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PRELIMINARY COMMUNICATIONS

The Role of Calcium and Magnesium in Insulin Secretion from Rabbit Pancreas Studied In Vitro

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Summary. Glucose, glucagon, tolbutamide and L-leucine stimulated insulin secretion from rabbit pancreas studied *in vitro*. In each case stimulation was inhibited by omitting calcium from the incubation medium. The omission of magnesium had no effect on glucose stimulated insulin secretion but 10 mM magnesium inhibited secretion. Optimal secretion of insulin occurred at an extracellular calcium concentration of 2.64 mM. The omission of calcium inhibited glucose-stimulated insulin secretion from pancreas of 27 day rabbit foetuses.

Influence du calcium et du magnésium sur la libération d'insuline par le pancréas de lapin in vitro

Résumé. Le glucose, le glucagon, le tolbutamide et la L-leucine stimulent la libération d'insuline par le pancréas de lapin étudié in vitro. Cette stimulation n'a pas lieu lorsque le milieu d'incubation est préparé sans calcium. L'absence de magnésium n'a pas d'effet sur la sécrétion insulinique stimulée par le glucose, alors que le magnésium à la concentration de 10mM exerce un effet inhibiteur. La concentration optimale du calcium extracellulaire pour la sécrétion insulinique est de 2.64 mM. La stimulation de la sécrétion insulinique par le glucose est également supprimée par l'absence de calcium pour le pancréas de foetus de lapin âgé de 27 jours.

Der Einfluß von Calcium und Magnesium auf die Freisetzung von Insulin aus Kaninchen-Pankreas, in virto.

Zusammenfassung. Die Freisetzung von Insulin aus Kaninchen-Pankreas in vitro wird durch Glucose, Glucagon, Tolbutamid oder L-Leucin stimuliert. In allen diesen Fällen bleibt die Stimulation in Abwesenheit von Calcium in Inkubationsmedium aus. In Abwesenheit von Magnesium stimulierte Glucose die Insulinsekretion weiter, währenddem eine Erhöhung der Magnesiumkonzentration auf 10 mM deutlich hemmend wirkte. Die optimale Sekretion von Insulin erfolgte bei einer Calciumkonzentration von 2.64 mM. In Abwesenheit von Calcium wurde die Freisetzung von Insulin auch aus dem Pankreas von 27 Tage alten Foeten gehemmt.

Key-words: Calcium, Magnesium, Insulin, Secretion Rabbit, Pancreas, Foetus.

In many cells that contain secretory products in the form of granules or vesicles extracellular calcium is a prerequisite for the secretion of the storage product. The release of acetylcholine from nerve endings at the neuro-muscular junction [1] or autonomic ganglion [9], the secretion of catecholamines from the adrenal medulla [4], of oxytocin and vasopressin from the neurohypophysis [3] and of amylase from the exocrine pancreas [7] are all calcium dependent.

Experiments have been performed to determine whether extracellular calcium is necessary for the secretion of insulin. Pieces of pancreas from 6—8 week-old rabbits have been incubated *in vitro* [2], and insulin secretion into the incubation medium has been measured [6] before and after exposure of the pancreas to stimuli in the presence or absence of calcium.

Glucose, glucagon, tolbutamide and L-leucine were selected as stimuli to provide a wide range of signals for insulin secretion. A difference in the mechanism of insulin secretion induced by glucose or tolbutamide is suggested by the observations that glucose-induced stimulation is inhibited by D-mannoheptulose [2] or diazoxide [8], whereas stimulation by tolbutamide is not. Glucose, glucagon and tolbutamide have been shown previously to stimulate insulin secretion from this preparation [2] and in the present experiments it has been possible for the first time to demonstrate stimulation of insulin secretion *in vitro* by L-leucine.

Pancreas from infant rabbits proved preferable to that from adults because it was thinner and less infiltrated with fat. The incubation of such pancreas, one piece in a flask, made it possible both to improve experimental precision and to express insulin secretion rates in absolute terms.

The general experimental design is illustrated in Table 1. Pieces of pancreas were incubated for nine 30 min periods. In periods 1, 4 and 7, which were to establish a steady state, no measurements were made.

Table 1. *General design of an experiment to investigate the relationship between stimulation of rabbit pancreas in vitro and the presence or absence of calcium in the incubation medium.*

30 min Period	1	2	3	4	5	6	7	8	9
Calcium (2.64 mM)	+	+	+	−	−	−	+	+	+
Stimulus	−	−	+	−	−	+	−	−	+

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In all experiments 3.3 mM glucose was added to the incubation medium, except in those where glucagon was the stimulus, when 16.5 mM glucose was added. In experiments where glucose was the stimulus the concentration was also raised to 16.5 mM. In other experiments, glucose-stimulated insulin secretion was studied while omitting or increasing the magnesium concentration of the incubation medium.

Table 2. *The effect of ionic changes in the incubation medium on different stimuli to insulin secretion from the rabbit pancreas studied in vitro*

Age of animal	Change in ionic composition Periods 4, 5, 6	Stimulus Periods 3, 6, 9	Number of Observations	Mean insulin secretion ± standard error of mean, ng/g wet weight pancreas/min Incubation Period 2	3	5	6	8	9
6–8 weeks postnatal	— Ca	Glucose 16.5 mM	4	12.4 ± 1.4	99.0 ± 7.8	16.0 ± 4.4	9.7 ± 2.4	14.0 ± 1.4	92.0 ± 11.7
	— Ca	Glucagon 5μg/ml	4	107 ± 26.7	218 ± 35.4	23.2 ± 7.3	14.7 ± 5.3	99.5 ± 21.9	328 ± 45.3
	— Ca	Tolbutamide 200μg/ml	5	9.8 ± 4.0	43.6 ± 5.2	7.6 ± 2.3	10.6 ± 1.8	15.3 ± 1.9	40.6 ± 5.8
	— Ca	L-Leucine 5 mM	10	7.0 ± 0.8	29.2 ± 5.8	3.3 ± 0.2	9.6 ± 1.3	4.6 ± 0.4	24.0 ± 2.3
	— Mg	Glucose 16.5 mM	10	8.7 ± 1.9	94.4 ± 11.5	5.1 ± 2.2	104 ± 24.0	3.8 ± 1.2	120 ± 14.7
	+ Mg 10 mM	Glucose 16.5 mM	10	2.9 ± 0.5	24.8 ± 4.1	3.2 ± 0.6	2.5 ± 0.2	3.1 ± 0.5	17.4 ± 4.6
27 day foetus	— Ca	Glucose 16.5 mM	10	62.0 ± 7.2	127 ± 18.7	29.0 ± 7.7	26.3 ± 4.2	100 ± 12.0	134 ± 13.7

The results of these experiments are shown in Table 2. The omission of calcium from the incubation medium inhibited the stimulation of insulin secretion (period 6) both in comparison with the response to the same stimulus in the presence of calcium (periods 3 and 9) and in comparison with basal secretion in the absence of calcium (period 5).

The small response to some stimuli, seen when calcium was not added to the incubation medium, may have been due to persistent traces of calcium. This response was only significant when L-leucine was the stimulus and, in other experiments using L-leucine, there was a total suppression of the response when calcium was omitted. Glucose-stimulated insulin secretion was unaffected by the omission of magnesium from the incubation medium but was inhibited when the magnesium concentration was raised to 10 mM.

The optimal extracellular calcium concentration for insulin secretion was studied by measuring secretion in response to 33 mM glucose in gradually increasing extracellular calcium concentrations for six successive half hour periods (Fig. 1).

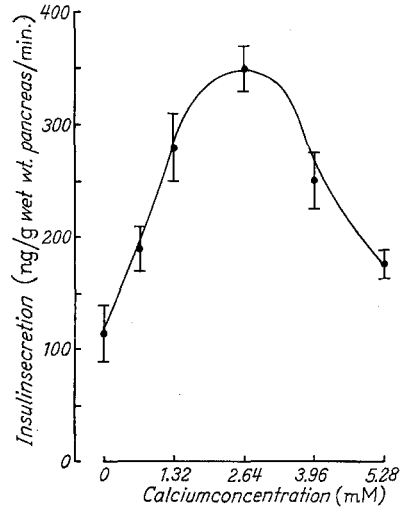

Fig. 1. Mean rate of insulin secretion (± S.E.M.) from rabbit pancreas *in vitro* in response to 33 mM glucose. The calcium concentration of the medium was increased in each successive 30 min period. The experiment was preceded by a 30 min preincubation period in the presence of 2.64 mM calcium

BENNETT and GRODSKY [5] have show that glucose-stimulated insulin secretion in the isolated perfused rat pancreas is inhibited by the omission of calcium [5]. The ubiquitous inhibition of insulin secretion by the omission of calcium from the incubation medium reported here suggests that this ion may play an important role in the mechanism of insulin release from the β-cell. The observation that secretion is also inhibited by an increase in the extracellular magnesium concentration suggests that calcium and magnesium may compete for a common receptor.

It is possible to test whether the insulin granule is the sole site of calcium action, for in the 27 day rabbit foetus the β-cells do not contain granules visible by light microscopy. In experiments using the pancreas from such foetuses glucose-stimulated insulin secretion was inhibited by the omission of calcium (Table 2).

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