Br. J. Pharmacol. (1988), **95**, 673–674

Calcitonin gene-related peptide desensitizes skeletal muscle arterioles to substance P *in vivo*

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Calcitonin gene-related peptide (CGRP) was tested for possible interactions with the effects of substance P on rabbit skeletal muscle arterioles *in vivo*. Both CGRP and substance P dose-dependently increased arteriolar diameter. However, pretreatment with CGRP made the arterioles insensitive to substance P, and also prevented the formation of aggregates of leukocytes and platelets normally seen after substance P. These results suggest that CGRP, in addition to its direct effects, may act as a modulator of the effects of substance P in neurogenic inflammation.

Introduction Calcitonin gene-related peptide (CGRP) has been shown to be a potent vasodilator in hamster cheek pouch, human and rabbit skin (Brain *et al.*, 1985), and in rabbit skeletal muscle (Öhlén *et al.*, 1987). In dorsal root ganglia and peripheral neurones in the rabbit, CGRP-like immunoreactivity (LI) is co-localized with substance P-LI (Öhlén *et al.*, 1987).

Substance P is a vasoactive neuropeptide present in sensory C-fibres throughout the body; it has been suggested that it may be responsible for vasodilatation and plasma extravasation following antidromic nerve stimulation (cf. Lembeck, 1983). Additional properties of substance P, such as its ability to modulate immediate hypersensitivity and stimulate T-cell proliferation indicate multiple roles for this peptide in inflammatory processes (Payan *et al.*, 1986).

The aim of this study was to elucidate the effect of pretreatment with CGRP on the vascular response to substance P *in vivo*. Intravital microscopy of the rabbit tenuissimus muscle was chosen as an experimental model because it allows monitoring of blood flow and blood cells in a tissue with intact nerve and vessel supply (Lindbom *et al.*, 1982).

Methods New Zealand white rabbits weighing 0.8–1.2 kg were anaesthetized with urethane (1.5 g kg^{-1}) via an ear vein. Catheters were inserted into the right carotid artery for blood pressure recordings and into the left jugular vein for supplementary injections of anaesthetic. Spontaneous breathing was facilitated by tracheotomy.

The tenuissimus muscle in the left hind leg was prepared for intravital microscopy as previously described (Lindbom *et al.*, 1982). During preparation and throughout the experiment the muscle was superfused with Krebs-Henseleit solution at 36°C, equilibrated with 5% CO_2 in N_2. The temperature of the animal was maintained at 38°C.

After mounting and identification of areas with well defined vessels, the preparations were allowed to stabilize for 30 min. Substance P, CGRP (10^{-9}–10^{-7} M; Peninsula Lab. Inc., Belmont, CA, U.S.A.) and acetylcholine (10^{-5} M; Sigma, St. Louis, MO, U.S.A.) were dissolved in saline and added with the buffer solution superfusing the muscle. The effect on arterioles (control diameter 14–28 μm) as well as leukocytes and platelets in venules (5–40 μm) was studied by intravital microscopy with a Leitz Intravital microscope and water immersion lens (Leitz SW × 25, NA 0.60).

Results Substance P and CGRP (10^{-9}–10^{-7} M; $n = 6$–7) caused dose-dependent increases of arteriolar diameters in the rabbit tenuissimus muscle (Figure 1). The effect of CGRP was slow in onset (5–10 min), and lasted for up to 30 min. The response to substance P was more prompt and of shorter duration. In addition to arteriolar dilatation, substance P, but not CGRP, caused the formation of aggregates of leukocytes and platelets in the venules (cf. Öhlén *et al.*, 1987).

When the tenuissimus muscle had been pretreated with CGRP (10^{-8} M; $n = 5$) for 10 min and the arteriolar diameters allowed to return to control values (30–45 min), application of substance P caused no increase in arteriolar diameter (Figure 1). Furthermore, the aggregates normally seen in the venules after application of substance P did not appear. However, the vascular response to repeated CGRP (10^{-8} M; $n = 3$) was not diminished (increase in diameter was 95 ± 14% and 87 ± 16%, respectively, after the first and second application of CGRP). Also, acetylcholine (10^{-5} M; $n = 3$) caused

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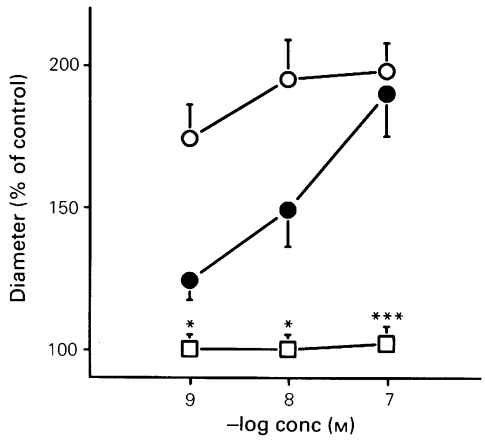

Figure 1 Increase of arteriolar diameter in response to topically applied calcitonin gene-related peptide (CGRP, ○) and substance P (●). Also shown (□) is the effect of substance P 30–45 min after 10 min pretreatment with CGRP 10^{-8} M. Each point represents the mean from 5–7 experiments, vertical bars show s.e.mean. Student's t test for unpaired variates: * $P < 0.05$; *** $P < 0.001$.

vasodilatation (102 ± 10% increase) after pretreatment with CGRP.

Discussion In agreement with previous findings, CGRP and substance P caused a dose-dependent increase in arteriolar diameter, and substance P caused the formation of aggregates of leukocytes and platelets (Öhlén *et al.*, 1987). Pretreatment with CGRP rendered the preparation insensitive to substance P. This is in agreement with *in vitro* data from guinea-pig femoral artery where CGRP abolished the inhibitory effect of substance P on neuroeffector transmission (Wiklund & Öhlén, unpublished). The mechanism for the antagonistic effect of CGRP is not clear. However, pretreatment with CGRP did not affect the vascular responsiveness to repeated application of CGRP or to acetylcholine. This implies that the inhibitory effect on substance P-vasodilatation was not due to a general desensitization and that pretreatment with CGRP does not interact with the vascular response to endothelium-derived relaxing factor(s).

Le Greves *et al.* (1985) suggested that CGRP may enhance the effect of substance P in the CNS by inhibiting substance P degradation. Furthermore, substance P has been shown to enhance markedly the vasodilator effect of CGRP in cat dental pulp (Gazelius *et al.*, 1987). Taken together, these findings suggest that CGRP and substance P, which are likely to be co-released during nerve activity, can interact in a complex manner. The present findings suggest that CGRP, in addition to its direct effect, may function as a modulator of the effects of substance P in neurogenic inflammation.

This work was supported by the Swedish MRC (14X-4342, X-7919), the National Environment Protection Board (5324067-7), the Swedish Society for Medical Research, and the Foundation Lars Hiertas Minne.

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(Received June 22, 1988
Accepted July 27, 1988)