pH dependence of inositol 1,4,5-trisphosphate-induced Ca^{2+} release in permeabilized smooth muscle cells of the guinea-pig

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1. The dependence on pH of inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release was studied in saponin-skinned smooth muscle cells from guinea-pig portal vein, using the indicator fura-2 to monitor Ca^{2+} release.

2. Increasing pH between 6·7 and 7·3 enhanced the rate of IP_3-induced Ca^{2+} release at all the Ca^{2+} concentrations above 30 nM without changing the bell-shaped dependence of the Ca^{2+} release rate on Ca^{2+} concentration with a peak near 300 nM.

3. The ascending limb of the biphasic Ca^{2+} dependence was shifted slightly toward the lower Ca^{2+} concentration at pH 7·3, suggesting an increase in the Ca^{2+} sensitivity of IP_3-induced Ca^{2+} release at the higher pH.

4. With the elevation in pH from 6·7 to 7·3 at 100 nM Ca^{2+}, about 7-fold higher IP_3 concentration was required to release half of the Ca^{2+} in the store within 15 s. This pH-dependent change in the IP_3 sensitivity was smaller at 1 μM Ca^{2+} and was indiscernible in the absence of Ca^{2+}.

5. These results suggest that H^+ may inhibit binding of IP_3 and Ca^{2+} to the modulator sites of the Ca^{2+} release mechanism. However, these effects on the binding sites may not fully explain the complex effect of pH, and there may be pH-dependent step(s) involved in the gating mechanism of IP_3 channels. The present study demonstrates the importance of pH as a modulator of IP_3-induced Ca^{2+} release.

In agonist-induced contractions of vascular as well as other smooth muscle cells, inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release from the intracellular Ca^{2+} store may play an important role in the regulation of cytoplasmic Ca^{2+} concentration (Suematsu, Hirata, Hashimoto & Kuriyama, 1984; Somlyo, Bond, Somlyo & Scarpa, 1985). IP_3 is a soluble product of the hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C, which is activated by G-protein-coupled agonist receptors (Berridge, 1993). IP_3 then diffuses inside the cell to bind to IP_3 receptors which are present in the non-mitochondrial membrane-bound Ca^{2+} stores and function as Ca^{2+} release channels (Ferris, Huganir, Supattapone & Snyder, 1989). We now know that IP_3 receptors have structural similarities to caffeine-sensitive Ca^{2+}-induced Ca^{2+} release channels, or ryanodine receptors, which are also present in the intracellular Ca^{2+} stores. Not only are both channels formed by tetramers of protein molecules (Saito, Inui, Radermacher, Frank & Fleischer, 1988; Lai, Erickson, Rousseau, Liu & Meissner, 1988; Chadwick, Saito & Fleischer, 1990), but there are several stretches of homologous amino acid sequence in the two Ca^{2+} release channel proteins (Furuichi, Yoshioka, Miyawaki, Wada, Maeda & Mikoshiba, 1989; Mignery, Newton, Archer & Südhof, 1990). Furthermore, the two channels share certain functional properties. Both are dependent on Ca^{2+} concentration in a biphasic manner, although the range of effective concentration is lower for the IP_3 receptor channels (Endo, 1985; Iino, 1990; Bezprozvanny, Watras & Ehrlich, 1991). Both Ca^{2+} release mechanisms are enhanced by adenine nucleotides (Endo, 1985; Ferris, Huganir & Snyder, 1990; Iino, 1991). Thus, IP_3- and Ca^{2+}-induced Ca^{2+} release mechanisms are closely related, and are co-expressed in smooth and cardiac muscle cells (Yamazawa, Iino & Endo, 1992; Kijima, Saito, Jetton, Magnuson & Fleischer, 1993) and in nerve cells (Walton *et al.* 1991).

It has been reported that binding of IP_3 to its receptors is pH dependent, and that the binding increases with the rise in pH (Worley, Baraban, Supattapone, Wilson & Snyder, 1987; Guillemette & Segui, 1988; Joseph, Rice & Williamson, 1989). It is also known that Ca^{2+}-induced Ca^{2+} release is sensitive to pH in such a way that an increase in pH sensitizes the channels to Ca^{2+} (Endo, 1985; Iino, 1989). On the basis of these findings and analogy, one might

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expect pH to be a modulator of IP_3-induced Ca^{2+} release. Although there have been several reports on the effect of pH on IP_3-induced Ca^{2+} release (Brass & Joseph, 1985; Clapper & Lee, 1985; Guillemette & Segui, 1988; Joseph *et al.* 1989), important modulating factors other than pH were not controlled during the Ca^{2+} release. Therefore, the effect of pH on the IP_3-induced Ca^{2+} release has not been quantitatively analysed. Since pH has profound effects on smooth muscle contraction (Wray, 1988), pH dependence of IP_3-induced Ca^{2+} release may be important for the regulation of the intracellular Ca^{2+} concentration in smooth muscle cells. We therefore studied the effect of pH on the rate of IP_3-induced Ca^{2+} release in permeabilized smooth muscle cells while keeping other modulating factors constant. The results indicate that pH is one of the important modulators and that IP_3-induced Ca^{2+} release is enhanced with increase in pH around neutral pH.

METHODS

Muscle preparation and experimental set-up

Thin smooth muscle strips (~70 μm thick, 200–300 μm wide, 3–5 mm long) were dissected from guinea-pig portal vein in a physiological salt solution containing (mM): NaCl, 150; KCl, 4; $CaCl_2$, 2; $MgCl_2$, 1; Hepes (*N*-2-hydroxyethylpiperazine-*N'*-ethanesulphonic acid), 5; pH 7·4. The guinea-pigs were killed by concussion and subsequent exsanguination. The muscle strips were tied with silk filaments at both ends to stainless-steel wires (100 μm in diameter). After treatment with saponin (50 μg ml^{-1}) for 35 min in a relaxing solution (solution 1, Table 1), fibre bundles attached to the metal wire were placed securely in a glass capillary cuvette (i.d., 400 μm; length, 32 mm). To study the IP_3-induced Ca^{2+} release mechanism independently of the Ca^{2+}-induced Ca^{2+} release, fibre bundles were treated with 30 μM ryanodine in the presence of 45 mM caffeine before each experiment to remove the function of the compartment with the Ca^{2+}-induced Ca^{2+} release mechanism that constitutes ~20 % of the total IP_3-sensitive store in this smooth muscle (Iino, 1991).

The experimental set-up uses a computer-controlled valve and pump system to change solutions in the cuvette and measures the fluorescence intensity at 510 nm, with excitation at 340 nm, of the Ca^{2+} indicator dye fura-2 (Grynkiewicz, Poenie & Tsien, 1985) bathing the preparation. Details of the set-up have been described previously (Iino, 1989). Experiments were carried out at room temperature (20–23 °C).

Experimental solutions

Table 1 shows the constituents of the solutions. Experimental solutions of various Ca^{2+} concentrations ($pCa = -\log[Ca^{2+}]$) were prepared by mixing two solutions, one containing 10 mM EGTA (ethylene glycol-bis (β-aminoethylether) *N,N,N',N'*-tetraacetic acid) or HEEDTA (*N*-hydroxyethylethlene diamine-*N,N',N'*-triacetic acid) without Ca^{2+} (solution 9) and the other both 10 mM EGTA (or HEEDTA) and 10 mM Ca^{2+} (solution 10) so that the total Ca^{2+} concentration had the values in Table 2. Ionic constituents were computed by solving multi-equilibrium equations using the binding constants compiled by Martell & Smith (1974–1989). pK_d (–log of the apparent dissociation constant) values thus obtained for Ca^{2+}-EGTA and Ca^{2+}-HEEDTA were 6·39 and 5·24, respectively, at 20 °C, pH 7·0.

Experimental protocol

The following protocol was used to study the Ca^{2+} release mechanism.

Procedure 1. To load the Ca^{2+} store with a fixed amount of Ca^{2+}, skinned fibres were incubated with 1 μM Ca^{2+} (solution 2) for 180 s (loading). Both Ca^{2+} and ATP were then washed with solution 6 for 120 s before Mg^{2+} was washed out by solution 8 (at pH 7·0 for 60 s).

Procedure 2. Following the change of pH to a desired value (solution 8 for 15 s), a test solution (Table 2) containing 0–30 μM IP_3 was applied in the absence of MgATP and hence in the absence of simultaneous Ca^{2+} uptake (test procedure).

Table 1. Composition of experimental solutions (mM)

Solution	EGTA or HEEDTA*	Ca-EGTA or Ca-HEEDTA*	$MgMs_2$	KMs	ATP	AMP	Mg^{2+}	pH
(1) G1	1	0	5·54	108·6	4·76	0	1·5	7·0
(2) Loading	0·298	0·702	5·51	108·6	4·76	0	1·5	7·0
(3) G0RMg0	0	0	0	142·1	0	0	0	7·0
(4) Assay	0	0	0	84·1	0	25	0	7·0
(5) Ryanodine	0·171	0·829	0	84·1	0	22·5	0	7·0
(6) G1R	1	0	1·54	134·5	0	0	1·5	7·0
(7) G10R†	10	0	1·90	107·1	0	0	1·5	7·0
(8) G1RMg0†	1	0	0	139·0	0	0	0	7·0
(9) Test Ca^{2+} free	10 or 10*	0	0	(see Table 2)	0	0	0	6·7–7·3
(10) Test Ca^{2+} plus	0	10 or 10*	0	(see Table 2)	0	0	0	6·7–7·3

All solutions contained 20 mM Pipes (piperazine-*N-N'*-bis(2-ethanesulphonic acid)) and 20 mM NaN_3. Solution 4 (Assay) contained 10 μM IP_3. Solution 5 (Ryanodine) contained 45 mM caffeine and 30 μM ryanodine. †Representative composition: the pH of these solutions was changed between 6·7 and 7·3, and HEEDTA was used instead of EGTA when necessary (see text). pH was adjusted at 20 °C. Ms, methanesulphonate. Mg^{2+} concentration was estimated by solving multi-equilibrium equations (see text). Total ionic strength was adjusted to 200 mM. Fura-2 and/or IP_3 were added to appropriate solutions (see text).

Table 2. Total Ca^{2+} and KMs concentrations in test solutions

	Total Ca^{2+} (mM)							KMs (mM)	
	pCa 8·0	7·5	7·0	6·5	6·0	5·5	5·0	Ca^{2+} free (soln 9)	Ca^{2+} plus (soln 10)
EGTA buffer									
pH 6·7	0·06	0·19	0·58	1·63	3·81	6·60	8·61	111·8	118·3
pH 7·0	0·24	0·71	1·96	4·34	7·09	8·85	9·61	111·8	112·0
pH 7·3	0·88	2·34	4·89	7·52	9·06	9·68	9·91	104·6	105·2
HEEDTA buffer									
pH 6·7	—	—	—	—	0·80	2·15	4·64	119·6	137·6
pH 7·0	—	—	—	—	1·51	3·60	6·40	112·7	131·4
pH 7·3	—	—	—	—	2·64	5·32	7·83	105·6	124·8

Test solutions with various pCa values were prepared by mixing solutions 9 and 10 (Table 1 with KMs concentrations shown in the right-hand columns of this table) in such a way that the total Ca^{2+} concentrations were equal to the values shown here.

The Ca^{2+} concentration of the test solutions was strongly buffered by 10 mM EGTA or HEEDTA to prevent Ca^{2+} concentration change due to Ca^{2+} release. Then, IP_3 and Ca^{2+} were washed out by solution 7 at the same pH before pH was brought back to 7·0 and the EGTA concentration was lowered to 1 mM (solution 6). When we used HEEDTA as the Ca^{2+} buffer, solutions 8 and 7 also had HEEDTA instead of EGTA.

Procedure 3. Then 35–40 μM fura-2 was introduced for 60 s in solution 6, and EGTA was removed in the continued presence of fura-2 for 60 s (solution 3). Finally, the remaining Ca^{2+} in the store was fully released by 10 μM IP_3, and the fluorescence intensity change of fura-2 was measured at pH 7·0 (assay, solution 4). The concentration of IP_3 in the assay solution was the maximum dose, and its increase to 30 μM induced no further increase in the amount of Ca^{2+} release (see Fig. 3 of Iino, 1991).

After the complete release of Ca^{2+}, procedure 3 was repeated to measure baseline intensity of fura-2 fluorescence, which was subtracted from the preceding response to obtain fluorescence intensity change due solely to Ca^{2+} release from the store (Iino, 1989, 1991). Because fura-2 has strong affinity for Ca^{2+}, most of the Ca^{2+} released from the store binds to fura-2 with 1:1 stoichiometry. Therefore, the fluorescence intensity change is proportional to the total amount of Ca^{2+} released (Iino, 1989).

The above sequence was run several times on one fibre bundle with different pH and/or different concentrations of Ca^{2+} during the test procedure. The amount of Ca^{2+} released during the test procedure was estimated by comparing the runs with the control runs as follows. The control runs were carried out in exactly the same way except for the omission of the application of the test solution containing IP_3, and the change in the pH in solutions 8 and 7 was always carried out. Usually two test runs were bracketed by a pair of control runs. If we take the amount of Ca^{2+} assayed in a test run as T, and the average of the control runs as C, the fractional amount of Ca^{2+} remaining in the store after the test application of IP_3 is expressed by $(T/C) \times 100$ % (Fig. 1) and the relative amount of Ca^{2+} released by the test application is expressed by $\{1-(T/C)\} \times 100$ % (Figs 2 and 3). Further details of the protocol are described elsewhere (Iino, 1991).

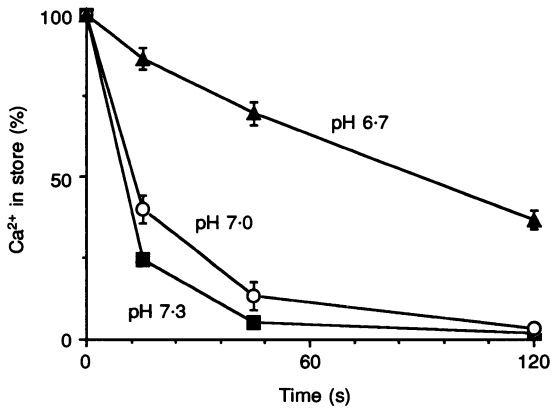

Figure 1. Increase in pH facilitated IP_3-induced Ca^{2+} release
Time course of IP_3-induced Ca^{2+} release at pH 6·7 (▲), pH 7·0 (○) and pH 7·3 (■). Relative amount of Ca^{2+} remaining in the store after application of 0·3 μM IP_3 at pCa 7 in the absence of ATP is plotted against the duration of the IP_3 application. Means ± S.E.M. ($n = 4$).

Chemicals

ATP was obtained from Boehringer Mannheim (FRG), saponin from ICN Pharmaceuticals Inc. (Cleveland, OH, USA), fura-2 from Molecular Probes, Inc. (Eugene, OR, USA). IP_3, EGTA and HEEDTA were from Dojindo Laboratories (Kumamoto, Japan). All the other chemicals were of the highest reagent grade.

RESULTS

Time course of IP_3-induced Ca^{2+} release at different pH levels

Figure 1 shows the time course of IP_3-induced Ca^{2+} release at pH 6·7, 7·0 and 7·3. The duration of the application of test solutions containing 0·3 μM IP_3 and 100 nM Ca^{2+} was varied as shown on the ordinate, and the amount of Ca^{2+} remaining in the store was plotted. The rate of Ca^{2+} release was slow at pH 6·7, and it took 90 s or more to release half of the Ca^{2+} in the store. In contrast, at pH 7·0 and 7·3 the rate of Ca^{2+} release was faster than at pH 6·7, and the half-time was less than 15 s. These results clearly show that the increase in pH results in faster release of Ca^{2+}. As is shown in Fig. 1, the effect of pH was more pronounced with the rise in pH from pH 6·7 to 7·0 than with the increase from pH 7·0 to 7·3 at pCa 7.

Dependence of Ca^{2+} release on Ca^{2+} concentration

The rate of IP_3-induced Ca^{2+} release is biphasically dependent on the cytoplasmic Ca^{2+} concentration (Iino, 1990; Bezprozvanny *et al.* 1991). We examined whether the Ca^{2+} dependence was altered by the change in pH. The amount of Ca^{2+} released by the test application of 0·3 μM IP_3 for 15 s was plotted against the Ca^{2+} concentration of the test solution at three different pH levels (Fig. 2). At pH 7·0, Ca^{2+} accelerated the IP_3-induced Ca^{2+} release in a concentration-dependent manner between pCa 7·5 and pCa 6·5. However, the increase in Ca^{2+} concentration beyond pCa 6·5 decreased the release of Ca^{2+}. Thus, the biphasic dependence of IP_3-induced Ca^{2+} release on the Ca^{2+} concentration is also observed in vascular smooth muscle cells as well as in intestinal smooth muscle cells (Iino, 1990) and in cerebellar microsomes (Bezprozvanny *et al.* 1991). Figure 2 clearly shows that the biphasic dependence on Ca^{2+} was retained at all the pH levels studied with the peak always obtained near pCa 6·5, but the rate of Ca^{2+} release was greater at higher pH.

There was a small but definite effect of pH on the Ca^{2+} sensitivity of the ascending limb of the biphasic Ca^{2+} dependence (Fig. 2*A*). At pH 7·3, Ca^{2+} significantly enhanced IP_3-induced Ca^{2+} release at pCa 8 compared with release in the absence of Ca^{2+} ($P < 0·01$, *t* test). However, no significant Ca^{2+}-dependent enhancement of the Ca^{2+} release was observed even at pCa 7·5, either at pH 7·0 or at pH 6·7 ($P > 0·4$, *t* test). Therefore, the ascending limb was shifted toward the lower Ca^{2+} concentration at the higher pH. On the other hand, there was little, if any, effect of pH in the absence of Ca^{2+}.

Ca^{2+} concentrations above 1 μM (pCa 6) are difficult to buffer adequately with EGTA at pH 7·3, because the pK_d of EGTA for Ca^{2+} is pH dependent, and is ~7 at pH 7·3. Therefore, we used another kind of Ca^{2+} buffer to study IP_3-induced Ca^{2+} release at higher Ca^{2+} concentrations. Panel *B* in Fig. 2 shows the Ca^{2+} dependence of IP_3-induced Ca^{2+} release at pH 7·3, 7·0 and 6·7 with HEEDTA as a Ca^{2+} buffer. The pK_d of this chelator is 5·5, 5·2 and 4·9 at pH 7·3, 7·0 and 6·7, respectively. It is clearly seen that the increase in Ca^{2+} concentration above 1 μM inhibited IP_3-induced Ca^{2+} release at all the pH levels, but there was no clear shift of the Ca^{2+} dependence.

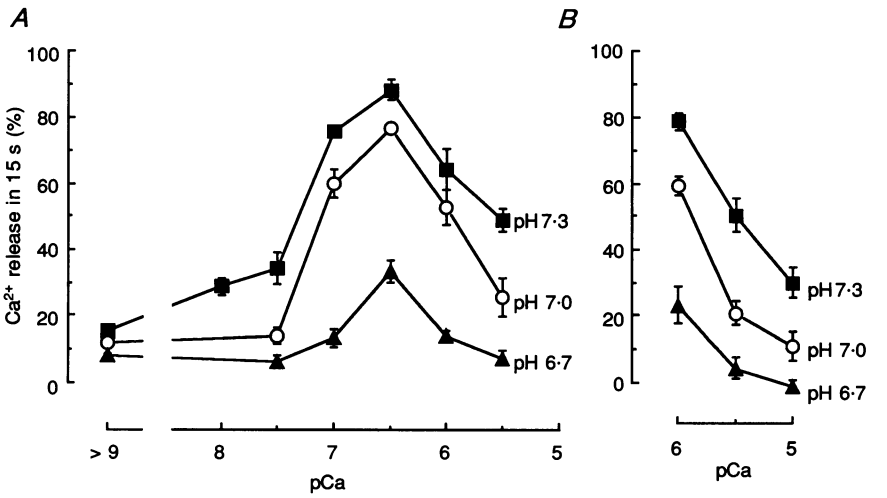

Figure 2. The peak of the biphasic Ca^{2+} dependence of IP_3-induced Ca^{2+} release was greater at higher pH, although little effect of pH was seen in the absence of Ca^{2+}

Ca^{2+} dependence of IP_3-induced Ca^{2+} release at pH values of 6·7 (▲), 7·0 (○) and 7·3 (■). Relative amount of Ca^{2+} released by 0·3 μM IP_3 in 15 s without ATP (means ± S.E.M.) is plotted against Ca^{2+} concentration. *A*, EGTA as the Ca^{2+} buffer ($n = 4$–9). *B*, HEEDTA as the Ca^{2+} buffer ($n = 4$–6).

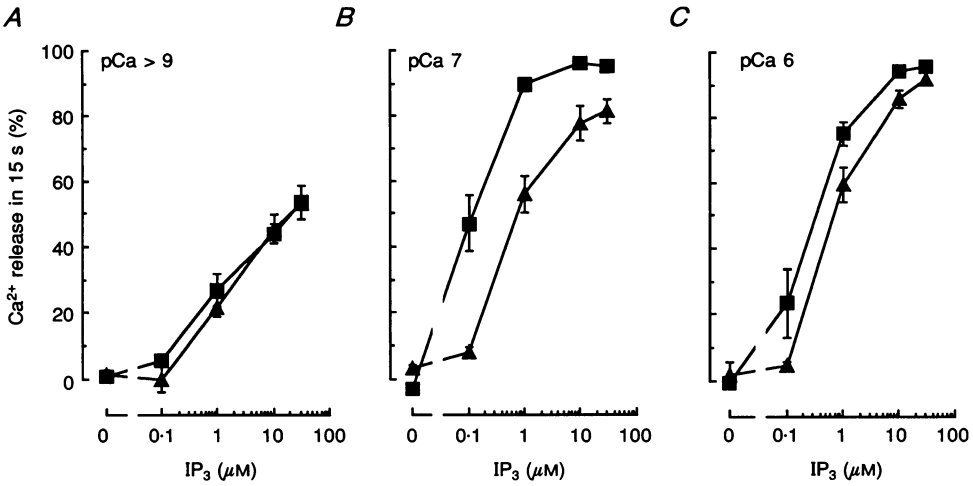

Figure 3. Effect of pH on the relationship between IP_3 concentration and Ca^{2+} release was observed only in the presence of Ca^{2+}

Relative amount of Ca^{2+} released as a function of IP_3 concentration at pH values of 6·7 (▲) and 7·3 (■). Data points were normalized by the control runs without a test procedure (means ± S.E.M.). Test IP_3 application was carried out in the absence of Ca^{2+} (*A*), at pCa 7 (*B*), and at pCa 6 (*C*) buffered with 10 mM EGTA ($n = 4$–7). Each fibre bundle was used to obtain complete IP_3 dependence at a fixed pH and pCa condition.

Dose–response relation of IP_3-induced Ca^{2+} release at high and low pH

While a significant effect of pH was observed in the presence of Ca^{2+}, there was little effect of pH in the absence of Ca^{2+} (Fig. 2*A*). We, therefore, studied the effect of pH on the dose–response relation of IP_3-induced Ca^{2+} release in the presence and absence of Ca^{2+}. The amount of Ca^{2+} released during test procedures with different concentrations of IP_3 for 15 s was plotted in Fig. 3. The dose–response relation was almost independent of pH between 6·7 and 7·3 in the absence of Ca^{2+} (Fig. 3*A*). At pCa 7, however, the relationship is significantly shifted toward the lower IP_3 concentration at pH 7·3 compared with that at pH 6·7, and about 7 times higher concentration of IP_3 was required to release half of the Ca^{2+} in the store within 15 s (Fig. 3*B*). Similar pH-dependent sensitization to IP_3 was observed at pCa 6, but the magnitude of the shift was smaller than that at pCa 7 (Fig. 3*C*). Figure 3 also shows that there is no significant effect of pH on the leak of Ca^{2+} from the store in the absence of IP_3.

DISCUSSION

The present study has demonstrated that pH is an important modulator of IP_3-induced Ca^{2+} release. We took careful measures to isolate the effects of pH from the effects of other modulating factors. If the experiments had been carried out in the presence of ATP, it would be difficult to separate the effects of pH on Ca^{2+} release from those on Ca^{2+} uptake (Watanabe, Lewis, Nakamoto, Kurzmack, Fronticelli & Inesi, 1981). It has been shown that the Ca^{2+} concentration on the cytoplasmic side of the Ca^{2+} release channels alters the rate of IP_3-induced Ca^{2+} release (Iino, 1990; Finch, Turner & Goldin, 1991; Bezprozvanny *et al.* 1991) and that Ca^{2+} released from the store exerts a feedback regulation on the rate of Ca^{2+} release (Iino & Endo, 1992). Since pH may affect the Ca^{2+}-buffering capacity, effects observed under a condition where the Ca^{2+} concentration is changing during Ca^{2+} release may not be ascribable to pH alone. To circumvent these problems we measured the amount of Ca^{2+} released upon application of IP_3 in the absence of ATP and with a high concentration of EGTA or HEEDTA to keep the ambient Ca^{2+} concentration constant. The present study, therefore, reports the first quantitative analysis of the effect of pH on IP_3-induced Ca^{2+} release.

Sensitivity of the Ca^{2+} release mechanism to IP_3 was increased at the higher pH in the presence of Ca^{2+}, but little effect was found without Ca^{2+} (Fig. 3). The IP_3 binding showed steep pH dependence above pH 7·5 but there was only weak dependence on pH around pH 7·0 in the absence of Ca^{2+} (Worley *et al.* 1987). The little effect of pH on IP_3 binding near neutral pH may correspond to the fact that very little effect of pH was found on the IP_3-induced Ca^{2+} release around pH 7·0 in the absence of Ca^{2+}. It will be interesting, therefore, to study if there is a steeper pH dependence of IP_3 binding near pH 7·0 in the presence of Ca^{2+}. The effect of pH on the IP_3 sensitivity was complex, and the difference between pH 6·7 and 7·3 became smaller at pCa 6 than at pCa 7.

The major effect of pH on the biphasic Ca^{2+} dependence of IP_3-induced Ca^{2+} release was the elevation of the rate of Ca^{2+} release at all the Ca^{2+} concentrations above pCa 7·5,

while little effect was found in the absence of Ca^{2+} (Fig. 2). Furthermore, the ascending limb of the biphasic Ca^{2+} dependence seems to be shifted toward the lower concentration of Ca^{2+} with increase in pH (Fig. 2*A*). The latter result suggests an increase in the Ca^{2+} sensitivity of IP_3-induced Ca^{2+} release at higher pH. However, as to the descending limb, we could not see a clear shift toward the lower Ca^{2+} concentration with the increase in pH.

Thus, the present study suggests that the changes in the rate of Ca^{2+} release observed with alteration in pH may involve alteration in the binding of IP_3 and Ca^{2+} to the IP_3 channels. However, a simple change in their binding may not fully explain the complex effects of pH on the Ca^{2+} release. For example, the effect of alteration in pH on the IP_3 sensitivity is observed only in the presence of Ca^{2+}. This suggests that there is a cross-talk among IP_3, Ca^{2+} and H^+ binding sites. Therefore, we may have to envisage pH-dependent step(s) between binding of IP_3 and/or Ca^{2+} and the gating of the channel. The IP_3 binding site on the IP_3 receptor protein has been shown to be present near the N-terminus (Mignery & Südhof, 1990; Miyawaki *et al.* 1991), while the channel-forming domain is predicted to reside toward the C-terminus (Furuichi *et al.* 1989; Mignery *et al.* 1990). Therefore, the ligand-binding site and the channel-forming pore are separated by a long stretch of polypeptide. A large conformational change of the IP_3 receptor proteins upon binding of IP_3 has been observed, and this has been implicated in the mechanism of the channel gating (Mignery & Südhof, 1990). It seems possible that some of the steps involved in that conformation change are pH dependent.

In summary, increase in pH enhances the rate of IP_3-induced Ca^{2+} release. The present results suggest that the pH dependence may partly involve increased affinity at higher pH for both IP_3 and Ca^{2+}, the latter of which is a positive modulator of the Ca^{2+} release mechanism. pH may also affect the step(s) between the binding of IP_3 and the gating of the channels.

The present results show that the effects of pH are greatest between 100 nM and 1 μM Ca^{2+}, concentrations which are within the physiological range of intracellular Ca^{2+} concentration, and suggest that a decrease in intracellular pH (pH_i) would tend to reduce IP_3-induced Ca^{2+} mobilization in smooth muscle cells. pH_i of smooth muscle cells may change under various conditions and has profound effects on the smooth muscle tone, although the effect of pH seems tissue specific (Wray, 1988). For example, increase in CO_2 tension resulted in a fall in the pH_i, and at the same time augmented electrically evoked contractions in detrusor muscle (Liston, Palfrey, Raimbach & Fry, 1991). On the other hand, CO_2 has a pronounced vasodilator action in cerebral circulation, and the main mechanism for this effect is regarded as being a direct action on cerebral vascular smooth muscle cells through lowering of pH_i (Kontos, 1981). The contraction of the detrusor muscle was evoked by depolarization-induced Ca^{2+} influx and IP_3 was unlikely to be involved in the Ca^{2+} regulation. Although it is not certain to what extent cerebral artery contraction depends on IP_3-induced Ca^{2+} mobilization, it seems possible that the tissue specificity of the pH effect may partly reflect different degrees of contribution of IP_3-sensitive Ca^{2+} mobilization to the Ca^{2+} regulation in various smooth muscle cells. Thus, the pH dependence of IP_3-induced Ca^{2+} release should be considered as one of the important factors that determine the intracellular Ca^{2+} rises in smooth muscle cells.

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