Prostaglandin analogue protects pancreatic B-cells against cyclosporin A toxicity

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Summary. Cyclosporin A toxicity on pancreatic B-cells and its prevention by rioprostil, a prostaglandin E_1 analogue, were studied in the model of the isolated perfused pancreas of rats treated with both compounds for 8 days. At toxic doses of cyclosporin (10 and 20 mg/kg b.wt), the B-cells showed severe hydropic degeneration of the endoplasmatic reticulum and slight degranulation of the B-cells. Accordingly, the insulin secretion was markedly impaired. Administration of rioprostil ameliorated the insulin secretion significantly, but not the ultrastructural changes. At therapeutic levels of cyclosporin (5 mg/kg b.wt), the hydropic degeneration and the drop in insulin secretion were completely prevented by rioprostil. This observation might have therapeutic implications in the treatment of patients, in particular those undergoing pancreatic transplantation.

Key words. Cytoprotection; cyclosporin; endocrine pancreas; insulin-secretion; electron microscopy; prostaglandin analogue; rioprostil.

The beneficial properties of cyclosporin A in preventing graft versus host reaction are hampered by severe side effects, including dose-related nephrotoxicity and hepatotoxicity[1,2]. In addition, it also effects the pancreatic B-cells causing severe degranulation and hydropic degeneration[3]. Accordingly, the B-cells show a reduced insulin content and glucose-induced insulin secretion[4,5]. The exocrine pancreas seems to be less sensitive to this drug[6].

Prostaglandins have been shown to prevent cytotoxic effects induced by various agents in different organs[7–9], in particular reducing the severity of diet-induced pancreatitis in mice[10]. Therefore, we were interested in knowing whether prostaglandins provide any cytoprotection for the pancreas under the influence of a cytotoxic agent such as cyclosporin. Recently, we demonstrated that rioprostil, a synthetic prostaglandin E_1 analogue[11], is able to prevent the noxious effect of cyclosporin on the rat endocrine and exocrine pancreas[12,13].

It was the aim of this study to investigate the structure of the endocrine pancreas using immunocytochemistry and electron microscopy in order to define the morphological basis of the functional findings observed after cyclosporin and rioprostil treatment.

Material and methods. Animals. Male Wistar rats (220–240 g) were used throughout the study. Cyclosporin (Sandimmun) was purchased from Sandoz (Basel, Switzerland). It was dissolved in olive oil and was applied intragastrically by a metal feeding tube once daily in the morning. Rioprostil was a generous gift from Bayer (Wuppertal, FRG). It was injected subcutaneously twice daily.

Design of the study. Eight groups of rats, each consisting of 8–10 animals, were treated for eight days as follows: olive oil only (group 1), cyclosporin 5 mg/kg b.wt (group 2), cyclosporin 10 mg/kg b.wt (group 3), cyclosporin 20 mg/kg b.wt (group 4), olive oil and rioprostil 2×7.5 µg/kg b.wt (group 5), cyclosporin 5 mg/kg b.wt and rioprostil 2×7.5 µg/kg b.wt (group 6), cyclosporin 10 mg/kg b.wt and rioprostil 2×7.5 µg/kg b.wt (group 7), cyclosporin 20 mg/kg b.wt and rioprostil 2×7.5 µg/kg b.wt (group 8). All animals had free access to food and water. Prior to operation on day 9, the rats fasted overnight with free access to water.

Isolated perfused pancreas preparation. The pancreas was isolated and perfused as described previously[14]. The preparation consisted of pancreas with a small remnant of duodenum. The preparation was perfused via the superior mesenteric artery and the celiac trunc at a constant flow rate of 4 ml/min without recirculation. The perfusate was collected in 1-min intervals by placing a second canula (PE 160) into the portal vein. The perfusate consisted of Krebs-Ringer bicarbonate buffer containing 0.2% bovine serum albumin, 3% dextran, 7.9 or 15.8 mM glucose. The perfusate was gassed with 95% O_2 5% CO_2 to achieve a final pH of 7.4.

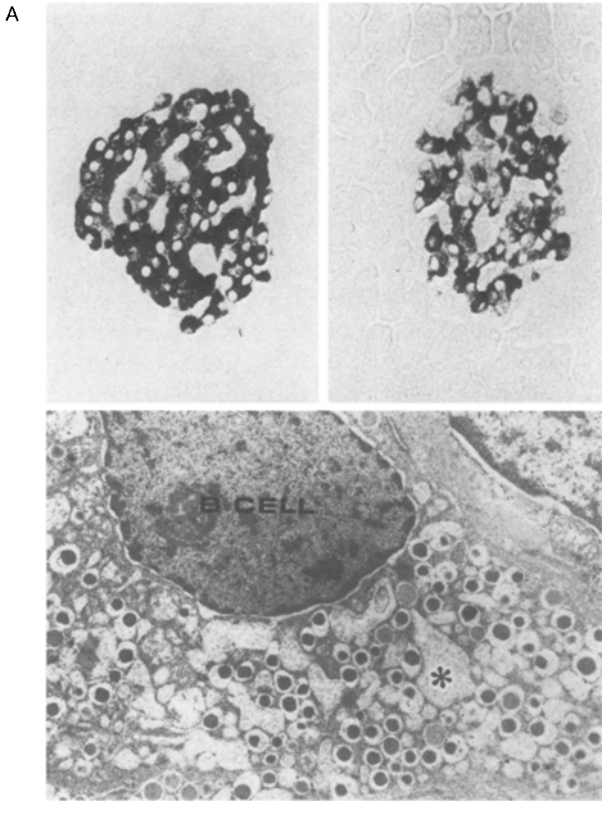
Design of the pancreatic perfusion. The preparation was equilibrated by perfusion for 15 min with a buffer containing 7.9 mM glucose. After equilibration, the buffer was switched to a glucose concentration of 15.8 mM glucose for 30 min. At the end of the perfusion study, pancreatic tissue was fixed in Bouin's solution and embedded in paraffin.

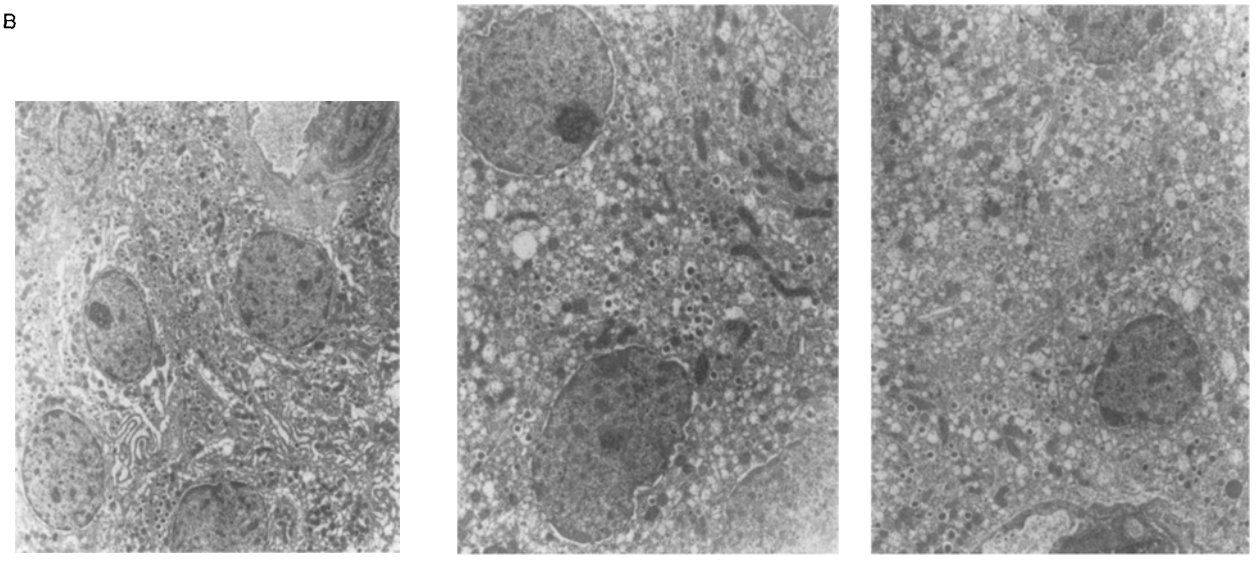
Light microscopy. Three µm-thick serial sections were cut and the first 2 sections were stained with hematoxylin and eosin (H & E) and periodic acid-Schiff (PAS). Immunocytochemistry was performed on the subsequent deparaffinized serial sections, using antibodies against insulin, glucagon, somatostatin and pancreatic polypeptide with the ABC method, as described previously[15].

Electron microscopy. For ultrastructural studies, pieces from the pancreas were immersion-fixed in 3% glutaraldehyde buffered with 0.1M sodium cacodylate (pH 7.4) for 2 h[16]. After rinsing in 0.1 M sodium cacodylate buf-

Total insulin output (mU) during glucose stimulation (means ± SE)

	Controls n = 10	Cyclosporin 5 mg/kg n = 10	Cyclosporin 10 mg/kg n = 8	Cyclosporin 20 mg/kg n = 8
No rioprostil	13.7±1.4	7.8±1.0	7.4±0.6	5.7±1.1
Rioprostil 7.5 µg/kg	15.2±1.8	13.4±1.3	11.0±1.6	9.1±1.5
Increase (%)		71	48	59
	N.S.	$p<0.005$	$p<0.05$	$p<0.05$





A Immunocytochemistry for insulin. Slightly reduced insulin staining in an animal treated with 20 mg/kg b.wt cyclosporin when compared with control pancreas (400×). Ultrastructurally, a hydropic degeneration of the endoplasmatic reticulum, in particular perinuclear, can be observed (8000×).

B Electron microscopic appearance of pancreatic B-cells of animals treated with olive oil (left), 5 mg/kg b.wt cyclosporin (middle) and 5 mg/kg b.wt cyclosporin plus rioprostil (right). The hydropic degeneration is completely abolished in the animal treated with rioprostil in addition to the cyclosporin (8000×). The micrographs in the figure depict representative examples of a series of 8 animals in each group.

fer, the cubes were postfixed in 1% osmium tetroxide buffered with 0.1 M sodium cacodylate for 90 min. The fixed tissue was dehydrated in ethyl alcohol and after passing propylenoxid embedded in Epon 812. Semithin sections were cut and screened for the presence of islets. 600–800 Å sections were cut on a Reichert ultramicrotome OM U2, double-stained with uranyl acetate and lead citrate and examined in a Phillips electron microscope EM 300 at 60 kV.

Analyses. Insulin was determined radioimmunologically in the perfusate as described previously[14]. Creatinine was determined in serum prior to operation using a Beckman analyzer. The animals were weighed daily. Histological samples were coded and evaluated blindly.

Calculations. Insulin secretion from the endocrine pancreas is presented as total output during stimulation with 15.8 mM glucose. Values were compared with Student's t-test for unpaired data and differences with p-values (two-tail) <0.05 were considered statistically significant[17].

Results. At all the dosages, cyclosporin treatment led to a reduced insulin secretion from the isolated perfused pancreas, when stimulated with 15.8 mM glucose. Rioprostil treatment of animals receiving 5 mg/kg cyclosporin resulted in a complete restoration of insulin secretion, reaching control levels (table). Rioprostil significantly improved insulin secretion in the animals treated with cyclosporin at 10 and 20 mg/kg b.wt. However, control rates could not be achieved in either case (table). All rats showed normal serum creatinine levels (mean $\pm$ SE $= 0.5 \pm 0.01$ mg/ml). Light microscopy revealed no differences in the structure of the endocrine pancreas between the rats treated with any dose of cyclosporin, rioprostil, or a combination of both. After immunocytochemical staining, however, a slight degranulation was present in animals receiving 20 mg/kg b.wt cyclosporin, irrespective of the rioprostil treatment (fig., A). At the electron microscopic level, a slight degranulation was noticed with dilatation of the perinuclear endoplasmatic reticulum, i.e. hydropic degeneration. (fig., B). There was almost no difference in these changes between the animals with and without rioprostil treatment in addition to the cyclosporin (10 and 20 mg/kg b.wt). This hydropic degeneration, found in animals receiving cyclosporin 5 mg/kg b.wt, was not observed when rioprostil was added (fig. B).

Discussion. The results of our study confirm the toxic effects of cyclosporin on pancreatic B-cells, demonstrated by an impaired insulin secretion and a hydropic degeneration of the rough endoplasmatic reticulum[3]. This toxicity is not selective for the endocrine pancreas, but this tissue is more sensitive for the noxious effects of cyclosporin than the acini[6]. In addition, our results show that treatment with rioprostil not only partially or even completely protects the functional capacity of the B-cells affected by cyclosporin[12,13] but also prevents the cyclosporin-induced alterations of the B-cell structure at least to some extent.

In the rats receiving high doses of cyclosporin and rioprostil, we observed only moderate improvement of glucose-induced insulin secretion and little if any change in the hydropic degeneration. However, if therapeutic doses of cyclosporin which are used for prevention of rejection were administered (i.e. 5 mg/kg b.wt[18]), a complete protection of the cyclosporin-induced changes in the B-cells treated with rioprostil could be observed.

Whether rioprostil has any potential in preventing the cyclosporin-induced cytotoxicity in other organs, e.g. liver and kidney, is not known yet. However, we assume that this might well be the case, since our studies in the pancreas also indicate prevention of cyclosporin-induced loss in exocrine pancreatic function[13], though we were unable to detect any abnormalities in morphology.

To date it is not clear on which level cyclosporin exerts its toxic effects on the pancreatic B-cells. Our results and those of others[3] indicate that the target site could be in the endoplasmatic reticulum, i.e. at least posttranscriptional. We can therefore not point out where rioprostil abolishes this toxicity. Preliminary results suggest a stabilizing effect of prostaglandins onto cell membranes[7] and lysosomal membranes[19]. The ability of rioprostil to reduce and even completely prevent cyclosporin-induced damage to the B-cell might have therapeutic implications for patients. It remains to be determined, however, whether the therapeutic effect of cyclosporin will be diminished by rioprostil.

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Action of juvenile hormone on the follicle cells of *Rhodnius prolixus:* Evidence for a novel regulatory mechanism involving protein kinase C

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Summary. Juvenile hormone (JH) is known to act on the membranes of the follicle cells of *Rhodnius,* activating a specific Na^+, K^+-ATPase. This leads to a decrease in volume of the cells and the appearance of spaces between them (patency). The addition of an inhibitor of protein kinase C, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7), to the medium in vitro inhibits the action of JH on the follicle cells. PDBU (phorbol-12,13-dibutyrate) mimics the action of JH in vitro and the response of the follicle cells to PDBU is blocked by ouabain. It is concluded that the activation of protein kinase C is a required step in the chain of events leading to activation of the JH-dependent ATPase and set in train by the binding of JH to the membrane.
Key words. Juvenile hormone; protein kinase C; Na^+, K^+-ATPase; follicle cells; patency; *Rhodnius.*

In many insect species, JH, a product of the corpus allatum, plays a significant role during vitellogenesis. While JH is known to initiate and sustain the synthesis and release of vitellogenin by the fat body [1], it also governs the uptake of vitellogenin by the oocyte from the hemolymph. Vitellogenin gains access to the oocyte surface via intercellular spaces of the follicular epithelium. The appearance of these spaces in *Rhodnius* is governed by JH [2]. The effect of JH on the follicle cells can be duplicated in vitro [3], and the opening of the spaces (patency) is rapid, reversible and unaffected by inhibitors of macromolecular synthesis [4]. Similarly, the effect of JH on patency, which involves a reduction in cell volume [5], is inhibited by ouabain, a specific inhibitor of the important membrane enzyme Na^+, K^+-ATPase [6]. Juvenile hormone stimulates Na^+, K^+-ATPase activity when applied directly to a microsomal preparation from follicle cells [7], and JH binding sites have been demonstrated in such membrane preparations [8]. These findings imply that JH specific receptors occur on the membrane of the follicle cells. The link between the binding of JH to the putative receptors and the activation of the JH-sensitive ATPase remains enigmatic. The present study provides an insight into the nature of that link.

Materials and methods. A modified method of Davey and Huebner [3] was used to determine the patency of the follicular epithelium. Ovaries were removed from 14-day-old mated females, fed on the 10th day of adult life. The vitellogenic oocytes were separated from the ovary and the ovarian sheath covering the follicle cells was removed. The follicles were incubated in Schneider's *Drosophila* medium (Gibco Laboratories) for 45 min at room temperature, with gentle mechanical agitation. Subsequently, JH I (Sigma Chemical Co.,) (10^{-7} M); JH I plus H-7 (10^{-4} M); PDBU (10^{-7} M); and PDBU plus ouabain (10^{-3} M) were added to the incubation medium (table). After additional 1-h incubation, the follicles were placed in a few drops of physiological saline containing 1% Evans' Blue dye on a microscope slide. The staining solution was removed and the degree of patency, as revealed by the penetration of the dye into the extracellular spaces, was estimated according to the scale devised by Davey and Huebner [3].

Results and discussion. The results, presented in the table, clearly show that exposure of vitellogenic follicles to JH in medium causes a significant increase in the patency index compared to those that were exposed to medium alone ($p < 0.001$). Addition of H-7, a potent inhibitor of protein kinase C [9, 10], to the medium prevents the JH-dependent rise in patency index ($p > 0.01$). This implies

The effect of JH, PDBU, ouabain and H-7 on the patency index of follicle cells in vitro

Treatment	Patency index ± SEM
Control (medium only)	0.50 ± 0.30 (n = 27)
JH	2.00 ± 0.38 (n = 15)
JH + H-7	0.67 ± 0.21 (n = 15)
PDBU	2.66 ± 0.37 (n = 15)
PDBU + Ouabain	0.75 ± 0.35 (n = 12)

n = total number of oocytes examined.