0022-3565/98/2872-0697$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 287:697–704, 1998

Vol. 287, No. 2
*Printed in U.S.A.*

# Acute and Chronic Effects of Capsaicin in Perfused Rat Muscle: The Role of Tachykinins and Calcitonin Gene-Related Peptide[1]

CORY D. GRIFFITHS, DOMINIC P. GERAGHTY,[2] TRISTRAM P.D. ELDERSHAW and ERIC Q. COLQUHOUN

*Division of Biochemistry, University of Tasmania, Hobart, Australia*

Accepted for publication June 10, 1998 This paper is available online at http://www.jpet.org

## ABSTRACT

In perfused rat skeletal muscle (hindlimb), capsaicin either stimulates (submicromolar concentrations) or inhibits (micromolar concentrations) oxygen consumption ($VO_2$). Both $VO_2$ effects are associated with vasoconstriction, evident as an increase in perfusion pressure (PP), under constant flow. We have proposed that these effects are mediated by two vanilloid receptor subtypes: $VN_1$ (stimulation of $VO_2$) and $VN_2$ (inhibition of $VO_2$) (Colquhoun *et al.*, 1995; Griffiths *et al.*, 1996). In the present study, the role of capsaicin-sensitive neurons and sensory neuropeptides in the $VN_1/VN_2$ receptor actions of capsaicin was investigated. The observed maximum stimulation of $VO_2$ by capsaicin (0.4 $\mu$M; $\Delta VO_2$, 1.35 $\pm$ 0.14 $\mu$mol $g^{-1}$ $h^{-1}$) was accompanied by mild vasoconstriction ($\Delta$PP, 5.8 $\pm$ 0.6 mm Hg). In contrast, 2 $\mu$M capsaicin produced strong inhibition of $VO_2$ ($\Delta VO_2$, $-2.25$ $\pm$ 0.23 $\mu$mol $g^{-1}$ $h^{-1}$) with pronounced vasoconstriction ($\Delta$PP, 28.0 $\pm$ 1.3 mm Hg). $VO_2$ stimulation was significantly inhibited ($P < .05$) by the selective NK1 receptor antagonist CP-99994 (1 $\mu$M) and the NK2 receptor antagonist SR 48968 (1 $\mu$M) (by 42% and 51%, respectively), but PP was not altered. Infused SP and neurokinin A (NKA) stimulated $VO_2$ (observed maximum $\Delta VO_2$, 0.52 $\pm$ 0.06 and 0.53 $\pm$ 0.08 $\mu$mol $g^{-1}$ $h^{-1}$, respectively; $EC_{50}$ values, 269 $\pm$ 23 and 21.2 $\pm$ 3.0 nM, respectively) and induced mild vasoconstriction (4.30 $\pm$ 0.33 and 6.75 $\pm$ 1.18 mm Hg, respectively; $EC_{50}$ values, 352 $\pm$ 25.7 and 25.5 $\pm$ 2.7 nM, respectively). Neurokinin B (NKB) also stimulated $VO_2$ (maximum not determined) and vasoconstriction (maximum $\Delta$PP, 3.40 $\pm$ 0.25 mm Hg; $EC_{50}$, 34.4 $\pm$ 5.2 nM). The rank order of potency for the tachykinins in this preparation was NKA > NKB > SP, which suggests stimulation primarily of NK2 receptors. Although infused calcitonin gene-related peptide (CGRP) did not alter hindlimb $VO_2$ or PP, the selective CGRP antagonist $CGRP_{(8-37)}$ markedly potentiated the inhibition of $VO_2$ produced by 1 $\mu$M capsaicin (84%) and the maximum capsaicin-induced vasoconstriction (57%), which indicates that endogenously released CGRP may act as a vasodilator. Hindlimbs perfused 1 day after capsaicin pretreatment showed attenuation of capsaicin-induced (0.4 $\mu$M) stimulation of $VO_2$ (92%) ($P < .05$) and vasoconstriction (64%), but this returned to normal after 7 days. The inhibition of $VO_2$ by 1 $\mu$M capsaicin was significantly ($P < .05$) enhanced 7 and 14 days after pretreatment (66% and 140%, respectively), as was the maximum vasoconstriction (64% and 68%, respectively). These data suggest that capsaicin-sensitive neurons, presumably *via* release of SP and NKA, are involved in $VN_1$ responses and that capsaicin pretreatment potentiates $VN_2$ responses, either by depletion of CGRP reserves or by up-regulation of putative $VN_2$ receptors.

The vanilloid spice principle capsaicin and its structural analogs (dihydrocapsaicin, resiniferatoxin, piperine, gingerols and shogaols) produce concentration-dependent vasoconstriction and a biphasic effect on skeletal muscle $VO_2$ in the constant-flow perfused rat hindlimb (Cameron-Smith *et al.*, 1990; Eldershaw *et al.*, 1992; Eldershaw *et al.*, 1994). Work from this laboratory suggests that the dual effect of vanilloids on $VO_2$ (stimulation and inhibition at low and high capsaicin concentrations, respectively) is mediated by at least two vanilloid receptor subtypes, designated $VN_1$ (stimulation of $VO_2$) and $VN_2$ (inhibition of $VO_2$) (Colquhoun *et al.*, 1995). This dual receptor hypothesis has recently been strengthened by the inhibition of the opposing $VO_2$ responses by selective competitive and noncompetitive vanilloid antagonists (Griffiths *et al.*, 1996). The putative $VN_1$ receptor appears to have a higher affinity for capsaicin and is more susceptible to blockade by capsazepine, a known competitive vanilloid antagonist (Urban and Dray, 1991; Bevan *et al.*, 1992). On the other hand, the $VN_2$ receptor has low affinity for capsaicin and capsazepine but is particularly sensitive to ruthenium red, a selective functional capsaicin antagonist at submicromolar concentrations (Amann and Maggi, 1991).

Although our previous findings show that the dual effects

Received for publication December 19, 1997.
[1] This work was supported in part by the National Health and Medical Research Council of Australia and the Australian Research Council.
[2] Present address: Department of Biomedical Science, University of Tasmania, Launceston, Australia 7250.

**ABBREVIATIONS:** SP, substance P; NKA, neurokinin A; NKB, neurokinin B; CGRP, calcitonin gene-related peptide; $VO_2$, oxygen consumption; $PO_2$, partial pressure of oxygen; PP, perfusion pressure; BSA, bovine serum albumin; $EC_{50}$, 50% of maximum response.

of capsaicin in perfused muscle are likely to be mediated by vanilloid receptor subtypes, the underlying mechanisms by which $VN_1$ and $VN_2$ receptors produce these responses are unknown. In other tissues, vanilloid receptors are thought to be coupled to nonselective cation channels on certain C-type and Aδ-type sensory neurons (James *et al.*, 1993). In fact, the recent cloning of a capsaicin receptor from dorsal root ganglia has revealed a 95-kD ion channel that is structurally related to members of the transient receptor potential (TRP) family of ion channels (Caterina *et al.*, 1997). Stimulation of these receptors facilitates the co-release of several neuropeptide transmitters, including the tachykinins SP and NKA, and CGRP (reviewed by Holzer, 1991). A hallmark of capsaicin action on peptide-containing neurons is its ability to induce a refractory state of sensory neuron block with prolonged or repeated *in vitro* application or after systemic administration (reviewed by Szolcsanyi, 1993).

Sensory neuropeptides released by capsaicin may produce a variety of biological responses, including changes in vascular tone and permeability, smooth muscle contraction, and inflammation (reviewed by Holzer, 1991). The actions of tachykinins are mediated by at least three receptor subtypes: SP-preferring NK1, NKA-preferring NK2 and NKB-preferring NK3 receptors (reviewed by Mussap *et al.*, 1993; Maggi *et al.*, 1993; Regoli *et al.*, 1994). These receptor preferences were originally based on the rank orders of potency of endogenous agonists, although each of the tachykinins will stimulate all three receptor types with varying affinity (Regoli *et al.*, 1994). NK1 receptors are widely distributed in both the CNS and peripheral tissues, whereas NK2 receptors are found mainly in peripheral tissues (predominantly on smooth muscle) and NK3 receptors in the CNS, although the latter are expressed in the rat portal vein and guinea pig myenteric plexus (Mastrangelo *et al.*, 1987; Guard *et al.*, 1990). At present there is little evidence for the presence of tachykinin receptors in skeletal muscle cells or skeletal muscle vasculature, although SP dilates the rat cremaster vasculature by a mechanism that is believed to involve the stimulation of NK1 receptors (Brock and Joshua, 1991), and vasodilation induced by stimulation of the rabbit tenuissimus muscle nerve is blocked by the SP antagonist spantide (Persson *et al.*, 1991).

Receptors for CGRP are tentatively divided into two distinct subtypes ($CGRP_1$ and $CGRP_2$) on the basis of the differing ability of C-terminal fragments of the peptide to antagonize the actions of intact CGRP in different preparations (reviewed by Poyner, 1995). CGRP receptors are expressed in cultured L6 rat skeletal muscle cells (Kreutter *et al.*, 1989; Poyner *et al.*, 1992) and whole rat skeletal muscle (Popper and Micevych, 1989; Pittner *et al.*, 1996). In addition, capsaicin has been shown to elicit vasodilation in a rat skeletal muscle preparation (cremaster) by stimulating the endogenous release of CGRP (White *et al.*, 1993).

The present study attempts to define a role for SP, NKA and CGRP in capsaicin-induced responses in the perfused hindlimb by 1) employing competitive NK1, NK2 and CGRP receptor antagonists (CP-99994, SR 48968 and $CGRP_{(8-37)}$), 2) examining the effects of SP, NKA, NKB and CGRP infusion and 3) examining the role of peptide-containing sensory neurons by investigating the effects of capsaicin pretreatment on hindlimb responses to infused capsaicin.

## Materials and Methods

**Rat hindlimb perfusion.** All experimental procedures used in this study were approved by the University of Tasmania Animal Ethics Committee under the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Australian Government Publishing Service, 1990).

Male Hooded-Wistar rats weighing 180 to 200 g were housed at 21 ± 1°C under a 12 h:12 h light:dark cycle and fed a commercial rat chow diet containing 21.4% protein, 4.6% lipid, 68% carbohydrate and 6% crude fiber with added vitamins and minerals. Water was supplied *ad libitum*.

Animals were anesthetized with pentobarbitone sodium (60 mg/kg) and their left hindlimbs perfused according to the method described previously (Colquhoun *et al.*, 1988). In brief, flow was isolated to the left hindlimb by cannulation of the abdominal aorta, posterior to the renal vessels, and ligation of the tail, right common iliac and cutaneous blood vessels. The hindlimb was perfused under constant-flow conditions (4.0 ± 0.1 ml/min) with a modified Krebs-Ringer bicarbonate buffer containing 8.3 mM glucose, 1.27 mM $CaCl_2$ and 2% BSA (fraction V) as an essential oncotic agent. All perfusions were conducted at 25°C, and the perfusate was continuously gassed with carbogen (95% $O_2$/5% $CO_2$) to ensure a constant arterial $PO_2$. The oxygen content of the venous effluent was measured continuously by directing outflow from the cannulated vena cava through an in-line 0.5-ml Clark-type oxygen electrode. PP was monitored by means of a pressure transducer adjoining the cannulated abdominal aorta.

The method of calculation of $VO_2$ has been described previously (Colquhoun *et al.*, 1988). Values for $VO_2$ calculation and perfusion pressure were taken only after steady-state conditions were obtained either under basal or drug-induced changes.

**Agent infusion.** Neuropeptides were dissolved into 20-μl aliquots using a 0.01 M acetic acid solution containing 1% β-mercaptoethanol and stored at −20°C to maintain chemical stability. The aliquots were then diluted, as needed, with 0.9% NaCl so that the acetate and β-mercaptoethanol concentrations were negligible. The neutral endopeptidase inhibitor phosphoramidon (5 μM) was co-infused with each neuropeptide (after the infusion of phosphoramidon alone for 5 min) to prevent enzymatic degradation. Because of the lipophilic nature of capsaicin, it was dissolved in 50% ethanol; thus care was taken to keep the infusion rates low (usually below 10 μl/min) to avoid vehicular perturbation. All other agents were dissolved in 0.9% saline. Capsaicin and the neuropeptides were infused with a syringe pump (Model 2620, Harvard Apparatus Inc., South Natick, MA) driving a 1.0-ml glass syringe (SGE, Australia) equipped with Teflon tubing. Other agents were infused with similar infusion pumps (Model 355, Sage Instruments, Orion Research Inc., (Beverly, MA or Model 11 microinfusion, Harvard Apparatus Inc.) also with an identical 1.0-ml glass syringe and Teflon tubing. All glass apparatus was silanized with Sigmacote before infusion to prevent peptide adhesion to glass surfaces.

In perfusions wherein CP-99994, SR 48968 or $CGRP_{(8-37)}$ was used, a control dose-response curve was first obtained by the cumulative infusion of increasing concentrations of capsaicin, followed by a period of recovery after drug removal. After re-establishment of basal $VO_2$ and PP, we infused CP-99994, SR 48968 or $CGRP_{(8-37)}$ alone for approximately 5 min, and then co-infused the antagonist while the capsaicin dose-response curve was repeated. When infused alone, none of the antagonists induced detectable changes in either basal $VO_2$ or PP.

**Capsaicin pretreatment.** Desensitization to capsaicin was induced by the method used previously by Cui and Himms-Hagen (1992), with a minor modification to the anesthetic used. Briefly, a total dose of 125 mg/kg capsaicin was administered, under anesthesia (40–60 mg/kg pentobarbitone), in four s.c. injections over a 3-day period (day 1, 12.5 mg/kg; day 2, 2 × 25 mg/kg; day 3, 62.5 mg/kg). Injections were given behind the neck or near the rump where s.c.

injection is easier because of the loose skin at these locations. Injections of the vehicle (10% Tween 80, 10% ethanol in normal saline) were given to control animals. The hindlimbs of all animals were perfused 1, 7 or 14 days after the final capsaicin (or vehicle) injection, and the responses to the infusion of the vanilloid were recorded.

**Drugs and chemicals.** SP, NKA, NKB, CGRP and $CGRP_{8-37}$ were purchased from Auspep (Australia); capsaicin, Sigmacote and phosphoramidon from the Sigma Chemical Company; BSA serum albumin (fraction V) from Boehringer Mannheim (Australia) and pentobarbitone sodium (Nembutal, 60 mg/ml) from Bomac Laboratories (Australia). Nonpeptide tachykinin antagonists were generous gifts: (2S,3S)-3-(2-methoxybenzyl)amino-2-phenylpiperidine (CP-99994) from Dr. S.B. Kadin, Pfizer Inc., Groton, CT, and (S)-N-methyl-N-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4dichlorophenyl) butyl]benzamide (SR 48968) from Dr. X. Emonds-Alt, Sanofi Recherche, Montpellier, France. All other reagents were of analytical grade.

**Data analysis.** Statistical analysis was performed by one-way analysis of variance (ANOVA) or ANOVA on ranks (Kruskal-Wallis analysis) where applicable. Paired data were analyzed by one-way repeated measures ANOVA or repeated measures ANOVA on ranks (Friedman analysis) where applicable. All ANOVAs were followed by multiple comparisons using the Student-Newman-Keuls method. $P < .05$ was considered statistically significant. The $EC_{50}$ and $E_{max}$ values for SP, NKA and NKB were estimated from $VO_2$ and PP concentration-response curves for individual experiments. For NKB, the maximum $VO_2$ effect was not obtained, so the $EC_{50}$ for this peptide was estimated by using the mean $E_{max}$ from the SP and NKA experiments. In capsaicin pretreatment experiments, the $EC_{50}$ for the acute effects of capsaicin was estimated from individual PP concentration-response curves and statistically analyzed by Student's *t* test.

## Results

**Effects of CP-99994.** Concentration-response curves for capsaicin were characteristically biphasic for $VO_2$, as seen previously (Colquhoun *et al.*, 1995; Griffiths *et al.*, 1996), with a concentration-dependent increase in PP that is indicative of vasoconstriction (fig. 1). Two consecutive concentration-response curves for capsaicin obtained in the same perfusion were very similar, as indicated by the data obtained using a low, ineffective concentration of CP-99994 (0.1 $\mu$M) (fig. 1A, D). However, there is occasionally mild sensitization to the $VO_2$ stimulatory response at a low capsaicin concentration (0.25 $\mu$M) (fig. 1B) that also occurs when a capsaicin dose-response curve is repeated in the absence of other agents (data not shown). The basis for this sensitization is unknown at present, but it may reflect an increase in the $VN_1$ receptor population or mild up-regulation of postreceptor cellular mechanisms. The observed maximum stimulation of $VO_2$ was produced by 0.4 $\mu$M capsaicin ($\Delta VO_2$, 1.35 ± 0.14 $\mu$mol $g^{-1}$ $h^{-1}$ above basal $VO_2$) followed by inhibition of $VO_2$ at concentrations above 1 $\mu$M, maximum inhibition occurring at 2 $\mu$M (−2.25 ± 0.35 $\mu$mol $g^{-1}$ $h^{-1}$ below basal $VO_2$; fig. 1C). The nonpeptide NK1 receptor antagonist CP-99994 (0.5 and 1 $\mu$M) selectively inhibited the stimulation of $VO_2$ produced by capsaicin ($\Delta VO_2$, 0.97 ± 0.03 and 0.78 ± 0.06 $\mu$mol $g^{-1}$ $h^{-1}$, respectively, $P < .05$; fig. 1B, C). Some statistically significant differences in capsaicin-induced PP changes were observed in the presence of CP 99994 (fig. 1, D, E, F), but these were not consistent over the three antagonist concentrations used.

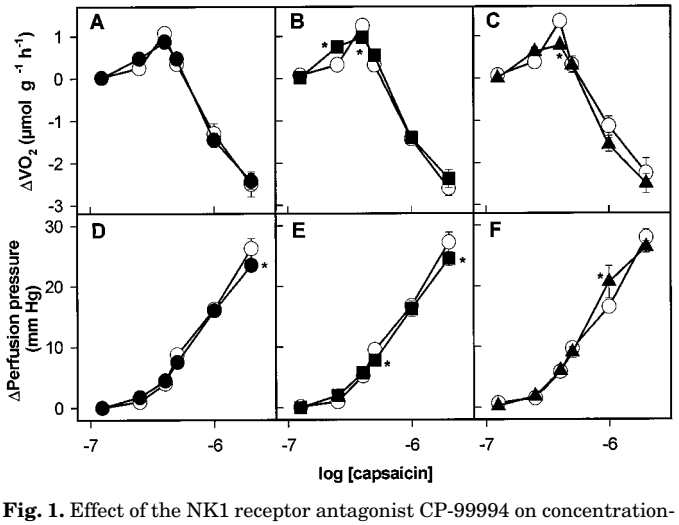


**Fig. 1.** Effect of the NK1 receptor antagonist CP-99994 on concentration-response curves for capsaicin-induced changes in oxygen consumption (panels A, B and C) and perfusion pressure (panels D, E and F) in the perfused rat hindlimb. Control (○), 0.1 $\mu$M (●), 0.5 $\mu$M (■) and 1.0 $\mu$M (▲) CP-99994. Statistical analysis was by one-way repeated measures ANOVA or repeated measures ANOVA on ranks (Friedman analysis) where applicable, followed by multiple comparisons (Student-Newman-Keuls method). * $P < .05$ from control. Values are mean ± S.E.M. of 5 to 6 experiments.

**Effects of SR 48968.** Consecutive concentration-response curves for capsaicin were very similar at an ineffective concentration of the selective NK2 receptor antagonist SR 48968 (fig. 2, A and D), a result that confirms the reproducibility of capsaicin-induced effects. At a concentration of 1 $\mu$M, SR 48968 significantly inhibited ($P < .05$) the maximum stimulation of $VO_2$ induced by 0.4 $\mu$M capsaicin ($\Delta VO_2$: control, 1.06 ± 0.13 $\mu$mol $g^{-1}$ $h^{-1}$; SR 48968, 0.52 ± 0.24 $\mu$mol $g^{-1}$ $h^{-1}$; fig. 2B). Although the stimulation of $VO_2$ at a lower concentration of capsaicin (0.25 $\mu$M) was potentiated in the presence of 1 $\mu$M SR 48968 (fig. 2B), this effect is likely to be caused not by the antagonist, but rather by the mild sensitization to capsaicin that occurs when doses of the vanilloid are repeated in a single perfusion (see above). Furthermore, there was not a statistically significant difference in the $VO_2$ response to 0.25 $\mu$M capsaicin when a higher concentration of SR 48968 (10 $\mu$M) was used (fig. 2C). However, at this concentration of SR 48968, further blockade of the maximum capsaicin-induced stimulation of $VO_2$ ($\Delta VO_2$: control, 1.03 ± 0.08 $\mu$mol $g^{-1}$ $h^{-1}$; SR 48968, 0.17 ± 0.30 $\mu$mol $g^{-1}$ $h^{-1}$, $P < .05$; fig. 2C) was evident, whereas the inhibition of $VO_2$ produced by a high concentration of the vanilloid (2 $\mu$M) was significantly enhanced ($\Delta VO_2$: control, −2.07 ± 0.20 $\mu$mol $g^{-1}$ $h^{-1}$; SR 48968, −3.04 ± 0.26 $\mu$mol $g^{-1}$ $h^{-1}$, $P < .05$). Vasoconstriction at all concentrations of capsaicin was also significantly ($P < .05$) enhanced by 10 $\mu$M SR 48968 (fig. 2F).

**Effects of $CGRP_{(8-37)}$.** Infusion of the CGRP antagonist $CGRP_{(8-37)}$ significantly ($P < .05$) increased the stimulation of $VO_2$ induced by 0.25 $\mu$M capsaicin ($\Delta VO_2$: control, 0.13 ± 0.06 $\mu$mol $g^{-1}$ $h^{-1}$; $CGRP_{(8-37)}$, 0.80 ± 0.09 $\mu$mol $g^{-1}$ $h^{-1}$) but did not significantly increase the observed maximum stimulation of $VO_2$ produced by the infusion of 0.4 $\mu$M capsaicin (fig. 3A). The inhibition of $VO_2$ induced by 1 $\mu$M capsaicin was significantly enhanced by the co-infusion of $CGRP_{(8-37)}$ ($\Delta VO_2$: control, −1.13 ± 0.29 $\mu$mol $g^{-1}$ $h^{-1}$; $CGRP_{(8-37)}$, −2.08 ± 0.15 $\mu$mol $g^{-1}$ $h^{-1}$, $P < .05$, fig. 3A), whereas ΔPP at 1 and 2 $\mu$M capsaicin was markedly increased (ΔPP: control, 16.5 ± 0.7 mm Hg and 29.3 ± 2.0 mm

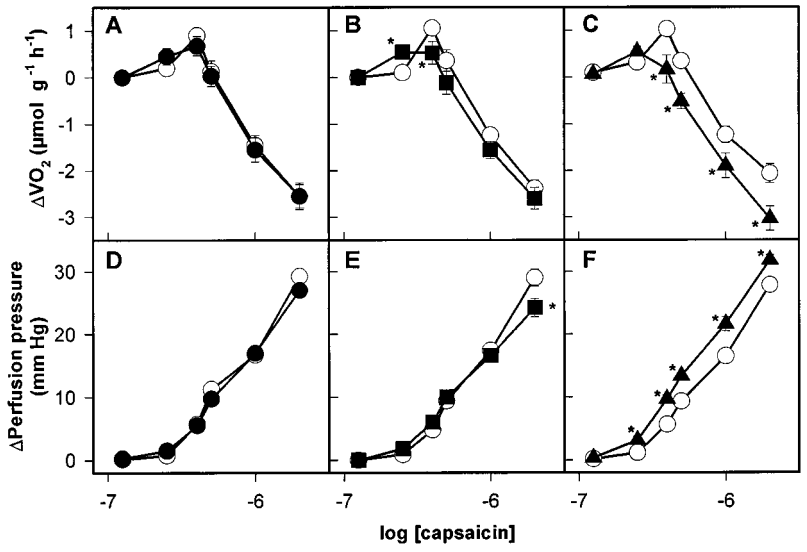


**Fig. 2.** Effect of the NK2 receptor antagonist SR 48968 on concentration-response curves for capsaicin-induced changes in oxygen consumption (panels A, B and C) and perfusion pressure (panels D, E and F) in the perfused rat hindlimb. Control (○), 0.1 $\mu$M (●), 1.0 $\mu$M (■) and 10.0 $\mu$M (▲) SR48968. Statistical analysis was by one-way repeated measures ANOVA or repeated measures ANOVA on ranks (Friedman analysis) where applicable, followed by multiple comparisons (Student-Newman-Keuls method). * $P < .05$ from control. Values are mean ± S.E.M. of 5 to 6 experiments.

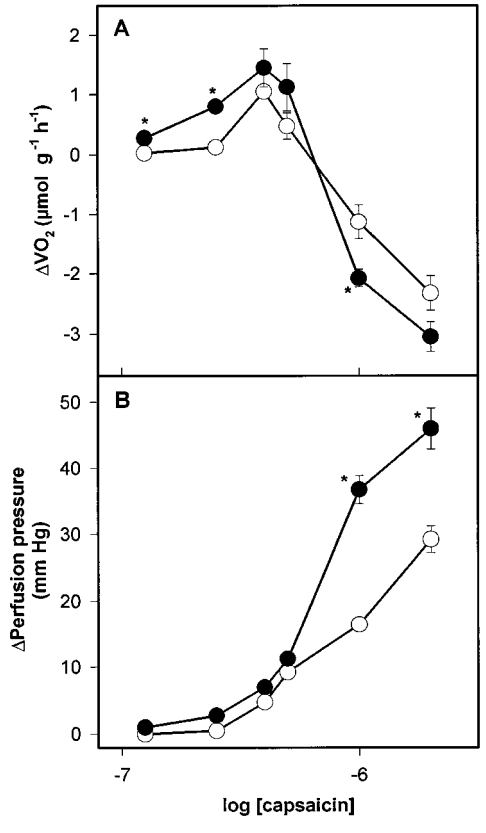


**Fig. 3.** Effect of the CGRP receptor antagonist $CGRP_{(8-37)}$ on concentration-response curves for capsaicin-induced changes in oxygen consumption (panel A) and perfusion pressure (panel B) in the perfused rat hindlimb. Control (○) and 1.0 $\mu$M $CGRP_{(8-37)}$ (●). Statistical analysis was by one-way repeated measures ANOVA or repeated measures ANOVA on ranks (Friedman analysis) where applicable, followed by multiple comparisons (Student-Newman-Keuls method). * $P < .05$ from control. Values are mean ± S.E.M. of 5 to 6 experiments.

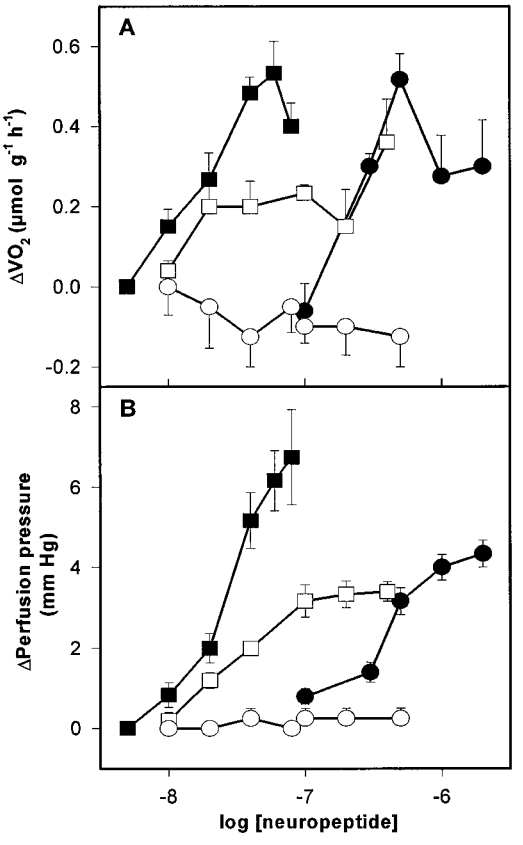


**Fig. 4.** Effect of SP (●), NKA (■), NKB (□) and CGRP (○) on oxygen consumption (panel A) and perfusion pressure (panel B) in the perfused rat hindlimb. In all experiments, SP, NKA, NKB and CGRP were co-infused with the neutral endopeptidase inhibitor phosphoramidon (5 $\mu$M). Values are mean ± S.E.M. of 4 to 6 experiments.

Hg, respectively; $CGRP_{(8-37)}$, 36.8 ± 2.1 mm Hg and 46.0 ± 3.1 mm Hg, respectively, $P < .05$).

**Effects of SP, NKA, NKB and CGRP.** Infusion of the neutral endopeptidase inhibitor phosphoramidon (5 $\mu$M) alone had no detectable effect on either basal $VO_2$ or PP. The co-infusion of increasing doses of SP with phosphoramidon produced a concentration-dependent increase in $VO_2$ (fig. 4A; table 1) and induced mild vasoconstriction (fig. 4B; table 1). Increasing the dose of SP to micromolar concentrations caused some attenuation of the $VO_2$ increase, whereas the effect on PP plateaued. NKA, also co-infused with phosphoramidon, produced similar effects on hindlimb $VO_2$ and PP but was approximately 10-fold more potent than SP (fig. 4; table 1). The infusion of NKB, with phosphoramidon, stimu-

TABLE 1

Maximum change in perfusion pressure (ΔPP) and oxygen consumption ($\Delta VO_2$), and concentration producing 50 percent of maximum response ($EC_{50}$) for SP, NKA and NKB in the perfused rat hindlimb

| Neuropeptide | $n$ | ΔPP | | $\Delta VO_2$ | |
|---|---|---|---|---|---|
| | | $E_{max}$ (mm Hg) | $EC_{50}$ (nM) | $E_{max}$ ($\mu$mol $g^{-1}$ $h^{-1}$) | $EC_{50}$ (nM) |
| SP | 5 | 4.33 ± 0.33 | 352 ± 26 | 0.52 ± 0.07 | 269 ± 23 |
| NKA | 4 | 6.75 ± 1.18 | 25.5 ± 2.7 | 0.53 ± 0.08 | 21.2 ± 3.0 |
| NKB | 5 | 3.40 ± 0.25 | 34.4 ± 5.2 | — | 71.8 ± 29.2† |

Values are mean ± S.E.M.
† $EC_{50}$ estimated using mean of $E_{max}$ ($\Delta VO_2$) for SP and NKA.

lated a small but reproducible change in $VO_2$; however, maximum $VO_2$ was not obtained using concentrations of NKB that induced a maximum change in vascular tone (fig. 4; table 1). On the other hand, the co-infusion of CGRP (10–500 nM) and phosphoramidon altered neither basal hindlimb $VO_2$ nor vascular tension.

**Effects of capsaicin pretreatment.** Figure 5 shows $VO_2$ and PP responses to capsaicin in hindlimbs perfused 1, 7 and 14 days after vehicle or systemic capsaicin pretreatment. The stimulation of $VO_2$ induced by submicromolar concentrations of capsaicin was significantly inhibited 1 day after capsaicin pretreatment (maximum $\Delta VO_2$: control, 0.98 ± 0.23 $\mu$mol $g^{-1}$ $h^{-1}$; capsaicin-pretreated, 0.08 ± 0.04 $\mu$mol $g^{-1}$ $h^{-1}$, $P < .05$; fig. 5A), whereas the increase in PP produced by 2 $\mu$M capsaicin was markedly enhanced (ΔPP: control, 23.2 ± 1.4 mm Hg; capsaicin-pretreated, 35.8 ± 3.3 mm Hg, $P < .05$; fig. 5D). Seven and 14 days after capsaicin pretreatment, the stimulation of $VO_2$ and the vasoconstriction induced by low concentrations of capsaicin was completely restored, whereas the maximum inhibition of $VO_2$ by 2 $\mu$M capsaicin was significantly enhanced compared with vehicle-pretreated controls ($\Delta VO_2$: 7 days, control, −3.18 ± 0.06, capsaicin-pretreated, −4.27 ± 0.46; 14 days, control, −3.02 ± 0.25, capsaicin-pretreated, −4.52 ± 0.40 $\mu$mol $g^{-1}$ $h^{-1}$; fig. 3B, C). The maximum vasoconstriction at micromolar concentrations of capsaicin was also greatly increased 7 days after capsaicin pretreatment, and it was increased further after 14 days (fig. 5; table 2). In addition, the $EC_{50}$ for capsaicin, estimated from the PP concentration-response curves, was significantly ($P < .01$) lower in animals perfused 7 and 14 days after capsaicin pretreatment (table 2).

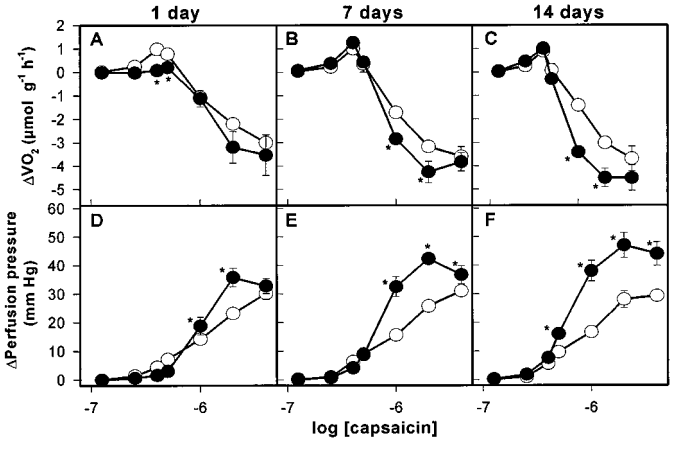


**Fig. 5.** Concentration-response curves for capsaicin-induced changes in oxygen consumption (panels A, B and C) and perfusion pressure (panels D, E and F) in the hindlimbs of rats perfused 1, 7 and 14 days after pretreatment with vehicle (○) or capsaicin (●). Statistical analysis was by one-way ANOVA or ANOVA on ranks (Kruskal-Wallis analysis) where applicable, followed by multiple comparisons (Student-Newman-Keuls method). * $P < .05$ from control. Values are mean ± S.E.M. of 4 to 6 experiments.

TABLE 2

Maximum change in perfusion pressure (ΔPP) and concentration producing 50 percent of maximum response ($EC_{50}$) for capsaicin in the perfused rat hindlimb, 1, 7 and 14 days after vehicle- or capsaicin-pretreatment

| Pretreatment | $n$ | Days after Pretreatment | $E_{max}$ (mm Hg) | $EC_{50}$ ($\mu$M) |
|---|---|---|---|---|
| Vehicle | 5 | 1 | 30.2 ± 2.2 | 1.07 ± 0.04 |
| Capsaicin | 5 | 1 | 35.8 ± 3.3 | 0.99 ± 0.07 |
| Vehicle | 4 | 7 | 31.0 ± 1.8 | 1.02 ± 0.05 |
| Capsaicin | 4 | 7 | 42.3 ± 0.3 | 0.74 ± 0.04** |
| Vehicle | 5 | 14 | 29.3 ± 1.8 | 0.96 ± 0.05 |
| Capsaicin | 5 | 14 | 47.0 ± 4.4 | 0.66 ± 0.05** |

Values are mean ± S.E.M. ** $P < 0.01$ (Student's $t$ test) from corresponding vehicle pretreated.

## Discussion

Capsaicin produced a powerful vasoconstrictor response and a biphasic effect on $VO_2$ in the perfused rat hindlimb, a result that confirmed previous data from this laboratory (Cameron-Smith *et al.*, 1990; Colquhoun *et al.*, 1995; Griffiths *et al.*, 1996). The main purpose of the present study was to investigate the role of sensory neurons and sensory neuropeptides (SP, NKA, NKB and CGRP) in capsaicin-induced changes in vascular resistance and $VO_2$ by studying the effects of capsaicin pretreatment and neuropeptide antagonists.

Stimulation of $VO_2$ produced by submicromolar concentrations of capsaicin ($VN_1$ response) was partly blocked by the selective NK1 receptor antagonist CP-99994 in a concentration-dependent manner (fig. 1). The NK2 receptor antagonist SR 48968 produced effects similar to those of CP-99994 but also enhanced the inhibition of $VO_2$ produced by micromolar concentrations of capsaicin ($VN_2$ response) and potentiated vasoconstriction over the entire capsaicin concentration range (fig. 2). Infusion of SP, NKA or NKB, in the presence of phosphoramidon, produced mild, concentration-dependent vasoconstriction and stimulated $VO_2$ (fig. 4). NKA was at least 10-fold more potent than SP at stimulating $VO_2$ and vasoconstriction (table 1), and its activity is comparable to that in an NK2 receptor bioassay (rabbit pulmonary artery) (Regoli *et al.*, 1987). The potency of SP in the present study is at least 1000-fold lower than in the NK1 receptor bioassay (dog carotid artery) and more closely resembles its activity on NK2 receptors in the rabbit pulmonary artery (Regoli *et al.*, 1987). However, the use of BSA as an essential colloid in the perfused hindlimb preparation may account for the apparent low potency of SP; this protein is known to bind numerous agents, including capsaicin. Taken together, these findings using neuropeptide agonists and antagonists provide evidence that stimulation of $VO_2$ by submicromolar concentrations of capsaicin is partly mediated by the endogenous release of SP and NKA, which then stimulate $VO_2$ *via* action on peripheral NK2 receptors and possibly NK1 receptors. However, the data obtained using nonpeptide tachykinin receptor antagonists should be interpreted with caution, because the submicromolar to micromolar concentrations required to alter the effects of capsaicin may not be specific for one tachy-

kinin receptor subtype and may induce nonspecific effects (Lombet and Spedding, 1994). Nonetheless, when taken in conjunction with the rank order of potency for the tachykinins in this preparation (NKA > NKB > SP), the present data support the notion of NK2 receptor involvement, although a role for NK1 receptors cannot be excluded because CP-99994 was also effective at blocking some actions of capsaicin. In addition, NKA is known to have a strong affinity for NK1 receptors, and preliminary autoradiographic studies indicate that NK1 receptors are present on blood vessels in hindlimb skeletal muscle (Griffiths, Mazzone, Geraghty and Colquhoun, unpublished observations). Although NKB stimulated $VO_2$ and vasoconstriction in the present study, it is unlikely that NK3 receptors play a role in the capsaicin-mediated effects in muscle, because their peripheral distribution is limited (Mastrangelo *et al.*, 1987; Guard *et al.*, 1990).

The potentiation of capsaicin-stimulated vasoconstriction by SR 48968 may indicate that endogenously released tachykinins, acting *via* NK2 receptors, are dilators of the perfused hindlimb vasculature, although the concentration of SR 48968 required for this effect may have also blocked NK1 receptors. Similarly, CGRP, which is released in skeletal muscle in response to capsaicin (Santicioli *et al.*, 1992), may act as a potent vasodilator in this preparation, because the CGRP receptor antagonist $CGRP_{(8-37)}$ greatly potentiated the capsaicin-induced vasoconstriction and inhibition of $VO_2$ (fig. 3). These hypotheses are not supported by the infusion, in the presence of phosphoramidon, of the tachykinins SP and NKA, which act as mild vasoconstrictors in this preparation (see above). Infused CGRP (also with phosphoramidon) did not produce a measurable effect on basal hindlimb $VO_2$ or vascular tone (fig. 4). This observation is unusual, given that CGRP has been shown to be a potent vasodilator in many tissues, including striated muscle (White *et al.*, 1993; Kim *et al.*, 1995). In addition, it has recently been shown that CGRP, released from capsaicin-sensitive primary afferents, contributes to the hyperemic response to skeletal muscle contraction (*via* sciatic nerve stimulation) in the rat hindlimb (Yamada *et al.*, 1997a, b). However, basal hindlimb PP in the present study probably represents near-maximum arteriolar dilation, because at the flow rate used (4 ml/min), the potent vasodilator nitroprusside has no measurable effect on vascular tone (Colquhoun *et al.*, 1988; Ye *et al.*, 1990). This may limit the scope of action of SP, NKA, NKB and/or CGRP such that any vasodilator action by these peptides would not be observed. The vasoconstriction induced by SP, NKA and NKB in the present study may have resulted from direct stimulation of smooth muscle cell NK receptors after diffusion of the peptides across the endothelium. It remains to be seen whether the neuropeptides used in the present study can significantly alter vascular tone in the constant-flow perfused-hindlimb preparation preconstricted with other vasoactive agents (*e.g.*, norepinephrine, serotonin and angiotensin II). Preliminary results obtained in the perfused rat hindlimb under norepinephrine-induced vascular tension indicate that these peptides may induce vasodilation, although it is not yet clear which receptors and mechanisms are involved in this response (Griffiths, Geraghty and Colquhoun, unpublished observations).

Capsaicin possesses a well-documented ability to stimulate and then desensitize peptide-containing sensory neurons with prolonged or repeated application or after systemic administration. Indeed, capsaicin is a widely used research tool that selectively blocks C-type and Aδ-type primary afferents. In the present investigation, we attempted to define a role for capsaicin-sensitive neurons in the acute metabolic and vascular effects of vanilloids in perfused muscle by studying the effects of systemic capsaicin pretreatment. Capsaicin pretreatment produced dramatically alters capsaicin-induced $VO_2$ and PP changes in the perfused hindlimb (fig. 5). One day after capsaicin pretreatment, the stimulation of $VO_2$ and the mild increase in PP produced by submicromolar concentrations of capsaicin ($VN_1$ response) were almost completely abolished. However, 7 days after capsaicin pretreatment, the $VN_1$ response had returned, and the magnitude of $VO_2$ stimulation was identical to that of the control.

Szolcsanyi (1993) describes four distinct actions of capsaicin pretreatment on sensory neurons: 1) release of neuropeptides within minutes; 2) "sensory neuron block," wherein sensory neurons are unresponsive to capsaicin (*i.e.*, neuropeptides are not released), which lasts for hours to several days; 3) recovery of function of some neurons and degeneration of others over several days to weeks and 4) complete degeneration of affected neurons over weeks to months. In the present study, acute sensory neuron block may explain the absence of the $VN_1$ response 1 day after capsaicin pretreatment. The re-establishment of the $VN_1$ response after 7 days may be due to a small population of intact C fibers that recover from the block and release sufficient neuropeptides to stimulate $VO_2$.

In contrast to the effects of capsaicin pretreatment on $VN_1$ responses, the inhibition of $VO_2$ ($VN_2$ response) was marginally enhanced 1 day, and significantly enhanced 7 and 14 days, after capsaicin pretreatment. A progressive increase in the vasoconstrictor response to capsaicin mirrored the enhancement of $VO_2$ inhibition, the maximum PP to 2 $\mu$M capsaicin infusion almost doubling 14 days after capsaicin pretreatment. Further analysis of the data revealed that the concentration of capsaicin producing a half-maximal increase in PP was significantly ($P < .01$) decreased 7 and 14 days after capsaicin pretreatment. Why the maximum vasoconstrictor response progressively increased in capsaicin-pretreated rats is unclear. This was an unexpected finding because capsaicin pretreatment normally leads to a blunting of nonvascular, smooth muscle responses to capsaicin (Maggi and Meli, 1988). This observation, when combined with the decrease in $EC_{50}$ for capsaicin, suggests either up-regulation of $VN_2$ receptors and/or sensitization of vascular smooth muscle to the direct constrictor action of capsaicin. Alternatively, the apparent increased sensitivity of the vasculature to constrict under capsaicin stimulation may be due to the absence of sufficient vasodilator peptides (*e.g.*, CGRP) to counteract the direct action of the vanilloid on vascular smooth muscle. In cats, "cold storage denervation" potentiates capsaicin-induced vasoconstriction of large cerebral arteries that correlates with degeneration of SP- and CGRP-containing perivascular nerves (Saito *et al.*, 1988). These authors suggested that although capsaicin releases vasodilator peptides (presumably SP, CGRP, etc.) from perivascular nerves of cat cerebral arteries, a direct vasoconstrictor effect of capsaicin predominates. This hypothesis is supported by the work of Edvinsson *et al.* (1990), who showed that the vasodilatation induced by capsaicin in cat cerebral arteries

was attenuated by repeated capsaicin application or by trigeminal ganglionectomy, whereas the vasoconstrictor effect was unaltered. Similarly, Duckles (1986) has shown that capsaicin applied to the isolated carotid artery and thoracic aorta of the guinea pig causes vasoconstriction, rather than dilation, after systemic *in vivo* capsaicin pretreatment. The apparent direct vasoconstrictor action observed in this study is also believed to be due to the absence of sufficient sensory vasodilator peptides after capsaicin pretreatment. However, the studies of Saito *et al.* (1988), Edvinsson *et al.* (1990) and Duckles (1986) suggest that the vasoconstrictor action of capsaicin occurs by a nonspecific effect on the plasma membrane of vascular smooth muscle cells. Conversely, the effects in the perfused hindlimb are believed to occur *via* the stimulation of specific vanilloid receptors because the vasoconstriction can be blocked by the competitive vanilloid receptor antagonist capsazepine (Griffiths *et al.*, 1996).

Exactly how capsaicin and the sensory neuropeptides produce their vascular and $VO_2$ effects in perfused muscle is unclear. The concept of site-specific vasoconstriction, leading to increased "nutritive" flow, has been proposed to explain the large increases in hindlimb $VO_2$ seen with the infusion of other potent vasoconstrictors, such as norepinephrine, angiotensin II and vasopressin (reviewed in Clark *et al.*, 1995; 1997). That is, vasoconstrictors that increase hindlimb $VO_2$ probably do so by redistributing perfusate flow to the network of vessels supplying skeletal muscle cells, which results in greater total nutrient exchange. On the basis of this flow redistribution model, it appears plausible that submicromolar concentrations of capsaicin may stimulate $VO_2$ ($VN_1$ response) by selectively constricting (*via* a direct effect) or dilating (by release of neuropeptides) blood vessels, leading to increased perfusate flow to "nutritive" vessels. However, a direct effect of capsaicin and the sensory neuropeptides to stimulate muscle $VO_2$ cannot be ruled out, because in the present study, NK1 and NK2 receptor antagonists decreased capsaicin-induced stimulation of $VO_2$ but did not cause appreciable changes in PP (fig. 1C, F; fig. 2, B and E).

On the other hand, there is convincing evidence that strong vasoconstrictors that inhibit $VO_2$ in the perfused hindlimb (*e.g.*, serotonin) do so by shunting perfusate away from nutritive vessels to non-nutritive vessels supplying hindlimb connective tissue (septa and tendons) (Newman *et al.*, 1997). Therefore, increased non-nutritive flow may explain the inhibition of $VO_2$ that accompanies the strong vasoconstriction induced by high concentrations of capsaicin. This hypothesis is strengthened by the current observation that the augmentation of capsaicin induced vasoconstriction 7 and 14 days after capsaicin pretreatment (fig. 5) produced a concomitant potentiation of $VO_2$ inhibition.

The results of the present study imply that capsaicin, when infused into the perfused rat hindlimb, stimulates higher-affinity vanilloid receptors ($VN_1$) that release thermogenic ($VO_2$-stimulating) peptides. These receptors appear to be neuronal (primary afferent C fiber), given that systemic capsaicin pretreatment ablates the acute $VO_2$ stimulation response to infused capsaicin. The stimulation of $VO_2$ by capsaicin is also selectively blocked by nonpeptide tachykinin antagonists of NK1 and NK2 receptors, and infused SP, NKA and NKB stimulate oxygen consumption and mild vasoconstriction with a rank potency order of NKA > NKB > SP. Hence, capsaicin may stimulate $VO_2$ by releasing endogenous tachykinins that interact primarily with NK2 receptors. Conversely, CGRP had no detectable effect on $VO_2$ or pressure, which may be due to the use of an almost fully dilated preparation. Indeed, the CGRP antagonist $CGRP_{(8-37)}$ enhanced capsaicin-induced vasoconstriction and inhibition of $VO_2$, which suggests that a direct vasoconstrictor action of capsaicin is opposed by the vasodilator action of CGRP. Consequently, the enhanced vasoconstrictor response to capsaicin in capsaicin-pretreated rats (7 and 14 days) may be due to a reduction in the release of CGRP from sensory neurons. Thus in the perfused rat hindlimb, the overall degree of capsaicin-induced vasoconstriction may be the sum of the indirect actions of vasoactive peptides (*e.g.*, SP, NKA and CGRP) released from sensory neurons, plus the direct vasoconstrictor action of capsaicin on vascular smooth muscle.

**Acknowledgments**

The authors wish to thank Dr. Jiming Ye for his assistance with the preparation of this paper. We also extend our thanks to Dr. S.B. Kadin (Pfizer Inc.) and Dr. X. Emonds-Alt (Sanofi Recherche) for their generous gifts of CP-99994 and SR 48968, respectively.

**References**

Amann R and Maggi CA (1991) Ruthenium red as a capsaicin antagonist. *Life Sci* **49:**849–856.

Australian Government (1990) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Australian Government Publishing Service, Canberra.

Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, Walpole CSJ and Yeats JC (1992) Capsazepine: A competitive antagonist of the sensory neuron excitant capsaicin. *Br J Pharmacol* **107:**544–552.

Brock JW and Joshua IG (1991) Substance P mechanisms in the regulation of striated muscle microcirculation. *Microvasc Res* **41:**24–28.

Cameron-Smith D, Colquhoun EQ, Ye J-M, Hettiarachchi M and Clark MG (1990) Capsaicin and dihydrocapsaicin stimulate oxygen consumption in the perfused rat hindlimb. *Int J Obesity* **14:**259–270.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD and Julius D (1997) The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature (Lond)* **389:**816–824.

Clark MG, Colquhoun EQ, Rattigan S, Dora KA, Eldershaw TPD, Hall JL and Ye J (1995) Vascular and endocrine control of muscle metabolism. *Am J Physiol* **268** (*Endocrinol Metab* 31):E797–E812.

Clark MG, Rattigan S, Dora KA, Newman JMB, Steen JT, Miller KA and Vincent MA (1997) Vascular and metabolic regulation of muscle, in *Physiology, Stress and Malnutrition: Functional Correlates, Nutritional Intervention* Kinney JM and Tucker HN (eds), pp 325–346, Lippincott-Raven Publishers, New York.

Colquhoun EQ, Hettiarachchi M, Ye J-M, Richter EA, Hniat AJ, Rattigan S and Clark MG (1988) Vasopressin and angiotensin II stimulate oxygen uptake in the perfused rat hindlimb. *Life Sci* **43:**1747–1754.

Colquhoun EQ, Eldershaw TPD, Bennett KL, Hall JL, Dora KA and Clark MG (1995) Functional and metabolic evidence for two different vanilloid ($VN_1$ and $VN_2$) receptors in perfused rat hindlimb. *Life Sci* **57:**91–102.

Cui J and Himms-Hagen J (1992) Rapid but transient atrophy of brown adipose tissue in capsaicin-desensitised rats. *Am J Physiol* **262** (*Regulatory Integrative Comp Physiol* **31**):R562–R567.

Duckles SP (1986) Effects of capsaicin on vascular smooth muscle. *Naunyn-Schmiedeberg's Arch Pharmacol* **333:**59–64.

Edvinsson L, Jansen I, Kingman TA and McCulloch J (1990) Cerebrovascular responses to capsaicin *in vitro* and *in situ*. *Br J Pharmacol* **100:**312–318.

Eldershaw TPD, Colquhoun EQ, Dora KA, Peng Z-C and Clark MG (1992) Pungent principles of ginger (*Zingiber officinale*) are thermogenic in the perfused rat hindlimb. *Int J Obesity* **16:**755–763.

Eldershaw TPD, Dora KA, Bennett K, Clark MG and Colquhoun EQ (1994) Resiniferatoxin and piperine: Capsaicin-like stimulators of oxygen uptake in the perfused rat hindlimb. *Life Sci* **55:**389–397.

Griffiths CD, Eldershaw TPD, Geraghty DP, Hall JL and Colquhoun EQ (1996) Capsaicin-induced biphasic oxygen uptake in rat muscle: Antagonism by capsazepine and ruthenium red provides further evidence for peripheral vanilloid receptor subtypes ($VN_1$/$VN_2$). *Life Sci* **59:**105–117.

Guard S, Watson SP, Maggio JE, Too HP and Watling KJ (1990) Pharmacological analysis of [$^3$H]-senktide binding to NK3 tachykinin receptors in guinea-pig longitudinal muscle-myenteric plexus and cerebral cortex membranes. *Br J Pharmacol* **90:**767–773.

Holzer P (1991) Capsaicin: Cellular targets, mechanisms of action and selectivity for thin sensory neurons. *Pharmacol Rev* **42:**143–201.

James IF, Ninkina NN and Wood JN (1993) The capsaicin receptor, in *Capsaicin in the Study of Pain.* (Wood JN ed) pp 83–102, Academic Press Ltd, London.

Kim C, Roberts AM and Joshua IG (1995) Differences in the capsaicin-induced dilation of arterioles and venules in rat striated muscle. *J Pharmacol Exp Ther* **273:**605–610.

Kreutter D, Orena SJ and Andrew KM (1989) Suppression of insulin-stimulated

glucose transport in L6 myocytes by calcitonin gene-related peptide. *Biochem Biophys Res Commun* **164:**461–467.
Lombet A and Spedding M (1994) Differential effects of non-peptide tachykinin antagonists on $Ca^{2+}$ channels. *Eur J Pharmacol* **267:**113–115.
Maggi CA and Meli A (1988) The sensory efferent function of capsaicin-sensitive sensory neurons. *Gen Pharmac* **19:**1–43.
Maggi CA, Patacchini R, Rovero P and Giachetti A (1993) Tachykinin receptors and tachykinin receptor antagonists. *J Auton Pharmacol* **13:**23–93.
Mastrangelo D, Mathison R, Huggel HJ, Dion S, D'Orleans-Juste P, Rhaleb N-E, Drapeau G, Rovero P and Regoli D (1987) The rat isolated portal vein: A preparation sensitive to neurokinins, particularly neurokinin B. *Eur J Pharmacol* **134:** 321–326.
Mussap CJ, Geraghty DP and Burcher E (1993) Tachykinin receptors: A radioligand binding perspective. *J Neurochem* **60:**1987–2009.
Newman JMB, Steen JT and Clark MG (1997) Vessels supplying septa and tendons as functional shunts in perfused rat hindlimb. *Microvasc Res* **54:**49–57.
Persson MG, Hedqvist P and Gustafsson LE (1991) Nerve-induced tachykinin-mediated vasodilatation in skeletal muscle is dependent on nitric oxide formation. *Eur J Pharmacol* **205:**295–301.
Pittner RA, Wolfe-Lopez D, Young AA and Beaumont K (1996) Different pharmacological characteristics in L6 and $C_2C_{12}$ muscle cells and intact skeletal muscle for amylin, CGRP and calcitonin. *Br J Pharmacol* **117:**847–852.
Popper P and Micevych PE (1989) Localisation of calcitonin gene-related peptide and its receptors in a striated muscle. *Brain Res* **496:**180–186.
Poyner D (1995) Pharmacology of receptors for calcitonin gene-related peptide and amylin. *Trends Pharmacol Sci* **16:**424–428.
Poyner D, Andrew DP, Brown D, Bose C and Hanley M (1992) Pharmacological characterisation of a receptor for calcitonin gene-related peptide on rat, L6 myocytes. *Br J Pharmacol* **105:**441–447.
Regoli D, Drapeau G, Dion S and D'Orléans-Juste P (1987) Pharmacological receptors for substance P and neurokinins. *Life Sci* **40:**109–117.
Regoli D, Boudon A and Fauchere J-L (1994) Receptors and antagonists for substance P and related peptides. *Pharmacol Rev* **46:**551–599.
Saito A, Masaki T, Lee TJ-F and Goto K (1988) Effects of capsaicin on the contractility and peptide-containing nerves of large cerebral arteries of the cat. *Arch Int Pharmacodyn* **295:**194–203.
Santicioli P, Del Blanco E, Geppetti P and Maggi CA (1992) Release of calcitonin gene-related peptide-like (CGRP-LI) immunoreactivity from rat isolated soleus muscle by low pH, capsaicin and potassium. *Neurosci Lett* **143:**19–22.
Szolcsanyi J (1993) Actions of capsaicin on sensory receptors, in *Capsaicin in the Study of Pain.* (Wood JN ed), pp 1–27, Academic Press Ltd, London.
Urban L and Dray A (1991) Capsazepine, a novel capsaicin antagonist, selectively antagonises the effects of capsaicin in the mouse spinal cord *in vitro. Neurosci Lett* **134:**9–11.
White CB, Roberts AM and Joshua IG (1993) Arteriolar dilation mediated by capsaicin and calcitonin gene-related peptide in rats. *Am J Physiol* **265** (*Heart Circ Physiol* **34**):H1411–1415.
Yamada M, Ishikawa T, Fujimori A and Goto K (1997a) Local neurogenic regulation of rat hindlimb circulation—role of calcitonin gene-related peptide in vasodilatation after skeletal muscle contraction. *Br J Pharmacol* **122:**703–709.
Yamada M, Ishikawa T, Yamanaka A, Fujimori A and Goto K (1997b) Local neurogenic regulation of rat hindlimb circulation—$CO_2$-induced release of calcitonin gene-related peptide from sensory nerves. *Br J Pharmacol* **122:**710–714.
Ye J-M, Colquhoun EQ, Hettiarachchi M and Clark MG (1990) Flow-induced oxygen uptake by the perfused rat hindlimb is inhibited by vasodilators and augmented by norepinephrine: A possible role for the microvasculature in hindlimb thermogenesis. *Can J Physiol Pharmacol* **68:**119–125.

**Send reprint requests to:** D.P. Geraghty, Department of Biomedical Science, University of Tasmania, Launceston, Australia 7250.