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Review

α-Endosulfine, a new entity in the control of insulin secretion

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Abstract. ATP-dependent potassium (K_{ATP}) channels occupy a key position in the control of insulin release from the pancreatic β cell since they couple cell polarity to metabolism. These channels close when more ATP is produced via glucose metabolism. They are also controlled by sulfonylureas, a class of drugs used in type 2 diabetic patients for triggering insulin secretion from β cells that have lost part of their sensitivity to glucose. We have demonstrated the existence of endogenous counterparts to sulfonylureas which we have called 'endosulfines.' In this review, we describe the discovery, isolation, cloning, and biological features of the high-molecular-mass form, α-endosulfine, and discuss its possible role in the physiology of the β cell as well as in pathology.

Key words. α-Endosulfine; sulfonylurea; ATP-dependent potassium channel (K_{ATP} channel); pancreatic β cell; insulin secretion; diabetes; human ENSA gene.

Introduction

Regulation of glycemia is an essential task of the endocrine system, since both hyperglycemia and hypoglycemia (a too high or a too low blood sugar level, respectively) are harmful to the organism [1]. Insulin, the product of the β cells in the pancreatic islets of Langerhans, has a major role in maintaining euglycemia (the level of blood sugar appropriate to a certain physiological situation), being the single hypoglycemic hormone [2]. A defect in insulin storage and release from β cells leads to diabetes mellitus. This pathology is either insulin-dependent (insulin-dependent diabetes mellitus, IDDM or type 1 diabetes) when this defect is total, or non-insulin dependent (non-insulin-dependent diabetes mellitus, NIDDM or type 2 diabetes) when this defect is partial [3]. In NIDDM, other phenomena, such as a lack of responsiveness to insulin of insulin-sensitive tissues (known as 'insulino-resistance') also usually occur.

Insulin secretion by β cells is under the control of many physiological parameters, the main one being the plasma glucose concentration [1, 2]. Others are stimulatory or inhibitory hormones or local regulators, such as neurotransmitters which, together, enable insulin secretion to be adapted to a given physiological situation. When plasma glucose increases (for instance after a meal), sensory mechanisms present mostly in the β cell detect the necessity for an insulin response that, in turn, forces plasma glucose to enter peripheral insulin-sensitive tissues (such as skeletal muscles), leading to a decrease in plasma glucose. In contrast, a decline in plasma glucose halts the insulin-secretory mechanisms and, at the same time, hyperglycemic mechanisms are triggered. Generally speaking, euglycemia, the normal situation that allows the whole organism to work properly with the correct fuel flow to the various cells and organs, is obtained mainly by the glucose-responsive

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insulin secretory system, while other mechanisms allow a more accurate tuning. The brain, the functions of which depend strongly on the amount of glucose available, is particularly sensitive to glucose variations and severe hypoglycemia may lead to coma and death.

Insulin secretion, glucose, sulfonylureas and K_{ATP} channels

Because of their major biological importance, the mechanisms that regulate glycemia have been the subject of a huge number of studies. Since the discovery of insulin, the molecular events through which the insulino-secretagogues (the molecules which trigger insulin secretion) control, directly or indirectly, the insulin-secreting β cell have been largely elucidated. It is now well established that most of the insulino-secretagogues have in common the ability to inhibit (to close) ionic channels which control potassium efflux from the β cell. This channel was recognized as being sensitive to the intracellular concentration of adenosine triphosphate (ATP) [4–8]. Since then, two proteins, derived from two separate adjacent genes on chromosome 11 in humans, were shown to form the β cell functional ATP-dependent potassium (K_{ATP}) channel.

These proteins are (fig. 1):

(1) Kir 6.2 [9], a member of the inwardly rectifying potassium channels, which forms the ionic pore of the complex and which displays ATP sensitivity on its own [10].

(2) SUR1 [11], a 17-transmembrane domain protein belonging to the ABC (ATP-binding cassette) family of proteins, which endows sensitivity to Mg-ADP and to K_{ATP} channel openers and which allows a higher sensitivity of the channel towards ATP [12]. SUR is an 'acronym for sulfonylurea receptor' since it was discovered as the protein carrying the binding site for sulfonylureas, a class of drugs widely used for managing type 2 diabetes thanks to their insulin-secreting activity.

According to the data available, the key importance of the K_{ATP} channels in the physiology of the β cell [7, 8] may be summarized as follows (fig. 2). The Na^+/K^+-ATPase, a major ATP-driven ionic pump present in all living cells, continuously pumps up potassium from the extracellular medium into the β cell (at the same time pumping down sodium ions). Most of this K^+ flux leaves the β cell through the K_{ATP} channels and the homeostasis of intracellular potassium is set by their degree of opening. Every subtle change in that parameter (the number of individual K_{ATP} channels that are

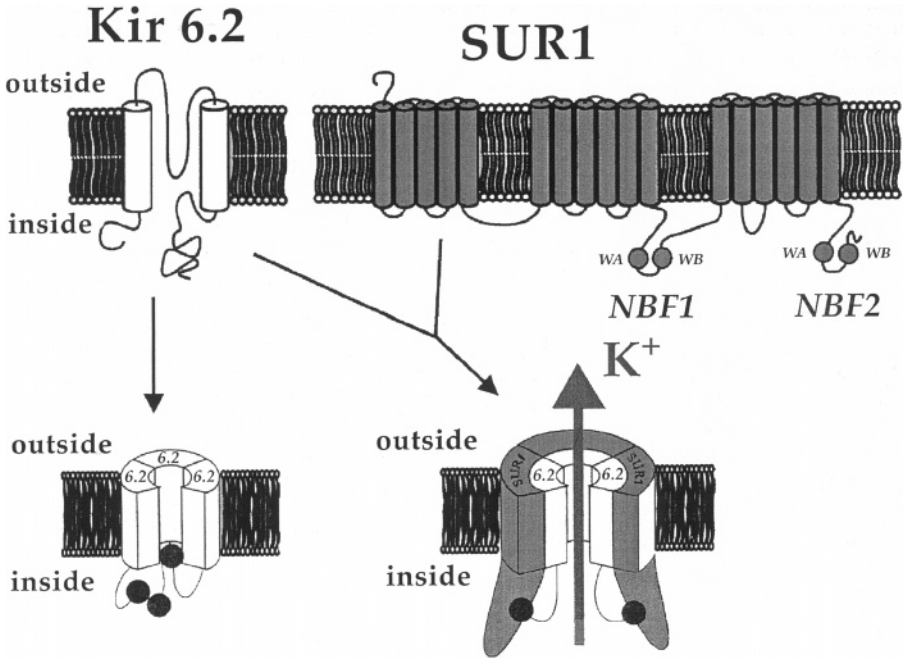

Figure 1. How the K_{ATP} channel works [7, 8]. The channel is made of two proteins, Kir 6.2 which forms the ionic pore, and SUR1 which endows the sensitivity to sulfonylureas and to diazoxide. It also contains two nucelotide-binding folds (NBF1 and NBF2), each formed by two 'Walker' sequences (WA and WB), which provide the channel with sensitivity to Mg-ADP and increase the initial sensitivity to ATP borne by Kir 6.2 [12]. No current is observed when Kir 6.2 is expressed alone, probably because the C-terminal tail blocks the pore (lower left). Kir 6.2 and SUR1 coassemble in a 4:4 stoichiometry, forming the active channel (lower right).

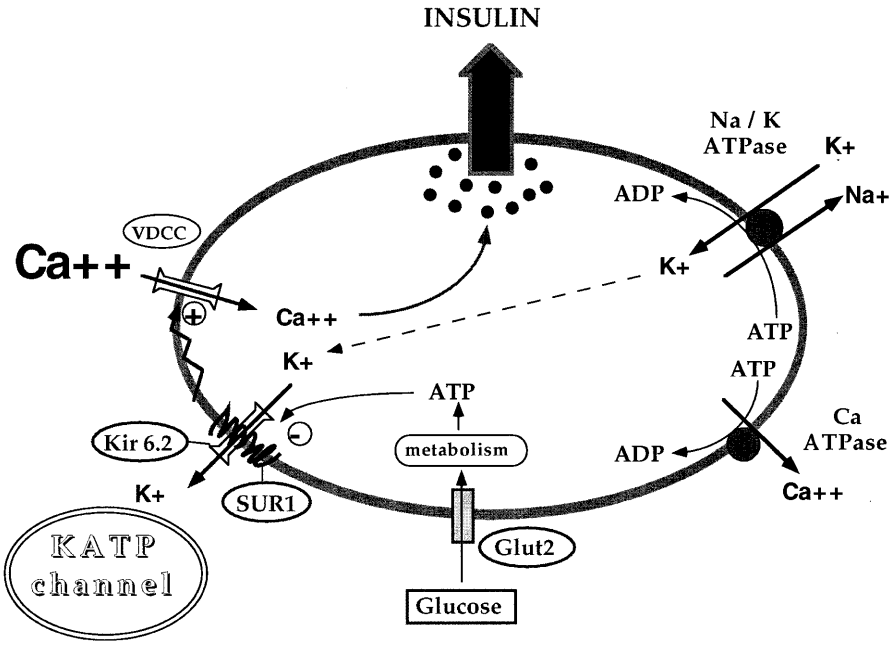

Figure 2. Central importance of the K_{ATP} channels in glucose-induced insulin release [7, 8]. Potassium ions (K^+) are pumped up continuously by the Na^+/K^+-ATPase and the intracellular potassium concentration is set by the degree of closure of the K_{ATP} channel made up from Kir 6.2 and SUR1 (see fig. 1), regulating the K^+ efflux and, thus, the intracellular K^+ concentration. Free cytosolic calcium is kept at low levels (below micromolar) compared to the extracellular medium (around 1.2 mM) by the calcium-ATPase (Ca^{2+}-ATPase). Under basal conditions (non-stimulatory glucose concentrations), 10–20% of the K_{ATP} channels are open, allowing a balance between potassium input and output, plasma membrane polarity is maintained around -70 mV and the voltage-dependent (membrane-polarity-dependent) calcium channels (VDCCs) are closed. When plasma glucose increases, more glucose enters the β cell via the Glut transporter (of the Glut2 type in the rat) and its metabolism raises the ATP level which will inhibit (close) more K_{ATP} channels inducing a depolarization of the membrane (more positive ions in the cell). When the polarity reaches around -40 mV, VDCCs open, allowing a burst of Ca^{2+} ions to enter the cell because of the huge concentration gradient (10^3–10^4) between the membrane sides.

opened or closed at a certain time) will control the intracellular free potassium concentration. Since these channels are ATP sensitive, a higher concentration of intracellular ATP will induce a higher number of K_{ATP} channels in the closed state, increasing the K^+ concentration. In fact, the parameter of importance is the ATP/ADP ratio. In a β cell at rest (basal secretion), 80–90% of the K_{ATP} channels are closed and the K^+ influx through the Na^+/K^+-ATPase is balanced by the K^+ efflux through the K_{ATP} channel molecules that are open. When plasma glucose increases, more glucose enters the β cell via the glucose transporters and is metabolized, leading to an increased production of ATP from ADP and thus an increase in the ATP/ADP ratio, closing more K_{ATP} channels. The resulting decreased K^+ leak rapidly induces an increase in free K^+ that will change the membrane polarity (around -70 mV at rest) to a less polarized state. When the polarity reaches about -40 mV, voltage-dependent Ca^{2+} channels (of the L type in mouse) start to open. Because of the huge Ca^{2+} gradient through the plasma membrane (around 1 mM outside and near 200 nM free Ca^{2+} inside), a burst of calcium entry occurs which, in turn mobilizes the insulin granules through Ca^{2+}-dependent processes. The system is set back to basal by various mechanisms and the cycle starts again.

The K_{ATP} channel is thus the pivot of this mechanism as it represents a major glucose sensor through the variations in the ATP/ADP ratio. Both the Kir 6.2 and the SUR1 proteins are involved in this sensing system, with ATP and Mg-ADP recognition sites, respectively [12]. The two proteins coassemble in a hetero-octamer with a 4:4 stoichiometry to form the functional channel [13]. It should be noted that other types of K_{ATP} channels exist that differ in their SUR type (for example, SUR2A and SUR2B in heart and vascular smooth muscles, respectively [8]).

One question is whether there are other mechanisms of regulation of this multimeric channel, besides the ATP/ADP ratio. The presence of a high-affinity binding site for sulfonylureas on the SUR1 protein suggests that there is at least another mechanism which controls the

channel activity. Indeed, it was known for many years that the drugs of the sulfonylurea family, used extensively for treating type 2 diabetes, act by releasing insulin from a pancreas which has partially lost its sensitivity to glucose. In some way, sulfonylureas may be seen as replacing glucose as the main stimulus. It was shown that sulfonylureas trigger insulin secretion in various in vitro and in vivo models, with the same pharmacology as that observed for their effects on blood sugar. The isolation of SUR1 (sulfonylurea receptor 1) on the basis of its interaction with glibenclamide (the most potent sulfonylurea) eventually proved that these drugs represent another way to control the K_{ATP} channel.

The endosulfine concept

After the first demonstration of the existence of sulfonylurea receptors in an insulin-secreting tissue [14, 15] and the demonstration of high-affinity binding sites for these drugs both in β cells [16] and in the central nervous system [16, 17], we hypothesized that the sulfonylureas, artificial ligands, may have natural, endogenous counterparts. Our hypothesis, strengthened by the discovery of the endorphines (peptides representing the endogenous ligands for the 'morphine receptors') [18] led us to look for peptides able to recognize the sulfonylurea-binding sites. For practical reasons, we extracted them from brain, a tissue that contains an important amount of sulfonylurea receptors and reasonable amounts of peptide-degrading activities. In 1988, we observed that rat brain extracts indeed contain an activity interfering with sulfonylurea binding [19]. In 1992, we were able to characterize the presence in ovine brains of two different molecules of peptidic nature, for which we coined the names α- and β-endosulfine [20]. Direct and indirect data suggested that β-endosulfine is smaller and has a higher isoelectric point. This molecule was obtained in a highly purified form and was found to stimulate insulin release in a manner similar to sulfonylureas [20]. β-Endosulfine, which we suspect to be the endogenous equivalent of sulfonylureas, has not, so far, been isolated in sufficient amounts for determining its chemical structure. Further studies, currently carried out in our laboratory, are necessary to address this important question. The high molecular form, α-endosulfine, has been isolated and partially characterized.

α-Endosulfine

Isolation of the α form of endosulfine [21] was obtained by a series of chromatographies from porcine brain extracts, obtained in cooperation with the Gastrointestinal Hormone Laboratory of the Karolinska Institute in Stockholm (Director Pr Viktor MUTT), by analyzing the ability of fractions to inhibit the binding of sulfonylureas to their sites. The resulting molecule, obtained as a single band in polyacrylamide gel electrophorersis with an apparent molecular mass of 19 kDa, turned out to be a 13-kDa protein according to mass spectrometry data [21].

From the partial peptidic sequences obtained, it was apparent that α-endosulfine strongly resembles a similarly sized peptide previously isolated from the bovine brain as a substrate for protein kinase-A. This peptide [22, 23] was called ARPP-19 (for cyclic AMP-regulated phosphoprotein of apparent molecular size 19 kDa) and, besides being phosphorylated when cellular cyclic AMP is raised, no biological role has been determined so far for this protein.

Since the differences in structures that were noted [21] might have been related to the fact that the two molecules originate from different animal species, the question arose as to whether α-endosulfine and ARPP-19 were the same entity or different proteins of the same family. Cloning of the α-endosulfine cDNA from the same source as ARPP-19 was necessary to address this issue. Using a RT-PCR approach, we isolated a partial cDNA clone corresponding to the bovine form of α-endosulfine [24]. From the sequences obtained, it was apparent that α-endosulfine and ARPP-19 are different molecular entities, encoded by different genes [24].

With the possible involvement of α-endosulfine in the physiology and pathophysiology of insulin secretion, we decided to analyze its structure in humans and in laboratory animals. The complete coding sequence for human α-endosulfine (EMBL X99906) has now been determined [25]. The nucleotide sequence predicts a polypeptide of 121 residues with a M_r of 13,517 Da. We have, in parallel, cloned the human counterpart of bovine ARPP-19 and found that it contains 112 amino acids, like the bovine molecule, with a M_r of 12,315. When aligned (fig. 3), the two proteins have 69.4% identities in their amino acid sequences, the main differences being found in the N terminus. The human gene encoding α-endosulfine was called (HSA)ENSA gene by the Human Gene Organization (HUGO) Nomeclature Committee.

From the cDNA sequence, we developed a strategy for producing recombinant α-endosulfine for functional studies. It was shown [25] that the protein inhibits the binding of [^{3}H]-glibenclamide to its receptors, as does the natural porcine molecule, and reduces K_{ATP} conductance in *Xenopus* oocytes injected with Kir 6.2/SUR1 when applied intracellularly. Although with slower dynamics, the peptide was also able to close the channel when applied extracellularly. The specificity of the interaction was demonstrated by the lack of effect of the peptide when Kir 6.2 was replaced by the non-β-cell-

pore-forming subunit Kir 1.1. It also stimulated insulin release from the MIN6 β cell line [25]. All the effects of α-endosulfine were obtained at micromolar concentrations.

We have also obtained quite recently [K. Peyrollier et al., unpublished data] the sequences of the translated regions of α-endosulfine from rat, mouse, porcine, and bovine species. α-Endosulfine is a highly conserved molecule: the mouse and human amino acid sequences, for example, are 100% and 98.3% identical to the rat sequence, respectively. ARPP-19, which was cloned from the same series of animal species, also shows a high degree of conservation.

Thanks to the development of antibodies against α-endosulfine and to the use of Northern blotting or quantitative relative PCR, we have been able to analyze the tissue distribution of the peptides [25; Peyrollier et al., unpublished data]. Of particular interest is the fact that α-endosulfine has a tissue distribution which resembles that of the K_{ATP} channel: most of the messenger, which exists as two forms of 1.4–1.5 and 2.5 kb, is found in brain, heart, skeletal muscle, islets of Langerhans, and lung. It is also present in several cell lines, in particular β cells lines such as MIN6. In sharp contrast, ARPP-19 is not detected in β cells, and the main sources are the olfactory bulb, kidney, and pancreas (most probably exocrine). Thus, it appears that, although obviously belonging to the same family of proteins, the two entities probably have different biological roles.

Possible biological role(s) of α-endosulfine

In searching for possible biological roles for the new peptide, several of its features have to be taken into account:

1) The peptide does not contain a signal peptide, suggesting that it is not secreted, at least in the regulatory pathway.

2) Its tissue distribution is similar to that of the K_{ATP} channels.

3) It contains a consensus sequence (K/R-K/R-X-S) for cyclic-AMP-dependent phosphorylation, allowing a level of regulation in the biological behavior of the peptide; we have confirmed that the pure molecule can indeed be phosphorylated in vitro by protein kinase A [25].

4) Its effect on cloned K_{ATP} channels injected into *Xenopus* oocytes is easier and more readily reversible when it is applied to the inner side of the membrane than to the outside.

5) The biological effects are observed at micromolar concentrations, rendering a hormonal status unlikely.

All these features are compatible with the idea that α-endosulfine is an intracellular protein acting as a regulatory component of the K_{ATP} channel activity. However, certain information needed to confirm this hypothesis is missing:

1) Does α-endosulfine close K_{ATP} channels when expressed in cells containing functional K_{ATP} channels? So far, only the results of actue experiments are available.

2) Is it possible, by adjusting the intracellular level of α-endosulfine expression, to set the degree of K_{ATP} channel activity to a level compatible with its modulation by ATP/ADP changes, as exist in a β cell?

3) Conversely, what happens to the channel activity when the level of α-endosulfine expression is decreased in a β cell, using, for example, an antisense approach?

4) Is the ability of α-endosulfine to be phosphorylated trivial or an important feature in the overall regulatory processes occurring at the level of K_{ATP} channels?

5) Does α-endosulfine also control the activity of non-β cell K_{ATP} channels?

When these issues are addressed, we will obtain a clear picture of the biological role of this peptide.

Possible role(s) of α-endosulfine in pathology

NIDDM (type 2 diabetes) is characterized by impaired insulin release in response to glucose. Sulfonylureas are used to compensate for this default. Because of the

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h α-endo      1-  MSQKQEEENPAEETGEEKQDTQEKEGILPERAEEAKLKAKYPSLGQKPGG
h ARPP-19     1-  MS***AEVPEAASAEEQKEMEDKV**TSPEKAEEAKLKARYPHLGQKPGG

h α-endo     51-  SDFLMKRLQKGQKYFDSGDYNMAKAKMKNKQLPSAGPDKNLVTGDHIPTP
h ARPP-19    46-  SDFLRKRLQKGQKYFDSGDYNMAKAKMKNKQLPTAAPDKTEVTGDHIPTP

h α-endo    101-  QDLPQRKSSLVTSKLAGGQVE
h ARPP-19    96-  QDLPQRKPSLVASKLAG****
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Figure 3. Amino acid sequences of human α-endosulfine (α-endo) and human ARPP-19. The differences between the two proteins are underlined. Gaps (*) in the ARPP-19 sequence were introduced for better alignment. The consensus sequences (basic-basic-X-S) for cyclic-AMP-dependent protein kinase are boxed. [Data from ref. 25.]

major importance of the K_{ATP} channel in glucose-stimulated insulin release, it is tempting to speculate that a dysfunction (due to mutation or inadequate expression) of a protein forming the channel (SUR1 or Kir 6.2) or a protein closely involved in its regulation may be responsible, in part or totally, for the NIDDM disorders. Similarly, certain forms of IDDM [26] may be related to genetic abnormalities other than those linked to autoimmune destruction of the β cells.

In the search for possible involvement of K_{ATP} channel mutations in pathology, a rare genetic disease called persistent hypoglycemic hyperinsulinemia of infancy was shown to be due to a mutation of the SUR1 gene which leads to permanent closure of the channel [27, 28] and a resultant non-regulatable and exaggerated insulin secretion. In one case, a Kir 6.2 mutation appears to be associated with a familial hyperinsulinism [29]. In the multigenic NIDDM disorder, it was observed that certain mutations of SUR1 occur slightly more frequently in NIDDM patients than in control subjects [30] leading, in some patients, to a decreased sensitivity to sulfonylureas [31]. On the other hand, no clear relation was found between polymorphism of the Kir 6.2 gene and diabetes [32, 33], although a recent paper suggests a link between missense mutations of Kir 6.2 and NIDDM [34]. Thus, generally speaking, mutations in the proteins forming the K_{ATP} channel do not explain the NIDDM syndrome. On the other hand, this lack of linkage leaves open the question as to whether mutations or an inappropriate expression of proteins regulating K_{ATP} channel activity, for instance α-endosulfine, may result in incorrect regulation of the channel that would lead to an inappropriate responsiveness of β cells to physiological stimuli. Studies are currently being carried out to address these important questions.

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