Br. J. Pharmacol. (1992), **106**, 331–334

Pituitary adenylate cyclase activating polypeptide is a potent vasodilator and oedema potentiator in rabbit skin *in vivo*

[1]J.B. Warren, S.W. Larkin, M. Coughlan, R. Kajekar & T.J. Williams

Department of Applied Pharmacology, National Heart & Lung Institute, Dovehouse Street, London, SW3 6LY

1 The effects of pituitary adenylate cyclase activating polypeptide (PACAP) on microvascular blood flow and plasma protein leakage were investigated in rabbit skin *in vivo*.

2 Intradermal injection of PACAP38, the 38 amino acid form of the peptide, caused a dose-dependent increase in blood flow measured by a ^{133}Xe clearance technique. An equivalent increase in blood flow was induced by 10^{-12} mol per site of PACAP38, 10^{-12} mol per site of human α-calcitonin gene-related peptide (CGRP) and 10^{-10} mol per site of vasoactive intestinal polypeptide (VIP).

3 The vasodilator activity of PACAP38 was not significantly different from that of the 27 amino acid form of the peptide, PACAP27, when measured with a laser Doppler flow meter, causing a 104 ± 14% compared with 110 ± 18% increase above basal blood flow at 10^{-12} mol per site respectively.

4 At 10^{-12} mol per site the effect of PACAP38 was longer lasting than that of CGRP. Blood flow remained significantly increased above control at 2 h with PACAP38 ($P<0.05$) whereas blood flow after intradermal CGRP had returned to control values by this time.

5 PACAP38 injected alone had no significant effect on microvascular leakage of ^{125}I-labelled albumin. However, PACAP38 significantly potentiated bradykinin-induced oedema where it was approximately 100 fold more potent than VIP.

6 Oedema potentiation induced by PACAP38 was not inhibited by indomethacin at a dose which did inhibit potentiation of bradykinin-induced oedema by arachidonic acid.

7 PACAP38 is at least as potent as other peptides which have been postulated to be involved in the inflammatory response when tested in rabbit skin *in vivo*. PACAP may contribute to both the hyperaemia and oedema components of inflammation.

Keywords: Pituitary adenylate cyclase activating polypeptide; vasodilatation; artery; vasoactive intestinal polypeptide; calcitonin gene-related peptide; adenylate cyclase

Introduction

Pituitary adenylate cyclase activating polypeptide (PACAP) was first isolated from ovine hypothalami. Its name derives from the observation that it is a powerful stimulant of adenylate cyclase in anterior pituitary cells in culture, being over 1000 times more potent than vasoactive intestinal polypeptide (VIP) in this system (Miyata *et al.*, 1989). The amino acid sequence of rat, ovine and human PACAP are identical (Ogi *et al.*, 1990).

A 38 amino acid and a 27 amino acid form of PACAP have been described (Miyata *et al.*, 1990). Both cause long lasting, endothelium-independent, relaxation of pre-contracted aortic rings and are more potent than VIP in stimulating adenylate cyclase in isolated vascular smooth muscle cells (Warren *et al.*, 1991). When injected intravenously in rats, both forms cause short lasting hypotension (Miyata *et al.*, 1990; Nandha *et al.*, 1991). The vasoactivity of the peptide appears to be incorporated within the first 27 amino acid sequence which is common to both PACAP38 and PACAP-27.

PACAP mRNA is expressed in several mammalian tissues (Kimura *et al.*, 1990; Koves *et al.*, 1990) indicating that its distribution is extensive. The cDNA's encoding the precursor of PACAP from the human testis and ovine hypothalamus are identical (Kimura *et al.*, 1990). Receptor binding studies show high levels of binding in rat lung and brain with low levels in the aorta (Lam *et al.*, 1990).

The N-terminal sequence of PACAP27 shows 68% homology with VIP (Miyata *et al.*, 1990) and there is evidence that both peptides can bind to the same receptor in the periphery (Gottschall *et al.*, 1990; Nandha *et al.*, 1991).

Specific receptors for PACAP exist in bovine and rat brain (Gottschall *et al.*, 1990; Lam *et al.*, 1990; Ohtaki *et al.*, 1990), a pancreatic cell line (Buscail *et al.*, 1990) and astrocytes (Tatsuno *et al.*, 1990). In human tissue, specific sites have also been demonstrated in the hypothalamus, brain stem, cerebellum, cortex and basal ganglia (Suda *et al.*, 1991) suggesting that it is likely to be a central neurotransmitter. Although PACAP binding to the sites mentioned above is not displaced by VIP, these two peptides probably share the same receptor in some blood vessels (Nandha *et al.*, 1991) and lung tissues (Gottschall *et al.*, 1990).

The clearance of ^{133}Xe from rabbit skin has been used to study a number of peptides with potential vasodilator activity in the microcirculation *in vivo*. With this method the most potent vasodilator peptides tested to date have been calcitonin gene-related peptide (CGRP, Brain *et al.*, 1985; Brain & Williams, 1989) and VIP (Williams, 1982). In this paper the vasodilator activity of PACAP in rabbit skin has been compared with VIP and CGRP. The ability of PACAP to potentiate bradykinin-induced oedema formation has also been tested by measuring the intradermal leakage of intravenously injected ^{125}I-labelled albumin.

Methods

All experiments were carried out in a room where the temperature was thermostatically controlled at 24–25°C. Intradermal injections were given into the clipped dorsal skin of anaesthetized (sodium pentobarbitone, 30 mg kg^{-1}, i.v.) male New Zealand White rabbits (3–4.5 kg).

[1] Author for correspondence.

Blood flow experiments

Blood flow was measured as the clearance of intradermally injected ^{133}Xe as previously described (Williams, 1979). VIP, CGRP or PACAP38 were added to ^{133}Xe in solution (activity 5–10 μCi per 100 μl) and injected intradermally in 100 μl volumes. In all experiments test agents were dissolved in phosphate buffered saline containing 0.3% albumin (PBS-albumin). Injections were given rapidly in a randomized block order according to a balanced site pattern. Six replicates were given per dose. Each animal was killed 15 min after the intradermal injections by an overdose of pentobarbitone. The dorsal skin was removed and the injection sites excised with a 17 mm diameter punch. Skin samples were placed under paraffin oil in sealed tubes and counted, together with samples of injection fluids, in an automatic gamma counter. The clearance per site was calculated as a percentage of the clearance from sites where PBS-albumin had been injected as control, as previously described (Williams, 1979).

The vasodilator effect of PACAP27 and PACAP38 was compared by measuring local blood flow in the dorsal skin with a Perimed II laser-Doppler flow meter (Brain *et al.*, 1986; Brain & Williams, 1989) connected to a computer recorder (Maclab, Apple Computer Inc, Cupertino, California). After clipping, the skin was depilated with a commercial depilatory cream (Immac) and the rabbit left for 1 h before starting the experiment. Each peptide was injected in a balanced site pattern at 10^{-14}, 10^{-13}, 10^{-12} and 10^{-11} mol per site and readings taken 30 min later. This was repeated 4 times in each of 5 rabbits. The laser-Doppler flow meter was used to compare the two peptides to confirm the ^{133}Xe clearance technique findings obtained with PACAP38. The laser-Doppler flow probe has the limitation of measuring red cell flux at only one depth but has the advantage that multiple measurements can be made at the same site allowing the determination of the duration of action.

To determine the duration of action, the laser-Doppler flow meter was used to compare PACAP38 and CGRP injected at 10^{-12} mol per site. The rabbit was prepared as above and the skin marked out with four sites per test agent in a balanced site pattern. Blood flow at each site was measured and then peptide or control (100 μl PBS-albumin) were injected at 1.5 min intervals. Measurements were repeated at 15 min intervals for 1 h and again at 2 h after injection. Each measurement was taken as the mean of three readings of 15 s each with 10 s between readings. The laser probe was held at right angles to the skin by a plastic guide. Results are presented as the percentage change in red cell flux above the pre-injection value for each site.

Oedema formation experiments

Local oedema formation was measured as the intradermal accumulation of intravenously injected ^{125}I-human serum albumin (5 μCi kg^{-1}) as previously described (Brain & Williams, 1989). Test agents were dissolved in 100 μl of PBS-albumin and injected intradermally in a balanced site pattern, 6 site replicates per rabbit. After 30 min, the animal was killed with an overdose of pentobarbitone and a 5 ml blood sample taken by cardiac puncture into heparin (10 uml^{-1} final concentration). The dorsal skin was removed and the injection sites punched out and counted as above. Oedema formation was expressed by dividing each skin ^{125}I count by the radioactivity in 1 μl of plasma at death.

Statistical analysis

Statistical analysis was performed by ANOVA and results termed significant if $P<0.05$.

Drugs and chemicals

PACAP27 and PACAP38 were obtained from Penninsula (Merseyside, UK). ^{133}Xe and ^{125}I-human serum albumin were from Amersham International, pentobarbitone sodium from May & Baker. Human αCGRP was from Bachem Feinchemikalien AG, (Bubendorf, Switzerland). All other drugs and chemicals were from the Sigma Chemical Co., (Poole, Dorset).

Results

PACAP38 caused a dose-related increase in skin blood flow as measured by ^{133}Xe clearance (Figure 1). The potency of the peptide was not significantly different from that of CGRP in this model but both peptides were approximately 100 fold more potent than VIP at both 10^{-12} and 10^{-11} mol per site ($P<0.05$ in each case). PACAP38 induced a significant response at a dose as low as 10^{-14} mol per site ($P<0.05$ compared with control).

The multiple site ^{133}Xe clearance technique enables the comparison of the quantitative effects of vasoactive substances on the microcirculation at the site of injection in the dermis. The laser-Doppler flow probe measures red cell flux in the superficial skin tissue at a depth of approximately 1 mm. The dose-response curve to PACAP38 measured by the laser-Doppler technique is shown in Figure 2. A significant change was induced by 10^{-13} mol per site by this method ($P<0.05$ compared with control). PACAP27 had similar effects on red cell flux and the dose-response to this peptide was not significantly different to that of PACAP38 (Figure 2).

With the laser-Doppler system, serial measurements can be made at the same site and thus the time course of action of vasoactive agents compared. Figure 3 shows a comparison of the effects of PACAP38 and CGRP given at the same dose (10^{-12} mol per site). Both peptides increased red cell flux, which was maximal at approximately 30 min. However, responses to PACAP38 were of longer duration; at 120 min PACAP38 still caused increased red cell flux whereas the response to CGRP had returned to baseline by this time. The

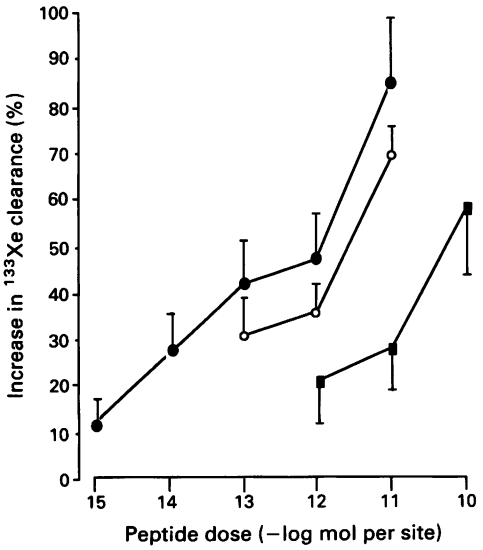

Figure 1 Effect of intradermal injections of the 38 amino acid form of pituitary adenylate cyclase activating polypeptide (PACAP38, ●), calcitonin gene-related peptide (CGRP, ○) and vasoactive intestinal polypeptide (VIP, ■) on rabbit skin blood flow measured by ^{133}Xe clearance. The dose-response to PACAP38 was performed 6 times in each rabbit and the data are the mean (± s.e.mean shown by vertical bars) of 5 rabbits. In a further 5 rabbits, 2 doses of PACAP38, 3 doses of CGRP and 3 doses of VIP were given to compare potency, each dose being repeated 5 times per rabbit. At 10^{-12} and 10^{-11} mol per site VIP was significantly less potent than the comparable dose of PACAP38 ($P<0.05$).

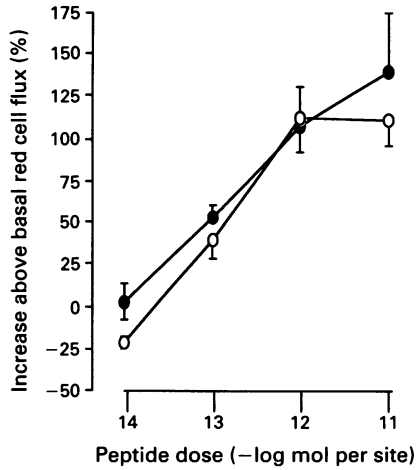

Figure 2 A comparison of the vasodilator effects of the 27 and 38 amino acid forms of pituitary adenylate cyclase activating polypeptide (PACAP). The peptides were injected in 100 µl volumes and measurements taken before and 30 min after injection. Blood flow was measured by laser Doppler flow meter and results are the mean ± s.e.mean of 5 rabbits, each experiment was repeated 4 times in each rabbit; PACAP38, (●); PACAP27 (○).

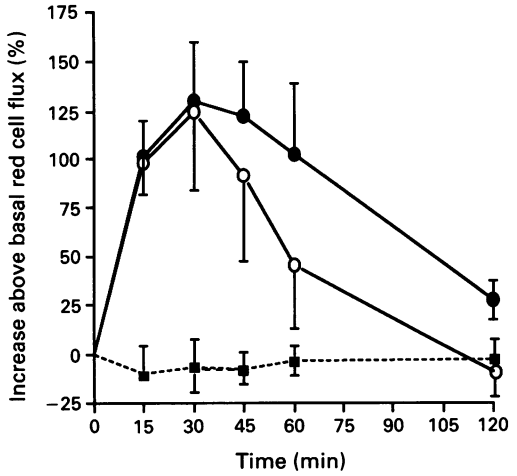

Figure 3 A comparison of the duration of action of pituitary adenylate cyclase activating polypeptide, 38 amino acid form (PACAP38, 10^{-12} mol per site) and calcitonin gene-related peptide (CGRP, 10^{-12} mol per site) in causing vasodilatation in rabbit skin. Peptides were injected intradermally in 100 µl volumes. Blood flow was measured by laser-Doppler flow meter which allows serial measurements at the same site. Results are the mean (s.e.mean shown by vertical bars) for 5 rabbits, each experiment was repeated 4 times in each rabbit. PACAP38: (●); CGRP (○); control (PBS + 0.3% albumin) (■). PACAP38 was significantly longer lasting than CGRP if the results from 45–120 min for each peptide were pooled, or if the slopes of the results from 30–120 min were compared.

duration of action of PACAP38 was significantly longer than CGRP ($P<0.05$) if the results were analyzed by testing for a significant difference between the slopes of the data, approximated to a straight line, from 30 to 120 min, or by comparing the pooled CGRP data from 45 to 120 min with that of the PACAP38 data over the same time course.

Figure 4 shows the potentiation by PACAP38 of bradykinin-induced microvascular plasma protein leakage in the skin. Bradykinin at 10^{-10} mol per site induced a response in this test that was approximately four fold greater than the PBS-albumin control value. PACAP38 alone induced little plasma protein leakage; skin sample plasma volumes were not significantly different from the PBS-albumin controls. However, PACAP38 did cause a marked dose-related potentiation of plasma protein leakage when injected in combination with bradykinin.

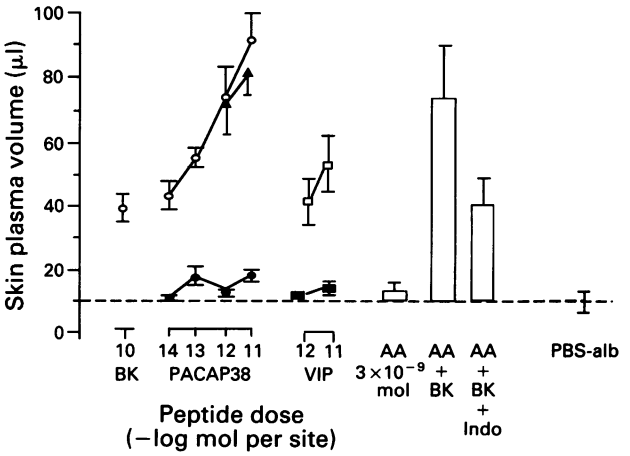

Figure 4 Potentiation of bradykinin-induced oedema formation in rabbit skin by pituitary adenylate cyclase activating polypeptide, 38 amino acid form (PACAP38). Local oedema formation was measured as the intradermal accumulation of intravenously injected ^{125}I-albumin. Test agents were dissolved in 100 µl of PBS-albumin and injected intradermally in a balanced site pattern. Each experiment was repeated in 6 sites per rabbit, each datum point is the mean of 6–10 rabbits. Bradykinin alone (BK, 10^{-10} mol per site) increased oedema formation ($P<0.05$ compared with control). This was potentiated by the co-injection of PACAP (○), ($P<0.05$ for 10^{-12} and 10^{-11} mol per site compared with bradykinin alone) in a dose-dependent manner whereas PACAP alone had no significant effect (●). The potentiation of bradykinin by PACAP at two doses was not significantly altered by the presence of indomethacin (3×10^{-9} mol/site, ▲). Intradermal injection of vasoactive intestinal polypeptide (VIP, ■) did not cause oedema formation whereas it potentiated bradykinin-induced oedema (□, $P<0.05$), where 10^{-11} mol per site had an equivalent effect to PACAP 10^{-13} mol per site. Arachidonic acid (3×10^{-9} mol per site, AA) had no significant effect on oedema formation but did significantly potentiate the effect of bradykinn (AA + BK, $P<0.05$, $n=7$ rabbits), an effect that was significantly inhibited by the presence of indomethacin (AA + BK + Indo, $P<0.05$, $n=7$ rabbits).

The potentiation of responses to bradykinin by PACAP38 was not affected significantly by indomethacin, suggesting cyclo-oxygenase was not involved in the response. In contrast, potentiation of responses to bradykinin by arachidonic acid were reduced by indomethacin to the level of the response to bradykinin alone ($P<0.05$, Figure 4).

VIP also potentiated plasma protein leakage induced by bradykinin ($P<0.05$ at each dose) but was 100 fold less potent than PACAP38 (Figure 4), the same potency difference as in the blood flow model (Figure 1).

Discussion

PACAP is a vasodilator neuropeptide with an amino acid sequence which shows similarities to that of VIP. The present experiments show PACAP to be approximately 100 fold more potent than VIP as a vasodilator in rabbit skin *in vivo* and of similar potency to CGRP. The 27 amino acid and the 38 amino acid forms of PACAP had similar activity. The observed effects of PACAP are in keeping with its ability to relax pre-contracted isolated rabbit aortic rings where it is also approximately 100 fold more potent than VIP (Warren *et al.*, 1991).

Although of similar potency to CGRP, the effect of PACAP38 was significantly longer lasting; vasodilatation was still measurable 2 h after the intradermal injection of 1 picomol. This long duration of action is in keeping with its prolonged effect in isolated aortic rings (Warren *et al.*, 1991).

However, when injected intravenously in whole animals, it is short acting. The hypotensive response to a bolus injection of PACAP in the rat lasts about 5 min (Nandha *et al.*, 1991), possibly because of clearance by organs such as the liver or lungs.

Although PACAP binding sites in the central nervous system are specific and cannot be displaced by VIP, it has been proposed that these two peptides share the same receptor in large blood vessels (Nandha *et al.*, 1991). Both are approximately equipotent at causing hypotension in the rat although PACAP was 100 fold more potent both in the present experiment and in isolated aortic rings (Warren *et al.*, 1991).

It is clear that PACAP is a potent activator of adenylate cyclase in both aortic smooth muscle and cultured neural cells, yet its intracellular vasodilator mechanism has not been confirmed. If PACAP relaxes vascular smooth muscle via adenylate cyclase, this is probably by triggering the sequestration of calcium ions into the sarcoplasmic reticulum; the subsequent lowering of intracellular calcium concentration reducing the formation of the calcium-calmodulin complex hence reducing actin-myosin interaction (MacDermot, 1990). An alternative explanation, that adenosine 3':5'-cyclic monophosphate (cyclic-AMP)-dependent phosphorylation of myosin light chain kinase causes relaxation, may not occur *in vivo* (Kamm & Stull, 1985). Interestingly, rabbit isolated aortic rings do not relax with iloprost, an adenylate cyclase-dependent vasodilator, whereas they do respond to PACAP (Gryglewski *et al.*, 1989; Warren *et al.*, 1991) suggesting that PACAP could act by a mechanism independent of adenylate cyclase.

Several vasodilators, such as CGRP, VIP and prostaglandins have been shown to potentiate oedema formation induced by bradykinin and other mediators of vascular permeability. In the present experiments PACAP potentiated bradykinin-induced oedema formation in a dose-dependent fashion, although it did not induce oedema formation alone. This effect was independent of cyclo-oxygenase as it was not inhibited by indomethacin. PACAP was again approximately 100 fold more potent than VIP in this system.

PACAP is a potent vasodilator in the rabbit skin. Binding sites are present at low levels in rat aorta (Lam *et al.*, 1990) and PACAP is found in close association with some blood vessels in the brain (Koves *et al.*, 1990). It is not known if PACAP is found in association with peripheral blood vessels *in vivo* and it remains to be ascertained whether sufficient endogenous peptide is present to affect vascular tone. Specific antagonists will be needed to determine if PACAP contributes to blood vessel tone *in vivo*. Our results suggest that picomolar amounts may have significant effects on both the hyperaemia and oedema response of inflammation.

J.B.W. is supported by the British Heart Foundation, S.L. and R.K. are supported by the Wellcome Trust, T.J.W. is supported by the National Asthma Campaign.

References

BRAIN, S.D., TIPPINS, J.R., MORRIS, H.R., MACINTYRE, I. & WILLIAMS, T.J. (1986). Potent vasodilator activity of calcitonin gene-related peptide in human skin. *J. Invest. Dermatol.*, **87**, 533–536.

BRAIN, S.D. & WILLIAMS, T.J. (1989). Interactions between the tachykinins and calcitonin gene-related peptide lead to the modulation of oedema formation and blood flow in rat skin. *Br. J. Pharmacol.*, **97**, 77–82.

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

BUSCAIL, L., GOURLET, P., CAUVIN, A., DE-NEEF, P., GOSSEN, D., ARIMURA, A., MIYATA, A., COY, D.H., ROBBERECHT, P. & CHRISTOPHE, J. (1990). Presence of highly selective receptors for PACAP (pituitary adenylate cyclase activating peptide) in membranes from the rat pancreatic acinar cell line AR 4-2J. *FEBS-Lett.*, **262**, 77–81.

GOTTSCHALL, P.E., TATSUNO, I., MIYATA, A. & ARIMURA, A. (1990). Characterization and distribution of binding sites for the hypothalamic peptide, pituitary adenylate cyclase activating polypeptide. *Endocrinology*, **127**, 272–277.

GRYGLEWSKI, R.J., KORBUT, R., TRABKA-JANIK, E., ZEMBOWICZ, A. & TRYBULEC, M. (1989). Interaction between NO donors and iloprost in human vascular smooth muscle, platelets and leukocytes. *J. Cardiovasc. Pharmacol.*, **14**, s124–128.

KAMM, W.E. & STULL, J.T. (1985). The function of myosin and myosin light kinase phosphorylation in smooth muscle. *Annu. Rev. Pharmacol. Toxicol.*, **25**, 593–598.

KIMURA, C., OHKUBO, S., OGI, K., HOSOYA, M., ITOH, Y., ONDA, H., MIYATA, A., JIANG, L., DAHL, R.R., STIBBS, H.H., ARIMURA, A. & FUJINO, M. (1990). A novel peptide which stimulates adenylate cyclase: molecular cloning and characterization of the ovine and human cDNA's. *Biochem. Biophys. Res. Commun.*, **166**, 81–89.

KOVES, K., ARIMURA, A., SOMOGYVARIA, V.A., VIGH, S. & MILLER, J. (1990). Immunohistochemical demonstration of a novel hypothalamic peptide, pituitary adenylate cyclase-activating polypeptide, in the ovine hypothalamus. *Endocrinology*, **127**, 264–271.

LAM, H.-C., TAKAHASHI, K., GHATEI, M.A., KANSE, S.M., POLAK, J.M. & BLOOM, S.R. (1990). Binding sites of a novel neuropeptide pituitary adenylate cyclase activating polypeptide in the rat brain and lung. *Eur. J. Biochem.*, **193**, 725–729.

MACDERMOT, J. (1990). Prostacyclin, cyclic AMP, and relaxation of vascular smooth muscle. *Clin. Pharmacol.*, **7**, 49–54.

MIYATA, A., ARIMURA, A., DAHL, R.R., MINAMINO, N., UEHARA, A., JIANG, L., CULLER, M.D. & COY, D.H. (1989). Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem. Biophys. Res. Commun.*, **164**, 567–574.

MIYATA, A., JIANG, L., DAHL, R.D., KITADA, C., KUBO, K., FUJINO, M., MINAMINO, N. & ARIMURA, A. (1990). Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). *Biochem. Biophys. Res. Commun.*, **170**, 643–648.

NANDHA, K.A., BENITO-ORFILA, M.A., SMITH, D.M., GHATEI, M.A. & BLOOM, S.R. (1991). Action of pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal polypeptide on the rat vascular system: effects on blood pressure and receptor binding. *J Endocrinol.*, **129**, 69–73.

OGI, K., KIMURA, C., ONDA, H., ARIMURA, A. & FUJINO, M. (1990). Molecular cloning and characterization of cDNA for the precursor of rat pituitary adenylate cyclase activating polypeptide (PACAP). *Biochem. Biophys. Res. Commun.*, **173**, 1271–1279.

OHTAKI, T., WATANABE, T., ISHIBASHI, Y., KITADA, C., TSUDA, M., GOTTSCHALL, P.E., ARIMURA, A. & FUJINO, M. (1990). Molecular identification of receptor for pituitary adenylate cyclase activating polypeptide. *Biochem. Biophys. Res. Commun.*, **171**, 838–844.

SUDA, K., SMITH, D.M., GHATEI, M.A., MURPHY, J.K. & BLOOM, S.R. (1991). Investigation and characterization of receptors for pituitary adenylate cyclase-activating polypeptide in human brain by radioligand binding and chemical cross-linking. *J. Clin. Endocrinol. Metab.*, **72**, 958–964.

TATSUNO, T., GOTTSCHALL, P.E., KOVES, K. & ARIMURA, A. (1990). Demonstration of specific binding sites for pituitary adenylate cyclase activating polypeptide (PACAP) in rat astrocytes. *Biochem. Biophys. Res. Commun.*, **168**, 1027–33.

WARREN, J.B., DONNELLEY, L.E., CULLEN, S., ROBERTSON, B.E., GHATEI, M., BLOOM, S.R. & MACDERMOTT, J. (1991). Pituitary adenylate cyclase activating polypeptide: a novel, long lasting, endothelium-independent vasorelaxant. *Eur. J. Pharmacol.*, **197**, 131–134.

WILLIAMS, T.J. (1979). Prostaglandin E_2, prostaglandin I_2 and the vascular changes in inflammation. *Br. J. Pharmacol.*, **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. Pharmacol.*, **77**, 505–509.

(Received January 8, 1992
Revised January 23, 1992
Accepted February 7, 1992)