# Resistance to alloxan of tumoral insulin-producing cells

Abdullah Sener and Willy J. Malaisse

*Laboratory of Experimental Medicine, Brussels Free University, Brussels B-1000, Belgium*

Received 17 October 1985

Rat pancreatic islets and insulin-producing cells of the RINm5F line were incubated for 5 min at 7 or 23°C in media containing $^3H_2O$ and either L-[1-$^{14}$C]glucose or [2-$^{14}$C]alloxan. In the islets the intracellular distribution space of [2-$^{14}$C]alloxan represented, at 7 and 23°C respectively, 11.4 ± 1.0 and 25.5 ± 2.3% of the intracellular $^3H_2O$ space. In the RINm5F cells, the distribution space of [2-$^{14}$C]alloxan failed to be affected by the ambient temperature and represented, after correction for extracellular contamination, no more than 5.2 ± 0.5% of the intracellular $^3H_2O$ space. Preincubation for 30 min at 7°C in the presence of alloxan (10 mM) failed to affect subsequent D-[U-$^{14}$C]glucose oxidation in the tumoral cells, whilst causing a 70% inhibition of glucose oxidation in the islets. It is proposed that RINm5F cells are resistant to the cytotoxic action of alloxan, this being attributable, in part at least, to poor uptake of the diabetogenic agent.

*Tumoral insulin-producing cell* *Pancreatic islet* *Alloxan*

## 1. INTRODUCTION

Cultured tumoral insulin-producing cells of the RINm5F line are currently used for the study of biochemical and biophysical events associated with insulin secretion. These tumoral cells differ, however, from normal pancreatic B-cells by their poor secretory response to D-glucose [1–3]. Such a situation coincides with an abnormal pattern of glucose metabolism in the RINm5F cells [3,4]. We have recently observed that a major difference between RINm5F and normal pancreatic B-cells consists in an impaired transport of hexose into the tumoral cells [5]. Thus, whereas the extracellular and intracellular concentrations of D-glucose rapidly reach close-to-equilibrium in normal B-cells [6], virtually no D-glucose accumulates in tumoral cells [5]. This unexpected finding led us to investigate whether the diabetogenic agent alloxan, which is thought to be transported into the normal B-cell by the same carrier as that responsible for the transport of hexoses [7], also accumulates to a lesser extent in tumoral than in normal insulin-producing cells. This report indicates that tumoral cells indeed take up alloxan less efficiently than normal islet cells and that this coincides with a resistance of the RINm5F cells to the cytotoxic action of alloxan.

## 2. MATERIALS AND METHODS

Pancreatic islets prepared by the collagenase method from fed albino rats [8] and RINm5F cells cultured and harvested as described [9] were placed in 60 μl of a bicarbonate-buffered medium [8] containing bovine albumin (5 mg/ml). Groups of 20 pancreatic islets or $3 \times 10^5$ RINm5F cells were incubated for 5 min at either 7 or 23°C in the presence of either $^3H_2O$ and 2.0 mM L-[1-$^{14}$C]glucose (control) or $^3H_2O$ and 10.0 mM [2-$^{14}$C]alloxan (alloxan). The distribution space of the labelled molecules was then measured as described in [10]. In table 1, the distribution space of the $^{14}$C-labelled compounds, i.e. L-[1-$^{14}$C]glucose in the control group and [2-$^{14}$C]alloxan in the alloxan group, is expressed in percent of the paired $^3H_2O$ space.

For measuring the oxidation of glucose, the islets or cells were preincubated for 30 min at 7°C with or without alloxan (10 mM), washed once



*Published by Elsevier Science Publishers B.V. (Biomedical Division)*
00145793/85/$3.30 

with an alloxan-free medium, and eventually incubated for 60 min at 37°C in the sole presence of 2.8 mM D-[U-$^{14}$C]glucose [11].

All results are expressed as the mean values (± SE) together with the number of individual observations (in parentheses). The statistical significance of differences between mean values was assessed by the Student's *t*-test.

## 3. RESULTS

To study the cellular uptake of alloxan, pancreatic islets or tumoral cells were incubated for 5 min, at 7 or 23°C, in media containing $^3H_2O$ and either L-[1-$^{14}$C]glucose or [2-$^{14}$C]alloxan. The distribution space of L-[1$^{14}$C]glucose in either the islets or tumoral cells was slightly higher at 23 than 7°C (table 1). The intracellular volume taken as the paired difference between the $^3H_2O$ and L-[1-$^{14}$C]glucose distribution spaces, averaged 4.68 ± 0.32 nl per islet and 1.14 ± 0.05 pl per tumoral cell ($n$ = 20 in each case; pooled data obtained at 7 and 23°C). Whether at 7 or 23°C, the $^3H_2O$ space was somewhat higher ($P < 0.02$) in islets exposed to alloxan (10 mM) than in control islets. Thus, in the presence of alloxan, the $^3H_2O$ space averaged 124.7 ± 7.9% of the mean corresponding control value (100.0 ± 4.7%; $n$ = 20 in each case). Our data do not allow one to decide whether this increase corresponds to an enlargement of the interstitial and/or the intracellular space(s). A comparable phenomenon was not observed in the tumoral cells in which the $^3H_2O$ space averaged, in the presence of alloxan, 104.5 ± 1.8% of the mean corresponding control value (100.0 ± 2.0%; $n$ = 20 in both cases; $P > 0.1$).

The distribution space of [2-$^{14}$C]alloxan exceeded that of L-[1-$^{14}$C]glucose under all experimental conditions (table 1). In the RINm5F cells, the alloxan space was unaffected by a rise in temperature from 7 to 23°C. In the islets, however, the alloxan space was almost doubled in response to the same increase in temperature (table 1). By comparison of the data obtained, within each experiment, with L-[1-$^{14}$C]glucose and [2-$^{14}$C]alloxan, it was calculated that the intracellular distribution space of [2-$^{14}$C]alloxan, in the tumoral cells, did not exceed 5.24 ± 0.47% of the intracellular space ($n$ = 20, pooled data obtained at 7 and 23°C). In the islets, however, the intracellular distribution space of [2-$^{14}$C]alloxan amounted to 11.35 ± 0.96% (at 7°C) and 25.48 ± 2.28% (at 23°C) of the intracellular space ($n$ = 10 in each case). In comparing the results obtained in the islets and tumoral cells, it should be kept in mind that the true uptake of alloxan by normal B-cells is probably higher than suggested by our data, since alloxan is virtually excluded from non-B islet cells [12].

The difference in alloxan uptake by tumoral and normal cells coincided with a difference in their sensitivity to the cytotoxic action of the diabetogenic agent, as assessed by their capacity to oxidize D-[U-$^{14}$C]glucose [13]. Preincubation of the islets with alloxan (10 mM) for 30 min at 7°C resulted in a severe reduction ($P < 0.001$) in D-[U-$^{14}$C]glucose oxidation (table 1). Under the same experimental conditions and, despite the high concentration of alloxan used in these experiments, the diabetogenic agent failed to affect glucose oxidation in the tumoral cells.

Table 1

Uptake and effect of alloxan in pancreatic islets and RINm5F

| Experimental conditions | Islets | | RINm5F cells | |
|---|---|---|---|---|
| | Control | Alloxan | Control | Alloxan |
| Distribution space of L-[1-$^{14}$C]glucose (control) or [2-$^{14}$C]alloxan (alloxan) expressed in % of $^3H_2O$ space | | | | |
| 7°C | 12.71 ± 2.03 (10) | 22.62 ± 1.83 (10) | 7.75 ± 0.44 (10) | 14.14 ± 0.81 (10) |
| 23°C | 16.84 ± 1.91 (10) | 38.03 ± 1.83 (10) | 10.18 ± 0.82 (10) | 13.37 ± 0.33 (10) |
| D-[U-$^{14}$C]Glucose oxidation (pmol/islet or $10^3$ cells per 60 min) | | | | |
| 37°C | 2.92 ± 0.11 (12) | 0.89 ± 0.05 (12) | 5.55 ± 0.13 (20) | 5.62 ± 0.17 (20) |

## 4. DISCUSSION

The present findings indicate that RINm5F cells are resistant to alloxan, and suggest that such a resistance is attributable, in part at least, to a poor uptake of the diabetogenic agent by the tumoral cells. This is consistent with the knowledge that the cellular accumulation of alloxan represents an essential, albeit not the sole, determinant of its cytotoxic action [14]. Incidentally, a modest uptake of alloxan by the RIN5mF cells is not incompatible with the integrity of glucose oxidation. Indeed, a restricted uptake of alloxan may not be sufficient to impair the function of islet cells. For instance, such a situation was already encountered in pancreatic islets exposed to alloxan in the presence of 3-*O*-methyl-D-glucose [15].

In conclusion, when taken together with our prior observations on the uptake of hexoses by RINm5F cells [5], the present results reinforce the view that an impaired transport of hexoses and structurally related molecules represents a fundamental defect of these tumoral, as distinct from normal, insulin-producing cells.

## ACKNOWLEDGEMENTS

We thank J. Schoonheydt and M. Urbain for technical help. This work was supported by grants from the Belgian Foundation for Scientific Medical Research and Belgian Ministry of Scientific Policy.

## REFERENCES

[1] Sopwith, A.M., Hutton, J.C., Naber, S.P., Chick, W.C. and Hales, C.N. (1981) Diabetologia 21, 224–229.
[2] Praz, G.A., Halban, P.A., Wollheim, C.B., Blondel, B., Strauss, A.J. and Renold, A.E. (1983) Biochem. J. 210, 345–352.
[3] Giroix, M.-H., Sener, A., Dufrane, S.P., Malaisse-Lagae, F. and Malaisse, W.J. (1985) Arch. Biochem. Biophys. 241, 561–570.
[4] Halban, P.A., Praz, G.A. and Wollheim, C.B. (1983) Biochem. J. 212, 439–443.
[5] Malaisse, W.J., Giroix, M.-H., Malaisse-Lagae, F., Leclercq-Meyer, V. and Sener, A. (1985) Excerpta Medica ICS, in press.
[6] Hellman, B., Sehlin, J. and Täljedal, I.-B. (1971) Biochim. Biophys. Acta 241, 147–154.
[7] Weaver, D.C., McDaniel, M.L., Naber, S.P., Barry, D.C. and Lacy, P.E. (1978) Diabetes 27, 1205–1214.
[8] Malaisse-Lagae, F. and Malaisse, W.J. (1984) in: Methods in Diabetes Research (Larner, R. and Pohl, S.L. eds) pp.147–152, John Wiley, New York.
[9] Giroix, M.-H., Malaisse-Lagae, F., Sener, A. and Malaisse, W.J. (1985) Mol. Cell. Biochem. 66, 61–64.
[10] Malaisse, W.J., Sener, A., Welsh, M., Malaisse-Lagae, F., Hellerström, C. and Christophe, J. (1983) Biochem. J. 210, 921–927.
[11] Carpinelli, A., Sener, A., Herchuelz, A. and Malaisse, W.J. (1980) Metabolism 29, 540–545.
[12] Gorus, F.K., Malaisse, W.J. and Pipeleers, D.G. (1982) Biochem. J. 208, 513–515.
[13] Malaisse, W.J. (1982) Biochem. Pharmacol. 31, 3527–3534.
[14] Malaisse, W.J., Malaisse-Lagae, F., Sener, A. and Pipeleers, D.G. (1982) Proc. Natl. Acad. Sci. USA 79, 927–930.
[15] Malaisse-Lagae, F., Sener, A. and Malaisse, W.J. (1983) Biochim. Biophys. Acta 762, 36–43.