Histamine-induced Ca^{2+} entry precedes Ca^{2+} mobilization in bovine adrenal chromaffin cells

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The relationship between histamine-induced Ca^{2+} mobilization and Ca^{2+} entry in bovine adrenal chromaffin cells has been investigated. Stopped-flow fluorimetry of fura-2-loaded chromaffin cell populations revealed that 10 μM histamine promoted entry of Ca^{2+} or Mn^{2+} without measurable delay (≤ 20 ms), through a pathway that was insensitive to the dihydropyridine antagonist nifedipine. In the absence of extracellular Ca^{2+} , or in the presence of 100 μM La^{3+} , a blocker of receptor-mediated Ca^{2+} entry, 10 μM histamine triggered an elevation in intracellular calcium concentration ($[\text{Ca}^{2+}]_i$), but only after a delay of approx. 200 ms, which presumably represented the time required to mobilize intracellular Ca^{2+} . These data suggested that histamine-induced bivalent-cation entry precedes extensive Ca^{2+} mobilization in chromaffin cells. In order to confirm that histamine can promote Ca^{2+} entry largely independently of mobilizing intracellular Ca^{2+} , the ability of histamine to promote

Ca^{2+} entry into cells whose intracellular Ca^{2+} store had been largely depleted was assessed. Fura-2-loaded chromaffin cells were treated with 10 μM ryanodine together with 40 mM caffeine, to deplete the hormone-sensitive Ca^{2+} store. This resulted in an approx. 95% inhibition of histamine-induced Ca^{2+} release. Under these conditions, histamine was still able to promote an entry of Ca^{2+} that was essentially indistinguishable from that promoted in control cells. In single cells, introduction of heparin (100 mg/ml), but not de-N-sulphated heparin (100 mg/ml), abolished the histamine-induced rise in $[\text{Ca}^{2+}]_i$. All these data suggest that histamine can induce G-protein- or inositol phosphate-dependent rapid (≤ 20 ms) Ca^{2+} entry without an extensive intracellular mobilization response in chromaffin cells, which points to activation of an entry mechanism distinct from the Ca^{2+} -release-activated Ca^{2+} channel found in non-excitable cells.

INTRODUCTION

Hormones that act via cell-surface receptors to generate inositol 1,4,5-trisphosphate (InsP_3) promote a transient rise in the intracellular concentration of free Ca^{2+} ($[\text{Ca}^{2+}]_i$) that is biphasic: Ca^{2+} release from the intracellular store is accompanied by some form of receptor-mediated Ca^{2+} entry at the plasma membrane (Berridge and Irvine, 1989; Putney, 1990). This entry of Ca^{2+} often occurs through a voltage-independent capacitative pathway that is thought to be activated by depletion of the intracellular Ca^{2+} store (Putney, 1986, 1990). How the emptying store communicates with the plasma membrane to activate the entry mechanism is unknown (Putney, 1990; Penner et al., 1993; Putney and Bird, 1993a). Entry could be mediated by a diffusible intracellular messenger (Fasolato et al., 1993; Parekh et al., 1993), such as the 500 Da ' Ca^{2+} -Influx Factor' (CIF) that has been proposed to be released into the cytoplasm upon Ca^{2+} store depletion (Randriamampita and Tsien, 1993). Alternatively, entry could be controlled by a few Ca^{2+} stores that are in direct communication with the plasma membrane by a conformational-coupling mechanism (Berridge, 1990) involving a protein–protein interaction (Irvine, 1990, 1991, 1992).

The biogenic amine histamine generates InsP_3 in adrenal chromaffin cells (Plevin and Boarder, 1988; Stauderman and Pruss, 1990), elevates $[\text{Ca}^{2+}]_i$, and triggers the exocytotic secretion of catecholamines (Stauderman et al., 1990; Cheek et al., 1993b). The secretory response is completely dependent upon a dihydropyridine-insensitive entry of external Ca^{2+} that occurs through a

Mn^{2+} -permeable channel localized over the entire cell surface (Cheek et al., 1993b). In order to gain greater insight into how histamine promotes Ca^{2+} entry in these cells, the relationship between histamine-induced Ca^{2+} -store depletion and Ca^{2+} entry has been investigated in detail by using microinjection, and fluorimetric and stopped-flow rapid kinetics techniques. Introduction of heparin, but not de-N-sulphated heparin, completely inhibits the histamine-induced rise in $[\text{Ca}^{2+}]_i$. In control cells, stopped-flow techniques reveal that histamine-induced bivalent-cation entry preceded the bulk mobilization of intracellular Ca^{2+} , and histamine is still able to promote Ca^{2+} entry after the intracellular store had been largely depleted and prevented from refilling. These results indicate that extensive bivalent-cation entry, while requiring either G-protein activation or inositol phosphates, can occur independently of global Ca^{2+} -store depletion in these cells. This suggests either that depletion of a specific limited Ca^{2+} store may be sufficient to control histamine-induced entry, or that InsP_3 can promote entry independently of its ability to mobilize intracellular Ca^{2+} .

MATERIALS AND METHODS

Materials

Fura-2 AM and fura-2 were from Molecular Probes (Eugene, OR, U.S.A.), and ryanodine was from ICN Biomedicals (High Wycombe, Bucks., U.K.). Unless otherwise stated, all other chemicals were from Sigma (St. Louis, MO, U.S.A.).

Abbreviations used: $[\text{Ca}^{2+}]_i$, intracellular free Ca^{2+} concentration; Ca^{2+}_o , extracellular Ca^{2+} ; NMDA, N-methyl-D-aspartate; ROC, receptor-operated channel; VOC, voltage-operated channel.

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Isolation of chromaffin cells

Chromaffin cells were dissociated from bovine adrenal medullas by enzymic digestion as described previously (Cheek et al., 1993a,b), and resuspended in Ca^{2+} -free Krebs–Ringer buffer consisting of 145 mM NaCl/5 mM KCl/1.3 mM MgCl_2 /1.2 mM NaH_2PO_4 /10 mM glucose/20 mM Hepes, pH 7.4 (buffer A). Cells were used freshly isolated, and within 5 h of preparation.

Fura-2 measurements of cell populations

Freshly isolated chromaffin cells were resuspended in 10 ml of buffer A containing 3 mM CaCl_2 and loaded with fura-2 by incubation with 2 μM fura-2 acetoxymethyl ester (fura-2 AM) for 35 min at room temperature (20 °C). Cells were then centrifuged (1000 g, 5 min), resuspended in fresh Ca^{2+} -containing buffer A, and left for 30 min to allow hydrolysis of the intracellular fura-2 AM. After 30 min, the cells were washed, resuspended in fresh Ca^{2+} -containing buffer A and transferred to a glass cuvette for fluorescence measurements. Fura-2 fluorescence was continuously monitored at 37 °C with a Perkin–Elmer LS-50 spectrophotometer with excitation and emission wavelengths of 340 nm and 510 nm respectively. $[\text{Ca}^{2+}]_i$ was calculated according to the following equations:

$$[\text{Ca}^{2+}]_i = K_d \cdot \frac{(F - F_{\min.})}{(F_{\max.} - F)}$$

and

$$F_{\min.} = \frac{1}{3}(F_{\max.} - F_{\text{Mn}}) + F_{\text{Mn}}$$

where F = fura-2 fluorescence from cells, $F_{\min.}$ = minimum fura-2 fluorescence, $F_{\max.}$ = maximum fura-2 fluorescence (determined by addition of 0.5 mg/ml saponin to permeabilize the cells), F_{Mn} = fluorescence after quenching of fura-2 (determined by addition of 4 mM MnCl_2 to the cells after obtaining $F_{\max.}$), and K_d = equilibrium dissociation constant of fura-2– Ca^{2+} . This was assumed to be 224 nM at 37 °C (Grynkiewicz et al., 1985).

Nominally Ca^{2+} -free media was obtained by adding 4 mM EGTA to cells in buffer A containing 3 mM CaCl_2 . This was calculated to give a free $[\text{Ca}^{2+}] < 2 \mu\text{M}$ (Maxchelator, Dr. C. Patton, Stanford University).

Use of caffeine and ryanodine to deplete the intracellular Ca^{2+} store

Caffeine was used instead of ryanodine to activate the putative Ca^{2+} -induced Ca^{2+} release channel in the cells because ryanodine binds very slowly to the inactivate channel ($t_{1/2} = 24.8$ min; Pessah et al., 1986). Caffeine is poorly soluble in water, so a stock of 400 mM caffeine in Krebs–Ringer buffer was prepared by shaking a solution warmed to approx. 60 °C, and when the caffeine had dissolved the solution was maintained at 37 °C.

The effect of ryanodine and caffeine in inhibiting the putative Ca^{2+} -induced Ca^{2+} release channel was achieved by incubating fura-2-loaded cell populations with 10 μM ryanodine (stock 100 mM in methanol) for 15 min, and then adding a maximal concentration of caffeine (40 mM; Cheek et al., 1993a) for a further 10 min. Cells were then centrifuged (1000 g, 5 min), resuspended in Ca^{2+} -containing buffer A without caffeine or ryanodine, and then stimulated with either 40 mM caffeine (Figure 4a) or 100 μM histamine (Figure 4b) after the medium

had been made nominally Ca^{2+} -free by the addition of 4 mM EGTA (see above).

Stopped-flow fura-2 measurements of cell populations

For measurement of sub-second kinetics, the fluorescence from fura-2-loaded chromaffin cells was recorded using a SFA-II Rapid Kinetic Stopped-flow Accessory (Hi-Tech Scientific, Salisbury, Wiltshire, U.K.) mounted in a Perkin–Elmer LS50 spectrophotometer, thermostatically controlled at 37 °C. Fura-2-loaded cells in buffer A with or without added CaCl_2 were injected through one port, and solution containing stimulus, MnCl_2 , LaCl_3 or EGTA (as required) was injected through the other. The resulting mixture of cells plus stimulus was termed the reaction solution. In this system, the stopped-flow cuvette was enclosed and only became full with reaction solution upon cell stimulation, at which time the recording began. As a result, the traces have no baseline. Fura-2 fluorescence was measured every 20 ms, and monitored using an excitation wavelength of 340 nm, or 360 nm for experiments with extracellular MnCl_2 , and an emission wavelength of 510 nm. It was not possible to calibrate fluorescence changes, because access to the cuvette only allowed replenishment of old reaction solution with new, and not the addition of stock solutions by pipette. However, our previous work has indicated that resting $[\text{Ca}^{2+}]_i$ in chromaffin cells lies in the range 10–120 nM, and after stimulation with histamine or high K^+ this rises to 300–1000 nM with no obvious difference in the magnitude of the responses elicited by these two stimuli (Stauderman et al., 1990; Burgoyne, 1991; Cheek et al., 1993b).

Each trace shows the average of at least five scans recorded from the same batch of cells (n value is given in the Figure legend). For averaging, corresponding time points from n single scans were summed and then divided by n , using Perkin–Elmer FLDM software. The record shown is typical of those obtained from at least three independent cell preparations.

Introduction of polyanions and measurement of $[\text{Ca}^{2+}]_i$ in single cells

Chromaffin cells, prepared and cultured as described previously (Pruss et al., 1985), were used after 24–48 h in culture. Cells were loaded with fura-2 by temporarily establishing a whole-cell patch with a pipette containing the following ingredients: 10 mM fura-2, 4 mM MgATP, 100 mM KCl, 20 mM NaCl, 1 mM MgCl_2 , 10 mM Mops, pH 7.1. After applying a brief pulse of positive pressure [0.7–2.1 kPa (0.1–0.3 lb/in²); Narishige IM 200 microinjector], the patch pipette was quickly withdrawn, allowing the cell membrane to reseal. The whole process of establishing the patch, pressure application, and withdrawal of the pipette took no more than 1 s. During the loading procedure, the cells were bathed in a Ca^{2+} -containing buffer consisting of (in mM): 118 NaCl, 4.6 KCl, 10 glucose, 25 Hepes (pH 7.4 with NaOH), 1.2 MgSO_4 , 1.1 CaCl_2 , and 0.1% (w/v) BSA (buffer B). When heparin (Calbiochem low molecular mass, average 5000) or de-N-sulphated heparin were used, they were present in the pipette at 100 mg/ml. After loading with fura-2, the cells were monitored for several minutes to determine if significant leakage of fura-2 occurred. Two criteria were used for selection of cells for experimental testing: first, the cell had to maintain a steady level of fluorescence intensity for at least 5 min, and second, the cell had to attain a level of $[\text{Ca}^{2+}]_i$ in the physiological range (100–200 nM), both of which were considered as indices of successful membrane resealing.

Measurements of fura-2 fluorescence in single cells were performed as described previously (Stauderman et al., 1990), using a Zeiss IM-35 inverted microscope attached to a photomultiplier

(Thorn EMI) that was part of a dual-excitation wavelength spectrofluorimeter (Deltascan I, Photon Technology). Using a pinhole coupler to the photomultiplier, the detection field was restricted to just the cytoplasmic region of a cell (30% of total area), avoiding the nucleus, which was often more fluorescent than the rest of the cell.

Cells were constantly bathed by superfusion with buffer B at a rate of 0.8–1.0 ml/min. Applications of 10 μM histamine and 10 mM caffeine were made via the superfusion system.

RESULTS

Histamine-induced bivalent-cation entry precedes intracellular Ca^{2+} mobilization

The sub-second kinetics of the stimulus-induced rise in $[\text{Ca}^{2+}]_i$ was investigated by monitoring the fluorescence change of fura-2-loaded cell populations with a time resolution of 20 ms. Depolarization of cells with high (55 mM) K^+ induced a rise in $[\text{Ca}^{2+}]_i$ that was activated within 20 ms of stimulation, and reached a peak within approx. 750 ms (Figure 1a). This $[\text{Ca}^{2+}]_i$ elevation was dependent upon the activation of dihydropyridine-sensitive voltage-operated Ca^{2+} channels, because pre-incubation of cells with 10 μM nifedipine blocked the response (Figure 1b). Stimulation of cells with 10 μM histamine induced an elevation in $[\text{Ca}^{2+}]_i$ that was also activated within 20 ms, but which did not reach a peak until approx. 1.5 s after stimulation (Figure 2a, i). Pretreatment of cells with 10 μM nifedipine did not attenuate the initial rate of rise of $[\text{Ca}^{2+}]_i$ in response to histamine (Figure 2a, ii), though there was a modest decrease in peak fluorescence attained. Histamine triggers the mobilization of intracellular Ca^{2+} and also Ca^{2+} entry, so in order to follow the kinetics of histamine-induced bivalent-cation entry more specifically, stopped-flow fluorimetry was combined with the Mn^{2+} -quench technique (Hallam and Rink, 1985; Sage et al., 1989). Cells were loaded with fura-2, and then stimulated with histamine in the presence of 1 mM external Mn^{2+} (without added Ca^{2+}). The resulting quench of fura-2 fluorescence was monitored at the isosbestic wavelength of 360 nm, where fura-2 fluorescence is independent of $[\text{Ca}^{2+}]_i$. Histamine (10 μM) evoked an immediate (≤ 20 ms) quench of fura-2 fluorescence, indicating the very rapid activation of a bivalent-cation entry pathway (Figure 2b, i). The activation of this entry pathway did not involve dihydropyridine-sensitive voltage-operated Ca^{2+} channels, because 10 μM nifedipine did not effect the initial kinetics of the response, though the extent of Mn^{2+} entry was decreased (Figure 2b, ii). These results suggest that dihydropyridine-sensitive voltage-operated channels are not involved in the rapid (≤ 20 ms) initiation of histamine-induced bivalent-cation entry, but may be recruited subsequently. All these data are consistent with previous data showing that K^+ -induced secretion is markedly attenuated by nifedipine, whereas histamine-induced secretion is relatively unaffected by this compound (Cheek et al., 1993b).

In order to study the kinetics of histamine-induced intracellular Ca^{2+} mobilization, it was necessary to block the rapid-entry component. This was achieved by two methods. Cells were stimulated with 10 μM histamine, either in EGTA-containing medium (Figure 3a) or in the presence of 3 mM Ca^{2+} and 100 μM La^{3+} (a blocker of receptor-mediated Ca^{2+} entry) (Stauderman and Pruss, 1989; Hardie and Minke, 1992; Hoth and Penner, 1993) (Figure 3b). In both experiments, histamine elevated $[\text{Ca}^{2+}]_i$, but only after a delay of approx. 200 ms (Figures 3a, ii, and 3b, ii). By contrast, when extracellular EGTA or La^{3+} was removed, and Ca^{2+} entry allowed to occur, histamine began to elevate $[\text{Ca}^{2+}]_i$ with no measurable delay (i.e. within 20 ms;

Figure 1 Stopped-flow fluorimetry of fura-2-loaded chromaffin cells depolarized with high K^+

(a) Chromaffin cells were suspended in Ca^{2+} -containing medium and mixed with 55 mM K^+ at zero time. (b) Cells were pre-incubated with 10 μM nifedipine for 10 min prior to stimulation with 55 mM K^+ . The traces show the average of 8 scans for (a) and (b).

Figure 2 Stopped-flow fluorimetry of fura-2-loaded chromaffin cells stimulated by histamine in the presence of nifedipine

(a) Chromaffin cells were suspended in Ca^{2+} -containing medium and mixed, at zero time, with 10 μM histamine in the absence (i) or presence (ii) of 10 μM nifedipine (10 min preincubation). The traces show the average of 7 (i) and 14 (ii) scans. (b) Cells were suspended in medium without added Ca^{2+} and mixed, at zero time, with 1 mM MnCl_2 (iii), 10 μM histamine in 1 mM MnCl_2 (i), or 10 μM histamine plus 10 μM nifedipine in 1 mM MnCl_2 (ii). Fura-2 fluorescence was excited at 360 nm. The traces show the average of 10 (i), (iii) and 13 (ii) scans.

Figures 3a, i, and 3b, i). These data suggest that histamine-induced Ca^{2+} entry precedes intracellular Ca^{2+} mobilization.

Interestingly, the rate of Ca^{2+} rise appeared to be the same in control and La^{3+} -treated cells after the initial delay (Figure 3b) whereas, in contrast, the rate of Ca^{2+} rise was slower in cells that were pretreated under Ca^{2+} -free conditions and then stimulated with histamine in the presence of EGTA (Figure 3a). This raises the possibility that the initial rate of intracellular Ca^{2+} mobili-

Figure 3 Stopped-flow fluorimetry of fura-2-loaded chromaffin cells stimulated by histamine in the absence of external Ca^{2+} , and in the presence of external Ca^{2+} and La^{3+}

(a) Chromaffin cells were suspended in buffer A (without added Ca^{2+}) and mixed, at zero time, with $10 \mu\text{M}$ histamine in either Ca^{2+} -containing (i) or EGTA-containing (ii) medium. The traces show the average of 5 (i) and 9 (ii) scans. (b) Cells were suspended in Ca^{2+} -containing medium and mixed, at zero time, with $10 \mu\text{M}$ histamine in the absence (i) and presence (ii) of $100 \mu\text{M}$ LaCl_3 . The traces show the average of 5 (i) and 11 (ii) scans. (c) Cells were suspended in medium without added Ca^{2+} and mixed, at zero time, with 1 mM MnCl_2 (iii), $10 \mu\text{M}$ histamine in 1 mM MnCl_2 (i), or $10 \mu\text{M}$ histamine plus $100 \mu\text{M}$ LaCl_3 in 1 mM MnCl_2 (ii). Fura-2 fluorescence was excited at 360 nm . The traces show the average of 5 (i), (iii) and 11 (ii) scans. Note the decrease in fluorescence after a 200 ms delay in the presence of La^{3+} (i, c, ii; see the text).

zation is attenuated by removal of extracellular Ca^{2+} , perhaps as a result of a decrease in endoplasmic-reticulum Ca^{2+} loading either lowering sensitivity of the release mechanism (Missiaen et al., 1992; Cheek et al., 1993a) or providing less Ca^{2+} for activation of phosphoinositidase C (Eberhard and Holz, 1988).

In addition to blocking histamine-induced rapid Ca^{2+} entry (Figure 3b), La^{3+} also blocked histamine-induced Mn^{2+} entry

Figure 4 Ryanodine inhibits the caffeine- and histamine-induced rise in $[\text{Ca}^{2+}]_i$ in a use-dependent manner

The Figure shows the Ca^{2+} responses of four populations of fura-2-loaded chromaffin cells to either (a) a maximal caffeine concentration (40 mM , arrows), or (b) $100 \mu\text{M}$ histamine (arrows), after various pretreatments. (a and b) (i) Control response of cells pre-incubated for 25 min in Ca^{2+} -containing buffer A. (a and b) (ii) Cells were pretreated for 25 min in Ca^{2+} -containing buffer A plus $10 \mu\text{M}$ ryanodine, before centrifugation (see the Materials and methods section) and resuspension in control buffer. (a and b) (iii) Cells were pretreated for 25 min in Ca^{2+} -containing buffer A plus 40 mM caffeine, before centrifugation and resuspension in control buffer. (a and b) (iv) Cells were incubated in Ca^{2+} -containing buffer A plus $10 \mu\text{M}$ ryanodine for 15 min , after which 40 mM caffeine was added and the cells were incubated for a further 10 min . The cells were then centrifuged and resuspended in Ca^{2+} -containing buffer A. The extracellular medium was made nominally Ca^{2+} -free by addition of 4 mM EGTA 30 s before the addition of stimulus in all experiments. These data are typical of 3 (a) and 6 (b) independent experiments (see the text).

(Figure 3c). In control cells, histamine ($10 \mu\text{M}$) induced an immediate ($\leq 20 \text{ ms}$) quench of fura-2 fluorescence (Figure 3c, i), indicating a rapid entry of Mn^{2+} , and this was blocked in the presence of extracellular La^{3+} (Figure 3c, ii).

In the presence of extracellular La^{3+} , Mn^{2+} may have entered cells approx. 200 ms after histamine addition (solid arrow, Figure 3c, ii). This could represent activation of a (La^{3+} -insensitive) Mn^{2+} -permeable entry pathway triggered by store depletion, because store depletion also requires approx. 200 ms (Figures 3a, ii and 3b, ii), but this possibility was not investigated further in this study.

These sub-second kinetics data demonstrate that histamine can trigger Ca^{2+} (and Mn^{2+}) entry before mobilizing intracellular Ca^{2+} in chromaffin cells, and therefore provide evidence that

Figure 5 Ryanodine pretreatment abolishes Ca^{2+} mobilization, but not Ca^{2+} influx, in response to histamine

(a) Fura-2-loaded chromaffin cells were stimulated with $100 \mu\text{M}$ histamine (arrow) in either Ca^{2+} -containing medium (i) or EGTA-containing medium (ii). The cells in (iii) were pretreated with ryanodine and caffeine (see Figures 4a, iv, and 4b, iv, and the Materials and methods section) to deplete the intracellular Ca^{2+} store, and then stimulated with $100 \mu\text{M}$ histamine (arrow) in the presence of extracellular Ca^{2+} . This treatment abolished Ca^{2+} mobilization, but not Ca^{2+} influx, as judged by a stimulus-induced elevation of $[\text{Ca}^{2+}]_i$ above the baseline (broken line). These data are typical of 6 independent experiments (see the text). (b) The external- Ca^{2+} dependence of the histamine-induced rise in $[\text{Ca}^{2+}]_i$ in ryanodine- and caffeine-pretreated cells, shown in (a) above. Chromaffin cells were pretreated with ryanodine and caffeine, and then stimulated with $100 \mu\text{M}$ histamine (arrow) in Ca^{2+} -containing (i) or EGTA-containing (ii) medium. The immediate, reversible, changes in fluorescence on histamine stimulation are addition artifacts. These data are typical of 6 independent experiments.

stimulus-induced Ca^{2+} entry and Ca^{2+} mobilization can be temporally dissociated in these cells.

Depletion of the intracellular Ca^{2+} store

If bivalent-cation entry precedes, and is therefore largely independent of, intracellular Ca^{2+} mobilization, it may be predicted that histamine should still be able to promote Ca^{2+} entry even after the intracellular Ca^{2+} store has been depleted and prevented from refilling. In order to compare the ability of histamine to promote Ca^{2+} entry into cells containing a Ca^{2+} -replete intracellular store with those containing a Ca^{2+} -deplete store, it was necessary to design an experimental protocol that would empty the Ca^{2+} store, and prevent it from refilling.

The hormone-sensitive intracellular Ca^{2+} store in intact chromaffin cells has been reported to be largely caffeine-sensitive (Stauderman and Murawsky, 1991). In order to deplete this store, and to prevent it from refilling, intact cells were preincubated with $10 \mu\text{M}$ ryanodine and 40 mM caffeine (Figure 4a). Confirmation that ryanodine and caffeine pretreatment depleted the caffeine-sensitive store in a use-dependent manner is shown in Figure 4(a). Pretreatment of a cell population with ryanodine ($10 \mu\text{M}$)

together with caffeine (40 mM) virtually abolished the subsequent response to 40 mM caffeine (Figure 4a, iv). Ryanodine alone gave a slight decrease in the Ca^{2+} response to 40 mM caffeine (Figure 4a, ii), while caffeine pretreatment alone gave only a 21 % decrease in the subsequent response (Figure 4, iii). These data indicate that only a combination of ryanodine plus caffeine was maximally effective at inhibiting caffeine-sensitive Ca^{2+} release, and this result is consistent with the concept that caffeine-pretreatment shifts the Ca^{2+} channel to an open sub-conductance state that is stabilized by ryanodine (Henzi and MacDermott, 1992).

Pretreatment of cells with ryanodine and caffeine in this way inhibited the subsequent Ca^{2+} response induced by $100 \mu\text{M}$ histamine in Ca^{2+} -free medium by $94 \pm 4\%$ ($n = 6$) (Figure 4b, iv), as compared with control cells pretreated with caffeine alone (Figure 4b, iii). These data suggested that the majority (approx. 95 %) of Ca^{2+} mobilized in response to histamine originates from a caffeine-sensitive Ca^{2+} store, which is in agreement with data obtained using single cells (Stauderman and Murawsky, 1991). Presumably the remaining Ca^{2+} is contained in a uniquely InsP_3 -sensitive store, which would be consistent with data obtained using permeabilized cells (Cheek et al, 1991; Robinson and Burgoyne, 1991; Stauderman et al., 1991).

Histamine promotes Ca^{2+} entry in stored Ca^{2+} -depleted cells

Cells were pretreated with ryanodine and caffeine, as described, to largely deplete the intracellular Ca^{2+} store, and then stimulated with histamine in the presence of extracellular Ca^{2+} (Ca^{2+}_o), to assess whether Ca^{2+} influx could still be stimulated. In control cells, histamine ($100 \mu\text{M}$) evoked a $[\text{Ca}^{2+}]_i$ transient [peak rise: $135 \pm 11 \text{ nM}$ ($n = 6$)] that was biphasic in the presence of Ca^{2+}_o . After the initial large transient, $[\text{Ca}^{2+}]_i$ declined to a plateau $23 \pm 6 \text{ nM}$ ($n = 6$) above the basal level (Figure 5a, i). This plateau was due to Ca^{2+} entry because, when cells were stimulated in the absence of Ca^{2+}_o , the initial transient returned to the baseline level (Figure 5a, ii). After ryanodine and caffeine pretreatment, the large transient rise in $[\text{Ca}^{2+}]_i$ due to the intracellular Ca^{2+} mobilization was absent, but the influx component remained intact, as judged by the ability of histamine to elevate $[\text{Ca}^{2+}]_i$ to $29 \pm 7 \text{ nM}$ ($n = 6$) above the basal level (Figure 5a, iii).

The external Ca^{2+} dependence of the histamine-induced rise in $[\text{Ca}^{2+}]_i$ in ryanodine- and caffeine-pretreated cells (Figure 5a, iii), is shown in detail in Figure 5(b). Cells were pretreated with ryanodine and caffeine to deplete the intracellular Ca^{2+} store, and then stimulated with $100 \mu\text{M}$ histamine. A rise in $[\text{Ca}^{2+}]_i$ was evoked in the presence (Figure 5b, i) but not the absence (Figure 5b, ii) of Ca^{2+}_o , indicating that the histamine-induced rise in $[\text{Ca}^{2+}]_i$ (Figure 5a, iii) was due to Ca^{2+} entry, and not the recycling of Ca^{2+} from the putative InsP_3 -sensitive store (see above).

All these data demonstrate that histamine is able to promote significant Ca^{2+} entry into chromaffin cells in the absence of a large Ca^{2+} -mobilization response, further indicating that hormone-induced Ca^{2+} entry and Ca^{2+} mobilization from the caffeine-sensitive store can be dissociated in chromaffin cells.

The histamine-induced rise in $[\text{Ca}^{2+}]_i$ requires G-protein activation or inositol phosphates

Histamine-induced Ca^{2+} entry could be independently regulated from Ca^{2+} mobilization in chromaffin cells if histamine activated non-selective cation entry through a receptor-operated channel (ROC), like ATP in PC12 cells (Nakazawa et al., 1990; Barry and Cheek, 1994a), or glutamate acting on the *N*-methyl-D-aspartate (NMDA) receptor (Moriyoshi et al., 1991). In these

Figure 6 Heparin abolishes the rise in $[Ca^{2+}]_i$ induced by histamine but not caffeine

(a) A single fura-2-loaded chromaffin cell was stimulated with $10 \mu M$ histamine and $10 mM$ caffeine, as shown, in Ca^{2+} -containing medium. (b) A single fura-2-loaded cell was stimulated with $10 \mu M$ histamine and $10 mM$ caffeine, as shown, in Ca^{2+} -containing medium after the introduction of heparin ($100 mg/ml$). The Ca^{2+} signal in response to histamine, but not caffeine, was abolished. (c) A single fura-2-loaded cell was stimulated with $10 \mu M$ histamine and $10 mM$ caffeine, as shown, in Ca^{2+} -containing medium after the introduction of de-N-sulphated heparin ($100 mg/ml$). At least five experiments were performed under each condition, and representative traces are shown (see the Results section). These data indicate that histamine-induced Ca^{2+} signals require either G-protein activation or inositol phosphate production.

examples, the receptor proteins themselves are also ligand-gated cation channels (Illes and Nörenberg, 1993). In order to test this hypothesis, the polyanion heparin was introduced into single chromaffin cells before stimulation with histamine. Heparin binds with relatively high affinity (approx. $2 \mu M$) to the $InsP_3$ receptor and thereby antagonizes the action of $InsP_3$ (Wakui et al., 1990; for review see Taylor and Richardson, 1991). Heparin may also directly uncouple the cell-surface receptor from its G-protein (Dasso and Taylor, 1991). The response of a single fura-2-loaded chromaffin cell to an application of $10 \mu M$ histamine followed by $10 mM$ caffeine is shown in Figure 6(a). Histamine promoted a rise in $[Ca^{2+}]_i$ that included mobilization of Ca^{2+} from the caffeine-sensitive store, because, when applied after histamine, caffeine was without effect on $[Ca^{2+}]_i$. This is consistent with previous suggestions that hormones release Ca^{2+} from the caffeine-sensitive store in these cells (Stauderman and Murawsky, 1991; Stauderman et al., 1991). In the presence of heparin, however, the rise in $[Ca^{2+}]_i$ evoked by $10 \mu M$ histamine was completely abolished (Figure 6b). This effect was observed in 8 of 10 heparin-containing cells. The two remaining cells responded to histamine, but the level of fura-2 in these cells inversely corresponded to their responsiveness to histamine. We therefore

believe that the level of fura-2 acted as an index of the amount of heparin that was inside the cell, and those cells with a low [fura-2] also contained little heparin and consequently were not inhibited. These data suggest that in most of our experiments we were near the threshold for heparin's inhibitory action, thus minimizing the possibility of a toxic effect by heparin. In support of this notion, cells whose histamine response had been inhibited by heparin remained responsive to a subsequent application of $10 mM$ caffeine (Figure 6b). Introduction of de-N-sulphated heparin ($100 mg/ml$), which does not bind to the $InsP_3$ receptor (Tones et al., 1989), did not inhibit the histamine-induced $[Ca^{2+}]_i$ signal and subsequent abolition of the caffeine-induced Ca^{2+} signal (Figure 6c), a response pattern similar to that observed in control cells (Figure 6a).

These results argue against the involvement of a ROC, because they indicate that histamine-induced Ca^{2+} entry, in addition to mobilization, requires either G-protein activation or inositol phosphate production.

DISCUSSION

The activation of H_1 histaminergic receptors on chromaffin cells results in generation of $InsP_3$, mobilization of intracellular Ca^{2+} , Ca^{2+} entry, and the triggering of exocytotic secretion (Plevin and Boarder, 1988; Stauderman and Pruss, 1990; Stauderman et al., 1990; Cheek et al., 1993b). The secretory response is completely dependent upon the stimulation of Ca^{2+} entry (Stauderman et al., 1990; Cheek et al., 1993b), but the mechanisms underlying the promotion of Ca^{2+} entry by histamine are unknown. In the present study, a combination of microinjection and fluorimetric and stopped-flow rapid kinetics techniques have been used to investigate the relationship between histamine-induced Ca^{2+} mobilization and Ca^{2+} entry. Studies using heparin suggest that the entry pathway requires either G-protein activation or inositol phosphate production, and rapid kinetics experiments indicate that Ca^{2+} (and Mn^{2+}) entry in response to histamine precedes bulk intracellular Ca^{2+} mobilization. Furthermore, histamine could still stimulate Ca^{2+} entry after the intracellular Ca^{2+} store had been largely emptied and prevented from refilling.

Ca^{2+} -entry pathways in chromaffin cells

In common with other electrically excitable cells, neuronally derived adrenal chromaffin cells possess voltage-operated Ca^{2+} channels (VOCs). Dihydropyridine-sensitive channels predominate, and are responsible for most of the Ca^{2+} entry that triggers secretion after electrical depolarization (Artalejo et al., 1994) or depolarization with either acetylcholine or high K^+ (Burgoyne, 1991). However, there are several reasons for supposing that these channels are not responsible for the Ca^{2+} entry stimulated by histamine in this study. Firstly, concentrations of the dihydropyridine antagonist nifedipine that blocked the K^+ -induced rise in $[Ca^{2+}]_i$ (Figure 1) did not affect the initial rate of histamine-induced Ca^{2+} (Figure 2a) or Mn^{2+} (Figure 2b) entry. Secondly, concentrations of nifedipine that inhibited K^+ -induced secretion had very little effect on histamine-induced secretion (Cheek et al., 1993b). Thirdly, histamine appears to hyperpolarize chromaffin cells, as judged by the use of the membrane-potential-sensitive dye bisoxonol (Cheek et al., 1993b) and whole-cell current-clamp electrophysiology (Artalejo et al., 1993). All these data suggest that histamine stimulates Ca^{2+} entry through a voltage-independent pathway in chromaffin cells.

The activation of voltage-independent Ca^{2+} entry is thought to be inextricably linked to Ca^{2+} -store depletion, so-called capacitative entry (Putney, 1990), but the underlying molecular mechan-

isms remain to be elucidated (Penner et al., 1993; Putney and Bird, 1993a). One possibility that has received experimental support is that entry is triggered by a globally diffusible intracellular messenger (Fasolato et al., 1993; Parekh et al., 1993), such as cyclic GMP in acinar cells (Pandolfi and Schoeffield-Payne, 1990; Bahnson et al., 1993), or a 500 Da ' Ca^{2+} -Influx factor' that appears to be released into the cytoplasm of stimulated cells as a consequence of intracellular store depletion (Randriamampita and Tsien, 1993). Such an entry pathway may require the regulatory involvement of a GTP-binding protein (Bird and Putney, 1993; Fasolato et al., 1993; Jaconi et al., 1993). An entry pathway linked to store depletion appears to exist in chromaffin cells, but some intriguing biochemical characteristics suggest that the relationship between store depletion and entry is not straightforward. For example, store depletion using the Ca^{2+} -ATPase inhibitor thapsigargin activated entry of both Ca^{2+} (Cheek and Thastrup, 1989) and Mn^{2+} (Robinson et al., 1992) whereas, in contrast, store depletion promoted by either ionomycin (Stauderman and Pruss, 1989) or caffeine (Cheek et al., 1990; Xu and Forsberg, 1993) triggered little or no Ca^{2+} entry in these cells. One possible explanation for these observations is that not all intracellular Ca^{2+} stores are equally linked to the promotion of Ca^{2+} entry. In support of this notion, bradykinin, but not caffeine, was reported to promote Ca^{2+} entry in vascular endothelial cells (Graier et al., 1994) and the caffeine-sensitive pool in excitable cells such as dorsal root ganglion (Thayer et al., 1988) and sympathetic (Friel and Tsien, 1992) neurons, and PC12 cells (Reber et al., 1993; Barry and Cheek, 1994b), has been reported to require depolarization to effectively refill with Ca^{2+} . Depolarization-induced Ca^{2+} entry through VOCs also refilled the caffeine-sensitive pool in chromaffin cells (Figure 6 in Liu et al., 1991). One possibility that is consistent with all these observations is that the promotion of Ca^{2+} entry may require the depletion of only a sub-population of (caffeine-insensitive) stores. To examine this possibility, we investigated the relationship between hormone-induced Ca^{2+} store depletion and the triggering of Ca^{2+} entry in greater detail.

Depletion of the intracellular Ca^{2+} store by ryanodine and caffeine pretreatment almost abolished histamine-induced intracellular Ca^{2+} mobilization (Figures 4b and 5a), but did not attenuate the ability of histamine to trigger Ca^{2+} entry (Figures 5a and 5b), demonstrating that hormone-induced Ca^{2+} entry and Ca^{2+} mobilization from the caffeine-sensitive store can be dissociated in these cells. A similar situation has been reported in smooth-muscle cells: ryanodine pretreatment inhibited internal Ca^{2+} release, but not Ca^{2+} influx, in response to acetylcholine (Katsuyama et al., 1991). Additionally, in chromaffin cells, stopped-flow rapid kinetics experiments revealed that histamine-induced entry of Ca^{2+} or Mn^{2+} preceded bulk intracellular Ca^{2+} mobilization. The mobilization of intracellular Ca^{2+} in response to a maximal concentration of histamine required approx. 200 ms to become activated (Figures 3, ii and 3b, ii). A similar delay was observed upon ADP stimulation of platelets (Sage et al., 1989). Flash photolysis of caged InsP_3 , to release a moderate concentration of InsP_3 into the cytoplasm, also required a 100–200 ms delay before initiating a rise in $[\text{Ca}^{2+}]_i$ in hepatocytes (Capiod et al., 1988), *Xenopus* oocytes (Miledi and Parker, 1989) and vascular smooth-muscle cells (Carter and Ogden, 1992), suggesting that the 200 ms latency observed in this study arises, in the main, from the processes within the cell responsible for InsP_3 -induced Ca^{2+} release. In agreement, experiments using InsP_3 microinjected into oocytes by pressure injection or ionophoresis showed a similar latency (Miledi and Parker, 1989). In contrast with the 200 ms delay that preceded intracellular Ca^{2+} mobilization histamine-induced Ca^{2+} or Mn^{2+} entry was activated

without any measurable delay (i.e. ≤ 20 ms; Figures 3a, i, 3b, i, and 3c, i), clearly demonstrating that extensive depletion of Ca^{2+} stores is not necessary to trigger bivalent-cation entry into chromaffin cells.

The mechanism underlying stimulus-induced rapid Ca^{2+} entry

Rapid (ms) Ca^{2+} entry requiring inositol phosphates occurs through the *trp*-encoded Ca^{2+} channel in *Drosophila* photoreceptors (Hardie, 1991; Hardie and Minke, 1992), and Ca^{2+} entry components that precede intracellular Ca^{2+} mobilization have been reported in platelets (Sage et al., 1989; Mahaut-Smith et al., 1992), adrenal glomerulosa cells (Ely et al., 1991) and hepatocytes (Bygrave et al., 1993; Striggow and Bohnensack, 1994). The mechanisms that underlie the activation of many of these entry pathways are, however, unknown. In chromaffin cells, two possibilities exist. Firstly, entry could be achieved through activation of a ROC, where the receptor protein itself is also the ion channel. Examples include the receptor responsible for ATP-induced Ca^{2+} entry in PC12 cells (Neuhaus et al., 1991; Barry and Cheek, 1994a) and the NMDA glutamate receptor (Moriyoshi et al., 1991). The involvement of a ROC seems unlikely, however, because the introduction of heparin, but not de-N-sulphated heparin, completely inhibited the histamine-induced rise in $[\text{Ca}^{2+}]_i$ in single chromaffin cells (Figure 6), suggesting a requirement for either G-protein activation or inositol phosphates in mediating the entry, in addition to the mobilization component of the histamine-induced $[\text{Ca}^{2+}]_i$ signal. A more likely possibility is that entry requires the action of InsP_3 at the plasma membrane. According to the conformational-coupling entry mechanism (Irvine, 1990; Berridge, 1990), InsP_3 binding causes a conformational change in the InsP_3 receptor on the stores next to the plasma membrane, and this conformational change is then transmitted to plasma-membrane Ca^{2+} channels either by direct coupling (Irvine, 1990, 1991, 1992) or via the cytoskeleton (Putney and Bird, 1993b). InsP_3 receptors are found at the plasma membrane of some cells (Fujimoto et al., 1992; Khan et al., 1992), and InsP_3 has been proposed to gate or modulate plasma-membrane Ca^{2+} channels by some direct means in Jurkat T cells (Kuno and Gardner, 1987), A431 cells (Mozhayeva et al., 1990, 1991) and olfactory neurones (Restrepo et al., 1990; Fadool and Ache, 1992). An alternative possibility is that InsP_3 acts at the plasma membrane in conjunction with limited Ca^{2+} -store depletion to control Ca^{2+} entry (Putney, 1990; Putney and Bird, 1993a). This possibility cannot be excluded, in view of the finding that ryanodine and caffeine pretreatment did not completely deplete the histamine-sensitive intracellular Ca^{2+} store (Figure 4b, iv). Presumably, the few Ca^{2+} stores remaining replete after ryanodine and caffeine pretreatment are uniquely InsP_3 -sensitive, as has been reported in PC12 cells (Barry and Cheek, 1994b). If these stores are localized at the plasma membrane, their depletion upon histamine stimulation may be sufficient to activate the entry pathway. Ca^{2+} release from such few stores may be so modest as to be undetectable using the stopped-flow technique (Figures 3a, ii, and 3b, ii), which may detect the global, but not local, release of stored Ca^{2+} .

Whatever the precise mechanism, if entry is associated with events occurring only at the sub-plasmalemmal stores, this may also explain the paradox (see above) that thapsigargin (Cheek and Thastrup, 1989; Robinson et al., 1992), but not caffeine (Cheek et al., 1990), was found to activate substantial Ca^{2+} entry in these cells, because the sub-plasmalemmal stores are presumed caffeine-insensitive.

At present, therefore, although InsP_3 has been shown to activate a plasma-membrane cation channel that gates Ca^{2+} in

excised patches from chromaffin cells (Mochizuki-Oda et al., 1991), whether in the intact cells InsP_3 triggers entry by direct means or in association with limited store depletion cannot be distinguished. Irrespective of the mechanism, however, the key point is that the hormone-induced rapid (≤ 20 ms) entry of Ca^{2+} clearly does not require extensive store depletion in chromaffin cells, which distinguishes this entry pathway from the Ca^{2+} -release-activated Ca^{2+} channel that appears to be responsible for the majority of hormone-induced Ca^{2+} entry in non-excitabile cells (Hoth and Penner, 1992, 1993; Penner et al., 1993). Voltage-independent Ca^{2+} entry can be achieved by a number of different pathways in cells (Fasolato et al., 1994) and it remains to be determined if, as suggested here, certain mechanisms predominate in certain cell types, for example excitable versus non-excitabile cells.

We are very grateful to Colin Taylor for the use of his Hi-Tech stopped-flow instrument, and to Martin Bootman for instruction in its use. Grateful thanks are also due to Stuart Gardiner and Richard Singleton (Canvin International) for adrenal tissue. We are also indebted to the following for their critical reading of the manuscript and ideas: Mike Berridge, Bob Burgoyne, Robin Irvine, Roger Hardie, Martin Bootman, Orla McGuinness, Vicky Barry and Carl Petersen. This work was funded in part by the A.F.R.C. and by the Royal Society. T.R.C. is a Royal Society University Research Fellow.

REFERENCES

- Artalejo, A. R., Garcia, A. G. and Neher, E. (1993) *Pflügers Arch.* **423**, 97–103
- Artalejo, C. R., Adams, M. E. and Fox, A. P. (1994) *Nature (London)* **367**, 72–76
- Bahnsen, T. D., Pandol, S. J. and Dionne, V. E. (1993) *J. Biol. Chem.* **268**, 10808–10812
- Barry, V. A. and Cheek, T. R. (1994a) *J. Cell Sci.* **107**, 451–462
- Barry, V. A., and Cheek, T. R. (1994b) *Biochem. J.* **300**, 589–597
- Berridge, M. J. (1990) *J. Biol. Chem.* **265**, 9583–9586
- Berridge, M. J. and Irvine, R. F. (1989) *Nature (London)* **341**, 197–205
- Bird, G. St. J. and Putney, J. W., Jr. (1993) *J. Biol. Chem.* **268**, 2146–2148
- Burgoyne, R. D. (1991) *Biochim. Biophys. Acta* **1071**, 174–202
- Bygrave, F. L., Gamberucci, A., Fulceri, R. and Benedetti, A. (1993) *Biochem. J.* **292**, 19–22
- Capiod, T., Ogden, D. C., Trentham, D. R. and Walker, J. W. (1988) *J. Physiol. (London)* **406**, 124P
- Carter, T. D. and Ogden, D. C. (1992) *Proc. R. Soc. London, Ser. B* **250**, 235–241
- Cheek, T. R. and Thastrup, O. (1989) *Cell Calcium* **10**, 213–221
- Cheek, T. R., O'Sullivan, A. J., Moreton, R. B., Berridge, M. J. and Burgoyne, R. D. (1990) *FEBS Lett.* **266**, 91–95
- Cheek, T. R., Barry, V. A., Berridge, M. J. and Missiaen, L. (1991) *Biochem. J.* **275**, 697–701
- Cheek, T. R., Moreton, R. B., Berridge, M. J., Stauderman, K. A., Murawsky, M. M. and Bootman, M. D. (1993a) *J. Biol. Chem.* **268**, 27076–27083
- Cheek, T. R., Morgan, A., O'Sullivan, A. J., Moreton, R. B., Berridge, M. J. and Burgoyne, R. D. (1993b) *J. Cell Sci.* **105**, 913–921
- Dasso, L. L. T. and Taylor, C. W. (1991) *Biochem. J.* **280**, 791–795
- Eberhard, D. A. and Holz, R. W. (1988) *Trends Neurosci.* **11**, 517–520
- Ely, J. A., Ambroz, C., Baukal, A. J., Christensen, S. B., Balla, T. and Catt, K. (1991) *J. Biol. Chem.* **266**, 18635–18641
- Fadool, D. A. and Ache, B. W. (1992) *Neuron* **9**, 907–918
- Fasolato, C., Hoth, M. and Penner, R. (1993) *J. Biol. Chem.* **268**, 20737–20740
- Fasolato, C., Innocenti, B. and Pozzan, T. (1994) *Trends Pharmacol. Sci.* **15**, 77–83
- Friel, D. D. and Tsien, R. W. (1992) *J. Physiol. (London)* **450**, 217–246
- Fujimoto, T., Nakade, S., Miyawaki, A., Mikoshiba, K. and Ogawa, K. (1992) *J. Cell Biol.* **119**, 1507–1513
- Graier, W. F., Simicek, S., Bowles, D. K. and Sturek, M. (1994) *Biochem. J.* **300**, 637–641
- Gryniewicz, G., Poenie, M. and Tsien, R. Y. (1985) *J. Biol. Chem.* **260**, 3440–3450
- Hallam, T. J. and Rink, T. J. (1985) *FEBS Lett.* **186**, 175–179
- Hardie, R. C. (1991) *Proc. R. Soc. London, Ser. B* **245**, 203–210
- Hardie, R. C. and Minke, B. (1992) *Neuron* **8**, 643–651
- Henzi, V. and MacDermott, A. B. (1992) *Neuroscience* **46**, 251–273
- Hoth, M. and Penner, R. (1992) *Nature (London)* **355**, 353–356
- Hoth, M. and Penner, R. (1993) *J. Physiol. (London)* **465**, 359–386
- Illes, P. and Nörenberg, W. (1993) *Trends Pharmacol. Sci.* **14**, 50–54
- Irvine, R. F. (1990) *FEBS Lett.* **263**, 5–9
- Irvine, R. F. (1991) *Bioessays* **13**, 419–428
- Irvine, R. F. (1992) *FASEB J.* **6**, 3085–3091
- Jaconi, M. E. E., Lew, D. P., Monod, A. and Krause, K.-H. (1993) *J. Biol. Chem.* **268**, 26075–26078
- Katsuyama, H., Ito, S., Itoh, T. and Kuriyama, H. (1991) *Pflügers Arch.* **419**, 460–466
- Khan, A. A., Steiner, J. P. and Snyder, S. H. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 2849–2853
- Kuno, M. and Gardner, P. (1987) *Nature (London)* **326**, 301–304
- Liu, P.-S., Lin, Y.-L. and Kao, L.-S. (1991) *J. Neurochem.* **56**, 172–177
- Mahaut-Smith, M. P., Sage, S. O. and Rink, T. J. (1992) *J. Biol. Chem.* **267**, 3060–3065
- Miledi, R. and Parker, I. (1989) *J. Physiol. (London)* **415**, 189–210
- Missiaen, L., De Smedt, H., Droogmans, G. and Casteels, R. (1992) *J. Biol. Chem.* **267**, 22961–22966
- Mochizuki-Oda, N., Mori, K., Negishi, M. and Ito, S. (1991) *J. Neurochem.* **56**, 541–547
- Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N. and Nakanishi, N. (1991) *Nature (London)* **354**, 31–37
- Mozhayeva, G. N., Naumov, A. P. and Kuryshv, Y. A. (1990) *FEBS Lett.* **277**, 233–234
- Mozhayeva, G. N., Naumov, A. P. and Kuryshv, Y. A. (1991) *J. Membr. Biol.* **124**, 113–126
- Nakazawa, K., Fujimori, A., Takanaka, A. and Inoue, K. (1990) *J. Physiol. (London)* **428**, 257–272
- Neuhaus, R., Reber, B. F. X. and Reuter, H. (1991) *J. Neurosci.* **11**, 3984–3990
- Pandol, S. J. and Schoeffield-Payne, M. S. (1990) *J. Biol. Chem.* **265**, 12846–12853
- Parekh, A. B., Terlau, H. and Stuhmer, W. (1993) *Nature (London)* **364**, 814–818
- Penner, R., Fasolato, C. and Hoth, M. (1993) *Curr. Opin. Neurobiol.* **3**, 368–374
- Pessah, I. N., Francini, A. D., Scales, D. J., Waterhouse, A. L. and Casida, J. E. (1986) *J. Biol. Chem.* **261**, 8643–8648
- Plevin, R. and Boarder, M. R. (1988) *J. Neurochem.* **51**, 634–641
- Pruss, R. M., Moskal, J. R., Eiden, L. E. and Beinfeld, M. C. (1985) *Endocrinology (Baltimore)* **117**, 1020–1026
- Putney, J. W., Jr. (1986) *Cell Calcium* **7**, 1–12
- Putney, J. W., Jr. (1990) *Cell Calcium* **11**, 611–624
- Putney, J. W., Jr. and Bird, G. St. J. (1993a) *Cell* **75**, 199–201
- Putney, J. W., Jr. and Bird, G. St. J. (1993b) *Endocr. Rev.* **14**, 610–631
- Randriamampita, C. and Tsien, R. Y. (1993) *Nature (London)* **364**, 809–814
- Reber, B. F. X., Stucki, J. W. and Reuter, H. (1993) *J. Physiol. (London)* **468**, 711–727
- Restrepo, D., Miyamoto, T., Bryant, B. P. and Teeter, J. H. (1990) *Science* **249**, 1166–1168
- Robinson, I. M. and Burgoyne, R. D. (1991) *J. Neurochem.* **56**, 1587–1593
- Robinson, I. M., Cheek, T. R. and Burgoyne, R. D. (1992) *Biochem. J.* **288**, 457–463
- Sage, S. O., Merritt, J. E., Hallam, T. J. and Rink, T. J. (1989) *Biochem. J.* **258**, 923–926
- Stauderman, K. A. and Murawsky, M. M. (1991) *J. Biol. Chem.* **266**, 19150–19153
- Stauderman, K. A. and Pruss, R. M. (1989) *J. Biol. Chem.* **264**, 18349–18355
- Stauderman, K. A. and Pruss, R. M. (1990) *J. Neurochem.* **54**, 946–953
- Stauderman, K. A., Murawsky, M. M. and Pruss, R. M. (1990) *Cell Regulation* **1**, 683–691
- Stauderman, K. A., McKinney, R. A. and Murawsky, M. M. (1991) *Biochem. J.* **278**, 643–650
- Strigrow, F. and Bohnsack, R. (1994) *Eur. J. Biochem.* **222**, 229–234
- Taylor, C. W. and Richardson, A. (1991) *Pharmacol. Ther.* **51**, 97–137
- Thayer, S. A., Perney, T. M. and Miller, R. J. (1988) *J. Neurosci.* **8**, 4089–4097
- Tones, M. A., Bootman, M. D., Higgins, B. F., Lane, D. A., Pay, G. F. and Lindahl, U. (1989) *FEBS Lett.* **252**, 105–108
- Wakui, M., Osipchuk, Y. V. and Petersen, O. H. (1990) *Cell* **63**, 1025–1032
- Xu, Y. and Forsberg, E. J. (1993) *J. Pharmacol. Exp. Ther.* **264**, 770–775