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THE ROLE OF SODIUM AND POTASSIUM IN INSULIN SECRETION FROM RABBIT PANCREAS

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SUMMARY

1. Insulin secretion from pieces of rabbit pancreas incubated *in vitro* was studied in media of different ionic composition and in response to different substances added to the media.

2. Experiments were performed which demonstrated that a sodium pump played a role in insulin secretion and that inhibition of the pump by ouabain, or by the omission of extracellular potassium, stimulated insulin secretion.

3. A rise in extracellular potassium concentration stimulated insulin secretion independently of changes in the osmolarity or sodium or chloride concentration of the incubation medium.

4. The role of extracellular sodium in insulin secretion was investigated. Extracellular sodium was a pre-requisite for insulin secretion stimulated by glucose, glucagon, L-leucine, tolbutamide, potassium or ouabain.

5. The presence of 3·3 mM glucose in the incubation medium was not essential for the stimulation of insulin secretion by L-leucine, tolbutamide or ouabain. Glucagon did not stimulate insulin secretion in the presence of 3·3 mM glucose but did so in the presence of 16·5 mM glucose.

6. The results obtained in these experiments suggested that a transmembrane sodium flux probably in the β cell was a fundamental event in the stimulation of insulin secretion by diverse stimuli.

INTRODUCTION

In recent studies of the role of extracellular ions in cellular excitation, cations have been extensively investigated. Normal nerve conduction or muscle contraction are associated with well documented movements of sodium and potassium across the cell membrane and depend, in a manner less well understood, on extracellular calcium (see review, Katz, 1966). Many different 'secretory' cells which store their product in granules or

vesicles need extracellular calcium for the normal release of the 'secretion'. The release of acetylcholine at the neuromuscular junction or synapse (del Castillo & Stark, 1952; Harvey & MacIntosh, 1940), catecholamines from neurones or the adrenal medulla (Kirkepar & Misu, 1967; Douglas & Rubin, 1961), vasopressin from the neurohypophysis (Douglas & Poisner, 1964), amylase from the exocrine pancreas (Hokin, 1966) and granule proteins from the polymorphonuclear leucocyte (Woodin & Wieneke, 1964) are all dependent on the presence of extracellular calcium.

Vasopressin secretion from the neurohypophysis may be stimulated by a rise in extracellular potassium (Douglas & Poisner, 1964), but the role of sodium and potassium in the release of granules from other cells has been little studied.

Investigations of ionic factors concerned in insulin secretion have so far been confined to cations. Calcium has been shown to be necessary for the secretion of insulin in response to glucose from the perfused rat pancreas (Grodsky & Bennett, 1966). Milner & Hales (1967*a*) demonstrated that calcium was necessary for a variety of substances to stimulate insulin secretion from rabbit pancreas *in vitro* and that secretion was inhibited by a high extracellular magnesium concentration.

This paper reports the results of experiments carried out to study the role of sodium and potassium in insulin secretion. Evidence is presented that a sodium pump plays a part in insulin secretion, that extracellular sodium is a pre-requisite for secretion in response to a variety of stimuli and that secretion may be associated with a rise in sodium concentration in the β cell. A preliminary report of some of these findings has been published (Milner & Hales, 1967*b*).

METHODS

Procedure. One or two rabbits aged 4–10 weeks were used in each experiment. The animals which were not of pure stock had been reared in the laboratory animal house. Each rabbit was killed by a sharp blow on the back of the neck. The tail and body of the pancreas were dissected out. After trimming the pancreas of blood vessels and mesentery it was divided into 5–16 pieces which usually weighed 20–30 mg each. Each piece was placed in a conical flask in a physiological buffer (see below) and was incubated with shaking at 37° C for 60 min before the experiment commenced. This incubation was found to be necessary to establish a steady rate of insulin secretion.

At the start of the experiment the pieces of pancreas were gently transferred with forceps to new flasks. In all experiments each period of incubation lasted 30 min or multiples thereof and at the end of each period the piece of pancreas was transferred to a fresh flask. Insulin which had been secreted from the pancreas into the medium was measured after removal of the pancreas from the pot. Insulin secretion was studied in response to various substances added to the medium and in media of different ionic compositions. Four to six pieces of pancreas were treated identically in each experiment. Different groups of pieces of pancreas in any experiment always contained a representative selection of pieces from different parts of the same pancreas. Each piece of pancreas was blotted carefully and weighed at the end

of the experiment, since in preliminary experiments no significant difference had been observed between the wet weight of pancreas before or after incubation.

Incubation media. The medium for all experiments (subsequently called the normal or Na medium) was a bicarbonate-buffered salt solution (Krebs, 1950) of the following final composition (m-equiv/l.) Na, 141·0; K, 5·9; Ca, 5·1; Mg, 2·4; PO_4, 2·2 (one P is taken as 1·8 equivalent); SO_4, 2·4; HCO_3, 24·9; Cl, 104·8; pyruvate, 4·9; glutamate, 4·9 and fumarate, 5·4; supplemented with 3·3 mM glucose and 1 mg/ml. bovine albumin, fraction V (Armour Pharmaceutical Co. Ltd., Hampden Park, Eastbourne, Sussex). In experiments where glucagon was used as a stimulus the glucose concentration in the medium was 16·5 mM as it was not possible to demonstrate stimulation by glucagon in 3·3 mM glucose.

In some experiments sodium was completely replaced by choline, potassium or lithium (subsequently referred to as choline, K or Li media). Lithium bicarbonate was prepared by bubbling CO_2 through lithium hydroxide. The salts of pyruvic, glutamic and fumaric acids were prepared by titrating the acid with the hydroxide or bicarbonate of the appropriate base. In some experiments potassium was completely replaced by equivalent amounts of sodium. In experiments to study the effect of different concentrations of potassium on insulin secretion the potassium concentration in the medium was raised either by adding the appropriate amount of 2·7 M-KCl or by mixing Na and K media in appropriate proportions. Using the former method, the effect of a high potassium concentration in the presence of 141 mM sodium in a hypertonic medium was studied. The latter method permitted the study of the effect of a high potassium concentration in an isotonic medium in which the sodium concentration had been reduced by an equivalent amount. Whenever possible all chemicals were of the analytical reagent grade. All solutions were equilibrated with 95 % O_2 and 5 % CO_2 (v/v).

Stimuli. The final concentration in the incubation medium and the source of the following substances, whose effect on insulin secretion was studied, was as follows: *glucose* 16·5 mM; *L-leucine* 5 mM, Koch Light Laboratories, Poyle Estate, Colnbrooke, Bucks; *tolbutamide* 200 μg/ml., Hoechst Pharmaceuticals Ltd., 11 Stoke Poges Lane, Slough, Bucks; *glucagon* 5 μg/ml., this preparation which was for therapeutic use contained less than 2·5 ng insulin/mg glucagon and was obtained from Eli Lilly and Co., Basingstoke; *ouabain* 10^{-9} to 10^{-3} M, Martindale Samoore Ltd., Norwich.

Experimental design. (*a*) The effect of different ionic media on insulin secretion or the prolonged effect of a stimulus was studied by measuring simultaneously the insulin secretion from alternate pieces of the same pancreas for successive periods in either the test or normal medium.

(*b*) In experiments designed to test the effect of a change in the ionic composition of the medium on different stimuli of insulin secretion, basal and stimulated insulin secretion was measured in the test medium and then in a normal medium. On changing the ionic composition of the medium a period of 30 min was always allowed to establish a new steady state. Experiments to test the effect of glucose on different stimuli were similar in design, basal and stimulated secretion was measured in the presence of 3·3 mM glucose, in its absence and finally in the presence of glucose again.

Measurement of insulin. The concentration of insulin in the incubation medium was measured by immuno-assay (Hales & Randle, 1963) by reference to standard solutions of ox insulin, employing an antiserum which had previously been demonstrated to cross react identically with rabbit and ox insulin (Coore & Randle, 1964). Rabbit anti-guinea-pig serum was kindly given by Dr B. A. L. Hurn of the Wellcome Research Laboratories, Beckenham, Kent and ^{125}I insulin was obtained from the Radiochemical Centre, Amersham, Bucks.

Calculation and expression of results. In all experiments insulin secretion was expressed as ng insulin/g wet wt. pancreas/min. In experiments in which insulin secretion from different groups of pieces of pancreas were compared, differences in mean rates of secretion were compared by Student's *t* test. In experiments where pieces of pancreas were studied under varying conditions the mean absolute change in secretion from the basal rate was so tested.

RESULTS

Effect of ouabain or potassium replacement on insulin secretion

The effect of 10^{-9}–10^{-3} M ouabain on insulin secretion was studied by measuring secretion from pieces of pancreas first under basal conditions and then after exposure to ouabain for 30 min. The results are illustrated in Fig. 1 and show that under these conditions 10^{-5}–10^{-3} M ouabain

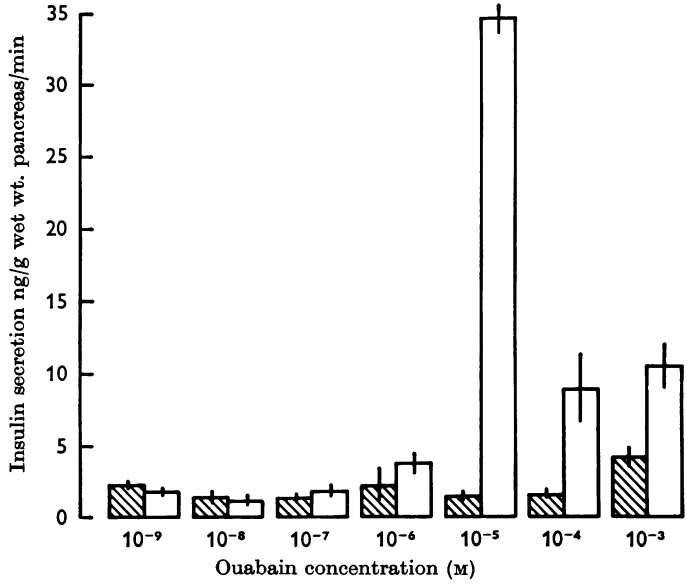

Fig. 1. Insulin secretion under basal conditions ▨ compared with secretion in response to 10^{-9}–10^{-3} M ouabain □. Each pair of histograms represents the mean (± S.E. of mean) secretion from a different group of six pieces of pancreas in successive 30 min periods.

stimulated insulin secretion. The effect of time on the pancreatic response to ouabain was studied by incubating pieces of pancreas for four successive periods in ouabain at concentrations of 10^{-8}, 10^{-7}, 10^{-6} or 10^{-5} M (Fig. 2). 10^{-8} and 10^{-7} M ouabain stimulated insulin secretion in the second to fourth periods. The response to 10^{-6} M ouabain was as great in the second period as that to 10^{-5} M ouabain in the first and after maximal stimulation by 10^{-6} or 10^{-5} M ouabain insulin secretion fell in subsequent periods.

If ouabain stimulated insulin secretion by inhibiting a sodium pump in the cell membrane, omission of potassium from the incubation medium should have a similar effect (Dunham & Glynn, 1961). Basal insulin secretion in a normally constituted medium was compared for four periods with that in a medium in which potassium had been replaced by sodium

(Fig 3). There was a sustained stimulation of insulin secretion in the potassium free medium. The reversibility of this stimulation was demonstrated in an experiment in which pieces of pancreas were incubated for two periods in potassium-free medium followed by two periods in a normally constituted medium (Fig. 4).

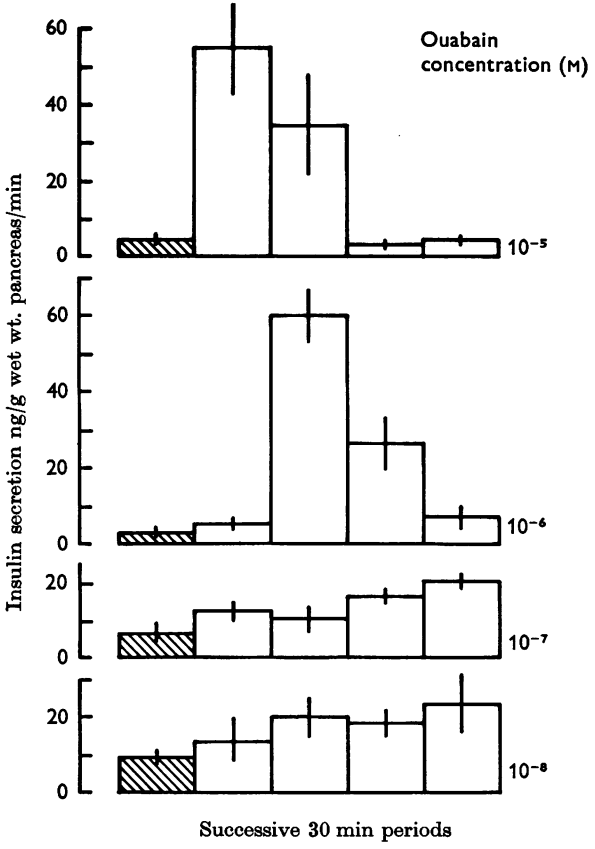

Fig. 2. Mean (±S.E. of mean) insulin secretion under basal conditions ▨ and in response to repeated exposure to 10^{-8}–10^{-5} M ouabain □. Each set of histograms depicts the results from a different group of five pieces of pancreas.

If ouabain and the replacement of potassium both stimulated insulin secretion by a common mechanism there should be no summation of their effects. This was investigated in the following manner: sixteen pieces from one pancreas were divided into groups of four and each group was incubated for four periods in either a normally constituted medium, a potassium-free medium (potassium replaced by sodium), a normal medium plus 10^{-6} M ouabain or a potassium-free medium plus 10^{-6} M ouabain. The

results (Fig. 5) confirmed the observation that replacement of potassium produced a sustained rise in insulin secretion and that 10^{-6} M ouabain caused a maximal rise in the second period. When both stimuli acted together maximal stimulation of insulin secretion occurred in the first

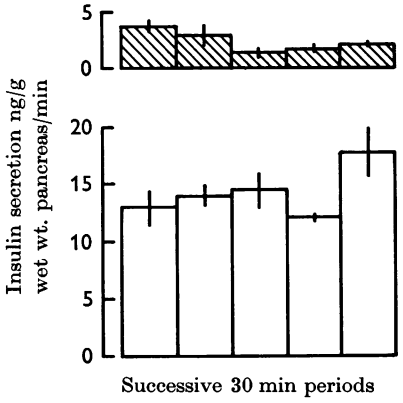

Fig. 3

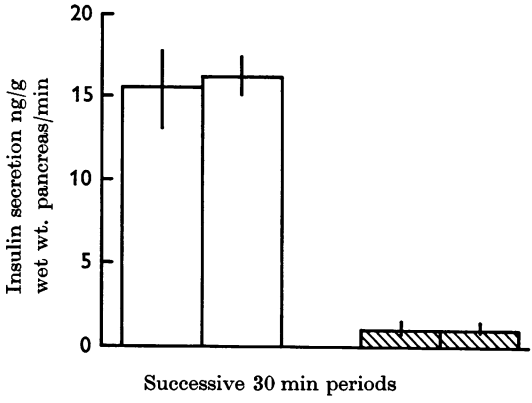

Fig. 4

Fig. 3. Mean (±S.E. of mean) insulin secretion from six pieces of pancreas under basal conditions ▨ compared with secretion from five pieces from adjacent regions of the same pancreas incubated in a medium in which all potassium had been replaced by sodium □.

Fig. 4. Mean (±S.E. of mean) insulin secretion from five pieces of pancreas incubated for two periods in a potassium-free medium □ compared with secretion from the same pieces of pancreas for two periods in a normal medium ▨. The experiment was preceded by 60 min incubation in a potassium-free medium and the two sets of observations were separated by a 30 min incubation in normal medium.

period and the degree of stimulation was similar to maximal stimulation caused by ouabain alone. This finding was consistent with the interpretation that ouabain and potassium replacement stimulated insulin secretion by a common mechanism and that when both stimuli acted together the speed of maximal stimulation increased but the magnitude of the stimulation remained unchanged.

Effect of sodium replacement by choline, potassium or lithium

The results of the experiments described in the previous section suggested that there might be a sodium pump in the cell membrane and that inhibition of the pump stimulated insulin secretion. If this were so an event in stimulation might be a rise in intracellular sodium concentration. The role of extracellular sodium in insulin secretion was therefore studied. Insulin secretion was measured first in a normal medium and then studied for four periods in choline, K or Li media. The experiment was repeated using media in which the glucose concentration was 16·5 mM throughout.

In each experiment four groups of four pieces of pancreas from one animal were used. In the experiment performed in 3·3 mM glucose there was a stimulation of insulin secretion on initial incubation in K medium and a smaller but significant stimulation in Li or choline medium (Fig. 6*a*). In

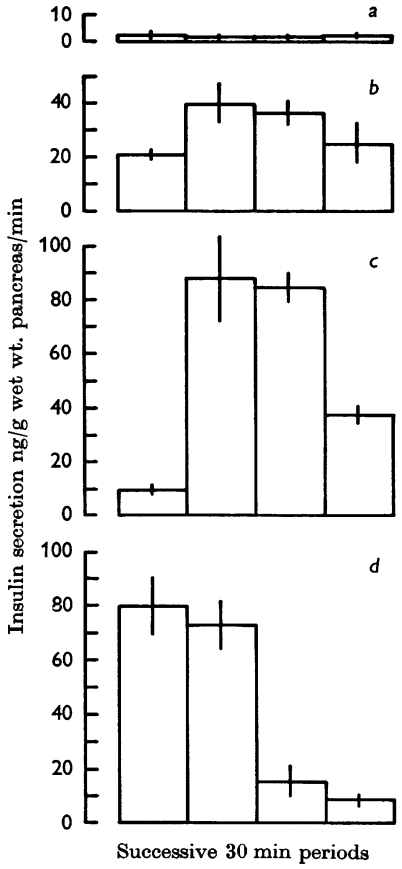

Fig. 5. Comparison of mean (±S.E. of mean) insulin secretion from pieces of pancreas incubated in normal medium (*a*), potassium free medium (*b*), normal medium + 10^{-6} M ouabain (*c*) or potassium-free medium + 10^{-6} M ouabain (*d*) for successive 30 min periods. Four groups of four pieces from one pancreas were employed. The experiment was preceded by a 60 min incubation in normal (*a* and *c*) or potassium-free medium (*b* and *d*).

16·5 mM glucose insulin secretion remained steady in Na medium but on transferring the pieces of pancreas to any of the other media, secretion fell to low levels (Fig. 6*b*). It was concluded that extracellular sodium was a pre-requisite for glucose stimulated insulin secretion.

It was postulated that the stimulation of insulin secretion on initial

incubation in K medium might be the result of cell membrane depolarization which permitted an influx of residual sodium from the interstitial space into the β cell. The possible depolarizing role of potassium was studied in two ways.

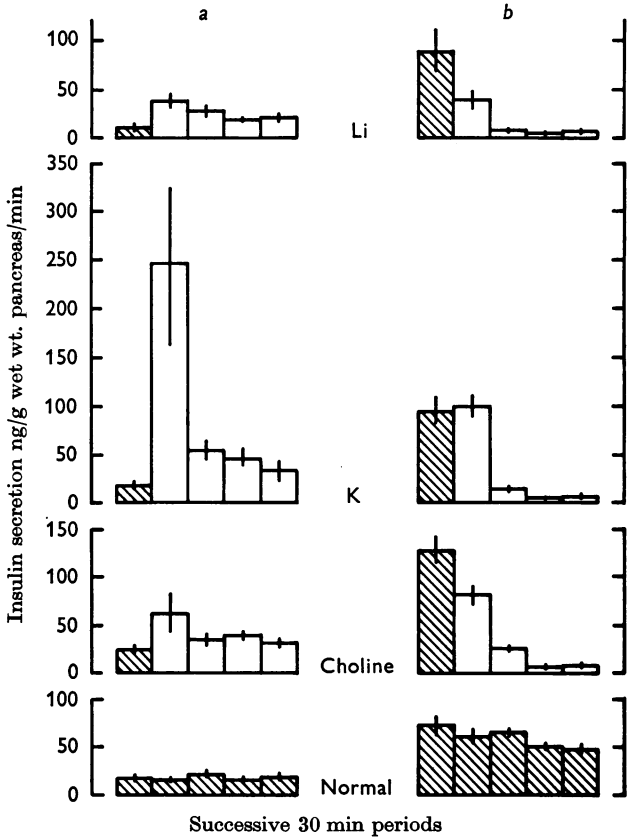

Fig. 6. Mean (± S.E. of mean) insulin secretion, □ in media in which Na had been replaced by choline, K or Li, or ▨ in normal medium, *a* under basal conditions and *b* supplemented with 16·5 mM glucose. Four groups of four pieces from one pancreas were employed in each case.

In one experiment the potassium concentration of the medium was raised by adding varying amounts of potassium chloride to a normal medium, in another the concentration was raised by replacing varying amounts of sodium chloride by potassium chloride. In the former experiment six pieces of pancreas were incubated for successive periods in increasing concentrations of potassium. Under these conditions the mean insulin secretion (± S.E. of mean) was 5·1 ± 0·7, 10·8 ± 2·9, 23·0 ± 5·5, 46·9 ± 7·3, 26·8 ± 10·7 and 14·0 ± 6·7 ng/g wet wt. pancreas/min in 5·6,

13·0, 18·7, 34·2, 59·5 and 90·0 m-equiv/l. potassium. In the latter experiment basal secretion of two groups of six pieces of pancreas was measured; then one group was incubated in a medium containing 17 m-equiv./l. potassium and the other in a medium containing 89·8 m-equiv./l. potassium. After a period to re-establish basal conditions and re-measurement

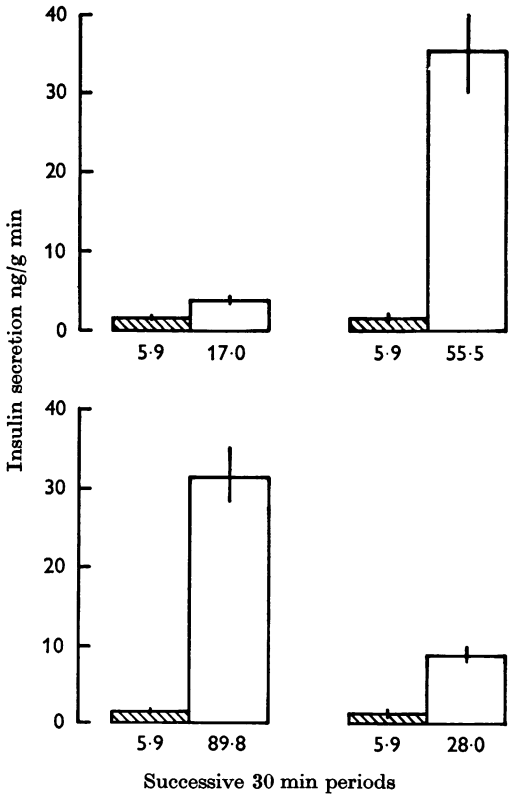

Fig. 7. Mean (±S.E. of mean) insulin secretion under basal conditions ▨ or in isotonic media with a high potassium concentration □. The potassium concentration of each medium is indicated beneath the histogram. The upper and lower row of histograms each depicts the mean insulin secretion from six pieces from adjacent regions of the same pancreas. The two sets of observations on a group of pieces were separated by a 30 min period in normal medium in which no measurements were made.

of basal secretion the former group was incubated in a medium containing 55·5 m-equiv./l. potassium and the latter group in a medium containing 28 m-equiv./l. potassium. The results of this experiment (Fig. 7) showed that a rise in the potassium concentration of the incubation medium caused a reversible stimulation of insulin secretion. The degree of stimulation was related to the potassium concentration in the medium and in

this experiment appeared to be maximal at an extracellular potassium concentration of 55·5 m-equiv/l. The stimulation of insulin secretion by potassium was not dependent on a rise in osmotic pressure or extracellular chloride concentration, nor on a fall in sodium concentration of the medium. The results suggested that the membrane potential of the β cell

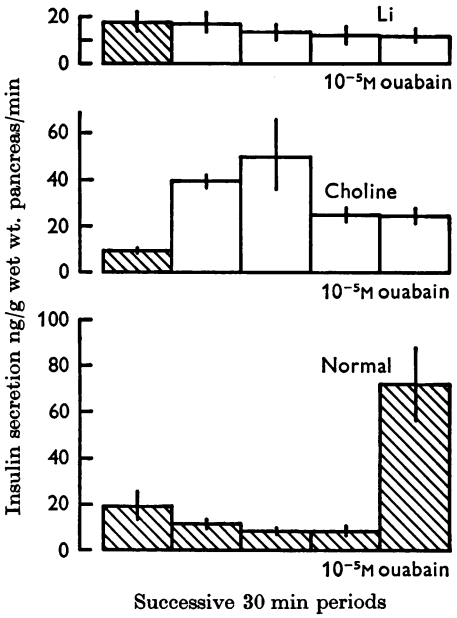

Fig. 8

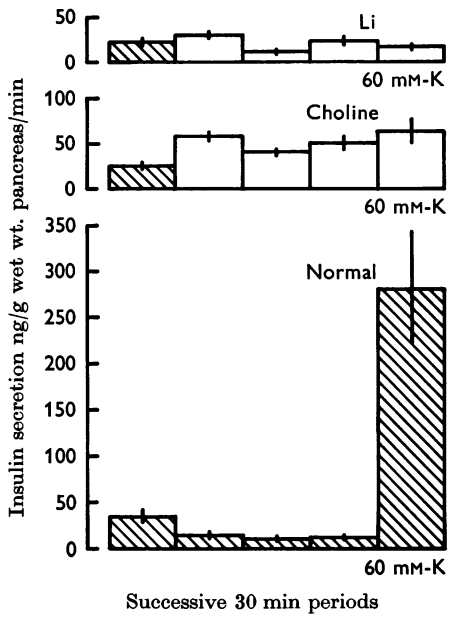

Fig. 9

Fig. 8. Mean (±S.E. of mean) insulin secretion in a normal medium ▨ or in media in which Na had been replaced by choline or Li □. In the final period 10^{-5} M ouabain was added to the incubation medium. Three groups of five pieces from one pancreas were employed.

Fig. 9. Mean (±S.E. of mean) insulin secretion in a normal medium ▨ or in media in which Na had been replaced by choline or Li □. In the final period the potassium concentration was raised to 60 mM. Three groups of five pieces from one pancreas were employed.

was potassium sensitive and that progressive depolarization of the membrane caused increasing stimulation of insulin secretion.

If insulin secretion were related to a rise in the intracellular sodium concentration which could be caused either by blocking the sodium pump or by depolarization of the cell membrane, the stimulatory effects of ouabain or potassium should be dependent on extracellular sodium. This was studied by experiments in which basal secretion in Na medium was measured in pieces of pancreas which were then incubated for four periods in Na, Li or choline medium. In the fourth period 10^{-5} M ouabain was

added or the pancreas was incubated in a medium containing 60 mM potassium (prepared by mixing K medium with Na, choline or Li medium). Figure 8 shows that 10^{-5} M ouabain stimulated insulin secretion only in the Na medium. Similar results were obtained when 60 mM potassium was the stimulus (Fig. 9).

Effect of sodium replacement on various stimuli of insulin secretion

Since sodium had been shown to be a pre-requisite for glucose, potassium or ouabain stimulation of insulin secretion, the role of sodium in pancreatic stimulation by other substances known to cause insulin secretion was investigated. Glucagon, L-leucine and tolbutamide were chosen as stimuli which would provide a wide range of signals for insulin secretion (Milner & Hales, 1967*a*).

The effect of replacing sodium by potassium, choline or lithium on the pancreatic response to the various stimuli was tested. The possibility that inhibition of insulin secretion in sodium replaced media in earlier experiments had been due to irreversible cell damage was controlled in these experiments by using each piece of pancreas as its own control. Basal and stimulated insulin secretion were measured first in a sodium free medium and then in normal medium. The results of these experiments are summarized in Table 1. The replacement of sodium by potassium, lithium or choline prevented the stimulation of insulin secretion by glucose, leucine, tolbutamide, glucagon or ouabain. The replacement of sodium by choline stimulated insulin secretion under basal conditions. The failure of the stimulus to augment insulin secretion in the choline medium was not due solely to the high basal secretion rate since the stimulated insulin secretion was greater in the presence of sodium. The inhibition of stimuli in K medium was reversible whereas in Li medium it was not. The effect of incubation in Li medium was therefore tested under conditions which were favourable for a return of the intracellular ionic environment to normal. Pieces of pancreas were initially incubated for 1 hr in Li medium, then for 3 hr in Na medium and were finally exposed to 16·5 mM glucose in Na medium. Their behaviour was compared with that of pieces which had been incubated in Na medium throughout (Table 2). The pieces of pancreas which had been initially incubated in lithium responded more poorly to glucose at the end of the experiment. These results demonstrated that in an experiment lasting $4\frac{1}{2}$ hr, the longest period for which we have studied insulin secretion, the effect of incubation in lithium was only partially reversible.

TABLE 1. The effect of Na replacement by choline, K or Li on insulin secretion from rabbit pancreas in response to various stimuli. Values indicate mean ± S.E. of mean absolute secretion rates (basal or stimulated) or mean ± S.E. of mean change in secretion rates (stimulated–basal). Significant ($P < 0.05$) changes in insulin secretion are indicated by an asterisk

	Stimulus	Number of observations	Insulin secretion (ng/g wet wt pancreas/min)					
				−Na			+Na	
			Basal	stimulated	Change	Basal	stimulated	Change
Choline	16·5 mM Glucose	6	22·7 ± 3·2	24·8 ± 7·7	+2·1 ± 8·6	16·6 ± 3·3	49·6 ± 8·7	+32·5 ± 8·0*
	5 mM L-leucine	5	20·1 ± 6·0	16·4 ± 3·6	−3·6 ± 5·3	4·1 ± 1·2	32·2 ± 7·6	+28·1 ± 6·3*
	5 μg/ml. Glucagon	5	16·5 ± 3·3	11·1 ± 2·9	−5·5 ± 0·7*	11·8 ± 2·9	43·3 ± 8·2	+31·4 ± 5·7*
	200 μg/ml. Tolbutamide	10	16·0 ± 1·7	14·8 ± 3·2	−1·2 ± 2·5	16·9 ± 3·2	35·2 ± 4·2	+18·3 ± 2·0*
	10 μM Ouabain	10	6·4 ± 0·6	4·8 ± 1·8	−0·6 ± 1·5	9·9 ± 1·0	18·6 ± 2·9	+ 8·6 ± 2·5*
K	16·5 mM Glucose	14	14·0 ± 3·5	19·8 ± 4·2	+5·6 ± 4·0	23·3 ± 10·3	79·8 ± 17·7	+56·5 ± 14·0*
	5 mM L-leucine	9	9·9 ± 2·3	10·0 ± 3·0	+0·1 ± 2·4	5·6 ± 1·0	15·5 ± 1·0	+9·9 ± 3·5*
	5 μg/ml. Glucagon	9	6·7 ± 1·7	7·6 ± 1·5	+0·7 ± 2·3	41·4 ± 5·1	77·2 ± 4·2	+35·8 ± 5·0*
	200 μg/ml. Tolbutamide	9	8·0 ± 2·1	8·7 ± 2·2	+0·6 ± 1·5	5·8 ± 0·8	11·6 ± 2·7	+5·8 ± 1·9*
	10 μM Ouabain	5	16·2 ± 5·0	18·0 ± 5·4	+1·7 ± 5·6	9·9 ± 2·6	85·3 ± 19·4	+75·4 ± 18·6*
Li	16·5 mM Glucose	12	58·3 ± 10·4	38·0 ± 4·9	−19·0 ± 8·0	60·0 ± 9·6	68·7 ± 13·2	+9·6 ± 8·3
	5 mM L-leucine	12	52·6 ± 8·8	41·9 ± 9·0	−10·9 ± 3·8	57·2 ± 8·6	65·4 ± 6·3	+8·2 ± 6·2
	5 μg/ml. Glucagon	5	52·5 ± 11·2	43·9 ± 4·0	−8·1 ± 3·9	33·2 ± 3·6	35·7 ± 5·4	+2·5 ± 7·9
	200 μg/ml. Tolbutamide	5	61·3 ± 16·0	34·9 ± 7·1	−24·1 ± 14·8	46·0 ± 12·5	31·1 ± 4·3	−14·9 ± 8·9
	10 μM Ouabain	5	56·3 ± 11·1	23·7 ± 3·6	−32·6 ± 7·5	35·4 ± 9·2	34·5 ± 8·8	−0·9 ± 12·8

TABLE 2. The effect of Na replacement by Li on insulin secretion from rabbit pancreas in response to 16·5 mM glucose. The incubation medium contained the ion shown in parentheses. Six pieces of pancreas were subjected to each treatment

Incubation period (min)	Glucose (mM)	Mean ± S.E. of mean insulin secretion (ng/mg wet wt. pancreas/min)	
30	3·3	18·7 ± 2·1 (*Li*)	18·0 ± 3·3 (*Na*)
30	3·3	10·7 ± 2·2 (*Li*)	12·8 ± 4·1 (*Na*)
30	3·3	9·2 ± 3·0 (*Na*)	6·2 ± 1·6 (*Na*)
30	3·3	8·5 ± 2·4 (*Na*)	4·8 ± 1·7 (*Na*)
90	3·3	4·6 ± 1·2 (*Na*)	4·2 ± 1·1 (*Na*)
30	3·3	8·2 ± 1·6 (*Na*)	5·8 ± 1·5 (*Na*)
30	16·5	16·6 ± 1·8 (*Na*)	35·9 ± 3·3 (*Na*)

Control experiments

Effect of glucose omission on insulin secretion. Glucose 3·3 mM had been added empirically to the incubation medium in all experiments in order to produce an extracellular glucose concentration which approximated to that *in vivo*. Stimulation of insulin secretion by glucagon was dependent on a high extracellular glucose concentration (16·5 mM) and could not be elicited in 3·3 mM glucose. It was possible that other stimuli which were tested had a common action on glucose metabolism which led to their ability to act as stimuli. In this case any experimental condition which affected the glucose stimulation of insulin secretion would also affect the action of other stimuli without necessarily reflecting anything other than their glucose dependence. This possible dependence was tested by experiments in which basal and stimulated insulin secretion were measured in media which contained no glucose or 3·3 mM glucose (Table 3). Leucine, tolbutamide, ouabain and potassium each stimulated insulin secretion in the presence or absence of glucose.

Effect of atropine on potassium or ouabain stimulation. The possibility that potassium or ouabain stimulation of insulin secretion might be mediated via parasympathetic neurones which innervate the islets of Langerhans (Coupland, 1958) was tested by comparing basal and stimulated secretion in the presence and absence of atropine. Atropine 10^{-7} or 10^{-6} g/ml. had no significant effect on insulin secretion stimulated by 60 mM potassium or 10^{-5} M ouabain respectively (Table 4).

Effect of different ionic media on insulin recovery. Previous studies have demonstrated that when ox insulin was incubated with pieces of rabbit pancreas or in medium which had previously contained rabbit pancreas, 20–30 % of the exogenous insulin was destroyed (Coore & Randle, 1964). Because of the possibility that the failure of stimuli to act in choline, K or Li media was due to an increased rate of insulin destruction in these media, the effect of incubation of ox insulin with rabbit pancreas in various media was studied. Three groups of five pieces of pancreas from one rabbit were employed. The recovery of insulin from each group of pieces was measured first after incubation in choline, K or Li medium and then after incubation in reconstituted medium. After measuring basal secretion in choline, K or Li medium, each group of pieces was incubated in the same medium to which 150 ng/ml. ox insulin had been added. The whole procedure was then repeated in normal medium. After correction for endogenous insulin secretion the recovery of ox insulin in sodium-free and normal media was calculated (Table 5).

Insulin recovery calculated in this way was of the same order as that found by Coore & Randle (1964). Similar results were obtained in an experiment in which 20 ng/ml. ox insulin was added to the media.

TABLE 3. The effect of omission of 3·3 mM glucose on insulin secretion from rabbit pancreas in response to various stimuli. Values indicate mean ± S.E. of mean absolute secretion rates (basal and stimulated) or mean ± S.E. of mean change in secretion rates (stimulated–basal). Significant changes ($P < 0.05$) in insulin secretion are indicated by an asterisk

		Insulin secretion (ng/g wet wt. pancreas/min)								
		+Glucose			−Glucose			+Glucose		
Stimulus	Number of observations	Basal	Stimulated	Change	Basal	Stimulated	Change	Basal	Stimulated	Change
5 mM L-leucine	12	5·5 ± 1·4	19·7 ± 5·6	+14·0 ± 5·0*	5·3 ± 2·3	19·2 ± 5·3	+13·9 ± 3·5*	4·8 ± 0·9	13·9 ± 3·3	+8·9 ± 3·6*
200 μg/ml. Tolbutamide	12	7·8 ± 1·6	15·9 ± 1·3	+ 8·0 ± 1·7*	3·7 ± 0·9	11·0 ± 1·3	+7·3 ± 1·4*	3·8 ± 0·8	8·0 ± 1·8	+4·2 ± 1·8*
10^{-5} M Ouabain	6	—	—	—	3·9 ± 1·0	50·9 ± 6·7	+47·0 ± 7·4*	7·2 ± 2·8	19·4 ± 6·1	+12·1 ± 4·1*
60 mM Potassium	6	—	—	—	4·3 ± 1·2	54·6 ± 11·4	+50·5 ± 10·7*	2·1 ± 0·4	42·0 ± 6·9	+39·9 ± 6·5*

TABLE 4. The effect of atropine on basal and stimulated insulin secretion in response to potassium or ouabain. Values indicate mean ± S.E. of mean secretion rates. Each group was composed of six pieces of pancreas

		Insulin secretion (ng/g wet wt. pancreas/min)	
Stimulus	Atropine (g/ml.)	Basal	Stimulated
60 mM Potassium	—	3·2 ± 1·2	39·5 ± 8·7
	$+10^{-7}$	1·1 ± 0·2	52·6 ± 14·3
10^{-5} M Ouabain	—	3·1 ± 0·6	83·6 ± 13·4
	$+10^{-6}$	1·4 ± 0·3	104·3 ± 12·2

TABLE 5. The destruction of ox insulin by incubation with pieces of rabbit pancreas in different ionic media. Values indicate mean ± S.E. of mean percentage recovery of insulin. Five pieces of pancreas were subjected to each treatment

	Insulin recovery (%)	
Sodium replacement	−Na	+Na
Choline	98·1 ± 9·9	71·3 ± 9·6
Potassium	107·1 ± 10·0	74·0 ± 8·9
Lithium	86·9 ± 4·2	101·4 ± 13·2

DISCUSSION

The release of insulin from pieces of rabbit pancreas *in vitro* has been shown to provide a simple and reproducible model for the behaviour of β cells *in vivo* (Coore & Randle, 1964). The simplicity of the preparation has certain advantages in comparison with the use of isolated islets of Langerhans (Lacy, 1967) or a perfused pancreas preparation (Grodsky, Batts, Bennett, Vcella, McWilliams & Smith, 1963) to study insulin *in vitro*. There is an important species difference between the rabbit and the rat when studying insulin secretion from pieces of pancreas. Ox insulin is rapidly destroyed on incubation with rat pancreas (Malaisse, Malaisse-Lagae & Wright, 1967) whereas the insulin degrading activity of rabbit pancreas under similar conditions is much less. Assuming that the destruction of endogenously released insulin is similar to that of added ox insulin, the loss of endogenous insulin will not exceed 30 %. This loss is small in comparison to the increase in the rate of insulin secretion induced by various stimuli and it cannot account for the changes observed. On the other hand an increase in the rate of insulin destruction could account for an apparent inhibition of insulin secretion. The recovery experiments exclude this possibility.

Each of the preparations which has been used to study insulin secretion *in vitro* is composed of a mixed cell population. The islets of Langerhans make up approximately 1 % of the pancreas and are innervated by parasympathetic nerves (Coupland, 1958). In the experiments reported here the possibility that changes in insulin secretion were mediated by the autonomic nervous system or by other cells in the islets of Langerhans rather than by a direct effect on the β cell was considered. Changes in the ionic composition of the incubation medium could have influenced insulin secretion by altering the secretion of autonomic transmitters or by altering secretion from cells within the islet. Mallaisse, Malaisse-Lagae, Wright & Ashmore (1967) demonstrated that insulin secretion was stimulated from rat pancreas *in vitro* by cholinergic drugs and that this stimulation was blocked by atropine. The possibility that some of the changes in insulin secretion in the present experiments were mediated by ionic fluxes in the autonomic nervous system appeared to be excluded by the fact that potassium and ouabain remained effective stimuli in the presence of atropine. Furthermore, Coore & Randle (1964) were unable to demonstrate any effect of parasympathomimetic drugs on insulin secretion from pieces of rabbit pancreas, whereas they were able to demonstrate an inhibition of secretion by catecholamines.

Glucagon has been shown to stimulate insulin secretion from pieces of rabbit pancreas (Turner & McIntyre, 1966), but the part played by α and

other islet cells in the release of insulin from the β cell cannot be analysed until it is possible to study ionic fluxes in β cells directly. Since glucagon is an effective stimulator of insulin secretion only in the presence of a high glucose concentration, it is unlikely that ionic changes which are active in the absence of extracellular glucose are mediated by glucagon.

The possibility that various substances stimulated insulin secretion by altering the rate of β cell uptake of glucose was tested. Extracellular glucose was not essential for stimulation by tolbutamide, leucine, ouabain or potassium. Tolbutamide had been shown previously to stimulate insulin secretion *in vitro* in the absence of glucose (Mehnert, Schäfer, Kaliampetsos, Stuhlfauth & Engelhardt, 1962).

The effect of ouabain on insulin secretion was studied because this glycoside had been shown to inhibit specifically a sodium pump in a wide variety of cells (Schatzmann, 1953; Skou, 1965). The dose–time relation of ouabain causing maximal stimulation of insulin secretion was similar to that of glycoside inhibition of a sodium pump in red blood cells (Glynn, 1957). Further exposure to 10^{-6} or 10^{-5} M ouabain after maximal stimulation of insulin secretion was accompanied by a progressive fall in insulin secretion. This suggested that although secretion was stimulated by a rise of intracellular sodium concentration, prolongation of the rise with an accompanying fall of intracellular potassium concentration caused ionic changes which inhibited secretion. Alternatively, secretion might have been stimulated by the rate of rise rather than by the absolute level of intracellular sodium.

The postulate that ouabain stimulated insulin secretion by inhibiting a sodium pump was tested by omitting potassium from the incubation medium. Active pumping of sodium across the red cell membrane is inhibited by the absence of extracellular potassium (Dunham & Glynn, 1961; Whittam, 1962). Insulin secretion was stimulated by incubation in a potassium-free medium, but the stimulation was sustained and not so great as that caused by ouabain. This was thought to be due to partial pump inhibition due to the presence of a low concentration of potassium in the incubation medium. This was measured at the end of some of these experiments and was 0·01–0·20 mM. The failure of omission of potassium to augment the stimulation caused by 10^{-6} M ouabain also suggested that both stimuli acted through a common mechanism.

The indirect evidence that a rise in the sodium concentration of the β cell stimulated insulin secretion led to a study of the effect on insulin secretion of replacing sodium in the medium by choline, potassium or lithium. The discovery that complete replacement of sodium by potassium initially stimulated insulin secretion in 3·3 mM glucose suggested a further characteristic of the β cell. If the resting potential of the cell were potas-

sium-dependent, transfer of pancreas to K medium would be followed by cell membrane depolarization, influx of residual sodium from the interstitial space and stimulation of insulin secretion. This sequence of events would not continue in successive periods because of the progressive elution of sodium from the tissue into the incubation medium. This interpretation was supported by two further observations. Stimulation of insulin secretion by potassium depended on the extracellular potassium concentration and therefore on the degree of cell membrane depolarization and sodium influx. Grodsky & Bennett (1966) had also demonstrated in the perfused rat pancreas that insulin secretion was stimulated by a rise in the concentration of circulating potassium from 4 to 8 m-equiv/l. In pieces of pancreas which had been incubated in a sodium-free medium for three periods and from which all extracellular sodium had presumably been removed secretion was not stimulated by potassium.

The discovery that replacement of sodium by choline, potassium or lithium progressively inhibited insulin secretion stimulated by glucose promoted further study of the role of extracellular sodium in the stimulation of insulin secretion by different substances. In these experiments the reversibility of inhibition of insulin secretion in sodium-free media was also studied. In a preliminary communication experiments were reported in which sodium was replaced by choline for the middle 90 min of experiments lasting 270 min (Milner & Hales, 1967*b*). These experiments served to define the viability of the preparation we have used. In the present experiments inhibition of secretion in response to all stimuli occurred in sodium-free media. When sodium was replaced by choline or potassium the inhibition was reversible, but when sodium was replaced by lithium it was not. A possible reason for this was that, as in red blood cells and muscle (Maizels, 1954; Keynes & Swan, 1959), lithium entered the cell like sodium, but was pumped out more slowly. Passive efflux of potassium from the cell might have been accompanied by an intracellular accumulation of lithium which was only slowly reversible. That this sequence of events might have occurred in these experiments was suggested by the finding that the effect of incubation in Li medium for 1 hr was only partly reversible 3 hr later.

A study of the part played by sodium and potassium in the secretion of insulin is, at present, necessarily an indirect one. The results of these experiments show that extracellular sodium is a pre-requisite for the stimulation of insulin secretion by a wide variety of stimuli and suggest that depolarization and/or influx of sodium across the β cell membrane leads to insulin secretion.

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