Amylin-amide displays a proliferative effect on human umbilical vein endothelial cells

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Amylin, also known as diabetes-associated peptide and islet amyloid polypeptide, is a 37 amino acid peptide which was recently isolated from pancreatic amyloids of type II diabetics [1]. Amylin shares 46% amino acid sequence identity with calcitonin gene-related peptide (CGRP) [2]. The human amylin gene is located on the short arm of chromosome 12 [3] and may share a common origin with the genes for CGRP, insulin and insulin-like growth factors. The latter peptides have been assigned to human chromosomes 11 and 12 and are thought to be derived from a common ancestral gene [5]. In view of the findings that CGRP and amylin share a common binding site on human osteosarcoma cells [6], and that the CGRP possesses a proliferative effect [7], we have examined the growth-factor-like effects of amylin-amide on human umbilical vein endothelial cells.

Umbilical vein endothelial cells were isolated as described elsewhere [8]. Fresh human umbilical veins were thoroughly rinsed with phosphate-buffered saline (37°C), filled with 10 min. Cells were harvested by gentle centrifugation at 1000 g for 3 min and resuspended in medium 199 (M 199; Gibco) containing fetal calf serum (20%, v/v, heat inactivated), and antibiotics (penicillin 100 IU/ml; streptomycin 100 μg/ml; streptomycin 50 units/ml). The cells were grown on fibronectin-coated plates (2 μg/cm²) and their viability was assessed by measuring the production of factor VIII-related antigen and cell proliferation. Cells were plated at 4 × 10⁴/cm² and, after overnight growth, the cells were growth-arrested by being grown in serum-free medium. The growth-arrested cells were subsequently exposed to amylin-amide, CGRP, eel-calcitonin, porcine-insulin, met-enkephalin, substance P, and somatostatin at a range of concentrations (0.1 nm–100 nm) in M199 containing 5% (v/v) FCS. The rate of increase in the number of endothelial cells was determined after six days of exposure to the peptides, the medium containing the appropriate peptides being renewed after 24 h. The cells used for DNA synthesis were also serum-starved overnight and the incorporation of 5-bromo-2-deoxyuridine was measured by the e.l.i.s.a. method with monoclonal antibodies following 2 h of exposure to various peptides in M199 containing FCS (5%, v/v). The cells grown in M199 containing FCS (20%, v/v) were used as controls. Insulin was found to increase cell number by 71% ± 8% (P<0.001), but met-enkephalin, substance P, and somatostatin were found to be without proliferative effect. Amylin-amide (10 nm), CGRP (1 nm) and eel-calcitonin (1 nm) caused a significant increase in the cell numbers; 56% ± 14% (P<0.01), 50% ± 9% (P<0.01) and 63% ± 14% (P<0.01), respectively. The stimulation of proliferation was found to be concentration-dependent, but concentrations higher than the optimal concentration led to a slight fall in the proliferation. The rate of DNA synthesis, assessed by the increased incorporation of 5-bromo-2-deoxyuridine, was significantly stimulated by amylin-amide (1.0 nm) (24% P<0.05) and CGRP (1 nm) (22% P<0.05). Insulin and calcitonin were also found to increase DNA synthesis significantly. We have demonstrated similar effects of amylin-amide and CGRP on human osteogenic osteosarcoma cells [6].

As would be expected from purely structural considerations, amylin and CGRP display a considerable receptor cross-reactivity [6]. Therefore, both amylin and CGRP share a number of physiological actions, though the exact function of amylin-amide remains a matter of speculation. Since amylin is thought to be co-secreted with insulin [9] recent studies have focused on the possible interaction between amylin and insulin secretion [10], and amylin and the peripheral actions of insulin [11]. All studies demonstrating the inhibition of insulin-induced glucose uptake and glycogen biosynthesis [11], and inhibition of insulin secretion [10] by amylin have used pharmacological concentrations of amylin. Although high concentrations of amylin cannot be ruled out around the β-cells, such high circulating concentrations are unlikely to exist in vivo [12]. However, when studies were conducted at concentrations that are likely to occur in vivo, amylin-amide failed to alter blood glucose and insulin levels. In contrast, at a similar dose, amylin and amylin-amide exhibited a profound hypocalcaemic effect [13]; the amidated form of amylin being more potent than the non-amidated form [14].

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Abbreviation used: CGKP, calcitonin gene-related peptide.


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