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EFFECTS OF ACETYLCHOLINE AND CAERULEIN ON $^{86}Rb^+$ EFFLUX IN THE MOUSE PANCREAS

EVIDENCE FOR A SODIUM-POTASSIUM-CHLORIDE COTRANSPORT SYSTEM

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The effects of acetylcholine and the cholecystokinin-like peptide, caerulein on the fractional efflux of $^{86}Rb^+$ from preloaded isolated segments of mouse pancreas were studied. Both secretagogues evoked a marked transient increase in $^{86}Rb^+$ efflux. The removal of Ca^{2+} from the superfusing medium and addition of 10^{-4} M EGTA, markedly reduced, but did not abolish the responses to either acetylcholine or caerulein. Furosemide (10^{-5}–10^{-3} M) or piretanide (10^{-4} M) reduced the basal efflux and inhibited the secretagogue-elicited responses. Stimulus-induced $^{86}Rb^+$ outflow was abolished when the Cl^- component of the superfusing solution was replaced by either NO_3^-, SO_4^{2-} or I^- but not in case of replacement by Br^-. When Na^+ was replaced with either Li^+ or choline$^+$ both acetylcholine and caerulein failed to elicit any detectable increase in $^{86}Rb^+$ outflow. However, when Tris$^+$ was substituted for Na^+, acetylcholine caused a moderate increase in $^{86}Rb^+$ efflux which was abolished by either furosemide (10^{-4} M) or chloride depletion (nitrate substitution). The removal of extracellular K^+ or pretreatment with 10^{-3} M ouabain had little effect on secretagogue-evoked $^{86}Rb^+$ efflux. These results indicate the presence of a diuretic-sensitive Na^+-K^+-Cl^- cotransport system in the mouse pancreatic acinar cell membrane.

Introduction

The transmembrane movements of K^+ in a variety of cell types is regulated by the ATP-driven ($Na^+ + K^+$)-ATPase pump located in the plasma membrane [1], a Na^+-K^+-Cl^- cotransport system which is highly Cl^- dependent and inhibited by loop diuretics such as furosemide and piretanide [2–6] and by the Ca^{2+}- and voltage-activated K^+ channels [7–12].

In the exocrine pancreas both tracer and electrophysiological studies have shown that the acinar plasma membrane is permeable to Na^+, K^+ and Cl^- [13–15]. Potassium ions are transported into the acinar cells by the ATP-driven ($Na^+ + K^+$)-ATPase pump [16] and released through the Ca^{2+}-activated channels [7–10,12]. The existence of a common diuretic-sensitive, cation- and anion-dependent cotransporter in the acinar plasma membrane mediating transmembrane movements of ions has not yet been demonstrated.

The present study was designed to determine whether such a cotransport system exists in the mouse pancreactic acinar plasma membrane. The approach was to investigate the ionic dependency and diuretic sensitivity of secretagogue-evoked K^+ transport using $^{86}Rb^+$ as a tracer.

A preliminary account of some aspect of this work was presented to the British Physiological Society [17].

Methods

All experiments were performed on isolated segments of mouse pancreas. Adult animals (30–50 g) were killed by a blow on the head and the pancreas rapidly removed and placed in a modified Krebs-Henseleit solution of the following composition: 103 mM NaCl/4.7 mM KCl/2.56 mM $CaCl_2$/1.13 mM $MgCl_2$/25 mM $NaHCO_3$/1.15 mM NaH_2PO_4/2.8 mM glucose/4.9 mM sodium pyruvate/2.7 mM sodium fumarate/4.9 mM sodium glutamate. The solution was gassed with 95% O_2 and 5% CO_2. The pancreas was cut into small segments (5–10 mg) and a total mass of 100–150 mg was loaded for 30 min in 1 ml Krebs-Henseleit solution containing 10 μCi $^{86}Rb^+$ at room temperature. The procedure was similar to that described for mouse parotid gland by Gallacher [18]. Atropine (10^{-5} M) was present in all the experiments involving caerulein stimulation.

After loading, the tissues were transferred to a perspex flow chamber (volume 1 ml) and superfused with non-radioactive physiological salt solution at the rate of 1 ml · min^{-1} at 37 °C using a 2132 microperspex peristaltic pump (LKB, Broma). The flow cell was perfused with the control solution for 19 min prior to stimulation with secretagogues. During stimulation, the fluid flowing through the chamber was replaced with Krebs-Henseleit solution containing appropriate concentrations of secretagogues. The duration of each experiment was 45 min. In some experiments the physiological salt solution was modified in several ways and further tested for Na^+ and K^+ concentrations using flame photometry (Corning; type 480). To Ca^{2+} free solution, 10^{-4} M EGTA (ethylene glycol bis(β-amino-ethyl ether)-*N*,*N*′-tetra-acetic acid) (Sigma) was added. In Na^+ free solutions all the Na^+ was replaced by either Li^+, choline$^+$ or Tris$^+$ and the substrates were added as the acids rather than the Na^+ salts and pH adjusted to 7.4. The Na^+-free Tris$^+$ medium was gassed with pure O_2. Atropine (10^{-4} M) was present in the solution containing choline chloride. In K^+-free solution, all KCl was replaced by NaCl. In Cl^--free solutions all NaCl, $MgCl_2$, KCl and $CaCl_2$ were replaced by equivalent amounts of either bromides, iodides, nitrates or sulphates to achieve appropriate osmolarity.

Effluent fractions were collected at 1 min intervals directly into scintillation vials using the 2112 Redirac Fraction Collector (LKB Broma). A volume of 10 ml distilled water was added to each vial. The total $^{86}Rb^+$ content remaining in the tissues at the end of each experiment was measured by digesting the tissue in 1 ml conc. HNO_3 and counting in 10 ml distilled water. The radioactivity of the samples and tissues was determined by liquid scintillation counting (Packard Tri-Carb 300) and the fractional efflux calculated using an on-line pre-programmed computer (Apple II). Values were obtained for fractional efflux ($(\Delta x/\Delta t)x_t$ min^{-1}, where Δx represents cpm of $^{86}Rb^+$ released in the time interval Δt and x_t the tissue $^{86}Rb^+$ content at the mid-point of interval Δt) as a function of time.

Results

(i) Effects of acetylcholine and caerulein on $^{86}Rb^+$ efflux in presence and absence of Ca^{2+}

The fractional efflux of $^{86}Rb^+$ from preloaded superfused mouse pancreatic fragments declined over a period of time leading to a relatively constant level after 16–19 min (Fig. 1A). Addition of either 10^{-5} M acetylcholine (open squares) or 10^{-9} M caerulein (solid squares) caused a rapid and marked increase in $^{86}Rb^+$ efflux (Fig. 1A). The responses to both secretagogues were transient and the fractional efflux returned to the prestimulation level after about 10–12 min. The removal of Ca^{2+} from the superfusing medium and addition of 10^{-4} M EGTA, markedly reduced, but did not abolish the responses to either acetylcholine (open squares) or caerulein (solid squares) (Fig. 1B).

(ii) Diuretic-sensitive $^{86}Rb^+$ efflux

Fig. 2 shows the effects of furosemide (10^{-5}–10^{-3} M) and piretanide (10^{-4} M) on the resting and acetylcholine-evoked $^{86}Rb^+$ fractional efflux. The preparations were pretreated with the diuretics 12 min prior to acetylcholine stimulation and the drug remained in the superfusing medium throughout the secretagogue application. Fig. 2A shows the effects of 10^{-5} M acetylcholine alone (solid circles) and the same concentration of acetylcholine in combination with either 10^{-5} M (open circles), 10^{-4} M (solid triangles) or 10^{-3} M

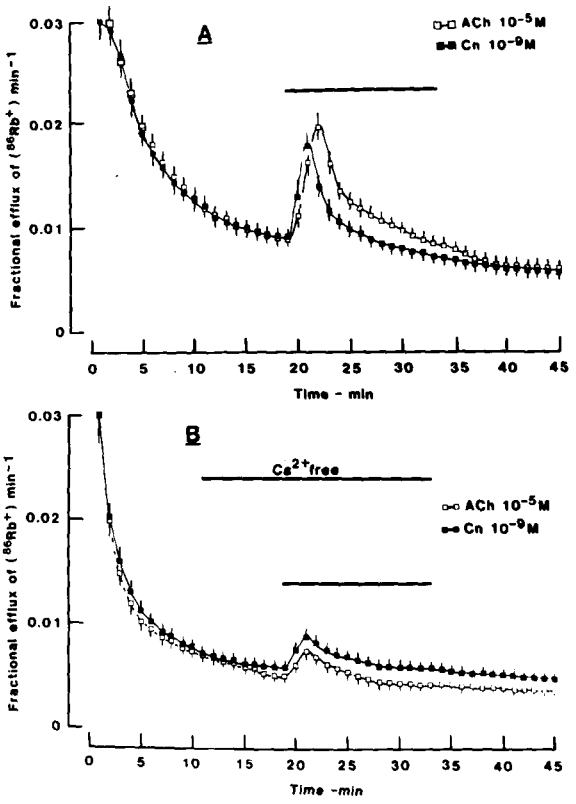

Fig. 1. (A) Effects of 10^{-5} M acetylcholine (ACh; open squares) and 10^{-9} M caerulein (Cn; solid squares) on the fractional efflux of $^{86}Rb^{+}$ from preloaded superfused fragments of mouse pancreas. In this and subsequent figures each point is the fractional efflux for 1-min collection period plotted as a function of time. The horizontal bar indicates the duration of secretagogue stimulation. Each point is mean ± S.E. ($n = 6$). (B) Effects of 10^{-5} M acetylcholine (ACh; open squares) and 10^{-9} M caerulein (Cn; solid squares) on $^{86}Rb^{+}$ efflux in the absence of Ca^{2+}. EGTA (10^{-4} M) was present in the Ca^{2+}-free medium. The horizontal bars indicate the period of stimulation and Ca^{2+} removal. Each point is mean ± S.E. ($n = 6$).

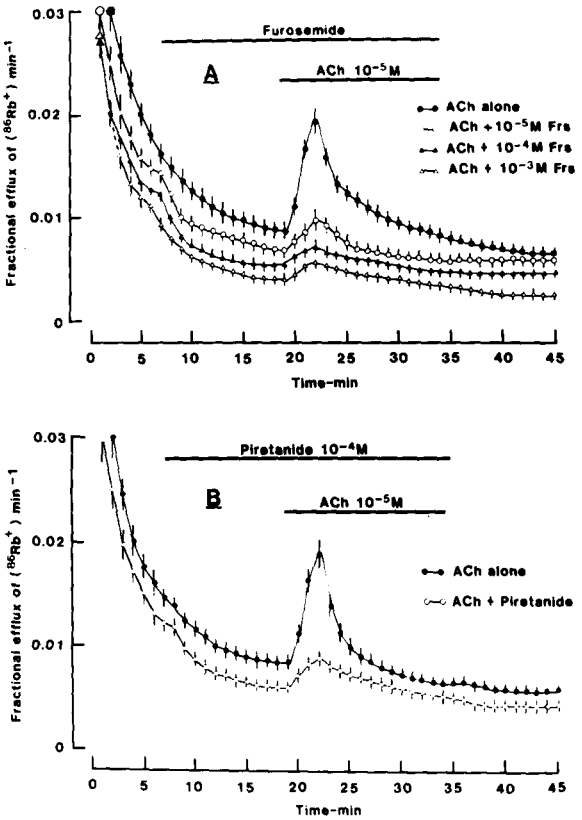

Fig. 2. (A) Effects of varying concentrations (10^{-5}–10^{-3} M) of furosemide on acetylcholine (ACh)-evoked $^{86}Rb^{+}$ fractional efflux. (solid circles), 10^{-5} M acetylcholine alone; (open circles), 10^{-5} M acetylcholine + 10^{-5} M furosemide; (solid triangles), 10^{-5} M acetylcholine + 10^{-4} M furosemide; (open triangles), 10^{-5} M acetylcholine + 10^{-3} M furosemide. (B) Effects of piretanide on acetylcholine-induced $^{86}Rb^{+}$ efflux. (solid circles), 10^{-5} M ACh alone; (open circles), 10^{-5} M ACh + 10^{-4} M piretanide. In both (A) and (B) the diuretics and acetylcholine were added to the superfusing medium as indicated by the horizontal bars. All points are mean ± S.E. ($n = 6$).

(open triangles) furosemide. The diuretic caused a marked reduction in both the basal and the acetylcholine-evoked $^{86}Rb^{+}$ efflux. The inhibitory effect of furosemide was dose-dependent. The action of piretanide on the acetylcholine-induced $^{86}Rb^{+}$ outflow was also examained. Fig. 2B shows the response produced by 10^{-5} M acetylcholine in the absence (solid circles) and presence (open circles) of 10^{-4} M piretanide. Here also, the diuretic caused a marked reduction in both the basal and the acetylcholine-elicited $^{86}Rb^{+}$ efflux. These effects of piretanide were similar to those obtained by the same concentration of furosemide.

The effects of varying the concentration of acetylcholine (10^{-8}–10^{-5} M) on $^{86}Rb^{+}$ efflux in the absence and presence of either 10^{-4} M furosemide or 10^{-4} M piretanide were also investigated. All measurements were made at the peak of the response, 2–4 min after commencing treatment with acetylcholine. Log-dose response curves showing the effects of differing concentrations of acetylcholine on the peak increase in $^{86}Rb^{+}$ outflow in the absence (solid circles) and presence of either furosemide (open circles) or piretanide (solid

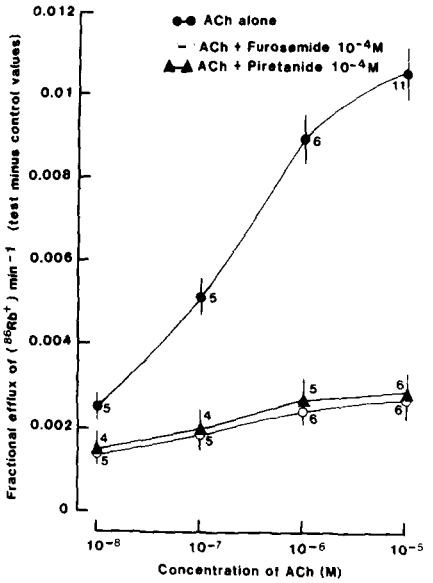

Fig. 3. Dose-response curves showing the effects of differing concentrations of acetylcholine (ACh; range: 10^{-8}–10^{-5} M) on the fractional efflux of $^{86}Rb^+$ in the absence (solid circles) and presence of either 10^{-4} M furosemide (open circles) or 10^{-4} M piretanide (solid triangles). Each point represents the $^{86}Rb^+$ efflux (test-control values) at the peak of the response, 2–4 min after the application of acetylcholine. The preparations were pretreated with the diuretics 12 min prior to acetylcholine stimulation. Each point is mean ± S.E. (*n* is shown besides each point).

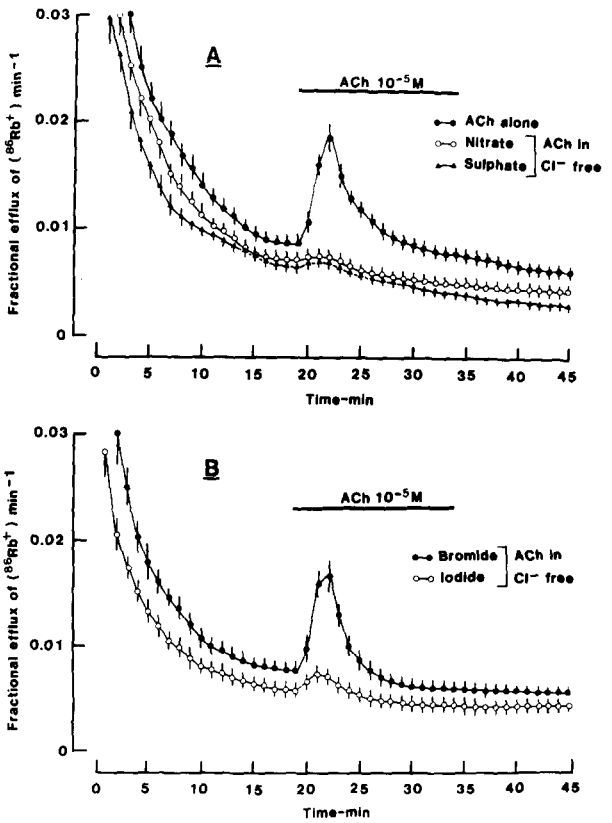

Fig. 4. Effects of Cl^- removal on acetylcholine (ACh)-evoked $^{86}Rb^+$ efflux. All results are the mean ± S.E. of six experiments. (A) (solid circles), 10^{-5} M acetylcholine in normal physiological salt solution containing chloride; (open circles), 10^{-5} M acetylcholine in Cl^--free medium (nitrate substitution); (solid triangles), 10^{-5} M acetylcholine in Cl^--free medium (sulphate substitution). (B) Effects of 10^{-5} M acetylcholine in Cl^--free medium containing either bromide (solid circles) or iodide (open circles).

triangles) are presented in Fig. 3. The data emphasise two important points. First, acetylcholine alone produced a dose-dependent increase in $^{86}Rb^+$ fractional efflux. Second, the same concentration (10^{-4} M) of either furosemide or piretanide caused closely similar reductions in $^{86}Rb^+$ efflux following stimulation with varying concentrations of acetylcholine.

(iii) Anion-dependent $^{86}Rb^+$ efflux

The nature of the secretagogue-evoked $^{86}Rb^+$ efflux was examined in more detail by carrying out a number of anion replacement experiments. Chloride in the superfusing medium was replaced at the start of each experiment by either nitrate, sulphate, iodide or bromide. Fig. 4A shows the effect of 10^{-5} M acetylcholine in the control solution containing chloride (solid circles) and in a physiological salt solution in which chloride was replaced by either nitrate (open circles) or sulphate (solid triangles). The results show that acetylcholine evoked a clear increase in $^{86}Rb^+$ efflux in the presence of chloride. However, when chloride was replaced by either nitrate or sulphate the response to acetylcholine was almost abolished.

The effects of replacing chloride with either bromide or iodide on the acetylcholine-induced $^{86}Rb^+$ outflow was also tested. Fig. 4B shows the response produced by 10^{-5} M acetylcholine in the physiological salt solution containing either bromide (solid circles) or iodide (open circles). The data show that when iodide was substituted for chloride, the acetylcholine-evoked $^{86}Rb^+$ efflux

was reduced by around 80–90%. This contrasts with the results obtained with bromide in which the acetylcholine-elicited $^{86}Rb^+$ outflow was reduced by only 5–10%.

(iv) Cation dependency of secretagogue-evoked $^{86}Rb^+$ efflux

The effects of Na^+ replacement on secretagogue-evoked $^{86}Rb^+$ efflux were investigated. Na^+ was replaced from the beginning of each experiment with either Li^+, choline$^+$ or $Tris^+$. Fig. 5A shows the effect of 10^{-5} M acetylcholine (solid circles) and 10^{-9} M caerulein (open circles) in a nominally Na^+ free (about 0.3–0.5 mM) physiological salt solution containing Li^+ instead. Both secretagogues failed to elicit any detectable increase in $^{86}Rb^+$ outflow. Fig. 5B shows the effects of 10^{-9} M caerulein on $^{86}Rb^+$ efflux in Na^+ free (about 0.1–0.3 mM) medium containing choline$^+$ (solid squares) and in normal Krebs-Henseleit solution (open squares) containing Na^+. Atropine (10^{-4} M) was present in both solutions. The data show that the caerulein-evoked response was unaffected in the presence of Na^+ but almost abolished on replacing Na^+ with choline$^+$. Fig. 6 shows the effect of acetylcholine on $^{86}Rb^+$ efflux in a physiological salt solution in which $Tris^+$ was substituted for Na^+. Acetylcholine (10^{-5} M) caused a moderate increase in $^{86}Rb^+$ outflow (open squares) which was abolished by either 10^{-4} M furosemide (solid square) or chloride depletion (nitrate substitution; solid triangles).

The effect of K^+ removal (Na^+ substitution) was investigated in a number of experiments mainly to test whether the secretagogue-evoked $^{86}Rb^+$ transport was due to a $^{86}Rb^+$-K^+ exchange process. The results are shown in Fig. 7A. The removal of K^+ from the physiological salt solution had virtually no effect on acetylcholine (open squares)- and caerulein (solid squares)-induced

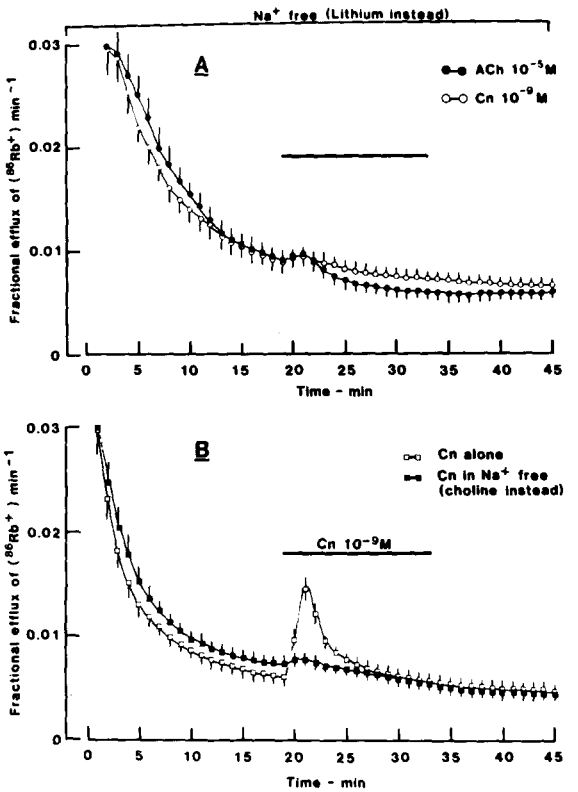

Fig. 5. Effects of Na^+ removal on secretagogue-induced $^{86}Rb^+$ fractional efflux. All points are mean ± S.E. of six experiments. (A) Effects of 10^{-5} M acetylcholine (ACh; solid circles) and 10^{-9} M caerulein (Cn; open circles) on $^{86}Rb^+$ outflow in Na^+ free medium (Li^+ substitution). The horizontal bar indicates the duration of secretagogue stimulation. (B) Effects of 10^{-9} M caerulein on $^{86}Rb^+$ efflux in normal Krebs-Henseleit solution (open squares) and in the absence of Na^+ (solid circles; choline$^+$ substitution). Atropine (10^{-4} M) was present in these experiments.

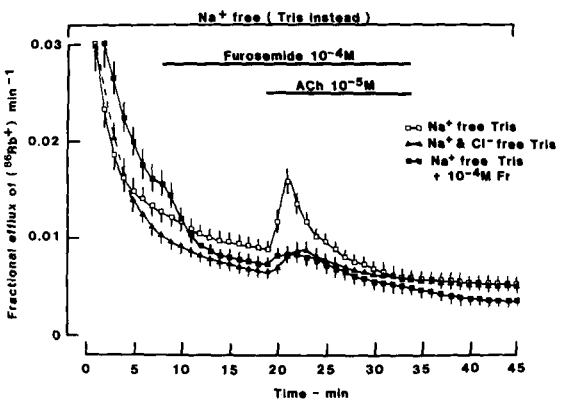

Fig. 6. Effects of Na^+ removal ($Tris^+$ substitution) on acetylcholine (ACh)-elicited $^{86}Rb^+$ fractional efflux. (open squares), 10^{-5} M acetylcholine in Na^+-free ($Tris^+$ substitution) medium; (solid triangles), 10^{-5} M acetylcholine in Na^+-free ($Tris^+$ substitution) and Cl^--free (nitrate substitution) medium; (solid squares), 10^{-5} M acetylcholine in the Na^+-free medium ($Tris^+$ substitution) containing 10^{-4} M furosemide. The horizontal bars represent the duration of exposing the tissue to acetylcholine and furosemide. Each point is mean ± S.E. ($n = 6$).

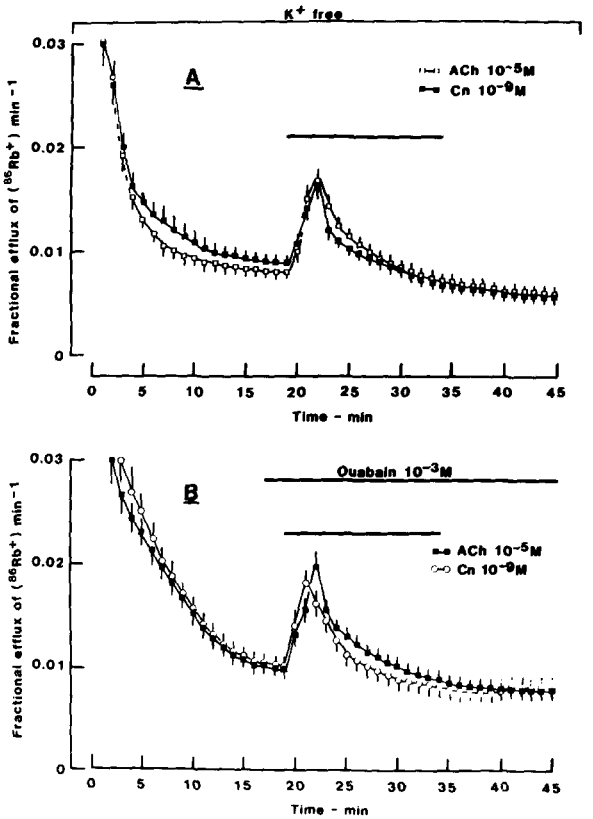

Fig. 7. Effects of K^+ removal (A) and ouabain (B) on secretagogue-induced Rb^+ efflux. All results are the mean ± S.E. of five or six experiments. The horizontal bars represent the duration of ouabain application and secretagogues stimulation. (A) Effects of K^+ removal (Na^+ substitution) in the presence of either 10^{-5} M acetylcholine (ACh; open squares) or 10^{-9} M caerulein (Cn; solid squares). (B) Effects of 10^{-5} M acetylcholine (solid squares) and 10^{-9} M caerulein (open circles) in the presence of 10^{-3} M ouabain.

$^{86}Rb^+$ efflux. The effect of ouabain (10^{-3} M) on secretagogue-elicited $^{86}Rb^+$ transport was also investigated. Ouabain was added 2 min prior to the application of either acetylcholine or caerulein and remained throughout the experiment. Fig. 7B shows that ouabain had no effect on the responses to either 10^{-5} M acetylcholine (solid squares) or 10^{-9} M caerulein (open circles). Exposure of preparations to ouabain for 50 min prior to stimulation also had no effect on secretagogue-evoked response.

(v) Effects of diuretics and Cl^- removal on caerulein-evoked $^{86}Rb^+$ efflux

The ability of either chloride depletion or the diuretics to reduce caerulein-induced $^{86}Rb^+$ efflux was also investigated. Fig. 8 shows the control response produced by 10^{-9} M caerulein (solid circles) and the responses obtained in the presence of either 10^{-4} M furosemide (open triangles) or 10^{-4} M piretanide (solid circles) or replacing chloride with nitrate (open circles). Atropine (10^{-5} M) was present throughout these experiments. Here also, the data show that either the diuretics or chloride removal caused a substantial reduction in both the basal and secretagogue-evoked $^{86}Rb^+$ efflux.

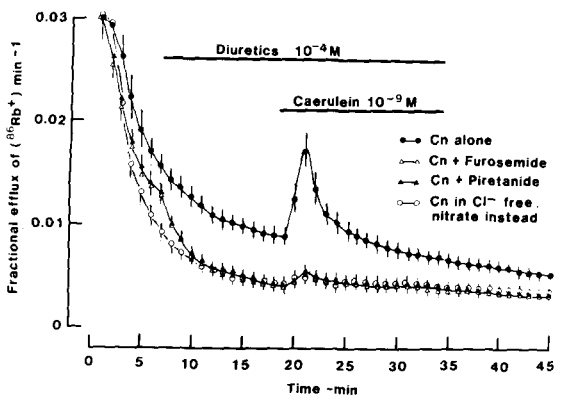

Fig. 8. Effects of 10^{-9} M caerulein (Cn) on $^{86}Rb^+$ efflux. (solid circles), caerulein alone in normal physiological salt solution; (open triangles), caerulein in the presence of 10^{-4} M furosemide; (solid triangles), caerulein in the presence of 10^{-4} M piretanide; (open circles) caerulein in Cl^--free medium (nitrate substitution). Cl^- was removed at the start of the experiments. Atropine (10^{-5} M) was present throughout. The horizontal bars represent the duration of diuretics application and caerulein stimulation. Each point is mean ± S.E. ($n = 6$).

Discussion

The present results demonstrate a diuretic-sensitive, cation- and anion-dependent secretagogue-evoked K^+ transport in the isolated mouse pancreas. Both acetylcholine and caerulein produced a marked transient increase in the fractional efflux of $^{86}Rb^+$ from preloaded tissue. The addition of loop diuretics such as furosemide and piretanide or the removal of either Na^+, Cl^- or Ca^{2+} from the superfusing medium markedly reduced the responses to acetylcholine and caerulein.

Both electrophysiological and tracer studies have shown that pancreatic secretagogues can

stimulate K^+ transport across the acinar plasma membrane [13–15]. Recent single-channel current recordings, using the patch-clamp technique, have demonstrated a non-discriminatory Ca^{2+} -activated cation channel in mouse pancreatic acinar cells. This channel is permeable to Na^+, K^+ and Rb^+ and possibly also, to a limited extent, to Ca^{2+}. The question arising from the observations presented here is whether the secretagogue-induced $^{86}Rb^+$ outflow is associated with either the Ca^{2+}-activated cation channel or with a cotransport system or with both? The results with Ca^{2+} removal, in which stimulus-evoked $^{86}Rb^+$ was markedly reduced, indicate a possible link between the channel and K^+ transport. A Ca^{2+}-dependent K^+ efflux in response to a α-adrenergic agonist has also been demonstrated in the parotid gland [19].

Stimulus-induced $^{86}Rb^+$ efflux was markedly reduced when chloride was replaced with either nitrate, sulphate or iodide but not bromide. In the presence of bromide the acetylcholine-elicited $^{86}Rb^+$ outflow was almost unaffected. These findings are consistent with previous observations in erythrocytes [3,20,21] and cultured cells [6,22] where only bromide would substitute for chloride in the operation of Na^+-K^+-Cl^- cotransport systems or in sustaining acetylcholine-evoked salivary secretion [23] although all the other anions can be equilibrated across the cell membrane by the anion (Cl^--HCO_3^-) exchanger [3,24]. It is now well established that in several different cell types the movement of either Na^+ or K^+ or both, which is highly chloride dependent, is markedly inhibited by diuretics [2–6,25,26]. The results of the present study show that furosemide and piretanide reduced both the basal and the secretagogue-induced $^{86}Rb^+$ transport. A similar observation with regard to the basal efflux was also made recently by Chipperfield [27]. The results described in this paper suggest that $^{86}Rb^+$ is extruded from the acinar cells by a process which requires chloride and that furosemide and piretanide selectively inhibit the process resulting in a decrease in $^{86}Rb^+$ transport. More recent flame photometric measurements of net K^+ efflux have also demonstrated an acetylcholine-evoked K^+ release which is chloride dependent, and diuretic sensitive [28]. These results strongly suggest the existence of a Cl^--dependent and diuretic-sensitive carrier system for K^+ transport.

The present results also show that secretagogue-evoked $^{86}Rb^+$ transport was sensitive to reductions in extracellular Na^+. When either Li^+ or choline$^+$ was substituted for Na^+, the response to either acetylcholine or caerulein respectively, was almost abolished. In some cell systems there is much evidence that the transport of K^+ is dependent upon extracellular Na^+ [6,25,29] whereas in others a Na^+-independent cotransport of K^+ and Cl^- has been demonstrated [21,24,30,31]. However, when Na^+ was replaced with Tris$^+$ the acetylcholine-elicited $^{86}Rb^+$ efflux was reduced but not abolished. These findings contrast with those observed when using either Li^+ or choline$^+$. The stimulus-induced $^{86}Rb^+$ outflow in the presence of Tris$^+$ was abolished in a Cl^--free medium (nitrate substitution) or in the presence of furosemide. These findings indicate that Tris$^+$ can exert an effect almost similar to Na^+. It is well known that Tris$^+$ can penetrate cell membrane [32] and produce marked physiological effects [33,34]. The removal of extracellular K^+ had virtually no effect on secretagogue-induced $^{86}Rb^+$ efflux. Moreover, acetylcholine is known to elicit marked depolarization and input resistance reduction during K^+ omission [35]. This observation does not support the presence of a quantitatively important K^+-$^{86}Rb^+$ exchange process. It further suggests that agents of depolarization may provide the driving force for K^+ transport. The results with ouabain, in which both acetylcholine and caerulein evoked $^{86}Rb^+$ outflow remained unaltered, suggest that K^+ extrusion operates independently of the ($Na^+ + K^+$)-ATPase pump. These observations, taken together, are consistent with the idea that the movement of K^+ goes by a Na^+-K^+-Cl^- cotransport system. The results of the experiments with Tris$^+$ would suggest that Tris$^+$ can move with the cotransport of K^+ in the mouse pancreas. In this study the stoichiometry of the Na^+-K^+-Cl^- cotransport cannot be assessed since only K^+ was measured. However, in other cell systems some workers have shown that Na^+, K^+ and Cl^- move simultaneously as a neutral complex with a stoichiometry of 1 Na^+, 1 K^+ and 2 Cl^- [4,21,36].

It has long been known that K^+ moves out of

mouse pancreatic acinar cells by a electrodiffusion process [37] through a Ca^{2+}-activated non-discriminatory cation channel [11,12]. This channel has a conductance of about 30–35 pS and can be stimulated by acetylcholine and caerulein in intact cells. Secretagogue-evoked increase in intracellular Ca^{2+} opens up the cation channel resulting in marked Na^+ influx and probably some K^+ efflux. The channel is insensitive to furosemide (Maruyama, Y., personal communication) and operates independently of chloride [7]. The results described in this paper suggest that in addition to the Ca^{2+}-activated cation channel, K^+ extrusion from acinar cells may also be associated with an additional route especially since the removal of chloride and the presence of either furosemide or piretanide markedly inhibit secretagogue-evoked $^{86}Rb^+$ release.

The most straightforward interpretation of the data is presented in a simplified model accounting for K^+ transport (Fig. 9). In this scheme it is envisaged that acetylcholine and caerulein first stimulate their respective receptors leading to an increase in intracellular Ca^{2+} which opens Ca^{2+}-activated non-selective cation channels in the baso-lateral plasma membrane giving rise to Na^+ influx and depolarization. While there would still be a Na^+ gradient in an inward direction this might have been reduced by the stimulation. Hence, the total balance of the electrochemical gradient for Na^+, K^+ and Cl^- could now promote transport in the opposite direction resulting in K^+ extrusion mainly through a K^+-Na^+-Cl^- cotransport carrier system. The cotransporter is sensitive to diuretics and dependent upon Na^+ and Cl^- for its operation. Na^+ extrusion from the acinar cell and K^+ influx are driven by the (Na^+ + K^+)-ATPase pump localized in the acinar cell membrane [16]. At present there is no evidence of location of the Na^+-K^+-Cl^- cotransporter. If it is present in the baso-lateral plasma membrane then it would seem to work against secretion. On the other hand, if it is located in either the apical membrane or both the apical and basolateral membranes then it may play a role in the secretory process in the mouse pancreas. This is an important area which deserves further investigation.

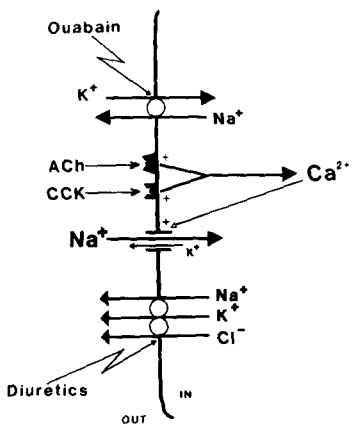

Fig. 9. A possible model to account for K^+ transport across mouse pancreatic acinar cell membrane. In this simplified scheme acetylcholine (ACh) and CCK (caerulein) first elevate intracellular Ca^{2+} which in turn opens Ca^{2+}-activated channels in the baso-lateral membrane resulting in Na^+ influx and depolarization. The latter facilitate K^+ extrusion via a Na^+-K^+-Cl^- cotransport system which is diuretic-sensitive and anion and cation-dependent.

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References

1 Skou, J.C. (1965) Physiol. Rev. 45, 596–617
2 Chipperfield, A.R. (1980) Nature 286, 281–282
3 Chipperfield, A.R. (1981) J. Physiol. 312, 435–444
4 Geck, P., Pietrzyk, C., Burckhardt, B.-C. Pfeiffer, B. and Heinz, E. (1980) Biochim. Biophys. Acta 600, 432–447
5 Dunham, P.B., Stewart, G.W. and Ellory, J.D. (1980) Proc. Natl. Acad. Sci. USA 77, 1711–1715
6 Aiton, J.F., Chipperfield, A.R., Lamb, J.F., Ogden, P. and Simmons, N.L. (1981) Biochim. Biophys. Acta 646, 389–398
7 Maruyama, Y. and Petersen, O.H. (1982) Nature 299, 159–161
8 Maruyama, Y. and Petersen, O.H. (1982) Nature 300, 61–63
9 Maruyama, Y., Gallacher, D.V. and Petersen, O.H. (1983) 302, 827–829
10 Maruyama, Y., Petersen, O.H., Flanagan, P. and Pearson, G.T. (1983) 305, 228–232
11 Petersen. O.H. and Maruyama, Y. (1983) Pflügers Arch. 396, 82–84
12 Petersen, O.H. and Maruyama, Y. (1984) Nature 307, 693–696

13 Case, R.M. and Clausen, T. (1973) J. Physiol. 235, 75–102
14 McCandless, M., Nishiyama, A., Petersen, O.H. and Philpot, H.G. (1981) J. Physiol. 318, 57–71
15 Petersen, O.H. (1982) Biochim. Biophys. Acta 694, 163–184
16 Hootman, S.R., Ernest, S.A. and Williams, J.A. (1983) Am. J. Physiol. G339–G346
17 Singh, J. (1984) J. Physiol. 346, 113P
18 Gallacher, D.V. (1982) Nature 296, 83–86
19 Selinger, Z., Batzri, S., Eimerl, S. and Schramm, M. (1973) J. Biol. Chem. 248, 369–372
20 Bourne, P.K. and Cossins, A.R. (1984) J. Physiol. 347, 361–375
21 Haas, M., Schmidt W.F., III and McManus, T.J. (1982) J. Gen. Physiol. 50, 125–147
22 Simmons, N.L. (1981) Biochim. Biophys. Acta 646, 231–242
23 Lundberg, A. (1956) Nature 177, 1080–1081
24 Kregenow, F.A. (1981) Annu. Rev. Physiol. 43, 493–505
25 Frizell, R.A., Field, M. and Schultz, S.G. (1979) Am. J. Physiol. 236, F1-8
26 Hannafin, J., Kinne-Saffran, E., Friedman, D. and Kinne, R. (1983) J. Membrane Biol. 75, 73–83
27 Chipperfield, A.R. (1984) J. Physiol. 346, 114P
28 Singh, J. (1984) J. Physiol. 353, 82P
29 Petersen, O.H. (1970) J. Physiol. 208, 431–447
30 Dunham, P.B. and Ellory, J.C. (1981) J. Physiol. 318, 511–530
31 Reuss, L. (1983) Nature 305, 723–726
32 Ligou, J.C. and Nahas, G.G. (1960) Am. J. Physiol. 98, 1201–1206
33 Mattiazzi, A.R., Cingolani, H.E. and Gonzalez, N.C. (1972) Am. J. Physiol. 222, 10–15
34 Gillespie, J.S. and McKnight, A.T. (1976) J. Physiol. 259, 561–573
35 Nishiyama, A. and Petersen, O.H. (1975) J. Physiol. 244, 431–465
36 Greger, R. and Schlatter, E. (1983) Pflügers Arch. 396, 325–334
37 Petersen, O.H., Maruyama, Y., Graf, J., Laugier, R., Nishiyama, A. and Pearson, G.T. (1981) Phil. Trans. R. Soc. (Lond) B 296, 151–166