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Desensitization of capsaicin-evoked neuropeptide release – Influence of Ca^{2+} and temperature

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Summary. Capsaicin-induced stimulation and desensitization of neuropeptide release from primary afferent neurons was investigated in the rat urinary bladder in-vitro. The capsaicin (5 min contact time)-evoked release of calcitonin gene-related peptide-like immunoreactivity (CGRP-IR) was dose-dependent; threshold to produce detectable release was 0.1 μmol/l, the EC_{50} was 0.17 μmol/l.

Pre-exposure of tissues to capsaicin (0.1 – 1.0 μmol/l, 5 min contact time) caused a dose-dependent reduction of the amount of CGRP-IR which was released by a second exposure to capsaicin. At 0.1 and 0.3 μmol/l, capsaicin was less effective to inhibit the subsequent K^+-evoked release than that evoked by a second capsaicin exposure. Pre-exposure to 1 μmol/l capsaicin completely prevented subsequent K^+- or capsaicin-evoked release of CGRP-IR.

Exposure of the preparation to capsaicin (0.3 μmol/l) in a Ca^{2+}-free, EDTA-containing medium did not produce release of CGRP-IR. A subsequent stimulation with capsaicin in a 2.5 mmol/l Ca^{2+}-containing superfusion solution was not less effective to release CGRP-IR than in tissues which had not been pre-exposed to capsaicin.

At 18° C, the capsaicin-evoked release of CGRP-IR was reduced to 20% of the value obtained by the same dose (0.3 μmol/l for 5 min) of capsaicin at 37° C.

Comparison of the desensitizing effect of 0.3 and 0.1 μmol/l capsaicin at 18° C and 37° C, respectively, showed significant inhibition of desensitization at 18° C. Inhibition of desensitization was also observed when the amount of CGRP-IR, which was released during pre-exposure to capsaicin (0.3 μmol/l for 10 min) at 18° C, was 3-fold higher than that produced by pre-exposure to capsaicin (0.1 μmol/l for 5 min) at 37° C.

The present results show that in a narrow range of concentrations, capsaicin induces "selective" desensitization which is entirely dependent on the presence of external Ca^{2+} – and which is attenuated at low temperature.

Key words: Capsaicin – Primary afferents – Desensitization – Temperature-dependency

Introduction

Capsaicin is regarded as a selective stimulant of primary afferent C/A-delta fibers (cf. Holzer 1988; Maggi and Meli 1988). The mode of action of capsaicin has been subject of a number of studies, the results of which indicate that on afferent neurons, capsaicin acts on a specific binding site (Szolcsányi and Jáncso-Gábor 1975; James et al. 1989; Szallasi and Blumberg 1989a), activates cation conductances (Heyman and Rang 1985; Marsh et al. 1987; Wood et al. 1988) and thus produces depolarization and neuropeptide release. In addition to this acute effect, capsaicin also is known to produce "desensitization" of primary afferent neurons (cf. Lembeck 1988; Szolcsányi 1985).

The initial stage of desensitization, which has been described as "sensory neuron blocking effect of capsaicin", develops immediately after capsaicin exposure (Szolcsányi 1985). In-vitro, this initial stage of desensitization has been investigated by, direct or indirect, determination of evoked neuropeptide release from peripheral afferent terminals (Dray et al. 1989; Håkanson et al. 1987; Maggi et al. 1989, 1990).

It has been suggested that capsaicin produces a non-selective blockade of neuropeptide release from afferent terminals, either by exhaustion of releasable pools (Håkanson et al. 1987), or by accumulation of intracellular Ca^{2+} (Maggi et al. 1989). On the other hand, recent reports suggest that non-selective blockade of afferent terminals only occurs when supramaximal concentrations of capsaicin are used (Dray et al. 1989). At lower concentrations, desensitization seems to be selective for capsaicin-induced stimulation of mediator release (Dray et al. 1989).

Another controversial point is the potency of capsaicin to desensitize as compared to its potency to stimulate afferent neurons. Dray et al. (1989) have shown that desensitization to capsaicin in the guinea-pig ureter in-vitro only occurs at doses which are considerably higher than those necessary to produce neuropeptide release. On the other hand, Maggi et al. (1990) who used a

Table 1. Evoked release of CGRP-IR in the isolated rat urinary bladder 60 min after a 5-min exposure to different concentrations of capsaicin. Number of experiments given in parenthesis. Mean ± SEM; * $P < 0.05$ compared to vehicle treatment

Pre-exposure	Release of CGRP-IR (fmol/g) induced 60 min later by				
	Capsaicin (μmol/l)				60 mM K^+
	0.03	0.1	0.3	1.0	
Vehicle	−5 ± 3.9 (4)	660 ± 76 (10)	2298 ± 220 (10)	2574 ± 157 (13)	602 ± 110 (8)
0.03 μM capsaicin	–	593 ± 77 (4)	–	–	–
0.1 μM capsaicin	–	128 ± 43 (4)*	1261 ± 250 (5)*	–	556 ± 81 (5)
0.3 μM capsaicin	–	5.3 ± 7.3 (4)*	363 ± 31 (6)*	–	245 ± 50 (4)*
1.0 μM capsaicin	–	–	−0.8 ± 9.1 (4)*	17.7 ± 17.3 (4)*	10.1 ± 5.4 (4)*

similar experimental protocol as Dray et al. (1989) in the rat urinary bladder, report that a dose of capsaicin, which corresponds to a threshold dose for stimulation of release, already produces significant desensitization. In view of reports on the effect of temperature on excitability of primary afferents (Szolcsányi 1977), a possible explanation for this discrepancy may be that in the above mentioned studies experiments were carried out at different temperatures (Dray et al.: 22° C ± 2, Maggi: ≥ 26° C).

The aim of the present study was to compare the potency of capsaicin to evoke neuropeptide release and to induce desensitization, and to determine which concentrations of capsaicin produce "selective" desensitization. Additional experiments were carried out to evaluate the Ca^{2+}- and temperature-dependency of capsaicin-induced desensitization.

To determine the stimulatory and desensitizing effect of capsaicin, the evoked release of CGRP-IR from the isolated rat urinary bladder was determined, because in this tissue CGRP is nearly exclusively stored in capsaicin-sensitive afferent neurons (Su et al. 1986) and is released by capsaicin in a Ca^{2+}-dependent manner (Amann et al. 1990).

Methods

Strips (85–100 mg wet weight) of the rat urinary bladder were placed in a chamber (1 ml) and superfused (0.6 ml/min) with gassed (95% O_2, 5% CO_2) physiological salt solution (NaCl 118, KCl 4.6, $MgSO_4$ 1.17, $CaCl_2$ 2.5, NaH_2PO_4 1.17, $NaHCO_3$ 25, glucose 10 mmol/l) containing 1 mg/ml bovine serum albumin at 37° C or at 18° C. The following experimental protocol was used:

A) After an equilibration period of 20 min, capsaicin or vehicle (NaCl containing 0.003% DMSO) was added to the superfusion solution for 5 min. 60 min later, stimulation (5 min) was performed with capsaicin or K^+ (60 mmol/l, isotonically replacing NaCl). "Desensitization" was determined by comparison of the amount of stimulus-evoked CGRP-IR release in preparations which had not been exposed to capsaicin, to that evoked in tissues which had been exposed to capsaicin (0.03–1.0 μmol/l).

B) For experiments with Ca^{2+}-free solution, $CaCl_2$ was replaced by NaCl and 1 mmol/l ethylenediaminotetra-acetic acid (EDTA) was added to the solution. After an equilibration period of 20 min, capsaicin was added to the superfusion solution for 5 min. 25 min later (30 min before second exposure to capsaicin), the superfusion solution was replaced by a Ca^{2+}-containing solution (composition see above). In control experiments, no capsaicin was added during the Ca^{2+}-free superfusion period.

C) Temperature-dependency of desensitization was determined by comparing the amount of CGRP-IR released by the first exposure to capsaicin (37° C: 0.1 μmol/l; 18° C: 0.3 μmol/l) to that which was produced 60 min later by a second exposure to the same concentration of capsaicin.

The perfusate was collected in 5-min fractions, the samples were lyophilized and then assayed for CGRP-IR using antiserum RAS 6009 N (Peninsula) raised against human CGRP. The crossreactivities are: rat-CGRP: 100%, rat calcitonin C-terminal adjacent peptide: <0.001%, calcitonin <0.001%, katacalcin: <0.001%, Arg-vasopressin: 0, oxytocin 0. (2-(I 125) iodohistidyl 10) CGRP, human (Amersham, UK) was used as radioligand and synthetic human alpha-CGRP (Peninsula, Merseyside, UK) as standard. Release was expressed as fmol CGRP-IR/g tissue released during a 5-min fraction. Total evoked release was calculated by subtracting basal value from the total release occurring in response to stimulation. The detection limit of the assay was 1.3 fmol/tube corresponding to 13–15 fmol/g tissue per 5-min fraction.

For high pressure liquid chromatography (HPLC) analysis, samples were injected on a reversed-phase nucleosil C_{18} column (Waters). Elution was performed by a 85-min gradient from 20% to 60% acetonitrile at a flow rate of 1.5 ml/min. The fractions were lyophilized and assayed for CGRP-IR as described above.

Tissue content of CGRP-IR was determined in a separate set of experiments. Strips of the rat urinary bladder were superfused as described above. 60 min after a 5-min exposure to vehicle or capsaicin, bladder strips were homogenized in 2 N acetic acid. After centrifugation, the supernatant was lyophilized and assayed as described above.

Materials and statistics. Capsaicin was obtained from Sigma (St. Louis, MO, USA). Stock solutions (30 mmol/l) were prepared in dimethylsulfoxide (DMSO) and diluted in physiological salt solution to final concentrations. Values are expressed as mean ± SEM. Unless stated otherwise, statistical significance was evaluated using one way analysis of variance and Newman-Keul's test.

Results

In the superfusate of the rat urinary bladder, basal efflux of CGRP-IR (17–45 fmol/g × 5-min) in control preparations was close to the detection limit of the assay. This value was not significantly changed in a Ca^{2+}-free, EDTA (1 mmol/l)-containing superfusion solution, at 18° C, nor

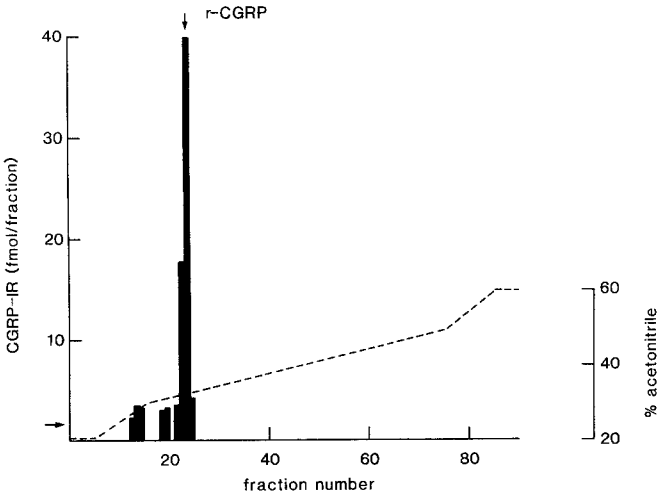

Fig. 1. Efflux of CGRP-IR from the isolated rat urinary bladder. HPLC separation of CGRP-IR which was released by capsaicin. Detection limit of the assay indicated by *horizontal arrow*. Position of synthetic rat-CGRP (r-CGRP) as indicated

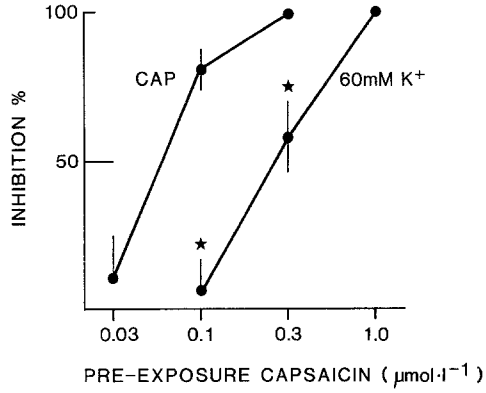

Fig. 2. Percent inhibition of the capsaicin (0.1 μmol/l) – or K^+ (60 mmol/l)-evoked release of CGRP-IR 60 min after exposure of the urinary bladder to different concentrations of capsaicin (given on the *abscissa*). In vehicle pre-exposed preparations, capsaicin (0.1 μmol/l) and K^+ (60 mmol/l) evoked the release of 660 ± 76 and 602 ± 110 fmol/g, respectively (see Table 1). Mean ± SEM; *n* as indicated in Table 1; $^*P < 0.05$ as compared to corresponding inhibition of the capsaicin-evoked release

Table 2. Tissue content (pmol/g) of CGRP-IR in the rat urinary bladder. Bladder strips were superfused according to protocol A, B and C (see methods), and CGRP-IR was determined 60 min after exposure to vehicle (0.003% DMSO) or capsaicin. Mean ± SEM; number of experiments in parenthesis

Pre-exposure protocol		CGRP-IR content (pmol/g)
A	(37°C) + vehicle	64.9 ± 4.9 (6)
B	(Ca^{2+}-free) + vehicle	72.9 ± 8.0 (5)
C	(18°C) + vehicle	63.9 ± 4.6 (4)
A	(37°C) + capsaicin (0.3 μmol/l)	56.8 ± 6.0 (4)
A	(37°C) + capsaicin (1.0 μmol/l)	59.7 ± 6.7 (6)

after different pre-exposure protocols. Therefore basal efflux of CGRP-IR is not likely to be caused by an active release process.

In vehicle pre-exposed preparations (protocol A), addition (5 min) of capsaicin to the superfusion solution caused a dose-dependent release of CGRP-IR (Table 1). The threshold concentration to produce significant release of CGRP-IR was 0.1 μmol/l capsaicin; the EC_{50} was 0.17 μmol/l (0.056 – 0.417 μmol/l, 95% confidence interval). A maximal effect was reached at 1.0 μmol/l capsaicin. HPLC analysis showed that more than 80% of the CGRP-IR which was released by capsaicin eluted at the position of synthetic rat-CGRP (Fig. 1).

Determination of the tissue content of CGRP-IR (Table 2) showed that the amount of CGRP-IR which was released by 1 μmol/l capsaicin (about 4% of content), was not sufficient to cause detectable depletion of CGRP-IR ($n = 6$).

Concentration-dependency of capsaicin-induced desensitization

Pre-exposure (5 min, 60 min before) of the tissues to 0.03 μmol/l capsaicin did not reduce the amount of CGRP-IR which was released by a subsequent stimulation (5 min) with 0.1 μmol/l capsaicin. Pre-exposure to higher concentrations of capsaicin (0.1 – 1.0 μmol/l) dose-dependently attenuated the capsaicin-evoked release of CGRP-IR (Table 1).

Superfusion of bladder strips with a solution containing 60 mmol/l K^+ released about the same amount of CGRP-IR (602 ± 110 fmol/g, $n = 8$) as 0.1 μmol/l capsaicin (660 ± 76 fmol/g, $n = 10$; Table 1).

Figure 2 shows the comparison of the effect of capsaicin pre-exposure on capsaicin (0.1 μmol/l)- and K^+-stimulated release. Pre-exposure to low concentrations of capsaicin attenuated the K^+-evoked release significantly less than that evoked by capsaicin.

Influence of external Ca^{2+} on capsaicin-induced desensitization

Further experiments were performed to determine the Ca^{2+}-dependency of desensitization to 0.3 μmol/l capsaicin. Superfusion of the bladder strips with a Ca^{2+}-free, EDTA (1 mmol/l)-containing solution completely prevented the capsaicin (0.3 μmol/l)-evoked release of CGRP-IR ($n = 6$, data not shown).

In one group of experiments ($n = 6$), tissues were exposed to 0.3 μmol/l capsaicin (5 min) during the Ca^{2+}-free superfusion period, while in control experiments ($n = 4$) vehicle was used instead of capsaicin. Thereafter, the Ca^{2+} concentration in the superfusion solution was adjusted to 2.5 mmol/l, and the capsaicin (0.3 μmol/l) evoked release of CGRP-IR determined. The amount of CGRP-IR which was released was not significantly affected by pre-exposure to capsaicin during Ca^{2+}-free superfusion period (control: 2315 ± 409 fmol/g, $n = 4$; capsaicin preexposed: 2254 ± 276 fmol/g, $n = 6$).

Control experiments showed that this protocol (reintroduction of Ca^{2+} after Ca^{2+}-free superfusion) did not

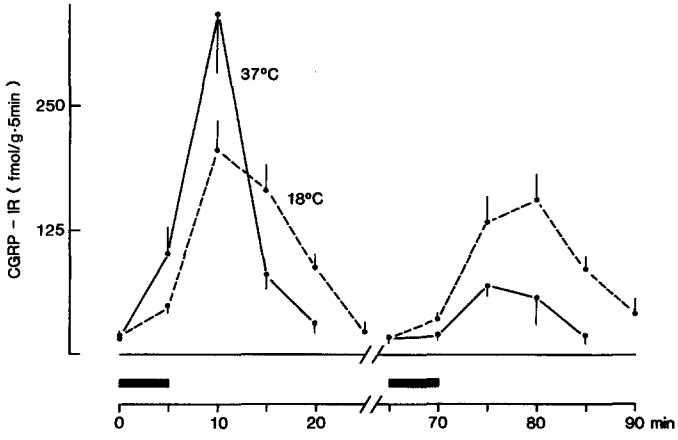

Fig. 3. Time-course of the capsaicin-evoked release of CGRP-IR from the isolated rat urinary bladder at 37° C (*solid line*, $n = 4$) and at 18° C (*broken line*, $n = 10$). Stimulation (5 min) with capsaicin (0.1 μmol/l at 37° C, 0.3 μmol/l at 18° C, indicated by *bars*) was repeated after a 60 min-interval. Values are mean ± SEM of CGRP-IR (fmol/g) released during a 5-min collection period

Table 3. Desensitization of capsaicin-evoked CGRP-IR release at 37° C and at 18° C. Amount of CGRP-IR which was released by capsaicin (concentration and exposure time as indicated) by first and by a second stimulation which were separated by a 60-min interval. The difference of the amount which was released by first and second stimulation was calculated for each experiment as % of the amount released during first stimulation. "Desensitization" is expressed as mean ± SEM of % reduction. The reduction was significant ($P < 0.05$) in all groups (*t*-test for paired data)

Stimulation by capsaicin	Total evoked release of CGRP-IR (fmol/g)		
	1st stimulation	2nd stimulation	% reduction
0.1 μmol/l, 5 min, 37° C	548 ± 102	119 ± 39**	−74.8 ± 11.3 (4)
0.3 μmol/l, 5 min, 18° C	499 ± 69	380 ± 65	−25.8 ± 5.1* (10)
0.3 μmol/l, 10 min, 18° C	1701 ± 202*	916 ± 163*,**	−46.3 ± 8.4* (4)

* $P < 0.05$ as compared to corresponding value at 37° C
** $P < 0.05$ as compared to first stimulation

significantly alter CGRP-IR content of the preparation (Table 2).

Influence of temperature on capsaicin-induced desensitization

In these set of experiments, "desensitization" was evaluated by comparison of the amount of CGRP-IR which was released by two consecutive (60 min interval) stimulations with capsaicin. The time-course of the evoked CGRP-IR release is shown in Fig. 3.

At 37°, 0.1 μmol/l capsaicin caused pronounced desensitization (Table 3), which is in agreement with the results of experiments described above (Table 1).

Superfusion of bladder strips at 18° C did not significantly change tissue content of CGRP-IR (Table 2). However, at this temperature, K^+ (60 mmol/l, 5 min) failed to evoke significant release of CGRP-IR ($n = 4$, data not shown). At 18° C, stimulation with capsaicin (0.3 μmol/l, 5 min) evoked the release of 499 ± 69 fmol/g ($n = 10$), only about 20% of the value obtained by the same dose of capsaicin at 37° C (Table 1).

Comparison of the amount of CGRP-IR which was released by first and second stimulation at 18° C showed that desensitization was significantly reduced as compared to 37° C (Table 3). When the duration of stimulation periods was increased to 10 min, the amount of CGRP-IR which was released by the first exposure to capsaicin (0.3 μmol/l) was about 3-fold higher as that released by 0.1 μmol/l (5 min) at 37° C. But nevertheless, desensitization was less pronounced than at 37° C (Table 3).

Discussion

"Desensitization" of primary afferent neurons by capsaicin has recently attracted increased interest especially because of its possible therapeutic value (cf. Lynn 1990). The term desensitization has been used to describe a number of changes in neuronal function and morphology which time-dependently develop after application of capsaicin (Szolcsányi 1985).

Studies in rodents demonstrate that immediately after capsaicin exposure primary afferents show a reduced sensitivity to further application of capsaicin (Heyman and Rang 1985; Hayes et al. 1984; Lembeck and Donnerer 1981; Maggi et al. 1987). Capsaicin-induced C-fiber conduction block which develops in rats already at micromolar concentrations of capsaicin (Baranowski et al. 1986; Waddell and Lawson 1989) may contribute to this initial blockade of afferent neuron function. Approximately 4 h after capsaicin administration, tissue levels of neuropeptides decline (Maggi et al. 1987) and ultrastructural changes are visible (Hoyes and Barber 1979; Marsh et al. 1987). This degenerative effect of capsaicin seems related to intraneuronal accumulation of Ca^{2+} (Jancsó et al. 1984; Marsh et al. 1987). In vivo, capsaicin-induced degeneration of primary afferent C-fibers was reduced by administration of nerve growth factor (Otten et al. 1983), which suggests that multiple factors contribute to the long-lasting effects of capsaicin in-vivo.

In the present study the initial stage of capsaicin-induced desensitization of evoked CGRP-IR release was investigated in the rat urinary bladder. Upon first administration of capsaicin, CGRP-IR release was dose-dependent. The EC_{50} (0.17 μmol/l) is in the range of the value given by Wood et al. (1988) for capsaicin-induced Ca^{2+} uptake in dorsal root ganglion cells.

To determine capsaicin-induced "desensitization" of CGRP-IR release, the preparations were pre-exposed to different concentrations of capsaicin 60 min before the evoked release of CGRP-IR was determined. The results (Table 1) indicate that the threshold dose of capsaicin to stimulate neuropeptide release (0.1 μmol/l) already produces desensitization.

Pre-exposure to 1 μmol/l capsaicin already caused complete and non-selective blockade of evoked CGRP-IR release. In preparations which had been pre-exposed to lower concentrations of capsaicin, K^+ (60 mmol/l)-stimulated release was significantly less attenuated than that which was produced by stimulation with capsaicin (0.1 μmol/l, Fig. 2). In control preparations, this concentration of capsaicin was equally effective as 60 mmol/l K^+ to stimulate CGRP-IR release (Table 1). These results are in agreement with Dray et al. (1989), who have demonstrated "selective" desensitization to capsaicin in the guinea-pig ureter.

Several mechanisms may contribute to the desensitizing effect of low concentrations of capsaicin.

a) It has been shown that capsaicin causes "desensitization" of neuropeptide release by depletion of releasable pools (Håkanson et al. 1987). The present observation that capsaicin did not cause detectable depletion of tissue content of CGRP-IR (Table 3) is in agreement with previous studies (Dray et al. 1989; Maggi et al. 1987). But certainly these results do not exclude the possibility that depletion of releasable stores, the size of which may be small as compared to total content, had occurred. The observation that a Ca^{2+}-free, EDTA-containing superfusion solution which prevented capsaicin-evoked release also prevented desensitization, seems in good agreement with the "depletion" theory.

On the other hand, the selective inhibition of capsaicin- but not of K^+-stimulated release by pre-exposure to submaximal concentrations of capsaicin (Fig. 2) is difficult to explain by capsaicin-induced depletion of releasable CGRP-IR. One had to assume a pool which is accessible to a depolarizing K^+ stimulus, but which is resistant to low concentrations of capsaicin. Additional indication, that depletion of releasable CGRP-IR is not the only mechanism by which desensitization to capsaicin is produced, was provided by the results of experiments at 18° C. Desensitization was significantly attenuated at 18° C, although the initial exposure to capsaicin released more CGRP-IR than at 37° C (Table 3).

b) Capsaicin-induced Ca^{2+} influx into afferent neurons seems closely related to its desensitizing effect. Exposure of dorsal root ganglion cells (DRG) of the rat to 1 μmol/l capsaicin for 5 to 10 min caused already a maximal Ca^{2+} uptake (Wood et al. 1988). This massive accumulation of Ca^{2+} (about 12 mmol/l) is likely to exhaust intracellular buffering capacity and thus leads to morphological signs of degeneration (Marsh et al. 1987). It seems reasonable to assume that these "capsaicin-poisoned" (Marsh et al. 1987) neurons fail to secrete neuropeptides in response to stimulation. This mechanism is likely to explain complete blockade of evoked neuropeptide release after high concentrations of capsaicin (Maggi et al. 1989), but not partial and selective desensitization which is produced by low concentrations of capsaicin (Dray et al. 1989; present results). In this context it seems important that the dose-response curve for capsaicin-induced Ca^{2+} uptake in DRG has been reported to be very steep, thus 0.1 μmol/l capsaicin produced only about 15% of the maximal response (Wood et al. 1988).

The present observation that desensitization by low concentrations of capsaicin was completely prevented in a Ca^{2+}-free, EDTA-containing solution confirms the importance of external Ca^{2+} in desensitization and suggests that capsaicin-induced Ca^{2+} entry is essential for the development of desensitization.

Recently it has been suggested that in rat DRG, capsaicin induced Ca^{2+} entry causes a long-lasting inactivation, of voltage-gated Ca^{2+} channels (Bevan and Szolcsányi 1990). Since Ca^{2+} entry constitutes an essential link in excitation-secretion coupling (Katz and Miledi 1967), this effect of capsaicin can explain capsaicin-induced desensitization of evoked release.

Several studies have indicated that capsaicin-induced Ca^{2+} influx in afferent neurons is not primarily mediated by voltage-gated channels (Donnerer and Amann 1990; Maggi et al. 1988; Marsh et al. 1987; Wood et al. 1988). Therefore, "selective" desensitization could be explained by the assumption that capsaicin-activated Ca^{2+} channels are more susceptible to inactivation than voltage-gated Ca^{2+} channels.

c) The effect of low temperatures on primary afferents has been reported by Szolcsányi (1977), who observed that cooling the receptive field to about 20° C prevents capsaicin-induced excitation. The present results demonstrate that low temperature also attenuates the stimulus-evoked neuropeptide release from peripheral afferent terminals. At the same time, low temperature inhibited capsaicin-induced desensitizing of CGRP-IR release (Table 3). This observation seems to resolve the apparent discrepancy (see introduction) between the results of Dray et al. (1989) and Maggi et al. (1990).

Dray et al. (1989) have suggested that, under appropriate conditions, low concentrations of capsaicin which produced submaximal release of neuropeptides, were not sufficient to induce desensitization. The present data indicate that the low temperature (20 ± 2° C) at which experiments were carried out in the study of Dray et al. (1989), was considerably influencing the stimulating as well as the desensitizing effect of capsaicin.

For reasons outlined above, it seems not likely that reduced depletion of releasable CGRP-IR at low temperature was responsible for inhibition of desensitization at 18° C. Capsaicin-induced Ca^{2+}-entry is also not likely to be reduced at low temperature (Wood et al. 1988). Thus, it seems conceivable that low temperature interfered with another step in the capsaicin-induced desensitization process. In this respect it seems interesting to note that desensitization of the muscarinic acetylcholine receptor in intact mouse neuroblastoma cells showed marked temperature-dependency between 20° C and 37° C. This phenomenon has been ascribed to reduced membrane phospholipid fluidity at low temperature (El-Fakahany and Richelson 1980).

In conclusion, the present results suggest that low concentrations of capsaicin which stimulate primary afferent terminals, at the same time also cause selective desensitization of the capsaicin-evoked release of CGRP-IR by a Ca^{2+}- and temperature-dependent mechanism.

In theory, it should therefore be possible to use "selective" desensitization to determine to which extent other sensory neuron stimulants activate a similar mechanism as capsaicin. However, the range of concentrations of capsaicin, for which this selectivity can be demonstrated was very narrow thus limiting this approach. It remains to be investigated to which extent capsaicin analogues, such as olvanil (Campbell et al. 1989; Dray et al. 1990) or resiniferatoxin (Szallasi and Blumberg 1989b) provide a better tool to produce selective desensitization.

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References

Amann R, Maggi CA, Giuliani S, Donnerer J, Lembeck F (1990) Effects of carbonyl cyanide p-trichloromethoxy-phenylhydrazone (CCCP) and of Ruthenium Red (RR) on capsaicin-induced neuropeptide release from peripheral terminals of primary afferent neurones. Naunyn-Schmiedeberg's Arch Pharmacol 341: 534–537

Baranowski R, Lynn B, Pini A (1986) The effects of locally applied capsaicin on conduction in cutaneous nerves in four mammalian species. Br J Pharmacol 89:267–276

Bevan S, Szolcsányi J (1990) Sensory neuron-specific actions of capsaicin: mechanisms and applications. Trends Pharmacol Sci 11:330–333

Campbell EA, Dray A, Perkins MN (1989) Comparison of capsaicin and olvanil as antinociceptive agents in vivo and in vitro. Br J Pharmacol 98:907P

Donnerer J, Amann R (1990) Capsaicin-evoked neuropeptide release is not dependent on membrane potential changes. Neurosci Lett (in press)

Dray A, Bettaney J, Rueff A, Walpole C, Wrigglesworth R (1990) NE-19550 and NE-21610, antinociceptive capsaicin analogues: studies on nociceptive fibres of the neonatal rat tail in vitro. Eur J Pharmacol 181:289–293

Dray A, Hankins MW, Yeats JC (1989) Desensitization and capsaicin-induced release of substance P-like immunoreactivity from guinea-pig ureter in vitro. Neuroscience 31:479–483

El-Fakahany E, Richelson E (1980) Temperature dependence of muscarinic acetylcholine receptor activation, desensitization, and resensitization. J Neurochem 34:1288–1295

Håkanson R, Beding E, Ekman R, Heilig M, Wahlestedt C, Sundler F (1987) Multiple tachykinin pools in sensory nerve fibres in the rabbit iris. Neuroscience 21:943–950

Hayes AG, Oxford A, Reynolds M, Shingler AH, Skingle M, Smith C, Tyers MB (1984) The effects of a series of capsaicin analogues on nociception and body temperature in the rat. Life Sci 34:1241–1248

Heyman I, Rang HP (1985) Depolarizing responses to capsaicin in a subpopulation of dorsal root ganglion cells. Neurosci Lett 56:69–75

Holzer P (1988) Local effector function of capsaicin-sensitive sensory nerve endings: Involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 24:739–768

Hoyes AD, Barber P (1979) In vitro effect of capsaicine on the axons in the subepithelial nerves of the rat ureter. J Anat 128:438–439

James IF, Walpole CSJ, Coote PR, Wood JN (1989) Immunochemical detection of photoaffinity-labelled capsaicin binding proteins from sensory neurons. Abstract to the International Workshop on Sensory Neuropeptides, Florence

Jancsó G, Karcsú S, Király E, Szebeni A, Tóth L, Bácsy E, Joó F, Párducz A (1984) Neurotoxin induced nerve cell degeneration: Possible involvement of calcium. Brain Res 295:211–216

Katz B, Miledi R (1967) A study of synaptic transmission in the absence of nerve impulses. J Physiol (Lond) 192:407–436

Lembeck F (1988) The 1988 Ulf von Euler Lecture – Substance P: From extract to excitement. Acta Physiol Scand 133:435–454

Lembeck F, Donnerer J (1981) Time course of capsaicin-induced functional impairements in comparison with changes in neuronal substance P content. Naunyn-Schmiedeberg's Arch Pharmacol 316:240–243

Lynn B (1990) Capsaicin: actions on nociceptive C-fibres and therapeutic potential. Pain 41:61–69

Maggi CA, Meli A (1988) The sensory-efferent function of capsaicin-sensitive sensory neurons. General Pharmacol 19:1–43

Maggi CA, Astolfi M, Donnerer J, Amann R (1990) Which mechanisms account for the sensory neuron blocking action of capsaicin on primary afferents in the rat urinary bladder. Neurosci Lett 110:267–272

Maggi CA, Patacchini R, Giuliani S, Santicioli P, Meli A (1988) Evidence for two independent modes of activation of the "efferent" function of capsaicin-sensitive nerves. Eur J Pharmacol 156:367–373

Maggi CA, Santicioli P, Geppetti P, Giuliani S, Patacchini R, Frilli S, Grassi J, Meli A (1987) Involvement of a peripheral site of action in the early phase of neuropeptide depletion following capsaicin desensitization. Brain Res 436:402–406

Maggi CA, Santicioli P, Geppetti P, Parlani M, Astolfi M, DelBianco E, Patacchini R, Giuliani S, Meli A (1989) The effect of calcium free medium and nifedipine on the release of substance P-like immunoreactivity and contractions induced by capsaicin in the isolated guinea-pig bladder. Gen Pharmacol 40:445–456

Marsh SJ, Stansfield CE, Brown DA, Davey R, McCarthy D (1987) The mechanism of action of capsaicin on sensory C-type neurons and their axons in vitro. Neuroscience 23:275–289

Otten U, Lorez HP, Businger F (1983) Nerve growth factor antagonizes the neurotoxic action of capsaicin on primary afferent sensory neurones. Nature 301:515–517

Su HC, Wharton J, Polak JM, Mulderry PK, Ghatei MA, Gibson SJ, Terenghi G, Morrison JFB, Ballesta J, Bloom SR (1986) Calcitonin gene-related peptide immunoreactivity in afferent neurons supplying the urinary tract: Combined retrograde tracing and immunohistochemistry. Neuroscience 18:727–747

Szallasi A, Blumberg PM (1989a) Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, to dorsal root ganglia membranes. Pharmacologist 31:353

Szallasi A, Blumberg PM (1989b) Resiniferatoxin, a phorbol-related diterpene, acts as an ultrapotent analog of capsaicin, the irritant constituent in red pepper. Neuroscience 30:515–520

Szolcsányi J (1977) A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain. J Physiol (Paris) 73:251–259

Szolcsányi J (1985) Sensory receptors and the antinociceptive effects of capsaicin. In: Hakanson R, Sundler F (eds) Tachykinin antagonists. Elsevier, pp 45–54

Szolcsányi J, Jancsó-Gabór A (1975) Sensory effects of capsaicin congeners. Drug Res 25:1877–1881

Waddell PJ, Lawson SN (1989) The C-fibre conduction block caused by capsaicin on rat vagus nerve in vitro. Pain 39:237–242

Wood JN, Winter J, James JF, Rang HP, Yeats J, Bevan S (1988) Capsaicin-induced ion fluxes in dorsal root ganglionic cells in culture. J Neurosci 8:3208–3220