A comparison of the effects of human alpha calcitonin gene-related peptide and glyceryl trinitrate on regional blood velocity in man

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1 Human alpha calcitonin gene-related peptide (hαCGRP) is a potent vasodilator which in doses up to $1.5 \mu\text{g min}^{-1}$ i.v. produces little or no fall in blood pressure in normal volunteers, but does cause a substantial tachycardia.

2 We have explored the underlying mechanism of this effect by comparing hαCGRP infused so as to maintain heart rate 25–30% above baseline with glyceryl trinitrate (GTN) in a dose sufficient to maintain a throbbing headache.

3 Ten normal volunteers were studied. In addition to blood pressure and heart rate, blood velocity and pulsatility index (PI) were determined from Doppler signals recorded from the internal and external carotid, renal and femoral arteries.

4 Following hαCGRP blood pressure (mean $\pm$ s.d., mm Hg) did not significantly change: $120 \pm 10/70 \pm 7$ before compared with $121 \pm 12/67 \pm 7$ during hαCGRP infusion. Heart rate (mean $\pm$ s.d., beats min^{-1}) increased from 62 ± 8 to 86 ± 10 ($P < 0.0001$). In contrast the blood pressure fell following GTN: $124 \pm 12/74 \pm 8$ before compared with $111 \pm 13/62 \pm 6$ following treatment ($P < 0.02$). Heart rate did not change following GTN: 64 ± 9 compared with 69 ± 10 .

5 GTN significantly increased PI (mean $\pm$ s.d.) in the common carotid artery from 2.8 ± 0.5 to 3.4 ± 0.5 ($P < 0.003$) while hαCGRP increased PI in the internal carotid from 1.3 ± 0.2 to 2.1 ± 0.4 ($P < 0.001$). There were no other changes in PI. hαCGRP increased velocity (cm s^{-1}) in the common carotid artery from 17 ± 5 to 31 ± 8 ($P < 0.0001$) and in the internal carotid artery from 17 ± 5 to 24 ± 9 ($P < 0.01$). There were no other changes in velocity.

6 We conclude that hαCGRP is able to increase selectively the velocity of blood to the head at a dose which does not cause a fall in systemic blood pressure.

Keywords Doppler CGRP blood pressure

Introduction

The mammalian calcitonin gene encodes peptides other than calcitonin (Jacobs *et al.*, 1981). One of these, calcitonin gene-related peptide (CGRP) is found in the circulation at concentrations often exceeding those of calcitonin, and through-

out the central and peripheral nervous system in nerve endings associated with vascular smooth muscle (Mulder *et al.*, 1985). The peptide exists in two forms, alpha and beta (Fisher *et al.*, 1983). CGRP is exciting considerable interest

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because of its variety of potent circulatory effects, suggesting physiological and possible therapeutic roles in blood flow regulation.

In animals, central administration of human α CGRP increases plasma noradrenaline and produces prolonged tachycardia with transient blood pressure elevation (Steenbergh *et al.*, 1985). Peripheral administration induces dose related tachycardia and hypotension. Positive chronotropic and inotropic effects are mediated through specific sites in the myocardium (Haass & Skofitsch, 1985).

In isolated vessels α CGRP induces smooth muscle relaxation (Kubota *et al.*, 1985; Thom *et al.*, 1987). Both endothelium dependent and independent relaxation have been recorded in different vessels (Brain *et al.*, 1985; Hughes *et al.*, 1987), indicating that α CGRP may act by more than one mechanism. α CGRP is also a potent vasodilator in man where studies have been performed largely with the alpha peptide. Dose dependent increases in forearm blood flow during brachial artery infusion and vasodilatation of epicardial vessels in patients undergoing coronary angiography have been demonstrated (McEwan *et al.*, 1986, 1987). However, few studies of systemic administration in normal subjects have been published. We recently assessed the haemodynamic and neuroendocrine effects of α CGRP given by intravenous bolus injection to 10 normal males (Howden *et al.*, 1988). α CGRP produced a dose related tachycardia of rapid onset and an increase in plasma noradrenaline concentration but blood pressure was unaffected over a 10 fold dose range.

We have now extended our observation by studying the action of systemically administered human alpha CGRP on a number of vessels in normal males and by comparing its effects with those of glyceryl trinitrate (GTN).

Methods

Ten healthy male volunteers aged 25–32 years were recruited to the study which was approved by the local Research and Ethics Committee. Each volunteer was investigated on one day only. Sublingual GTN and α CGRP by intravenous infusion were administered to each subject in random order.

Following an overnight fast, subjects attended the Clinical Investigation Unit and initially lay supine for 30 min. A Venflon intravenous catheter was then inserted into an antecubital vein. Baseline blood velocity measurements were performed on common and internal carotid, femoral and renal arteries. Supine blood pressure

and heart rate were recorded during each measurement. Subjects then received sublingual GTN in a dose sufficient to produce and sustain a headache or α CGRP by intravenous infusion of $1.5 \mu\text{g min}^{-1}$ until the heart rate had increased by 25–30% above baseline. The GTN was given in the dose of one tablet (300 μg) every 5 min until headache was produced and additional tablets were given as required. The rate of α CGRP infusion was adjusted to maintain the heart rate at this level. Blood velocity studies were repeated in each vessel with simultaneous recording of blood pressure and heart rate. The infusion was then stopped. Measurements were repeated 30–45 min later when heart rate had returned to baseline following α CGRP or the headache had resolved following GTN. After the first study period subjects had a light lunch and a rest for 2–2.5 h before the sequence was repeated with the other preparation. All haemodynamic variables had returned to baseline values before commencing the second study period.

Blood pressure and heart rate were recorded with a semi-automated sphygmomanometer (Sentron, Bard Biomedical, Illinois). Blood velocity indices were recorded with a Doppler, Duplex Scanner (Doptek Ltd, Chichester, UK). Doppler waveform analysis provides a simple and reproducible method of studying indices related to blood flow. The difference between emitted and received frequencies is used to calculate blood velocity. If the vessel diameter is also known then it is theoretically possible to calculate volume flow. However, it is our experience and that of others that measurement of vessel diameter is too inaccurate to provide reproducible measurements of flow and we therefore prefer to use indices of blood velocity as an indirect measure of impedance to flow. In general, provided other factors remain unchanged, an increase in downstream impedance to flow will result in a decrease in blood velocity and increase in the ratio of systolic to diastolic velocity. Various indices and velocity ratios have been used. In this study we have used the pulsatility index (PI) and maximum (centre stream) velocity. PI is a quantification of the blood velocity waveform and is derived from the peak to peak amplitude of the waveform divided by the mean amplitude over the cardiac cycle (Gosling & King, 1974) and is directly proportional to downstream impedance to flow (Trudinger, 1987). The PI was calculated as the mean of five measurements in consecutive waveforms.

The angle of insonation of the Doppler probe to the vessel was kept as close to 0° as possible for each measurement. An angle of insonation no

greater than 20° results in an error of no more than 6% in estimation of velocity. In the case of measurements from the renal artery, the vessel was first visualised by real time scanning while other vessels were easily identified anatomically. One way analysis of variance showed no difference between baseline recordings made in the afternoon compared with the morning. The overall within subject, within day coefficients of variation were 14.4% for PI and 15.7% for velocity.

Statistical analysis was by repeated measures one way analysis of variance with paired *t*-test being used to test significance between individual points if an overall difference was observed.

Results

Subjects all completed the study uneventfully. In order to produce a headache, six subjects required 1 mg (2 tablets) of GTN, two subjects required 2 mg, one subject received 2.5 mg and one subject 4.5 mg. 25% increase of heart rate occurred following an average hαCGRP infusion of 21.6 µg, the range being 10.5–40.5 µg at the rate of 1.5 µg min⁻¹. Recordings were made between 7–30 min following sublingual administration of the first GTN tablet. With hαCGRP recordings were all made within 25 min of attaining the target heart rate in 9 of the 10 subjects. In the remaining subject difficulty with recording from the renal artery extended this final

measurement to 60 min after reaching the target heart rate. In all subjects the heart rate was maintained at its desired level by adjusting the infusion rate of hαCGRP. Similarly, in all subjects headache was present throughout the recording period following the use of GTN. During the hαCGRP infusion all subjects experienced marked facial flushing which resolved 30–40 min following the end of drug administration. In keeping with the facial flushing, subjects felt substantially warmer with hαCGRP than GTN. No change in skin colour was seen with GTN. hαCGRP produced feelings of anxiety while both compounds were reported as producing an increase in a general feeling of unwellness.

Blood pressure and heart rate data are shown in Table 1. GTN significantly lowered both systolic and diastolic blood pressure while heart rate showed only a modest and statistically insignificant change. In contrast, hαCGRP had no effect on blood pressure but heart rate increased substantially.

The data from the Doppler recordings are presented in Tables 2 and 3. With GTN pulsatility index showed a reversible increase in the common carotid artery. No significant change occurred in the other vessels. Velocity did not change significantly in any vessel.

Following hαCGRP PI showed a reversible increase in the internal carotid artery. None of the other vessels showed a significant change. Velocity increased in both common carotid and internal carotid arteries and showed a trend towards increase in the femoral artery.

Table 1 Heart rate and blood pressure (mean ± s.d.)

	Heart rate (beats min ⁻¹)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
Baseline	64.3 ± 9.1	124.2 ± 12.5	74.3 ± 7.9
GTN	69.3 ± 9.6	111.4 ± 13.2* ²	62.4 ± 5.8* ³
Baseline	62.7 ± 7.7	120.7 ± 10.0	70.1 ± 6.9
hαCGRP	86.0 ± 10.4* ¹	121.2 ± 12.5	67.3 ± 7.4

* significantly different from baseline.

¹ *P* < 0.0001, ² *P* < 0.02, ³ *P* < 0.001.

Table 2 Pulsatility index (mean ± s.d.)

	Common carotid	Internal carotid	Renal	Femoral
Baseline	2.8 ± 0.5	1.5 ± 0.5	1.1 ± 0.3	12.7 ± 4.7
GTN	3.4 ± 0.5* ¹	2.0 ± 0.8	0.9 ± 0.2	16.9 ± 8.8
Baseline	2.9 ± 0.5	1.3 ± 0.2	1.0 ± 0.2	19.9 ± 13.8
hαCGRP	2.7 ± 0.4	2.1 ± 0.4* ²	1.2 ± 0.5	15.8 ± 8.0

* significantly different from baseline.

¹ *P* < 0.003, ² *P* < 0.001.

Table 2 Velocity (cm s⁻¹) (mean $\pm$ s.d.)

	Common carotid	Internal carotid	Renal	Femoral
Baseline	19.0 $\pm$ 4.1	16.7 $\pm$ 5.2	28.1 $\pm$ 10.2	5.4 $\pm$ 2.8
GTN	16.3 $\pm$ 1.6	14.7 $\pm$ 3.9	25.1 $\pm$ 10.7	4.2 $\pm$ 2.5
Baseline	17.2 $\pm$ 5.3	17.3 $\pm$ 5.3	30.6 $\pm$ 14.5	3.9 $\pm$ 2.2
h α CGRP	30.7 $\pm$ 8.0* ¹	24.3 $\pm$ 9.0* ²	29.4 $\pm$ 13.0	5.6 $\pm$ 4.1

* significantly different from baseline.

¹ $P < 0.0001$, ² $P < 0.01$.

Discussion

In a previous study we had shown that h α CGRP produces a substantial tachycardia with no change in blood pressure when administered in doses up to 25 μ g by intravenous bolus injection to normal volunteers (Howden *et al.*, 1988). Since there is no doubt that h α CGRP is a vasodilator there were various possible explanations for our observations. One possibility was simply that the compensatory response to a fall in blood pressure in these young subjects was sufficient to prevent any hypotension, and explained the tachycardia. Another possible explanation was that h α CGRP acts as a vasodilator only in certain vascular beds. A further explanation is that h α CGRP was having direct inotropic and chronotropic effects as described both in animal studies and *in vitro* (Steenbergh *et al.*, 1985; Haass & Skofitsch, 1985). In order to derive further information about the actions of h α CGRP in man, the present study was devised. The aim was to compare the actions of h α CGRP with those of another vasodilator both in terms of effect on blood pressure and heart rate and with regard to actions on different vascular territories. This latter aim in particular required that h α CGRP be given by infusion in order to allow time for all the various measurements to be made.

In studies such as this, comparability of dose is clearly of major importance as is comparability of study environment. Since our observations had not only physiological but possibly therapeutic applications the dose of GTN chosen was that which produced and sustained a throbbing headache. In the case of h α CGRP, selection of the appropriate dose is somewhat more difficult. In our earlier study facial flushing, a sensation of swelling in the head and tachycardia were consistent findings except at the lowest dose. In addition, pilot observations with h α CGRP infusions (unpublished) suggested an increased heart rate of 25–30% as the most reliable and reproducible indicator that h α CGRP was having a pharmacological effect. In the event, facial

flushing and symptoms referable to the head accompanied this target and in particular the headache was comparable to that of GTN.

Against this background, GTN and h α CGRP produced widely different effects. Perhaps most striking was that GTN lowered systemic blood pressure with an accompanying modest increase in heart rate while h α CGRP had no effect on blood pressure but produced a substantial tachycardia suggesting direct positive chronotropic properties. The failure of h α CGRP to lower the blood pressure in normal volunteers cannot therefore be explained simply on compensatory mechanisms. As indicated above there is no doubt that h α CGRP can act as a vasodilator when administered locally in man (McEwan *et al.*, 1986, 1987). In addition, the marked facial flushing presumably reflects cutaneous vasodilation. One possible explanation for h α CGRP not reducing blood pressure is that the peptide had different effects in different vascular beds. This is supported by our own observations and the findings of others. In the present study h α CGRP was found to increase selectively the blood velocity in common and internal carotid arteries (Table 3) and to increase PI in the internal carotid arteries (Table 2). The increased velocity could be attributed to positive inotropic effects as described *in vitro* (Haass & Skofitsch, 1985) combined with vasodilatation in the vessels concerned. The different PI measurements in the two carotid arteries presumably reflect differences in the extent of vasodilatation.

Differential pharmacological effects of this kind have been reported before. For example, serotonin infused directly into the internal or external carotid artery in anaesthetized baboons produces opposite effects, increasing blood flow through the external carotid and decreasing blood flow in the internal carotid (Deshmukh & Harper, 1973). More recently, phenytoin and dihydropyridines have been shown to relax the basilar artery selectively (Gerthoffer *et al.*, 1987). Further support for this possibility comes from observations that in the Long Evans rats, the rat form of CGRP causes differential vaso-

dilatation (Struthers *et al.*, 1986). We would therefore, propose that human CGRP in the doses used in this study produces selective vasodilation in man with the affected vessels including cerebral arteries and those supplying the skin of the head and neck.

This study indicates that blood velocity waveform data can be used to provide interesting information with regard to clinical pharmacology. However, it must be remembered that conventional interpretation of changes in PI with regard to pathological situations cannot be readily transferred to interpretation of pharmacology. Thus, an increase in PI is generally taken to indicate an increase in downstream impedance to flow, with consequent reduction in diastolic velocity and increase in the PI ratio. In this situation the abnormal vessel is downstream to that which is being insonated with the Doppler beam. However, when a drug is given systemically far more general effects can be expected: in

particular, the vessel being insonated might itself be influenced by the drug or the entire vascular bed might have an increased or decreased resistance to flow. In this case the systolic and diastolic velocities may change quite considerably but in the same proportion which will result in no change in PI. In conclusion, we have found that in contrast to GTN which lowers blood pressure with a very modest change in heart rate, CGRP produces no change in blood pressure at a time when there is a substantial tachycardia. It is possible that the tachycardia is purely the result of compensatory mechanisms but we suggest that hαCGRP also has chronotropic and inotropic effects in man. In addition, we propose that hαCGRP acts as a selective vasodilator in man.

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References

- Brain, S. D., Williams, T. J., Tippins, J. R., Morris, H. R. & McIntyre, I. (1985). Calcitonin gene-related peptide is a potent vasodilator. *Nature*, **313**, 54–56.
- Deshmukh, V. D. & Harper, A. M. (1973). The effect of serotonin on cerebral blood flow with possible implications in migraine. *Acta Neurol. Scand.*, **49**, 649–658.
- Fisher, L. A., Kikikiwa, D. O., Rivier, J. E., Amara, S. A., Evans, R. M., Rosenfield, M. G., Vale, W. W. & Brown, M. R. (1983). Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide. *Nature (London)*, **305**, 534–536.
- Gerthoffer, W. T., Shafer, P. G. & Taylor, S. (1987). Selectivity of phenytoin and dihydropyridine calcium channel blockers for relaxation of the basilar artery. *J. cardiovasc. Pharmac.*, **10**, 9–15.
- Gosling, R. G. & King, D. H. (1974). Arterial assessment by Doppler Shift Ultrasound. *Proc. Roy. Soc. Med.*, **67**, 447–449.
- Haass, M. & Skofitsch, G. (1985). Cardiovascular effects of calcitonin gene-related peptide in the pithed rat: comparison with substance P. *Life Sci.*, **37**, 2085–2090.
- Howden, C. W., Logue, C. M., Gavin, K., Colvie, L. & Rubin, P. C. (1988). Haemodynamic effects of intravenous human calcitonin gene-related peptide in man. *Clin. Sci.*, **74**, 413–418.
- Hughes, A., Nielson, H., Thorns, E., Martin, G. & Sever, P. (1987). Differences between human resistance and conduit arteries. *J. Hypertension Abstracts: Annual Scientific Meeting of British Hypertension Society*.
- Jacobs, J. W., Goodman, R. H., Chin, W. W., Dee, P. C., Habener, J. F., Bell, N. M. & Potts, J. T. (1981). Calcitonin messenger RNA encodes multiple polypeptides in a single precursor. *Science*, **213**, 457–459.
- Kubota, M., Moseley, J. M., Butters, S. B., Dusting, G. J., Macdonald, P. J. & Martin, T. J. (1985). Calcitonin gene-related peptide stimulates cyclic AMP formation in rat aortic smooth muscle cells. *Biochem. Biophys. Res. Comm.*, **132**, 88–94.
- McEwan, J., Larkins, S., Davies, G., Chierchia, S., Brown, M., Stevenson, J., MacIntyre, I. & Maseri, A. (1986). Calcitonin gene-related peptide: a potent dilator of human epicardial coronary arteries. *Circulation*, **14**, 1243–1247.
- McEwan, J. R., Benjamin, N., Larkins, T., Fuller, R. W., MacIntyre, I. & Dollery, C. T. (1987). A comparison of the effects of substance P and calcitonin gene-related peptide in resistance vessels and veins in man. *Br. Heart J.*, **57**, 586.
- Mulderry, P. K., Ghatei, M. A. & Rodrigo, J. (1985). Calcitonin gene-related peptide in cardiovascular tissues of the rat. *Neuroscience*, **14**, 947–954.
- Steenbergh, P. H., Hoppener, J. W. M., Zandberg, J., Lips, C. J. H. & Jansz, H. S. (1985). A second human calcitonin/CGRP gene. *FEBS Letters*, **183**, 403.
- Struthers, A. D., Brown, M. J., MacDonald, D. W. R., Beacham, J. L., Stevenson, J. C., Morris, H. R. & MacIntyre, I. (1986). Human calcitonin-gene related peptide: a potent endogenous vasodilator in man. *Clin. Sci.*, **10**, 389–393.
- Thom, S. McG., Hughes, A. D., Goldberg, P., Martin, G., Schachter, M. & Sever, P. S. (1987). The actions of calcitonin gene-related peptide and vasoactive intestinal peptide as vasodilators in man *in vivo* and *in vitro*. *Br. J. clin. Pharmac.*, **24**, 139–144.
- Trudinger, B. J. (1987). Umbilical artery flow velocity waveforms in high-risk pregnancy. *Lancet*, **i**, 188–190.

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