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Tritiated Taurine Handling by Isolated Rat Pancreatic Islets

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A gating of volume-sensitive anion channels may participate in the depolarization of the plasma membrane caused by high concentrations of D-glucose in insulin-producing B-cells of the endocrine pancreas. The efflux of tritiated taurine from prelabeled cells is currently used to assess changes in the activity of such channels. The handling of [1,2-^{3}H]taurine by isolated rat pancreatic islets was therefore investigated. The net uptake of [1,2-^{3}H]taurine was found to represent a concentration-, time-, and temperature-dependent process. It was progressively increased in the range of D-glucose concentrations between 2.8 and 8.3 m*M*, but no further increase was observed at 16.7 m*M* D-glucose. Over 15 min incubation, the efflux of radioactivity from prelabeled islets was inhibited by MK571 (1.0 m*M*). It was increased in response to hypoosmolarity both in the presence and absence of extracellular Na^+. Whether in salt-balanced or Na^+-deprived media, the efflux of radioactivity from prelabeled islets increased in response to a rise in D-glucose concentration from 2.8 to 5.6 or 8.3 m*M*, but decreased when the concentration of the hexose was further increased from 8.3 to 16.7 m*M*. In perifused islets, however, the radioactive efflux from prelabeled islets was inhibited, in a concentration-related manner, when islets first deprived of D-glucose for 45 min were then exposed to 2.8, 5.6, or 16.7 m*M* D-glucose. Likewise, in prelabeled and perifused islets first exposed for 45 min to 4.0 m*M* D-glucose, a later rise in hexose concentration to 8.3 m*M* failed to affect significantly effluent radioactivity, while an increase in hexose concentration from 4.0 to 16.7 m*M* inhibited the radioactive outflow. In these perifusion experiments, the rise in D-glucose concentration provoked the expected changes in insulin output. The findings obtained in islets examined immediately after preincubation in the presence of [1,2-^{3}H]taurine are consistent with the presence of volume-sensitive anion channels in islet cells and with a gating of such channels in response to a rise in D-glucose concentration from 2.8 to 5.6–8.3 m*M*. However, the radioactive fractional outflow rate from prelabeled islets seems to reach its highest value at about 8.3 m*M* D-glucose, being unexpectedly decreased at a higher concentration (16.7 m*M*) of the hexose. In conclusion, the pleiotropic effects of D-glucose upon tritiated taurine outflow from prelabeled rat islets, which could conceivably be ascribed to differences in the handling of this amino sulfonic acid by distinct islet cell types, indicates that the present approach is far from optimal to characterize unambiguously the regulation by the hexose of volume-sensitive anion channel activity in insulin-producing islet cells.

Key Words: Volume-sensitive anion channels; hypoosmolarity; rat pancreatic islets; [1,2-^{3}H]taurine uptake and efflux; insulin release.

Introduction

Glucose-induced insulin release from the pancreatic B-cell is triggered by an increase in cytosolic Ca^{2+} concentration, itself mainly attributable to the opening of voltage-sensitive Ca^{2+} channels resulting from depolarization of the plasma membrane *(1)*. A current consensus model ascribes such a depolarization to the closure of ATP-sensitive K^+ channels *(2)*. However, evidence is now accumulating from several laboratories indicating that at least one additional and possibly more important mechanism couples D-glucose metabolism with B-cell electrical activity. To cite only one example, the rate of $^{86}Rb^+$ efflux from prelabeled and perifused islets reaches its nadir value at a D-glucose concentration close to 5 m*M*, while stimulation of insulin release only occurs at D-glucose concentrations in excess of a threshold value itself close to 5 m*M* *(3)*.

As the additional process, it is currently considered that a gating of volume-sensitive anion channels may participate in the depolarization of the plasma membrane in pancreatic islet B-cells exposed to high concentrations of D-glucose *(4–17)*. The following findings support such a concept. D-glucose stimulates $^{36}Cl^-$ efflux from prelabeled islets *(18, 19)*. Insulin-secretory cells are equipped with volume-regulated anion channels *(4,6)*. By activation of these channels, hypotonic swelling of B-cells causes depolarization of the plasma membrane, leading to electrical activity and insulin

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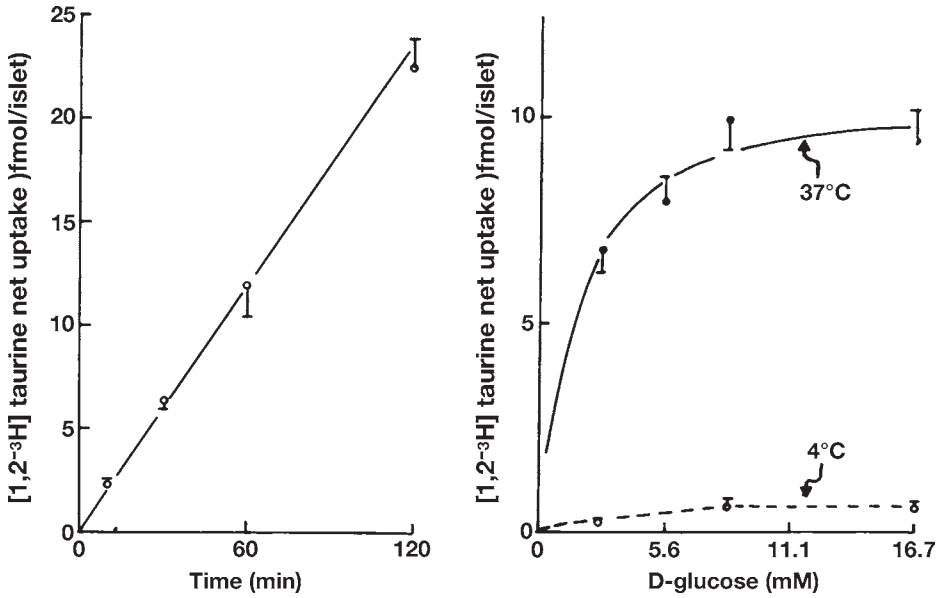

Fig. 1. Left panel: time course for the net uptake of [1,2-^{3}H]taurine (0.31 μM) by islets incubated at 37°C in the presence of 8.3 mM D-glucose. Right panel: effects of increasing concentrations of D-glucose upon the net uptake of [1,2-^{3}H]taurine (0.24–0.31 μM) by islets incubated for 60 min at either 4°C or 37°C. Mean values (± SEM) refer to 5–8 observations (left panel), 4–5 measurements (lower curve in the right panel), and 12–25 determinations (upper curve in the right panel).

release *(5,10)*. D-glucose (4–20 mM) and other nutrient secretagogues also activate the volume-sensitive anion channels, both at the whole-cell *(7,12)* and the single-channel *(11,17)* levels. This coincides with B-cell swelling, possibly linked to the intracellular accumulation of D-glucose metabolites *(9)*.

The measurement of tritiated taurine outflow from prelabeled cells represents one approach to document changes in the activity of volume-sensitive anion channels *(20–23)*. Indeed, an increase in the unidirectional efflux of taurine, like that of D-aspartate, from prelabeled cells informs on the loss of organic osmolytes resulting from the gating of these channels.

In the present study, therefore, the handling of [1,2-^{3}H] taurine by isolated rat pancreatic islets was investigated, with emphasis on the effects of increasing concentrations of D-glucose on effluent radioactivity from prelabeled islets.

Results

Taurine Uptake

Over 60 min incubation at 37°C in the presence of 8.3 mM D-glucose, the net uptake of [1,2-^{3}H]taurine (0.24–0.31 μM) averaged 9.95 ± 0.72 fmol/islet (n = 25) Under these conditions and in the restricted range of [1,2-^{3}H] taurine concentrations used in the present experiments, its net uptake was proportional to its concentration averaging, at 0.24 and 0.31 μM, respectively, 36.4 ± 3.7 (n = 13) and 35.9 ± 2.5 (n = 12) fmol·islet^{-1}·μM^{-1}.

As illustrated in Fig. 1 (left panel), the net uptake of [1,2-^{3}H]taurine (0.31 μM) was, at 37°C and in the presence of 8.3 mM D-glucose, virtually proportional to the length of incubation (10–120 min). At all concentrations of D-glucose (2.8–16.7 mM), it was suppressed when the incubation was conducted at only 4°C (Fig. 2, right panel).

Relative to the mean corresponding values found within the same experiments in the presence of 8.3 mM D-glucose, the net uptake of [1,2-^{3}H]taurine after 60 min incubation at 37°C averaged 75.1 ± 4.7% (n = 18) at 2.8 mM D-glucose, 80.2 ± 6.3% (n = 12) at 5.6 mM D-glucose, and 95.6 ± 6.8% (n = 17) at 16.7 mM D-glucose. The net uptake of the tritiated amino sulfonic acid was thus significantly lower at 2.8 mM D-glucose ($p < 0.005$) and 5.6 mM D-glucose ($p < 0.05$), but not so at 16.7 mM D-glucose ($p > 0.6$), than at 8.3 mM D-glucose. Similar statistical significance was reached when the overall absolute mean values for [1,2-^{3}H]taurine net uptake, as derived from all available data, were compared at the four concentrations of D-glucose used in the present experiments.

Effect of D-Glucose upon Taurine Outflow and Insulin Release in Perifused Islets

In the two next sets of experiments, groups of 100 islets each were preincubated for 60 min at 37°C in the presence of D-glucose (16.7 mM) and [1,2-^{3}H]taurine (0.94 μM), and then placed in a perifusion chamber.

In islets exposed for the first 45 min of perifusion to a glucose-free medium, the fractional outflow rate of [1,2-^{3}H] taurine averaged, between min 31 and 45, 2159 ± 43 10^{-6}/min (n = 12). The administration of D-glucose (2.8, 5.6, or 16.7 mM) always caused a rapid, sustained, and reversible decrease in effluent radioactivity (Fig. 2). The magnitude of such a decrease was closely related to the concentration

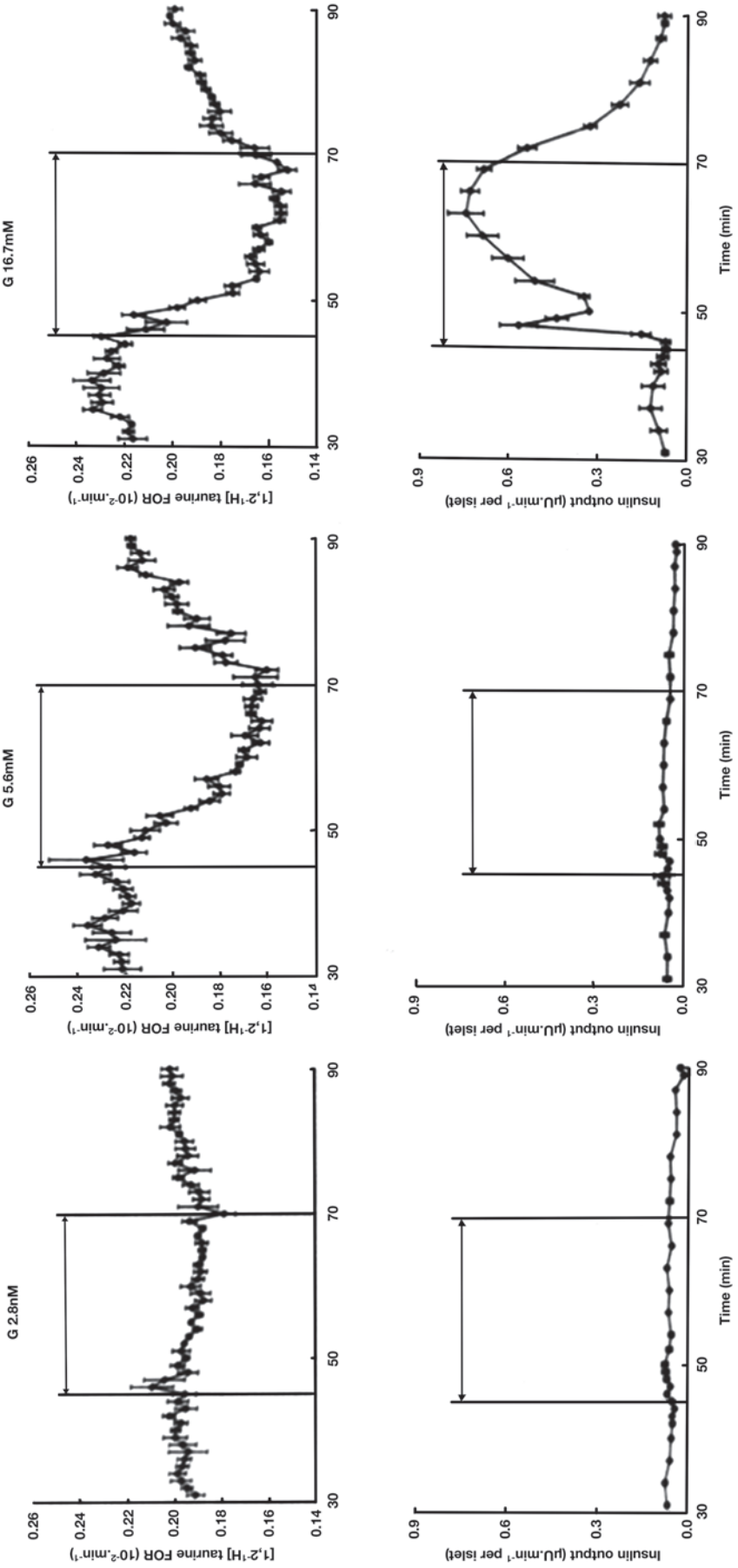

Fig. 2. Effects of a rise in D-glucose concentration from zero to 2.8 (left), 5.6 (middle), or 16.7 m*M* (right) between min 46 and 70 upon [1,2-^{3}H]taurine fractional outflow rate (upper panels) and insulin output (lower panels). Mean values (± SEM) refer to four individual experiments in all cases.

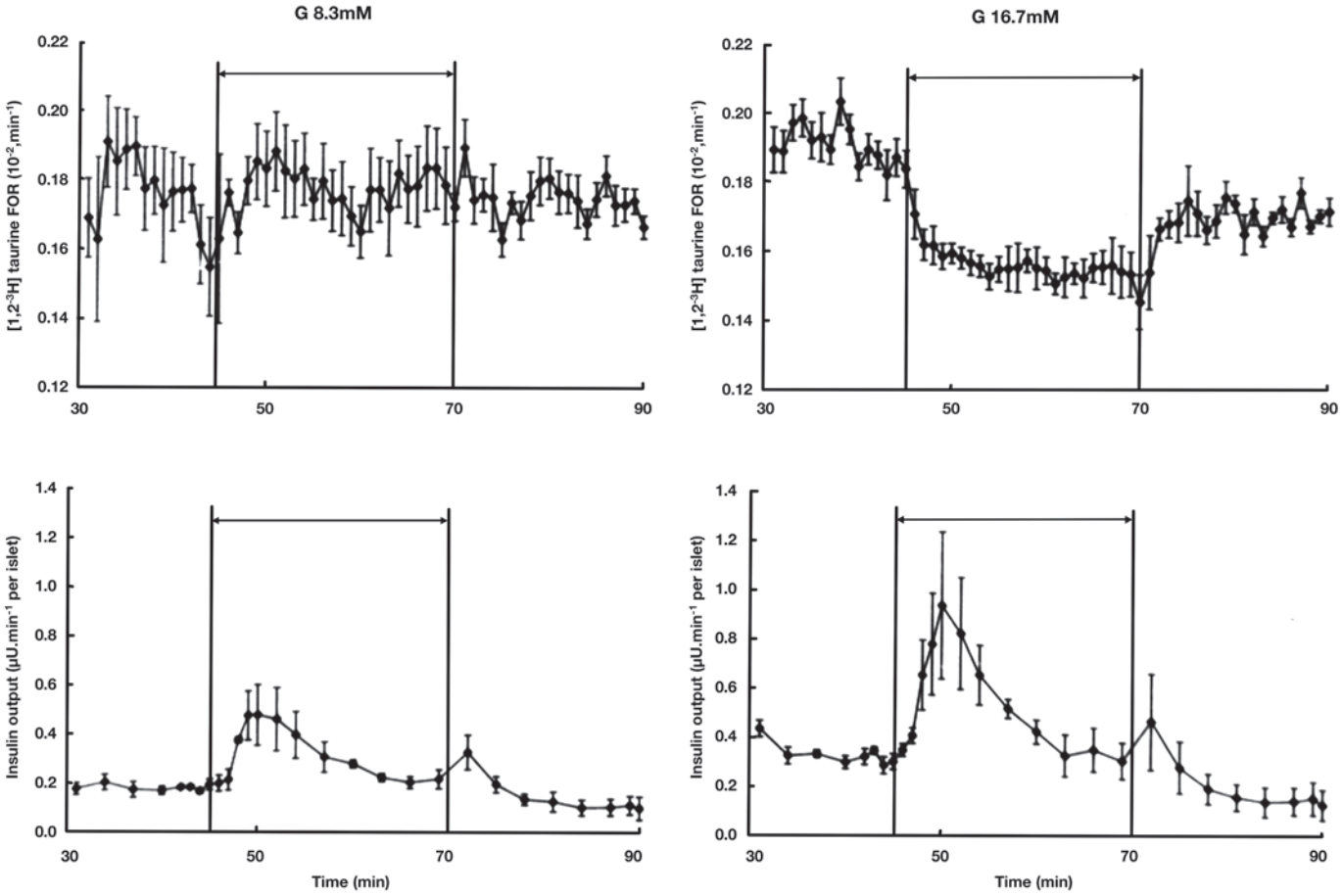

Fig. 3. Effects of a rise in D-glucose concentration from 4.0 to either 8.3 m*M* (left) or 16.7 m*M* (right) between min 46 and 70 upon [1,2-^{3}H]taurine fractional outflow rate (upper panels) and insulin output (lower panels). Mean values (± SEM) refer to four individual experiments in all cases.

of D-glucose present in the perifusate from min 46 to min 70. Thus, the paired difference in [1,2-^{3}H]taurine fractional outflow rate before (min 31–45) and after (min 46–70) the rise in hexose concentration averaged 44 ± 33, 392 ± 48, and 535 ± 43 10^{-6}/min ($n = 4$ in all cases) in islets exposed from min 46 to min 70 to 2.8, 5.6, and 16.7 m*M* D-glucose, respectively. When the effect of the hexose was judged from the difference between the mean fractional outflow rate of [1,2-^{3}H]taurine during exposure to D-glucose (min 46 to min 70) and the paired theoretical value calculated by exponential extrapolation of the 15 measurements made before introduction of D-glucose (min 31–45) and the last five measurements made at the end of the experiment (min 86–90), the hexose-induced decrement in effluent radioactivity averaged 54 ± 12 10^{-6}/min in islets exposed to 2.8 m*M* D-glucose ($n = 4$; $p < 0.02$), 355 ± 30 10^{-6}/min in islets exposed to 5.6 m*M* D-glucose ($n = 4$; $p < 0.005$) and 424 ± 20 10^{-6}/min in islets exposed to 16.7 m*M* D-glucose ($n = 4$; $p < 0.001$). The absolute value for [1,2-^{3}H]taurine fractional outflow rate averaged 1928 ± 4, 1856 ± 21, and 1722 ± 15 10^{-6}/min ($n = 4$ in each case) during exposure to 2.8, 5.6, and 6.7 m*M* D-glucose, respectively. These three mean values were significantly different ($p < 0.02$ or less) from one another.

The [1,2-^{3}H]taurine fractional outflow rate in islets exposed to 4.4 m*M* D-glucose for the first 45 min of perifusion averaged, between min 31 and 45, 1828 ± 66 10^{-6}/min ($n = 8$). It was not significantly different ($p > 0.25$) in the islets later exposed to either 8.3 or 16.7 m*M* D-glucose (Fig. 3). The paired difference in [1,2-^{3}H]taurine fractional outflow rate before (min 31–45) and after (min 46–70) the rise in D-glucose concentration was not significantly different from zero (–24 ± 199 10^{-6}/min; $n = 4$; $p > 0.9$) in the islets exposed to 8.3 m*M* D-glucose from min 46 to min 70, while being significantly different from zero (+340 ± 73 10^{-6}/min; $n = 4$; $p < 0.02$) in islets exposed to 16.7 *M* D-glucose over the same period. As judged by paired comparison of data recorded in each individual experiment, the glucose-induced change in [1,2-^{3}H]taurine fractional outflow rate was indeed 364 ± 106 10^{-6}/min different ($n = 4$; $p < 0.05$) under these two experimental conditions. This indicates that a rise in D-glucose concentration from 4.0 to 8.3 m*M* failed to affect significantly [1,2-^{3}H]taurine outflow, while it was significantly decreased in response to a rise in hexose concentration from 4.0 to 16.7 m*M*.

In these experiments, the output of insulin between min 42 and 45 averaged in the absence of glucose 64 ± 8 nU/min

per islet ($n = 12$), as distinct ($p < 0.001$) from 250 ± 27 nU/min per islet ($n = 8$) in the presence of 4.0 m*M* D-glucose. When the islets were first perifused for 45 min in the absence of the hexose, the administration of either 2.8 or 5.6 m*M* D-glucose only caused a minor increase in insulin output. Thus, the secretory rate recorded during exposure to the hexose was only 20 ± 4 nU/min per islet higher ($n = 8$; $p < 0.005$) than the paired theoretical value calculated by exponential extrapolation of the eight readings made before D-glucose administration (min 31–45) and the four measurements made at the end of the experiments (min 84–90). However, when the islets were exposed to 16.7 m*M* D-glucose from min 46 to 70, the increment in insulin output, calculated as just indicated, averaged 464 ± 50 nU/min per islet ($n = 4$; $p < 0.005$), with a typical biphasic pattern for the secretory response to the hexose. In the islets first exposed for 45 min to 4.0 m*M* D-glucose, the secretory rate recorded during exposure to 8.3 and 16.7 m*M* D-glucose averaged 306 ± 29 and 506 ± 33 nU/min per islet ($n = 4$ in both cases), indicating a concentration-related effect ($p < 0.005$) of the hexose upon insulin release. The latter value was not significantly different ($p > 0.3$) from that found in the first series of experiments during administration of 16.7 m*M* D-glucose to islets first deprived of any exogenous nutrient for the first 45 min of perifusion (552 ± 33 nU/min per islet; $n = 4$).

Effect of MK571 upon Taurine Efflux

Over 15 min incubation in the presence of 8.3 m*M* D-glucose, MK571 (0.1 m*M*), an inactivator of volume-sensitive anion channels *(24)*, failed to affect significantly the outflow of [1,2-^{3}H]taurine from prelabeled islets as judged from either the absolute values for such an efflux or the fractional outflow rate of the tritiated amino sulfonic acid. For instance, the latter fractional outflow rate averaged, in the presence of MK571, 98.5 ± 7.2% ($n = 13$) of the mean value recorded in its absence (100.0 ± 8.6%; $n = 12$). When the concentration of MK571 was raised to 1.0 m*M*, however, it decreased ($p < 0.05$) [1,2-^{3}H]taurine fractional outflow rate to 66.6 ± 12.4% ($n = 8$) of the mean corresponding values found, within the same experiment(s), in its absence (100.0 ± 9.6%; $n = 9$). The relative extent of such an inhibition was not significantly affected by the length of incubation. Thus, after only 5 min incubation in the presence of 8.3 m*M* D-glucose, the fractional outflow rate of [1,2-^{3}H]taurine was also decreased ($p < 0.01$) by 1.0 m*M* MK571 to 53.9 ± 8.9% ($n = 5$) of the mean corresponding value found in its absence (100.0 ± 11.0%; $n = 5$).

Effect of Hypotonicity upon Taurine Efflux

In experiments conducted in salt-balanced media containing 8.3 m*M* D-glucose, a reduction in osmolarity of the final incubation medium by 100 mOsmol/L, as achieved by decreasing the NaCl concentration by 50 m*M*, increased ($p < 0.02$) over 15 min incubation [1,2-^{3}H]taurine fractional outflow rate to 141.8 ± 12.2% ($n = 14$) of its control value

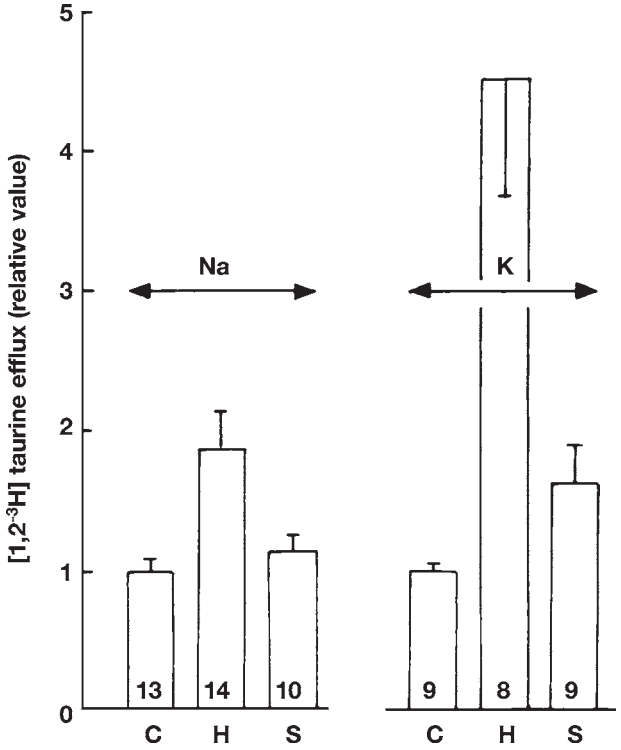

Fig. 4. Effect of hypotonicity upon [1,2-^{3}H]taurine efflux from prelabeled islets. The outflow of tritiated taurine (fmol/islet) over 15 min incubation in the presence of 8.3 m*M* D-glucose, as recorded in the presence of Na^+ (Na) or its absence (K), was measured either in a hypotonic medium (H) or a medium containing sucrose (S) in order to restore iso-osmolarity. Mean values (± SEM) are expressed relative to the mean corresponding control value (C) found, within the same experiment(s) in a salt-balanced medium and are shown together with the number of individual observations (at the bottom of each column).

(100.0 ± 8.6%; $n = 12$). Restoration of normal osmolarity, by incorporation of 100 m*M* sucrose to the final incubation medium, decreased ($p < 0.05$) [1,2-^{3}H]taurine fractional outflow rate to 77.3 ± 4.3% ($n = 10$) of the mean value found in the absence of the disaccharide (100.0 ± 8.6%; $n = 14$). As a matter of fact, in the presence of sucrose, the [1,2-^{3}H]taurine fractional outflow rate was no more significantly different ($p > 0.3$) from the value recorded, within the same experiments, in a salt-balanced medium (Fig. 4).

Comparable, but not identical, results were observed in Na^+-deprived media, in which NaCl and NaHCO3 were replaced by equimolar amounts of KCl and KHCO3. Thus, in such a case, the decrease in osmolarity by 100 mOsmol/L increased [1,2-^{3}H]taurine outflow ($p < 0.001$) over 15 min incubation in the presence of 8.3 m*M* D-glucose to 447.3 ± 72.7% ($n = 8$) of the mean control values recorded, within the same experiment(s), in the salt-balanced medium (100.0 ± 4.1%; $n = 9$). The incorporation of sucrose (100 m*M*) to the incubation medium decreased ($p < 0.001$) the outflow of tritiated taurine to 37.4 ± 6.4% ($n = 9$) of the mean corresponding values recorded in its absence (100.0 ± 12.7%;

$n = 8$). The relative magnitude of the increase in effluent radioactivity due to hypotonicity was thus much higher ($p < 0.001$) in the Na^+-depleted medium than in the presence of Na^+ (Fig. 4). Moreover, in the former case, even in the presence of 100 m*M* sucrose, the outflow of [1,2-^{3}H]taurine remained higher ($p < 0.03$) than that recorded, within the same experiments, in a salt-balanced medium, it averaging 163.2 ± 25.3% ($n = 9$) of the latter value (100.0 ± 4.1%; $n = 9$).

In these experiments, the islet radioactive content after 60 min incubation in the presence of 8.3 m*M* D-glucose averaged, when expressed as [1,2-^{3}H]taurine equivalent 14.19 ± 0.49 fmol/islet ($n = 75$).

Effect of D-Glucose upon Taurine Efflux

After 60 min preincubation at 37°C in the presence of 8.3 m*M* D-glucose and 0.31 μM [1,2-^{3}H]taurine, the islet radioactive content, as judged from the radioactivity recovered in both the final incubation medium and islet pellet, averaged 19.28 ± 0.94 fmol/islet ($n = 36$). It failed to be significantly different in the islets exposed during the final incubation of 15 min at 37°C to increasing concentrations of D-glucose. At the end of such an incubation, the effluent radioactivity increased ($p < 0.005$ or less) from 2.37 ± 0.18 fmol/islet at 2.8 m*M* D-glucose to 3.77 ± 0.35 and 3.92 ± 0.21 fmol/islet at 5.6 and 8.3 m*M* D-glucose, respectively ($n = 9$ in all cases). At 16.7 m*M* D-glucose, however, it was lower ($p < 0.02$ or less) than at either 5.6 or 8.3 m*M* D-glucose, averaging only 2.57 ± 0.23 fmol/islet ($n = 9$), a value no more significantly different ($p > 0.45$) from that recorded at 2.8 m*M* D-glucose.

In a second series of experiments, the final incubation medium contained no Na^+, NaCl and $NaHCO_3$ being replaced by equimolar amounts of KCl and $KHCO_3$. Under these conditions, the efflux of [1,2-^{3}H]taurine again increased ($p < 0.005$) from 3.56 ± 0.30 fmol/islet ($n = 8$) at 2.8 m*M* D-glucose to 4.93 ± 0.22 ($n = 10$) and 4.76 ± 0.20 ($n = 12$) fmol/islet at 5.6 and 8.3 m*M* D-glucose, respectively. At 16.7 m*M* D-glucose, the mean value for [1,2-^{3}H]taurine was lower than that recorded at 5.6 or 8.3 m*M* D-glucose. Such a difference failed, however, to achieve statistical significance ($p > 0.1$). Nevertheless, at 16.7 m*M* D-glucose, the efflux of tritiated taurine was no more significantly higher ($p > 0.2$) from that measured at 2.8 m*M* D-glucose. Despite the fact that the total radioactive content of the islets happened to be somewhat lower ($p < 0.03$) in this second series of experiments (16.83 ± 0.59 fmol/islet; $n = 42$) than in the first series of experiments (19.28 ± 0.94 fmol/islet; $n = 36$), the absolute values for tritiated taurine efflux at each concentration of D-glucose were always higher ($p < 0.02$ or less) in the former than the latter experiments, averaging in the Na^+-deprived media 140.8 ± 5.6% ($n = 42$; $p < 0.001$) of the corresponding mean values (100.0 ± 3.8%; $n = 36$) found at the same hexose concentration in the salt-balanced media (Fig. 5). When the results obtained in these two series of experiments at increasing concentrations of D-glucose were expressed in percent of their respective overall mean values, the increase in [1,2-^{3}H]taurine efflux caused by a rise in hexose concentration from 2.8 m*M* to 5.6 and 8.3 m*M* D-glucose became highly significant ($p < 0.001$), while the results recorded at 16.7 m*M* D-glucose remained both lower ($p < 0.005$ or less) than those found at 5.6 or 8.3 m*M* D-glucose and not significantly higher ($p > 0.1$) than those observed at 2.8 m*M* D-glucose (Fig. 5).

Discussion

The present study affords the following novel pieces of information. First, it documents that the uptake of taurine by rat pancreatic islets represents a concentration-, time-, and temperature-dependent process, modulated in a concentration-related manner by the extracellular concentration of D-glucose.

Second, the experiments conducted in perifused islets might suggest a dual effect of D-glucose upon effluent radioactivity from prelabeled islets. In this respect, it should be emphasized that, in these and further experiments, it was assumed that such a radioactive outflow corresponds to the exit of [1,2-^{3}H]taurine from the prelabeled islets. The molecular identity of the effluent radioactivity was not assessed, however. The first effect of D-glucose, documented in islets deprived of the hexose for 45 min, consisted in a concentration-related decrease in radioactive efflux. Likewise, in islets exposed for the first 45 min to 4.0 m*M* D-glucose, the [1,2-^{3}H]taurine fractional outflow rate was, between min 30 and 45, lower ($p < 0.001$) than that recorded, at the same time, in glucose-deprived islets. Finally, after prior perifusion at 4.0 m*M* D-glucose, the [1,2-^{3}H]taurine outflow rate was lower in islets exposed to 16.7 m*M*, rather than 8.3 m*M*, D-glucose. This inhibitory action of the hexose on taurine outflow could also participate to the concentration-related increase in [1,2-^{3}H]taurine net uptake recorded in the first series of experiments.

In considering the results of the experiments conducted in perifused islets, it should be kept in mind that the effect of changes in D-glucose concentration was explored at a relatively late time, i.e., 45 min after preincubation of the islets with the tritiated amino sulfonic acid. At that time, the apparent fractional outflow rate of [1,2-^{3}H]taurine outflow was quite low, close to no more than 0.2 percent/min. It was investigated, therefore, whether a different situation may prevail when the efflux of radioactivity is measured immediately after the preincubation in the presence of [1,2-^{3}H]taurine. Under these conditions, the fractional outflow rate of the tritiated amino sulfonic acid, as measured over 15 min incubation in a salt-balanced medium containing 8.3 m*M* D-glucose, was indeed about seven times higher, averaging 1.40 ± 0.09 percent/min ($n = 30$).

In the latter experimental design, evidence was obtained for the presence of volume-sensitive anion channels in the

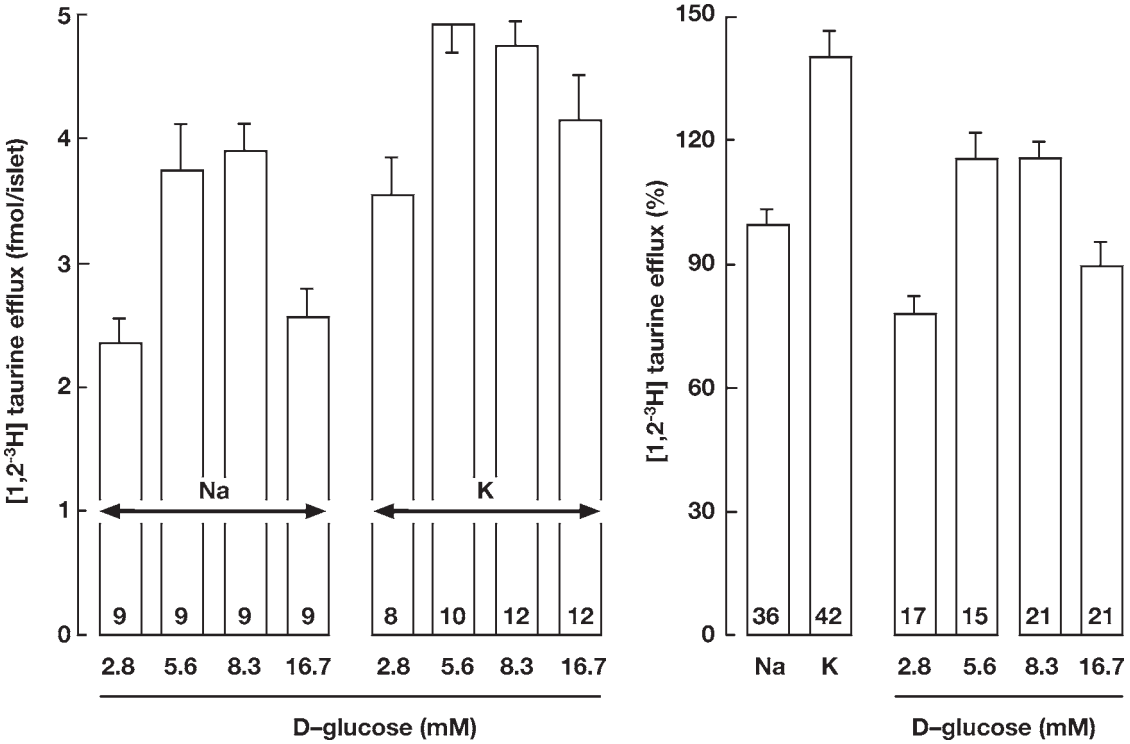

Fig. 5. Effect of increasing concentrations of D-glucose upon [1,2-^{3}H]taurine efflux from prelabeled islets. Left panel: absolute value (fmol/islet per 15 min) recorded in either a salt-balanced medium (Na) or in a medium deprived of Na^+ (K). Right panel: comparison between Na and K media, all results being expressed in percent of those recorded in the Na medium at the same concentration of D-glucose, and effects of increasing concentrations of D-glucose (pooled data obtained in both Na and K media expressed relative to their overall respective mean values). In all cases, mean values are shown together with their SEM and the corresponding number of individual measurements (at the bottom of each column).

islet cells. Thus, on the one hand, the outflow of radioactivity from the prelabeled islets was inhibited by MK571 (1.0 *M*), considered as an inactivator of such channels *(24)*. On the other hand, the efflux of [1,2-^{3}H]taurine from the prelabeled islets was increased when they were exposed to a medium of low osmolarity, this effect being opposed when a normal osmolarity was restored by incorporation of sucrose (100 m*M*) in the incubation medium (Fig. 4).

In the same experimental design, a second effect of D-glucose upon taurine outflow, namely, a hexose-induced increase in taurine efflux, was convincingly documented, whether at normal Na^+ extracellular concentration or in the absence of this cation (Fig. 5). Thus, the effluent radioactivity over 15 min incubation was significantly higher at 5.6 and 8.3 m*M* than at 2.8 m*M* D-glucose. Nevertheless, even in these experiments, a further rise in hexose concentration up to 16.7 m*M* invariably provoked a sizable decrease in [1,2-^{3}H] taurine outflow.

Taken as a whole, these findings indicate that a rise in D-glucose concentration from 2.8 m*M* to 5.6 or 8.3 m*M* increases effluent radioactivity from prelabeled islets, as if the hexose were to cause the gating of voltage-sensitive anion channels. However, the decrease in such an outflow recorded at 16.7 m*M* D-glucose, as well as the findings made in perifused islets, indicate that D-glucose may also decrease the radioactive outflow from prelabeled islets. The interpretation of the latter unexpected finding is open to speculation. It is unlikely to be due to a further decrease in plasma-membrane polarization. Indeed, taurine is poorly ionized at close-to-physiological pH and the decrease in its outflow recorded in the presence of 16.7 m*M* D-glucose persisted in islets exposed to a high concentration of extracellular K^+ (145 m*M* instead of 5 m*M*), sufficient to cause depolarization of the plasma membrane at all concentrations of D-glucose. It is conceivable, however, that the increase in cytosolic pH normally caused by D-glucose *(25)* tended to decrease taurine outflow by augmenting the positive charges of the amino sulfonic acid.

An alternative explanation for the pleiotropic effects of D-glucose upon tritiated taurine outflow from prelabeled islets is inspired by the recent finding that the taurine content of pancreatic islet cells is much higher in glucagon- and somatostatin-producing cells than in insulin-producing cells *(26)*. The striking difference in the effects of D-glucose upon tritiated taurine outflow found between the experiments conducted in prelabeled and perifused islets (Figs. 2 and 3), on the one hand, and those performed immediately after preincubation in the presence of [1,2-^{3}H]taurine (Fig. 5), on the other hand, could indeed be considered in the framework of a dissimilarity in taurine handling by insulin-pro-

ducing vs glucagon-producing cells. Thus, the results obtained in the second set of experiments (Fig. 5) may well relate preferentially to the outflow of tritiated taurine from insulin-producing cells, because such cells are apparently unable to maintain a high taurine content. If so, the rise in [1,2-^{3}H]taurine net uptake found at increasing concentrations of D-glucose (Fig. 1) could also indicate facilitated entry of the amino sulfonic acid in islet B-cells through gated volume-sensitive anion channels. Inversely, the inhibitory action of D-glucose upon [1,2-^{3}H]taurine outflow from prelabeled and perifused islets (Figs. 2 and 3) might concern preferentially glucagon-producing cells able to maintain for a longer period a sufficient intracellular concentration of the amino sulfonic acid. The postulated opposite effects of D-glucose on tritiated taurine outflow from insulin- and glucagon-producing cells would then coincide with the well known opposite effects of the hexose on hormonal release from these two islet cell types.

The highly speculative nature of the above comments lead us to conclude that the characterization of the effects of D-glucose on taurine handling by rat islets, especially the outflow of [1,2-^{3}H]taurine from prelabeled islets, does not provide unambiguous information on the regulation by the hexose of volume-sensitive anion channel activity. As a matter of fact, it is striking that some other research teams, which documented the increase in taurine outflow from islets in response to hypoosmolarity, either did not provide any information on the effect of D-glucose upon the same variable *(14,27)* or failed to detect any significant effect of the hexose *(26)*. In this respect, the measurement of $^{36}Cl^-$ efflux from prelabeled islets would appear as a more reliable approach *(18,19)*.

Materials and Methods

All experiments were conducted in isolated pancreatic islets prepared from fed female Wistar rats (Iffa Credo, L'Arbresle, France) by the collagenase procedure and collected under control of a dissecting microscope *(28)*. Tritiated taurine ([1,2-^{3}H]taurine) was purchased from Amersham (UK). The leukotriene LTD4 receptor antagonist MK571 (3-([{3-(2-[7-chloro-2-quinolinyl]ethenyl)phenyl}-{3-dimethylamino-3-oxopropyl)-thio}-methyl]thio)proprionic acid, sodium salt) was kindly provided by Prof. R. Beauwens (Brussels Free University).

For the study of [1,2-^{3}H]taurine net uptake, groups of 20 islets each were incubated for 10–120 min at 4°C or 37°C in 40 μL of a HEPES- and bicarbonate-buffered medium containing bovine serum albumin (5 mg/mL), D-glucose (2.8–16.7 m*M*) and [1,2-^{3}H]taurine (0.24–0.31 μM; 40.7 ± 2.5 cpm/fmol; $n = 5$) and equilibrated against a mixture of O_2/CO_2 (95/5, v/v). This salt-balanced medium consisted of a solution of HEPES (20 m*M*), NaCl (112 m*M*), $NaHCO_3$ (24 m*M*), KCl (5 m*M*), $MgCl_2$ (1.0 m*M*), and $CaCl_2$ (1.0 m*M*). Taking into account the pKa for HEPES and the amount of NaOH used to bring the pH of the medium to 7.4, its osmolarity was estimated to be close to 315 mOsmol/L. After incubation, the islets were separated from the incubation medium by centrifugation through an oil layer *(29)*. In this procedure, the blank value (no islet) represented no more than 0.57 ± 0.10 ‰ ($n = 26$) of the total radioactive content of the incubation medium.

In the experiments conducted in perifused islets, groups of 110 islets each were preincubated for 60 min at 37°C in 240 μL of a salt-balanced medium containing 16.7 m*M* D-glucose and 0.94 μM [1,2-^{3}H]taurine. The islets were then placed in a perifusion chamber. The effluent radioactivity and output of insulin were measured as described elsewhere *(30)*.

In order to measure the efflux of [1,2-^{3}H]taurine from prelabeled islets, they were preincubated, in groups of 20 islets each, for 60 min at 37°C in a salt-balanced medium (40 μL) containing 8.3 m*M* D-glucose and the tritiated amino sulfonic acid (0.31 μM). After removal of the preincubation medium, the islets were washed in 150 μL of a non-radioactive salt-balanced medium and eventually incubated for 5–15 min at 37°C in 40 μL of different media defined in the Results section. The islets were then separated from the incubation medium as described above. Both the islet pellet and an aliquot part (20 μL) of the incubation medium were examined by liquid scintillation for their radioactive content.

All results, including those already mentioned, are presented as mean values (± SEM) together with the number of individual observations. The statistical significance of differences between mean values was assessed by use of Student's *t*-test and, when so required, confirmed by covariance analysis.

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