Vasodilatation by calcitonin gene–related peptide and by substance P: a comparison of their effects on resistance and capacitance vessels of human forearms


ABSTRACT A comparison has been made of the effects of the potent vasodilating peptide calcitonin gene–related peptide (CGRP) and substance P (SP) on resistance and capacitance vessels of normal subjects. Brachial artery infusion of 1.25 to 10 pmol/min CGRP and of 0.25 to 1.5 pmol/min SP produced maximal increases in forearm blood flow (177 ± 75% and 198 ± 50%, respectively), as measured by venous occlusion plethysmography. The vasodilatation due to CGRP was prolonged, with a half-life of biological effect of approximately 18 min, while that due to SP was of short duration, with a half-life of biological effect of approximately 15 sec. There was rapid development of tachyphylaxis to the effects of arterial infusion of SP, but not of CGRP, during a prolonged infusion at one dose. CGRP did not alter the diameter of a superficial hand vein, either at rest or when the vein was constricted by a simultaneous infusion of norepinephrine or by the single deep breath reflex. In contrast, SP caused dilatation of veins preconstricted with norepinephrine, although the effect was only transient and dose-response curves could not be constructed. The vеноconstrictor response to a single deep breath was abolished by SP. Simultaneous arterial infusion of both peptides produced at least additive, and possibly synergistic, effects on forearm blood flow. We propose that both CGRP and SP have a role in the regulation of vascular smooth muscle tone. *Circulation* 77, No. 5, 1072–1080, 1988.

IT HAS BEEN proposed that peptidergic nerves in the heart and around blood vessels have a role in the regulation of the cardiovascular system. Calcitonin gene–related peptide (CGRP) is a 37-amino acid peptide resulting from alternative processing of the RNA during calcitonin gene expression.1 Its existence was first predicted by analysis of a new RNA in a rat cell line and subsequently a similar peptide was isolated from human medullary carcinoma of thyroid and sequenced by FAB spectroscopy.2 CGRP is a potent vasodilator in vitro and in vivo in the rat.3–5 It has also been demonstrated that CGRP will dilate human epicardial coronary arteries in vivo.6 Extensive histochemical studies in the rat and guinea pig have shown a widespread distribution of CGRP and substance P (SP) in the perivascular nerves and atrium, and in the conducting tissue of the heart.7, 8 SP is an 11-amino acid peptide that was first isolated over 50 years ago.9 In both central and peripheral nerves it is found to colocalize with CGRP.8, 10 Studies using extracted and synthetic SP have shown it to be a potent arterial dilator in man but the duration of the responses has not been studied in any detail.11, 12

CGRP and SP will each produce a fall in diastolic blood pressure and an associated tachycardia when infused systemically in man.13, 14 The systemic infusion of each of these peptides suggested that while of comparable potency as vasodilators, the rates of onset and offset of effects were different. However, the blood pressure effects of a systemic infusion of a vasodilator are masked in young fit people by prompt baroreflex responses, resulting in norepinephrine release and an increased heart rate, and the systolic blood pressure is thus maintained.15 For this reason, we have now compared the effects of CGRP with those of SP on two
different vascular beds in man: the forearm resistance vessels and the superficial veins of the hand. By performing the studies with doses of peptides that were sufficiently low that no systemic effect would be expected, we were able to study the rates of onset and offset of the local responses. The effects of CGRP and SP on forearm resistance vessels were assessed by measuring forearm blood flow in response to a brachial artery infusion of peptide. The effect of the peptides on the superficial veins of the hand was assessed by measuring venous diameter during infusion of the peptide, either at rest or when the vein was preconstricted with norepinephrine or by the single deep breath reflex.

Methods

Subjects. The brachial artery infusion studies were performed on eight normal individuals, all men, four of whom participated in both studies so that there were six studies of dose-response for each peptide. Three subjects also received two prolonged infusions, one of each peptide at one dose, and four also participated in the study on the effects of simultaneous infusion of both peptides. Each infusion study was performed on a different day. Eight subjects (one woman) participated in the venous studies, four receiving both peptides on different days. There were therefore six studies of the venous effects of each peptide. The age range in all groups was the same, 28 to 32 years. Experiments were performed with the subjects supine; laboratory temperature was constant during any one study, but varied between 24°C and 28°C on different occasions. All studies were approved by the local Ethical Committee and written informed consent was obtained.

Materials. Synthetic human CGRP (Bachem, Saffron Walden, U.K.) was the generous gift of Dr. Jan Pless of Sandox Ltd., Basel, Switzerland. CGRP and synthetic SP (Cambridge Research Biochemicals, Cambridge, U.K.) were each dissolved in 10% acetic acid with 25 μl/ml sterile heat-treated human serum albumin, to give a concentration of 0.05 mg/ml, and were stored at −80°C before use. Both peptides were diluted in Haemaccel (Hoechst, London, U.K.) before infusion. Norepinephrine (Levophed, Winthrop, U.K.) was diluted in normal saline containing 5 to 10 μg/ml of ascorbic acid to prevent oxidation.

Forearm resistance vessels. Forearm blood flow was measured by venous occlusion plethysmography with use of a temperature-compensated mercury-in-silicone rubber strain gauge with a Devices amplifier and recorder. A wrist occlusion cuff pressure of 180 to 200 mm Hg and an upper arm collecting cuff pressure of 35 to 45 mm Hg were used; flows were recorded for 10 sec in every 15 sec. A 26 SWG needle was introduced into the brachial artery of one arm under local anaesthesia (Lignocaine 1%).

Comparison of SP and CGRP. A 15 min infusion of the vehicle solution, Haemaccel, was followed by incremental doses of peptide, infused at rates varying from 0.25 to 1.0 ml/min, by a Harvard constant-rate infusion pump. The dose of peptide infused was increased every 8 min by increasing the concentration or rate of infusion. Flows were recorded during the last 3 min of each infusion period. The average of the last eight measurements of flow at each dose was taken as the response. After stopping the highest dose of peptide, flows were recorded at intervals until control values had been achieved. On a separate occasion, after the infusion of control vehicle solution, peptide was infused at one dose only (SP at 1 pmol/min, CGRP at 7.5 pmol/min) but for 30 min, with measurement of flow at intervals during this time.

Coinfusion of SP and CGRP. A dose-response curve was first constructed with incremental doses of SP for 5 min at each dose, with flow recordings during the last 3 min of each infusion. The peptide was then replaced with control vehicle solution and flows were allowed to return to baseline. An infusion of CGRP (7.5 pmol/min) was commenced and continued for 25 min, by which time the increase in flow had reached a plateau. The SP dose-response study was then repeated during the CGRP infusion. The volume of infusion was kept constant throughout the study by the use of a parallel infusion of control vehicle solution when only one peptide was being infused. After the second SP dose-response study the CGRP infusion was continued, with measurement of blood flow at intervals until blood flow had become constant.

Superficial veins of hand. The hand and forearm rested on a stable support that was inclined at an angle of about 30% to the horizontal and the position of the subject was arranged so that the hand was above the level of the central venous pressure and the veins appeared fully collapsed when the congesting cuff

\[ \text{FIGURE 1. Tracing from Devices amplifier and recorder attached to the lever balanced lightly on a superficial hand vein. At D the vein has been distended by inflation of a cuff at 25 mm Hg around the upper arm. At C the cuff has been released and the vein has been allowed to collapse and then was further emptied by the stroking of the vein with a suitable bent needle. The difference between the distended and collapsed state is the maximum venous diameter at rest for a distending pressure of 25 mm Hg.} \]
was not inflated. A lightweight lever was balanced on a selected
dorsal vein of the hand. The lever was attached to a Harvard
displacement transducer and measured changes in the size of the
selected vein. A cuff on the upper arm was inflated to the
congesting pressure of 25 mm Hg and the vein was allowed to
fill. When a steady state had been maintained for at least 1 min,
the cuff was deflated and when the vein had collapsed it was
gently stroked two to three times in the direction of flow with
a suitable bent needle to ensure that emptying was complete
(figure 1). The change in size from the congested to the collapsed state is the index of distensibility. Suitable calibration allows the
distension of the vein to be measured in absolute terms, but our results have been expressed as the percentage change in vein size. The distending pressure was kept constant and the arm and hand were immobilized so that the pressure within the vein was maintained constant for any one experiment and would be very similar for any series of experiments.

The peptides were infused into the vein at 0.125 ml/min through
a 23 SWG butterfly needle (Abbot, Sligo, Republic of Ireland) that
was introduced without local anesthesia and positioned with its tip
5 to 10 mm upstream from the point of the measurement. Each
dose of peptide was infused for a period of 6 min with the
distending cuff inflated. The cuff was then deflated and the vein
diameter was measured. Since veins of the dorsum of the hand
have no resting tone in a relaxed and warm subject, in some experiments the veins were preconstricted by a continuous infusion
of norepinephrine (0.125 ml/min; figure 2). The infusion of norepinephrine was adjusted to produce a vein diameter of approximately 30% of maximum (8 to 64 ng/min). Peptides were then
infused simultaneously with norepinephrine.

The effects of the peptides on reflex venoconstriction induced by endogenous norepinephrine was assessed by measuring
the diameter of the vein after a single deep breath (figure 3) before
and after peptide infusion.

Data analysis. Results in the text and figures are expressed as
the mean ± SE. Statistical analysis was by two-way analysis of variance and differences were tested by a Bonferroni modi-
fication of Student’s t test to allow for multiple comparisons.

Results

Forearm resistance vessels

Dose response. Figure 4 shows the effect of the incremen-
tal increases in infusions of CGRP or SP on forearm blood flow. Forearm blood flow increased during infusion of CGRP from a mean value of 3.28 ± 0.56
to 9.08 ± 2.12 ml/min/100 ml at the highest dose of
10 pmol/min (a rise of 177 ± 75%). Infusion of SP
caused a dose-dependent increase in forearm blood flow of from 2.18 ± 0.39 to 6.41 ± 1.42 ml/min/100
ml at 1.5 pmol/min (a rise of 198 ± 50%). Thus, the
dose of SP required to produce equivalent increases in
forearm blood flow was approximately one-tenth of the
dose of CGRP. The dose-dependent increase in forearm
blood flow was significant (p < .05) for CGRP at doses
of greater than 1.25 pmol/min and for SP infusion at
doses greater than 0.5 pmol/min.

Duration of effect. When the infusion of CGRP was
replaced with control vehicle solution, the increased
blood flow returned slowly toward the starting value
(figure 5). The half-time of the vasodilatation was
approximately 18 min. This was in marked contrast to
the short duration of action of SP. Preliminary ex-
periments suggested that the half-time of biological
action of SP was less than 5 min. For this reason, the
SP infusion was simply stopped, rather than replaced by
control vehicle solution, thus avoiding infusion of dead
space volume (approximately 2 min of infusion) con-
taining SP. The results for one individual are seen in

![Figure 2](http://circ.ahajournals.org/doi/abs/10.1161/CIRCULATIONAHA.107.658588)

**Figure 2.** Incremental doses of vehicle solution of norepinephrine were infused locally for periods of
5 min and the vein gradually constricted, as can be seen by the gradual reduction in the distended diameter. The dose of norepinephrine producing a reduc-
tion in venous diameter to 30% of that at rest was selected for subsequent experiments to examine the
effects of potential vasodilators. For all measure-
ments the distending pressure was 25 mm Hg.
FIGURE 3. A single deep breath taken at the point indicated by the arrow (DB) produced a reduction in venous diameter of approximately 70% of maximum, with a slow relaxation over the next 3 min. Paper speed was 2.5 cm/min (large box = 1 cm).

the insert in figure 2. The flow returned to basal levels in 30 sec, with a half-time of biological effect of approximately 15 sec. The fall in blood flow after cessation of peptide infusion was significant (p < .05) within 4 min for SP, but not until 28 min for CGRP.

During a 30 min infusion of CGRP (7.5 pmol/min) blood flow increased rapidly during the initial 5 min and then flow increased more slowly. Although maximum flow was not achieved until 25 min, about 80% of the maximum increase in flow was achieved by 8 min, the time used during the dose-response studies (figure 6). The blood flow increase in response to SP (1 pmol/min) had reached a maximum within 5 min, but then declined toward baseline. However, approxi-

imately 80% of the maximum increment in flow was present at 8 min.

During the prolonged infusion of CGRP, each subject developed a few well-circumscribed areas of skin reddening near to the site of the needle that persisted for at least 1 hr after the end of the infusion. These areas were of a deep red hue (like a port wine stain nevus) and were similar to those described after the intradermal injection of CGRP. No such skin change occurred during infusion of SP.

There was no significant change in heart rate or blood pressure during the prolonged infusion of SP or CGRP, suggesting that there were no systemic effects of the doses used.

Effect of coinfusion. In this study, the initial response to incremental doses of SP was similar to that found in the first study, with flow increasing from 5.5 ± 1.4 to 11.8 ± 2.8 ml/min/100 ml at 1 pmol/min of SP (n = 3). After the flow returned to basal levels, the infusion of CGRP (3.75 pmol/min) increased blood flow to 8.4 ± 2.3 ml/min/100 ml. SP infused with CGRP produced a further increase in the flow to 17.3 ± 4.7
FIGURE 6. Forearm blood flow during a 30 min infusion of 1 pmol/min SP (△) and 7.5 pmol/min CGRP (▲) (mean ± SEM, n = 3).

ml/min/100 ml at the highest dose of 1 pmol/min, this increase being slightly greater than that achieved with the initial response to SP alone (figure 7).

Superficial veins of hand. Infusion of CGRP at doses of up to 5 pmol/min had no effect on the diameter of superficial veins of the hand at rest, when the vein was preconstricted by norepinephrine, or when the vein was stimulated to constrict by a single deep breath. Infusion of SP into a vein had no effect on its resting diameter but consistently abolished the venoconstrictor response to a single deep breath (figure 8). Infusion of SP caused a variable degree of vasorelaxation of the vein when partially constricted by a simultaneous infusion of norepinephrine. Between 30% and 100% relaxation was achieved on each occasion, which was rapidly reversed by stopping the SP while continuing the norepinephrine infusion (figure 9). A similar degree of relaxation was not always achieved when SP was reintroduced. Higher doses of SP did not increase relaxation. In five of six studies the change in vein diameter was transient, with reconstriction of the vein despite continued infusion of SP and no dilatation when the SP was discontinued for 5 min and then restarted. The lowest dose of SP that was found to be effective was 100 fmol/min, but the rapid development of tachyphylaxis meant that dose-response curves of vein dilatation in response to SP could not be constructed.

Discussion

Our results show that both CGRP and SP are potent arteriolar dilators in the human forearm and that SP, but not CGRP, relaxes the dorsal hand veins when they are constricted by norepinephrine or by a deep breath reflex. As suspected from the previous systemic infusion studies in man, the effects of CGRP are prolonged, persisting for at least 40 min after cessation of the infusion. SP was approximately 10 times more potent than CGRP in producing arteriolar dilatation.
However, the effect of SP was of much shorter duration and within 30 sec of stopping the infusion of SP, blood flow had returned to basal levels. The reasons for this difference in duration of the effects of the two peptides is unknown, but might be due to differences in the rate of metabolism of the peptides within the local tissues or differences in the rate of dissociation of agonist and receptor.

Both resistance vessels and veins show tachyphylaxis to SP. Others have shown that during systemic infusion of SP in dogs and in man the maximum effect is achieved rapidly, followed by a gradual decline in the degree of hypotension and tachycardia. It is thought unlikely that this loss of the cardiovascular effects is due to physiologic antagonism by increased sympathetic activity since no similar loss of effect is seen with other vasodilators, such as nitroprusside. Our demonstration of a decline in the degree of vasodilation during prolonged infusion into the isolated forearm vascular bed confirms that this is due to tachyphylaxis rather than to systemic reflexes or a central action of the SP. Tachyphylaxis to the venodilator response to SP was also shown. Interestingly, tachyphylaxis also occurs in vitro to the vasorelaxant effects of both SP and CGRP, but we were unable to demonstrate rapid development of any significant tachyphylaxis to CGRP in vivo.

In a warm relaxed subject, the superficial veins are without tone. To study the effects of a vasodilator it is necessary to induce tone either by infusion of a constrictor agent, such as norepinephrine, or by reflex vasoconstriction. Transient vasoconstriction occurs in response to a single deep breath. The reflex can be blocked by ganglion-blocking drugs such as tetraethyl ammonium bromide. With repeated deep breaths at rapid intervals the constriction is not sustained. CGRP had no effect on this reflex, but it was abolished by infusion of SP into a vein at a concentration so low as to be effective only locally. The lowest effective dose of SP was 100 fmol/min, while CGRP was ineffective at doses up to 5 pmol/min. If CGRP were equipotent in arteries and veins then it might be expected to be effective in veins at 0.25 pmol/min since veins respond to a tenth of the dose that causes significant vasodilation in arteries. The mechanism of the inhibition by SP of reflex vasoconstriction has not been elucidated, but the relaxation of veins preconstricted by exogenous norepinephrine suggests that SP is acting postjunctionally. However, others have suggested that SP can directly modulate sympathetic nerves, and certainly prejunctional inhibition of local norepinephrine release, which would account for the loss of the single deep breath reflex, cannot be excluded. A direct inhibition of sympathetic nerves was proposed to explain why a systemic SP infusion produced less of a rise in plasma norepinephrine than was observed with a similar degree of vasodilation due to CGRP, nitroprusside, or histamine. Our observations on the effects of SP on veins are in contrast to the lack of significant effects described by Eklund et al. This may
CGRP has been identified in normal human and rat plasma (25 pmol/liter), and it has been proposed that circulating CGRP may control vascular smooth muscle. In contrast, plasma levels of SP are below 5 pmol/ml. In the present study, plasma levels of peptides were not measured. In previous studies the assay of levels of peptide in the infusate, which was prepared by a method identical to that used here, showed that the infusate contained approximately 66% of that estimated and it is presumed that adhesion to syringes and tubing accounts for the losses. It is possible to make estimates of the concentration of peptide in the forearm blood during infusion studies. Thus, 50 pmol/liter of CGRP and 10 pmol/liter of SP produce significant vasodilatation in the forearm. Plasma levels of CGRP of 0.84 to 45 mmol/liter have been reported in patients who have medullary thyroid carcinomas. Medullary carcinoma is frequently associated with flushing attacks and so CGRP may be involved in the pathogenesis of these symptoms. The origin of circulating CGRP is not yet clear in man. In the rat CGRP has a dual origin from perivascular nerves and from thyroid, but whether its presence in plasma reflects a spillover phenomenon or the true secretion of a hormone is not known.

CGRP-induced vasodilatation is not mediated by histaminergic, cholinergic, or adrenergic receptors, and the positive inotropic and chronotropic effects of CGRP on the isolated perfused guinea pig heart are unaffected by metoprolol. The CGRP-induced vasodilatation in rabbit skin is unaffected by indomethacin, but in man aspirin may reduce the initial flare induced by intradermal injection. SP acts independently of cholinergic, adrenergic, or histaminergic receptor stimulation. It would appear that the effects of CGRP and SP are mediated by specific receptors for each.

SP has been shown to colocalize with CGRP in both nerve cell bodies and peripheral nerve fibers. Double staining immunohistochemical studies of the trigeminal ganglion show that 20% of neurons within it are positive for SP immunoreactivity and that these cells also contain CGRP. Coexistence of the two peptides has also been shown in cell bodies in the dorsal root ganglia and nodose ganglia. Nerve fibers positive for both peptides are seen in the celiac, superior cervical myenteric, and autonomic ganglia. A similar distribution of nerve fiber networks containing each peptide has been demonstrated in the walls of rodent blood vessels and the colocalization of CGRP and SP in the perivascular nerve networks has been confirmed in the cerebral arteries of the rat by double-staining techniques. The fibers that contain CGRP and SP are largely from primary sensory afferent nerves, since they are sensitive to capsaicin, a neurotoxin selective for C and Aδ fibers. A few fibers appear more resistant to the neurotoxin and, although this may be a dose-dependent phenomenon, it could also suggest the presence of CGRP in fibers subserving different functions.

The presence of a long- and a short-acting vasodilator in the forms of CGRP and SP would appear an attractive option, giving good physiologic control of
blood flow. These two peptides may, however, act synergistically. CGRP alone has no effect on vessel permeability when injected into the dorsal skin of the rabbit, but it will amplify the edema caused by intradermal injection of SP when given simultaneously. The interpretation of the vasodilator effects of brachial infusion of the two peptides simultaneously is difficult. Increased blood flow due to infusion of one would be expected to reduce the blood concentration of the second, and therefore reduce the response to the second infusion. Here, we have shown a small increase in the response to SP during CGRP infusion. Although the peptides may be acting synergistically, other factors may account for the effect. For example, the first vasodilator may open up the access for the second to the resistance vessels.

Although CGRP and SP are both vasodilators, the differences in rates of onset and offset of action and in the development of tachyphylaxis suggests different modes of action. The potencies of each as vasodilators supports their involvement in the control of vascular smooth muscle tone, and make their respective receptors promising targets for pharmacologic intervention.

We are grateful to Miss B. Edinborough for excellent secretarial assistance.

References


36. Brain SD, William TJ: Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. Br J Pharmacol 86: 855, 1985
Vasodilatation by calcitonin gene-related peptide and by substance P: a comparison of their effects on resistance and capacitance vessels of human forearms.
J R McEwan, N Benjamin, S Larkin, R W Fuller, C T Dollery and I MacIntyre

_Circulation_. 1988;77:1072-1080
doi: 10.1161/01.CIR.77.5.1072

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/77/5/1072

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/