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European Journal of Pharmacology 397 (2000) 11–17

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Inhibition of mechanotransducer currents in crayfish sensory neuron by CGS 9343B, a calmodulin antagonist

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Received 28 October 1999; received in revised form 21 March 2000; accepted 24 March 2000

Abstract

The effects of CGS 9343B (zaldaride maleate), a calmodulin antagonist, on mechanosensitive channels were examined in crayfish slowly adapting sensory neurons using the two-electrode voltage clamp technique. In addition to its inhibition of voltage-gated Na^+ and K^+ currents, CGS 9343B (< 30 μM) blocked reversibly the receptor current in a dose-dependent and voltage-dependent manner with a dissociation constant (K_d) of 26.8 μM. The time course of the block was 265 s. Within the extension range of 3–30%, the reduction in receptor current was stimulus-independent and the gating mechanisms were not affected. Extracellular Ca^{2+} was not necessary for its blocking effects. No changes in passive muscle tension were observed in the presence of 20 μM CGS 9343B. These results suggest that CGS 9343B, as a calmodulin antagonist, can also block mechanosensitive channels, possibly by being incorporated into the lipid membrane and/or interacting with the channel protein.

Keywords: Calmodulin antagonist; Mechanosensitive channel; Stretch receptor; Voltage clamp

1. Introduction

Calmodulin antagonists are pharmacological agents that are supposed to bind to the Ca^{2+}-calmodulin complex and antagonize Ca^{2+}-calmodulin mediated functions. However, various biological processes, such as ion channel functions, that are not entirely or directly related to calmodulin have also been reported to be affected by different calmodulin antagonists. Klöckner and Isenberg (1987) observed that calmodulin antagonists (trifluoperazine, calmidazolium and chlorpromazine) reduced the Ca^{2+}, Na^+ and K^+ currents in ventricular and vascular myocytes, and that the suppression of I_{Ca} and I_K did not require interaction with the Ca^{2+}-calmodulin complex. In a study of the maxi-K^+ channel in dog airway smooth muscle, the calmodulin antagonists trifluoperazine, chlorpromazine, thioridazine and haloperidol were shown to reduce the open probability and mean open duration by directly acting on channel proteins instead of via calmodulin (McCann and Welsh, 1987). Na^+ channels, K^+ channels and Na^+ gating currents in giant axon of the squid (Ichikawa et al., 1991), glibenclamide-sensitive K^+ channels in *Xenopus* oocytes (Sakuta et al., 1992), Ca^{2+}-activated-maxi-K^+ channels of the *chara* protoplasmic drop (Laver et al., 1997), and Ca^{2+}-activated K^+ channels in rat myometrium (Kihira et al., 1990) were also reported to be blocked by various calmodulin antagonists. The blocking effects of these calmodulin antagonists on membrane channels are suggested to be due to direct binding to the channel protein.

Since calmodulin is known to interfere with the cytoskeletal function of biological membranes, and mechanosensitive channels are thought to be modified by cytoskeletal components (Sachs, 1997), calmodulin antagonists may affect mechanosensitive channels. Furthermore, no studies on the action of calmodulin antagonists on mechanosensitive channels, which play important roles in various cellular processes, have so far been reported. Therefore, we investigated the effects of calmodulin antagonists on mechanotransducer currents in stretch receptor sensory neurons of crayfish. The antagonist tested is CGS 9343B, which was introduced by Norman et al. (1987). CGS 9343B is considered to be a potent and selective calmodulin antagonist because it does not inhibit protein kinase C and postsynaptic dopaminergic receptors (Norman et al., 1987; Das et al., 1989). However, Neuhaus and

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00256-9

Reber (1992) showed that CGS 9343B affects both the Ca^{2+} channels and the subsequent changes in intracellular Ca^{2+} concentration, as well as the voltage-gated Na^+, K^+ and nicotinic acetylcholine receptor channels in PC12 cells. In our study, we show that CGS 9343B (< 30 μM), in addition to its inhibition of voltage-gated Na^+ and K^+ currents, blocks reversibly the stretch-activated current in a voltage-dependent and concentration-dependent manner in the slowly adapting stretch receptor neuron of crayfish. The block of the mechanotransducer by CGS 9343B is not mediated through gating mechanisms and does not require extracellular Ca^{2+}.

2. Materials and methods

2.1. Preparation

The experiments were performed on slowly adapting stretch receptors of the crayfish *Pacifastacus leniusculus*. The sensory neuron, together with its muscle, was isolated and transferred to a small chamber. The cut ends of the muscle were then tied to thin tungsten rods attached to a stretching device (Brown et al., 1978). The chamber was perfused at a rate of up to 6 mL/min. A complete exchange of drug solution could be obtained within 5 s. The experiments were carried out at room temperature (20–22°C). The normal astacus saline (NAS) contained (in mM): NaCl 207, KCl 5.4, $CaCl_2$ 13.5, $MgCl_2$ 2.6, Hepes 10. The Ca^{2+}-free solution contained (in mM): NaCl 207, KCl 5.4, $MgCl_2$ 14.7, Hepes 10. All solutions were buffered to pH = 7.4 and had an osmolality of 420 ± 15 mOsmol kg^{-1}.

CGS 9343B or zaldaride maleate, 1,3-dihydro-1-[1-(4-methyl-4H,6H-pyrrolo[1,2-alpha][4,1]-benzoxazepin-4-yl-methyl)-4-piperidinyl-2H-benzimidazole-2-one (1:1) maleate (generously provided by Novartis, Switzerland), was prepared as a 10-mM stock solution in dimethyl sulfoxide (DMSO). When used, aliquots were diluted with NAS or Ca^{2+}-free solution to the final concentration, in which DMSO was less than 0.5%, and the changes in pH and osmolality were small enough to be neglected. Further tests were also done using the same amount of DMSO in NAS. No significant effects of DMSO on stretch receptor currents were observed even after 30 min of its application (data not shown).

2.2. Recordings and stimulation

The apparatus and the methods for stimulation and recording have been described previously (Brown et al., 1978). Briefly, the receptor muscle was stretched using ramp (1500% s^{-1}) and hold pulses. The amplitude of extension is given as a percentage of resting length, as measured under the dissecting microscope. The micropipette (GC 150F, Clarke Electromedical Instruments, UK) for membrane potential measurements and current injection was filled with 3 M KCl (2–5 MΩ). The reference electrode consisted of a pipette filled with 3 M KCl–agar mixture and was connected to an Ag/AgCl pin.

In the present experiments, data were recorded with two electrodes in current clamp or voltage clamp modes using an Axoclamp 2B amplifier. Signals were obtained by a computer-aided stimulation and sampling system (Digi-Data 1200, pClamp6, Axon Instrument, CA, USA), and stored on a hard disk of a computer. The sampling frequency was 20 kHz. Analysis of the data was done using the pClamp6 programs.

Tension measurements were made as described previously using an Ackers 804 strain gauge calibrated with weights from 1 to 5 g (Rydqvist et al., 1990; Lin and Rydqvist, 1999).

Values are expressed as means ± S.E.M. unless otherwise stated. Student's *t*-test was used for statistical evaluation of the means.

3. Results

Tension measurements were performed to study possible effects of this calmodulin antagonist on the viscoelastic properties of the receptor muscle when a ramp (1500% s^{-1}) and hold stimulus from 3% to 30% was applied. No changes in passive tension could be detected after treatment with 20 μM CGS 9343B for up to 30 min (data not shown), and no muscle contractures were observed, suggesting that the viscoelastic properties of the muscle could be excluded when effects of CGS 9343B on membrane currents were considered.

When 20 μM CGS 9343B was applied, the sensory neuron depolarized gradually. In five minutes, the membrane potential decreased from -58.3 ± 0.9 to -38.8 ± 1.4 mV (18 cells), and the leakage conductance increased from 0.35 ± 0.04 to 0.45 ± 0.06 nS ($n = 10$). When depolarizing voltage steps were applied, 20 μM CGS 9343B reduced the maximal peak Na^+ current to 17.1 ± 5.0 % ($n = 7$). The maximal K^+ current at 80 mV depolarization was decreased to 55.5 ± 3.7% ($n = 8$). Thus, CGS 9343B blocked the voltage-gated Na^+ and K^+ currents in this sensory neuron, in agreement with the previous observations in giant axon of squid, PC12 cells, ventricular and vascular myocytes (Ichikawa et al., 1991; Neuhaus and Reber, 1992; Klöckner and Isenberg, 1987). The results also indicated that the action of CGS 9343B on Na^+ channels might be more specific than its effect on K^+ channels, as suggested in squid giant axon (Ichikawa et al., 1991).

3.1. Effects of CGS 9343B on receptor current

Fig. 1a shows the time course of the stretch receptor current at 24% extension when the neuron was exposed to

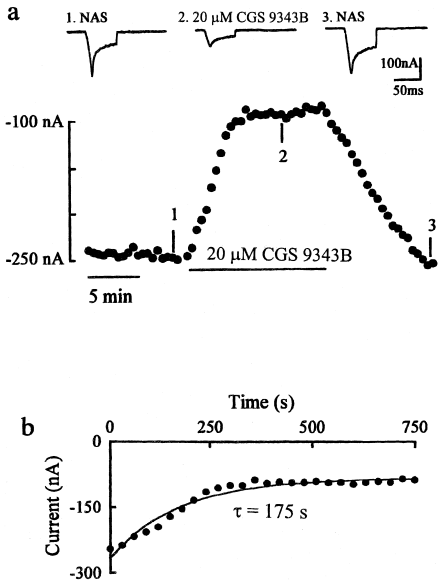

Fig. 1. Time course of reduction and recovery of the receptor current during application and washing out of 20 μM CGS 9343B. (a) Receptor currents initiated using a 66-ms ramp and hold extension of 24% (1500 % s^{-1}) applied every 30 s. The cell was held at resting membrane potential of −64 mV. Inserts are the current recordings from the points 1, 2 and 3, which are −250, −93 and −267 nA, respectively. (b) A first-order exponential $f = a + b(\exp(-T/\tau))$ was assumed to fit for the time constant, giving $\tau = 175$ s for the cell shown.

20 μM CGS 9343B. The amplitude of the receptor current decreased gradually from −250 nA, and then stabilized at a steady state value of −93 nA. The speed of recovery when CGS 9343B was washed out was slower than that of the blocking process, and 5–15 min was necessary to reverse its effects on receptor currents. The time constant of block, presupposing a first-order exponential, was 175 s for the cell shown (Fig. 1b). In six cells exposed to 20 μM CGS 9343B, the mean time constant was 265 ± 40 s at 24% extension.

In the following experiments, measurements were made 5 min after the drug solution was applied to the cell. At least 30 min was allowed to wash away CGS 9343B and let the neuron recover completely.

Exposure to 20 μM CGS 9343B for 5 min reversibly suppressed the receptor current over the whole extension range. Fig. 2a shows the current responses to ramp and hold extension from 3% to 30% of receptor muscle resting length in steps of 3% in control (NAS) and 20 μM CGS 9343B solution. The receptor current at the largest extension (30%) was reduced from −120 to −65 nA, a reduction of 54%. The steady state current measured at the end of stimulation was decreased to 53% from −38 to −20 nA at 30% extension. The results for the nine cells exposed to 20 μM CGS 9343B are summarized in Fig. 2b. The receptor currents were proportionally reduced over the whole extension range and no shift of the stimulus–response curve could be detected. We chose 15% and 27% as representative points. At 15% extension, 20 μM CGS 9343B reduced the receptor current by 56 ± 5% ($n = 14$) while the reduction was 59 ± 4% ($n = 14$) at 27% extension. No significant difference in the reduction of the receptor current between 15% and 27% extension was observed ($P > 0.05$; Fig. 2c). The steady state current in the presence of 20 μM CGS 9343B was decreased to 49 ± 6% ($n = 7$) at 15% extension and to 55 ± 5% ($n = 7$) at 27% extension, with no significant difference ($P > 0.05$; Fig. 2c). Therefore, the block of both the peak and steady

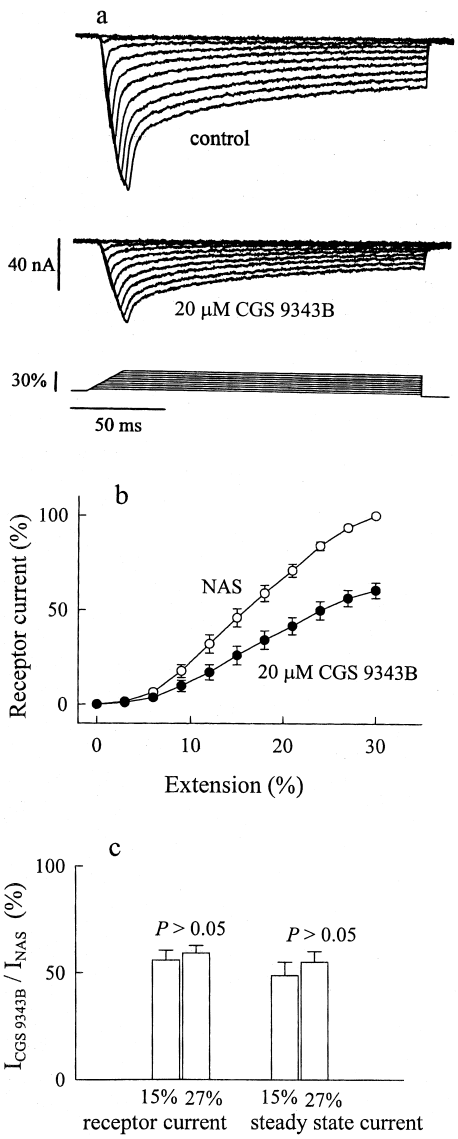

Fig. 2. Stimulus–response relationship of 20 μM CGS 9343B. (a) Receptor currents in response to ramp (1500 % s^{-1}) and hold extension from 3% to 30% of the resting muscle length (bottom) in control (NAS) and 20 μM CGS 9343B solution. The cell was held at its resting potential (−57 mV). (b) Plot of relative receptor currents vs. extensions using records from (a) and eight other cells. (c) Ratios of $I_{CGS\ 9343B} / I_{NAS}$ for the receptor current ($n = 14$) and the steady state current ($n = 7$) at 15% and 27% extension in exposure to 20 μM CGS 9343B were plotted. Data are expressed as means ± S.E.M.

state currents induced by CGS 9343B was stimulus independent, suggesting that no gating mechanism was affected by CGS 9343B.

3.2. Concentration dependence of CGS 9343B block

At pH = 7.4, CGS 9343B is mainly present as a base and is very insoluble in aqueous media (data from Novartis). Therefore, it was difficult to prepare a high concentration of CGS 9343B in NAS with the same low percentage of DMSO and the same pH and osmolality as the control solution. In the present study, 30 μM CGS 9343B in NAS was the highest final concentration that could be prepared. CGS 9343B at increasing concentrations was applied to the same cell several times, providing that washing away of CGS 9343B for more than 30 min restored the receptor current to the control value.

Fig. 3 shows the dose–response relationship of the effect of CGS 9343B on the receptor current at 15% and 27% extension. The receptor current was reduced in a dose-dependent manner. The reduction at 15% or 27% extension was similarly independent of extension at all concentrations tested, as indicated by the overlapping of control and CGS 9343B data points. Fitting the data obtained for the 27% extension according to the Michaelis–Menten theory, $I_{CGS\ 9343B}/I_{NAS} = 1/(1 + ([CGS\ 9343B]/K_d)^{n_H})$, yielded a dissociation constant (K_d) of 26.8 μM and a Hill coefficient (n_H) of 1.7. It is thus likely that at least two molecules of CGS 9343B are necessary to block the stretch-activated channel, and that co-operative interaction between these binding sites might exist.

3.3. Effects of CGS 9343B do not need extracellular Ca^{2+}

Since calmodulin antagonists bind to calmodulin only when Ca^{2+} is bound to calmodulin (Roufogalis et al., 1983), calcium ions play an important role in the interaction between calmodulin antagonists and the Ca^{2+}-calmodulin complex. Ca^{2+}-free extracellular solution was therefore used to elucidate whether CGS 9343B could exert its membrane effects independent of extracellular Ca^{2+}. Removal of Ca^{2+} from the extracellular solution increased reversibly the amplitude of the receptor current (Fig. 4a,b), as previously reported (Brown et al., 1978, Rydqvist and Purali, 1993). This increase in receptor current occurred within 10 s. Then, the receptor current saturated and little change was observed during a further 30 min of exposure to extracellular Ca^{2+}-free solution (Fig. 4a).

In Ca^{2+}-free solution, 20 μM CGS 9343B, measured 5 min after its application, decreased the receptor current from −147 (Ca^{2+}-free solution) to −73 nA at 24% extension, a reduction of 50% (Fig. 4b). In the same cell as shown in Fig. 4b, 20 μM CGS 9343B in NAS solution resulted in a similar reduction in the receptor current of 49% from −134 (NAS) to −65 nA at 24% extension.

Since the effect of CGS 9343B was measured 5 min after its exposure when the action of Ca^{2+}-free solution on receptor current was already in a steady state, we determined the action of extracellular Ca^{2+} on the effect of CGS 9343B by comparing the ratios of receptor currents induced by 20 μM CGS 9343B with or without extracellular Ca^{2+}, as demonstrated in Fig. 4c. A similar block was seen over the whole extension range no matter whether extracellular Ca^{2+} was present or not (four cells). These results suggest that the effects of CGS 9343B on the mechanosensitive channels were independent of extracellular Ca^{2+}, and also that Ca^{2+}-activated calmodulin is not involved in the Ca^{2+}-dependent control of the mechanosensitive channels.

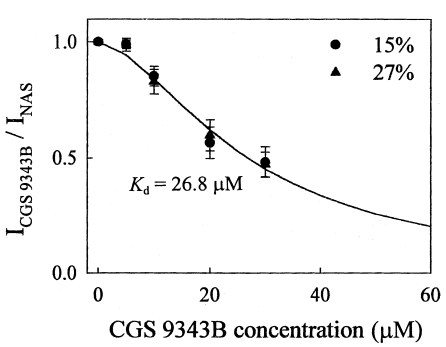

Fig. 3. The dose–response relationship of CGS 9343B block on the receptor current at 15% (●) and 27% (▲) extension. The fitted curve is drawn with data for 27% extension according to the equation $I_{CGS\ 9343B}/I_{NAS} = 1/(1+([CGS\ 9343B]/K_d)^{n_H})$ with $K_d = 26.8$ μM and $n_H = 1.7$. Points represent means ± S.E.M from four to six cells.

3.4. Voltage dependence of CGS 9343B block

The voltage dependence of the CGS 9343B block of receptor currents is illustrated in Fig. 5a. 20 μM CGS 9343B changed the reversal potential of the receptor current by −13 mV, indicating an increase in the ratio of the permeability of P_K/P_{Na} for the stretch receptor channels. In the positive potential range, 20 μM CGS 9343B either shifted the receptor current from inward to outward or increased the outward receptor current (eight cells). However, 20 μM CGS 9343B decreased the receptor current (inward) throughout the entire negative potential range with a larger reduction in hyperpolarized potentials.

The relative current blocked by 20 μM CGS 9343B in the negative potential range was plotted as a function of voltage in Fig. 5b. The continuous line was calculated using the following equations: $E_D = E_{DO}\exp(-FzV/RT)$

$$I_{CGS\,9343B}/I_{NAS} = 1/\left(1+[\mathrm{CGS\,9343B}]/K_d\left(1+E_D[\mathrm{CGS\,9343B}]\right)\right)$$

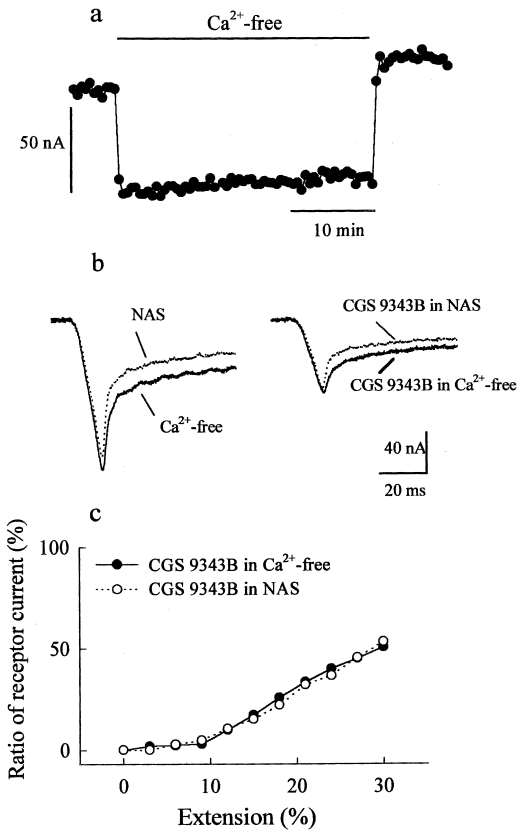

Fig. 4. Action of extracellular Ca^{2+} on the blocking effects of CGS 9343B. (a) Time course of the increase and recovery of the receptor current to the treatment with Ca^{2+}-free extracellular solution at 24% extension. 66-ms pulses were applied every 30 s. The cell was held at resting potential (−65 mV). (b) Representative recordings showing the effects of 20 μM CGS 9343B on receptor currents with or without Ca^{2+} in extracellular solution at 24% extension. (c) Representative plot of the ratios of receptor currents vs. extensions induced by 20 μM CGS 9343B in NAS solution (○: $I_{CGS\ 9343B}/I_{NAS}$) or in Ca^{2+} free solution (●: $I_{CGS\ 9343B-Ca^{2+}\text{-free}}/I_{Ca^{2+}\text{-free}}$). Ramp (1500% s^{-1}) and hold extension from 3% to 30%. Same cell as in (b) with resting potential of −55 mV.

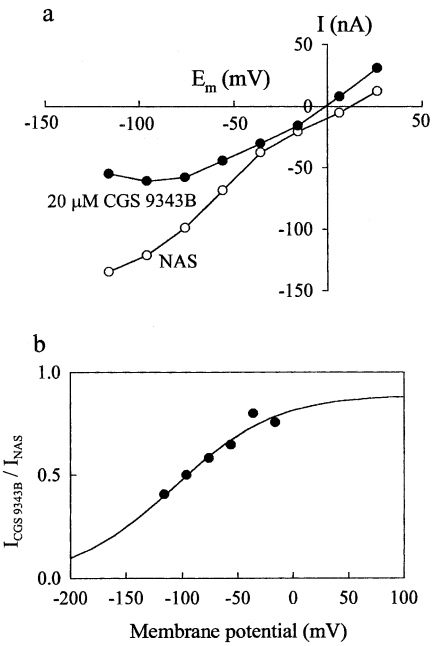

Fig. 5. Voltage-dependent block of CGS 9343B. (a) $I-V$ relationship of the peak receptor current (leakage current subtracted) before (○) and after (●) treatment with 20 μM CGS 9343B at 24% extension. The neuron was held at its resting potential (−56 mV). (b) Ratios of receptor currents ($I_{CGS\ 9343B}/I_{NAS}$) in the negative potential range were plotted vs. membrane potentials. The continuous curve was the fit of the equations described in text, producing the value of the equivalent valency $z = 0.57$. Same cell as in (a).

In these equations (Rusch et al., 1994), E_{DO} is defined as the equilibrium constant at 0 mV membrane potential, while E_D is the equilibrium constant of the conformational change which is assumed to be a function of the membrane potential. z is the equivalent valency for the voltage dependence of the block; K_d is the dissociation constant; F, R and T have their usual meanings. The continuous line in Fig. 5b yielded an equivalent valency z of 0.57 in this cell and the average value of z from three neurons was 0.55 ± 0.04, corresponding to a voltage dependence of CGS 9343B block of 46 mV per e-fold change of $I_{CGS\ 9343B}/I_{NAS}$ at negative potentials.

3.5. CGS 9343B could delay the adaptation of the transducer current

The transducer current in this sensory neuron, after it reaches a peak, starts to relax or adapt until it attains its steady state value. The process of adaptation is complex and can be attributed to two factors: the viscoelastic properties of muscles and the intrinsic properties of mechanosensitive channels. A second-order exponential

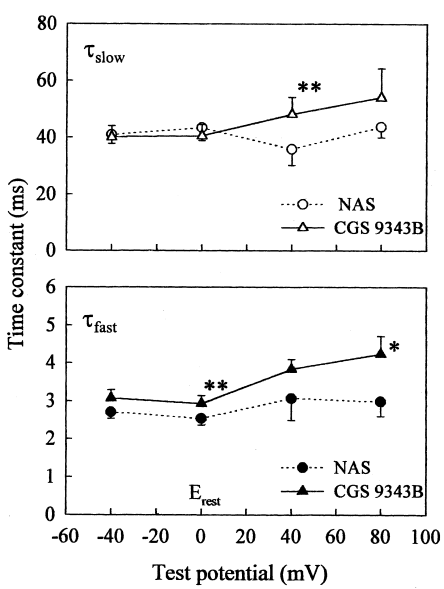

Fig. 6. Effects of CGS 9343B on the adaptation of the transducer current. τ_{slow} and τ_{fast} were plotted vs. the test potentials (relative to resting potentials). Points represent means ± S.E.M. from five to seven cells. $^{*}P < 0.05$ and $^{**}P < 0.01$.

function could be used to fit the adaptation process, yielding two time constants (τ_{fast} and τ_{slow}) (Swerup et al., 1983).

As shown above, CGS 9343B had no effects on the viscoelastic properties of the muscle, and therefore, the difference in the time constants of the adaptation between NAS and CGS 9343B is mainly due to the effects of CGS 9343B on mechanosensitive channels. Because cells had different resting potentials, these two time constants were plotted vs. the test potentials, as illustrated in Fig. 6. 20 μM CGS 9343B had no significant effects on τ_{fast} or τ_{slow} when the cell was hyperpolarized. At the resting potential, 20 μM CGS 9343B increased τ_{fast} significantly whereas τ_{slow} remained unchanged. However, when depolarized potentials were applied, 20 μM CGS 9343B increased both τ_{fast} and τ_{slow}. At 40 mV depolarization, the increase induced by 20 μM CGS 9343B was 3.8 ± 0.3 ms for τ_{fast} and 48.3 ± 6.0 ms for τ_{slow} in comparison with $\tau_{fast} = 3.1 \pm 0.6$ ms and $\tau_{slow} = 36.0 \pm 5.8$ ms in NAS solution (seven cells). These results suggest that, in addition to its suppression of the receptor current, CGS 9343B also delays the adaptation of the transducer current at depolarized potentials.

4. Discussion

In this study, the blocking properties of a calmodulin antagonist, CGS 9343B, on the transducer current of the slowly adapting sensory neuron of crayfish were examined. 20 μM CGS 9343B had no effects on muscle passive tension, and therefore, viscoelastic properties of the muscle could be excluded regarding its inhibition of the mechanotransducer. CGS 9343B decreased reversibly the receptor current in a dose-dependent manner with a dissociation constant (K_d) of 26.8 μM (Fig. 3). The CGS 9343B block was stimulus-independent and no shift of the stimulus–response curve in either direction was observed (Fig. 2), suggesting that gating mechanisms were not affected by CGS 9343B. In the negative potential range, 20 μM CGS 9343B was found to suppress the receptor current with a voltage dependence of 46 mV per e-fold change (equivalent to $z = 0.55$) (Fig. 5b). The time course of CGS 9343B block was slow, with a time constant of 265 s at 24% extension (Fig. 1). Extracellular Ca^{2+} was not necessary for the CGS 9343B block (Fig. 4). In addition to the suppression of receptor currents, CGS 9343B could also retard the adaptation of the transducer current at depolarized potentials (Fig. 6).

No other studies about the effects of calmodulin antagonists on mechanosensitive channels have so far been reported. However, the blocking effects of calmodulin antagonists on voltage-gated channels have been described previously. CGS 9343B, when studied in a rat pheochromocytoma cultured cell line (PC12) (Neuhaus and Reber, 1992), blocked Ca^{2+} currents reversibly with an IC_{50} of 3.0 μM. Trifluoperazine, when applied intracellularly to the squid giant axon, blocked the Na^+ current with $IC_{50} = 16$ μM and the K^+ current with $IC_{50} = 62$ μM, while W-7 reduced the Na^+ and K^+ currents with $IC_{50} = 35$ and 36 μM, respectively (Ichikawa et al., 1991). In ventricular myocytes, the EC_{50} of calmidazolium block on Ca^{2+} current was 1 μM, whereas in vascular myocytes, the EC_{50} was 0.3 μM (Klöckner and Isenberg, 1987). Therefore, the K_d value for the CGS 9343B block of mechanosensitive channels in the present study was comparable to that for the block elicited by other calmodulin antagonists on Na^+, K^+ and Ca^{2+} channels.

Some properties shared by these calmodulin antagonists are their nonspecific blocking effects on ion channels (Klöckner and Isenberg, 1987; Ichikawa et al., 1991; Neuhaus and Reber, 1992). Their block of voltage-gated and ligand-gated channels has already been reported, and our studies now show that the mechanosensitive channels could also be blocked by the calmodulin antagonist CGS 9343B. One explanation for such non-specificity may be related to certain common domains within all channel macromolecules or some common proteins bound to all channels, with which these antagonists could bind. The other explanation for the nonspecific reduction of membrane currents may result from the disturbance of the lipid membrane into which calmodulin antagonists partition and accumulate because of their high lipid-to-water partition coefficient.

In addition to CGS 9343B, gadolinium (Gd^{3+}) (Swerup et al., 1991) and the local anesthetics, bupivacaine and tetracaine (Lin and Rydqvist, 1999), which can block voltage-gated channels, have also been demonstrated to inhibit the transducer current in the stretch receptor neuron. Gd^{3+} inhibits the receptor current with $K_d = 395$ μM, and the K_d value for bupivacaine and tetracaine is larger than 4 mM. With a K_d of 26.8 μM, CGS 9343B was the most effective inhibitor compared to Gd^{3+}, tetracaine and bupivacaine. All these agents reversibly blocked the receptor current in a dose-dependent and voltage-dependent manner, and shifted the reversal potential by 8–13 mV towards the negative direction. However, there are some differences among their inhibition: (1) a stoichiometry of 1:1 is assumed for the Gd^{3+} block while the Hill coefficient was 1.7 for the CGS 9343B block; (2) bupivacaine and tetracaine shifted the stimulus–response curve towards the right, whereas no shift of the stimulus–response curve in either direction was caused by CGS 9343B; (3) the effects of Gd^{3+} and the local anesthetics are sensitive to extracellular Ca^{2+}, whereas for CGS 9343B, extracellular Ca^{2+} had no effect on the CGS 9343B block. CGS 9343B, Gd^{3+} and local anesthetics are different regarding their structure and electrical charge, and therefore, it is unlikely that they share common binding sites. The local anesthetic effect is suggested to be mediated by the changes in the lipid phase of the membrane (Lin and Rydqvist, 1999; Martinac et al., 1990). The block of Gd^{3+}

is proposed to be due to both its interaction with the lipid bilayer and its binding to fixed negative charges on the membrane surface (Swerup et al., 1991; Yang and Sachs, 1989). As for CGS 9343B, in addition to its possible interaction with channel proteins, it may also affect the mechanotransducer by accumulating in the lipid membrane as a result of its high hydrophobicity.

In addition to CGS 9343B, Gd^{3+}, tetracaine and bupivacaine, many substances have been shown to block mechanosensitive channels in different cell systems (Hamill and McBride, 1996), such as amiloride-like substances, aminoglycosides antibiotics, lanthanides, Na^+, K^+ and Ca^{2+} channel blockers. However, none of these substances have been demonstrated to be a specific and general blocker of mechanosensitive channels.

In summary, the calmodulin antagonist CGS 9343B blocks the transducer current of the stretch receptor neuron in a reversible dose- and voltage-dependent manner. The block is unlikely to be due to interference with the gating process of the mechanosensitive channels. The mechanisms of action could involve nonspecific accumulation of CGS 9343B in the lipid membrane and/or interactions with the channel proteins.

Acknowledgements

We thank Dr. Joe Bruton for his critical reading of the manuscript. This work was supported by grants from the Swedish Medical Research Council (Project No. 6838) and Karolinska Institutet.

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