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The effect of ABT-702, a novel adenosine kinase inhibitor, on the responses of spinal neurones following carrageenan inflammation and peripheral nerve injury

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1 Adenosine (ADO) receptor activation modulates sensory transmission in the dorsal horn. Little is known about the circumstances underlying release of the purine. The present study was conducted to investigate the effect of a novel and potent non-nucleoside adenosine kinase (AK) inhibitor, ABT-702, on the responses of dorsal horn neurones to selected peripheral stimuli. ABT-702 is orally effective to reduce behavioural signs of nociception in models of acute, inflammatory, and neuropathic pain.

2 Electrophysiological recordings were made from wide dynamic range (WDR) neurones in halothane-anaesthetized rats. ABT-702 was given subcutaneously following either carrageenan inflammation or peripheral nerve injury (L5/L6 spinal nerve ligation). Comparisons were made between carrageenan and uninjected control animals, and similarly between spinal nerve ligated (SNL) and sham operated animals.

3 ABT-702 produced inhibition of the postdischarge, wind-up and C-fibre evoked responses in both carrageenan and nerve-injured animals. Furthermore, the mechanical and thermal evoked responses were similarly reduced in SNL rats. Overall, ABT-702 produced a significantly greater inhibition of these responses in SNL rats as compared to sham controls. Similarly ABT-702 tended to produce greater effects after carrageenan inflammation, however this did not reach significance.

4 Protection of endogenous adenosine by ABT-702 therefore produces a marked inhibition of the noxious evoked neuronal activity in inflamed and neuropathic rats. Our results demonstrate a plasticity in the endogenous adenosine-mediated inhibitory system following SNL and provide a possible basis for the use of this compound for the treatment of neuropathic and other persistent pain states.

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Abbreviations: AK, adenosine kinase; ADO, adenosine; SNL, spinal nerve ligated; WDR, wide dynamic range

Introduction

Substantial evidence exists for a physiological role of ADO in the modulation of primary afferent transmission in the spinal cord (Dickenson *et al*., 2000; Sawynok, 1998; Sawynok & Sweeney, 1989). The antinociceptive properties of ADO and P1 receptor agonists have been demonstrated across a wide range of animal models, including acute nociceptive tests (Sawynok, 1999), models of inflammation (Karlsten *et al*., 1992; Poon & Sawynok, 1998) and neuropathy (Lavand'homme & Eisenach, 1999; Lee & Yaksh, 1996; Sjolund *et al*., 1998; von Heijne *et al*., 1998), and there has been a considerable interest in the development of these compounds as potential analgesics for the treatment of various pain states. The spinal antinociceptive actions of ADO are mediated through interaction with various cell surface receptor subtypes, predominantly through the A_1 receptor. One limitation in the use of ADO receptor agonists is the occurrence of side effects (e.g. motor dysfunction and cardiac effects) which are associated with higher doses of these compounds (Karlsten *et al*., 1990; Lee & Yaksh, 1996; von Heijne *et al*., 1999).

ADO has a very short half-life (s) in physiological fluids (Moser *et al*., 1989), thus restricting its actions to tissues and cellular sites where it is released. ADO kinase (AK; ATP:adenosine 5′-phosphotransferase, EC 2.7.1.20) catalyses the phosphorylation of ADO to AMP (adenosine 5′-monophosphate), and is the primary enzyme regulating ADO metabolism under physiological conditions (Arch & Newsholme, 1978). Thus, inhibition of AK represents an alternative strategy for taking advantage of the beneficial actions of ADO by selectively increasing local concentration of endogenous ADO in a site and event specific manner. Inhibitors of this enzyme have been demonstrated to modulate ADO release from the spinal cord (Golembiowska *et al*., 1995; 1996) and to selectively increase ADO concentrations in traumatised neural tissue *in vivo* (Britton *et al*., 1999). The pharmacologic manipulation of the endogenous ADO system produces antinociception in

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nociceptive assays (Keil & DeLander, 1992; 1994) and decreases pain behaviours induced by substance P and kainic acid (Keil & DeLander, 1996). Similarly, the administration of AK inhibitors has been shown to effectively reduce nociception after inflammation (Poon & Sawynok, 1995) and nerve injury (Lavand'homme & Eisenach, 1999).

The spinal cord appears to be a key site of ADO modulation of nociceptive signalling since ADO receptors, ADO transport sites, and AK are localized in spinal cord (Kowaluk *et al*., 1999). Electrophysiological studies also indicate that ADO A_1 receptor agonists modulate acutely evoked and inflammation-evoked responses and also those after nerve injury in spinal cord dorsal horn neurones (Reeve & Dickenson, 1995). Additionally, the antinociceptive effects of ADO A_1 receptor agonists and AK inhibitors can be blocked by intrathecal administration of theophylline, a non-selective ADO receptor antagonist (Sawynok *et al*., 1986).

The present studies were conducted to examine the effect of ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl) pyrido[2,3,-d] pyrimidine), a novel, potent and selective, non-nucleoside AK inhibitor (Jarvis *et al*., 2000), on the responses of spinal neurones to peripheral electrical and/or natural stimuli. ABT-702 is orally effective to reduce nociception in animal models of acute nociceptive, inflammatory, and neuropathic pain (Jarvis *et al*., 2000; Kowaluk *et al*., 2000) but has never been studied using electrophysiological approaches. We aimed to investigate whether there is plasticity in the spinal purinergic system following carrageenan inflammation or peripheral nerve injury (Kim & Chung, 1992), and furthermore provide a possible basis for the treatment of the multiple symptoms of these persistent pain states.

Methods

All experimental procedures were approved by the UK Home Office and follow the guidelines under the International Association for the Study of Pain (Zimmermann, 1983).

Surgical procedures for selective spinal nerve ligation

Rats (male Sprague-Dawley, 130–250 g; Central Biological Services, University College London, U.K.) were divided into a sham operated ($n=9$) and L5/L6 spinal nerve ligated group ($n=9$). Neuropathic surgery was carried out as previously described (Kim & Chung, 1992). Briefly, the left L5 and L6 spinal nerves were isolated and tightly ligated with 6-0 silk thread under halothane anaesthesia (50% O_2: 50% N_2O). The surgical procedure for the sham operated group was identical to that of the SNL group, except that the L5/L6 spinal nerves were not ligated.

After surgery, the foot posture and general behaviour of the operated rats were monitored throughout the post-operative period. Behavioural testing was carried out over a 2-week period following procedures previously described to confirm the success of the surgery (Kim & Chung, 1992). Mechanical sensitivity was assessed through the measurement of foot withdrawal frequencies to a series of calibrated von Frey filaments (1, 5 and 9 g; 9.9, 49.5, 89.1 mN, respectively) applied to the plantar surface of the foot. Similarly, cooling sensitivity was assessed through the application of a drop of acetone to the hindpaw. Sufficient time interval was allowed between each stimulus application to ensure that no sensitization was observed following repeated mechanical stimulation. The occurrence of foot withdrawal for each trial was quantified as previously described (Suzuki *et al*., 2000). Rats were subsequently used for electrophysiological studies.

Electrophysiological studies

Two weeks after surgery, electrophysiological studies were conducted on the operated rats as previously described (Suzuki *et al*., 2000). Briefly, anaesthesia was induced in rats with 2.0–2.5% halothane (66% N_2O and 33% O_2). The spinal cord was exposed and extracellular recordings were (1.2–1.8% halothane) made from ipsilateral convergent dorsal horn neurones with defined receptive fields in the toe regions of the hindpaw. Spontaneous neurones with no clear receptive fields were not employed for the pharmacological study. The core body temperature of the rat was monitored and maintained (36.5–37°C) by means of a heating blanket connected to a rectal thermal probe *via* an automatic feedback control unit.

A train of sixteen transcutaneous electrical stimuli (2-ms wide pulses, 0.5 Hz) was applied at three times the threshold current for C-fibres and a post-stimulus histogram was constructed. Aβ-, Aδ- and C-fibre evoked neuronal responses were separated and quantified on the basis of latency. Neuronal responses occurring after the C-fibre latency band resulting from the hyperexcitability of the neurone (300–800 ms) were taken to be the postdischarge of the neurone. Wind-up was calculated as the total number of action potentials evoked at three times the C-fibre threshold after all 16 stimuli, minus the baseline response. The peripheral neuronal receptive field was also stimulated using a range of noxious and innocuous natural stimuli (mechanical punctate and heat) over a period of 10 s. Heat was applied with a constant water jet onto the centre of the receptive field. Data was captured and analysed using Spike 2 software.

Control responses to peripheral electrical and selected natural stimuli (von Frey 9 g, 50 g and heat 45°C) were established prior to drug administration.

Carrageenan inflammation

A separate group of animals (200–250 g; $n=16$) was prepared for electrophysiological recordings as described above. In eight of these animals, inflammation was induced by the intraplantar injection of 100 μl of 2% carrageenan into the ipsilateral hindpaw and the electrically evoked response of the neurone followed for 3 h at 10-min intervals. In these animals, the three controls immediately prior to drug administration were used as controls for the subsequent drug effect. Previous reports from behavioural studies have demonstrated the presence of hyperalgesia 2 h after carrageenan injection (Kowaluk *et al*., 2000), and effects appear to last up to 96 h (Kayser & Guilbaud, 1987). Furthermore, using this present electrophysiological approach robust changes in spinal opioid controls are seen at 3 h post-carrageenan (Stanfa & Dickenson, 1993).

Drug administration

ABT-702 was synthesized as described by Lee *et al.* (submitted) (Figure 1) and given subcutaneously to the scruff of the neck in increasing doses (cumulative doses of 0.1, 1 and 10 mg kg^{-1}). Tests were made every 10 min for 60 min per dose. Drug vehicle was saline. ABT-702 has been shown to readily cross the blood brain barrier and antinociceptive efficacy has been observed at plasma concentrations as low as 20 ng ml^{-1} (unpublished observations). Brain levels of ABT-702 are approximately 1/3 of plasma levels.

Data are presented as mean ± standard error of mean (s.e.mean) unless stated otherwise. Drug effects are expressed as mean maximal percentage of the pre-drug control value. Drug effects were analysed with the Student's paired *t*-test using Statview 4.5. Unpaired *t*-test was employed for the comparison of drug effects between the groups. Level of significance was taken to be $*P \leqslant 0.05$.

Results

Mechanical allodynia following spinal nerve (L5/L6) ligation

Following surgery, rats maintained good health, exhibiting normal weight gain and general level of activity with no signs of distress. SNL, but not sham operated rats, exhibited guarding behaviour of the ipsilateral hindpaw.

Consistent with previous studies (Suzuki *et al.*, 2000), all SNL rats displayed behavioural signs of mechanical allodynia of the ipsilateral hindpaw, which was prominent as early as 2 days after surgery (Figure 2). Application of innocuous von Frey filaments (weights 1, 5 and 9 g) produced exaggerated responses on the ipsilateral paw and mechanical allodynia was maintained throughout the whole of the behavioural testing period (14 days). Similarly, the application of a drop of acetone to the plantar surface of the ipsilateral hindpaw evoked aversive behaviours in SNL rats, and this was often accompanied by licking and shaking of the hindpaw. In complete contrast, the contralateral hindpaw displayed no signs of mechanical or cooling allodynia and rarely responded to acetone or von Frey filaments (weights 1–9 g). Similarly, sham operated rats did not develop mechanical/cold sensitivity. Full behavioural changes accompanying SNL have been characterized in our previous study (Suzuki *et al.*, 2000): here a figure of the

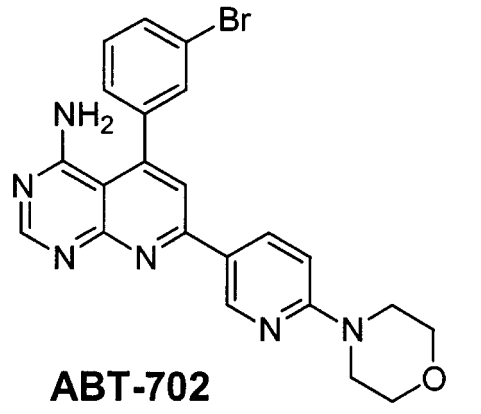

Figure 1 Chemical structure of ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl) pyrido[2,3,-d] pyrimidine).

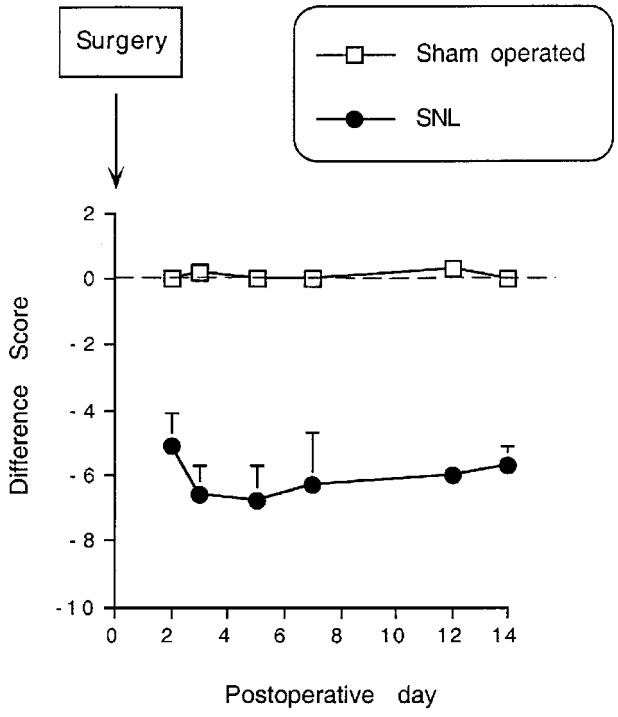

Figure 2 The difference score of foot withdrawal frequencies to the application of von Frey 5 grams in SNL ($n=9$) and sham operated rats ($n=9$). Each trial consisted of application of the mechanical stimulus 10 times onto the plantar surface of each hindpaw. Difference scores were calculated as: difference score = no. of foot withdrawals on contralateral paw − no. of foot withdrawals on ipsilateral paw. Negative values indicate a greater frequency of foot withdrawal on the ipsilateral hindpaw, which was interpreted to be a manifestation of mechanical allodynia. Data is presented as the mean foot withdrawal frequency ± s.e.mean.

Table 1 A comparison of the pre-drug neuronal evoked responses to electrical stimuli in uninjected, carrageenan, SNL and sham operated rats. AP represents the mean number of action potentials evoked by a train of 16 electrical stimuli at three times C-fibre threshold

	Pre-drug control responses (AP)			
	Uninjected	*Carrageenan*	*SNL*	*Sham operated*
Aβ-fibre evoked response	94 ± 13	118 ± 13	102 ± 12	112 ± 9
Aδ-fibre evoked response	70 ± 16	62 ± 16	87 ± 15	72 ± 6
C-fibre evoked response	318 ± 28	322 ± 54	331 ± 39	305 ± 36
Postdischarge	193 ± 35	219 ± 42	229 ± 48	190 ± 31

responses to von Frey 5 g has been included (Figure 2). The application of acetone, von Frey 1 g and 9 g produced similar behavioural responses to those evoked by von Frey 5 g,

Electrophysiological studies

Recordings were made from a total of 34 dorsal horn neurones in SNL ($n=9$), sham operated ($n=9$), uninjected ($n=8$) and carrageenan-inflamed rats ($n=8$), with mean depths from the surface of the dorsal horn of 722 ± 54, 733 ± 33, 830 ± 43, and 680 ± 61 μm respectively. This corresponded to laminae V of the spinal cord and the neuronal depths of the neurones were comparable for all animal groups.

The pre-drug control responses of spinal neurones to electrical and natural stimuli were comparable for all animal groups (Tables 1 and 2). Thus, there was no significant difference in the baseline responses between control (uninjected/sham operated) and carrageenan or SNL animals. Table 3 shows a summary of the effects of ABT-702 on the responses of spinal neurones following peripheral nerve injury and carrageenan inflammation.

Table 2 A comparison of the pre-drug neuronal evoked responses to natural stimuli in SNL and sham operated rats, 2 weeks after surgery. AP represents the mean number of action potentials evoked by each stimulus. Each stimulus was applied for 10 s onto the centre of the peripheral receptive field

	Pre-drug control responses (AP)	
	SNL	*Sham operated*
9 g von Frey evoked response	236 ± 61	166 ± 47
50 g von Frey evoked response	401 ± 79	361 ± 72
Heat (45°) evoked response	357 ± 87	280 ± 80

The effect of ABT-702 on the responses of dorsal horn neurones following peripheral nerve injury

ABT-702 significantly reduced the postdischarge (10 mg kg^{-1}, $P=0.0001$), and C-fibre evoked responses (10 mg kg^{-1}, $P=0.0001$) of spinal neurones in SNL rats (Figure 3A,B). In sham operated rats, however, the postdischarge was facilitated following the administration of ABT-702. The effects of ABT-702 were significantly different between the two animal groups for postdischarge (0.1 mg kg^{-1}, $P=0.007$; 1 and 10 mg kg^{-1}, $P=0.02$) and C-

Table 3 The effect of ABT-702 on the responses of spinal neurones to electrical and natural stimuli. ABT-702 was administered cumulatively in increasing doses (0.1, 1 and 10 mg kg^{-1}) to the scruff of the neck. The values indicate the maximal percentage inhibition of the pre-drug control responses following ABT-702 administration. A negative value indicates a facilitation of the responses compared to pre-drug control values

	% inhibition of pre-drug control responses following ABT-702 administration		
	(0.1 mg kg^{-1})	(1 mg kg^{-1})	(10 mg kg^{-1})
Aβ-fibre evoked response			
Uninjected	6 ± 8	13 ± 8	13 ± 1
Carrageenan	10 ± 7	0.2 ± 13	-0.8 ± 13
Sham operated	15 ± 7	12 ± 7	20 ± 6
SNL	19 ± 4.3	21 ± 4	19 ± 5
Aδ-fibre evoked response			
Uninjected	3 ± 16	7 ± 19	23 ± 22
Carrageenan	39 ± 13	31 ± 9	48 ± 12
Sham operated	-16 ± 10	-26 ± 12	4 ± 13
SNL	30 ± 11	26 ± 11	44 ± 6
C-fibre evoked response			
Uninjected	3 ± 10	10 ± 8	21 ± 15
Carrageenan	22 ± 6	24 ± 6	33 ± 6
Sham operated	-4 ± 10	-4 ± 11	11 ± 11
SNL	10 ± 8	14 ± 8	36 ± 3
Postdischarge			
Uninjected	-9 ± 21	21 ± 22	24 ± 27
Carrageenan	44 ± 10	53 ± 9	70 ± 9
Sham operated	6 ± 18	12 ± 19	36 ± 15
SNL	31 ± 12	31 ± 15	58 ± 8
9 g von Frey evoked response			
Sham operated	-2 ± 20	11 ± 22	44 ± 9
SNL	21 ± 22	62 ± 8	71 ± 4
50 g von Frey evoked response			
Sham operated	15 ± 5	28 ± 5	33 ± 6
SNL	43 ± 10	53 ± 10	67 ± 9
Heat (45°) evoked response			
SNL	38 ± 6	60 ± 5	69 ± 10
Sham operated	29 ± 14	44 ± 3	45 ± 19

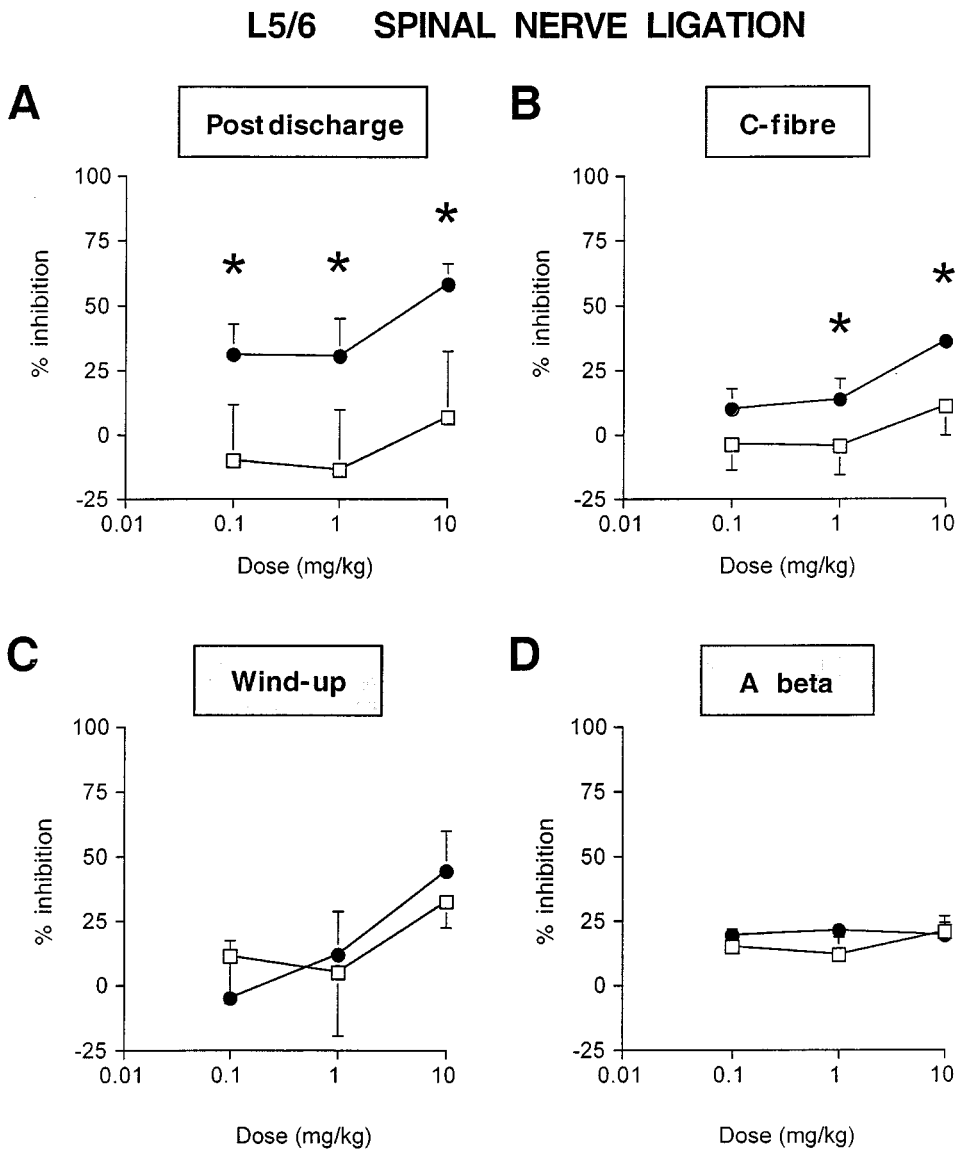

Figure 3 The effect of ABT-702 on the (A) postdischarge, (B) C-fibre evoked response, (C) wind-up and (D) Aβ-fibre evoked response of spinal neurones in SNL (closed circles, $n=9$) and sham operated rats (open squares, $n=9$) at PO 14–17 days. Data are presented as a percentage inhibition of the pre-drug control value$\pm$s.e.mean. *$P<0.05$.

fibre evoked responses (1 mg kg^{-1}, $P=0.01$; 10 mg kg^{-1}, $P=0.02$). The wind-up of spinal neurones was also reduced by ABT-702 in SNL rats (10 mg kg^{-1}, $P=0.02$) and sham operated rats (10 mg kg^{-1}, $P=0.01$) (Figure 3C). This effect of ABT-702 was comparable between the animal groups. ABT-702 reduced the Aδ-fibre evoked response of SNL rats (maximal inhibition: 44$\pm$6%), but produced little effect in sham operated rats (maximal inhibition: 4$\pm$13%) (data not shown). The inhibitions were significantly greater after nerve injury (0.1 mg kg^{-1}, $P=0.01$; 1 and 10 mg kg^{-1}, $P=0.009$).

On the contrary, ABT-702 produced only a minor insignificant reduction of the Aβ-fibre evoked response in both SNL and sham operated rats, and this effect was comparable in both groups of animals (Figure 3D).

Both the innocuous (von Frey 9 g) and noxious (von Frey 50 g) mechanical evoked responses were reduced following the administration of ABT-702 (Figure 4A,B). ABT-702 produced a significant inhibition of the 9 g von Frey evoked response in SNL (1 mg kg^{-1}, $P=0.04$; 10 mg kg^{-1}, $P=0.02$) and sham operated rats (10 mg kg^{-1}, $P=0.004$). Similarly, the 50 g von Frey evoked response was reduced by ABT-702 (SNL: 0.1 mg kg^{-1}, $P=0.004$; 1 mg kg^{-1}, $P=0.0002$; 10 mg kg^{-1}, $P<0.0001$; sham operated: 1 mg kg^{-1}, $P=0.006$; 10 mg kg^{-1}, $P=0.001$). Overall, ABT-702 produced a significantly greater inhibitory effect in SNL rats, as compared to sham operated rats (von Frey 9 g, 1 mg kg^{-1}, $P=0.03$; 10 mg kg^{-1}, $P=0.01$; von Frey 50 g, 0.1 mg kg^{-1}, $P=0.03$; 1 mg kg^{-1}, $P=0.04$; 10 mg kg^{-1}, $P=0.01$).

The thermal evoked response of spinal neurones was similarly reduced after ABT-702 in SNL (0.1 mg kg^{-1}, $P=0.04$; 1 mg kg^{-1}, $P=0.008$, 10 mg kg^{-1}, $P=0.03$) and sham operated rats (0.1 mg kg^{-1}, $P=0.02$; 1 mg kg^{-1}, $P=0.03$) (Figure 4C). Again, the inhibition was greater after nerve injury (1 mg kg^{-1}, $P=0.02$).

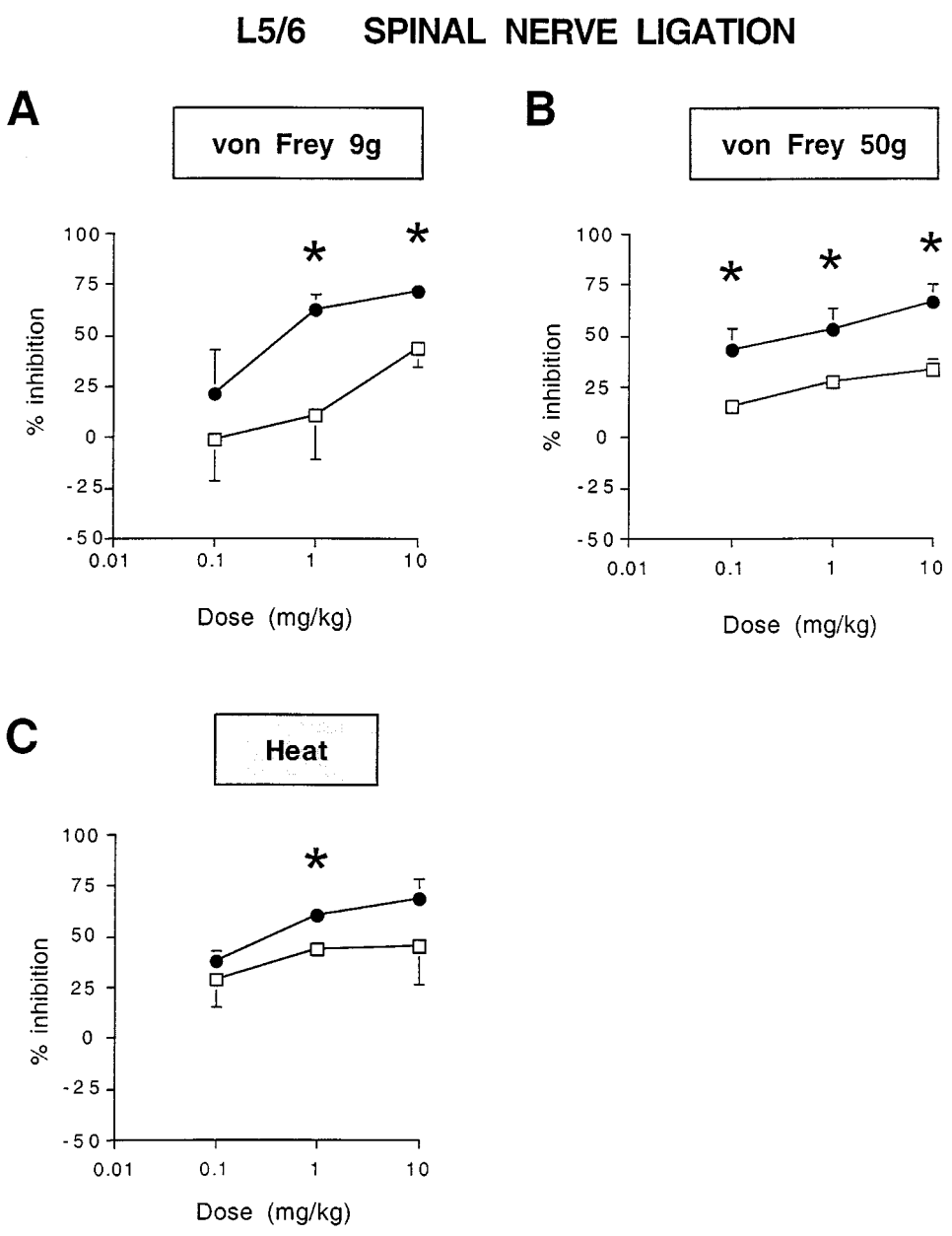

Figure 4 The effect of ABT-702 on the responses of spinal neurones to (A) von Frey 9 g, (B) von Frey 50 g and (C) heat (45°) in SNL (closed circles, $n=9$) and sham operated rats (open squares, $n=9$) at PO 14–17 days. Data are presented as a percentage inhibition of the pre-drug control value ± s.e.mean. $^*P<0.05$.

The effect of ABT-702 on the responses of dorsal horn neurones during carrageenan inflammation

ABT-702 significantly reduced the postdischarge (0.1 mg kg^{-1}, $P=0.02$; 1 mg kg^{-1}, $P=0.007$; 10 mg kg^{-1}, $P=0.001$), C-fibre evoked response (0.1 mg kg^{-1}, $P=0.03$; 1 mg kg^{-1}, $P=0.002$; 10 mg kg^{-1}, $P=0.001$), Aδ-fibre evoked response (0.1 mg kg^{-1}, $P=0.04$; 1 and 10 mg kg^{-1}, $P=0.01$; maximal inhibition 48 ± 12%, data not shown) and wind-up (1 and 10 mg kg^{-1}, $P=0.04$) of spinal neurones after carrageenan inflammation (Figure 5A–C). The Aβ-fibre evoked response of spinal neurones, however, remained relatively unaffected (Figure 5D).

In contrast to these inhibitory effects seen in carrageenan animals, ABT-702 produced non-significant effects on the postdischarge, wind-up, Aδ-fibre (maximal inhibition 23 ± 20%) and C-fibre evoked responses of uninjected rats. Overall, the inhibition tended to be greater after inflammation, however these differences were non-significant.

Discussion

Abundant evidence exists for the role of ADO in the modulation of primary afferent transmission to spinal neurones of the dorsal horn (Sawynok, 1998). Previous studies have demonstrated the existence of an endogenous purinergic tone in the spinal cord that acts to modulate sensory input (Keil & DeLander, 1994). Extracellular ADO

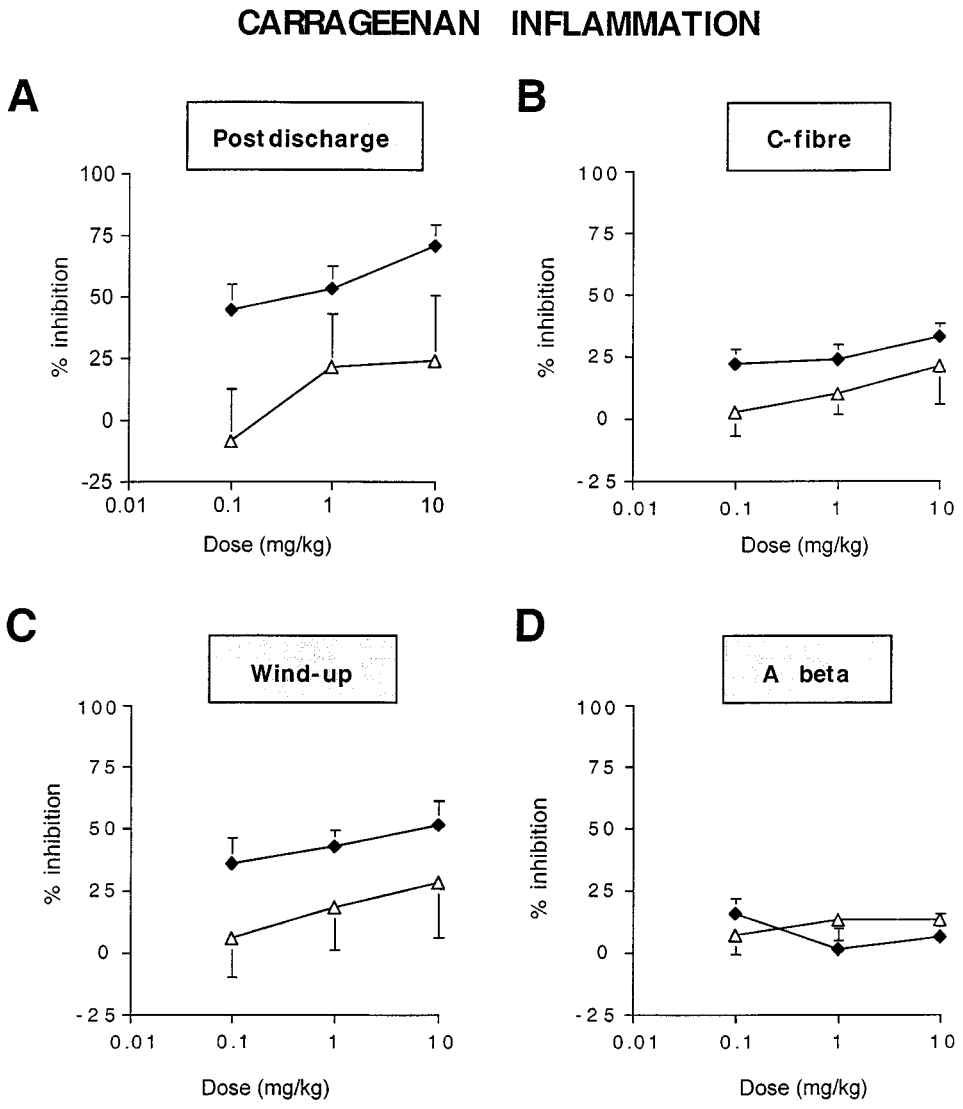

Figure 5 The effect of ABT-702 on the (A) postdischarge, (B) C-fibre evoked response, (C) wind-up and (D) Aβ-fibre evoked response of spinal neurones in carrageenan (closed diamonds, $n=8$) and uninjected control animals (open triangles, $n=8$). Data are presented as a percentage inhibition of the pre-drug control value$\pm$s.e.mean.

levels are regulated by phosphorylation by AK, the primary metabolic enzyme for intracellular ADO (Golembiowska *et al*., 1996). Intrathecal administration of AK inhibitors produces antinociception, and this is thought to result indirectly from the activation of ADO (A_1) receptors by extracellular ADO that accumulates following the inhibition of the enzyme (Poon & Sawynok, 1998). Recently, ABT-702, a novel and potent AK inhibitor which possess high specificity for the enzyme has been synthesized and thereby allows the investigation of the therapeutic potential of protecting adenosine in various pain states (see Kowaluk *et al*., 2000; Jarvis *et al*., 2000).

The present data provide further evidence for a role of ADO in nociceptive transmission. We have demonstrated that ABT-702, a novel and potent AK inhibitor, produces inhibition of the evoked neuronal responses after carrageenan inflammation, as well as after peripheral nerve injury. When given systemically, ABT-702 reduced the electrical evoked responses (postdischarge, wind-up and C-fibre evoked responses) of spinal neurones in SNL and carrageenan animals and furthermore, produced strong inhibition of the natural (mechanical/thermal) evoked responses in SNL rats. The inhibition produced by ABT-702 following carrageenan inflammation although marked and significant was not as profound as that seen after peripheral nerve injury. This does not exclude an important role for adenosine after inflammation and indeed, in a previous behavioural study, it has been demonstrated that ABT-702 produces effective analgesia following formalin and carrageenan inflammation (Kowaluk *et al*., 2000). The present electrophysiological results therefore suggest that ADO modulation plays an important role in nociceptive transmission during inflammatory and neuropathic pain states.

The greater effectiveness of ABT-702 observed after peripheral nerve injury suggests for an enhanced role of the ADO under this persistent pain state and may reflect an increased release of ADO in the spinal cord. The sustained afferent drive from the periphery after inflammation or nerve

injury gives rise to spinal hyperexcitability and may evoke a greater release of ADO, either from primary afferent fibres or intrinsic cells in the spinal cord. Consistent with this hypothesis are data indicating that systemic administration of an AK inhibitor can significantly enhance extracellular ADO concentration in traumatised neural tissue in a site and event specific fashion (Britton *et al*., 1999). Evidence suggests that there is a possible interaction between adenosine and glutamate in the spinal cord and between NMDA-receptor activation and adenosine release elsewhere in the brain (Dickenson *et al*., 2000). The A1 receptor has been implicated in indirectly controlling the spinal NMDA polysynaptic nociceptive pathway and this is suggested to be one mechanism underlying adenosine-mediated antinociception at the spinal level (Reeve & Dickenson, 1995; Suzuki *et al*., 2000). Following neuropathy, there is an enhanced role of the NMDA-receptor system in both low- and high-threshold signalling in the spinal cord (Qian *et al*., 1996; Suzuki *et al*., 2001). Conceivably, increased NMDA receptor activation may provoke elevations in extracellular adenosine level or release, thereby producing the enhanced inhibitions seen with ABT-702 in the present study.

Furthermore, the enhanced effect of ABT-702 after L5/L6 spinal nerve ligation may possibly indicate an upregulation of ADO A_1 receptors. This would also support the observation of a greater effectiveness of adenosine analogues following spinal nerve ligation (Suzuki *et al*., 2000). Previous studies have suggested the existence of an endogenous ADO-mediated tone in the spinal cord which modulates sensory transmission (Keil & DeLander, 1994; 1996). It could be envisaged that any disruption of the endogenous ADO inhibitory tone as a result of nerve injury would augment spinal nociceptive transmission, thus contributing to facilitated sensory transmission (hyperalgesia) and miscoding of innocuous information (allodynia). Any alteration in the level or release of ADO is therefore likely to induce changes in receptor density. To date, changes in ADO receptor expression following nerve injury have not been reported.

Our results are consistent with previous behavioural studies which demonstrate that AK inhibitors attenuate acute thermal nociception (Kowaluk *et al*., 1999) and pain behaviours induced by putative pain neurotransmitters (Keil & DeLander, 1996). The administration of AK inhibitors has also been shown to be effective in inflammatory pain assays (Poon & Sawynok, 1999) and against allodynia in SNL (Lavand'homme & Eisenach, 1999) and streptozocin-induced diabetic rats (Lynch *et al*., 1999). To date, there is little evidence from electrophysiological studies demonstrating the effects of AK inhibitors on neurotransmission. The present findings support these behavioural studies where AK inhibitors have been found to be effective against some of the symptoms of persistent pain states, and possibly provide a neuronal basis for these findings.

Clinically, there has been a considerable interest in the development of ADO agonist compounds for the treatment of various pain states (Belfrage *et al*., 1995; 1999; Karlsten & Gordh, 1995; Sollevi *et al*., 1995). The use of these agents is, however, largely limited by their narrow therapeutic window, thereby producing side effects at analgesic doses. The spinal administration of AK inhibitors, on the other hand, has been reported to be devoid of side effects (Lavand'homme & Eisenach, 1999). The use of ADO metabolism inhibitors allows the selective recruitment of the endogenous adenosine system and so this approach could potentially minimize side effects and represent a more favourable therapy in areas of the CNS where ADO is released, than the administration of direct-acting ADO agonists.

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