5'-GUANYLYLIMIDODIPHOSPHATE:A MODULATOR OF GLUCAGON-INDUCED INSULIN RELEASE FROM ISOLATED RAT PANCREATIC ISLETS

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SUMMARY

GMP-PNP(0.1 or 0.01 mM) enhanced the glucagon-induced (5 µg/ml) IRI release from isolated rat pancreatic islets. Glucose-induced(8 or 16.6 mM) and theophylline-induced(0.5 mM) IRI release were unaffected.The modulatory effect of GMP-PNP on glucagon-induced IRI release might be explained by an alteration of the adenylate cyclase system in B-cells.Phosphotransferase reactions do not seem to be involved in this process.

The stimulatory action of glucagon on IRI release from islets of Langerhans in vivo and in vitro(1,2) is currently thought to be mediated via stimulation of the adenylate cyclase system in B cells(3,4).Glucagon-induced stimulation of adenylate cyclase from isolated liver plasma membranes was recently shown to be amplified by GTP,as well as by GMP-PNP,a GTP analogue resistent to cleavage by phosphohydrolases(5-7).

We have studied the effect of GMP-PNP on glucagon-mediated IRI release from isolated rat pancreatic islets.A marked stimulatory effect of GMP-PNP on glucagon-induced IRI release was observed,while glucose- and theophylline-induced IRI release remained unaffected.

MATERIALS AND METHODS

Fed,male Wistar rats(200-250 g) were used throughout the study. Bovine serum albumin(lot ORHD 20) was purchased from Behringwerke

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Abbreviations used:GMP-PNP,5'-guanylylimidodiphosphate
IRI,immunoreactive insulin

A.G. Marburg,FRG;125-J porcine insulin(specific activity 150-200 mC/mg) from Farbwerke Hoechst A.G Frankfurt,FRG.Crystalline rat insulin was a gift from Novo industri A.S.Copenhagen,Denmark; crystalline porcine glucagon(lot 258-D30-128-4) a gift from Eli Lilly,Indianapolis USA.Further reagents used are listed in a previous report(8).

Islets were isolated from rat pancreas by collagenase(9) and incubated by batches of 10 in 500 ul Krebs-Ringer bicarbonate buffer as previously described(8) with glucose,glucagon,theophylline and GMP-PNP added as indicated in the tables.

Insulin content of the medium was determined by radioimmunoassay with rat insulin as reference standard(10).IRI release was expressed as ng/10 islets/45 min.

RESULTS

Glucagon,in the presence of a high glucose concentration (16.6 mM),stimulated IRI release from isolated rat pancreatic islets(Table 1;line 7 vs line 11).This stimulatory effect was amplified by GMP-PNP(Table 1;line 12 and 13).At lower glucose concentrations(8 mM) glucagon stimulated IRI release only in the presence of GMP-PNP(Table 1;line 3,5 and 6).Neither basal IRI release(Table 1;line 1 and 2) nor glucose-induced(8 or 16.6 mM) IRI release were affected by GMP-PNP(Table 1; line 3,4 and 7-10).Theophylline(0.5 mM) stimulated IRI release in the presence of 8,12 or 16.6 mM glucose but was not effective at 2 mM glucose. Theophylline-induced stimulation was not further enhanced by GMP-PNP(Table 2).

DISCUSSION

The data confirm previous studies(3) indicating that glucagon, in the presence of a high glucose concentration(16.6 mM),stimulates IRI release in vitro(Table 1).This effect of glucagon is amplified by GMP-PNP.At a lower glucose concentration glucagon stimulates IRI release only if GMP-PNP is present.GMP-PNP thus seems to increase the response of the glucagon-sensitive mechanism in B-cells.

GMP-PNP alone neither stimulates basal IRI release nor does it influence theophylline- or glucagon-induced IRI release.Therefore,under the present experimental conditions, GMP-PNP seems to modulate specifically glucagon-induced IRI release.

It has been reported recently(11) that GTP stimulates glucose-induced(3.3 mM) IRI release from segments of pancreas from

Glucose (mM)	GMP-PNP (mM)	Glucagon (ug/ml)	IRI release (ng/10 islets/45 min)	
----	----	----	6.0 ± 0.5	(20)
----	0.10	----	6.2 ± 0.7	(20)
8.0	----	----	23.9 ± 1.1	(42)
8.0	0.10	----	23.7 ± 1.7	(17)
8.0	----	5	25.8 ± 1.3	(43)
8.0	0.10	5	28.8 ± 1.6	(42)+
16.6	----	----	42.6 ± 2.1	(38)
16.6	0.01	----	45.4 ± 2.0	(29)
16.6	0.10	----	45.6 ± 1.8	(28)
16.6	1.00	----	44.8 ± 1.7	(20)
16.6	----	5	50.6 ± 2.0	(36)+
16.6	0.01	5	56.6 ± 1.7	(46)+§
16.6	0.10	5	60.9 ± 2.6	(46)+§

Table 1. Effect of 5'-guanylylimidodiphosphate on glucagon-induced and glucose-induced IRI release from isolated rat pancreatic islets. Mean values ± SEM are shown with the number of individual observations obtained from islets of 5-12 pancreata in parentheses. + indicates a significant difference from the control value in line 3 and line 7 respectively ($P < 0.01$). +§ indicates a significant difference from the control value in line 11 ($P < 0.01$). Values for "P" were calculated by the "t" test based on nonpaired comparisons.

golden hamster, however, at a much higher concentration (10 mM) than those used in this study. In addition, GTP is catabolized by phosphohydrolases whereas GMP-PNP is not affected (7). GTP might thus serve as a phosphate donor. This considerably complicates interpretation of the results.

Indeed, using GMP-PNP instead of GTP as in this study confers the advantage that the effect of the nucleotide on IRI release can be assessed at low concentrations, with certainty that the observed effects are not due to phosphotransferase reactions. How GMP-PNP stimulates glucagon-induced IRI release remains to be established, but a possible explanation may be the modulation of the effect of glucagon on the adenylate cyclase system in islet tissue. GMP-PNP, as well as GTP, have been shown to amplify the glucagon-

Glucose (mM)	GMP-PNP (mM)	Theophylline (mM)	IRI release (ng/10 islets/45 min)	
2.0	----	----	6.5 ± 0.5	(20)
2.0	----	0.5	6.1 ± 0.6	(20)
2.0	0.1	0.5	7.4 ± 0.6	(20)
8.0	----	----	20.3 ± 1.7	(18)
8.0	----	0.5	34.1 ± 1.5	(20)+
8.0	0.1	0.5	35.3 ± 2.9	(20)
12.0	----	----	31.9 ± 2.2	(34)
12.0	----	0.5	46.0 ± 2.1	(34)+
12.0	0.1	0.5	46.1 ± 1.9	(34)
16.6	----	----	43.1 ± 2.4	(18)
16.6	----	0.5	59.7 ± 2.9	(18)+
16.6	0.1	0.5	60.0 ± 1.5	(18)

Table 2. Ineffectiveness of 5'-guanylylimidodiphosphate to influence the theophylline-induced IRI release from isolated rat pancreatic islets. Mean values ± SEM are shown with the number of individual observations obtained from islets of 5-8 pancreata. + indicates a significant difference from the respective control value ($P < 0.01$). Values for "P" were calculated by the "t" test based on nonpaired comparisons.

mediated increase in the activity of the adenylate cyclase system in liver tissue, possibly involving changes of the affinity of glucagon with the enzyme system(5,6).

Evidence presently available indicates that glucagon-stimulated insulin release is initiated by binding of the hormone to specific receptors in B-cells(12), followed by activation of the adenylate cyclase system and elevation of cyclic 3'-5' adenosine monophosphate(3,4,13-15). Our study suggests that the GTP analogue GMP-PNP modulates glucagon-induced IRI release. The exact nature of this effect remains to be established. However, GMP-PNP does not appear to serve as a phosphate donor since the terminal phosphate of this guanine nucleotide does not seem to be utilized in phosphotransferase reactions(7).

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