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Are Cl^- Mechanisms in Mouse Pancreatic Islets involved in Insulin Release?

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INTRODUCTION

A number of ion mechanisms are involved in the stimulus-secretion coupling in the pancreatic β-cells. We have previously produced evidence that transmembrane Cl^- fluxes may be important for normal insulin release (11, 12) and that disturbed Cl^- fluxes may be connected with the deterioration of insulin release in diabetic mouse β-cells (2). The Cl^- mechanisms operating in mouse pancreatic islets appear to consist of both a metabolism-dependent inward transport of Cl^- against its electrochemical potential gradient and an efflux controlled by the islet cell Cl^- permeability and the magnitude of an outwardly directed electrochemical potential gradient (11, 12). The work presented here tested further the possible relationship between transmembrane Cl^- fluxes and insulin release by using either insulin secretagogues, i.e. L-leucine, glibenclamide or theophylline or the Cl^- transport inhibitor furosemide.

METHODS

Adult, non-inbred, obese-hyperglycemic (ob/ob) mice of the Umeå colony were starved overnight. Pancreatic islets were isolated by free-hand microdissection (10) and exposed to a preliminary incubation for 30 min at 37°C. The basal medium in all incubations had approximately the same composition as Krebs-Ringer bicarbonate medium except that the bicarbonate was replaced by 20 mM 2-(N-hydroxyethylpiperazine-N'-yl)ethane-sulphonic acid (Hepes) and 3 mM D-glucose was added. The gas phase was ambient air. When insulin release was studied, bovine serum albumin (1 mg/ml) was added. The Cl^- concentration was 139.8 mM.

After the preliminary incubation, batches of 2–5 islets were incubated for various periods of time in basal medium supplemented as described in the Results and Discussion. In studies of $^{36}Cl^-$ uptake, this medium was labelled

with $^{36}Cl^-$ (The Radiochemical Centre, Amersham, Bucks., England) and at the end of incubation the islets were washed for 2 min at 0°C as previously described (11). For measurement of $^{36}Cl^-$ efflux, islets were labelled with $^{36}Cl^-$ as described above for 30 or 60 min (apparent isotope equilibrium; reference 11) and then transferred to large volumes (5 ml) of non-radioactive medium with or without test substances. After the incubations the islets were transferred to pieces of aluminium foil, freed of contaminating medium and frozen in melting isopentane (-165°C). They were then freeze-dried overnight, weighed on a glass-fibre balance and their radioactivity measured in a liquid-scintillation spectrometer. Aliquots (5 µl) of each incubation medium were used as external standards to determine the specific radioactivity. Data were expressed as mmol of Cl^- labelled with $^{36}Cl^-$ to the same specific radioactivity as in the incubation medium. Insulin was determined radioimmunologically using crystalline mouse insulin for reference.

RESULTS AND DISCUSSION

Previous results showed that D-glucose or D-mannose but not L-glucose or 3-O-methyl-D-glucose accelerated $^{36}Cl^-$ efflux from prelabelled ob/ob-islets (11). The D-glucose effect was reproduced in islets from lean C57BL/KsJ mice (2). The action of two other stimulators of insulin release, 20 mM L-leucine or 20 or 50 µM glibenclamide, is depicted in Table 1. Pancreatic islets were prelabelled with $^{36}Cl^-$ for 30 min and then transferred to 5 ml of non-radioactive medium with 3 mM D-glucose and with or without test substance. Data show amount of labelled Cl^- after the 5-min-efflux period. As shown, both

Table 1. Effects of L-leucine or glibenclamide on islet efflux of labelled Cl^-

Test substance	Islet content of labelled Cl^- (mmol/kg dry islet weight)	
	Primary data	Difference from control
None (control)	40.04 ± 3.69	-
L-leucine, 20 mM	26.87 ± 2.77	-13.16 ± 4.16**
Glibenclamide, 20 µM	29.52 ± 2.89	-10.52 ± 2.98***
Glibenclamide, 50 µM	29.31 ± 2.16	-10.73 ± 3.94*

Mean values ± SEM for 8 experiments. The effect of test substance was evaluated by t-testing differences between parallel incubations. *P<0.05, **P<0.02, ***P<0.01.

L-leucine and glibenclamide stimulated the efflux, thus strengthening the proposition that there is a correlation between insulin release and $^{36}Cl^-$ efflux.

Cl^- transport in some other tissues, such as frog cornea (1, 8), intestinal

mucosa (9) or blowfly salivary glands (3) is stimulated by cyclic AMP. Since cyclic AMP is also known to be involved in the regulation of insulin release, the effect of theophylline-induced change in cyclic AMP concentrations on $^{36}Cl^-$ fluxes in islet cells was tested. Table 2 confirms previous results (11, 12) showing that D-glucose increases the rate of $^{36}Cl^-$ efflux. However, 2 mM theophylline did not change the $^{36}Cl^-$ efflux at 3 mM D-glucose and slightly reduced the efflux at 20 mM D-glucose. Table 3 indicates that 1-5 mM theophylline did not affect short-term uptake of $^{36}Cl^-$ nor the equilibrium con-

Table 2. Effects of theophylline on basal and D-glucose-induced $^{36}Cl^-$ efflux for 5 min.

D-glucose concn.	Islet content of labelled Cl^- (mmol/kg dry islet weight)		
	No theophylline	2 mM theophylline	Effect of theophylline
3 mM	41.07 ± 0.96	41.68 ± 2.47	0.61 ± 2.28
20 mM	23.75 ± 1.26	25.06 ± 1.23	1.31 ± 0.42*
Effect of D-glucose	-17.31 ± 1.15**	-16.61 ± 1.74**	0.70 ± 2.20

Mean values ± SEM for 6 experiments. Statistical significances tested as in Table 1. *P<0.05, **P<0.001.

Table 3. Effect of theophylline on islet uptake of labelled Cl^-.

Theophylline concn.	Islet content of labelled Cl^- (mmol/kg dry islet weight)
	Uptake for 3 min
0 (control)	38.80 ± 2.83
1 mM	38.30 ± 5.88
2 mM	37.18 ± 2.52
5 mM	37.64 ± 3.69
	Uptake for 60 min
0 (control)	92.67 ± 1.35
5 mM	90.27 ± 4.07

Mean values ± SEM for 5 experiments. Statistical significances tested as in Table 1. All P>0.05.

centration of $^{36}Cl^-$ in islet cells. This lack of effect of theophylline may indicate a difference between the Cl^- transporting systems in islet cells as compared with those in corneal cells, intestinal epithelium or blowfly salivary glands. Additional experiments may, however, be necessary to rule out the possibility of cAMP-sensitive Cl^- transport in islet cells.

Furosemide, a potent diuretic drug, is thought to exert its diuretic action mainly by blocking active Cl^- transport in the thick ascending limb of the loop of Henle (5, 6). Furosemide also inhibits active Cl^- transport in the cornea (7) and Cl^- exchange across the erythrocyte membranes (4). It was there-

fore of interest to test whether furosemide would affect $^{36}Cl^-$ fluxes and insulin release in isolated pancreatic islets. Fig. 1 shows that the 5-min-uptake of $^{36}Cl^-$, representing the Cl^- influx rate, was reduced by 10-1000 μM furosemide, whereas the rate of efflux was not affected. This resulted in a considerable drop (about 40 %) in the islet cellular concentration of labelled Cl^- at apparent isotope equilibrium (data not shown). The capacity of D-glucose to increase $^{36}Cl^-$ efflux rate was not affected by 100 μM furosemide (data not shown).

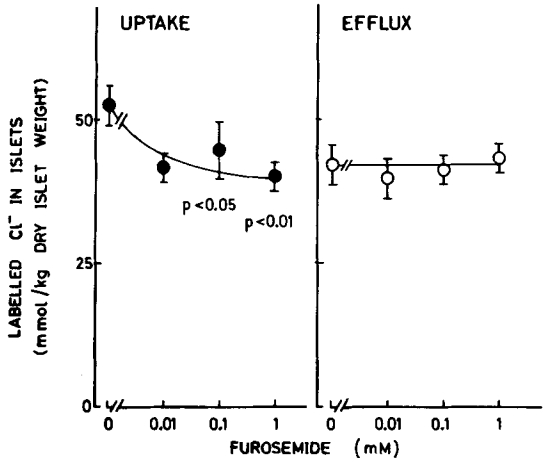

Fig. 1. Effect of furosemide on islet uptake and efflux of labelled Cl^-. Pancreatic islets were preincubated for 30 min in basal medium. In uptake experiments they were then incubated for 5 min in the same type of medium also supplemented with $^{36}Cl^-$ and different concentrations of furosemide. In efflux experiments the islets were labelled for 30 min in basal medium supplemented with $^{36}Cl^-$ and then transferred to large volumes (5 ml) of non-radioactive medium and incubated with different concentrations of furosemide for 5 min. Data denote islet content of labelled Cl^- after completed incubation. Mean values ± SEM for 4 (uptake) or 6 (efflux) experiments.

As suggested by previous results (11), the pancreatic β-cells may have an inward transport of Cl^- against its electrochemical gradient and a Cl^- efflux, the rate of which is determined by the electrochemical gradient and the β-cell Cl^- permeability. Data have also suggested that Cl^- efflux may be electrogenic and that secretagogue-induced increase of Cl^- permeability may be a link in the β-cell stimulus-secretion coupling (11, 12). If this hypothesis were correct, a reduction by furosemide of the inward Cl^- pumping, leading to a reduction of the electrochemical potential gradient for Cl^-, would be expected also to inhibit insulin release. Figure 2 shows that this is indeed the case. Exposing ob/ob-islets to 10-100 μM furosemide markedly reduced insulin release induced by D-glucose but did not markedly influence basal

insulin release at 3 mM D-glucose.

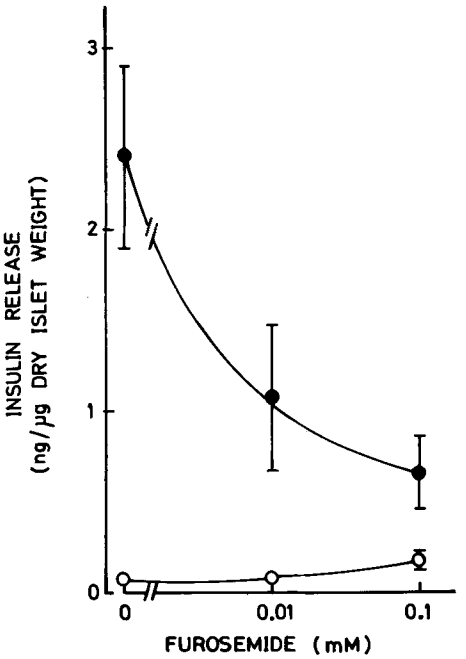

Fig. 2.Effect of furosemide on insulin release. Isolated pancreatic islets were first preincubated for 30 min in basal medium. They were then incubated for 60 min in the same type of medium containing 3 or 20 mM D-glucose and different concentrations of furosemide. Insulin release into this medium was determined. Mean values ± SEM for 4-8 experiments.

The present data support the view that transmembrane Cl^- transport and Cl^- permeability in the pancreatic β-cells are involved in the regulation of insulin release.

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