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## Pharmacology and regulation of ATP-sensitive K<sup>+</sup> channels

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### Introduction

A new class of K<sup>+</sup> channels that link membrane potential to the bioenergetic situation of the cell has been recently discovered (Noma 1983). These K<sup>+</sup> channels (K<sub>ATP</sub>) are normally closed at physiological intracellular ATP concentrations and open upon a diminution of [ATP]<sub>in</sub>. These channels have been shown to be present in pancreatic β-cells (Ashcroft et al. 1984; Cook and Hales 1984; Rorsman and Trube 1985a; Rorsman and Trube 1985b), cardiac ventricular cells (Noma 1983; Trube and Hescheler 1984) and skeletal muscle (Spruce et al. 1985; Spruce et al. 1986; Spruce et al. 1987). They might also be present in the central nervous system (Ashford et al. 1988; Bernardi et al. 1988) and in smooth muscle (Quast 1988; Quast and Cook 1988).

### The ATP-sensitive K<sup>+</sup> channel is the receptor for sulfonylureas

Sulfonylureas are hypoglycemic agents that are widely used in the treatment of diabetes mellitus (Loubatières 1977). It has been shown that sulfonylureas act principally by decreasing K<sup>+</sup> permeability in the β-cell membrane (Ferrer et al. 1984; Gylfe et al. 1984; Henquin, 1977; Henquin 1980; Matthews and Shotton 1982; Meissner et al. 1979; Rorsman and Trube 1985a), thus leading to β-cell membrane depolarization, increased Ca<sup>2+</sup> influx and insulin secretion (Gylfe et al. 1984; Henquin and Meissner, 1984; Lambert et al. 1986; Boyd, 1988). Both <sup>86</sup>Rb<sup>+</sup> flux techniques and electrophysiological methods have recently demonstrated that sulfonylureas block K<sub>ATP</sub> channels in pancreas and cardiac ventricular cells with high specificity (Belles et al. 1987; Dunne et al. 1987; Fosset et al. 1988a; Schmid-Antomarchi et al. 1987a; Schmid-Antomarchi et al. 1987b; Sturgess et al. 1985; Trube et al. 1986) (Fig. 1A and B). However, all sulfonylureas currently used in the treatment of type 2 diabetes are not equally active. Concentrations for half-maximum inhibition of <sup>86</sup>Rb<sup>+</sup> efflux from β-cells are found over 6 orders of magnitude depending on the structure of the sulfonylurea used (K<sub>0.5</sub> = 0.06 nM for glibenclamide and 40 mM for carbutamide) (Schmid-Antomarchi et al. 1987a). The rank order of potency to block the K<sub>ATP</sub> channel from ATP-depleted insulinoma cells, determined for a series of sulfonylureas, is glibenclamide > glipizide > gliquidone > glisoxepide > glibornuride > gliclazide > chlorpropamide and tolbutamide > carbutamide (Schmid-Antomarchi et al. 1987a) (Fig. 1C). Glibenclamide, because it is a very

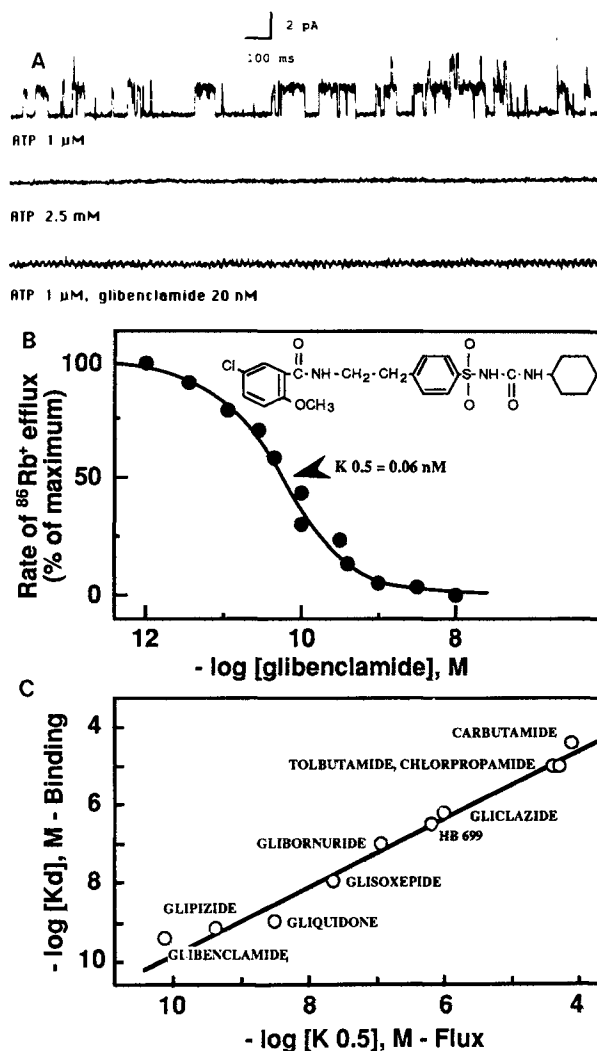


Fig. 1. Insulinoma RINm5F cells. A Single channel currents recorded from inside-out patches. An upward deflection in the traces corresponds to an inward current. The membrane potential was -60 mV. ATP-regulated K<sup>+</sup> channels are opened at low concentrations of ATP and could be blocked at high concentration (2.5 mM) of [ATP]<sub>in</sub> or by glibenclamide. B Inhibition by glibenclamide of ATP-sensitive <sup>86</sup>Rb<sup>+</sup> efflux from RIN cells. Time of ATP depletion was 20 min ([ATP]<sub>in</sub> = 0.4 mM). Time of <sup>86</sup>Rb<sup>+</sup> efflux was 1 min. C Correlation between inhibition by various hypoglycemic drugs of [<sup>3</sup>H]glibenclamide binding and <sup>86</sup>Rb<sup>+</sup> efflux in RINm5F insulinoma cells. K<sub>d</sub> (half-inhibition of [<sup>3</sup>H]glibenclamide binding by hypoglycemic drugs is plotted versus K<sub>0.5</sub> (half-inhibition of ATP-sensitive rate of <sup>86</sup>Rb<sup>+</sup> efflux) (Schmid-Antomarchi et al. 1987a).

potent blocker of  $K_{ATP}$  channels (Schmid-Antomarchi et al. 1987a, b), more potent than tetrodotoxin or saxitoxin for the voltage-sensitive  $Na^+$  channel, has been used successfully to identify biochemically the sulfonylurea receptor. Affinities ( $K_d$ ) for the different sulfonylureas measured with the [ $^3H$ ]glibenclamide binding assay were found to be nearly perfectly correlated to efficacy ( $K_{0.5}$ ) for  $K_{ATP}$  channel blockade (Schmid-Antomarchi et al. 1987a) (Fig. 1). Sulfonylurea receptors with very similar properties have also been identified in chicken and guinea-pig cardiac ventricular microsomes (Fosset et al. 1988a). These results suggest that the sulfonylurea receptor is closely linked, if not identical, to the  $K_{ATP}$  channel. The notion that the  $K_{ATP}$  channel is the receptor for sulfonylureas is consistent with findings showing that the  $K_{ATP}$  channel in excised membrane patches is readily blocked by the very potent sulfonylurea glibenclamide or by the less potent tolbutamide, i.e. without the need of soluble second messengers (Dunne et al. 1987; Fosset et al. 1988a; Schmid-Antomarchi et al. 1987a; Schmid-Antomarchi et al. 1987b; Sturgess et al. 1985; Trube et al. 1986).

Activation of the  $K_{ATP}$  channel by uncoupling of oxidative phosphorylation or by directly lowering  $[ATP]_i$  reduces the duration of the action potential and is believed to induce arrhythmia in the heart (Trube and Hescheler 1984; Fosset et al. 1988a). These deleterious effects are reversed by treatment with sulfonylureas (Fosset et al. 1988a). Under physiological conditions however, sulfonylurea treatment does not appear to have any effect on the cardiac action potential, indicating that the channel is then permanently closed by the high intracellular ATP concentration and casting doubt on a role of the  $K_{ATP}$  channel in this tissue as a sensor of intracellular ATP variations as in pancreatic  $\beta$ -cells.

Sulfonylurea receptors are also present in brain cells. Rat and pig microsomes specifically bind sulfonylureas with half-maximum values that correlate closely with the values found for  $\beta$ -cell microsomes (Bernardi et al. 1988; Geisen et al. 1985; Lupo and Bataille 1987; Mourre et al. 1989) (Fig. 2A), suggesting the presence of  $K_{ATP}$  channels in the central nervous system. The five main structures containing the highest concentrations of receptors were the substantia nigra, the globus and central pallidus, the motor neocortex and the molecular layer of the cerebellar cortex. In the hippocampus, the highest level of binding sites was found in stratum lucidum of CA<sub>3</sub> where the mossy fibers make synaptic contacts with the proximal part of the apical dendrite of CA<sub>3</sub> neurons. There were high densities of receptors in CA<sub>3</sub> and intermediate densities in CA<sub>1</sub> and CA<sub>2</sub> (Fig. 2B). The high affinity of glibenclamide for its receptor in pig brain (0.8 nM) has been used to both affinity label and purify

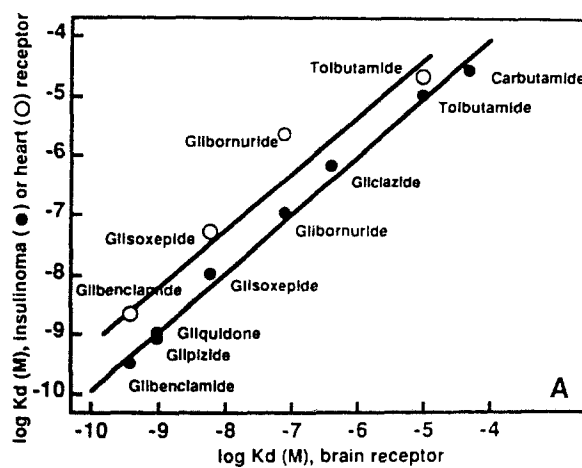
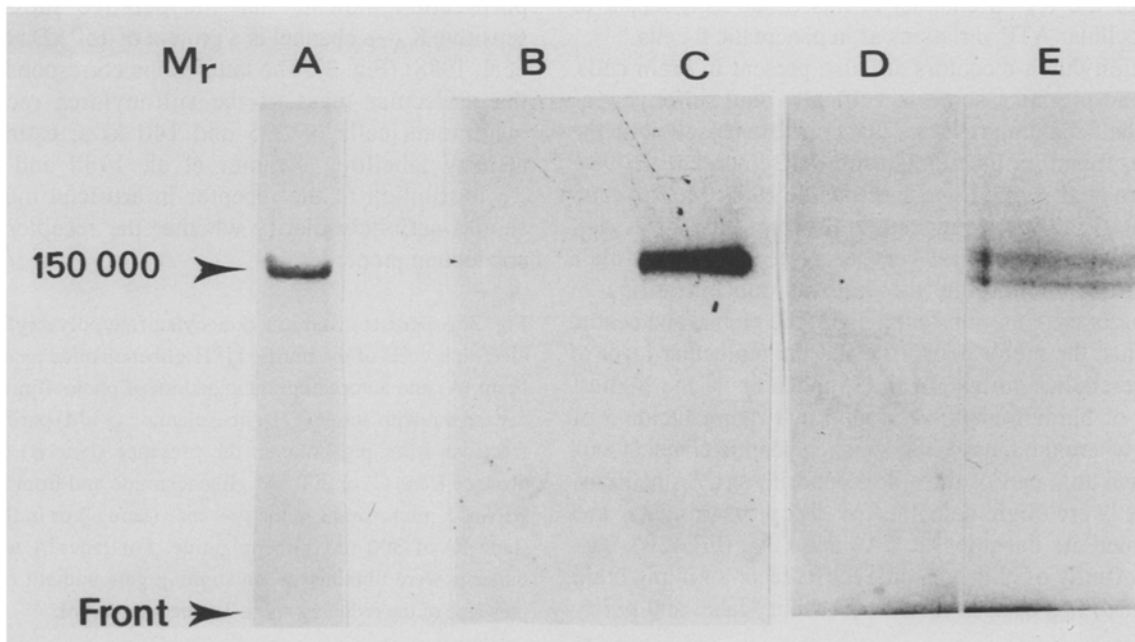
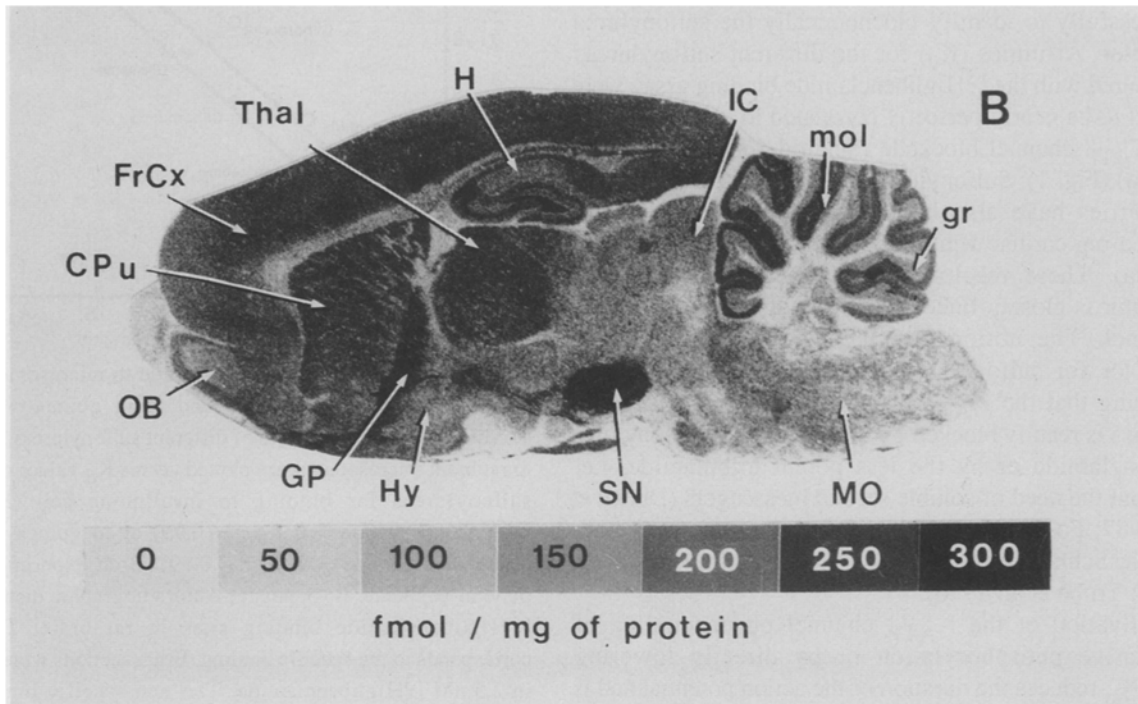


Fig. 2. A Binding of [ $^3H$ ]glibenclamide to microsomes from rat brain, from insulinoma cells and from guinea-pig hearts. Correlation curve.  $K_d$  values of different sulfonylureas for binding to rat brain microsomes were plotted versus  $K_d$  values of different sulfonylureas for binding to insulinoma cell (RINm5F) microsomes (slope = 0.98;  $r = 0.99$ ) or to guinea-pig heart microsomes (slope = 0.98;  $r = 0.99$ ). Both experiments were done in duplicate. B (opposite) Autoradiographic distribution of [ $^3H$ ]glibenclamide binding sites in rat brain. The scale corresponds to the specific binding. Brain sections were incubated in 2.5 nM [ $^3H$ ]glibenclamide. The non-specific binding was identical to the background of the tritium-sensitive film (not shown). CPu, caudate putamen; FrCx, frontal cortex; GP, globus pallidus; Gr, granular layer of the cerebellar cortex; H, hippocampus; Hy, hypothalamus; IC, inferior colliculus; Mo, medulla oblongata; mol, molecular layer of the cerebellar cortex; OB, olfactory bulb; SN, substantia nigra; Thal, thalamus.

the sulfonylurea receptor. Both affinity labelling and purification indicate that the putative sulfonylurea-sensitive  $K_{ATP}$  channel is a protein of 150 kDa (Bernardi et al. 1988) (Fig. 3). The latter value corresponds well to the molecular mass of the sulfonylurea receptor in insulinoma cells of 125 and 140 kDa, estimated by affinity labelling (Kramer et al. 1988 and Fig. 3). Reconstitution of the receptor in artificial membranes should definitely clarify whether the receptor has  $K^+$  conducting properties.

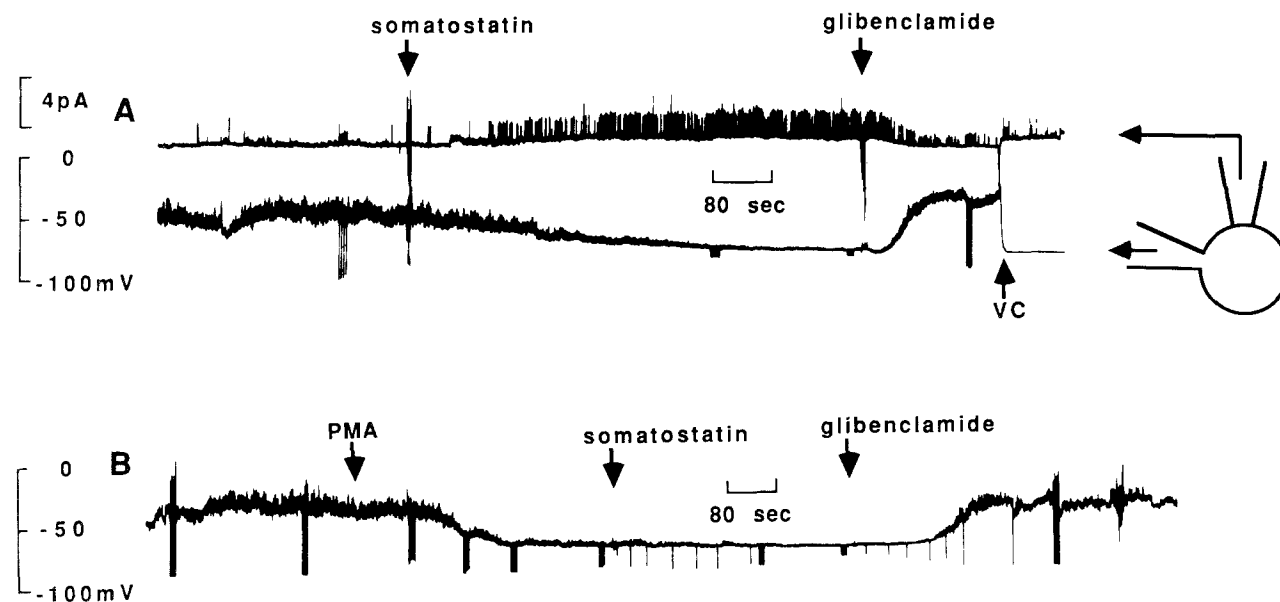
Fig. 3. (opposite) Sodium dodecylsulfate/polyacrylamide gel electrophoresis of the purified [ $^3H$ ]glibenclamide receptor in pig brain (A) and autoradiographic pattern of photoaffinity labelling carried out with active [ $^3H$ ]glibenclamide (3 nM) purified binding fractions from pig brain in the presence (lane B) and in the absence (lane C) of 300 nM glibenclamide and from insulinoma RINm5F microsomes in the presence (lane D) or in the absence (lane E) of 300 nM glibenclamide. For lanes A to E, same patterns were obtained when running gels without or with the presence of the reducing agent 2-mercaptoethanol.



### Phosphorylation of the ATP-sensitive K<sup>+</sup> channel by kinase C

Stimulation of insulin secretion by carbohydrates such as glucose and glyceraldehyde is accompanied by depolarization of the  $\beta$ -cell membrane and by an increase of diacylglycerol synthesis (Wollheim et al. 1988). Exogenous kinase C stimulators such as 4- $\beta$ -12-phorbol-myristate-13-acetate (PMA) and 1,2-didecanoylglycerol (DC<sub>10</sub>) also depolarize  $\beta$ -cells, raise free cytosolic Ca<sup>2+</sup> and stimulate insulin secretion. The membrane depolarization induced by PMA and DC<sub>10</sub> is transient and lasts 2 to 3 minutes. In cell-attached membranes patches, it was shown that membrane depolarization was paralleled by a transient reduction of the probability of K<sub>ATP</sub> channel opening. As during a period of 5 min after kinase C activation by PMA or DC<sub>10</sub>, intracellular ATP levels did not diminish significantly, it was concluded that membrane depolarization is not the result of K<sub>ATP</sub> channel block by raised intracellular ATP, but is mediated by a kinase C catalyzed phosphorylation leading to K<sub>ATP</sub> channel blockade. These observations obviously raise questions about the regulatory role of intracellular ATP in the control of  $\beta$ -cell membrane potential and insulin secretion (Wollheim et al. 1988).

When the  $\beta$ -cell is treated with PMA for a period longer than 5 min, the initial channel inhibition is followed by a marked increase of K<sub>ATP</sub> channel activity in cell-attached patches (Ribalet et al. 1988; de Weille et al. 1989). Also in insulinoma cells that were intracellularly perfused with a solution containing 2 mM ATP, it was shown that both PMA and 1-oleyl-2-acetylglycerol (OAG), induce membrane hyperpolarization (Fig. 4B) and reduce membrane resistance. These effects are due to K<sub>ATP</sub> channel activation and are antagonized by subsequent treatment with glibenclamide (de Weille et al. 1989). Interestingly, K<sub>ATP</sub> channel activity induced by "long-term" treatment with PMA or OAG (>5 min, in our experiments) is observed even in the presence of an otherwise blocking concentration of 2 mM ATP. Once activated by kinase C, the channel loses the property of being regulated by [ATP]<sub>in</sub>. At first sight, K<sub>ATP</sub> channel activation as a result of long-term kinase C stimulation seems difficult to reconcile with a regulatory role of glycolysis. However, it is shown that cytosolic-free Ca<sup>2+</sup> may activate phospholipase C resulting in diacylglycerol synthesis and kinase C activation (Eberhard and Holz 1988). If it is assumed that an increase of diacylglycerol synthesis following glucose stimulation in  $\beta$ -cells is the



**Fig. 4.** Somatostatin hyperpolarizes RINm5F cells by activating K<sub>ATP</sub> channels. Membrane potentials of RINm5F were recorded using the whole-cell tight-seal technique. The recording pipette contained 150 mM KCl, 2 mM ATP, 2 mM EGTA, 0.5 mM MgCl<sub>2</sub> and 100 mM GTP. Membrane resistance was estimated by a series of 5 small hyperpolarizing current pulses. A Single channel currents were recorded by a second pipette in the cell-attached configuration clamped at 0 mV (see inset). The somatostatin-evoked hyperpolarization was seen to be paralleled by the activation of K<sup>+</sup> channels that could be blocked by 100 nM of glibenclamide. After cell membrane depolarization induced by glibenclamide, the dialyzing pipette was voltage-clamped to -70 mV (vc) showing that channel block by glibenclamide was not voltage-dependent. **B** Protein phosphorylation. Stimulation of kinase C activity by 1 mM PMA hyperpolarizes the  $\beta$ -cell membrane. Additional application of 100 nM somatostatin does not further reduce membrane resistance. 2 mM intracellular ATP.

result rather than the cause of  $K_{ATP}$  channel closure, then phosphorylation-induced  $K_{ATP}$  channel reopening may be thought to limit glucose-induced  $Ca^{2+}$  influx. According to this view, intracellular ATP and free  $Ca^{2+}$  have opposite effects on  $K_{ATP}$  channel activation and insulin secretion. Alternatively, the phosphorylation pathway may form part of a regulatory system that is controlled by extracellular factors that have not yet been identified.

The role of kinase C in the regulation of the  $K_{ATP}$  channel and cell function is apparently rather complex. However, similar types of effects of PMA involving both activation or inhibition of channel activity have now also been observed for L-type  $Ca^{2+}$  channels in cardiac and smooth muscle cells (Lacerda et al. 1988; Van Renterghem et al. 1988; Fish et al. 1988).

#### Regulation of the ATP-sensitive $K^+$ channel in $\beta$ -cells by hormonal peptides

Galanin is a 29 amino acid peptide that was isolated initially from porcine intestine (Tatemoto et al. 1983). The peptide is also present in the pancreas (Amiranoff et al. 1987; Dunning et al. 1986) and in the central nervous system (Ahrén et al. 1988; Rökaeus 1987). In the pancreas, galanin inhibits insulin secretion *via* membrane hyperpolarization and lowering of cytosolic free  $Ca^{2+}$  (Ahrén et al. 1986; McDonald et al. 1985).

It has been demonstrated that the galanin-induced membrane hyperpolarization is the result of  $K_{ATP}$  channel opening, which could be blocked by glibenclamide (de Weille et al. 1988). As  $K_{ATP}$  channel activation by galanin could be obtained both with internally perfused  $\beta$ -cells and excised patches, in the absence of cAMP, it seems improbable that the adenylate cyclase system is involved in the activation of the  $K_{ATP}$  channel by galanin (de Weille et al. 1988). It was shown that pretreatment of insulinoma cells by pertussis toxin also prevented activation of the  $K_{ATP}$  channel by galanin (Dunne et al. 1989). Hence, galanin probably inhibits insulin secretion from pancreatic  $\beta$ -cells by opening the  $K_{ATP}$  channel *via* an activation of a pertussis toxin sensitive G protein, leading to hyperpolarization and inhibition of  $Ca^{2+}$  influx. Intracellular cAMP levels and hence adenylate cyclase activity are reduced by galanin *via* a pertussis toxin sensitive G protein (Amiranoff et al. 1988). This effect may also contribute to the inhibition of insulin secretion by a yet unknown mechanism not directly involving  $K_{ATP}$  channel regulation.

Another important hormone with hyperglycemic properties is somatostatin, a 14 amino acid that inhibits secretion from pituitary (Lewis et al. 1986; Pennefather et al. 1988; Yamashita et al. 1988) and pancreatic cells (Pace and Tarvin 1981) and serves as a neurotransmitter in the central nervous system (Epelbaum 1986). The somatostatin-evoked inhibition of secretion from

pituitary and possibly pancreatic cells may pass via multiple pathways including (i) inhibition of voltage-dependent  $Ca^{2+}$  channels (Lewis et al. 1986; Luini et al. 1986), (ii) inhibition of adenylate cyclase activity (Lebrun et al. 1982) and (iii) activation of an inwardly rectifying  $K^+$  conductance (Lewis et al. 1986; Luini et al. 1986).

In whole cell recordings from insulinoma cells intracellularly perfused with a solution containing 2 mM ATP and 100 mM GTP (Fig. 4A), it was shown that somatostatin induced membrane hyperpolarization by an activation of  $K_{ATP}$  channel (de Weille et al. 1989). Somatostatin failed to hyperpolarize the  $\beta$ -cell when GTP was absent in the perfusate. Furthermore, somatostatin-stimulated  $^{86}Rb^+$  efflux from  $\beta$ -cells was prevented by pertussis toxin pretreatment, suggesting that a G protein mediates the somatostatin response (Fosset et al. 1988b). As electrophysiological experiments were done in the absence of cAMP, it is clear that the adenylate cyclase system is not involved in the coupling between somatostatin receptor occupancy and  $K_{ATP}$  channel activation (de Weille et al. 1989). As is the case for galanin, inhibition of insulin secretion by somatostatin is due to an activation of the  $K_{ATP}$  channel *via* a pertussis toxin sensitive G protein. Somatostatin reduction of intracellular cAMP may also play a role in the inhibition of secretion. It is not yet known whether the voltage-dependent L-type  $Ca^{2+}$  channel in  $\beta$ -cells is affected by somatostatin.

In summary, it has been shown that the  $K_{ATP}$  channel plays a central role in the functioning of the pancreatic  $\beta$ -cell, as both intracellular and extracellular factors lead to the modulation of its activity. It is not known whether the  $K_{ATP}$  channel plays as important a role in other tissues in which it has been identified such as the brain, the cardiac muscle or the striated muscle, since modulation of the channel in those tissues by (neuro) peptides has not yet been demonstrated.

#### Physiological role of sulfonylurea-sensitive $K^+$ channels

The physiological role of  $K_{ATP}$  channels in the pancreatic  $\beta$ -cell seems clear. In the absence of extracellular glucose, the pancreatic  $\beta$ -cell is electrically silent. At physiological glucose concentrations,  $K_{ATP}$  channels close and membrane resistance increases (Atwater et al. 1978), giving rise to generation of action potentials and  $Ca^{2+}$  ion influx. The ensuing secretion of insulin by  $\beta$ -cells ultimately leads to a reduction of blood glucose levels.

In brain and muscle, a negative feedback system requiring a sensor of intracellular ATP is difficult to envisage, as the channel only seems to be expressed in those tissues under extreme conditions such as ischemia

or uncoupling of oxydative phosphorylation. It has been suggested (Spruce et al. 1987) that the  $K_{ATP}$  channel in muscle might serve to reduce  $Ca^{2+}$  influx and contraction during periods of exhaustion, thus limiting further ATP utilization. However during ischemia of the heart, shortening of the action potential and of the refractory period as a result of  $K_{ATP}$  channel activation, although it prevents excessive  $Ca^{2+}$  entry which may lead to cell death, also leads to arrhythmia and fibrillation, which can hardly be considered as a physiologically useful response. Moreover, pretreatment with sulfonylureas apparently protects the heart against these deleterious effects, indicating that the supposed beneficial role of the  $K_{ATP}$  channel in counteracting effects of a sharp decrease of intracellular ATP may even turn out to be counterproductive.

Induction of ischemia in the brain rapidly creates an hyperpolarizing phase which is probably associated with conscience loss (Hansen 1985; Fujiwara et al. 1987). This hyperpolarization is eliminated by glibenclamide (Mourre et al. 1989) and is probably due to the activation of a  $K_{ATP}$  channel following a decrease of  $[ATP]_{in}$  due to brain ischemia. In the brain, because the interstitial space is much smaller than the intracellular volume, opening of  $K_{ATP}$  channels will lead to sustained  $K^+$  efflux which would create extracellular  $K^+$  accumulation, depolarization of synaptic terminals, and release of citotoxic amino acids such as glutamate which will lead to death of hippocampal neurones. These effects again, which would be directly linked to opening of  $K_{ATP}$  channels following decrease of ATP, are not particularly favorable.

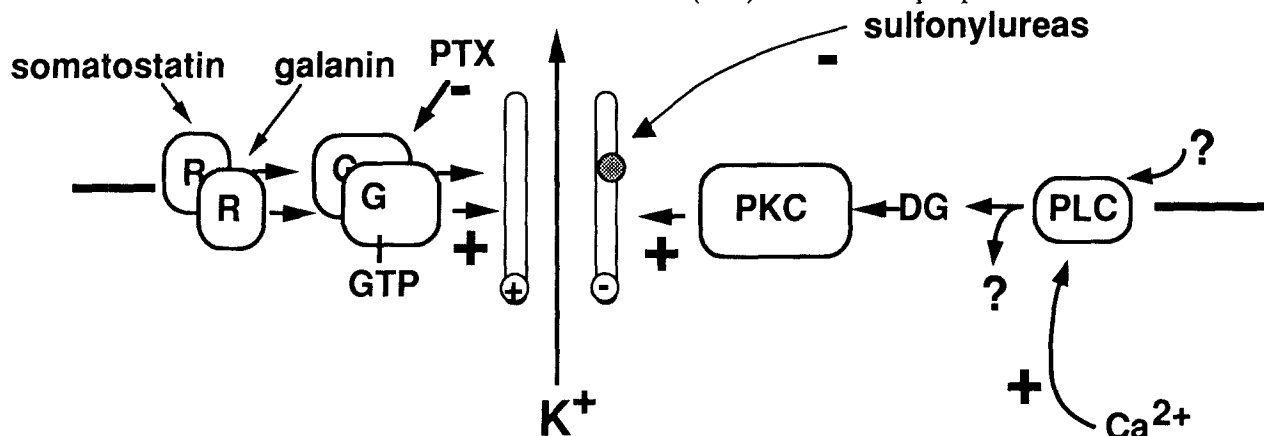
All the considerations mentioned above suggest that the  $K_{ATP}$  channel may generally serve other goals than making a link between membrane polarization and intracellular ATP levels.

In view of the findings that  $K_{ATP}$  channel activity in  $\beta$ -cells is modulated by hormones such as galanin and somatostatin (Fig. 5), it now seems possible that  $K_{ATP}$  channels are the targets of extracellular messengers in heart and brain tissue as well. Future research will tell us whether  $K_{ATP}$  channels actually constitute a class of sulfonylureas-sensitive and peptide-activatable  $K^+$  channels that also turn out to have ATP-sensitivity.

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**Fig. 5.** Different types of regulation of the  $K_{ATP}$  channel. The  $K_{ATP}$  channel is inhibited (-) by sulfonylureas. Somatostatin and galanin activate the  $K_{ATP}$  channel (in  $\beta$ -cells) via pertussis toxin (PTX) sensitive G-proteins (G). Stimulation and diacylglycerol (DG) synthesis activated the  $K_{ATP}$  channel. Phospholipase C (PLC) may be stimulated by either intracellular  $Ca^{2+}$  or extracellular factors.

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