after 30 min felbamate	% Response present 1 min after trauma	% Response 60 min after trauma (final recovery)
Orthodromic CA1 population spike				
Trauma	6.4 ± 0.8 mV	100 ± 0	0	2 ± 2
Trauma, felbamate	6.4 ± 1.4	100 ± 0	0	98 ± 5 [a]
CA3 fiber volley				
Trauma	0.8 ± 0.6 mV	100 ± 0	80 ± 20	70 ± 15
Trauma, felbamate	0.7 ± 0.3	100 ± 0	56 ± 6	89 ± 11
CA1 e.p.s.p. slope				
Trauma	0.5 ± 0.1 mV/mS	100 ± 0	0	0
Trauma, felbamate	0.5 ± 0.2	100 ± 0	0	87 ± 13 [a]
Antidromic CA1 population spike				
Trauma	6.7 ± 0.7	–	–	15 ± 6
Trauma, felbamate	6.5 ± 1.1	–	–	94 ± 1 [a]

The findings of this table indicate that initial potentials were well matched, and that felbamate administration alone produced little change. Trauma produced severe loss of evoked response. Pre-trauma treatment with felbamate did not prevent early loss of these potentials after trauma. However, felbamate did promote striking recovery of these potentials 60 min after trauma. Felbamate exposure was initiated 30 min prior to trauma and continued through the first 15 min of recovery. Values represent mean ± S.E.M. percentage of initial amplitude of CA1 orthodromic population spike, CA1 antidromic population spike and CA3 fiber volley response; in the case of CA1 e.p.s.p., values represent mean ± S.E.M. percentage of initial slope. For all values, $n = 3$ paired trials. Final recoveries were assessed 60 min after recovery. [a] $P < 0.05$, Student's correlated t-test.

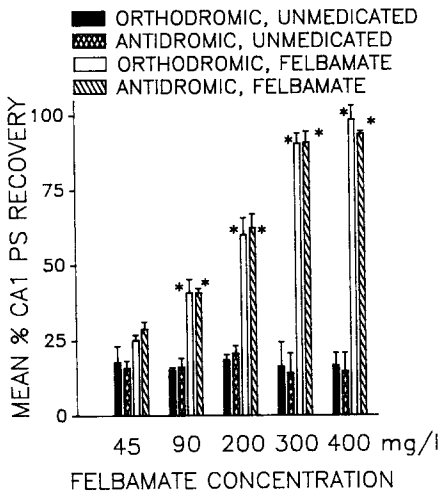

Fig. 2. Neuroprotection with felbamate pre-treatment against CA1 traumatic neuronal injury is concentration-dependent. Graph illustrates effect of increasing concentrations of felbamate pre-treatment upon recovery of CA1 population spike amplitude in studies of paired hippocampal slices after trauma. Felbamate treatment was begun 30 min before fluid percussion trauma and continued through the first 15 min of recovery. Final recovery of CA1 orthodromic and antidromic population spike was assessed 60 min after trauma. Abscissa represents increasing felbamate treatment concentrations; ordinate represents recovery of CA1 population spike amplitude. Vertical bars show mean % ± S.E.M. Filled bars indicate CA1 orthodromic population spike in unmedicated slices; cross-hatched bars indicate CA1 antidromic population spike in unmedicated slices. Clear bars indicate CA1 orthodromic population spike in felbamate-treated slices; single-hatched bars indicate CA1 antidromic population spike in felbamate-treated slices. * $P < 0.05$, Student's correlated t-test.

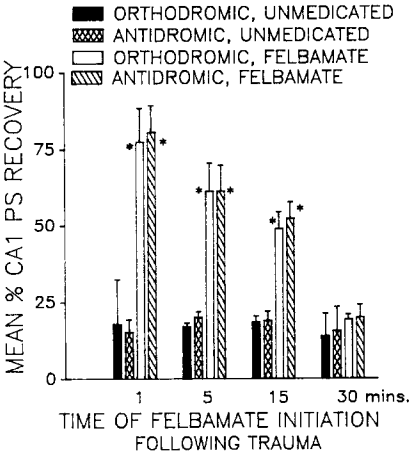

Fig. 3. CA1 neuroprotection against trauma with felbamate post-treatment begun after trauma. Graph demonstrates the effect of felbamate post-trauma treatment upon recovery of CA1 population spike amplitude. Abscissa indicates delay of felbamate exposure after trauma. All felbamate exposures were given for a duration of 35 min with artificial cerebral spinal fluid containing felbamate 300 mg/l. Final CA1 orthodromic and antidromic population spike amplitudes were assessed 95 min after trauma. Abscissa represents delay time in initiating felbamate after trauma; ordinate represents recovery of CA1 population spike amplitude. Vertical bars show mean % ± S.E.M. Filled bars indicate CA1 orthodromic population spike in unmedicated slices; cross-hatched bars indicate CA1 antidromic population spike in unmedicated slices. Clear bars indicate CA1 orthodromic population spike in felbamate-treated slices; single-hatched bars indicate CA1 antidromic population spike in felbamate-treated slices. * $P < 0.05$, Student's correlated t-test. Significant protection is seen with felbamate treatment begun at 1, 5 and 15 min after trauma. No protection is detected when felbamate treatment was initiated 30 min after trauma.

bamate treatment also resulted in an average CA1 antidromic population spike recovery of $80 \pm 9\%$. In comparison, paired unmedicated slices recovered only $18 \pm 8\%$ and $14 \pm 4\%$ of original CA1 orthodromic and antidromic population spike amplitude respectively ($P < 0.05$). The CA1 e.p.s.p. response was similarly protected by 300 mg/l post-trauma treatment. When this treatment was begun within 1 min after trauma, CA1 e.p.s.p. slope recovered to an average $71 \pm 11\%$ of original response. Without felbamate treatment, no recovery of CA1 e.p.s.p. slope occurred in any unmedicated slice subjected to trauma.

Significant protection was also seen with application of felbamate as late as 15 min after trauma (Fig. 3). With felbamate exposure begun at that point, CA1 orthodromic and antidromic population spike amplitudes recovered to a mean of $49 \pm 6\%$ and $52 \pm 5\%$ respectively (Fig. 3), while paired unmedicated slices recovered to only $14 \pm 7\%$ and $16 \pm 8\%$ ($P < 0.05$). With felbamate post-trauma exposures initiated 30 min after trauma, no protective effects were detected.

The protection provided by felbamate against CA1 traumatic injury appeared to be long-lasting and was

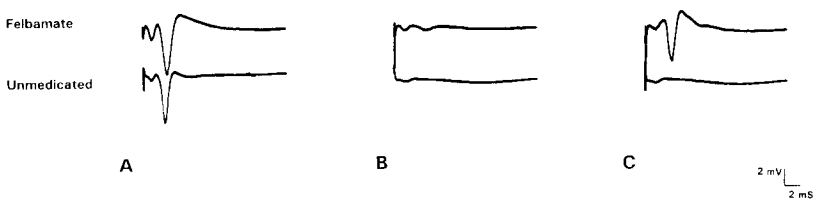

Fig. 4. Protection with post-trauma felbamate treatment. Panel A: Initial CA1 orthodromic evoked response in two paired hippocampal slices taken from the same dissection. Panel B: Trauma induces loss of evoked responses for both slices at 1 min following fluid percussion trauma. Treatment with 300 mg/l felbamate was initiated within 1 min after trauma for the slice in the upper tracing of panel B, and continued for 35 min thereafter. Panel C: Final responses of both slices are seen 95 min after trauma. The upper tracing slice which received felbamate-treatment after trauma demonstrates substantial recovery of CA1 population spike amplitude after trauma, while poor recovery is seen in unmedicated slice.

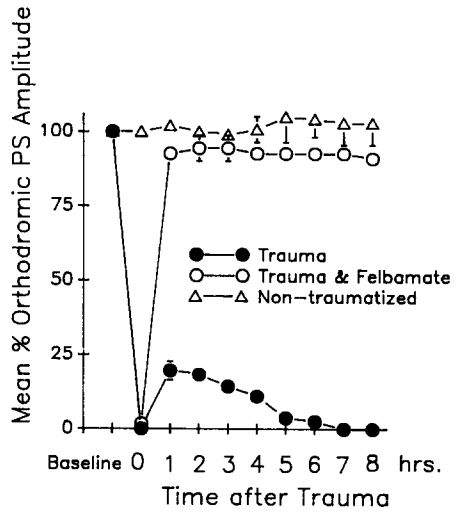

Fig. 5. Extended electrophysiological monitoring shows persistence of neuroprotection after brief felbamate post-trauma treatment. Results of extended electrophysiological monitoring over 8 h are illustrated. Felbamate-treated slices received exposure with felbamate beginning 1 min after trauma and continuing for 35 min thereafter. Recovery was assessed 1 h after the cessation of felbamate exposure. Abscissa indicates time after trauma; ordinate represents mean % recovery of CA1 orthodromic population spike amplitude. Data points indicate mean % ± S.E.M. Filled circles indicate response of traumatized slices. Open circles indicate response of slices treated with felbamate 300 mg/l initiated 1 min after trauma and continued for 35 min. Open triangles indicate responses of slices which were not traumatized and received no felbamate treatment. Slices traumatized without felbamate treatment demonstrated significant injury at all time points after trauma, when compared to non-traumatized slices. In contrast, felbamate-treated slices maintained recovery of CA1 evoked response throughout 8 h of monitoring. Felbamate-treated slices which were traumatized differed significantly from unmedicated traumatized slices at all time points after trauma ($P < 0.05$, Wilcoxon rank-sums test).

sustained over the course of 8 h of extended electrophysiological monitoring. In slices given this monitoring, post-trauma treatment with 300 mg/l felbamate yielded recovery of CA1 orthodromic population spike amplitude at 95 min after trauma which averaged 93 ± 3% of initial amplitude (Fig. 5). This recovery of electrophysiological function showed little change in subsequent observation. At 8 h after trauma, CA1 orthodromic population spike still showed 91 ± 3% of original amplitude. In comparison, unmedicated slices showed initial recovery of only 20 ± 3% orthodromically and 13 ± 1% antidromically. Over 8 h of subsequent monitoring, only gradual deterioration was seen with no further recovery observed in these slices traumatized without medication.

Slices protected with transient exposure to felbamate after trauma, later showed preservation of synaptic plasticity as demonstrated by the ability to induce long-term potentiation. In three slices traumatized without felbamate treatment, tetanic stimulation 1 h after trauma produced no increase in synaptic response, consistent with a loss of long-term potentiation. This response was not improved by delaying tetanic stimulation for 8 h after trauma in three additional slices (Fig. 6). In contrast, five slices given post-trauma 300 mg/l felbamate treatment begun within 1 min after trauma, showed preservation of long-term potentiation when given tetanic stimulation after 8 h of extended monitoring (Fig. 6). This stimulation increased CA1 orthodromic population spike amplitude by 138 ± 5%, a significant difference from the baseline seen at 8 h after trauma. This increase was similar to that seen in four non-traumatized unmedicated slices

Table 2
Neuroprotection with post-treatment of 300 mg/l felbamate against CA1 traumatic neuronal injury

	Initial potential	% Response present 1 min after trauma	% Response 95 min after trauma (final recovery)
Orthodromic CA1 population spike			
Trauma	4.8 ± 0.7 mV	0	18 ± 8
Trauma, felbamate	4.8 ± 0.5	4 ± 4	77 ± 11 [a]
CA3 fiber volley			
Trauma	0.7 ± 0.1 mV	37 ± 5	41 ± 21
Trauma, felbamate	0.6 ± 0.1	46 ± 4	88 ± 25
CA1 e.p.s.p. slope			
Trauma	0.4 ± 0.4 mV/mS	0	0
Trauma, felbamate	0.4 ± 0.1	0	71 ± 11 [a]
Antidromic CA1 population spike			
Trauma	5.2 ± 0.7 mV	–	14 ± 4
Trauma, felbamate	4.9 ± 0.5	–	80 ± 9 [a]

Felbamate and unmedicated slices show similar initial potential and early loss of CA1 e.p.s.p. and CA1 orthodromic population spike after trauma. Treatment with felbamate initiated after trauma produced significant recovery of evoked response, when assessed 60 min after felbamate was discontinued. Felbamate exposure was initiated within 1 min following trauma and continued for 35 min. Values represent mean ± S.E.M. percentage of initial amplitude of CA1 orthodromic population spike, CA1 antidromic population spike and CA3 fiber volley response; in the case of CA1 e.p.s.p., values represent mean ± S.E.M. percentage of initial slope. Final recoveries were assessed 95 min after trauma. For all values, $n = 3$ paired trials. [a] $P < 0.05$, Student's correlated t-test.

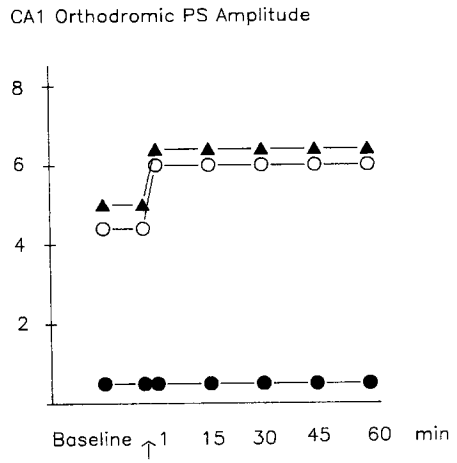

Fig. 6. Long-term potentiation response is preserved in slice given brief felbamate treatment after trauma. Responses to CA1 orthodromic stimulation are seen in a pair of slices previously traumatized. Abscissa represents time in min after tetanus; ordinate represents CA1 orthodromic population spike amplitude in mV. Filled circles indicate response of slice traumatized 8 h earlier. Open circle indicates response of slice traumatized 8 h earlier and receiving brief exposure to felbamate 300 mg/l for 35 min. Filled triangle represents response of non-traumatized unmedicated slice given extended monitoring. Tetanus, indicated by arrow, consisted of stimulation at increased frequency of 100 Hz given for 1 s, with no change in current strength. Stimulation frequency thereafter was returned to pre-tetanus rate of one stimulation every 30 s (0.033 Hz). The unmedicated traumatized slice shows no response to tetanus with no long-term potentiation. The slice receiving brief felbamate exposure after trauma regained 96% of initial population spike amplitude. When given tetanus, this slice demonstrates a good long-term potentiation response with a 128% increase in CA1 orthodromic population spike amplitude (123% of initial amplitude before trauma). Non-traumatized, unmedicated slice shows similar increase in population spike amplitude after tetanus (136% increase).

given 8 h of extended monitoring, which demonstrated a $130 \pm 9\%$ increase in amplitude. These responses were also similar to those of four slices given 1 h of monitoring, which showed increases of $133 \pm 4\%$. In all cases, increases in synaptic response persisted through 60 min of additional recording, as would be expected with long-term potentiation.

Analysis of initial electrophysiological responses demonstrated no significant differences between slice groups later treated with or without felbamate (Tables 1 and 2). Additionally, pre-trauma treatment with felbamate alone for 30 min produced no detected change in monitored evoked responses (Table 1).

4. Discussion

The findings of this study indicate that felbamate provides robust protection against CA1 traumatic neuronal injury in the hippocampal slice. Although the most potent felbamate protection was seen at higher concentrations, significant protective effects were also seen at felbamate concentrations as low as 90 mg/l, which is well within the range of felbamate serum concentrations reported during monotherapy for epilepsy (Wagner et al., 1990). Felbamate was most strongly protective when given before trauma, but significant protection was also seen with felbamate treatment begun as late as 15 min after trauma. These results suggest that acute treatments with felbamate, or other dicarbamates, may represent a potential therapeutic strategy in the treatment of traumatic brain injury.

The CA1 injury produced by fluid percussion trauma to hippocampal slices appeared to be severe and long-lasting. In each case, trauma produced major loss of both the CA1 orthodromic population spike and the CA1 antidromic population spike response. This concomitant loss of both population spike responses signifies the occurrence of severe neuronal cell body dysfunction, as would be expected in true neuronal injury. In addition, this pattern of electrophysiological loss helps exclude synaptic depression, which would be expected to produce a more transient evoked response loss.

The CA1 neuronal injury produced in this preparation showed several features similar to that found in traumatic brain injury. Traumatic injury was generated by transmission of mechanical force through fluid waves to brain tissue, similar to the generation of neuronal injury postulated to occur with head trauma (Toulmond et al., 1993). In addition, CA1 neurons showed susceptibility to traumatic injury, similar to the vulnerability reported for this neuronal population with observations from traumatic brain injury seen in humans (Kotapka et al., 1992). Additionally, trauma in these studies caused a loss of the ability to induce long-term potentiation in CA1 cells, which is analogous to the loss of long-term potentiation reported with traumatic brain injury (Miyazaki et al., 1992). Long-term potentiation is a process of synaptic plasticity thought to be involved in memory and learning (Bliss and Collingridge, 1993), and loss of this process has been thought to be a contributing factor to memory deficits occurring after head trauma (Levin et al., 1982; Eiben et al., 1984).

The electrophysiological distribution of the injury created by trauma suggests that excitotoxic mechanisms were a factor in the development of that damage. CA1 e.p.s.p. and population spike responses, indicative of dendritic and cell body function, were affected to the greatest degree. This post-synaptic pattern of injury is consistent with the damage seen after direct glutamate application (Olney, 1978) and underscores the probable importance of excitotoxicity in the evolution of traumatic injury in this neuronal population.

The felbamate concentrations needed to protect

against CA1 traumatic neuronal injury were similar to those needed for protection against hypoxia. The EC_{50} for protection of CA1 orthodromic population spike response was 138 mg/l felbamate. This value is comparable to the EC_{50} of 199 mg/l felbamate seen for protection of CA1 orthodromic population spike response against hypoxia in this preparation (Wallis and Panizzon, 1993). This similarity suggests that both forms of neuroprotection may occur through similar mechanisms.

Although the precise mechanism of felbamate hypoxic protection is not well understood, multiple lines of evidence point to a glycine interaction, possibly at the glycine binding site associated with the *N*-methyl-D-aspartate (NMDA) receptor (McCabe et al., 1993). With both hypoxic-ischemic and traumatic injury to the central nervous system, elevation in the extracellular concentration of several amino acids occurs, including glycine (Nilsson et al., 1990). The significance of this glycine elevation during pathological circumstances is suggested by the fact that small quantities of glycine specifically reverse felbamate neuroprotection against hypoxia (Wallis and Panizzon, 1993). In addition, histidine, a blocker of glycine uptake, also specifically reverses felbamate hypoxic neuroprotection (Panizzon and Wallis, 1993). Furthermore, felbamate has been shown to displace the highly specific ligand of the NMDA-associated glycine site, 5,7-dichlorokynurenic acid (McCabe et al., 1993), and felbamate protects against the injury produced by direct glycine application (Wallis et al., 1994).

In these studies, felbamate was also found to protect against the loss of the ability to induce long-term potentiation in CA1 neurons by trauma. Since synaptic plasticity has been postulated to be a fundamental element of memory and learning (Bliss and Collingridge, 1993), felbamate protection of long-term potentiation with post-traumatic application suggests that this drug could be useful in preventing the memory deficits frequently seen with head trauma (Eiben et al., 1984; Levin et al., 1982).

In recent reports, felbamate has been associated with development of aplastic anemia (Nightingale, 1994). However, this has primarily been reported with chronic use. The use of drugs as neuroprotectants will likely occur with acute treatment protocols which would be expected to have much less cumulative exposure. Therefore, the use of felbamate, or other dicarbamates, as neuroprotectants may be possible.

In conclusion, these experiments demonstrate that felbamate provides neuroprotection against CA1 traumatic neuronal injury at concentrations similar to those seen with felbamate anticonvulsant protocols. Additionally, they suggest that in vivo testing is warranted, and that dicarbamates, such as felbamate, may be a useful therapeutic strategy for traumatic brain injury.

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