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Heparin inhibits IP_3-induced Ca^{2+} release in permeabilized pancreatic β-cells

Thomas Nilsson, Jean Zwiller+, Alton L. Boynton+ and Per-Olof Berggren

Department of Medical Cell Biology, University of Uppsala, Biomedicum, Box 571, S-751 23 Uppsala, Sweden and *+Cancer Research Center of Hawaii, 1236 Lauhala St., Honolulu, Hawaii 96813, USA*

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Heparin was found to inhibit the Ca^{2+} release induced by inositol 1,4,5-trisphosphate (IP_3) in permeabilized pancreatic β-cells obtained from obese hyperglycemic mice. The effect of heparin was dose-dependent and not due to inhibition of Ca^{2+} uptake into the IP_3-sensitive pool. The effect appeared specific for heparin and was not reproduced by other polysaccharides such as chondroitin sulfates. Heparin might consequently be a useful tool when investigating the molecular mechanism whereby IP_3 mobilizes Ca^{2+}.

Ca^{2+} release; Inositol 1,4,5-trisphosphate; Inositol 1,3,4,5-tetrakisphosphate; Heparin; (Permeabilized pancreatic β-cell)

1. INTRODUCTION

In a variety of cells certain agonists activate phospholipase C, promoting the hydrolysis of inositol 4,5-bisphosphate with the subsequent formation of diacylglycerol and inositol 1,4,5-trisphosphate (IP_3) [1]. Whereas diacylglycerol is an activator of protein kinase C, IP_3 releases Ca^{2+} from intracellular stores [1]. The target for IP_3 is most likely the endoplasmic reticulum [1], although it has recently been proposed that the trisphosphate-sensitive pool represents a morphologically distinct organelle [2]. The IP_3-induced Ca^{2+} release is well characterized in many different cell types, but so far the underlying mechanism is unknown. The trisphosphate is believed to activate a membrane bound receptor regulating the opening of some sort of Ca^{2+} channel [3]. Specific binding sites for IP_3 have been described in many tissues [4-6], but little is known about the nature of the putative Ca^{2+} channel. After having fulfilled its role as second messenger, IP_3 can either be stepwise dephosphorylated or converted to inositol 1,3,4,5-tetrakisphosphate (IP_4) by a specific inositol 1,4,5-trisphosphate kinase [7-10]. Although the physiological role of IP_4 is somewhat unclear, it has been suggested that it opens up channels for Ca^{2+} in the plasma membrane [11].

Recently two of us discovered that IP_3 and IP_4 activate a protein phosphatase isolated from rat brain (Zwiller and Boynton, unpublished). This enzyme was active in the absence of metal ions, preferentially membrane bound and inhibited by heparin, indicating that it may correspond to a protein phosphatase type-1 [12]. Such an enzyme might consequently be involved in the mechanism whereby IP_3 releases Ca^{2+}. In the present study, we decided to investigate whether heparin interferes with IP_3-induced Ca^{2+} release in permeabilized pancreatic β-cells.

Correspondence address: P.-O. Berggren, Department of Medical Cell Biology, University of Uppsala, Biomedicum, Box 571, S-751 23 Uppsala, Sweden

2. MATERIALS AND METHODS

Pancreatic islets were obtained from obese hyperglycemic mice (*ob/ob*) taken from a local non-inbred colony [13]. A β-cell suspension was prepared and cultured as described [14]. The cells were permeabilized with electrical discharges (5 × 2.5 kV/cm) and suspended in 25 μl medium containing 110 mM KCl, 10 mM NaCl, 2 mM KH_2PO_4, 1 mM $MgCl_2$, 2 mM

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MgATP, 10 mM phosphocreatine, 20 U/ml creatine phosphokinase, 0.2 μM antimycin and 1 μg/ml oligomycin [15,16]. pH was 7.0 and the ambient Ca^{2+} concentration was monitored with a Ca^{2+} selective electrode [17]. Test substances were added as 100 × concentrated stock solutions with constant volume pipettes [18]. All experiments were performed with magnetic stirring at room temperature. The traces shown are typical for experiments repeated with at least three different cell preparations. Heparin, chondroitin sulfate A and C were bought from Sigma. IP_3 was from Amersham and IP_4 was a gift from Dr R.F. Irvine, Cambridge, England.

3. RESULTS AND DISCUSSION

When electrically permeabilized pancreatic β-cells were incubated in a Ca^{2+}-deficient medium containing ATP, an ATP regenerating system and mitochondrial inhibitors, there was a marked sequestration of Ca^{2+} (fig.1A), most likely promoted by the endoplasmic reticulum [19]. Under these conditions, addition of IP_3 evoked a pronounced mobilization of Ca^{2+}. In fig.1B the cells were incubated in the presence of 50 μg/ml heparin. Whereas there was almost no response to IP_3, a pulse addition of Ca^{2+} still elicited a transient rise in the ambient Ca^{2+} concentration. This effect of heparin could not be explained in terms of an inhibition of Ca^{2+} uptake into the IP_3-sensitive pool, since heparin added just prior to IP_3 produced the same effect (fig.1C). As demonstrated in fig.2, the effect of heparin was dose-dependent. Stimulation of Ca^{2+} release with 6 μM IP_3 was slightly attenuated by 1 μg/ml heparin and completely inhibited at 100 μg/ml. Approx. 5–10 μg/ml heparin caused a 50% inhibition of the IP_3-induced Ca^{2+} release. Previous studies have shown that the activity of protein phosphatase-1, isolated from rabbit skeletal muscle and liver, is inhibited by heparin at concentrations similar to those used in the present study, but not by chondroitin sulfate A and C [20,21]. In accordance with these results, neither chondroitin sulfate A (fig.3A) nor C (not shown) had any effect, even at concentrations as high as 100 μg/ml. Since the stimulatory effect of IP_3 on the phosphatase activity was inhibited by Mg^{2+} (Zwiller and Boynton, unpublished), we investigated the trisphosphate-induced Ca^{2+} release in the presence of high concentrations of this divalent cation. However, as is shown in fig.3B, 10 mM Mg^{2+} neither inhibited the Ca^{2+} release promoted by IP_3 nor interfered with the effect of heparin (not shown). Although IP_4 was found to activate the phosphatase, 10 μM of the tetrakisphosphate was ineffective in releasing Ca^{2+} in permeabilized β-cells, as has previously been reported for other cell types, including insulin pro-

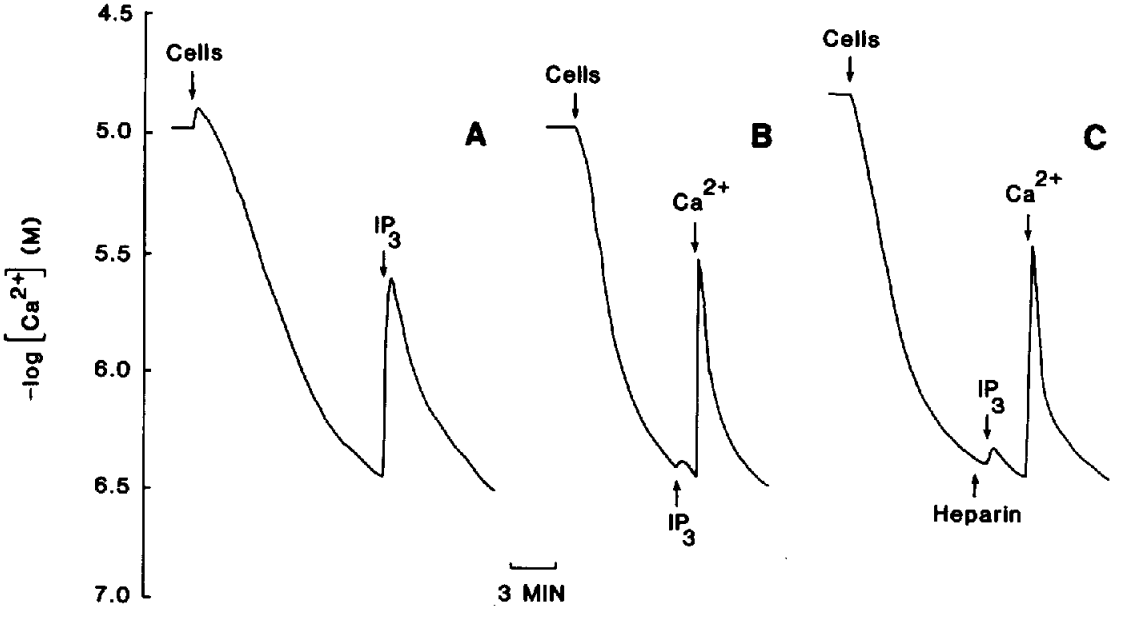

Fig.1. Effects of 6 μM IP_3, 50 μg/ml heparin and 0.25 nmol Ca^{2+} on the ambient Ca^{2+} concentration maintained by permeabilized pancreatic β-cells. In C, 50 μg/ml heparin was present throughout the experiment.

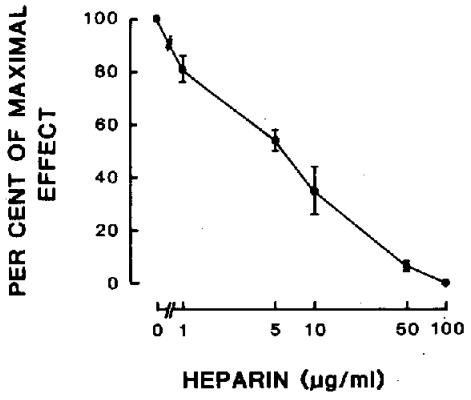

Fig.2. Effect of increasing concentrations of heparin on Ca^{2+} release induced by 6 μM IP_3. Mean ± SE for 3–7 experiments.

ducing RINm5F cells [10,22,23]. It is therefore not likely, at least in pancreatic β-cells, that IP_3 promotes Ca^{2+} release by activating a protein phosphatase.

When looking for possible inhibitors of the IP_3-induced Ca^{2+} release, it is of interest to note that monoclonal antibodies, which effectively inhibit the action of IP_3 on platelet membranes, have been produced [24]. Morever, the IP_3-induced Ca^{2+} release has been found to be suppressed by micromolar concentrations of Cd^{2+} in permeabilized β-cells [15]. The effect of Cd^{2+} might reflect its binding to IP_3 and thereby changing the binding properties of the trisphosphate to its receptor [25]. We cannot exclude that heparin also exerts its effect by binding IP_3, but it should be remembered that both the polysaccaride and the trisphosphate are negatively charged [26]. Recently it was demonstrated that heparin interacts with receptors for IP_3 in cerebellar membranes [27]. It is possible that such a mechanism also operates in pancreatic β-cells.

Heparin is known to interact, not only with protein phosphatases, but with a variety of different proteins [26,28,29]. The effect of heparin on the IP_3-induced Ca^{2+} release is probably of unspecific nature and most likely of no physiological relevance. However, by using heparin and/or fragments of this polysaccharide, it should be possible to clarify more specifically the molecular identity of the inhibitory site and thereby get a better understanding of the intricate mechanism whereby IP_3 promotes Ca^{2+} release.

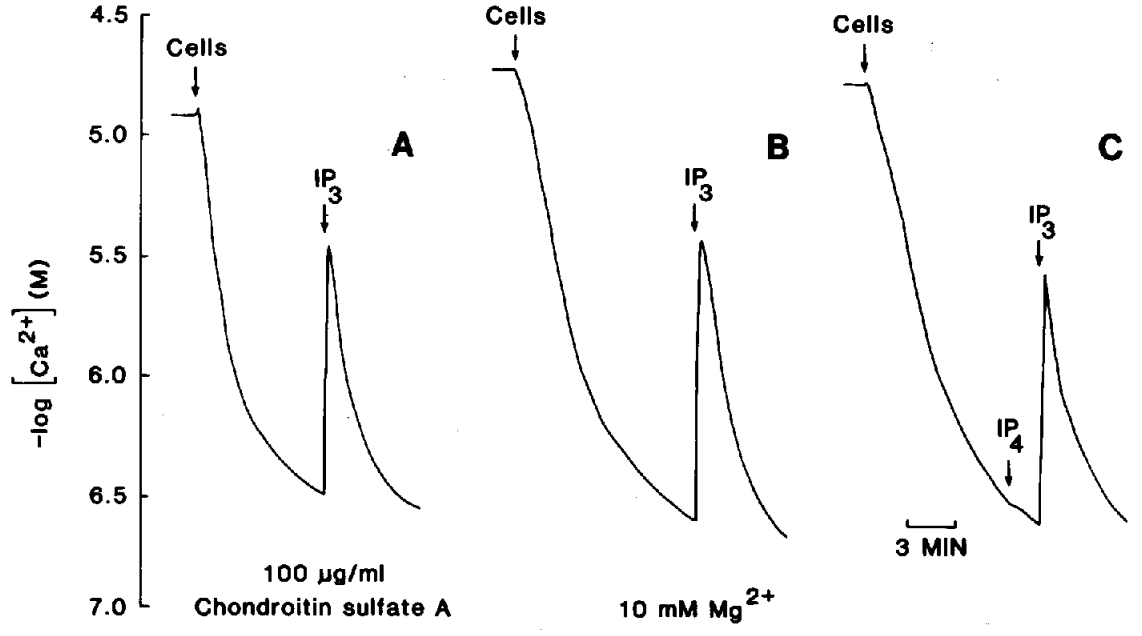

Fig.3. Effects of 6 μM IP_3 and 10 μM IP_4 on the ambient Ca^{2+} concentration maintained by permeabilized β-cells. In A and B, the incubations were performed in the presence of 100 μg/ml chondroitin sulfate A and 10 mM $MgCl_2$, respectively.

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