

## Capsaicin-induced Relaxation in Rabbit Coronary Artery

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**ABSTRACT.** In the present study mechanism of inhibitory effects of capsaicin on the contractility of rabbit coronary artery were studied by measurement of isometric tension and intracellular  $Ca^{2+}$  concentration. Capsaicin (1  $\mu$ M to 30  $\mu$ M) relaxed the coronary artery pre-contracted with prostaglandin (PG)  $F_{2\alpha}$  (1  $\mu$ M) in a concentration-dependent manner. The  $PGF_{2\alpha}$ -induced increase in intracellular  $Ca^{2+}$  concentration was also inhibited. The effects of capsaicin were readily reversed by washing capsaicin from the bath. Capsaicin-induced relaxation was not attenuated by pretreatment with capsazepine (1  $\mu$ M), a blocker of vanilloid receptor or ruthenium red (1  $\mu$ M), a blocker of non-selective cation channel. Previous exposure to a high concentration of capsaicin (100  $\mu$ M) or repeated application of capsaicin did not eliminate the relaxation response to subsequent application of capsaicin. Increasing the external  $K^+$  concentration to 80 mM significantly attenuated the capsaicin-induced relaxation with simultaneous change in intracellular  $Ca^{2+}$  concentration. Pretreatment with iberiotoxin (100 nM), a blocker of  $Ca^{2+}$ -activated  $K^+$  channel, only partially inhibited the capsaicin-induced relaxation. However, application of 4-aminopyridine (4-AP, 1 mM), a blocker of delayed rectifier  $K^+$  current significantly inhibited the capsaicin-induced relaxation with concomitant attenuation of the effect on intracellular  $Ca^{2+}$  concentration. These results indicate that capsaicin may have a direct relaxing effect on the smooth muscle contractility, and relaxation may be due to activation of the 4-AP-sensitive, delayed rectifier  $K^+$  channels in the rabbit coronary artery.

**KEY WORDS:** capsaicin, coronary smooth muscle, delayed rectifier  $K^+$  current, relaxation.

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Capsaicin is a pungent constituent of red peppers and known to activate sensory nerve fibers via vanilloid receptor [20, 22–23]. Acute administration of capsaicin releases neuropeptide from sensory nerve endings, such as substance P, calcitonin gene related peptide (CGRP) and neurokinin A [7, 17]. These neuropeptides seem to play a role in the regulation of vascular and airway smooth muscle tone [8, 16, 21]. In addition to this neuropeptide-mediated effect, capsaicin has a diverse effect on smooth muscle contractility and ion channel activity, depending on the species and preparations. Capsaicin relaxes bronchial smooth muscle through activation of  $Ca^{2+}$ -activated  $K^+$  channels [5, 24], or constricts cerebral arteries by increasing  $Ca^{2+}$  influx [4]. The inhibitory effect of capsaicin on  $Ca^{2+}$  and  $K^+$  current in cultured aortic smooth muscle cells has also been reported [15].

In coronary artery, capsaicin has been reported to increase coronary flow and decrease ischemic ventricular tachycardia which may be related to the decrease in  $Ca^{2+}$  influx [2]. The authors suggest a protective role of capsaicin on the ischemic cardiac damage through maintaining adequate coronary flow. However, it is uncertain whether capsaicin relaxes coronary artery through the release of neuropeptide such as CGRP, or through the direct inhibition of coronary smooth muscle [3, 8, 15]. Thus, the primary goal of the present study was to examine the involvement of neuropeptides on capsaicin-induced coronary relaxation, and then elucidate the mechanism underlying the capsaicin-induced coronary relaxation by measuring vascular tone and intracellular  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) in the presence of

various blockers. The present results provide a evidence that the 4-AP sensitive, delayed rectifier  $K^+$  current might be responsible for the capsaicin-induced relaxation and  $[Ca^{2+}]_i$  decrease in the rabbit coronary artery.

### MATERIALS AND METHODS

*Tissue preparation and measurement of tension:* After anesthetizing a rabbit with pentobarbital sodium (60 mg/Kg), the heart was extracted and placed in a preparation chamber containing a physiological salt solution (PSS). In the preparation chamber, meticulous dissection of the coronary arteries from neighboring tissues was done with ophthalmologic scissors and forceps. Coronary artery rings were suspended in a organ bath under a resting tension of 0.8 g. After equilibration for 1 hr in PSS, each rings was repeatedly exposed to 80 mM KCl solution until the responses became stable. The high  $K^+$  solution was prepared by replacing NaCl with equimolar KCl. Muscle contraction was recorded isometrically with a force-displacement transducer and recorded on a computer.

*Fura-2 loading and simultaneous measurements of tension and  $[Ca^{2+}]_i$ :*  $[Ca^{2+}]_i$  and muscle contraction were measured simultaneously as described by Kwon *et al.* [12]. Muscle strips were exposed to acethoxymethyl ester of fura-2 (fura-2/AM, 5  $\mu$ M) in the presence of 0.01% cremophore EL for 5–6 hr at room temperature (22–24°C). After the fura-2 loading, coronary strip was placed in a experimental chamber and illuminated alternatively (48 Hz) with 340 nm and 380 nm light. The ratio of 500 nm fluorescence induced by 340 nm excitation (F340) and that induced by 380 nm excitation (F380) was detected with a spectrophotometer

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(CAF 110, Japan Spectroscopic, Tokyo, Japan). Increase in the ratio due to 70 mM KCl was considered as a reference response (100 %).

**Solutions and chemicals:** PSS had following composition (mM): NaCl 118; KCl 4.8; CaCl<sub>2</sub> 2.5; KH<sub>2</sub>PO<sub>4</sub> 1.2; MgSO<sub>4</sub> 1.5; NaHCO<sub>3</sub> 25; Glucose 11. PSS was saturated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub> to adjust pH to 7.4. Temperature was maintained at 37°C. Capsaicin, capsazepine, ruthenium red, and 4-AP was purchased from Sigma Chemical Co. All the drugs were prepared on the day of the experiment. Stock solutions of capsaicin were prepared in ethanol and were diluted with PSS as appropriate. Capsazepine was dissolved in dimethylsulfoxide to a concentration of 10 mM and further diluted with PSS solution.

**Statistics:** Results of experiments are expressed as means  $\pm$  S.E.M. Student's *t*-test was used for statistical analysis of the results and the number of preparations taken from separate animals was indicated by *n*. *P* values less than 0.05 were considered to be significantly different.

## RESULTS

**Capsaicin-induced relaxation with or without depletion of sensory nerve ending:** Capsaicin relaxed the prostaglandin (PG) F<sub>2 $\alpha$</sub>  (1  $\mu$ M)-induced contraction in a concentration-dependent manner. The threshold concentration of capsaicin to induce relaxation was approx. one  $\mu$ M and maximum relaxation induced by capsaicin (30  $\mu$ M) was  $91.5 \pm 1.6\%$  of the PGF<sub>2 $\alpha$</sub> -induced contraction. The depletion of sensory nerve endings by prolonged treatment with a high concentration of capsaicin (100  $\mu$ M) had no apparent effect on the capsaicin-induced relaxation of rabbit coronary artery. The maximum relaxation response was  $91.5 \pm 1.6\%$  and  $91.8 \pm 1.6\%$  with and without pretreatment with 100  $\mu$ M capsaicin, respectively (*n*=7). In addition, repeated application of capsaicin to the same coronary ring elicited the same magnitude of relaxation. Pretreatment with capsazepine (1  $\mu$ M) or ruthenium red (1  $\mu$ M) did not alleviate the relaxation response to capsaicin (Fig. 1).

**Capsaicin-induced relaxation in agonist- or high K<sup>+</sup>-induced contraction:** As shown in Fig. 2A, capsaicin produced only slight relaxation in the high K<sup>+</sup>-induced contraction compared to the PGF<sub>2 $\alpha$</sub> -induced contraction. Capsaicin (10  $\mu$ M)-induced relaxation was  $14.9 \pm 3.3\%$  and  $91.8 \pm 1.6\%$  in 80 mM K<sup>+</sup>- and PGF<sub>2 $\alpha$</sub> -induced contraction, respectively (*n*=7). We also measured the effect of capsaicin on [Ca<sup>2+</sup>]<sub>i</sub>. As shown in Fig. 2B, application of capsaicin (10  $\mu$ M) at the plateau of the PGF<sub>2 $\alpha$</sub> -induced contraction significantly lowered [Ca<sup>2+</sup>]<sub>i</sub>. However, in 70 mM K<sup>+</sup>-induced contraction, capsaicin had no apparent effect on plateau level of [Ca<sup>2+</sup>]<sub>i</sub>. Figures 2C & D show the summarized results of capsaicin-induced decrease of [Ca<sup>2+</sup>]<sub>i</sub> and contractility due to high K<sup>+</sup> and PGF<sub>2 $\alpha$</sub> .

**Potassium channels involved in the capsaicin-induced relaxation:** Because the relaxing effect of capsaicin was effectively antagonized by high extracellular K<sup>+</sup>, we investigated the types of potassium channels involved in the capsa-

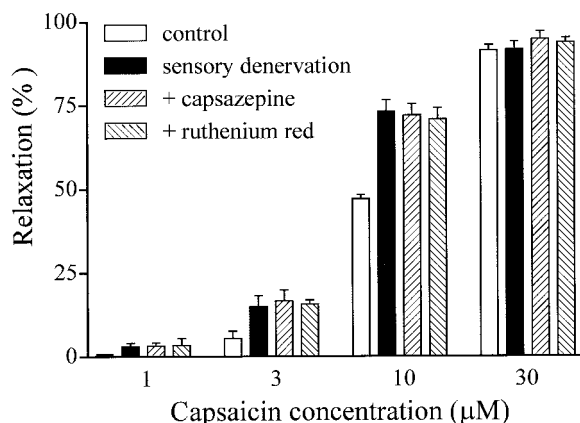


Fig. 1. Effect of pretreatment with a high concentration capsaicin, capsazepine, and ruthenium red on the capsaicin-induced relaxation in the rabbit coronary artery. Relaxation induced by 1, 3, 10 and 30  $\mu$ M capsaicin in the absence (open bar) and after 100  $\mu$ M capsaicin treatment (filled bar), pretreatment with 1  $\mu$ M capsazepine (left hatched bar), and 1  $\mu$ M ruthenium red (right hatched bar). Relaxation is expressed as a percentage of 80 mM K<sup>+</sup> contraction. Values are expressed as mean  $\pm$  SEM (*n*=7).

icin-induced relaxation. We first examined the effect of iberiotoxin, a specific blocker of Ca<sup>2+</sup>-activated K<sup>+</sup> channel, on capsaicin-induced relaxation. As shown in Fig. 3A, pretreatment with iberiotoxin had no apparent effect on the capsaicin-induced relaxation. Relaxation induced by capsaicin (10  $\mu$ M) was  $73.2 \pm 3.3\%$  and  $70.5 \pm 5.1\%$  before and after pretreatment with iberiotoxin, respectively (*n*=7). Pretreatment with 1 mM 4-aminopyridine (4-AP), a blocker of the delayed rectifier K<sup>+</sup> channel, significantly inhibited the relaxation due to capsaicin. Relaxation induced by capsaicin (10  $\mu$ M) was  $73.2 \pm 3.4\%$  and  $17.5 \pm 3.8\%$  before and after pretreatment with 4-AP, respectively (*n*=7). Capsaicin showed a similar change in [Ca<sup>2+</sup>]<sub>i</sub> to those in contractility. As shown in Fig. 3B, pretreatment with iberiotoxin had no apparent effect on the effect of capsaicin on [Ca<sup>2+</sup>]<sub>i</sub>. However, pretreatment with 4-AP significantly attenuated the effect of capsaicin on [Ca<sup>2+</sup>]<sub>i</sub> (*n*=5).

## DISCUSSION

Immunohistochemical investigations have revealed that neuropeptides such as CGRP and substance P are present in the cardiac C fiber afferents in a variety of species [7]. Activation of cardiac C fiber results in the release of CGRP, which induces coronary vasodilation in several animal species including rat and pig [6, 9]. Therefore, it seems likely that activation of cardiac C fiber by capsaicin may relax coronary artery by releasing CGRP. In our present studies, pretreatment with a high concentration of capsaicin, which causes a chemical desensitization of sensory nerves and renders them insensitive to further exposure to capsaicin [4, 24], did not change the relaxation responses to subsequently

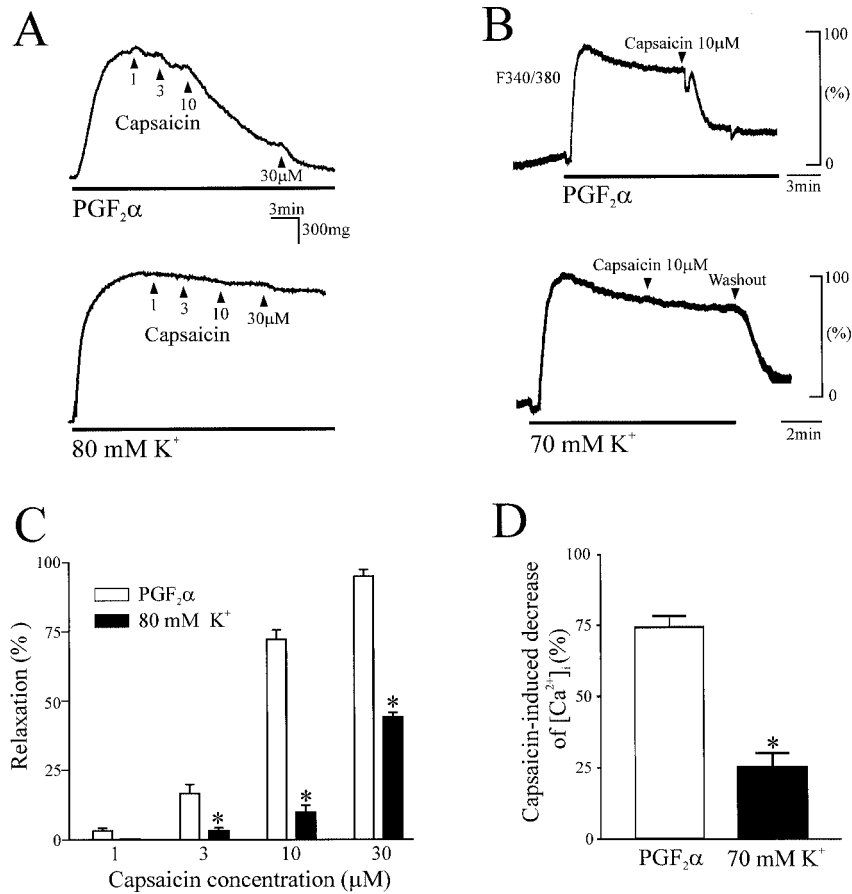


Fig. 2. Typical recordings of the inhibitory effect of capsaicin on the contractions (A) and increase in the intracellular  $\text{Ca}^{2+}$  concentration (B) due to  $\text{PGF}_2\alpha$  and 80 mM  $\text{K}^+$ . (C) Summarized results of the inhibitory effect of capsaicin on  $\text{PGF}_2\alpha$ - and 80 mM  $\text{K}^+$ -induced contraction. Relaxation is expressed as a percentage of 80 mM  $\text{K}^+$  contraction. Values are expressed as mean  $\pm$  SEM (n=7). (D) Summarized results of the capsaicin-induced change in  $[\text{Ca}^{2+}]_i$  in  $\text{PGF}_2\alpha$ - and 80 mM  $\text{K}^+$ -induced contraction. Changes in  $[\text{Ca}^{2+}]_i$  is expressed as a percentage of the 70 mM  $\text{K}^+$ -induced increase in  $[\text{Ca}^{2+}]_i$ . Values are expressed as mean  $\pm$  SEM (n=5). Asterisk represents  $P < 0.01$ .

applied capsaicin. Furthermore, pretreatment with capsaizepine or ruthenium red, both of which block the release of neuropeptides from sensory nerve [14, 22], did not alter the relaxation response to capsaicin. These results suggest that capsaicin-induced relaxation does not involve the sensory neuropeptides in our experimental condition. It is therefore plausible to hypothesize that capsaicin may have a direct effect on smooth muscle cells.

There are several reports showing that capsaicin can change the activity of ion channels in vascular smooth muscle cells, which is related to the relaxant effect of capsaicin [5, 15, 24]. In our present study, capsaicin-induced relaxation was depend on the type of contracting agents. As shown in Fig. 2A, capsaicin-induced relaxation was significantly smaller in high  $\text{K}^+$ -induced contraction than in  $\text{PGF}_2\alpha$ -induced contraction. Raising the extracellular  $\text{K}^+$  concentration decreases the driving force for  $\text{K}^+$  efflux

because the electromotive force for  $\text{K}^+$  flux becomes smaller when external  $\text{K}^+$  concentration is higher. In this case, activating or opening of the  $\text{K}^+$  channels has a lesser effect on the membrane potential and tension [1, 18]. The attenuation of capsaicin-induced relaxation in high  $\text{K}^+$  medium suggests that the capsaicin-induced relaxation involves the activation of  $\text{K}^+$  channels. At least four different types of macroscopic  $\text{K}^+$  currents have been identified in the coronary smooth muscle cells [10, 11, 13, 19]. Among these currents,  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  current and delayed rectifier  $\text{K}^+$  current have been considered as the important effector systems that mediate the agonist-induced change in vascular tone by altering the membrane potential in the rabbit coronary artery [13, 19]. Therefore, it may be possible that capsaicin can active these  $\text{K}^+$  channels resulting in relaxation of the pre-contracted artery. In our present studies, pretreatment with 100 nM iberiotoxin (a blocker of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  current) had

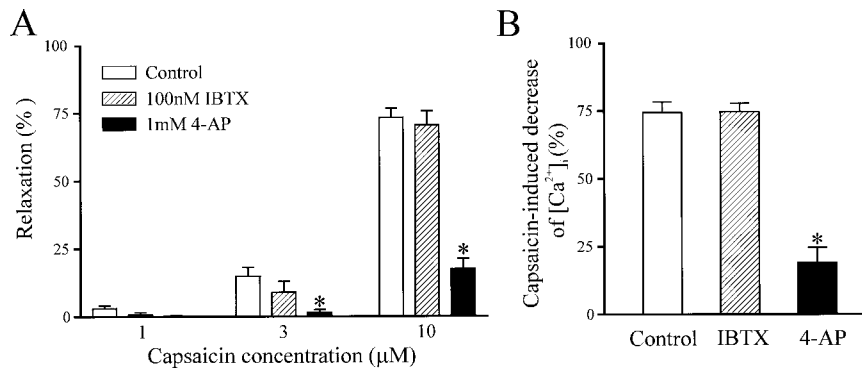


Fig. 3. Effect of pretreatment with 4-AP and iberiotoxin on the capsaicin-induced relaxation and intracellular  $\text{Ca}^{2+}$  concentration. (A) Relaxation induced by 1, 3, and 10  $\mu\text{M}$  capsaicin in the absence (open bar) and after treatment with 100 nM iberiotoxin (hatched bar) and 1 mM 4-AP (filled bar). Relaxation is expressed as a percentage of 80 mM  $\text{K}^+$  contraction. Values are expressed as mean  $\pm$  SEM ( $n=7$ ). (B) Summarized results of the capsaicin-induced decrease in  $[\text{Ca}^{2+}]_i$  in the pre-contracted coronary strips (control, open bar), and after treatment with 100 nM iberiotoxin (IBTX, hatched bar), and 1 mM 4-AP (4-AP, filled bar). Values are expressed as mean  $\pm$  SEM ( $n=5$ ). Asterisk represents  $P<0.01$ .

only a small effect on the capsaicin-induced coronary relaxation. However, pretreatment with 4-AP (a blocker of delayed rectifier  $\text{K}^+$  current) significantly attenuated the capsaicin-induced coronary relaxation with simultaneous attenuation of the effect of capsaicin on  $[\text{Ca}^{2+}]_i$ . These results best fit the hypothesis that capsaicin relaxes the coronary artery by activating the 4-AP sensitive, delayed rectifier  $\text{K}^+$  currents in the coronary smooth muscle cells.

In summary, our present study has demonstrated that in rabbit coronary artery 1) capsaicin may have a direct relaxing effect on coronary smooth muscle cells, and 2) capsaicin can relax the coronary artery by activating the 4-AP sensitive  $\text{K}^+$  channels accompanied by the decrease intracellular  $\text{Ca}^{2+}$  concentration.

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#### REFERENCES

- Benham, C.D., Bolton, T.B., Lang, R.J. and Takewaki, T. 1986. Calcium-activated potassium channels in single smooth muscle cells of rabbit jejunum and guinea pig mesenteric artery. *J. Physiol.* 371: 45–67.
- D'Alonzo, A.J., Grover, G.J., Darbenzio, R.B., Hess, T.A., Slep, P.G., Dzwonczyk, S., Zhu, J.L. and Sewter, J.C. 1995. *In vitro* effects of capsaicin: antiarrhythmic and antiischemic activity. *Eur. J. Pharmacol.* 272: 269–278.
- Donnerer, J. and Lembeck, F. 1982. Analysis of the effects of intravenously injected capsaicin in the rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 320: 54–57.
- Edvinsson, L., Jansen, I., Kingman, T.A. and McCulloch, J. 1990. Cerebrovascular responses to capsaicin *in vitro* and *in situ*. *Br. J. Pharmacol.* 100: 312–318.
- Ellis, J.L., Sham, J.S. and Udem, B.J. 1997. Tachykinin-independent effects of capsaicin on smooth muscle in human isolated bronchi. *Am. J. Respir. Crit. Care Med.* 155: 751–755.
- Ezra, D., Laurindo, F.R.M., Goldstein, D.S., Goldstein, R.E. and Feuerstein, G. 1986. Calcitonin gene-related peptide: a potent modulator of coronary blood flow. *Eur. J. Pharmacol.* 137: 101–105.
- Franco-cereceda, A., Lundberg, J.M., Saria, A., Schreiber, W. and Tritthart, H.A. 1988. Calcitonin gene related peptide: release by capsaicin and prolongation of the action potential in the guinea pig heart. *Acta Physiol. Scand.* 132: 181–190.
- Franco-cereceda, A. and Rudehill, A. 1989. Capsaicin-induced vasodilation of human coronary arteries *in vitro* is mediated by calcitonin gene-related peptide rather than substance P or neurokinin A. *Acta Physiol. Scand.* 136: 575–580.
- Holman, J.J., Craig, R.K. and Marshall, I. 1986. Human alpha and beta CGRP are coronary vasodilators in the rat. *Peptides* 7: 231–235.
- Ishizaka, H. and Kuo, L. 1996. Acidosis-induced coronary arteriolar dilation is mediated by ATP-sensitive potassium channels in vascular smooth muscle. *Circ. Res.* 78: 50–57.
- James, A.F., Okada, T. and Horie, M. 1995. A fast transient outward current in cultured cells from human pulmonary artery smooth muscle. *Am. J. Physiol.* 268: H2358–H2365.
- Kwon, S.C., Park, K.Y., Ahn, D.S., Lee, H.Y. and Kang, B.S. 2000. The effect of NO donor on contraction, cytosolic  $\text{Ca}^{2+}$  level and ionic currents in guinea pig ileal smooth muscle. *Korean J. Physiol. Pharmacol.* 4: 33–40.
- Leblanc, N., Wan, X. and Leung, P.M. 1994. Physiological role of  $\text{Ca}^{2+}$ -activated and voltage-dependent  $\text{K}^+$  currents in rabbit coronary myocytes. *Am. J. Physiol.* 266: C1523–C1537.
- Liu, L. and Simon, S.A. 1997. Capsazepine, a vanilloid receptor antagonist, inhibits nicotinic acetylcholine receptors in rat trigeminal ganglia. *Neurosci. Lett.* 228: 29–32.
- Lo, Y.C., Wu, S.N., Wu, J.R. and Chen, I.J. 1995. Effect of capsaicin on membrane currents in cultured vascular smooth muscle cells of rat aorta. *Eur. J. Pharmacol.* 292: 321–328.
- Lundberg, J.M. and Saria, A. 1987. Polypeptide-containing neurons in airway smooth muscle. *Ann. Rev. Physiol.* 49: 557–572.
- Mitchell, R.W., Ndukwu, I.M., Herrreiter, A., Uzendowski, K., Gitter, B., Solway, J. and Leff, A.R. 1995. Differential tachyki-

- nin receptor subtype activation in capsaicin and KCl contractions of guinea pig trachealis. *Am. J. Physiol.* 269: L837–L842.
18. Nelson, M.T., Patlak, J.B., Worley, J.F. and Standen, N.B. 1990. Calcium channels, potassium channels and voltage dependence of arterial smooth muscle tone. *Am. J. Physiol.* 259: C3–C18.
  19. Nelson, M.T. and Quayle, J.M. 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.* 268: C799–C822.
  20. Oh, U. Hwang, S.W. and Kim, D. 1996. Capsaicin activates a nonselective cation channel in cultured neonatal rat dorsal root ganglion neurons. *J. Neurosci.* 16: 1659–1667.
  21. Saito, A., Kimura, S. and Goto, K. 1986. Calcitonin gene related peptide as potential neurotransmitter in guinea pig right atrium. *Am. J. Physiol.* 250: H693–H698.
  22. Wardle, K.A., Furey, G. and Sanger, G.J. 1996. Pharmacological characterization of the vanilloid receptor in the rat isolated vas deferens. *J. Pharmacy Pharmacol.* 48: 285–291.
  23. Wood, J.N. 1997. Chemical activators of sensory neurons. *Ann. Rev. Physiol.* 59: 457–482.
  24. Zhu, F.X., Zhang, X.Y., Olszewski, M.A. and Robinson, N.E. 1997. Mechanism of capsaicin-induced relaxation in equine tracheal smooth muscle. *Am. J. Physiol.* 273: L997–1001.