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Neurokinin-induced changes in pial artery diameter in the anaesthetized guinea-pig

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1 The effects of selective neurokinin agents on pial artery diameter, measured with an on-line image analyser, have been studied in anaesthetized guinea-pigs in order to characterize the neurokinin receptors present on pial arteries.

2 Perivascular injection of either substance P (0.01–1 μM) or the selective NK_1 receptor agonists, substance P methyl ester (SPOMe, 0.01–1 μM) and GR73632 (0.1 μM), increased pial artery diameter.

3 In contrast, the selective NK_2 receptor agonist, GR64349 (1 μM), produced a small vasoconstriction while the NK_3 receptor-selective agonist, senktide (1 μM) was inactive.

4 Co-administration of GR82334 (1 μM), a selective NK_1 receptor antagonist, inhibited the vasodilatation produced by SPOMe (0.1 μM) but not that caused by calcitonin gene-related peptide (CGRP, 0.01 μM).

5 The results are consistent with an involvement of NK_1 receptors in the neurokinin-induced increase in guinea-pig pial artery diameter.

Keywords: Pial artery; vasodilatation; substance P; neurokinin receptors; calcitonin gene-related peptide (CGRP); GR73632; GR64349; GR82334

Introduction

The neuropeptides, substance P and calcitonin gene-related peptide (CGRP), have been implicated in the pathogenesis of vascular headache (Moskowitz, 1984; Moskowitz *et al.*, 1989). Cranial blood vessels possess a dense distribution of substance P- and CGRP-like immunoreactive nerve fibres originating in the trigeminal ganglion (Edvinsson *et al.*, 1983; Liu-Chen *et al.*, 1983; Skofitsch & Jacobowitz, 1985) and stimulation of these fibres results in a release of substance P and CGRP (Goadsby *et al.*, 1988; Moskowitz *et al.*, 1983). Substance P causes both local oedema and plasma protein extravasation by increasing vascular permeability (Markowitz *et al.*, 1987) and, in common with CGRP, produces vasodilatation of the intracranial vasculature (Edvinsson *et al.*, 1981; McCulloch *et al.*, 1986). This vasodilatation may result in the initiation of local axon reflexes and thus further activation of trigeminal perivascular sensory nerves contributing to the perception of pain (Humphrey & Feniuk, 1991).

It is now clear that neurokinin receptor-mediated responses, such as those implicated in vascular headache, may involve at least three different receptors, classified NK_1, NK_2 and NK_3 according to relative potencies of neurokinin agonists (Lee *et al.*, 1986; Watson *et al.*, 1983). Although substance P has greater affinity for NK_1 receptors (Regoli *et al.*, 1989; Guard & Watson, 1991), compounds with a much greater selectivity for each neurokinin receptor type have recently been introduced, allowing a more definitive classification of neurokinin-induced responses. Thus, there are selective neurokinin receptor agonists, such as SPOMe (Watson *et al.*, 1983) and GR73632 (both NK_1), GR64349 (NK_2) (Hagan *et al.*, 1991b) and senktide (NK_3) (Wormser *et al.*, 1986), and antagonists, such as the NK_1-sensitive, GR82334 (Hagan *et al.*, 1991a), each exhibiting 100–1000 fold selectivity for the particular receptor type implicated in, for example, neurokinin-induced constriction of the rat portal vein (NK_3) or plasma protein extravasation (NK_1). GR73632, GR64349 and GR82334 are resistant to metabolism by peptidases (Hagan *et al.*, 1991a,b) and are therefore useful for *in vivo* investigation of neurokinin-mediated effects. The use of these compounds in an earlier study (Stubbs *et al.*, 1992) indicated that substance P-induced relaxations of the dog middle cerebral artery, *in vitro*, were mediated via NK_1 receptor activation. The aim of the present study was to characterize the neurokinin receptor(s) mediating dilatation of guinea-pig pial arteries *in vivo* by use of these selective drug tools.

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Methods

Adult male Dunkin-Hartley guinea-pigs (300–450 g) were anaesthetized (i.p.) with ketamine (40 mg kg^{-1}), pentobarbitone (18 mg kg^{-1}) and xylazine (6 mg kg^{-1}). The trachea was cannulated and animals artificially respired (60 strokes min^{-1}, 12 ml kg^{-1}) with room air. The right carotid artery and jugular vein were cannulated to permit, respectively, the continuous measurement of arterial blood pressure and infusion of anaesthetic (15 mg kg^{-1} h^{-1} pentobarbitone). The acid/base status in the arterial blood was checked during the experiment by blood gas analysis (Radiometer ABL 30) and pH, $PaCO_2$ and PaO_2 maintained at physiological levels (7.36 ± 0.01, 38.2 ± 2.0 mmHg and 98.3 ± 5.2 mmHg respectively (mean ± s.e.mean, $n = 53$)) by adjusting the stroke volume and/or supplementing the inspired air with O_2. Rectal temperature was monitored and maintained at 37–38°C with a heated blanket and thermistor (CFP 8185).

The head of the guinea-pig was placed in a stereotaxic frame (David Kopf) and a longitudinal incision made in the scalp. A craniotomy measuring, approximately, 1 cm × 0.5 cm was made over the left parietal cortex with a saline-cooled dental drill and the area covered with mineral oil (37°C). The dura was then carefully removed to expose the underlying pial blood vessels. Changes in pial artery calibre were recorded with an automatic image analyser (HVS SP143) attached via a camera (Ikegami) to a binocular microscope (Meiji) and displayed, together with blood pressure on a chart recorder (Lectromed M19). The image was continuously displayed on a videomonitor (Hitachi VM-910E/K).

Drugs were dissolved in artificial cerebrospinal fluid (ACSF) immediately before use and were applied (approx. 0.5 μl in 10 s) by pressure ejection (WPI PV 830) through glass micropipettes (6–8 μm tip, outer diameter) placed, by use of a micromanipulator, in the perivascular space close to the artery under investigation. ACSF was applied to each artery prior to any drug application, and in most experiments 10 mM K^+ was ejected to ascertain the normal vessel reactivity. Responses were expressed as a percentage change from the pre-injection diameter. Antagonist/agonist interactions were studied by co-administration from the same micropipette after having first determined, on different arteries, the responses to the agonist and antagonist alone.

Drugs and solutions

The ACSF had the following composition (mmol l^{-1}): Na^+ (159.5), K^+ (2.5), Ca^{2+} (1.5), Cl^- (150.5), HCO_3^- (14.5) (Wahl *et al.*, 1989) and was bubbled with 5% CO_2 in O_2 giving a pH of 7.2. The following drugs were used in the study: substance P (Peninsula Laboratories), human α-calcitonin gene-related peptide (CGRP, Peninsula Laboratories), substance P methyl ester (SPOMe, Cambridge Research Biochemicals Ltd), senktide (Cambridge Research Biochemicals Ltd). GR73632 (δAva [L-Pro^9, N-MeLeu^{10}] substance P (7–11)), GR64349 ([Lys^3,Gly^8-**R**-γ-Lactam-Leu^9] neurokinin A (3–10)) and GR82334 ([D-Pro^9[Spiro-γ-Lactam]Leu^{10},Trp^{11}] physalaemin (1–11)) were synthesized by the Medicinal Chemistry Department, Glaxo Group Research Ltd, Greenford. Each compound was dissolved in 10% (v:v) acetic acid (10 mM) in distilled water and stock solutions (1 or 5 mM) stored in aliquots at −20°C until needed. Solutions were diluted to the final concentrations with ACSF.

Statistical analysis

Values shown are expressed as means ± s.e.mean and n values quoted refer to the number of arteries studied, from at least 3 animals (exact number shown in square brackets in the figure legends). Comparisons between treatments were made by Student's t test for unpaired samples.

Results

The guinea-pigs had a mean resting blood pressure of 44 ± 1 mmHg (n = 40). Following perivascular administration, none of the compounds used in the study had any effect on systemic blood pressure. The diameter of pial arteries studied ranged from 22 to 110 μm (mean = 58 ± 2 μm, n = 96) and responses elicited by either the neurokinin agonists or CGRP did not appear to be dependent on initial artery calibre. Under resting conditions, the diameter of the majority of arteries remained constant, although in approximately 10% of the vessels small spontaneous myogenic changes in tone were apparent. Arteries which were spontaneously active responded to CGRP and neurokinins in a similar manner to those vessels which demonstrated no myogenic activity.

The perivascular injection of ACSF (0.5 μl in 10 s) produced little change (2.0 ± 0.8%, n = 39) in diameter of most (>90%) pial arteries (Figure 1). Vessels which exhibited dilatation in response to ACSF, probably reflecting effects of the pressure ejection *per se*, were excluded from any further study. Ejection of 10 mM K^+ in ACSF produced a transient, reproducible vasodilatation (Figure 1).

The neuropeptides, substance P (0.01–1 μM) and CGRP (0.01 and 0.1 μM) dose-dependently increased pial artery diameter (Figure 1). Substance P and CGRP had similar potencies, the concentrations (with 95% confidence limits) producing a 30% increase in pial artery diameter were 0.04 (0.02–0.09) and 0.01 (0.01–0.03) μM, respectively. While the vasodilatations produced by both peptides were immediate in onset, the duration of their responses differed markedly (Figure 2); those evoked by substance P were short lasting with the time required to reach a 50% reduction in response amplitude ($t_{1/2}$) of approximately 0.5 min at 0.1 μM, while those to CGRP were of a much longer duration ($t_{1/2}$ > 15 min at 0.01 μM). Dose-response curves to either compound were not constructed from the same artery as repeated application of substance P and to a lesser extent CGRP resulted in desensitization. In addition, the long duration of the CGRP-induced response made further injections of this agent at the same site impractical. The effects of only a single concentration of each drug were therefore examined in any one vessel.

Selective neurokinin receptor agonists were used to characterize the neurokinin-induced responses. The NK_1 receptor agonists, SPOMe (0.01–1 μM) and GR73632 (0.1 μM), but not GR64349 or senktide (NK_2 and NK_3 agonists, respec-

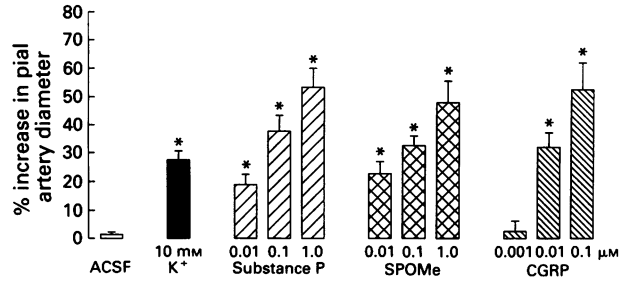

Figure 1 The effect of ACSF (n = 39 [19]), K^+ (10 mM, n = 25[13]), substance P (0.01 (n = 12 [5]), 0.1 (n = 6 [4]) and 1 μM (n = 3 [3])), substance P methyl ester (SPOMe, 0.01 (n = 5[3]), 0.1 (n = 4 [3]) and 1 μM (n = 4 [3])) and calcitonin gene-related peptide (CGRP, 0.001 (n = 6 [3]), 0.01 (n = 10 [6]) and 0.1 μM (n = 7 [3])), injected perivascularly, on guinea-pig pial artery diameter, expressed as a percentage increase (mean ± s.e.mean, vertical bars) (n = number of arteries [number of guinea-pigs])) over pre-injection tone. K^+ (10 nM), substance P (0.01–1 μM), SPOMe (0.01–1 μM) and CGRP (0.01–0.1 μM) induced a significant increase in pial artery diameter (*P<0.05 compared to ACSF).

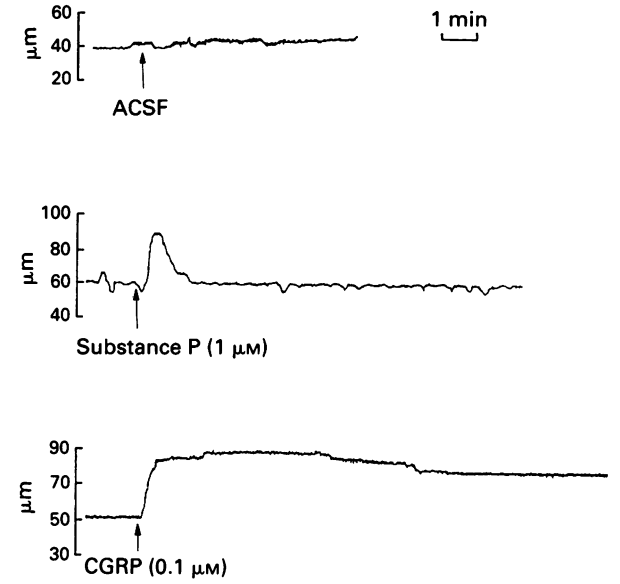

Figure 2 Responses evoked by ACSF, substance P (1 μM) and calcitonin gene-related peptide (CGRP, 0.1 μM) in guinea-pig pial arteries (resting diameters 40, 60 and 52 μm, respectively), following perivascular bolus injection of ACSF or drug in ACSF. ACSF had no effect on artery diameter, while substance P produced a short lasting, and CGRP a maintained vasodilatation.

tively, both 1 μM), significantly increased pial artery diameter (Figures 1 and 3). Indeed, GR64349 (1 μM) significantly reduced artery calibre while senktide (1 μM) was relatively inactive, producing an increase in diameter of 8.0 ± 3.7% (not significantly different from ACSF injection). The vasoconstriction produced by GR64349 had a slow onset (15–30 s), relative to the neurokinin-induced vasodilatations and a $t_{1/2}$ of approximately 3 min at 1 μM. The vasodilatations evoked by SPOMe and GR73632 had a $t_{1/2}$ of approximately 0.5 min at 0.1 μM. The NK_1 receptor antagonist, GR82334 (1 μM), had no apparent effect on artery calibre in its own right, but significantly attenuated vasodilator responses to SPOMe (0.1 μM). In contrast, GR82334 (1 μM) had no effect on the vasodilatation produced by CGRP (0.01 μM) (Figure 4).

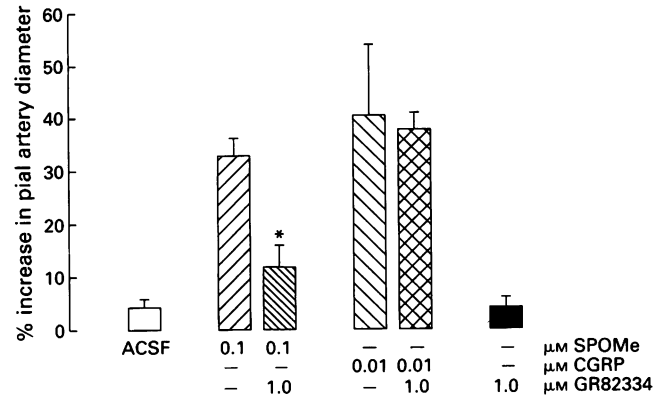

Figure 4 The effect of co-administration of GR82334 (1 μM, $n = 3,4$ [3]) on the percentage increase in pial artery diameter produced by substance P methyl ester (SPOMe, 0.1 μM, $n = 4$ [3]) and calcitonin gene-related peptide (CGRP, 0.01 μM, $n = 3$ [3]). GR82334 reduced significantly ($^*P < 0.05$) SPOMe-, but not CGRP-, induced vasodilatation. GR82334 (1 μM, $n = 7$ [4]) alone had no effect on artery calibre.

Discussion

A number of nociceptive agents, such as substance P, CGRP, bradykinin, prostaglandins and 5-hydroxytryptamine may be involved in the pathogenesis of vascular headache (Moskowitz, 1984; Moskowitz *et al.*, 1989). Evidence for the involvement of substance P is particularly strong; it is stored in, and released from, trigeminal nerve terminals in the intracranial artery wall where it produces plasma protein extravasation and vasodilatation (Edvinsson *et al.*, 1983; Moskowtiz *et al.*, 1989). In the present study, substance P markedly dilated guinea-pig pial arteries when injected perivascularly, an effect consistent with its vasodilator activity in a variety of species, both *in vitro* and *in situ* (Regoli *et al.*, 1989; Jansen *et al.*, 1991; Stubbs *et al.*, 1992). Moreover, the potency with which substance P increased pial artery diameter in the guinea-pig was similar to that observed in cat cerebral vessels (Edvinsson *et al.*, 1981; Jansen *et al.*, 1991). Such a vasodilatation could activate trigeminal nerve endings and via the initiation of local axon reflexes lead to the sensation of pain (Humphrey & Feniuk, 1991).

CGRP, which is co-localized with substance P in trigeminal sensory nerves (Skofitsch & Jacobowitz, 1985; Goadsby *et al.*, 1988), also increased pial artery diameter, in keeping with its recognized potent vasodilator activity in the cerebrovasculature of the cat (McCulloch *et al.*, 1986) and in other tissues (Brain *et al.*, 1985). As in the cat (McCulloch *et al.*, 1986), CGRP-induced responses in the guinea-pig had a longer duration than those produced by substance P.

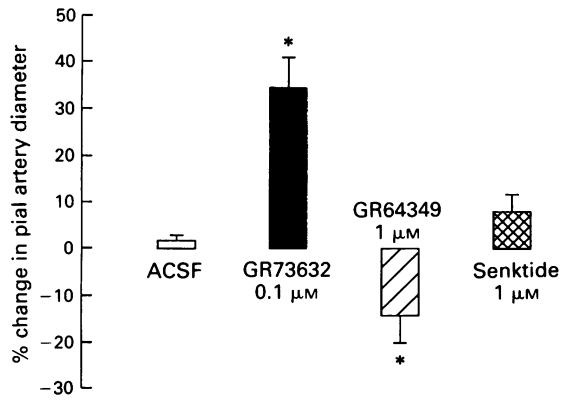

Figure 3 The effect of perivascular injections of receptor-selective neurokinin agonists on guinea-pig pial artery diameter, expressed as the percentage change from resting levels. GR73632 (0.1 μM, $n = 6$ [3]) increased while GR64349 (1 μM, $n = 13$ [4]) decreased and senktide (1 μM, $n = 8$ [3]) had no effect on artery calibre. $^*P < 0.05$ compared to ACSF.

The ability of the selective NK_1 receptor agonists, SPOMe and GR73632 (Guard & Watson, 1991; Hagan *et al.*, 1991b), to dilate pial arteries suggested the involvement of NK_1 receptors. Consistent with this proposal, the selective NK_1 receptor antagonist, GR82334 (Hagan *et al.*, 1991a), inhibited the SPOMe-induced vasodilatation. GR82334 is a highly selective NK_1 receptor antagonist, having little affinity for other peptide receptors, such as those activated by neurokinins A and B, cholecystokinin, bradykinin or bombesin (Hagan, personal communication). The specificity of the compound was also evident in this study, as CGRP-induced vasodilatation was unaffected by GR82334 at a concentration that caused a marked reduction in the response to SPOMe.

The results obtained with selective NK_2 and NK_3 receptor agonists suggested that neurokinin-induced dilatation of pial arteries may not involve NK_2 or NK_3 receptors. Indeed, while senktide (1 μM), a NK_3 receptor agonist (Laufer *et al.*, 1986) was inactive, the selective NK_2 receptor agonist, GR64349 (Hagan *et al.*, 1991b) reduced guinea-pig pial artery diameter. This, however, contrasts with the finding (Jansen *et al.*, 1991) that in cat pial arteries, neurokinin A (0.01–1 μM), a high affinity NK_2 receptor agonist produced only dilatation. This apparent anomaly may suggest that species differences exist but seems more likely to reflect the poor selectivity of neurokinin A for NK_2 receptors (Guard & Watson, 1991). In support of the present findings, NK_2 receptor activation resulted in contraction of the rabbit pulmonary artery (D'Orléans-Juste *et al.*, 1986) and the endothelium-denuded rabbit thoracic aorta (Ireland *et al.*, 1991). The relevance of the vasoconstriction noted in guinea-pig pial arteries, which occurred at only a high concentration (1 μM), remains to be determined.

The results of this study suggest therefore, that NK_1, and possibly not NK_2 or NK_3, receptors mediate the neurokinin-induced vasodilatation of guinea-pig pial arteries and in so doing raises the possibility that NK_1 receptors may have a role in the pathogenesis of vascular headache.

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