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Cytoplasmic Ca^{2+} Signals Evoked by Activation of Cholecystokinin Receptors: Ca^{2+}-Dependent Current Recording in Internally Perfused Pancreatic Acinar Cells

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Summary. The effects on the cytosolic Ca^{2+} concentration of activating cholecystokinin receptors on single mouse pancreatic acinar cells have been investigated using patch-clamp whole-cell recording of Ca^{2+}-dependent Cl^- current. We used the nonsulphated octapeptide of cholecystokinin (CCK8-NS) since the effects of even high concentrations were rapidly reversible which was not the case for the sulphated octapeptide. A submaximal concentration of CCK8-NS (10 nM) evoked a current response consisting of short-lasting (a few seconds) spikes, and some of these spikes were seen to trigger larger and longer (about half a minute) current pulses. At a higher concentration (100 nM) CCK8-NS evoked smooth and sustained responses. The effect of CCK8-NS was almost abolished when the internal perfusion solution contained a high concentration of the Ca^{2+} chelator EGTA (5 mM). The responses evoked by CCK8-NS were independent of the presence of Ca^{2+} in the external solution at least for the first 5 min of stimulation. Internal perfusion with GTP-γ-S markedly potentiated the effect of CCK8-NS or at a higher concentration itself induced responses very similar to those normally evoked by CCK8-NS. Caffeine added to the external solution at a low concentration (0.2–1 mM) enhanced weak CCK8-NS responses, whereas high caffeine concentrations always inhibited the CCK8-NS-evoked responses. These inhibitory caffeine effects were quickly reversible. Forskolin evoked a similar inhibitory effect. Intracellular heparin (200 μg/ml) infusion markedly inhibited the response to CCK8-NS stimulation. We conclude that the primary effect of activating CCK receptors is to induce inositoltrisphosphate (IP_3) production. IP_3 evokes a small and steady Ca^{2+} release, and this in turn evokes pulsatile release of a larger magnitude from a caffeine-sensitive Ca^{2+} pool. The action of CCK is thus very similar to that previously established for muscarinic receptor activation in the same cells. Nevertheless, the pattern of the cytosolic Ca^{2+} fluctuations are different, and the basic process of Ca^{2+}-induced Ca^{2+} release and Ca^{2+} signal spreading must therefore be modulated by a messenger yet unknown.

Key Words cholecystokinin · Ca^{2-} signal · caffeine · heparin · G protein

Introduction

Neurotransmitters and hormones often evoke rises in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) seen as repetitive spikes (Berridge & Irvine, 1989; Petersen & Wakui, 1990). In pancreatic acinar cells it has been shown that activation of muscarinic (Yule & Gallacher, 1988) cholecystokinin (CCK) and bombesin (Pralong, Wollheim & Bruzzone, 1988) receptors evoke fluctuations in $[Ca^{2+}]_i$.

The mechanisms underlying the acetylcholine (ACh)-evoked cytoplasmic Ca^{2+} spiking has been investigated in some detail in studies on single internally perfused pancreatic acinar cells in which $[Ca^{2+}]_i$ has been assessed either by microfluorimetry using the fluorescent probe fura-2 and/or by measurement of the Ca^{2+}-dependent Cl^- current in the patch-clamp whole-cell current recording configuration (Osipchuk et al., 1990). The ACh-evoked cytoplasmic Ca^{2+} spikes in the pancreatic acinar cells are mediated by the Ca^{2+}-releasing messenger inositol (1,4,5) trisphosphate (IP_3) (Streb et al., 1983) since intracellular IP_3 infusion can evoke repetitive Ca^{2+} spikes (Wakui, Potter & Petersen, 1989; Osipchuk et al., 1990), and the effect of ACh can be blocked by intracellular infusion of the IP_3 antagonist heparin (Wakui, Osipchuk & Petersen, 1990). The effect of intracellular IP_3 infusion can be mimicked by intracellular Ca^{2+} infusion (Osipchuk et al., 1990; Wakui et al., 1990), but whereas the Ca^{2+} spiking evoked by IP_3 infusion can be blocked by

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internal heparin application, this is not the case for the spiking induced directly by Ca^{2+} (Wakui et al., 1990). Caffeine has also been shown to evoke Ca^{2+} spiking when applied during subthreshold intracellular Ca^{2+} infusion (Wakui et al., 1990). These results indicate that ACh-evoked Ca^{2+} oscillations are due to repetitive pulses of Ca^{2+} release through a caffeine-sensitive channel in an internal store triggered by a small and steady IP_3-evoked Ca^{2+} flow through a heparin-sensitive channel in another internal store (Wakui et al., 1990) in accordance with the quantitative two-pool model (Goldbeter, Dupont & Berridge, 1990; Dupont, Berridge & Goldbeter, 1991).

In contrast to the relatively clear results of the investigations into the ACh-evoked Ca^{2+}-spiking mechanisms in pancreatic acinar cells the information about the action of CCK is more confusing. It has not been possible to demonstrate IP_3 production after stimulation with low (physiological) concentrations of CCK or the CCK analogue CCK-JMV-180 which evoke fluctuations in $[Ca^{2+}]_i$ (Matozaki et al., 1990; Rowley et al., 1990), and it has been suggested that release of intracellular Ca^{2+} in this case is mediated by an as yet unidentified messenger (Saluja, Powers & Steer, 1989). On the other hand, the original work of Streb et al. (1985) had shown a good correlation between CCK-evoked Ca^{2+} release and IP_3 production in permeabilized pancreatic acinar cells.

It is known that the pattern of cytoplasmic Ca^{2+} fluctuations evoked by CCK is different from that induced by ACh (Osipchuk et al., 1990; Yule, Lawrie & Gallacher, 1991), and since it has recently been shown that various stimuli not working via IP_3 production can induce regular cytoplasmic Ca^{2+} oscillations in liver cells (Capiod et al., 1991*a*,*b*) CCK could evoke cytoplasmic Ca^{2+} signals via a mechanism separate from that involved in the ACh effect. Nevertheless Petersen, Toescu and Petersen (1991*a*) showed that the CCK-evoked Ca^{2+}-oscillation pattern was a variant of that induced by ACh, and on the basis of experiments demonstrating marked Ca^{2+}-signal pattern variations with different intracellular Ca^{2+} buffer composition proposed that ACh and CCK did not act by separate mechanisms, but that there might be a messenger(s) other than IP_3 involved in regulation of the spreading of the Ca^{2+} signal and that CCK might generate more of this unknown factor than ACh.

The present work was undertaken to investigate the mechanisms underlying the action of CCK. In order to gain direct access to the cell interior we used internally perfused single acinar cells and monitored $[Ca^{2+}]_i$ by measurement of the Ca^{2+}-dependent current. Our results show that CCK-evoked Ca^{2+} signals are generated by a mechanism that is not fundamentally different from that involved in the ACh-evoked signals.

Materials and Methods

Materials

Mice were obtained from Bantin & Kingman (Hull). Collagenase (high purity) was from Worthington. Cholecystokinin octapeptide (sulphated and nonsulphated), acetylcholine, caffeine, EGTA, heparin (Na-salt, mol. wt. = 3000) were from Sigma. Guanosine 5′-[γ-thio] triphosphate (GTP-γ-S) was from Boehringer. Theophylline was from B.D.H. All other reagents were of analytical grade.

Methods

Fragments of mouse pancreas were digested by pure collagenase, washed and pipetted to produce single acinar cells as previously described (Wakui et al., 1989, 1990). The tight-seal, whole-cell current configuration of the patch-clamp technique was used for the measurement of the transmembrane current from single cells (Hamill et al., 1981; Marty & Neher, 1983) as previously described in detail for studies on pancreatic acinar cells (Jauch, Petersen & Lauger, 1986). In some experiments internal perfusion of the pipette tip via a thin polythene tube was used (Osipchuk et al., 1990; Wakui et al., 1990). The Ca^{2+}-dependent Cl^- currents were measured with the two-voltage pulse protocol as described by Wakui et al. (1989, 1990) in which acinar cells were voltage clamped at a holding potential of −30 mV and depolarizing voltage jumps of 100-msec duration to a membrane potential of 0 mV were applied repeatedly throughout the experiments. The control extracellular solution contained in (mM): NaCl 140, KCl 4.7, $CaCl_2$ 1.0, MgCl 1.13, HEPES 10 (pH 7.2) and glucose 10. In some cases no $CaCl_2$ was present and 0.5–1 mM EGTA was added (Ca^{2+}-free extracellular solution). The cell under investigation was continuously exposed to a flow of control solution or control solution containing cholecystokinin octapeptide, nonsulphated (CCK). The control intracellular pipette solution contained (in mM): KCl 140, Na_2ATP 5, MgCl 1.13, glucose 10 and HEPES 10 (pH 7.2). The pipette solution normally contained 0.25 mM EGTA, but in some experiments EGTA was present in a concentration of 5 mM. All experiments were carried out at room temperature.

Results

The Effects of Nonsulphated and Sulphated Cholecystokinin Octapeptide

In preliminary experiments using sulphated CCK octapeptide (CCK8) we established that it was necessary to use a 100-pM concentration in order to obtain a sustained increase in Ca^{2+}-dependent current. The effect was, however, not immediately

reversible since oscillatory currents (mixtures of spikes and slow waves) were still observed several minutes after removal of the hormone. The nonsulphated cholecystokinin octapeptide (CCK8-NS) was in this respect easier to use since the sustained effect of a 100-nM concentration was almost immediately reversible upon removal of the peptide (Fig. 1). It is known from receptor-binding studies on isolated pancreatic acinar cells that the concentration of CCK8-NS needed to displace 50% of the bound ^{125}I-Bolton-Hunter-CCK8 is close to 1000 times higher than that of CCK8 itself (Yu et al., 1990). Our data are in agreement with these receptor-binding studies since the effect of 100 nM CCK8-NS corresponded to that of 100 pM CCK8. The effects of 10 nM CCK8-NS shown in Fig. 1 are also very similar to those of 10 pM CCK8 previously described (Petersen et al., 1991*a*). The lowest concentration of CCK8-NS tried was 1 nM. Only one out of the five cells tested responded to this low concentration. In another five cells tested with 1 pM CCK8 no responses were found. These results are in agreement with those of Petersen et al. (1991*a*) who reported that 5 pM CCK8 was a threshold concentration for evoking fluctuations in the Ca^{2+}-dependent Cl^- current. We therefore decided to use 10 nM CCK8-NS as the standard concentration for evoking fluctuating responses and 100 nM as the standard concentration for evoking sustained responses.

Figure 1 shows responses to stimulation with 10 nM CCK8-NS. These responses are typical for the more than 20 experiments carried out. The responses consisted of a mixture of narrow free-standing spikes and spikes followed by broader pulses of currents (waves). The duration of the spikes was about 3–8 sec, whereas the broader waves lasted about 20–30 sec. The wave frequency was about 0.5 to 2 per min, and there were normally two to five spikes between waves. The amplitude of the waves was normally larger than that of the spikes (Fig. 1). This response pattern is virtually identical to that obtained with 10 pM CCK8 in the study of Petersen et al. (1991*a*). There were a few cases where the spikes between the waves were missing (*see also* Fig. 5 in Osipchuk et al., 1990).

In all the seven experiments where 100 nM CCK8-NS was tested, sustained responses of the type shown in Fig. 1*b* were obtained. In four experiments where a relatively high concentration of EGTA (5 mM) was present in the pipette solution, 100 nM CCK8-NS only evoked a very small and entirely transient response (Fig. 1*c*).

The fluctuating current responses evoked by 10 nM CCK8-NS were not acutely dependent on the presence of Ca^{2+} in the extracellular solution since

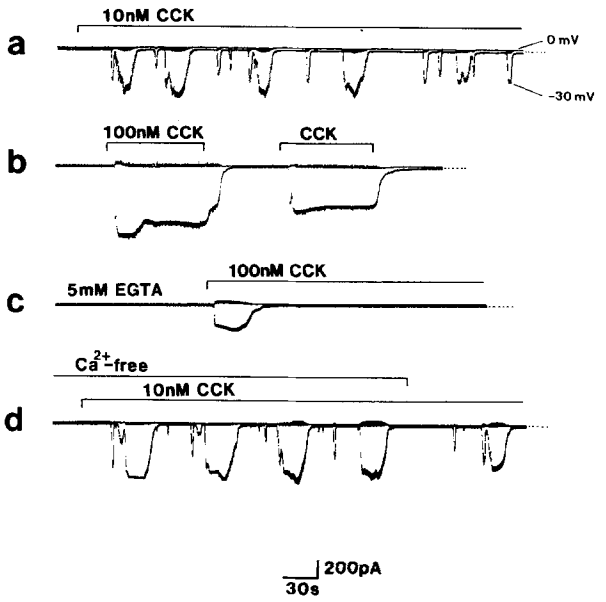

Fig. 1. The effects of CCK8-NS (CCK) on transmembrane current in single internally perfused mouse pancreatic acinar cells. At a membrane potential of −30 mV CCK8-NS evokes inward currents, whereas there is virtually no current flow at 0 mV. (*a*) Typical trace showing pattern of the Ca^{2+}-dependent Cl^- current response to 10 nM CCK8-NS. (*b*) Typical responses to 100 nM CCK8-NS. (*c*) This cell was internally perfused with an intracellular solution containing 5 mM EGTA, and 100 nM CCK8-NS only evoked a brief and small response. (*d*) At the start of this experiment the cell was exposed to an external solution containing no Ca^{2+}, but 10 nM CCK8-NS evoked its usual effect. Readmission of external Ca^{2+} did not produce any effect

very similar responses could be seen in the six experiments with Ca^{2+}-free extracellular solution (Fig. 1*d*).

Interaction between CCK and GTP-γ-S

It has previously been shown that GTP-γ-S applied to the cell interior via a patch pipette can evoke fluctuations in the Ca^{2+}-dependent Cl^- current as well as in $[Ca^{2+}]_i$ measured by microfluorimetry of fura-2 (Osipchuk et al., 1990). GTP-γ-S also markedly potentiates the action of ACh (Wakui et al., 1989). When 50 μM GTP-γ-S was present in the pipette solution, responses of the type shown in Fig. 2 (*a* and *d*) were seen in eight experiments, whereas in the remaining four cases no effects of GTP-γ-S alone could be observed (Fig. 2*c*). The GTP-γ-S-induced responses gradually changed with time. Initially free-standing short-lasting spikes were observed; thereafter, some of the spikes were seen to trigger waves, and finally, repetitive waves were seen alone (Fig. 2*a* and *d*). When GTP-γ-S was used at the slightly higher

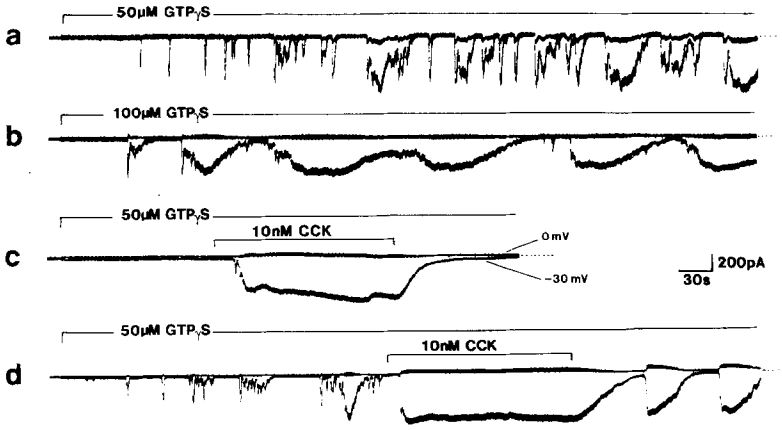

Fig. 2. The effects of internal perfusion with GTP-γ-S. (*a*) GTP-γ-S (50 μM) was present in the pipette solution. Trace shows gradual slow increase in activity with more and more spikes triggering long current pulses. (*b*) GTP-γ-S (100 μM) in pipette solution evoking long waves of Ca^{2+}-dependent Cl^- current. (*c*) In this case 50 μM GTP-γ-S did not evoke any effect, but 10 nM CCK8-NS induced a sustained response. (*d*) GTP-γ-S (50 μM) evokes a fluctuating current response, and 10 nM CCK8-NS makes the response sustained

concentration of 100 μM all seven cells investigated responded. In four cases wave responses (Fig. 2*b*) or completely sustained responses were seen after only a few minutes of stimulation. In the three other cases responses similar to those described with 50 μM GTP-γ-S were obtained.

In three of the experiments in which 50 μM GTP-γ-S failed by itself to evoke a response, application of 10 nM CCK8-NS evoked a sustained increase in the current which was immediately reversible after removal of the peptide (Fig. 2*c*).

In three of the experiments where 50 μM GTP-γ-S evoked fluctuating current responses, application of 10 nM CCK8-NS induced a sustained response which was reversible upon removal of the hormone (Fig. 2*d*).

Interaction between CCK and Caffeine

Caffeine alone has no effect on $[Ca^{2+}]_i$ as measured with fura-2 microfluorimetry or as assessed by measurement of Ca^{2+}-dependent Cl^- current (Osipchuk et al., 1990) except under the special circumstance when the Ca^{2+} chelator EGTA is omitted from the pipette solution (Wakui et al., 1990).

When 0.2 mM caffeine was applied externally in cases where 10 nM CCK8-NS evoked a normal response no effects were seen ($n = 2$). However, in two cases where 10 nM CCK8-NS evoked a relatively weak response, 0.2 mM caffeine clearly enhanced the current fluctuations mainly by increasing the probability that spikes triggered waves (Fig. 3*a*). Similar effects were obtained in four experiments with 1 mM caffeine.

When 10 nM CCK8-NS evoked normal responses 2 mM caffeine applied externally markedly and reversibly inhibited the responses mainly by reducing the probability that spikes triggered waves ($n = 3$) (Fig. 3*b*).

A high concentration of caffeine (20 mM) abolished the responses evoked by 10 nM CCK8-NS in 8 out of the 10 experiments carried out (Fig. 3*c*). In the remaining two cases marked inhibitions were seen. The effect of even this high caffeine concentration was completely and immediately reversible (Fig. 3*c*).

In four experiments we initially applied 20 nM caffeine without any effect, but when 10 nM CCK8-NS was applied on top of caffeine no response was seen until caffeine had been removed. Readmission of 20 mM caffeine in the continued presence of 10 nM CCK8-NS immediately abolished the response ($n = 4$) (Fig. 4*a*).

We also investigated the effects of caffeine applied during sustained responses evoked by 100 nM CCK8-NS. Caffeine in a concentration of 1 mM generally evoked only a brief transient inhibition ($n = 4$) (Fig. 5*a*), but in two experiments this caffeine concentration transformed a sustained response into an oscillatory response, and a similar result was obtained with 2 mM caffeine ($n = 3$) (Fig. 5*b*). With 20 mM caffeine, more pronounced inhibitions were observed. In six experiments sustained responses were transformed into patterns of repetitive waves ($n = 6$), and this effect was immediately reversible (Fig. 5*c*).

Forskolin evoked effects similar to those seen with caffeine. Figure 6*a* shows that 0.2 mM forskolin markedly and reversibly inhibited the oscillatory response to 10 nM CCK8-NS ($n = 4$). Forskolin also transformed the sustained response to 100 nM CCK8-NS to an oscillating one ($n = 2$). Since forskolin is a powerful activator of adenyl cyclase we wanted to test whether the action of forskolin was due to an increase in intracellular cyclic AMP concentration. We therefore carried out experiments with 1 mM cyclic AMP in the pipette solution. In these cases 100 nM CCK8-NS evoked the usual sustained responses. In all the

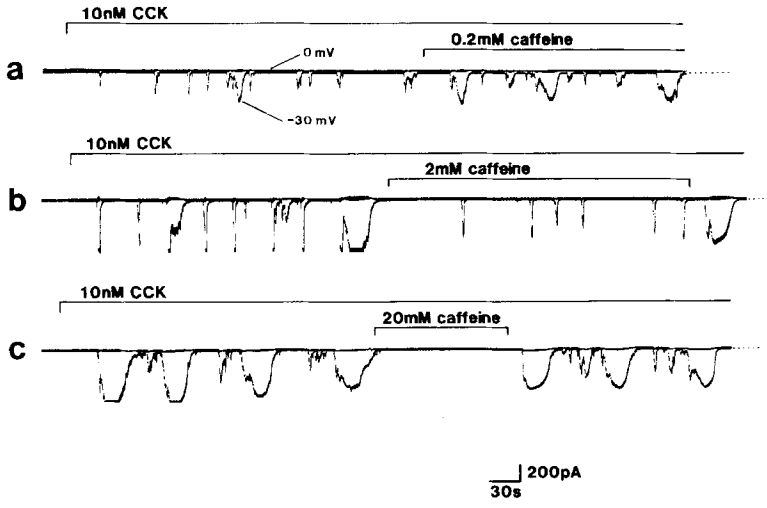

Fig. 3. The effects of caffeine on CCK-evoked responses. (*a*) In this experiment 10 nM CCK8-NS evoked only a weak response, and 0.2 mM caffeine added to the external solution prolonged the duration of the current pulses. (*b*) Normal response to 10 nM CCK8-NS. Caffeine (2 mM) markedly and reversibly inhibits the response. (*c*) A high caffeine concentration (20 mM) immediately abolishes the response to 10 nM CCK8-NS, and this effect is reversible

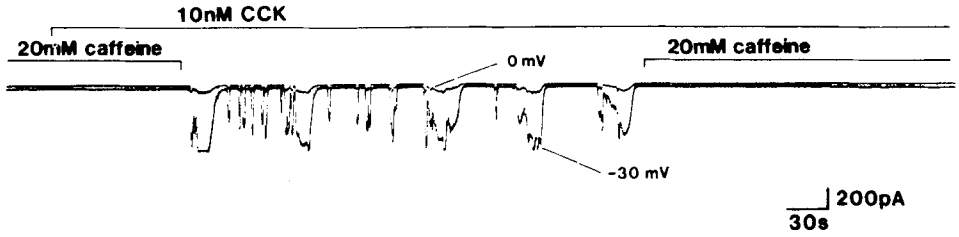

Fig. 4. Caffeine (20 mM) prevents CCK8-NS from evoking its normal response, but as soon as the drug is removed the response is seen, only to be abolished later when caffeine is reintroduced

three experiments of this type subsequent application of 0.2 mM forskolin transformed the sustained responses to oscillatory ones (Fig. 6*b*), exactly as observed in the absence of cyclic AMP.

The Effect of Intracellular Heparin Infusion on CCK-Evoked Responses

In three experiments we infused a heparin-containing intracellular solution into the patch pipette tip during sustained stimulation with 50 nM CCK8-NS and observed in all cases a marked inhibition of the current response (Fig. 7). After heparin had inhibited the CCK response, application of 50 nM ACh—a concentration that normally evokes repetitive spikes (Wakui et al., 1990; Petersen et al., 1991*a*)—failed to evoke any current changes (*not shown*). Heparin did not abolish the responses evoked by 50 nM CCK8-NS, but induced a very marked inhibition so that only solitary waves occurred at intervals of several minutes (Fig. 7).

Discussion

The results described in this paper indicate that the basic mechanism by which activation of CCK receptors induces cytoplasmic Ca^{2+} signals is very similar to that previously described for muscarinic receptors. The cytoplasmic Ca^{2+} spikes evoked by CCK stimulation are due to repetitive pulses of Ca^{2+} release from intracellular stores since the responses, at least during the first 5 min of stimulation, are independent of the presence of extracellular Ca^{2+} (Fig. 1*d*) as previously shown for the ACh- (Wakui et al., 1989) and IP_3- (Petersen & Wakui, 1990) evoked responses. The CCK-evoked responses are potentiated by the presence of GTP-γ-S in the cell (Fig. 2*c* and *d*) as previously shown for ACh (Wakui et al., 1989), and the effect of CCK stimulation is also inhibited by intracellular infusion of the IP_3 antagonist heparin (Fig. 7) as previously described in the case of ACh stimulation (Wakui et al., 1990). Our results therefore do not give support to the hypothesis proposed by Saluja et al. (1989) that cytoplasmic Ca^{2+} signals induced by CCK stimulation are not mediated by IP_3, but are in agreement with the original work of Streb et al. (1985) in which a close correlation between CCK-evoked IP_3 production and release of Ca^{2+} from nonmitochondrial stores was demonstrated.

In spite of the similar basic mechanism by which ACh and CCK produce cytoplasmic Ca^{2+} signals, the pattern of $[Ca^{2+}]_i$ fluctuations evoked by submaximal stimulation is different for the two agonists.

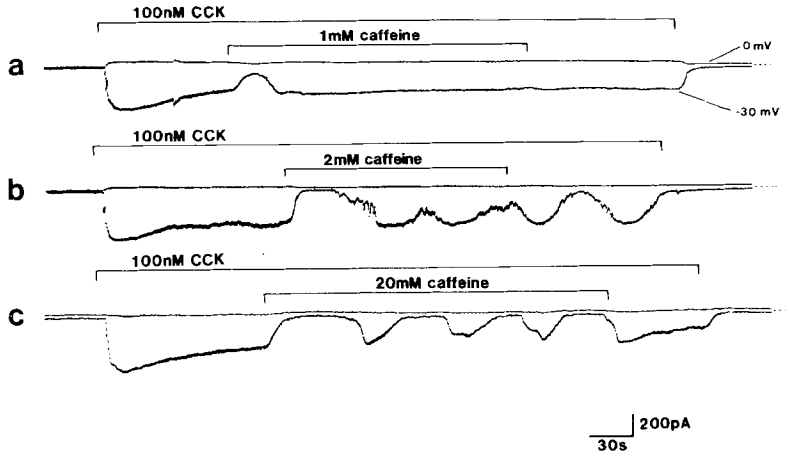

Fig. 5. Dose-dependent inhibitory effects of caffeine on the responses induced by 100 nM CCK8-NS

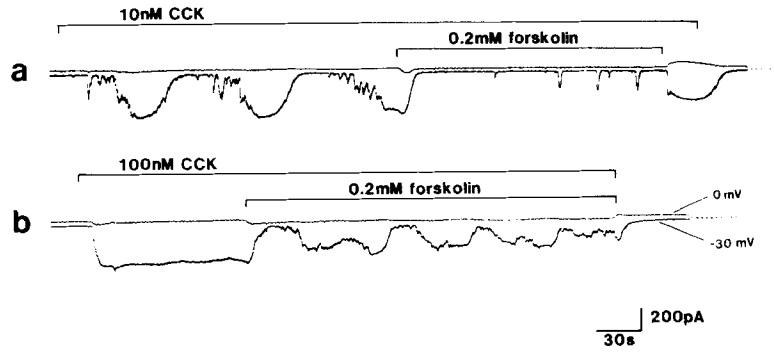

Fig. 6. Inhibitory effects of 0.2 mM forskolin added to the external solution on responses to 10 and 100 nM CCK8-NS. In the experiment represented by trace *b*, 1 mM cyclic AMP was present in the pipette solution

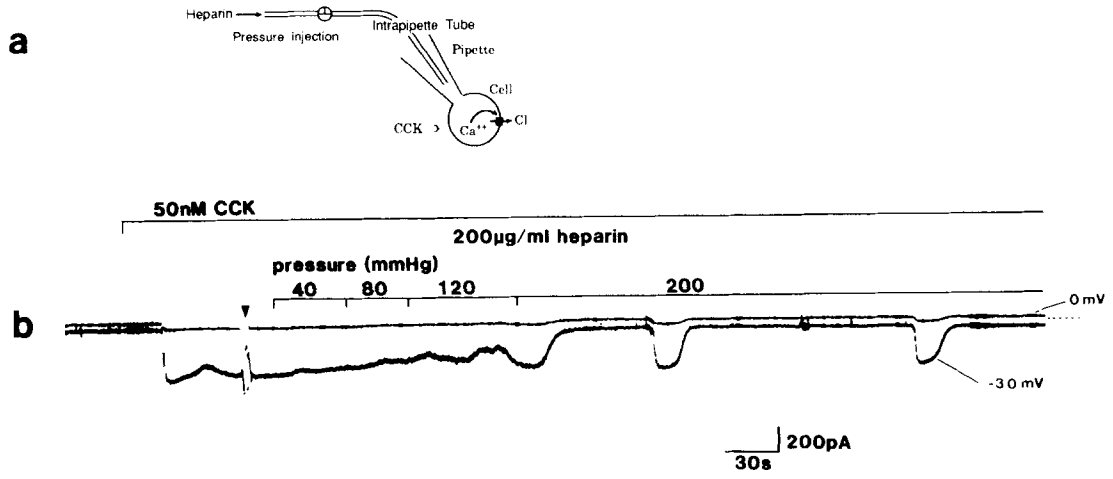

Fig. 7. The effect of intrapipette infusion of a heparin-containing intracellular solution on the response to 50 nM CCK8-NS. (*a*) Cartoon illustrating the experimental set-up. (*b*) Initially 50 nM CCK8-NS evokes a sustained response. At the arrowhead the intrapipette infusion line is opened, and thereafter pressure is applied to induce movement of heparin-containing solution into the pipette tip. The pressures applied are indicated. When a high pressure is applied the sustained response is rapidly abolished, but short current pulses occur at relatively long intervals

The short-lasting spikes of Ca^{2+}-dependent Cl^- current evoked by CCK stimulation are as previously pointed out (Petersen et al., 1991*a*) similar to those induced by stimulation with a low ACh concentration, but the relatively broad waves of about 0.5-min duration with intervals of more than 1 min between them observed during submaximal CCK stimulation have not been described for ACh stimulation (Wakui et al., 1989, 1990; Osipchuk et al., 1990; Petersen et al., 1991*a*). Infusion of a solution with a submaximal IP_3 concentration produces a $[Ca^{2+}]_i$ fluctuation pattern which is much closer to that obtained at low ACh concentrations than what can be achieved with CCK (Osipchuk et al., 1990). The only type of stimulation that can produce a fluctuation pattern similar to that seen after exposure to submaximal CCK concentrations is internal perfusion with GTP-γ-S since this agent can also evoke the peculiar mixture of short-lasting spikes and broad waves (Figs. 1 and 2). In the cases of both CCK and GTP-γ-S stimulation it is clear from studies with simultaneous measurements of $[Ca^{2+}]_i$ by fura-2 microfluorimetry and electrical recording of Ca^{2+}-dependent Cl^- current that the broad waves are seen near the cell membrane and in the cell at large, whereas the short spikes are only seen near the plasma membrane (Osipchuk et al., 1990). Since it is known that CCK receptors functionally interact with six G_s proteins as well as with G_i1, G_i2 and G_i3 proteins (Schnefel et al., 1990) it seems possible that CCK stimulation could, in addition to forming IP_3, produce effects not mediated by phosphoinositidase C (phospholipase C) activation. One possibility is that one of the messages generated is a spreading factor responsible for the formation of Ca^{2+} waves, bringing the Ca^{2+} signal to all parts of the intracellular space (Petersen et al., 1991*a*,*b*).

The effects of caffeine on the CCK-evoked responses are similar to those previously described for ACh- and IP_3-induced Ca^{2+} signals (Osipchuk et al., 1990; Wakui et al., 1990). In cases where CCK evoked weak effects low caffeine concentrations enhanced the current responses, whereas in cases of vigorous responses to CCK higher caffeine concentrations always evoked inhibition (Fig. 3). The stimulatory effects of caffeine can be explained by enhancement of Ca^{2+}-induced Ca^{2+} release (Osipchuk et al., 1990; Petersen & Wakui, 1990; Dehlinger-Kremer, Zeuzem & Schulz, 1991), but the mechanism underlying the acute and acutely reversible inhibitions of Ca^{2+}-signal generation are obscure. It has previously been shown that the inhibitory effect of caffeine on the repetitive cytosolic Ca^{2+} spikes evoked by intracellular IP_3 infusion are not mediated by cyclic AMP (Wakui et al., 1990), and the same conclusion has also been reached in recent studies on oocytes where the caffeine-evoked inhibition of IP_3-induced Ca^{2+} liberation could not be mimicked by theophylline or 3-isobutyl-1-methylxanthine (IBMX) (Berridge, 1991; Parker & Ivorra, 1991). The finding that forskolin, a powerful activator of adenyl cyclase, can mimick the inhibitory caffeine action on the CCK-evoked responses suggests that caffeine works by inhibition of the phosphodiesterase to increase the cyclic AMP concentration. The inability of internally perfused cyclic AMP to block the effects of CCK or to occlude the inhibitory effect of forskolin should not be regarded as strong evidence that forskolin is not acting by increasing cyclic AMP. The forskolin action could result in an increase in cyclic AMP in a cell compartment that is accessible to the CCK-triggered cascade but inaccessible to the compartment that is in contact with the content of the pipette. Recent data obtained in internally perfused pancreatic acinar cells show that dibutyryl cyclic AMP applied to the bath reversibly inhibits Ca^{2+} oscillations evoked by ACh but not by intracellular IP_3 (H. Kase, M. Wakui and O.H. Petersen, *submitted*). Caffeine therefore most likely has two separate effects on Ca^{2+} signaling: it inhibits in some way IP_3-evoked Ca^{2+} spiking (Wakui et al., 1990; Berridge, 1991; Parker & Ivorra, 1991) and via cyclic AMP formation there is inhibition of receptor-activated IP_3 production.

A simple explanation for the caffeine-evoked inhibitions of receptor-activated Ca^{2+} signals would be that this drug in some way directly inhibits IP_3-induced opening of Ca^{2+} channels in the endoplasmic reticulum (Parker & Ivorra, 1991). However, caffeine does not inhibit IP_3-evoked Ca^{2+} release from membrane vesicles containing the IP_3-sensitive endoplasmic reticulum Ca^{2+} pool (Dehlinger-Kremer et al., 1991). It is also important to note that a high caffeine concentration can inhibit cytosolic Ca^{2+} spiking initiated by a low caffeine concentration (Wakui et al., 1990), a finding not easily explained by assuming that the inhibitory caffeine effect is exclusively due to block of the action of IP_3.

It may seem surprising that caffeine added outside the cells can have such acute and acutely reversible effects (Figs. 3 and 4), but it is known that caffeine crosses the muscle cell membrane easily and that equilibration between the intra- and extracellular compartments occurs very rapidly (O'Neill, Donoso & Eisner, 1990). Recent studies have shown that this is also the case in pancreatic acinar cells (E.C. Toescu, S. O'Neill, D.A. Eisner and O.H. Petersen, *in preparation*).

Agonist-specific cytosolic Ca^{2+}-oscillation patterns were first described in liver cells (Woods, Cuthbertson & Cobbold, 1987; Rooney, Sass & Thomas, 1989), and thereafter, in pancreatic acinar

cells (Osipchuk et al., 1990; Petersen et al., 1991*a*; Yule et al., 1991). In microfluorimetric studies using fura-2 to monitor $[Ca^{2+}]_i$ in single pancreatic acinar cells, the essential difference between the patterns evoked by submaximal concentrations of CCK and ACh is that CCK evokes cytosolic Ca^{2+} transients of about 30-sec duration with about 1-min intervals between them, whereas ACh evokes a sustained rise in $[Ca^{2+}]_i$ with superimposed sinusoidal oscillations occurring at a frequency of 4–6 per min (Osipchuk et al., 1990; Yule et al., 1991). In the terminology used by Berridge (1990), ACh evokes sinusoidal, whereas CCK evokes transient oscillations. In the recordings of Ca^{2+}-dependent Cl^- current (Figs. 1, 3, and 4) the 30-sec pulses (waves) occurring with intervals of 1–2 min between them correspond to the transients seen in fluorimetric recordings (Matozaki et al., 1990; Osipchuk et al., 1990), but it is also clear that there are mostly several short-lasting spikes in each interval between two waves (Petersen et al., 1991*a*). Furthermore, it would appear that each wave is initiated by a short-lasting spike (Figs. 1, 3 and 4) (Petersen et al., 1991*a*). These short-lasting spikes can only be seen with electrophysiological methods during ACh stimulation using lower concentrations than those needed to evoke measurable changes in $[Ca^{2+}]_i$ as assessed with microfluorimetry, and this effect of ACh can be mimicked by intracellular perfusion with IP_3 (Osipchuk et al., 1990). It would appear that local cytosolic Ca^{2+} spikes can occur close to the Cl^--conducting plasma membrane probably in the luminal pole of the cells (Kasai & Augustine, 1990). Petersen et al. (1991*a*) have proposed that the broader Ca^{2+} pulses initiated by the short spikes in the presence of CCK stimulation represent spreading of the Ca^{2+} signal throughout the cell by wave formation (Meyer, 1991). The crucial question is what controls the ability of the Ca^{2+} signal to spread, but so far we have no evidence on this point, and further work is needed before a solution can be found.

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