

Capsaicin-Induced Membrane Currents in Cultured Sensory Neurons of the Rat

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Summary

Membrane currents induced by capsaicin (CAPS) in cultured sensory neurons from 1- to 2-day-old rats were studied. Responses to CAPS ($10\ \mu\text{M}$) exceeding 1 nA at $-50\ \text{mV}$ were found in smaller, usually bipolar or tripolar neurons in which GABA ($30\ \mu\text{M}$) induced small or no response. Large, unipolar neurons, which exhibited large responses to GABA, were completely insensitive to CAPS ($10\ \mu\text{M}$). In contrast to GABA, responses to CAPS exhibited a slow rise and slow decay and a marked tachyphylaxis after repeated CAPS applications at high concentrations which made it difficult to study the concentration-response relationship. In partially run-down neurons, which exhibited quasi stable responses, the slope of the ascending phase was concentration-dependent with an apparent association rate constant $K_1\ 9 \times 10^4\ [\text{M}^{-1}\text{s}^{-1}]$. The time constant of the decay was 3.5 s, and was concentration-independent. However, in 5 neurons the EC_{50} measured from the first series of CAPS applications at increasing concentrations was $0.31 \pm 0.5\ \mu\text{M}$ with a Hill coefficient 1.66 ± 0.35 . The responses to CAPS reversed at $+10.4 \pm 2.5\ \text{mV}$ suggesting that the current is carried nonselectively by monovalent cations and Ca^{2+} . The channel conductance of CAPS-gated channels at $-50\ \text{mV}$ calculated from the mean membrane current and variance of the current noise in outside-out patches or measured directly was 28 pS ($n=5$). It is suggested that the CAPS-gated channels are either controlled by receptors with a very high affinity or that the channels are controlled by membrane-bound protein(s) which do not depend in their function on the supply of GTP or other intracellular metabolites.

Key words

Sensory neurons – Rat – Capsaicin – GABA – Membrane currents – Primary culture

Introduction

Capsaicin (8-methyl-N-vanillyl-6-nonamide), a pungent component of red pepper and similar plants, has been shown to be excitatory and toxic on a population of sensory neurons which include nociceptors (Bevan and Forbes 1988, Fitzgerald 1983, Lynn *et al.* 1984, Petersen and LaMotte 1991, Szolcsanyi 1983) and thermosensitive neurons (Coleridge *et al.* 1965, Lundberg 1985).

In neonatal rats, CAPS damages the "small dark" neurons (Jancso *et al.* 1977) termed "B" neurons (Andres 1961, Lawson 1979) which are the cell bodies of C and A δ afferents. There is less conclusive evidence about the action of CAPS on "large pale" neurons, termed "A", which are the cell bodies of fast conducting

afferents. They were reported to suffer a 30 % loss after prolonged CAPS treatment (Lawson and Harper 1984); in culture about 28 % of large cells were found to respond to CAPS (Petersen and LaMotte 1991).

The original idea that CAPS receptors must exist in a distinct subpopulation of sensory neurons (Szolcsanyi and Jancso-Gabor 1975) was supported by the later finding that CAPS induces an inward current which is carried by cations (Bevan and Forbes 1988, Bevan and Szolcsanyi 1990, Forbes and Bevan 1988, Petersen and LaMotte 1991, Winter *et al.* 1990, Wood *et al.* 1988). The aim of this study was to analyze the CAPS-induced membrane current in more detail.

Methods

Cell culture

The experiments were performed on primary cultures of dorsal root ganglion (DRG) neurons from rats 1–2 days old which were cultivated on a feeder layer of glial cells prepared from hippocampi of newborn mice (Vyklický *et al.* 1990). The rationale for this procedure was that the glial monolayer supported the growth of DRG neurons without nerve growth factors and that the neurones developed only short branches which made them easier to clamp. In addition, the neurones adhered firmly to the bottom of the dish which was essential for a rapid exchange of the solutions produced by employing our drug application system. DRG neurones cultivated on polylysine or collagen in the presence of nerve growth factor did not adhere to the dish and could not be used. The possibility of an admixture of hippocampal neurones in the glial monolayer was excluded before adding dissociated DRG neurones to the culture by checking that the monolayer was formed exclusively by glial cells. If any neuron was found to be vacuolated and shrunken it was not suitable for patch clamping already at this stage of cultivation.

Primary cultures of DRG neurons were prepared in two steps. In the first step, hippocampi from newborn BALB/c mice were dissociated with trypsin and plated at 5×10^5 cells per 35 mm collagen-coated Petri dish in a nutrient medium composed of 90 % Eagle's minimum essential medium (MEM) and 10 % fetal bovine serum. When the glial cultures became confluent, usually after 6–9 days, the medium was switched to nutrient-supplemented MEM with 5-fluoro-2-deoxyuridine and uridine and 10 % horse serum (Guthrie *et al.* 1987) to suppress cell division. In the second step, dorsal root ganglia were dissected from 1- to 2-day-old Sprague-Dawley rats and incubated for 1–2 hours at 37 °C in a phosphate-buffered solution (PBS) containing 2.5 % collagenase. The ganglia were then rinsed with a calcium- and magnesium-free PBS. Dissociation into single cells was achieved by trituration through a Pasteur pipette.

DRG cells were plated at a density 10^4 cells per dish on the glial feeder layer cultures and grown in a medium composed of 90 % MEM and 10 % fetal calf serum and maintained at 37 °C in a water-saturated atmosphere with 5 % CO₂. No antibiotics and nerve growth factors were added. After 3 days this medium was changed for nutrient supplemented MEM containing 5 % horse serum and 5-fluoro-2-deoxyuridine (15 mg/ml) and uridine (35 mg/ml). The nutrient supplement contained transferrin, insulin, selenium, corticosterone, triiodothyronine, progesterone and putrescine (Guthrie *et al.* 1987). Newborn mice and rats were killed by cervical dislocation.

Recording and perfusion techniques

Experiments were performed at room temperature (22–23 °C) 4–7 days after DRG cells had been plated. Whole-cell membrane current and single-channel recordings were carried out with an Axopatch 1D amplifier (Axon Instruments). The series resistances of the recording pipettes were usually about 10 MΩ.

The system for fast superfusion of the neurons consisted of a planar array of eight parallel glass tubes 0.4 mm in diameter. The orifice of one of the tubes was placed at a distance of about 150 μm from the soma of a selected neuron which allowed a complete exchange of the solutions in 30–50 ms. The step motor for changing the position of the tubes and the time of opening of the solenoid valves which allowed the flow of the solutions by hydrostatic pressure were controlled by a microcomputer. Before and after application of any of the test solutions the neurons were superfused by solution (ECS) of following composition (in mM): NaCl 160, KCl 2.5, CaCl₂ 1, MgCl₂ 2, HEPES 10, glucose 10, pH was adjusted to 7.3 with NaOH. The culture dishes, 35 mm in diameter, were independently perfused by ECS 2 ml/min.

The pipette solution contained (in mM): CsCl 140, KCl 2.5, CaCl₂ 0.5, MgCl₂ 1, EGTA 5, HEPES 10, pH was adjusted to 7.3 with CsOH. CAPS and GABA were added to the ECS solution. CAPS was dissolved in 100 μl 96 % ethanol and then diluted with water to a final concentration 10^{-3} M which was then used to prepare the test solutions. CAPS and GABA were purchased from Sigma.

Membrane current recording and variance analysis

Control and capsaicin-induced membrane currents were stored on video-tape after digital sampling at 20 kHz (adapted Sony PCM-501ES), while the Axopatch-1D low-pass filter was set at 10 kHz. The replayed single-channel signal was low-pass filtered at 1.4 kHz (-3dB, 8-pole Bessel) and digitized with 10 kHz sampling rate by LABMASTER TL-1 (Axon Instruments), for variance analysis filtered at 1 kHz (-3dB, 8-pole Butterworth) and digitized with 2 kHz sampling rate. The variances of the current noise were computed using a program SPAN kindly provided by Dr. John Dempster. Data are given as means ± S.E.M.

Results

Cell specificity of the responses to CAPS and GABA

The 115 DRG neurons showed two subpopulations in agreement with other studies (Andres 1961, Harper and Lawson 1985, Lawson 1979, Petersen and LaMotte 1991). In the "large" cells

($n=49$) which were round, unipolar, with cell body diameters of about $30\ \mu\text{m}$, GABA ($30\ \mu\text{M}$) induced large membrane currents which desensitized to a plateau of about 30% of the peak current in about 3 s. These DRG neurons were completely insensitive to CAPS at concentrations as high as $10\ \mu\text{M}$ (Fig. 1A). In the "small" neurons ($n=66$) which were usually bipolar or tripolar, with the cell body diameters in two axes of $10\times 20\ \mu\text{m}$ or less, GABA ($30\ \mu\text{M}$) induced a small membrane current which usually did not exceed 50 pA at a membrane potential of $-50\ \text{mV}$. In some "small" neurons ($n=4$) the responses to GABA were virtually non-existent. In all of these neurons the responses to CAPS were large, and the responses to the first application of $10\ \mu\text{M}$ CAPS always exceeded the amplitude of the GABA responses (Fig. 1B).

There was an overlap among the DRG neurons in respect to their size as noted by Harper and Lawson (1985) which sometimes made it difficult to predict to which group the neuron would belong according to the morphological criteria. This, however, became evident after the first exposure to CAPS which revealed to which category the neuron belongs. The responses to CAPS ($10\ \mu\text{M}$) were either large or absent.

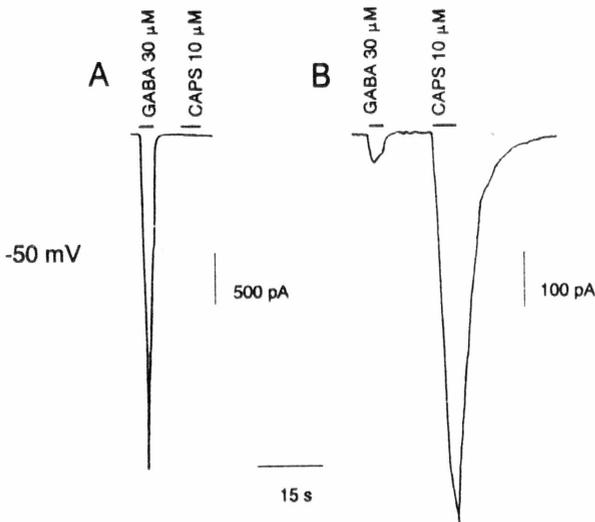


Fig. 1
Whole-cell membrane currents induced by rapid application of GABA and capsaicin in two different DRG neurons in the same culture. Membrane potential $-50\ \text{mV}$.

A: Recording from unipolar cell, $30\ \mu\text{m}$ in diameter. Large response to GABA ($30\ \mu\text{M}$) and no response to CAPS ($10\ \mu\text{M}$). **B:** Bipolar neuron, diameter $10\ \mu\text{m}$ and $20\ \mu\text{m}$ in two axes. Small response to GABA ($30\ \mu\text{M}$) and large response to CAPS ($10\ \mu\text{M}$). The bars above the records indicate the time of drug application.

Tachyphylaxis of responses to CAPS

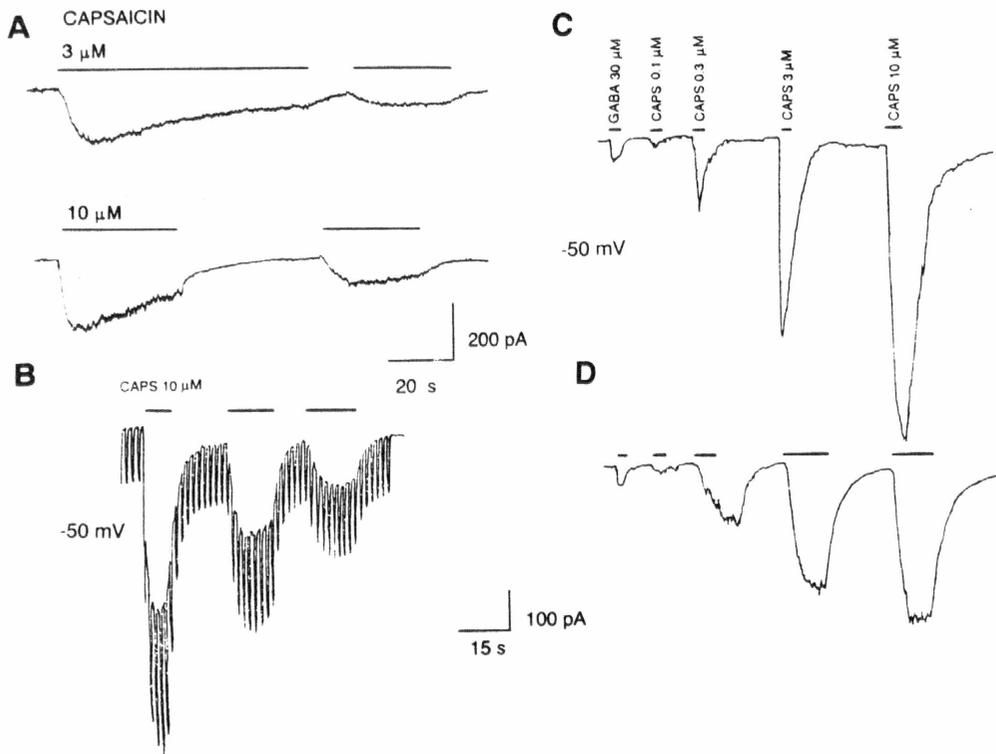
The amplitudes of the responses to high concentrations ($>1\ \mu\text{M}$) of CAPS exhibited a progressive decrease (Fig. 2A). The magnitudes of the increase of the conductance during application of CAPS also decreased as judged from the amplitudes of the deflections produced by $10\ \text{mV}$ hyperpolarizing pulses applied through the microelectrode (Fig. 2B). After several series of CAPS applications in which the dose-response relationship was tested, there was no obvious change in the threshold CAPS concentration that usually remained at $0.1\ \mu\text{M}$; in contrast, the maximum response was much smaller (Fig. 2C,D). The responses to CAPS, however, did not disappear completely in any neuron examined during the whole time of recording, lasting usually 30–50 min.

Concentration-response relationship of the responses to CAPS

It was difficult to study the dose-response relationship of the responses to CAPS because of extensive tachyphylaxis observed when high concentrations were applied for a sufficiently long time to reach the maximum membrane current. Very different curves were found in which EC_{50} varied from $0.2\ \mu\text{M}$ to $2.8\ \mu\text{M}$ and the Hill coefficient from 0.7 to 4.5. Such variations of the dose-response relationship were observed not only in different neurones but also in the same neuron during repeated series of CAPS applications. This made it difficult to draw any conclusions. However, in 5 neurons the dose-response curves for the responses to the first series of CAPS applications revealed EC_{50} of $0.31\ \mu\text{M} \pm 0.5\ \mu\text{M}$ with a Hill coefficient 1.8 ± 0.4 ($n=5$). This might suggest that at least two molecules of CAPS must bind to the receptor to activate the channel.

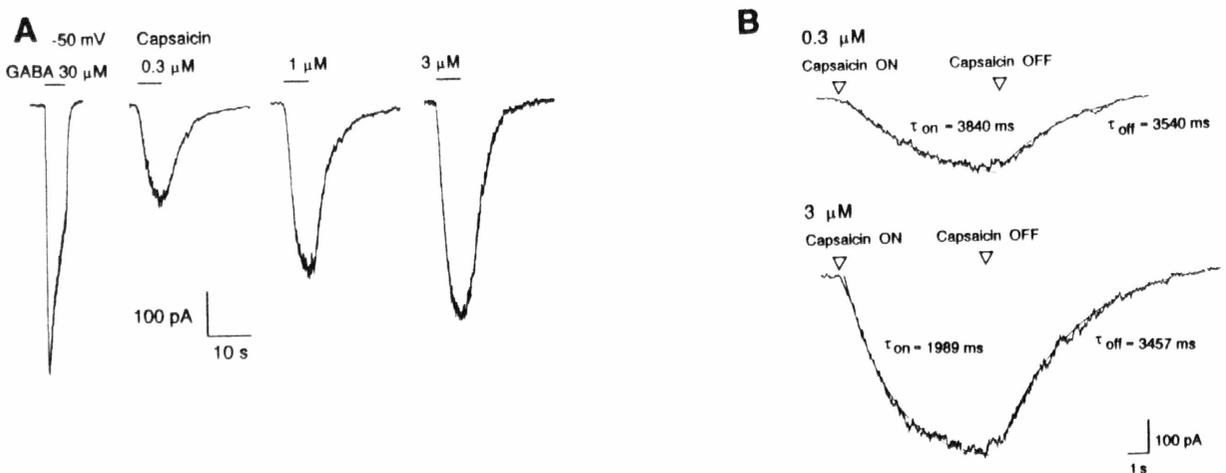
The time course of responses to CAPS

The time courses of the responses to GABA and to CAPS showed a striking differences. The onset and offset of the responses to GABA were fast, apparently approaching the limits of the drug application system. In contrast, it took several seconds for the response to CAPS to reach its maximum amplitude and for the return to the baseline after the neuron had been rapidly washed by the control ECS (Fig. 3A). The rise times of the responses were concentration-dependent and were prolonged after repeated CAPS applications. In the third series of CAPS applications in the neuron shown in the Fig. 3B, the time constants of the ascending phase of the responses to CAPS at $0.3\ \mu\text{M}$ and $3\ \mu\text{M}$ were 3.8 and 2 s, respectively. The association rate constant (K_1) calculated from these values was $9 \times 10^4\ [\text{M}^{-1}\ \text{s}^{-1}]$.

**Fig. 2**

Tachyphylaxis of the responses to CAPS.

A: Whole cell membrane currents induced by two successive applications of CAPS at concentrations 3 μM and 10 μM in a neuron at membrane potential -50 mV. **B:** Three successive applications of CAPS (10 μM) in a neuron at -50 mV. 10 mV hyperpolarizing pulses of 1 s duration were applied at 0.33 Hz. **C and D:** Whole cell currents induced by GABA (30 μM) and CAPS at concentrations 0.1 μM , 0.3 μM , 3 μM and 10 μM as indicated. **C**, the first application series and **D**, the third application series in the same neuron. Membrane potential -50 mV. Calibration 200 pA, 20 s applies to **A**. Calibration 100 pA, 15 s applies to **B**, **C** and **D**.

**Fig. 3**

Differences in the time course of the responses to GABA and CAPS.

A: GABA 30 μM , capsaicin 0.3 μM , 1 μM and 3 μM . Bars above the records indicate the time of application. **B:** Responses to 0.3 μM and 3 μM capsaicin with a faster time scale. The solid lines drawn through the data points are single-exponential functions fitted to the ascending and descending phase of the responses with the indicated time constants (τ).

The time constant of relaxation after rapid washing of the neuron with ECS was 3.5 s at all concentrations tested. This value corresponds to an apparent dissociation rate constant K_{-1} 0.29 s^{-1} . If one neglects the diffusion time of the application system necessary to reach the maximum concentration or wash the drug away (about 50 ms) and assumes that channel opening and closing are highly dependent on

binding and unbinding of the agonist, then the K_d calculated as K_{-1}/K_1 was $3 \mu\text{M}$ which corresponded to the EC_{50} computed from the same series of responses used to construct a concentration-response curve. This is about ten times greater than the value calculated from the first trial of CAPS applications at increasing concentrations.

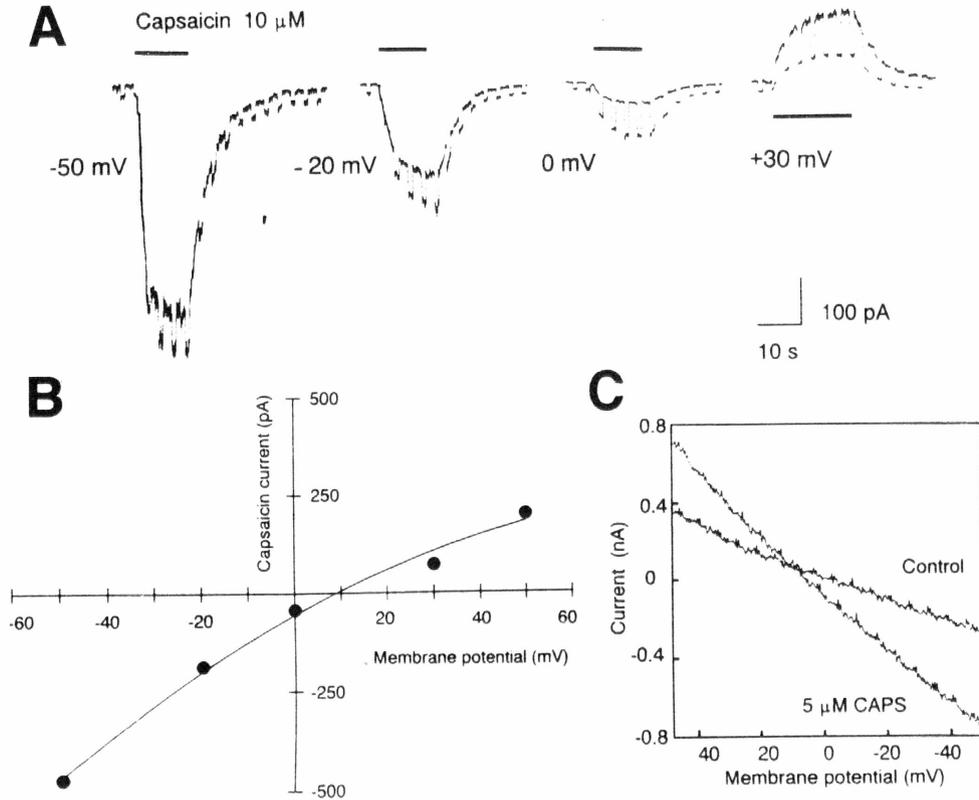


Fig. 4 Current-voltage relationship of CAPS-induced membrane currents.

A: Four records of responses to $10 \mu\text{M}$ CAPS at different membrane potentials. 10 mV hyperpolarizing pulses 1 s in duration at 0.33 Hz were applied to the microelectrode. **B:** The diagram shows current-voltage relation for the cell shown above. Solid line: least square regression line fitted to the data points, gives the current reversal potential $+10 \text{ mV}$. **C:** Estimation of reversal potential. Voltage ramp $+100 \text{ mV}$ of 2 s duration was applied at -50 mV membrane potential and 2 s back from $+50 \text{ mV}$ to -50 mV in control and at maximum of the membrane current induced by CAPS ($5 \mu\text{M}$) containing extracellular solution. Only the descending phase of current responses was used for estimating the zero current. The estimated reversal potential was $+10 \text{ mV}$.

Current-voltage relationship of the responses to CAPS

The current-voltage relationship at membrane potentials between -50 mV and $+50 \text{ mV}$ (Fig. 4) was slightly concave upwards. The polarity of the membrane current reversed at $+10.4 \pm 2.5 \text{ mV}$ ($n=5$).

To minimize possible effects of tachyphylaxis on the reversal potential of the CAPS-induced responses we applied $+100 \text{ mV}$ voltage ramps of 2 s duration from the holding potential -50 mV and then back to the original membrane potential. To avoid

activation of voltage-dependent calcium channels which produced an inflection more pronounced on the ascending phase of the ramp, CdCl_2 (1 mM) was added both to the control and CAPS-containing superfusing solutions. The effects of activation of voltage-dependent channels were minimized by adding $1 \mu\text{M}$ tetrodotoxin (TTX). However, as this would still not exclude the possibility of activation of TTX-insensitive voltage-gated sodium channels only the descending phase was used for estimation of the zero current during activation of the CAPS-gated channels.

The membrane current responses in the control and 5 μM CAPS solutions to the voltage ramp of -100 mV from $+50$ mV to -50 mV (Fig. 4C) showed the membrane current to be zero at 10 mV. This suggested that this membrane potential represented reversal potential for the CAPS membrane current. In four other cells in which the control ramp current did not change after CAPS application the mean reversal

potential was 15 ± 5 mV (S.D. $n=4$). In view of the study of Winter *et al.* (1990) who estimated the reversal potential for the CAPS-induced current to be close to zero membrane potential when CaCl_2 was omitted from the extracellular solution, our finding suggests that a substantial part of CAPS-induced current was carried by Ca^{2+} in addition to the monovalent cations.

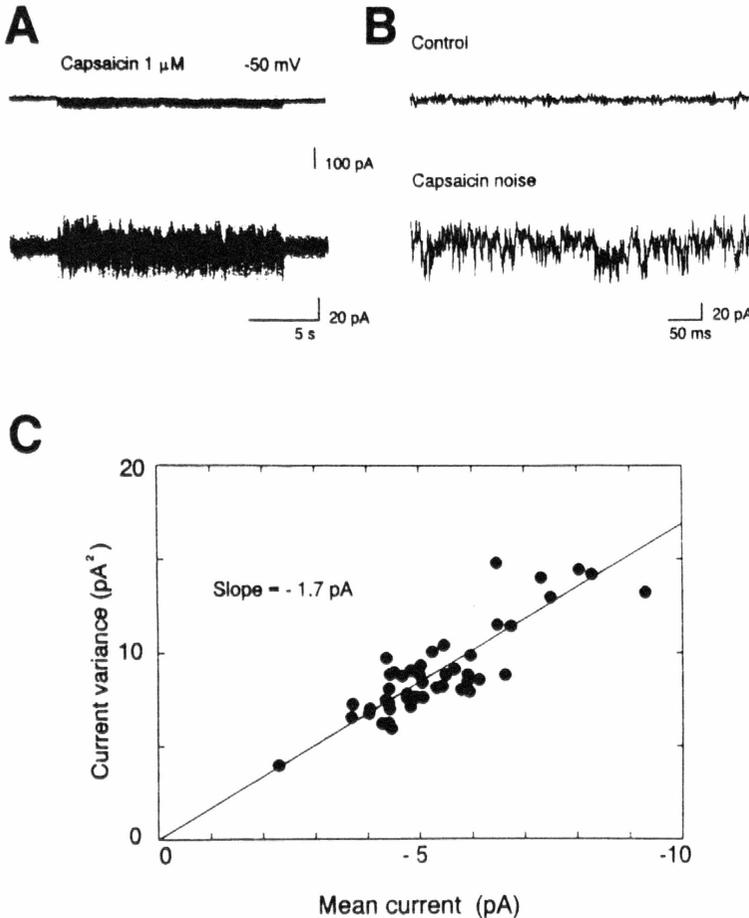


Fig. 5

Capsaicin-induced current noise in an outside-out patch.

A: Inward membrane current evoked by steady application of $1 \mu\text{M}$ capsaicin. D.C. shift of the mean current (upper trace) and A.C. record of current noise (lower trace). Calibration: 100 pA, 20 pA and 5 s. **B:** The same experiment as in A. The control (upper trace) and capsaicin induced currents (lower trace). Calibration: 20 pA and 50 ms. **C:** Relationship between mean inward current, μ_I , and the variance of the associated increase in current noise, $\text{var}(I)$, induced by application of $1 \mu\text{M}$ capsaicin. The slope of the line fitted by linear regression to the points gave the single channel current ($i = \text{var}(I)/\mu_I$) $i = -1.7$ pA at -50 mV, which is consistent with a minimum single channel conductance 28 pS. Bandwidth: 0.2 – 1000 Hz, -3 dB (8-pole Butterworth).

Single channel conductance

The conductances of the single channels involved in the CAPS-induced membrane currents were estimated from outside-out patches in which the mean membrane current produced by a low concentration of CAPS ($1 \mu\text{M}$) were relatively small with respect to the induced current noise (Fig. 5A,B). When the probability of the channel opening was low (e.g. for 0.1 – $1 \mu\text{M}$ CAPS), the variance of the capsaicin-induced current noise ($\text{var}(I)$) was presumed to vary linearly with the mean membrane current μ_I

($\text{var}(I) = i \cdot \mu_I$) where i is single channel current. The slope of this linear relationship gives the single-channel current (Anderson and Stevens 1973). Assuming the reversal of the CAPS-induced responses to be at $+10$ mV, the slope of current noise variance found at -50 mV of membrane potential, 1.7 pA, indicated a single channel conductance of 28 pS (Fig. 5C). This value was similar to that which was measured directly from transitions of the membrane current induced by a low concentration of CAPS ($0.1 \mu\text{M}$) in a patch in which at least four active channels were present (Fig 6).

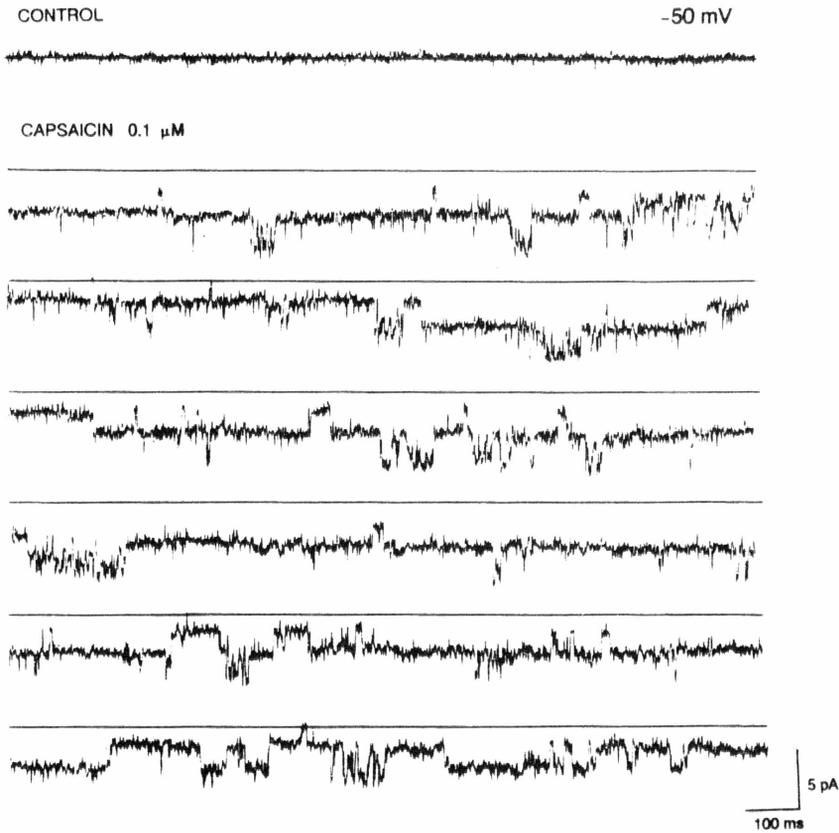


Fig. 6

Capsaicin-activated single-channel currents in outside-out recording mode. The first control trace was taken in ECS solution. Holding potential: -50 mV. After application $0.1 \mu\text{M}$ capsaicin superimposed channel openings can be distinguished as discrete steps in the current. Single-channel current estimated from the records was -1.7 pA which is consistent with a single channel conductance 28 pS. The lines above each record indicate zero current, i.e. the state when all channels present in the patch were closed (presumably four).

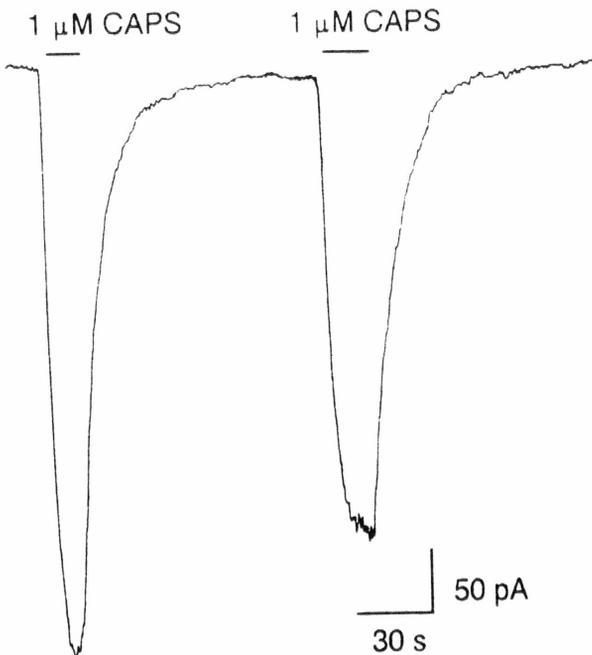


Fig. 7

Records of two consequent responses to CAPS ($1 \mu\text{M}$). The pipette solution used for whole cell recording contained $50 \mu\text{M}$ CAPS.

Lack of the effect of CAPS from intracellular side

The striking difference between the action of CAPS and conventional agonists as acetylcholine, glutamic acid and GABA is the presence of very slow rising and decay phases of the responses. One possibility considered was that CAPS recognition sites might be localized on the inside of the plasma membrane and that CAPS has to cross the outer layer to reach the lipophilic part in which it might act. This idea was substantiated by the fact that in contrast to conventional transmitters, CAPS is very poorly soluble in water and easily in alcohol or dimethylsulfoxide. Therefore we tested this idea by adding either 10 μM CAPS or 50 μM CAPS to the pipette solution used for whole cell recording. If this hypothesis was correct it could be expected that CAPS acts also from the intracellular side and desensitizes the receptors. However, in 5 neurons tested with 10 μM ($n=2$) and 50 μM ($n=3$) in the pipette solution no changes of the sensitivity of the neurones to CAPS (1 μM) were found during the whole time of recording (Fig. 7). Smaller amplitude of the second response results from the tachyphylaxis described earlier. This finding excludes the possibility that CAPS might act from the intracellular side of the plasma membrane and makes it very unlikely that the CAPS recognition site of the receptors are localized on the intramembrane part of the protein complex.

Discussion

Our results demonstrate that, in cultured small DRG neurons of the rat, CAPS activates a distinct class of cationic channels which discriminate poorly between monovalent and divalent cations and exhibit slow kinetics of activation and inactivation and a progressive run-down after repeated application of CAPS at high concentrations.

Correlation of CAPS sensitivity with morphology of DRG neurons, GABA sensitivity, and voltage-dependent currents

Our results agree with other studies (Baccaglioni and Hogan 1983, Bevan and Forbes 1988, Heyman and Rang 1985, Petersen and LaMotté 1991, Winter *et al.* 1990, Wood *et al.* 1988) which have shown that CAPS induces membrane currents in a distinct group of DRG neurons which are small and apparently correspond to the B-type (Andres 1961, Harper and Lawson 1985, Winter *et al.* 1990). Morphologically, these neurons are bipolar or tripolar and can usually be easily distinguished from the large unipolar neurons which were completely insensitive to CAPS at concentrations as high as 10 μM .

Our results also confirm the observation that GABA induces much smaller membrane currents in the CAPS-sensitive DRG neurons than in the large, CAPS-insensitive neurons (White 1990). In our experiments some small neurons in which CAPS induced large membrane currents, the GABA responses were virtually absent. On the other hand, it seemed that the responses to GABA of neurons of the overlapping subpopulation as judged by size, were intermediate in magnitude. This suggests that there is no strict separation of DRG neurons with respect to GABA sensitivity and supports earlier evidence that GABA_A receptors are unevenly distributed on cell bodies of primary afferents and that a much higher density may exist on the central terminals of the fast conducting afferent fibers than on the C-afferents (Desarmenien *et al.* 1984).

Reversal potential of CAPS-induced responses

The slight rectification of membrane currents seen at negative membrane potentials was very likely an artifact because of the tachyphylaxis of the responses after repeated CAPS application and because it was not observed in voltage ramp experiments. The reversal of the polarity of CAPS-induced responses at around +10 mV suggested that the CAPS-induced membrane currents were carried nonspecifically by monovalent cations and Ca^{2+} . This would explain the massive calcium accumulation in DRG neurons after prolonged treatment with CAPS (Wood *et al.* 1988). The difference with the findings of Winter *et al.* (1990) who estimated the reversal of CAPS-induced responses at close to zero membrane potential can be fully explained by the fact that these authors did not use Ca^{2+} in the extracellular solution. However, a more detailed study is needed to elucidate the proportion of cations that contribute to the CAPS-induced membrane currents as it has been reported that the single channel conductance is voltage-dependent (Forbes and Bevan 1988).

Tachyphylaxis and concentration-response relationship

Physiological effects of CAPS are studied mostly in the micromolar range. However, depolarizing effects at concentration as low as 50 nM have been reported (White 1990). Our results demonstrate that the responses to CAPS at high concentrations exhibited small desensitization and a progressive run-down which was accompanied by a reduction of the increased membrane conductance (Fig. 2). Therefore our values of EC_{50} and of the Hill coefficient derived from concentration-response curves should be considered with caution. In spite of the difficulty imposed by tachyphylaxis the value of EC_{50} 0.3 μM found in the first series of CAPS applications at increasing concentrations is not very different from the value

0.2 μM obtained by measurements of CAPS-induced calcium uptake (Wood *et al.* 1988). However, EC₅₀ exceeding 3 μM was observed in other neurones or at certain stages of recording from the same neuron. Although a Hill coefficient of 1.7 was found in the cells in which the responses to CAPS were rather stabilized, values ranging from 0.7 to 4.0 were observed. This variability may reflect difficulties in constructing dose-response curves imposed by the progressive run-down of the responses. However, the possibility cannot be excluded that there are additional factors other than affinity of the receptors to CAPS and the number of agonist recognition sites which control the opening of the channels (see later).

Conductance of CAPS-gated channels

Assuming a reversal potential of +10 mV, variance analysis of the noise in outside-out patches induced by low concentrations of CAPS indicated that the conductance of the ionic channels was about 28 pS. A similar value of single channel conductance was obtained by measuring the amplitude of single channel activity which could be recovered from the noise. This value is in good agreement with the single channel conductances evoked by CAPS at negative membrane potentials of about 25–30 pS reported by Forbes and Bevan (1988). A detailed analysis of single channel activity is needed to learn more about the kinetics of CAPS-gated channels. The long time constant of relaxation exceeding 3 s suggests that the mean time of their opening is unusually long.

What type of channels CAPS-gated channels may represent?

One of the characteristic features of the responses to CAPS was that their rise and decay time constants were several seconds. It can be excluded that this is due to a slowly increasing concentration of CAPS because the drug application system ensured that the maximum concentration around the neuron was reached in about 50 ms. Indeed, the responses to GABA were fast both in their rise and decay using the same application system in one neuron (see Fig. 3A).

There are several possibilities which should be considered to explain the slow kinetics of CAPS-gated channels. Firstly, the channels may possess several receptor sites with very high affinity for CAPS which after binding results in a high probability of opening the channel lasting for as long as the agonist is bound. A very long time constant of relaxation supports this idea. The progressive run-down of the responses would then reflect an inherent property of the CAPS receptors.

It is unlikely that CAPS recognition site(s) are located on the intramembrane part of the receptor-protein complex. This idea was considered because

reduced diffusion of CAPS through the upper layer of the plasma membrane might restrict the access of the agonist to the receptor recognition site and thus reduce the speed of the onset and the decay of the responses to CAPS. However, our finding that CAPS up to the 50 μM in the intracellular solution did not change the responses produced by extracellular application of CAPS even after prolonged recording times, makes this hypothesis extremely unlikely (see Fig. 7).

It seems unlikely that the run-down of the CAPS response was due to deprivation of energy from intracellular sources, i.e. ATP which has been shown to attenuate the run-down of NMDA receptors when added to the pipette solution (Mody *et al.* 1988), because a progressive decrease of the sensitivity of nociceptors to repeated applications of CAPS has been well documented *in vivo* (Fitzgerald 1983, Szolcsanyi 1983).

The slow kinetics and run-down of the CAPS-induced responses raises a question as to whether the agonist receptor sites could not be localized on a separate protein complex in the membrane which would interact with the ionic channel either directly or indirectly by means of another protein similar to G-proteins (Brown and Birnbaumer 1988, Linder 1992, Ross 1992). In contrast to G-proteins, however, a remote receptor or intermediate protein would not need to be supported in the function by a fast supply of GTP or other metabolites depending on soluble intracellular enzymes because the responses to CAPS were recorded in the whole cell mode with electrodes that allow fast washing out of the interior of the cells and because the responses were also present in outside-out patches (Hamill *et al.* 1981, Neher 1988). This, however, does not exclude a possibility that without the support of GTP the protein which binds CAPS or the protein which mediates the effects undergo much faster run-down. A possible involvement of protein kinase C was excluded because of staurosporine, an inhibitor of protein kinases, does not inhibit ion fluxes induced by resiniferatoxin which is a highly potent CAPS analogue (Winter *et al.* 1990).

A possible physiological role of CAPS-gated channels

Experimental evidence that CAPS receptors are present not only at the peripheral terminals but also on the cell bodies of a distinct group of DRG neurons in culture may suggest that these cells are a suitable model for studying molecular mechanisms of nociception (Baccaglini and Hogan 1983). There is general agreement that the CAPS receptors are expressed on mammalian sensory neurons which are smaller and whose action potential possesses a substantial calcium component (Petersen and LaMotte 1991, White 1990). It is, however, unlikely that the CAPS receptors serve as a common sensor for all chemical agents which produce pain. Several algogens,

i.e. weak acids and serotonin, have been shown to employ other mechanisms (Bevan and Yeats 1991, Handwerker *et al.* 1990, Konnerth *et al.* 1987, Krishtal and Pidoplichko 1981, Neto 1978).

With respect to the integrative mechanisms involved in nociception it is of interest that CAPS-sensitive DRG neurons exhibit very low sensitivity to GABA because it suggests that presynaptic inhibition could be much less effectively exerted on a slow conducting afferent system than on the fast conducting system in which presynaptic inhibition was mainly studied (Eccles 1964).

In vivo, some CAPS-sensitive afferents represent mechano-heat sensitive nociceptors in the C fiber and A β fiber range (Szolcsanyi *et al.* 1988). It seems likely that other afferents subserving pain sensation, in particular polymodal C type, also belong to this category of DRG neurons (Szolcsanyi 1973, Szolcsanyi 1987). However, it is unlikely that CAPS induces activity exclusively in primary afferents which subserve pain because some thermosensitive neurons

were also found to be sensitive to CAPS (Coleridge *et al.* 1965, Lundberg *et al.* 1985). Nor are all afferents subserving pain capsaicin-sensitive because the A β high threshold mechanoreceptors, which also subserve nociception, were found insensitive to CAPS (Foster and Ramage 1981, Kenins 1982, Szolcsanyi *et al.* 1988). Apparently, a great mosaic of receptors and ionic channels in the membrane controls the excitability in different classes of peripheral neurons which serve distinct modalities of sensation including pain with great specificity.

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