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Inhibition by hydrochlorothiazide of insulin release and calcium influx in mouse pancreatic β-cells

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1 The effect of hydrochlorothiazide on insulin release, $^{36}Cl^-$ fluxes and $^{45}Ca^{2+}$ uptake was tested in β-cell-rich mouse pancreatic islets.

2 At high glucose concentrations (10 and 20 mmol l^{-1}), low concentrations of hydrochlorothiazide (0.1–1.0 μmol l^{-1}) reduced insulin release by 22–42%. At lower glucose concentrations (3–8.5 mmol l^{-1}) insulin release was not affected by the drug.

3 Neither short-term influx (3 min) nor net accumulation (60 min) of $^{36}Cl^-$ in the islets was affected by hydrochlorothiazide (0.1–500 μmol l^{-1}).

4 Glucose-stimulated $^{45}Ca^{2+}$ uptake was significantly reduced by hydrochlorothiazide (1–10 μmol l^{-1}).

5 The data suggest that the diabetogenic effect of hydrochlorothiazide, at least in part, can be mediated by direct inhibition of insulin release from the pancreatic β-cells. The inhibition is not mediated by reduced chloride fluxes but may rather be caused by inhibition of calcium uptake.

Keywords: Hydrochlorothiazide; diuretics; β-cells; diabetes mellitus; insulin release; Cl^-; Ca^{2+}

Introduction

Thiazide diuretics may aggravate or even induce diabetes mellitus (Wilkins, 1959; Goldner *et al.*, 1960; Amery *et al.*, 1978) but the underlying mechanisms are largely unknown. Pancreatic as well as extra pancreatic mechanisms have been suggested to explain this diabetogenic action (for review, see Furman, 1981; Brass, 1984).

From studies with human patients it was proposed that thiazide-induced impairment in glucose tolerance is caused by a reduced β-cell response to glucose that is secondary to diuresis-induced hypokalaemia (Helderman *et al.*, 1983). However, it has also been suggested that hypokalaemia may be an aggravation factor rather than a primary factor for development of diuretic-induced disturbances in glucose tolerance (for review, see Furman, 1981).

The few *in vitro* studies on the effect of thiazides on insulin release are contradictory. Malaisse & Malaisee-Lagae (1968) suggested that insulin release is not affected, but it has also been proposed that it can be stimulated by thiazide diuretics (Hermansen *et al.*, 1985). Thus, it is not known whether thiazide-induced impairment of glucose tolerance can be explained by a direct effect on the pancreatic β-cells. Therefore, in the present study, the effect of hydrochlorothiazide on insulin release from isolated β-cell-rich mouse pancreatic islets and the effect on key processes in the stimulus-secretion coupling pathway were investigated.

Methods

Animals and isolation of islets

Eight months old ob/ob mice from the Umeå colony were used as source of pancreatic islets. The hyperplastic islets of these mice contain a high proportion of normal β-cells (>90%, Hellman, 1965) that respond adequately to various stimulators and inhibitors of insulin release (Hahn *et al.*, 1974; Hellman *et al.*, 1974). After overnight food deprivation to normalize the blood sugar (Sandström & Sehlin, 1988a) their pancreatic islets were dissected out without the use of enzymes.

Solutions

A Hepes-buffered Krebs-Ringer solution with the following composition was used as basal medium (mmol l^{-1}): NaCl 130, KCl 4.7, $CaCl_2$ 2.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES 20 and D-glucose 3. The medium was supplemented with 1 mg ml^{-1} bovine serum albumin and the pH was adjusted to 7.4 with NaOH.

Hydrochlorothiazide was obtained from Sigma (St. Louis, MO, U.S.A.) and dissolved in basal medium. The pH of the basal medium was not changed by the addition of the drug.

Insulin release

After a preincubation for 30 min in basal medium, groups of 2–3 islets were incubated for 60 min in 300 μl basal medium with or without test substance. The islets were then freeze-dried (−40°C, 0.1 Pascal) and weighed on a quartz-fibre balance. Samples from the incubation medium were assayed for insulin by standard radioimmunoassay (Heding, 1966), using crystalline mouse insulin as standard.

$^{36}Cl^-$ uptake

After preincubation for 30 min in basal medium, groups of 4–5 islets were incubated for 3 or 60 min in control or test medium labelled with $^{36}Cl^-$ (specific radioactivity, 1.9–2.6 GBq mol^{-1}). Then, the islets were washed for 2 min in ice-cold basal medium (0°C) to remove extracellular radioactivity. The islets were freeze-dried and weighed as above and the islet radioactivity was measured by liquid scintillation counting. The method is described in detail elsewhere (Sehlin, 1978).

$^{45}Ca^{2+}$ uptake

The islets were pre-incubated in basal medium as above and then groups of 4–5 islets were incubated for 10 min in control or test medium labelled with $^{45}Ca^{2+}$ (specific activity, 0.3 TBq/mol). Contaminating extracellular radioactivity was removed by washing for 60 min in 2 mmol l^{-1} $LaCl_3$. After freeze-drying and weighing as above, the islet radioactivity was measured by liquid scintillation counting. The method is described in detail elsewhere (Hellman *et al.*, 1976).

Statistics

In the experimental situation, control and test islets were handled in parallel. The differences between control and test groups were analysed by Student's paired *t* test for the number of experiments indicated in the figures and table.

Results

Effect on insulin release

As shown in Figure 1, the effect of hydrochlorothiazide on insulin release was complex. At 10 mmol l^{-1} D-glucose, the release was reduced by 42% by 1 μmol l^{-1} hydrochlorothiazide. At an intermediate concentration (10–100 μmol l^{-1}) no effect was observed, and at a high concentration of hydrochlorothiazide (1 mmol l^{-1}), a potentiation of insulin release was registered. At the higher glucose concentration (20 mmol l^{-1}) a similar pattern was observed. Thus, at 20 mmol l^{-1} D-glucose, a low hydrochlorothiazide concentration (0.1 μmol l^{-1}) reduced insulin release by 22%, whereas at higher concentrations no effect was observed.

At lower glucose concentrations (3–8.5 mmol l^{-1}) hydrochlorothiazide (0.1–1000 μmol l^{-1}) had no significant effect on insulin release (data not shown).

Effect on $^{36}Cl^-$ influx

Inhibition of $^{36}Cl^-$ accumulation in the β-cells is associated with reduced insulin release (Sehlin, 1981; Sandström & Sehlin, 1988b; Sandström, 1990). Since hydrochlorothiazide is an inhibitor of Cl^- transport (Na^+, Cl^- co-transport) in other cell types (for review, see O'Grady *et al.*, 1987) its effect on

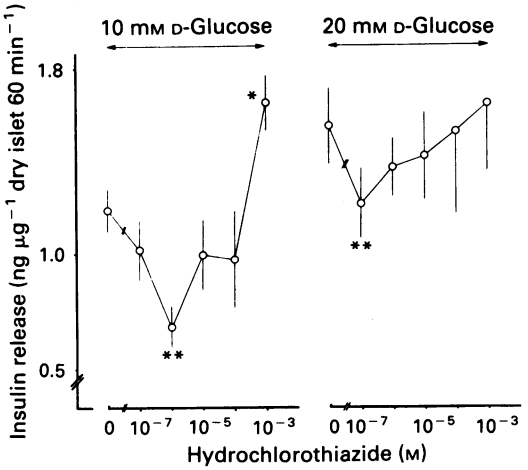

Figure 1 Effect of hydrochlorothiazide on insulin release. After a preliminary incubation for 30 min in basal medium, groups of 2–3 islets were incubated for 60 min in basal medium containing the concentrations of D-glucose and hydrochlorothiazide indicated in the figure. Data are expressed as means ± s.e.mean for 16 experiments. Differences from control were evaluated by Student's *t* test for paired data. $*P<0.05$, $**P<0.025$.

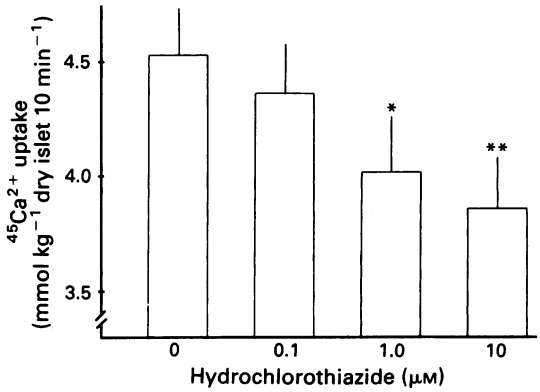

Figure 2 Effect of hydrochlorothiazide on $^{45}Ca^{2+}$ uptake. After a preliminary incubation for 30 min in basal medium, groups of 4–5 islets were incubated for 10 min in basal medium supplemented with $^{45}Ca^{2+}$, 10 mmol l^{-1} glucose and the concentrations of hydrochlorothiazide indicated. Data are expressed as means ± s.e.mean for 20 experiments. Differences from control were evaluated by Student's *t* test for paired data: $*P<0.05$, $**P<0.025$.

$^{36}Cl^-$ influx was also investigated in the β-cells. As shown in Table 1 hydrochlorothiazide had no effect on short-term uptake (3 min) or the accumulation (60 min) of $^{36}Cl^-$ in the islets.

Effect on $^{45}Ca^{2+}$ uptake

Islet uptake of calcium is one of the most distal steps in the stimulus-secretion coupling sequence in the pancreatic β-cells (for review, see Wollheim & Sharp, 1981), and therefore the effect of hydrochlorothiazide on this process was investigated. As shown in Figure 2, the $^{45}Ca^{2+}$ uptake was reduced in a dose-dependent manner by 0.1–10 μmol l^{-1} hydrochlorothiazide.

Discussion

The data in the present work suggest that the adverse effect on carbohydrate metabolism by thiazide diuretics can be mediated by a direct effect on the β-cells. An important observation was that only low concentrations of hydrochlorothiazide inhibited insulin release and that this was observed only when hormone release was stimulated by high (10 and 20 mmol l^{-1}) glucose concentrations. This may help to explain that previous *in vitro* studies with thiazide diuretics showed no effect (Malaisse & Malaisse-Lagae, 1968) or even a stimulation of insulin release (Hermansen *et al.*, 1985). Thus, in the study by Hermansen and co-workers (1985), a

Table 1 Effect of hydrochlorothiazide on $^{36}Cl^-$ influx

	$^{36}Cl^-$ *influx* (mmol kg^{-1} dry islet) *Hydrochlorothiazide* (μmol l^{-1})					
Influx period (min)	*0 (control)*	*0.1*	*1.0*	*10*	*100*	*500*
3 (exptl. series I)	51.65 ± 3.45 (12)	47.32 ± 2.40 (12)	49.62 ± 2.35 (12)	48.89 ± 2.27 (12)		
3 (exptl. series II)	45.05 ± 2.18 (10)			41.02 ± 2.34 (10)	41.061 ± 1.75 (10)	45.59 ± 2.18 (10)
60	82.93 ± 1.78 (14)	80.53 ± 2.50 (14)	84.62 ± 1.40 (12)	80.67 ± 2.63 (14)		

After a preliminary incubation for 30 min in basal medium, groups of 4–5 islets were incubated for 3 or 60 min in basal medium supplemented with $^{36}Cl^-$ and the concentrations of hydrochlorothiazide indicated. Data are expressed as means ± s.e.mean for the number of experiments indicated in parentheses. Differences from control were evaluated by use of Student's *t* test for paired data.

low concentration of thiazide (3 μmol l^{-1}) was tested only at 5.5 mmol l^{-1} D-glucose and no effect was detected. At a higher glucose concentration, where an inhibitory effect of 0.1–1 μmol l^{-1} thiazide was observed in the present study, only high thiazide concentrations (150 μmol l^{-1}) were tested and a stimulation was registered.

In patients treated with hydrochlorothiazide (75 mg/day), the steady state plasma concentration is about 0.4 μmol l^{-1} (Beerman & Groschinsky-Grind, 1978). Therefore, it seems reasonable to believe that the inhibitory effect of low thiazide concentrations on stimulated insulin release observed here could help to explain the adverse action on carbohydrate metabolism seen in clinical practice. Since hydrochlorothiazide never reaches millimolar concentrations in human patients, the stimulatory effect of the high thiazide concentrations on insulin-release is probably not of clinical interest.

The exocytotic release of insulin stimulated by D-glucose is preceded by a number of steps involving depolarization of the plasma membrane by inhibition of K^+ channels, opening of Cl^- channels, and finally the opening of voltage-regulated Ca^{2+} channels, resulting in an increase in $[Ca^{2+}]_i$ (Sehlin, 1978; Wollheim & Sharp, 1981; Pedersen & Findlay, 1987). Thiazide diuretics are sulphonamides, and it is well known that a structurally related compound, diazoxide, hyperpolarizes the β-cells by opening K^+ channels, and that this leads to reduced insulin release (Malaisse & Malaisse-Lagae, 1968; Henquin & Meissner, 1982; Trube *et al.*, 1986). In a recent study it was shown that thiazide diuretics do not affect the activity of K^+ channels in the β-cell plasma membrane (Gillis *et al.*, 1989), and therefore, it seems likely that the inhibitory action of thiazides is not mediated by a hyperpolarization of the β-cell plasma membrane.

It was proposed that the inhibitory effects of loop diuretics (frusemide or bumetanide) on insulin release is due to reduced intracellular chloride levels in the β-cells before stimulation with high glucose concentration (Sandström & Sehlin, 1988b; Sandström, 1990). This reduction is mediated by inhibition of a loop diuretic-sensitive Na^+, K^+, Cl^- co-transport system that functions as a chloride pump in the β-cell plasma membrane (Lindström *et al.*, 1988; Sandström & Sehlin, 1988b; Sandström, 1990; Sandström & Sehlin, 1990). Since thiazide diuretics may reduce chloride transport by inhibition of a Na^+, Cl^- co-transport system (Stokes, 1984), it could be speculated that the inhibitory effect on insulin release is mediated by inhibition of chloride transport. However, in the present study neither the influx nor the accumulation of $^{36}Cl^-$ in the β-cells was affected by hydrochlorothiazide. This shows that the effect of thiazide diuretics on insulin release is not mediated by changed chloride transport and suggests that thiazides and loop diuretics inhibit insulin release by different mechanisms. The lack of effect of hydrochlorothiazide on chloride transport in β-cell-rich islets also shows that a Na^+, Cl^- co-transport system is not present in the β-cell plasma membrane.

The uptake of calcium is one of the distal steps preceding the exocytotic release of insulin (Wollheim & Sharp, 1981). In the present study it was found that the uptake of calcium was significantly reduced by thiazide concentrations that inhibit glucose-induced insulin release. Therefore, it is tempting to speculate that the effect of low concentrations of hydrochlorothiazide on insulin release is, at least in part, mediated by an effect on calcium uptake in the β-cells.

The data in the present study show that thiazide diuretics, in concentrations that are clinically relevant, inhibit glucose-induced insulin release by a direct effect of the pancreatic β-cells. This observation may help to explain the adverse effect on carbohydrate metabolism in human patients. It is suggested that the inhibitory effect of low thiazide concentrations is mediated by an effect on calcium influx in the β-cells.

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