Rapid Publications

Stimulation of Adenylate Cyclase by Ca^{2+} and Calmodulin in Rat Islets of Langerhans

Explanation for the Glucose-induced Increase in Cyclic AMP Levels

GEOFFREY W. G. SHARP, DAYNA E. WIEDENKELLER, DENISE KAELIN, EBERHARD G. SIEGEL, AND CLAES B. WOLLHEIM

SUMMARY

The effect of Ca^{2+} and calmodulin has been studied on adenylate cyclase activity in homogenates of rat islets of Langerhans. EGTA had a stimulatory effect on the enzyme in accord with the known inhibitory effect of Ca^{2+}. In contrast, the addition of Ca^{2+} together with calmodulin is stimulatory and demonstrates the existence of a Ca^{2+}-dependent adenylate cyclase in islets of Langerhans. It is suggested that the glucose-induced increase in cyclic AMP concentrations in intact islets is a secondary consequence of the glucose-induced increase in cytosol free-Ca^{2+} concentrations which, with calmodulin, causes an increase in the activity of adenylate cyclase. DIABETES 29:74–77, January 1980.

Cyclic AMP has been shown to have a marked potentiating effect on the ability of glucose to stimulate insulin release from islets of Langerhans.[1] In addition, intracellular concentrations of cyclic AMP in islets can be modestly increased by stimulatory levels of glucose.[2,3] These two effects, and a variety of studies that demonstrate a strong correlation between the glucose-induced increase in cyclic AMP and the rate of insulin release,[4–8] suggest a role for cyclic AMP in the mechanism of glucose-stimulated insulin release. However, agents such as theophylline can cause a greater increase in intracellular cyclic AMP concentration than glucose and have only minor effects on insulin release at nonstimulatory glucose concentrations. For this and other reasons, a straightforward association of glucose causing stimulation of insulin release by raising cyclic AMP levels is unlikely. It seems more likely that the glucose-induced rise in cyclic AMP has a self-potentiating effect on the glucose-stimulated insulin release.[2]

From the Department of Physiology, Tufts University Schools of Medicine, Dental Medicine, and Veterinary Medicine, Boston, Massachusetts 02111, and the Institut de Biochimie Clinique, University of Geneva, Geneva, Switzerland.
Address reprint requests to Geoffrey W.G. Sharp, Department of Physiology, Tufts University School of Medicine, Boston, Massachusetts 02111.
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The mechanism by which glucose increases intracellular cyclic AMP is not understood. Only one report[9] out of eight published reports[1] claims that glucose does stimulate adenylate cyclase in broken cell preparations of islets. Repeated studies in this laboratory have also failed to detect a stimulatory effect (unpublished work). An explanation involving inhibition of cyclic nucleotide phosphodiesterase activity seems unlikely as no such effect has been demonstrated.[10–13]

In seeking an explanation for the effect of glucose to elevate cyclic AMP levels, the possibility was considered that raised intracellular Ca^{2+} concentrations in response to glucose could be responsible for the stimulation of adenylate cyclase. The effect of glucose to raise cyclic AMP levels, like that of insulin release, depends on the presence of extracellular calcium,[14–16] but calcium, by itself, is inhibitory to adenylate cyclase in islets.[1] The possibility that a factor important for the regulation of adenylate cyclase in the intact cell is missing when the enzyme is studied in the broken cell situation was suggested by the fact that calmodulin, a calcium-dependent regulator protein, activates adenylate cyclase in the brain.[17–19] Thus we have studied the possibility that calcium, in the presence of calmodulin, might stimulate adenylate cyclase in islets of Langerhans and thereby afford an explanation for the glucose-induced rise in cyclic AMP levels.

METHODS

Pancreatic islets were isolated from male Wistar rats by the collagenase digestion technique of Lacy and Kostianovsky.[20] Adenylate cyclase activity was measured by the method of Salomon et al.[21] and protein by the method of Lowry et al.[22] Islets were assayed for adenylate cyclase activity under two sets of conditions: (1) Approximately 1500 islets were sonicated in 500 μl of a buffer solution (A) containing 75 mM Tris-HCl (pH 7.5) and 5 mM $MgCl_2$. Twenty microliters of this sonicate was then mixed with 20 μl of the appropriate reaction mixture and 10 μl of test agents or control solution. (2) Approximately 2000 islets were homogenized in a ground glass hand homogenizer in 1 ml of 10 mM

TABLE 1
The effects of fluoride, calcium, calmodulin, and the combination of calcium and calmodulin on adenylate cyclase activity of rat islets in the presence of 200 μM EGTA (expressed as pmol · cyclic AMP · mg^{-1} · min^{-1})

Basal	F^- (10 mM)	Cal (6.6 μM)	Ca^{2+} (150 μM)	Cal + Ca^{2+}	Δ	SEM	P	% change	N
1.93	8.22				6.29	1.04	<0.001	326	10
1.93		2.19			0.26	0.14	<0.1		10
1.93			1.87		−0.06	0.16	<0.8		10
1.93				2.57	0.64	0.15	<0.01	33	10

imidazole buffer (pH 7.5) containing 1 mM EDTA. The homogenate was centrifuged for 15 min at 3600 rpm in a Beckman JS-4.0 rotor at 4°C. The supernatant was discarded and the pellet resuspended in 600 μl of a buffer solution (B) containing 125 mM glycylglycine, 10 mM imidazole, 12.5 mM $MgCl_2$, 2.5 mM dithiothreitol, and 0.5 mM EGTA at pH 7.5. Twenty microliters of this suspension was then mixed with 20 μl of the appropriate reaction mixture and 10 μl of water or test agents for assay. The final assay mixture contained, in addition to the islet suspensions, α-^{32}P-ATP, 0.1 mM; cyclic AMP, 0.1 mM; and an ATP regenerating system of phosphoenolpyruvate, myokinase, and pyruvate kinase. Protein concentration was approximately 20 μg per assay and activity was measured over 20 min at 37°C. Under these conditions the reaction rate is linear for at least 30 min. Blanks were always close to background levels of radioactivity. Statistics were performed using Student's *t* test.

RESULTS

In the first group of studies, removal of Ca^{2+} by the addition of 500 μM EGTA had a stimulatory effect on adenylate cyclase activity. The basal activity in the absence of EGTA (buffer solution A) was 1.00 ± 0.24 pmol · mg^{-1} · min^{-1} and increased to 1.49 ± 0.24 in the presence of EGTA (Δ = 0.49 ± 0.12, $P < 0.01$, N = 8). Assuming that each islet has a calcium content of 11 pmol,[23] the concentration of Ca^{2+} in the assay could be as high as 13 μM. Simple addition of calmodulin to the sonicate had no effect on the activity of adenylate cyclase; in these experiments basal activity was 0.94 ± 0.27 pmol · mg^{-1} · min^{-1}, and in the presence of calmodulin the activity was 0.88 ± 0.23 (Δ = −0.06 ± 0.10, $P < 0.6$, N = 7). Because of the possible inhibitory effects of Ca^{2+} and the lack of response to calmodulin, the second group of experiments was performed with a buffer containing EGTA (buffer solution B) in the presence or absence of Ca^{2+}. These conditions were similar to those used by Brostrom et al.[17,18] in their studies on calmodulin stimulation of adenylate cyclase in brain tissue. The results are presented in Table 1. Calmodulin alone (6.6 μM) caused a small but nonsignificant increase in activity. Ca^{2+} (150 μM), in the presence of 200 μM EGTA, did not affect the activity of the enzyme, whereas the combination of calmodulin and 150 μM Ca^{2+} caused a reproducible and highly significant 33% increase in adenylate cyclase activity. NaF (10 mM) caused, as expected, a marked stimulation of adenylate cyclase.

Further studies were performed with higher concentrations of free Ca^{2+} by carrying out experiments in the 200 μM EGTA buffer, but increasing the Ca^{2+} concentration to 200 and 250 μM. These results are shown in Table 2. No significant effects were detected, although the activity decreased from 1.76 to 1.34 pmol · mg^{-1} · min^{-1} with 250 μM Ca^{2+}.

DISCUSSION

Calmodulin is a ubiquitous protein involved in a variety of calcium-dependent control mechanisms. With Ca^{2+} it has been shown to stimulate adenylate cyclase,[17–19] cyclic nucleotide phosphodiesterase,[24–26] Ca^{2+}-dependent ATPase,[27] NAD-kinase,[28] myosin light chain kinase,[29] glycogen synthase phosphorylation,[30] and membrane protein phosphorylation.[31] The results presented here demonstrate the existence of a Ca^{2+}-dependent adenylate cyclase in rat islets of Langerhans which is dependent on calmodulin for its expression. Ca^{2+} alone is inhibitory to adenylate cyclase, as has been demonstrated directly (results not shown) and as can be deduced from the stimulatory effect of EGTA. Calmodulin alone was without effect. This is similar to the result obtained with brain tissue, and presumably reflects the need for careful control of free Ca^{2+} to strike a balance between the Ca^{2+} concentration that interacts with calmodu-

TABLE 2
The effects of calmodulin in the presence of 200 μM EGTA and two different concentrations of Ca^{2+} (200 and 250 μM) (expressed as pmol · cyclic AMP · mg^{-1} · min^{-1})

Basal	Cal (6.6 μM)	Ca^{2+} (200 μM)	Cal + Ca^{2+}	Δ	SEM	P	N
1.76	2.05			0.29	0.21	<0.3	5
1.76		1.74		−0.02	0.42	>0.9	5
1.76			1.85	0.09	0.23	<0.8	5
		Ca^{2+} (250 μM)					
1.76	2.05			0.29	0.21	<0.3	5
1.76		1.34		−0.42	0.37	<0.4	5
1.76			1.78	0.02	0.23	>0.9	5

lin and stimulates adenylate cyclase and the concentration that directly inhibits adenylate cyclase. That the Ca^{2+} concentration has to be maintained between critical constraints can be seen from the results presented. In the presence of EGTA-Ca^{2+} buffer, comprising 200 μM EGTA and 150 μM Ca^{2+}, calmodulin caused a significant stimulation of adenylate cyclase. With 200 μM Ca^{2+} no effect was observed. With 250 μM Ca^{2+} there was a tendency toward inhibition by Ca^{2+}.

This demonstration of a Ca^{2+}-calmodulin-dependent adenylate cyclase in islets of Langerhans could provide an explanation for the glucose-induced rise in cyclic AMP levels. Given that glucose stimulates insulin release by a mechanism that involves increased cytosol Ca^{2+} concentrations, the increased Ca^{2+} could, with calmodulin, cause a secondary rise in adenylate cyclase activity and an increase in the cyclic AMP content. This proposed mechanism is in accord with the following observations: (1) the Ca^{2+}-dependency of the effect,[14,15] (2) the similar anomeric specificity of glucose-induced insulin release and elevation of cyclic AMP,[5-8] (3) the increase in cyclic AMP which has been observed during treatment of islets with ionophore A23187 in the presence of Ca^{2+},[32] and (4) the reduced insulin release and reduced cyclic AMP responses to glucose under fasting conditions,[33,34] in neonatal rats,[5] and in islets from diabetic animals.[35-37] Furthermore, as glucose causes only a small increase in cyclic AMP levels in intact islets, the relatively small (30%) increase in adenylate cyclase activity seen with Ca^{2+} and calmodulin would be appropriate for such a response. It suggests that, of the total adenylate cyclase in the islet, only a small portion is calmodulin-sensitive. The presence of calmodulin-sensitive and -insensitive adenylate cyclases in tissues has already been demonstrated.[38] It should be noted that as rat islets of Langerhans are composed of approximately 80% β-cells, the conclusions on the mechanism of the glucose-induced increase in cyclic AMP levels depend on the absence of a large effect of calcium and calmodulin on the remaining 20% non-β-cells. Further studies on the role of calmodulin with respect to Ca^{2+}-dependent adenylate cyclase and the control of insulin release are in progress.

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Note added in proof. Two papers dealing with the roles of calmodulin in islets of Langerhans were published recently.[39,40]

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