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Effects of ethosuximide, a T-type Ca^{2+} channel blocker, on dorsal horn neuronal responses in rats

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Abstract

Plasticity in transmission and modulatory systems are implicated in mechanisms of neuropathic pain. Studies demonstrate the importance of high voltage-activated Ca^{2+} channels in pain transmission, but the role of low voltage-activated, T-type Ca^{2+} channels in nociception has not been investigated. The Kim and Chung rodent model of neuropathy [Pain 50 (1992) 355] was used to induce mechanical and cold allodynia in the ipsilateral hindpaw. In vivo electrophysiological techniques were used to record the response of dorsal horn neurones to innocuous and noxious electrical and natural (mechanical and thermal) stimuli after spinal nerve ligation. Spinal ethosuximide (5–1055 μg) exerted dose-related inhibitions of both the electrically and low- and high-intensity mechanical and thermal evoked neuronal responses and its profile remained unaltered after neuropathy. Measures of spinal cord hyperexcitability were most susceptible to ethosuximide. This study, for the first time, indicates a possible role for low voltage-activated Ca^{2+} channels in sensory transmission.

Keywords: Ca^{2+} channels, voltage-dependent; Neuropathic pain; T-type channel; Analgesia; Nociception; Dorsal horn

1. Introduction

Neuropathic pain, arising from injury- or disease-evoked damage to the peripheral or central nervous system, often responds poorly to traditional analgesics. Patients often experience sensory deficits, persistent and stimulus-evoked pain (allodynia and hyperalgesia). Peripheral nerve damage provides abnormal input into the central nervous system and then leads to dorsal horn hyperexcitability. Under the influence of both excitatory and inhibitory neurotransmitter systems, the dorsal horn is a site of peripheral input modulation before projection to higher brain centres, thus it controls the stimulus–response relationship. Reduction of this excitability is a possible key to neuropathic pain management.

Animal models of neuropathy have been critical in elucidating its complex causal mechanisms, involving plasticity in nociceptive transmission and modulating systems. The rat spinal nerve ligation model (Kim and Chung, 1992) involves tight ligation of two (L5 and L6) of the three spinal nerves that form the sciatic nerve. Behavioural consequences include thermal hyperalgesia, and mechanical and cooling allodynia (Kim and Chung, 1992; Chaplan et al., 1997).

High voltage-activated Ca^{2+} channels (L-, N-, P/Q- and R-types), consisting of a pore-forming $\alpha 1$ subunit and modulatory accessory subunits, β, α_2-δ and γ (Walker and De Waard, 1998), are widely expressed throughout the brain and spinal cord (Kerr et al., 1988; Mintz et al., 1992; Gohil et al., 1994). They are activated by relatively strong membrane depolarisation and permit Ca^{2+} influx in response to action potentials. Consequential secondary actions include neurotransmitter release; thus these channels establish a major link between neuronal excitability and synaptic transmission. For these reasons high voltage-activated Ca^{2+} channels have been the focus of both acute and persistent pain transmission studies. Animal models have demonstrated the antinociceptive abilities of antagonists specific for L-, N- and P/Q-type Ca^{2+} channels, highlighting the differential role each subtype plays in

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nociception, often dependent on the nature of the pain state (Vanegas and Schaible, 2000).

In addition to high voltage-activated Ca^{2+} channels, kinetically distinct low voltage-activated Ca^{2+} channels, or T-type channels, also exist both in neuronal and non-neuronal cells. They activate at voltages near the resting membrane potential, inactivate rapidly, deactivate slowly and have a small single channel conductance (Huguenard, 1996). These unique gating properties prohibit T-type channels alone to support neurotransmission, however they permit their involvement in low-amplitude oscillations, neuronal bursting, synaptic signal boosting, Ca^{2+} entry promotion and lowering threshold for high-threshold spike generation. This Ca^{2+} current appears to play an important physiological role in near-threshold phenomena and regulation of neuronal excitability.

Until recently, the low voltage-activated current was considered a single entity. However, α1G, α1H and α1I subunits have now been cloned, showing 30% homology to high voltage-activated channel forming α1 subunits (Cribbs et al., 1998; Perez-Reyes, 1998; Lee et al., 1999) and hallmark native T-type Ca^{2+} channel properties when expressed heterologously. In situ hybridisation studies on the rat brain have shown that these channels have unique distributions, including the dorsal horn of the spinal cord and sensory ganglia (Talley et al., 1999). This is complimented by reported T-type currents in primary sensory neurones (Carbone and Lux, 1984; Kostyuk et al., 1992; Scroggs and Fox, 1992; Todorovic and Lingle, 1998) and some superficial rat dorsal horn neurones (Ryu and Randic, 1990), an important site for the processing and integration of sensory information, including pain. Unlike the high voltage-activated Ca^{2+} channels, the involvement of T-type channels in pain-related central sensitisation has been hindered by a scarcity of specific pharmacological agents.

Neuropathic pain and epilepsy both share neuronal hyperexcitability as a common underlying mechanism. There are established antiepileptic drugs that target the generation of neuronal hyperexcitability in the brain and some of these have been proven effective in the treatment of various forms of neuropathic pain (Swerdlow and Cundill, 1981; McQuay et al., 1995). The succinimde derivative ethosuximide, or 2-ethyl-2-methylsuccinimide, is an anticonvulsant (Macdonald and McLean, 1986) effective in the treatment of absence epilepsy (Coulter et al., 1989b); a condition characterised by spike-wave rhythm likely generated by T-type Ca^{2+} current. Ethosuximide has been demonstrated to be a relatively specific T-type channel antagonist in thalamic (Coulter et al., 1989a) and dorsal root ganglion neurones (Kostyuk et al., 1992). This study uses the spinal nerve ligation model, confirmed by behavioural testing, to induce a neuropathic state, subsequent to which electrophysiological studies of dorsal horn spinal neurones were made to investigate the effects of spinally delivered ethosuximide on a wide range of electrical and natural-evoked neuronal activity.

2. Materials and methods

2.1. Spinal nerve ligation

Male Sprague–Dawley rats, initially weighing 130–150 g, were used in this study. All experimental procedures were approved by the Home Office and follow the guidelines under the International Association for the study of Pain (Zimmermann, 1983). Selective tight ligation of spinal nerves L5 and L6, and a sham procedure were performed as first described by Kim and Chung (1992). For details, see Chapman et al. (1998).

2.2. Behavioural testing

For 2 weeks following surgery, the rats were housed in groups of 4, in plastic cages under a 12/12 h day/night cycle and their general health monitored. Successful reproduction of the neuropathic model was confirmed by behavioural testing (post-operative days 2, 3, 5, 7, 9, 12 and 14) assessing the sensitivity of both the ipsilateral and contralateral hindpaws to normally non-noxious punctate mechanical (von Frey filaments) and cooling (acetone) stimuli. Rats were placed in transparent plastic cubicles on a mesh floor and allowed to acclimatise before initiating any tests. Foot withdrawals to von Frey filaments 1, 5 and 9 g (trials of 10) and acetone (trials of 5) were quantified as described in Chapman et al. (1998) and expressed as Difference Scores = Ipsilateral response − contralateral response.

2.3. Spinal cord electrophysiology

Subsequent to behavioural testing (post-operative days 14–17), the operated rats were used for electrophysiological studies (Dickenson and Sullivan, 1986). Briefly, anaesthesia was induced with 3% halothane in a mixture of 66% N_2O and 33% O_2 and a cannula inserted into the trachea. A laminectomy was performed (vertebrae L1–L3) to expose segments L4–L5 of the spinal cord and the level of halothane was reduced to 1.8%. Extracellular recordings of single convergent neurones, located deep within the dorsal horn (>500 μm), receiving input from the toe region ipsilateral to the spinal nerve ligation or sham procedure, were made using a parylene coated tungsten electrode. Neurones selected responded to both noxious (pinch) and non-noxious (touch) stimuli.

2.3.1. Cell characterisation

Spontaneous activity exhibited by a neurone was recorded over 10 min. Action potentials evoked by natural stimuli applied constantly over 10 s were quantified by the application of both punctate mechanical (von Frey fila-

ments 9 and 75 g) and thermal (constant water jet at 45°C) stimuli applied to the centre of the neurone's receptive field. The thermal response to 45°C was determined by subtracting the response to 32°C (a non-noxious temperature so as to ascertain any mechanical response evoked by the water jet) from the response to 45°C. All responses to natural stimuli were normalised by the subtraction of any spontaneous activity measured before the application of each stimulus. Response of the neurone to transcutaneous electrical stimulation was established by insertion of two fine needles into the centre of its peripheral receptive field. A test consisted of a train of 16 stimuli (2 ms wide pulse at 0.5 Hz at three times the threshold required to evoke a C-fibre response), and a post-stimulus histogram was constructed. The thresholds were determined by increasing the electrical stimulus from 0 mA until an action potential was evoked in the corresponding latency band. Electrically evoked action potentials were separated on a latency basis into Aβ-fibres (0–20 ms), Aδ-fibres (20–90 ms), C-fibres (90–300 ms) and post-discharge (300–800 ms). The 'input' is the number of action potentials (90–800 ms) evoked by the first stimulus of the train. 'Excess spikes' is a measure of 'wind-up', which is increased neuronal excitability to repeated constant stimulation. Excess spikes was calculated as the total action potentials (90–800 ms) after 16 stimulus train minus the input × 16. Wind-up graphs for individual neurones show how the combined number of evoked C-fibre and post-discharge action potentials (i.e., 90–800 ms) increases with each repeated electrical stimulation.

2.3.2. Pharmacological studies

The testing protocol, initiated every 10 min, consisted of an electrical test followed by the natural stimuli, as described above. Stabilisation of the neuronal responses was confirmed with at least three consistent pre-drug responses (< 10% variation), for all measures. These values were then averaged to generate pre-drug control values with which to compare the effect of ethosuximide (Sigma-Aldrich, Poole, Dorset, UK) administration on subsequent evoked responses. Ethosuximide was dissolved in saline and applied directly onto the spinal cord in 50 μl volumes. Each dose (5, 55, 555, 1055 μg) was followed until maximum effects were exerted (a minimum of 50 min), when the next dose would be applied cumulatively. The results were calculated as maximum percentage inhibition from the averaged pre-drug value for each neurone and the overall results for each dose were expressed as means ± standard error of mean (S.E.M.) of the normalised data. Statistical analysis of maximal drug effects at each dose compared to the averaged pre-drug value was determined by paired *t*-test on raw data. An unpaired *t*-test on the normalised data was used for the comparison of drug effects between different experimental groups. The level of significance was taken as $P \leq 0.05$.

3. Results

3.1. Behavioural studies

During the post-operative period the animals showed normal weight gain and maintained good general health. Rats subjected to spinal nerve ligation exhibited abnormal foot posture ipsilateral to nerve injury whereby toes were held together in a 'guarding' behaviour. This did not occur in either the contralateral hindpaw, or in the sham-operated rats. Successful replication of the nerve injury model was confirmed by behavioural testing which demonstrated the development of mechanical and cooling allodynia of the injured hindpaw of spinal nerve ligated rats. Evoked allodynia, in response to innocuous mechanical (von Frey filaments bending force 1–9 g) and cooling (acetone) stimuli, was displayed as a brisk withdrawal, accompanied in some cases by shaking and licking of the foot ipsilateral to spinal nerve ligation. This was evident at post-operative day 2, reached maximum at days 7–12 and still maintained at day 14 (Fig. 1). Consistent withdrawal responses were never exhibited by the control group or by the contralateral hindpaw of the experimental group, and when present were never accompanied by the pain-like behaviours displayed by the lesioned hindpaw of spinal nerve ligated rats.

3.2. Spinal cord electrophysiology

3.2.1. Cell characterisation

The numbers of ipsilateral dorsal horn neurones characterised in each group were 11 in spinal nerve ligated rats, 6

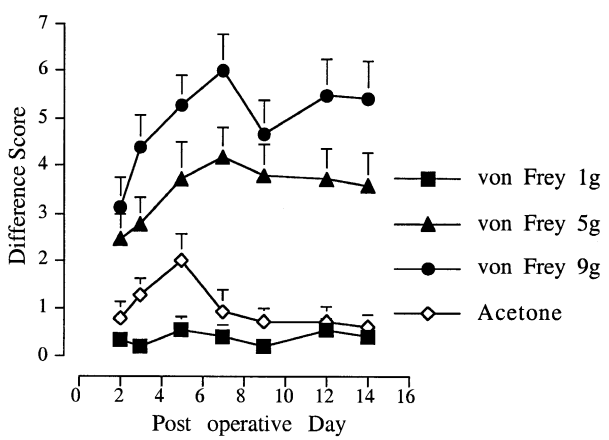

Fig. 1. Development of mechanical and cooling allodynia in the ipsilateral hindpaw over the 2-week period following spinal nerve ligation. Data is presented as the mean difference score ± S.E.M. ($n = 11$) in the withdrawal response to punctate mechanical stimuli (von Frey filaments) and cooling stimulus (drop of acetone) applied to the plantar surface of the hindpaws (trials of 10 for the mechanical and 5 for the cooling). Difference score = (ipsilateral response frequency) − (contralateral response frequency). No nociceptive behaviour was observed in the sham-operated rats (data not shown).

in sham-operated rats and 8 in naïve rats. All neurones had a receptive field over the left ipsilateral hindpaw. No significant differences were found between experimental groups in the mean values of recorded neurone depth, responses evoked by electrical and natural stimulation, and level of ongoing spontaneous activity. It is worth noting that 45% of neurones characterised in spinal nerve ligated rats exhibited spontaneous activity at a rate greater than 0.1 Hz in comparison to only 17% of characterised neurones in sham-operated rats and 25% of naive.

3.2.2. Pharmacological studies

The effect of ethosuximide (5–1055 μg), applied directly onto the spinal cord, on the electrically and naturally evoked dorsal horn neuronal responses was tested in spinal nerve ligated, sham-operated and naïve animals. Since the sham operation is the appropriate control for spinal nerve ligation, and for clarity, the results obtained from the naïve, non-operated, group shall not be displayed on the graphs. However, no difference in the effects of ethosuximide was observed between sham and naïve groups. Ethosuximide produced a dose-related inhibition of the electrically and naturally evoked responses in neurones in all experimental groups (Figs. 2, 3 and 4) and there was no difference in the extent of its effects between groups. Clear effects were seen around 40 min, with maximal inhibitions established at around 60 min. For sham and spinal nerve ligated groups, all doses of ethosuximide (5–1055 μg) elicited statistically significant inhibitions of the electrically evoked responses, compared to pre-drug control (Fig. 2; $P \leq 0.05$; $n = 6–11$). For naïve animals, statistically significant inhibitions were elicited by ethosuximide: at all doses (5–1055 μg) for the Aβ-fibre, Aδ-fibre, post-discharge and excess spikes measurements; at 55–1055 μg ethosuximide for the C-fibre response; and at 555 and 1055 μg for the input response ($P \leq 0.05$; $n = 7$). In all three groups, the Aβ-fibre response was least affected with mean maximal inhibitions at top dose ranging from 17 ± 6% to 26 ± 8% (Fig. 2F). In all three groups, the C-fibre and Aδ-fibre responses reached similar mean maximal inhibitions at top dose ranging from 33 ± 13% to 47 ± 17% (Fig. 2D and E, respectively). Greater inhibitory effects

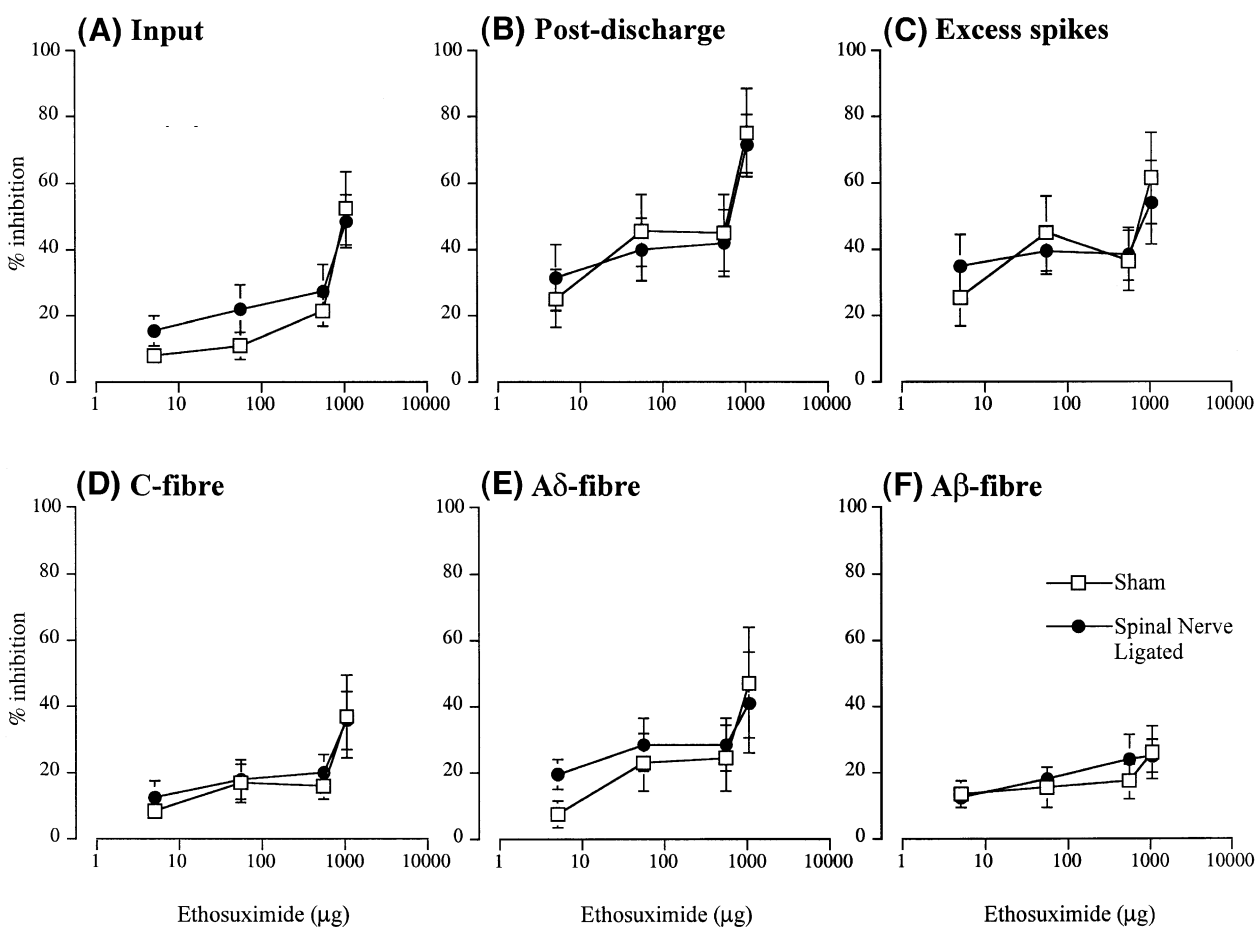

Fig. 2. Effect of spinally applied ethosuximide on the electrically evoked dorsal horn neuronal responses (see Materials and methods) recorded from spinal nerve ligated ($n = 6–11$) and sham-operated ($n = 6$) rats at post-operative days 14–17. Data is expressed as maximal mean % inhibition of the pre-drug values ± S.E.M.

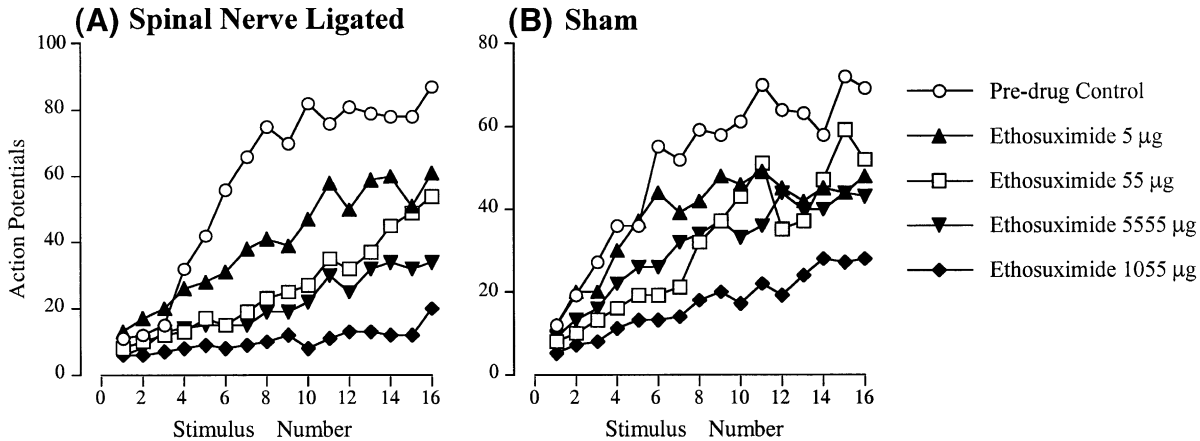

Fig. 3. Examples of the inhibitory effect of spinally applied ethosuximide on individual neurones exhibiting wind-up recorded from (A) spinal nerve ligated and (B) sham-operated rats.

over the entire ethosuximide concentration range were observed on the input, post-discharge and excess spikes measurements (Fig. 2A, B and C, respectively). At 1055 μg, ethosuximide maximally inhibited the input and excess spikes to within the range $48 \pm 8\%$ to $61 \pm 14\%$. The greatest effect was observed on post-discharge, which was maximally inhibited in spinal nerve ligated, sham and naïve rats ranging from $58 \pm 10\%$ to $75 \pm 13\%$.

Fig. 3A and B shows examples of the effects of ethosuximide on the wind-up of an individual neurone recorded from a spinal nerve ligated and sham rat, respectively. It is clear that ethosuximide produces a dose-dependent inhibition from the control response of both the wind-up (evident by a flattening of the curve) and to a lesser extent, the input, with little difference seen between experimental animal groups.

Ethosuximide also produced an inhibitory effect on the naturally evoked neuronal responses (Fig. 4). In spinal nerve ligated animals this was significant ($P \leq 0.05$; $n = 6–11$) for all concentrations of ethosuximide employed in this study on the response to non-noxious mechanical stimulation (von Frey 9g, Fig. 4A), noxious mechanical stimulation (von Frey 75g, Fig. 4B) and noxious thermal stimulation (water jet at 45°C, Fig. 4C). In sham rats the von Frey 9g and heat responses were significantly inhibited at all concentrations of ethosuximide, and the von Frey 75g response was inhibited by 555 and 1055 μg ($P \leq 0.05$; $n = 5–6$). In naïve animals responses to both von Frey hairs were significantly inhibited by all concentrations of ethosuximide, and inhibition of the heat response reached significance at 555 and 1055 μg ($P \leq 0.05$; $n = 5–7$). No differences in the effects of the drug were

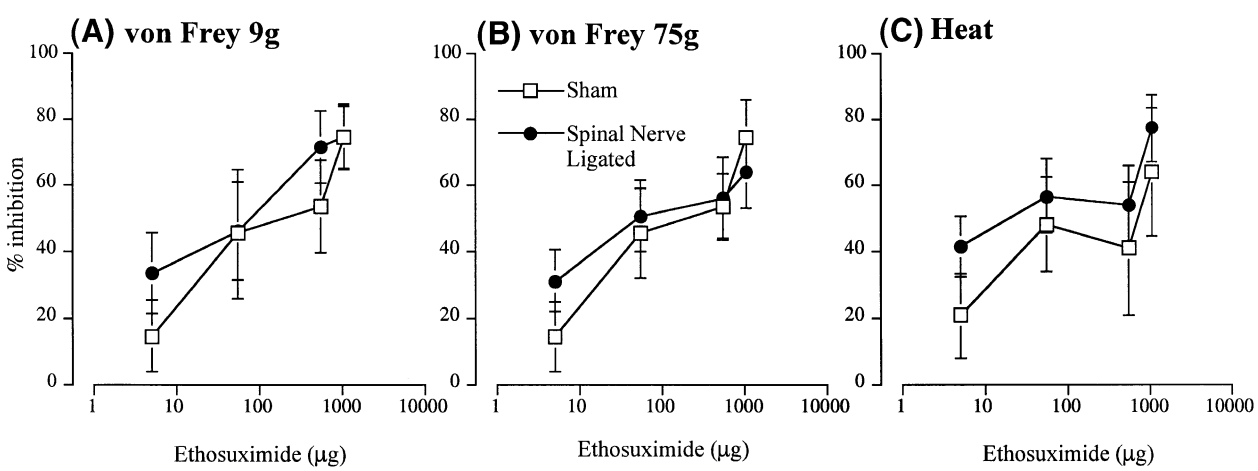

Fig. 4. Effect of spinally applied ethosuximide on the naturally evoked dorsal horn neuronal responses (see Materials and methods) recorded from spinal nerve ligated ($n = 6–11$) and sham-operated rats ($n = 4–6$). (A) Innocuous punctate mechanical stimulus (von Frey 9g), (B) noxious punctate mechanical stimulus (von Frey 75g) and (C) noxious heat stimulus (water jet at 45°C normalised by response to water jet at 32°C). Data is expressed as maximal mean % inhibition of the pre-drug values ± S.E.M.

apparent between experimental groups and the mean maximal inhibitions established at top dose were in the range $61 \pm 11\%$ to $77 \pm 10\%$.

4. Discussion

Unilateral tight ligation of L5 and L6 spinal nerves produced reproducible nociceptive syndromes in the lesioned hindpaw. A clear withdrawal reflex with associated aversive behaviours, indicative of the development of mechanical and cooling allodynia, was produced as previously described by Chapman et al. (1998). Thus, all spinal nerve ligated animals used for the electrophysiology and subsequent pharmacology exhibited neuropathic signs; sham-operated did not. This is the first electrophysiological study addressing the role of low voltage-activated T-type Ca^{2+} channels in the spinal processing of sensory information after nerve injury. Spinal ethosuximide, a relatively specific T-type channel antagonist, mediated significant inhibition of the electrical and natural (innocuous and noxious) evoked rat dorsal horn neuronal responses, suggesting some role for T-type Ca^{2+} channels in sensory transmission.

Extensive behavioural and electrophysiological nociceptive studies have demonstrated an important role for high voltage-activated Ca^{2+} channels in the processing of pain (Vanegas and Schaible, 2000). The contribution of N-, P/Q- or L-type channels appears to alter depending on the nature of the pain (acute or chronic, inflammatory or neuropathic in origin), and this has been established by the use of specific channel antagonists. A predominant nociceptive role for N-type channels has been established, which is enhanced after neuropathy (Chaplan et al., 1994; Xiao and Bennett, 1995; Bowersox et al., 1996; Brose et al., 1997; White and Cousins, 1998; Matthews and Dickenson, 2000). Upon substantial membrane depolarisation N- and P-type voltage-activated Ca^{2+} channels mediate the release of excitatory neurotransmitters, such as glutamate, substance P and calcitonin gene-related peptide, critical for wind-up and central sensitisation, in the presence of constant afferent input (Dickenson, 1994).

This study highlights the role of Ca^{2+} influx via T-type channels in the nociception pathway, within which ethosuximide may be exerting its effects at a number of sites. The biophysical characteristics of the T-type channel have led to its implication mainly in the regulation of cell excitability. The broad action of ethosuximide, with no marked selectivity for a particular modality or evoked response, is in keeping with an action on postsynaptically located Ca^{2+} channels. Since T-type channel activation occurs close to resting potential, they allow Ca^{2+} influx when cells are at rest (Magee and Johnston, 1995) or in response to subthreshold synaptic inputs (Markram and Sakmann, 1994; Magee and Johnston, 1995). Thus, these channels enhance neuronal excitability and contribute to the generation of subthreshold membrane potential oscillations that lead to bursts of sodium dependent action potentials (Huguenard, 1996). Whilst unable to mediate synaptic transmission alone, T-type channels do serve to boost synaptic inputs and lower threshold for high-threshold spike generation. Their block would result in an overall reduction in the underlying level of neuronal excitability, rendering the achievement of threshold levels of membrane depolarisation less likely. Postsynaptically, NMDA receptor activation would be reduced as would the consequential development of central sensitisation.

In this study, ethosuximide exerted its greatest inhibitory effects upon the input, post-discharge and excess spikes. Each of these electrical measures can be related to a specific part of the nociceptive pathway. The non-potentiated input response can be related to the level of synaptic transmission between the central terminals of primary afferents and the neurones of the spinal cord dorsal horn. Thus, blockade of T-type channels at this location is likely causing a reduction in the exocytosis of excitatory neurotransmitters by prohibiting the depolarisation required to activate high voltage-activated Ca^{2+} channels. Alternatively, ethosuximide could be exerting its effects directly on neurones located early in polysynaptic pathways. Even more susceptible to the actions of ethosuximide were the postsynaptic NMDA receptor-mediated post-discharge and excess spike measurements. These are indicative of central sensitisation and neuronal hyperexcitability, and since T-type Ca^{2+} channels are heavily linked to the level of neuronal excitability it follows that they would have a greater functional role here.

What is surprising is that we saw no difference in the effects of ethosuximide after the establishment of neuropathy, especially since neuronal hyperexcitability is a key underlying factor. Interestingly it has been shown that although unilateral cortical ablation causes a 68% increase in T-current measured from isolated rat thalamic relay neurones, *a*-methyl-*a*-phenylsuccimide (another related T-type channel antagonist) was more effective in reducing T-current in normal rats compared to axotomised animals (Chung et al., 1993). The authors suggest an injury induced alteration in the pharmacological properties the T-type channels either by de novo synthesis and/or modification. In this study, it may be that there is indeed no increase in the functional role of T-type Ca^{2+} channels after nerve injury. Alternatively, the specificity and/or potency of ethosuximide may be such that subtle differences in T-type channel function were not highlighted.

Peripheral nerve injury results in reduced afferent input via L5 and L6 spinal nerves, yet as observed here, the magnitude of neuronal responses recorded was not diminished in comparison to sham and normal rats. Conversely, increased frequency and occurrence of spontaneous activity was observed. This suggests that perhaps compensatory increases in peripheral and/or spinal neuronal activity are in play after neuropathy. Ectopic C-fibre activity originat-

ing within the dorsal root ganglion, the nerve injury site, or residual intact afferents provides an ongoing, continual barrage of neuronal activity via primary afferents into the spinal cord (Wall and Devor, 1983; Kajander et al., 1992). Evidence suggests that this is one possible source for the initiation and maintenance of central sensitisation and therefore the positive sensory symptoms observed after nerve damage (Devor and Seltzer, 1999). Interestingly, spinal nerve ligation has been shown to increase the prevalence of subthreshold membrane potential oscillations in dorsal root ganglion neurones which augments ectopic discharge (Liu et al., 2000). It does not seem unreasonable to postulate a role for T-type Ca^{2+} channels in this underlying oscillatory behaviour, which may imply increased activity via T-type channels in the remaining afferents tantamount to a restoration of excitability to that in the non-injured situation.

The existence of a neuronal Ca^{2+} current elicited just above the resting potential was first established in primary sensory neurones (Carbone and Lux, 1984; Bossu et al., 1985; Fedulova et al., 1985; Nowycky et al., 1985), and low voltage-activated current has since been observed in a wide variety of cell types (Huguenard, 1996). The presence of a relatively large T-type current in some superficially located rat spinal dorsal horn neurones is of much interest because this region is involved in processing and integration of sensory information, including pain (Ryu and Randic, 1990). T-type channels are pharmacologically and physiologically heterogeneous (Akaike, 1991; Huguenard, 1996; Tarasenko et al., 1997), which may reflect differential expression of the three known subtypes (α1G, H and I). The regional and cellular distribution of gene expression for the different T-type Ca^{2+} channel family members in the rat central and peripheral nervous systems has recently been determined using in situ hybridisation (Talley et al., 1999). All three transcripts were detected in sensory areas and in the dorsal horn of the spinal cord where in particular α1H was mainly restricted to the outermost laminae I and II. In the dorsal root ganglion high levels of α1H and moderate levels of α1I mRNA were found restricted to small and medium sized neurones, whereas the extremely large were not labelled. This correlates with substantial T-type current observed in medium-diameter dorsal root ganglion neurones isolated from adult rats that is absent in larger dorsal root ganglion cells (Scroggs and Fox, 1992). Since dorsal root ganglion cell body diameter is correlated to axon conduction velocity and sensory modality (Yaksh and Hammond, 1982), this evidence is indicative of T-type current specifically localised to smaller Aδ- and C-type sensory neurones that convey thermal and nociceptive information and not to larger Aβ-type neurones that subserve tactile and proprioceptive pathways. In the present study the extent of inhibition observed with the highest dose of ethosuximide was Aδ-fibre > C-fibre > Aβ-fibre, which fits well with these studies. However, the Aδ- and C-fibre responses were not markedly inhibited over the Aβ-fibre response, as one might expect if smaller diameter sensory neurones exhibited substantial low voltage-activated Ca^{2+} current. Also no difference in the extent of inhibition was observed for the evoked responses to innocuous and noxious mechanical and thermal stimuli. This may again be explained by the existence of the three α1 subunits, each with a unique distribution, encoding for T-type Ca^{2+} channels. Data suggests that diversity exists between T-currents of different cell types both in terms of kinetics and pharmacological sensitivity (Huguenard, 1996). For example, the current clinical use of ethosuximide to treat epilepsy is via block of T-current in thalamic neurones (Coulter et al., 1989a,b). However, T-current in GH3 cells is relatively resistant to block by ethosuximide (Herrington and Lingle, 1992) and in dorsal root ganglion neurones ethosuximide is over an order of magnitude less effective (Coulter et al., 1989a,b). Furthermore, the blockade is complete in dorsal root ganglion but only partial (40%) in thalamic neurones. α1G is the predominant subtype found in thalamic relay neurones, and therefore may be more sensitive to the effects of ethosuximide in comparison to the α1H T-type channel which is more abundant than α1G in the outer lamina of the spinal cord and dorsal root ganglia (Talley et al., 1999).

Two other licensed anticonvulsants, carbamazepine and gabapentin, have been investigated using the same experimental protocol as the present study (Chapman et al., 1998). Carbamazepine, a sodium channel blocker, and gabapentin, thought to act via Ca^{2+} channels (see Matthews and Dickenson, 2000) were found to have similar efficacy and range of effectiveness as ethosuximide. Although both gabapentin and ethosuximide were equally effective in sham and neuropathic animals, carbamazepine was only effective in the latter group and this could possibly be a consequence of differential regulation of Ca^{2+} and sodium channels following nerve injury. To our knowledge the present study is the first to demonstrate a possible role of T-type Ca^{2+} channels in the spinal processing of sensory information related to pain. Given the parallels between epilepsy and pain, the likelihood of common causal mechanisms and the ability of antiepileptic drugs to be effective in neuropathic pain states, the results indicate that ethosuximide may merit both behavioural testing in animals and human studies.

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