Effect of perchlorate on calcium uptake and insulin secretion in mouse pancreatic islets

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Microdissected β -cell-rich pancreatic islets of non-inbred *ob/ob* mice were used in studies of how perchlorate (ClO_4^-) affects stimulus–secretion coupling in β -cells. ClO_4^- at 16 mM potentiated D-glucose-induced insulin release, without inducing secretion at non-stimulatory glucose concentrations. The potentiation mainly applied to the first phase of stimulated insulin release. In the presence of 20 mM-glucose, the half-maximum effect of ClO_4^- was reached at 5.5 mM and maximum effect at 12 mM of the anion. The potentiation was reversible and inhibitable by D-mannoheptulose (20 mM) or Ca^{2+} deficiency. ClO_4^- at 1–8 mM did not affect glucose oxidation. The effects on secretion were paralleled by a potentiation of glucose-induced $^{45}\text{Ca}^{2+}$ influx during 3 min. K^+ -induced insulin secretion and $^{45}\text{Ca}^{2+}$ uptake were potentiated by 8–16 mM- ClO_4^- . The spontaneous inactivation of K^+ -induced (20.9 mM- K^+) insulin release was delayed by 8 mM- ClO_4^- . The anion potentiated the $^{45}\text{Ca}^{2+}$ uptake induced by glibenclamide, which is known to depolarize the β -cell. Insulin release was not affected by 1–10 mM-trichloroacetate. It is suggested that ClO_4^- stimulates the β -cell by affecting the gating of voltage-controlled Ca^{2+} channels.

INTRODUCTION

Insulin secretion is controlled by the cytoplasmic Ca^{2+} activity in the β -cells (for review, see Wollheim & Sharp, 1981). Voltage-dependent Ca^{2+} channels in the β -cell plasma membrane have a key role in translating primary signals in the stimulus–secretion coupling sequence into stimulated Ca^{2+} influx in the β -cells (Wollheim & Sharp, 1981; Satin & Cook, 1985; Rorsman & Trube, 1986). Like voltage-controlled Ca^{2+} channels in other cells, the channel in the pancreatic β -cells can be inhibited (Henquin *et al.*, 1985) as well as being stimulated (Malaisse-Lagae *et al.*, 1984; Henquin *et al.*, 1985) by pharmacological agents. Studies on frog skeletal muscle have suggested that the inorganic anion perchlorate (ClO_4^-) at millimolar concentrations improves excitation–contraction coupling by interfering with the gating of Ca^{2+} channels (Gomolla *et al.*, 1983; Lüttgau *et al.*, 1983). To test whether ClO_4^- affects stimulus–secretion coupling in the pancreatic β -cells in a similar way, we have measured the effects of this anion on insulin secretion and $^{45}\text{Ca}^{2+}$ uptake by isolated mouse pancreatic islets stimulated by D-glucose, elevated extracellular K^+ concentrations, or glibenclamide.

MATERIALS AND METHODS

Animals

Pancreatic islets were isolated by microdissection (Hellerström, 1964) from adult non-inbred *ob/ob* mice (Umeå *ob/ob*). Islets from these animals are large and contain an unusually high proportion of β -cells (> 90 %; Hellman, 1965). Although the mice from this stock are metabolically abnormal, with a mild hyperglycaemia, which may be due to a peripheral insulin resistance and hyperphagia (Stauffer *et al.*, 1967; Stauffer & Renold, 1969), their islets show a normal regulation of insulin secretion *in vitro* (Hahn *et al.*, 1974; Hellman *et al.*, 1974; Idahl *et al.*, 1976).

Incubation medium

The basal incubation medium had the same composition as Krebs–Ringer medium, except that bicarbonate was omitted and replaced by 20 mM-Hepes, adjusted with NaOH to pH 7.40. The gas phase was ambient air. The basal medium was supplemented with 1 mg of bovine serum albumin/ml.

Incubations

Insulin secretion was measured either in static incubations or in a perfusion system (Asplund *et al.*, 1979; Lindström & Sehlin, 1982). Briefly, batches of three (static incubations) or about 30 islets (perfusions) were pre-incubated for 30 min at 37 °C in 1 ml of basal medium. In static incubations, they were then incubated for 10 or 60 min in 300 μl of incubation medium, as specified in the legends to Tables and Figures. In perfusions, the islets were placed in a perfusion chamber and medium was pumped at a rate of 0.56 ml/min. The time required for new medium to be pumped through the flow system was 120 s. No correction for this lag time has been made in the Figures.

Measurements of $^{45}\text{Ca}^{2+}$ uptake with the La^{3+} -wash technique were performed essentially as described by Hellman *et al.* (1976), except that the temperature during washing was 0 °C (Hellman, 1978). Incubated pancreatic islets were freeze-dried overnight (0.1 Pa, –40 °C), weighed on a quartz-fibre balance, and their radioactive contents were analysed by liquid-scintillation spectrometry.

Glucose oxidation was measured as the conversion of [^{14}C]glucose into $^{14}\text{CO}_2$, as previously described (Hellman *et al.*, 1974).

Chemicals

$^{45}\text{CaCl}_2$ and D-[U- ^{14}C]glucose were from Amersham International, Amersham, Bucks., U.K., and ^{125}I -insulin was from Hoechst A.G., Frankfurt/Main, Germany. Bovine serum albumin (fraction V) was from Miles

Fig. 1. Effect of ClO_4^- on insulin release

After preincubation for 30 min in basal medium, islets were incubated for 10 min in the same medium with modifications as indicated in the Figure. Values represent means $\pm$ S.E.M. (vertical lines) for eight experiments. * $P < 0.05$ for difference from control, by using Student's t test for paired data.

Fig. 2. Concentration-dependence of ClO_4^- -induced potentiation of insulin release

Pancreatic islets were preincubated and incubated as described in the legend to Fig. 1. $\circ$, Incubation in the presence of 3 mM-glucose; $\bullet$, incubation with 20 mM-glucose. The values represent means $\pm$ S.E.M. (vertical lines) for eight experiments.

Laboratories, Stoke Poges, Bucks., U.K. Other chemicals were commercially available reagents of analytical grade. Double-quartz-distilled water was used throughout.

RESULTS

Effect of ClO_4^- on glucose-induced insulin secretion

Fig. 1 shows the effect of 16 mM- ClO_4^- on glucose-induced insulin release. The anion did not significantly stimulate the basal insulin release in the presence of 0–4 mM-glucose, but potentiated the glucose-stimulated secretion at 7.5–20 mM-glucose. ClO_4^- did not change the glucose concentration required for half-maximum stimulation of insulin release.

The concentration-dependence of the ClO_4^- effect in the presence of 20 mM-glucose is shown in Fig. 2. Half-maximum potentiation was obtained by 5.5 mM- and maximum effect by 12 mM- ClO_4^- .

Pancreatic islets stimulated by 20 mM-D-glucose reacted with a biphasic insulin response (Fig. 3), similar to previously published data on this type of islet preparation (Lindström, 1984). ClO_4^- (8 mM) together with 20 mM-glucose markedly enhanced the stimulated secretion during the initial 20 min (cumulative secretion during min 0–20 increased by 42%; $P < 0.01$; $n = 7$), whereas the insulin release during the subsequent 40 min was much less, if at all, affected by the anion (cumulative secretion during min 20–60 increased by 8.5%; $P > 0.05$).

The reversibility of the potentiation of insulin secretion was measured by incubating pancreatic islets during two successive 10 min periods (Table 1). During the first 10 min period, 12 mM- ClO_4^- was added together with 20 mM-glucose. As shown in Table 1, the anion aug-

mented the glucose-induced secretion. During the second 10 min period, ClO_4^- was omitted from the incubation medium, which completely abolished the potentiation of the hormone release. This suggests that the effect of ClO_4^- is completely and rapidly reversible.

To test further whether the ClO_4^- -potentiated insulin release is physiologically regulated, the effect of D-mannoheptulose and Ca^{2+} deficiency was tested. Previous studies have established that mannoheptulose inhibits glucose-stimulated insulin release by blocking the β -cell glucose metabolism (Hellman *et al.*, 1972), whereas Ca^{2+} deficiency blocks insulin secretion distally in the stimulus-secretion coupling sequence (for review, Wollheim & Sharp, 1981). As shown in Table 2, the combined effects of 20 mM-glucose and 16 mM- ClO_4^- were almost completely blocked by either 20 mM-mannoheptulose or Ca^{2+} deficiency.

Effect of ClO_4^- on glucose-induced $^{45}\text{Ca}^{2+}$ uptake

To test the idea that ClO_4^- potentiates glucose-induced insulin secretion by improving the stimulus-secretion coupling in the pancreatic β -cells, the $^{45}\text{Ca}^{2+}$ uptake by pancreatic islets was studied. In order to measure preferentially influx of Ca^{2+} into the islet cells, we used short incubation times and washed the islets with La^{3+} (Hellman *et al.*, 1976; Hellman, 1978). Fig. 4 shows that the $^{45}\text{Ca}^{2+}$ uptake during the initial 3 min was stimulated by increasing concentrations of glucose, similar to what was previously shown for net uptake during longer

Fig. 3. Effect of ClO_4^- on the dynamics of glucose-induced insulin release

Pancreatic islets were perfused with glucose ('G') and ClO_4^- as indicated in the Figure. The values denote means $\pm$ S.E.M. (vertical lines) for seven experiments. Statistical significance was tested by Student's *t* test for paired data.

periods of time (120 min) (Hellman *et al.*, 1977). At 16 mM, ClO_4^- did not affect the basal $^{45}\text{Ca}^{2+}$ uptake in the absence of glucose, but amplified the glucose-stimulated uptake, resulting in significant potentiation in the presence of 8–20 mM-glucose. Similar effects were

observed with 8 mM- ClO_4^- [0 mM-glucose, 1.68 ± 0.18 ; 0 mM-glucose plus 8 mM- ClO_4^- , 1.59 ± 0.16 ; 6 mM-glucose, 1.81 ± 0.18 ; 6 mM-glucose plus 8 mM- ClO_4^- , 1.95 ± 0.31 ; 10 mM-glucose, 2.39 ± 0.17 ; 10 mM-glucose plus 8 mM- ClO_4^- , 2.97 ± 0.19 ($P < 0.01$ for difference from 10 mM-glucose alone); results denote means $\pm$ S.E.M. for eight separate experiments measuring $^{45}\text{Ca}^{2+}$ uptake during 3 min].

Effect of ClO_4^- on K^+ -induced insulin secretion

Acute increases in the extracellular K^+ concentration depolarize the pancreatic β -cells (Dean & Matthews, 1970), leading to increased Ca^{2+} influx (Malaisse-Lagae & Malaisse, 1971) and stimulation of insulin secretion (Grodsky & Bennett, 1966). Fig. 5 shows how an increase in the K^+ concentration from 5.9 to 20.9 mM in the presence of 3 mM-glucose resulted in a rapid increase in the insulin-secretory rate, with a maximum after 7 min, followed by a decline, and after about 30 min of perfusion an almost stable but still elevated rate of insulin secretion was reached (about 3 times the basal secretory rate). ClO_4^- (8 mM) potentiated the effect of K^+ , both the initial peak stimulation and the secondary, more persistent, secretory phase.

The effects of different concentrations of extracellular K^+ on the cumulative insulin secretion during 60 min was measured with or without 16 mM- ClO_4^- (Fig. 6). ClO_4^- did not affect insulin secretion in the presence of 3 mM-glucose and the normal concentration (5.9 mM) of extracellular K^+ . However, the elevated insulin secretion induced by K^+ was markedly amplified by ClO_4^- .

The transient appearance of the insulin secretion

Table 1. Reversibility of ClO_4^- -induced potentiation of insulin release

Islets were preincubated and incubated as described in the legend to Fig. 1, except that after the incubation for 10 min (Period I) the islets were rapidly transferred to new incubation vials (completed within 20 s) and incubated for another 10 min (Period II). The values represent means $\pm$ S.E.M. for seven experiments. * $P < 0.025$ for difference from 319, by using Student's *t* test for paired data.

Glucose concn. (mM)	ClO_4^- concn. (mM)	Insulin release (pg/ μ g dry wt.)	
		Period I	Period II
3	0	32 ± 7	65 ± 12
20	0	319 ± 42	712 ± 68
20	12	$574 \pm 81^*$	690 ± 99

Table 2. Effect of D-mannoheptulose or Ca^{2+} deficiency on ClO_4^- -induced potentiation of insulin release

Pancreatic islets were incubated as described in the legend to Fig. 1. Ca^{2+} deficiency was obtained by omitting CaCl_2 from the medium. Values denote means $\pm$ S.E.M. for the numbers of experiments given in parentheses. * $P < 0.001$ for differences from 481, by using Student's *t* test for paired data.

ClO_4^- concn. (mM)	Glucose concn. (mM)	Test situation	Insulin release (pg/ μ g dry wt.)
0	3	—	71 ± 12 (23)
0	20	—	315 ± 27 (29)
16	20	—	481 ± 41 (29)
16	20	20 mM-mannoheptulose	$148 \pm 29^*$ (14)
16	20	Ca^{2+} deficiency	$94 \pm 14^*$ (12)

Fig. 4. Effect of ClO_4^- on $^{45}\text{Ca}^{2+}$ uptake

Pancreatic islets were preincubated and incubated as described in the legend to Table 3. The results represent mean values $\pm$ S.E.M. (vertical lines) for 16–25 experiments. * $P < 0.05$ for differences from control by using Student's t test for paired data.

Fig. 6. Effect of ClO_4^- on K^+ -induced insulin release

Pancreatic islets were preincubated and incubated as described in the legend to Fig. 1, except that the incubation time was 60 min. The extracellular K^+ concentration was elevated by replacing NaCl by KCl. The values denote means $\pm$ S.E.M. (vertical lines) for eight experiments. * $P < 0.05$ for effect of ClO_4^- , by using Student's t test for paired data. G, glucose.

Fig. 5. Effect of ClO_4^- on the dynamics of K^+ -induced insulin release in the presence of 3 mM-glucose

After preincubation for 30 min in basal medium, pancreatic islets were pre-perfused for 45 min in the same type of basal medium. After 45 min (time 0), the medium was changed to one containing elevated K^+ concentration (15 mM-KCl substituted for NaCl) with or without ClO_4^- as indicated in the Figure. The glucose concentration ('G') was 3 mM throughout. The values denote means $\pm$ S.E.M. (vertical lines) for six experiments.

induced by increased extracellular K^+ is thought to be due to spontaneous inactivation of voltage-controlled Ca^{2+} channels (Henquin & Lambert, 1974). It has previously been shown that the presence of glucose at low concentrations, which by itself does not induce insulin secretion (Fig. 1), but is enough to decrease substantially the K^+ permeability in the β -cells (Henquin, 1980a; Sehlin, 1980), interferes with the phasic appearance of the K^+ -induced insulin secretion (Henquin & Lambert, 1974). Therefore, in a separate series of experiments (Fig. 7) glucose was omitted from the extracellular medium. In the absence of glucose, increase in K^+ concentration from 5.9 to 20.9 mM caused a much sharper peak increase in insulin release than that seen in the presence of 3 mM-glucose (cf. Fig. 5). This was followed by a rapid decline in the secretory rate, which could be fitted almost perfectly to a first-order function (inset in Fig. 7). The addition of 8 mM- ClO_4^- together with the elevated K^+ caused a rather modest increase in the peak height of the K^+ -induced insulin release. Thus the secretory rate at 4 min was increased by about 40%, but the effect did not reach a high statistical significance

Fig. 7. Effect of ClO_4^- on the dynamics of K^+ -induced insulin release in the absence of glucose

Perfusion experiments were performed as described in Fig. 5, except that the perfusion medium was glucose ('G')-free. The inset shows values of the rate of insulin release during min 5–9, transformed into natural logarithms. The rate coefficient for the fall in insulin release (k) was computed by using the method of least squares. The values represent means $\pm$ S.E.M. for eight experiments. Statistical analysis of data was done by Student's t test for paired data.

($0.05 < P < 0.1$). However, ClO_4^- significantly increased the integrated insulin release during the first 10 min by 53% ($P < 0.01$). The inset in Fig. 7 depicts the slope of the decay in insulin secretion during min 5–9. ClO_4^- significantly decreased the rate coefficient for decrease in insulin release ($P < 0.025$; $n = 8$).

Effect of ClO_4^- on K^+ -induced $^{45}\text{Ca}^{2+}$ uptake

Fig. 8 shows the dose-dependent effects of elevated extracellular K^+ concentrations on the short-term $^{45}\text{Ca}^{2+}$ uptake into pancreatic islet cells. In accordance with previous observations on this type of islet preparation (Hellman *et al.*, 1978), increase in extracellular K^+ by 10–15 mM increased the $^{45}\text{Ca}^{2+}$ uptake in the presence of 3 mM-glucose. At 16 mM, ClO_4^- did not affect the $^{45}\text{Ca}^{2+}$ uptake in the presence of 5.9–8.4 mM- K^+ , but significantly amplified the stimulatory effect of elevated extracellular K^+ concentrations in the range 10.9–20.9 mM.

Effect of ClO_4^- on glibenclamide-induced $^{45}\text{Ca}^{2+}$ uptake

Hypoglycaemic sulphonylureas are known to stimulate insulin secretion (Bouman & Goorenstroom, 1961),

Fig. 8. Effect of ClO_4^- on K^+ -induced $^{45}\text{Ca}^{2+}$ uptake

Islets were preincubated and incubated as in Table 3. The glucose ('G') concentration was 3 mM throughout, and K^+ was increased by replacing different concentrations of NaCl by KCl. The results denote means $\pm$ S.E.M. for 9–19 experiments. * $P < 0.05$ for effect of ClO_4^- , by using Student's t test for paired data.

probably by depolarizing the pancreatic β -cells and thereby activating voltage-controlled Ca^{2+} channels, leading to cellular uptake of Ca^{2+} (for review, see Gylfe *et al.*, 1984). In accordance with this model, it has previously been shown that the sulphonylurea glibenclamide induces an increase in $^{45}\text{Ca}^{2+}$ uptake, that is spontaneously inactivated (Norlund & Sehlin, 1985). Table 3 shows that 1 μM -glibenclamide increased $^{45}\text{Ca}^{2+}$ uptake in the presence of 3 mM-glucose; ClO_4^- at 8 or 16 mM augmented the $^{45}\text{Ca}^{2+}$ uptake further in a dose-dependent manner.

Effect of ClO_4^- on glucose oxidation

At 1–16 mM, ClO_4^- did not significantly affect the rate of oxidation of 20 mM-glucose, measured as the production of $^{14}\text{CO}_2$ from D-[U- ^{14}C]glucose during 60 min [control, 28.1 ± 2.8 ; 1 mM- ClO_4^- , 25.8 ± 3.7 ; 8 mM- ClO_4^- , 26.9 ± 3.2 ; 16 mM- ClO_4^- , 22.6 ± 3.9 (mmol of glucose equiv./kg; $n = 6$ –7)].

Table 3. Effect of glibenclamide on the $^{45}\text{Ca}^{2+}$ uptake

Pancreatic islets were first exposed to preincubation for 30 min in 1 ml of basal medium, followed by incubation for 3 min in 200 μl of the same medium supplemented with $^{45}\text{Ca}^{2+}$ (0.3 TBq/mol) and test substances as required. The results denote mean values $\pm$ S.E.M. for 12 experiments. * $P < 0.001$ for difference from control, and † $P < 0.05$ for difference between 2.23 and 1.87, by using Student's t test for paired data.

Glibenclamide concn. (μM)	ClO_4^- concn. (mM)	$^{45}\text{Ca}^{2+}$ uptake (mmol/kg dry wt.)
None	None	1.87 ± 0.11
1 (control)	None	$2.23 \pm 0.11^\dagger$
1	8	$2.58 \pm 0.12^*$
1	16	$2.90 \pm 0.12^*$

Effect of trichloroacetate on insulin release

Under the experimental conditions as in the experiments with ClO_4^- (cf. Fig. 1), trichloroacetate at 0.1–10 mM did not affect insulin release at 3 mM- or 20 mM-glucose [3 mM-glucose, 0.049 ± 0.016 ($n = 10$); 3 mM-glucose plus 1 mM-trichloroacetate, 0.037 ± 0.010 ($n = 11$); 3 mM-glucose plus 10 mM-trichloroacetate, 0.027 ± 0.011 ($n = 11$); 20 mM-glucose, 0.213 ± 0.028 ($n = 16$); 20 mM-glucose plus 1 mM-trichloroacetate, 0.194 ± 0.027 ($n = 16$); 20 mM-glucose plus 10 mM-trichloroacetate, 0.245 ± 0.030 ($n = 16$) ng/10 min per μg dry wt.].

DISCUSSION

Perchlorate potentiated the insulin release and $^{45}\text{Ca}^{2+}$ uptake induced by D-glucose or elevated extracellular K^+ concentrations as well as the $^{45}\text{Ca}^{2+}$ uptake induced by glibenclamide. The effect on secretion was reversible and could be inhibited by D-mannoheptulose or extracellular Ca^{2+} deficiency, suggesting that the ClO_4^- -potentiated insulin release was based on physiologically regulated exocytotic secretion.

Chaotropic anions, such as ClO_4^- , can adsorb to phospholipid membranes and induce a negative electrostatic potential at the membrane surface (McLaughlin *et al.*, 1975). If such binding were preferentially located to the outer surface of the β -cells, it should shift the voltage-dependence of the Ca^{2+} channels and other channels to more negative potentials, which could open the Ca^{2+} channels or potentiate the effect of primary depolarizing stimuli. However, several lines of evidence suggest that ClO_4^- adsorption to the β -cell plasma membrane is not a major factor in the present stimulatory effects. Firstly, ClO_4^- did not induce insulin release or $^{45}\text{Ca}^{2+}$ uptake in the resting β -cells at 3 mM-glucose, suggesting that any electrostatic potential created is not enough to open Ca^{2+} channels from the resting state. Secondly, the potentiation of insulin secretion at 20 mM-glucose showed a maximum at 12 mM- ClO_4^- . At such a relatively low concentration, electric adsorption effects on artificial phospholipid membranes are small (McLaughlin *et al.*, 1975) and considered not to be significantly contributing to the regulation of membrane mechanisms in frog muscle fibres (Gomolla *et al.*, 1983). Thirdly, any contribution of small electrostatic potentials would be

most effective at threshold concentrations of glucose or depolarizing K^+ . Instead, we found the maximum potentiating effects in the presence of maximally depolarizing glucose concentrations (20 mM), and the potentiation increased with increasing extracellular K^+ concentrations. Fourthly, trichloroacetate, an anion with stronger chaotropic potency than ClO_4^- (Hanstein, 1979), which would be expected to adsorb even more strongly to the β -cell membrane, neither induced insulin release at 3 mM-glucose nor potentiated the secretion at 20 mM-glucose.

The regulation of glucose-induced insulin release consists of many steps. We cannot exclude the possibility that ClO_4^- potentiates $^{45}\text{Ca}^{2+}$ uptake and insulin secretion by interacting with some proximal steps in the signal chain. However, the anion did not affect the rate of islet glucose metabolism, which is considered to be associated with the initial secretory signal(s) (Ashcroft, 1980), and it also potentiated depolarization-induced secretion (increased extracellular K^+) and $^{45}\text{Ca}^{2+}$ uptake induced by K^+ or glibenclamide. Taken together, these results may indicate a direct effect on Ca^{2+} influx.

Previous studies on frog skeletal muscle have shown that ClO_4^- improves excitation-contraction coupling, probably by interfering with the gating mechanism that activates Ca^{2+} release from the sarcoplasmic reticulum (Gomolla *et al.*, 1983; Lüttgau *et al.*, 1983). It was suggested that ClO_4^- acts on charged membrane particles, serving as voltage sensors associated with the Ca^{2+} -channel gating, by obstructing their movement from the active to the resting position. Insulin secretion by pancreatic β -cells is stimulated by increase in the cytoplasmic Ca^{2+} concentration, and this increase, at least in part, is due to the gating of voltage-controlled Ca^{2+} channels (for review, see Wollheim & Sharp, 1981). Not much is known about the mechanisms controlling the gating of the Ca^{2+} channel in the β -cells. Considering the central role that this channel may have in the regulation of insulin secretion, it is of interest to consider the possibility that the anion ClO_4^- interacts directly with gating of the Ca^{2+} channel in the β -cell plasma membrane.

In the absence of glucose, increase in the extracellular K^+ concentration caused a transient peak of insulin release (cf. Grodsky & Bennett, 1966; Henquin & Lambert, 1974). This transiency is likely to be caused by spontaneous inactivation of the voltage-controlled Ca^{2+} channels, by the depolarization itself or by the increased intracellular Ca^{2+} concentration (Henquin & Lambert, 1974; Henquin, 1980b; Hagiwara & Byerly, 1981), leading to decreased inflow of Ca^{2+} and a gradual decrease in the cytoplasmic Ca^{2+} concentration. Apart from producing a modest increase in the peak height, ClO_4^- decreased the slope of the fall in the K^+ -induced hormone release. These results indicate that ClO_4^- may counteract the spontaneous inactivation of the channel.

The lack of effect of ClO_4^- on either insulin release or $^{45}\text{Ca}^{2+}$ uptake in the absence of primary depolarizing stimuli suggests that the anion cannot induce opening of the Ca^{2+} channel. It has been shown that the gating of Ca^{2+} channels can be stimulated by drugs structurally similar to known Ca^{2+} -channel blockers (Malaisse-Lagae *et al.*, 1984; Henquin *et al.*, 1985; Lebrun & Atwater, 1985; Morgan *et al.*, 1985). Such Ca^{2+} -channel agonists cannot open the channels from the resting state, but act by increasing the mean open time after primary stimu-

lation (Kokubun & Reuter, 1984). This is consistent with the observation that a moderate depolarizing stimulation of the β -cells is required for a stimulating action of the pharmacological Ca^{2+} agonists (Malaisse-Lagae *et al.*, 1984; Henquin *et al.*, 1985; Lebrun & Atwater, 1985; Morgan *et al.*, 1985). There are striking similarities between the secretory effects of these drugs and the effects of ClO_4^- as presented here. It therefore seems possible that these chemically quite different compounds affect the gating of Ca^{2+} channels by similar mechanisms. It has been suggested that the voltage-controlled Ca^{2+} channel in the β -cell may be anion-dependent with a specific anion selectivity (Sehlin, 1986). It is therefore possible that a number of naturally occurring anions can modulate the secretory response by affecting the properties of the gating process.

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