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Differential sensitivity to tetrodotoxin and lack of effect of prostaglandin E_2 on the pharmacology and physiology of propagated action potentials

[1]K.J. Farrag, [1,2]S.K.P. Costa & *,[1]R.J. Docherty

[1]Sensory Function Group, Centre for Neuroscience, Hodgkin Building, King's College London, Guy's Campus, London Bridge, London SE1 1UL

1 We have studied the effects of prostaglandin E_2 (PGE_2) on action potential propagation in the isolated, desheathed vagus and saphenous nerves of rats using an extracellular grease gap recording method.

2 PGE_2 evoked a small depolarization of vagus nerves but had no effect on the stimulation threshold, size or latency of either the A wave (corresponding to conduction in A fibres) or the C wave (corresponding to conduction in C fibres) of the compound action potential (CAP) recorded from either vagus or saphenous nerves.

3 Lidocaine (0.01–10 mM) reduced all components of the CAP of both vagus and saphenous nerves. PGE_2 had no significant effect on the sensitivity of any component of the CAP to lidocaine.

4 Tetrodotoxin (TTX, 10 μM) blocked completely both the A wave and the C wave of the CAP in either vagus or saphenous nerves.

5 In saphenous nerve preparations the A wave was blocked by lower concentrations of TTX than the C wave or any component of the CAP in vagus nerve preparations which suggests that somatosensory A fibres express a different sub-type of TTX-sensitive voltage-gated sodium channel (VGSC) than somatosensory C-fibres or visceral sensory fibres.

6 Chemical activation of VGSCs with veratridine (10 or 50 μM) induced a depolarization in either nerve. The depolarization induced by 50 μM veratridine was blocked by 10 μM TTX.

7 Although TTX-insensitive VGSCs are expressed by some vagal and some somatosensory neurones they do not appear to be expressed functionally in the axons.

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Abbreviations: CAP, compound action potential; DRG, dorsal root ganglion; PGE_2, prostaglandin E_2; TTX, tetrodotoxin; TTX-I, tetrodotoxin-insensitive; TTX-S, tetrodotoxin-sensitive; VGSC, voltage-gated sodium channel

Introduction

Prostaglandin E_2 (PGE_2) produces acute excitatory effects on sensory neurones by at least three different mechanisms. Firstly, PGE_2 changes the voltage dependence of tetrodotoxin-insensitive (TTX-I) voltage-gated Na^+ channels (VGSCs) in dorsal root ganglion (DRG) cell somata (England *et al*., 1996; Gold *et al*., 1996; 1998) so that they open at more negative potentials. The TTX-I channels have been identified as the SNS1/PN3 channel subtype (Akopian *et al*., 1996; Sangameswaran *et al*., 1996) with a possible contribution from SNS2 (Gold, 1999; Waxman *et al*., 1999). SNS1/PN3 and SNS2 have recently been renamed as $Na_V1.8$ and $Na_V1.9$ respectively (Goldin *et al*., 2000). Secondly, PGE_2 causes inhibition of voltage-gated K^+ channels in DRG (England *et al*., 1996; Nicol *et al*., 1997; Evans *et al*., 1999) and Ca^{2+}-dependent K^+ channels in nodose ganglion neurones (Weinreich & Wonderlin, 1987) and thirdly, prostaglandins including PGE_2, directly depolarize axons in the vagus nerve (Smith *et al*., 1998). The eicosanoids may also affect excitability in sensory neurones by effects on other ion channels such as those associated with vanilloid receptors (Lopshire & Nicol, 1998).

If PGE_2 modifies the behaviour of ion channels in sensory nerves, especially VGSCs, then it is reasonable to suppose that it might have an effect on action potential generation and propagation in the nerve axons. Additionally the pharmacology of the fibres with respect to state-dependent sodium channel blockers such as lidocaine would probably be changed. Accordingly, in this study, we have used extracellular recording techniques to study the effects of PGE_2 on action potential generation and propagation and on the sensitivity to lidocaine. The nerve trunks chosen for the study were the vagus nerve which contains a high proportion of visceral, sensory fibres including capsaicin-sensitive C-fibres (Marsh *et al*., 1987) and the saphenous nerve which contains predominantly somatosensory fibres. Since one of the mechanisms by which PGE_2 enhances sensory neurone excitability involves modulation of TTX-I sodium channels we have also studied the TTX sensitivity of the different fibre populations in the nerves. Preliminary

*Author for correspondence; E-mail: reginald.docherty@kcl.ac.uk
[2]Current address: Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, CP 6111, 13081-970, Campinas (SP), Brazil

results from this study have already appeared in abstract form (Farrag & Docherty, 2001).

Methods

Adult rats (Sprague-Dawley, either sex, $\geqslant$200 g) were killed humanely by asphyxiation in a chamber filled with a slowly rising concentration of CO_2 gas. For experiments on isolated nerves both vagus nerves and both saphenous nerves were dissected and the perineural sheaths were removed. Each nerve was mounted in a custom made vaseline-gap recording apparatus that was made according to the design described by Rang & Ritchie (1988) save that the 10 mm long channel containing the vaseline was 1.0 mm rather than 0.3 mm in diameter. Bipolar electrical stimuli (Grass S88) were applied to the nerve *via* a pair of Pt-Ir wires (Goodfellow, 0.2 mm diameter, 1 mm apart) placed such that the cathodal wire was closest to and 10 mm from the vaseline gap. The extracellular DC potential across the vaseline gap was recorded *via* Ag/AgCl pellet electrodes that were in contact with the bathing solution (see below) around the nerve, one on each side of the vaseline gap. The signal was amplified (WPI, DAM50, 3 kHz low-pass) and relayed to a chart recorder or AC-coupled (Axon Instruments, Cyberamp 320, hi-pass 0.1 Hz) and recorded on a personal computer *via* an A/D interface (Axon Instruments, TL-1) under the control of pClamp (v5.5) software (Axon Instruments). Data acquired to the computer were sampled at 10 kHz. For measurement of A-fibre compound action potentials (CAPs) nerves were stimulated with a pulse of 1–70 V of width 0.05 ms applied at a frequency of 0.1 Hz. For measurement of C-fibre CAPs nerves were stimulated with a pulse of 1–70 V of width 0.5 ms applied at a frequency of 0.1 Hz. The electrical artefacts associated with stimulation were isolated at the end of the experiment by applying 10–100 mM lidocaine to the nerve and these data were digitally subtracted from experimental data to isolate the CAP. One end of the nerve that emerged from the vaseline gap was immersed in a stagnant pool of a physiological solution and the other end (the end that was electrically stimulated) was superfused at room temperature (20–22°C) with a physiological solution that had the following composition (in mM): NaCl 130; KCl 3; $CaCl_2$, 1; $MgCl_2$, 1; glucose 11; HEPES 5; pH = 7.4, adjusted with NaOH). Drugs were applied to one end of the nerve by addition to the superfusate.

The threshold for a response to electrical stimulation was defined as the minimum voltage of stimulation that evoked a response that was time-locked to the stimulus and had an amplitude of greater than 25 μV. Data are expressed as mean $\pm$ s.e.mean for n experiments. Statistics were calculated using Microsoft Excel software and data were compared using Student's t-test as indicated in the text. Values for the IC_{50} of drugs were determined in each experiment by fitting a standard logistic equation to the data (response $= R_{max}/(1 + (([drug]/IC_{50})^p) + R_{max})$; where Rmax is the maximum response and p is the slope factor for the line) using MicroCal Origin (v4.1) and extracting the IC_{50} from the fitted line. Values for IC_{50}s were then averaged and presented in the text as mean $\pm$ s.e.mean. DC potential evoked by drugs were not absolute values but were measured as a deflection from the baseline. Drugs used were TTX (Alomone), lidocaine hydrochloride, PGE_2 and veratridine (Sigma). Dose-response curves to lidocaine and TTX were constructed by cumulative addition of the blocking agent to the isolated nerve preparation.

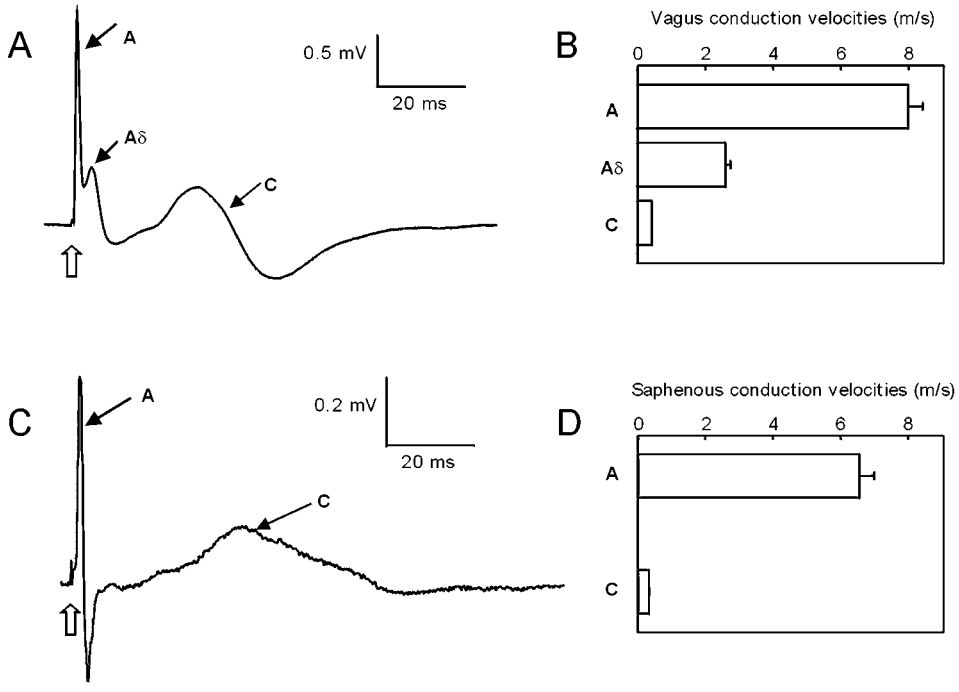

Figure 1 Typical compound action potentials (CAPs) evoked by electrical stimulation of the vagus and the saphenous nerves. (A) Shows the vagus CAP and (B) shows the conduction velocities (mean $\pm$ s.e.mean) of the components of the vagus CAP (n = 27). (C and D) Show the corresponding data for the saphenous CAP (n = 33). An electrical shock (0.5 ms) was applied to the nerve at the point indicated by the open arrow on each trace. Conduction velocity was measured along a 1 cm conduction path.

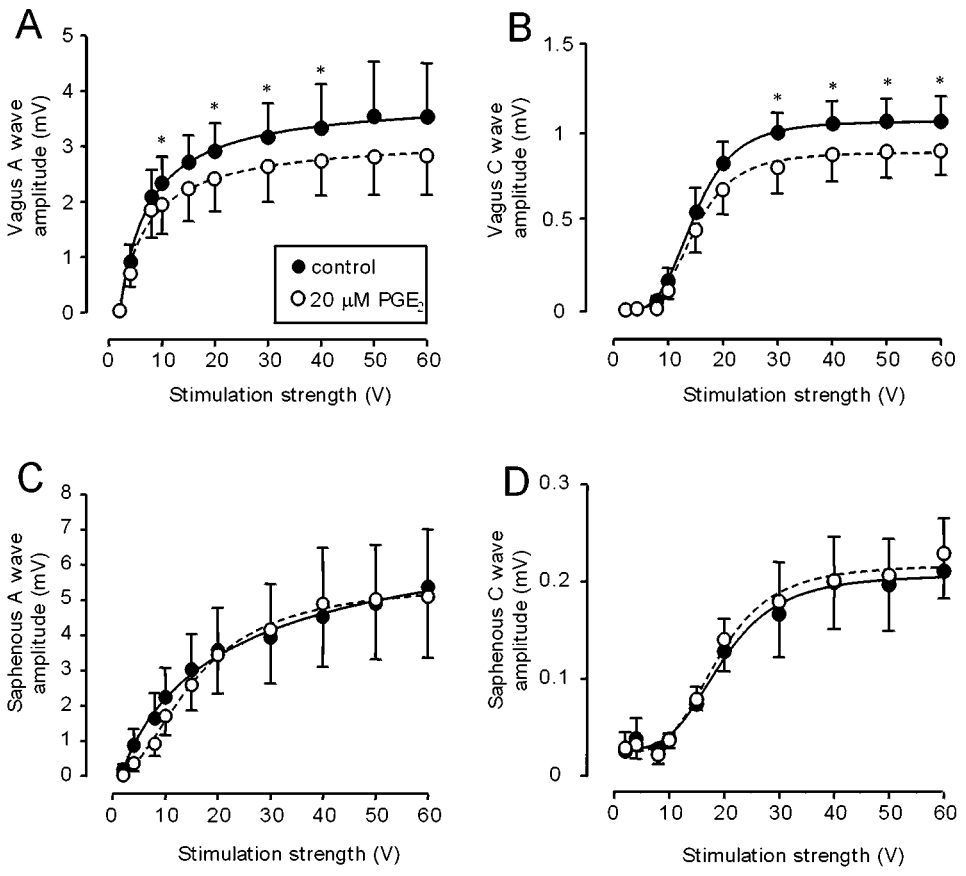

Figure 2 The effect of PGE_2 on the CAP of the vagus and saphenous nerves. (A and B) Show the relationship between the stimulus strength and the amplitude of the A and C components of the vagus CAP before and during application of PGE_2 ($n=21$). (C and D) Show the corresponding data for the saphenous nerve CAP ($n=10$). All data are mean$\pm$s.e.mean. Solid and broken lines were fitted by eye to the data from control and PGE_2 treated nerves respectively. The asterisks indicate data points where PGE_2 caused a significant reduction ($P<0.05$) in amplitude (2-tailed paired Student's t-test).

Table 1 Minimum voltages required to evoke threshold and maximal amplitudes of compound action potentials in the absence or in the presence of PGE_2

		Threshold (V)	*Maximum*(V)	n
Vagus nerve				
	A	4.4±0.4	36.0±4.4	21
control	Aδ	6.3±0.8	45.0±3.8	21
	C	11.1±1.5	51.4±2.2	21
	A	4.3±0.4	41.8±4.2	21
PGE_2	Aδ	6.3±1.3	40.8±4.2	21
(20 μM)	C	11.2±1.6	51.0±2.6	21
Saphenous nerve				
control	A	2.4±0.3	54.0±4.3	10
	C	5.7±1.5	46.1±6.1	10
PGE_2	A	3.2±0.6	54.0±2.7	10
(20 μM)	C	6.8±1.7	49.8±5.1	10

Electrical stimuli of 0.05 ms or 0.5 ms width were used to evoke A fibre or C fibre responses respectively. There were no significant effects of PGE_2 on threshold or maximum responses. Values are mean±s.e.mean.

Results

Electrical stimulation of either the vagus nerve (Figure 1A,B) or the saphenous nerve (Figure 1C,D) gave rise to a multiphasic CAP that could be recorded stably for several hours. Characteristically the vagus CAP comprised an A-wave that occurred at a latency of 1.4±0.1 ms ($n=26$), an Aδ-notch (latency = 4.1±0.3 ms, $n=24$) on the falling phase of the A-wave and a biphasic C-wave (latency to first peak = 24.7±0.7 ms, $n=27$). Over the 10 mm conduction path in the apparatus these data correspond to conduction velocities (at 20–22°C) of 7.99±0.43 m.s^{-1}, 2.60±0.14 m.s^{-1} and 0.42±0.01 m.s^{-1} for A-, Aδ- and C-fibres respectively. Since the Aδ- wave coincided with the falling phase of the A wave it was difficult to determine its amplitude in isolation so we have focused our attention on the A- and C- waves and do not consider the Aδ- component further. The saphenous nerve CAP comprised an A-wave and a C-wave (Figure 1D) at latencies of 1.8±0.1 ms ($n=33$) and 33.1±1.8 ms ($n=23$) corresponding to conduction velocities of 6.55±0.45 m.s^{-1} and 0.32±0.02 m.s^{-1} respectively. Usually, we could not discriminate a measurable Aδ-component in recordings from saphenous nerves. The conduction velocities were significantly slower in the saphenous than in the vagus nerve ($P<0.05$ for the A wave and $P<0.001$ for the C wave).

Effect of PGE_2

PGE_2 (20 μM) caused a small DC depolarization of 74±15 μV ($n=11$) in vagus and 70±13 μV ($n=7$) in

saphenous nerve. By comparison, increasing extracellular K^+ to 10 mM (by adding KCl to the extracellular medium) caused a DC depolarization of 334 ± 29 μV ($n=5$) in vagus and 384 ± 125 μV ($n=5$) in saphenous nerves. CAPs were evoked at a range of stimulation voltages in the absence or presence of PGE_2 (20 μM) which was allowed to act for at least 5 min before any measurements were taken. The stimulus strength-response data for the A- and C- components of the vagus and saphenous nerves are shown in Figure 2 and quantitative estimates for the threshold and maximum voltages for each component of each type of nerve before and after application of PGE_2 are given in Table 1. The threshold voltage required to evoke either an A wave or a C wave was significantly lower for saphenous than for vagus ($P=0.001$ for A wave and 0.039 for C wave). PGE_2 caused a small but significant reduction in the maximum amplitude of the CAP for both A and C fibre components (Figure 2A,B) of the vagus nerve but had no significant effect on the amplitude of either A or C fibre components of the saphenous nerve CAP. There were no significant differences in the stimulation threshold or minimum supramaximal stimulus voltage for any component of the CAP in either type of nerve nor did PGE_2 have any effect on the latency of the A or C waves.

Table 2 IC_{50}s for lidocaine alone, for lidocaine in the presence of PGE_2, and for TTX for the reduction of amplitude of the A and C waves of the compound action potential

	A fibre IC_{50} (μM)	*C fibre* IC_{50} (μM)	n	P
Vagus				
lidocaine	84 ± 22	274 ± 129	5	n.s.
lidocaine + PGE_2 (20 μM)	97 ± 27	321 ± 107	5	n.s.
TTX	0.113 ± 0.033	0.233 ± 0.054	6	n.s.
Saphenous				
lidocaine	119 ± 46	63 ± 16	7	n.s.
lidocaine + PGE_2 (20 μM)	43 ± 8	183 ± 87	7	n.s.
TTX	0.057 ± 0.018	0.373 ± 0.082	9	$P<0.005$

Values presented are mean $\pm$ s.e.mean for n experiments. The P values (fourth column) are calculated by 2-tailed, unpaired Student's t-test and compare data for A fibres (first column) with data for C fibres (second column) for each condition (n.s. = no significant difference).

Effect of lidocaine

Lidocaine (0.01 – 10 mM) caused a dose-dependent inhibition of all phases of the CAP in either vagus or saphenous nerves. The IC_{50}s for the inhibitory effect of lidocaine either in control nerves or in nerves that had been exposed to PGE_2 are given in Table 2 and the data are shown in Figure 3. There were no significant differences between data obtained for the sensitivity of the A wave to lidocaine compared to the sensitivity of the C wave in either nerve. Nor were there any significant differences when data were compared in the absence or in the presence of PGE_2 or when data from the the vagus were compared to the corresponding data obtained in the saphenous nerve.

Effect of TTX

TTX (0.005 – 0 μM) caused a dose-dependent inhibition of all phases of the CAP in both vagus and saphenous nerves (Figure 4). All phases of the CAP were blocked completely

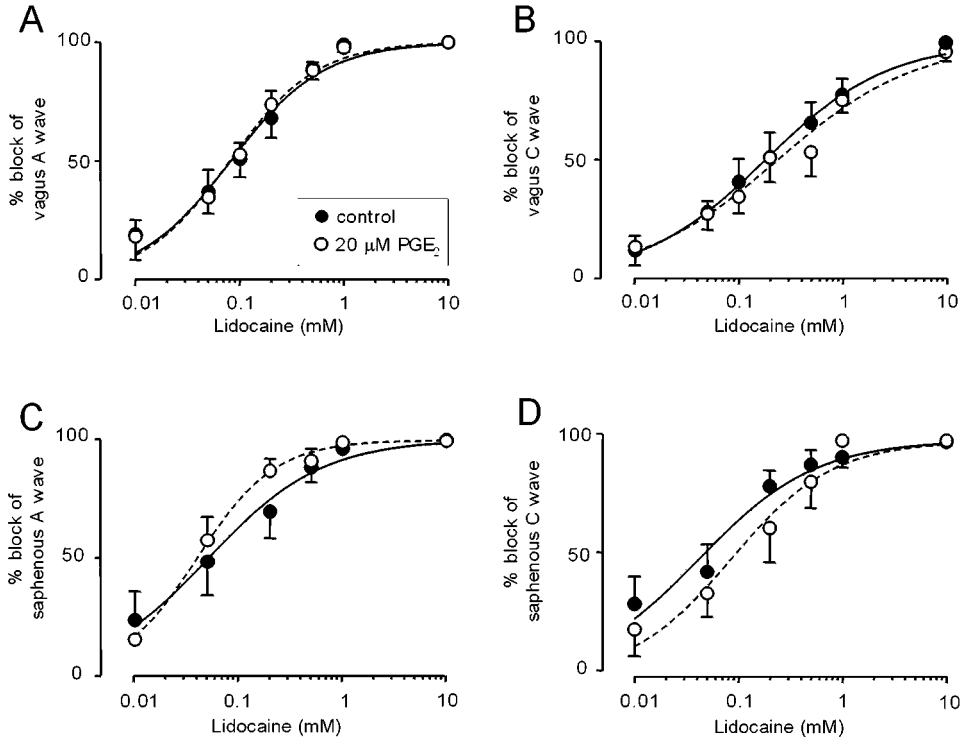

Figure 3 PGE_2 does not affect the sensitivity of the CAP to lidocaine. (A and B) Show the effect of lidocaine on the amplitude of the A and C components of the vagus CAP before and during application of PGE_2 ($n=5$). (C and D) Show the corresponding data for the saphenous nerve CAP ($n=7$). Lines were fitted to the data using a standard logistic equation (see Methods). All data are mean $\pm$ s.e.mean.

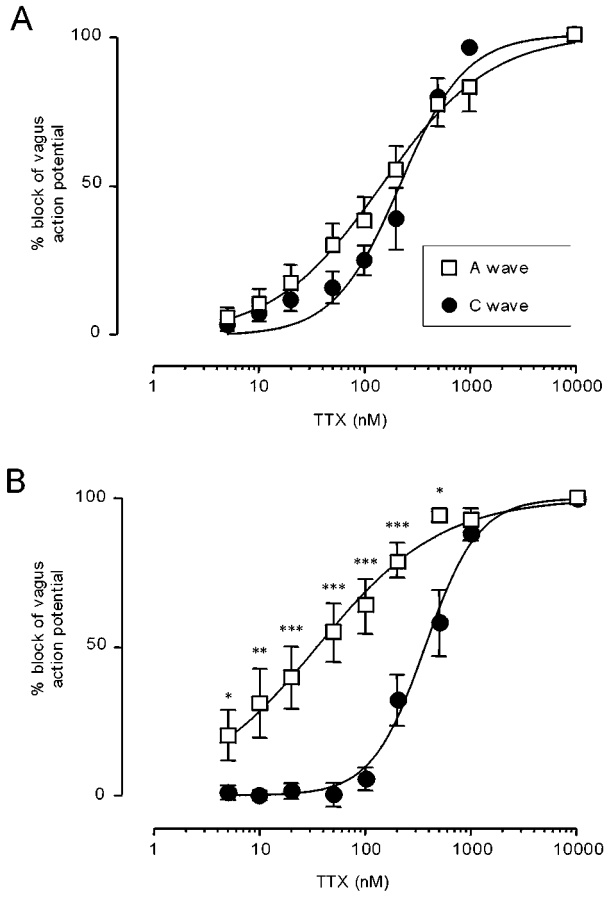

Figure 4 Effect of tetrodotoxin (TTX) on the CAP. (A) Shows the effect of TTX on the amplitude of the A ($n=6$) and C ($n=5$) components of the vagus CAP. (B) Shows the corresponding data for the A ($n=7$) and C ($n=9$) components of the saphenous nerve CAP. Lines were fitted to the data using a standard logistic equation (see Methods). All data are mean ± s.e.mean. The asterisks indicate data points where there was a significant difference (*indicates $P<0.05$, **indicates $P<0.01$, ***indicates $P<0.005$; 2-tailed unpaired Student's t-test).

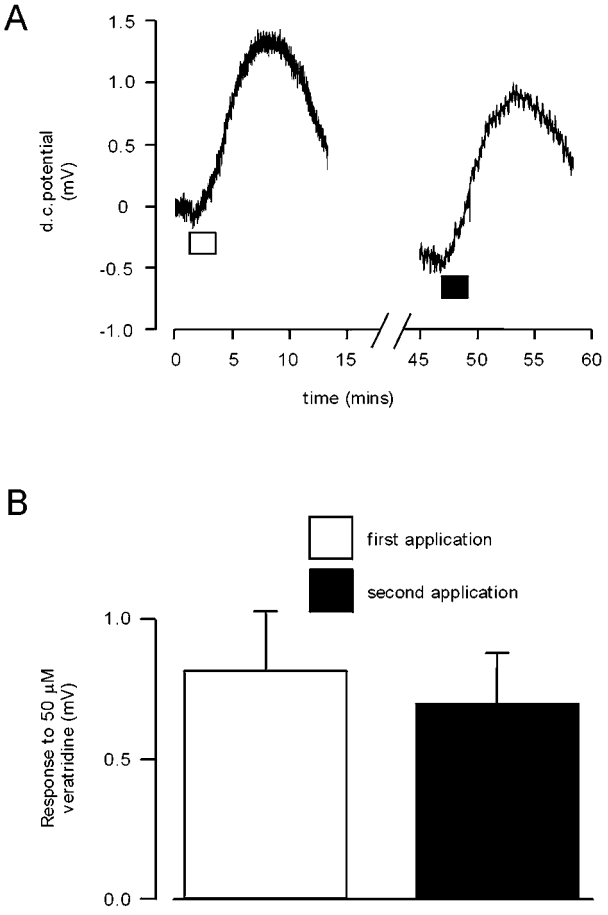

Figure 5 Responses to veratridine in isolated rat vagus and saphenous nerves. (A) Shows chart records from an experiment to test the reproducibility of repeated applications of veratridine to vagus nerve and (B) shows mean data obtained from seven similar experiments.

by TTX ($\geqslant 10\ \mu$M). The IC_{50}s for the inhibitory effect of TTX are given in Table 2. There was no significant difference between the sensitivity of the A- and C- waves of the vagus nerve CAP or between these values and the C-wave of the saphenous nerve CAP. The A-wave of the saphenous nerve CAP was significantly more sensitive to block by TTX than the C-wave of saphenous nerve CAP or either the A- or C-waves of the vagus nerve CAP.

Effect of veratridine

Veratridine (10 or 50 μM) depolarized either vagus or saphenous nerves. The veratridine-induced depolarization recovered on washing and was reproduced when veratridine was re-applied (Figure 5). The veratridine-induced depolarization in saphenous nerve was blocked completely within 5 min by application of 1 or 10 μM TTX (see Figure 6A,B). The effects of TTX on the vagus nerve developed more slowly than the corresponding effects on the saphenous nerve so that vagus tissue had to be incubated with TTX for 20–30 min before the full effects of the drug were observed. After 30 min incubation with TTX the veratridine-induced depolarization in vagus nerve was only partly blocked at a concentration of 1 μM (see Figure 6A) but was almost abolished by 10 μM TTX (see Figure 6B).

Discussion

In the present study we have confirmed that PGE_2 depolarizes peripheral nerve axons. Data from a more extensive study of eicosanoid-induced vagus nerve depolarization by Smith *et al.* (1998) suggested that the receptors mediating the depolarizing response are likely to be of the IP subtype and that the response is mediated, at least partly, by the adenylate cyclase/protein kinase A signalling system that also mediates the effects on VGSCs (England *et al.*, 1996; Gold *et al.*, 1998; Fitzgerald *et al.*, 1999). We cannot be sure that the depolarization is due to a direct effect of PGE_2 on the nerve axons since the whole nerve contains other cell types including satellite cells such as Schwann cells which might be responsive to eicosanoids and could depolarize nerve axons by an indirect mechanism. Irrespective of the cellular mechanism of the small depolarization, PGE_2 alone did not provide a sufficient stimulus to cause any measurable

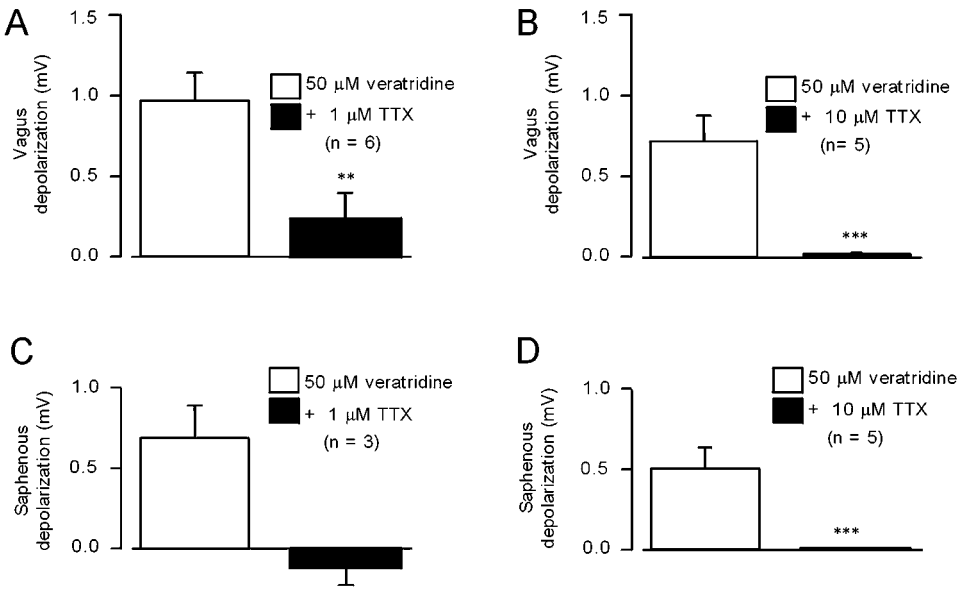

Figure 6 (A and B) Show the effect of TTX on the response of the vagus nerve to veratridine and (C and D) show the effect of TTX on the response of the saphenous nerve. All data are mean ± s.e.mean. The asterisks indicate data where there was a significant difference (**indicates $P<0.01$, ***indicates $P<0.005$; two-tailed unpaired Student's *t*-test).

increase in the excitability of the fibres, even though the concentration used was sufficient to cause a substantial enhancement of TTX-I sodium currents in DRG neuronal somata (England *et al*., 1996). If anything, PGE_2 impaired conduction of action potentials in vagal fibres since it caused a small reduction of the amplitude of the CAP. These data suggest that PGE_2 mediated increases in the excitability of neurones do not occur in the nerve trunks but rather are restricted to neuronal somata and possibly to the terminal regions of the nerve. This is important since it suggests that fibres in passage through regions of inflammation are not likely to be affected by PGE_2 and the fibres will therefore retain their specificity with respect to the location of their terminals.

In mammals, VGSCs are essential for the propagation of action potentials along axons. There is clear and abundant evidence that supports a role for TTX-S sodium channels in axons (e.g. see Villiere & McLachlan, 1996) but whether TTX-I channels such as $Na_V1.8$ are involved in action potential conduction is more controversial. Jeftinija (1994) has presented evidence that suggests that TTX-I channels are capable of supporting action potential conduction in sensory nerve roots and there is evidence that TTX-I channels support action potential conduction in amphibian peripheral nerves (Buchanan *et al*., 1996; Kobayashi *et al*., 1996), rat (Steffens *et al*., 2001) and human tissue (Quasthoff *et al*., 1995). Certainly, $Na_V1.8$ mRNA is expressed in the cell bodies of nociceptive somatosensory neurones (Akopian *et al*., 1996; Sangameswaran *et al*., 1996) and in nodose ganglion neurones (Chen *et al*., 1997) and TTX-I currents (Arbuckle & Docherty, 1995) and TTX-I somatic action potentials have been described in nociceptive neurones (e.g. see England *et al*., 1996). So, if the cognate VGSC protein is also expressed in the axons of the somatosensory DRG neurones and in the visceral sensory fibres of the vagus then TTX-I action potentials would be expected, at least in the sensory C-fibre populations. In our experiments TTX blocked completely all components of the CAP in both the vagus and in the saphenous nerve which suggests that TTX-I channel protein is not expressed in the axons in sufficient amounts to support action potential conduction. It is conceivable that the channels are present in the nerve axons, perhaps contributing to excitability, but cannot support electrogenesis of action potentials because of the relatively slow kinetics of the channels, especially at room temperature. On the other hand veratridine – at a concentration that has been shown to be effective on TTX-I currents in HEK cells expressing recombinant $Na_V1.8$ (Fitzgerald *et al*., 1999) or on TTX-I currents in neuronal somata (Farrag and Docherty, unpublished observations) – induced a depolarization of either saphenous or vagus nerves that was completely blocked by TTX which suggests strongly that functional TTX-I channels are not expressed in the somatosensory axons and that this explains why PGE_2 does not affect axonal excitability and action potential propagation in the isolated nerve preparations. Consistent with this, there is evidence that TTX-I channels are only present at sensory nerve terminals and not axons in somatosensory neurones (Villiere & McLachlan, 1996) and in corneal C-fibres (Brock *et al*., 1998). Further, $Na_V1.8$ (i.e. SNS/PN3) protein is expressed at low levels in normal peripheral sensory axons (Novakovic *et al*., 1998). These data support the suggestion that TTX-I VGSCs do not contribute significantly to electrogenesis or propagation of action potentials in peripheral nerve axons.

Although TTX blocked completely all components of the CAP in vagus and saphenous nerve in our experiments, the TTX sensitivity of all components was not equal. The A fibre wave in saphenous nerve was significantly more sensitive to TTX than the A fibre wave in vagus or the C fibre waves in saphenous or vagus nerves. The saphenous fibres also conducted at a slower velocity than the vagus fibres. While the difference in conduction velocity might easily be explained by differences in fibre diameter or degree of myelination the difference in sensitivity to TTX suggests a more fundamental

difference in the physiology and pharmacology of the visceral and somatosensory A fibre populations. The difference in TTX-sensitivity is not simply due to differences in safety factor in the two nerves since there was no significant difference in their sensitivity to lidocaine. Similar data have been reported by Strassman & Raymond (1999) for sensory fibres innervating the dura. It is possible that the two nerve populations express different species of TTX-sensitive (TTX-S) channels, one being more resistant to TTX than the other. This has important implications for interpretation of the effects of sodium channel blocking drugs *in vivo*.

Sodium channel blocking agents such as anti-convulsants, anti-dysrhythmics, some anti-depressants and local anaesthetics have very different pharmacological profiles *in vivo* but the pharmacology of such drugs with respect to sodium channel subtypes is largely unexplored.

In summary, we have shown that the inflammatory mediator PGE_2 has no measurable effect on action potential propagation in peripheral nerve trunks *in vitro*, and we suggest that the most likely reason for this is very low or the absence of expression of TTX-I sodium channels in the axons. Absence of TTX-I channels in the axons or, conversely, selective expression of these channels in terminal regions, may contribute to the location specificity of nociceptive sensory nerves in inflammatory hyperalgesia. We have also presented evidence that suggests that somatosensory A fibre axons may express a species of TTX-S VGSCs that can be distinguished pharmacologically form C fibre VGSCs.

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References

AKOPIAN, A.N., SIVILOTTI, L. & WOOD, J.N. (1996). A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. *Nature,* **379,** 257–262.

ARBUCKLE, J.B. & DOCHERTY, R.J. (1995). Expression of tetrodotoxin-resistant sodium channels in capsaicin-sensitive dorsal root ganglion neurons of adult rats. *Neurosci. Lett.*, **185,** 70–73.

BROCK, J.A., MCLACHLAN, E.M. & BELMONTE, C. (1998). Tetrodotoxin-resistant impulses in single nociceptor nerve terminals in guinea-pig cornea. *J. Physiol.,* **512,** 211–217.

BUCHANAN, S., HARPER, A.A. & ELLIOTT, J.R. (1996). Differential effects of tetrodotoxin (TTX) and high external K^+ on A and C fibre compound action potential peaks in the frog sciatic nerve. *Neurosci. Lett.,* **219,** 131–134.

CHEN, J., IKEDA, S.R., LANG, W., ISALES, C.M., & WEI, X. (1997). Molecular cloning of a putative tetrodotoxin-resistant sodium channel from dog nodose ganglion neurons. *Gene,* **202,** 7–14.

ENGLAND, S., BEVAN, S. & DOCHERTY, R.J. (1996). PGE_2 modulates the tetrodotoxin-resistant current in neonatal rat dorsal root ganglion neurones via the cyclic cAMP-protein kinase A cascade. *J. Physiol.,* **495,** 429–440.

EVANS, A.R., VASKO, M.R. & NICOL, G.D. (1999). The cAMP transduction cascade mediates the PGE_2-induced inhibition of potassium currents in rat sensory neurones. *J. Physiol.,* **516,** 163–178.

FARRAG, K.J. & DOCHERTY, R.J. (2001). Differential TTX sensitivity of A and C fibre populations of the rat saphenous nerve *in vitro*. *J. Physiol.,* **531P,** 221P.

FITZGERALD, E.M., OKUSE, K., WOOD, J.N., DOLPHIN, A.C. & MOSS, S.J. (1999). cAMP-dependent phosphorylation of the tetrodotoxin-resistant voltage-dependent sodium channel SNS. *J. Physiol.,* **516,** 433–446.

GOLD, M.S. (1999). Tetrodotoxin-resistant Na+ currents and inflammatory hyperalgesia. *Proc. natl. Acad. Sci. U.S.A.,* **96,** 7645–7649.

GOLD, M.S., LEVINE, J.D. & CORREA, A.M. (1998). Modulation of TTX-R INa by PKC and PKA and their role in PGE2-induced sensitization of rat sensory neurons in vitro. *J. Neurosci.,* **18,** 10345–10355.

GOLD, M.S., REICHLING, D.B., SHUSTER, M.J. & LEVINE, J.D. (1996). Hyperalgesic agents increase a tetrodotoxin-resistant Na+ current in nociceptors. *Proc. natl. Acad. Sci. U.S.A.,* **93,** 1108–1112.

GOLDIN, A.L., BARCHI, R.L., CALDWELL, J.H., HOFMANN, F., HOWE, J.R., HUNTER, J.C., KALLEN, R.G., MANDEL, G., MEISLER, M.H., NETTER, Y.B., NODA, M., TAMKUN, M.M., WAXMAN, S.G., WOOD, J.N., & CATTERALL, W.A. (2000). Nomenclature of voltage-gated sodium channels. *Neuron,* **28,** 365–368.

JEFTINIJA, S. (1994). The role of tetrodotoxin-resistant sodium channels of small primary afferent fibers. *Brain Res.,* **639,** 125–134.

KOBAYASHI, J., OHTA, M. & TERADA, Y. (1996). Tetrodotoxin-resistant Na^+ spikes of C fibers have at least two subtypes in the isolated bullfrog sciatic nerve. *Neurosci. Lett.,* **221,** 9–12.

LOPSHIRE, J.C. & NICOL, G.D. (1998). The cAMP transduction cascade mediates the prostaglandin E_2 enhancement of the capsaicin-elicited current in rat sensory neurons: whole-cell and single-channel studies. *J. Neurosci.,* **18,** 6081–6092.

MARSH, S.J., STANSFELD, C.E., BROWN, D.A., DAVEY, R. & MCCARTHY, D. (1987). The mechanism of action of capsaicin on sensory C-type neurons and their axons *in vitro*. *Neuroscience,* **23,** 275–289.

NICOL, G.D., VASKO, M.R. & EVANS, A.R. (1997). Prostaglandins suppress an outward potassium current in embryonic rat sensory neurons. *J. Neurophysiol.,* **77,** 167–176.

NOVAKOVIC, S.D., TZOUMAKA, E., MCGIVERN, J.G., HARAGUCHI, M., SANGAMESWARAN, L., GOGAS, K.R., EGLEN, R.M. & HUNTER, J.C. (1998). Distribution of the tetrodotoxin-resistant sodium channel PN3 in rat sensory neurons in normal and neuropathic conditions. *J. Neurosci.,* **18,** 2174–2187.

QUASTHOFF, S., GROSSKREUTZ, J., SCHRODER, J.M., SCHNEIDER, U. & GRAFE, P. (1995). Calcium potentials and tetrodotoxin-resistant sodium potentials in unmyelinated C fibres of biopsied human sural nerve. *Neuroscience,* **69,** 955–965.

RANG, H.P.R. & RITCHIE, J.M. (1988). Depolarization of non-myelinated fibers of the rat vagus nerve produced by activation of protein kinase C. *J. Neurosci.,* **8,** 2606–2617.

SANGAMESWARAN, L., DELGADO, S.G., FISH, L.M., KOCH, B.D., JAKEMAN, L.B., STEWART, G.R., SZE, P., HUNTER, J.C., EGLEN, R.M. & HERMAN, R.C. (1996). Structure and function of a novel voltage-gated, tetrodotoxin-resistant sodium channel specific to sensory neurons. *J. Biol. Chem.,* **271,** 5953–5956.

SMITH, J.A., AMAGASU, S.M., EGLEN, R.M., HUNTER, J.C. & BLEY, K.R. (1998). Characterization of prostanoid receptor-evoked responses in rat sensory neurones. *Br. J. Pharmacol.,* **124,** 513–523.

STEFFENS, H., HOHEISEL, U., EEK, B. & MENSE, S. (2001). Tetrodotoxin-resistant conductivity and spinal effects of cutaneous C-fibre afferents in the rat. *Neurosci. Res.,* **39,** 413–419.

STRASSMAN, A.M. & RAYMOND, S.A. (1999). Electrophysiological evidence for tetrodotoxin-resistant sodium channels in slowly conducting dural sensory fibres. *J. Neurophysiol.,* **81,** 413–424.

VILLIERE, V. & MCLACHLAN, E.M. (1996). Electrophysiological properties of neurons in intact rat dorsal root ganglia classified by conduction velocity and action potential duration. *J. Neurophysiol.,* **76,** 1924–1941.

WAXMAN, S.G., DIB-HAJJ, S., CUMMINS, T.R. & BLACK, J.A. (1999). Sodium channels and pain. *Proc. natl. Acad. Sci. U.S.A.,* **96,** 7635–7639.

WEINREICH, D. & WONDERLIN, W.F. (1987). Inhibition of calcium-dependent spike after-hyperpolarization increases excitability of rabbit visceral sensory neurones. *J. Physiol.,* **394,** 415–427.

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