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Interaction with the inositol 1,4,5-trisphosphate receptor promotes Ca^{2+} sequestration in permeabilised insulin-secreting cells

Md. Shahidul Islam, Thomas Nilsson, Patrik Rorsman* and Per-Olof Berggren

The Rolf Luft Center for Diabetes Research, Department of Endocrinology, Karolinska Institute, Box 60500, Karolinska Hospital, S-104 01 Stockholm, Sweden

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Electropermeabilised insulin-secreting RINm5F cells sequestered Ca^{2+}, resulting in a steady-state level of the ambient free Ca^{2+} concentration corresponding to 723 ± 127 nM (mean ± SEM, $n = 10$), as monitored by a Ca^{2+}-selective minielectrode. Inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) promoted a rapid and pronounced release of Ca^{2+}. This Ca^{2+} was resequestered and a new steady-state Ca^{2+} level was attained, which was always lower (460 ± 102 nM, $n = 10$, $P < 0.001$) than the steady-state Ca^{2+} level maintained before the addition of $Ins(1,4,5)P_3$. Whereas the initial reuptake of Ca^{2+} subsequent to $Ins(1,4,5,)P_3$ stimulation was relatively slow, the later part of reuptake was fast as compared to the reuptake phases of a pulse addition of extraneous Ca^{2+}. In the latter case the uptake of Ca^{2+} resulted in a steady-state level similar to that found in the absence of $Ins(1,4,5)P_3$. Addition of $Ins(1,4,5)P_3$ under this condition resulted in a further Ca^{2+} uptake and thus a lower steady-state Ca^{2+} level. Heparin, which binds to the $Ins(1,4,5)P_3$ receptor, also lowered the steady-state free Ca^{2+} concentration. In contrast to $Ins(1,4,5)P_3$, inositol 1,3,4,5-tetrakisphosphate was without effect on Ca^{2+} sequestration. These findings are consistent with the presence of a high-affinity $Ins(1,4,5)P_3$ receptor promoting continuous release of Ca^{2+} under basal conditions and/or the $Ins(1,4,5)P_3$ receptor being actively involved in Ca^{2+} sequestration.

Inositol 1,4,5-trisphospate; Inositol 1,4,5-trisphosphate receptor; Intracellular Ca^{2+}-transport; Insulin-secreting cell

1. INTRODUCTION

Not only inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) but also inositol 1,3,4,5-tetrakisphosphate ($Ins(1,3,4,5)P_4$), one of its metabolites, is known to be involved in the generation of intracellular Ca^{2+} signals [1–3]. $Ins(1,4,5)P_3$ rapidly mobilizes intracellular Ca^{2+} stores in a wide variety of permeabilized cells, including insulin secreting RINm5F cells [4] and normal pancreatic β-cells [5]. The resulting rise in Ca^{2+} is short-lived and the ambient free Ca^{2+} concentration is eventually returned to basal levels reflecting reuptake into intracellular Ca^{2+} pools. Regulation of Ca^{2+} reuptake into intracellular Ca^{2+} pools following $Ins(1,4,5)P_3$-induced Ca^{2+} release is poorly understood. $Ins(1,3,4,5)P_4$ has been suggested to act in concert with $Ins(1,4,5)P_3$ in the mobilization of intracellular Ca^{2+} [2,3] and it has also been suggested that the tetrakisphosphate induces Ca^{2+} sequestration [6].

Using electropermeabilised insulin-secreting RINm5F cells and Ca^{2+} selective minielectrodes, we have investigated the reuptake of Ca^{2+} following its release by $Ins(1,4,5)P_3$. We now demonstrate that interaction with the $Ins(1,4,5)P_3$ receptor, in addition to releasing Ca^{2+}, also promotes the reuptake of this ion, resulting in a lowered ambient steady-state Ca^{2+} concentration.

Correspondence address: P.-O. Berggren, The Rolf Luft Center for Diabetes Research, Department of Endocrinology, Karolinska Institute, Box 60500, Karolinska Hospital, S-104 01 Stockholm, Sweden

* Permanent address: Department of Medical Physics, Gothenburg University, Box 33031, S-400 33, Gothenburg, Sweden

2. MATERIALS AND METHODS

Clonal insulin-secreting RINm5F cells were maintained in culture in RPMI 1640 medium supplemented with 10% foetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml), all from Flow Laboratories (Scotland).

$Ins(1,4,5)P_3$ (potassium salt) was purchased from Sigma (St. Louis, USA). HPLC pure $Ins(1,3,4,5)P_4$ and $Ins(2,4,5)P_3$ were generous gifts from Dr R.F. Irvine, Cambridge, UK. Calcium ionophore cocktail, containing neutral carrier ETH 1001 was from Fluka. All other chemicals were of highly purified grade and were either from Sigma or Merck.

Cells were detached from culture flasks using Trypsin-EDTA. They were then washed twice with culture medium and twice with a cold nominally Ca^{2+} free buffer, containing 110 mM KCl, 10 mM NaCl, 2 mM KH_2PO_4, 1 mM $MgCl_2$, 0.5 mg/ml bovine serum albumin and 25 mM HEPES, pH 7.0 (adjusted with KOH). After washing the cells were permeabilised by exposure to high-voltage discharges (six pulses of 3.2 kV/cm). This treatment resulted in more than 90% permeabilised cells, as verified by Trypan blue uptake. After permeabilisation, cells were centrifuged and the pellet was kept on ice until use.

8 μl of cell pellet was added to a plexiglass chamber containing 52 μl of incubation buffer. The incubation buffer was supplemented with 2 mM MgATP and an ATP regenerating system, consisting of 10 mM phosphocreatine and 20 U/ml of creatine kinase. The incubation buffer also contained mitochondrial inhibitors consisting of 0.2 μM antimycin and 1 μg/ml of oligomycin. Experiments were carried out at room temperature and the cell suspension was stirred continuously, using a small magnetic bar. Additions were made from 100–200 times concentrated solutions. Changes in the ambient free Ca^{2+} concentra-

tion were recorded using a Ca^{2+}-selective minielectrode constructed and calibrated essentially as described by Tsien and Rink [7]. Calibration of the electrode was done at the beginning and at the end of each experiment. All data on Ca^{2+} concentrations are given as mean values ± SEM and statistical significances were judged by Student's *t*-test for paired data.

3. RESULTS AND DISCUSSION

Addition of permeabilised RINm5F cells (4.7×10^7 cells/ml) resulted in a lowering of the ambient free Ca^{2+}-concentration to a steady-state level of 723 ± 127 nM, from the initial Ca^{2+} level of 4.5 ± 0.18 μM ($n = 10$). Stimulation with 5 μM $Ins(1,4,5)P_3$ induced a prompt release of Ca^{2+} reaching a value of 2.72 ± 0.16 μM ($n = 10$), which was slowly taken up again (Fig. 1A). The new steady-state Ca^{2+} level obtained was lower ($P < 0.001$) than prior to stimulation with $Ins(1,4,5)P_3$ and corresponded to 460 ± 102 nM ($n = 10$).

When a pulse of $CaCl_2$ (0.6 nmol) was added to the cells, the initial uptake of the ion was rapid but the terminal part of the uptake phase was slow and the Ca^{2+} concentration was maintained at a slightly elevated level, during the period of observation (Fig. 1B). $Ins(1,4,5)P_3$ added under these conditions induced a normal release of Ca^{2+}. In this case the initial phase of reuptake of Ca^{2+} was relatively slow, as compared to the initial rapid uptake following an extraneous Ca^{2+} pulse. However, the later part of the reuptake phase was rapid, reaching a steady-state level that was lower than that prior to the addition of $CaCl_2$ or $Ins(1,4,5)P_3$. Previous studies that have addressed the effect of $Ins(1,4,5)P_3$ on Ca^{2+} handling in permeabilised cells have not discussed the lowering effect of the trisphosphate on the steady-state Ca^{2+} level [8–13]. This effect is easy to miss if $Ins(1,4,5)P_3$ is added during continuing Ca^{2+}-uptake and if enough time is not allowed before and after the actual additions [8–12]. In neoplastic rat liver epithelial cells $Ins(1,3,4,5)P_4$ has been found to promote sequestration of pulse additions of Ca^{2+} or Ca^{2+} released by $Ins(2,4,5)P_3$ [6]. However, under these conditions 2 μM $Ins(1,3,4,5)P_4$ did not lower the steady-state free Ca^{2+} concentration and the intracellular Ca^{2+} pools had to be saturated prior to the addition of $Ins(1,3,4,5)P_4$. We were unable to demonstrate any lowering effect of 2.5 μM $Ins(1,3,4,5)P_4$ on the steady-state Ca^{2+} level (Fig. 2). Rather, at a concentration of 5 μM, $Ins(1,3,4,5)P_4$ induced a small increase in Ca^{2+} (data not shown). It is therefore unlikely, that the increased Ca^{2+} sequestration following $Ins(1,4,5)P_3$-induced Ca^{2+} release is mediated through metabolism of $Ins(1,4,5)P_3$ to $Ins(1,3,4,5)P_4$.

In the presence of heparin (200 μg/ml), which binds to the $Ins(1,4,5)P_3$ receptor [14] and blocks the trisphosphate-mediated release of Ca^{2+} [15–17], $Ins(1,4,5)P_3$ failed to induce a rise in the Ca^{2+} concentration, but the lowering effect on the steady-state Ca^{2+} concentration was still evident, starting approximately a minute after addition of $Ins(1,4,5)P_3$ (Fig. 3A). As evident from Fig. 3B, heparin also blocked the Ca^{2+} release evoked by $Ins(2,4,5)P_3$, a non-metabolisable analogue of $Ins(1,4,5)P_3$, resulting in a similar lowering in Ca^{2+} as that obtained in the presence of heparin plus $Ins(1,4,5)P_3$. Interestingly, it was observed that heparin alone also lowered the steady-state Ca^{2+} level (Fig. 4). That heparin by itself may cause increased sequestration of Ca^{2+} is apparent from at least one other study [17]. The same study also demonstrated that heparin-induced reuptake of $Ins(1,4,5)P_3$-released Ca^{2+} was extremely rapid. Noteworthy is that the effects of $Ins(1,4,5)P_3$ and heparin on Ca^{2+} sequestration were not additive (cf. Figs. 1, 3 and 4), suggesting that these agonists act through the same mechanism.

The mechanism(s) by which $Ins(1,4,5)P_3$ and heparin induce sequestration of Ca^{2+} can only be speculated upon at this stage. One possibility is the existence of high-affinity $Ins(1,4,5)P_3$ receptors. Under basal condi-

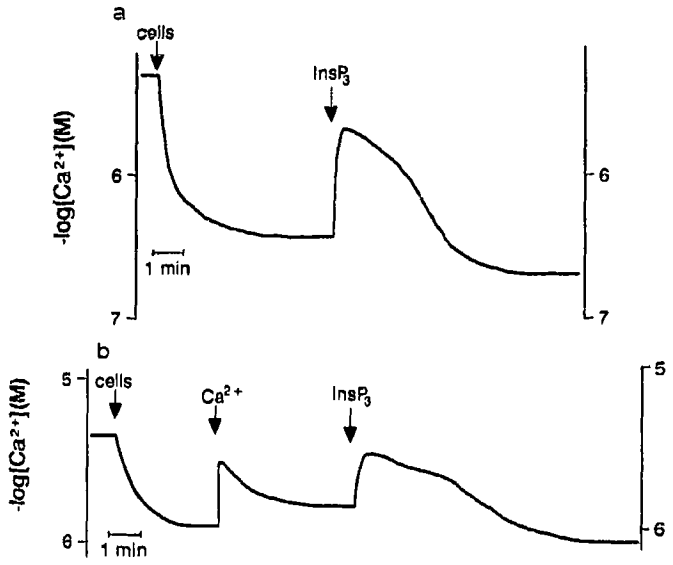

Fig. 1. Effect of $Ins(1,4,5)P_3$ on the steady-state free Ca^{2+} concentration. The figure shows Ca^{2+}-electrode traces obtained under conditions described in materials and methods section. A. At the point indicated $Ins(1,4,5)P_3$ (5 μM, final concentration) was added. The trace is typical of 6 independent experiments. B. At points indicated $CaCl_2$ (0.6 nmol) or $Ins(1,4,5)P_3$ (5 μM, final concentration) was added. The trace is representative of 4 different experiments.

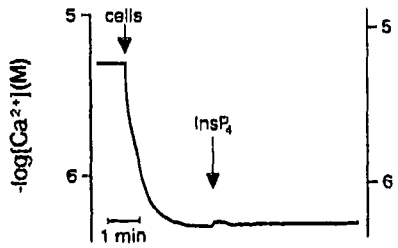

Fig. 2. Effect of $Ins(1,3,4,5)P_4$ on the steady-state free Ca^{2+} concentration. At the point indicated $Ins(1,3,4,5)P_4$ (2.5 μM, final concentration) was added. The trace is typical of three different experiments.

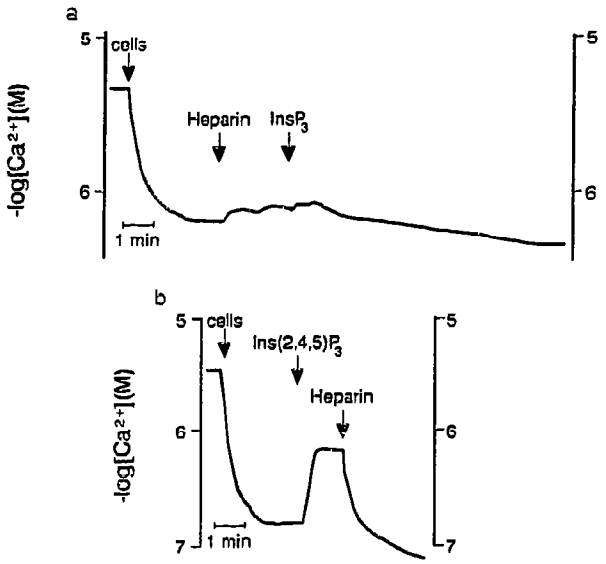

Fig. 3. Effect of Ins(1,4,5)P_3, in the presence of heparin, on the steady-state free Ca^{2+} concentration (A). Heparin (200 μg/ml) and Ins(1,4,5)P_3 (5 μM) were added as indicated. The trace is representative of three different experiments. B. Effect of adding heparin after Ins(2,4,5)P_3-induced Ca^{2+} release. As indicated Ins(2,4,5)P_3 (5 μM) or heparin (200 μg/ml) were added. The trace is representative of three different experiments.

tions low levels of Ins(1,4,5)P_3 [11] mediate continuous mobilisation of Ca^{2+} from endoplasmic reticulum, thus balancing the uptake of the ion. Following exposure to high concentrations of Ins(1,4,5)P_3 these receptors may become down-regulated and therefore Ca^{2+} uptake is not counteracted, resulting in a more pronounced buffering of Ca^{2+}. By binding to these high-affinity Ins(1,4,5)P_3 receptors heparin will block the Ca^{2+} release pathway operating under basal conditions, the net effect also in this case being an increased uptake of Ca^{2+}. Prentki et al. proposed a role for basal Ins(1,4,5)P_3 in regulating Ca^{2+} cycling across endoplasmic reticulum [11] and it appears that various cells indeed contain low levels of Ins(1,4,5)P_3 even under basal conditions [18]. Hence, the continuous presence of low concentrations of Ins(1,4,5)P_3 will enable continuous activation of the high-affinity Ins(1,4,5)P_3 receptors. Spät et al. described a type of high-affinity Ins(1,4,5)P_3 receptor of unknown functional significance in neutrophils [19]. Whether such receptors also exist in RINm5F cells merits further investigations.

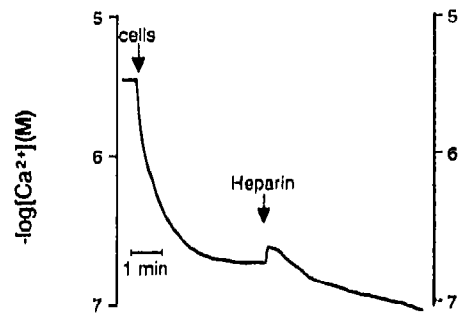

Fig. 4. The effect of heparin on the steady-state free Ca^{2+} concentration. Heparin (200 μg/ml) was added as indicated. The trace is typical of three different experiments.

Another possibility whereby Ins(1,4,5)P_3 and heparin promote increased Ca^{2+} sequestration may be through the activation of receptors distinct from the traditional Ins(1,4,5)P_3 receptors. In this case heparin, by being a structural analogue of Ins(1,4,5)P_3, may actually promote Ca^{2+} uptake by acting like a partial agonist.

We have now demonstrated that the presence of Ins(1,4,5)P_3 not only leads to Ca^{2+} mobilisation but also a more effective Ca^{2+} buffering. Hence, under physiological conditions the trisphosphate may induce sequential both increase and decrease in cytoplasmic free Ca^{2+}, enabling complex regulation of intracellular processes dependent on Ca^{2+}.

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