Lowering of the extracellular Na⁺ concentration enhances high-K⁺-induced formation of inositol phosphates in the guinea-pig ileum

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1. Formation of inositol phosphates (InsPs) was measured in cross-chopped slices or dispersed cells, isolated by collagenase treatment, of guinea-pig ileum longitudinal smooth muscle pre-labelled with [³H]inositol.
2. Elevation of the extracellular K⁺ concentration by equimolar replacement of Na⁺ induced accumulation of InsPs in the dispersed cells and in the tissue slices. These effects were blocked by neither tetrodotoxin (1 μM) nor atropine (10 μM), and were approximately additive with carbachol-induced accumulation.
3. In the tissue slices, the response to K⁺ was partially inhibited by nifedipine (10 μM) and by CdCl₂ (0.3 mM), but the carbachol-induced response was not altered.
4. Accumulation of InsPs induced by KCl-excess solution (high-K⁺ solution without Na⁺ replacement) was suppressed strongly by nifedipine and completely by CdCl₂. The response to KCl excess was approx. 40% of that to high K⁺ with Na⁺ replacement.
5. Low-NaCl solution (replacement of NaCl with equimolar sucrose) also produced InsPs, and this was not blocked by either nifedipine (10 μM) or CdCl₂ (0.3 mM).
6. The formation of InsPs by a maximally effective concentration of carbachol (1 mM) in the presence of KCl excess or low NaCl was greater than the additive effect of the two stimuli on their own. Enhancement of the carbachol-induced response by KCl excess disappeared in the presence of CdCl₂ (0.3 mM).
7. These data suggest that formation of InsPs induced by high-K⁺ solution with equimolar replacement of Na⁺ consists of two components, i.e. high-K⁺-induced inositol-phospholipid hydrolysis by Ca²⁺ entry through voltage-sensitive channels, and low-Na⁺-induced formation of InsPs, insensitive to Ca²⁺ antagonists, but that both of them do not contribute significantly to the activation of phospholipase C by muscarinic stimuli.

INTRODUCTION

Elevation of the extracellular K⁺ concentration induces the hydrolysis of inositol phospholipids in the guinea-pig ileum longitudinal smooth muscle (Jafferji & Michell, 1976*b*; Watson & Downes, 1983; Best & Bolton, 1986), as well as in some other tissues, including sympathetic ganglia (Bone & Michell, 1985), brain (Kendall & Nahorski, 1985; Habermann & Laux, 1986) and pancreatic islets (Biden *et al.*, 1987). The mechanism of this effect, however, has remained controversial.

High-K⁺-induced accumulation of InsPs in rat brain is enhanced by Bay K8644 and suppressed by PN-200-110, suggesting that Ca²⁺ entry through dihydropyridine-sensitive channels contributes to the response (Kendall & Nahorski, 1985). This observation was extended to two other main chemical classes of Ca²⁺-channel blockers, i.e. phenylalkylamines and benzothiazepines, by Zernig *et al.* (1986). Contrary to these observations, in the particulate preparation of the same tissue Habermann & Laux (1986) showed that accumulation of InsPs by K⁺ depolarization was not influenced by dihydropyridine derivatives.

In the guinea-pig ileum, we have hypothesized that high-K⁺-induced accumulation of InsPs is caused by the release of endogenous neurotransmitters such as acetylcholine from enteric neurons (Watson & Downes, 1983). On the other hand, Best & Bolton (1986) suggested that

this hydrolysis is triggered by membrane depolarization, because other conditions which also cause depolarization, e.g. the administration of ouabain, induce accumulation of InsPs. Those authors suggested that Ca²⁺ entry is not essential for the effect on inositol phospholipid hydrolysis, since the response to K⁺ was not affected by the Ca²⁺-channel blocker D600 and was only partially inhibited in the presence of Mn²⁺.

In the experiments in the guinea-pig ileum described above, K⁺ was increased by equimolar replacement of Na⁺. According to Best & Bolton (1986), however, the removal of Na⁺ from the incubation medium induces accumulation of InsPs. It is therefore likely that Na⁺ replacement influences the accumulation of InsPs induced by increased K⁺.

In the present work, we have investigated the mechanism of inositol phospholipid hydrolysis induced by high K⁺ and low Na⁺, with special regard to the dependence on voltage-sensitive Ca²⁺ entry. The role of Ca²⁺ entry on the accumulation of InsPs induced by carbachol has also been discussed.

METHODS

Preparation of [³H]inositol-labelled tissue slices or dispersed cells

Hartley-strain guinea pigs of either sex (300–500 g) were killed by cervical dislocation. The whole length of

the small intestine except for the duodenum and the ileocaecal region was removed into pre-gassed (O_2/CO_2 , 19:1) Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4 , 1.2; glucose, 10; pH 7.4, at room temperature. Longitudinal smooth-muscle strips were prepared essentially as described by Rang (1964).

Cross-chopped slices ($350\ \mu m \times 350\ \mu m$) of the muscle were prepared with a McIlwain tissue chopper. The slices were washed twice with 10 ml of the Krebs solution and incubated in 5 ml of Krebs solution containing $30\ \mu Ci$ of *myo*-[3H]inositol under O_2/CO_2 (19:1) for 1 h in a shaking water bath at $37^\circ C$. The prelabelled slices were washed five times with 10 ml of solution A [5 mM-Hepes/Tris buffer (pH 7.4), 135 mM-NaCl, 4 mM-KCl, 2 mM- $MgCl_2$, 2 mM- $CaCl_2$, 1 mM- KH_2PO_4 and 12 mM-glucose].

To prepare dispersed cells, longitudinal muscle strips dissected as described above were incubated in 40 ml of solution B [5 mM-Hepes/Tris buffer (pH 7.4), 135 mM-NaCl, 4 mM-KCl, 1 mM- KH_2PO_4 and 12 mM-glucose] for 1 h at $37^\circ C$, with oxygenation with 100% O_2 . The tissue strips were then dispersed into isolated cells by incubating in 10 ml of solution B containing collagenase (2 mg/ml), trypsin inhibitor (0.5 mg/ml) and bovine serum albumin (1 mg/ml), for 1 h at $37^\circ C$; the tissue was gently agitated with a polyethylene pipette at intervals of 10 min during this period. After addition of 40 ml of solution A, the suspension was filtered through a nylon gauze (mesh size $250\ \mu m$) on which the undigested residue was trapped. Dispersed cells were collected by centrifugation at $30 g$ for 5 min, followed by washing once with 50 ml of solution A. The cells were labelled with $30\ \mu Ci$ of *myo*-[3H]inositol in 2.5 ml of solution A for 1 h at $37^\circ C$. After one washing with 50 ml of solution A, prelabelled cells were suspended in 2.5 ml of solution A. Approx. 10^7 cells were obtained from one animal. They appeared morphologically homogeneous, and few non-muscle cells could be seen under a microscope. The cell viability, as assessed by the Trypan Blue exclusion test, was over 90%.

Assay of inositol phosphates

All the reactions were performed in $250\ \mu l$ volumes. At zero time, $35\ \mu l$ portions of the tissue or cell suspension were transferred to plastic tubes containing LiCl (11.6 mM) dissolved in $215\ \mu l$ of solution A. The concentrations of NaCl and/or KCl were changed, and other reagents were added, as indicated in the Results section. The tubes were gassed and incubated in a shaking water bath at $37^\circ C$ for 30 min, followed by addition of $940\ \mu l$ of chloroform/methanol/12 M-HCl (50:100:1, by vol.) to terminate the reactions. [3H]InsPs were extracted essentially as described by Berridge *et al.* (1982). After chloroform ($310\ \mu l$) and water ($310\ \mu l$) were added, the samples were vortex-mixed and the phases were separated by centrifugation at $1000 g$ for 5 min. A portion of the upper aqueous phase ($950\ \mu l$) was applied to a glass column containing 1 ml of an approx. 1:1 (w/v) slurry of Dowex AG 1-X8 resin (100–200 mesh, formate form) and water. After washing with 16 ml of water, [3H]glycerophosphoinositol was removed with 16 ml of 5 mM-sodium tetraborate/60 mM-ammonium formate. Total [3H]InsPs (inositol mono-, bis- and trisphosphates) were eluted with 6 ml of 0.8 M-ammonium formate/0.1 M-formic acid. The eluate was collected in scintillation vials and counted in the gel phase for

radioactivity after addition of 10 ml of Liquiscint. Data were normalized by the radioactivities in total lipid (lower organic phase of the tube described above), and are shown as percentages of the control (Watson & Downes, 1983).

Preliminary experiments revealed that formation of InsPs induced by carbachol or high K^+ (with equimolar replacement of Na^+) was approximately linear throughout the 30 min, and that no inositol tetrakisphosphate was detected by elution with 6 ml of 1.2 M-ammonium formate/0.1 M-formic acid (Batty *et al.*, 1985). The combined amounts of inositol mono-, bis- and trisphosphates were measured rather than the individual phosphates, because, although inositol trisphosphate formed a detectable peak at 15 s after stimulation with 0.1 mM-carbachol ($122 \pm 8.7\%$ relative to basal; $n = 7$) or 112 mM- K^+ with equimolar replacement of Na^+ ($117 \pm 3.0\%$; $n = 4$) in dispersed cells, these increases appeared to be too small to be used as the parameters. After a 30 min incubation with excess KCl, low NaCl or carbachol, more than 90% of the stimulated increase in InsPs could be found in the inositol monophosphate fraction.

Chemicals

myo-[2- 3H]inositol (17.9 Ci/mmol) was purchased from Amersham International and cleaned before use by passage through a column of Dowex-1 resin (formate form). Collagenase from *Clostridium histolyticum* (0.44 unit/mg) was purchased from Boehringer Mannheim. Other compounds were purchased from Sigma.

RESULTS

Comparison of the accumulation of InsPs in dispersed cells and in tissue slices

Carbachol induced accumulation of InsPs in the collagenase-treated single-cell preparation in a concentration-dependent fashion, with an EC_{50} of $11.2\ \mu M$ (Fig. 1a). The response to 0.1 mM-carbachol was completely blocked by $10\ \mu M$ -atropine (Fig. 1a). The maximal response to carbachol in the dispersed cells was $640 \pm 56\%$ of the control (mean $\pm$ S.E.M.; $n = 3$), which is similar to the maximal response observed in the tissue slices ($555 \pm 11\%$; $n = 3$). This suggests that the dispersal of the longitudinal muscle cells by collagenase treatment maintains cell viability.

The effect of changing ambient K^+ concentration on accumulation of InsPs was investigated in the dispersed cells by replacement of equimolar Na^+ (Fig. 1b). Lowering the K^+ concentration from 5 mM (normal concentration) to 0.5 mM had no effect on accumulation of InsPs. Higher concentrations of K^+ resulted in a concentration-dependent increase in InsPs, with a response of $309 \pm 32\%$ to 112 mM- K^+ . The response to 112 mM- K^+ was not altered significantly in the presence of atropine ($10\ \mu M$) or tetrodotoxin ($1\ \mu M$) (Table 1).

The response to carbachol ($10\ \mu M$ and 0.1 mM) was approximately additive with the high- K^+ -induced response for each K^+ concentration tested (Fig. 1b). Atropine ($10\ \mu M$) suppressed the elevation induced by carbachol (0.1 mM) in the presence of 112 mM- K^+ to $332 \pm 16\%$ ($n = 3$); this value is not significantly different from the response to 112 mM- K^+ (Table 1).

The response to high K^+ (112 mM) in the tissue slices

Fig. 1. Formation of InsPs induced by carbachol and high K⁺ with replacement of Na⁺ in the single cells

(a) The dispersed cells labelled with [³H]inositol were stimulated by the indicated concentration of carbachol in the presence of 5 mM-Hepes/Tris buffer (pH 7.4), 135 mM-NaCl, 4 mM-KCl, 2 mM-MgCl₂, 2 mM-CaCl₂, 1 mM-KH₂PO₄, 12 mM-glucose and 10 mM-LiCl for 30 min at 37 °C. ○, No atropine; ●, 10 μM-atropine. (b) The cells were incubated at various concentrations of K⁺. K⁺ concentration was lowered by decreasing KCl concentration, in the presence of 1 mM-NaH₂PO₄ instead of 1 mM-KH₂PO₄. Higher concentration of K⁺ was obtained by the addition of KCl replacing equimolar NaCl. ○, No carbachol; △, 10 μM-carbachol; □, 0.1 mM-carbachol. Vertical bars indicate S.E.M. of three separate experiments in quadruplicate.

agrees well with the response observed with the dispersed cells (Table 1). This response was not altered significantly in the presence of atropine or tetrodotoxin (Table 1), suggesting that the response is not mediated through the release of acetylcholine and is not dependent on axonal excitation leading to the release of other transmitters. We therefore used tissue slices rather than dispersed cells in the following experiments.

Accumulation of InsPs caused by high K⁺ with Na⁺ replacement

To investigate the role of Ca²⁺ entry in high-K⁺-induced accumulation of InsPs, the effects of nifedipine and CdCl₂ were observed (Fig. 2). For the high-K⁺

Table 1. Effect of atropine or tetrodotoxin on the accumulation of InsPs induced by high K⁺ with Na⁺ replacement

The isolated cells or the tissue slices pre-labelled with [³H]inositol were incubated in normal-K⁺ (5 mM) or high-K⁺ (112 mM) solution in the presence or absence of atropine or tetrodotoxin. Na⁺ concentration was lowered to 28 mM in the 112 mM-K⁺ solution. Results are means ± S.E.M. of three experiments in triplicate.

	[³ H]Inositol phosphates (% of control)	
	Dispersed cells	Tissue slices
Control	100	100
Atropine (10 μM)	96.0 ± 4.0	97.5 ± 2.5
Tetrodotoxin (1 μM)	98.8 ± 1.4	91.0 ± 8.3
K ⁺ (112 mM)	309 ± 32	357 ± 8.5
K ⁺ (112 mM) + atropine (10 μM)	309 ± 18	360 ± 12.4
K ⁺ (112 mM) + tetrodotoxin (1 μM)	317 ± 9.3	341 ± 1.6

solutions used here, K⁺ replaced equimolar Na⁺. Nifedipine (10 μM) decreased the resting amount of InsPs (K⁺ = 5 mM) by 5.6 ± 1.2% (*P* < 0.05) and inhibited the response to high K⁺ (56 mM or 112 mM) by 45.3% or 43.6% respectively (Fig. 2a). CdCl₂ (0.3 mM) increased the resting amounts of InsPs by 16 ± 5.5% (*P* < 0.05), but inhibited the high-K⁺ (56 mM or 112 mM)-induced response by 61.6% or 49.6% respectively (Fig. 2a). On the other hand, the response to 0.1 mM-carbachol (445 ± 7.4% relative to basal; *n* = 3), was not altered in the presence of 10 μM-nifedipine (432 ± 5.7%), but was enhanced by approx. 20% in the presence of 0.3 mM-CdCl₂ (524 ± 11.4%). The accumulation of InsPs induced by carbachol at very early times, i.e. 15, 30 or 60 s, was also not decreased by nifedipine or CdCl₂ (results not shown).

These data show that, although the entrance of Ca²⁺ through voltage-sensitive Ca²⁺ channels accounts partially for the accumulation of InsPs induced by high K⁺ with Na⁺ replacement, it does not contribute significantly to the carbachol-induced response.

Accumulation of InsPs caused by KCl excess

Best & Bolton (1986) showed that Na⁺-free medium stimulated accumulation of InsPs in guinea-pig ileum. To remove the influence of the lower concentration of Na⁺ from high-K⁺-induced accumulation of InsPs, the effect of KCl excess, i.e. a simple addition of KCl without replacing NaCl, was examined (Fig. 2b).

The accumulation of InsPs increased as the KCl concentration was elevated. However, the maximal response to KCl excess was approx. 40% of that observed after K⁺ was increased through Na⁺ replacement (compare Figs. 2a and 2b). To evaluate the influence of the increase of osmolarity caused by addition of KCl, the effect of NaCl excess was investigated. In the solution containing 242 mM-NaCl (107 mM above normal), the osmolarity of which corresponds to that of the solution containing 112 mM-KCl, the amount of InsPs was decreased to 11.3 ± 2.7% (*P* < 0.05) below basal value (Fig. 2b).

Fig. 2. Effect of nifedipine or CdCl₂ on the formation of InsPs induced by high K⁺ with or without replacement of Na⁺

(a) The tissue slices pre-labelled with [³H]inositol were incubated in the solution containing various concentrations of K⁺. K⁺ concentration was raised by the addition of KCl replacing equimolar NaCl. ○, No Ca²⁺-channel blocker; ▲, 10 μM-nifedipine; ■, 0.3 mM-CdCl₂. (b) The abscissa indicates K⁺ concentration, except for closed circles (●). K⁺ concentration was raised by the addition of KCl without replacement of NaCl. ○, No Ca²⁺-channel blocker; ▲, 10 μM-nifedipine; ■, 0.3 mM-CdCl₂. Closed circles (●) show the addition of excess NaCl, which gives the same osmolarity as KCl excess on the same abscissa. Bars indicate S.E.M. of three experiments in triplicate.

Nifedipine (10 μM) inhibited KCl-excess-induced accumulation of InsPs to nearly basal values, although slight elevations still remained at K⁺ concentrations over 56 mM (Fig. 2b). In the presence of 0.3 mM-CdCl₂, the stimulatory effect of KCl excess on the accumulation of InsPs disappeared completely (Fig. 2b).

Effect of low NaCl on accumulation of InsPs

To investigate whether the replacement of Na⁺ by K⁺ contributes to inositol phospholipid metabolism, the effect of lowered concentrations of Na⁺ was studied by

Fig. 3. Effect of nifedipine or CdCl₂ on the formation of InsPs induced by low NaCl

The abscissa indicates the concentration of sucrose added to maintain the osmolarity, to facilitate comparison of this Figure with Fig. 2(a). Each Na⁺ concentration used here (135, 115, 84 or 28 mM) corresponds to that in the 5 mM-, 25 mM-, 56 mM- or 112 mM-K⁺ solution in Fig. 2(a) respectively. ○, No Ca²⁺-channel blocker; ▲, 10 μM-nifedipine; ■, 0.3 mM-CdCl₂. Vertical bars show S.E.M. of three experiments in triplicate.

Table 2. Additive effects of various stimuli on the carbachol-induced response

The tissue slices labelled with [³H]inositol were incubated under various conditions in the absence or presence of 1 mM-carbachol. Results show means ± S.E.M. of three experiments in triplicate.

	[³ H]Inositol phosphates (% of control)	
	No addition	Carbachol (1 mM)
Control	100	555 ± 11.0
K ⁺ (112 mM) with Na ⁺ replacement	360 ± 2.9	1059 ± 54.3
KCl excess (112 mM)	209 ± 3.2	774 ± 6.9
Low NaCl (28 mM)	148 ± 7.6	645 ± 6.9
Ionomycin (1 μM)	224 ± 3.2	764 ± 9.3

using low-NaCl solutions in which iso-osmotic sucrose was substituted for NaCl. Each Na⁺ concentration used in Fig. 3 (135, 115, 84 or 28 mM) corresponds to that in the 5 mM-, 25 mM-, 56 mM- or 112 mM-K⁺ solutions in Fig. 2(a) respectively.

The amounts of InsPs increased as the Na⁺ concentration was decreased. Simple addition of 214 mM-sucrose, the same concentration of sucrose as in the 28 mM-Na⁺ solution in Fig. 3, without decreasing NaCl, had no significant effect on the amount of InsPs (90.3 ± 4.5%; *n* = 3).

Nifedipine (10 μM) did not affect low-NaCl-induced accumulation of InsPs (Fig. 3). CdCl₂ (0.3 mM), which increased basal InsPs by 20 ± 4.5% (*P* < 0.05) on its

own, had an approximately additive effect with each concentration of Na⁺ tested (Fig. 3). Therefore the entry of Ca²⁺ through voltage-sensitive channels does not appear to be required for this effect.

Effect of KCl excess or low NaCl on the carbachol-induced accumulation of InsPs

The effect of KCl excess or low NaCl on carbachol-induced accumulation of InsPs was examined in order to investigate the mechanism underlying the carbachol-induced response. As shown in Table 2, KCl excess, low NaCl and high K⁺ (with substitution for Na⁺) all enhanced the carbachol-induced response, with the total increase being approximately equal to the sum of the individual responses. In the presence of CdCl₂ (0.3 mM), the enhancement of carbachol (0.1 mM)-induced response by KCl excess disappeared (results not shown).

Effect of Ca²⁺ ionophore on accumulation of InsPs

Since the entry of extracellular Ca²⁺ appears to be required for inositol phospholipid hydrolysis in the KCl-excess solution, the effect of the Ca²⁺ ionophore ionomycin on accumulation of InsPs was examined. Ionomycin (0.1–10 μ M) induced formation of InsPs in a concentration-dependent manner, with a maximal increase of $323 \pm 20\%$ and an EC₅₀ of 0.81 μ M. The response to ionomycin (1 μ M) was approximately additive with that to carbachol (1 mM), as shown in Table 2.

Ionomycin (1 μ M)-induced accumulation of InsPs was not inhibited by the cyclo-oxygenase inhibitor indomethacin ($211 \pm 14.4\%$ or $225 \pm 13.5\%$ in the absence or presence of 10 μ M-indomethacin respectively; $n = 3$). Similarly, indomethacin had no effect on the response to high K⁺ (112 mM) with Na⁺ replacement ($367 \pm 12.7\%$ or $394 \pm 17.6\%$ in the absence or presence of 10 μ M-indomethacin respectively; $n = 3$). Indomethacin (10 μ M) did not influence the resting amount of InsPs on its own ($104 \pm 3.1\%$; $n = 3$). Preincubation (30 min) with aspirin (1 mM), another cyclo-oxygenase inhibitor, also failed to block the response to ionomycin (1 μ M) and high K⁺ (112 mM) with Na⁺ replacement (results not shown).

Since Ca²⁺-ionophore-induced accumulation of InsPs was also observed in the dispersed cells (results not shown), this effect is unlikely to be a result of neurotransmitter release caused by Ca²⁺ mobilization.

DISCUSSION

Mechanism of inositol phospholipid hydrolysis by high K⁺ and low Na⁺

The present paper shows clearly that high-K⁺-induced inositol phospholipid hydrolysis in the guinea-pig ileum smooth muscle is attributable to voltage-sensitive Ca²⁺ entry. In experiments involving the substitution of K⁺ for equimolar Na⁺, the formation of InsPs was inhibited partially by nifedipine or CdCl₂. In contrast, when the K⁺ concentration was elevated without lowering the Na⁺ concentration, the response was almost fully inhibited by nifedipine and blocked completely by CdCl₂. The explanation for the difference in these two experiments appears to be related to the ability of low-Na⁺ medium to induce the hydrolysis of inositol phospholipids also. This was confirmed in studies in which the Na⁺ concentration was lowered without a

concomitant elevation of the K⁺ concentration (sucrose was added to maintain osmolarity). The response to high K⁺ with equimolar replacement of Na⁺ therefore appears to consist of two components which are approximately additive, i.e. high-K⁺-induced inositol phospholipid hydrolysis, mediated by Ca²⁺ entry through voltage-sensitive channels, and low-Na⁺-induced inositol phospholipid hydrolysis, independent of voltage-operated Ca²⁺ entry. This conclusion also explains why Mn²⁺ only partially inhibited the formation of InsPs induced by high K⁺ with Na⁺ replacement (Best & Bolton, 1986). The observation that D600 has no significant effect on the formation of InsPs by high K⁺ (Best & Bolton, 1986) is in contrast with the present results with nifedipine; the explanation for this discrepancy is unclear, although Best & Bolton (1986) did in fact observe a decrease of 28% in the response to high K⁺ with equimolar Na⁺ substitution in the presence of D600, but this did not reach statistical significance.

Best & Bolton (1986) listed two other possible mechanisms by which high K⁺ might stimulate inositol phospholipid hydrolysis, i.e. the release of neurotransmitters and membrane depolarization. It is unlikely that the release of neurotransmitters plays a significant role, however, because the formation of inositol phosphates induced by high K⁺ was similar in the dispersed cells, which removes close synaptic contact, to that obtained with the cross-chopped slices. This is further supported by the lack of effect of tetrodotoxin or atropine on the response to high K⁺. There remains the possibility that the release of transmitters other than acetylcholine may contribute to this response, but this seems unlikely, since acetylcholine is the major excitatory transmitter in the ileum smooth muscle. The response to high K⁺ is not a direct effect of membrane depolarization, because Ca²⁺-channel blockers inhibit the formation of InsPs by high K⁺, but have little effect on membrane potential.

The mechanism of low-NaCl-induced formation of InsPs appears to be unrelated to Ca²⁺ entry through voltage-sensitive channels, because the response was not altered significantly in the presence of nifedipine or CdCl₂, despite the observation that low NaCl, with sucrose replacement, causes a small elevation of resting membrane potential (Bolton, 1972). The response to low NaCl could be accounted for if we postulate the existence of Na⁺/Ca²⁺ exchange leading to an increase in intracellular Ca²⁺. Brading (1978) has shown that lowering the extracellular Na⁺ concentration induces contraction of the guinea-pig taenia coli, and suggested the involvement of a Na⁺/Ca²⁺ exchange mechanism in this response. It is not known whether Na⁺/Ca²⁺ exchange also occurs in the ileum.

The possibility that increased intracellular Ca²⁺ may activate phospholipase C in the guinea-pig ileum is supported by the studies with the Ca²⁺ ionophore ionomycin, which stimulated a marked increase in InsPs similar to that seen with high K⁺ or low Na⁺. This result, however, contradicts that of Best & Bolton (1986), who showed that the Ca²⁺ ionophore A23187 or the Ca²⁺-channel activator CGP28392 did not increase InsPs. The explanation for this discrepancy is not known.

Role of Ca²⁺ entry in carbachol-induced accumulation of InsPs

Since the Ca²⁺-channel antagonists nifedipine and CdCl₂ had no apparent effect on the carbachol-induced

formation of InsPs, thereby confirming the observation by Jafferji & Michell (1976a), it appears that Ca^{2+} entry through voltage-sensitive channels does not contribute significantly to the response to carbachol, although carbachol causes depolarization in this tissue (Bolton, 1972). This is supported by the observation that the responses to maximally effective concentrations of carbachol and KCl excess are approximately additive, thereby implying that these two stimuli induce formation of InsPs by distinct mechanisms.

The formation of InsPs mediated by muscarinic receptor is thought to involve the activation of a guanine nucleotide-binding protein (Evans *et al.*, 1985; Sasaguri *et al.*, 1985, 1986), and this process may not require an increase in intracellular Ca^{2+} (Sasaguri *et al.*, 1985). The conclusion, however, that Ca^{2+} entry through voltage-sensitive channels does not contribute to this response is surprising, given that carbachol has been shown to cause a transient mobilization of intracellular Ca^{2+} in this tissue (Himpens & Casteels, 1987), and that Ca^{2+} mobilization in itself may be able to produce InsPs, as shown in the present study. It may be that carbachol does not increase intracellular Ca^{2+} to a sufficient amount or for a sufficient length of time to activate phospholipase C by this route. In contrast, KCl excess may cause a much larger or a much longer increase in intracellular Ca^{2+} , which is sufficient to bring about the activation of phospholipase C. Work is needed to answer the important question, i.e. whether the hydrolysis of inositol phospholipid by KCl excess, low NaCl or carbachol contributes to the development of contractile force in these conditions.

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