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BA^{++} STIMULATES ACCUMULATION OF CYCLIC AMP IN RAT PANCREATIC ISLETS

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SUMMARY: In pancreatic islets prelabelled with (^3H) adenine, Ba^{++} augmented (^3H) cyclic AMP in 1-10 min incubations. 3-isobutyl-1-methylxanthine markedly enhanced and prolonged the Ba^{++}-induced nucleotide as well as the insulin response. In the presence of the methyl xanthine 1.6 mM Ba^{++} was a maximally and 0.4 mM a submaximally effective concentration both for the stimulation of (^3H) cyclic AMP and insulin. A 5-fold excess of Ca^{++} partly inhibited the Ba^{++}-induced nucleotide and - more profoundly - the insulin response. Increasing Mg^{++} from 2 to 10 mM was also inhibitory. Stimulation by Ba^{++} was observed in the absence as well as in the presence of D-glucose. It is concluded that the insulinotropic action of Ba^{++} is at least partly mediated by cyclic AMP.

INTRODUCTION: Insulin secretion involves changes in cation fluxes over the B-cell membrane. Increases of the cell-membrane bound and/or intracellular concentrations of Ca^{++} are thought by most investigators to mediate the effect of insulinogogues on the secretory process (for review see 1). Also cyclic AMP is important for insulin secretion since a) the nucleotide enhances secretion, b) glucose - the most important regulator of insulin secretion - augments cyclic AMP in intact islets in parallel with insulin secretion (1). Since Ca^{++} is a necessary requirement for the glucose action on cyclic AMP as well as for stimulation of insulin release (1) it is conceivable that an increased availability of Ca^{++} causes activation of cyclic AMP. However, in physiological concentrations and in the absence of other stimulatory agents, Ca^{++} neither elevates cyclic AMP nor insulin secretion (1) leaving open the possibility that Ca^{++} plays a permissive as distinct from an initiating role in these respects.

Ba^{++} is a potent stimulator of insulin secretion (2,3). It may serve as

Abbreviations used: 3-isobutyl-1-methylxanthine; IBMX.

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an, at least partial, analogue of Ca^{++} since a) Ba can substitute for Ca^{++} in the secretory process, b) the effects of Ba^{++} can be inhibited by divalent cations such as Ca^{++} or Mg^{++} (2,3). The purpose of the present study was to investigate whether Ba^{++} could initiate stimulation of cyclic AMP and,if so, in which way such a stimulation would be related to the Ba^{++}-induced insulin response. A prelabelling method was used to assess changes in cyclic AMP metabolism. The validity of this method has recently been evaluated (4).

MATERIAL AND METHODS: Material Collagenase was obtained from Worthington Biochemical corporation, Freehold N.J., U.S.A., 3-isobutyl-1-methylxanthine (IBMX) from Aldrich Co., Milwaukee, Wis., U.S.A.; (2-^{3}H)-adenine (specific activity 25 Ci/mmole) from New England Nuclear, Boston, Mass., U.S.A. ^{131}I labelled pork insulin was from the Radiochemical Centre, Amsterdam. Rat insulin, used as standard in the immunoassay, was a kind gift of Dr J. Schlichtkrull, Novo Research Institute, Bagsvaerd, Denmark.

Methods: The procedures for preparation of islets, prelabelling with (2-^{3}H)-adenine and incubation of islets have been described in detail elsewhere. Briefly, pancreatic islets were isolated by collagenase (5) from fed male Sprague-Dawley rats weighing 120-200 g. Islets were pulse-labelled with (2-^{3}H)-adenine (100/uCi/ml) for 60 min, and washed 4 times with centrifugation. They were finally placed in groups of 10-15 in "baskets" consisting of small plastic cylinders capped with nylon gauze (4). The final incubations were started by transferring the baskets to small incubation tubes containing 0.5 ml of pre-warmed buffer medium with additions according to the experimental protocol. Incubations were carried out at 37° and were terminated by boiling. During isolation and preincubation the buffer consisted of KHB with 2 mg/ml bovine albumin, 10 mM HEPES and 3.3 mM glucose. In the final incubations this buffer was modified by replacing $MgSO_4$ with $MgCl_2$ and by changing the glucose, and ionic concentrations according to the experimental protocol. Isoosmolarity was maintained by varying the NaCl concentration. Measurement of islet (^{3}H) cyclic AMP: Incubations were terminated by transferring the baskets containing the islets to new tubes containing a mixture of 0.4 ml of 0.9 % NaCl and 100/ug of cyclic AMP kept at boiling temperature in a waterbath. The samples were then boiled for 5 min. (^{3}H) cyclic AMP was purified as described (6) and the radioactivity counted by liquid scintillation. The purity of (^{3}H) cyclic AMP was checked by paper chromatography and phosphodiesterase treatment as described (7). Insulin assay: Insulin was measured using a charcoal method of radioimmunoassay (8).

Presentation of results: Because of the considerable inter-experimental variations encountered with the present in vitro system, all comparisons were calculated as paired differences (Student's t-test) within experiments.

RESULTS: Time-course of effects of Ba^{++}: In a medium devoid of added Ca^{++}, Ba^{++} (2,5 mM) rapidly increased islet (^{3}H) cyclic AMP levels. A peak response was recorded after 1 min of incubation which was the earliest time of measurements (Fig 1). After 3 and 10 min a smaller stimulation (approximately 40 % of basal) was observed. In a parallel fashion, insulin release

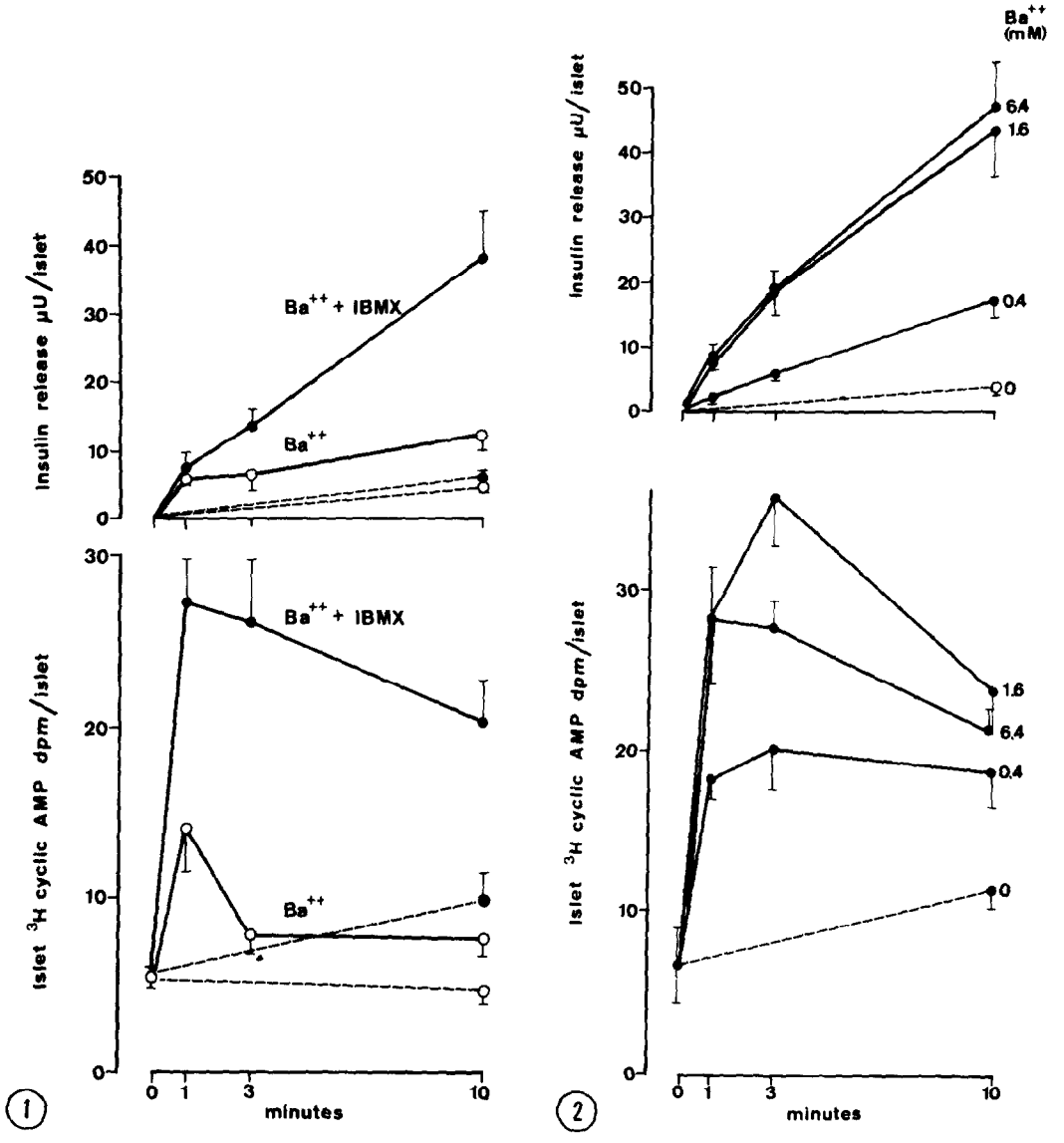

Fig. 1 Effects of 2.5 mM Ba^{++} on the (^3H) cyclic AMP and insulin responses in the absence or presence of 0.1 mM IBMX. ---o--- = basal medium (including 3.3 mM glucose), ---●--- = basal + IBMX. Mean ± S.E.M. of 6 complete experiments.

Fig. 2 Dose-response dependency of Ba^{++} effects on the (^3H) cyclic AMP and insulin responses. 0.1 mM IBMX and 3.3 mM glucose were included in all incubations. Mean ± S.E.M. of 6 complete experiments.

increased rapidly in response to Ba^{++}. Addition of 0.1 mM IBMX greatly enhanced the peak nucleotide response and delayed the subsequent decline of

(^{3}H) cyclic AMP. In parallel with this potentiation by IBMX, Ba^{++}-induced insulin release was greatly enhanced, particularly the "late" (3-10 min) secretory response. Since the potentiation by IBMX seemed to permit a more detailed analysis of the Ba^{++} effects the methylxanthine was included in all following experiments.

Dose-dependency of the Ba^{++} effects: The effects of 0.4, 1.6 and 6.4 mM Ba^{++} were compared (Fig 2). 1.6 mM of Ba^{++} elicited maximal (^{3}H) cyclic AMP and insulin responses, 0.4 mM Ba^{++} was submaximally effective in both these respects.

Effects of Ca^{++}: The (^{3}H) cyclic AMP response induced by 0.4 mM Ba^{++} was moderately inhibited by a 5-fold excess of Ca^{++}. Thus a 30 % inhibition of (^{3}H) cyclic AMP was observed after 3 min of stimulation ($p < 0.01$) while no significant inhibition by Ca^{++} was seen after 1 or 10 min of incubation (right part of Fig 3). By contrast insulin release was profoundly inhibited by at least 75 % after 3 and 10 min of incubation. The inhibitory action of Ca^{++} on the effects induced by Ba^{++} was concentration dependent as shown by the fact that inclusion of a low concentration of Ca^{++} (0.4 mM) did not affect insulin or (^{3}H) cyclic AMP responses to 2.5 mM Ba^{++} (left part of Fig 3).

Effects of Mg^{++}: Increasing Mg^{++} from 2 to 10 mM profoundly suppressed both (^{3}H) cyclic AMP and insulin responses induced by Ba^{++} when measured after 10 min of incubation (Table 1).

Effects of glucose: Ba^{++} stimulated (^{3}H) cyclic AMP in the complete absence of glucose (Table 2). Ba^{++} was also tested together with concentrations of glucose and Ca^{++} known to give optimal hexose stimulation (7). Under these conditions Ba^{++} effects on (^{3}H) cyclic AMP were additive to those of glucose, while an effect of Ba^{++} on insulin release was seen only after 1 min of incubation (Fig 4).

DISCUSSION: The present results clearly show that Ba^{++} is a potent stimulator of cyclic AMP in pancreatic islets. Since a majority of the cells of rat islets consist of B-cells (9) it is likely - but not definitive proven - that

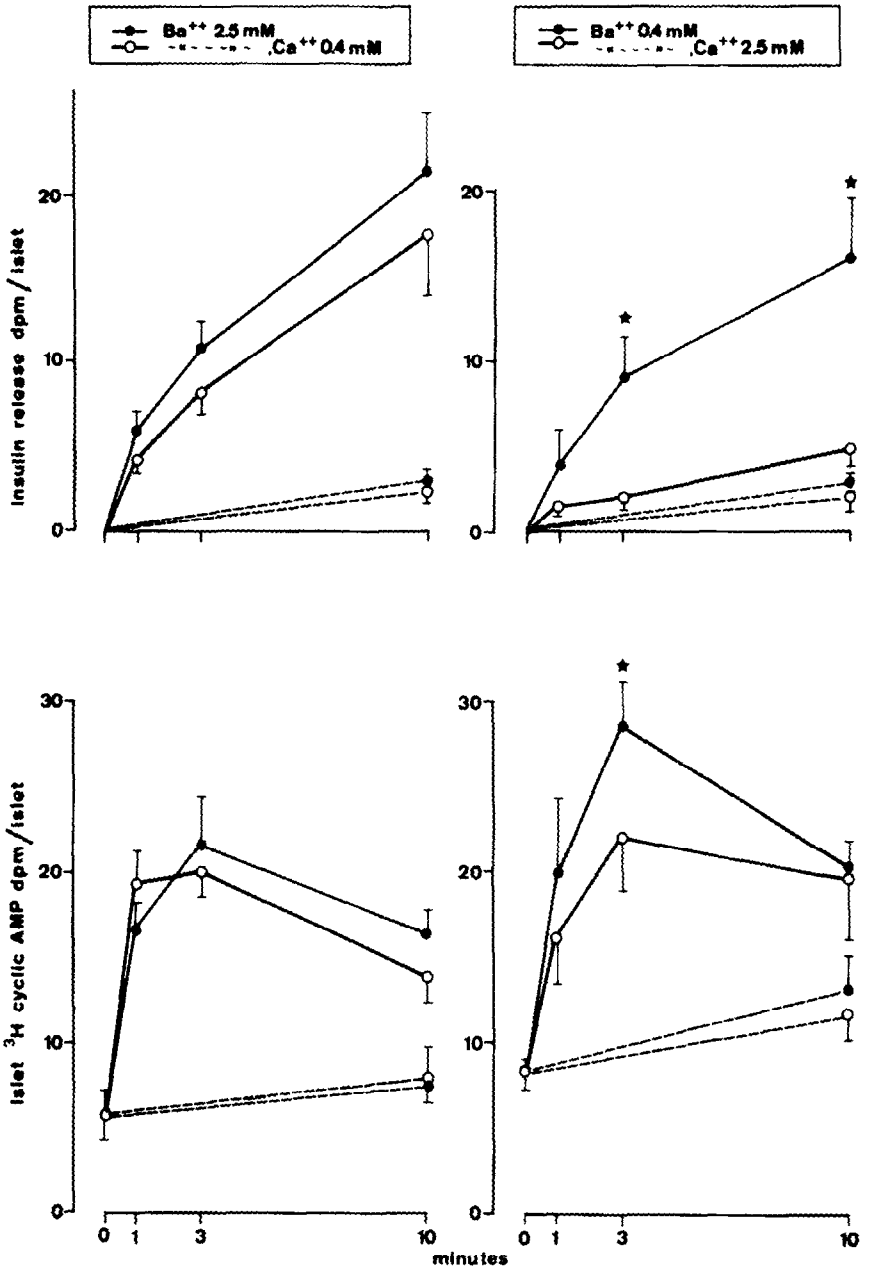

Fig. 3 Inhibitory effect of Ca^{++} on the Ba^{++}-induced (^3H) cyclic AMP and insulin response. Mean ± S.E.M. of 2 separate sets of experiments consisting of 7 (left part of figure) or 6 (right part of figure) complete experiments. 0.1 mM IBMX and 3.3 mM glucose were included in all incubations. ---●--- control, no Ca^{++}. ---o--- control + Ca^{++}. —●— Ba^{++}, —o— Ba^{++} + Ca^{++}. The concentrations of Ba^{++} were 2.5 and 0.4 and those of Ca^{++} 0.4 and 2.5 in the left and right part of the figure respectively. * = $p < 0.05$ or less, significance of difference for inhibition by Ca^{++}.

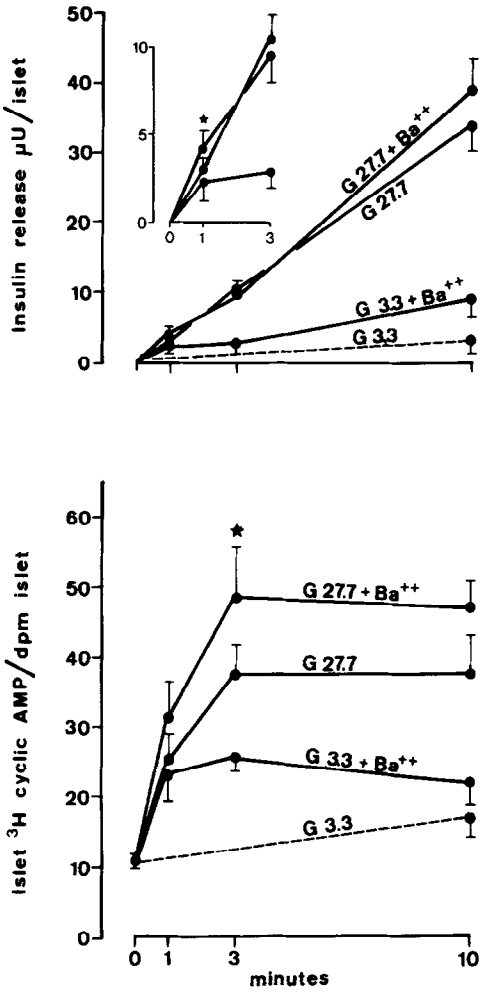

Fig. 4 Effect of Ba^{++} on the glucose-induced (^3H) cyclic AMP and insulin responses. The concentration of glucose was 3.3 or 27.7 mM, that of Ba^{++} 0.4 mM. 0.1 mM IBMX and 2.5 mM Ca^{++} was present in all incubations. Mean + S.E.M. of 6 complete experiments. * = $p < 0.05$, significance of difference for the barium effect in the presence of 27.7 mM glucose.

Ba^{++} increases cyclic AMP in the insulin-producing cells. With this reservation in mind, the findings support an important role of cyclic AMP in the insulinotropic action of Ba^{++}. Thus the time-course, dose-dependency as well as the potention by a phosphodiesterase inhibitor were similar for barium-induced cyclic AMP and insulin response, indicating a close coupling of these two actions of Ba^{++}.

Table 1 Effects of Mg^{++} on Ba^{++}-induced (^3H) cyclic AMP and insulin responses. Incubation time 10 min. Mean ± S.E.M. of 6 complete experiments. All incubations contained 3.3 mM glucose and 0.1 mM IBMX.

Mg (mM)	Ba^{++} (mM)	(^3H) cyclic AMP dpm/islet	Insulin release µU/islet/10 min
2	-	9.2 ± 1.3	3.7 ± 0.8
10	-	12.9 ± 1.9	5.1 ± 1.2
2	2.5	27.1 ± 2.9	38.1 ± 7.8
10	2.5	13.8 ± 2.1*	20.6 + 3.2*

* = $p < 0.05$ or less for difference line 3 vs 4.

The action of Ba^{++} on the insulinproducing cells may well be complex, the ion exerting effects both at the cell-membrane and at intra-cellular sites. Thus, Ba^{++} depolarizes the B-cell membrane, induces spike activity and prevents repolarization (10,11). These actions may involve substituting for Ca^{++} as a current carrier as well as blocking a Ca^{++}-activated K^+-permeability (10). An action on intracellular organelles is indicated by the fact that Ba^{++} readily permeates the cell membrane and is taken up into the mitochondria (12). Ba^{++} may thus influence insulin secretion in more than one way, other factors in addition to cyclic AMP being importance for the insulinotropic effect. This notion is supported by the present findings where the effects of Ca^{++} on Ba^{++} action were tested (Fig 3). In these experiments Ca^{++} inhibited the Ba^{++}-induced insulin release much more profoundly than the (^3H) cyclic AMP response.

The action of Ba^{++} on cyclic AMP and insulin did not require the presence of glucose, nor did Ba^{++} and glucose show any positive interaction at the concentrations tested. The present results are, however, insufficient to rule out interaction between these insulinogogues. At least it can be safely

Table 2 Effects of Ba^{++} in the absence of glucose. Incubation time 3 min. 0.1 mM IBMX was present in all incubations. Mean ± S.E.M. of 4 complete experiments.

Ba^{++} (mM)	(^3H) cyclic AMP dpm/islet	Insulin release µU/islet/3 min
-	17.2 ± 2.0	1.2 ± 0.5
2.5	43.4 ± 5.4*	10.4 ± 3.2*

* = $p < 0.05$ significance of difference for incubations with and without Ba^{++}.

concluded that Ba^{++} does not mimick the characteristics of many insulinogogues, including phosphodiesterase inhibitors, which amplify in a multiplicate fashion the maximal effect of glucose.

The mechanism by which Ba^{++} increases cyclic AMP is unknown. As pointed out above the action of Ba^{++} does not resemble that of a phosphodiesterase inhibitor. Activation by hormones of adenylate cyclase entails binding to a receptor or the outer side of the cell membrane, the receptor in turn activating a catalytic subunit of the enzyme which is probably located at the inner side of the membrane (13,14). It is tempting to speculate that the naked ion could directly activate the catalytic subunit. Such a mechanism may involve displacement of other cations from the adenylate cyclase or from its substrate. Alternatively, Ba^{++} may act by increasing intracellular levels of Ca^{++} which, in its turn, could activate the adenylate cyclase of the B-cell, again by so far unknown mechanisms.

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