SODIUM UPTAKE BY MICRODISSECTED PANCREATIC ISLETS: EFFECTS OF OUABAIN AND CHLOROMERCURIBENZENE-*p*-SULPHONIC ACID

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1. Introduction

Stimulus-secretion coupling may involve the movement of ions across the plasma membrane. Milner and Hales [1] proposed that in pancreatic β-cells the transport of Ca^{2+} is regulated by Na^+; intracellular Na^+ was assumed to promote the net uptake of Ca^{2+} (and hence insulin release), while extracellular Na^+ would have inhibitory effects. This hypothesis has gained some indirect support from studies on the effects of Na^+ on the electrical activity in [2] and the uptake of $^{45}Ca^{2+}$ by [3] pancreatic islet cells, as well as from studies on the effects of ouabain [1] and diphenylhydantoin [4] on insulin release. It is not known, however, whether stimulators of insulin release affect the β-cell uptake of Na^+. We therefore studied the uptake of $^{22}Na^+$ by islets microdissected from the pancreatic glands of obese-hyperglycemic mice; these islets contain more than 90% β-cells. Equilibrium was reached after about 60 min with values suggesting an intracellular Na^+ concentration as high as 95 mEq/litre. Glucose (20 mM) had no convincing effect, while ouabain (0.1 mM) or chloromercuribenzene-*p*-sulphonic acid (0.1 and 1.0 mM) increased the rate of $^{22}Na^+$ uptake. The results suggest that the β-cells are permeable to Na^+. Moreover, they conform with the hypotheses that ouabain stimulates insulin release by increasing Na^+ net uptake in the β-cells [1], and that the secretagogic action of sulphydryl reagents is mediated by effects on the transmembrane distribution of ions [5, 6].

2. Experimental

$^{22}NaCl$ was obtained from the Radiochemical Centre, Amersham, England, and ouabain as well as chloromercuribenzene-*p*-sulphonic acid from Sigma Chemical Co., St. Louis, Mo., USA. Adult *ob/ob*-mice were taken from the non-inbred Umea colony. Fresh pancreatic islets were microdissected freehand at 2°C and subsequently incubated at 37°C, using Krebs–Ringer bicarbonate medium equilibrated with O_2–CO_2 (95:5) as the basal medium. The Na^+ concentration of the medium was 143.5 mEq/litre. All experiments were started by preliminary incubation of the isolated islets for 30 min in basal medium supplemented with 3 mM glucose. The islets were then transferred to fresh basal medium labelled with $^{22}NaCl$ to give a specific radioactivity of 0.13 mCi/mEq of Na^+. The length of the incubation time and the presence of test substances were as described in the Results section. In most experiments the incubated islets were washed at 0°C in non-radioactive Krebs–Ringer bicarbonate medium before being freeze-dried (– 40°C, 0.1 N/m^2) overnight and weighed on a quartz-fibre balance. In one series of experiments the islets were incubated in medium containing both $^{22}Na^+$ and 0.1 mM [6, 6′-^{3}H]sucrose (150 mCi/mmole). These islets were not washed prior to freeze-drying and weighing but simply freed rapidly of adhering fluid with the aid of a micropipette. Dry islets were dissolved in Hyamine and analyzed for radioactivity in a liquid scintillation spectrometer, using 5μl samples of incubation medium as external standard. Relevant methodological details have been described [5].

3. Results

In several previous uptake studies, islets were incubated in media containing both the radioactive test so-

Table 1

Islet uptake of $^{22}Na^+$ in relation to that of [6, 6'-^{3}H] sucrose

Glucose in medium (mM)	pEq of labelled $Na^+/\mu g$ dry wt. of islets	
	Total	In excess of sucrose space
0	365 ± 17	63 ± 20**
20	352 ± 18	79 ± 14***

After preliminary incubation, islets were incubated for 10 min in $^{22}Na^+$-labelled Krebs–Ringer bicarbonate medium supplemented with 0.1 mM [6, 6'-^{3}H] sucrose (150 mCi/mmole). ^{22}Na and ^{3}H in islets was then determined without prior washing. The total content of labelled Na^+ (with same specific activity as in medium) as well as that in excess of the sucrose space are given as mean values ± S.E.M. for 12 different observations.

** $p < 0.01$.

*** $p < 0.001$ for difference versus zero.

lute and a differently labelled space marker. This experimental design made possible the correction for extracellular and contaminating radioactivity without the necessity of washing the islets after incubation [5]. In preliminary experiments we used the same technique for measuring the β-cell uptake of $^{22}Na^+$. Islets were incubated for 10 min in basal medium labelled with $^{22}Na^+$ and supplemented with 0.1 mM [6, 6'-^{3}H] sucrose as extracellular space marker [7]. The results of these experiments are shown in table 1. Although the uptake of $^{22}Na^+$ significantly exceeded the sucrose space, the difference amounted to only about one-fifth of the total content of labelled Na^+ in the unwashed islet specimens. In relation to the random errors associated with the determinations of sucrose and Na^+ uptake by whole islets, the difference between $^{22}Na^+$ and [6, 6'-^{3}H] sucrose may be too small to permit fair estimates of the cellular Na^+ uptake within a reasonable number of experiments. It was therefore decided to reduce the extracellular radioactivity by washing and to omit [6, 6'-^{3}H] sucrose from the incubation medium.

Fig. 1 shows the effect of washing on the radioactivity of islets that had been preloaded by incubation with $^{22}Na^+$ for 10 min. More than 80% of the radioactivity of the unwashed specimens disappeared during washing at 0°C for 2 min, after which time the islet radioactivity decreased only slowly. Washing for 4 min was therefore employed in the following experiments.

Fig. 2 shows the radioactivity in islets that had been washed for 4 min after being loaded with $^{22}Na^+$ for different periods of time. A time-dependent increase of the islet radioactivity was observed for up to 60 min.

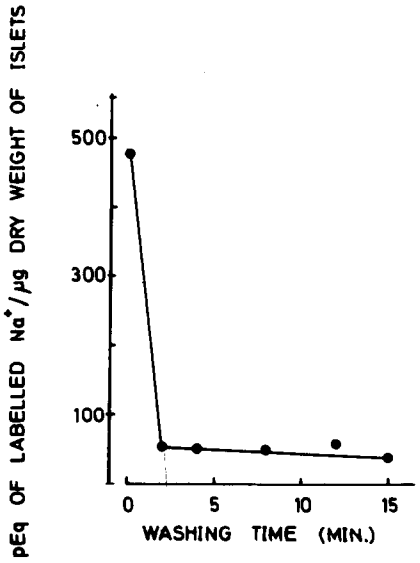

Fig. 1. Effect of washing on the radioactivity of islets preloaded with $^{22}Na^+$. After preliminary incubation, islets were incubated for 10 min in $^{22}Na^+$-labelled Krebs–Ringer bicarbonate medium. They were then washed for different periods of time in non-radioactivity Krebs–Ringer medium at a temperature of about 0°C. The content of labelled Na^+ (with same specific activity as in medium) in unwashed and washed islet specimens is given as the mean values for 2–3 different experiments. The unwashed specimens were merely freed of incubation medium by means of a micropipette. Note, however, that this procedure does not remove all contamination, and that the total radioactivity of unwashed specimens therefore does not represent the true amount of $^{22}Na^+$ in islet tissue.

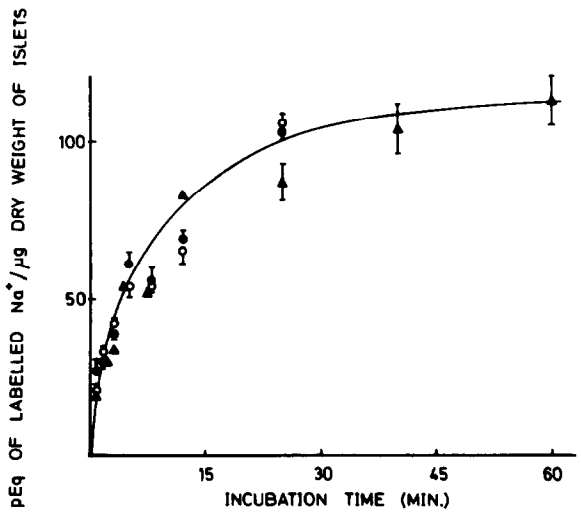

Fig. 2. Effect of incubation time on the islet uptake of $^{22}Na^{+}$. After preliminary incubation, islets were incubated for different periods of time in $^{22}Na^{+}$-labelled Krebs–Ringer bicarbonate medium. Two series of experiments were performed with medium containing 3 mM D-glucose (○ and ▲), one of which series (○) also included parallel incubations at 20 mM D-glucose (●). After incubation the islets were washed for 4 min as described in fig. 1. The content of $^{22}Na^{+}$ (with same specific activity as in medium) is given as the mean values for 2–9 different experiments. Bars denote ± S.E.M. for mean values based on more than 4 experiments.

At that time the radioactivity corresponded to about 114 pEq of labelled Na^{+} per μg dry weight of islets. If all this $^{22}Na^{+}$ were located within the islet cells and isotopic equilibrium is assumed, the intracellular concentration of Na^{+} would be about 95 mEq/litre [7]. As is also shown in fig. 2, the islet uptake of $^{22}Na^{+}$ was not demonstrably different in media containing 3 mM and 20 mM glucose.

Because ouabain is a classic inhibitor of the enzyme-catalyzed extrusion of Na^{+} from other cells, we tested it for effects on the $^{22}Na^{+}$ uptake by pancreatic β-cells. Ouabain (0.1 mM) caused a significant increase of the radioactivity of islets incubated for 10 min in $^{22}Na^{+}$-labelled medium (table 2). Significant stimulation of $^{22}Na^{+}$ uptake was also observed on the addition of 0.1 mM or 1.0 mM chloromercuribenzene-*p*-sulphonic acid to the medium (table 2).

Control experiments revealed that islets microdissected at 37°C took up $^{22}Na^{+}$ to about the same extent as those microdissected at 2°C and subsequently preincubated for 30 min at 37°C. In other control experiments it was found that the presence of 0.1 mM ouabain during washing at 0°C did not affect the amount of $^{22}Na^{+}$ retained by the islets.

4. Discussion

The clearly biphasic time-curve for $^{22}Na^{+}$ wash-out indicates that, prior to washing, the $^{22}Na^{+}$ was located in two different pools. Although it may be hasty to equate these pools with the extra- and intracellular islet spaces, it is noteworthy that the amount of $^{22}Na^{+}$ retained after 4 min of washing was very similar to that taken up in excess of the sucrose space. That $^{22}Na^{+}$ penetrated into the β-cells is also suggested by the slow uptake leading to equilibrium after about 60 min. In rat adenohypophysial cells, which may be relatively impermeable to Na^{+}, the uptake of $^{22}Na^{+}$ did not change significantly after 3 min [8].

For the intracellular concentration of Na^{+} in mammalian cells, values ranging from a few mEq/litre (e.g. neurons and muscle cells) to almost (calf adrenal) or even more than (cat erythrocytes) 100 mEq/litre have been reported [9]. Therefore it is not inconceivable that the β-cells may contain Na^{+} at a concentration not far from the 95 mEq/litre suggested by the present radioisotope uptake studies. This high value would seem to indicate that the β-cells possess a Na^{+} extrusion mechanism of low activity in comparison with nerve and muscle cells. The occurence in the β-cells of a Na^{+}/K^{+}-activated 'pump' adenosine triphoshatase has not yet been reported, and our unpublished attempts to demonstrate it have so far been in vain. Since the Na^{+}/K^{+}-activated adenosine triphosphatase is commonly implicated with the establishment of transmembrane electric potentials, it is noteworthy that the resting potential in islet cells is only about −20 mV [2]. It must be emphasized, however, that until microdeterminations of native Na^{+} in free β-cells and nonincubated islet specimens are available, our results should be interpreted with caution as regards the physiological Na^{+} concentration in the β-cells. One source of uncertainty is the fact that we do not know the extent to which $^{22}Na^{+}$ may be bound to islet structures other than β-cells or to the surface of the β-cells themselves.

The fact that 0.1 mM ouabain stimulated the rate of $^{22}Na^{+}$ uptake may be regarded as indirect evidence for the existence in the β-cells of a Na^{+} extrusion mechanism

Table 2

Effects of ouabain and chloromercuribenzene-*p*-sulphonic acid (CMBS) on $^{22}Na^+$ net uptake

Test compound		pEq of labelled $Na^+/\mu g$ dry wt. of islets		
		Test value	Control	Test minus control
Ouabain, 0.1 mM	8	83.8 ± 7.3	69.6 ± 4.4	14.1 ± 5.8*
CMBS, 0.1 mM	6	71.6 ± 2.2	61.8 ± 4.2	9.8 ± 3.5*
CMBS, 1.0 mM	6	138.3 ± 6.0	61.8 ± 4.2	76.5 ± 6.5***

After preliminary incubation, islets were incubated for 10 min in ^{22}Na-labelled Krebs–Ringer bicarbonate medium supplemented with 3 mM glucose and one of the test compounds indicated. Parallel control incubations were performed without test compound in the medium. The islets were then washed for 4 min at 0°C and their radioactivity determined. The residual $^{22}Na^+$ (expressed as pEq of labelled Na^+ with same specific activity as in medium) is given as mean values ± S.E.M.

*$p < 0.05$.

***$p < 0.001$.

with properties similar to those of the classical Na^+/K^+-activated adenosine triphosphatase [9]. Kizer et al. [4] reported that diphenylhydantoin, an activator of the Na^+/K^+-activated adenosine triphosphatase in other cells, diminished the $^{22}Na^+$ uptake by rat islets. As proposed by Milner and Hales [1], the mechanism for Na^+ extrusion may affect insulin release indirectly by regulating the concentration of Na^+ in the β-cells. By competing with Ca^{2+} for a hypothetical extrusion site, intracellular Na^+ could perhaps stimulate the net uptake of Ca^{2+} [1], an ion thought to play a causal role in the triggering of insulin discharge [1, 10]. Na^+-depleted β-cells exhibit diminished secretory responses [11–13] as well as partial inhibition of glucose metabolism [12, 13].

We previously suggested that the marked insulin-releasing action of *p*-chloromercuribenzoic acid and chloromercuribenzene-*p*-sulphonic acid may be due to these thiol reagents altering the ion distribution across the β-cell plasma membrane [5]. This idea remains conjectural until more is known about the role of thiol groups in secretagogic ionic fluxes. However, it is encouraged by the fact that we have now uncovered one effect of chloromercuribenzene-*p*-sulphonic acid on the cation distribution in pancreatic islets.

The stimulation of $^{22}Na^+$ uptake observed in responce to chloromercuribenzene-*p*-sulphonic acid could reflect an increased influx or an inhibition of Na^+ extrusion. By reacting with membrane thiol groups chloromercuribenzene-*p*-sulphonic acid increases Na^+ permeability in erythrocytes [14, 15]. Studies on the effects of organic mercurials on membrane permeability in β-cells showed that exposing the islets to 0.1 mM chloromercuribenzene-*p*-sulphonic acid for 60 min had no significant effect on the uptake of organic extracellular space markers such as sucrose and mannitol [5]. With 1.0 mM thiol reagent, however, there was evidence of an increased membrane permeability to the same space markers.

In contrast to 0.1 mM ouabain or 0.1 mM chloromercuribenzene-*p*-sulphonic acid, 20 mM glucose had no convincing effect on the $^{22}Na^+$ uptake. It cannot be decided whether this was due to a real failure of glucose to affect Na^+ uptake by the β-cells, or whether the present technique is not sufficiently sensitive to detect small changes induced by glucose. Studies on the adenohypophysis revealed no uptake of $^{22}Na^+$ in response to stimulators of secretion [8]. However, if an increase of Na^+ uptake is an obligatory accompaniment to the stimulation of insulin release, the effect of a physiological stimulus such as glucose may be smaller than that of the more potently secretagogic organic mercurial. It seems impossible to decide how much the uptake of Na^+ would have to increase in order to raise the juxta-membrane concentration sufficiently for an effect on Ca^{2+} exit to occur in accordance with the model of Milner and Hales [1].

Acknowledgements

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