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Ionic requirements in histamine-evoked potassium efflux in guinea pig pancreas

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Guinea pig pancreatic segments were superfused during 10 min with physiological saline solutions containing 10^{-6} M acetylcholine (ACh) or histamine (10^{-3}-10^{-6} M) and the potassium concentration in the effluent ($[K^+]_o$) was measured by flame photometry. Histamine evoked a transient increase in $[K^+]_o$. The removal of calcium from the superfusing solution and addition of 10^{-4} M EGTA caused a significant reduction in the histamine-evoked potassium outflow. Replacement of chloride (Cl^-) in the physiological salt solution by nitrate (NO_3^-) caused a significant reduction in the histamine-evoked potassium release. However, when Cl^- was replaced by bromide (Br^-) the response to histamine was unaffected. Pre-treatment of pancreatic segments with furosemide (10^{-4} M) or ouabain (10^{-3} M) caused a marked reduction in the histamine-induced potassium release. The results suggest that ionic requirements in histamine-evoked potassium release are the same as those in acetylcholine-evoked potassium efflux.

Key words: Histamine, Exocrine pancreas, Potassium, Guinea pig.

Pancreatic acinar cells are known to possess different potassium channels localized in the basolateral plasma membrane which can be activated by intracellular calcium $[Ca^{2+}]_i$ and by membrane depolarization (8).

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Mouse and rat pancreatic acinar cells have Ca^{2+}-activated non selective cation channels that are voltage-insensitive (9, 10, 11). Pig and man pancreatic acinar cells have Ca^{2+}-activated potassium-selective channels voltage-sensitive (16). There are three kinds of potassium channels in the basolateral plasma membrane of guinea pig pancreatic acinar cells: Ca^{2+}-activated non selective cation and two kinds of

Ca^{2+}-activated potassium-selective channels voltage-sensitive, with high and low conductance (22).

Some studies have provided the presence of a Na^+:K^+:Cl^- co-transport in the pancreatic acinar cells of mouse (2, 17), pig and man (16) and guinea pig (18, 19). One study (21) indicates that in the mouse pancreas the co-transport was activated by acetylcholine (ACh) to extrude Na^+, K^+ and Cl^- from the cells whereas the models proposed for the guinea pig, man and pig pancreas (2, 16, 18, 19) were based on this co-transporter mainly functioning as a device for cellular Cl^- accumulation.

Exogenous application of histamine seems to have stimulatory effects on pancreatic secretion in dog (7, 23), pig (3) and *in vitro* rabbit pancreas (6). Activation of H1 receptors in anaesthetized rabbit is also known to be associated with a stimulation of pancreatic exocrine secretion compared to the inhibitory effects observed with H2 receptor activation (13).

In a previous study (18) the histamine-evoked potassium release in guinea pig and mouse pancreatic segments was examined. The use of histamine revealed its dose-dependent stimulatory action on the potassium efflux in both, mouse and guinea pig, species. However, histamine-evoked potassium output was smaller than ACh-evoked potassium efflux; moreover, the responses to both secretagogues were much higher in the mouse than in the guinea pig pancreatic segments.

The present study was designed to test the hypothesis that the ionic requirements for potassium efflux in isolated superfused segments of guinea pig pancreas stimulated by histamine are the same as when these are stimulated by ACh (19).

Materials and Methods

Materials.– Histamine dihydrochloride, furosemide, ethylene-glycol-bis-(β-aminoethylether) N,N'-tetraacetic acid (EGTA), acetylcholine and ouabain were purchased from Sigma. All the other reagents were of the highest commercially available grade.

Animals and experimental design.– Adult male guinea-pigs (250-400 g) were stunned and sacrificed by cervical dislocation, and the pancreas was quickly removed and placed in a modified Krebs-Henseleit solution of the following composition (mM): NaCl, 103; KCl, 4.7; $CaCl_2$, 2.56; $MgCl_2$, 1.13; $NaHCO_3$, 25; $NaHPO_4$, 1.15; D-glucose, 2.8; Na-pyruvate, 4.9; Na-fumarate, 2.7 and Na-glutamate, 4.9. The solution was continuously gassed with 95 % O_2 - 5 % CO_2 mixture and maintained at 37 °C.

The pancreas was cut into small segments (3-5 mg) and a total weight of 50-80 mg was placed into a Perspex flow chamber (0.5 ml volume) and superfused with Krebs-Henseleit solution at a flow rate of 150 µl/min at 37 °C. The effluent from the flow chamber passed directly to an on-line flame photometer (Corning-480) for measurements of potassium concentration at 40 second intervals.

The tissue was superfused for 30-40 min prior to experimentation, the potassium concentration in the effluent ($[K^+]_o$) being stabilized to a steady-state value. During stimulation, the fluid flowing through the chamber was replaced with the appropriate physiological salt solution containing histamine (10^{-3}-10^{-6} M) or ACh (10^{-6} M). The results were expressed as the change in $[K^+]_o$ in mM (100 mg tissue)$^{-1}$ from the steady-state value. The potassium concentrations of all solutions used in every experimental protocol were identical so that changes in potassium concentration in the effluent indicated changes in potassium transport in the pancreatic tissue.

In some experiments the physiological salt solution was modified in several ways.

10^{-4} M EGTA was added to calcium-free solutions. All NaCl, KCl and $MgCl_2$ in Cl—free solutions were replaced by equivalent amounts of either nitrates (NO_3^-) or bromides (Br^-) to attain appropriate osmolarity.

Statistical treatment.– Results are expressed as means ± standard errors of the mean (SEM). The resting basal values for K^+ efflux were obtained from the mean (±SEM) of the three points (e.g. 40 s, 80 s and 120 s) prior to secretagogue application. The maximal increase was calculated at the peak of the response, 2 to 3 min after the stimulus application and expressed as mean ± SEM. Significance of data was assessed by Student's *t* -test, and a level of $P<0.05$ was considered to be significant.

Results

Effect of histamine on potassium transport.– Fig. 1 shows time course changes in the potassium concentration in the effluent $[K^+]_o$ from the tissue flow chamber in response to 10^{-4} M of histamine and 10^{-6} M of acetylcholine. Stimulation of guinea-pig pancreatic segments with ACh (10^{-6} M) resulted in a rapid and marked transient potassium release. The maximal increase in $[K^+]_o$ obtained at the peak of the response after application of 10^{-6} M of ACh was 0.12 ± 0.02 mM (100 mg tissue)$^{-1}$. However, the value obtained at the peak of the response after stimulation with 10^{-4} M of histamine (0.068 ± 0.006 mM (100 mg tissue)$^{-1}$) was significantly smaller than the response obtained with 10^{-6} M of ACh. The maximal efflux occurs after 2–4 min of stimulation with both secretagogues.

Fig. 2 shows the maximal responses obtained with different concentrations of histamine. The highest response was obtained at 10^{-4} M histamine which was not significantly different from 10^{-3} M or 10^{-5} M-evoked potassium release (0.054 ± 0.01 or 0.056 ± 0.014 mM (100 mg tissue)$^{-1}$, respectively). However, these val-

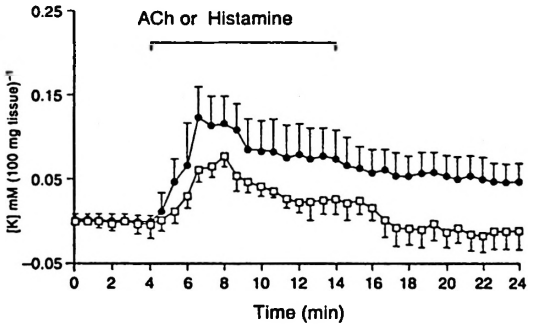

Fig 1. *Time course effect of 10^{-6} M ACh (solid circles) or 10^{-4} M histamine (open squares) on the potassium concentration between inflowing and outflowing fluid of a superfusion chamber containing segments of guinea-pig pancreas.*
Positive values indicate that the concentration of K^+ in effluent is higher (i.e.: K^+ release or efflux). ACh and histamine was added to the superfusion medium as indicated by the horizontal bar. Each point is mean ± SEM (n = 7).

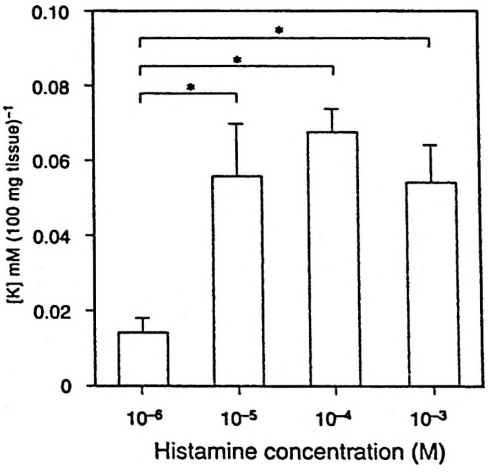

Fig 2. *Maximal increases in potassium concentration in the effluent in response to varying concentrations of histamine (10^{-3} - 10^{-6} M).*
Values are mean ± SEM (n = 6). Asterisk indicates significant differences between the doses of histamine. *$p<0.05$.

ues are statistically higher than the 10^{-6} M histamine-evoked potassium release (0.014 ± 0.004 mM (100 mg tissue)$^{-1}$). Due to this, the nature of the histamine-induced response by using the dose that caused the maximal increase in $[K^+]_o$, 10^{-4} M of histamine was examined in greater detail.

Effect of Ca^{2+} removal on histamine evoked potassium efflux.– The time course effect of 10^{-4} M of histamine on the mean (± S.E.M.) change in $[K^+]_o$ in the absence of extracellular calcium is shown in fig. 3. The maximal increase obtained in this condition was 0.049 ± 0.006 mM (100 mg tissue)$^{-1}$; this value was significantly different from the resting basal values. The response obtained in the absence of extracellular calcium was significantly smaller than that obtained in a normal superfusion medium.

Effect of Cl^- removal on histamine-evoked potassium efflux.– The effect of Cl^- substitution on the histamine-evoked potassium release was investigated. Fig. 4 shows the time course changes in $[K^+]_o$ following 10^{-4} M histamine application in the absence of Cl^-. Cl^- in the superfusing medium was replaced by either NO_3^- or

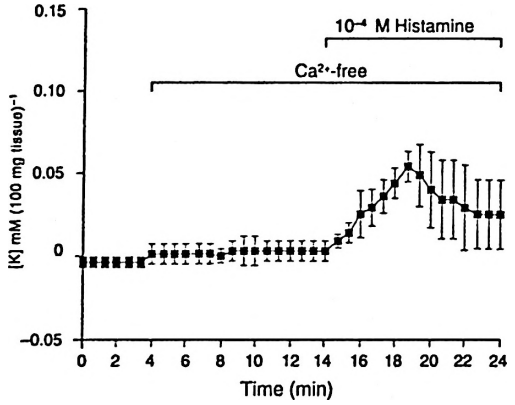

Fig 3. *Effect of 10^{-4} M histamine on the potassium concentration in the effluent in the absence of extracellular Ca^{2+}.*
EGTA (10^{-4}M) was present in the Ca^{2+}-free medium. Horizontal bars indicate the period of stimulation and Ca^{2+} removal. Each point is mean ± SEM (n = 5).

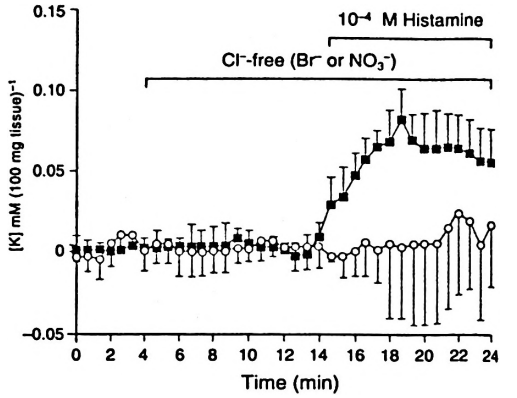

Fig 4. *Effect of 10^{-4} M histamine on the potassium concentration in the effluent in the absence of Cl^-. (Br^- substitution, solid squares and NO_3^- substitution, open circles). Horizontal bars indicate the duration of ACh stimulation and Cl^- removal.* Each point is mean ± SEM (n = 4-6).

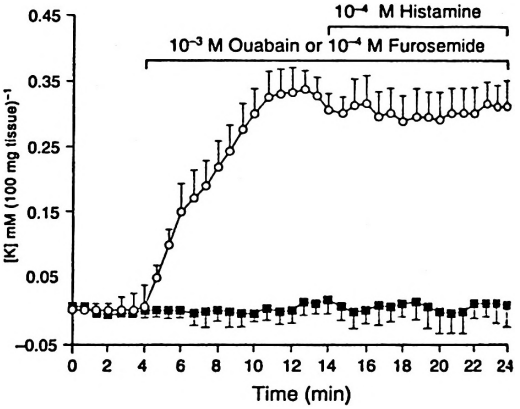

Fig 5. *Effect of 10^{-4} M histamine on the potassium concentration in the effluent in presence of 10^{-3} M ouabain (open circles) or 10^{-4} M furosemide (solid squares) in the superfusion medium.*
The tissue was pretreated with ouabain or furosemide for 10 min prior to histamine application. Horizontal bars indicate the duration of histamine stimulation and ouabain or furosemide perfusion. Each point is mean ± SEM (n = 5).

Br^-. The substitution of Cl^- by NO_3^- completely inhibited the histamine-evoked potassium release. When Br^- was substituted for Cl^- the histamine-evoked potassium outflow remained virtually unchanged (0.073 ± 0.009 mM (100 mg tissue)$^{-1}$). Substitution of Cl^- by NO_3^- or Br^- did not modify the resting $[K^+]_o$.

Effect of furosemide or ouabain on histamine-evoked potassium efflux.– The effect of the presence of ouabain (10^{-3} M) or the loop diuretic furosemide (10^{-4} M) in the superfusing medium on histamine-evoked potassium efflux is shown in fig. 5. Ouabain itself caused a sustained and significant increase in $[K^+]_o$ and inhibited the histamine-induced increase in $[K^+]_o$, indicating that activity of the Na^+-K^+ ATPase pump is associated with histamine-induced potassium release. On the other hand, 10^{-4} M of the loop diuretic furosemide itself had no effect on potassium concentration of the effluent, but abolished the histamine-evoked potassium release ($p<0.05$).

Discussion

Histamine has long been recognized as an important chemical messenger communicating information from one cell to another. Contrary to initial studies suggesting that histamine had no secretagogue effects on the guinea pig pancreas (6), posterior findings have demonstrated clearly that histamine can, indeed, stimulate amylase secretion (13). Moreover, the present study demonstrates that histamine may evoke marked potassium release from guinea pig pancreatic segments, although being not as potent a secretagogue as ACh.

In the absence of any stimuli in guinea pig pancreatic segments there is a passive potassium efflux continuously balanced by the activity of a Na^+:K^+ pump (19).

Previous studies (17, 18, 19) showed that stimulation of pancreatic segments with ACh causes a net potassium efflux. Present and previous (18) results demonstrate that histamine can elicit a marked potassium transport in guinea pig pancreatic segments. Both, ACh- and histamine receptor activation releases calcium from an intracellular store (14), and the resulting increase in cytosolic calcium concentration opens calcium-dependent potassium channels on the basolateral membrane (15). Some authors (22) have previously described three types of potassium channels in the guinea pig pancreatic acinar cells: a calcium activated non-selective cation channels and two kinds of calcium-activated potassium selective channels with 200 and 30 pS of conductance, respectively.

The most important channels responsible for the secretagogue-evoked potassium efflux are the calcium activated potassium selective ones (22). The present results show that histamine-evoked potassium release was significantly reduced during exposure of the pancreatic segments to Ca^{2+}-free solution. The histamine-evoked amylase release was associated with a small elevation in cytoplasmic free Ca^{2+} concentration $[Ca^{2+}]_i$ and a transient efflux of $^{45}Ca^{2+}$ (20). These findings support the hypothesis that in guinea pig exocrine pancreas histamine acts via Ca^{2+}-activation of potassium selective channels leading to potassium efflux.

As in ACh-evoked potassium release, histamine-evoked potassium transport in guinea pig pancreas compresses an efflux potassium pathway following secretagogue stimulation. This secretagogue-evoked potassium release is sensitive to the replacement of Cl^- by NO_3^-, which suggests that a potassium extrusion is linked to the movements with other ions.

In previous studies (15) different models of ion co-transports have been proposed for distinct animal species; for example, in rat the ionic transport occurs by a combination of Na^+:H^+ and Cl^-:HCO_3^- exchanges, but in mouse (17), pig (15) and guinea pig (19) the ionic co-transport can be explained by a Na^+:K^+:$2Cl^-$ co-transport. The present results show that Cl^- and Br^- can support this histamine evoked potassium efflux, whereas NO_3^- cannot. Moreover the loop diuretic furosemide completely abolished the potassium release evoked by histamine. Br^- is as good an exchanger as Cl^- in the Na^+:K^+:$2Cl^-$ co-transport, NO_3^- being much less efficient (17). With regard to the Cl^-:HCO_3^- exchanger it is well known that NO_3^- is an equally good exchanger partner for Cl^- as Cl^- itself while Br^- is less efficient (15, 17). The present results are most easily interpreted by assuming that histamine-evoked potassium efflux, as well as ACh, requires the existence of linked Na^+, K^+ and Cl^- movements through a Na^+:K^+:$2Cl^-$ co-transporter which moves the ions from the cell exterior to the interior.

Ouabain, a Na^+:K^+ ATPase pump inhibitor, was found to enhance potassium release and to abolish potassium efflux in the presence of 10^{-4} M histamine, which is consistent with the findings where carbachol stimulates Na^+:K^+ pump activity, as measured by changes in the kinetics of [$^3H^+$]-ouabain binding to dispersed guinea pig pancreatic acinar cells (4, 5). As it occurs in histamine-evoked potassium release, ouabain completely inhibited the potassium efflux in the presence of 10^{-6} M ACh (19).

This study shows that both, ACh and histamine-evoked potassium release, have the same ionic requirements and can be explained by the previously described model (19). Briefly, activation in guinea pig receptor causes the opening of a large calcium-activated potassium channel on the basolateral membrane leading to potassium efflux. Released potassium is then introduced into the acinar cell by the Na^+:K^+ ATPase pump and the electroneutral, diuretic sensitive Na^+:K^+:$2Cl^-$ cotransporter localized in the basolateral membrane. In the steady secreting state, potassium, as well as Na^+, recirculates via the channel, cotransporter and pump. The only net transport is that of Cl^- uptake where the cotransporter acts as a chloride pump, using the energy of the Na^+ gradient to increase the intracellular Cl^- concentration above electrochemical equilibrium. Cl^- leaves the cell via the luminal Ca^{2+}-activated Cl^- channels (15) which causes an electronegative gradient in the lumen allowing Na^+ to move between the cells through the narrow intracellular spaces and the tight junctions placed at their luminal end (15). Cholecystokinin in guinea pig pancreas is also known to evoke the secretion of a large volume of HCO_3^- -rich fluid (12). Moreover, some authors (1) have demonstrated the existence of a Na^+:H^+ antiporter activated by cerulein and gastrin in isolated acini prepared from guinea pig pancreas. The Na^+:H^+ antiporter activation is likely coupled to an HCO_3^- secretion. The HCO_3^- ions are then thought to exit across the apical membrane probably by a Cl^-:HCO^- exchanger.

The net result of all these transport events, together with water movements by osmosis, is the formation of an isotonic Na^+-HCO_3^- -rich fluid (12).

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J. A. ROSADO, L. J. GARCÍA y G. M. SALIDO. *Requerimientos iónicos en la salida de potasio estimulada por histamina en páncreas de cobaya.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (2), 231-238, 1997.

En segmentos pancreáticos de cobaya perfundidos durante 10 minutos en una solución salina fisiológica con ACh (10^{-6} M) o histamina (10^{-3}-10^{-6} M) se mide por fotometría de llama la concentración de potasio en el efluente ($[K^+]_o$). La histamina produce un incremento transitorio en $[K^+]_o$, que es significativamente disminuído por la ausencia de calcio con adición de EGTA (10^{-4} M) en el medio de perfusión. La sustitución del Cl^- por el ion nitrato en el medio de perfusión causa una reducción significativa en la salida de K^+ estimulada por histamina, en tanto que la sustitución por el bromuro no modifica la respuesta. El tratamiento de los segmentos pancreáticos con furosemida (10^{-4} M) u ouabaina (10^{-3} M) causa una marcada reducción de la liberación de K^+ estimulada por histamina. Estos resultados sugieren que los requerimientos iónicos necesarios para el proceso de liberación de potasio estimulada por histamina son los mismos que los que intervienen en la salida de potasio provocada por la acetilcolina.

Palabras clave: Histamina, Páncreas exocrino, Potasio, Cobaya.

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