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Selective uptake of alloxan by pancreatic B-cells

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Alloxan rapidly binds to or accumulates in pancreatic B-cells as distinct from non-B-cells. The selective uptake of this cytotoxic agent by the insulin-producing B-cells might account for its well-known diabetogenic effect.

Alloxan, which induces diabetes mellitus in animals (Dunn *et al.*, 1943; Dunn & McLetchie, 1943), selectively destroys the insulin-producing pancreatic B-cells without affecting other islet cells. It has been suggested (Malaisse *et al.*, 1982) that this cytotoxic effect involves both a rapid uptake of alloxan (Weaver *et al.*, 1978; Malaisse *et al.*, 1982) and a drug-induced generation of oxygen-containing radicals and peroxide (Heikkila *et al.*, 1976; Grankvist *et al.*, 1979, 1981*a,b*; Fisher & Hamburger, 1980; Malaisse *et al.*, 1982). Furthermore, it is conceivable that the exquisite sensitivity of the B-cell is caused by a preferential uptake of the drug by B-cells as distinct from islet non-B-cells. Alloxan uptake was therefore measured in B- and non-B-cell preparations purified from the rat endocrine pancreas.

Experimental

Materials

Alloxan and bovine serum albumin (fraction V) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); [^{14}C]urea, [6,6′(*n*)-^{3}H]sucrose and 3-*O*-methyl-D-[U-^{14}C]glucose were from Amersham International (Amersham, Bucks., U.K.); and [2-^{14}C]alloxan has from California Bionuclear Corp. (Sun Valley, CA, U.S.A.). Both labelled and unlabelled alloxan preparations contained less than 5% alloxanic acid as judged by t.l.c. (Weaver *et al.*, 1978).

Methods

Islets of Langerhans were isolated by collagenase digestion (Lacy & Kostianovsky, 1967) from the pancreas of adult fed Sprague–Dawley rats and were dissociated by trypsin treatment in a Ca^{2+}-free Krebs medium (Pipeleers & Pipeleers-Marichal, 1981). Differences in islet-cell size were used to separate single B-cells (>95% B-cells) from single non-B-cells (<5% B-cells) (Pipeleers & Pipeleers-Marichal, 1981; Van De Winkel *et al.*, 1982). After preincubation of the cells for 30 min at 23°C in glucose-free Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]-buffered Krebs–Ringer medium (KRH) containing 10 g of bovine serum albumin/litre, uptake experiments were performed with 100 μl samples containing 75000 cells incubated for 5 min at 23°C in 0.6 mM-[6,6′(*n*)-^{3}H]sucrose (25 μCi/ml) and 0.6 mM-[^{14}C]urea (12.5 μCi/ml), 0.6 mM-[2-^{14}C]-alloxan (1.8 μCi/ml) or 0.6 mM-3-*O*-methyl-D-[U-^{14}C]glucose (12.5 μCi/ml). Some experiments were conducted in the presence of unlabelled D-glucose (20 mM), alloxan (0.6 mM) or urea (0.6 mM). After 5 min, the cells were separated from the medium by a 4 s centrifugation at 8000 **g** (Microfuge B; Beckman Instruments, Fullerton, CA, U.S.A.) through 100 μl of di-n-butyl phthalate (BDH Chemicals, Poole, Dorset, U.K.; *d* 1.045) and the radioactivity of the cell pellets was counted (Malaisse *et al.*, 1978). From these data the apparent distribution space of the ^{14}C-labelled compounds was calculated; results were always corrected for extracellular contamination as judged by the [^{3}H]sucrose space measured in the same sample (Malaisse *et al.*, 1978). In view of the short half-life of alloxan at 23°C and pH 7.4 (Weaver *et al.*, 1978), the drug was kept in 1 mM-HCl until start of the experiment.

Statistical analysis

Results were expressed as mean values ± S.E.M. for the numbers of experiments stated in parentheses. The statistical significance of differences between experimental groups was assessed by Student's *t* test for unpaired data.

Results and discussion

Cytosolic volume was measured through the [^{14}C]urea space, which was corrected for extracellular contamination (Malaisse *et al.*, 1978), and

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Table 1. *Distribution of various ^{14}C-labelled compounds in purified islet-cell preparations*
After a 30min preincubation at 23°C, purified B- and non-B-cells were incubated for 5min at 23°C in KRH medium containing various ^{14}C-labelled and unlabelled compounds. Results are expressed as mean values ± S.E.M. for the numbers of experiments stated in parentheses, and are corrected for the corresponding [^3H]sucrose space. The sucrose space averaged 377 ± 43 fl/cell for single non-B-cells ($n = 10$) and 431 ± 25 fl/cell for single B-cells ($n = 16$) and was not significantly altered by 5min exposure to 0.6mM-alloxan at 23°C. No experiments were carried out at 37°C in view of the short half-life (1.38min) of alloxan under such conditions *in vitro* (Weaver *et al.*, 1978).

^{14}C-labelled compound	Unlabelled compound	Single non-B-cells (fl/cell)	Single B-cells (fl/cell)
Urea (0.6mM)	—	177 ± 25 (7)	733 ± 37 (10)
Urea (0.6mM)	Alloxan (0.6mM)	182 ± 23 (5)	777 ± 28 (6)
Alloxan (0.6mM)	Urea (0.6mM)	28 ± 7 (5)	474 ± 29 (11)
Alloxan (0.6mM)	Urea (0.6mM) + glucose (20mM)	54 ± 7 (6)	425 ± 22 (8)
3-*O*-Methyl-D-glucose (0.6mM)	—	56 ± 11 (7)	759 ± 47 (7)

was 3–4 times larger for B-cells than for non-B-cells (Table 1); this ratio is higher than the ratio of the corresponding cell volumes as measured by Coulter analysis (Pipeleers & Pipeleers-Marichal, 1981), which is consistent with our observation that B- and non-B-cells mainly differ in their cytoplasmic volumes, rather than in the size of their nuclear compartment. In single B-cells, the apparent space of alloxan distribution averaged 60% of the corresponding urea space, which contrasts with the 15% found in non-B-cells (Table 1). The addition of 0.6mM-alloxan has no effect on the urea space, as corrected for the corresponding sucrose space (Table 1), and is therfore unlikely to increase membrane permeability of rat B-cells; these results confirm earlier observations in intact rat pancreatic islets (McDaniel *et al.*, 1975), but are at variance with studies on toadfish islets (Watkins *et al.*, 1973).

In contrast with B-cells, the 3-*O*-methyl-D-glucose space of non-B-cells differed significantly from the corresponding urea space (Table 1), indicating that the transport of both alloxan and 3-*O*-methyl-D-glucose occurs more slowly in non-B-cells than in B-cells. This identical behaviour of stereomeric analogues suggests that hexose transport in islet non-B-cells becomes a rate-limiting step for their metabolism, but not in islet B-cells (Hellman *et al.*, 1971).

Under the present conditions, alloxan uptake by B-cells was unaltered by 20mM-D-glucose (Table 1), which is rather unexpected if glucose would really interact with alloxan binding to or its entry into the B-cell membrane (Watkins *et al.*, 1973). It is, however, more likely that glucose protects the B-cell via its metabolism, e.g. by increasing the generation of reducing equivalents (Sener *et al.*, 1982). Incidentally, a modest but significant ($P < 0.025$) enhancement of alloxan uptake by non-B-cells was noted in the presence of 20mM-D-glucose.

In conclusion, our data demonstrate that alloxan rapidly and selectively accumulates in pancreatic B-cells, which contrasts with a low uptake in islet non-B-cells. So far, no difference has been observed in the protection mechanisms of the various islet cell types against alloxan (Malaisse *et al.*, 1982). It is therefore conceivable that, by analogy with muscle cells, non-B-cells are protected against alloxan (Cooperstein & Watkins, 1981) through a low degree of drug uptake, rather than through the existence of enzymic protection mechanisms. The association of a high rate of alloxan uptake and a poor enzymic protection seems, until now, to be a unique feature of the islet B-cells and might fully account for their exquisite vulnerability to alloxan. It is, however, not excluded that variations in intracellular degradation of alloxan or in its generation of oxygen-containing radicals also contribute to the observed variability in tissue sensitivity to alloxan.

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