Vol. 112, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 29, 1983 Pages 614-620

DIBUTYRYL CYCLIC AMP TRIGGERS Ca^{2+} INFLUX AND Ca^{2+}-DEPENDENT ELECTRICAL ACTIVITY IN PANCREATIC B CELLS

Jean-Claude HENQUIN *† and Hans Peter MEISSNER †

*Unité de Diabète et Croissance, University of Louvain School of Medicine, 1200 Brussels, Belgium ; and †I Physiologisches Institut und Medizinische Klinik, University of Saarland, 6650 Homburg/Saar, Germany

Received March 7, 1983

SUMMARY: In the presence of 7 mM glucose, dibutyryl cyclic AMP induced electrical activity in otherwise silent mouse pancreatic B cells. This activity was blocked by cobalt or D600, two inhibitors of Ca^{2+} influx. Under similar conditions, dibutyryl cyclic AMP stimulated $^{45}Ca^{2+}$ influx (5-min uptake) in islet cells; this effect was abolished by cobalt and partially inhibited by D600. The nucleotide also accelerated $^{86}Rb^{+}$ efflux from preloaded islets, did not modify glucose utilization and markedly increased insulin release. Its effects on release were inhibited by cobalt, but not by D600. These results show that insulin release can occur without electrical activity in B cells and suggest that cyclic AMP not only mobilizes intracellular Ca, but also facilitates Ca^{2+} influx in insulin secreting cells.

There is now almost general agreement (1,2) that cyclic AMP is not the key mediator in the stimulus-secretion coupling in pancreatic B cells. It rather seems to play a role of potentiator, i.e. to amplify the release of insulin in response to a primary stimulus. However, the molecular mechanisms whereby the nucleotide modulates secretion are only partially defined. Much attention has been paid to the complex interplay between Ca^{2+} and cyclic AMP. It is currently thought that the glucose-induced elevation of cyclic AMP levels in islet cells results from the activation of a calmodulin-sensitive adenylate cyclase by the rise in cytoplasmic Ca^{2+} brought about by the sugar (3,4). Evidence has also accumulated suggesting that cyclic AMP contributes to the rise in cytoplasmic Ca^{2+} by mobilizing intracellular bound Ca or by preventing Ca^{2+} sequestration

Abbreviation: Dibutyryl cyclic AMP : DbcAMP

0006-291X/83 $1.50

in cellular organelles (see ref.5, for a review). On the other hand, it is widely admitted (5) that cyclic AMP does not influence Ca^{2+} entry in B cells.

In this report, we show that dibutyryl cyclic AMP (DbcAMP) triggers Ca^{2+} influx and induces Ca^{2+}-dependent electrical activity in mouse B cells perifused with solutions containing glucose concentrations close to the threshold for stimulation of insulin release. It is suggested that cyclic AMP could also influence the Ca-permeability of the plasma membrane in B cells.

MATERIALS AND METHODS: All experiments were performed with islets of fed female NMRI mice. The membrane potential of single B cells was continuously recorded with microelectrodes in partially microdissected islets (6). For the other experiments, islets were isolated after collagenase digestion of the pancreas. The technique to study insulin release by incubated or perifused islets (7), to measure $^{45}Ca^{2+}$ uptake by islet cells (7,8), to monitor the efflux of $^{86}Rb^{+}$ from preloaded islets (9) and to evaluate glucose utilization by the production of tritiated water from [5-^{3}H]-glucose (8,10) have been described in detail.

The solutions used had the following ionic composition (mM): NaCl,118; KCl, 4.8; $CaCl_2$,2.5; $MgCl_2$,1.2; $NaHCO_3$,25. They were gassed with O_2/CO_2 (94:6) and the pH was 7.4. Except for electrophysiological experiments, they were supplemented with 5 mg/ml of bovine serum albumin. For uptake experiments, they also contained 10 mM Hepes. DbcAMP was from Sigma Chemical Co. (St Louis, MO, USA) and D600 (methoxyverapamil) was a gift of Knoll A.G. (Ludwigshafen, Germany).

RESULTS: B cells were identified by the typical electrical activity (6,11) that they exhibit in the presence of 10 or 15 mM glucose (Fig.1A). When the medium contained only 7 mM glucose, the membrane potential was stable in most B cells and no activity was recorded. Addition of DbcAMP caused a slow depolarization of 3-5 mV, followed by appearance of electrical activity after a delay of 3-6 min (Fig.1A and C). This activity consisted of slow waves of the membrane potential with spikes superimposed on the plateau, and was similar to that induced by a higher concentration of glucose. It was not affected by 5 µM atropine and propranolol and slowly disappeared after withdrawal of DbcAMP (not shown). When electrical activity was already induced by 7 mM glucose alone (in 1 of 6 cells), DbcAMP markedly increased the frequency and the duration of the slow waves (not shown). Cobalt and D600, two inhibitors of Ca^{2+} entry in islet cells (7,12), blocked the electrical activity induced by DbcAMP; the effect of cobalt was rapid and reversible (Fig.1B), whereas that of D600 was slightly slower and not reversible (Fig.1D).

Since the effects of DbcAMP on the membrane potential were slightly delayed, $^{45}Ca^{2+}$ influx (5-min uptake) in islet cells was measured 20 min after addition

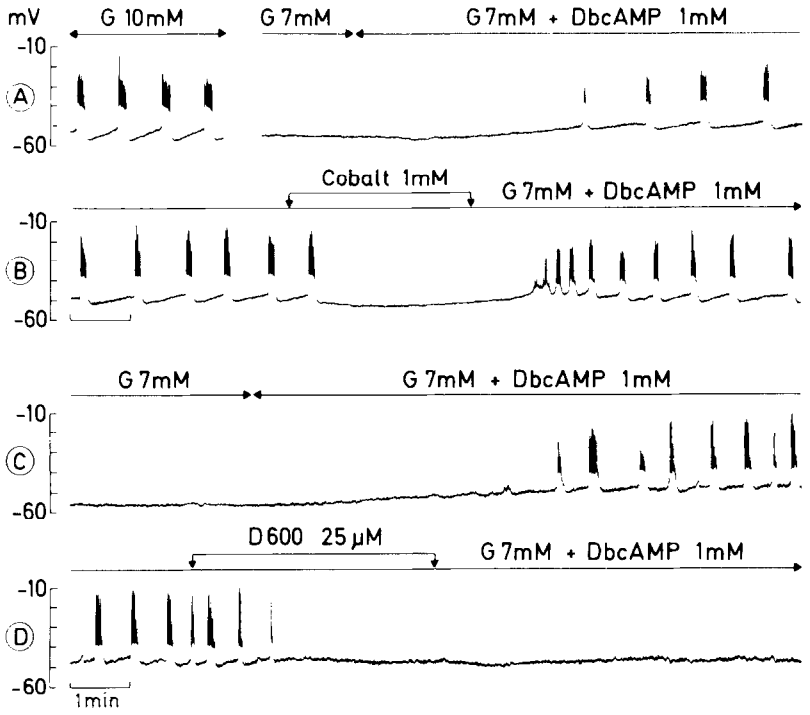

Figure 1. Effects of dibutyryl cyclic AMP (DbcAMP) on the membrane potential of single mouse B cells. Records A, B and records C, D were obtained in two different cells; records B and D are the direct continuation of records A and C, respectively. The left part of record A shows the electrical activity induced by 10 mM glucose, and the right part starts 8.5 min after the concentration of glucose was decreased from 10 to 7 mM. DbcAMP, cobalt and D600 were added as indicated. These records are representative of six experiments (different animals).

of the nucleotide. DbcAMP increased $^{45}Ca^{2+}$ influx in the presence of 7 or 8 mM glucose and 1 or 2.5 mM Ca^{2+} (Table 1). The absolute increase in influx was more marked at 8 than at 7 mM glucose, but was little affected by the concentration of extracellular Ca^{2+}. Cobalt completely prevented the effect of DbcAMP on $^{45}Ca^{2+}$ influx whatever the composition of the medium, whereas the inhibitory effect of D600 was only partial in the presence of 2.5 mM Ca^{2+} and 7 mM glucose (Table 1). It should also be noted that cobalt, but not D600, inhibited $^{45}Ca^{2+}$ influx in the absence of DbcAMP.

Thirty min after addition of 1 mM DbcAMP to a medium containing 7 mM glucose and 2.5 mM Ca^{2+}, the fractional efflux rate of $^{86}Rb^{+}$ from preloaded islets (0.0120 ± 0.0004) was significantly higher ($P < 0.001$) than in the absence of the nucleotide (0.0102 ± 0.0004; means ± SEM, n=6). This acceleration of $^{86}Rb^{+}$ efflux is

TABLE 1. Effects of dibutyryl cyclic AMP on $^{45}Ca^{2+}$ uptake by incubated mouse islets

Composition of medium (mM)			N	$^{45}Ca^{2+}$ uptake (pmol/islet/5 min)		Effect of DbcAMP
Glucose	Ca^{2+}	Test agent		Controls	+ DbcAMP	
7	1	—	20	3.46 ± 0.12	4.37 ± 0.13 †	+ 0.86
7	2.5	—	25	5.60 ± 0.17	6.56 ± 0.18 †	+ 0.96
7	2.5	D600	17	5.52 ± 0.15	6.12 ± 0.16 *	+ 0.60
7	2.5	Cobalt	17	4.45 ± 0.13	4.55 ± 0.11	—
8	1	—	25	3.70 ± 0.12	5.10 ± 0.12 †	+ 1.40
8	1	D600	17	3.49 ± 0.14	3.69 ± 0.12	—
8	1	Cobalt	17	2.57 ± 0.09	2.60 ± 0.09	—
8	2.5	—	20	6.01 ± 0.19	7.32 ± 0.19 †	+ 1.31

Islets were first preincubated for 30 min in a medium containing the same concentrations of glucose and calcium as during the subsequent incubation period. Batches of seven islets were then incubated for 20 min in 50 µl medium without or with DbcAMP (1 mM), D600 (25 µM) and cobalt (1 mM). Finally, 50 µl medium of the same composition, but supplemented with 0.5 mM [6,6']-sucrose (0.1 Ci/mmol) and 2 µCi $^{45}CaCl_2$, were added for 5 min. The islets were then separated from the radioactive solution by centrifugation through the silicone oil on which the incubation medium was layered. Each experimental condition was tested in at least three separate experiments. Values are means ± SEM of N batches of islets.
* $P < 0.05$ and † $P < 0.001$ for the effect of DbcAMP (unpaired t test).

likely due to activation of Ca-sensitive K channels (13) by the incoming Ca^{2+} and/or the mobilization of cellular Ca^{2+}.

Addition of DbcAMP to a medium containing 7 mM glucose and 2.5 mM Ca^{2+} markedly increased insulin release by perifused islets (Fig. 2). This increase was again

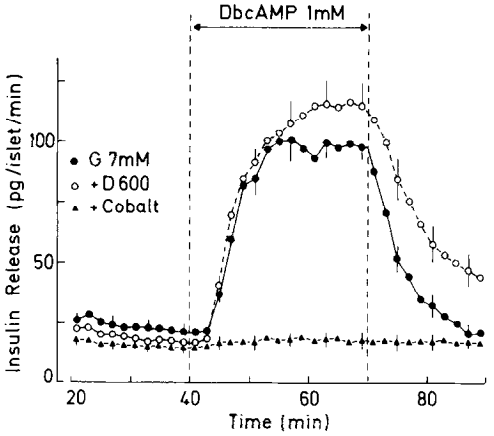

Figure 2. Effects of dibutyryl cyclic AMP (DbcAMP) on insulin release by mouse islets perifused with a medium containing 7 mM glucose (G) and 2.5 mM calcium. In two series, 25 µM D600 (○) or 1 mM cobalt (▲) were also present during the whole experiment. Values are means ± SEM of 4 experiments.

TABLE 2. Effects of dibutyryl cyclic AMP on insulin release by incubated mouse islets

Composition of medium (mM)			Insulin release (ng/islet/60 min)		Effect of DbcAMP
Glucose	Ca^{2+}	Test agent	Controls	+ DbcAMP	
7	1	–	0.33 ± 0.02	0.98 ± 0.06 †	+ 0.65
7	1	D600	0.28 ± 0.02	0.93 ± 0.06 †	+ 0.65
7	1	Cobalt	0.24 ± 0.02	0.30 ± 0.03	–
7	2.5	–	0.36 ± 0.02	1.42 ± 0.08 †	+ 1.06
7	2.5	D600	0.34 ± 0.03	2.11 ± 0.13 †	+ 1.77
7	2.5	Cobalt	0.32 ± 0.02	0.43 ± 0.03 *	+ 0.11
8	1	–	0.41 ± 0.03	1.94 ± 0.08 †	+ 1.53
8	1	D600	0.31 ± 0.02	1.45 ± 0.08 †	+ 1.14
8	1	Cobalt	0.24 ± 0.02	0.29 ± 0.02	–
8	2.5	–	0.54 ± 0.03	4.42 ± 0.22 †	+ 3.88
8	2.5	D600	0.37 ± 0.03	4.89 ± 0.39 †	+ 4.52
8	2.5	Cobalt	0.32 ± 0.02	0.53 ± 0.03 †	+ 0.22

Islets were first preincubated for 30 min in a medium containing the same concentrations of glucose and calcium as during the subsequent incubation period. Batches of three islets were then incubated for 60 min, in 1 ml medium without or with DbcAMP (1 mM), D600 (25 µM) and cobalt (1 mM). Each experimental condition was tested in at least three separate experiments. Values are means ± SEM of 24 batches of islets. * $P < 0.01$ and † $P < 0.001$ for the effect of DbcAMP (unpaired t test).

slightly delayed and slowly reversible; it was completely prevented by cobalt, but not by D600. As shown in Table 2, DbcAMP augmented insulin release by incubated islets. The effect of the nucleotide was more important in the presence of 8 than 7 mM glucose and, at each concentration of the sugar, more marked in the presence of 2.5 than 1 mM Ca^{2+}. When the medium contained only 1 mM Ca^{2+}, cobalt suppressed the stimulation of release by DbcAMP, whereas D600 had no effect (glucose 7 mM), or reduced it partially (glucose 8 mM). In the presence of 2.5 mM Ca^{2+}, the releasing effect of DbcAMP was strongly inhibited by cobalt and either not affected (glucose 8 mM) or paradoxically enhanced (glucose 7 mM) by D600.

After 60 min of incubation in a medium containing 7 mM glucose and 2.5 mM Ca^{2+}, glucose utilization by islet cells was similar without (56.1 ± 1.6 pmol/islet) or with DbcAMP (57.6 ± 1.1 pmol/islet). In the presence of 8 mM glucose and 1 mM Ca^{2+}, glucose utilization amounted to 60.2 ± 1.3 and 64.4 ± 1.8 pmol/islet (means ± SEM for 18 batches of 10 islets) without and with the nucleotide, respectively.

DISCUSSION: This study shows that, in the presence of threshold concentrations of glucose, DbcAMP increases $^{45}Ca^{2+}$ influx in islet cells and induces electrical activity in B cells. This activity is similar to that triggered by stimulatory concentrations of glucose and, like this latter (14), is suppressed by agents that block Ca^{2+} entry. These observations suggest that the role of cyclic AMP in the stimulus-secretion coupling might not be limited to mobilization of intracellular calcium (5), but that the nucleotide could also facilitate Ca^{2+} influx in B cells. However, the data also indicate that the increase in insulin release brought about by DbcAMP is unlikely to result solely from the acceleration of Ca^{2+} entry in B cells. Thus, in the absence of cobalt and D600, there was no quantitative correlation between both phenomena : the increase in release, but not in influx, was much higher in the presence of 2.5 than 1 mM Ca^{2+}. The involvement of intracellular calcium pools is also supported by the ability of DbcAMP to increase insulin release in the presence of D600, which inhibited, at least partially, the stimulation of Ca^{2+} influx. Cobalt, on the other hand, almost completely suppressed all effects of DbcAMP. It is possible that the stronger inhibition of Ca^{2+} influx by cobalt, already present under control conditions, eventually caused depletion of the intracellular pool from which DbcAMP derives Ca^{2+}.

The present results (with DbcAMP and D600) and our recent study with methylxanthines (15) also establish that insulin release may occur in the absence of electrical activity in B cells, if the stimulatory agents are able to mobilize Ca^{2+} from intracellular stores. It is thus obvious that electrical activity in B cells is not the mere consequence of exocytosis.

The mechanisms whereby DbcAMP produces its effects are still unclear. Although the nucleotide did not increase glucose utilization, one may not exclude that acceleration of metabolic fluxes, e.g. glycogenolysis (16,17), contributes to the stimulation of Ca^{2+} influx. On the other hand, DbcAMP could modify the ionic permeabilities of the plasma membrane more directly, through phosphorylation of certain proteins (18). Thus, a cyclic AMP-activated protein kinase and its substrate are present in the plasma membrane of mouse islet cells (19). Whatever the exact mechanism, the stimulation of Ca^{2+} influx by DbcAMP does not appear to result

from a decrease in K permeability of the B cell membrane since it was accompanied by an acceleration of $^{86}Rb^{+}$ efflux. We rather propose that cyclic AMP could modulate the permeability of Ca^{2+} channels in B cells as in heart muscle cells (20-21). Further experiments will be necessary, however, to assess the importance of such an effect for the stimulus-secretion coupling. In particular, it will be important to determine whether cyclic AMP plays a role in the lengthening of the slow waves of membrane potential that occurs when the concentration of glucose increases (11).

ACKNOWLEDGMENTS: This work was supported by grant 3.4552.81 from the FRSM, Brussels and by the Deutsche Forschungsgemeinschaft, SFB 38. J.C. Henquin is "Chercheur Qualifié" of the FNRS, Brussels. We thank M. Nenquin, W. Schmeer and F. Mathot for skilled assistance and M. Nenquin for editorial help.

REFERENCES

1. Sharp, G.W.G. (1979) Diabetologia 16, 287-296.
2. Hedeskov, C.J. (1980) Physiol. Rev. 60, 442-509.
3. Valverde, I., Vandermeers, A., Anjaneyulu, R., and Malaisse, W.J. (1979) Science 206, 225-227.
4. Sharp, G.W.G., Wiedenkeller, D.E., Kaelin, D., Siegel, E.G., and Wollheim, C.B. (1980) Diabetes 29, 74-77.
5. Wollheim, C.B., and Sharp, G.W.G. (1981) Physiol. Rev. 61, 914-973.
6. Meissner, H.P., and Schmelz, H. (1974) Pflügers Arch. 351, 195-206.
7. Henquin, J.C., and Lambert, A.E. (1975) Am. J. Physiol. 228, 1169-1177.
8. Henquin, J.C., and Lambert, A.E. (1976) Am. J. Physiol. 231, 713-721.
9. Henquin, J.C. (1977) Biochem. Biophys. Res. Commun. 77, 551-556.
10. Ashcroft, S.J.H., Weerasinghe, L.C.C., Bassett, J.M., and Randle, P.J. (1972) Biochem. J. 126, 525-532.
11. Meissner, H.P. (1976) J. Physiol. (Paris) 72, 757-767.
12. Malaisse, W.J., Devis, G., Pipeleers, D.G., and Somers, G. (1976) Diabetologia 12, 77-81.
13. Henquin, J.C. (1979) Nature 280, 66-68.
14. Meissner, H.P., and Schmeer, W. (1981) In : The mechanism of gated calcium transport across biological membranes, pp 157-165. Ohnishi, S.T. and Endo, M. (eds) Academic Press, New York.
15. Henquin, J.C., and Meissner, H.P. (1982) Diabetologia 23, 173 (Abstract).
16. Hellman, B., and Idahl, L.A. (1970) In : The structure and metabolism of the pancreatic islets, pp 253-262. Falkmer, S., Hellman, B., and Täljedal, I.-B. (eds) Pergamon Press, Oxford.
17. Malaisse, W.J., Sener, A., Koser, M., Ravazzola, M., and Malaisse-Lagae, F. (1977) Biochem. J. 164, 447-454.
18. Greengard, P. (1976) Nature 260, 101-108.
19. Davis, B., and Lazarus, N.R. (1975) J. Membr. Biol. 20, 301-318.
20. Reuter, H.A. (1979) Rev. Physiol. 44, 415-424.
21. Osterrieder, W., Brum, G., Hescheler, J., Trautwein, W., Flockerzi, V., and Hofmann, F. (1982) Nature 298, 576-578.