BIOCHEMICAL BASIS OF A SPECIES DIFFERENCE IN SENSITIVITY TO ALLOXAN

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1. Introduction

Selective toxicity of alloxan towards the pancreatic B-cell can be explained by the coincidence of two biochemical features: the capacity of alloxan to accumulate rapidly into the B-cell [1,2]; and the high sensitivity of the B-cell towards peroxides (unpublished). In other tissues, either alloxan does not enter rapidly into the cell (e.g., in muscle) or the activity of enzymes involved in the reduction of peroxides is sufficiently high to ensure resistance against these agents (e.g., in liver).

Guinea pigs are more resistant than rats towards the diabetogenic action of alloxan [3]. Here, we have investigated whether this species difference could be accounted for by a difference in sensitivity of pancreatic islets removed from guinea pigs and rats, respectively, towards t-butyl hydroperoxide.

2. Materials and methods

Pancreatic islets were isolated by the collagenase method [4] from fed guinea pigs or albino rats. Groups of 10–15 islets each were preincubated for 30 min at 37°C in 30 μl bicarbonate-buffered medium [5] containing bovine albumin (5 mg/ml), D-glucose (11.1 mM) and, as required, *tert*-butyl hydroperoxide (0.005–2.0 mM; Aldrich-Europe, Beerse). The preincubation medium was then brought to 60 μl by addition of an identical medium enriched with D-[U-^{14}C]glucose (10 μCi/ml). The oxidation of glucose was measured as in [6] over 60 min incubation at 37°C and expressed as pmol . h^{-1} . $islet^{-1}$. The statistical significance of differences between mean values was assessed by use of the Student's *t*-test.

3. Results

In the absence of t-butyl hydroperoxide, the oxidation of D-[U-^{14}C]glucose (11.1 mM) was not significantly different in rat and guinea pig islets, averaging, respectively, 28.7 ± 1.2 (n = 34) and 23.9 ± 3.4 (n = 11) pmol . h^{-1} . $islet^{-1}$. Up to 0.1 mM, t-butyl peroxide failed to significantly affect glucose oxidation by rat islets (fig.1). At higher concentrations (0.02–2.0 mM), t-butyl peroxide caused a dose-related inhibition of glucose oxidation by rat islets. Guinea

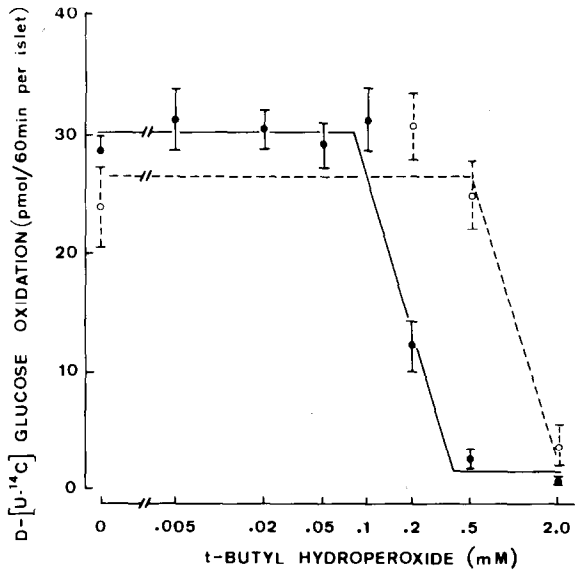

Fig.1. Effect of increasing concentrations of t-butyl hydroperoxide (logarithmic scale) upon the oxidation of D-[U-^{14}C]-glucose by islets of rats (●——●) and guinea pigs (○– – –○). Mean values (±SEM) refer to 10–34 individual measurements. In each species, the horizontal lines were drawn at the mean level derived from all sets of data which were not significantly different from one another.

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pig islets were more resistant than rat islets to t-butyl peroxide. For instance, at 0.5 mM, t-butyl peroxide failed to significantly affect glucose oxidation by guinea pig islets and virtually abolished oxidation in rat islets. Thus, relative to the mean control value, the rate of glucose oxidation in the presence of 0.5 mM t-butyl peroxide averaged 104.6 ± 12.1% (n = 13) and 9.1 ± 3.1% (n = 10) in guinea pig and rat islets, respectively ($P < 0.001$). The dose–action relationship suggested that guinea pig islets are ~5-times more resistant than rat islets towards t-butyl hydroperoxide (fig.1).

4. Discussion

It is thought that the cytotoxic effect of alloxan is related to the generation of reactive compounds such as hydrogen peroxide, superoxide anion radicals and hydroxyl radicals [7–9]. However, the selectivity of the cytotoxic effect of alloxan towards the pancreatic B-cell remained so far unexplained. In the rat, using t-butyl hydroperoxide as substrate, glutathione peroxidase activity was found to be much lower in islets than in such tissues as liver, kidney or exocrine pancreas [10]. To assess the functional significance of this finding, we investigated whether the difference in enzymatic activity was reflected by a difference in the sensitivity towards exogenous peroxides: We examined the influence of t-butyl hydroperoxide upon D-[U-^{14}C]glucose oxidation by several tissues. t-Butyl hydroperoxide was used instead of alloxan, on the assumption that the entry of the former drug into the cell would not display any marked tissue specificity, in contrast to alloxan which, shortly after its injection in vivo, accumulates preferentially in such tissues as liver and pancreatic islets [2,11,12]. Using glucose oxidation as an indicator of the sensitivity of intact cells towards t-butyl hydroperoxide, we observed, in good agreement with the enzymic data, that islets were much less resistant to peroxide than other tissues (unpublished). This study indicates that the lesser sensitivity of guinea pigs, as distinct from rats, towards the diabetogenic action of alloxan [3] coincides with a lesser sensitivity of the pancreatic islets towards t-butyl hydroperoxide. Thus, this finding supports the view that the selective toxicity of alloxan towards the pancreatic B-cell is attributable, in addition to the cellular uptake of the drug, to the poor capacity of islet cells to protect themselves against the deleterious effects of peroxides.

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