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Neurogenic vasodilation in rabbit basilar isolated artery: involvement of calcitonin-gene related peptide

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Abstract

Neurogenic vasodilation in cranial arteries may be an important mechanism in the pathogenesis of migraine headache. We describe a novel, in vitro assay to characterise neurogenic vasodilator responses in endothelium-denuded segments of rabbit isolated basilar artery, with particular focus on calcitonin-gene related peptide (CGRP). In arterial segments precontracted with prostaglandin $F_{2\alpha}$, relaxations evoked by exogenously applied αCGRP (EC_{50} = 2.9 nM) were inhibited by αCGRP-(8–37) (pA_2 = 6.49) or by desensitisation resulting from prior exposure to αCGRP. Relaxations evoked by exogenously applied vasoactive intestinal polypeptide (VIP) (EC_{50} = 2.5 nM) were inhibited by VIP-(7–28) 1 μM. The 5-HT_1 receptor agonists L-771,331 ((3*S*)-3[*N*-(*S*)-α-methylbenzyl]aminomethyl-(*S*)-1-[2-(5-(2-oxo-1,3-oxazolidin-4-ylmethyl)-1*H*-indol-3-yl)ethyl]pyrrolidine) and sumatriptan exerted contractile effects (EC_{50} = 293 and 95 nM, respectively). In neurogenic experiments, vasodilation evoked by electrical field stimulation was markedly attenuated by pre-treatment with capsaicin (10 μM) or by prior CGRP receptor desensitisation and to a lesser extent by pre-treatment with VIP-(7–28) 1 μM. L-771,331 (100 nM) exerted a weak inhibitory effect, marked only by a short reduction in the recovery time (post-electrical stimulation) and sumatriptan (30 nM) had no effect. The neurogenic response was potentiated by αCGRP-(8–37) 1 μM (reversible on wash-out). Short application (5–10 min) of capsaicin (10 μM) produced vasodilation that was inhibited by αCGRP-(8-37) 1 μM. These data suggest that electrically evoked neurogenic vasodilation in rabbit basilar artery has a large component resulting from the release of sensory neuropeptides in particular CGRP and a smaller component involving the release of VIP.

Keywords: Vasodilation; Sensory nerve fibre; CGRP (calcitonin-gene-related peptide); Migraine

1. Introduction

Cranial blood vessels are innervated by a dense network of sympathetic, parasympathetic and trigeminal sensory nerve fibres and these systems act in concert to regulate craniovascular tone. While activation of the sympathetic system is pro-constrictor, vasodilation can result from activation of the trigeminal sensory and/or parasympathetic systems (Edvinsson and Jansen, 1993). Changes in craniovascular tone seem to be important mechanisms in the pathogenesis of migraine headache pain and the trigeminal system is now recognised to play a critical role. Both experimental and clinical research indicate that distension or vasodilation of extracerebral, intracranial blood vessels and the activation of trigeminal system are key factors in the pathogenesis of migraine headache pain (Ray and Wolff, 1940; Uddman and Edvinsson, 1989; Humphrey and Feniuk, 1991; Moskowitz, 1992). The stimulus for dilation of the cranial vasculature may be neurogenic in origin and involve the release of endogenous pro-dilator peptides from trigeminal nerve fibres, with calcitonin gene-related peptide (CGRP), a powerful, vasodilator, being of particular importance. In several species including humans, stimulation of the trigeminal ganglion has been shown to increase cerebral and extracerebral blood flow, and in man, this response was accompanied by increases in local blood levels of CGRP and substance P (Lambert et al., 1984; Goadsby et al., 1986, 1990, 1997; Tran-Dinh et al., 1992). Furthermore, in migraineurs experiencing an attack, increased levels of CGRP and neurokinin A have been detected in jugular blood (i.e. in blood returning from the cranium) and in the case of CGRP, these elevated levels were normalised on administration of sumatriptan

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concomitant with relief of headache pain (Goadsby and Edvinsson, 1993). This action of sumatriptan has been suggested to be related, at least in part, to an action at inhibitory prejunctional 5-$HT_{1B/1D}$ receptors located on trigeminal terminals thereby preventing CGRP release and inhibiting neurogenic vasodilation (Humphrey and Feniuk, 1991; Williamson et al., 1997a). There is no evidence for activation of the parasympathetic nervous system during migraine headache, levels of vasoactive intestinal polypeptide (VIP, found in parasympathetic fibres) are unchanged during an attack (Goadsby et al., 1990). On the other hand, blood levels of both CGRP and VIP are increased in cluster headache sufferers reflecting activation of the trigeminal and parasympathetic systems (Goadsby and Edvinsson, 1994).

The aim of the present study was to develop an in vitro model to characterise the neurogenic vasodilation response in rabbit isolated basilar artery, with the primary focus on the effects of endogenously released CGRP and secondly on the involvement of VIP as a marker for parasympathetic activation. A series of preliminary experiments was conducted to fully characterise the vasodilator effects of exogenously applied neuropeptides (i.e. the post-synaptic effects). Then, neurogenic vasodilation was evoked by electrical stimulation of the arteries. We examined the inhibition of this neurogenic response by (a) blockade of post-synaptic receptors by exogenously applied neuropeptide (CGRP and VIP) antagonists; (b) depletion of endogenous neuropeptides by pre-treatment with the sensorotoxin capsaicin; and (c) inhibition of neuropeptide release by activation of inhibitory pre-junctional receptors evoked by exogenous application of 5-HT_1 receptor agonists (sumatriptan and L-771,331 ((3*S*)-3[*N*-(*S*)-*a*-methylbenzyl]aminomethyl-(*S*)-1-[2-(5-(2-oxo-1,3-oxazolidin-4-ylmethyl)-1*H*-indol-3-yl)ethyl]pyrrolidine), Sternfeld et al., 1999). Since activation of sensory fibres can result in the release of several neuropeptides including CGRP and substance P, endothelium-denuded vessels were used in order to preferentially study the effects of CGRP since these responses, unlike those of substance P, are endothelium-independent (Van Rossum et al., 1997).

2. Material and methods

Basilar arteries were obtained from male New Zealand White rabbits (1.5–2 kg) euthanased by barbiturate overdose. The arteries were cleaned of connective tissue and cut into three- or four-ring segments, which were slipped onto two wire supports under a binocular microscope. One of the supports was connected to the 3 ml organ bath and the other to an isometric transducer. The organ bath contained a modified Krebs solution, which was continuously oxygenated (95% O_2/5% CO_2) and maintained at pH 7.4, 37°C. A resting tension of 2 g was applied. Two platinum stimulating electrodes were inserted at the top and bottom of the organ bath facing each other directly above and below the basilar artery segments and connected to an electrical stimulator (MultiStim D330 System). The tissues were equilibrated (1 h) and then the successful removal of the endothelium was assessed by precontracting the tissues with KCl (45 mM) and ensuring there was no relaxation response on addition of acetylcholine (1 μM).

2.1. Characterisation of the post-synaptic receptor mediated effects

The relaxant effects of exogenously applied peptides (i.e. human αCGRP or VIP) were measured by first pre-contracting the tissues with prostaglandin $F_{2\alpha}$ (10 μM) and then a cumulative relaxant concentration–effect curve to either human αCGRP or VIP was performed. At the end of the concentration–effect curve, forskolin 5 μM was added to establish the maximum relaxation response. For some experiments, the tissues were pretreated with vehicle or the CGRP receptor antagonist human αCGRP-(8–37) 1 μM or the VIP receptor antagonist fragment VIP-(7–28) 1 μM for 15 min prior to the agonist concentration–effect curve. Since the responses showed rapid desensitisation, each arterial segment was exposed to a single agonist concentration–effect curve and antagonist experiments were performed using a matched pairs protocol, i.e. one arterial segment was exposed to peptide agonist alone (control) and a second segment from the same artery was exposed to the peptide antagonist (for details see Longmore et al., 1994).

The vasoconstrictor effects of sumatriptan and L-771,331 were assessed by obtaining cumulative concentration–effect curves (0.1 nM–3 μM).

2.2. Characterisation of neurogenic evoked vasodilation

Quiescent tissues were electrically stimulated at 2 Hz, 0.5 ms pulse width, for 10-s at 15-min intervals. Each tissue was initially stimulated with 30 V and the voltage was increased in 5-V steps (usually to 35 or 40 V) until vasodilatation was observed and two identical relaxant responses were obtained. Tissues were stimulated at that voltage for the rest of the experiment. Immediately following each stimulation, the tissues were washed by perfusion of the organ bath (2 ml/min) for 5 min. Then, perfusion was stopped and the tissues rested for 14 min prior to the next stimulus. Firstly, the mean response to three consecutive stimuli was determined and this was considered as ''control''. Then, a test stimulation was applied following incubation with the following drugs (note the time in brackets refers to the incubation period prior to the stimulus); sumatriptan 30 nM (5 min), L-771,331 100 nM (5 min), human αCGRP-(8–37) 1 μM (15 min), VIP-(7–28) 1 μM (15 min) or capsaicin 10 μM (> 30 min). In addition, desensitisation of post-synaptic CGRP responses

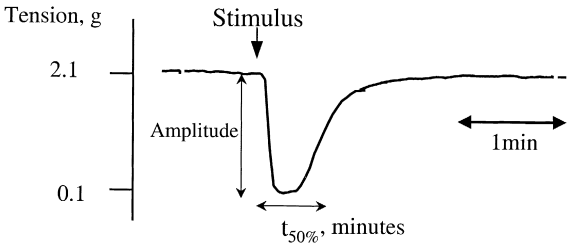

Fig. 1. Experimental recording showing a typical relaxation response in rabbit isolated basilar artery evoked by electrical field stimulation. The data were analysed firstly by determining the effect of the drug treatment on the amplitude of the response (g tension) and secondly on the time taken to achieve 50% recovery (min).

was used as an alternative way to characterise whether neurogenic vasodilation response had a component resulting from CGRP release. It was established in preliminary experiments that repeated exogenous application of 300 nM CGRP produced marked desensitisation (see below) and therefore the electrical stimulation paradigm was applied under ''desensitised'' conditions (i.e. following prior exposure to CGRP 300 nM). For some experiments, arterial segments were exposed to more than one test drug, in this case, the tissues were washed and ''control'' levels of the neurogenic vasodilation response was re-established before application of the next test drug.

Experiments were conducted to examine whether neurogenic vasodilation evoked by capsaicin involved a CGRP component. An experimental paradigm was established in which repeated, short-duration application of capsaicin (5–10 min) produced consistent vasodilator responses. The tissues were precontracted with 45 mM KCl and when this contraction reached a plateau, capsaicin 10 μM was added to the organ bath (control response = 100%). To test for the involvement of CGRP, this procedure was repeated following pre-treatment of the tissues with human αCGRP-(8–37) 1 μM or vehicle prior to addition of KCl.

2.3. Analysis of data

For the concentration–effect curves to exogenously applied CGRP or VIP, the relaxant responses were expressed relative to the size of the maximum relaxant response evoked by forskolin. Contractile responses to 5-HT_1 receptor agonists were expressed relative to the size of the maximum of the curve to each agonist. The amplitude of the neurogenic vasodilation evoked by short application of capsaicin was expressed as a percentage of the pre-contraction evoked by 45 mM KCl (= 100%). Concentration–effect curves were fitted to the mean data using weighted least squares non-linear regression analysis and the equation:

$$E = E_{\max}/\left(1 + (\mathrm{EC}_{50}/\text{agonist concentration})^{\mathrm{nH}}\right)$$

where $E_{\max}$ is the maximum contraction evoked by each agonist (relative to reference response = 100%), EC_{50} is the half maximally effective concentration and nH is the Hill coefficient (GraphPad Prism, Version 2.0b). The ap-

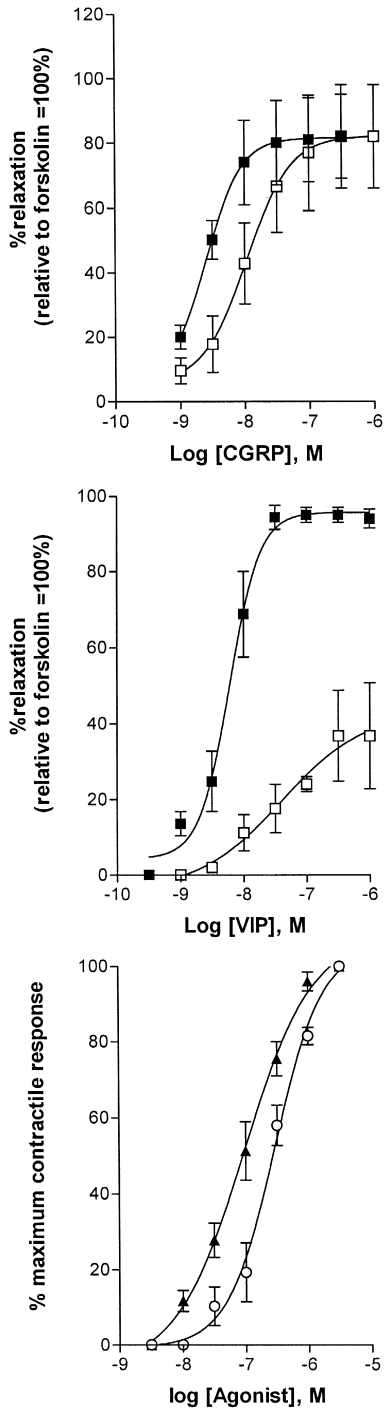

Fig. 2. The upper panel shows the relaxant effect of exogenously applied CGRP (post-synaptic effects) in the absence (closed squares $n = 5$) and presence of human αCGRP-(8–37) 1 μM (open squares $n = 4$). The middle panel shows the relaxant effect of exogenously applied VIP (post synaptic effects) in the absence (closed square $n = 4$)) and presence of VIP-(7–28) 1 μM (open squares $n = 5$). The lower panel shows the vasoconstrictor effects (post-synaptic) of exogenously applied sumatriptan (triangles $n = 5$) and L-771,331 (open circles $n = 4$). Points represent mean values and the vertical bars indicate ± S.E.M. Curves were fitted using nonlinear regression analysis (GraphPad Prism).

parent pA_2 values were calculated using the following equation:

$$\text{apparent } pA_2 = \log(\text{dose ratio} - 1) / \text{antagonist concentration}$$

where the dose-ratio is the concentrations required to produce half maximal response in the presence and absence of the antagonist.

The ability of drugs to inhibit the neurogenic vasodilation responses was assessed in two ways to take into account the possibility that the electrical stimulus may be supra-maximal (see Fig. 1). In the first instance, the amplitude (g tension) of the neurogenic vasodilation response obtained under control conditions (= 100%) was compared to the magnitude of the response obtained following drug treatment and this was regarded as the most stringent test. Secondly, if no significant effect on amplitude was observed, the effect on the kinetics, i.e. time to 50% recovery (a second measure of the level of neuropeptide release) was also examined (see Fig. 1).

2.4. Drugs and solutions

The modified Kreb's solution had the following composition mM: NaCl 118; KCl 4.7; $NaHCO_2$ 25.3; KH_2PO_4 1.19; $CaCl_2$ 2.5; glucose 11.1. CGRP peptides were obtained from California peptides; capsaicin, forskolin, prostaglandin $F_{2\alpha}$ and VIP peptides were from Sigma; sumatriptan and L-771,331 ((3*S*)-3[*N*-(*S*)-α-methylbenzyl]aminomethyl-(*S*)-1-[2-(5-(2-oxo-1,3-oxazolidin-4-ylmethyl)-1*H*-indol-3-yl)ethyl]pyrrolidine) were synthe-

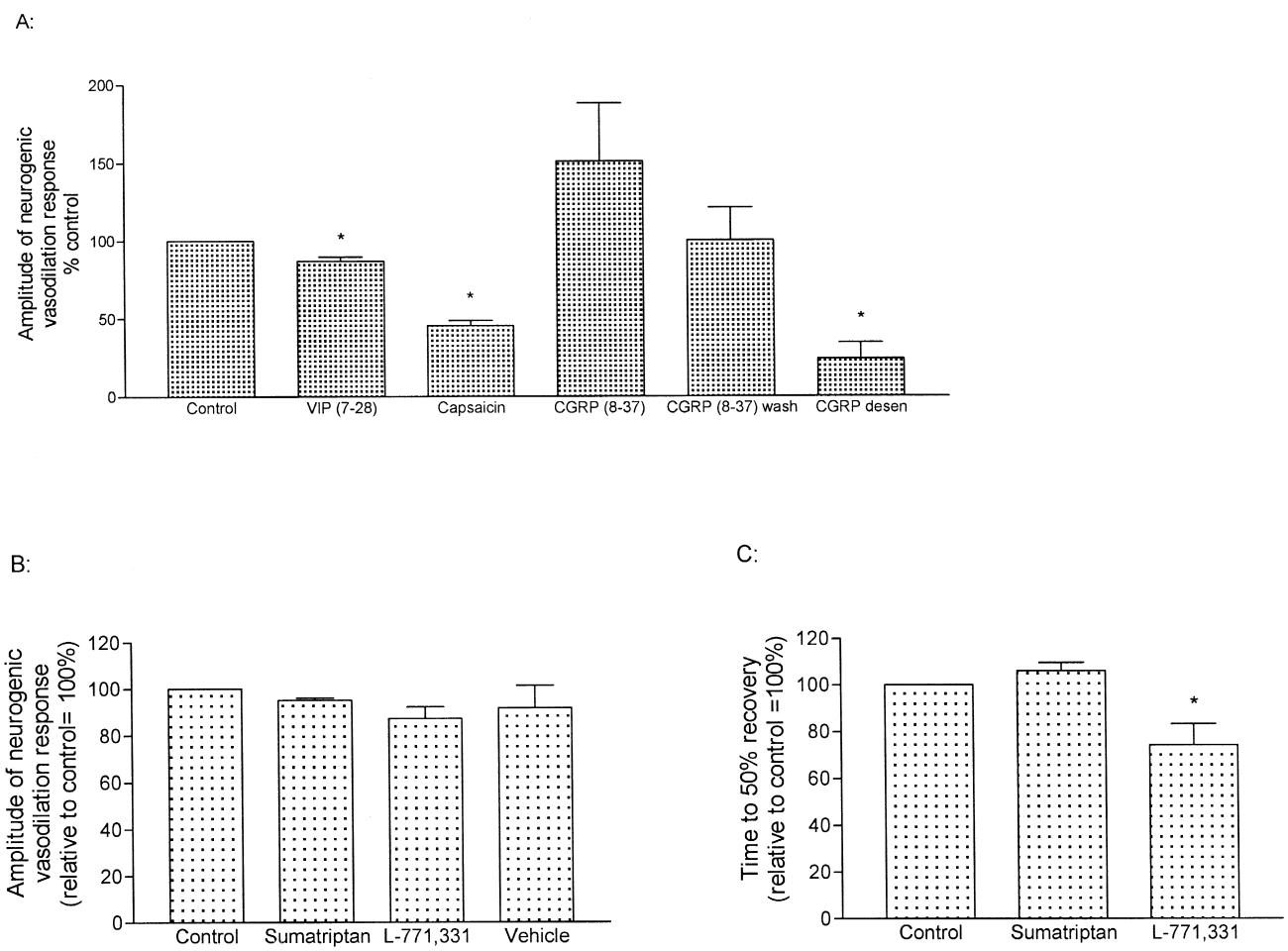

Fig. 3. Electrically evoked neurogenic vasodilation. (A) shows the amplitude of the neurogenic relaxation responses (relative to control = 100%, column 1) in the presence of VIP-(7–28) (1 μM, column 2), capsaicin (10 μM long application > 30 min, expected to deplete endogenous sensory peptides, column 3), human αCGRP-(8–37) (1 μM, column 4) and recovery of the response following wash-out of human αCGRP-(8–37) (column 5) and following CGRP-receptor desensitisation (column 6). (B) shows the amplitude of the neurogenic vasodilation response under control conditions (= 100%, column 1) and following treatment with sumatriptan (30 nM, column 2), L-771,331 (100 nM, column 3) or vehicle (column 4). (C) shows the time taken to achieve 50% recovery of the neurogenic vasodilation response under control conditions (= 100%, column 1) and following treatment with sumatriptan (30 nM, column 2) and L-771,331 (100 nM, column 3). The histobars represent mean values and the vertical bars indicate + S.E.M. (n = 4–5). Asterisks denote significant differences from control ($P < 0.05$; Student's t-test, paired comparisons).

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3. Results

All experiments were conducted in endothelium-denuded segments of rabbit isolated basilar artery as determined by a lack of a relaxant response on addition of acetylcholine (10 μM, data not shown). In segments pre-contracted with prostaglandin $F_{2\alpha}$, human αCGRP acted as a potent vasodilator, producing endothelium-independent relaxations with a pEC_{50} value of 8.53 ± 0.08 (equivalent to 2.9 nM). This response was inhibited by human αCGRP-(8–37) with an apparent pA_2 value of 6.49 (see Fig. 2a). Repeated exposure at 30-min intervals to 300 nM CGRP (representing a supramaximal concentration) resulted in desensitisation with the response to second and third exposures being reduced to $31.0 \pm 6.8\%$ and $19.0 \pm 7.8\%$, respectively ($n = 7$ segments from 5 rabbits) relative to the first exposure to CGRP (= 100%). VIP was a potent relaxant with pEC_{50} value of 8.60 ± 0.02 (equivalent to 2.5 nM) and this response was inhibited in a non-competitive fashion by VIP-(7–28) 1 μM (see Fig. 2b). Sumatriptan and L-771,331 produced contractile responses with pEC_{50} values of 7.02 ± 0.06 and 6.5 ± 0.08 (equivalent to 95 and 293 nM, respectively, see Fig. 2c).

Fig. 1 shows the typical relaxation response of rabbit isolated basilar artery evoked by electrical stimulation of quiescent tissues (mean amplitude of the control responses was 0.22 ± 0.01 g, $n = 5$). Using this electrical stimulation paradigm only relaxation responses were observed. The amplitude of the vasodilation response was reduced by prior CGRP receptor desensitisation ($24.0 \pm 10.5\%$, see Fig. 3a) and by pre-treatment (> 30 min) with capsaicin (10 μM, mean ± S.E.M. = $45.5 \pm 3.02\%$, see Fig. 3a). Following pre-treatment with human αCGRP-(8–37), the responses to electrical stimulation were increased by 51.21% compared with the control response ($n = 6$ segments from 4 rabbits, see Fig. 3a) and the response returned to control levels following washout of human αCGRP-(8-37). The concentrations of sumatriptan and L-771,331 tested on neurogenic vasodilation were 30 and 100 nM, respectively (representing EC_{25} for vasoconstriction) and were selected on the basis that these concentrations produced a small vasoconstrictor effect which did not contaminate the neurogenic vasodilator response. Sumatriptan (30 nM) had no significant effect on the amplitude or kinetics of the neurogenic vasodilation response whereas L-771,331 (100 nM) did not reduce amplitude but it significantly reduced recovery time (see Fig. 3b and c).

Under conditions of short application, capsaicin (10 μM) was shown to evoke reproducible relaxation responses of tissues pre-contracted with 45 mM KCl (amplitude mean ± S.E.M. = 0.42 ± 0.06 g, $n = 6$) with a second application of capsaicin producing similar responses (i.e. $92.6 \pm 8.2\%$ compared to the first exposure). This response to capsaicin was significantly inhibited by human αCGRP-(8–37) 1 μM (i.e. $28.53 \pm 4.4\%$ compared to the vehicle control $92.6 \pm 8.2\%$, $n = 6$ segments from 4 rabbits, see Fig. 4).

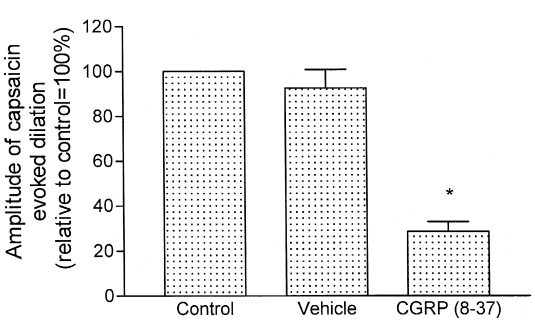

Fig. 4. Vasodilation responses evoked by short application (5–10 min) of capsaicin (10 μM). Column 1 shows the response to the first application of capsaicin (control = 100%). Columns 2 and 3 show the response to the second application of capsaicin following incubation with vehicle or αCGRP-(8–37) 1 μM, respectively. The histobars represent mean values and the vertical bars indicate + S.E.M. ($n = 4$–5). Asterisks denote significant differences from control ($P < 0.05$; Student's *t*-test, paired comparisons).

4. Discussion

The aim of this study was to characterise an in vitro model of neurogenic vasodilation using rabbit isolated basilar artery. In order to focus on the role of CGRP, endothelium-denuded segments were used. Neurogenic vasodilation was evoked by electrical field stimulation using a stimulus paradigm expected to activate sensory nerve fibres (Williamson et al., 1997a). It was possible to evoke reproducible responses that allowed the study of pharmacological agents. Preliminary experiments were first performed to (a) characterise the post-synaptic relaxant effects of neuropeptides by using exogenously applied agonists, and in line with studies in other blood vessels, CGRP and VIP were shown to be potent vasodilators in rabbit isolated basilar artery (Edvinsson et al., 1985; Van Rossum et al., 1997); (b) to confirm that concentrations of the peptide receptor antagonists used in the neurogenic experiments (see below) were effective in antagonising the post-synaptic responses. In this case, human αCGRP-(8–37) inhibited relaxations evoked by exogenously applied CGRP, however, it was weak antagonist (apparent pA_2 = 6.49) and VIP (7–28) markedly antagonised VIP-induced relaxations and acted as a non-competitive antagonist; (c) to select concentrations of sumatriptan (a mixed 5-$HT_{1B/1D}$ receptor agonist, Pauwels et al., 1997) and L-771,331 (a selective agonist at human 5-HT_{1D} receptors, Sternfeld et al., 1999) to be used in the neurogenic experiments to examine the role of pre-junctional 5-$HT_{1B/1D}$ receptors in regulating neuropeptide release.

The results from the present study show that the vasodilator response in rabbit basilar artery evoked by electrical stimulation is likely to be a neuronally mediated event and not due to direct stimulation of the smooth muscle cells. First, applications of the same electrical stimuli to non-cranial vessels produced contractile responses (data not shown). Also, capsaicin, (prolonged application, expected to deplete sensory neuropeptides) partially blocked electrically evoked relaxations. Capsaicin is a sensorotoxin shown to act on vanalloid receptors located on sensory neurones (Caterina et al., 1997) and in rabbits, it is selective for C-fibres (Lynn, 1996). Capsaicin has dual activity and it is recognised that at low doses it produces a vasodilator response mediated through the release of pro-dilator sensory peptides whereas at higher doses capsaicin causes desensitisation by depleting peptides from sensory afferents (Lynn, 1996).

The observation that in rabbit isolated basilar artery desensitisation of CGRP receptors significantly reduced electrically evoked vasodilation suggests a large CGRP component to this response. Interestingly, CGRP receptor desensitisation was more effective than capsaicin in preventing neurogenic vasodilation and the possibility that the electrical stimulus evokes CGRP release from an additional population of capsaicin-insensitive fibres (possibly Aδ-fibres) cannot be ruled out. The lack of inhibitory effect of human αCGRP-(8–37), and indeed its potentiating effect on neurogenic vasodilation, was surprising, particularly in view of the marked reduction in neurogenic vasodilation following CGRP receptor desensitisation suggesting this response is CGRP-receptor mediated. Human αCGRP-(8–37) was shown to block off the effects of exogenously applied human αCGRP and to antagonise capsaicin evoked vasodilation indicative of the blockade of endogenously released rabbit CGRP. The unexpected finding that neurogenic vasodilation was not sensitive to human αCGRP-(8–37) may reflect that this peptide fragment is a weak antagonist and a poor pharmacological tool, possibly with residual partial agonist activity (Longmore et al., 1994; Poyner, 1995). It would be interesting to determine whether a non-peptide, high affinity CGRP receptor antagonist could inhibit the neurogenic responses in a similar way as CGRP-receptor desensitisation.

VIP-(7–28) 1μM produced a small but significant reduction in the amplitude of the neurogenic vasodilation response indicating the release of VIP, presumably from parasympathetic nerve fibres (Edvinsson and Jansen, 1993). VIP release seems to represent only a minor component of the neurogenic response. These observations in rabbit isolated basilar artery are in general agreement with the findings from in vivo studies. It has been shown that in anaesthetised rats electrical stimulation of the dura mater (using a stimulation paradigm similar to the one used in the present study) produced a neurogenic vasodilation response that was abolished by αCGRP-(8–37) indicating that this response is entirely CGRP-mediated (Williamson et al., 1997b). Kurosawa et al. (1995), using higher stimulation frequencies, suggested that meningeal neurogenic vasodilation has a large CGRP component but also a smaller VIP component.

Sumatriptan (an agonist with activity at human recombinant 5-HT_{1D} and 5-HT_{1B} receptors, with EC_{50} values of 9 and 150 nM, respectively, Shaw et al., 1996) and L-771,331 (a selective agonist at human recombinant 5-HT_{1D} receptors, EC_{50} = 1.7 nM compared to 1650 nM for human 5-HT_{1B} receptors (Sternfeld, personal communication) caused contraction in rabbit basilar artery. In human blood vessels, contraction is selectively mediated via 5-HT_{1B} receptors (for review see Longmore et al., 1999) whereas in rabbit blood vessels there is the possibility that 5-HT_{1D} receptors may also be involved (Tilford and Baxter, 1994; Razzaque et al., 1995). Although the affinity of L-771,331 for rabbit receptors is unknown and can only be inferred from human receptor binding data, the relatively weak vasoconstrictor potency of L-771,331 in the present study is consistent with its lower affinity at 5-HT_{1B} receptors.

Sumatriptan tested at a concentration of 30 nM (representing the EC_{25} in terms of vasoconstriction) was without effect on neurogenic vasodilation. It was not possible to test higher concentrations due to profound vasoconstrictor effects, which were at least fivefold greater in magnitude than the relaxation response, and swamped the response to the electrical stimulus such that it was not possible to distinguish whether the change in the neurogenic response was due to a pharmacological effect on neuropeptide release or due to change in the underlying basal tension. Also, preliminary studies showed that it was not possible to obtain consistent and reproducible neurogenic responses in the presence of elevated tone. In anaesthetised rats, sumatriptan has been shown to abolish neurogenic vasodilation evoked by electrical stimulation of the dura mater, since sumatriptan was devoid of intrinsic vasoconstrictor effects in these experiments it was possible to test relatively high doses (i.e. 3 and 10 mg/kg i.v., Williamson et al., 1997a). L-771,331 (100 nM, representing EC_{25} in terms of vasoconstriction) had no effect on the amplitude of the neurogenic vasodilation response; however, it reduced the recovery time indicating inhibition of neuropeptide release. Thus, when tested at equiactive vasoconstrictor concentrations L-771,331, but not sumatriptan, produced a small inhibition of neurogenic vasodilation. Assuming that in rabbit, as in man, 5-HT_{1D} receptors are present on trigeminal terminals (Longmore et al., 1997), then this may reflect the higher affinity of L-771,331 at 5-HT_{1D} receptors compared to sumatriptan (Sternfeld et al., 1999). These findings suggest that the electrical stimulus used in the present experiments evokes substantial release of endogenous neuropeptides which produces a powerful vasodilation and that selective activation of prejunctional inhibitory 5-HT_{1D} receptors is a weak mechanism by which to inhibit this response. LY334370 (4-

luoro-*N*-[3-(1-methyl-4-piperidinyl)-1*H*-indol-5-yl]-benzaide, selective 5-HT_{1F} receptor agonist) also failed to inhibit the magnitude of the neurogenic vasodilation response (unpublished observations this laboratory). Furthermore, it has been reported that in small-scale Phase IIa clinical trials PNU-142633 ((*s*)-3,4,dihydro-1-[2-[4-[4-aminocarbonyl0phenyl]-1 piperazinyl]ethyl]-*N*-methyl-1*H*-2-benzopyran-6-carboximide, a selective 5-HT_{1D} receptor ligand) lacks clinical efficacy as an acute abortive antimigraine therapy (McCall, 1999).

In conclusion, the electrically induced neurogenic vasorelaxation of rabbit isolated basilar artery described in this study provides a useful experimental assay to investigate the pharmacology of endogenously released neuropeptides. The present results show that depletion of sensory peptides by capsaicin or the blockade of the post-synaptic effects of neuropeptides (and in particular the antagonism of the effects of CGRP via post-synaptic receptor desensitisation) are powerful mechanisms by which to prevent neurogenic vasodilation. In contrast, the inhibition of neurotransmitter release via an action at pre-synaptic 5-HT_1 receptors is less powerful.

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