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Evidence for the involvement of endogenous substance P in the motor effects of capsaicin on the rat urinary bladder

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[pro^4,trp^{7,9},Leu^{11}]SP-(4-11), a substance P (SP) antagonist, selectively antagonized contractions produced by either capsaicin or SP on the rat isolated urinary bladder. These experiments provide direct evidence indicating that the motor effects of capsaicin on rat urinary bladder are attributable, at least in part, to the release of endogenous SP.

We have recently shown that topical application of capsaicin to the urinary bladder of urethane-anaesthetized rats activates the micturition reflex (Maggi et al 1984). Since the effects of capsaicin were selectively mimicked by exogenous substance P (SP) we hypothesized that the acute effects of capsaicin might be mediated through the release of SP from afferent nerve endings in the bladder wall (Maggi et al 1984). Since SP does not produce an appreciable tachyphylaxis in the rat urinary bladder (Sjogren et al 1982; Maggi et al 1984), the existence of a potential cross desensitization between SP and capsaicin, as a means of testing the above hypothesis, could not be determined (Maggi et al 1984). In this communication evidence is presented indicating that capsaicin-induced contractions of the rat isolated urinary bladder are selectively blocked by [pro^4,trp^{7,9},Leu^{11}]SP-(4-11) (ptLSP), a SP antagonist recently described by Regoli et al (1984).

Materials and methods

Male albino rats of Wistar-Morini strain, 340–360 g, were killed by a blow to the back of the head. The urinary bladder was removed, and freed of mucosa. A strip (about 1 cm long and 2 mm wide) of the detrusor muscle was excised and placed, under the constant load of 1 g, in a heated (37 °C) 5 ml organ bath containing an oxygenated (96% O_2 + 4% CO_2) Krebs solution of the following composition (mM): NaCl 119, KCl 4·7, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25, $CaCl_2$ 2·5 and glucose 11.

Contractile tone was recorded by means of an isometric strain gauge connected to a Basile 7050A polygraph. Field stimulation of the strips was performed by means of a GRASS S11 stimulator at a frequency of 0·1 Hz (60 V, 1 ms) by means of two wire platinum electrodes placed at the top and the bottom of the organ bath. To allow comparisons between contractile responses produced by capsaicin in different preparations, the strips were stimulated, during the equilibration period, for 1 h or more, until uniform responses were obtained. This was considered as an indication that the reactivity of the preparation had reached a steady value. At this time field stimulation was stopped and the strips exposed, at 15 min intervals, to either SP (10 nM) or acetylcholine (ACh, 1 μM) until steady contractile responses were obtained. The effects of various substances was investigated after 5–15 min contact. In experiments with ptLSP contact time was 2 min. Capsaicin (0·1 μM) was tested only once in each preparation either in presence or absence of ptLSP or of other antagonists. SP was dissolved in bidistilled water and then diluted in Krebs solution; ptLSP was dissolved in 2% acetic acid and then diluted in Krebs solution. A stock solution of capsaicin (100 mM) was prepared with absolute ethanol and then diluted in Krebs solution.

To minimize binding of polypeptides to glassware, both the organ bath and the microsyringe used for drug administration were treated with 5% dichloromethylsilane in benzene for 20 min and then rinsed in water before use.

Statistical analysis of the data was performed by means of the Student's *t*-test for paired or unpaired data, when applicable.

Results

Capsaicin (0·1 μM) induced contractions of the isolated rat urinary bladder which amounted to 84 ± 8% of those produced by SP (10 nM, n = 8) and to 80 ± 5% of those produced by supramaximal field stimulation at 0·1 Hz (n = 8). In the presence of tetrodotoxin (0·5 μM) or atropine (3 μM) the amplitudes of capsaicin-induced contractions were similar to those of controls (n = 8). A second administration of capsaicin (0·1 μM) 1 h after the first challenge was ineffective. SP (10 nM) and ACh (1 μM) induced contractions that were unaffected by tetrodotoxin (n = 5). ACh- but not SP-induced contractions were suppressed by atropine (n = 9).

The SP antagonist ptLSP (8·5 μM) produced a rapid phasic contraction with an amplitude that amounted to 35 ± 4% of those produced by SP (n = 11) and which returned to baseline within 1 min. In the presence of ptLSP, 2 min before, SP (10 nM)- or capsaicin (0·1 μM)-induced contractions amounted to the 68 ± 3 and 63 ± 6% of controls (n = 14), while ACh (1 μM) induced contractions were unaffected (Fig. 1, n = 6).

To rule out that the antagonistic effect of ptLSP toward SP or capsaicin was due to its agonistic proper-

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ties, we investigated the effects of a concentration of SP (2 nM) which produced contractions of similar amplitude to those produced by ptLSP (8·5 μM). Previous exposure to SP (2 nM), 2 min before, did not affect amplitude of contractions produced by either SP (10 nM), capsaicin (0·1 μM) or ACh (1 μM) (n = 6).

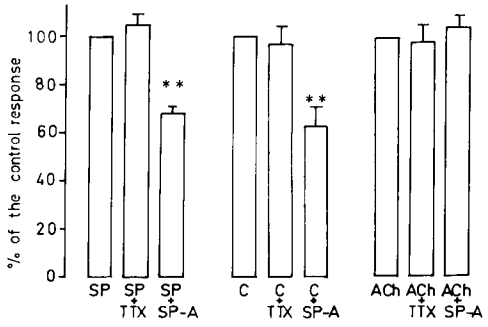

FIG. 1. Effect of tetrodotoxin (TTX, 0·5 μM) and the substance P antagonist, [pro^4,trp^{7,9},Leu^{11}]SP-(4-11) (SP-A, 8·5 μM) on contractions of isolated rat urinary bladder produced by substance P (SP, 10 nM), capsaicin (C, 0·1 μM) or acetylcholine (ACh, 1 μM). Each value is the mean ± s.e. of at least 6 experiments. ** Significantly different from controls, $P < 0·001$.

Discussion

Immunoreactive SP nerve fibres are present in the wall of rat urinary bladder and SP neurons have been identified in dorsal ganglia receiving afferents from the bladder (Sharkey et al 1983). Systemic pretreatment with high doses of capsaicin produces urinary retention and bladder hypertrophy, along with disappearance of SP nerve fibres and depletion of SP in the bladder wall (Holzer et al 1982; Sharkey et al 1983). These findings suggest that endogenous SP might modulate the micturition reflex (Sharkey et al 1983).

A distinctive feature of capsaicin's action on various reflex responses is to produce excitation on acute administration and functional impairment on systemic pretreatment with large doses (Nagy 1982). Accordingly we observed that, in adult rats, topical capsaicin activates, while systemic pretreatment impairs, the micturition reflex (Maggi et al 1984). The acute excitatory effect of capsaicin on afferent fibres is paralleled (and might be mediated) by the release of stored neuropeptides such as SP and somatostatin (Nagy 1982). Since SP mimicked the acute excitatory effects of capsaicin, we hypothesized that capsaicin activates the micturition reflex by releasing endogenous SP (Maggi et al in press). Accordingly the impairment of the micturition reflex produced by systemic pretreatment with a high dose of capsaicin could be related to a neurotoxic effect of capsaicin on SP-containing nerve fibres in the bladder wall. Our present findings with [pro^4,trp^{7,9},Leu^{11}] SP-(4-11) one of the most potent antagonists of SP thus far available (Regoli et al 1984) provide direct evidence that capsaicin-induced contractions of the rat urinary bladder are mediated, at least in part, by the release of endogeneous SP and support the hypothesis of an involvement of SP in the regulation of micturition in this species.

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