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Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability

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1 The potent vasodilator calcitonin gene-related peptide (CGRP, human synthetic), when mixed with histamine and injected intradermally in the rabbit, induced a marked potentiation of local oedema.

2 CGRP also potentiated oedema induced by other mediators of increased microvascular permeability in the rabbit; bradykinin, platelet-activating factor (Paf), C5a des Arg, *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) and leukotriene B_4 (LTB_4).

3 Substance P alone, or mixtures of substance P and CGRP, failed to induce oedema in rabbit skin. In rat skin, however, substance P induced oedema and this was potentiated by CGRP.

4 CGRP had a protracted potentiating action following intradermal injection in the rabbit. The time for half loss of activity for CGRP was 40.1 ± 7.5 min compared to 18 ± 1 min for prostaglandin E_2 (PGE_2).

5 No loss of potentiating activity was detected after incubation of CGRP in rabbit plasma or blood for 60 min.

6 We postulate that endogenous CGRP, if released locally from nerve endings, could have a marked enhancing effect on oedema induced by other mediators in an inflammatory reaction.

Introduction

The gene which encodes for calcitonin also encodes for other peptides, one of which has been termed calcitonin gene-related peptide, CGRP, (Amara *et al.*, 1982; Rosenfeld *et al.*, 1983). Rat CGRP (37 amino acids) has been synthesized according to its predicted structure. Immunocytochemical studies carried out in the rat have revealed immunoreactive CGRP in discrete areas of the central and peripheral nervous system (Rosenfeld *et al.*, 1983). In the peripheral nervous system immunoreactive CGRP has been found in thin beaded fibres that are associated with the smooth muscle of blood vessels. CGRP is thought to result from alternative processing of mRNA from the calcitonin gene, such that calcitonin is predominantly formed in normal thyroid whilst in certain neural tissue CGRP is formed (Rosenfeld *et al.*, 1983). An antibody to part of the predicted sequence of rat CGRP has been used to extract material from thyroid tissue taken from patients with medullary thyroid carcinoma (MTC). From the purified extract the structure of human CGRP has been elucidated (Morris *et al.*, 1984).

In the rat intracerebroventricular administration of CGRP was found to cause an increased sympathetic outflow and a pressor response, but in control experiments where CGRP was given intravenously a depressor response was observed (Fisher *et al.*, 1983). This depressor response can be explained by our recent findings that CGRP is a potent vasodilator, which has been shown in four unrelated species (Brain *et al.*, 1985a). Both human CGRP, extracted from the thyroid tissue of patients with MTC, and synthetic rat CGRP, synthesized according to its predicted structure, have potent effects in increasing microvascular blood flow in rabbit skin, as measured using a multiple site xenon clearance technique (Williams, 1979). Additionally, synthetic CGRP when injected intradermally in man causes a prolonged erythema with a threshold effect with doses as low as 15 fmol. CGRP relaxes rat aorta *in vitro* by a mechanism dependent on the presence of endothelial cells, but whether the same mechanism is involved in arteriolar dilatation *in vivo* is conjectural (Brain *et al.*, 1985a). The potency of CGRP as a vasodilator and its localization in nerves associated with blood vessels led us to suggest that CGRP could have a role in the local regulation of blood flow.

Studies carried out in animal skin into the mechan-

isms underlying inflammatory oedema have shown how oedema induced by mediators of increased vascular permeability can be regulated by vasodilators (see Williams, 1983). In guinea-pig and rabbit skin, where basal blood flow is low, vasodilator prostaglandins have a pronounced potentiating effect on oedema formation induced by mediators of increased vascular permeability (Williams & Morley, 1973; Williams & Peck, 1977; Kopaniak *et al.*, 1978). The potentiating ability of different prostaglandins is proportional to their activity as vasodilators and it has been demonstrated that endogenous vasodilator prostaglandins and vascular permeability-increasing mediators can act synergistically to produce oedema in experimental inflammatory reactions (Williams & Peck, 1977; Williams 1979; Williams & Jose, 1981). This hypothesis is supported by the observation that vasoactive intestinal polypeptide (VIP), a potent vasodilator in rabbit skin (Williams, 1982) is also a potent potentiator of oedema formation.

In this study, using seven chemically-distinct mediators of increased vascular permeability, we have investigated whether CGRP could be involved in the regulation of inflammatory oedema.

A preliminary account of some of this work was presented to the British Pharmacological Society, Cardiff, April 10–12th 1985 (Brain *et al.*, 1985b).

Methods

Male New Zealand White (NZW) rabbits (specific pathogen free, 2.5–3 kg) were purchased from Froxfield Ltd, Petersfield, Hampshire. Local oedema formation was measured in the skin of these rabbits in response to intradermal injections of test agents, as previously described (Williams, 1976; 1979). The rabbits were anaesthetized by intravenous injection of Saffan (1 ml kg^{-1}, Glaxovet, Harefield, Middx.) into the marginal ear vein. [^{125}I]-human serum albumin (1.5 μCi kg^{-1}; Amersham International, Amersham, Bucks.) and Evans Blue (0.5 ml kg^{-1} of 2.5% w/v; B.D.H., Poole, Dorset) were injected by the same route. The agents under test were made up in saline and injected in 100 μl volumes into the shaved dorsal skin according to a balanced site pattern with six replicates per dose. After a 30 min accumulation period each rabbit was killed by a barbiturate overdose. Similar experiments were performed on male Lewis rats (specific pathogen free, 200–250 g) which were bred at the Clinical Research Centre. Rats were anaesthetized by intraperitoneal injection of a mixture of Hypnorm (Crown Chemical Co. Ltd, Lamberhurst, Kent)/Hypnovel (Roche Products Ltd, Welwyn Garden City, Herts)/water (1 : 1 : 2; 2.7 ml kg^{-1}). [^{125}I]-human serum albumin (10 μCi kg^{-1}) and Evans Blue (0.5 ml kg^{-1} of 2.5% w/v) were injected intravenously via the tail vein. Test agents were made up in phosphate buffered saline and injected in volumes of 50 μl into the shaved dorsal skin according to a balanced site pattern with six replicates for each treatment. After a 30 min accumulation period, the rats were killed by cervical dislocation. For both rabbits and rats, the dorsal skin was removed and injection sites were punched out (17 mm and 12 mm diameter in rabbits and rats, respectively). Oedema responses were expressed as equivalent plasma volumes by dividing each skin sample count by the count of 1 μl of plasma. The effects of human synthetic CGRP (Bachem, Saffron Walden, Essex) were compared with the established effects of the potent vasodilator prostaglandin E_2 (PGE_2; Sigma Chemical Company, Poole, Dorset). The ability of CGRP to potentiate oedema induced by different mediators of increased vascular permeability was examined, i.e. histamine (Macarthys Ltd, Romford, Essex), rabbit C5a des Arg (purified in our laboratory by Dr P.J. Jose), leukotriene B_4 (LTB_4; gift from Miles, Stoke Poges, Bucks), platelet-activating factor (Paf; gift from Professors Godfroid and Benveniste, France), substance P (Bachem, Saffron Walden, Essex), bradykinin and *N*-formyl-methionyl-leucyl-phenylalanine (FMLP; Sigma Chemical Company, Poole,

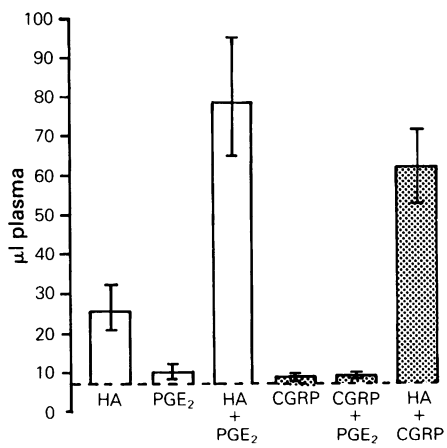

Figure 1 The effect of human synthetic CGRP and prostaglandin E_2 (PGE_2) on histamine-induced oedema in rabbit skin. Histamine (HA; 1.6×10^{-8} mol per site), CGRP (10^{-11} mol per site) and PGE_2 (3×10^{-10} mol per site), when injected alone, produced little oedema formation. Combination of CGRP with PGE_2 also induced little oedema. When PGE_2 or CGRP was injected with histamine, a potentiation of histamine-induced oedema was seen. The stippled columns denote oedema induced at sites which had received CGRP. The dashed line represents sites injected with saline. The results are expressed as mean values from 4 rabbits and the bars represent s.e.mean.

Dorset). The duration of the vasodilator activity of CGRP was investigated in experiments where the dilators were injected at pre-selected times before an intravenous injection of [^{125}I]-albumin, followed immediately by superimposed local injections of bradykinin, so that all sites received a total of 200 µl injection fluid. Responses were then measured over a 30 min period, as above. The stability of CGRP and PGE_2 in plasma and blood was investigated by incubating CGRP (1 nmol ml^{-1}) or PGE_2 (10 nmol ml^{-1}) with freshly prepared heparinised (10 u ml^{-1}; Payne and Byrne Ltd, Greenford, Middx.) rabbit plasma, whole blood or saline (for controls) at 37°C for 1 h with occasional shaking. Incubations in saline and plasma were terminated by cooling to 4°C and for blood, centrifugation (7,800 *g* for 1 min) to remove cells, followed by cooling. All samples were then diluted 1 : 10 in saline and histamine was added. An equivalent volume of plasma was added to control samples immediately before intradermal injection.

Results

The effects of human synthetic CGRP were compared with those of the established vasodilator PGE_2 in rabbit skin. Figure 1 shows that CGRP potentiated histamine-induced oedema in a manner analagous to PGE_2. CGRP, like PGE_2, induced little oedema when injected alone into the skin, although the doses used are known to have potent vasodilator effects in the rabbit (Williams & Peck, 1977; Brain *et al.*, 1985a). The simultaneous injection of either PGE_2, or CGRP, together with histamine caused a marked potentiation of the histamine-induced oedema. It has been suggested that this phenomenon results from arteriolar dilatation which produces increased intravenular hydrostatic pressure and passive venular distension leading to increased protein leakage in regions of enhanced venular permeability (Williams, 1982). The simultaneous injection of CGRP with PGE_2 did not induce oedema formation. This is evidence that CGRP does not directly act to increase vascular permeability itself, or induce the release of a mediator of increased vascular permeability.

The ability of CGRP to potentiate oedema formation induced by inflammatory mediators which increase vascular permeability by two different mechanisms was examined. Agents such as histamine, bradykinin and Paf act to increase vascular permeability by a direct effect on post-capillary venules whilst LTB_4, FMLP and C5a, or its physiologically more stable

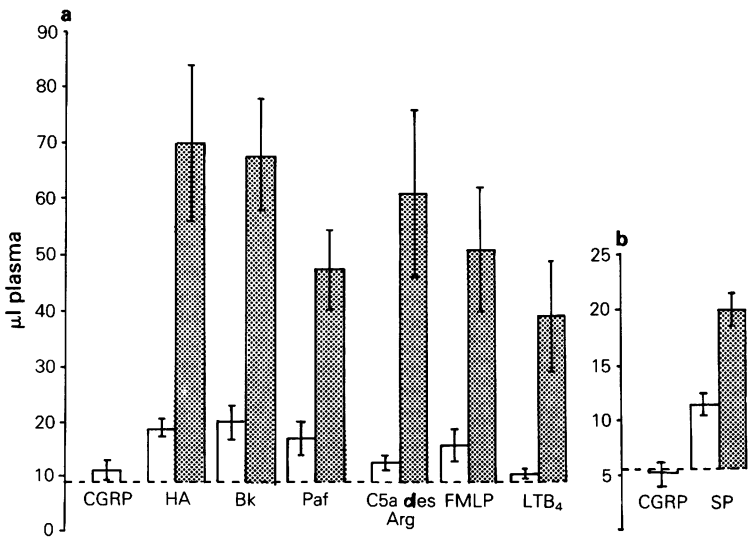

Figure 2 Potentiating effect of CGRP on oedema formation induced by different mediators of increased vascular permeability. (a) Oedema was induced in rabbit skin by histamine (HA; 10^{-8} mol per site), bradykinin (Bk, 10^{-10} mol per site), platelet-activating factor (Paf; 10^{-9} mol per site), C5a des Arg (5×10^{-11} mol per site), FMLP (5×10^{-11} mol per site) and leukotriene B_4 (LTB_4 3×10^{-10} mol per site). Each agent when tested alone, produced a small amount of oedema (open columns). In every case the simultaneous injection of CGRP (10^{-11} mol per site) led to a potentiation of plasma exudation (stippled columns). The dashed line represents sites injected with saline and results are expressed as mean values from 7 rabbits and the bars represent s.e.mean. (b) Oedema was induced in rat skin by substance P (SP; 2.5×10^{-11} mol per site). The injection of CGRP (5×10^{-12} mol per site) with substance P induced a potentiation of plasma exudation (stippled column). The dashed line represents sites injected with phosphate buffered saline and results shown are the mean values from 4 rats and the bars represent s.e.mean.

form C5a des Arg, act via a mechanism dependent on the presence of circulating polymorphonuclear leukocytes (Wedmore & Williams, 1981). Figure 2 shows that when each of these mediators was injected alone, a small amount of oedema formation was observed and that in every case the concomitant injection of CGRP led to a potentiation of oedema. The results are consistent with the hypothesis that if endogenous CGRP is released at an inflammatory site it could act to potentiate oedema induced by any one of these mediators of increased vascular permeability.

Our previous experiments have shown that substance P is a weak vasodilator in rabbit skin (Brain *et al.*, 1985a). In the present experiments we found that substance P is also very weak in affecting vascular permeability in rabbit skin. In an experiment in which the injection of bradykinin (10^{-10} mol per site) with CGRP (10^{-11} mol per site) gave a response of $57.5 \pm 4.6\,\mu l$ plasma exudate (mean ± s.e. of six replicates), the injection of substance P (10^{-10} mol per site) with CGRP (10^{-11} mol per site) gave a response of $10.0 \pm 1.7\,\mu l$ compared with a saline control level of $4.4 \pm 0.5\,\mu l$. We therefore tested the ability of CGRP to potentiate substance P-induced plasma extravasation in the skin of rat, a species in which substance P is known to induce increased vascular permeability. The results are shown in Figure 2b. CGRP alone (5×10^{-12} mol per site) did not induce detectable oedema responses. The injection of substance P (2.5×10^{-11} mol per site) caused a small response which was potentiated by the simultaneous injection of CGRP. This suggests that if substance P is active in inducing increased vascular permeability in a particular vascular bed, then CGRP can potentiate oedema formation.

Experiments in human skin demonstrated that CGRP has a long duration of vasodilator action (Brain *et al.*, 1985a). Figure 3 shows the results of experiments designed to determine the duration of the oedema-potentiating activity of CGRP. These experiments were performed by measuring bradykinin-induced exudation in sites pre-injected at selected time points with either PGE_2 or CGRP. No significant potentiating activity was observed 40 min after an injection of PGE_2, whereas a significant effect was still apparent with CGRP at this time. The time for CGRP to lose half its activity was 40.1 ± 7.5 min whereas the time calculated for PGE_2 in these experiments was 18 ± 1 min. These results show that CGRP has a relatively long duration of action in increasing blood flow in rabbit skin.

The long duration of action of CGRP suggests that it may be metabolized slowly *in vivo*. We therefore investigated the stability of CGRP in plasma and blood. The activity of CGRP was assayed by its ability to potentiate histamine-induced oedema. Figure 4 shows the results of these experiments. Oedema induced by a mixture of histamine and CGRP was similar both before and after 1 h incubation of CGRP at 37°C in plasma and blood, suggesting that, like PGE_2 (Ferreira & Vane, 1967) the dilator activity of CGRP is stable in blood. For these experiments submaximal concentrations of CGRP and PGE_2 were used in order that any loss of activity of CGRP or PGE_2 would be detected. Thus CGRP is not inactivated in blood in terms of vasodilator activity, although the peptide may be metabolized to smaller vasodilator peptides.

Discussion

The results suggest that exogenous CGRP, as a consequence of its potent vasodilator activity, can potentiate oedema induced by agents which increase microvascular permeability. This is demonstrated here using seven chemically distinct agents of both the direct-acting and polymorphonuclear leukocyte-dependent type. This is consistent with the hypothesis that any endogenous CGRP released locally from nerve endings could contribute to vasodilatation in inflammatory reactions and have a marked enhancing effect on oedema formation. We have previously provided evidence of synergism between endogenous vasodilator prostaglandins and mediators such as C5a

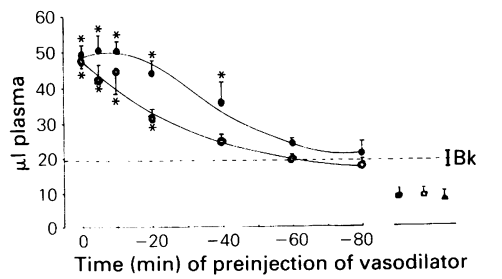

Figure 3 Comparison of the duration of oedema potentiating action of CGRP and prostaglandin E_2 (PGE_2). CGRP (10^{-11} mol per site, ●) and PGE_2 (10^{-10} mol per site, ○) were injected into each rabbit at selected times (− 80, − 60, − 40, − 20, − 10, − 5 and 0 min) before a superimposed injection of bradykinin (10^{-10} mol per site). [^{125}I]-albumin was given i.v. immediately before intradermal bradykinin and plasma accumulation was then monitored as before. The dashed line shows the response to bradykinin (Bk) alone. Each point is the mean with s.e.mean shown by vertical lines; $n = 3-5$ rabbits. The other symbols represent (■) CGRP alone, (□) PGE_2 alone and (▲) saline alone. The significance of differences between individual treatments and control (Bk) at each time were assessed by Dunnet's t test (Gill, 1978) using the standard error estimate from the analysis of variance to take account of the fact that multiple tests were being performed. * $P < 0.01$.

to induce oedema in certain inflammatory models. Vasodilatation and oedema in these models are clearly sensitive to inhibition by non-steroidal anti-inflammatory compounds which suppress prostaglandin synthesis (Williams & Peck, 1977; Williams & Jose, 1981). If CGRP has a role as a vasodilator in other types of inflammatory reactions, this may explain why vasodilatation and oedema are sometimes unaffected by non-steroidal anti-inflammatory compounds. In this respect, we have shown that the vasodilator action of CGRP is unaffected by indomethacin (Brain *et al.*, 1985a), although the possibility remains that indomethacin may affect the synthesis or release of CGRP.

There is a growing literature on the role of substance P, both as a mediator of the axon-reflex flare in human skin (Foreman *et al.*, 1983) and as a neurogenic component of local oedema in various experimental models (Lembeck & Holzer, 1979; Gamse *et al.*, 1980; Lembeck & Gamse, 1982). This is based on four pieces of evidence: (i) the activity of exogenous substance P, (ii) the effect of nerve sectioning and degeneration or local anaesthetics, (iii) the effect of local neuropeptide depletion using capsaicin and (iv) the effect of substance P antagonists. Since (ii) and (iii) are not specific for substance P and substance P antagonists have limited specificity, we have proposed that CGRP is a reasonable alternative candidate as the mediator of the axon reflex flare in human skin (Brain *et al.*, 1985a). Further, CGRP may have a role in the animal models of neurogenic inflammation which have been previously described, perhaps in combination with substance P. In the rat immunoreactive CGRP has been recently shown to be localized in the same nerve endings as substance P (Lundberg *et al.*, 1985). In this context, we have described synergism between exogenous CGRP and exogenous substance P to induce oedema in rat skin in this paper.

Nothing is known about the metabolism of CGRP *in vivo*. Low doses of CGRP induce transient vasodilatation in human skin, whereas higher doses induce very persistent vasodilatation (Brain *et al.*, 1985a). In this paper we show that the effects of CGRP, when matched for potency with PGE_2, are more persistent than the prostaglandin in rabbit skin. Further, we demonstrate that the activity of CGRP is stable in plasma and blood at 37°C for 60 min *in vitro*. If CGRP is an important regulator of blood vessel tone *in vivo* an efficient inactivating mechanism would

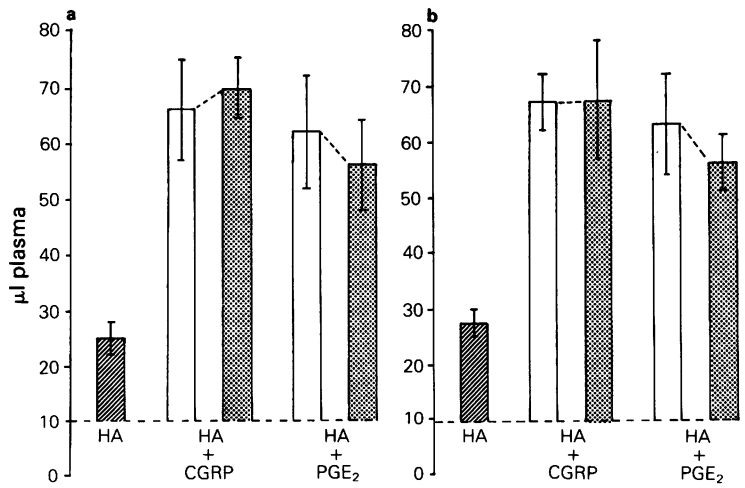

Figure 4 The stability of CGRP in (a) plasma and (b) blood. For incubation procedure and preparation of samples see Methods section. (a) CGRP (10^{-11} mol per site) and prostaglandin E_2 (PGE_2; 10^{-10} mol per site), that had previously been incubated in rabbit plasma, potentiated oedema induced by histamine (HA; 10^{-8} mol per site) (stippled columns), in a similar manner to the control CGRP and PGE_2 (open columns). The hatched column shows the response to histamine alone. The dashed line represents the response to sites injected with saline. The results are expressed as mean, with s.e.mean shown by bars, $n = 7$ rabbits for CGRP and $n = 8$ rabbits for PGE_2. (b) CGRP (10^{-11} mol per site) and PGE_2 (10^{-10} mol per site), that had previously been incubated in rabbit whole blood, potentiated oedema induced by histamine (10^{-8} mol per site) (stippled columns), in a similar manner to CGRP and PGE_2 controls (open columns). The hatched column shows the response to histamine alone. The dashed line shows the response to sites injected with saline. The results are expressed as mean, with s.e.mean shown by bars, $n = 4$ rabbits.

be expected. Perhaps this is located close to the site of CGRP liberation and action.

Thus, CGRP is a potent vasodilator and exhibits marked synergism with inflammatory mediators to induce local oedema. CGRP may provide an important neurogenic component in certain inflammatory reactions. This postulate, however, awaits direct evidence of CGRP release in inflammatory reactions.

Note added in proof
Synergism between CGRP and substance P in the rat has now been observed by Gamse & Saria (1985, *Eur. J. Pharmac.* **114,** 61–66).

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References

AMARA, S.G., JONAS, V., ROSENFELD, M.G., ONG, E.S. & EVANS, R.M. (1982). Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature*, **298,** 240–244.

BRAIN, S.D., WILLIAMS, T.J., TIPPINS, J.R., MORRIS, H.R. & MACINTYRE, I. (1985a). Calcitonin gene-related peptide is a potent vasodilator. *Nature*, **313,** 54–56.

BRAIN, S.D., GIRGIS, S., MACINTYRE, I., MORRIS, H.R. TIPPINS, J.R. & WILLIAMS, T.J. (1985b). Local oedema induced by synergism between calcitonin gene-related peptide and mediators of increased vascular permeability. *Br. J. Pharmac. Proc. Suppl.*, **85,** 233P.

FERREIRA, S.H. & VANE, J.R. (1967). Prostaglandins: Their disappearance from and release into the circulation. *Nature*, **216,** 868–873.

FISHER, L.A., KIKKAWA, D.O., RIVIER, J.E., AMARA, S.G., EVANS, R.M., ROSENFELD, M.G., VALE, W.W. & BROWN, M.R. (1983). Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide. *Nature*, **305,** 534–536.

FOREMAN, J.C., JORDAN, C.C., OEHME, P. & RENNER, H. (1983). Structure-activity relationships for some substance P-related peptides that cause wheal and flare reactions in human skin. *J. Physiol.*, **335,** 449–465.

GAMSE, R., HOLZER, P. & LEMBECK, F. (1980). Decrease of Substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. *Br. J. Pharmac.*, **68,** 207–213.

GILL, J.L. (1978). *Design and analysis of experiments in the animal and medical sciences.* Vol. 1. pp. 183–185, Ames, Iowa (USA): Iowa State U.P.

KOPANIAK, M.M., HAY, J.B. & MOVAT, H.Z. (1978). The effect of hyperemia on vascular permeability. *Microvasc. Res.*, **15,** 77–82.

LEMBECK, F. & GAMSE, R. (1982). Substance P in peripheral sensory processes. In *Substance P in the nervouse system* ed. Porter, R. & O'Connor, M. pp. 35–54. London: Pitman Books Ltd.

LEMBECK, F. & HOLZER, P. (1979). Substance P as a mediator of antidromic vasodilatation and neurogenic plasma extravasation. *Naunyn-Schmiedebergs Arch. Pharmak.*, **310,** 175–183.

LUNDBERG, J.M., FRANCO-CERECEDA, A., HUA, X., HOKFELT, T. & FISCHER, J.A. (1985). Co-existence of substance P and calcitonin gene-related peptide like-immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. *Eur. J. Pharmac.*, **108,** 315–319.

MORRIS, H.R., PANICO, M., ETIENNE, T., TIPPINS, J.R., GIRGIS, S.I. & MACINTYRE, I. (1984). Isolation and characterization of human calcitonin gene-related peptide. *Nature*, **308,** 746–748.

ROSENFELD, M.G., MERMOD, J.J., AMARA, S.G., SWANSON, L.W., SAWCHENKO, P.E., RIVIER, J., VALE, W.W. & EVANS, R.M. (1983). Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. *Nature*, **304,** 129–135.

WEDMORE, C.V. & WILLIAMS, T.J. (1981). Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature*, **289,** 646–650.

WILLIAMS, T.J. (1976). Simultaneous measurement of local plasma exudation and blood flow changes induced by intradermal injection of vasoactive substances, using ^{131}I albumin and ^{133}Xe. *J. Physiol.*, **351,** 48P.

WILLIAMS, T.J. (1979). Prostaglandin E_2, prostaglandin I_2 and the vascular changes of inflammation. *Br. J. Pharmac.*, **65,** 517–524.

WILLIAMS, T.J. (1982). Vasoactive intestinal polypeptide is more potent than prostaglandin E_2 as a vasodilator and oedema potentiator in rabbit skin. *Br. J. Pharmac.*, **77,** 505–509.

WILLIAMS, T.J. (1983). Interactions between prostaglandins, leukotrienes and other mediators of inflammation. *Br. med. Bull.*, **39,** 239–242.

WILLIAMS, T.J. & JOSE, P.J. (1981). Mediation of increased vascular permeability after complement activation: Histamine-independent action of rabbit C5a. *J. exp. Med.*, **153,** 136–153.

WILLIAMS, T.J. & MORLEY, J. (1973). Prostaglandins as potentiators of increased vascular permeability in inflammation. *Nature (Lond.)*, **246,** 215–217.

WILLIAMS, T.J. & PECK, M.J. (1977). Role of prostaglandin-mediated vasodilatation in inflammation. *Nature*, **270,** 530–532.

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