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A comparison of the anti-nociceptive effects of voltage-activated Na^+ channel blockers in the formalin test

Gordon Blackburn-Munro*, Nete Ibsen, Helle K. Erichsen

Department of Pharmacology, NeuroSearch A/S, 93 Pederstrupvej, DK-2750, Ballerup, Denmark

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Abstract

We have used the rat formalin test to compare the anti-nociceptive properties of several voltage-activated Na^+ channel blockers. The antiarrhythmic mexiletine (37.5 and 50 mg/kg, i.p.) attenuated flinching behaviour in both first and second phases of the test compared with vehicle ($P<0.05$). The anti-convulsants lamotrigine (15 and 30 mg/kg, i.p.) and carbamazepine (20 mg/kg, i.p.) also inhibited second phase flinching behaviour compared with vehicle ($P<0.05$), although phenytoin (up to 40 mg/kg, i.p.) was without effect. Riluzole (5 mg/kg, i.p.), in contrast to lubeluzole (up to 10 mg/kg, i.p.) also inhibited second phase flinching behaviour compared with vehicle ($P<0.05$). When tested against an acute thermal nociceptive stimulus mexiletine, lubeluzole and riluzole exhibited anti-nociceptive effects. The anti-nociceptive doses used in the formalin test produced no motor impairment in the rotarod test. Thus, voltage-activated Na^+ channel blockers can attenuate nociceptive behaviour in the formalin test, and a specific mechanism of action on Na^+ channel function may be required for this to occur.

Keywords: Formalin test; Hyperalgesia; Inflammation; Ion channels; Neuropathic pain

1. Introduction

Noxious insult to the peripheral and central nervous systems arising as a result of inflammation or nerve damage can induce a state of prolonged neuronal hyperexcitability within the spinal cord (Woolf and Salter, 2000). A consequence of this is the expression of accentuated pain-related behaviours, reflecting hyperalgesia and allodynia in response to thermal and mechanical stimulation. Inflammatory pain is relatively successfully treated with non-steroidal anti-inflammatory drugs (Moore et al., 1998). However, chronic inflammatory disorders and neuropathic pain disorders respond poorly to conventional treatment with analgesics (Arner and Meyerson, 1988). This latter observation has prompted the need to identify drugs with alternative mechanisms of action from standard analgesics, which can provide adequate relief from treatment-resistant chronic pain.

The formalin test assesses the behavioural response of an animal to a chemical nociceptive stimulus and is routinely used to identify drugs with potential anti-nociceptive activity. The test consists of two distinct phases of behaviour both of which can be blocked by morphine, whilst only the second phase is blocked by anti-inflammatory drugs such as indomethacin (Hunskaar and Hole, 1987). This, combined with other cellular and electrophysiological evidence (Dickenson and Sullivan, 1987) suggests that the two phases probably reflect different types of pain, induced as a result of differential activation of peripheral nociceptive mechanisms and subsequent central sensitising events involved in nociceptive transmission (Juilius and Basbaum, 2001).

Voltage-activated Na^+ channels consist of a pore-forming α-subunit of which nine have been cloned ($Na_v1.1$–1.9), as well as up to three associated β subunits (β1–3) which can regulate α subunit function (Baker and Wood, 2001). Several α subunits and all three β subunits have been detected in dorsal root ganglion neurones, contributing in turn to the two types of Na^+ current which have been defined in accordance with their pharmacological sensitivity to tetrodotoxin; a fast tetrodotoxin-sensitive current and a slow tetrodotoxin-resistant Na^+ current which is

* Corresponding author. Tel.: +45-44-60-8333; fax: +45-44-60-8080.
E-mail address: gbm@neurosearch.dk (G. Blackburn-Munro).

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activated at more depolarised membrane potentials (Baker and Wood, 2001). Dynamic changes in Na^+ channel function appear to contribute to the increased sensitivity of primary afferents following injury (Gold et al., 1996; Waxman, 1999), and these changes may also explain the efficacy of voltage-dependent Na^+ channel blockers in the treatment of some chronic pain conditions (Tanelian and Victory, 1995; Nakamura-Craig and Follenfant, 1995; Canavero and Bonicalzi, 1996; Hunter et al., 1997, Backonja, 2001). However, the relative usefulness of available Na^+ channel blocking drugs is somewhat restricted by their inability to discriminate adequately between the various Na^+ channel subtypes (Baker and Wood, 2001). In addition, there is a relative deficit of studies, which have attempted to compare the effects of drugs which preferentially block Na^+ channel function with their potential anti-nociceptive activity.

To address this issue, the current study has evaluated the effects of a range of Na^+ channel blockers on nociceptive behaviours displayed by rats in response to formalin injection. These included the class 1B antiarrhythmic mexiletine, the anticonvulsants lamotrigine, carbamazepine and phenytoin, and the neuroprotective drugs lubeluzole and riluzole. Each drug was also tested in a model of acute thermal nociceptive pain to assess for any effects on reflex nociceptive responses. Finally, to ensure that any attenuation of flinching behaviour was not due to drug effects on motor activity, the maximal dose of drug tested in the formalin test was also tested in the rotarod test.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (body weight, 180–300 g, Möllegaard, Denmark) were housed together in groups of four animals under standard conditions with unrestricted access to food and water. Rats were housed in the room in which the testing procedure was performed to try and minimise any stress response to novel environmental cues. All experiments were conducted according to the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983) and the procedures were approved by the Danish Committee for Experiments on Animals (Dyreforsøgstilsynet).

2.2. Formalin test

Separate groups of rats were used for the different experiments such that each animal was used on one occasion only. All experiments for a given drug treatment were performed using age matched animals in an attempt to avoid variability between experiments (except in the carbamazepine experiment which was performed on two separate occasions). Prior to initiation of the formalin test, four rats were habituated in separate testing cages for 15–20 min. The testing cages were constructed from open mesh steel (L × H × W: 29 × 16 × 22 cm), with angled mirrors placed underneath and behind the cages to allow for an unimpeded view of the animals paws. Animals were then given an injection of either drug or vehicle as appropriate for the testing paradigm. Subsequently, they were gently restrained and formalin (5%, 50 μl, s.c.; formaldehyde solution minimum 37%, diluted 1:20 in saline; Merck, Germany) was carefully injected into the plantar surface of the hindpaw using a 27-gauge needle. After injection, each rat was placed back in its testing cage and measurement of flinching behaviour was initiated by a skilled observer blinded to drug treatment. On the basis of the response pattern, two distinct phases of nociceptive behaviour characterised by flinching of the affected paw were identified and scored (Abbott et al., 1995; Simmons et al., 1998). The first phase was recorded 0–5 min after injection of formalin and the second phase was recorded 15–40 min after the injection. Each flinch was registered on-line by the observer into a DOS-based PC programme. Each rat was observed for 15 s in sequence and the 15 s bins were then collated for each rat to obtain 5 min data bins for the 60 min duration of the experiment.

2.3. Acute nociception (thermal latency)

Reflex nociceptive pain was assessed using the rat plantar test (Ugo Basile, Comerio, Italy) following a modified method of Hargreaves et al. (1988). Single rats were placed in individual perspex boxes on a glass platform and allowed to habituate for 15 min. A mobile radiant heat source was located under the platform and focussed onto the plantar surface of each hindpaw in turn, enabling a paw withdrawal latency value to be recorded. The apparatus was calibrated to give a paw withdrawal latency of approximately 5–6 s prior to drug injection. A total of eight paw withdrawal latencies (four for each hindpaw, each separated by at least 5 min) were measured prior to drug injection and a baseline mean paw withdrawal latency calculated. Although not used for statistical comparisons, these baseline measurements were made based on observations in our laboratory which show an initial increased sensitivity of the hindpaw to repeated nociceptive thermal stimulation, which might be expected to mask any obvious increase in paw withdrawal latency (indicative of anti-nociceptive activity) after drug treatment. The total pre-administration time for each drug was determined by adding 30 min (the midpoint of the second phase of the formalin test) to the preadministration time used in the formalin test. Following drug administration, four further paw withdrawal latencies were measured and a test mean value calculated as before. Once the last paw withdrawal latency had been measured the same groups of animals were tested for drug-induced sedation in the rotarod test.

2.4. Rotarod (ataxia)

The propensity for the Na^+ channel blockers used in the formalin test to induce sedation/ataxia was examined using an automated rotarod (Erichsen and Blackburn-Munro, in press). The rotarod consisted of a wooden rod (length: 49 cm, diameter: 11 cm) divided into four compartments (width: 11.5 cm) by plastic discs (diameter: 41 cm). Rats were placed on the rotating rod, at 4 rpm, and were required to walk against the motion. The day prior to drug testing, rats were given a training trial to learn to maintain posture on the revolving drum. In the testing situation, three animals were tested simultaneously. They were placed on the drum for 2 min and the number of times they fell down was counted. Impaired motor coordination was determined to be present if any rat fell down more than two times during the recording period. The effective dose (ED_{50}) of a drug required to induce ataxia was calculated as the dose that induced motor impairments in 50% of the animals.

2.5. Drugs

The dose ranges for drugs used in the formalin test were estimated from preliminary observational effects on general behaviour of the animals. Thus, drug doses which did not exceed the maximum tolerated dose based on these observations were used.

Morphine hydrochloride was obtained from Mecobenzon (Denmark) and lamotrigine was kindly supplied by Glaxo Wellcome (UK). Mexiletine hydrochloride, carbamazepine and phenytoin (5,5-diphenylhydantoin) were purchased from Sigma (Denmark). Riluzole and lubeluzole were kindly supplied by Pierre Fabre (France). Lubeluzole, riluzole and phenytoin were all dissolved in 10% Tween 80. Mexiletine and morphine were dissolved in 0.9% saline. Lamotrigine was suspended in 0.5% carboxymethylcellulose/0.5% Tween 80 and carbamazepine were dissolved in 20% hydroxypropyl-β-cyclodextrin (HPCD). Morphine was administered s.c. in a volume of 1 ml/kg and all the other compounds were administered i.p. in a volume of 2 ml/kg.

2.6. Analysis

All data were analysed using Sigmastat 2.03 statistical software (Jandel Scientific, Germany) and expressed as mean ± S.E.M. Two-way repeated measures analysis of variance (two-way RM ANOVA) was used to analyse formalin test data and where applicable, this was followed by post-hoc analysis using Bonferroni's *t*-test to allow comparisons between groups. Comparisons of total number of flinches recorded during the first and second phase of the formalin test between groups were made using one-way ANOVA. Data for paw withdrawal latencies obtained using the Hargreaves test were compared by making post drug comparisons between vehicle and drug treated groups using the Student's *t*-test. $P < 0.05$ was considered statistically significant in all cases.

3. Results

3.1. Morphine

Administration of morphine (0.25 and 1 mg/kg, s.c.) 30 min prior to injection of formalin had no effect on nociceptive behaviour compared with vehicle in the first phase of the test (0–5 min). In contrast, morphine (0.25 and 1 mg/kg) dose-dependently inhibited flinching behaviour from 20 to 40 min during the second phase (both $P < 0.05$, two-way RM ANOVA followed by Bonferroni's *t*-test) and this effect continued for the duration of the experiment. This finding agrees with previously reported anti-nociceptive effects for morphine in the formalin test (Malmberg and Yaksh, 1995), confirming that the testing conditions used were comparable to those reported previously. At the highest dose tested, morphine (1 mg/kg) inhibited the total number of flinches recorded during the second phase by 93% ($P < 0.05$, one-way ANOVA) compared with injection of vehicle (Table 1).

Injection of morphine (1 mg/kg) 60 min before testing with an acute thermal stimulus significantly prolonged ($P < 0.05$, Student's *t*-test) the paw withdrawal latency compared with vehicle treated animals (Table 2). When subsequently tested in the rotarod test, the morphine treated animals showed no signs of motor disturbance as compared with the vehicle treated animals ($ED_{50} > 1$ mg/kg).

Table 1
Summary of effects of Na^+ channel blockers on nociceptive behaviour in the formalin test

Treatment	Dose (mg/kg)	% Inhibition of 1st phase (vs. vehicle)	% Inhibition of 2nd phase (vs. vehicle)
Morphine	0.25	5.7 (13.1 ± 2.4)	56.8 (134.1 ± 10.3)[a]
	1	26.7	93.2[a]
Mexiletine	25	25.8 (28.5 ± 4.1)	32.8 (192.0 ± 14.5)[a]
	37.5	61.8[a]	55.8[a]
	50	72.4[a]	76.1[a]
Lamotrigine	15	4.7 (26.8 ± 4.1)	43.8 (157.0 ± 23.5)[a]
	30	17.3	60.6[a]
Carbamazepine	10	− 45.2 (17.8 ± 2.5)	3.4 (170.6 ± 18.6)
	20	7.9	36.1[a]
Phenytoin	20	− 62.3 (18.6 ± 3.1)	− 9.6 (137.4 ± 14.7)
	40	− 45.6	− 8.3
Lubeluzole	2.5	33.3 (30.4 ± 6.7)	35.1 (173.9 ± 20.9)
	5	44.0	30.9
	10	52.3	41.6
Riluzole	2.5	8.2 (27.4 ± 2.6)	1.9 (169.3 ± 17.4)
	5	− 7.7	34.0[a]

Numbers in parentheses refer to the raw data (expressed as mean number of total flinches ± S.E.M.) for the corresponding vehicle treated group, against which statistical comparisons were made to estimate drug effects on flinching behaviour. All treatment groups $n = 8$ animals (except for carbamazepine/vehicle groups, $n = 7–10$ animals).

[a] $P < 0.05$ one-way ANOVA.

Table 2
Summary of effects of Na^+ channel blockers on reflex nociceptive behaviour in response to an acute thermal stimulus

Treatment	Dose (mg/kg)	Vehicle PWL (s)	Treatment PWL (s)
Morphine	1	4.7 ± 0.3	5.9 ± 0.3[a]
Mexiletine	37.5	4.9 ± 0.2	7.0 ± 0.3[a]
	50		8.5 ± 0.5[a]
Lamotrigine	30	5.2 ± 0.3	4.6 ± 0.3
Carbamezepine	20	6.2 ± 0.3	7.2 ± 0.4
Phenytoin	20	5.3 ± 0.3	5.2 ± 0.3
	40		6.0 ± 0.3
Lubeluzole	10	4.4 ± 0.2	6.9 ± 0.4[a]
Riluzole	5		5.4 ± 0.3[a]

Data are represented as the latency to respond to the thermal stimulus and are expressed as mean ± S.E.M. (s). Post treatment comparisons between drug treated and vehicle treated animals were made using Student's *t*-test. All groups $n = 8$ animals.
[a] $P < 0.05$.

3.2. Mexiletine

Intraperitoneal administration of the class 1B antiarrhythmic mexiletine at 37.5 and 50 mg/kg 10 min prior to injection of formalin, significantly inhibited flinching behaviour compared with administration of vehicle during the first phase ($P < 0.05$, Fig. 1 and Table 1). For all three doses tested (25, 37.5 and 50 mg/kg), mexiletine significantly attenuated flinching behaviour during the second phase in a dose-dependent manner compared with injection of vehicle ($P < 0.05$, Fig. 1). Furthermore, at the highest dose tested, mexiletine significantly inhibited the total number of flinches recorded during the second phase by 76% compared with vehicle ($P < 0.05$, Table 1).

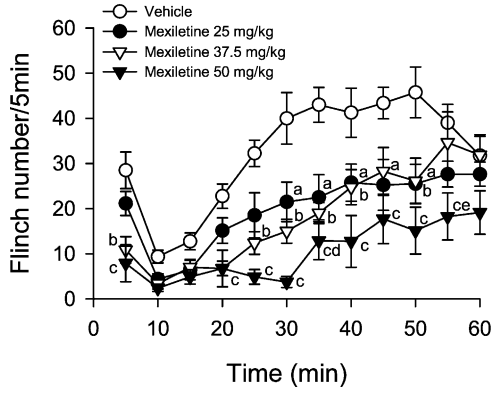

Fig. 1. Time-course of mexiletine effects in the formalin test. Mexiletine (25, 37.5 and 50 mg/kg, i.p., $n = 8$ each group) was administered 10 min prior to injection of formalin (5%, 50 μl). In the first phase of the formalin test, flinching behaviour was inhibited by the two highest doses of mexiletine compared with vehicle (0.9% saline, $n = 8$). In the second phase, mexiletine dose-dependently inhibited flinching behaviour compared with vehicle and this effect continued for a further 15 min. [a,b,c]All $P < 0.05$ vs. vehicle; [d]$P < 0.05$ vs. 25 mg/kg mexiletine; [e]$P < 0.05$ vs. 37.5 mg/kg mexiletine.

Injection of mexiletine (37.5 and 50 mg/kg) 40 min before testing with an acute thermal stimulus significantly prolonged (both $P < 0.05$, Student's *t*-test) the paw with-

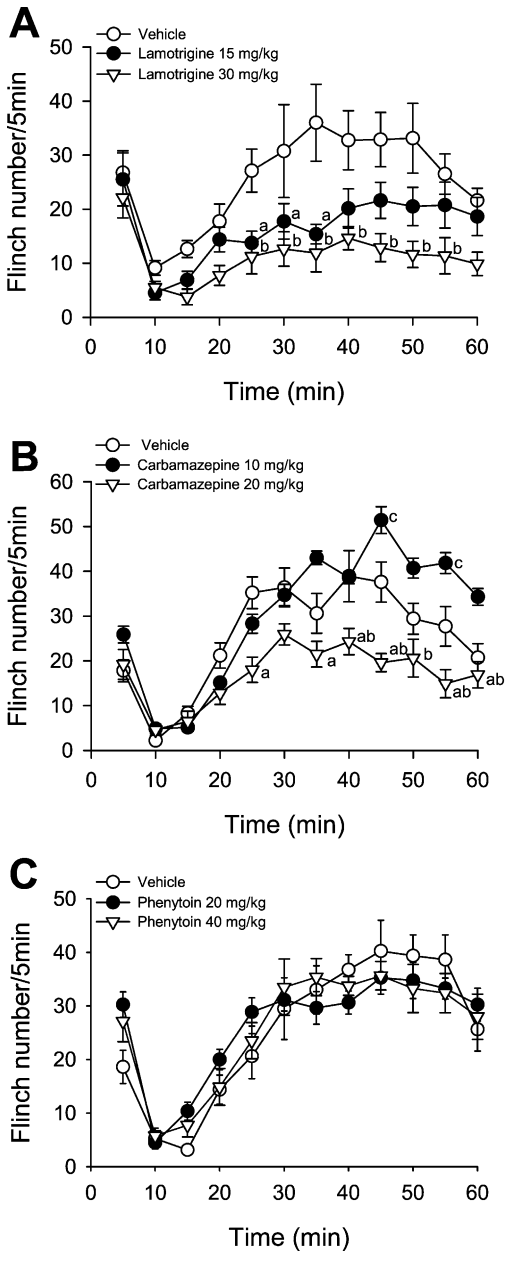

Fig. 2. Time-course of lamotrigine, carbamazepine and phenytoin effects in the formalin test. (A) Lamotrigine (15 and 30 mg/kg, i.p., $n = 8$ each group) was administered 10 min prior to injection of formalin (5%, 50 μl) and dose-dependently inhibited flinching behaviour compared with vehicle (0.5% carboxymethylcellulose/0.5% Tween 80, $n = 8$) during the second phase of the formalin test. [a,b]$P < 0.05$ vs. vehicle. (B) Carbamazepine (10 and 20 mg/kg, i.p., $n = 7–10$ each group) was administered 30 min prior to injection of formalin and significantly inhibited second phase flinching behaviour compared with vehicle (20% HPCD, $n = 7–10$) only at the highest dose tested. [a,b]$P < 0.05$ vs. vehicle, [c]$P < 0.05$ vs. 20mg/kg carbamazepine. (C) Phenytoin (20 and 40 mg/kg, i.p., $n = 8$ each group) was also administered 30 min before injection of formalin but had no effect on flinching behaviour during the second phase of the formalin test compared with vehicle (10% Tween 80, $n = 8$).

drawal latency compared with vehicle treated animals (Table 2). Subsequent testing in the rotarod test demonstrated that mexiletine treated animals had no signs of motor disturbance as compared with the vehicle treated animals (ED_{50}>50 mg/kg).

3.3. Lamotrigine, carbamazepine and phenytoin

Injection of the anticonvulsant drug lamotrigine (15 and 30 mg/kg, i.p.) 10 min prior to injection of formalin had no effect on nociceptive behaviour compared with vehicle in the first phase of the test. In contrast, lamotrigine significantly attenuated flinching behaviour during the second phase in a dose-dependent manner ($P<0.05$, Fig. 2A). At the highest dose tested, lamotrigine significantly inhibited the total number of flinches recorded throughout the duration of the second phase (15–40 min) by 61% compared with vehicle ($P<0.05$, one-way ANOVA; Table 1). Similarly, administration of carbamazepine (10 and 20 mg/kg, i.p.) and phenytoin (20 and 40 mg/kg, i.p.) 30 min prior to injection of formalin had no significant effect on flinching behaviour compared with vehicle during the first phase of the test (Table 1). Injection of carbamazepine (20 mg/kg) significantly attenuated flinching behaviour at 25 and 40 min in the second phase compared with injection of vehicle ($P<0.05$, Fig. 2B), and this effect was reinforced by a 36% inhibition of the total number of flinches recorded during the second phase compared with vehicle ($P<0.05$, Table 1). After completion of the second phase, the lowest dose of carbamazepine (10 mg/kg) actually increased flinching behaviour at $t=45$ and 55 min, compared with both vehicle and animals injected with 20 mg/kg carbamazepine. Administration of phenytoin (20 and 40 mg/kg) had no effect on flinching behaviour compared with vehicle during the second phase (Fig. 2C). Furthermore, when a comparison was made on the total number of flinches recorded during the second phase (15–40 min) with vehicle, phenytoin was shown to be devoid of anti-nociceptive effects (Table 1).

Injection of lamotrigine (30 mg/kg) 40 min prior to testing with an acute thermal stimulus had no effect on the paw withdrawal latency compared with vehicle treated animals (Table 2). Similarly, injection of carbamazepine (20 mg/kg) and phenytoin (20 and 40 mg/kg) 60 min prior to testing with an acute thermal stimulus had no effect on the paw withdrawal latency compared with vehicle treated animals (Table 2). When subsequently tested in the rotarod test, none of the three treatment groups (lamotrigine, phenytoin and carbamazepine) showed any signs of motor disturbance as compared with the vehicle treated animals (ED_{50}>30, 20 and 40 mg/kg, respectively).

3.4. Lubeluzole and riluzole

Administration of the neuroprotective drug lubeluzole (2.5, 5 and 10 mg/kg, i.p.) 30 min prior to injection of formalin had no effect on flinching behaviour compared with vehicle in either the first or second phases of the formalin test at any timepoint examined (Fig. 3A). Although lubeluzole appeared to dose-dependently inhibit the total number of flinches recorded during the second phase compared with vehicle, this effect was not significant (Table 1). Riluzole (2.5 and 5 mg/kg, i.p.) was also administered 30 min before injection of formalin and had no effect on flinching behaviour in the first phase compared with vehicle (Fig. 3B). Although the lowest dose of riluzole tested had no effect on flinching behaviour during the second phase compared with vehicle, administration of 5 mg/kg riluzole significantly attenuated flinching behaviour from 40 to 60 min compared with vehicle ($P<0.05$, Fig. 3B). More importantly, this dose significantly inhibited the total number of flinches observed throughout the second phase by 34% compared with vehicle ($P<0.05$, Table 1).

Injection of lubeluzole and riluzole (10 and 5 mg/kg) 60 min before testing with an acute thermal stimulus significantly prolonged (both $P<0.05$) the paw withdrawal latency

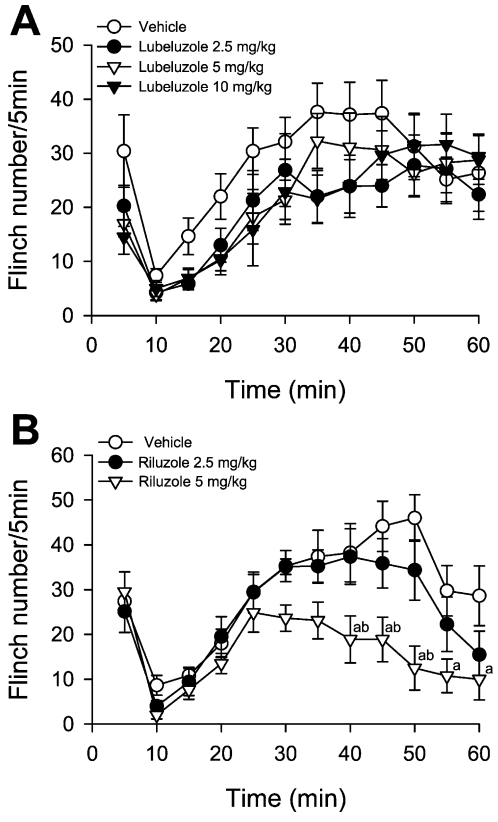

Fig. 3. Time-course of lubeluzole and riluzole effects in the formalin test. (A) Lubeluzole (2.5, 5 and 10 mg/kg, i.p., $n=8$ each group) was administered 30 min prior to injection of formalin (5%, 50 μl), and had no effect on flinching behaviour compared with vehicle (10% Tween 80, $n=8$) at any timepoint. (B) Riluzole (2.5 and 5 mg/kg, i.p., $n=8$ each group) was administered 30 min prior to injection of formalin, but only the highest dose significantly inhibited flinching behaviour compared with vehicle during the second phase of the formalin test. [a]$P<0.05$ vs. vehicle; [b]$P<0.05$ vs. 2.5 mg/kg riluzole.

compared with vehicle treated animals (Table 2). Subsequent testing in the rotarod test demonstrated that neither lubeluzole- nor riluzole-treated animals had any signs of motor disturbance as compared with the vehicle treated animals (ED_{50}>10 and 5 mg/kg, respectively).

4. Discussion

The present study has compared the relative anti-nociceptive properties of a range of drugs capable of blocking voltage-activated Na^+ channels. Mexiletine and lamotrigine at non-sedative doses induced a robust dose-dependent attenuation of second phase flinching behaviour in the formalin test in a manner comparable to that of morphine. Carbamazepine and riluzole also attenuated second phase flinching behaviour albeit to a lesser extent. These drugs have proven analgesic efficacy in other animal pain models and in humans (Tanelian and Victory, 1995; Nakamura-Craig and Follenfant, 1995; Canavero and Bonicalzi, 1996; Hunter et al., 1997; Backonja, 2001), although of the drugs tested only mexiletine has previously been shown to alleviate pain behaviour in response to formalin injection (Jett et al., 1997).

Sodium channel blocking agents such as the anticonvulsants lamotrigine, carbamazepine and phenytoin and the antiarrhythmic mexiletine preferentially bind to the inactivated state of voltage-activated Na^+ channels to prevent Na^+ influx into cells (Catterall, 1987). This similar binding mechanism endows these compounds with the ability to depress sustained firing of neurones in response to depolarising stimuli whilst leaving the first action potential in a train unimpaired (Ragsdale and Avoli, 1998), a mechanism of action referred to as use- or state-dependent block. However, other more recent studies have revealed that these structurally diverse drugs bind to specific amino acid residues in an overlapping but non-identical binding site located on the inner surface of the channel pore (Ragsdale and Avoli, 1998), with differing affinities and binding rates (Kuo et al., 2002) and which might in turn account for their differing therapeutic profiles.

Mexiletine has recently been shown to be antihyperalgesic in the complete Freund's adjuvant model of hindpaw inflammatory pain (Laird et al., 2001), and increased mRNA expression of the tetrodotoxin-resistant Na^+ channel subtype $Na_v1.8$ within dorsal root ganglion cells in response to this inflammatory stimulus has also been shown (Tate et al., 1998). We observed in the current study that mexiletine, lamotrigine and to a lesser extent carbamazepine and riluzole, all attenuated flinching behaviour in the second phase of the formalin test. As intimated above, all of the drugs tested have in common the ability to block tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels in a use dependent fashion. As the membrane potential becomes more depolarised, as might be expected to occur in the setting of tissue injury, lamotrigine and mexiletine appear to be more effective use-dependent blockers of tetrodotoxin-resistant currents (Xie et al., 1996; Brau et al., 2001), than either carbamazepine or phenytoin (Rush and Elliott, 1997; Brau et al., 2001). This mechanism of action may in turn account for their greater capacity to attenuate second phase nociceptive behaviour, where it might be expected that phosphorylation of $Na_v1.8$ by cAMP-dependent protein kinase A would occur in response to inflammatory mediators such as prostglandin E_2. Although the increased activation of tetrodotoxin-resistant Na^+ channel subtypes has been proposed to be ideally suited for sustaining repetitive firing of injured neurones at depolarised potentials (Gold et al., 1996; Eglen et al., 1999), to date selective blockers of these channels have not been identified. One study has reported that lubeluzole in contrast to riluzole, does not appear to affect high frequency firing of neurones in response to sustained depolarisation, at concentrations which prevent Na^+ influx in response to veratradrine induced stimulation (Ashton et al., 1997). Whether this explanation accounts for the lack of anti-nociceptive effect observed for lubeluzole in the formalin test remains to be tested.

It is generally agreed that the formalin test reproduces various aspects of acute inflammatory pain (Malmberg and Yaksh, 1995). Administration of the *N*-methyl D-aspartate (NMDA) receptor antagonist MK-801 attenuates nociceptive behaviour in the second phase of the formalin test (Coderre and Melzack, 1992; Chaplan et al., 1997), indicative of a role for spinal NMDA receptors in contributing to persistent nociception. Furthermore, pretreatment with MK-801 can also attenuate the intracellular translocation of protein kinase C in response to both noxious chemical injury with formalin and nerve injury (Yashpal et al., 2001). These key changes in nociceptive glutamatergic signalling if sensitive to voltage-activated Na^+ block as has been shown for lamotrigine (Lees and Leach, 1993) may partially explain the observed correlation for lamotrigine, mexiletine and carbamazepine in alleviating nociceptive transmission in both inflammatory and neuropathic pain models (Nakamura-Craig and Follenfant, 1995; Blackburn-Munro and Fleetwood-Walker, 1997; Chapman et al., 1998; Laird et al., 2001; Erichsen and Blackburn-Munro, in press). Of course separate actions at Na^+ channel subtypes sensitive to tetrodotoxin may account for some of the anti-nociceptive actions of lamotrigine, mexiletine and carbamazepine in neuropathic pain models (Xie et al., 1996; Brau et al., 2001), since neuropathic pain has been suggested to be more sensitive to tetrodotoxin than inflammatory pain (Baker and Wood, 2001).

In the present study, all drugs were administered systemically prior to formalin injection, and would thus have been expected to inhibit peripherally located Na^+ channels activated after formalin administration. However, lamotrigine, carbamazepine and phenytoin were intially developed as antiepileptics, whilst the neuroprotectants riluzole and lubeluzole have been developed with cerebral ischaemia as

a primary indication (Culmsee et al., 1998), thereby necessitating that these drugs have actions within the central nervous system. In this regard, spinal application of lamotrigine and mexiletine has been shown to selectively inhibit the activity elicited by sustained activation of nociceptive primary afferent fibres in neurones within the dorsal horn (Blackburn-Munro and Fleetwood-Walker, 1997; Chapman et al., 1998). Thus, it is possible that the anti-nociceptive effects of any of the drugs tested may have been mediated by both peripheral and central sites of action (Blackburn-Munro and Fleetwood-Walker, 1997; Chapman et al., 1998). However, the relative contribution of peripheral versus central Na^+ channels in formalin-induced nociceptive transmission would need to be verified by comparing the present results with those where the same drugs were administered by intrathecal application. The deficit of anti-nociceptive effects observed for lubeluzole and phenytoin in the formalin test are unlikely to have been a result of the difference in dose administered for each drug, since all drugs were administered at maximal tolerated dose levels as estimated from preliminary experiments on general behaviour and from lack of effect on motor disturbance as determined by the rotarod test.

None of the anti-convulsant compounds tested (lamotrigine, carbamazepine, phenytoin) affected paw withdrawal latency in response to an acute thermal nociceptive stimulus which is in general agreement with previously published observations (Hunter et al., 1997). For lamotrigine and carbamazepine this attractive ability to distinguish between phasic and tonic pain might be attributed to their use-dependent block of Na^+ channels. However, this explanation may be overly simplistic since mexiletine when tested i.p. at 37.5–50 mg/kg increased the latency to respond to a thermal stimulus by up to 43%. Thus, the ability to block voltage-activated Na^+ channels in a use-dependent manner may be insufficient in itself to discriminate between phasic and tonic pain behaviour. Although the nature of the chemical versus thermal nociceptive stimuli used in making this comparison might be regarded as clouding this issue, mexiletine was the only drug tested which significantly inhibited first phase nociceptive behaviour. Nevertheless, a more direct comparison of mexiletine with lamotrigine on voltage-activated Na^+ channel functioning in response to uniform depolarising stimuli in the acute nociceptive range would help to address this problem.

In summary, the results of the present study have shown that various Na^+ channel blockers (primarily lamotrigine and mexiletine), can robustly attenuate pain behaviour in the rat induced as a result of formalin injection into the hindpaw. The marked differences of all the Na^+ channel blockers tested in producing anti-nociceptive effects may reflect both their use-dependence and selective binding properties to voltage-activated Na^+ channels. This further supports the argument for selective targeting of Na^+ channel functioning for the treatment of chronic pain.

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