

Elevated serum levels of calcitonin gene-related peptide in Hymenoptera sting-induced anaphylaxis

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Calcitonin gene-related peptide (CGRP) is a 37 amino acid polypeptide that can be alternatively spliced from the primary RNA transcript of the calcitonin gene. CGRP is predominantly synthesized and stored in sensory neurons and can be released from both central and peripheral axons in which it is often colocalized with other neuropeptides, including the tachykinins-substance P and neurokinin A. In many tissues the CGRP-containing nerves perivascularly form a dense sensory network that appears to account for CGRP's effect on the vascular system. Nonvascular effects include relaxation of smooth muscle in gastrointestinal and urogenital tracts, cotransmission with acetylcholine in some motor neurons, and central and peripheral neurotransmitter activity.¹

The cardiovascular effects associated with CGRP include hypotension, tachycardia, and vasodilation of the coronary arteries. The hypotension mediated by CGRP appears to be the result of the direct interaction of CGRP with its receptors on vascular smooth muscle, whereas its tachycardic effect involves reflex activation of the sympathetic nervous system in addition to its direct positive chronotropic effect. Mechanisms of the response to CGRP include elevation in cyclic adenosine monophosphate levels, nitric oxide production, and direct activation of potassium adenosine triphosphate channels of vascular smooth muscle.² During stepwise continuous infusion of human CGRP into six normal volunteers, Struthers et al.³ noted a drop in diastolic pressure, tachycardia, and increased levels of adrenaline and noradrenaline with increasing doses of CGRP. The half-life was calculated to be 9.7 minutes. On a molar basis, CGRP was found to be a more potent vasodilator in humans than any other endogenous or synthetic compound, including prostacyclin, adrenaline, histamine, vasoactive intestinal polypeptide, and sodium nitroprusside. Butterfield et al.⁴ noted increased levels of CGRP during hypotensive events in a patient with mastocytosis undergoing aspirin desensitiza-

Abbreviation used

CGRP: Calcitonin gene-related peptide

tion. The elevated levels in the blood were thought to represent a *spillover* phenomenon from the CGRP released from the nerve terminals.

Receptors for inflammatory mediators, including bradykinin and histamine, are present on sensory neurons and may further increase CGRP release. CGRP is thought to be the principal vasodilator released after capsaicin stimulation of sensory nerves. However, the mechanisms underlying CGRP release *in vivo* are poorly understood.

We are not aware of CGRP levels measured during allergen-induced anaphylaxis in humans. Because of CGRP's potent vasodilatory effects, perivascular distribution, and elevated levels in patients undergoing desensitization with aspirin in mastocytosis, we wished to extend our observations using stored serum from patients allergic to Hymenoptera venom who had undergone deliberate sting challenges after discontinuing venom immunotherapy.

METHODS

CGRP levels were measured on the stored serum of seven patients who had undergone deliberate sting challenges after stopping venom immunotherapy of 2 to 10 years duration. The sera were stored at -70° C for 13 years. Before the sting challenge, catheters were placed in the hand distal to the sting challenge site, in the antecubital vein proximal to the sting challenge site, and in the contralateral antecubital vein.⁵ Serum samples were obtained at time 0 (immediately before the sting), 2.5, 5, 10, 30, 60, 120, and 240 minutes after the sting. Histamine and tryptase levels were measured in two patients. We have now subsequently measured CGRP levels in these samples.

CGRP levels were measured by radioimmunoassay with a 1:7000 dilution of a locally developed rabbit antibody. The tracer is made from a chloramine-T radiolabelling of synthetic (Tyr-O) rat CGRP-37 (Peninsula Lab, Belmont, Calif.).⁴ Synthetic rat CGRP-37 is used as a standard. The molar cross-reactivity is 100% with human CGRP II, 50% with human CGRP I, 1.4% with CGRP 23-37, and 0.3% with CGRP 28-37. The assay precision is 10.9% at 128 pg/ml and 13.8% at 57 pg/ml. Serum tryptase levels were measured at Virginia Commonwealth University by a previously described method.⁴ Histamine levels were determined by a double isotopic radioenzyme technique.⁵

Because Hymenoptera venom contains vasoactive substances, honeybee dried whole venom obtained from Sigma Chemical Company in 1979 and 1996 was diluted in phosphate-buffered saline in

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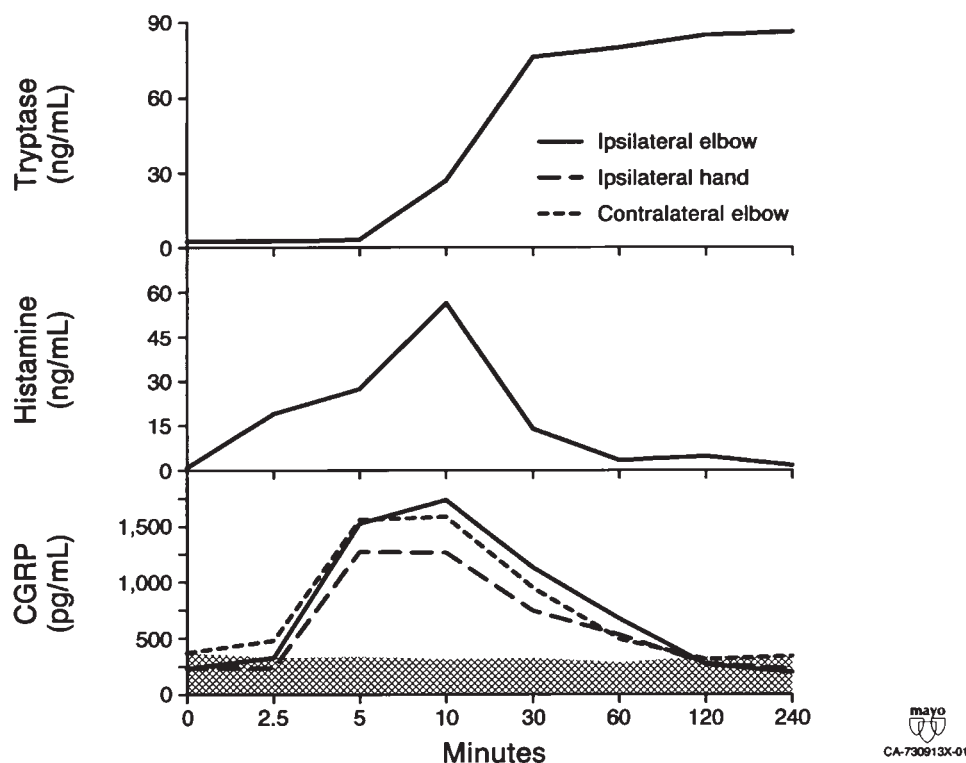


FIG. 1. Serum CGRP levels from three separate sites and serum tryptase and histamine levels from ipsilateral antecubital vein in patient who had anaphylaxis. Shaded area represents average serum CGRP level from three sites for each time point in nonreactors. Samples were available for all six nonreactors at time points 0 to 30 minutes, for five at 60 minutes, for four at 120 minutes, and for one at 240 minutes.

5- to 10-fold dilutions from 10 mg/ml to 1 μ g/ml and also assayed for CGRP.

RESULTS

The CGRP levels in the one patient who had anaphylaxis to the deliberate sting challenge were markedly elevated compared with the levels found in nonreactors (Fig. 1). Her symptoms consisted of oral pruritis at 2 minutes and tongue edema at 5 minutes, and they progressed to wheezing, dyspnea, tachycardia, and marked hypotension, with a systolic blood pressure of 50 mmHg at 10 minutes. She received intravenous fluids and subcutaneous epinephrine, and her vital signs stabilized at 40 minutes. The other six patients did not have any systemic symptoms. The levels of CGRP in each sampling site at each time point for these six patients were averaged and are represented by the shaded area in Fig. 1.

Histamine and tryptase levels were elevated in the patient who had anaphylaxis to the challenge (Fig. 1). The histamine and tryptase levels were not elevated in the nonreactor.

The CGRP levels measured on the bee venom diluted in phosphate-buffered saline at a dilution of 10 mg/ml from 1979 and 1996 were 207 and 251 pg/ml, respectively. Further dilutions revealed a corresponding decrease in CGRP levels and were less than 20 pg/ml (same as control phosphate-buffered saline) in dilutions less than or equal to 500 μ g/ml.

DISCUSSION

The markedly elevated CGRP peaked at the time the patient's symptoms were most severe. In normal subjects the basal level of CGRP ranges from 81 to 367 pg/ml.² Although the sera were stored for 13 years, the levels in the nonreactors were in the normal range, suggesting that there was not a significant decay or additive effect to the CGRP during the storage. CGRP, known for its potent vasodilating effects, likely played a significant role in the hypotension in this patient. The elevation of CGRP was six times the normal value. The values in the reactor were higher than those seen during intravenous infusion of CGRP in volunteers in whom values of 250 pmol/L (987 pg/ml) were noted with a small but statistically significant drop in diastolic pressure and tachycardia.³ The time course of the rise and fall of CGRP paralleled that of histamine, although the CGRP reached near peak levels within 5 minutes and had a slower return to baseline. Although CGRP was present in the venom tested, the quantities were too low to suggest that the elevated serum CGRP level was venom-derived. Interestingly, the elevations in CGRP were nearly equal in the antecubital vein on the side of the sting site and the contralateral arm. This finding suggests a systemic release of the CGRP as opposed to only a localized release at the site of the sting. Although the stimuli for CGRP release are unknown, theoretically the release could be either a direct consequence of the antigen-antibody reaction or result from

the release of primary mediators, which in turn stimulate the sensory nerves to release CGRP. In a study of allergen-induced anaphylaxis in a guinea pig heart, histamine and products of the cyclooxygenase pathway appeared to initiate release of CGRP that doubled from baseline and correlated with hypotension.⁶ In that study prechallenge administration of H₁ and H₂ antagonists and the cyclooxygenase inhibitor indomethacin attenuated the release of CGRP, whereas pretreatment with the 5-lipoxygenase inhibitor had no significant effect. Taken together, it is possible that histamine and prostaglandins released after an allergic response are significant triggers for the release of CGRP.

Our findings suggest that CGRP may play a pathophysiologic role in human anaphylaxis. Further knowledge of the mechanisms of its release and subsequent attenuation will be valuable in the understanding and treatment of anaphylaxis. More definitive data may be obtained in future studies involving larger numbers of patients.

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