GLUCOSE-DEPENDENT EFFECT OF METHYLXANTHINES ON THE ^{45}Ca DISTRIBUTION IN PANCREATIC β-CELLS

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1. Introduction

The concentration of free Ca^{2+} in a critical cytosolic pool close to the β-cell plasma membrane might regulate insulin secretion [1–3]. Under a large number of experimental conditions a close relationship exists between intracellular ^{45}Ca uptake and insulin release [4,5]. It has therefore been suggested that changes of Ca^{2+} inward transport may play a vital role in providing the appropriate secretory response to a given stimulus.

Methylxanthines, like theophylline or 3-isobutyl-1-methylxanthine (IBMX), potentiate insulin secretion without increasing ^{45}Ca uptake [6,7]. The present investigation evaluates the effects of methylxanthines on ^{45}Ca distribution among the organelles of β-cell-rich pancreatic islets labelled in situ. In the presence of glucose, methylxanthines are shown to inhibit the incorporation of ^{45}Ca into a mitochondria-rich fraction, thus probably providing more free Ca^{2+} for the critical regulatory pool.

2. Methods

Chemicals of analytical grade, deionized water and siliconized glass were used. β-Cell-rich islets were isolated by collagenase digestion from pancreatic glands taken from overnight starved ob/ob mice of a non-inbred colony [8]. The islets were loaded for 120 min at 37°C with 1.28 mmol/l ^{45}Ca (15.6 Ci/mol, NEN Chemicals GmbH, Dreieich) in a modified Krebs-Ringer medium under a gas phase of oxygen [9]. Control media contained either 0 or 20 mmol/l glucose whereas the test media were also supplemented with 1 mmol/l IBMX or 2 mmol/l theophylline. Loading was terminated by transfer to an ice-bath followed by 3 washings in 0.25 mmol/l sucrose buffered with 5 mmol/l Hepes to pH 6.0 and supplemented with 1 mmol/l EDTA to remove extracellular Ca^{2+}. The islets were homogenized in 200 μl of the EDTA-containing medium under conditions preventing intracellular redistribution of Ca^{2+} [10,11]. Subcellular fractions were prepared essentially as in [12], using a Beckman J-21C centrifuge equipped with a JA-20 rotor for isolation of nuclei-cell debris (600 × g 5 min), mitochondria (5500 × g for 5 min) and secretory granules (24 000 × g for 10 min) and a Beckman Airfuge® for pelleting the microsomal fraction (110 000 × g for 60 min). The fractions were resuspended in 100 μl 0.1% (v/v) Triton® X-100 and aliquots taken for determination of protein [13] and for counting of the radioactivity in a liquid scintillation spectrometer.

The ^{45}Ca content of the homogenates was expressed as mmol/kg protein assuming the same specific labelling as in the medium. The incorporation of radioactivity into the subcellular fractions is presented as percentage distribution to give estimates about relative pool sizes. Statistical significances were calculated from differences between paired test and control experiments using the two-tailed Student's distribution.

3. Results and discussion

In addition to electron microscopy, the sub-

Table 1
Contents of ^{45}Ca and protein in islet homogenates

Glucose (mmol/l)	Other additives	Recovery (%)		Protein (μg)	^{45}Ca content (mmol/kg protein)
		Protein	^{45}Ca		
0		93.2 ± 2.9	93.0 ± 5.8	300.1 ± 45.4	7.43 ± 1.45
0	Theophylline	97.8 ± 4.1	91.3 ± 3.4	312.1 ± 45.6	6.58 ± 0.65
0	IBMX	90.6 ± 3.2	96.4 ± 4.6	335.8 ± 49.8	6.21 ± 0.40
20		93.1 ± 4.6	86.9 ± 2.6	316.8 ± 44.8	14.04 ± 1.96
20	Theophylline	94.5 ± 2.4	90.2 ± 3.9	298.7 ± 28.2	14.04 ± 2.28
20	IBMX	92.3 ± 3.6	91.2 ± 1.9	286.6 ± 63.8	13.38 ± 1.71

Islets were loaded for 120 min with ^{45}Ca at 37°C in the presence and absence of glucose (20 mmol/l), theophylline (2 mmol/l) and IBMX (1 mmol/l). The islets were then homogenized and subjected to differential centrifugation. Samples of the homogenates and subcellular fractions were taken for measurements of protein and ^{45}Ca. The recoveries from the subcellular fractions are given as percent of the homogenate values. The results represent the mean ± SEM of 6 (no glucose) or 8 (20 mmol/l glucose) experiments

cellular fractions were characterized by determination of marker enzymes and insulin content [11]. Whereas the secretory granule fraction was fairly pure, the mitochondrial one was contaminated with secretory granules as reported in other investigations [6,12,14,15]. The microsomal fraction consisted of small vesicles of smooth-surfaced or rough-surfaced membranes together with free ribosomes.

The reproducibility of the experimental procedure is indicated in tables 1,2. There were no significant differences in the amounts of tissue (protein in the homogenates) used in the various experimental series (table 1). Neither did the protein distribution among the β-cell fractions differ within an experimental protocol nor between the experimental series (table 2). The percentage recoveries ranged from 72–117 for radioactivity and from 76–113 for protein with mean values ± SEM depicted in table 1.

Exposure of the islets to glucose resulted in enhanced homogenate content of ^{45}Ca ($P < 0.01$, table 1), analogous to what has been observed with other experimental approaches [4,5,9]. Methylxanthines had no significant effects on the ^{45}Ca content of homogenates when added to loading media supplemented or not with glucose, confirming previous ^{45}Ca uptake data [6,7].

Neither IBMX nor theophylline affected the isotope distribution among subcellular fractions prepared from islets loaded with ^{45}Ca in the absence of glucose (fig.1). The addition of 20 mmol/l glucose

Table 2
Percentage distribution of protein among subcellular islet fractions

Glucose (mmol/l)	Other additives	Percentage distribution of protein				
		Nuclei + cell debris	Mitochondria	Granules	Microsomes	Supernatant
0		38.5 ± 1.4	18.6 ± 1.8	13.9 ± 1.1	2.5 ± 0.5	26.5 ± 0.8
0	Theophylline	36.7 ± 3.2	14.8 ± 1.3	14.5 ± 1.4	2.5 ± 0.6	31.5 ± 2.9
0	IBMX	35.0 ± 1.5	14.9 ± 4.9	16.2 ± 0.9	2.7 ± 0.7	31.2 ± 1.2
20		40.2 ± 2.8	16.0 ± 1.1	12.1 ± 1.1	3.8 ± 0.8	27.9 ± 2.6
20	Theophylline	40.9 ± 1.5	14.8 ± 1.4	14.9 ± 3.2	3.0 ± 0.7	26.4 ± 1.7
20	IBMX	40.5 ± 2.5	13.6 ± 1.0	14.4 ± 1.3	2.6 ± 0.8	28.9 ± 1.7

The figures denote the percentage (mean ± SEM) of total amount of protein present in the fractions of the islets referred to in table 1

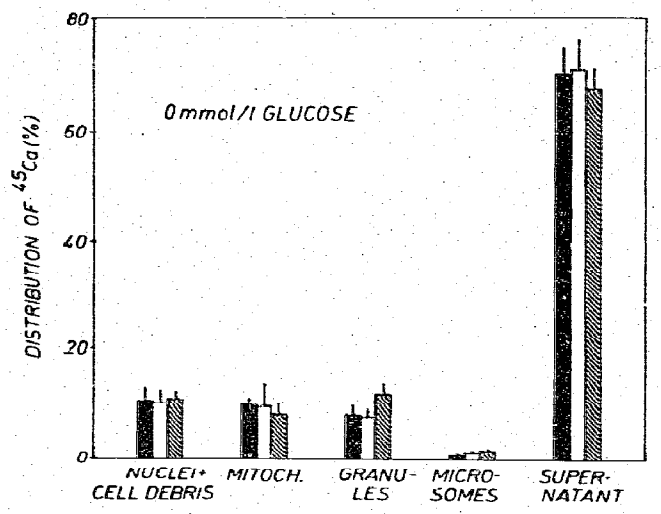

Fig.1. Effect of methylxanthines on the percentage distribution of radioactivity among subcellular fractions of islets loaded with ^{45}Ca in the absence of glucose. The islets were incubated with ^{45}Ca for 120 min at 37°C in media supplemented or not with 2 mmol/l theophylline (□) or 1 mmol/l IBMX (▨). After homogenization of the islets, subcellular fractions were prepared by differential centrifugation. The results represent the mean ± SEM of 6 expt.

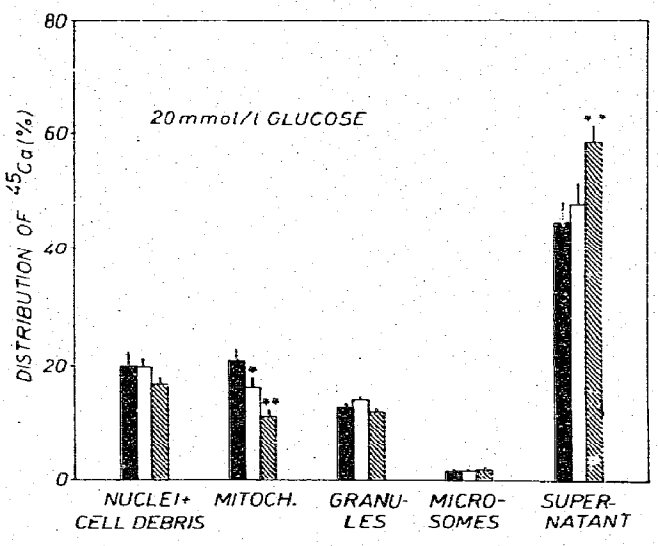

Fig.2. Effect of methylxanthines on the percentage distribution of radioactivity among subcellular fractions of islets loaded with ^{45}Ca in the presence of 20 mmol/l glucose. The islets were incubated with ^{45}Ca for 120 min at 37°C in media supplemented or not with 2 mmol/l theophylline (□) or 1 mmol/l IBMX (▨). After homogenization of the islets, subcellular fractions were prepared by differential centrifugation. The results represent the mean ± SEM of 8 expt. $*P < 0.02$, $**P < 0.001$.

resulted in enhanced relative amounts of ^{45}Ca in the nuclei-cell debris, mitochondria and secretory granule fractions and consequently in a reduction of incorporation into the supernatant (fig.1,2). The presence of methylxanthines resulted in decreased amounts of mitochondrial ^{45}Ca taken up in response to glucose (fig.2). The effect observed could not be attributed to contamination of the mitochondrial fraction with secretory granules since the granule fraction remained unaffected after addition of methylxanthines (fig.2).

It has been postulated that cyclic AMP increases the concentration of cytoplasmic Ca^{2+} by preventing uptake into organelles or by mobilizing Ca^{2+} from such intracellular pools [16]. Evidence suggesting that cAMP induces a redistribution of calcium in pancreatic β-cells was obtained by studying the effect of methylxanthines on efflux of ^{45}Ca from pancreatic islets [7] and uptake of ^{45}Ca in broken cell preparations [17–19]. The present analysis of in situ labelled β-cell organelles provides direct evidence that methylxanthines inhibit glucose-induced accumulation of Ca^{2+} in mitochondria. Preliminary data indicate that dibutyryl-cAMP exerts a similar action on the ^{45}Ca content of mitochondria, suggesting that the methylxanthine effect is mediated by cAMP. Since glucose is a prerequisite for the insulin secretagogic effect of cAMP, it is pertinent to note that the methylxanthine effect on mitochondria also required the presence of glucose. We propose that the potentiating effect of cAMP on glucose-stimulated insulin secretion is due to interference with the calcium buffering by mitochondria after opening of calcium channels in the β-cell membrane by glucose.

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