

Inositol trisphosphate accumulation by high K^+ stimulation in cultured adrenal chromaffin cells

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Stimulation with high K^+ (KCl, 56 mM) of *myo*- $[^3H]$ inositol-prelabelled cells increased Ca^{2+} uptake and $[^3H]$ inositol trisphosphate (IP_3) accumulation in a concentration-dependent manner. Nifedipine, a Ca^{2+} channel antagonist, inhibited high K^+ -induced $[^3H]IP_3$ accumulation and $^{45}Ca^{2+}$ uptake with a similar potency. Furthermore, ionomycin (1 μM), a Ca^{2+} ionophore, also induced $^{45}Ca^{2+}$ uptake and $[^3H]IP_3$ accumulation. These results indicate the existence of the Ca^{2+} uptake-triggered mechanism of IP_3 formation in cultured adrenal chromaffin cells.

Inositol trisphosphate accumulation; Ca^{2+} uptake; High K^+ stimulation; (Adrenal chromaffin cell)

1. INTRODUCTION

In various types of cells including adrenal chromaffin cells, it appears established that mobilization of Ca^{2+} from its intracellular stores following receptor stimulation is the consequence, not the cause, of inositol trisphosphate (IP_3) formation [1–5]. However, the role of extracellular Ca^{2+} and its influx into cells in controlling IP_3 formation remains unclarified [6,7]. High K^+ stimulation which provokes membrane depolarization is the most common method to introduce Ca^{2+} into the cells without receptor stimulation. It has been reported that in some tissues, such as rabbit iris smooth muscle [8] and GH_3 pituitary tumor cells [9], high K^+ stimulation is unable to induce IP_3 accumulation.

We report here that high K^+ stimulates IP_3 accumulation in cultured adrenal chromaffin cells.

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2. EXPERIMENTAL

2.1. Primary culture of bovine adrenal chromaffin cells

According to our previous reports [10,11], chromaffin cells were isolated and plated on 35 mm diameter dishes (2.5×10^6 cells/dish) and a 16 mm diameter 4-well dish (1×10^6 cells/well) in a volume of 3 ml and 1 ml of minimum essential medium (Gibco; Grand Island, NY), supplemented with 10% fetal calf serum (Hyclone; Logan, UT), respectively. The chromaffin cells were used for experiments 5 days after plating.

2.2. Measurement of inositol phosphate accumulation

3 days after the cell preparation, 2 ml of culture medium was removed from the dishes. *myo*- $[2-^3H]$ inositol (17 Ci/mmol, Amersham International Inc., Buckinghamshire, England) was added (10 $\mu Ci/ml$) to each dish and the cells were cultured further for 2 days. The cells were washed three times with 3 ml of Locke's solution of the following composition: 154 mM NaCl, 5.6 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 3.6 mM

NaHCO₃, 5.6 mM glucose, and 10 mM Hepes (pH 7.4). The cells were preincubated with 1 ml of Locke's solution supplemented with 0.1% bovine serum albumin and 10 mM LiCl at 37°C for 20 min. Stimulant was added to the incubation mixture and the cells were incubated for the indicated time periods. Antagonist was added, when necessary, 3 min before the addition of stimulants. At the end of the incubation, the incubation mixture was aspirated and 1.5 ml of 10% trichloroacetic acid was added to culture dishes. The attached cells were homogenized by sonication (30 s) and the homogenate was transferred to 1.5 ml plastic tubes followed by centrifugation at 12000 rpm for 60 s. The supernatant was transferred to 10 ml glass tubes and trichloroacetic acid was extracted four times with 4 ml water-saturated diethyl ether. Inositol phosphates were separated by ion-exchange chromatography according to the method of Berridge et al. [12].

2.3. Measurement of $^{45}\text{Ca}^{2+}$ uptake

Cellular $^{45}\text{Ca}^{2+}$ uptake was measured as described in [10,11].

3. RESULTS AND DISCUSSION

Fig.1 shows the time course of [^3H]inositol phosphate accumulation and $^{45}\text{Ca}^{2+}$ uptake stimulated by high K⁺. [^3H]IP₃ accumulation increased significantly 30 s after high K⁺ stimulation and reached maximum level at 2 min. The accumulation of [^3H]inositol phosphate did not increase significantly at 15 s after stimulation. By 5 min, the accumulation of IP₃ gradually decreased and declined to basal level at 15 min. This pattern was also seen with [^3H]inositol bisphosphate and [^3H]inositol monophosphate. The cellular content of inositol phosphates in non-stimulated cells was not changed significantly during the whole experimental period (fig.1). The bottom panel shows the time course of high K⁺-induced $^{45}\text{Ca}^{2+}$ uptake. The $^{45}\text{Ca}^{2+}$ uptake increased significantly at 15 s and reached maximum level at 1 min. The intracellular free Ca²⁺ concentration was also measured by using the fluorescent Ca²⁺ indicator fura-2. High K⁺ stimulation induced a rapid rise in intracellular free Ca²⁺ concentra-

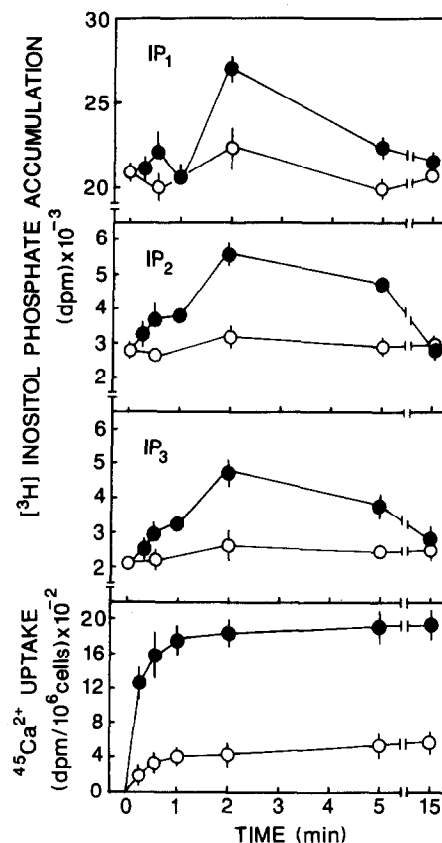


Fig.1. Time course of the high K⁺-induced inositol phosphate accumulation in cultured adrenal chromaffin cells. The cells were treated with or without KCl (56 mM) for the indicated time period. For the determination of the cellular $^{45}\text{Ca}^{2+}$ uptake, the cells (1×10^6 cells/well) were preincubated with Locke's solution for 3 min. The cells were then stimulated by KCl (56 mM) at time zero. The results shown are mean \pm SE of quadruplicate determinations. Typical data obtained from three different cell preparations are presented. (○) Basal, (●) KCl (56 mM).

tion to maximum level within 5 s (not shown) and the result is consistent with our previous observations by use of quin-2 [13].

As shown in table 1, KCl induced a concentration-dependent increase in [^3H]IP₃ accumulation and $^{45}\text{Ca}^{2+}$ uptake with a similar potency. Nifedipine (30 μM), a Ca²⁺ channel antagonist, completely inhibited high K⁺-induced [^3H]IP₃ accumulation and $^{45}\text{Ca}^{2+}$ uptake. The apparent IC₅₀ of nifedipine for these cellular

Table 1

Effects of KCl, ionomycin and nifedipine on IP₃ accumulation and ⁴⁵Ca²⁺ uptake

| Additions | IP ₃ accumulation (dpm) | ⁴⁵ Ca ²⁺ uptake (dpm) |
|-----------------------------|------------------------------------|---|
| None | 1740 ± 170 | 880 ± 90 |
| KCl (28 mM) | 2400 ± 120 ^a | 1200 ± 80 ^a |
| KCl (56 mM) | 3350 ± 180 ^a | 2100 ± 110 ^a |
| Nifedipine (30 μM) | 1610 ± 70 | 810 ± 30 |
| KCl (56 mM) + nifedipine | 1670 ± 130 | 910 ± 40 |
| Ionomycin (1 μM) | 2580 ± 60 ^a | 2370 ± 180 ^a |

Determination of [³H]IP₃ accumulation and cellular ⁴⁵Ca²⁺ uptake for 2 min was carried out as described in section 2. Nifedipine was added to the preincubation medium 3 min before the addition of 56 mM KCl. The results shown are mean ± SE of quadruplicate determinations. Typical data obtained from three different cell preparations are presented. ^a *P* < 0.05 vs none

responses was 5 μM. Nifedipine affected neither basal [³H]IP₃ accumulation nor basal ⁴⁵Ca²⁺ uptake at the concentrations tested. Furthermore, ionomycin, a Ca²⁺ ionophore, also induced an increase in [³H]inositol phosphate accumulation and ⁴⁵Ca²⁺ uptake.

Since the [³H]IP₃ accumulation was intimately related to cellular ⁴⁵Ca²⁺ uptake, the effect of extracellular Ca²⁺ on high K⁺-induced [³H]inositol phosphate accumulation was examined. High K⁺ failed to increase [³H]inositol phosphate accumulation in Ca²⁺-depleted medium and the basal level of accumulation was also reduced slightly (not shown).

The above evidence indicates the existence of a Ca²⁺ entry-dependent mechanism of IP₃ formation in cultured adrenal chromaffin cells. These results also indicate that depolarization of the cell membrane itself does not lead to the stimulation of IP₃ formation in these cells. Although the physiological significance of this Ca²⁺ entry-dependent IP₃ formation is unknown, it is possible that phospholipase C with low sensitivity to Ca²⁺, which is activated by massive Ca²⁺ entry, is involved in the amplification system of receptor stimulation-mediated IP₃ formation in these cells.

It has been reported that in some tissues such as

visceral smooth muscles [14] and pancreatic islets [15–17], high K⁺ stimulates the accumulation of inositol phosphates. Thus, it might be possible that IP₃ accumulation is also induced by high K⁺ through a Ca²⁺-dependent mechanism in these tissues. Alternatively, it is also possible that since the bulk of tissues may contain nerve endings, neurotransmitters released by high K⁺ stimulation may cause IP₃ accumulation [7,8].

In order to exclude the possibility that the IP₃ accumulation is induced by some substances which are secondarily released from chromaffin cells by high K⁺ stimulation, we have performed the following experiments. The cells were stimulated with high K⁺ for 2 min either in 1 ml or 1000 ml of the incubation medium which were stirred continuously, and the amount of accumulated [³H]IP₃ determined thereafter. High K⁺ stimulated [³H]IP₃ accumulation either in 1 ml or 1000 ml medium to a similar extent, i.e. 156% in 1 ml medium and 165% in 1000 ml medium compared to the prestimulatory level (100%). It is highly unlikely that the high K⁺-induced IP₃ accumulation is due to some substances which are secondarily released from chromaffin cells by high K⁺ stimulation.

In conclusion, our present results demonstrate the existence of a Ca²⁺ uptake-dependent mechanism of IP₃ formation in cultured adrenal chromaffin cells.

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