Possible role of carbonic anhydrase in rat pancreatic islets: enzymatic, secretory, metabolic, ionic, and electrical aspects


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The presence of carbonic anhydrase (type V) was recently documented in rat and mouse pancreatic islet β-cells by immunostaining and Western blotting. In the present study, the activity of carbonic anhydrase was measured in rat islet homogenates and shown to be about four times lower than in rat parotid cells. The pattern for the inhibitory action of acetazolamide on carbonic anhydrase activity also differed in islet and parotid cell homogenates, suggesting the presence of different isoenzymes. NaN3 inhibited carbonic anhydrase activity in islet homogenates and both n-[U-14C]glucose oxidation and glucose-stimulated insulin secretion. Acetazolamide (0.3–10.0 mM) also decreased glucose-induced insulin output but failed to affect adversely n-[U-14C]glucose oxidation, although it inhibited the conversion of n-[5-3H]glucose to [3H]OH and that of n-[U-14C]glucose to acidic metabolites. Hydrochlorothiazide (3.0–10.0 mM), which also lyzes the reversible dehydration of HCO3−caused a concentration-related inhibition of the secretory response, which could well be due to inhibition of carbonic anhydrase, might in part affect the activity of volume-sensitive anion channels in β-cells but lowered intracellular pH and adversely affected both the bioelectrical response to n-glucose and its effect on the cytosolic concentration of Ca2+ in these cells. The lowering of cellular pH by acetazolamide, which could well be due to inhibition of carbonic anhydrase, might in turn account for inhibition of glycolysis. The perturbation of stimulus-secretion coupling in the β-cells exposed to acetazolamide may thus involve impaired circulation in the pyruvate-malate shuttle, altered mitochondrial Ca2+ accumulation, and perturbation of Cl−fluxes, resulting in both decreased bioelectrical activity and insulin release.

Acetazolamide; d-glucose metabolism; insulin secretion; bioelectrical activity; bicarbonate production

CARBONIC ANHYDRASE (carbonic hydrolase; E.C. 4.2.1.1) catalyzes the reversible dehydration of HCO3−in solution. Several isoforms of this zinc metalloenzyme have been described. They differ from one another by their kinetic properties, sensitivity to various inhibitors such as acetazolamide, and subcellular localization (9, 18). A recent report (19) has drawn attention to the presence in rat pancreatic insulin-producing cells of the mitochondrial carbonic anhydrase isoenzyme V. On the basis of the inhibition of glucose-stimulated insulin secretion by acetazolamide, it was proposed that carbonic anhydrase V may be functionally linked to the regulation of insulin release (19), either by providing HCO3−for pyruvate carboxylase, which is thought to participate in a pyruvate-malate shuttle, itself considered as the major means for generating cytosolic NADPH from the metabolism of d-glucose in the islet cells (11), or by regulation of mitochondrial Ca2+ concentration, which is known to increase in glucose-stimulated INS-1 cells (8).

The major aims of the present study were to provide quantitative information about the activity of carbonic anhydrase in rat pancreatic islet homogenates and to compare it, as well as its sensitivity to acetazolamide, with those found in rat parotid cell homogenates and to evaluate the effects of acetazolamide on secretory, metabolic, ionic, and electrical events in intact pancreatic islets or islet β-cells.

MATERIALS AND METHODS

Female Wistar rats (Ifa Credo, L’Arbresle, France) were given free access to food (KM-04-k12; Pavan Service, Oud Turnhout, Belgium) and tap water up to the time of death. This study was approved by the Animal Care and Use Committee of the Université Libre de Bruxelles (Brussels, Belgium).

Pancreatic islets (14), purified B and non-B islet cells (7), and parotid cells (15) were prepared by methods described in the cited references.

For measurement of carbonic anhydrase activity, pancreatic islets and parotid cells were ultrasonicated in 50 mM HEPES buffer (pH 8.1). An aliquot (50 μl) of the homogenate (from 30 to 60 islets or from 50 to 250 × 103 cells per assay) was placed in a glass microtube kept in a plugged scintillation vial. The reaction was started by injection of 50 μl of a 100 μM or 1.0 mM solution of bicarbonate, which was mixed with a tracer amount of H14CO3−into the microtube. After a 3- to 9-min incubation at 20°C, the reaction was stopped by injection of 1 M NaOH (50 μl) into the microtube. 14CO2 was then trapped by hyamine hydroxide (500 μl) injected into the vial bottom. After 60 min, the assay tube was removed from the vial and scintillation liquid was added for radioactivity counting.

In homogenates of parotid cells or pancreatic islets, the protein content was measured by the method of Lowry et al. (10). The methods used to measure insulin release (14) and d-glucose metabolism (13) in isolated rat pancreatic islets were identical to those described in the cited references.

For measurement of H14CO3−production by isolated rat islets, groups of 60 islets each were incubated for 90 min at 37°C in 0.1 ml of a HEPES- and bicarbonate-buffered medium (12) containing d-glucose (16.7 mM) mixed with a tracer amount of n-[U-14C]glucose (4.0 μCi/ml) in Eppendorf tubes (1.5 ml), which were themselves placed in counting vials (20 ml). At the end of incubation, the vials were opened to eliminate over 10 min at room temperature the 14CO2 formed during incubation. NaOH (0.1 ml; 0.1 M) was then added to...
each tube, and the islets were sonicated (3 × 10 s). The islet homogenates were then transferred on Pasteur pipettes containing 1.0 ml of AG1X8 resin. The tubes were washed twice with 0.5 ml H$_2$O, which was also placed on the column. The column was then washed with distilled water (5 × 1.0 ml), and the H$^4$CO$_3$ was eventually eluted with 3.0 ml of 0.3 M NaCl. This eluate was placed in a vial, together with a glass tube containing 0.5 ml of hyamine hydroxide. After vials were closed with rubber covers, 0.1 ml HCl (0.1 M) was injected in each vial, and $^{14}$CO$_2$ was collected over 30 min at 37°C. Control samples (no islets) and reference solutions of H$^4$CO$_3$ (0.2 μCi/ml), prepared in the HEPES- and bicarbonate-buffered incubation medium and used to evaluate the recovery of H$^4$CO$_3$ (51.6 ± 3.7%; n = 7) under these experimental conditions, were treated in the same manner.

Electrophysiological experiments and intracellular pH measurements were carried out with dispersed rat islet cells, prepared essentially as described previously (2) and cultured for 2–10 days in MEM supplemented with 5% (vol/vol) FCS. β-cells were identified by their size, granular appearance, and electrical response to increased concentrations of β-glucose.

β-Cell membrane potential was monitored with the perforated-patch configuration of the patch-clamp technique, as described previously (2). Activity of the volume-sensitive anion channel was assessed from conventional whole cell recordings (3). Briefly, the cells were voltage clamped at 0 mV and subjected to 200-ms voltage pulses of ±100 mV at 4-s intervals. A hypertonic CsCl-rich pipette solution was used to activate the channel.

Intracellular pH was assessed from the 500 nm-to-450 nm fluorescence ratio in cells loaded with the pH-sensitive dye BCECF (5). Cytosolic Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) was measured as previously described (20).

All results are presented as means ± SE together with the number of individual determinations (n). The statistical significance of differences between mean values was assessed by Student’s t-test.

**RESULTS**

**Enzymatic data.** As illustrated in Fig. 1, top, the generation of $^{14}$CO$_2$ in the assay of islet carbonic anhydrase was proportional to both the length of incubation (3, 6, and 9 min) and the number of islets (30, 48, and 60 islets) with respective variation coefficients of 6.1% (n = 15) and 6.3% (n = 9). In the experiments illustrated in Fig. 1, top, the absolute value for reaction velocity averaged, at 50 μM HCO$_3^-$, 335 ± 18 fmol·min$^{-1}$·islet$^{-1}$ (n = 9). At the same HCO$_3^-$ concentration, as little as 25 μM acetazolamide decreased the reaction velocity by 50.1 ± 7.2% (n = 3; P < 0.025) of paired control value (Fig. 1, bottom). The residual value for reaction velocity was not significantly different at 25, 50, 100, or 200 μM acetazolamide, with an overall mean value for the relative extent of acetazolamide inhibition representing 56.3 ± 3.9% (n = 20; P < 0.001) of the paired control reaction velocity (420 ± 67 fmol·min$^{-1}$·islet$^{-1}$; n = 7). Essentially comparable results were obtained at a HCO$_3^-$ concentration of 0.5 mM, with the residual value for reaction velocity averaging in the presence of 50–200 μM acetazolamide 20.3 ± 6.1% (n = 8; P < 0.001) of the paired control value (5.71 ± 1.75 pmol·min$^{-1}$·islet$^{-1}$; n = 4). NaN$_3$ (0.05 mM) also inhibited carbonic anhydrase activity in islet homogenates incubated in the presence of 0.5 mM HCO$_3^-$ by 30.7 ± 3.9% (n = 5; P < 0.05) of paired control value (6.57 ± 1.25 pmol·min$^{-1}$·islet$^{-1}$; n = 5). Such an inhibitory action was not enhanced at higher concentrations of NaN$_3$. For instance, the relative extent of the decrease in reaction velocity averaged 22.2 ± 3.5% (n = 5; P < 0.005) of the paired control value at 0.2 mM NaN$_3$. When we pooled all available results, the reaction velocity for carbonic anhydrase averaged 372 ± 32 fmol·min$^{-1}$·islet$^{-1}$ (n = 16) at 50 μM HCO$_3^-$, compared (P < 0.001) with 5.10 ± 0.69 pmol·min$^{-1}$·islet$^{-1}$ (n = 15) at 0.5 mM HCO$_3^-$ (Fig. 1, bottom). These mean values are not incompatible (P > 0.07) with a rule of proportionality between reaction velocity and HCO$_3^-$ concentration in the range of substrate concentrations under consideration.

Further experiments aimed at comparing the absolute values for carbonic anhydrase activity in purified B vs. non-B islet cell homogenates and in islets vs. parotid cell homogenates and to compare the pattern of acetazolamide inhibitory action on carbonic anhydrase activity in islet vs. parotid cell homogenates.

Relative to cell number, the activity of carbonic anhydrase at 50 μM HCO$_3^-$ was not significantly different (P > 0.9) in B and non-B islet cells, with an overall mean value of 144 ± 23 amol·min$^{-1}$·cell$^{-1}$ (n = 6). Assuming a mean number of 2,000 cells per islet, this would correspond to 288 ± 46 fmol·min$^{-1}$·islet$^{-1}$, a value not significantly different (P > 0.25) from that indeed recorded under the same experimental conditions in islet homogenates (335 ± 18 fmol·min$^{-1}$·islet$^{-1}$). In the presence of acetazolamide (20–200 μM), the reaction velocity in B and non-B islet cell
homogenates was decreased, respectively, by 54.5 ± 9.7% and 41.6 ± 12.9% (n = 6 and P < 0.025 in both cases) of paired control value (no acetazolamide), which is also in fair agreement with the data recorded in islet homogenates.

In parotid cell homogenates, the reaction velocity for carbonic anhydrase was again virtually proportional to HCO₃⁻ concentration, increasing (P < 0.05) from 1.11 ± 0.04 (n = 4) to 11.68 ± 3.01 (n = 8) mmol·min⁻¹·mg⁻¹ protein as the concentration of HCO₃⁻ was raised from 50 μM to 0.5 mM. In the presence of 50 μM HCO₃⁻, the activity of the enzyme, expressed per milligram of protein, was four to five times higher (P < 0.03), however, in parotid cell homogenates than in islet homogenates of the same animals. In these experiments, conducted in the presence of 50 μM HCO₃⁻, the control value for carbonic anhydrase activity averaged 216.4 ± 25.4 fmol·min⁻¹·10³ cells⁻¹ (n = 9).

The inhibition of carbonic anhydrase in parotid cell homogenates by acetazolamide, as well as by hydrochlorothiazide, represented a concentration-relative phenomenon, with inhibition constant values close to 2.2 and 27.8 μM, respectively (Fig. 2). Acetazolamide in high concentrations (25–200 μM) decreased carbonic anhydrase activity, as measured in the presence of 50 μM HCO₃⁻, by no more than 56.3 ± 3.9% (n = 20) in islet homogenates and 54.5 ± 9.7% (n = 6) in B islet cell homogenates; however, as little as 10 μM acetazolamide decreased the activity of the enzyme at the same concentration of HCO₃⁻ by 84.3 ± 3.0% (n = 5) in parotid cell homogenates. Such a difference between islet β-cell and parotid cell homogenates, in terms of the maximal relative extent of carbonic anhydrase activity inhibition by acetazolamide, achieved statistical significance (P < 0.025).

**Secretory data.** At a low D-glucose concentration (2.8 mM), the output of insulin in the presence of 0.1 and 5.0 mM acetazolamide averaged, respectively, 97.1 ± 13.9 and 119.9 ± 19.5% (n = 30 and P > 0.3 in both cases) of the mean corresponding value found, within the same experiments, in the absence of acetazolamide (100.0 ± 6.4%; n = 30). Such a reference value represented no more than 39.3 ± 4.4 μU/islet per 90 min.

As illustrated in Fig. 3, NaN₃ (0.03–5.0 mM) caused a concentration-related inhibition of insulin release evoked over 90-min incubation by 16.7 mM D-glucose. In these experiments, the control value for insulin secretion averaged 345.6 ± 29.2 μU/islet per 90 min (n = 39). As little as 0.1 mM NaN₃ was sufficient to cause a significant decrease in insulin output (P < 0.05) relative to the value recorded at 0.03 mM NaN₃. At the highest concentration of NaN₃ tested in these experiments (5.0 mM), the release of insulin only represented 3.7 ± 2.2% (n = 23) of the mean control value found within the same experiment(s). Low concentrations of acetazolamide (0.03–0.3 mM) tended to augment glucose-induced insulin release; this effect only achieved statistical significance (P < 0.02) at the lowest drug concentration (0.03 mM). In the concentration range between 0.3 and 10.0 mM, however, acetazolamide progressively decreased the output of insulin (P < 0.02). In the ranges of concentrations in which the concentration-response relationships illustrated in Fig. 3 could be considered as running in parallel, acetazolamide was ~40 times less potent than NaN₃ as an inhibitor of glucose-stimulated insulin release. Hydrochlorothiazide, like acetazolamide, tended to increase insulin output at the lowest concentration of the benzothiadiazide diuretic tested in the present study (1.0 mM). At higher concentrations (3.0–10.0 mM), however, hydrochlorothiazide caused a concentration-related inhibition of glucose-induced insulin secretion. In the range of concentrations in which the inhibitory action of NaN₃ and hydrochlorothiazide ran in parallel, the latter drug was about nine times less potent than NaN₃ (Fig. 3).

**Metabolic data.** NaN₃, when tested in the range from 0.3 to 1.0 mM, failed to affect significantly the metabolism of...
D-glucose (16.7 mM) by the islets (Table 1). At 5.0 mM, however, NaN3 decreased (P < 0.001) the oxidation of D-[U-14C]glucose and the paired ratio between such an oxidation and D-[5-3H]glucose utilization. Acetazolamide in vitro increased the total production of 14CO2 by islets (Table 1). At 5.0 mM, however, NaN3 decreased (P < 0.02) in the net generation of 14C-labeled acidic metabolites from D-[U-14C]glucose. In the range from 0.1 to 5.0 mM, acetazolamide exerted an effect opposite to that of NaN3 on the paired ratio between D-[U-14C]glucose oxidation and D-[5-3H]glucose utilization. Acetazolamide indeed caused a progressive increase in such a ratio from a control value of 24.1 ± 2.2% (n = 19) to 28.7 ± 1.8% (n = 19; P > 0.1), 31.8 ± 1.4% (n = 18; P < 0.01), and 34.6 ± 1.7% (n = 17; P < 0.001) at concentrations of 0.1, 1.0, and 5.0 mM, respectively. This increase was mainly attributable to a progressive fall in the utilization of D-[5-3H]glucose (from 30.5 ± 1.6% to 20.3 ± 1.4%; n = 19 in both cases). This decrease in D-[U-14C]glucose oxidation coincided with a significant increase (P < 0.02) in the production of [14C]CO2 and acidic metabolites from D-[U-14C]glucose (Table 1). In the range from 0.1 to 5.0 mM, acetazolamide exerted an effect opposite to that of NaN3 on the paired ratio between D-[U-14C]glucose oxidation and D-[5-3H]glucose utilization. Acetazolamide indeed caused a progressive increase in such a ratio from a control value of 24.1 ± 2.2% (n = 19) to 28.7 ± 1.8% (n = 19; P > 0.1), 31.8 ± 1.4% (n = 18; P < 0.01), and 34.6 ± 1.7% (n = 17; P < 0.001) at concentrations of 0.1, 1.0, and 5.0 mM, respectively. This increase was mainly attributable to a progressive fall in the utilization of D-[5-3H]glucose (P < 0.02), coinciding with a parallel decrease in the generation of 14C-labeled acidic metabolites from D-[U-14C]glucose (P < 0.005).
Acetazolamide (5 mM) did not appear to affect directly the activity of the volume-sensitive anion channel under whole cell recording conditions where the channel was activated by the use of a hypertonic pipette solution (Fig. 7).

As shown in Fig. 8, a rise in D-glucose concentration from 4 to 16 mM depolarized the cells and evoked electrical activity. Acetazolamide (5 mM) suppressed this electrical activity and repolarized the cells (Fig. 8, top trace). In a number of cells, this repolarization was preceded by a transient period of silent depolarization (Fig. 8, bottom trace). In either case, inhibition of electrical activity by 5 mM acetazolamide was essentially irreversible, at least within a “washout” period of 5–10 min. However, when acetazolamide was used at a concentration of 1 mM, the inhibitory effect of the drug was reversible following its removal from the bath solution (Fig. 9). In addition, no silent depolarization preceding repolarization was observed with this lower concentration of the drug.

When the cells were preincubated for 30 min with acetazolamide (5 mM), the depolarization otherwise caused by a rise in D-glucose concentration was severely blunted and was insufficient to result in electrical activity (not shown). When acetazolamide (5 mM) was applied to cells in the presence of 4 mM D-glucose, a transient depolarization sometimes accompanied by electrical activity was observed (Fig. 10). In the continued presence of acetazolamide, a subsequent rise in D-glucose concentration to 16 mM resulted in a transient period of electrical activity before a period of silent depolarization (Fig. 10, bottom trace).

DISCUSSION

The present data indicate that the specific activity of carbonic anhydrase in rat islet homogenates is about four times lower than that found in parotid cell homogenates. The relative extent of the inhibitory action of acetazolamide on carbonic anhydrase was also vastly different in islet and parotid cells, suggesting the participation of different isoenzymes in these two secretory cell types. Parotid glands indeed contain mainly carbonic anhydrase VI (16, 17, 24), whereas the isoenzyme V prevails in pancreatic islets (19).

The presence of the mitochondrial carbonic anhydrase V in insulin-producing islet cells may be relevant to the secretory activity of these cells in several respects. As already mentioned in the introduction, it may both provide HCO₃⁻/H⁺ for pyruvate carboxylase and participate in the glucose-induced increase of mitochondrial Ca²⁺ concentration (19). It may also provide HCO₃⁻ for a bicarbonate-sensitive ATPase, previously shown to be present in rat pancreatic islets (23), and play a role in the regulation of intracellular pH and ionic fluxes in the islet β-cells.

For instance, our findings suggest that carbonic anhydrase activity in islet homogenates could be sufficient to ensure full conversion of glucose-derived CO₂ to bicarbonate. Thus, at a concentration of HCO₃⁻ as low as 0.5 mM, well below the

Fig. 5. Effects of acetazolamide (5 mM; ACZ) and sodium acetate (20 mM, Ac⁻) on intracellular pH (pHi), assessed by BCECF fluorescence in rat pancreatic β-cells. This recording is representative of 6 experiments with similar results.

Fig. 6. Effects of ACZ (5 mM) on cytosolic Ca²⁺ concentration, assessed from the 350 nm-to-380 nm ratio in fluorescence (F) of rat pancreatic β-cells loaded with fura 2 and exposed to 4 or 16 mM glucose (G). Also shown is the response to a rise in extracellular K⁺ concentration to 25 mM. Recordings are representative of 5 experiments with similar results.

Fig. 7. Effect of ACZ (5 mM) on the activity of the volume-sensitive anion channel. Whole cell recording of channel activation was done with use of hypertonic pipette solution. Currents are shown in response to 500-msec voltage pulses of +100 and −100 mV. This recording is representative of 4 experiments with similar results.

Fig. 8. A rise in D-glucose concentration from 4 to 16 mM depolarized the cells and evoked electrical activity. Acetazolamide (5 mM) suppressed this electrical activity and repolarized the cells (Fig. 8, top trace). In a number of cells, this repolarization was preceded by a transient period of silent depolarization (Fig. 8, bottom trace). In either case, inhibition of electrical activity by 5 mM acetazolamide was essentially irreversible, at least within a “washout” period of 5–10 min. However, when acetazolamide was used at a concentration of 1 mM, the inhibitory effect of the drug was reversible following its removal from the bath solution (Fig. 9). In addition, no silent depolarization preceding repolarization was observed with this lower concentration of the drug.

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intracellular concentration of the anion as judged from the influx of $\text{H}^{14}\text{CO}_3^-$/H$^{10}\text{CO}_2$ in rat pancreatic islets (6), the carbonic anhydrase activity averaged $5.10 \pm 0.69$ pmol·min$^{-1}$·islet$^{-1}$, compared with a rate of $\text{CO}_2$ generation from $\text{d-}[\text{U-14C}]$glucose (16.7 mM) close to $3.18 \pm 0.03$ pmol·min$^{-1}$·islet$^{-1}$ (see Table 1).

The secretory data obtained in a previous report (19) and in the present study support the view that the integrity of carbonic anhydrase activity is required to allow a normal stimulation of insulin release by $\text{d}$-glucose. In this respect, the results collected in islets exposed to NaN$_3$ are poorly contributive because of the lack of selectivity toward carbonic anhydrase. Indeed, NaN$_3$ inhibits several enzymes, such as catalase and iron enzymes, and uncouples phosphorylation. It also decreased $\text{d-}[\text{U-14C}]$glucose oxidation and the paired ratio between such an oxidation and $\text{d-}[\text{5-3H}]$glucose utilization in the rat islets. At variance, acetazolamide is considered as a rather selective inhibitor of carbonic anhydrase. It increased the paired ratio between $\text{d-}[\text{U-14C}]$glucose oxidation and $\text{d-}[\text{5-3H}]$glucose utilization. This coincided, at low concentrations of acetazolamide (0.03–0.3 mM), with an increase in glucose-induced insulin release. Higher concentrations of acetazolamide (0.3–5.0 mM) were required to cause a progressive decrease in insulin output. At these high concentrations (1.0–5.0 mM), acetazolamide caused a modest decrease in intracellular $\text{pH}$ and adversely affected glucose-induced bioelectrical activity. Thus, in $\beta$-cells exposed to 16 mM $\text{d}$-glucose, it suppressed electrical activity and repolarized the cells, with this repolarization being often preceded by a transient period of silent depolarization. Acetazolamide also lowered $[\text{Ca}^{2+}]_i$ in cells exposed to a high concentration of $\text{d}$-glucose. Incidentally, the high concentrations of carbonic anhydrase inhibitors required to decrease insulin secretion from intact islets, compared with those required to inhibit the activity of the enzyme in islet homogenates, could reflect the concentration dependency for the cellular uptake of these inhibitors.

The modest lowering of intracellular $\text{pH}$ caused by acetazolamide could conceivably be related to the fact that it behaves as a weak acid ($pK_a$ close to 7.1) and, more likely, to the ionic consequences of carbonic anhydrase inhibition. In turn, the lowering of intracellular $\text{pH}$ could be responsible for inhibition of glycolysis, as indicated by the decrease in the conversion of both $\text{d-}[\text{5-3H}]$glucose to $\text{H}_2\text{O}$ and $\text{d-}[\text{U-14C}]$glucose to acidic $\text{14C}$-labeled metabolites (e.g., pyruvic and lactic acid). The fall in intracellular $\text{pH}$ might also account for the occurrence of a transient depolarization and transient burst of spiking electrical activity in $\beta$-cells exposed to 4 mM $\text{d}$-glucose, with these

![Fig. 8. Effect of ACZ (5 mM) on membrane potential in isolated $\beta$-cells, using the perforated-patch technique. The concentration of $\text{d}$-glucose (G) in the medium was raised from 4 to 16 mM, as indicated in the top rectangle. The break in the lower trace corresponds to a period of ~6 min in the continued presence of 16 mM $\text{d}$-glucose and 5 mM ACZ, during which the cell gradually repolarized. Recordings are representative of 4 experiments with similar results.](image)

![Fig. 9. Effect of ACZ (1 mM) on membrane potential in isolated $\beta$-cells. Presentation is the same as in Fig. 8. Recordings are representative of 3 experiments with similar results.](image)

![Fig. 10. Effect of ACZ (5 mM) on membrane potential in cells exposed to 4 mM $\text{d}$-glucose. In the bottom trace, the concentration of $\text{d}$-glucose was raised to 16 mM, as indicated in the top rectangle. Recordings are representative of 3 experiments with similar results.](image)
effects being reminiscent of those otherwise seen after addition of 5–10 mM acetate.

Despite inhibition of both carbonic anhydrase in islet homogenates and \( \text{HCO}_3^- \) generation in intact islets, acetazolamide failed to affect significantly the total production of \( \text{^{14}CO}_2 \) from \( \text{D-[^{14}C]} \)glucose. Even \( \text{Na}_2\text{CO}_3 \) only inhibited total \( \text{^{14}CO}_2 \) production at the highest concentration (5.0 mM) tested in the present experiments. These findings suggest that any decrease in \( \text{HCO}_3^- \) production attributable to inhibition of carbonic anhydrase is soon masked by initiation of compensatory mechanisms, as also known to occur when considering the effect of carbonic anhydrase inhibitors on pulmonary \( \text{CO}_2 \) elimination (1).

The concentration-dependent dual effect of both acetazolamide and hydrochlorothiazide on glucose-stimulated insulin release, as illustrated in Fig. 3, was found to coincide within the same range of concentrations with a dual effect of the same two drugs on \(^{86}\text{Rb} \) outflow from prelabeled islets (unpublished observation). Moreover, the present experiments and unpublished results indicated that, at a given concentration, the effect of acetazolamide on selected islet variables may differ as a function of the concentration of \( \text{D}- \)glucose. For instance, at 4 mM \( \text{D}- \)glucose, acetazolamide (5 mM) caused, on occasion, a modest and gradual increase in \( \text{[Ca}^{2+}] \text{c} \), whereas the sulfonamide obviously decreased \( \text{[Ca}^{2+}] \text{c} \), at 16 mM \( \text{D}- \)glucose. Likewise, at the same 5 mM concentration, acetazolamide provoked, in cells exposed to 4 mM \( \text{D}- \)glucose, a transient depolarization sometimes accompanied by electrical activity, while suppressing electrical activity in cells exposed to 16 mM \( \text{D}- \)glucose. These converging observations point to a pleiotropic response of ionic and secretory variables to acetazolamide as well as hydrochlorothiazide as a function of both the relative extent of carbonic anhydrase inhibition and the metabolic stimulation of islet cells.

In the liver, \( \text{HCO}_3^- \) generated by carbonic anhydrase serves as a substrate in the reactions catalyzed in the mitochondria by either pyruvate carboxylase or carbamylphosphate synthase 1 and, by doing so, contributes to both gluconeogenesis and the urea cycle. However, neither gluconeogenesis (22) nor the urea cycle (21) is operative in pancreatic islets. The relevance of carbonic anhydrase to the biochemical organization of islet cells remains therefore, a matter of speculation. As proposed by Parkkila et al. (19), the perturbation of stimulus-secretion coupling in the \( \beta \)-cells exposed to acetazolamide may involve both impaired circulation in the pyruvate-malate shuttle and altered mitochondrial accumulation of \( \text{Ca}^{2+} \). Moreover, although no direct effect of acetazolamide on the activity of the volume-sensitive anion channel was apparent, a decreased generation of pyruvate and lactate from \( \text{D}- \)glucose could impair activation of the channel and subsequently alter the remodeling of \( \text{Cl}^- \) fluxes, recently proposed to play a key role in the control of plasma membrane potential and electrical activity in the range (4–16 mM) of insulinotropic \( \text{D}- \)glucose concentrations (4).

In conclusion, therefore, the present findings reinforce the view that carbonic anhydrase \( V \) plays a key role in the normal process of glucose-stimulated insulin secretion.

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