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α-ADRENERGIC INHIBITION OF RAT PANCREATIC ß-CELL REPLICATION AND INSULIN SECRETION IS MEDIATED THROUGH A PERTUSSIS TOXIN-SENSITIVE G-PROTEIN REGULATING ISLET cAMP CONTENT

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The rate of DNA synthesis, insulin secretion and cAMP content in isolated pancreatic islets were markedly inhibited by long-term exposure to the α_1-adrenoceptor agonist phenylephrine, the α_2-adrenoceptor agonist clonidine and the ß-adrenoceptor antagonist propranolol. Pertussis toxin or the stimulatory cAMP analog Sp-cAMPS increased DNA synthesis and insulin secretion in the absence of the adrenergic agents. Pertussis toxin blocked the inhibitory actions of these agents on DNA synthesis, insulin secretion and cAMP content, and a similar protection was imposed by Sp-cAMPS. Thus, long-term α-adrenergic stimulation interferes with signaling through pertussis toxin-sensitive G-protein(s) and, by decreasing the islet cAMP content, inhibits ß-cell DNA synthesis and insulin secretion.

The pancreatic islets form a highly innervated organ, receiving sympathetic neural inflow via the splanchnic nerves (1). Ever since the original discovery by Coore and Randle (2) that the sympathetic neurotransmittor epinephrine inhibits insulin secretion from pieces of rabbit pancreas, there has been a great number of studies investigating the effects of adrenergic factors on insulin secretion (reviewed in (1,3)). Because epinephrine and norepinephrine exhibit overlapping activities on different adrenoceptors and are unstable in culture, the true significance of interference with these receptors is usually studied by using highly specific and stable receptor agonists or antagonists. It has thus been shown that the α_2-adrenoceptor agonist clonidine suppresses insulin secretion from islets *in vitro* (4). This effect was paralleled by a reduction in cytoplasmic free Ca^{2+} concentrations, and was prevented by pertussis toxin pretreatment (4). The aim of the present study was to evaluate the long-term influence of α-adrenergic stimulation on DNA synthesis and insulin secretion by fetal rat islets

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containing a high fraction of rapidly proliferating ß-cells. Attempts were also made to elucidate by what intracellular signaling systems the effects of the adrenergic agents were conveyed.

MATERIALS AND METHODS

MATERIALS: Antibovine insulin serum was supplied by Miles-Yeda, Rehovot, Israel. Crystalline mouse insulin and ^{125}I-insulin were from Novo, Copenhagen, Denmark. Pertussis toxin, clonidine, ±propranolol and phenylephrine were obtained from Sigma Chemicals, St. Louis, MO. [*Methyl*-^{3}H]thymidine (5 Ci/mmol), the cAMP (Cat. # RPA.509) assay kit were purchased from Amersham International, Bucks., U.K.

METHODS: Fetal rat islets were prepared from pancreatic glands as described (5). At the end of the culture period, groups of 50 islets were transferred to fresh media containing 1 % fetal calf serum, supplemented with the desired test substance(s) and cultured free-floating for an additional 3-day period. Pertussis toxin was added on day 1 and the other test substances on day 2.
For DNA synthesis measurements, 1 µCi/ml of [*methyl*-^{3}H]thymidine was present in culture media during the last 5 h. At the end of the labeling period the islets were washed in PBS, sonicated in redistilled water and precipitated in ice-cold 10 % trichloroacetic acid. The precipitate was washed twice in trichloroacetic acid and dissolved in 50 µl of Soluene. The radioactivity incorporated was determined by scintillation counting after addition of 1 ml of Unisolve. Duplicate samples of the homogenate were analyzed fluorometrically for DNA (6,7)
The islet insulin content in aqeuos homogenates extracted overnight in acid ethanol (8) and insulin secretion to culture media during the last 24 h of culture were determined radioimmunologically (9).
For cAMP measurements, cultured islets in groups of 50 were quickly washed in PBS and then transferred to tubes containing 150 µl 6% trichloroacetic acid. These were immediately sealed, plunged into liquid nitrogen and stored at -80 °C pending analysis. The samples were thawed by sonication on ice and centrifuged at 2,000 *g* (4 °C) for 15 min. The pellet was re-sonicated in 200 µl of redistilled water and used for measurements of DNA, [^{3}H]thymidine incorporation and insulin as detailed above. The supernatant was washed 4 times with 5 volumes of water-saturated diethyl ether. The aqeuos extract was freeze-dried and the content of cAMP in acetylated samples measured by RIA (using ^{125}I-cAMP) exactly as described by the manufacturer of the assay kit.

RESULTS AND DISCUSSION

The α_1-agonist phenylephrine, the α_2-agonist clonidine and the ß-adrenoceptor antagonist propranolol were all potent inhibitors of islet cell DNA synthesis, while phenylephrine and clonidine also suppressed long-term insulin secretion (Table 1).

These findings add further credence to the suggested role of α-adrenergic stimulation as an inhibitor of short-term insulin release (4,10), whereas the observed antiproliferative effects are novel. In search for possible intracellular signaling systems conveying these effects, interest was focussed on cAMP. This nucleotide is known to increase in concentration in islets exposed to high glucose, a cardinal stimulator of both ß-cell

TABLE 1. Effects of adrenoceptor stimulation and inhibition, pertussis toxin and Sp-cAMPS on islet DNA synthesis, insulin secretion and cyclic AMP content

Islet culture			DNA synthesis	Insulin secretion	cAMP content
Addition	PTX	Sp-cAMPS	(c.p.m./μg DNA)	(ng/μg DNA per ml x 24 h)	(fmol/μg DNA)
Nil	-	-	1273 ±144	185 ±26	97 ±11
Phe	-	-	814 ±67*	73 ±14*	58 ±7*
Clon	-	-	471 ±33*	64 ±8*	33 ±5*
Propr	-	-	330 ±46*	173 ±19	36 ±6*
Nil	+	-	2444 ±219#	332 ±29#	154 ±25#
Phe	+	-	1497 ±164#§	198 ±24#	123 ±16#
Clon	+	-	1611 ±178#§	211 ±26#§	118 ±23#
Propr	+	-	1412 ±98#§	318 ±29#	139 ±18#
Nil	-	+	2520 ±294$	361 ±47$	N.D.
Phe	-	+	1931 ±211$	199 ±41$†	N.D.
Clon	-	+	1611 ±173$†	212 ±18$†	N.D.
Propr	-	+	1840 ±219$†	333 ±27$	N.D.

Islets were exposed for 24 h to phenylephrine (Phe, 10 μM), clonidine (Clon, 10 μM), propranolol (Propr, 10 μM) or Sp-cAMPS (50 μM). Twentyfour hours prior to and during exposure to phenylephrine, clonidine and propranolol, islets were either treated (+) or not (-) with 50 ng/ml pertussis toxin (PTX). Values are means ±SEM for 4-8 experiments. N.D., not determined. * (*vs.* control without PTX and Sp-cAMPS), # (*vs.* corresponding group without PTX), § (*vs.* controls with PTX), $ (*vs.* corresponding group without Sp-cAMPS) and † (*vs.* controls with Sp-cAMPS) denote 95 % multicomparison significance level using 1-way ANOVA.

proliferation and insulin secretion (11). It is thus possible that cAMP mediates part of the effect of glucose on these parameters. Such a role is supported by the present finding that the cAMP analog Sp-cAMPS increased both DNA synthesis and long-term insulin secretion by the ß-cells (Table 1). The membrane-permeant Sp-cAMPS is a stimulatory analog of the natural signal molecule cAMP in which one of the two exocyclic oxygen atoms in the cyclic phosphate moiety is replaced by sulfur by axial modification. It mimics all biological effects of natural cAMP, is extremely resistant to cyclic nucleotide phosphodiesterases and is an agonist of cAMP-dependent protein kinases I and II (12-15).

The presently reported reduction in the islet content of cyclic AMP by adrenergic factors (Table 1) might be suspected to contribute to the inhibitory actions of these agents on insulin secretion and DNA synthesis. One way by which a decrease in cyclic AMP can be brought about is through interference with GTP-binding proteins that

connect a cell surface receptor to adenylyl cyclase (16). Certain of these inhibitory GTP-binding proteins are sensitive to the toxin of *Bordetella pertussis*, which alleviates the adenylyl cyclase from an inhibitory constraint (16). Interference at the GTP-binding protein level by the adrenergic factors is supported by the present finding of a prevention of the inhibitory actions of these agents by pertussis toxin pretreatment (Table 1). The finding that this treatment also prevented the decrease in cAMP evoked by the adrenergic agents further supports a regulatory role of cAMP in this context. This notion is further strengthened by the observation that a similar protection was imposed by Sp-cAMPS (Table 1).

It is concluded that long-term α-adrenergic stimulation interferes with signaling through pertussis toxin-sensitive G-protein(s), which, by suppressing cAMP synthesis, inhibits ß-cell DNA synthesis and insulin secretion.

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