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Actions of the anaesthetic Saffan on rat sympathetic preganglionic neurones *in vitro*

[1]M.F. Nolan, I.C. Gibson & S.D. Logan

Department of Biomedical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, Scotland AB25 2ZD

1 Whole-cell patch-clamp recordings were used to investigate the effects of the anaesthetic Saffan on the electrophysiological properties of sympathetic preganglionic neurones (SPNs) in rat spinal cord slices.

2 Saffan (1–54 μM) abolished or reduced the frequency of spontaneous action potential firing and abolished spontaneous, sub-threshold membrane potential oscillations. Saffan caused dose-dependent decreases in input resistance and depending upon the initial resting membrane potential, either a depolarization, a hyperpolarization or no change in membrane potential.

3 Responses to Saffan were blocked by the $GABA_A$ receptor antagonists bicuculline (5–20 μM) and picrotoxin (20 μM), but not by the glycine receptor antagonist strychnine (20 μM) indicating that they were mediated by $GABA_A$ receptors.

4 Changes in the properties of SPN action potentials were also observed. In the presence of Saffan the amplitude and duration of the action potential after-hyperpolarization were reduced and larger depolarizations were required in order to evoke trains of action potentials.

5 To examine the effects of Saffan on electrotonic coupling between SPNs, experiments were performed with the Na^+ channel blocker QX-314 in the intracellular solution and antidromic oscillations were evoked by ventral root stimulation. Saffan failed to abolish antidromic oscillations, but reduced their amplitude and duration. This indicates that the abolition of spontaneous membrane potential oscillations was not a direct effect on the coupling between SPNs, but was a result of the abolition of spontaneous activity by Saffan.

6 The responses to Saffan occurred within the plasma concentration range of Saffan during anaesthesia, suggesting that the electrophysiological properties of SPNs may be altered during anaesthesia with Saffan. This would be expected to lead to changes in sympathetic tone and in the integration of sympathetic output.

Keywords: Sympathetic; preganglionic neurones; anaesthetic; Saffan; autonomic nervous system; GABA

Introduction

Changes in the functioning of the autonomic nervous system often accompany general anaesthesia. Such changes include modification of basal sympathetic discharge and of sympathetic reflexes (Blake & Korner, 1981; Kollai & Koizumi, 1981; Quail *et al*., 1985; Paton & Gilbey, 1992). In order to understand how these changes occur it is important to determine the actions of anaesthetics on individual neurones. One possible site for these effects is at sympathetic preganglionic neurones (SPNs). As SPNs mediate the sympathetic output from the central nervous system (CNS) to the periphery and integrate information from spinal and supraspinal regions involved in sympathetic control (Coote, 1988), any alteration of their membrane properties by an anaesthetic may have profound effects on the functioning of the sympathetic nervous system.

Saffan is an anaesthetic commonly used in experiments examining autonomic function (e.g. Hayes & Weaver, 1992; Louwerse & Marshall, 1993; McAllen & May, 1994). It contains alphaxalone and alphadolone in a 3:1 ratio by mass. Alphaxalone is the more active constituent, alphadolone which is added to improve the solubility of alphaxalone, is half as potent and additive to alphaxalone in its effects (Davis & Pearce, 1972). Alphaxalone-based anaesthetics have the advantage of a high therapeutic index, short action and quick recovery (Davis & Pearce, 1972). However, they have undesirable autonomic effects, including a rise in heart rate (Kollai & Koizumi, 1981), dose-dependent changes in mean arterial pressure (Quail *et al*., 1985) and modification of autonomic reflexes (Paton & Gilbey, 1992). A number of these effects have been suggested to result from actions within CNS regions involved in sympathetic regulation (Quail *et al*., 1985). Alphaxalone acts at $GABA_A$ receptors *in vivo* at similar concentrations to which it occurs *in vivo* during anaesthesia with Saffan (reviewed in Lambert *et al*., 1987). GABAergic synapses have been demonstrated impinging directly onto SPNs (Bogan *et al*., 1989), and GABA inhibits electrical activity in SPNs (Yoshimura & Nishi, 1982) by a $GABA_A$ mediated Cl^- current (Krupp & Feltz, 1993). SPNs are therefore a potential target site for the effects of Saffan. In this study we have used whole-cell patch-clamp recording to investigate the effects of Saffan on SPNs. A preliminary account of some of the data has been published (Nolan *et al*., 1996).

Methods

Preparation and maintenance of slices

Sprague-Dawley rats (7–16 days old) were anaesthetized with Enflurane (Abbott), decapitated, the spinal cord removed and 300–500 μm transverse thoracolumbar slices cut with a vibratome (Spanswick & Logan, 1990). The slices were kept in artificial cerebrospinal fluid (aCSF) of composition (mM): NaCl 127, KCl, 1.9, KH_2PO_4 1.2, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHCO_3$ 26, D-glucose 10, equilibrated with 95% O_2/5% CO_2 and were used between 1 and 24 h after preparation. During recording, slices were held in place between two grids, in a chamber continuously perfused with aCSF at a rate of 4–8 ml min^{-1}.

[1] Author for correspondence.

Recording methods

Whole-cell recordings were made in current-clamp mode from neurones in the intermediolateral column identified as SPNs as described previously (Pickering *et al*., 1991). The patch electrodes had resistance 3–12 MΩ and were filled with the following solution (mM): potassium gluconate 130, KCl 10, $MgCl_2$ 2, $CaCl_2$ 1, EGTA-Na 11, HEPES 10, Na_2ATP2 and either Lucifer yellow 2 or biocytin 5, pH adjusted to 7.4 with NaOH, osmolarity adjusted to 315 mOsm with sucrose. In some experiments 2 mM lignocaine N-ethyl bromide (QX-314) was also included in the patch electrode solution to block Na^+ channels in the recorded cell. In 9 experiments the concentration of EGTA-Na was reduced to 1 mM. This had no effect on the results described here. Recordings were made by use of either List EPC7 (List-medical) or Axopatch 1D (Axon Instruments) amplifiers. Data were displayed on-line on an oscilloscope (Gould DSO 1602) and chart recorder (Electrtomed, Multipulse 2) and digitized (Sony, PCM-701ES) and stored on videotape (Sony, SL-F25UB) for later playback and analysis. In some experiments data were also stored on a personal computer (Research Machines, 486DX) via a DigiData 1200 interface with pClamp6 software (Axon Instruments). Electrical stimulation of the ventral horn was performed with bipolar stimulating electrodes (Clarke Electromedical) in conjunction with an isolated stimulator (Digitimer DS2; stimulus intensity 2–12 V, pulse duration 1 ms). Antidromic responses were identified as such by a constant latency between the stimulus artefact and the base and peak of the response, a stable all or none threshold and faithful responses to high frequency stimulation.

Histological processing

The morphology of the neurones was examined to confirm their identity as SPNs. The histological techniques for processing of the slices were as described previously (Pickering *et al*., 1991; Logan *et al*., 1996). Neurones were viewed under a microscope (Zeiss, Axioskop) and identified by their location and axo-dendritic architecture (Pickering *et al*., 1991).

Drugs

The following drugs were used: Saffan (Pitman-Moore, U.K.) QX-314 (Research Biochemicals Inc., via Semat, U.K.), muscimol (Tocris Cookson, U.K.), γ-aminobutyric acid (GABA), strychnine, picrotoxin and bicuculline (Sigma, U.K.). In all experiments the stated concentration for Saffan, is that of the more active constituent alphaxalone. All drugs were applied to the slice by perfusion. Saffan was perfused for at least 5 min (responses generally reached a steady state within 4 min). To determine the effects of antagonists on Saffan responses, either: a control response to Saffan was obtained, Saffan was washed from the slice, then the antagonist was perfused alone for at least 5 min and then together with Saffan for a similar period to that used to obtain the control response; or Saffan was perfused onto the slice for at least 5 min then the antagonist was co-applied with Saffan for at least a further 5 min.

Data analysis

Membrane potential responses to agonists were identified as either depolarizations or hyperpolarizations if the agonist caused a shift in the membrane potential of >3 mV in the direction stated. Data were analysed off-line by the pClamp6 suite of programmes or Axoscope (Axon Instruments). Statistical analysis was carried out with StatView2 (Abacus Concepts Inc.). Mean values are presented as mean ± s.e.mean (n = number of neurones). The significance of differences between results was determined by Student's 2-tailed t test.

Results

Effects of Saffan on spontaneous activity

The effects of Saffan on the activity of 27 SPNs were examined by whole-cell recordings. They were divided into three groups with respect to spontaneous activity (see also Logan *et al*., 1996). Silent SPNs had a mean resting potential of -61 ± 2.4 mV ($n=10$, range -50 to -70 mV), did not fire action potentials at rest, but did in response to injection of depolarizing current; active SPNs had a mean resting membrane potential of -49 mV ± 1.1 mV ($n=9$, range -45 to -55 mV) and fired action potentials spontaneously; oscillating SPNs had a mean resting membrane potential of -52 mV ± 3.3 mV ($n=8$, range -40 to -70 mV) and showed sub-threshold oscillations in membrane potential which could result in action potential firing at their peak. Perfusion of Saffan to give steady-state alphaxalone concentrations of 1–54 μM abolished ($n=8$) or reduced the frequency ($n=1$) of spontaneous action potential firing in active SPNs and abolished oscillatory activity in spontaneously oscillating SPNs ($n=8$, Figure 1). Silent SPNs remained so in the presence of Saffan ($n=10$, Figure 1).

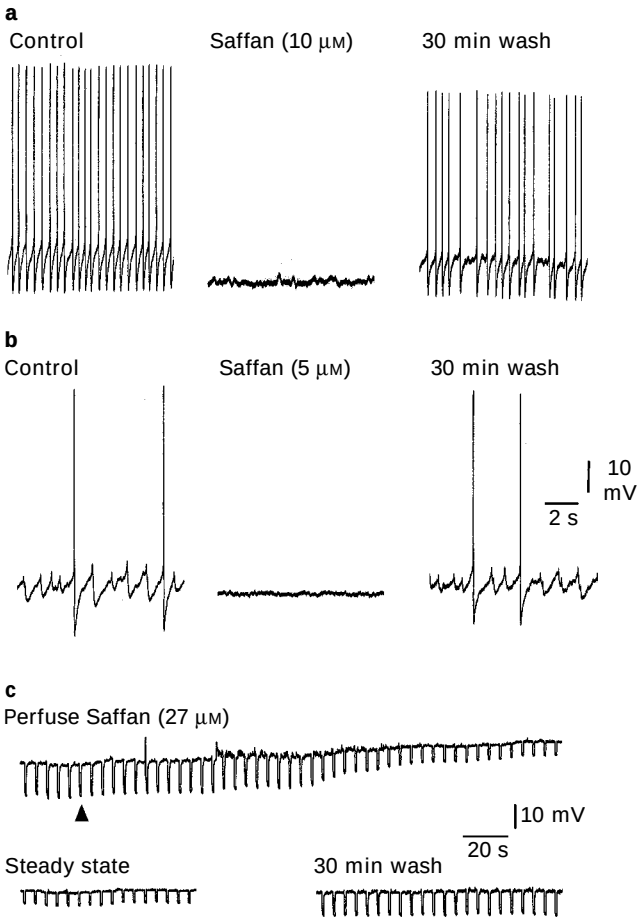

Figure 1 Current-clamp recordings from three different sympathetic preganglionic neurones (SPNs) demonstrating the effects of Saffan on spontaneous activity. (a) Saffan (10 μM) reversibly abolished spontaneous action potential firing in an active SPN. Scale bars as for (b). (b) Saffan (5 μM) reversibly abolished spontaneous subthreshold membrane potential oscillations. (c) Change in membrane potential and input resistance of a silent SPN during onset of perfusion, at steady state (5 min perfusion) and following wash (30 min) of Saffan (27 μM). The regular downward membrane potential deflections are caused by injection of negative current pulses down the recording electrode (amplitude 28 pA, duration 800 ms) every 5s. The decrease in the amplitude of the membrane potential response to the current pulses indicates a decrease in input resistance of the cell (from 536 MΩ to 326 MΩ).

Effects of Saffan on membrane potential and input resistance

Input resistances were calculated from the membrane potential responses to injection of small negative current steps (amplitude 10–30 pA, duration 800–1200 ms). During responses involving a membrane polarization, the membrane potential was returned to control level by injection of appropriate current, before the input resistance was calculated. The SPNs had a mean input resistance, determined at their initial resting membrane potential in the absence of drugs, of 570 ± 60 MΩ ($n = 18$, range 257–1150 MΩ). Saffan (2.7–54 μM) caused a dose-dependent decrease in input resistance in all three groups of SPN ($n = 17$ of 18 SPNs, Figures 2 and 3). During anaesthesia the concentration of alphaxalone in the plasma is in the range of 2–12 μM (Sear & Prys-Roberts, 1979). The decrease in input resistance of SPNs in response to Saffan occurred within a similar concentration range (Figure 3). Perfusion of Saffan to give an alphaxalone concentration in the bath of 10 μM or 5.4 μM, caused a $36.8 \pm 1.6\%$ ($n = 3$) or a $22.3 \pm 11.5\%$ ($n = 4$) decrease in input resistance, respectively. The majority of SPNs demonstrate a delay during depolarization from negative membrane potentials caused by activation of a transient outwardly rectifying conductance (Pickering *et al*., 1991). In the presence of Saffan the duration of the delay was reduced (Figure 2). SPNs also show inward rectification following injection of large negative current pulses (Pickering *et al*., 1991). This also appeared to be reduced in the presence of Saffan (not shown).

Perfusion of Saffan was accompanied by changes in membrane potential. The direction of the membrane potential response to Saffan was either a depolarization ($n = 4$), hyperpolarization ($n = 14$) or no change ($n = 9$). Measuring the reversal potential of responses to Saffan within the time course of one experiment was impractical as the neurones took at least 30 min to recover from the response to the anaesthetic. However, depolarizations were observed in neurones with resting potentials below −60 mV and hyperpolarizations in neurones with resting potentials of −50 mV and above, suggesting that Saffan was opening an ion channel with a reversal potential between −50 and −60 mV. Furthermore, when the direction of the membrane polarization was compared to that of responses to perfusion of GABA, which activates $GABA_A$ receptor-gated Cl^- currents on SPNs (Krupp & Feltz, 1993), it was the same for responses to both drugs in 5 of 6 neurones (under the recording conditions used Cl^- had an estimated reversal potential of −55 mV). In one cell in which the sign of the responses differed, GABA (5 s, 200 μM) caused a depolarization with a reversal potential of 0 mV, indicating it was not activating a

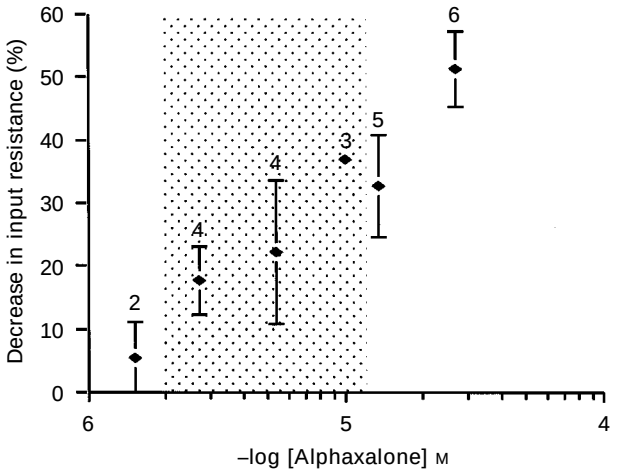

Figure 3 Dose-dependence of the reduction in input resistance by Saffan. The input resistance in the presence of Saffan is expressed as a percentage of the control input resistance for concentrations of alphaxalone between 1 and 27 μM. The shaded area represents the plasma concentration range of alphaxalone during anaesthesia. Vertical lines show s.e.mean, numbers are the number of responses measured. The s.e.mean for the effects of 10 μM Saffan were too small to be shown.

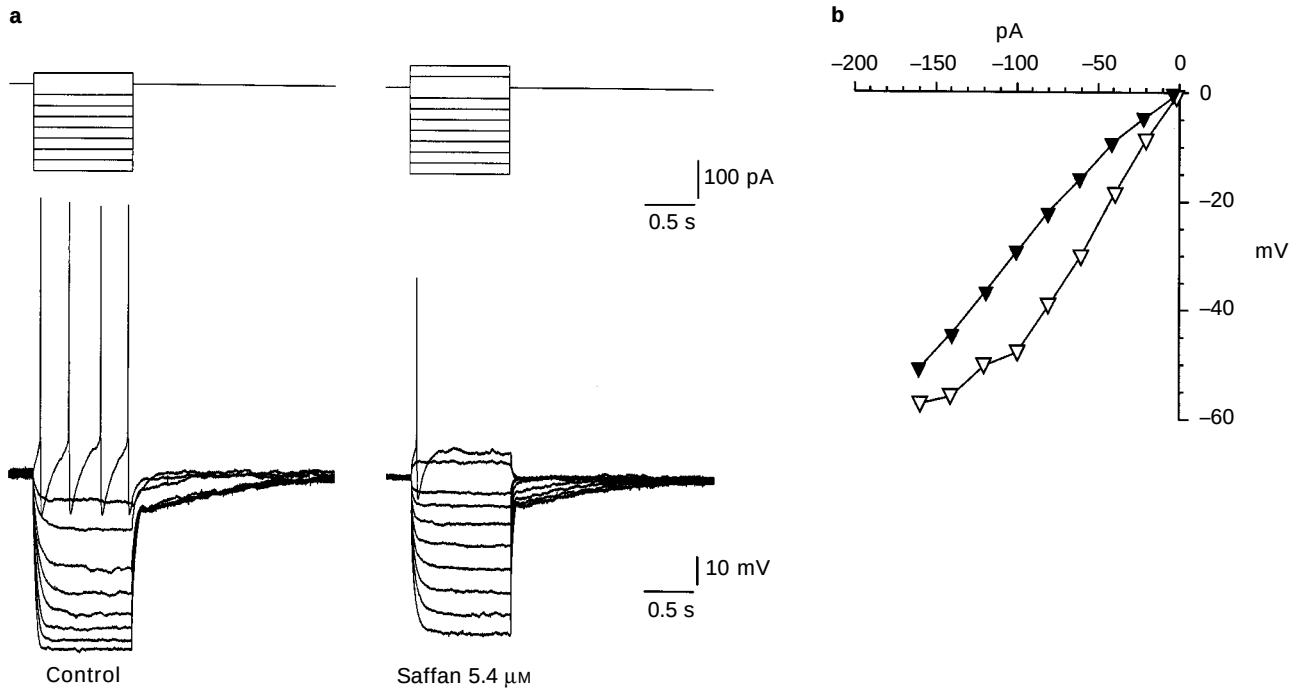

Figure 2 Effects of Saffan on membrane properties of SPNs. (a) Membrane potential responses of an SPN (bottom) to injection of current steps down the recording electrode (top), showing control responses left and the effects of Saffan (5.4 μM) right. In the presence of Saffan the amplitude of the membrane potential response to the current steps was reduced indicating a decrease in input resistance. The delay before repolarization following larger negative current steps, indicating activation of a subthreshold transient outward rectification, was also reduced. (b) Current-voltage relationship obtained from the neurone above demonstrating the decrease in input resistance caused by Saffan 5.4 mM (▼); (▽) control.

Cl^- conductance and Saffan (2.7 μM) caused a hyperpolarization from an initial resting potential of -50 mV.

The membrane potential responses to Saffan suggest that it was acting on a channel with a reversal potential similar to that of Cl^-. SPNs express both GABA- and glycine-gated Cl^- currents (Krupp & Feltz, 1993). The changes in membrane potential and input resistance caused by Saffan were unaffected by the glycine receptor antagonist strychnine (20 μM, $n=2$), but were blocked by the $GABA_A$ receptor antagonists picrotoxin (20 μM, $n=1$) and bicuculline (5–20 μM, $n=5$, Figure 4). None of the antagonists had any direct effect on membrane potential or input resistance in the absence of Saffan. Perfusion of the $GABA_A$ receptor agonist muscimol (duration 5–30 s, concentration 2–10 μM) induced similar effects to Saffan (data not shown): spontaneous action potential firing ($n=4/4$) and spontaneous membrane potential oscillations ($n=2/2$) were abolished; the input resistance was reduced ($n=9$); when applied at the resting potential, either a depolarization ($n=4$), a hyperpolarization ($n=5$) or no change in membrane potential ($n=2$) was observed; when applied at a series of potentials set by current injection, the muscimol response was shown to have a reversal potential of -50 to -55 mV ($n=4$). The Saffan responses described therefore appear to be mediated by $GABA_A$ receptors.

Effects of Saffan on action potentials

The effects of Saffan on single and trains of action potentials were examined. Positive current pulses sufficient to reach action potential threshold and pulses that evoked continuous trains of spikes throughout their duration (>500 ms) were used as test pulses. During perfusion of Saffan the amplitudes of the current pulse and the holding current were adjusted in order to compensate for the changes in input resistance and membrane potential. The ability of SPNs to fire trains of action potentials and the amplitude and duration of the action potential after hyperpolarization (AHP) were reversibly reduced in concentrations of Saffan above 10 μM (Figure 5). In the presence of 10–13.5 μM Saffan the threshold for a single action potential was unaltered ($n=6$). However, trains of action potentials could only be evoked at very depolarized

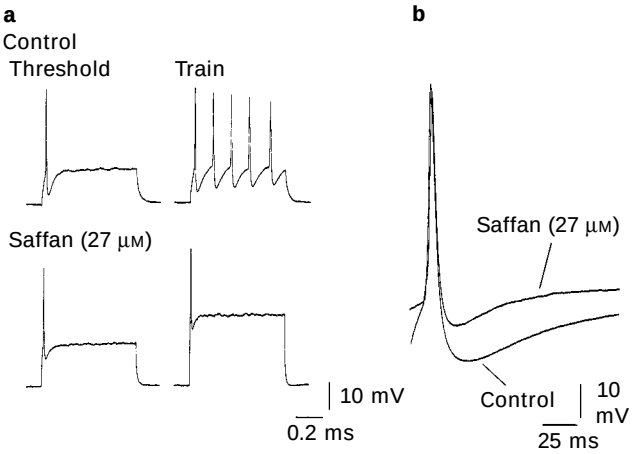

Figure 5 Effects of Saffan on action potentials. Action potentials were evoked by injection of positive current pulses as described in the text. (a) Saffan (27 μM) had no effect on the threshold for action potential firing, but reduced the amplitude and duration of the after-hyperpolarization. In this neurone during perfusion of Saffan trains of action potentials could not be evoked even following large depolarizations. (b) Action potentials shown on an expanded time base. In the presence of Saffan the action potential duration is unaffected, but the duration and amplitude of the after hyperpolarization are reduced.

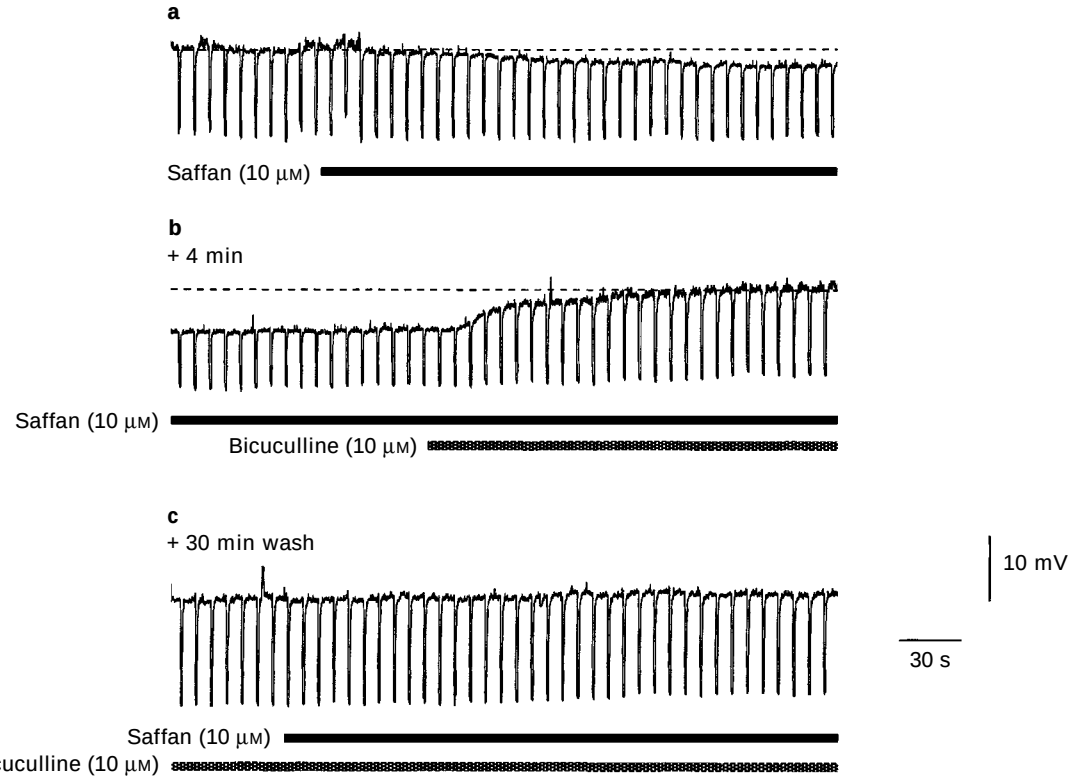

Figure 4 Antagonism of responses to Saffan by bicuculline. Perfusion of Saffan onto a silent SPN caused an hyperpolarization and decrease in input resistance (a). After continuous perfusion of Saffan for 4 min the response reached a steady state, co-application of bicuculline along with Saffan reversed the Saffan response (b). Following wash of both drugs for 30 min bicuculline was applied for 5 min before co-applying both Saffan and bicuculline (c). In the presence of bicuculline, Saffan had no effect on the membrane potential or input resistance confirming that bicuculline had antagonized the responses to Saffan. The regular downward membrane potential deflections are caused by injection of negative current pulses down the recording electrode (30 pA, 1 s) and are proportional to the input resistance of the neurone.

membrane potentials ($n=3$) or could not be evoked at all ($n=3$). In the same neurones, the duration of the AHP was reversibly reduced from a control value of 194 ± 56.6 ms to 107 ± 26.3 ms ($P<0.05$) and following a wash period of at least 20 min returned to 174 ± 40 ms ($n=6$). The amplitude of the AHP was also reversibly reduced (control 15.1 ± 1.9 mV, Saffan 11.4 ± 3.3 mV and wash 12.3 ± 2 mV, $n=6$), although this effect failed to reach significance. The action potential amplitude was reduced slightly and the duration increased slightly, but neither effect was significant ($n=6$).

Effects of Saffan on electrotonic coupling

The membrane potential oscillations described above are a consequence of electrotonic coupling between SPNs (Logan *et al*., 1996). Hence a single oscillation occurs as a result of spread across electrotonic junctions of an action potential fired by an adjoining SPN (Logan *et al*., 1996). As some anaesthetics have been shown to disrupt gap-junction coupling (Perachhia, 1991; Lazrak *et al*., 1994), the mechanism by which alphaxalone abolished oscillations was examined. Spontaneous oscillations could be abolished either by uncoupling the cells or by abolishing spontaneous action potential firing in other SPNs that were coupled to the cell from which the recordings were being made. The intracellularly-acting, Na^+ channel blocker QX-314 was included in the patch pipette solution. Five minutes after commencement of whole-cell recording, QX-314 had blocked somatic and antidromically-evoked action potentials in the cell recorded from. Antidromic stimulation then evoked a remnant of similar waveform to a single spontaneous oscillation, consisting of a fast depolarization followed by a slow hyperpolarization (Figure 6, and see Logan *et al*., 1996). The remnant or antidromically-evoked oscillation was a result of an antidromic action potential fired in an adjoining neurone conducting across electrotonic junctions to the recorded neurone (Logan *et al*., 1996). Perfusion of Saffan (27 μM) abolished the spontaneous oscillations and reduced, but did not abolish the slow hyperpolarizing component of the antidromically-evoked oscillation (Figure 6). The ability to maintain an antidromically evoked oscillation in the presence of Saffan indicates that Saffan does not uncouple the electrotonic junctions between SPNs and that the abolition of spontaneous oscillations is a consequence of abolition of spontaneous action potential firing by Saffan.

Discussion

These results demonstrate that the anaesthetic Saffan is able to alter the electrophysiological properties of SPNs *in vitro* at concentrations at which it acts during anaesthesia *in vivo*. Perfusion of Saffan onto SPNs depressed spontaneous activity, reduced the input resistance and altered the properties of action potential firing. *In vivo* these changes could have profound effects on the basal level of sympathetic activity and on the integration of synaptic inputs to SPNs.

Mechanisms mediating the actions of Saffan on SPNs

The more active constituent of Saffan, alphaxalone, has potent effects *in vitro* on $GABA_A$ receptors (Lambert *et al*., 1987). Studies with a number of different preparations have shown that at concentrations above 1 μM, alphaxalone opens $GABA_A$ receptor channels causing an increase in chloride conductance and at lower concentrations (above 30 nM) alphaxalone potentiates responses to $GABA_A$ agonists by prolonging the mean open time of GABA activated Cl^- channels (Barker *et al*., 1987; Cottrell *et al*., 1987). The effects of Saffan on SPN were consistent with an effect on $GABA_A$ receptors. Perfusion of Saffan to give alphaxalone concentrations between 2.7 and 54 μM caused a decrease in input resistance and changes in membrane potential towards the Cl^- reversal potential and of the same direction as responses to GABA. These effects were seen at similar concentrations of alphaxalone to those at which it activates $GABA_A$ receptors (Barker *et al*., 1987; Cottrell *et al*., 1987). Furthermore, responses to Saffan were mimicked by the $GABA_A$ receptor agonist muscimol, blocked by $GABA_A$ receptor antagonists picrotoxin and bicuculline, but were in-

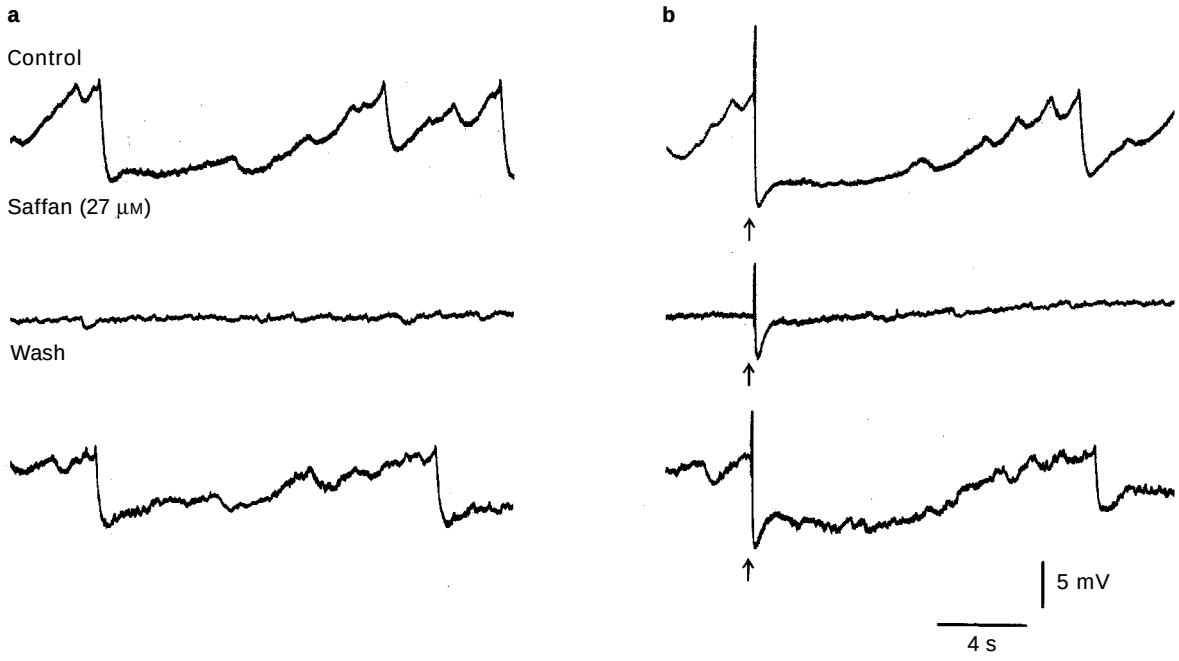

Figure 6 Effects of Saffan on electrotonic oscillations. The Na^+ channel blocker QX-314 was included in the intracellular solution to prevent firing of action potentials in the neurone recorded from. (a) Control (top) trace shows spontaneous membrane potential oscillations from an electrotonically-coupled SPN. The oscillations were inhibited by 27 μM Saffan (middle trace), and returned gradually during a 30 min wash period (bottom trace). (b) In the same cell the effect of Saffan on antidromically evoked oscillations is shown (arrow corresponds to time of ventral horn stimulus). Compared to the control response (top) the antidromically evoked oscillation was reduced in amplitude and duration in the presence of Saffan (middle). This also recovered following a 30 min wash period (bottom).

sensitive to the glycine receptor antagonist strychnine. The responses to Saffan were unlikely to have been caused by potentiation of responses to tonically released GABA as perfusion of $GABA_A$ antagonists alone had no effect, consistent with previous findings (Krupp & Feltz, 1993). Therefore at concentrations above 1 μM Saffan appears to act as an agonist at $GABA_A$ receptors on SPNs.

Saffan reduced repetitive action potential firing and the action potential AHP evoked by depolarizing current pulses. A similar effect of alphaxalone was seen on spikes evoked by current injection in neocortical neurones (El-Beheiry & Puil, 1989). At the frog node of Ranvier, 50 to 1000 μM alphaxalone inhibits Na^+ and K^+ currents (Benoit *et al*., 1988), and in axons of olfactory cortex neurones 5–50 μM alphaxalone inhibits Na^+ currents (McGivern & Scholfield, 1991). The effects of Saffan on the action potentials in SPNs may therefore be caused by inhibition of Na^+ and K^+ currents. Alternatively, $GABA_A$ receptor activation by Saffan may interfere with action potential generation in SPNs. Iontophoretic application of GABA or glycine prevents antidromic action potentials from invading the soma of SPNs (Backman & Henry, 1983). Furthermore, glycine is able to cause shunting of the action potentials of SPNs (Spanswick *et al*., 1994), suggesting that $GABA_A$ receptor activation, which also causes an increase in Cl^- conductance, may have a similar effect.

Saffan abolished spontaneous action potential firing and spontaneous membrane potential oscillations. These actions occured at concentrations of alphaxalone which open $GABA_A$ receptor gated Cl^- channels and were therefore most likely caused by $GABA_A$ receptor activation. Furthermore, the $GABA_A$ receptor agonist muscimol had similar inhibitory effects on SPNs. The inhibition of repetitive action potential firing described above may also have contributed to the abolition of spontaneous activity. Spontaneous oscillations occur as the result of action potentials fired by active SPNs crossing electrotonic junctions with other SPNs where they appear as oscillations (Logan *et al*., 1996). The inability of Saffan to block antidromically-evoked oscillations indicates that it did not uncouple the cells. The amplitude and duration of the antidromically-evoked oscillation were reduced by Saffan, probably as a result of the reduction of the AHP in the active cell and also increased shunting of the action potentials and oscillations due to the decrease in input resistance. Saffan therefore appears to have blocked spontaneous oscillations by abolishing spontaneous activity in cells electrotonically coupled to the recorded cell.

Implications for experiments performed during anaesthesia

During anaesthesia with alphaxalone-based anaesthetics reductions in hind limb conductance and mean arterial pressure (Quail *et al*., 1985), modification of baroreceptor reflexes (Blake & Korner, 1981), Valsalva-heart rate reflexes (Blake *et al*., 1982) and of sympathetic responses evoked by cerebellar stimulation (Paton & Gilbey, 1992) have been observed. The plasma concentration of alphaxalone during anaesthesia is in the range 2–12 μM (Sear & Prys-Roberts, 1979). The effects of Saffan on SPNs *in vitro* occurred at similar concentrations and were consistent with its effects *in vivo*: reductions in spontaneous activity, input resistance and repetitive action potential firing could all potentially modify ongoing and reflex discharges from SPNs. Saffan is likely also to affect other central neurones involved in autonomic control which could facilitate or oppose its effects on SPNs. Changes in hind limb conductance and arterial pressure occurring during Saffan anaesthesia have been localized to central sympathetic sites and appear to require the presence of noradrenergic and 5-hydroxytryptaminergic neurones (Quail *et al*., 1985), both of which synapse onto SPNs (Chiba & Masuko, 1986; Bacon & Smith, 1988). Further experiments *in vivo* would be required to show exactly which neurones are affected during anaesthesia.

Other anaesthetics acting through $GABA_A$ receptors (Franks & Lieb, 1994), or at other targets, may have similar effects on SPNs to Saffan. It is therefore useful to compare the electrophysiological properties of SPNs recorded *in vivo* from anaesthetized animals and *in vitro* from slice preparations. Electrophysiological recordings from SPNs *in vivo* have suggested that action potential discharge is driven by tonically active synaptic inputs and that SPNs themselves do not possess pacemaker activity or fire action potentials spontaneously (McLachlan & Hirst, 1980; Dembowsky *et al*., 1985). This is in contrast to recordings from SPNs *in vitro* which show that up to one third of SPNs are spontaneously active (Logan *et al*., 1996), and that sub-populations of SPNs demonstrate pacemaker-like activity in the form of rhythmic burst firing (Yoshimura *et al*., 1987) or spontaneous membrane potential oscillations (Spanswick & Logan, 1990; Logan *et al*., 1996). One interpretation of these differences is that during *in vivo* experiments performed under anaesthesia, spontaneous activity endogenous to SPNs may be depressed. Furthermore, intracellular recordings made from SPNs also show that *in vivo* they have lower input resistances (*cf*. McLachlan & Hirst, 1980; Yoshimura & Nishi, 1982; Dembowsky *et al*., 1986; Sah & McLachlan, 1995), suggesting more ion channels are open *in vivo*, possibly as a result of the actions of anaesthetics. In support of this, we have shown that a number of anaeshetics including α-chloralose (Logan *et al*., 1996) and Enflurane (MFN, T. Nicholson and SDL unpublished observations) as well as Saffan abolish membrane potential oscillations and spontaneous action potential firing and decrease the input resistance of SPNs *in vitro*. The effects of anaesthetics may therefore explain some of the discrepancies between the properties of SPNs *in vivo* and *in vitro*.

The results described here also have implications for experiments attempting to identify the source of tonic sympathetic output. This is often attributed to neurones in the brain stem on the basis of experiments performed on anaesthetized animals (e.g. Sun, 1995). However, experiments on unanaesthetized, acutely decapitated cats show that SPNs are capable of generating spontaneous action potential discharges in the absence of descending inputs (Beacham & Perl, 1964). Furthermore, even in the presence of anaesthetic a small proportion of SPNs from spinalectomized, deafferented cats fire spontaneous action potentials (Mannard & Polosa, 1973). If anaesthetics act *in vivo* to reduce sympathetic output cardiovascular variables such as blood pressure and heart rate, would tend to be reduced. Reflex responses to reductions in these parameters would then take place, causing activation of brainstem neurones with excitatory inputs onto SPNs. The resulting increase in the excitatory drive to SPNs would tend to restore the disturbances in the cardiovascular system and also increase the contribution of descending inputs to the generation of tonic sympathetic output. In our opinion the importance of spinal mechanisms for the maintenance of tonic sympathetic discharges may have been underestimated.

The actions of anaesthetics at the spinal level may be as important in producing unconsciousness and analgesia as their actions on higher centres in the brain (Collins *et al*., 1995). The results shown here suggest that the autonomic effects of anaesthetics may also be partly mediated at the spinal level. In the presence of Saffan the electrophysiological properties of SPNs were more like those described in anaesthetized animals in that the input resistance was reduced and spontaneous action potential firing and membrane potential oscillations were not seen. The actions of anaesthetics may therefore account for these differences. It follows that SPN may play a different role in synaptic integration and generation of rhythmic activity than has previously been suggested by experiments carried out *in vivo* under anaesthesia.

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