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Cyclic AMP-evoked oscillations of intracellular [Ca^{2+}] in guinea-pig hepatocytes

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The effects of the β-adrenoceptor agonist isoprenaline and cyclic AMP (cAMP) on cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) were studied in the single guinea-pig hepatocyte. In common with $InsP_3$-dependent agonists such as noradrenaline or angiotensin II, isoprenaline (0.5–10 μM) and cAMP (50–100 mM, perfused into the cell via the patch-pipette), were able to generate fast and slow fluctuations of $[Ca^{2+}]_i$. Responses to isoprenaline and cAMP also were observed in the absence of external Ca^{2+}. Isoprenaline-evoked $[Ca^{2+}]_i$ rises were not blocked by the intracellular perfusion of heparin, suggesting that these fluctuations are independent of the binding of $InsP_3$ to its receptor.

INTRODUCTION

Recent studies have revealed that a wide range of non-excitable cells, including liver, display oscillations of cytosolic free [Ca^{2+}] ($[Ca^{2+}]_i$) in response to $InsP_3$-dependent hormones and neurotransmitters [1,2]. A distinction has been made between sinusoidal (type I: oscillations on an elevated plateau phase) and base-line spiking (type II: transient rising from resting $[Ca^{2+}]_i$) behaviour corresponding to fast and slow fluctuations of $[Ca^{2+}]_i$ respectively [3]. Also, recent work has shown that oscillations can be triggered directly by Ca^{2+} [4] or by bile acids in the absence of $InsP_3$ [5]. We have shown previously that glucagon, isoprenaline and dibutyryl cAMP increase $[Ca^{2+}]_i$ in rat liver cells, probably by releasing Ca^{2+} from the same internal store as that permeabilized by vasopressin [6,7]. In the present work, we investigated the effects of isoprenaline, a β-adrenoceptor agonist, and cAMP on $[Ca^{2+}]_i$ by monitoring the Ca^{2+}-dependent K^+ conductance in single guinea-pig hepatocytes. The results presented here show, first, that isoprenaline and intracellularly applied cAMP can evoke fast (type I) and slow (type II) fluctuations of $[Ca^{2+}]_i$, and second, that isoprenaline-induced type I responses are insensitive to

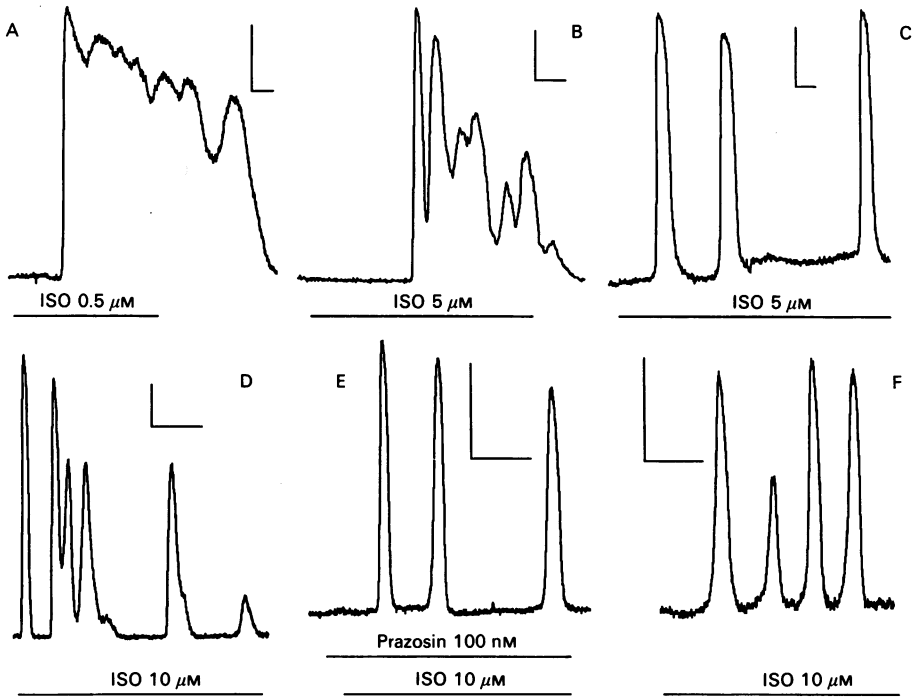

Fig. 1. Outward Ca^{2+}-dependent K^+ current evoked by the external application of isoprenaline (ISO) in single guinea-pig hepatocytes

Data were obtained from five different cells voltage-clamped at 0 mV. (A) Application of isoprenaline (0.5 μM) along the horizontal bar elicited a large and fast rise in K^+ conductance, followed by an oscillating plateau phase (type I oscillations). (B) and (C), type I (B) and type II (C) oscillations evoked by an application of 5 μM-isoprenaline in two different cells. (D) and (E), regular type II oscillations recorded on the same cell in the absence (D) or presence (E) of 100 nM-prazosin (0.01 % ethanol) applied shortly before 10 μM-isoprenaline. (F) Type II oscillations recorded during a prolonged application of 10 μM-isoprenaline. The response consisted of cyclical activations of the Ca^{2+}-dependent K^+ current arising from a resting baseline level. Scale bars: vertical, 200 pA; horizontal, 20 s (A, B, C), 100 s (D, E, F).

Abbreviations used: $[Ca^{2+}]_i$, cytosolic free [Ca^{2+}]; cAMP, cyclic AMP.

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heparin (known to block $InsP_3$ binding to its receptor [8,9]) perfused into the cell via the patch-pipette.

EXPERIMENTAL

Materials

Collagenase was obtained from Worthington Biochemical Corp. or Boehringer Mannheim, and heparin was from Prolabo. All other reagents were of the highest purity commercially available and were obtained from Sigma or Boehringer Mannheim.

Methods

All the procedures utilized in this study described here, including cell preparation, cell incubation and whole-cell recording techniques, were as described in [5]. Experiments were made in chloride-free conditions, with gluconate replacing chloride. The external solution contained (mM): sodium gluconate, 145; potassium gluconate, 5.6; $CaSO_4$, 5; $MgSO_4$, 1.2; NaH_2PO_4, 0.4; Hepes, 8 (pH 7.3). In Ca^{2+}-free experiments, the external medium contained (mM): sodium gluconate, 149; potassium gluconate, 5.6; $MgSO_4$, 3; NaH_2PO_4, 0.8; EGTA, 0.04 (*a*) and none (*b*); Hepes, 8 (pH 7.3). Patch-pipettes contained (mM): potassium gluconate, 153; Na_2ATP, 3; $MgSO_4$, 3; Na_2GTP, 0.3; Hepes, 8 (pH 7.3). The dish was continuously perfused, and hormones or drugs were added to the external medium. All solutions were Millipore-filtered (0.22 μm pore size).

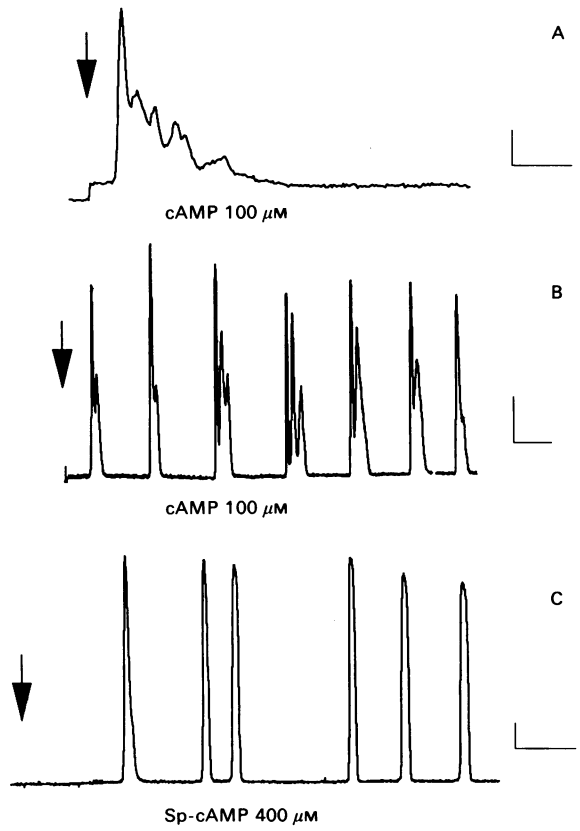

Fig. 2. Ca^{2+}-dependent K^+ outward current evoked by internal perfusion of single guinea-pig hepatocytes with cAMP and Sp-cAMP

Traces were obtained from three different cells, with membrane potentials held at 0 mV. The arrows indicate the rupture of the patch of membrane underneath the pipette on going to whole-cell recording conditions. This also represents the beginning of the internal perfusion with the cyclic nucleotides. (A) Single response to 100 μM-cAMP, displaying type I oscillations on the declining phase. (B) Type II oscillations evoked by 100 μM-cAMP. (C) Type II response induced by 400 μM-Sp-cAMP. Scale bars: vertical, 200 pA; horizontal, 20 s (A), 100 s (B, C).

RESULTS AND DISCUSSION

Guinea-pig liver cells possess a Ca^{2+}-dependent K^+ conductance that has been characterized in detail [10–12] and provides a measure of hormone-mediated $[Ca^{2+}]_i$ rises in single cells. Control experiments on a suspension of guinea-pig liver cells showed that isoprenaline promoted a time- and dose-dependent accumulation of cAMP, with an EC_{50} (concn. giving 50% of maximum effect) of about 30 nM and maximal response between 0.3 and 1 μM (results not shown). Fig. 1 shows that maximal concentrations of the β-adrenoceptor agonist isoprenaline (0.5–10 μM) initiated cyclical fluctuations of the Ca^{2+}-dependent K^+ current. Several patterns of oscillations were seen and were not dependent on the agonist concentration. Some 60% of the cells (15 cells tested) showed a fast peak, followed by a slow decline exhibiting oscillations of elevated frequency (type 1, 5–10 min^{-1}; Fig. 1, traces A and B). The remaining 40% of cells displayed more regular spikes of slow frequency (type 2, 0.25–1 min^{-1}; Fig. 1, traces C–F). This remarkable heterogeneity of the cellular response to a given stimulation has previously been observed in single hepatocytes treated with $InsP_3$-dependent agonists and attributed to cell-to-cell variations [5,13,14]. The possibility of an indirect effect of isoprenaline on α_1-adrenoceptors could be ruled out by the lack of effect of the α_1-

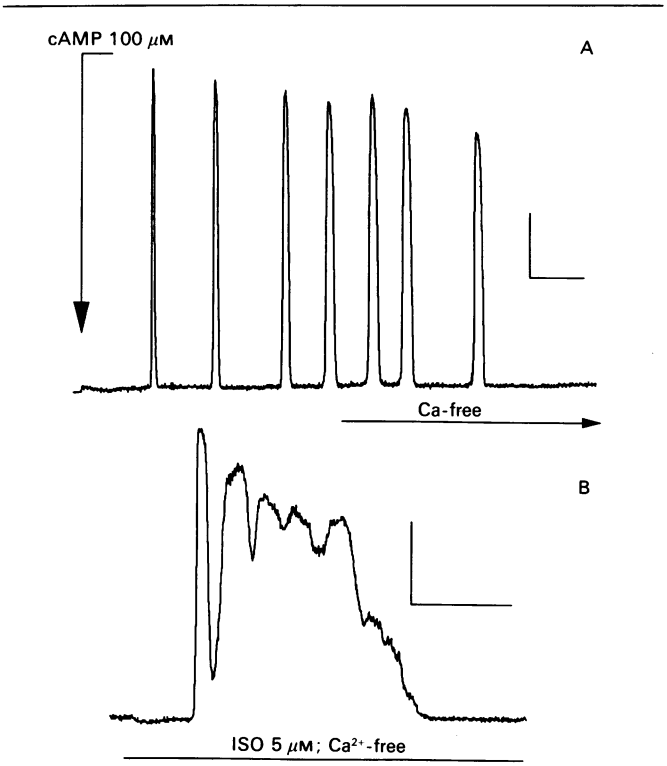

Fig. 3. (*a*) Internal perfusion with cAMP (100 μM, starting as indicated by the vertical arrow) evoked type II oscillations maintained for at least 3 min in Ca^{2+}-free medium applied along the horizontal arrow; (*b*) isoprenaline (ISO, 5 μM) applied along the bar induced a large rise in K^+ conductance in absence of external Ca^{2+}

Membrane potentials were held at 0 mV. Scale bars: vertical, 200 pA; horizontal, 100 s (A), 60 s (B).

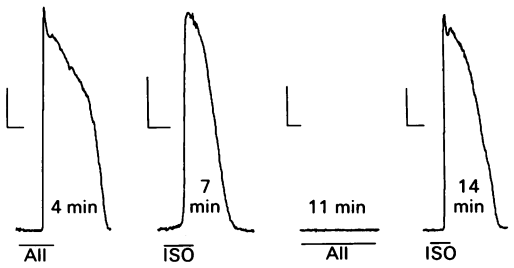

Fig. 4. Effect of heparin on the rise in Ca^{2+}-dependent K^+ conductance evoked by an $InsP_3$-dependent agonist (angiotensin II; AII) and a cAMP-dependent agonist (isoprenaline, ISO) on the same guinea-pig hepatocyte

The patch-pipette contained 150 μg of heparin/ml, which diffused into the cell. The time elapsed since the beginning of the internal perfusion after going to whole-cell recording conditions is indicated inside each trace. The hormones (AII, 100 μM; ISO, 5 μM) were applied to the cell as indicated by the horizontal bars. Owing to the rather long duration of this experiment, they were washed off the external solutions as soon as the first phase of the response was obtained. These four traces show that the angiotensin II-evoked rise K^+ conductance was blocked after 10 min of internal perfusion of heparin, whereas the response to isoprenaline of the same cell remained unaffected after 13 min of the same treatment. Further application of isoprenaline gave a similar rise in conductance, and control experiments on heparin-free cells showed no desensitization of the angiotensin II-induced responses with time (results not shown). Scale bars: vertical, 200 pA; horizontal, 20 s. membrane potential, 0 mV.

adrenoceptor antagonist prazosin (Fig. 1, trace E; 0.1 μM) on the isoprenaline-evoked K^+ conductance increase.

The isoprenaline-evoked rises in K^+ conductance were mimicked by internal perfusion of the cell cAMP and Sp-cAMP, a specific non-hydrolysable protein kinase A activator [15]. Both types of $[Ca^{2+}]_i$ fluctuations seen with β-stimulation were reproduced by the second messenger (Fig. 2), suggesting that these fluctuations do not arise from fluctuations in cAMP concentrations. Type I and type II oscillations were observed respectively in 9 and 12 of the cells tested with the second messenger and the non-hydrolysable derivative. We have shown previously that the cAMP-evoked responses were not produced through a direct effect of the second messenger on the K^+ channels [16]. Ca^{2+} withdrawal did not abolish the responses (Fig. 3), suggesting that the isoprenaline- and cAMP-evoked K^+-conductance increases were mainly due to the release of Ca^{2+} from internal stores. on a longer time scale, it must be noted that the Ca^{2+} signal decreases and disappears, reflecting the emptying of the Ca^{2+} stores. Also, experimental evidence indicates that the isoprenaline-evoked rises in $[Ca^{2+}]_i$ in suspension of rat liver cells are comparable with those measured with vasopressin, a Ca^{2+}-mobilizing hormone [6,17,18]. Preliminary results on single guinea-pig hepatocytes with fura2 have shown that, on the same cell, maximal concentrations of angiotensin II and isoprenaline induced similar rises in $[Ca^{2+}]_i$ (results not shown).

Controversies about a possible cAMP-mediated $InsP_3$ synthesis in liver cells have been reported [6,19–22]. To test this possibility, we used heparin, which inhibits both $InsP_3$ binding and the resulting $InsP_3$-induced Ca^{2+} release [8,9]. Heparin (150 μg/ml) was perfused internally into the cell via the patch-pipette. As shown in Fig. 4, a delay of approx. 10 min was required to observe an inhibitory effect of heparin on the angiotensin II-induced rise in Ca^{2+}-dependent K^+ conductance. This probably resulted from the slow diffusion of the high-molecular-mass heparin. In these conditions, despite the loss of the $InsP_3$-dependent response produced by a maximal dose of angiotensin II (100 nM), the cell remained responsive to 5 μM-isoprenaline (Fig. 4, far right). Similar results were found in six other cells from five different cell preparations, strongly suggesting an action of cAMP on a site different from the $InsP_3$-binding site itself.

The mechanism by which isoprenaline and cAMP can mobilize internal Ca^{2+} remains unclear. It has been suggested that β- and α_1-adrenoceptor agonists mobilize Ca^{2+} from the same internal compartment(s) in rat hepatocytes [6,7,19–22]. We have performed experiments on a suspension of saponin-permeabilized guinea-pig hepatocytes, showing that, unlike $InsP_3$, cAMP could not directly promote any Ca^{2+} release (results not shown). However, these results do not allow us a definitive conclusion, since the intracellular mechanism coupling cAMP to Ca^{2+} release could have been lost during the permeabilizing process. Since cAMP generates Ca^{2+} responses in heparin-loaded cells, it may be postulated that β-adrenergic agonists open Ca^{2+} channels different from those opened by $InsP_3$, either in the endoplasmic reticulum or in a second Ca^{2+} pool. We suggest that β-adrenoceptor agonists and cAMP could either open Ca^{2+} channels located in the $InsP_3$-sensitive Ca^{2+} store or increase the Ca^{2+}-sensitivity of a Ca^{2+}-induced Ca^{2+}-release mechanism in some other Ca^{2+} stores in the endoplasmic reticulum, thus inducing the release of Ca^{2+} from the internal stores even at resting $[Ca^{2+}]_i$. In this way, it has been shown that cAMP facilitates the Ca^{2+}-induced Ca^{2+}-release mechanism of the intracellular store in the skinned fibre preparation of smooth muscle [23]. However, there is still no evidence of a Ca^{2+}-induced Ca^{2+} release in liver cells.

The unexpected finding that cAMP can induce oscillating Ca^{2+} releases from an internal compartment through an $InsP_3$-independent mechanism implies a new approach for the strong interactions that exist between α_1- and β-pathways in liver cells [19–22,24,25].

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