High affinity binding sites for proinsulin on human umbilical vein endothelial cells (HUVEC)

Dear Sir,

We read with great interest the paper by Du et al. [1] demonstrating that supraphysiological concentrations of proinsulin (100 nmol/l) induced apoptosis in human umbilical vein endothelial cells (HUVEC). Data about the concentration dependency of proinsulin and the mechanism by which proinsulin stimulates apoptosis (e.g. receptor binding) are, however, missing. The stimulation of apoptosis by proinsulin contrasts with the biological action of the structurally related peptides insulin and insulin-like growth factor I (IGF-I), both inducing their mitogenic effects, at least in part, by inhibition of apoptosis [2, 3]. Specific receptors for insulin and IGF-I are expressed in many cell types, including endothelial cells, transducing the biological effects via specific tyrosine phosphorylation of downstream signalling proteins [4, 5]. There is no study investigating proinsulin binding in endothelial cells. In human IM-9 lymphoblasts, we recently provided evidence for the existence of specific proinsulin binding sites with differences in signal transduction from insulin [6]. Furthermore, we found proinsulin binding sites in intestinal epithelial crypt cells (IEC-6), human placental membranes and in adipocyte precursor cells [7].

To assess whether proinsulin mediates its biological effects in HUVEC via specific receptors, we did saturation and competition-inhibition binding studies with ^{125}I-labelled and unlabelled proinsulin as described previously [6]. As biological effects of proinsulin, we assessed ^{3}H thymidine incorporation, cell proliferation, and PAI-1 activity. All data are given as means ± SD of at least three different experiments. Binding assays showed the presence of a single class of high affinity proinsulin receptors on HUVEC (Fig. 1). Saturation binding data (Fig. 1 a) were analysed using computer-assisted Scatchard analysis showing 43 300 ± 12 900 receptors per cell with a dissociation constant [K_d] of 6.1 ± 1.5 nmol/l (Fig. 1 b). The high affinity of proinsulin binding was confirmed by the IC_{50}-value of 2.2 ± 1.2 nmol/l obtained in the competition-inhibition binding studies (Fig. 1 c). In contrast to proinsulin binding, Scatchard analysis of ^{125}I-insulin binding showed a typical curvilinear plot ($K_{d/1}$ 26 pmol/l, 200 receptors per cell; $K_{d/2}$ 120 nmol/l, 117 000 receptors per cell). Consistent with the binding affinity, proinsulin concentration-dependently stimulated both DNA synthesis and cell number with half-maximum effects between 1 and 10 nmol/l (^{3}H thymidine incorporation/cell number after 3 days of incubation: 98 ± 5/104 ± 6 % (1 nmol/l), 107 ± 7/108 ± 8 (10 nmol/l), 115* ± 8/127* ± 10 % (100 nmol/l); four independent experiments, values in triplicate, *$p < 0.05$ vs control (= 100 %)). The activity of PAI-1 was measured in HUVEC conditioned medium after 24-h incubation with different proinsulin concentrations. Proinsulin concentration-dependently increased PAI-1 activity (per 10^5 cells) from a basal level of 4.95 ± 0.9 to 7.22 ± 2.29 (0.1 nmol/l) and 9.19 ± 2.9 AU/ml (100 nmol/l), consistent with results published previously [8].

Our data extend those presented by Du's group indicating that proinsulin binds with high affinity and in a different manner from insulin to human endothelial cells. Proinsulin induces various biological effects. Lower concentrations of proinsulin were already effective to stimulate PAI-1 activity. In accordance with our previous findings in IM-9 cells [9], the physiological concentration of proinsulin (0.01 nmol/l) slightly inhibited cell growth. Significant growth stimulation was found in nanomolar concentrations, consistent with investigations on other cell types [10, 11]. As apoptosis and cell proliferation are contradictory events, it would have been interesting to have cell counts of Du's experiments. The microscopical appearance, however, suggests a decrease in cell number rather than an increase. The net balance between proliferation and apoptosis could therefore depend on the exact experimental conditions, e.g. the concentration of proinsulin and glucose and the presence of other factors such as the antiapoptotic

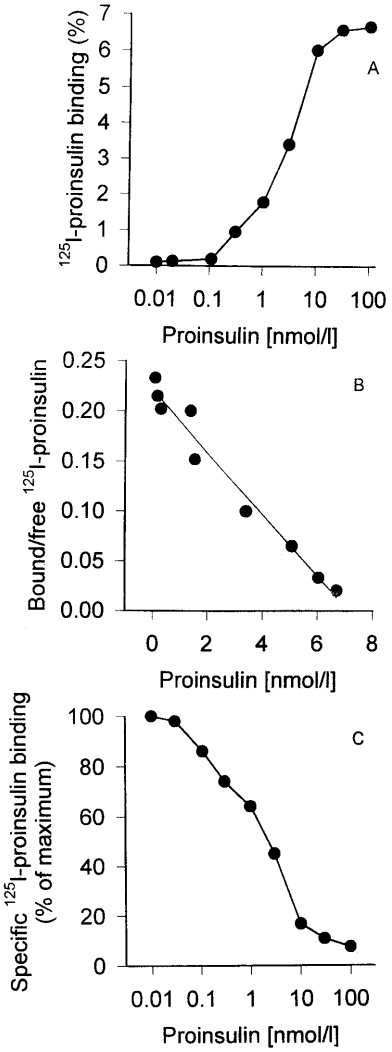

Fig. 1A–C. Proinsulin binding to human umbilical vein endothelial cells (HUVEC). Three separate experiments were done using HUVEC passage 4–10 from 3 different cell isolates. **A** Saturation binding of proinsulin was determined by adding increasing concentrations of ^{125}I-labelled peptide to confluent cell monolayers. **B** Scatchard analysis of the saturation binding data: a single class of binding sites was detected (K_d: 6.1 ± 1.5 nmol/l, receptors/cell: 43 300 ± 12 900). **C** Competition-inhibition binding of proinsulin was done by adding 10 pmol/l of ^{125}I-labelled proinsulin and increasing concentrations of unlabelled peptide to confluent cell monolayers. Half-maximum competition of radioligand was obtained by 2.2 ± 1.2 nmol/l unlabelled proinsulin

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IGF-I which could be released by endothelial cells and act in an auto-/paracrine manner [11]. In conclusion, it is of particular importance to view apoptosis in line with other known biological effects of proinsulin such as the stimulation of cell proliferation and PAI-1 activity and to compare the effects of proinsulin with those of structurally related peptides (e. g. insulin and IGF-I). It is essential to further elucidate the molecular mechanism by which proinsulin acts to understand its physiological and pathophysiological role.

Yours sincerely,
M. Faehling, R. D. Fussgaenger, P. M. Jehle

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Capsaicin-induced sensory denervation increases glucose elimination in rodents

Dear Sir,
It is well known that adrenergic (sympathetic) and cholinergic (parasympathetic) nerves are of importance for islet hormone secretion and glucose homeostasis [1–3]. In contrast, the involvement of the sensory nerves in the regulation of glucose homeostasis has not been established, which could be partially ascribed to lack of good and reliable experimental methods to study this topic. One useful technique, however, is treating neonatal animals with capsaicin, which results in permanent destruction of sensory nerves [4, 5].

In the July 1998 issue of Diabetologia, Dr Koopmans and collaborators reported a study in neonatally capsaicin-treated rats [6]. They found that capsaicin-treated animals developed increased insulin sensitivity as judged by euglycaemic clamp studies in the adult rats and based on these observations they suggested that sensory nerves are involved in glucose metabolism by reducing insulin sensitivity and thereby inhibiting glucose elimination. In the article, the authors state that "only one previous study has examined the involvement of capsaicin-sensitive sensory nerves in the regulation of glucose homeostasis" and they then refered to the important work by Zhou and collaborators, showing failure of normal recovery from insulin-induced hypoglycaemia after capsaicin in rats [7]. The statement that this is the only study examining the influence of capsaicin-induced sensory denervation on glucose homeostasis is not correct, however, since a growing body of evidence in the literature has already arrived at the same conclusion, i. e. that increased glucose elimination is seen after neonatal capsaicin treatment. For example, we have shown previously that giving capsaicin to neonatal mice increases glucose elimination following an i. v. glucose tolerance test in adulthood [8]. More importantly, Guillot and collaborators have, likewise in rats of the same species as studied by Dr Koopmans and collaborators, shown that capsaicin increases glucose elimination by an action independent of insulin secretion [9]. Therefore, the work by Dr Koopmans and collaborators [6] supports the previously stated suggestion [5, 7, 9] that sensory nerves are of importance for glucose metabolism and they provide evidence that this is due to an action on insulin sensitivity. It is now important to delineate in detail the mechanism of such an action.

Yours sincerely,
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