Distinct effects of acetylcholine and glucose on ^{45}calcium and ^{86}rubidium efflux from mouse pancreatic islets

M. Nenquin, P. Awouters, F. Mathot and J.C. Henquin*

Unité de Diabétologie et Nutrition, University of Louvain Faculty of Medicine, UCL 54.74, B 1200 Brussels, Belgium

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The similarities between the effects of acetylcholine and glucose on phospholipid metabolism in pancreatic islet cells prompted the comparison of their effects on ionic fluxes. Acetylcholine (1 μM) consistently increased $^{45}Ca^{2+}$ efflux from mouse islets, whereas glucose increased it in the presence, but decreased it in the absence of extracellular Ca^{2+}. Acetylcholine consistently accelerated $^{86}Rb^{+}$ efflux, and this effect was augmented by Ca^{2+} omission. On the other hand, glucose markedly inhibited $^{86}Rb^{+}$ efflux, except when its concentration was raised from 10 to 15 mM in the presence of Ca^{2+}. Unlike their effects on phospholipid metabolism, the ionic effects of the two insulin-secretagogues are thus very different.

Pancreatic islet *Acetylcholine* *Glucose* *Insulin release* *Calcium* *Ion efflux*

1. INTRODUCTION

Activation of phospholipid turnover is an early event of the stimulus-secretion coupling in many gland cells [1-3]. Hormones and neurotransmitters, which utilize Ca^{2+} as second messenger, often induce hydrolysis of membrane phosphoinositides. Several mechanisms may link this reaction to the rise in cellular free Ca^{2+}, but recently much attention has been focused on the mobilization of Ca^{2+} from intracellular pools by inositol trisphosphate [4].

Various insulin-secretagogues stimulate phospholipid metabolism in pancreatic islet cells, but the significance of this activation is still controversial [5-10]. Analogies exist between the effects of glucose and cholinergic agonists [9,10], which appear to cause a similar hydrolysis of phosphoinositides by a phospholipase C mechanism [11]. This, obviously, raises the possibility that an activation of phospholipid turnover is involved in the regulation of ionic fluxes in B cells by both the nutrient and the neurotransmitter insulin-secretagogues. Such a speculation is reinforced by the recent observation that inositol trisphosphate mobilizes Ca^{2+} from microsomes of tumoural B cells [12]. We have thus compared the effects of glucose and acetylcholine on insulin release, $^{45}Ca^{2+}$ and $^{86}Rb^{+}$ efflux from isolated mouse islets.

2. MATERIALS AND METHODS

All experiments were performed with islets isolated by collagenase digestion of the pancreas of fed female NMRI mice. The medium used contained 122 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$ and 25 mM $NaHCO_3$. It was gassed with O_2/CO_2 (94%/6%) to maintain pH at 7.4, and was supplemented with 1 mg bovine serum albumin/ml. In certain experiments, $CaCl_2$ was omitted and was substituted by $MgCl_2$. The perifusion technique used to monitor the efflux of $^{45}Ca^{2+}$ or $^{86}Rb^{+}$ (used as tracer for K^{+}) from preloaded islets has been detailed in [13,14]. The only significant differences were that the loading with the isotopes lasted only 90 min and was carried out in the presence of 10 mM glucose. A portion of the effluent fractions collected during the experiment of $^{86}Rb^{+}$ efflux was drawn for measurement of immunoreactive insulin. Acetylcholine chloride was

* To whom correspondence should be addressed

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obtained from Sigma (St. Louis, USA), atropine sulfate from Merck (Darmstadt, FRG) and the radioisotopes from the Radiochemical Centre (Amersham, England).

3. RESULTS

Addition of 1 μM acetylcholine to a medium containing 3 mM glucose and 2.5 mM Ca^{2+} slightly increased the rate of $^{45}Ca^{2+}$ and $^{86}Rb^{+}$ efflux from the islets (fig.1). The acceleration of $^{45}Ca^{2+}$ efflux was biphasic, the largest increase occuring during the first 4 min. Acetylcholine failed to induce insulin release under these conditions. As also shown by fig.1, 15 mM glucose produced a transient inhibition, followed by a large stimulation of $^{45}Ca^{2+}$ efflux and a marked decrease in $^{86}Rb^{+}$ efflux. The sugar also triggered a biphasic release of insulin. The effects of both acetylcholine and glucose were reversible upon return to the control medium.

When the same experiments were carried out in the absence of extracellular Ca^{2+}, acetylcholine

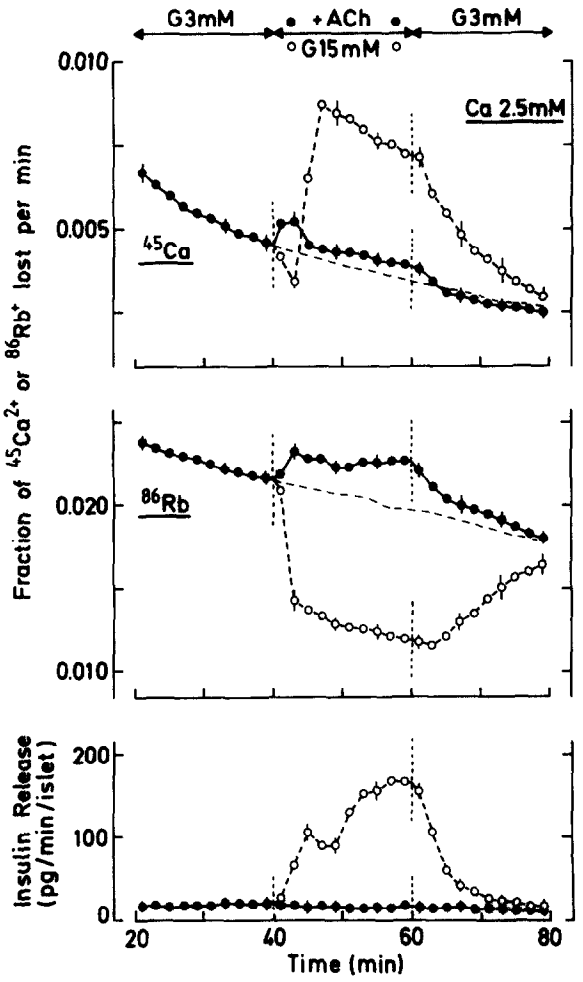

Fig.1. Effects of acetylcholine (ACh) and glucose (G) on $^{45}Ca^{2+}$ or $^{86}Rb^{+}$ efflux, and on insulin release from perifused mouse islets. Experiments were started in the presence of 3 mM glucose; between 40 and 60 min, 1 μM acetylcholine was added to the medium (●) or the concentration of glucose was raised to 15 mM (○). The concentration of Ca^{2+} was 2.5 mM throughout. In all figures, control experiments are shown by the broken lines, and values are means ± SE for 4–5 experiments.

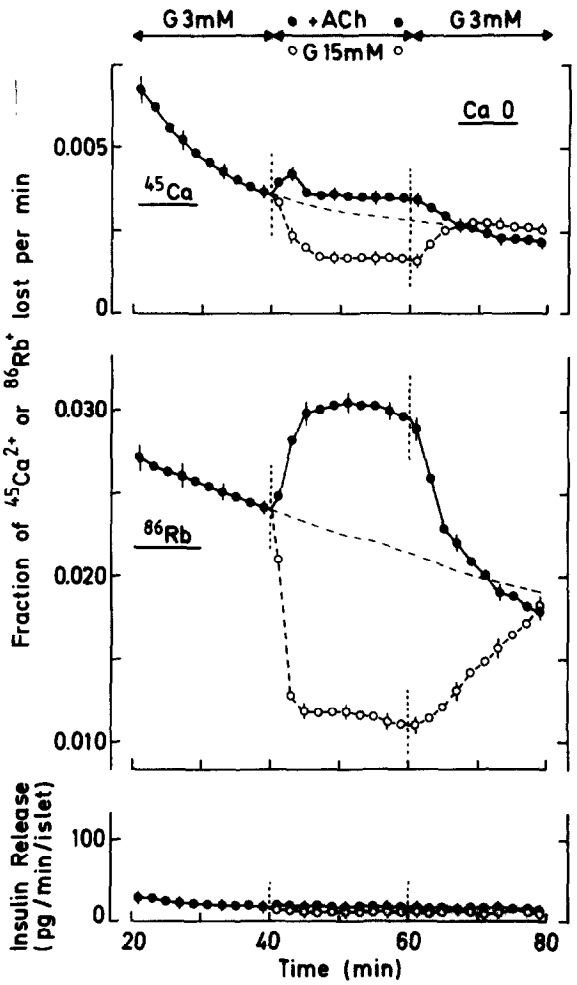

Fig.2. Effects of acetylcholine (ACh) and glucose (G) on $^{45}Ca^{2+}$ or $^{86}Rb^{+}$ efflux, and on insulin release from perifused mouse islets. Same experimental conditions as in fig.1, except for omission of extracellular Ca^{2+} throughout.

still reversibly accelerated $^{45}Ca^{2+}$ and $^{86}Rb^{+}$ efflux (fig.2). The effect on $^{86}Rb^{+}$ efflux was even larger than in the presence of extracellular Ca^{2+}. By contrast, glucose only produced a sustained and reversible inhibition of both $^{45}Ca^{2+}$ and $^{86}Rb^{+}$ efflux. Neither agent increased insulin release in a Ca^{2+}-free medium.

In the presence of 10 mM glucose and 2.5 mM Ca^{2+}, the rate of $^{45}Ca^{2+}$ efflux was higher, whereas that of $^{86}Rb^{+}$ efflux was lower than in the presence of only 3 mM glucose. Insulin release was stimulated approximately 4-fold (fig.3). Addition of 1 μM acetylcholine caused a rapid biphasic increase in $^{45}Ca^{2+}$ efflux, $^{86}Rb^{+}$ efflux and insulin release. Raising the concentration of glucose to 15 mM was followed by qualitatively similar changes, the amplitude of which was less marked, however, than after acetylcholine. All these effects were reversible upon return to the control medium.

If the same experiments were performed in a medium containing 10 mM glucose, but no Ca^{2+}, acetylcholine still accelerated $^{45}Ca^{2+}$ and $^{86}Rb^{+}$ efflux (fig.4). The effect on $^{45}Ca^{2+}$ efflux was

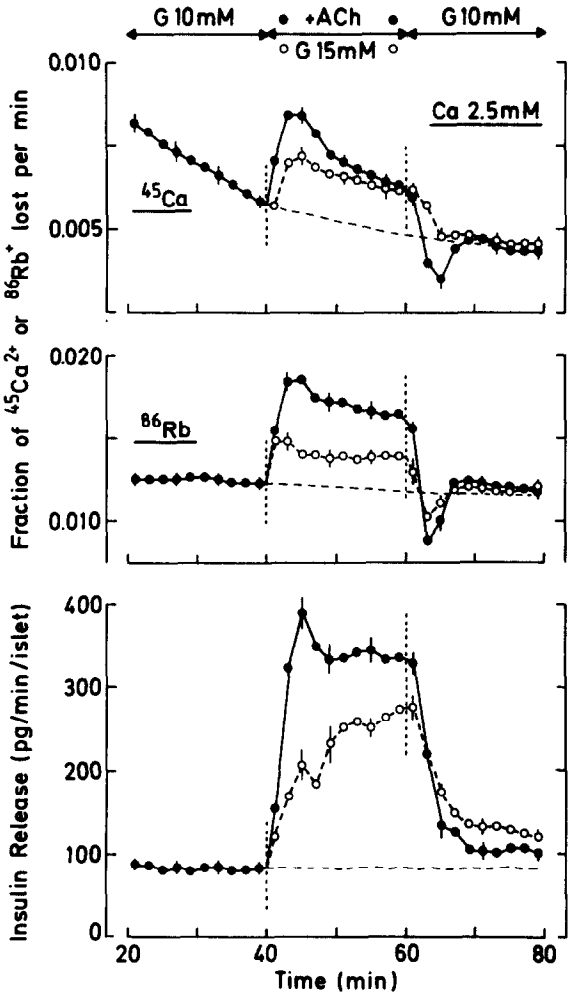

Fig.3. Effects of acetylcholine (ACh) and glucose (G) on $^{45}Ca^{2+}$ or $^{86}Rb^{+}$ efflux, and on insulin release from perifused mouse islets. The experiments started in the presence of 10 mM glucose; between 40 and 60 min, 1 μM acetylcholine was added to the medium (●) or the concentration of glucose was raised to 15 mM (○). The concentration of Ca^{2+} was 2.5. mM throughout.

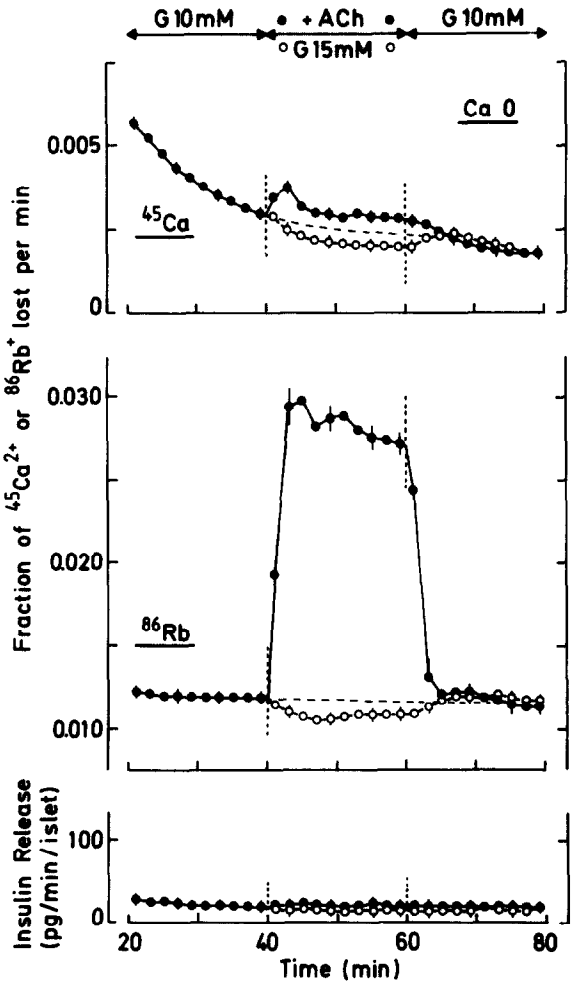

Fig.4. Effects of acetylcholine (ACh) and glucose (G) on $^{45}Ca^{2+}$ or $^{86}Rb^{+}$ efflux, and on insulin release from perifused mouse islets. Same experimental conditions as in fig.3, except for omission of extracellular Ca^{2+} throughout.

smaller, but that on $^{86}Rb^+$ efflux considerably larger than in the presence of extracellular Ca^{2+}. On the other hand, 15 mM glucose no longer increased, but slightly decreased both $^{45}Ca^{2+}$ and $^{86}Rb^+$ efflux. Neither agent induced insulin release under these conditions (fig.4).

Atropine (5 μM) was without effect alone, but abolished all the changes in $^{45}Ca^{2+}$ or $^{86}Rb^+$ efflux and in insulin release otherwise brought about by 1 μM acetylcholine (not shown).

4. DISCUSSION

The changes in $^{45}Ca^{2+}$ efflux brought about by glucose in islet cells show a much greater dependence on extracellular Ca^{2+} than the effects of acetylcholine. Thus, the sugar stimulated $^{45}Ca^{2+}$ efflux if extracellular Ca^{2+} was present, but decreased it if extracellular Ca^{2+} was absent. The mechanism of this inhibition is still controversial, but the acceleration of efflux is thought to reflect the stimulation of Ca^{2+} influx through voltage-dependent Ca-channels [15-17]. By contrast, acetylcholine increased $^{45}Ca^{2+}$ efflux in the absence as well as in the presence of external Ca^{2+}. The magnitude of this stimulation was not influenced by external Ca^{2+} in the presence of 3 mM glucose, but was increased by external Ca^{2+} in the presence of 10 mM glucose. Interestingly, it was only under these latter conditions that acetylcholine triggered insulin release. Taken together these observations suggest that acetylcholine is able to mobilize cellular Ca^{2+}, but that such mobilization is not sufficient to stimulate exocytosis. The releasing effect of the neurotransmitter appears to depend entirely on an influx of Ca^{2+}, that can be stimulated only in the presence of a sufficient concentration of glucose, perhaps because the membrane is depolarized by the sugar. These conclusions are in keeping with the evidence that acetylcholine increases the electrical activity triggered by 11 mM glucose in mouse B cells [18,19], but fails to induce it in resting B cells perifused with only 3 mM glucose [18].

Acetylcholine consistently increased the rate of $^{86}Rb^+$ efflux from islet cells, an effect that escaped detection by the less sensitive measurement of $^{86}Rb^+$ retention of preloaded islets [20]. This acceleration of $^{86}Rb^+$ efflux cannot be the mere consequence of membrane depolarization, since the neurotransmitter does not change the membrane potential of B cells in the presence of 3 mM glucose [18]. Activation of Ca-sensitive K channels [14] is possible, as in pancreatic acinar cells [21]. Surprisingly however, the stimulation of $^{86}Rb^+$ efflux by acetylcholine was larger in the absence than in the presence of extracellular Ca^{2+}, and not directly proportional to the magnitude of the concomitant increase in $^{45}Ca^{2+}$ efflux. Therefore, the participation of non-voltage, non-Ca-dependent K channels cannot be ruled out. Again, these effects of acetylcholine sharply contrast with the effects of glucose on $^{86}Rb^+$ efflux. As previously reported [13], raising the concentration of glucose from 3 to 15 mM markedly lowered the rate of $^{86}Rb^+$ efflux in the presence or absence of Ca^{2+}. This effect reflects the decrease in K^+-permeability whereby the sugar depolarizes the B cell membrane (review, [22]). A slight acceleration of $^{86}Rb^+$ efflux was observed only when the concentration of glucose was raised from 10 to 15 mM, and this increase was completely abolished by omission of extracellular Ca^{2+}.

In conclusion, acetylcholine and glucose produce distinct changes in Ca^{2+} and K^+ ($^{86}Rb^+$) fluxes, the control of which is essential for insulin release [15,22]. It is thus unlikely that their effects on islet phospholipid turnover, though similar, have the same significance for the remodelling of the ionic fluxes involved in the stimulus-secretion coupling in B cells. Further experiments will be necessary, however, to determine the possible links between these respective events.

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