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BINDING OF OUABAIN TO NORMAL AND TUMORAL PANCREATIC ISLET CELLS

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Abstract—The binding of [^{3}H(G)]ouabain was examined in both rat pancreatic islets and tumoral islet cells (RINm5F line). In the islets, the specific binding was considered too low to allow for extensive investigations, taking into account the limited number of islets readily available. In the RINm5F cells, which bound more ouabain than normal islet cells, the time course for binding, its temperature dependency, its competitive inhibition by K^+, the number of binding sites, their affinity, and the turnover of ATP per binding site were all similar to those found in other cell types. An incomplete dissociation of [^{3}H(G)]ouabain by unlabelled glycoside and the stimulation of binding by D-glucose were noticed, both phenomena being possibly attributable, in part at least, to the functional response of the RINm5F cells to D-glucose with resulting activation of exocytosis–endocytosis coupling.

Ouabain has been used for several decades to interfere with the process of insulin release [1–3]. The glycoside inhibits both ^{86}Rb uptake and ^{22}Na outflow in rat pancreatic islets [4, 5], these cationic changes coinciding with an enhancement of glucose-induced insulin secretion. However, to our knowledge, the binding of the glycoside to pancreatic islet cells had not yet been investigated. The present study provides information on the binding of tritiated ouabain to both normal and tumoral islet cells.

MATERIALS AND METHODS

Pancreatic islets isolated from fed albino rats [6] and tumoral islet cells of the RINm5F line [7] were prepared as described in the cited references. Except if otherwise mentioned, groups of 15 to 60 islets or 0.5×10^6 RINm5F cells were incubated for 30 min at 37° in a K^+-free and bicarbonate-buffered medium (0.1 ml) equilibrated against CO_2/O_2 (1/19, v/v) and containing 120 mM NaCl, 24 mM $NaHCO_3$, 1 mM $MgCl_2$, 1 mM $CaCl_2$, D-glucose (16.7 mM in the experiments with islets and 2.8 mM in the experiments with RINm5F cells), [^{3}H(G)]ouabain (31.5–39.7 Ci/mmol, NEN Research Products) and, as required, unlabelled ouabain. When KCl was added to the medium, its NaCl content was decreased in order to maintain the same osmolarity. After incubation, the islets or cells were washed rapidly (< 0.5 min) and twice with 0.5 ml of non-radioactive medium, the islet or cell pellet being eventually examined for its radioactive content by liquid scintillation.

The non-specific binding was measured in the presence of 0.1 mM unlabelled ouabain in both the incubation and washing medium and subtracted from all other readings.

In both pancreatic islets and RINm5F cells, the radioactivity recovered in the second wash was virtually identical after incubation in either the sole presence of a tracer concentration of [^{3}H(G)]ouabain (e.g. 7.5 nM) or concomitant presence of unlabelled ouabain (0.1 mM). Although trapping of extracellular material in the compartmentalized interstitial domain of islets [8] cannot be ruled out, these data suggest that the specific binding of [^{3}H(G)]ouabain was little affected by the washing procedure. This was confirmed by direct comparison (Table 1) of results obtained either in the washing technique or by separation of the RINm5F cells from the incubation medium by centrifugation through a layer of oil [9]. In the latter procedure, the blank readings recorded in the absence of cells did not exceed 7.7 ± 2.2 cpm per sample ($N = 8$). As expected from the incomplete removal of extracellular radioactivity in the washing technique, the apparent non-specific binding was three to four times higher in the latter technique than in the oil procedure. However, after correction for such a non-specific binding (including extracellular contamination), the specific binding of [^{3}H(G)]ouabain (75 nM) was not significantly lower in the washing technique than oil procedure ($P > 0.25$). Although the data documented in Table 1 suggest that the oil procedure may provide more precise results than the washing technique, the latter approach was nevertheless followed in all further experiments because the size of the extracellular space in islets separated from the incubation medium by the oil technique (approximately 1.0 nl/islet), relative to the actual binding of [^{3}H(G)]ouabain (see below) precluded the use of this technique in the case of normal islets (as distinct from RINm5F cells).

In order to measure ^{86}Rb net uptake, groups of 1.6 to 1.7×10^6 RINm5F cells each were incubated for 60 min at 37° in 0.1 ml of a salt-balanced bicarbonate-buffered medium placed on 0.15 ml of silicone oil [9] itself layered on 30 μl of a solution of

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Table 1. Binding of [^{3}H(G)]ouabain to RINm5F cells*

Separation procedure	Washing technique		Oil procedure	
[^{3}H(G)]ouabain (nM)†	75	75	75	75
Unlabelled ouabain (mM)	—	0.1	—	0.1
Bound radioactivity (cpm)‡	1936 ± 138	1143 ± 138	1344 ± 49	330 ± 17
Specific binding (amol/10^3 cells)	57.0 ± 14.0		72.8 ± 3.8	

* The cells were incubated for 30 min at 37° in a K^+-free bicarbonate-buffered medium containing 2.8 mM D-glucose.

† The radioactive content of the incubation medium (2.38 μCi/ml) yielded a mean reading of 1465.7 ± 26.4 cpm/μl (N = 8).

‡ The number of cells amounted to 712.5 × 10^3 cells per sample; each mean value is derived from six individual measurements.

HCl (50 mM) and CsCl (0.64 M). After incubation, the tubes were centrifuged for 2 min (Beckman Microfuge, Palo Alto, CA) and the tip of the tube containing the cell pellet and CsCl solution examined for its radioactive content. Blank readings recorded in the absence of cells represented no more than 0.08 ± 0.02% (N = 8) of the mean experimental values found in the presence of RINm5F cells. No correction for extracellular contamination was introduced since this would not exceed, under the present experimental conditions 0.2 pmol/10^3 cells [9], i.e. less than 0.3% of the actual net uptake of ^{86}Rb by the tumoral cells.

All results, including those already mentioned, are expressed as the mean value (± the range of individual variation, if N = 2, or SE, if N ≥ 3). The statistical significance of differences was assessed by use of Student's *t*-test.

RESULTS

Pancreatic islets

The non-specific binding of [^{3}H(G)]ouabain to pancreatic islets was close to the limit of detection, not exceeding 16 ± 4 fmol/islet (N = 9) after 30 min incubation when expressed by reference to the specific radioactivity of the glycoside (0.1 mM). As shown in Fig. 1, under the present experimental conditions, the specific binding of [^{3}H(G)]ouabain was grossly proportional to both its concentration, in the 50 to 100 nM range, and the number of islets (15–60 islets/0.1 ml). It averaged, at a concentration of 100 nM, 17.2 ± 3.1 amol/islet (N = 18). Even so, the recovery of cell-associated radioactivity was considered too low and, hence, not precise enough to allow for more detailed investigations.

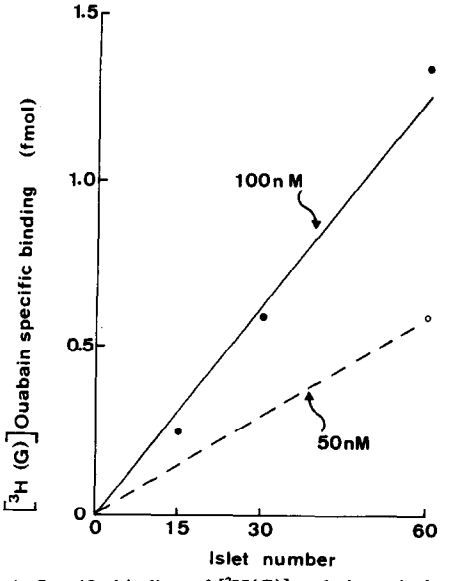

Fig. 1. Specific binding of [^{3}H(G)]ouabain to isolated rat islets over 30 min incubation, as a function of both the concentration of the glycoside and the number of islets. Mean values are derived from duplicate measurements in a representative experiment.

RINm5F cells

Over 60 min incubation at 37° in a salt-balanced (K^+: 5.0 mM) bicarbonate-buffered medium containing 2.8 mM D-glucose, ouabain (0.1 mM) decreased the net uptake of ^{86}Rb (12.5 μCi/ml; 0.07 mM), expressed as K^+ equivalent with the same ^{86}Rb/^{39}K ratio as in the incubation medium, from a control value of 79.1 ± 2.0 to 58.7 ± 2.3 pmol/10^3 RINm5F cells (N = 6 in both cases; $P < 0.001$). Taking into account the intracellular H_2O space of RINm5F cells (510 pl/10^3 cells; see Ref. 9), the control value for ^{86}Rb net uptake would yield an intracellular K^+ concentration close to 155 mM.

The non-specific binding of [^{3}H(G)]ouabain to RINm5F cells averaged, after 30 min incubation at 37° in the presence of 2.8 mM D-glucose, 38 ± 8 fmol/10^3 cells (N = 52) when expressed by reference to the specific radioactivity of the glycoside (0.1 mM). Such a non-specific binding was little affected by factors such as the temperature or length of incubation and the absence or presence of D-glucose (data not shown).

The specific binding of [^{3}H(G)]ouabain to RINm5F cells (0.5 × 10^6 cells/0.1 ml) measured over 30 min incubation at 37° in the presence of 2.8 mM D-glucose averaged, when the glycoside was tested at a concentration of 7.5 nM, 26.0 ± 2.4 amol/10^3 cells (N = 56).

At the same concentration of [^{3}H(G)]ouabain, the specific binding of the glycoside was grossly proportional to the number of RINm5F cells and mark-

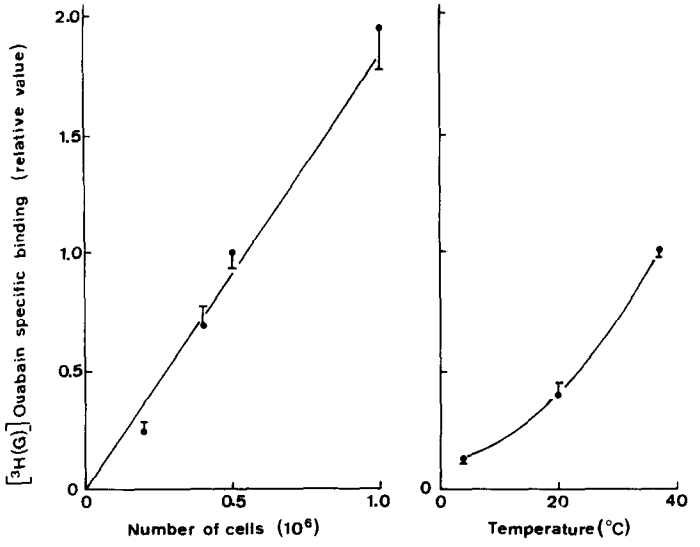

Fig. 2. Relationship between the specific binding of [^{3}H(G)]ouabain (7.5 nM) and either the number of RINm5F cells (left panel) or temperature (right panel). Mean values (± range of individual variation) refer to two observations and are expressed relative to the mean reference value found, within the same experiment, after 30 min incubation at 37° in the presence of 0.5×10^6 cells.

edly decreased at low temperatures (Fig. 2). It reached a close-to-equilibrium value after 30 min incubation (Fig. 3, upper panel). When the cells were first exposed for 30 min at 37° to [^{3}H(G)]ouabain (7.5 nM) and then incubated for 10 to 60 min at 37° in the presence of unlabelled glycoside (0.1 mM), a progressive decline in cell-associated radioactivity, as measured after correction for non-specific binding, was observed (Fig. 3, lower panel). However, even after 60 min incubation in the presence of unlabelled ouabain, the cell-associated specific binding still represented 43.1 ± 2.1% (N = 3) of the mean initial reading recorded immediately after the period of exposure to [^{3}H(G)]ouabain.

When increasing concentrations of unlabelled ouabain in the 50 to 100 nM range were present together with the tracer tritiated glycoside (7.5 nM), a concentration-related decrease in the bound/free ratio was observed (Fig. 4). At higher concentrations of unlabelled ouabain (1.0 to 10.0 μM), the cell-associated radioactivity was barely higher than the non-specific binding (measured in the presence of 0.1 mM unlabelled ouabain). The data were compatible, at least as far as a high-affinity binding process is concerned, with a maximal specific binding close to 0.1 amol/cell (6.0×10^4 binding sites per cell) and a half-maximal binding at a concentration of free ouabain close to 21 nM.

At a concentration of 0.1 mM (or less), K^+ decreased the specific binding of [^{3}H(G)]ouabain (7.5 nM) by no more than about 25% (Fig. 5, left panel). In the millimolar range (1–5 mM), however, K^+ provoked a marked and dose-related inhibition of [^{3}H(G)]ouabain specific binding, down to 15.1 ± 3.4% of the control value found in the absence of K^+. A Dixon plot yielded an apparent K_i close to 1.0 mM.

Over 30 min incubation at 37°, the specific binding of [^{3}H(G)]ouabain was lower ($P < 0.025$) in the

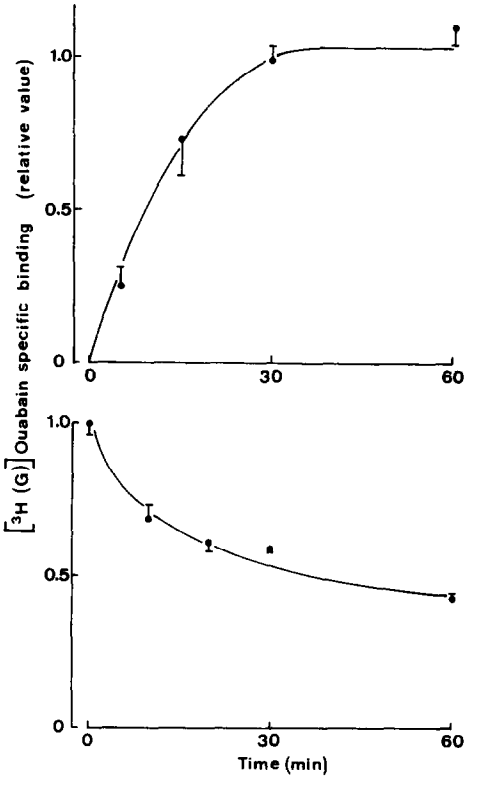

Fig. 3. Upper panel: time course for the specific binding of [^{3}H(G)]ouabain (7.5 nM) to RINm5F cells. Lower panel: time course for the decrease in binding observed in cells incubated in the presence of 0.1 mM unlabelled glycoside after an initial exposure of 30 min to [^{3}H(G)]ouabain (7.5 nM). Same presentation as in Fig. 2. Mean values refer to two to five individual measurements and are shown together with either the range of variation (N = 2) or SE ($N \geq 3$).

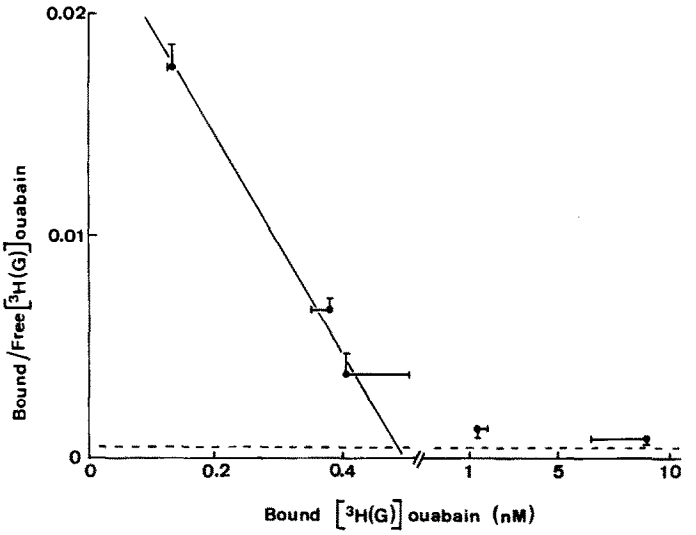

Fig. 4. Scatchard plot for the specific binding of [^{3}H(G)]ouabain to RINm5F cells (0.5 × 10^6 cells/0.1 ml) over 30 min incubation at 37°. Mean values (± SE) refer to 6 ± 1 individual measurements. The regression line was calculated from the mean readings recorded at the three lowest concentrations of the glycoside. The horizontal dotted line corresponds to the SE value for the non-specific binding (measured in the presence of 0.1 mM unlabelled ouabain).

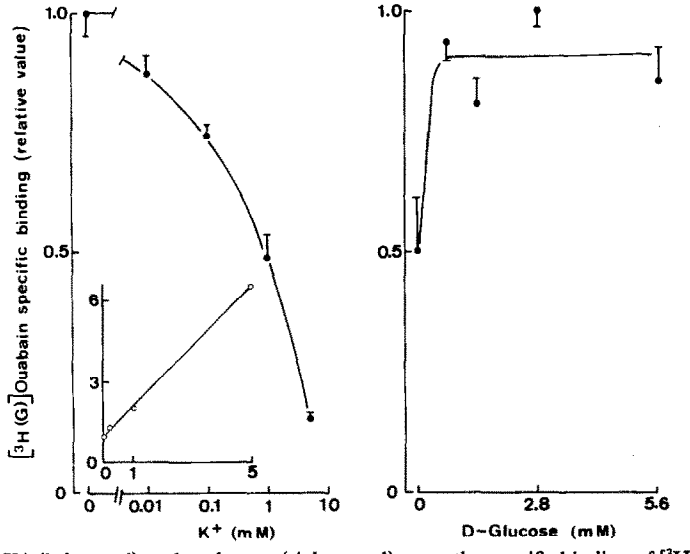

Fig. 5. Effect of K^+ (left panel) and D-glucose (right panel) upon the specific binding of [^{3}H(G)]ouabain (7.5 nM) to RINm5F cells. Same presentation as in Fig. 2. Mean values refer to 4–6 (left panel) or 5–14 (right panel) individual measurements. In the left panel, the inset refers to a Dixon plot of the same data as those illustrated in semi-logarithmic coordinates in the main figure.

absence than presence of D-glucose (Fig. 5, right panel). A concentration of D-glucose as low as 0.7 mM was sufficient to augment the binding of the glycoside above basal value ($P < 0.005$), no further significant increase in specific binding being observed at higher concentrations of the hexose (1.4–5.6 mM).

DISCUSSION

The present results indicate that tritiated ouabain binds in a specific manner to both normal and tumoral islet cells. With the limited number of isolated islets readily available, the specific binding of the glycoside to normal islet cells was considered too low, however, to allow for extensive investigations. At the same concentration of [^{3}H(G)]ouabain (0.1 μM), its specific binding appeared higher, relative to either the cell surface or protein content [9, 10], in tumoral than normal islet cells, as already observed in other rapidly dividing cells [11, 12]. For instance, in the presence of 0.08–0.10 μM [^{3}H(G)]ouabain, the specific binding of the glycoside

per μg protein was about one order of magnitude higher in RINm5F cells (412 ± 108 amol/μg) than pancreatic islets (23 ± 5 amol/μg).

The time course for the specific binding of [^{3}H(G)]ouabain to RINm5F cells, its temperature dependency, and its competitive inhibition by K^+ were all similar to those found in other cell types [12–16]. The number of binding sites per cell was also comparable to that previously measured in other cells [11, 12].

In RINm5F cells exposed to D-glucose, the steady-state K^+ content is close to 75 pmol/10^3 cells and the K^+ fractional outflow rate close to 0.02/min [17]. These data yield an outflow rate of K^+ close to 1.5 pmol per min and per 10^3 cells. Assuming that the latter value is also representative of the rate of K^+ inflow and that all influent K^+ would be transported by an ouabain-sensitive Na–K–ATPase with a 3/2/1 stoichiometry for Na^+/K^+/ATP [4], the ATP consumption linked to the active transport of monovalent cations would be close to 0.8 pmol/min per 10^3 cells, representing 6 to 7% of the total rate of ATP generation, i.e. approximately 11.6 pmol/min per 10^3 cells in RINm5F cells exposed to D-glucose [18]. From these data and the number of ouabain binding sites, the active transport of K^+ was estimated to consume about 7,500 molecules of ATP per min and per pump, in fair agreement with data collected in mammalian intestine, kidney, brain and heart [13].

Two less classical features of the binding of ouabain to RINm5F cells consisted in the apparently incomplete dissociation of [^{3}H(G)]ouabain in the presence of unlabelled glycoside and the stimulation of binding by D-glucose. The concentration dependency of the latter phenomenon was similar to that observed for other biophysical and biochemical variables in RINm5F cells [10, 17] and, hence, its occurrence may be linked to the functional response of these islet cells to D-glucose, including changes in membrane viscosity [19] and activation of exocytosis–endocytosis coupling [20]. The latter process, if coupled with the internalization of [^{3}H(G)]ouabain, could account, in part at least, for the incomplete release of cell-associated radioactivity after a first exposure of the RINm5F cells to the tritiated glycoside in the presence of D-glucose. Alternatively, ouabain may be taken up by RINm5F cells by a carrier-mediated process comparable to that found in rat hepatocytes [21].

In conclusion, the present data based mainly but not exclusively on the use of tumoral islet cells, indicate that the binding of ouabain to these cells displays, from both the qualitative and quantitative standpoints, essentially the same characteristics as those encountered in other cell types. The identification and quantification of ouabain binding sites in islet cells are not solely relevant to the effect of the glycoside upon cationic movements and insulin release, but may also represent a useful tool in exploring the mechanism(s) responsible for alteration of islet cell function, as suggested for instance by the higher binding capacity of tumoral than normal islet cells.

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