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Cardiovascular Action of Calcitonin Gene-Related Peptide in Humans

Carlo Gennari and Jan A. Fischer

Institute of Medical Semeiotics, University of Siena, 53100 Siena, Italy, and Research Laboratory for Calcium Metabolism, Departments of Orthopedic Surgery (Balgrist) and Medicine, University of Zurich, 8008 Zurich, Switzerland

Summary. Calcitonin gene-related peptide (CGRP) has been localized in cardiac nerve fibers and blood vessels from which it may be released as neurotransmitter or neuromodulator. Acute cardiovascular effects of i.v. administered CGRP have been studied in human subjects. CGRP (25.3 nmol) caused a mean maximal increase of the heart rate of 41 beats per min ($P < 0.01$) and lowered arterial systolic and diastolic pressures by 26 mm Hg and 20 mm Hg, respectively ($P < 0.01$) (n = 6 subjects). These effects were associated with facial flushing, and a rise of plasma levels of norepinephrine and epinephrine of 257 pg/ml and 9 pg/ml, respectively ($P < 0.01$). Administration of equimolar amounts of human calcitonin caused no cardiovascular effects except for minor facial flushing. Serum calcium was marginally lowered with both CGRP (0.2 mg/100 ml) and calcitonin (0.4 mg/100 ml) ($P < 0.05$). Furthermore, CGRP (12.7 nmol) reduced the preejection period and duration of the electromechanical systole by 26 msec and 66 msec, respectively ($P < 0.001$ and $P < 0.01$), presumably acting as positive inotropic agent. Labetalol, blocking adrenergic receptors, obliterated these inotropic effects, whereas the positive chronotropic and hypotensive actions of CGRP remained unchanged.

Key words: Calcitonin — Calcitonin gene-related peptide — Heart — Hypotension.

Calcitonin gene-related peptide (CGRP) is a unique 37 amino acid neuropeptide with potent cardiovascular effects. The existence of CGRP has been predicted by analysis of the nucleotide sequence of the rat and human calcitonin (CT) genes [1, 2], and the amino acid sequence of the human peptide was subsequently derived from extracts of medullary thyroid carcinoma tissue [3]. CGRP has been recognized in the central and peripheral nervous system including the heart [4–7]. Indeed, a specific radioimmunoassay in combination with high-performance liquid chromatography revealed the presence of CGRP predominantly in the rat atrium (unpublished data), and CGRP stained by immunohistochemistry was abundant in nerve fibers around coronary vessels, especially those of the right atrium [5, 7]. Moreover, specific binding sites of CGRP have been recognized in corresponding parts of the heart, notably the atrium (unpublished data). Recently, intracerebroventricular administration of synthetic CGRP has been shown to raise the arterial pressure in rats, concomitant with a stimulation of noradrenergic sympathetic outflow [8]. Intravenous injections and administration of CGPR *in vitro* caused hypotension and a positive inotropic effect in rats [7–9]. Through intracerebroventricular, i.v., and local injections the heart rate was increased in the rat. Intradermal administration in man and rabbits evoked vasodilatation [10].

Here we have assessed cardiovascular effects of i.v. administered CGRP in man through the use of noninvasive procedures, and have recognized positive chronotropic and inotropic effects associated with hypotension and a rise of plasma norepinephrine and epinephrine levels.

Subjects and Methods

Subjects

Twelve subjects were selected for study. Serum levels of calcium, phosphate, alkaline phosphatase, and creatinine were normal and administration of all drugs was stopped at least 4 days prior to the i.v. injection of CGRP or CT. Six subjects (age 41–77 years, 3 females with postmenopausal osteoporosis, 3 males with osteoarthrosis) received 100 μg synthetic human CGRP and 100 μg synthetic human CT, freshly dissolved in 1

Send reprint requests to J. A. Fischer, Klinik Balgrist, Forchstrasse 340, 8008 Zurich, Switzerland.

ml 0.9% NaCl (Table 1). Injections of CGRP preceded those of CT by 3 days in 4 subjects and followed CT in the remaining 2. Six different subjects (age 29–70 years, 1 normal female and 2 females with postmenopausal osteoporosis, 2 normal males and 1 male with osteoporosis) received 50 μg human CGRP, infused in 50 ml 0.9% NaCl from 0–10 min in the absence, and 3 days later in the presence, of 100 mg labetalol infused in 100 ml 0.9% NaCl from minus 15 to 60 min (Fig. 1). Informed consent about the experimental procedure was obtained from all the subjects.

The heart rate and the arterial pressure and facial flushing were monitored, and blood (15 ml) was collected 15 min before, at time "0", and 15, 30, 60, and 120 min after the start of the administration of CGRP and CT. The intensity of readily observable facial flushing was evaluated in arbitrary units according to a severity score ranging from 0 (no effect) to 4 (maximal discomfort).

Ten milliliters of blood was transferred to a plastic tube containing 50 U (USP) of heparin and after centrifugation at 4°C, the plasma was separated and kept frozen at −20°C until assayed within 4 weeks for norepinephrine and epinephrine. The remaining 5 ml of blood was separated after clot retraction and centrifugation, and used for the determination of serum calcium and inorganic phosphorus. In one group of subjects (Fig. 1), the heart rate was also electrocardiographically monitored and the preejection period and duration of the electromechanical systole evaluated [11].

Reagents

Synthetic human CGRP was obtained from Peninsula Laboratories, Belmont, CA, and synthetic human CT from Ciba-Geigy, Basel, Switzerland. Labetalol (Trandate) was purchased from Glaxo, Greenford, UK.

Methods of Analysis

Plasma catecholamines (norepinephrine, epinephrine) were determined by a specific radioenzymatic method (CAT-A-Kit, Upjohn, Kalamazoo, MI). Total serum calcium was measured by atomic absorption spectrophotometry and inorganic phosphorus according to Fiske and Subbarow [12].

Results are expressed as mean values ± SEM. Statistical analysis was done by paired t test and one-way analysis of variance [13].

Results

In response to i.v. injections of CGRP (25.3 nmol), the heart rate was stimulated and the arterial pressure was decreased ($P < 0.01$) (Table 1). Associated with these effects was severe facial flushing ($P < 0.001$), and plasma levels of norepinephrine and epinephrine were raised ($P < 0.01$). In response to 12.7 nmol CGRP rather than 25.3 nmol, the rise of the heart rate and plasma norepinephrine levels, the fall of the blood pressure, and facial flushing were less pronounced, but the difference was only statistically significant for facial flushing ($P < 0.05$) (Table 1, Fig. 1). The structurally related CT (29.3 nmol) caused mild and transient facial flushing, and small rises of plasma norepinephrine ($P < 0.05$) and epinephrine levels ($P < 0.1$), and the heart rate and arterial pressure remained unaltered. Analysis of variance revealed that the CGRP responses at 15, 30, and 60 min were different from those obtained with CT ($P < 0.01$ for systolic arterial pressure and $P < 0.05$ for all remaining parameters). In response to CGRP and CT, marginal falls of serum calcium levels occurred ($P < 0.05$) which could not be statistically differentiated, and serum phosphate remained unchanged (not shown).

Furthermore, i.v. CGRP (12.7 nmol) caused a decrease of the preejection period and of the duration of the electromechanical systole ($P < 0.01$) which reflect enhanced myocardial contractility, and CGRP can be considered as a positive inotropic agent (Fig. 1) [11]. These latter effects were suppressed in the presence of labetalol. The chronotropic effect of CGRP, hypotension and facial flushing, on the other hand, were not affected by labetalol. The rise of plasma levels of norepinephrine and epinephrine, and the small fall of serum calcium concentrations were similar in the presence and absence of labetalol (not shown).

Discussion

Calcitonin gene-related peptide is a neuropeptide recognized in the central and peripheral nervous system, including the heart, of man and rats [4–7]. The presence of CGRP [5, 7] and of its binding sites (unpublished data) has recently been revealed in cardiac tissue, and they are predominantly localized in the atrium. Intravenous injections of CGRP in rats evoked hypotension accompanied by an increase of the heart rate [8]. Both capsaicin which stimulates the release of CGRP from nerve fibers, and CGRP have been shown to cause hypotension and positive inotropic and chronotropic effects in the isolated heart of guinea-pigs, and auricles of rats and man [7, 14]. Intravenous administration of capsaicin was associated with a depletion of immunoreactive CGRP from nerve fibers in the myocardium and around coronary vessels. Recently, CGRP has been reported to cause relaxation of rat aorta strips and an increase of the blood flow in rabbit skin and local reddening in human skin [10]. It appears possible that endogenous CGRP released from nerve fibers is responsible for the characteristic cardiovascular actions.

Here we report potent cardiovascular effects of CGRP *in vivo* in humans. The use of a noninvasive procedure has allowed us to assess left ventricular performance [11], and the findings are compatible with positive inotropic effects of CGRP previously recognized in the isolated perfused guinea-pig heart

Table 1. Comparison of heart rate, arterial pressure, flushing, plasma norepinephrine and epinephrine and serum calcium responses to CGRP and CT

Peptide	Time (min) −15	0	15	30	60	120
Heart rate (min^{-1})						
CGRP	70 ± 3	69 ± 4	111 ± 4[b]	90 ± 5[a]	86 ± 6	75 ± 5
CT	71 ± 4	67 ± 3	71 ± 4	68 ± 4	69 ± 4	69 ± 3
Arterial pressure, systolic (mm Hg)						
CGRP	139 ± 3	135 ± 3	111 ± 4[b]	116 ± 4[b]	116 ± 3[b]	126 ± 4[a]
CT	135 ± 4	135 ± 6	133 ± 5	134 ± 6	131 ± 5	136 ± 4
Arterial pressure, diastolic (mm Hg)						
CGRP	84 ± 4	83 ± 3	63 ± 3[b]	68 ± 3[a]	75 ± 4	74 ± 4[a]
CT	81 ± 4	83 ± 4	83 ± 3	84 ± 4	82 ± 4	83 ± 3
Flushing (arbitrary units)						
CGRP	0	0	3.50 ± 0.22[b]	2.67 ± 0.21[c]	1.67 ± 0.33[c]	0.33 ± 0.21
CT	0	0	0.83 ± 0.31[a]	0.17 ± 0.16	0	0
Plasma norepinephrine (pg/ml)						
CGRP	270 ± 60	266 ± 45	359 ± 50	525 ± 49[b]	550 ± 89[a]	381 ± 59
CT	223 ± 40	238 ± 45	240 ± 24	259 ± 43[a]	240 ± 49	259 ± 51
Plasma epinephrine (pg/ml)						
CGRP	51 ± 5	51 ± 5	60 ± 3[b]	62 ± 7[a]	44 ± 5	42 ± 6
CT	33 ± 8	34 ± 4	37 ± 5	38 ± 5	34 ± 5	35 ± 5
Serum calcium (mg/dl)						
CGRP	9.3 ± 0.2	9.2 ± 0.1	9.1 ± 0.1[a]	9.0 ± 0.2[a]	9.1 ± 0.1	9.1 ± 0.1
CT	9.2 ± 0.2	9.1 ± 0.2	9.0 ± 0.2	8.8 ± 0.2	8.7 ± 0.2[a]	8.9 ± 0.2

I.V. injection of 100 μg CT CGRP and 100 μg CT at time "0" in 6 subjects
Values significantly different from preinjection periods ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$)

[7]. Direct pressure recordings through heart catheterization are required in the future to confirm our findings. The positive inotropic effects (reduction of preejection period and electromechanical systole) were totally suppressed in the presence of labetalol blocking α and β sympathetic receptors [15] and could therefore be caused also by reflex stimulation. Moreover, CGRP induced stimulation of the heart rate, prolonged hypotension and facial flushing, were presumably caused by vasodilatation. In contrast to the positive inotropic effects of CGRP obliterated by labetalol, these later effects were unaltered.

In isolated rat auricles, propranolol, a specific β adrenergic blocking agent [15], was shown to lower the rate and force of contraction, but CGRP reversed the effects in a similar dose-dependent manner as in the absence of propranolol [9]. Metoprolol blocking more selectively β_1 adrenergic receptors [15] did not affect similar CGRP responses in the isolated perfused heart of guinea-pigs [7]. In the same preparation, norepinephrine also caused marked positive inotropic and smaller chronotropic effects than CGRP which were completely suppressed by metoprolol. The associated rise of plasma norepinephrine levels is not necessarily involved in the actions of CGRP, but the detailed mechanism remains to be delineated.

The structurally related CT (see ref. 4) is known to cause facial flushing in man [16]. The action of equimolar amounts of human CT on facial flushing was much less marked than that of human CGRP. Besides, human CT did not exert any other cardiovascular effects when used in the same quantity. The more potent salmon CT, which shares closer structural homology to human CGRP (30%) than human CT (19%), had only marginal inotropic effects in normal subjects and in contrast to CGRP, a small negative chronotropic effect [17]. In the isolated dog atrium, salmon CT had negative chronotropic and inotropic effects [18].

In conclusion, CGRP is a novel neuropeptide with potent cardiovascular properties. The pharmacological and perhaps physiological effects of CGRP remain to be further characterized. To this end, CGRP is released from cardiac nerve fibers in response to i.v. capsaicin which, much like CGRP, has positive inotropic and chronotropic effects [7, 14]. Conceivably, CGRP may act in the heart as neurotransmitter or neuromodulator.

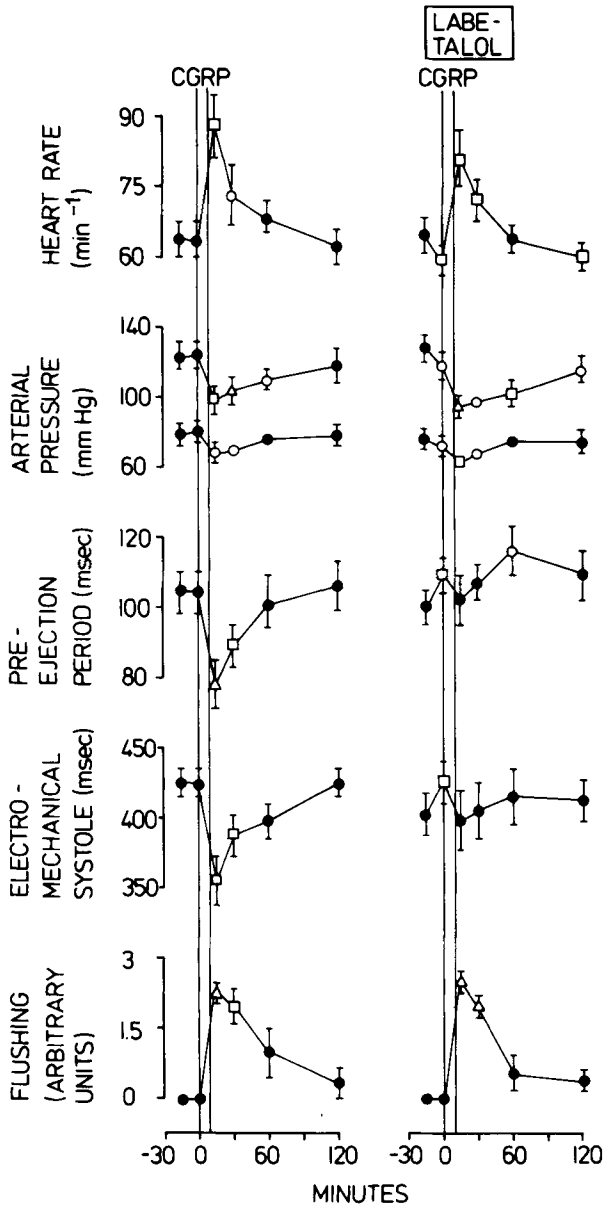

Fig. 1. Effects of CGRP (50 μg, infused from 0–10 min) on 6 subjects in the absence and presence of labetalol (100 mg, infused from minus 15 to 60 min) on heart rate, arterial pressure, preejection period, electromechanical systole, and flushing. *Open symbols* represent statistically significant changes from preinfusion levels (○ = $P < 0.05$, □ = $P < 0.01$, △ = $P < 0.001$), *closed symbols* (● = $P > 0.05$).

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