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Rapid Communication

The Demonstration of Vasodilator Activity of Pancreatic Amylin Amide in the Rabbit

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Amylin amide, a 37-amino acid peptide that is a major component of amyloid deposits in the diabetic pancreas, possesses vasodilator activity. Human synthetic amylin amide (30 to 300 pmol/site) stimulated a dose-dependent increase in blood flow after intradermal injection in rabbit skin. Amylin amide was 100 times less active than the structurally related potent vasodilator neuropeptide calcitonin gene-related peptide. Amylin amide did not induce edema formation; however, as a consequence of its vasodilator activity, amylin amide potentiated edema formation induced in rabbit skin by bradykinin. The intravenous injection of amylin amide (10 nmol) caused a systemic drop in blood pressure. This study demonstrates that amylin amide elicits vasodilator responses in vivo. ***It is possible that the release of amylin amide from the pancreas in type II diabetes could lead to changes in vascular tone. (Am J Pathol 1990, 136:487–490)***

Amyloid deposits are found in the islets of Langerhans of people suffering from type II diabetes.[1] A major component of amyloid tissue recently has been shown to be the novel peptide amylin amide (also known as diabetes-associated peptide [DAP] and islet-amylated polypeptide [IAPP]).[2–6] Amylin amide has a 46% structural similarity with the neuropeptide calcitonin gene-related peptide (CGRP).[2] CGRP is a 37-amino peptide produced by alternative processing of the RNA during calcitonin gene expression.[7,8] Unlike calcitonin, CGRP is a neuropeptide and widespread distribution of CGRP has been demonstrated in both the central and peripheral nervous system.[9] The potent vasodilator activity of CGRP was discovered when CGRP was injected intradermally into animal skin.[10] Subsequent studies have revealed its activity in human skin[10,11] and when infused intra-arterially into the human forearm and human epicardial coronary arteries.[12–14] When infused intravenously CGRP can stimulate a hypotensive response in humans.[15] CGRP is considered to play an important role in the control of blood flow and pressure. In this study we have investigated the possibility that amylin amide could possess vasodilator activity, based on the structural similarity to CGRP.

Materials and Methods

Animals

Male New Zealand White rabbits were purchased from Regal Rabbits, Great Bookham, Surrey, UK. For all experiments rabbits were anesthetized with pentobarbitone sodium (Sagatal, 30 mg/kg intravenously) and anesthesia was maintained with pentobarbitone sodium (10 mg/kg intravenously every 30 minutes).

Measurement of Microvascular Blood Flow in Skin

Blood flow changes in response to the intradermal injection of agents under test were measured in the shaved dorsal skin of New Zealand White rabbits (2 to 2.5 kg) using a multiple-site xenon clearance technique.[16] Briefly, human amylin amide (30 to 300 pmol/site) and human alpha CGRP (1 to 10 pmol/site) were prepared immediately before use in sterile saline. An equal amount of ^{133}Xe (5 to 10 μCi) was added to each 1-ml injection sample

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and test agents were then rapidly injected into rabbit skin with six replicate injections for each treatment. The dorsal skin was divided into six blocks with an injection site for each treatment in each block. Injection sites were at least 30 mm apart and treatments were allocated to sites using a balanced site pattern. Animals were killed by sodium pentobarbitone overdose after a clearance period of 15 minutes and skin punch sites (16-mm diameter) were counted for radioactivity. Blood flow was calculated as the percentage change compared with blood flow at saline control-injected sites.[16]

Measurement of Edema Formation in Skin

Edema formation was measured in multiple sites in skin. Edema formation in response to intradermally injected agents was measured during a 30-minute period as the local extravascular accumulation of intravenously injected ^{125}I-albumin.[16]

Measurement of Blood Pressure

The central ear artery was cannulated using a 20-gauge plastic catheter. Blood pressure was monitored continuously *via* a pressure transducer attached to a Lectromed multi-trace recorder. Test agents were composed in sterile saline and injected *via* the marginal ear vein of the corresponding ear. Bolus injections of test agents were injected in 100 μl volumes during a 15-second period followed in each case by 400 μl of saline.

Materials

Lyophilised diabetes-associated peptide amide, also known as amylin amide, was purchased from Bachem Ltd. (Essex, UK). The amylin amide was dissolved and diluted in sterile saline immediately before experiments. Human alpha CGRP was a gift from Dr. U. Ney (Celltech, Bucks, UK) and was synthesized according to the established structure of human alpha CGRP extracted and purified from human medullam thyroid carcinoma tissue.[17] Substance P was purchased from Sigma Chemical Co. Ltd. (Dorset, UK). Sterile pyrogen-free saline was from Travenol Laboratories (Thetford, UK). ^{133}Xenon (10 mCi in 3 ml sterile saline) was from Amersham International (Bucks, UK).

Results

Synthetic amylin amide (30 to 300 pmol/site) produced a dose-dependent increase in microvascular blood flow as shown in Figure 1. Responses to amylin amide were compared with responses to human alpha CGRP. Amylin amide was 100 times less potent on a molar basis than CGRP. In a second series of experiments, samples were assayed after freeze/thawing; amylin amide, unlike CGRP, was not active after freeze/thawing. After freeze/thawing CGRP at 3 pmol/site produced an increased flow of 57.0% ± 12.0% compared to amylin amide at 300 pmol/site, which produced a nonsignificant increase of 10.6% ± 11.6%; results were expressed as mean ± SEM, n = 4 rabbits, and as percentage increase in blood flow when compared with saline-injected sites. In experiments described below amylin amide was composed immediately before injection.

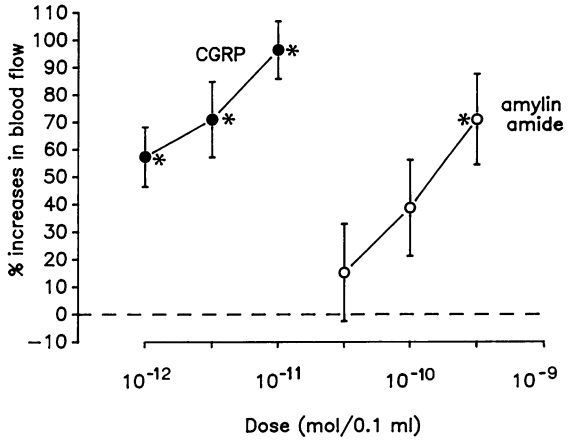

Figure 1. *Effect of human synthetic amylin amide (30 to 300 pmol/site ○) and human synthetic alpha CGRP (1 to 10 pmol/site ●) on blood flow in rabbit skin. The dotted line represents the control saline-injected sites. Results are expressed as mean ± SEM for n = 4 rabbits where each result is the average of six injections in each rabbit. The significance of differences between individual treatments and control (saline) blood flow was assessed by Bonferroni's modified t-test using the standard error estimate from the analysis of variance to account for the fact that multiple tests were performed.* * $P < 0.01$.

The effect of amylin amide was compared with that of CGRP on bradykinin-induced edema by coinjecting with bradykinin intradermally. Amylin amide potentiated bradykinin-induced edema as shown in Figure 2. Previous studies have shown that vasodilators, as a consequence of their vasodilator activity, act in a synergistic manner with mediators of vascular permeability to potentiate edema formation. Accordingly, amylin amide was 100 times less potent than CGRP in terms of blood flow (Figure 1) and potentiation of edema formation (Figure 2). Amylin amide did not stimulate edema formation when injected alone (Figure 2).

Experiments were designed to examine the effect of amylin amide when given intravascularly. Figure 3 shows that amylin amide caused a drop in systemic blood pressure when injected intravenously. CGRP causes a long-lasting effect on blood pressure when injected intravascularly, as shown in Figure 3, especially when compared

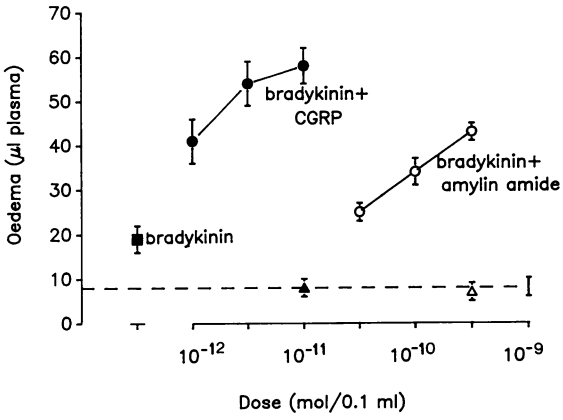

Figure 2. *The effect of human synthetic amylin amide and human synthetic alpha CGRP on bradykinin-induced edema in rabbit skin. The response of bradykinin (10^{-10} mol/site) when injected alone (■) and in combination with increasing concentrations of amylin amide (○) and CGRP (●) is shown. The effect of amylin amide (300 pmol/site) (▲), and CGRP (10 pmol/site) (△) when injected alone is shown. The dotted line represents site injected with saline. The results are expressed as mean values for n = 4 and the bar represents SEM.*

with other vasodilators such as substance P. The trace shows that, in a similar manner to CGRP, amylin amide caused a similar long-lasting drop in blood pressure (amylin amide, 10 nmol; drop in blood pressure, −11.0 ± 1.9 mm Hg; $P < 0.05$, paired *t*-test when compared with resting blood pressure; duration of hypotensive response, 3.5 ± 0.6 minutes; results mean + SEM n = 4).

Discussion

The results demonstrate that intradermally injected synthetic amylin amide induces vasodilatation *in vivo* and, when administered intravenously, the vasodilator effect of amylin amide results in a systemic hypotensive response. In rabbit skin, human synthetic amylin amide is 100 times less potent than human alpha CGRP in increasing microvascular blood flow and in potentiating edema formation after intradermal injection. Previously, in studies when the vasodilator potency of CGRP has been compared with other endogenous vasodilators, CGRP has been described as one of the most potent known endogenous vasodilators.[10,15] It is equipotent in increasing blood flow after intradermal injection in rabbit skin with the neuropeptide vasoactive intestinal peptide (VIP), 3 to 10 times more potent than the prostanoid vasodilators prostaglandins $(PG)E_2$ and I_2, and 10 to 100 times more potent than isoprenaline.[10,17] The vasodilators acetylcholine and substance P, although potent hypotensive agents when injected intravascularly in the rabbit, are extremely weak when injected intradermally in rabbit skin.[10,18] Thus, although amylin amide was less potent than CGRP, a highly significant vasodilator effect of amylin amide was observed in rabbit skin.

The finding that amylin amide has a high structural similarity with CGRP has led to the use of CGRP to examine for possible activities of amylin amide that are relevant to the pathology of diabetes. This led to the demonstration that rat synthetic alpha CGRP and human purified amylin amide, extracted from diabetic pancreas, were equipotent in inhibiting basal and insulin-stimulated glycogen synthesis in rat muscle *in vitro*.[20,23]

CGRP is contained in and released from capsaicin-sensitive C-fiber nerves, which are often found in close association with the smooth muscle of blood vessels.[9,21] Intradermal CGRP can have protracted vascular effects and then be inactivated locally, without reaching blood.[22] In contrast, amylin amide is colocalized with insulin, a circulating hormone, in the beta cells of the pancreas.[3,4] If amylin amide is released into blood, high local concentrations could have vasodilator effects in the pancreas. In addition amylin amide could circulate in an active form, as has recently been suggested.[23] The structural similarity of amylin amide to CGRP and the similar long-lasting hypotensive response to both amylin amide and CGRP indicates that CGRP and amylin amide could exert their vasodilator activity by similar mechanisms, perhaps acting on the same receptors. If so, it is possible that amylin amide can affect the responses of vascular smooth muscle to nervously released CGRP in the diabetic. Alternatively endogenous amylin amide could, by itself, stimulate vasodilator responses. Both mechanisms could be relevant to the changes in microvascular tone and permeability observed in diabetic patients.[24]

Amylin amide used in this study was synthesized according to the sequence of amylin amide extracted and purified from diabetic pancreas and the experiments were

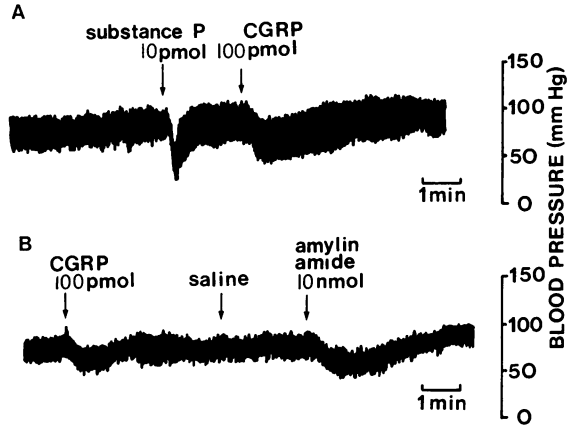

Figure 3. *The effect of intravenous substance P, CGRP, and amylin amide on arteriolar blood pressure in the rabbit.* **A**: *A typical tracing showing arterial blood pressure responses to substance P and CGRP.* **B**: *A trace to show the effect of CGRP and amylin amide.*

performed in the rabbit. Our previous studies with human synthetic CGRP sugest that results from studies carried out in the rabbit are highly relevant to the action of CGRP-like peptides in humans.[10,22] In conclusion, the present results suggest that further studies into mechanisms by which amylin amide can affect vascular tone are potentially important to understanding the pathology of type II diabetes.

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