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# Uptake of Tritiated Glibenclamide by Endocrine and Exocrine Pancreas

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**Tritiated glibenclamide binds to specific receptors and is internalized in pancreatic insulin-producing B-cells. We investigated, therefore, whether tritiated glibenclamide could be used to preferentially label the endocrine, as distinct from exocrine, pancreas. In isolated rat pancreatic islets, the net uptake of $^3$H-glibenclamide reached within 30 min of incubation a near-equilibrium value, corresponding to an apparent distribution space close to three to four times the islet volume. In pieces of pancreas exposed up to 1 h to $^3$H-glibenclamide, however, its apparent distribution space progressively increased and, even at the min 60 of incubation, did not exceed a third of the wet weight of the pieces. Yet, no significant difference could be detected between the time course for $^3$H-glibenclamide uptake by pancreatic pieces from either control animals or rats injected with streptozotocin a few days before the experiments. Likewise, no significant difference in the paired ratio between the radioactive content of the pancreas and plasma could be found between the control and diabetic rats when examined 1, 5, or 24 h after the IV administration of $^3$H-glibenclamide. These findings indicate that the sulfonylurea does not represent a suitable tool for preferential labeling of the endocrine pancreas in the perspective of its imaging by a noninvasive procedure.**

**Key Words:** $^3$H-Glibenclamide; isolated islets; pancreas.

## Introduction

Hypoglycemic sulfonylureas bind to specific receptors located at the plasma membrane of insulin-producing pancreatic islet cells and some other cell types *(1)*. Moreover, hypoglycemic sulfonylureas of the so-called second generation, with high insulinotropic efficiency, such as glibenclamide and glimepiride, were shown to be internalized in islet cells *(2,3)*. In the present study, we investigated whether tritiated glibenclamide could be used for preferential labeling of pancreatic islets, as distinct from acinar cells, with the perspective of developing a noninvasive imaging technique for the endocrine pancreas.

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## Results

### *In Vitro Experiments*

Figure 1 illustrates the time course for the net uptake of $^3$H-glibenclamide (0.1 μ*M*) by groups of 20 islets each incubated in the presence of 8.3 m*M* D-glucose and then submitted to four successive washes (0.1 mL/wash) at 4°C. A near-equilibrium value was reached within 30 min of incubation. The apparent distribution space of the tritiated sulfonylureas averaged, between min 30 and 60 of incubation, 19.5 ± 2.1 nL/islet ($n$ = 24), largely exceeding the intracellular $^3$HOH space of the islets *(4)*. When the islets were successively incubated for 30 min at 37°C in the presence of $^3$H-glibenclamide, washed at 4°C, incubated for 60 min at 37°C in the absence of the sulfonylurea, and eventually washed again, the apparent distribution space was decreased ($p < 0.001$) to 4.4 ± 1.2 nL/islet ($n$ = 12). The inset in Fig. 1 documents the decrease in radioactivity of the successive washing media. The radioactive content of the last washing medium did not exceed 2.9 ± 0.5% ($n$ = 18) of the paired final radioactive content of the islets.

When pancreatic islets were first preincubated for 60 min at 37°C in the absence or presence of streptozotocin (3.8 m*M*), then incubated for 30–90 min in the presence of $^3$H-glibenclamide (0.1 μ*M*) and D-glucose (8.3 m*M*), and eventually washed four times at 4°C, their final radioactive content, expressed as an apparent distribution space, was not affected by the prior exposure to the β-cytotoxic agent, averaging 15.0 ± 2.0 and 15.9 ± 1.0 nL/islet ($n$ = 12 in both cases) after preincubation in the absence and presence of streptozotocin, respectively.

Further experiments were aimed at investigating whether the islets contribute to any detectable extent to the overall uptake of $^3$H-glibenclamide by the pancreatic gland. For such a purpose, the time course for $^3$H-glibenclamide was first examined in pieces of pancreatic tissue from control

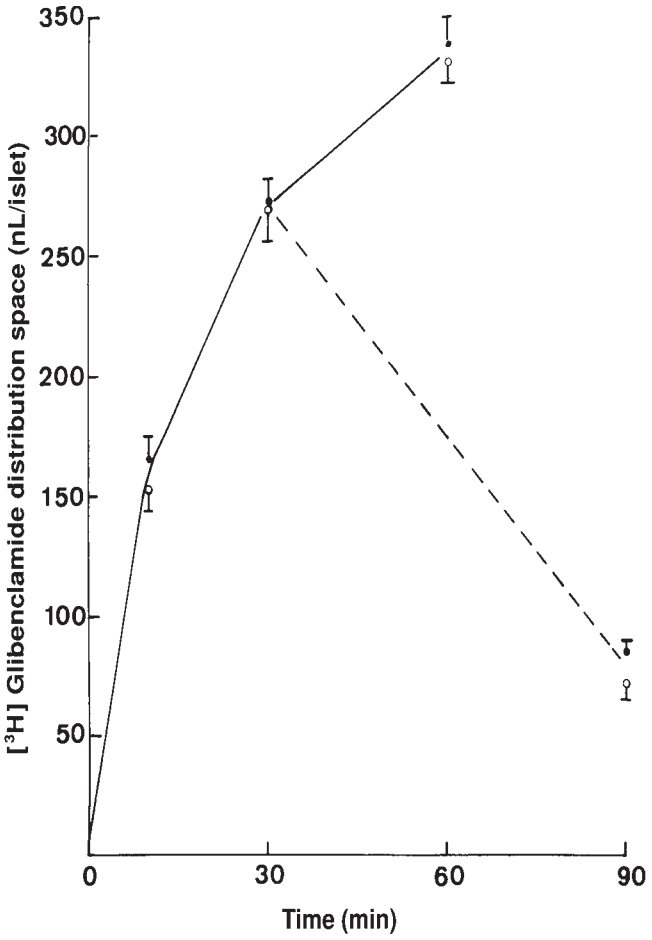


Fig. 1. Time course for the net uptake of $^3$H-glibenclamide by isolated islets (solid line). The dashed line refers to the decrease in the radioactive content of islets first incubated for 30 min in the presence of $^3$H-glibenclamide and then further incubated for 60 min in its absence. The inset illustrates the decrease in the radioactive content of the incubation medium (IM) and successive washing media (logarithmic scale) during the washing procedure. All results are expressed as the apparent distribution space of $^3$H-glibenclamide (nanoliters/islet) and represent mean values (±SEM) derived from 12 (main graph) or 18–24 (inset) individual observations.

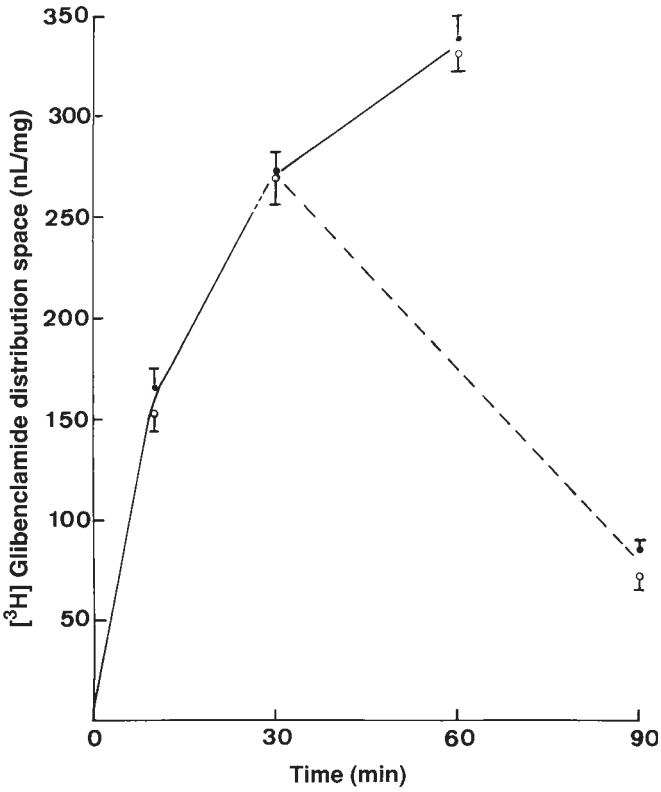


Fig. 2. Time course for the net uptake of $^3$H-glibenclamide (solid line) by pieces of pancreas from either control rats (s ) or STZ rats (d ). The dashed line refers to the decrease in the radioactive content of the pieces first incubated for 30 min in the presence of $^3$H-glibenclamide and then further incubated for 60 min in its absence. All results are expressed as the apparent distribution space of $^3$H-glibenclamide (nanoliters/mg of wet weight) and represent mean values (±SEM) derived from 12–18 individual measurements.

rats and animals injected intravenously with streptozotocin (0.25 μmol/g body weight) 4–6 d before the experiments. At sacrifice, the plasma D-glucose concentration averaged 8.01 ± 0.98 and 22.12 ± 2.84 m*M* ($n$ = 3 in both cases; $p <$ 0.01) in these control and STZ rats, respectively. As shown in Fig. 2, no significant difference could be detected between control and diabetic rats in terms of $^3$H-glibenclamide uptake and release by the pancreatic pieces. Thus, in both types of rats, the apparent distribution space of $^3$H-glibenclamide in the pancreatic pieces progressively increased over 1 h of incubation at 37°C, reached after 60 min of incubation a value close to one third of the wet weight of the pieces, and markedly decreased when the pieces of pancreas were incubated for 60 min in the absence of the sulfonylurea after a first incubation of 30 min in its presence. In these experiments, the radioactive content of the last washing medium corresponded to a $^3$H-glibenclamide apparent distribution space not exceeding 343.1 ± 24.5 nL/sample ($n$ = 120), a value indeed quite low when considering that the wet weight of the pancreatic pieces averaged 20.4 ± 0.3 mg ($n$ = 120). In fact, the radioactive content of the last wash represented no more than 1.01 ± 0.07% ($n$ = 120) of the paired final radioactive content of the pancreatic pieces. We then investigated whether a comparable situation prevails in vivo after intravenous administration of the tritiated sulfonylurea.

### *In Vivo Experiments*

Over the 7 d following the injection of streptozotocin, the diabetic rats gained only 8 ± 3 g, as distinct ($p <$ 0.05) from 19 ± 4 g over the same period in the control animals ($n$ = 9 in both cases). The plasma D-glucose concentration averaged 9.08 ± 0.26 and 32.35 ± 1.20 m*M* in the control and STZ rats, respectively ($n$ = 9; $p <$ 0.001). The plasma insulin concentration was much lower ($p <$ 0.001) in the STZ rats (23.7 ± 2.7 μU/mL; $n$ = 8) than in the control animals (61.8 ± 8.9 μU/mL; $n$ = 9).

The apparent distribution space of $^3$H-glibenclamide, as judged from its plasma concentration, tended to be higher in the STZ rats than in the control animals; the difference achieved or was close to achieving statistical significance ($p < 0.06$) 1 and 24 h after administration of $^3$H-glibenclamide. When all the available results were pooled, the apparent distribution space of the tritiated sulfonylurea was 26.5 ± 14.7% higher (df = 14; $p < 0.07$) in the STZ than in the control rats examined at the same time after the injection of $^3$H-glibenclamide. Such an apparent distribution progressively increased ($p < 0.001$) from 72.0 ± 8.5 to 98.5 ± 12.7 and 129.6 ± 7.0% ($n = 6$ in each case) of the overall mean value found in the same type of rats, as the time after $^3$H-glibenclamide injection increased from 1 to 5 and 24 h. No significant amount of radioactivity could be detected in erythrocytes, whether in control or STZ rats and whether 1, 5, or 24 h after administration of $^3$H-glibenclamide.

Whether expressed in absolute terms (8.581 ± 0.188 vs 9.129 ± 0.129 g) or relative to paired body wt. (4.02 ± 0.08 vs 4.42 ± 0.07%), the wet weight of the liver was lower ($p <$ 0.05 or less) in the control rats than in the diabetic animals ($n$ = 9 in all cases). One to five hours after $^3$H-glibenclamide injection, the radioactive content of the liver was comparable in control and STZ rats. Relative to that of plasma, it increased ($p < 0.05$) from 36.1 ± 4.6 to 51.4 ± 3.6 ($n = 6$ in both cases) between the first and fifth hour after administration of the tritiated sulfonylurea. However, 24 hours after such an administration, the radioactive content of the liver was much higher ($p < 0.005$) in control animals (776.9 ± 32.2 cpm/mg) than in STZ rats (349.5 ± 30.0 cpm/mg). Even when expressed relative to paired plasma radioactivity, it remained significantly higher ($p < 0.05$) in the control rats than in the diabetic animals (Table 1).

The absolute value for the wet weight of the pancreatic gland was not significantly different in control and STZ rats. When expressed relative to paired body weight, however, it was higher ($p < 0.05$) in the STZ rats (4.29 ± 0.15‰; $n$ = 9) than in the control animals (3.73 ± 0.17‰; $n$ = 9). Relative to paired plasma radioactivity, that of the pancreas was never significantly different in control and STZ rats. In absolute terms, however, it was somewhat higher ($p < 0.06$) in control animals than STZ rats. Indeed, the values recorded in the control animals averaged 133.5 ± 18.2% (df = 14) of the mean corresponding value found, at the same time, in the STZ rats. Moreover, in the control animals, the mean paired ratios between pancreas and plasma radioactivity progressively increased (r = 0.600; $n$ = 9; $p < 0.09$; double logarithmic coordinates) during the experiments, whereas this was not the case in STZ rats.

The paired ratio between pancreatic and hepatic radioactivity (counts per minute/milligram) failed to differ significantly in control and STZ rats. One to five hours after the injection of $^3$H-glibenclamide, it averaged 0.645 ± 0.064% ($n = 12$), as distinct ($p < 0.02$) from 1.059 ± 0.173% ($n = 6$), 24 h after the administration of the tritiated sulfonylurea.

## Discussion

The present results confirm that the apparent distribution space of $^3$H-glibenclamide in isolated pancreatic islets rapidly reaches a near-equilibrium value largely in excess of the islet total volume *(5)*. A different situation was found in pieces of pancreatic tissue, in which case the apparent distribution space of the tritiated sulfonylureas progressively increased over 1 h of incubation and, even at min 60, represented no more than about one third of the wet weight of the pieces. In considering these findings, bear in mind that the apparent uptake of radioactive hypoglycemic sulfonylureas by living cells does not necessarily imply the presence of specific binding sites. Indeed, such an uptake process can be simulated in protein-free multilamellar liposomes *(6)*. In the latter model, the time course and concentration dependency, as well as the characterization of so-called specific and nonspecific binding components, are all similar to those found in intact cells, emphasizing the quantitatively important contribution of the drug insertion into the phospholipid bilayer domain of cell membranes to its overall uptake.

Despite the obvious differences in the time course for $^3$H-glibenclamide uptake by isolated islets and pieces of pancreas, no significant difference could be observed between control and STZ rats in terms of the uptake of $^3$H-glibenclamide by pieces of pancreas. This situation seems unlikely to be attributable to an interference of streptozotocin with the specific or nonspecific binding of the tritiated glibenclamide to the plasma membrane. Indeed, when islets were preincubated with streptozotocin, it failed to affect significantly the subsequent uptake of $^3$H-glibenclamide, although these experiments were conducted according to a protocol previously used to document the alteration of glucose-stimulated insulin release by the β-cytotoxic agent *(7)*.

The sole indication of an impaired uptake of $^3$H-glibenclamide by the endocrine pancreas of STZ rats was obtained in vivo. Thus, after IV administration of $^3$H-glibenclamide, the radioactive content of the pancreas was lower in STZ rats than in control animals. The most obvious difference between control and STZ rats, however, consisted in the much lower hepatic accumulation of radioactivity in the liver of the latter, as distinct from former, animals, when examined 1 d after the IV of $^3$H-glibenclamide. Whether such a difference reflects a relatively lower contribution of the liver to the clearance of the tritiated sulfonylurea in the diabetic rats, as could result from a larger elimination of the hypoglycemic agent by osmotic diuresis, remains to be investigated.

In conclusion, the present study clearly indicates that $^3$H-glibenclamide does not represent a suitable tool for the preferential labeling of the endocrine pancreas, at least in the perspective of its imaging by a noninvasive procedure.

## Materials and Methods

Tritiated glibenclamide, labeled at positions 2 and 3 in the cyclohexyl ring (52 Ci/mmol), was purchased from

**Table 1**
Biological Data on Control and STZ Rats Injected with $^{3}$H-Glibenclamide

| | Control rats | | | STZ rats | | |
|---|---|---|---|---|---|---|
| | 1h | 5h | 24h | 1h | 5h | 24h |
| Body weight (g) | | | | | | |
| Day 0 | 185 ± 2 | 182 ± 4 | 215 ± 3 | 187 ± 3 | 193 ± 6 | 215 ± 6 |
| Day 7 | 207 ± 2 | 207 ± 1 | 226 ± 6 | 197 ± 4 | 208 ± 10 | 216 ± 2 |
| Paired change | +23 ± 1 | +24 ± 3 | +11 ± 3 | +10 ± 4 | +14 ± 6 | +1 ± 5 |
| Plasma | | | | | | |
| D-Glucose (m*M*) | 9.75 ± 1.31 | 8.53 ± 0.44 | 8.97 ± 0.20 | 35.33 ± 1.50 | 29.75 ± 1.43 | 31.97 ± 2.25 |
| Insulin (μU/mL) | 96 ± 18 | 58 ± 6 | 42 ± 8 | 20 ± 4 | 31 ± 1 | 21 ± 6[a] |
| $^{3}$H-Glibenclamide space (mL/g) | 1.65 ± 0.26 | 3.00 ± 0.51 | 3.23 ± 0.32 | 2.78 ± 0.24 | 2.64 ± 0.29 | 4.41 ± 0.24 |
| Liver | | | | | | |
| Wet wt (g) | 8.389 ± 0.253 | 8.540 ± 0.229 | 8.814 ± 0.513 | 8.959 ± 0.041 | 8.886 ± 0.171 | 9.542 ± 0.198 |
| Wet wt (% of body weight) | 4.04 ± 0.13 | 4.13 ± 0.14 | 3.89 ± 0.16 | 4.54 ± 0.10 | 4.29 ± 0.15 | 4.43 ± 0.06 |
| Liver:plasma radioactive ratio | 36.5 ± 5.3 | 52.0 ± 7.3 | 45.0 ± 5.0 | 35.7 ± 9.4 | 50.9 ± 3.5 | 26.3 ± 3.0 |
| Pancreas | | | | | | |
| Wet wt (mg) | 768 ± 95 | 801 ± 73 | 835 ± 41 | 902 ± 41 | 844 ± 94 | 932 ± 62 |
| Wet wt (‰ of body weight) | 3.64 ± 0.51 | 3.86 ± 0.13 | 3.79 ± 0.23 | 4.56 ± 0.12 | 4.02 ± 0.28 | 4.30 ± 0.28 |
| Pancreas:plasma radioactive ratio (%) | 22.4 ± 5.4 | 33.2 ± 5.1 | 43.0 ± 14.3 | 29.0 ± 2.8 | 27.5 ± 2.1 | 30.9 ± 2.1 |
| Pancreas:liver radioactive ratio (%) | 0.61 ± 0.14 | 0.64 ± 0.04 | 0.95 ± 0.33 | 0.80 ± 0.29 | 0.54 ± 0.04 | 1.17 ± 0.07 |

[a] Mean value derived from only two observations, as distinct from three measurements in all other cases.

NEN Life Science (Boston, MA). All experiments were conducted in female Wistar rats (Iffa Credo, L'Arbresle, France) given free access to food (KM-04-k12; Pavan Service, Oud Turnhout, Belgium) and tap water up to the time of the experiments. Some investigations were performed in rats that had been injected intravenously with streptozotocin (0.25 μmol/g of body weight; Sigma, St. Louis, MO) a few days before the experiments.

The methods used to measure the apparent distribution space of $^{3}$H-glibenclamide in either pancreatic islets, isolated by the collagenase method, or pieces of pancreas were comparable to those recently reported for assessing the net uptake of $^{65}$Zn *(8)*. Briefly, after incubation at 37°C in the presence of $^{3}$H-glibenclamide, the islets or pieces of pancreas were submitted to repeated washes at 4°C and eventually examined for their radioactive content by liquid scintillation.

Likewise, the procedure used to measure the radioactive content of plasma, erythrocytes, liver, and pancreas after IV injection of $^{3}$H-glibenclamide was similar to that recently reported for assessing the net uptake of $^{65}$Zn by different organs *(8)*.

All results are presented as mean values (±SEM) together with either the number of individual observations (*n*) or degree of freedom (df). In the case of data representing the ratio between two independent variables, a geometric, rather than arithmetic, mean was calculated. In such a case, the SEM given in Results represents the mean of the upper and lower deviation from the geometric mean *(9)*.

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