

Somatostatin activates glibenclamide-sensitive and ATP-regulated K^+ channels in insulinoma cells via a G-protein

Michel Fosset, Heidy Schmid-Antomarchi, Jan R. de Weille and Michel Lazdunski

Centre de Biochimie, Centre National de la Recherche Scientifique, Parc Valrose, 06034 Nice Cedex, France

Received 31 October 1988

Somatostatin, an hyperglycemia-inducing hormone, was studied in rat insulinoma (RINm5F) cells using $^{86}Rb^+$ efflux techniques. $^{86}Rb^+$ efflux is stimulated by somatostatin in a dose-dependent manner. The half-maximum value of activation is 0.7 nM. Somatostatin-induced $^{86}Rb^+$ efflux is abolished by the hypoglycemia-inducing sulfonylurea, glibenclamide, a known blocker of ATP-regulated K^+ channels. Somatostatin activation is prevented by pretreatment of insulinoma cells with pertussis toxin. $^{86}Rb^+$ efflux studies show that somatostatin activates an ATP-dependent K^+ channel.

Somatostatin; Glibenclamide; $^{86}Rb^+$ efflux; G-protein; (RINm5F cell)

1. INTRODUCTION

Insulin secretion from pancreatic β -cells is stimulated by glucose [1]. Glucose application causes a slow depolarization, due to the closure of ATP-regulated K^+ channels, which then evokes a repetitive pattern of electrical activity during which Ca^{2+} flows in through voltage-dependent Ca^{2+} channels and triggers insulin release [2–4].

Important progress has recently been made in the pharmacology of the ATP-regulated K^+ channel. This channel has been shown to be the target of antidiabetic sulfonylureas [5–8]. One of them, glibenclamide, appears to be a very potent blocker (K_d in the 0.1–1 nM range) of the ATP-regulated K^+ channel (K_{ATP}) [6,7].

This channel has also been shown to be activated by a hyperglycemia-inducing hormone, galanin [9]. Somatostatin is an important hormone in the control of inhibition of insulin secretion by the pancreatic β -cell [10].

We demonstrate in this paper that somatostatin, like galanin, activates sulfonylurea-sensitive K^+

channels and that this activation involves a G-protein.

2. MATERIALS

2.1. Materials

Glibenclamide was from Hoechst, somatostatin-14 from Bachem and pertussis toxin from List Biological Laboratories (Campbell, CA).

2.2. Cell culture

RINm5F cells, an insulin-producing cell line derived from a rat islet cell tumor, were grown as described in [11]. Cells were plated at a density of 200 000 cells per well (Falcon 24-well tissue culture plates).

2.3. $^{86}Rb^+$ efflux experiments

Efflux studies were done according to [7,9] in 24-well culture plates at 37°C and after overnight equilibration of cells in RPMI 1640 medium supplemented with 10% fetal calf serum, 0.1 μ Ci of $^{86}RbCl$ per ml (1 Ci = 37 GBq), and 0.2 μ Ci of L-[3H]leucine per ml (internal marker of cell recovery). After removing the medium, cells were preincubated, for various times as indicated, in a medium of 120 mM NaCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 10 mM KCl containing 20 mM Hepes/NaOH buffer, pH 7.5, supplemented by 0.1 μ Ci of $^{86}RbCl$ per ml without (5 mM glucose added) or with 0.24 μ g of oligomycin per ml, 1 mM 2-deoxy-D-glucose, and ligands as indicated in the figures. $^{86}Rb^+$ efflux studies were initiated by removing the preincubation medium and incubating the cells with 200 μ l per cell of the same medium without $^{86}Rb^+$, and without or with oligomycin, and 2-deoxy-D-glucose. Efflux was

Correspondence address: M. Lazdunski, Centre de Biochimie, Centre National de la Recherche Scientifique, Parc Valrose, 06034 Nice Cedex, France

stopped by removing the latter medium and washing the cells three times with 1 ml of 0.1 M MgCl_2 at 37°C . Cells were extracted with two 1-ml portions of 0.1 M NaOH, and radioactivity was counted.

2.4. Pertussis toxin treatment

RINm5F cells were incubated for 15 h in complete culture medium with 100 ng of pertussis toxin per ml at 37°C before $^{86}\text{Rb}^+$ efflux experiments.

3. RESULTS AND DISCUSSION

This laboratory has previously shown that the $^{86}\text{Rb}^+$ efflux technique can be very useful to study the activity of ATP-regulated K^+ channels in insulinoma RINm5F cells [7,9]. Lowering of intracellular ATP by oligomycin and 2-deoxy-D-glucose treatment reveals an important component of the $^{86}\text{Rb}^+$ efflux as can be seen again in fig.1A. This component of the $^{86}\text{Rb}^+$ efflux is completely

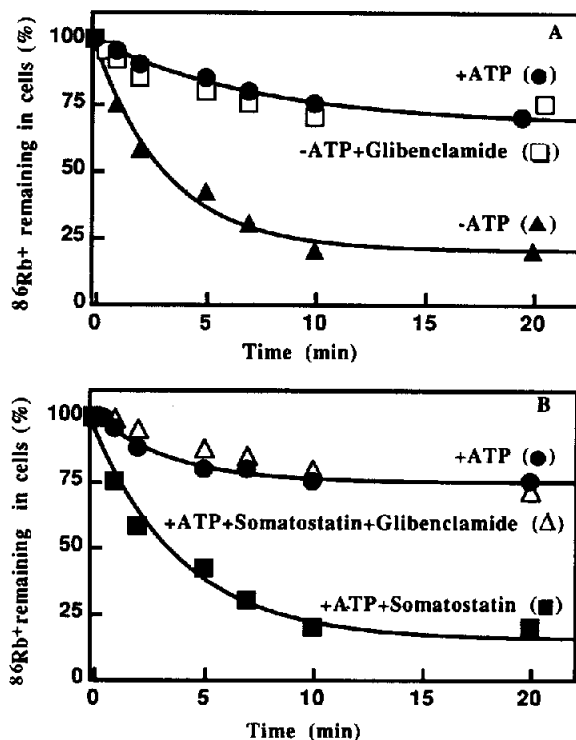


Fig.1. Kinetics of $^{86}\text{Rb}^+$ efflux from RINm5F cells: effect of ATP depletion and somatostatin. (A) Kinetics of $^{86}\text{Rb}^+$ efflux without depletion of $[\text{ATP}]_{\text{in}}$ (●) or after depletion of $[\text{ATP}]_{\text{in}}$ for 15 min in the absence (▲) or presence (□) of 100 nM glibenclamide. (B) Kinetics of activation of $^{86}\text{Rb}^+$ efflux without depletion of $[\text{ATP}]_{\text{in}}$ by 100 nM somatostatin in the absence (■) or presence (Δ) of 100 nM glibenclamide. (Control) Kinetics of $^{86}\text{Rb}^+$ efflux without depletion of $[\text{ATP}]_{\text{in}}$ (●).

eliminated by the potent sulfonylurea glibenclamide [7].

Interestingly somatostatin also stimulates an increase of $^{86}\text{Rb}^+$ efflux but then it is not necessary to decrease the intracellular ATP concentration to observe the hormonal activation of ATP-regulated K^+ channels. The activation presented in fig.1B was observed with an intracellular ATP concentration of 4.0 ± 0.5 mM.

Somatostatin activation of $^{86}\text{Rb}^+$ efflux was eliminated by the blocker of ATP-regulated K^+ channels, glibenclamide.

The concentration dependence of the somatostatin effect on the activity of the glibenclamide-sensitive K^+ channel is presented in fig.2. Half-maximal activation by the hormone was observed at $K_{0.5} = 0.7 \pm 0.2$ nM which is similar to the value ($K_{0.5} = 1.6$ nM) for half-maximal activation of K_{ATP} channels by galanin [9].

A very interesting aspect of the effect of somatostatin on glibenclamide- (and ATP-) sensitive K^+ channels is that the hormonal effect is abolished by pretreatment with pertussis toxin (fig.3). This observation clearly indicates that the somatostatin control of ATP-regulated K^+ channels in insulinoma cells involves a G-protein. It remains to be seen whether the pertussis toxin-sensitive G-protein is involved in a direct coupling between the somatostatin receptor and the ATP-regulated K^+ channel or whether the key

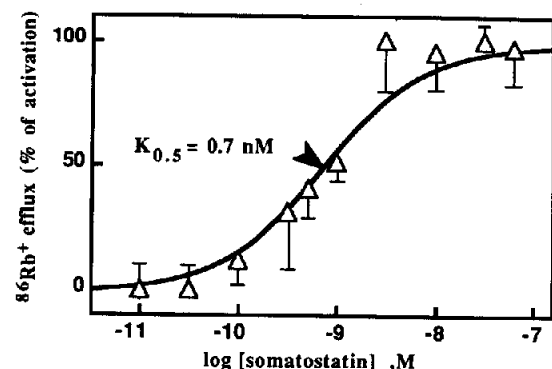


Fig.2. Dose-response curve for the activation of the glibenclamide-sensitive $^{86}\text{Rb}^+$ efflux by somatostatin without $[\text{ATP}]_{\text{in}}$ depletion. $^{86}\text{Rb}^+$ efflux was measured at 1 min. Somatostatin was pre-incubated for 3 min before the start of the flux experiments. Bars indicate means $\pm$ SE ($n = 5$ experiments in duplicate).

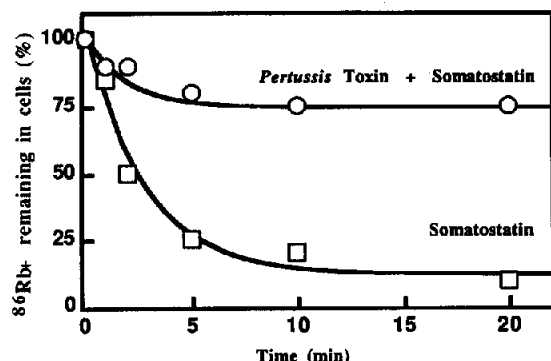


Fig.3. Kinetics of activation of $^{86}\text{Rb}^+$ efflux without depletion of $[\text{ATP}]_{\text{in}}$ by 10 nM of somatostatin in the absence ($\square$) or after treatment ($\circ$) with 100 ng/ml of pertussis toxin.

G-protein is associated with the regulation of levels of an intracellular messenger which, in turn, can regulate the activity of the channel. Involvement of a G-protein in the galanin activation of ATP-regulated K^+ channel [9] has also been demonstrated independently (Petersen, O.H., personal communication).

Somatostatin after galanin [9] is the second hormone described which decreases insulin release and which apparently does it, at least partly, by activating a K_{ATP} channel. This observation definitely shows that the K_{ATP} channel plays a key role in insulin secretion. It is activated by hyperglycemia-inducing hormones and inhibited by sulfonylureas [5–8] that are so largely utilized in the treatment of type II diabetes.

It has been shown previously that somatostatin induces hyperpolarization in a corticotropin-secreting cell line and that this event is correlated with an activation of an inward rectifying K^+ conductance [12–15]. It will be important to see whether this K^+ channel is also ATP-regulated and sensitive to sulfonylureas.

The other information of importance in this paper is that there are clearly two different ways to activate the sulfonylurea-sensitive K^+ channel. The first one is the now classical regulation associated to a decrease of ATP concentration. The second one occurs at high intracellular ATP concentration and is associated to an activation of the channel with hormones. Therefore the physiological con-

trol of the activity of this channel is probably not limited to a control via variations of intracellular ATP concentrations (which probably relates insulin secretion to glucose perfusion). Opening of the sulfonylurea-sensitive channel under the influence of peptide hormones without changing intracellular ATP might be the really important physiological event. It would be interesting to analyze the properties of somatostatin activation of the sulfonylurea-sensitive K^+ channel in β -cells from diabetic pancreas.

Acknowledgements: We are grateful to Hoechst for a gift of glibenclamide and to Y. Lemarchand-Brustel (Nice) for the gift of RINm5F cells. Thanks are due to M.-T. Bohn, C. Roulinat-Bettelheim and F. Aguila for expert assistance. This work was supported by the Centre National de la Recherche Scientifique, the Fondation pour la Recherche Médicale, the Institut National de la Santé et de la Recherche Médicale (grant CRE 88.2007).

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