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Suramin Affects Capsaicin Responses and Capsaicin-Noxious Heat Interactions in Rat Dorsal Root Ganglia Neurones

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Summary

The effect of suramin, an inhibitor of G protein regulated signalling, was studied on the membrane currents induced by noxious heat and by capsaicin in cultured dorsal root ganglia neurones isolated from neonatal rats. Whole-cell responses induced by a heat ramp (24-52 °C) were little affected by suramin. The noxious heat-activated currents were synergistically facilitated in the presence of 0.3 μM capsaicin 13.2-fold and 6.3-fold at 40 °C and 50 °C, respectively. In 65% of neurones, the capsaicin-induced facilitation was inhibited by 10 μM suramin to 35±6 % and 53±6 % of control at 40 °C and 50 °C (S.E.M., *n*=15). Suramin 30 μM caused a significant increase in the membrane current produced by a nearly maximal dose (1 μM) of capsaicin over the whole recorded temperature range (2.4-fold at 25 °C and 1.2-fold at 48 °C). The results demonstrate that suramin differentially affects the interaction between capsaicin and noxious heat in DRG neurones and thus suggest that distinct transduction pathways may participate in vanilloid receptor activation mechanisms.

Key words

Dorsal root ganglia neurones • Vanilloid receptor • Capsaicin • Noxious heat • Whole-cell

Introduction

A subset of small diameter (<20 μm) primary sensory neurones with somata in dorsal root ganglia (DRG) fulfils the criteria to provide an experimental model for the study of nociception. These DRG neurones highly express capsaicin (vanilloid) receptors whose activation by noxious stimuli, primarily capsaicin and noxious heat, opens channels non-selectively permeable to cations (Wood *et al.* 1988). Increasing temperature to the noxious range (43-52 °C) produces in these neurones a membrane current (I_{heat}) (Cesare and McNaughton 1996) characterized by an unusually high temperature coefficient (Q_{10}) ~18 (Vyklický *et al.* 1999). This current is potentiated by protons, capsaicin and protein kinase C (PKC) activators (Caterina *et al.* 1997, Cesare and McNaughton 1996).

A functional vanilloid receptor termed VR1 (vanilloid receptor type 1) has been cloned recently (Caterina *et al.* 1997). Despite the growing number of contemporary investigations, little is known about the structural basis and molecular mechanisms involved in the agonist- and noxious heat-induced activation of this receptor. VR1 belongs to the superfamily of cation

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channels with six transmembrane domains, a putative pore-loop region and the cytoplasmic amino and carboxyl termini. Within this superfamily, VR1 is most closely related to the transient receptor potential (TRP) family of ion channels, which play crucial roles in photo-transduction. Both when expressed endogenously in cultured dorsal root ganglion cells or when expressed heterologously in transfected human cells or frog oocytes, VR1 exhibits sensitivity to capsaicin, acids and noxious heat (Caterina *et al.* 1997) and all these stimuli act synergistically.

The complex interaction of algogenic stimuli on the capsaicin receptor, together with a delayed onset and slow activation/deactivation kinetics of the capsaicin induced responses, (Koplas *et al.* 1997, Vlachová and Vyklický 1993) prompted us to explore whether capsaicin receptor activation involves second messenger pathways at the G-protein level. We used suramin, a drug that inhibits G protein regulated signalling (Freissmuth *et al.* 1996, Freissmuth *et al.* 1999, Hohenegger *et al.* 1998, McCleskey and Gold 1999), in an attempt to determine a possible role of G proteins in the effects of capsaicin and noxious heat as well as in their interaction.

Methods

Cell cultures

Primary cultures of DRG neurones were prepared from new-born rats (2-4D) as previously described (Vyklický *et al.* 1999). Nerve growth factor (mNGF 7S, Alomone, Israel) (30 ng/ml) was added to the nutrient medium.

Membrane current recording

Whole cell membrane currents were recorded by employing an Axopatch-1D amplifier, and pCLAMP6 programs (Axon Instruments). Electrodes were pulled from borosilicate glass; after fire polishing and filling they had a resistance of 2-4 MΩ. The series resistance was usually less than 10 MΩ and was not compensated. For drug application, a system for fast superfusion of the neurones was used. It consisted of a manifold of 7 fused silica capillaries (0.36 mm, inner diameter) connected to a common outlet made from a glass capillary covered by a layer of platinum used for heating (Dittert *et al.* 1998). The orifice of the outlet capillary was placed less than 100 μm from the soma of a selected neuron. Ramps of heat applied were 3 s. Each of the 7 tubes was connected to a reservoir containing solution separated from the tube by a valve that was closed under resting conditions. The opening and closing of the solenoid valves and heating were controlled by a microprocessor. Before and after the test solutions, the neurones were superfused with control extracellular solution (ECS) of following composition (in mM): NaCl 160, KCl 2.5, $CaCl_2$ 1, $MgCl_2$ 2, HEPES 10, glucose 10; pH was adjusted to 7.3 with NaOH. All experiments were performed at room temperature (22–24 °C). The intracellular pipette solution (ICS) contained (in mM): KCl 140, $CaCl_2$ 0.5, $MgCl_2$ 2, MgATP 2, GTP 0.2, EGTA 10, HEPES 10; pH was adjusted to 7.3 with KOH. Capsaicin was dissolved in DMSO 100 μl and diluted with 0.9 ml of distilled water to make a stock solution of 1 mM. Suramin was dissolved in distilled water and kept as a 10 mM stock solution. All drugs were purchased from Sigma.

Only small neurones (<20 μm in diameter) were selected for recording. These neurones had a high probability of being sensitive to capsaicin and noxious heat. The experiments were performed between two and four days after plating. If any chemical was used, only one neurone was tested per cover slip. Data are expressed as means ± S.E.M. For statistical comparisons one-way ANOVA and t-test were performed, significant differences were accepted at $P<0.05$. All experiments were performed according to the European Community's Council Directive and with approval of the Institutional Animal Care and Use Committee.

Results

In ~80 % of small DRG neurones, a 3 s heat ramp (24-52 °C) induced a steeply rising inward current (I_{heat}) characterised by a high temperature coefficient, Q_{10}, of 17.5±1.7 (n=31). At a membrane potential of -70 mV, the average maximum current at 49 °C was 401±60 pA (n=47). In about one half of the cells, at room temperature (~24 °C), capsaicin 0.3 μM induced an inward current when applied in the first trial (188±25 pA, n=66). In the other half of the cells, capsaicin induced little or no current (<50 pA) at room temperature. However, capsaicin facilitated I_{heat} in all cells (Vlachová *et al.* 2001). As reported previously (Vyklický *et al.* 1999), there was no correlation between the magnitudes of the capsaicin response recorded at room temperature and of I_{heat} at 49 °C.

Superfusion with suramin (5-30 μM) did not significantly affect I_{heat} in 21/27 and 12/26 neurones tested at 10 μM and 30 μM, respectively. A small increase in I_{heat} was observed in 6 cells exposed to 10 μM suramin (39±8 % at 49 °C) and in 14 cells tested with 30 μM suramin (25±6 % at 49 °C). Figure 1A shows an example of the lack of effect of 30 μM suramin on I_{heat}.

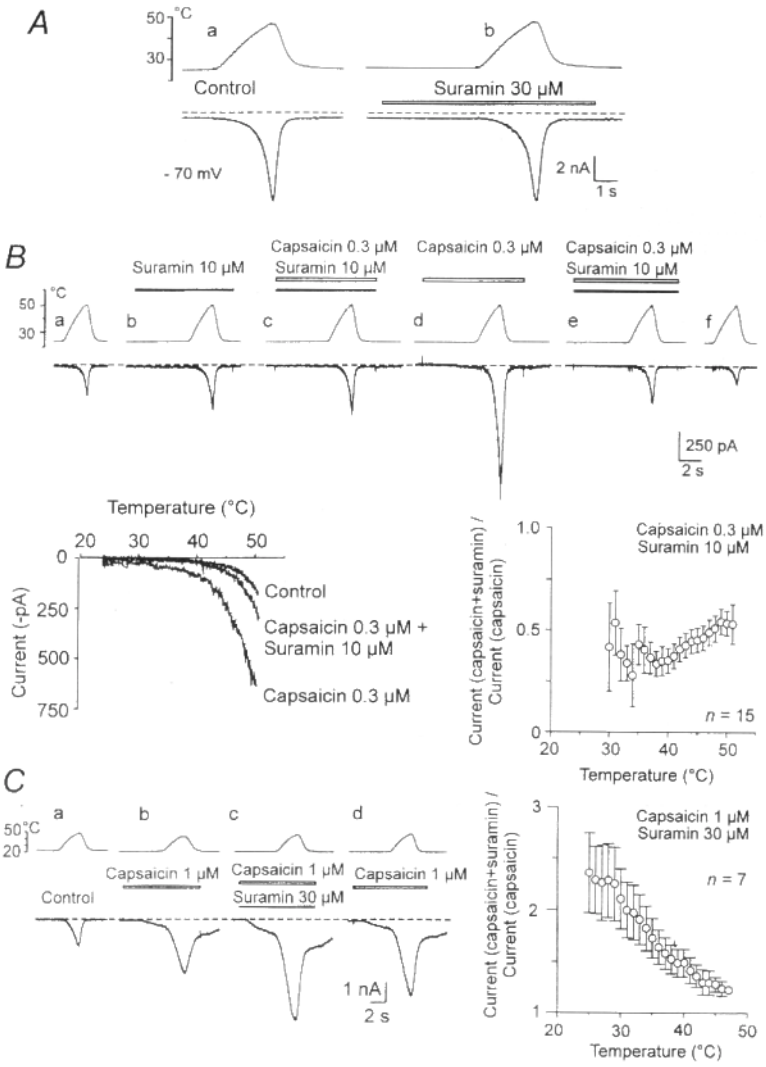

Fig. 1. (A) *Suramin does not affect membrane currents induced by noxious heat. Upper traces, temperature of the superfusing solution measured by a miniature thermocouple at the outlet. Lower trace, I_{heat} at a membrane potential of -70mV recorded in the whole-cell configuration of the patch clamp technique, induced by a 3 s ramp of increased temperature from 24 °C to 47 °C in control extracellular solution (a). I_{heat} in the same cell, 30 s later in 30 µM suramin as indicated by the bar (b). The dashed line indicates zero current.* ***(B)*** *The effects of suramin on whole-cell membrane currents induced by noxious heat in the presence and absence of capsaicin. Membrane current responses induced by a 3 s ramp of increasing temperature from 24 °C to 50 °C: (a) in control solution, (b) in the presence of 10 µM suramin (c) capsaicin 0.3 µM together with suramin 10 µM. (d) The same heat stimulus applied in capsaicin (0.3 µM) alone. (e) Capsaicin and suramin were applied together 30 s later. (f) Control I_{heat}. The diagram below on the left shows temperature-current relationships of responses (a, c) and (d). The diagram on the right shows the inhibition of the capsaicin (0.3 µM) induced facilitation of I_{heat} produced by suramin (10 µM) (mean ± S.E.M., n=15). Note the more pronounced inhibition at 40 °C than at 50 °C.* ***(C)*** *Suramin increased 1 µM capsaicin induced currents over the whole temperature range. (a) control I_{heat}, (b) capsaicin 1 µM (c) 1 µM capsaicin and 30 µM suramin and (d) capsaicin 1 minute later. The graph on the right shows the average peak current in the presence of 1 µM capsaicin and 30 µM suramin divided by the preceding current in capsaicin alone. The extent of suramin-induced potentiation of capsaicin responses decreased between 25 °C and 48 °C from 2.4±0.4 to 1.2±0.1 (n=7). The vertical bars indicate S.E.M.*

Whole-cell current responses induced by a heat ramp (24-52 °C) were facilitated by 0.3 µM capsaicin. The mean relative increase of the inward membrane current recorded in the presence of capsaicin compared with the control I_{heat} was 13.2±3.5 and 6.3±2.0 at 40 °C and 50 °C, respectively (n=15). This dramatic increase of I_{heat} was inhibited by 10 µM suramin (Fig. 1B). To circumvent the effect of desensitization (Docherty *et al.* 1996, Koplas *et al.* 1997), the response induced by capsaicin in the presence of suramin was recorded first (Fig. 1B, c) and the effect of capsaicin alone was tested in the following trial (Fig. 1B, d). The inhibition produced by suramin was also observed after application of capsaicin alone 30 s later (Fig.1B, e). After this treatment, a desensitization of I_{heat} was observed (Fig 1B, f). The mean inward current induced by 0.3 µM capsaicin at 24 °C during and 30 s after 10 µM suramin exposure was 17±5 pA and 61±13 pA (n=27), respectively. This inhibitory effect of suramin was significant according to paired Student's t-test ($P<0.05$). Suramin, 30 µM, had no measurable effect on the 0.3 µM capsaicin-induced current at 24 °C. The average capsaicin evoked inward

current recorded in the presence and the absence of suramin 30 μM was 29±16 pA and 27±17 pA, respectively (n=12).

The graph below the records (Fig. 1B, left) compares the rising phase of the current-temperature relationships of the first control (a), the first application of capsaicin with suramin (c) and capsaicin applied alone (d). Statistical evaluation (Fig. 1B, right) shows that the inhibition of capsaicin-induced responses by suramin was temperature-dependent. In the presence of 10 μM suramin, the heat responses facilitated by capsaicin were reduced to 35±6 % and 53±6 % of the control value at 40 °C and 50 °C (S.E.M., linear regression coefficient for 40-50 °C, r=0.98, n=15). We did not observe significant differences in the effect of different concentrations of suramin (5, 10 and 30 μM) on the inhibition of the facilitatory effects of 0.3 μM capsaicin on I_{heat}. The inhibitory effect of 30 μM suramin was 28±8 % and 58±7 % of the control value at 40 °C and 50 °C (n=4). The difference between the 30 μM and 10 μM suramin effect at these two temperatures was not significant (one-way ANOVA, P=0.58 and P=0.69).

Two arguments led us to use a submaximal dose of capsaicin (0.3 μM) in these experiments. First, we intended to minimise the effect of capsaicin-induced desensitization. Second, we attempted to distinguish the sensitisation effect of capsaicin on I_{heat} from thermally increased capsaicin-induced current (assuming that these two phenomena are distinct; Vlachová *et al.* 2001). In order to elucidate further the pharmacology of suramin, another series of experiments was performed, in which the effect of suramin (30 μM) was tested in the presence of a nearly maximal dose of capsaicin (1 μM).

Surprisingly, in all 7 cells tested, suramin caused a significant increase in the capsaicin-induced membrane current (Fig. 1C) over the whole temperature range. The average inward membrane current induced by 1 μM capsaicin was significantly increased by 30 μM suramin from 244±67 pA to 456±82 pA at 24 °C (the mean difference; 212±74 pA) and from 1918±223 pA to 2524±227 pA at 43 °C (the mean difference; 606±242 pA, paired Student's t-test, P<0.05). Moreover, a reverse temperature dependency of the suramin effect was evident when compared with the effect on 0.3 μM capsaicin responses (Fig. 1C, right). The extent of suramin-induced potentiation of capsaicin responses decreased from an average relative value of 2.4±0.4 at 25 °C to 1.2±0.1 at 48 °C (n=7). In three neurones, we recorded both suramin effects described above: the inhibition of the 0.3 μM capsaicin induced facilitation of I_{heat} and the potentiation of the 1 μM capsaicin response.

Discussion

The results indicate that suramin affects the interaction between capsaicin and noxious heat in a complex manner. This is not surprising in view of our observation that the interaction of capsaicin and heat is in itself complex. We have previously found that when capsaicin induces a significant current (>50 pA) at room temperature, this current increases approximately twice every 10 °C over the whole temperature range (24-50 °C). When capsaicin produces little or no current at room temperature, it greatly facilitates I_{heat} in the noxious temperature range (Vlachová *et al.* 2001). Suramin is an unspecific G protein inhibitor that restrains the rate limiting step in the activation of the G-alpha subunit i.e. the exchange of GDP for GTP (Freissmuth *et al.* 1999, Hohenegger *et al.* 1998). To explain our results one would have to assume that there are at least two distinct G protein controlled pathways acting on the capsaicin receptor with opposing effects.

Our results demonstrate that suramin (5-30 μM) does not significantly affect inward membrane currents induced by noxious heat (43-50 °C). This finding suggests that the ion channels gated by noxious heat are not directly controlled by suramin-sensitive G proteins. Suramin, however, strongly influences the response of the cells to capsaicin. In 65 % of cells, suramin effectively inhibited capsaicin induced facilitation of I_{heat} (42-51 °C) and the degree of the inhibition exerted by 10 μM suramin slightly decreased with increasing temperature (from 35 % at 40 °C to 53 % at 50 °C). This temperature dependence most likely reflects thermally induced shifts in the equilibrium of the receptor-capsaicin-suramin interaction.

In contrast, suramin 30 μM increased responses to 1 μM capsaicin over the whole temperature range 24-48 °C. The existence of two distinct suramin effects on capsaicin induced responses prohibits a simple explanation based on a single mechanism by which suramin inhibits desensitization (or accelerates resensitization) of the capsaicin receptor.

Suramin is a drug with a broad spectrum of biological and therapeutic properties (Voogd *et al.* 1993). The multiple biological effects of suramin complicate the interpretation of the results described here. Suramin acts both on extracellular and intracellular receptors (Beindl

et al. 1996, Nakazawa *et al.* 1990, Novotny *et al.* 1999). However the degree to which it enters DRG neurones is not known. Suramin acts as a non-specific purinergic antagonist (McCleskey and Gold 1999, Nakazawa *et al.* 1990) and, at low micromolar concentrations, suramin has also been shown to inhibit protein-tyrosine phosphatases (Zhang *et al.* 1998).

There is considerable evidence that maximal VR1 activity requires a combination of protein kinase C-related signalling events (Premkumar and Ahern 2000). Suramin in concentrations 10-40 μM is capable of reducing the activity of Ser/Thr kinases such as protein kinase C (Mahoney *et al.* 1990). Suramin has been reported to differentially affect the activated protein kinase C isoforms (Gschwendt *et al.* 1998). PKC isoenzymes are activated by suramin in the absence of any cofactors. However, once activated by phosphatidylserine or phorbol esters, PKC isoforms α, β1, β2, γ and δ are inhibited by suramin, whereas the activity of PKCζ is further stimulated. A twofold increase of PKCζ activity by 10 μM suramin is inhibited with higher concentrations of suramin (100-200 μM); this was explained by a concomitant inhibitory action on the kinase activity (Gschwendt *et al.* 1998). In analogy to the PKCζ isoform, a similar mechanism might be one of the factors controlling the activity of PKCε isoform, that is known to be specifically involved in the bradykinin induced sensitization of noxious heat responses (Cesare *et al.* 1999). Based on our findings of both inhibitory and stimulatory effects of suramin, we must consider the possibility that suramin differentially affects several PKC isoforms in signalling pathways involved in either capsaicin or noxious heat responsiveness in DRG neurones.

Our recent findings demonstrate that the native vanilloid receptor can exist in at least two distinct inactive conformational states (Vlachová *et al.* 2001). At one extreme, the binding of capsaicin brings the majority of the channels into their open state and causes an inward cationic current to flow (see Fig. 1C, b). In this state, elevation of temperature, without an obvious threshold, increases this membrane current over the innocuous and noxious range of temperature and exhibits temperature coefficient Q_{10} of 2 to 4. Thus, the capsaicin activated receptor does not respond with a high Q_{10} to noxious thermal stimuli as it is the case in the control. At the other extreme, a majority of receptors bind capsaicin but no current is produced at room temperature (see Fig. 1B, d). Under these conditions, the receptors are sensitised to noxious heat and respond with a high Q_{10} >10 and with the threshold similar to the control.

The presented results suggest that, depending on the mode of activation, capsaicin receptors can be differentially affected by suramin through distinct modulatory pathways. Based on the findings mentioned above, our conclusion is consistent with the view that suramin inhibits the modulatory effect exerted by capsaicin on I_{heat} while agonist induced receptor activity is, on the contrary, increased by suramin. To elucidate possible mechanisms underlying those effects of suramin requires more experimentation, for which the data presented here may serve as a basis.

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Reprint requests

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