3664 Journal of Physiology (1995), 484.3, pp. 549–555

Release of Ca^{2+} by noradrenaline and ATP from the same Ca^{2+} store sensitive to both $InsP_3$ and Ca^{2+} in rat portal vein myocytes

Pierre Pacaud and Gervaise Loirand *

*Laboratoire de Physiologie, Faculté de médecine Victor Pachon, Université de Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux and *INSERM U-390, Hôpital Arnaud de Villeneuve, 34295 Montpellier, France*

1. Changes in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) induced by noradrenaline (NA) and ATP were investigated using indo-1 microspectrofluorimetry in single smooth muscle cells of rat portal vein.

2. Activation of P_{2x}-purinoceptors by ATP (10 μM) increased $[Ca^{2+}]_i$ from 92 ± 7 nM ($n = 18$) to 557 ± 30 nM ($n = 11$). In the presence of NA (10 μM), the ATP-induced rise in $[Ca^{2+}]_i$ was reduced to $23 \cdot 6 \pm 1 \cdot 5$% ($n = 7$) of the control response (in the absence of NA).

3. Tetracaine (10 μM to 2 mM) inhibited in a concentration-dependent manner the Ca^{2+}-induced Ca^{2+} release (CICR) evoked by 5 mM caffeine. In the presence of 1 mM tetracaine, the rise in $[Ca^{2+}]_i$ induced by ATP (10^{-8}–10^{-4} M) was strongly inhibited. A tetracaine-resistant rise in $[Ca^{2+}]_i$, corresponding to $26 \cdot 4 \pm 2 \cdot 3$% ($n = 14$) of control values, was recorded in response to 10 μM ATP.

4. The amplitude of the NA-induced $[Ca^{2+}]_i$ rise depended on NA concentrations (10^{-8}–10^{-5} M) and was not modified by tetracaine (1 mM).

5. This study suggests that Ca^{2+} ions released through the $InsP_3$ receptor–channel upon NA application do not activate CICR and that the $InsP_3$- and Ca^{2+}-sensitive Ca^{2+} store appears to represent, at least functionally, a single releasable Ca^{2+} pool.

In smooth muscle cells, as in skeletal and cardiac myocytes, the sarcoplasmic reticulum (SR) is a major source contributing to the increase in the free cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) upon excitation (Somlyo, 1985). The mobilization of Ca^{2+} from SR by inositol 1,4,5-trisphosphate ($InsP_3$) is now widely accepted as a principal link between cell surface receptor activation, coupled to stimulation of phosphoinositidase C, and the increase in $[Ca^{2+}]_i$ (Berridge & Irvine, 1989). Upon binding $InsP_3$, the $InsP_3$ receptor of the SR membrane undergoes a large conformational change, leading to channel opening.

However, another intracellular Ca^{2+} channel, a member of the ryanodine receptor family, has been identified in SR of smooth muscle (Hermann-Frank, Darling & Meissner, 1991). This Ca^{2+} release channel–receptor complex is activated by Ca^{2+} and caffeine. There is now evidence that Ca^{2+} entry through the voltage-gated Ca^{2+} channel is able to activate Ca^{2+} release independently of the $InsP_3$ receptor in smooth muscle cells (Ganitkevich & Isenberg, 1992; Grégoire, Loirand & Pacaud, 1993).

In portal vein smooth muscle cells, the membrane of the SR contains both $InsP_3$ receptors, which can be activated by noradrenaline (NA) (Pacaud, Loirand, Grégoire, Mironneau & Mironneau, 1993; Loirand, Grégoire & Pacaud, 1994), and ryanodine receptors, which can be opened following activation of P_{2x}-purinoceptors by ATP (Pacaud, Grégoire & Loirand, 1994). The present experiments were designed to define whether the $InsP_3$ and ryanodine receptor–channels regulate Ca^{2+} release from different or identical functional compartments in portal vein smooth muscle cells, and whether Ca^{2+} released through the $InsP_3$ receptor might stimulate Ca^{2+} release via the ryanodine receptor.

METHODS

Cell preparation

Wistar rats (150 g) were stunned by a blow to the head and then killed by cervical dislocation. The portal vein was cut into several pieces, incubated for 10 min in low-Ca^{2+} (40 μM) physiological saline solution (PSS; composition given below) and then incubated in low-Ca^{2+} PSS containing 1·2 mg ml^{-1} collagenase, 0·5 mg ml^{-1} pronase and 1 mg ml^{-1} bovine serum albumin at 37 °C for 20 min. After this time, the solution was removed and the pieces of vein were incubated again in a fresh enzyme-free solution and triturated using a fire-polished Pasteur pipette to release cells. Cells were stored on glass coverslips at 4 °C in PSS containing 0·8 mM Ca^{2+} and used on the same day.

Membrane current and fluorescence measurements

Voltage clamp and membrane current recordings were made with standard patch-clamp techniques using a Biologic RK400 patch-clamp amplifier (Biologic Co., Claix, France). Whole-cell membrane currents were recorded with borosilicate patch pipettes of 1–4 MΩ resistance. The series resistances (5–8 MΩ) were not corrected. The liquid junction potentials were corrected with an offset circuit before each experiment. Membrane potential and current records were stored and analysed using an IBM-PC computer.

Measurement of $[Ca^{2+}]_i$ was carried out as described previously (Pacaud *et al.* 1993). Cells were loaded with indo-1, either by addition of 50 μM indo-1 in the pipette solution, which thereby entered cells following establishment of the whole-cell recording mode, or by incubation in PSS containing 1 μM indo-1 penta-acetoxymethyl ester (indo-1 AM) for 25 min at room temperature. The fluorescence signal from cells loaded with indo-1 AM indicated an internal indo-1 concentration in the range 40–70 μM, from comparison with cells loaded with indo-1 using a patch pipette. $[Ca^{2+}]_i$ was estimated from the fluorescence ratio at 405 and 480 nm (F_{405}/F_{480}) (Grynkiewicz, Poenie & Tsien, 1985). Results are expressed as the mean values ± S.E.M., with n the sample size. Significance was tested by means of Student's unpaired t test.

Solutions

During the experiments, the cells were continuously superfused with a physiological saline solution (PSS) that contained (mM): 130 NaCl, 5·6 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 11 glucose, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (Hepes); pH adjusted to 7·4 with NaOH. The pipette solution was composed of (mM): 120 CsCl, 10 NaCl, 10 Hepes; brought to pH 7·3 with the addition of approximately 5 mM NaOH. ATP, NA and caffeine were usually applied to the recorded cell by pressure ejection from a glass pipette for the period indicated on the records. No change in the holding current was observed during ejection of the physiological saline solution. All experiments were done at room temperature and in the presence of 10 μM methoxyverapamil hydrochloride (D600), a voltage-dependent Ca^{2+} channel blocker.

Chemicals and drugs

Collagenase was from Worthington Biochemical Corp., Freehold, NJ, USA. Pronase (type E), bovine serum albumin, adenosine 5′-triphosphate (disodium salt; ATP), heparin (from porcine intestinal mucosa; molecular weight, 4000–6000), D600, NA and tetracaine were purchased from Sigma. Indo-1 (pentasodium salt) and indo-1 AM were from Calbiochem, France Biochem, Meudon, France. Caffeine was from Merck, Darmstadt, Germany.

RESULTS

Effect of ATP during NA application

As described previously (Pacaud, Loirand, Bolton, Mironneau & Mironneau, 1992), NA (10 μM) induced a transient increase in $[Ca^{2+}]_i$ from 92 ± 7 nM ($n = 18$) to 610 ± 96 nM ($n = 7$), followed by a plateau phase, maintained at a $[Ca^{2+}]_i$ of about 130 nM (Fig. 1*A*). At a holding potential of −60 mV, the NA-induced $[Ca^{2+}]_i$ transient activated a Ca^{2+}-dependent Cl^- current (Pacaud *et al.* 1992). In the continuous presence of NA, ATP (10 μM, 3 s) applied after the termination of the NA-induced $[Ca^{2+}]_i$ transient increased $[Ca^{2+}]_i$ to only 202 ± 7 nM ($n = 7$) whereas, under control conditions (Fig. 1*B*), the peak of the ATP-induced $[Ca^{2+}]_i$ rise which occurred for the same ATP concentration (i.e. 10 μM) averaged 557 ± 30 nM ($n = 11$). At a holding potential of −60 mV, ATP induced an inward current corresponding to cation entry through P_{2x}-receptor–channels (Pacaud *et*

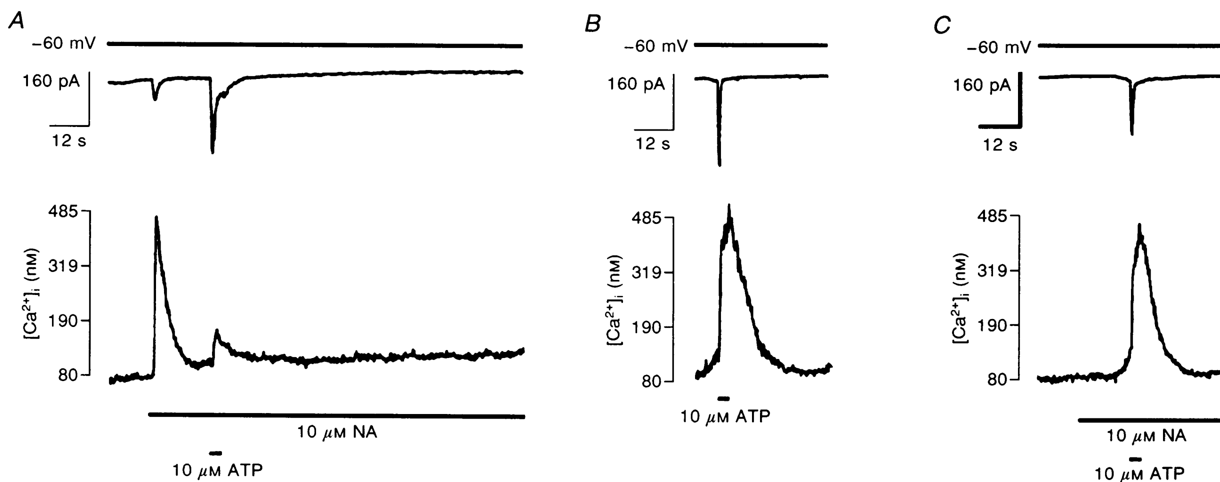

Figure 1. Effect of ATP in a cell maintained at a holding potential of −60 mV

Traces represent the membrane potential (top), the membrane current (middle) and the $[Ca^{2+}]_i$ (bottom). *A*, NA (10 μM) induced a transient rise in $[Ca^{2+}]_i$ followed by a sustained rise. The NA-induced $[Ca^{2+}]_i$ transient activated a Ca^{2+}-dependent Cl^- current. ATP (10 μM), applied during the sustained NA-induced $[Ca^{2+}]_i$ rise, evoked a transient inward cation current and a small increase in $[Ca^{2+}]_i$. *B*, effect of ATP (10 μM) under control conditions. *C*, during a maintained application of NA (10 μM) and in the presence of heparin (1 mM) in the pipette solution, the response evoked by ATP (10 μM) was not modified.

al. 1994). In the presence of the InsP_3 receptor blocker heparin (1 mM; Worley, Baraban, Supattapone, Wilson & Snyder, 1987) in the pipette solution, NA (10 μM) was not able to increase $[Ca^{2+}]_i$, but a transient rise in $[Ca^{2+}]_i$ was still induced upon addition of 10 μM ATP (Fig. 1*C*). This suggests that the inhibition of the ATP-induced $[Ca^{2+}]_i$ rise by NA did not result from the inhibition of the purinoceptors or intracellular mechanisms responsible for the ATP-induced $[Ca^{2+}]_i$ rise. Since InsP_3-induced Ca^{2+} release and Ca^{2+}-induced Ca^{2+} release (CICR) are involved in the response evoked by NA (Pacaud *et al.* 1993) and ATP (Pacaud *et al.* 1994), respectively, at least two hypotheses could explain the inhibition of the ATP-induced $[Ca^{2+}]_i$ rise in the presence of NA. First, the Ca^{2+} released via NA-induced InsP_3 receptor activation could activate the CICR and thus deplete the Ca^{2+}-sensitive store. Secondly, InsP_3 and ryanodine receptors could be located at the site of the same functional intracellular Ca^{2+} compartment.

We therefore attempted to determine whether NA activates the CICR, by examining the effect of the local anaesthetic tetracaine, a CICR blocker, on the NA-induced $[Ca^{2+}]_i$ rise.

Effect of tetracaine on the caffeine-induced Ca^{2+} release

It was first verified that tetracaine does inhibit CICR in portal vein smooth muscle cells by demonstrating its inhibitory effect on the $[Ca^{2+}]_i$ rise induced by caffeine, a known potentiator of CICR (Endo, 1977).

Caffeine (5 mM, 6 s) induced a transient $[Ca^{2+}]_i$ rise of 404 ± 59 nM ($n = 12$) (Fig. 2*A*). In addition to the change in 480 and 405 nm signals caused by variations in $[Ca^{2+}]_i$, caffeine decreased the fluorescence by the same factor at both wavelengths. The quench produced by caffeine did not depend on either the wavelength or $[Ca^{2+}]_i$ and thus did not affect the ratio trace (O'Neill, Donoso & Eisner, 1990). This effect of caffeine was still observed in the presence of tetracaine (0·3 and 2 mM, Fig. 2*A*), although tetracaine inhibited the caffeine-induced $[Ca^{2+}]_i$ rise in a concentration-dependent manner from 10 μM to 2 mM. The apparent inhibitory constant was 190 μM and complete blockade was achieved at 2 mM (Fig. 2*B*, ●). In addition, to reduce the amplitude of the caffeine-induced $[Ca^{2+}]_i$ rise, tetracaine increased the time to peak, measured as the delay between the onset of the quench and the peak $[Ca^{2+}]_i$ (Fig. 2*A*, dashed lines; Fig. 2*B*, ■).

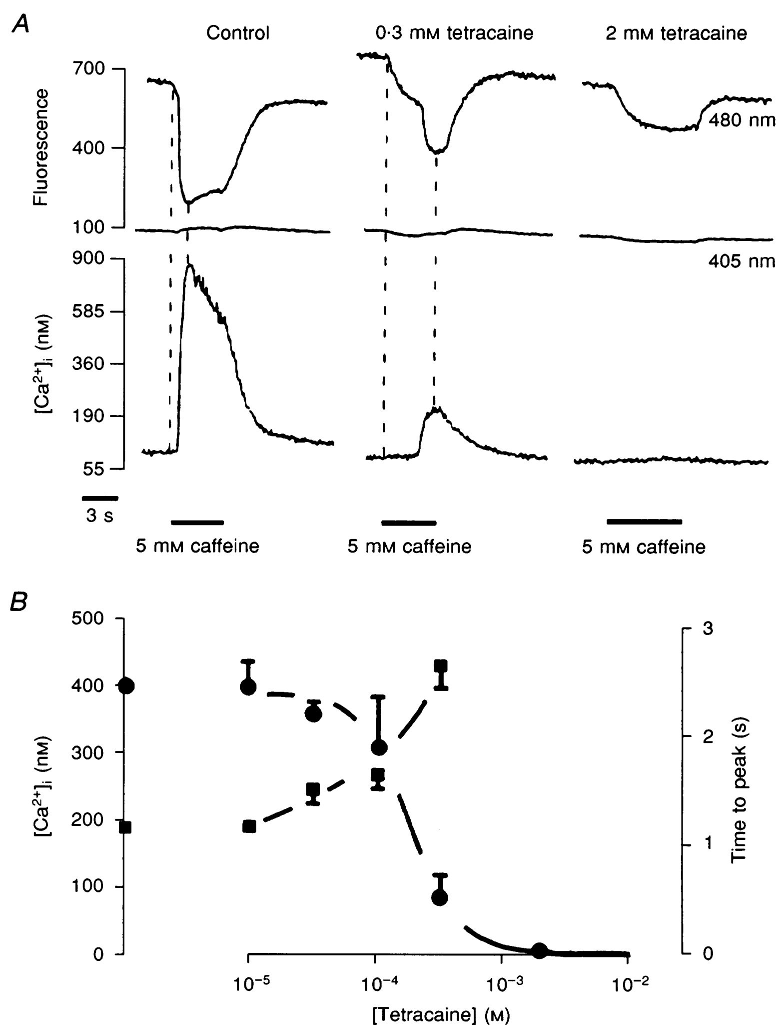

Figure 2. Effect of tetracaine on the caffeine-induced $[Ca^{2+}]_i$ rise in cells loaded with indo-1 AM

A, traces show fluorescence signal at 480 and 405 nm (top) and the $[Ca^{2+}]_i$ estimated from the F_{405}/F_{480} ratio (bottom). The increase in $[Ca^{2+}]_i$ elicited by 5 mM caffeine was inhibited in a concentration-dependent fashion by tetracaine (0·3 and 2 mM). The time to peak, defined as the time between the onset of the quench induced by caffeine and the peak of the response (dashed lines) was increased by tetracaine. *B*, the magnitude (left axis, ●) and the time to peak (right axis, ■) of the caffeine-induced $[Ca^{2+}]_i$ rise were plotted against tetracaine concentration. Data (●) were fitted by a Michaelis–Menten equation. Half-maximal inhibition was obtained with a tetracaine concentration of 190 μM. Each point represents the mean value recorded in 4–12 cells.

These results confirm that CICR in portal vein myocytes is sensitive to tetracaine, as in skeletal muscle cells (Györke & Palade, 1992).

Effect of tetracaine on ATP-induced $[Ca^{2+}]_i$ rise

The amplitude of the ATP-induced $[Ca^{2+}]_i$ rise increased with ATP concentration (Fig. 3*A*). The maximal response (557 ± 30 nM, $n = 11$) was achieved at an ATP concentration of 10 μM, and the concentration of ATP that induced a half-maximal rise in $[Ca^{2+}]_i$ was 0·30 μM (Fig. 3*C*, ■). Responses induced by 1 and 10 μM ATP often appeared as a biphasic rise in $[Ca^{2+}]_i$. The peak of the $[Ca^{2+}]_i$ rise induced by 10 μM ATP occurred $3{\cdot}03 \pm 0{\cdot}23$ s ($n = 11$) after the onset of the response. In the presence of 1 mM tetracaine, the ATP-induced $[Ca^{2+}]_i$ rise was strongly reduced, whatever the ATP concentration (Fig. 3*B*; Fig. 3*C*, □). The peak $[Ca^{2+}]_i$ induced by 10 μM ATP was decreased to 215 ± 11 nM ($n = 14$), although the mean inward current recorded at -60 mV was similar (349 ± 77 pA, $n = 6$, $P > 0{\cdot}2$) to that recorded under control conditions (246 ± 36 pA, $n = 8$). In addition, tetracaine modified the time course of the response. The delayed phase of the ATP-induced $[Ca^{2+}]_i$ rise was not observed in the presence of 1 mM tetracaine, leading to a reduction of the time to peak, measured from the onset of the response induced by 10 μM ATP, to $0{\cdot}85 \pm 0{\cdot}11$ s ($n = 14$, $P < 0{\cdot}005$). The half-maximal rise in $[Ca^{2+}]_i$ was obtained for an ATP concentration of 0·26 μM in the presence of tetracaine, which was not different from that in the absence of tetracaine, i.e. tetracaine did not shift the concentration–response curve. This result confirms the involvement of CICR in the ATP-induced $[Ca^{2+}]_i$ rise in rat portal vein smooth muscle cells.

Effect of tetracaine on NA-induced $[Ca^{2+}]_i$ rise

The amplitude of the $[Ca^{2+}]_i$ rise in response to NA depended on the concentration used (Fig. 4*A*). The threshold concentration was 0·01 μM and the maximal $[Ca^{2+}]_i$ rise, obtained in response to 10 μM NA, reached 610 ± 96 nM ($n = 7$) (Fig. 4*C*, ■). The NA concentration that induced a half-maximal $[Ca^{2+}]_i$ rise corresponded to 0·16 μM. None of these values were significantly modified in the presence of 1 mM tetracaine (Fig. 4*B*; Fig. 4*C*, □). The time course of the NA-induced $[Ca^{2+}]_i$ transient was not changed either. The times corresponding to 90% of the decline from the peak $[Ca^{2+}]_i$ induced by 1 and 10 μM

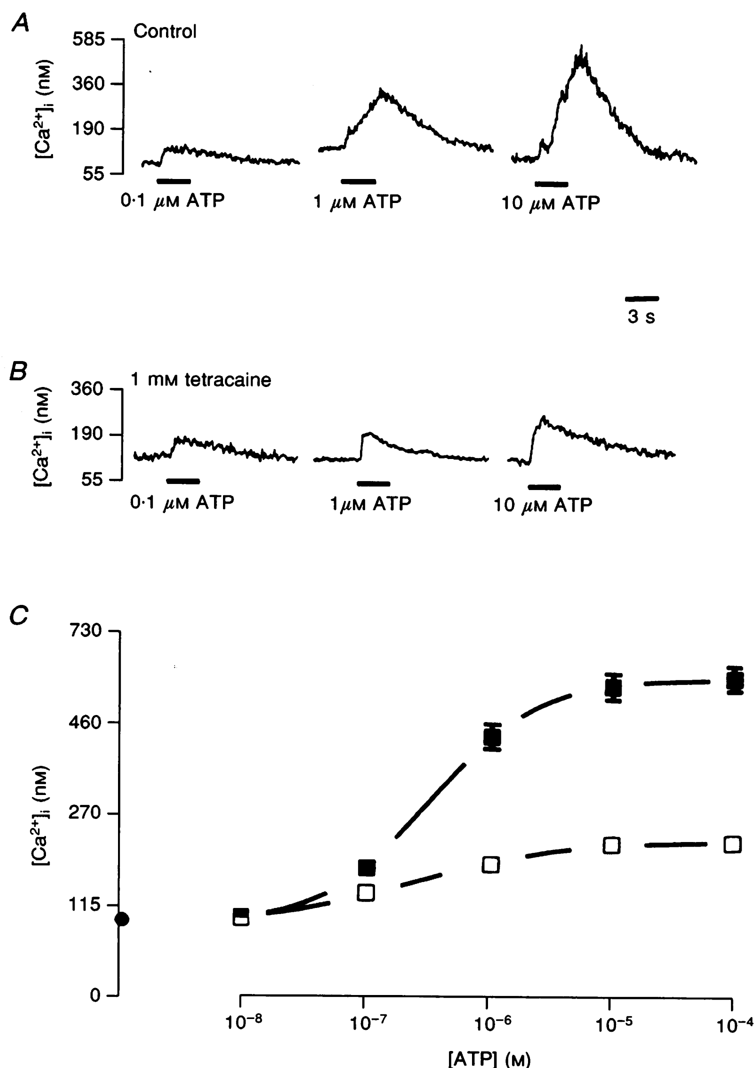

Figure 3. Concentration dependence of the ATP-induced $[Ca^{2+}]_i$ rise in cells loaded with indo-1 AM

A, the maximal amplitude of the rise in $[Ca^{2+}]_i$ increased with the concentration of ATP (0·1, 1 and 10 μM). *B*, in the presence of 1 mM tetracaine, the $[Ca^{2+}]_i$ rises induced by 0·1, 1 and 10 μM ATP were reduced. *C*, the peak of the ATP-induced $[Ca^{2+}]_i$ rise under control conditions (■) and in the presence of 1 mM tetracaine (□) was plotted against ATP concentration and fitted by a Michaelis–Menten equation. The ATP concentration that induced a half-maximal rise corresponded to 0·30 and 0·26 μM under control conditions and in the presence of tetracaine, respectively. Each point represents the mean value recorded in 9–15 cells.

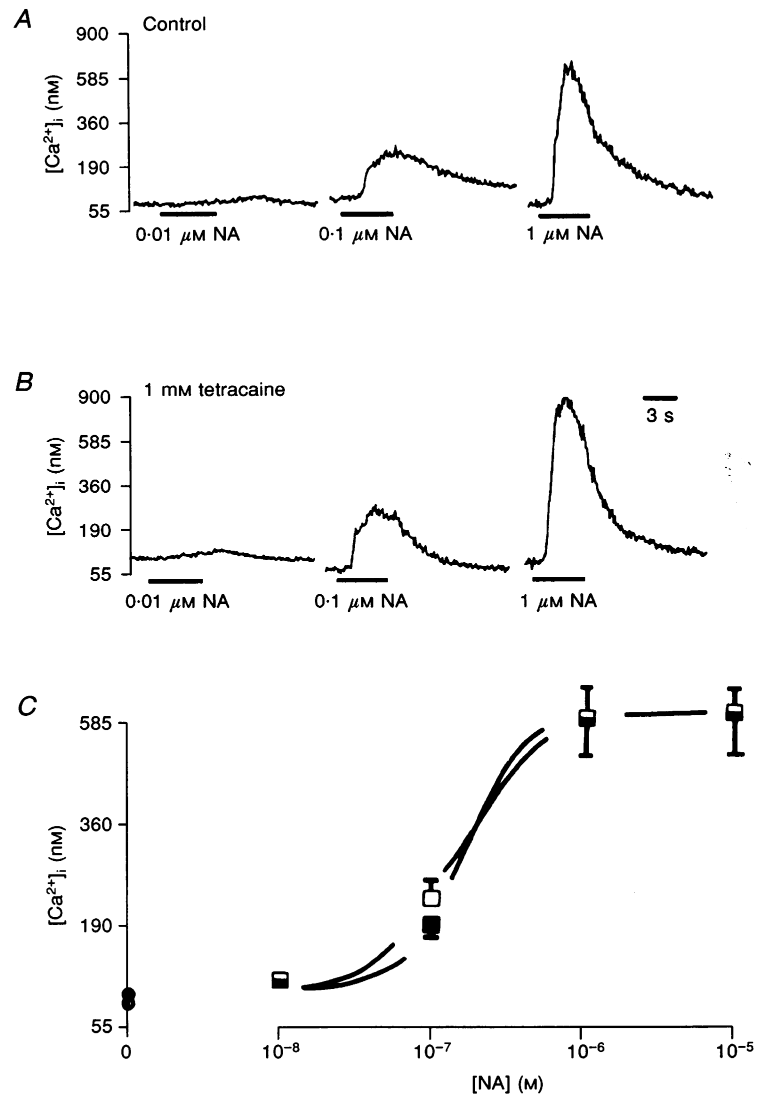

Figure 4. Concentration dependence of the NA-induced $[Ca^{2+}]_i$ rise in the cells loaded with indo-1 AM

A, the maximal amplitude of the rise in $[Ca^{2+}]_i$ increased with the concentration of NA (0·01, 0·1 and 1 μM). *B*, in the presence of 1 mM tetracaine, the $[Ca^{2+}]_i$ rises induced by 0·01, 0·1 and 1 μM NA were not modified. *C*, the peak of the NA-induced $[Ca^{2+}]_i$ rise under control conditions (■) and in the presence of 1 mM tetracaine (□) was plotted against NA concentration and fitted by a Michaelis–Menten equation. The NA concentration that induced a half-maximal rise corresponded to 0·16 and 0·15 μM under control conditions and in the presence of tetracaine, respectively. Each point represents the mean value recorded in 7–15 cells.

NA were 6·07 ± 0·46 s ($n = 7$) and 7·54 ± 0·51 s ($n = 7$), respectively, under control conditions and 6·94 ± 0·47 s ($n = 8$, $P > 0{\cdot}2$) and 8·01 ± 0·94 s ($n = 6$, $P > 0{\cdot}5$), respectively, in the presence of tetracaine (1 mM). The threshold concentration was still 0·01 μM, the maximal $[Ca^{2+}]_i$ rise induced by 10 μM NA reached 613 ± 63 nM ($n = 9$, $P > 0{\cdot}5$) and the half-maximal $[Ca^{2+}]_i$ rise was obtained in response to 0·15 μM NA.

DISCUSSION

The data presented here suggest that, in portal vein smooth muscle cells, CICR is not involved in the NA-induced $[Ca^{2+}]_i$ rise and that the InsP_3- and Ca^{2+}-sensitive Ca^{2+} store appears to represent functionally a single releasable Ca^{2+} pool.

Experiments with caffeine provide evidence for the sensitivity of the CICR to tetracaine in portal vein smooth muscle cells. Complete inhibition was achieved with tetracaine concentrations in the millimolar range. Therefore, tetracaine is a useful tool to differentiate the contributions of CICR, InsP_3-induced Ca^{2+} release and Ca^{2+} influx to $[Ca^{2+}]_i$ rises induced by ATP and NA. The major part of the $[Ca^{2+}]_i$ rise induced by ATP (10^{-8}–10^{-4} M) was inhibited by 1 mM tetracaine. This confirms previous results (Pacaud *et al.* 1994), indicating that CICR mostly contributes to the ATP-induced $[Ca^{2+}]_i$ rise. The tetracaine-resistant rise in $[Ca^{2+}]_i$, corresponding to 26·4 ± 2·3% ($n = 14$) of the control response for 10 μM ATP, was likely to be caused by Ca^{2+} influx through P_{2x}-purinoceptor ion channels (Pacaud *et al.* 1994). This ATP-induced $[Ca^{2+}]_i$ rise recorded in the presence of tetracaine was similar to that recorded after depletion of the intracellular Ca^{2+} store by either caffeine or ryanodine (Pacaud *et al.* 1994) as well as after InsP_3-induced Ca^{2+} release produced by NA (23·6 ± 1·5%, $n = 7$; Fig. 1*A*).

On the contrary, $[Ca^{2+}]_i$ rises evoked by NA concentrations ranging between 10^{-8} and 10^{-5} M were similar under control conditions (with 10 μM D600) and in the presence of tetracaine (Fig. 4). In view of this experiment, it appears that the time to peak decreased when the NA concentration increased and that the

amplitude of the $[Ca^{2+}]_i$ response in an individual cell was graded. This result does not agree with the agonist-induced all-or-none $[Ca^{2+}]_i$ mobilization recently described in single smooth muscle cells (Iino, Yamazawa, Miyashita, Endo & Kasai, 1993). It was reported that, because of the Ca^{2+}-dependent positive feedback control of the InsP_3-induced Ca^{2+} release, carbachol concentrations above the threshold value induced a constant peak $[Ca^{2+}]_i$ in intestinal smooth muscle cells. It seems that this behaviour should not be extended to all smooth muscle cell types. It is well known that the InsP_3 receptor is subject to negative as well as positive feedback by cytoplasmic Ca^{2+} (Iino, 1990), in addition to regulation by luminal Ca^{2+} concentration (Missiaen, De Smedt, Droogmans & Casteels, 1992); the increase in $[Ca^{2+}]_i$ first enhances and then inhibits response to InsP_3, and the fall in luminal Ca^{2+} decreases the sensitivity of the receptor to InsP_3. Thus, it is likely that, depending on factors including the amount of calcium in intracellular stores, number of InsP_3 receptors and Ca^{2+} pump activity, the contribution of these positive and negative controls to the regulation of the InsP_3-mediated agonist response may change. This variation could be a possible explanation for the existence of either all-or-none or graded $[Ca^{2+}]_i$ response to InsP_3-generating agonists in different smooth muscle cells.

The failure of tetracaine to reduce the $[Ca^{2+}]_i$ transient induced by NA indicates that the CICR was not involved in the NA-induced Ca^{2+} release. Therefore, the inhibition of the ATP-induced $[Ca^{2+}]_i$ rise in the presence of NA (Fig. 1) suggests that NA and ATP release Ca^{2+} from the same intracellular store, containing both InsP_3 and ryanodine receptors. However, although the NA-induced $[Ca^{2+}]_i$ rise was graded, i.e. NA concentrations below 1 μM did not release the entire Ca^{2+} pool, the absence of a CICR component in the $[Ca^{2+}]_i$ transient induced by NA would also indicate that the Ca^{2+}-sensitive channel is curiously insensitive to the Ca^{2+} that has just been released through InsP_3 receptors. This result is consistent with the absence of effect of procaine on the Ca^{2+} release induced by photoreleased InsP_3 (Loirand *et al.* 1994). This behaviour can be explained if the InsP_3 and the ryanodine receptors are localized in different regions of the store, so that the Ca^{2+} released through InsP_3 receptors moves away from the Ca^{2+}-sensitive activation sites of the ryanodine receptors. An alternative explanation would be that, as a consequence of the InsP_3-induced Ca^{2+} release, the ryanodine receptor responds to the fall in luminal Ca^{2+} concentration by decreasing its sensitivity to cytosolic Ca^{2+} (Nelson & Nelson, 1990; Grégoire *et al.* 1993) before the released Ca^{2+} can open the channel. The distribution of InsP_3 and ryanodine receptors as well as the characterization of the functional properties of Ca^{2+} stores require further investigation, according to the type of smooth muscle cells.

Berridge, M. J. & Irvine, R. F. (1989). Inositol phosphates and cell signalling. *Nature* **341**, 497–508.

Endo, M. (1977). Calcium release from the sarcoplasmic reticulum. *Physiological Reviews* **57**, 71–108.

Ganitkevich, V. Ya. & Isenberg, G. (1992). Contribution of Ca^{2+}-induced Ca^{2+} release to the $[Ca^{2+}]_i$ transients in myocytes from guinea-pig urinary bladder. *Journal of Physiology* **458**, 119–137.

Grégoire, G., Loirand, G. & Pacaud, P. (1993). Ca^{2+} and Sr^{2+} entry induced Ca^{2+} release from the intracellular Ca^{2+} store in smooth muscle cells of rat portal vein. *Journal of Physiology* **472**, 483–500.

Grynkiewicz, G., Poenie, M. & Tsien R. Y. (1985). A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *Journal of Biological Chemistry* **260**, 3440–3450.

Györke, S. & Palade, P. (1992). Calcium-induced calcium release in crayfish skeletal muscle. *Journal of Physiology* **457**, 195–210.

Herrmann-Frank, A., Darling, E. & Meissner, G. (1991). Functional characterization of the Ca^{2+}-gated Ca^{2+} release channel of vascular smooth muscle sarcoplasmic reticulum. *Pflügers Archiv* **418**, 353–359.

Iino, M. (1990). Biphasic Ca^{2+} dependence of inositol 1,4,5-trisphosphate induced Ca^{2+} release in smooth muscle cells of the guinea-pig taenia caeci. *Journal of General Physiology* **95**, 1103–1122.

Iino, M., Yamazawa, T., Miyashita, Y., Endo, M. & Kasai, H. (1993). Critical intracellular Ca^{2+} concentration for all-or-none Ca^{2+} spiking in single smooth muscle cells. *EMBO Journal* **12**, 5287–5291.

Loirand, G., Gregoire, G. & Pacaud, P. (1994). Photoreleased inositol 1,4,5-trisphosphate-induced response in single smooth muscle cells of rat portal vein. *Journal of Physiology* **479**, 41–62.

Missiaen, L., De Smedt, H., Droogmans, G. & Casteels, R. (1992). Ca^{2+} release induced by inositol 1,4,5-trisphosphate is a steady phenomenon controlled by luminal Ca^{2+} in permeabilized cells. *Nature* **357**, 599–602.

Nelson, T. E. & Nelson, K. E. (1990). Intra- and extraluminal sarcoplasmic reticulum membrane regulatory sites for Ca^{2+}-induced Ca^{2+} release. *FEBS Letters* **263**, 292–294.

O'Neill, S. C., Donoso, P. & Eisner, D. A. (1990). The role of $[Ca^{2+}]_i$ and $[Ca^{2+}]$ sensitization in the caffeine contracture of rat myocytes: measurement of $[Ca^{2+}]_i$ and $[caffeine]_i$. *Journal of Physiology* **425**, 55–70.

Pacaud, P., Grégoire, G. & Loirand, G. (1994). Release of Ca^{2+} from intracellular store in smooth muscle cells of rat portal vein by ATP-induced Ca^{2+} entry. *British Journal of Pharmacology* **113**, 457–462.

Pacaud, P., Loirand, G., Bolton, T. B., Mironneau, C. & Mironneau, J. (1992). Intracellular cations modulate noradrenaline-stimulated calcium entry into smooth muscle cells of rat portal vein. *Journal of Physiology* **456**, 541–556.

PACAUD, P., LOIRAND, G., GRÉGOIRE, G., MIRONNEAU, C. & MIRONNEAU, J. (1993). Noradrenaline-activated heparin-sensitive Ca^{2+} entry after depletion of intracellular Ca^{2+} store in portal vein smooth muscle cells. *Journal of Biological Chemistry* **268**, 3866–3872.

SOMLYO, A. P. (1985). Excitation–contraction coupling and the ultrastructure of smooth muscle. *Circulation Research* **57**, 497–508.

WORLEY, P. F., BARABAN, J. M., SUPATTAPONE, S., WILSON, V. S. & SNYDER, S. H. (1987). Characterization of inositol trisphosphate receptor in brain. Regulation by pH and calcium. *Journal of Biological Chemistry* **262**, 12132–12136.

Acknowledgements

This work was supported by a grant from the Conseil Régional d'Aquitaine (9203023). We thank Drs R. Marthan and J. P. Savineau for helpful advice in the preparation of this manuscript.

Received 29 July 1994; accepted 13 October 1994.