



Effects of potassium channel and $\text{Na}^+ - \text{Ca}^{2+}$ exchange blockers on the responses of slowly adapting pulmonary stretch receptors to hyperinflation in flecainide-treated rats

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1 The effects of K^+ channel blockers, such as 4-aminopyridine (4-AP) and tetraethylammonium (TEA), and a reverse-mode $\text{Na}^+ - \text{Ca}^{2+}$ exchange blocker, 2-[2-[4-(4-nitrobenzyloxy) phenyl] ethyl] isothiouria methanesulphonate (KB-R7943), on the responses of slowly adapting pulmonary stretch receptor activity to hyperinflation (inflation volume=3 tidal volumes) were investigated in anaesthetized, artificially ventilated, unilaterally vagotomized rats after pretreatment with a Na^+ channel blocker flecainide. The administration of flecainide (9 mg kg^{-1}) at a dose greater than that which abolished $50 \mu\text{g kg}^{-1}$ veratridine-induced SAR stimulation also inhibited hyperinflation-induced stimulation of SARs.

2 In flecainide-treated animals, administration of 4-AP (0.7 and 2 mg kg^{-1}) stimulated SAR activity during normal inflation and also caused a partial blockade of hyperinflation-induced SAR inhibition.

3 The discharges of SARs during normal inflation in flecainide-treated animals were not significantly altered by administration of either TEA (2 and 7 mg kg^{-1}) or KB-R7943 (1 and 3 mg kg^{-1}), but both K^+ channel and $\text{Na}^+ - \text{Ca}^{2+}$ exchange blockers partially attenuated hyperinflation-induced SAR inhibition.

4 These results suggest that hyperinflation-induced SAR inhibition in the presence of flecainide (9 mg kg^{-1}) involves the activation of several K^+ conductance pathways.

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Keywords: Slowly adapting pulmonary stretch receptor; Na^+ channel blocker; hyperinflation; K^+ channel blocker; $\text{Na}^+ - \text{Ca}^{2+}$ exchange blocker

Abbreviations: 4-AP, 4-aminopyridine; I_A , a fast-inactivating A-type current; I_K , a sustained K^+ current of delayed-rectifier type; KB-R7943, 2-[2-[4-(4-nitrobenzyloxy) phenyl] ethyl] isothiouria methanesulphonate; MBP, mean blood pressure; P_T , tracheal pressure; SAR, slowly adapting pulmonary stretch receptor; TEA, tetraethylammonium; V_T , tidal volume

Introduction

During the generation of action potentials, Na^+ conductance plays an important role in the depolarization of the nerve cell membranes. The Na^+ channel opener veratridine in neural tissues is voltage-dependent (Corbett & Kreuger, 1989; Castillo *et al.*, 1992) and tetrodotoxin (TTX)-sensitive (Narahashi, 1974; Gatteral, 1992). Pretreatment with flecainide, one of the Na^+ channel blockers (Banitt *et al.*, 1977; Campbell & Williams, 1983), prevents veratridine-induced slowly adapting pulmonary stretch receptor (SAR) stimulation in the rabbit (Matsumoto & Shimizu, 1995; Matsumoto *et al.*, 1998) and rat (Matsumoto *et al.*, 2000).

The ion channels responsible for mechanically activated currents in spider mechanoreceptors are mainly selective for Na^+ (Juusola *et al.*, 1994; Höger *et al.*, 1997). The mechanical deformation through stretch or distention of the airway smooth muscle is thought to stimulate SARs which have stretch-activated (SA) channels on the receptor endings

but the mechanism mediating the mechanosensitivity of SARs remains to be determined. In the rat, flecainide treatment (6 mg kg^{-1}) sufficient to abolish $50 \mu\text{g kg}^{-1}$ veratridine-induced SAR stimulation has no significant effect on excitation of the receptor activity seen during hyperinflation (Matsumoto *et al.*, 2000). The explanation for such an effect is that flecainide-resistant Na^+ channels in SARs contribute to excitation of the receptor activity during hyperinflation.

Three prevalent types of K^+ channels, characterized by kinetic and pharmacological properties, have been identified as 'fast transient outward' (Connor & Stevens, 1971), 'delayed rectifier' (Hodgkin & Huxley, 1952) and ' Ca^{2+} -activated K^+ ' (Meech & Standen, 1975) currents. In both the repolarization and after-hyperpolarization phases of action potentials, K^+ conductances have the ability to regulate the number of spikes with a cycle of Na^+ inflow and K^+ outflow. Both a fast-inactivating A-type current (I_A) and a sustained K^+ current of delayed-rectifier type (I_K) in addition to a Ca^{2+} -activated K^+ current (I_{KCa}) are responsible for the hyperpolarizing effect which inhibits the number of action

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potentials. When considering together the hyperpolarization caused by the activation of I_A , I_K , or I_{KCa} and the inhibitory effect of flecainide (9 mg kg⁻¹) on hyperinflation-induced SAR stimulation (Matsumoto *et al.*, 2000), it can be assumed that hyperinflation exerts an inhibitory effect on the activity of SARs through modification of K⁺ currents, particularly under conditions in which Na²⁺-dependent veratridine-induced SAR stimulation was blocked with flecainide.

To test this assumption, we performed three different types of experiments in anaesthetized, artificially ventilated rats after unilateral vagotomy. First, the responses of SARs to hyperinflation were examined before and after administration of 4-aminopyridine (4-AP), a K⁺ channel blocker inhibiting the I_A , in the rats after flecainide treatment. Secondly, the responses of SARs to hyperinflation were compared before and after administration of tetraethylammonium (TEA), a K⁺ channel blocker inhibiting the I_K , in flecainide-treated rats. Thirdly, in rats after flecainide treatment the changes in SAR activity in response to hyperinflation were examined before and after administration of 2-[2-[4-(4-nitrobenzyloxy) phenyl] ethyl] isothiurea methanesulphonate (KB-R7943), which inhibits the inward Na⁺-Ca²⁺ exchange current more potently than the outward Na⁺-Ca²⁺ exchange current in guinea-pig ventricular cells (Watano *et al.*, 1996). We selected either mostly inflationary SARs, firing throughout the whole respiratory cycle, or inflationary SARs, as described previously (Tsubone, 1986; Bergren & Peterson, 1993).

Methods

Animal preparation

The experiments were performed on 18 Wistar rats (280–350 g). All experimental protocols used in this study were approved by the Animal Use and Care Committee of Nippon Dental University. Each animal was first anaesthetized with sodium pentobarbital (45–50 mg kg⁻¹) intraperitoneally. The trachea was reached through a middle incision in the neck and cannulated below the larynx. In order to obtain sufficient space for liquid paraffin, the trachea and esophagus were dissected free and retracted rostrally. Tracheal pressure (P_T) was measured by connecting a polyethylene catheter inserted into the tracheal tube to a pressure transducer. The right carotid artery was cannulated for continuous monitoring of blood pressure (BP). The left vagus nerve was exposed and sectioned, whereas the right vagus nerve was left intact. Rectal temperature was maintained at around 37°C by means of a heating pad or lamp. The animals were paralyzed with intravenous administration of gallamine (5–10 mg kg⁻¹) for 30–60 min, and additional doses (3–5 mg kg⁻¹) of gallamine were administered to eliminate spontaneous respiratory movements, as required. The stroke volume of the respirator was set at 10 ml kg⁻¹ and its frequency ranged from 50 to 60 cycles min⁻¹. Tracheal CO₂ was monitored with a CO₂ gas analyser (Respina IH 26, Sanei) and kept at approximately 32–35 mmHg. Anaesthesia was maintained with additional doses of 15–20 mg kg⁻¹ h⁻¹ through a cannula inserted into the right jugular vein. In order to assess the level of anaesthesia, we used corneal and tail-pinch reflexes. Abrupt changes in BP induced by these two manoeuvres

were monitored to ensure that the animals remained fully anaesthetized during neuromuscular blockade.

Measurement of slowly adapting pulmonary stretch receptors

The peripheral end of the cut left vagus nerve was desheathed. To record the single unit activity of SARs, thin strands containing afferent nerve fibres were separated, placed on a unipolar silver electrode and submerged in a pool of liquid paraffin (37–38°C). The SARs were identified, on the basis of their firing behaviour during inflation, as follows: (1) The most inflationary SARs increased their activity during inflation and decreased their discharge during deflation, and in the case of inflationary SARs, those receptors increased their activity during inflation only. (2) The increase in SAR activity was proportional to that in the inflation volume of the respirator. (3) The discharge of SARs continued as long as the tracheal tube was occluded in a hyperinflated condition. The SAR activity was amplified and selected by means of a window discriminator for counting the number of impulses.

Experimental design

The experiments were designed to test the roles of generalized K⁺ channels and a reverse-mode Na⁺-Ca²⁺ exchanger in the SAR responses to hyperinflation, particularly under conditions in which flecainide treatment was more than sufficient to abolish veratridine-induced SAR stimulation. (1) In six SAR fibres in case of six rats, the effects of hyperinflation for approximately 10 respiratory cycles on SAR activity and P_T were determined. Ten minutes after flecainide administration at a dose (9 mg kg⁻¹) sufficient to block 50 µg kg⁻¹ veratridine-induced SAR stimulation, the same sets of experiments were repeated, and we confirmed hyperinflation-induced SAR inhibition, which fell below the discharge rate of SARs during normal inflation in flecainide-treated animals. Finally, 10 min after 4-AP administration (0.7 and 2 mg kg⁻¹) in flecainide-treated animals, the same tests were repeated under the same conditions. (2) In six SAR fibres in case of six rats, the changes in SAR activity and P_T in response to hyperinflation were examined before and after administration of flecainide (9 mg kg⁻¹) and, subsequently, after TEA (2 and 7 mg kg⁻¹) in the presence of flecainide. (3) In six SAR fibres in case of six rats, the responses of SARs and P_T to hyperinflation were determined. The absence of an effect of veratridine (50 µg kg⁻¹) on SAR activity and the occurrence of hyperinflation-induced SAR inhibition were confirmed after administration of flecainide (9 mg kg⁻¹). Subsequently, after KB-R7943 administration (1 and 3 mg kg⁻¹) the effects of hyperinflation on SAR activity and P_T were examined under the same conditions. In these experiments, the effectiveness of flecainide was indicated by the absence of increased SAR activity after veratridine administration.

Drugs

4-AP and TEA were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 4-AP (10 mg) and TEA (10 mg) were dissolved in and diluted with 0.9% NaCl. Veratridine (Sigma)

was dissolved in a small amount of weak HCl and diluted with 0.9% NaCl. Flecainide (10 mg, Eizai Pharmaceutical Co. Ltd., Tokyo, Japan) was dissolved in 5.0% glucose solution. KB-R7943 (10 mg, Kanebo, Osaka, Japan) was dissolved in dimethylsulphoxide (DMSO) and the final concentration of DMSO was 0.1% or less.

Statistical analysis

Under control conditions, the firing rates of SARs in each whole respiratory cycles were measured over several respiratory cycles and expressed as imp s^{-1} . The SAR responses to hyperinflation (inflation volume = $3 V_T$) for approximately 10 respiratory cycles and to veratridine administration ($50 \mu\text{g kg}^{-1}$) were obtained by counting the firing rates of receptors during hyperinflation and between the onset of increased receptor activity and recovery to the control level, respectively, and the average activities of SARs during one whole respiratory cycle were expressed as imp s^{-1} . Similarly, control values for P_T were averaged over several respiratory cycles and expressed as cmH_2O . The responses of P_T to hyperinflation and veratridine administration were obtained by measuring the respiratory parameter, as described above, and the average values for P_T were expressed as cmH_2O . The statistical significance of the effects of flecainide (9 mg kg^{-1}) on the responses of SAR activity and P_T to veratridine administration ($50 \mu\text{g kg}^{-1}$), normal inflation and hyperinflation was first calculated by a one-way ANOVA for repeated measurements. In flecainide (9 mg kg^{-1})-treated animals before and after administration of 4-AP (0.7 and 2 mg kg^{-1}), TEA (2 and 7 mg kg^{-1}) and KB-R7943 (1 and 3 mg kg^{-1}), the changes in SAR activity during hyperinflation were compared those during normal inflation, by using a paired *t*-test. All values were expressed as the means \pm s.e.means. A value of less than 0.05 was considered statistically significant.

Results

Effects of flecainide on responses of SARs to veratridine administration and hyperinflation

Typical examples of the effects of veratridine on SAR activity and P_T before and after pretreatment with flecainide are shown in Figure 1a,b. Intravenous administration of veratridine ($50 \mu\text{g kg}^{-1}$) caused vigorous stimulation of the SAR activity during both inflation and deflation, and this effect lasted for about 100 s. Under these conditions, the value for P_T did not change significantly (Figure 1a). Flecainide treatment at a dose which blocked veratridine-induced SAR stimulation decreased the discharge of SARs during normal inflation (Figure 1b). In the same preparation, before flecainide treatment, the SAR activity increased during hyperinflation (Figure 1c). As shown in Figure 1d, in a flecainide-treated animal the magnitude of decreased SAR activity provoked by hyperinflation became more prominent than that seen during normal inflation. Figure 2a,b summarize the effects of flecainide on the SAR and P_T responses to veratridine administration ($50 \mu\text{g kg}^{-1}$) during normal inflation ($1 V_T$). During normal inflation, veratridine administration ($50 \mu\text{g kg}^{-1}$) caused approximately 2.4 fold

increase in the SAR activity and this increase was abolished by pretreatment with flecainide (9 mg kg^{-1}), which significantly reduced the baseline discharges of SARs. The changes in P_T in responses to normal ventilation were not significantly altered by administration of either flecainide or veratridine. The responses of 18 different SAR fibres to hyperinflation before and after pretreatment with flecainide at 9 mg kg^{-1} are summarized in Figure 2c. Before flecainide treatment, hyperinflation caused an approximately 3.6 fold increase in SAR activity but pretreatment with flecainide (9 mg kg^{-1}), which resulted in a significant inhibition of SAR activity during normal inflation, caused a greater inhibition on hyperinflation-induced SAR stimulation. The Na⁺ channel blocker flecainide at a dose of 9 mg kg^{-1} inhibited the SAR responses to hyperinflation more efficaciously than that to normal inflation but had no significant effect on the responses of P_T to normal inflation and hyperinflation (Figure 2c,d). The decreases in BP occurred during hyperinflation as well as after flecainide administration in normal inflation, but the hypotension evoked by hyperinflation in the presence of flecainide was small. The mean BP (MBP) values were 98.7 ± 2.6 and 81.2 ± 2.1 mmHg during normal inflation and hyperinflation, respectively, and in the presence of flecainide, 83.4 ± 2.9 and 80.9 ± 2.3 mmHg during normal inflation and hyperinflation, respectively.

Effects of 4-AP on responses of SARs to hyperinflation in flecainide-treated animals

Figure 3a shows the responses of SAR activity and P_T to normal inflation and hyperinflation in a flecainide (9 mg kg^{-1})-treated animal. Flecainide treatment caused hyperinflation-induced SAR inhibition. Under normal inflation, subsequent administration of 4-AP (2 mg kg^{-1}) increased SAR activity during deflation but did not significantly alter P_T (Figure 3b). In the presence of flecainide, the inhibitory response of SAR activity to hyperinflation was partially attenuated by 4-AP treatment (Figure 3c). Figure 4a summarizes the effect of 4-AP (0.7 and 2 mg kg^{-1}) on the responses of SARs to hyperinflation in the presence of flecainide (9 mg kg^{-1}). The K⁺ channel blocker 4-AP (0.7 and 2 mg kg^{-1}) significantly reversed the inhibitory effect of hyperinflation on SAR activity in flecainide-treated animals (per cent change: absence, -41.6 ± 5.2 , $n=6$; in the presence of 4-AP, 0.7 mg kg^{-1} , -7.8 ± 1.2 , $n=6$, $P<0.05$; 2 mg kg^{-1} , $+51.1 \pm 5.5$, $n=6$, $P<0.05$). The administration of 4-AP in flecainide-treated animals caused an increase in BP, but this effect was short-lasting. During hyperinflation the MBP values were 80.4 ± 4.9 , 81.2 ± 4.4 and 82.4 ± 3.8 mmHg after flecainide treatment without and with 4-AP administration at 0.7 and 2 mg kg^{-1} , respectively. No significant effects of 4-AP (0.7 and 2 mg kg^{-1}) on the P_T responses to hyperinflation were found in flecainide-treated animals (Figure 4b).

Effects of TEA on responses of SARs to hyperinflation in flecainide-treated animals

After administration of flecainide (9 mg kg^{-1}), hyperinflation inhibited SAR activity. Subsequent administration of TEA (7 mg kg^{-1}) that did not change the discharge rate of SARs during normal inflation caused a partial blockade of hyperinflation-induced SAR inhibition in a flecainide-treated

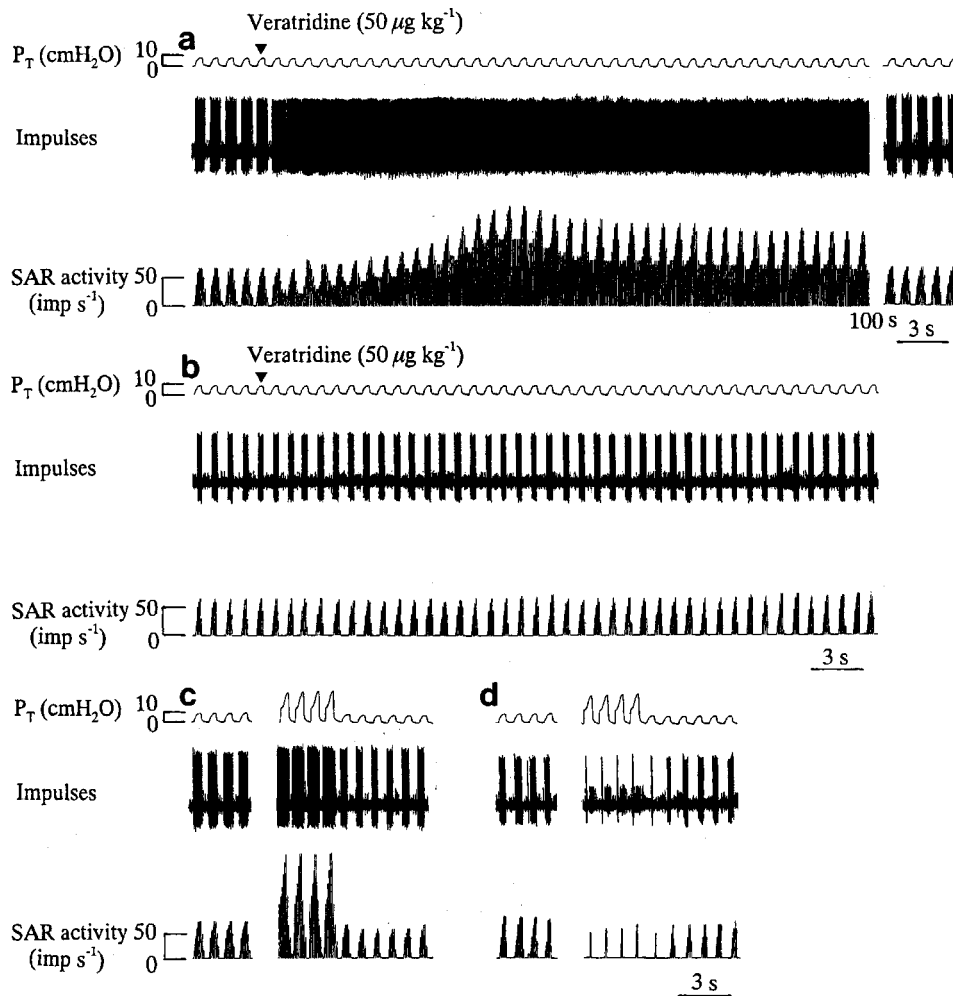


Figure 1 Responses in P_T and SAR activity to veratridine before (a) and after (b) administration of flecainide (9 mg kg⁻¹). 100 s indicates the elapsed time between recordings. Responses in P_T and SAR activity to control inflation (inflation volume = 1 V_T) and hyperinflation (inflation volume = 3 V_T) before (c) and after (d) administration of flecainide (9 mg kg⁻¹) in the same rat.

animal (Figure 5a,b). In six rats, pretreatment with flecainide (9 mg kg⁻¹) greatly inhibited stimulation of SAR activity by hyperinflation. As shown in Figure 5c, subsequent administration of TEA (7 mg kg⁻¹) in those animals partially attenuated hyperinflation-induced SAR inhibition (per cent change; absence, -56.3 ± 6.2 , $n=6$; in the presence of TEA, 2 mg kg⁻¹, -49.8 ± 5.4 , $n=6$, $P>0.05$; 7 mg kg⁻¹, $+7.0 \pm 1.1$, $n=6$, $P<0.05$). The MBP values were 82.6 ± 3.5 , 80.9 ± 3.8 and 80.2 ± 4.3 mmHg during hyperinflation after flecainide treatment without and with TEA administration 2 and 7 mg kg⁻¹, respectively. TEA treatment had no significant effect on the P_T and MBP responses to hyperinflation in flecainide-treated animals (Figure 5d).

Effects of KB-R7943 on responses of SARs to hyperinflation in flecainide-treated animals

As shown in Figure 6a,b, hyperinflation caused inhibition of the SAR activity in a flecainide (9 mg kg⁻¹)-treated animal, and this inhibitory effect was partially attenuated by subsequent administration of a reverse-mode Na⁺-Ca²⁺ exchange blocker, KB-R7943 (3 mg kg⁻¹). Figure 6c summarizes the effects of KB-R7943 (1 and 3 mg kg⁻¹) on the

responses of SAR activity to hyperinflation in six flecainide (9 mg kg⁻¹)-treated animals. The inhibitory effect of hyperinflation on SAR activity in the presence of flecainide was partially attenuated by the Na⁺-Ca²⁺ exchange blocker KB-R7943 (1 and 3 mg kg⁻¹) (per cent change: absence, -57.4 ± 6.3 , $n=6$; in the presence of KB-R7943, 1 mg kg⁻¹, -26.1 ± 3.4 , $n=6$, $P<0.05$; 3 mg kg⁻¹, $+43.2 \pm 5.5$, $n=6$, $P<0.05$). In addition, no significant effects of KB-R7943 on the P_T responses to normal inflation and hyperinflation were found in flecainide-treated animals (Figure 6d). During hyperinflation the MBP values were 81.1 ± 3.9 , 80.8 ± 3.8 and 80.5 ± 4.2 after flecainide treatment without and with KB-R7943 administration at 1 and 3 mg kg⁻¹. In flecainide-treated animals, administration of DMSO, a solvent of KB-R7943, at a final concentration (0.1%) did not cause any significant change on SAR activity (Figure 6e).

Discussion

In the present study we demonstrated that K⁺ channel blockers and a reverse mode Na⁺-Ca²⁺ exchange blocker attenuated flecainide-induced inhibition of hyperinflation-

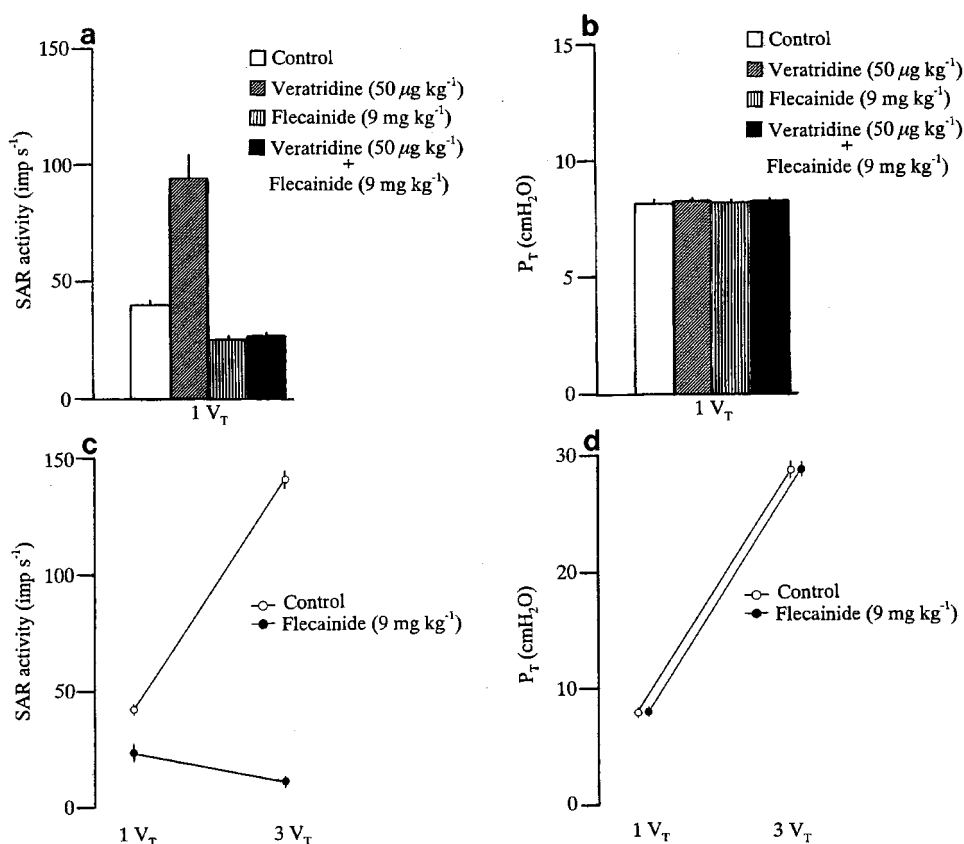


Figure 2 Responses in SAR activity (a) and P_T (b) to veratridine at control inflation (inflation volume = $1 V_T$). Changes in SAR activity (c) and P_T (d) in response to control inflation and hyperinflation (inflation volume = $3 V_T$) before and after pretreatment with flecainide (9 mg kg^{-1}). Values are the means for 18 animals and the vertical bars show the s.e.means.

induced SAR stimulation in rats. Our data suggest that K⁺ conductance pathways are involved in the hyperpolarization of SARs in the presence of flecainide.

Because TTX can block veratridine-induced increases in intracellular Na⁺ concentration $[Na^+]_i$ in cultured rat hippocampal neurons (Rose & Ransom, 1997) and because vigorous stimulation in SARs by veratridine is completely blocked by flecainide treatment in the rabbit (Matsumoto & Shimizu, 1995; Matsumoto *et al.*, 1998) and rat (Matsumoto *et al.*, 2000), veratridine-induced SAR stimulation appears to be mediated by TTX-sensitive Na⁺ channels, but not by TTX-insensitive Na⁺ channels. In a previous study in rats, we found that flecainide treatment at a dose (6 mg kg^{-1}), which abolished 50 µg kg^{-1} veratridine-induced SAR stimulation, had no significant effect on the excitation of SAR activity during hyperinflation (inflation volume = $3 V_T$) (Matsumoto *et al.*, 2000). This finding may be consistent with the observation that both TTX-sensitive and TTX-insensitive Na⁺ channel currents are present in the peripheral terminals of primary afferent nociceptive-like neurons (Brock *et al.*, 1998). Presumably, TTX-resistant Na⁺ channels in SARs contribute at least to hyperinflation-induced stimulation of the receptor activity in flecainide (6 mg kg^{-1})-treated rats. However, the mechanism mediating the mechanosensitivity of SARs needs further elucidation. In *in vivo* preparations, the different flecainide concentration at the site of SAR endings is thought to be changed by several factors, for example, connective tissue barrier, dilution of tissue fluid

and transport in the circulation. In this study, flecainide administration at a dose (9 mg kg^{-1}) which blocked 50 µg kg^{-1} veratridine-induced SAR stimulation usually inhibited the basal discharge of SARs. Under these conditions, the magnitude of decreased SAR activity during hyperinflation became more prominent than that during normal inflation, indicating that hyperinflation itself in flecainide-treated animals may elicit the hyperpolarizing response which inhibits SAR activity.

Two broad classes of voltage-gated K⁺ channel currents have been distinguished on the basis of differences in time- and voltage-dependent properties: (1) a typical fast-inactivating A-type current (I_A) and (2) a more sustained K⁺ current of the delayed-rectifier type (I_K) (Halliwell, 1990; Storm, 1988). Two pharmacologically different types of K⁺ channels have also been identified in mammalian myelinated axons in the peripheral (Baker *et al.*, 1987; Kocsis *et al.*, 1987) and central (Gordon *et al.*, 1989) nervous system: I_A is blocked by 4-AP and I_K is blocked by TEA. There is a functional difference between I_A and I_K in the myelinated axons of the rat sciatic nerve fibres because I_A is responsible for action potential repolarization and I_K causes the afterhyperpolarization (AHP) after repetitive firing (Kocsis *et al.*, 1987). Although the application of 4-AP is known to elicit the broad spike of action potentials (Kocsis *et al.*, 1987; Poulter & Padjen, 1995), such an effect could not be confirmed in this study because we were measuring extracellular action potentials. The administration of 4-AP in the presence of

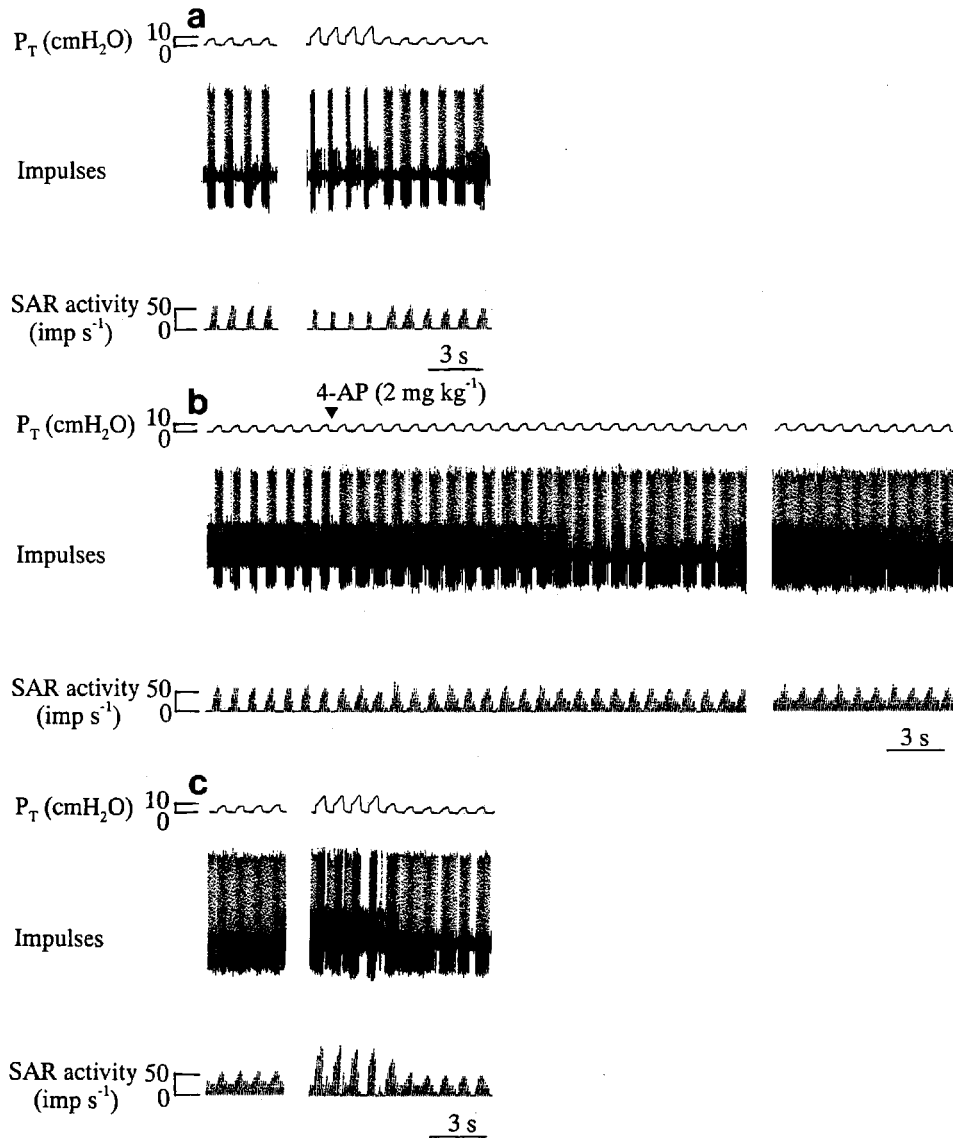


Figure 3 Responses in P_T and SAR activity to control inflation (inflation volume = 1 V_T) and hyperinflation (inflation volume = 3 V_T) before (a), during (b) and after administration of 4-AP (2 mg kg⁻¹) in a flecainide (9 mg kg⁻¹)-treated animal.

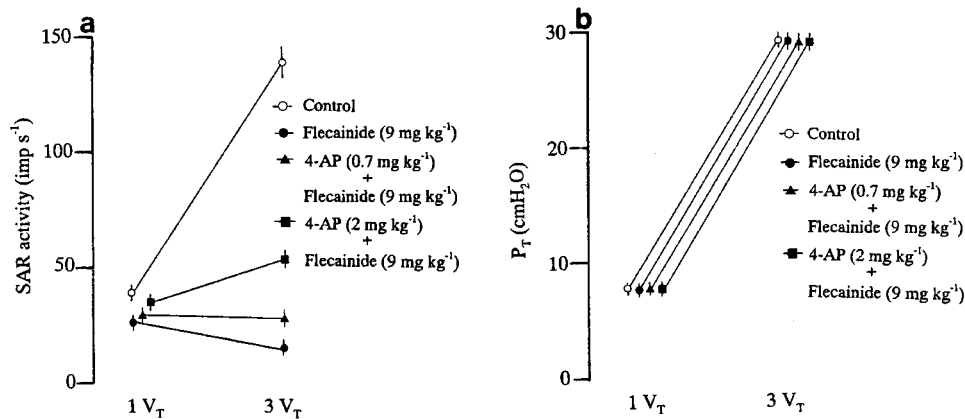


Figure 4 Changes in SAR activity (a) and P_T (b) in response to control inflation (inflation volume = 1 V_T) and hyperinflation (inflation volume = 3 V_T) before and after pretreatment with 4-AP. Values are the means for six flecainide (9 mg kg⁻¹)-treated animals and the vertical bars shown are the s.e.means.

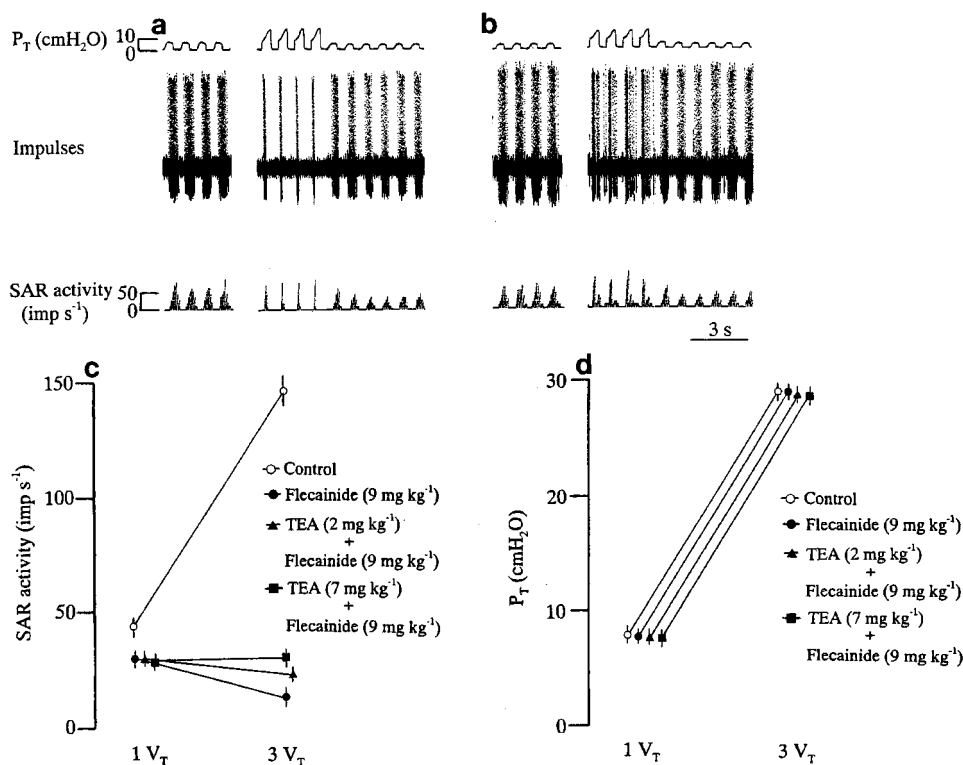


Figure 5 Responses in P_T and SAR activity to control inflation (inflation volume = 1 V_T) and hyperinflation (inflation volume = 3 V_T) before (a) and after (b) administration of TEA (7 mg kg⁻¹) in a flecainide (9 mg kg⁻¹)-treated animal. Changes in SAR activity (c) and P_T (d) in response to control inflation and hyperinflation before and after pretreatment with TEA. Values are the means for six flecainide (9 mg kg⁻¹)-treated animals and the vertical bars show the s.e.means.

flecainide increased the discharge of SARs, particularly in the deflation phase, in a dose-dependent manner, as reported in a previous study in the rabbit without flecainide treatment (Matsumoto *et al.*, 1999), and caused a pressor effect (Chang *et al.*, 1996; Matsumoto *et al.*, 1999), which was short lasting. Because 4-AP can elicit both membrane depolarization and repetitive firing in squid axons (Yeh *et al.*, 1976a,b), blockade of I_A by 4-AP, even in the presence of Na⁺ channel blocker flecainide, could increase the expiratory discharge of SARs, in which there was no mechanical deformation due to lung inflation. We also found that 4-AP treatment resulted in a partial blockade of hyperinflation-induced SAR inhibition in flecainide-treated animals. This finding may be similar to a report demonstrating that administration of 4-AP antagonizes saxitoxin (STX)- and TTX-induced cardiorespiratory depression in anaesthetized guinea-pigs (Chang *et al.*, 1996). Accordingly, a partial blockade of hyperinflation-induced SAR inhibition by 4-AP in the presence of flecainide is mediated by attenuation of the hyperpolarizing effect.

There are some difficulties in separating I_A and I_K, pharmacologically but the slow TEA-sensitive K⁺ conductance channel is prevalent in the nodal membrane of rat myelinated nerve fibres (Roper & Schwartz, 1989). Baker *et al.* (1987) also reported a similar localization of TEA-sensitive K⁺ channels. In a study using sucrose gap and intra-axonal recording of the isolated sciatic nerve fibres, TEA application had little effect on the wave form of compound action potentials in neither immature nor mature rats but blocked 4-AP-induced postspike positivity, and a pronounced AHP seen after repetitive stimulation was

blocked by TEA (Eng *et al.*, 1988). From these observations, it is possible to conclude that the TEA-sensitive I_K does not activate the early phase of action potential repolarization but contributes to the later phase of AHP, back to the resting potential. Indeed, in this study the administration of TEA at any of the doses examined did not significantly alter the activity of SARs in flecainide-treated animals, but we found that TEA treatment caused a partial blockade of hyperinflation-induced SAR inhibition in those animals. The results indicate that blockade of TEA-sensitive K⁺ channels partially inhibits the hyperpolarizing response of SARs to hyperinflation in the presence of the Na⁺ channel blocker flecainide.

The existence of an endogenous Na⁺-Ca²⁺ exchanger has been demonstrated in myelinated axons (Stys *et al.*, 1991; 1992). The exchanger maintains intracellular Ca²⁺ concentration ([Ca²⁺]_i) homeostasis by pumping Ca²⁺ out of cells under normal conditions. However, when the Na ion gradient is reversed, the exchanger increases the level of [Ca²⁺]_i (Mullins *et al.*, 1985; DiPolo & Beaugé, 1987). In an anoxic optic nerve model, Stys *et al.* (1990) suggested that Ca²⁺ enters the intracellular component *via* a route that is independent of voltage-gated Ca²⁺ channels. Furthermore, *in vivo* experiments on guinea-pigs, KB-R7943 can block the inward Na⁺-Ca²⁺ exchange current providing protection of Ca²⁺ overload on ouabain-induced arrhythmias (Watano *et al.*, 1999). If Ca²⁺ influx into the SAR endings occurred during hyperinflation in flecainide-treated animals, one could expect that pharmacological inhibition of the inward Na⁺-Ca²⁺ exchange current should reduce Ca²⁺ entry responsible for the hyperpolarizing response stimulating Ca²⁺-activated

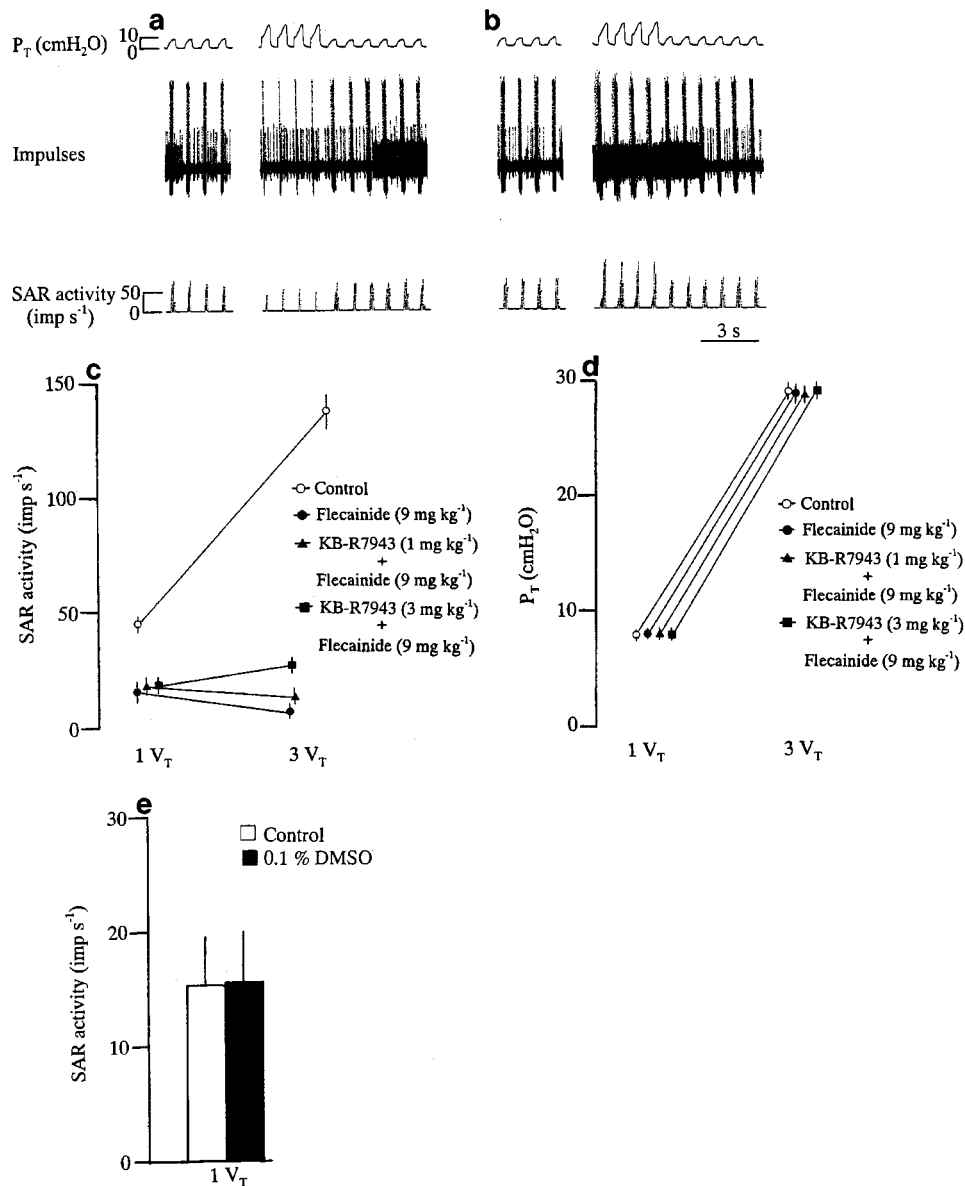


Figure 6 Responses in P_T and SAR activity to control inflation (inflation volume = 1 V_T) and hyperinflation (inflation volume = 3 V_T) before (a) and after (b) administration of KB-R7943 (3 mg kg^{-1}) in a flecainide (9 mg kg^{-1})-treated animal. Changes in SAR activity (c) and P_T (d) in response to control inflation and hyperinflation before and after pretreatment with KB-R7943. Changes in SAR activity (e) induced by DMSO administration at control inflation. Values are the means for six flecainide (9 mg kg^{-1})-treated animals and the vertical bars show the s.e.means.

K⁺ channel (Leblond & Krnjevic, 1989). In flecainide-treated rats, we found that administration of KB-R7943 (1 and 3 mg kg^{-1}), which had no significant effect on SAR activity during normal inflation, produced a partial blockade of hyperinflation-induced SAR inhibition. This implies that during hyperinflation in flecainide-treated animals, the presumed rise in free $[Ca^{2+}]_i$ in the SAR endings *via* the activation of a reverse-mode of the Na⁺-Ca²⁺ exchanger may stimulate Ca²⁺-activated K⁺ channels and, as a result, cause the hyperpolarizing effect of SARs. On the other hand, there is a report that the solvent DMSO of KB-R7943 has the ability to inhibit Na⁺-K⁺ ATPase activity (Chiou & Vesely, 1995). In this study we could not find any evidence that administration of DMSO alone at a final concentration (0.1%) alters the discharge of SARs.

In conclusion, we have shown that administration of flecainide at a dose sufficient to abolish veratridine-induced SAR stimulation resulted in strong inhibition of hyperinflation-induced SAR stimulation, and that the two K⁺ channel blockers (4-AP and TEA) and a reverse mode Na⁺-Ca²⁺ exchange blocker (KB-R7943) partially attenuated hyperinflation-induced SAR inhibition. The results suggest that the effect of hyperinflation on SAR activity in the presence of flecainide at a higher dose would promote hyperpolarizing response of SARs *via* the activation of K⁺ conductance pathways.

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