Calcitonin Gene-Related Peptide Inhibits Human Platelet Aggregation

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Calcitonin gene-related peptide (CGRP) is a potent vasodilator in humans. CGRP receptors have also been found in various tissues other than blood vessels, such as the central nervous system, kidney, and heart. However, little is known about the effects of CGRP on human platelets. We investigated the effect of CGRP (human α type) on platelet aggregation in 21 healthy subjects (9 men and 12 women, mean age 54.6 years). CGRP inhibited platelet aggregation in vitro in 19 of the subjects (90.5%) in a dose-dependent manner with 50% inhibitory doses of $1.6 \mu\text{mol/L}$ and $1.1 \mu\text{mol/L}$ for aggregation induced by epinephrine and collagen, respectively. ^{125}I -labeled CGRP bound specifically to intact platelets. The dissociation constant (K_d) was $61.9 \pm 17.7 \text{ pmol}$ and the maximum number of binding sites (B_{max}) was $6.4 \pm 3.9 \text{ pmol}/10^9$ platelets. The CGRP analogue (8-37)CGRP, but not calcitonin, inhibited the binding of ^{125}I -CGRP to platelets. CGRP ($5 \mu\text{mol/L}$), but not (8-37)CGRP or calcitonin, increased the platelet cyclic AMP (cAMP) concentration by $31.7 \pm 3.6\%$. Thus, CGRP inhibits platelet aggregation via a specific receptor and by increasing the platelet cAMP concentration. CGRP may play a role in the modulation of platelet function in humans.

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CALCITONIN gene-related peptide (CGRP) is a 37-amino acid peptide that is produced as a result of alternative processing of RNA transcribed from the calcitonin gene.^{1–3} Receptors for CGRP have been characterized in a variety of tissues, including the central nervous system, pituitary and adrenal glands, kidney, bone, pancreas, spleen, heart, and blood vessels.^{4–11} CGRP is a potent vasodilator in animal and human vessels,^{12–18} including the coronary

arteries.^{19,20} CGRP has been detected in picomolar concentrations in the circulation,¹⁸ suggesting that it affects vessels and other tissues. Infusion of CGRP into coronary arteries induces dilation of both large and small vessels.^{19,20} Since platelets express receptors for vasoactive peptides such as angiotensin II^{21,22} and vasopressin,²³ as well as α^2 -adrenergic receptors,²⁴ all of which are present in the extrasynaptic smooth muscle cell membrane, it is important to determine if CGRP affects platelet function. In this study, we investigated whether human platelets express CGRP binding sites and the effect of CGRP on platelet aggregation.

Key words:

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METHODS

Platelet Isolation

Twenty one healthy adult volunteers (9 men and 12 women; mean age 54.6 years, range 24 to 76 years) were included in the study. Except for borderline hypertension, the subjects had no other known chronic or acute disease, and they denied that they had taken drugs known to affect platelet function for at least 1 week prior to blood sampling. Blood was removed into a 1/10 volume of 3.8% sodium citrate to prevent coagulation. Platelet-rich and platelet-poor plasma were prepared by centrifugation of the blood at room temperature at 110 g for 10 min, as described elsewhere²⁵

Platelet Aggregation

Platelet aggregation was assayed turbidometrically with a computerized multi-channel analyzer (Niko Bioscience, Tokyo, Japan), and was induced by epinephrine (0.5 nmol/L to 5 mmol/L) or collagen (0.1 ng/ml to 10 µg/ml). Threshold values for aggregation, defined as the lowest concentration of epinephrine or collagen that induced irreversible aggregation which produced at least an 80% difference in light transmission between platelet-rich plasma and platelet-poor plasma, were determined. The 50% effective dose (ED₅₀) was determined as the concentration that induced 50% aggregation in the aggregometer.

The effect of CGRP on platelet aggregation was determined in a cuvette containing 200 µL of platelet-rich plasma. After preincubation with 25 µL of various concentrations (0.5 µmol/L to 0.5 mmol/L) of CGRP, (8-37) CGRP, calcitonin, CGRP plus (8-37) CGRP, or CGRP plus calcitonin for 5 to 10 min at room temperature, platelets were challenged with 25 µL of the various inducers at the respective ED₅₀ concentration, and platelet aggregation was recorded continuously. The 50% inhibitory dose (ID₅₀) was determined as the concentration that resulted in half the estimated maximum response (EMR/2).

Measurement of cAMP and cGMP

Platelets were incubated for 10 min at 37 °C in the presence of CGRP, (8-37) CGRP, CGRP plus (8-37) CGRP, calcitonin, or

CGRP plus calcitonin at a concentration of 5 µmol/L. The reaction was terminated by the addition of ice-cold trichloroacetic acid to a final concentration of 6% (w/v). After centrifugation (2500×g at 4 °C for 10 min), the acid was extracted from the supernatant by incubation four times with 5 volumes of water-saturated ether. Samples were then frozen and lyophilized to remove all remaining ether. After the samples were reconstituted in distilled water, cAMP and cGMP were determined by radioimmunoassay (Du Pont, Boston, USA)²⁶ with ¹²⁵I-labeled cyclic nucleotides as tracers. All measurements were performed in duplicate.

Binding Assay

In saturation binding experiments, platelets were incubated in a final volume of 250 µL with various concentrations of ¹²⁵I-labeled CGRP (0.5 to 100 pmol; specific activity, ~2000 Ci/mmol, Amersham Co). Nonspecific binding was measured in the presence of 50 µmol/L unlabeled CGRP. Incubations were performed at 22 °C for 60 min with constant agitation. Separation of bound and free radioactivity was achieved by rapid filtration through polyethylenimine-treated Whatman GF/C filters that had been previously soaked with 0.5 ml of assay buffer. Filters were washed twice with 3 ml of assay buffer and then allowed to dry. Filter-associated radioactivity was determined in a gamma counter. The overall procedure has been described previously²⁷

Materials

CGRP and (8-37) CGRP were synthesized by Asahi Chemical Co (Tokyo, Japan). Epinephrine and prostaglandin I₂ (PGI₂) were obtained from Sigma (St Louis, USA); collagen was from Hormon-chemie (Munich, Germany); and ¹²⁵I-CGRP and ¹²⁵I-labeled calcitonin were from Amersham (Amersham, UK).

Statistical Analysis

Data are presented as means ± SD unless otherwise indicated. Comparisons were made with Student's paired t test and the Mann-Whitney test. A p value of <0.05 was considered statistically significant.

Fig 1. Effects of epinephrine and collagen on platelet aggregation.

(A and C) Aggregation of platelets from a representative subject in the presence of four different concentrations of epinephrine and collagen, respectively.

(B and D) The corresponding dose-response curves.

Values are means $\pm$ SD of 21 subjects.

RESULTS

Platelet Aggregation

The aggregation of platelets from a representative subject in the presence of various concentrations of epinephrine and collagen is shown in Fig 1A and 1C, respectively. Platelet aggregation increased in a dose-dependent manner for both compounds. The ED_{50} of epinephrine and collagen was $5 \mu\text{mol/L}$ and $0.05 \mu\text{g/ml}$, respectively (Fig 1B and 1D).

Inhibition of Platelet Aggregation

CGRP at various concentrations ($0.5 \mu\text{mol/L}$ to 0.5mmol/L) inhibited epinephrine ($5 \mu\text{mol/L}$)- and collagen ($0.05 \mu\text{g/ml}$)-induced platelet aggregation in 19 (90.5%) of the 21 patients in a dose-dependent manner (Figs 2 and 3). Platelet aggregation was not inhibited by CGRP in the remaining

2 patients even at higher concentrations. Although the backgrounds of the inhibition and non-inhibition cases were nearly matched, including hematological characteristics, the level of fibrinogen was higher in the inhibition cases. The red blood cell count and hematocrit were higher in the non-inhibition cases (data is not shown). Neither the CGRP analogue (8-37) CGRP nor calcitonin aggregated platelets or inhibited platelet aggregation (calcitonin's data is not shown). The ID_{50} of CGRP for epinephrine- and collagen-induced aggregation was $1.6 \mu\text{mol/L}$ and $1.1 \mu\text{mol/L}$, respectively. PGI_2 ($5 \mu\text{mol/L}$ to 0.5mmol/L) also inhibited epinephrine- and collagen-induced platelet aggregation, with an ID_{50} of $4.4 \mu\text{mol/L}$ and $3.8 \mu\text{mol/L}$, respectively.

Cyclic AMP and cGMP Levels in Platelets

The effects of CGRP, (8-37) CGRP,

Fig 2. Effect of CGRP on platelet aggregation induced by epinephrine (A) or collagen (B).

Fig 3. Effects of CGRP and (8-37) CGRP on platelet aggregation induced by epinephrine and collagen. (□) CGRP plus collagen; (○) CGRP plus epinephrine; (●) (8-37) CGRP plus epinephrine; (■) CGRP plus (8-37) CGRP plus epinephrine. Values are means ± SD of 17 subjects. *p < 0.05, **p < 0.01 vs control.

CGRP plus (8-37) CGRP, calcitonin, and CGRP plus calcitonin, all at a concentration of 5 μmol/L, on the platelet cAMP concen-

Fig 4. Effects of CGRP, (8-37) CGRP, and calcitonin on platelet cAMP concentration. All agents were present at a concentration of 5 μmol/L. Values are means ± SD of 8 subjects. *p < 0.05 vs control.

tration were determined (Fig 4). CGRP increased the cAMP concentration by $31.7 \pm 3.6\%$ ($p < 0.05$). (8-37) CGRP alone and calcitonin each had no effect on the cAMP concentration. However, while (8-37) CGRP inhibited the CGRP-induced increase in this cyclic nucleotide, calcitonin did not. Isoproterenol and PGI₂ both increased the cAMP concentration (data not shown) in a manner that was not sensitive to (8-37) CGRP (16.1 ± 3.2 pmol/10⁹ platelets in the presence of (8-37) CGRP). The platelet cGMP concentration (control) was 2.6 ± 0.8 pmol/10⁹ platelets, and this was not affected by CGRP (2.7 ± 1.1 pmol/10⁹ platelets) or (8-37) CGRP (2.6 ± 0.6 pmol/10⁹ platelets).

¹²⁵I- CGRP Binding Assay

¹²⁵I-CGRP bound specifically to platelets (Fig 5). The maximum number of CGRP binding sites (B_{max}) was 6.4 ± 3.9 pmol/10⁹ platelets, and the dissociation constant (K_d) was 61.9 ± 17.7 pmol (values are means ± SD of six cases). Neither isoproterenol nor PGI₂ affected CGRP binding on platelets.

Displacement Curve

We examined the competition of CGRP, (8-37) CGRP, and calcitonin with 50 pmol ¹²⁵I-CGRP for binding to human platelets in six subjects (Fig 6). Whereas CGRP and (8-37) CGRP showed similar competition curves, calcitonin did not compete with ¹²⁵I-CGRP.

Fig 5. Binding of ^{125}I -CGRP to intact human platelets. (A) Representative saturation curve for the binding of ^{125}I -labeled CGRP to platelets. (B) Scatchard analysis. Nonspecific binding was determined in the presence of $50 \mu\text{mol/L}$ unlabeled CGRP.

Fig 6. Competition of CGRP, (8-37) CGRP, and calcitonin with $50 \text{ pmol } ^{125}\text{I}$ -CGRP for binding to human platelets. Values are means $\pm$ SD of 6 cases.

DISCUSSION

Intracoronary platelet aggregation and coronary vasospasm are important pathogenic factors that result in angina and myocardial infarction in humans. Thus, interactions between aggregating platelets and the vascular wall appear to be critical in these conditions.

Our results have shown that CGRP inhibits the aggregation of human platelets (in 90.5% of those incubated) induced by collagen and epinephrine in vitro. CGRP is

an extremely potent vasodilator. CGRP at 10^{-12} to 10^{-9} mol/L causes relaxation of isolated arteries without any interaction with the endothelium. CGRP inhibits platelet aggregation at higher concentrations of 10^{-7} to 10^{-5} mol/L , and is thus about as potent as PGI_2 .

We have shown that CGRP increases the cyclic AMP concentration in human platelets, while it has no effect on cyclic GMP accumulation. (8-37) CGRP, an inhibitor of CGRP, inhibited both cyclic AMP formation and the inhibitory effect of CGRP on platelet aggregation, while calcitonin had similar effects. As in smooth muscle and endothelial cells,²⁸ specific CGRP receptors in platelets appear to be functionally coupled to the adenylate cyclase system. Since prostacyclin increases the cellular cyclic AMP concentration, and the β -receptor agonist isoproterenol inhibits platelet aggregation²⁷ it seems likely that cyclic AMP contributes to the inhibitory effect of CGRP on platelet aggregation.

The B_{max} value in non-inhibition cases tended to be lower than that in inhibition cases. In addition, the dissociation constant for ^{125}I -CGRP in non-inhibition cases was similar to that in inhibition cases. There were no statistically significant differences between the B_{max} and K_d values in inhibition and non-inhibition cases because there were so few non-inhibition cases. However, these

data suggest that inhibition cases may have more platelet CGRP receptors than non-inhibition cases. We demonstrated a decrease in platelet CGRP receptor density and resistance to the antiaggregatory effects of CGRP in non-inhibition cases. Therefore, in non-inhibition cases, resistance to the antiaggregatory effects by CGRP may be related to receptor changes and low cyclic AMP production in platelets. Age and sex were not correlated with the inhibition of platelet aggregation or with receptor binding. However, the stimulatory effect of CGRP on cyclic AMP was less than that of isoproterenol. The partial success of inhibition (90.5%) in samples incubated with CGRP may suggest that an increase in cyclic AMP alone is not sufficient for the CGRP-mediated inhibition of platelet aggregation. Subjects with platelet inhibition had a higher level of plasma fibrinogen. These results suggest that CGRP may have another unknown mode of action, as has been suspected for other plasmic components.

Distinct receptors for CGRP have been identified in a variety of tissues and organs⁴⁻¹¹ including not only the heart, vessels, central nervous system, and kidneys, but also circulating blood cells such as lymphocytes and macrophages^{29,30}. Our present study demonstrates the presence of specific binding sites for CGRP in human platelets. The competitive binding experiment showed that CGRP and (8-37) CGRP exhibited similar binding affinities, whereas calcitonin did not inhibit the binding of ¹²⁵I-CGRP to platelets. Thus, CGRP appears to interact with specific receptors in human platelets, which, despite the structural similarity between CGRP and calcitonin molecules, do not recognize calcitonin. The interaction of calcitonin and CGRP with distinct receptor sites has been demonstrated in the central nervous system and kidney^{4,5}. The K_d values for the binding of ¹²⁵I-CGRP to CGRP binding sites, or the 50% inhibitory concentration of CGRP for inhibiting the binding of ¹²⁵I-CGRP, have been shown to be 0.4 to 20 nmol/L in peripheral tissues^{6,9}, 0.1 to 1 nmol/L or 9 to 15 pmol in the brain, 34 pmol in heart ventricle, and 85 pmol in heart atrium^{8,10}. Thus, our data demonstrate the presence of specific, high-affinity CGRP binding sites in human platelets. The

concentration of circulating CGRP has been reported to be 10 pmol to 100 pmol¹⁸ and is increased in congestive heart failure and myocardial infarction³¹⁻³³. The K_d values platelets were very close to the concentration of CGRP in blood. Hence, CGRP may prevent platelet aggregation in response to circulating catecholamine and angiotensin II, especially in myocardial infarction, in concert with a vasodilatory effect. Epinephrine and collagen produce strong aggregation *in vitro*. Therefore, a higher CGRP concentration is required to produce 50% inhibition of platelet aggregation against these potent aggregatory agents than that which is required for almost complete inhibition of ¹²⁵I-CGRP binding at a concentration of 10^{-7} mol/L.

CGRP has been successfully used to prevent vasospasm in subarachnoid bleeding³³⁻³⁵. In the heart, intracoronary CGRP infusions produce a dose-dependent increase in the diameter of coronary arteries, which suggests that CGRP regulates coronary vascular smooth muscle tone²⁰. The number of CGRP receptors is reduced in areas of atheroma in the main coronary arteries¹¹, which possibly predisposes these vessels to spasm and thrombosis. Since CGRP prevents platelet aggregation, as described in the present study, CGRP may inhibit vasospasm and thrombus formation, and should play a role in preventing coronary restenosis.

In summary, we have shown that CGRP inhibits the aggregation of human platelets, and that this effect may be mediated by interaction of the peptide with specific receptors, and by a subsequent increase in the platelet cyclic AMP concentration. CGRP may thus be an important modulator of platelet function.

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REFERENCES

1. Rosenfeld MG, Amara SG, Roos BA, Ong ES, Evans RM: Altered expression of the calcitonin gene associated with RNA polymorphism. *Nature* 1981; **290**: 63-65

2. Amara SG, Jonas V, Rosenfeld MG, Ong ES, Evans RM: Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature* 1982; **298**: 240–244
3. Rosenfeld MG, Mermod J, Amara SG, Swanson LW, Sawchenko PE, Rivier J et al: Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. *Nature* 1983; **304**: 129–135
4. Tschopp FA, Henke H, Petermann JB, Tobler PH, Janzer R, Hokfelt T et al: Calcitonin gene-related peptide and its binding sites in the human central nervous system and pituitary. *Proc Natl Acad Sci USA* 1985; **82**: 248–252
5. Goltzman D, Mitchell J: Interaction of calcitonin and calcitonin gene-related peptide at receptor sites in target tissues. *Science* 1985; **227**: 1343–1345
6. Seifert H, Sawchenko P, Chesnut J, Rivier J, Vale W, Pandolfi SJ: Receptor for calcitonin gene-related peptide; binding to exocrine pancreas mediates biological actions. *Am J Physiol* 1985; **249**: G147–151
7. Wohlwend A, Malmstrom K, Henke H, Murer H, Vassalli JD, Fisher JA: Calcitonin and calcitonin gene-related peptide interact with the same receptor in cultured LLC-PK1 kidney cells. *Biochem Biophys Res Commun* 1985; **131**: 537–542
8. Henke H, Tschopp FA, Fisher JA: Distinct binding sites for calcitonin gene-related peptide and salmon calcitonin in rat central nervous system. *Brain Res* 1985; **360**: 165–171
9. Sigrist S, Franco CA, Muff R, Henke H, Lundberg JM, Fischer JA: Specific receptor and cardiovascular effects of calcitonin gene-related peptide. *Endocrinology* 1986; **119**: 381–389
10. Yoshizaki H, Takamiya M, Okada T: Characterization of picomolar affinity binding sites for [¹²⁵I]-human calcitonin gene-related peptide in rat brain and heart. *Biochem Biophys Res Commun* 1987; **146**: 443–451
11. Coupe MO, Mak JCW, Yacoub M, Oldershaw PJ, Barnes PJ: Autoradiographic mapping of calcitonin gene-related peptide receptors in human and guinea pig hearts. *Circulation* 1990; **81**: 741–747
12. Fisher LA, Kikkawa DO, River JE, Amara SG, Evans RM, Rosenfeld MG et al: Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide. *Nature* 1983; **305**: 534–536
13. Toppins JR, Morris HR, Panico M, Etienne T, Bevis P, Girgis S et al: The myotropic and plasma-calcium modulating effects of calcitonin gene-related peptide (CGRP). *Neuropeptide* 1984; **4**: 425–434
14. Brain SD, Williams TJ, Toppins JR, Morris HR, MacIntyre I: Calcitonin gene-related peptide is a potent vasodilator. *Nature* 1985; **313**: 54–56
15. Edvinsson L, Fredholm BB, Hamel E, Jansen I, Verrecchia C: Perivascular peptides relax cerebral arteries concomitant with stimulation of cyclic adenosine monophosphate accumulation or release of an endothelium-derived relaxing factor in the cat. *Neurosci Lett* 1985; **58**: 213–217
16. Haass M, Skofitsch G: Cardiovascular effects of calcitonin gene-related peptide in the pithe rat. comparison with substance P. *Life Sci* 1985; **37**: 2085–2090
17. Kubota M, Moseley JM, Butera L, Dusing GJ, MacDonald PS, Maritin TJ: CGRP stimulated cyclic AMP formation in rat aortic smooth muscle cells. *Biochem Biophys Res Commun* 1985; **132**: 88–94
18. Struthers AD, Brown MJ, MacDonald DWR, Beacham JL, Stevenson JC, Morris HR, MacIntyre I: Human calcitonin gene-related peptide: a potent endogenous vasodilator in man. *Clin Sci* 1986; **70**: 389–393
19. Holman JJ, Craig RK, Marshall I: Human alpha and beta CGRP and rat alpha CGRP are coronary vasodilators in the rat. *Peptide* 1986; **7**: 231–235
20. McEvan J, Larkin S, Davies G, Chierchia S, Brown M, Stevenson J et al: Calcitonin gene-related peptide: a potent dilator of human epicardial coronary arteries. *Circulation* 1986; **74**: 1243–1247
21. Moore TJ, Williams GH: Angiotensin II receptors in human platelets. *Circ Res* 1982; **51**: 314–320
22. Ding YA, Kenyon CJ, Semple PF: Receptors for angiotensin II on platelets from man. *Clin Sci* 1984; **66**: 725–731
23. Thibonnier M, Bourne HR, Roberts JM: Human platelet vasopressin receptors are of the V1-vascular type [Abstract]. *Circulation* 1984; **70**(Suppl II): II-70
24. Motulsky HJ, Shattil SJ, Insel PA: Characterization of E₀₂-adrenergic receptors in human platelets using [³H]-yohimbine. *Biochem Biophys Res Commun* 1980; **97**: 1562–1570
25. Lou HC, Dalsgrand-Nielsen NJ, Bomholdt A, Gormsen J: Platelet hyperaggregability in young patients with completed stroke. *Acta Neurol Scand* 1978; **56**: 326–334
26. Steiner AL, Parker CW, Kipnis DM: Radioimmunoassay for cyclic nucleotides. I. preparation of antibodies and iodinated cyclic nucleotides. *J Biol Chem* 1972; **247**: 1106–1113
27. Kroll MH, Schafer AI: Biochemical mechanisms of platelet activation. *Blood* 1989; **74**: 1181–1195
28. Hirata Y, Takagi Y, Takata S, Fukuda Y, Yoshimi H, Fujita T: Calcitonin gene-related peptide in cultured vascular smooth muscle and endothelial cells. *Biochem Biophys Res Commun* 1988; **151**: 1113–1121
29. Wang F, Millet I, Bottomly K, Vignery A: Calcitonin gene-related peptide inhibits interleukin 2 production by murine T lymphocytes. *J Biol Chem* 1992; **267**: 21052–21057
30. Chiba T, Yamaguchi A, Yamatani T, Nakamura A, Morishita T, Inui T et al: Calcitonin gene-related peptide receptor antagonist human-CGRP- (8-37). *Am J Physiol* 1989; **256**: E331–335
31. Anand IS, Gurden J, Wander GS, O'Gara P, Harding SE, Ferrari R et al: Cardiovascular and hormonal effects of calcitonin gene-related peptide in congestive heart failure. *J Am Coll Cardiol* 1991; **17**: 208–217
32. Mair J, Lechleitner P, Langle T, Wiedermann C, Dienstl F, Saria A: Plasma CGRP in acute myocardial infarction. *Lancet* 1990; **335**: 168
33. Lechleitner P, Genser N, Mair J, Dienstl A,

- Haring C, Wiedermann CJ et al: Calcitonin gene-related peptide in patients with and without early reperfusion after acute myocardial infarction. *Am Heart J* 1992; **124**: 1433–1439
34. Johnston FG, Bell BA, Robertson IJA, Miller JD, Haliburn C, O'Shaughnessy D et al: Effect of calcitonin-gene-related peptide on postoperative neurological deficits after subarachnoid haemorrhage. *Lancet* 1990; **335**: 869–872
35. European CGRP in subarachnoid haemorrhage study group: Effect of calcitonin gene-related peptide in patients with delayed postoperative cerebral ischaemia after aneurysmal subarachnoid haemorrhage. *Lancet* 1992; **339**: 831–834