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Short communication

Capsaicin-induced nonneural vasoconstriction in canine mesenteric arteries

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Abstract

Prolonged cold storage (4 °C) of canine mesenteric arteries was used to reveal the role of nonneural mechanisms in capsaicin-induced vascular contraction. The EC_{50} values of capsaicin were 3.0 μM, 670 and 104 nM in preparations made fresh, after a 1- or 2-week period of cold storage, respectively, indicating an enhanced contractile responsiveness of the denervated tissue to capsaicin. A similar exaggerated contractile response was seen with phenylephrine exclusively after a 1-week cold storage. For fresh, 1- and 2-week cold-stored arteries, the EC_{50} of phenylephrine were 248, 38 and 30 nM, respectively. The maximum contraction produced by tyramine was decreased with time. The results suggest that capsaicin may attain vasoconstriction independent of neural elements.

Keywords: Capsaicin; Cold storage; Vasoconstriction

1. Introduction

Capsaicin, the pungent ingredient of red pepper is known to evoke vasodilatation by liberating vasoactive neuropeptides (calcitonin gene-related peptide (CGRP), tachykinins) from sensory nerve endings (Holzer, 1991). In contrast, the vasoconstrictory effect of capsaicin is not well understood and has not been investigated in detail. The phenomenon of capsaicin-induced vasoconstriction was revealed in various canine arteries in vitro (Toda et al., 1972) and it was confirmed by injecting capsaicin arterially into tracheal vascular bed (Salonen et al., 1988) as well. In spinalized cats treated with ganglion-blocking agents or after treatment with α-adrenoceptor antagonists, the rise in blood pressure evoked by capsaicin injection was not diminished (Pórszász et al., 1955). These results raised the possibility of a non-neural mode of action of capsaicin. Recently, the cloned vanilloid VR1 (capsaicin) receptor (Caterina et al., 1997) was identified on sensory neurons and in some brain areas (Mezey et al., 2000), but not in other tissues. However, the existence of functional nonneural vanilloid receptors was also put forward (Bíró et al., 1998). The aim of present study was therefore to analyse changes in isometric tension produced by capsaicin in isolated arterial segments stored cold for 1–2 weeks, a method known to destroy neural elements (Ito and Chiba, 1985; Maggi et al., 1986).

2. Materials and methods

The jejunal portion of canine mesenteric arteries were obtained from five dogs under halothane–N_2O anaesthesia according to the animal welfare law of Hungary. The arteries were used immediately or were stored for 1 or 2 weeks in cold (4 °C) Krebs solution. After the period of cold storage, segments were prepared for the experiments in the same manner as the freshly prepared specimens. Rings (5 mm in length) were mounted horizontally on a teflon

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tissue holder (OHO-02-V, Experimetria) between two stainless steel wires in 5 ml chamber volume of tissue bath system (TSZ-04/2, Experimetria). Vessels were maintained at 37 °C in Krebs solution bubbled with 5% CO_2 and 95% O_2 (pH 7.4) and the passive tension was set to 2 g. Prior to commencement of the experiments, the preparations were allowed to equilibrate over 60 min. At the end of experiment, the maximum contraction was evoked by phenylephrine.

The concentration of agonists, which increased maximum tension by 50%, was expressed (EC_{50}) and each point of concentration–response curves was statistically evaluated by Student's *t*-test for unpaired variables.

3. Results

In fresh arteries, capsaicin evoked a concentration-dependent vasoconstriction from 330 nM to 33 μM (EC_{50} = 3.0 μM, n = 7) (Fig. 1). Phenylephrine, an α-adrenoceptor agonist induced contractions in a concentration range of 30 nM to 3 μM (EC_{50} = 248 nM, n = 8) (Fig. 2A). Tyramine, an indirect sympathomimetic agent induced 30.9 ± 5.1% of maximum contraction in a concentration of 33 μM (Fig. 2B). After a 1-week period of cold storage, the potency of capsaicin and phenylephrine were enhanced: EC_{50} = 670 nM (n = 12) and EC_{50} = 38 nM (n = 14), respectively (Figs. 1 and 2A). After a 2-week period of cold storage, the potency of capsaicin was further enhanced (EC_{50} = 104 nM) (Fig. 1), whereas the effect of phenylephrine remained similar (EC_{50} = 30 nM, n = 7) (Fig. 2a). On the other hand, after cold storage, the concentration–response curve for tyramine was shifted to the right with a progressive decrease in E_{max} (Fig. 2B). The latency of the capsaicin responses at the lowest effective concentration were similar in the three kinds of preparation, i.e. it was 43.2 ± 5.1 s. Furthermore, the capsaicin-induced constrictions were also of long-lasting nature independent of preceding cold storage, i.e. changing the bathing solution was necessary at least three times until the recovery of resting tension approximately over an hour. It should also be mentioned that thoracic aortic rings of the rat were resistant to capsaicin, but KCl and phenylephrine evoked constrictions in a concentration-dependent manner.

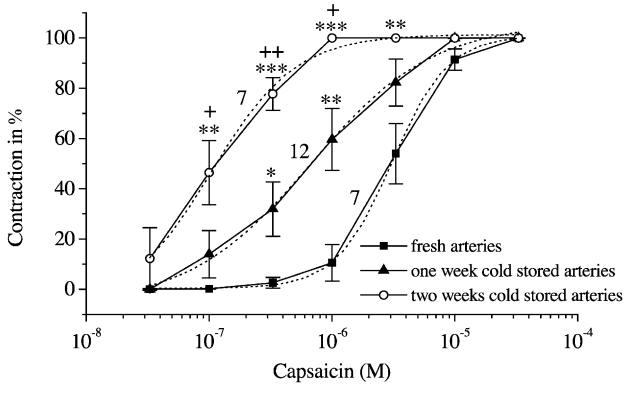

Fig. 1. Vasoconstrictory effect of capsaicin in canine mesenteric arteries. A significant difference is observed between the fresh, the 1 week cold-stored and the 2 weeks cold-stored arteries. The dotted lines represent the fitted concentration–response curves. The EC_{50} values of freshly used, 1 week cold-stored and 2 weeks cold-stored arteries were 3.0 μM, 670 and 104 nM, respectively. The values near to each curve represent the numbers of mesenteric artery rings used in the experiments. * Denotes values significantly different from those in freshly used arteries; + indicates values significantly different from 1 week cold-stored arteries. (* or +)$P < 0.05$; (** or ++)$P < 0.01$; * * * $P < 0.001$.

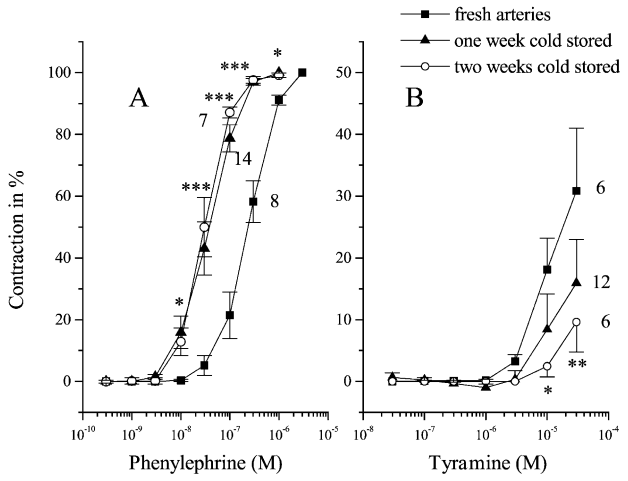

Fig. 2. Vasoconstrictory effect of phenylephrine and tyramine induced in freshly used mesenteric arteries and in cold-stored (1 or 2 weeks at 4 °C) ones. The n values represent the number of jejunal mesenteric artery rings used. The responsiveness of arterial segments to phenylephrine increased after the first week period of cold storage. Prolonging the cold storage period to 2 weeks yielded no further increase in amplitude of the contractile response (A). In contrast, the vasoconstrictory response to tyramine gradually decreased with time (B). * Denotes values significantly different from those in freshly used arteries. * $P < 0.05$; * * $P < 0.01$; * * * $P < 0.001$.

4. Discussion

In the present study, we demonstrated for the first time that destruction of the periarterial neural elements by cold storage (Ito and Chiba, 1985; Maggi et al., 1986) increased the vasoconstrictive responses to capsaicin, whereas the effect of tyramine was markedly decreased. These findings suggest that sensory nerve fibres are not involved in this vascular response to capsaicin. Previous studies suggesting a direct vascular effect of capsaicin (Toda et al., 1972) did not consider the possibility that a sensory neuropeptide with vasoconstrictive property could release from capsaicin-sensitive afferent fibers (Szolcsányi et al., 2001). Such a sensory neuropeptide release can be excluded in isolated tissues subjected to cold storage for 1 or 2 weeks because of the destruction of neural elements (Ito and Chiba, 1985; Maggi et al., 1986). The mechanism of this nonneural smooth muscle response of capsaicin is not known. Never-

theless, the fact that it was striking even in a nanomolar concentration range is suggestive of the presence of non-neural capsaicin receptors. The nature and cellular location of these receptors have not been investigated and further experiments are needed to decide whether the target of capsaicin is on the smooth muscle or on the endothelium. Immunoreactive vanilloid VR1 receptor or in situ hybridization of vanilloid VR1 mRNA have been hitherto identified only in neural tissues (Caterina et al., 1997; Mezey et al., 2000). On the other hand [H^3]resiniferatoxin binding studies indicated a vanilloid receptor site also on the cell surface of mast cells (Bíró et al., 1998). Identification of these binding sites with the VR1 receptor or its splice variants has not been tested. The present functional approach provides a clue for studying nonneural vanilloid receptors.

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References

Bíró, T., Maurer, M., Modarres, S., Lewin, N.E., Brodie, C., Ács, G., Ács, P., Paus, R., Blumberg, P.M., 1998. Characterization of functional vanilloid receptors expressed by mast cells. Blood 91, 1332–1340.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Holzer, P., 1991. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol. Rev. 43, 143–201.

Ito, T., Chiba, S., 1985. Effects of prolonged cold storage on the responsiveness of isolated and perfused canine intermediate auricular artery. Arch. Int. Pharmacodyn. 275, 13–21.

Maggi, C.A., Santicioli, P., Manzini, S., Meli, A., 1986. Capsaicin activates neurogenic non-adrenergic non-cholinergic relaxations of the isolated rat duodenum. Eur. J. Pharmacol. 120, 367–370.

Mezey, E., Tóth, Z.E., Cortright, D.N., Arzubi, M.K., Krause, J.E., Elde, R., Guo, A., Blumberg, P.M., Szállási, Á., 2000. Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc. Natl. Acad. Sci. U. S. A. 97, 3655–3660.

Pórszász, J., György, L., Pórszász-Gibiszer, K., 1955. Cardiovascular and respiratory effects of capsaicin. Acta Physiol. Acad. Sci. Hung. 8, 61–76.

Salonen, R.O., Webber, S.E., Widdicombe, J.G., 1988. Effects of neuropeptides and capsaicin on the canine tracheal vasculature in vivo. Br. J. Pharmacol. 95, 1262–1270.

Szolcsányi, J., Oroszi, G., Németh, J., Szilvássy, Z., Blasig, J.E., Tósaki, Á., 2001. Functional and biochemical evidence for capsaicin-induced neural endothelin release in isolated working rat heart. Eur. J. Pharmacol. 419, 215–221.

Toda, N., Usui, H., Nishino, N., Fujiwara, M., 1972. Cardiovascular effects of capsaicin in dogs and rabbits. J. Pharmacol. Exp. Ther. 181, 512–521.