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Cations and the secretion of insulin

Insulin secretion *in vitro* in response to glucose, leucine, glucagon and tolbutamide occurs only in the presence of extracellular calcium, and glucose-stimulated insulin secretion may be inhibited by a rise in the extracellular concentration of magnesium[1]. Extracellular sodium, like calcium, is necessary for these stimuli to be effective[2]. Insulin release in response to ouabain[2] or a high concentration of extracellular potassium[3] is thought to follow a rise in intracellular sodium concentration. The effect of barium on insulin secretion has been studied. This ion is a potent secretogogue in other cells in which it is thought to act *via* the same mechanism as calcium[4,5]. It was argued that should barium stimulate insulin secretion, it might prove a useful tool with which to study the interrelationship of sodium and calcium. Other stimuli depend on each of these cations.

Pieces of pancreas from young rabbits were incubated *in vitro*[6] and insulin secretion into the incubation medium was measured[7] in media of different ionic compositions. Krebs–Henseleit medium supplemented with sodium pyruvate, glutamate and fumarate[8] and 3.3 mM glucose was used for all basal measurements. In some experiments calcium was omitted from the medium or was replaced by an equivalent amount of barium. In others sodium was completely replaced by choline. Pancreas was incubated one piece in a flask and was transferred to fresh flasks at 30-min intervals.

Fig. 1 depicts the results of an experiment in which 12 pieces were cut from one pancreas and were incubated initially in a medium containing neither calcium nor barium. 6 pieces were then incubated in the presence of calcium and 6 in the presence of barium. After a further period in the absence of either ion the procedure

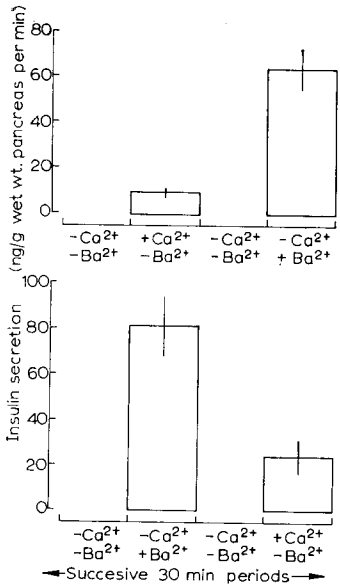
TABLE I

THE EFFECT OF IONIC CHANGES ON INSULIN SECRETION FROM RABBIT PANCREAS IN RESPONSE TO BARIUM OR POTASSIUM

The first incubation period was 60 min and subsequent periods 30 min. No measurements were made in Periods 1 and 4 which were to establish a steady state.

Stimulus	*Number of observations*	*Mean insulin secretion ± S.E. (ng/min per g wet wt. pancreas)*			
		1	*2* *Basal*	*3* *Stimulated*	*Stimulated −basal*
		$-Na^+$			
2.5 mM barium	5		16.8 ± 7.2	8.9 ± 2.6	− 7.9 ± 5.0
		$-Ca^{2+}$			
60 mM potassium	5		12.3 ± 3.4	16.3 ± 3.2	+ 4.0 ± 3.3
		4	*5* *Basal*	*6* *Stimulated*	*Stimulated −basal*
		$+Na^+$			
2.5 mM barium	5		10.6 ± 3.5	27.6 ± 8.5	+16.9 ± 5.8
		$+Ca^{2+}$			
60 mM potassium	5		7.6 ± 1.2	59.7 ± 7.1	+52.1 ± 7.1

was reversed. It was concluded that barium stimulated insulin secretion reversibly. The dependence of barium on the presence of sodium in the incubation medium was studied under conditions which have been described previously[2]. Replacement of sodium by choline blocked the action of barium which was restored upon reconstitution of the medium (Table I).



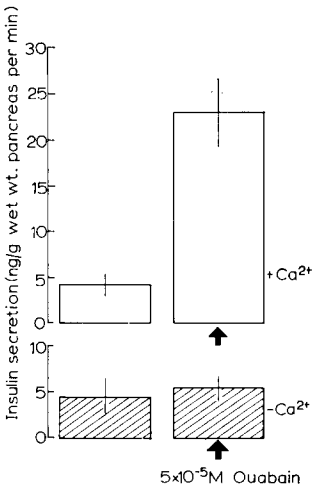


Fig. 1. Mean rate of insulin secretion (± S.E.) from rabbit pancreas *in vitro* in the presence or absence of calcium or barium. Horizontally adjacent histograms depict secretion from the same 6 pieces of pancreas.

Fig. 2. Mean rate of insulin secretion (± S.E.) from rabbit pancreas *in vitro* under basal conditions and in response to $5 \cdot 10^{-5}$ M ouabain in Krebs–Henseleit medium (open rectangles) or in medium from which calcium had been omitted (shaded rectangles). Each histogram depicts the mean of 5 observations and each experiment was preceded by a 60-min preincubation period.

The ability of ouabain and a high extracellular concentration of potassium to stimulate insulin secretion in the absence of extracellular calcium was studied. Basal and stimulated insulin secretion in response to ouabain was measured simultaneously in media which contained calcium or from which calcium had been omitted (Fig. 2). Ouabain only stimulated insulin secretion in the presence of extracellular calcium. The effect of potassium on insulin secretion in the absence and presence of calcium was studied in an experiment of the same design as that in which sodium was replaced (Table I). Like ouabain, potassium only stimulated insulin secretion in the presence of extracellular calcium.

The demonstration that barium stimulates insulin secretion only in the presence of extracellular sodium and that ouabain or potassium are only effective in the presence of extracellular calcium provides indirect evidence that sodium and calcium have an interrelated role in the mechanism of insulin release. Since either ion is necessary for a variety of stimuli to act *in vitro*[1,2], it is probable that both must act at the β cell membrane or enter the cell before insulin can leave it in response to these stimuli.

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Sodium-dependent uptake of calcium by crab nerve

The influx of Ca^{2+} into squid axons is dependent both on the Na^+ content of the sea water and also on the Na^+ concentration in the axoplasm[1]. These observations have been repeated and extended on nerves obtained from the limbs of the spider crab *Maia squinado*. In this preparation the nerves are too small to sample the axoplasm directly. The measurements of Ca^{2+} uptake were made on whole nerve trunks and presumably include contributions from axons and Schwann cells.

After dissection, nerves were kept for up to 2 h in artificial sea water (ASW) of composition (in mM): NaCl, 460; KCl, 10; $MgCl_2$, 55; $CaCl_2$, 11; $NaHCO_3$, 2.5. Nerves were loaded with Na^+ by tetanization at 30 impulses/sec for 5 min in K^+-free sea water. This increased the Na^+ content from about 25 mmoles/kg nerve to about 80 mmoles/kg nerve. Exposure to test solutions was made in a shaking water bath maintained at 16.5°. As ouabain (10^{-3} M) inhibits the Na^+–K^+ pump in crab nerve[2] without affecting Ca^{2+} uptake (see below), this drug was included in all solutions in order to slow down the rate of loss of Na^+ from the cells. Even in the presence of ouabain, the Na^+ content fell by about 25 % during a 7-min exposure to Li^+-ASW. Ca^{2+} uptake was followed by flame photometry and by use of ^{45}Ca. In both measurements great care was taken before analysis to wash the nerves free of extracellular Ca^{2+}. For flame photometry, nerves were washed for 10 min at 0° in 3 changes of Ca^{2+}-free choline–ASW followed by 2 washes in buffered isotonic choline and for tracer uptake the nerves were given five 2-min washes in K^+-free sea water at 0°. After being washed, the nerves were blotted on filter paper and their middle portions taken for analysis.

The Ca^{2+} content of nerves immersed in Na^+-ASW averaged 1.01 ± 0.18

Abbreviation: ASW, artificial sea water.