Vol. 164, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1989 Pages 212-218

# FREE AND BOUND SODIUM IN PANCREATIC β-CELLS EXPOSED TO GLUCOSE AND TOLBUTAMIDE

**Liaquat Ali, Eva Grapengiesser, Erik Gylfe, Bo Hellman and Per-Eric Lund**

Department of Medical Cell Biology, Uppsala University, Biomedicum,
Box 571, S-751 23 Uppsala, Sweden

Received August 30, 1989

The effects of glucose and tolbutamide on the sodium handling of the pancreatic β-cells were evaluated by measuring the total sodium content in intact islets from ob/ob-mice by integrating flame photometry and the free ion in individual β-cells by dual wavelength fluorometry. Whereas increasing the glucose concentration from 3 to 20 mM resulted in a lowering of sodium, the addition of 100 μM tolbutamide caused a rise. The above-mentioned effects were most marked (about 50%) for the physiologically significant free sodium. The data indicate a more important role for $Na^+$ in the regulation of insulin release than so far acknowledged. Increase of $Na^+$ may contribute to the secretory response to hypoglycemic sulfonylureas by providing an additional rise of cytoplasmic $Ca^{2+}$. 

Pancreatic β-cells differ from most excitable cells, including glucagon-producing $\alpha_2$-cells, in lacking prominent $Na^+$ channels (1,2). Nevertheless their content of $Na^+$ may be an important determinant of the $Ca^{2+}$ which regulates insulin release. Whereas a rise of cytoplasmic $Na^+$ can be expected to increase this $Ca^{2+}$ by mobilizing it from organelles and by inhibition of outward transport, lowering of $Na^+$ would favour $Ca^{2+}$ elimination (3).

Taking advantage of the sensitivity offered by integrating flame photometry it has been possible to measure the total sodium in islets isolated from ob/ob-mice (4,5) and rats (6-8). Contrary to previous assumptions this approach revealed that glucose does not increase but actually lowers the islet content of sodium. Although glucose and insulin-releasing sulfonylureas depolarize the β-cells by a common mechanism (9), the latter compounds were found to increase islet sodium. Since the presence of different cell types within the islets may contribute to this discrepancy, we now present data on free $Na^+$ ($Na^+_i$) in individual β-cells measured by dual wavelength microfluorometry. Evidence will be provided that the effects of glucose and tolbutamide on this physiologically important fraction of sodium in the β-cells are rapid, qualitatively similar and even more marked than those recorded for the total amount of this element in intact islets.

0006-291X/89 $1.50

## MATERIALS AND METHODS

Islets of Langerhans were isolated from the splenic part of the pancreas by collagenase digestion using 10 months old ob/ob-mice taken from a non-inbred colony (10). Previous studies have indicated that these islets contain more than 90% β-cells, which respond normally to glucose and other stimulators of insulin release (11). The isolated islets were either transferred directly to RPMI-1640 medium supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 µg/ml streptomycin and 30 µg/ml gentamycin or used for preparing single cells (12), which were allowed to attach to cover glasses during storage in the same type of medium. After culture for 1-2 days at 37°C in a humidified atmosphere of air-$CO_2$ subsequent experimental handling was performed in an albumin-containing (1 mg/ml) Hepes buffer physiologically balanced in cations with $Cl^-$ as the sole anion (13). When not otherwise stated this medium was supplemented with 3 mM glucose.

Measurements of total sodium. After 45 min of preincubation at 37°C in the presence of 3 mM glucose, intact islets were exposed for 15 min to a medium of similar composition (controls) or modified as indicated in Table 1. At the end of the experiments the islets were washed for 2 min with an ice-cold solution of sucrose (4). Subsequent freeze-drying and weighing of the islets made it possible to express the sodium contents in terms of dry weight. The individual islets were analyzed for sodium by integrating flame photometry using a previously described procedure (4).

Measurements of free sodium. Cells attached to cover glasses were loaded with the $Na^+$ indicator SBFI (14-16) during 1.5 - 2 hrs incubation with 5 µM of its acetoxymethyl ester (Molecular Probes, Eugene, Or.) in the presence of 11 mM glucose. Adequate uptake of the indicator was facilitated by also including 0.02% of the non-ionic dispersing agent Pluronic F-127 (BASF Wyandotte Corp., Wyandotte, Mi.) together with 2.5% fetal calf serum in the loading medium (17). The cover glasses with the SBFI-loaded cells were rinsed and used as the bottom of a perifusion chamber designed for microscopy (18). Individual β-cells were selected on the basis of previously described criteria (19,20), and their contents of free sodium continuously recorded by epifluorescence microfluorometry using the experimental set-up described for measurements of intracellular $Ca^{2+}$ (20,21). A xenon arc lamp combined with 5 nm half-bandwith interference filters in the rotating air-turbine filter changer of a time-sharing multichannel spectrofluorometer provided excitation light flashes of 1 ms duration at 340 and 380 nm every 10 ms. Emission at 510 nm was measured with a photomultiplier using a 30 nm half-bandwith filter. The electronically separated signals were fed into an analog ratio meter and the fluorescence excitation ratio used for calculating intracellular sodium. $Na^+_i$ was calibrated in situ by exposing the cells to different concentrations of $Na^+$ in the presence of gramicidin as shown in Fig. 1.

Evaluation of results. Statistical significances for differences between paired observations were evaluated by Student's t-test.

## RESULTS

The total content of sodium found in the islets in the presence of 3 mM glucose was 78 ± 6 mmol/kg dry weight (n = 7). The percentage changes obtained with alterations of the glucose concentration or addition of 100 µM tolbutamide are shown in Table 1. Whereas increasing the glucose concentration to 20 mM resulted in significant lowering of sodium, omission of the sugar was associated with a rise of the sodium content. Tolbutamide had the opposite effect to glucose and raised the islet content of sodium.

The concentration of free sodium in single cells exposed to 3 mM glucose was 13 ± 1 mM (n = 14). Omission of $Na^+$ from the medium caused a rapid decrease of $Na^+_i$, which increased above the original level on subsequent restoration of the external sodium concentration (Fig. 2). The effects of altering the glucose concentration on $Na^+_i$ are shown in Fig. 3. Whereas increase of the glucose concentration from 3 to

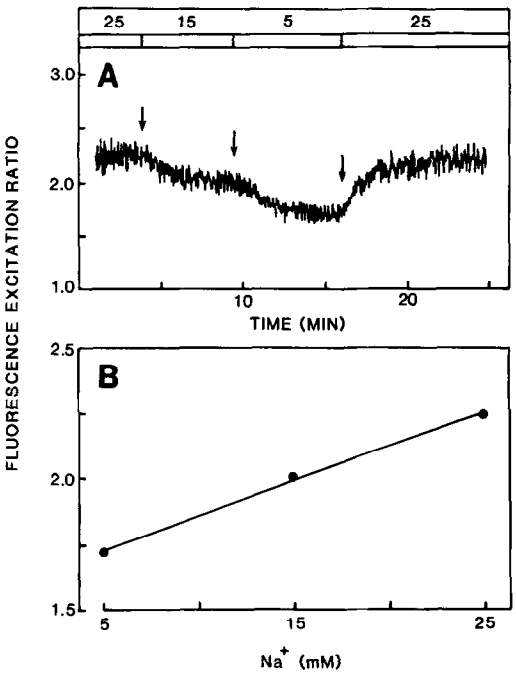


**Fig 1.** Calibration curves relating the fluorescence excitation ratio of intracellular SBFI to the concentration of free $Na^+$. **(A)** β-cells were loaded with SBFI and exposed for 5 min to 10 μg/ml gramicidin in Hepes-buffered medium to allow equilibration of extracellular and intracellular $Na^+$. The medium was then changed to one containing different concentrations of $Na^+$ maintaining the osmotic pressure by adjusting $K^+$. The figure shows the 340/380 nm fluorescence excitation ratio of an individual β-cell exposed to 5, 15 and 25 mM $Na^+$ as indicated. (B) The steady-state fluorescence ratios plotted against free intracellular $Na^+$.

20 mM was associated with a rapid lowering of the $Na^+$ activity, subsequent omission of glucose resulted in an increase of $Na^+_i$ above the original level. Tolbutamide had an action opposite to that of glucose in inducing prompt increase of $Na^+_i$ when added

TABLE 1

Changes of sodium contents after alterations of the glucose concentration or addition of tolbutamide

| Modifications to medium | Percentage changes | |
|---|---|---|
| | Total sodium in islets | Free sodium in β-cells |
| Omission of glucose | + 22 ± 4* | + 50 ± 5** |
| Addition of 17 mM glucose | - 15 ± 4* | - 46 ± 5** |
| Addition of 100 μM tolbutamide | + 27 ± 6* | + 57 ± 9** |

The figures denote the percentage changes (mean values ± SEM) in comparison to a control medium with 3 mM glucose for total (n = 7) and free sodium (n = 8 - 10). Statistical significances for effects are indicated by *P < 0.01; **P < 0.001.

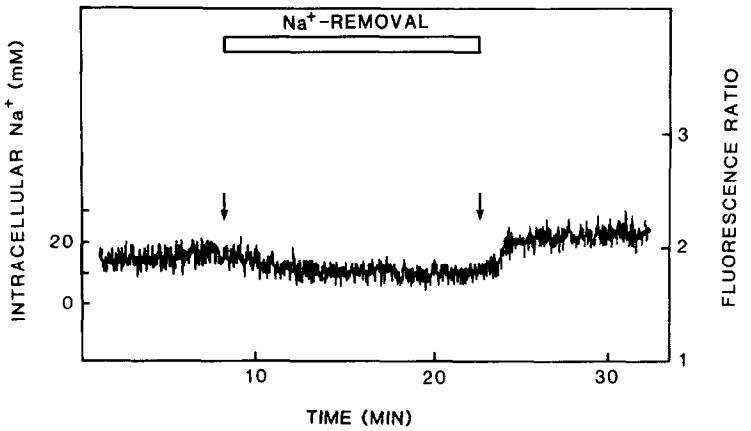


**Fig 2.** Effects of removing extracellular $Na^+$ on free $Na^+$ in an individual β-cell. The figure shows the 340/380 nm fluorescence excitation ratio and the corresponding values for $Na^+_i$. During the period indicated by the bar, $Na^+$ was omitted from the perifusion medium and replaced by equimolar N-methyl-D-glucamine. When repeated in a series of experiments the omission of $Na^+$ resulted in a lowering of $Na^+_i$ by 50 ± 4% (n = 8).

to a basal medium containing 3 mM glucose (Fig. 4). The effects of glucose and tolbutamide are summarized in Table 1. It is evident that the percentage alterations of $Na^+_i$ obtained in individual β-cells when modifying the medium with respect to glucose and tolbutamide are more pronounced than those recorded for the total sodium content of pancreatic islets.

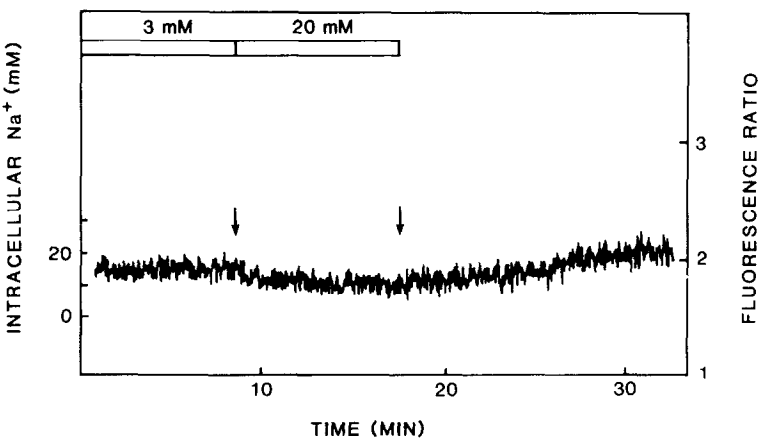


**Fig 3.** Effects of glucose on free $Na^+$ in an individual β-cell. The figure shows the 340/380 nm fluorescence excitation ratio and the corresponding values for $Na^+_i$. The presence of 3 and 20 mM glucose in the perifusion medium is indicated by the bars.

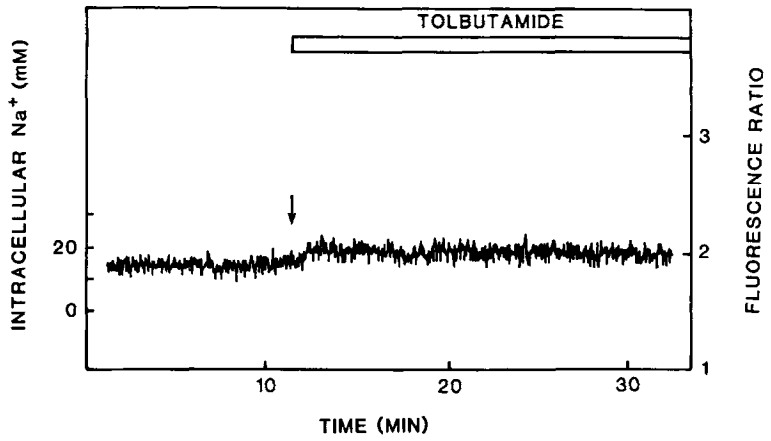


**Fig 4.** Effects of tolbutamide on free $Na^+$ in an individual β-cell. The figure shows the 340/380 nm fluorescence excitation ratio and the corresponding values for $Na^+_i$. The presence of 100 μM tolbutamide in the perifusion medium is indicated by the bar.

## DISCUSSION

The present study indicates that glucose and tolbutamide modify the sodium content in islets isolated from ob/ob-mice. The magnitude of the changes was similar to that previously observed for freshly isolated islets from these animals (4,5). The fact that the two secretagogues had opposite effects on sodium indicates that the observed changes of sodium do not simply result from exocytosis. Since both glucose and tolbutamide supposedly depolarize the β-cells by closing the ATP-regulated $K^+$ channels (9), it is also evident that a reduction of the $K^+$ permeability does not invariably result in a lowering of the islet content of sodium.

The islets from the ob/ob-mice contain up to 90% β-cells (10). Nevertheless, it was considered important to analyze whether the observed alterations in whole islets reflect those occurring in the β-cells. The significance of this approach was underlined by the observations that the $Na^+$ channels in pancreatic β-cells appear to be less active than in other islet cells (9). By measuring $Na^+_i$ in individual β-cells it became possible to analyze also the fraction of sodium directly involved in the regulation of the $Ca^{2+}$ responsible for insulin release. In demonstrating rapid and more marked effects of glucose and tolbutamide on the free $Na^+$ in individual β-cells than on total sodium in whole islets the present study reinforces previous arguments (4-8) for a modulatory effect of $Na^+$ on the release of insulin initiated by glucose and hypoglycemic sulfonylureas.

The fluorescent indicator SBFI represents a significant advance in measurements of $Na^+_i$. The relatively selective binding of $Na^+$ with a $K_d$ of 18 mM (14) is associated with a change of the 340/380 nm excitation ratio. It was therefore possible to measure $Na^+_i$ in individual β-cells adhering to the same procedure with dual wavelength fluorometry as employed in our laboratory for measuring cytoplasmic $Ca^{2+}$ with fura-2 (20,21).

In situ calibration indicated a linear relationship between the fluorescence excitation ratio and the concentration of $Na^+$ in the β-cells under the present experimental conditions. Since removal of extracellular $Na^+$, which causes decrease of the $Na^+_i$, results in acidification of the β-cells (22), it is also important that the fluorescence of SBFI remains relatively unaffected with lowering of pH down to 6.2.

Previous attempts to use SBFI for measuring $Na^+_i$ in single cells from gastric glands (15), smooth muscle (16) and liver (23) have shown resting concentrations between 8-20 mM. The present study reveals similar concentrations of free $Na^+$ in the pancreatic β-cells. Nevertheless, the measured values for $Na^+_i$ are unexpectedly low and should be regarded as provisional until confirmed by other techniques. Indirect estimates of the $Na^+$ activities based on the membrane potential have given twice as high values (24,25). Assuming that intracellular water corresponds to 1.2 l/kg dry weight (4), the total amounts of sodium in the islets exposed to 3 mM glucose were equivalent to a concentration of 65 mM. If the latter figure is representative for the β-cells, only 20% of the sodium is ionized. Various methodological inadequacies might contribute to an underestimate of $Na^+_i$ when β-cells are loaded with the hydrophobic acetoxymethyl ester of SBFI. One possibility is sequestration of the ester in the plasma membrane with incomplete hydrolysis.

With the demonstration of rapid and marked effects of glucose and tolbutamide on the functionally significant $Na^+_i$ in the β-cells it seems likely that this ion has a more important role in the regulation of insulin release than so far acknowledged. The glucose-induced lowering of $Na^+_i$ counteracts the sugar-induced rise of cytoplasmic $Ca^{2+}$ by favouring $Ca^{2+}$ uptake into organelles and outward transport (3). Such an action may be important for a sustained secretory response (26). Sulfonylureas other than tolbutamide also promote a rise of the islet content of sodium (7,8). Increase of $Na^+_i$ can therefore be supposed to be a mechanism, which provides an additional rise of the cytoplasmic $Ca^{2+}$ determining the secretory response of the sulfonylurea-stimulated β-cell.

## ACKNOWLEDGMENTS

This study was supported by grants from the Swedish Medical Research Council (12x-562; 12x-6240), the Swedish Diabetes Association, the Nordic Insulin Foundation, the Swedish Hoechst and Novo Companies and the Family Ernfors Foundation. L.A. is a lecturer from Sir Salimulla Medical College and research associate from BIRDEM, Dhaka, Bangladesh, working as a visiting research fellow sponsored by the International Program in the Chemical Sciences.

## REFERENCES

1. Plant TD (1988). Pflügers Arch 411:429-435.
2. Hiriart M and Matteson DR (1988). J Gen Physiol 91:617-639.
3. Hellman B (1986). Diab/Metab Rev 2:215-241.
4. Wesslén N, Bergsten P and Hellman B (1986). Biosci Rep 6:967-972.

5. Wesslén N, Bergsten P and Hellman B (1988). Pharmacology 37:125-131.
6. Ali L, Wesslén N and Hellman B (1988). Acta Endocrinol 118:227-231.
7. Ali L, Wesslén N and Hellman B (1988). Eur J Pharmacol 158:257-262.
8. Ali L, Wesslén N and Hellman B (1989). Exp Clin Endocrinol 93:299-306.
9. Hellman B, Gylfe E, Bergsten P, Johansson H and Wesslén N (1988). In: Pathogenesis of non-insulin dependent diabetes mellitus (Grill V and Efendić S, eds), pp 39-60. Raven Press, New York.
10. Hellman B (1965). Ann N Y Acad Sci 131:541-558.
11. Hahn HJ, Hellman B, Lernmark Å, Sehlin J and Täljedal IB (1974). J Biol Chem 249:5275-5284.
12. Lernmark Å (1974). Diabetologia 10:431-438.
13. Hellman B (1975). Endocrinology 97:392-398.
14. Harootunian A, Eckert B, Minta A and Tsien RY (1988). Faseb J 2;A728.
15. Negulescu PA, Harootunian A, Minta A, Tsien RY and Machen TE (1988). J Gen Physiol 92:26A.
16. Moore EDW, Minta A, Tsien RY and Fay FS (1989). Biophys J 55:471A.
17. Poenie M, Alderton J, Steinhardt R and Tsien R (1986). Science 233:886-889.
18. Sykes JA and Moore EB (1959). Proc Soc Exp Biol Med 100:125-127.
19. Grapengiesser E, Gylfe E and Hellman B (1988). Biochem Biophys Res Commun 150:419-425.
20. Grapengiesser E, Gylfe E and Hellman B (1989). Acta Physiol Scand 136:113--119.
21. Grapengiesser E, Gylfe E and Hellman B (1989). Arch Biochem Biophys 268:404--407.
22. Pace CS and Tarvin JT (1986). Metabolism 35:176-181.
23. Kawanishi T, Blank LM, Harootunian AT, Smith MT and Tsien RY (1989). J Biol Chem 264:12859-12866.
24. Atwater I, Ribalet B and Rojas E (1978). J Physiol 278:117-139.
25. Meissner HP, Henquin JC and Preissler M (1978). FEBS Lett 94:87-89.
26. Gylfe E (1989). Biochem Biophys Res Commun 159:907-912.