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THE PHARMACOLOGY OF ATP-SENSITIVE POTASSIUM CHANNELS

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INTRODUCTION

A potassium (K) channel that was inhibited by physiological (μM) concentrations of intracellular ATP ($[ATP]_i$) and that opened as $[ATP]_i$ decreased was first described in the heart (1). Subsequently, similar K-channels all with unitary conductances in the range of 40–80 pS (measured under symmetrical high K^+ conditions) were also found to exist in insulin-secreting cells and in skeletal muscle (2–5).[1] Such channels constitute what are classically described as ATP-sensitive K-channels and were termed Type 1 by Ashcroft & Ashcroft (6). In this review they are designated K_{ATP} and the current flowing through them is defined as $I_{K(ATP)}$. Type 1 K_{ATP} channels are essentially calcium- and voltage-independent, K^+-selective, and are half-maximally inhibited by $[ATP]_i$ in the range 10–100 μM. They exhibit inward rectification and a key pharmacological feature is their inhibition by agents like tolbutamide and glyburide. Other ATP-sensitive K-channels exist and these were designated Types 2, 3, 4, and 5 (6). Such channels vary not only in their sensitivity to calcium and to the inhibitory effects of $[ATP]_i$, but also in their selectivity for potassium and their susceptibility to pharmacological modulation.

[1]Unitary conductances are usually measured under quasi-physiological $[K^+]$ (6 mM outside, 140 mM inside) or symmetrical high $[K^+]$ (approximately 140 mM) conditions. To a first approximation, the unitary conductance in quasi-physiological conditions is half the value obtained in symmetrical high $[K^+]$ solutions.

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K_{ATP} channels are described variously as ATP-*sensitive* (4), ATP-*regulated* (1), or ATP-*dependent* (7). Davies and coworkers (7) used the term ATP-dependent since they felt that the *physiological* function of the channel was dependent on ATP. However, since phosphorylation via ATP can modify the opening of large conductance calcium-sensitive K-channels (BK_{Ca}) (8–11) or delayed rectifier K-channels (12), these channels could also be described as ATP-*dependent*. In addition, this term may imply too strong a role for ATP in the physiological modulation of channel opening in some tissues. Similarly, the term ATP-regulated (1) also implies that this adenine nucleotide has a physiological function in the modulation of channel activity. Except in the pancreatic β-cell, such a role in vivo is not certain and therefore we use the term ATP-*sensitive*. This is intended to convey that although the opening of K_{ATP} can be experimentally modulated by $[ATP]_i$, the physiological control of this channel in many tissues may be primarily associated with other nucleotides, G-proteins, various ligands, or even pH.

This review aims to describe the pharmacology of Type 1 ATP-sensitive K-channels (K_{ATP}) in a variety of tissues. The general biophysical properties of this channel have been comprehensively reviewed (6, 7, 13, 14). In addition, we describe the pharmacology of those K-channels in smooth muscle and neuronal cells that exhibit some of the characteristics of K_{ATP} and that may prove to be subtypes of this channel.

EFFECTS OF SYNTHETIC POTASSIUM CHANNEL OPENERS

Introduction

The K-channel openers are a diverse group of agents originally characterized by their ability to open smooth muscle K-channels (15, 16). However, it is now known that their K-channel opening action is additionally exerted in a variety of tissue types including the pancreatic β-cell/insulinoma cell (6, 17), neurones (18), skeletal muscle (19), and cardiac muscle (14, 20). Most of the electrophysiological and other effects of these agents can be inhibited by K-channel inhibitors like the sulfonylurea glyburide (see below), a pharmacological feature that led Quast & Cook (21) to propose that interaction with K_{ATP} formed the basis of the action of the K-channel openers in all tissues.

Chemical Classification

The classification of and inter-relationships between the various groups of K-channel opener have been detailed in several reviews (15, 22, 23; Figure 1.) No common pharmacophore linking the ability of all these agents to open K_{ATP} has yet been identified, although structure-activity relationships within

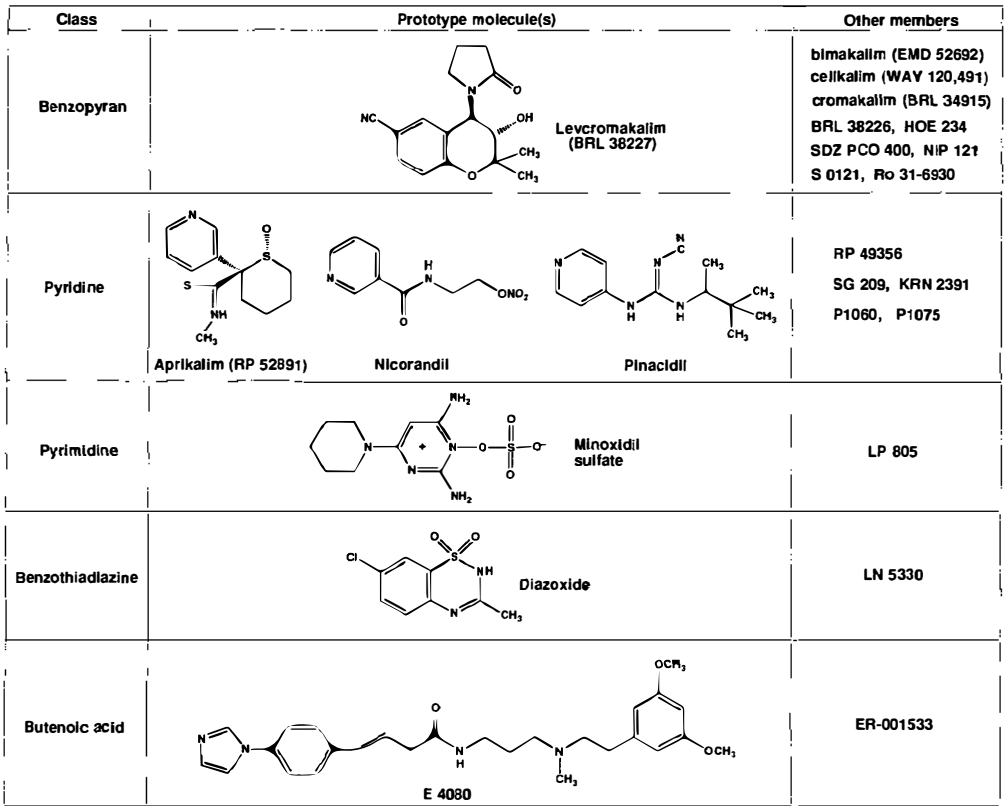

Figure 1 Chemical classification of K-channel openers. The agents listed all relax smooth muscle in vitro and are hypotensive in vivo, actions that can be inhibited by glyburide. The prototype molecules increase $^{86}Rb/^{42}K$ efflux from smooth muscle and enhance K-currents in a variety of cell types, effects that are inhibited by glyburide. In a few instances, the opening of K_{ATP} has been confirmed in patch/whole-cell clamp studies. It can thus be tentatively concluded that the characteristic pharmacological effects of all these agents are mediated, at least in part, via the opening of K_{ATP}. (Details derived from 15, 22, 23, 299).

the benzopyran group have been detailed (22). Until further data on the mode/site of action of these agents are available, some aspects of chemical classification are rather arbitrary. For simplification in this review, the openers formerly classified as pyridines (e.g. nicorandil), cyanoguanidines (e.g. pinacidil), and thioformamides (e.g. aprikalim) have all been re-designated as "pyridines" since this chemical nucleus is common to all these agents.

Effects of Openers on K_{ATP} in Various Tissue Types

Early in their development, it was recognized that the effects of the openers on whole tissues in vitro and in whole organisms in vivo could be antagonized by inhibitors of K_{ATP} like glyburide (24). Furthermore, subsequent patch-clamp studies showed that the K-channel openers did indeed open K_{ATP}, especially in insulinoma cells and in cardiac muscle (see 6). However, the absolute potency of the K-channel openers in these tissue types was lower than in smooth muscle and the relative order of potency of a series of K-channel openers in smooth muscle was significantly different from that in insulinoma cells.

PANCREATIC β-CELL/INSULINOMA CELLS K_{ATP} channels are thought to form the link between nutrient (glucose) availability and insulin secretion from the pancreas. In this tissue, K_{ATP} in the β-cell is predominantly closed in the presence of physiological concentrations of intracellular ATP and opens as $[ATP]_i$ falls. Despite this, it is the major K-channel responsible for setting the basal level of membrane potential and its modulation is thought to play an important role in the regulation of glucose-stimulated insulin secretion (25–28). Since the membrane resistance of the β-cell is very high, the closure of a few K_{ATP} channels has a pronounced effect on the membrane potential (29). An increase in extracellular glucose concentration produces an increase in $[ATP]_i$. The consequential closure of K_{ATP} depolarizes the cell, enhancing calcium influx that then stimulates insulin secretion from storage granules within the cell (see 17, 30 for reviews).

In both pancreatic β-cells and insulinoma cells, diazoxide stimulates the opening of K_{ATP}, inducing membrane hyperpolarization and inhibiting insulin secretion (31–34). The effective concentration of diazoxide in β-cells or insulinoma cells (RIN m5F; approximately 100 μM) is similar to that which exerts mechano-inhibitory effects in vascular smooth muscle (35). In contrast, in patch-clamp studies, other K-channel openers have proved to be less potent openers of K_{ATP} in either β-cells or insulinoma (RIN m5F) cells, (effective concentrations >100 μM) (36, 37) than in vascular smooth muscle cells (levcromakalim ≥ 100 nM) (38). In the CRI-G1 cell line, cromakalim (up to 100 μM) was without effect on the opening of K_{ATP} (39). The activation of channels by the K-channel openers is only evident in the presence of MgATP,

and several of these agents (pinacidil, RP 49356, nicorandil, and diazoxide) inhibit channel opening in the absence of MgATP (36, 39). The mechanism underlying this inhibitory action (see below) remains to be determined, although in the case of diazoxide, its irreversible nature has led to the suggestion that this agent may accelerate channel run-down (39).

The ability of the K-channel openers to enhance K_{ATP} opening is reflected by their ability to inhibit insulin release. Thus, the most potent K-channel opener, diazoxide, produces >90% inhibition of insulin release at a concentration of 100 μM, whereas a higher concentration of cromakalim (500 μM) produces only 35% inhibition (40). The rank order of potency of the K-channel openers for inhibition of insulin release is diazoxide>pinacidil>cromakalim (40).

CARDIAC MUSCLE K_{ATP} and the associated current $I_{K(ATP)}$ were first described in cardiac muscle by Noma (1) and Trube & Hescheler (41). The characteristics of this channel and current, together with those of K_{ATP} and $I_{K(ATP)}$ in the pancreatic β-cell, define what are classically described as Type 1 ATP-sensitive channels and currents. Thus, the cardiac K_{ATP} has a typical unitary conductance of 80 pS (symmetrical high potassium solution), and requires Mg-dependent phosphorylation to prevent run-down. It is normally closed in the presence of a physiological $[ATP]_i$ by a mechanism that does not involve a phosphorylation step (for details, see (6)).

The effects of K-channel openers on K_{ATP} or $I_{K(ATP)}$ in the heart have been widely studied. Direct effects of cromakalim on K_{ATP} were first described by Escande et al, Osterrieder, and Sanguinetti et al (42–44). These studies collectively showed that cromakalim shortened action potential duration without affecting upstroke velocity, an effect that was reversed by glyburide. No single investigation of the order of potency of a range of K-channel openers drawn from the different chemical subgroups has been published. However, the absolute potencies of individual agents are similar to those observed in β-/insulinoma cells and are approximately 10–50 times lower than those seen in smooth muscle.

The finding that both the effects of cromakalim and of hypoxia were glyburide-sensitive (44) and that ATP, like glyburide, inhibited the channel opened by cromakalim (42) strongly suggested that K_{ATP} was the target channel for the K-channel openers in cardiac muscle. Subsequent experiments (e.g. 45–47) have confirmed this view and shown that the effects of the K-channel openers can be opposed by increasing $[ATP]_i$ (47). In single cardiac myocytes, Thuringer & Escande (47) found that RP 49356 effectively decreased the sensitivity of K_{ATP} to closure by ATP and broadly similar results were obtained for nicorandil (14) and for pinacidil (48). Thuringer & Escande (47) concluded that the openers probably inhibited the binding of ATP to its

inhibitory site (see Figure 3, Site 2) whereas Fan et al (49) suggested that the openers bound to a different site from ATP and thereby reduced the affinity of Site 2 for ATP.

In excised patches, the K-channel openers are effective when applied on both the extra- and intracellular sides of the membrane (50, 51). However, higher concentrations of pinacidil must be applied to the outside compared with the inner surface (49), perhaps suggesting that the openers do not bind at an external site on the membrane.

SKELETAL MUSCLE K_{ATP} channels have been described in both mammalian and amphibian skeletal muscle cells (4, 52–54). They exhibit typical Type 1 characteristics (see 6), with unitary conductances in the range 40–90 pS (symmetrical high K-solution).

The effects of K-channel openers have been investigated in relatively few studies. In excised inside-out patches from normal muscle, Weik & Neumcke (55) found that diazoxide had little effect, whereas both pinacidil and cromakalim increased channel open probability in the presence of an inhibitory concentration of ATP. These agents were without effect in the absence of ATP at the cytoplasmic side of the membrane in contrast to those of RP 49356. This pyridine derivative increased the open probability of K_{ATP} in the absence of $[ATP]_i$ (55). These authors concluded that cromakalim, pinacidil, and RP 49356 each displaced ATP from the channel (see Figure 3, Site 2), while RP 49356 was additionally able to open previously inactive channels.

The effects of the K-channel openers on diseased skeletal muscle have been reviewed by Wareham (19). In general, these investigations have not been specifically concerned with linking the actions of the openers with K_{ATP} per se. However, since the effective concentrations of the agents are similar to those known to open K_{ATP}, this channel seems the likely target. In skeletal muscle bundles from patients with myotonia congenita, both cromakalim and bimakalim (EMD 52692) abolished spontaneous twitches and the after-contractions characteristic of electrically stimulated myotonic tissues (56). In skeletal muscle taken from patients with hyperkalemic paralysis, cromakalim increased macroscopic K-currents and restored the membrane potential of depolarized fibers (57). Using muscle from patients with hypokalemic periodic paralysis, cromakalim hyperpolarized the cells and increased the force of twitch contractions, effects that were inhibited by glyburide (58).

NEURONES Evidence in favor of the presence of K_{ATP} in neurones is essentially indirect. It is largely derived from studies of sulfonylurea binding (usually [^{3}H]-glyburide) to brain membranes and from the pharmacological effects of K-channel openers reducible by inhibitors of K_{ATP}. Using autoradiography, Mourre et al (59, 60) have recently described the location of

[^{3}H]-glyburide binding sites. The highest levels of binding were detected in the substantia nigra, the globus and ventral pallidus, the motor neocortex, and parts of the cerebellar cortex. High levels of binding were also detected in the limbic system. Complementary studies have been reported by Gehlert et al (61) following the use of [^{125}I]-glyburide and by Treherne & Ashford (62).

Initial investigations (63) showed that cromakalim decreased neuronal excitability and reduced epileptiform discharges in hippocampal slices. In subsequent experiments, a small membrane hyperpolarization and activation of an inwardly rectifying K-current was detected (64, 65). Whether this current was ATP-sensitive was not investigated. In cultured hippocampal neurones, cromakalim produced a sustained outward current that was enhanced by depolarization (66). Again, no specific attempt was made to link this effect to K_{ATP}. Further evidence consistent with K-channel opening by levcromakalim and RP49356 was described by Gandolfo et al (67) who showed that these agents inhibited seizures produced by mast-cell degranulating peptide.

Perhaps the best (but still indirect) evidence for the existence of brain K_{ATP} channels has been obtained in the substantia nigra. In this region, sulfonylureas or increasing extracellular glucose increase the release of [^{3}H]-GABA, effects which are inhibited by a range of K-channel openers (68, 69). In one study (69), the order of potency of the K-channel openers (levcromakalim >nicorandil>cromakalim>diazoxide>pinacidil) was found to be different from that in either the pancreatic β-cell or in smooth muscle, possibly indicating a difference between the target K-channel (presumably K_{ATP}) in this brain region and that in other tissues.

HYPOTHALAMUS Distinct regions of the hypothalamus, i.e. the ventromedial (VMH) and lateral, contain neurones that respond to changes in circulating glucose concentration (70, 71). The VMH can simplistically be regarded as the satiety center (72) and glucose-sensitive cells in this region are involved in the control of appetite (see 73 for review). Although the density of sulfonylurea-binding sites in the VMH is relatively low in comparison to other areas of the brain, it is proposed that in this tissue, as in the pancreatic β-cell, K_{ATP} plays an important role as the link between glucose availability and cellular activity (62, 74). Thus, it is thought that K_{ATP} channels are inhibited as $[ATP]_i$ in the VMH cell rises in response to an increase in blood glucose (74). The associated depolarization increases hypothalamic firing, which induces the sensation of satiety. However, electrophysiological evidence suggests that the glucose-sensitive K-channel in the VMH is quite different from K_{ATP}. The VMH channel has a high conductance (146 pS in symmetrical high K^+) (75) and does not rectify (76). This contrasts with K_{ATP} in the other tissues in which the channel is an inward rectifier (1, 2, 4), with a unitary conductance in the range 40–80 pS when measured with a symmetrical K^+

gradient. Furthermore, although in most tissues the concentration of ATP producing a half-maximal inhibition of K_{ATP} in inside-out patches is 20–200 μM (see 13), 2–3 mM ATP is required to produce a comparable inhibition of K_{ATP} in neurones from the VMH (74). In cell-attached patches, levcromakalim, pinacidil, minoxidil sulfate, and diazoxide were each also without effect (75). Furthermore, in experiments using conventional microelectrodes, cromakalim has no effect on the firing rate of gluco-receptive VMH neurones (75).

Using patch-clamp techniques, tolbutamide inhibits ATP-sensitive K-channels in rat glucoreceptive VMH neurones in cell-attached recordings, but has no effect on these channels in isolated patches (74). This contrasts with insulin-secreting cells, in which tolbutamide is capable of inhibiting K_{ATP} in isolated patches irrespective of whether the outer or inner face of the cell membrane is exposed to the drug, and in which there appears to be a tight coupling between the sulfonylurea-binding site and the channel (28, 32, 33). In addition, glyburide, which has no effect on the opening of the ATP-sensitive K-channel in the VMH when applied alone, antagonizes tolbutamide-induced inhibition of the channel (74).

These biophysical and pharmacological discrepancies are so numerous that it is difficult to consider the VMH channel as a close variant of the Type 1 K_{ATP} channel and suggests that it may indeed be a different K-channel type (see 6).

SMOOTH MUSCLE In smooth muscle, early attempts failed to show the existence of a K_{ATP} channel which was closed by raising $[ATP]_i$ (77). The first, apparently successful, series of experiments by Standen et al (78) in rat and rabbit mesenteric artery reported a K-selective channel, closed by $[ATP]_i$, opened by cromakalim, and inhibited by glyburide. However, the unitary conductance of this channel (135 pS at 0 mV in 60 mM/120 mM extra-/intracellular K^+) is high for a typical Type 1 K_{ATP} channel (6) and raises the possibility that the cromakalim-sensitive channel reported in Standen et al (78) was in fact a large conductance, Ca-sensitive K-channel. Similar channels (BK_{Ca}) have indeed been reported to be closed as $[ATP]_i$ increases (79), an action resulting not from closure by ATP but largely from the chelation of Ca^{2+} by the disodium salts of ATP used in the experiments (see 80). Under certain conditions, BK_{Ca} can be opened by cromakalim/levcromakalim (80–82), data consistent with the possibility that the "K_{ATP}" detected by Standen et al (78) was, in fact, a calcium-dependent K-channel.

More recently, results that seemed consistent with the presence of a levcromakalim-sensitive K_{ATP} in pulmonary artery were obtained (83). However, the use of Na_2ATP together with the presence of Ca^{2+} in this study means that apparent changes in ATP-sensitive K-currents could have been

due to Ca-chelation by ATP and subsequent reduction in a Ca-dependent rather than ATP-sensitive K-currents (see 80). An alternative approach designed to link the effects of K-channel openers with those of K_{ATP} in rabbit mesenteric artery was recently described by Silberberg & van Breemen (84). These workers showed similarities between an outward current stimulated by metabolic inhibition (and presumed reduction in $[ATP]_i$) and by the current induced by levcromakalim. Both currents were glyburide-sensitive, but the authors did not exclude the possibility that a Ca-dependent K-channel carried the observed currents and neither the K-selectivity nor the unitary conductance of the channel(s) involved was determined.

In contrast to the report that the K-channel openers interact with high conductance ATP-sensitive K-channel (78; see above), or with a small conductance ATP-insensitive K-channel (85), evidence is now accumulating that in smooth muscle the K-channel openers interact with a small-medium conductance ATP-sensitive K-channel within the conductance range typical of a Type 1 K_{ATP} channel. The first suggestion of an action of K-channel openers on such a K-channel was derived from whole-cell voltage-clamp studies on the action of cromakalim in portal veins (86, 87). Based largely on the low noise of cromakalim-induced current, it was shown that a channel of low unitary conductance was most likely involved. Such a view was reinforced by Kajioka et al (88) who found that in rat portal vein nicorandil opened an ATP- and Ca^{2+}-sensitive K-channel with a unitary conductance of 10 pS (quasiphysiological K^+ gradient). Vascular smooth muscle ATP-sensitive K-channels of similar conductance and opened by cromakalim (89), nicorandil (90, 91) and by pinacidil (92) were recently described. In the experiments described by Kajioka et al (92) this channel was only opened by pinacidil in cell-attached patches or after patch excision when GDP was present on the interior side of the membrane. The study performed by Kovacs & Nelson (89) was particularly interesting since it attempted to demonstrate that the sulfonylurea binding sites were associated with a K-channel that, after incorporation into a lipid bilayer, was inhibited by ATP (K_i 41 μM) applied to the cis (intracellular) side. In addition, the enhancement of channel opening by cromakalim (1 μM) was inhibited by glyburide, although a very high concentration was required (50 μM). This contrasts markedly with the dissociation constant for selective binding of glibenclamide (10 nM) to the membrane fraction from which the ATP-sensitive channels were derived. Further experimentation is clearly required to determine whether the incorporated channel really functions like a typical K_{ATP}.

The excised patch-clamp experiments described above have provided some evidence for the existence of a K_{ATP} in smooth muscle and for a role of this channel in the action of the K-channel openers. However, the concentrations of agents required to produce channel opening have been 10–50 times higher

(typically 10–100 μM) than those required to produce relaxation in whole smooth muscle tissues. Recently, however, whole-cell voltage-clamp studies in rat portal vein have convincingly shown that low concentrations of levcromakalim open a small conductance K-channel (unitary conductance 10–20 pS in quasi-physiological conditions). Furthermore, using rapid voltage-clamp–current-clamp switching, the associated membrane hyperpolarization was typical of that seen in whole smooth muscle tissues (38). A continuation study demonstrated that the channel opened by levcromakalim in rat portal vein was the same as that which opened on depletion of $[ATP]_i$ in this tissue. Furthermore, depletion of this nucleotide induced membrane hyperpolarization of a magnitude similar to that produced by maximally effective concentrations of levcromakalim (93).

Although further studies are required, reasonable evidence for the existence of K_{ATP} in smooth muscle has now been obtained and this channel can clearly be opened by the K-channel openers. However, the rank order of potency for inhibition of spontaneous activity in vascular smooth muscle is cromakalim (maximal effect produced by 1 μM)> pinacidil (maximally effective at 3 μM)>diazoxide (maximal effective concentration 100 μM) (35). This contrasts with the ratio diazoxide> pinacidil> cromakalim for inhibition of insulin release (40). In addition, minoxidil sulfate, which fully inhibits spontaneous activity in the rat portal vein at a concentration of 10 μM (35), *stimulates* insulin release (40). These findings, together with the different potencies of glyburide in β-cells compared with smooth muscle (see below), collectively suggest that some differences exist between the K_{ATP} channels or their regulatory sites in these two tissue types.

K_{ATP}-CHANNEL INHIBITORS

Agents that restrict the movement of K^+ through K_{ATP} (see Figure 2) have been variously described as *blockers, antagonists,* or *inhibitors*. With the possible exception of an agent like tetraethylammonium (TEA), which probably blocks the channel (94), the detailed mode and site of action of the other substances shown in Figure 2 is unclear. The term *inhibitor,* which has no mechanistic implications, has thus been adopted for all agents that reduce the flow of K^+ through K_{ATP}.

Hypoglycemic Agents

CHEMICAL CLASSIFICATION Sulfonylurea drugs, long used in the treatment of noninsulin-dependent diabetes mellitus, are now known to stimulate insulin secretion by inhibiting the opening of K_{ATP} in pancreatic β-cells (28, 32, 95, 96). Patch-clamp studies have shown that the sulfonylureas are also selective

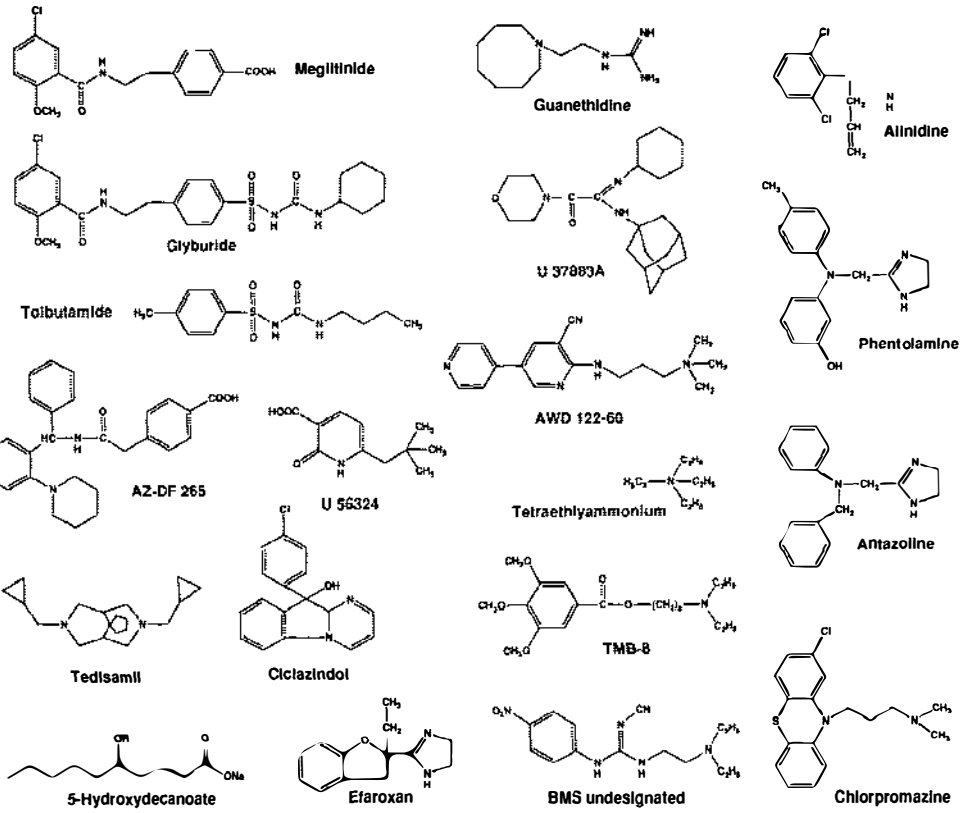

Figure 2 Chemical structures of molecules capable of reducing the flow of K^+ through K_{ATP} by various mechanisms. For further details, see text. BMS: Bristol Myers Squibb.

inhibitors of K_{ATP} in cardiac myocytes (96, 97) and skeletal muscle (5). Other structurally dissimilar agents that also stimulate insulin secretion by inhibiting K_{ATP} or inhibit the effects of K-channel openers in smooth muscle include the guanidine derivative linogliride (98, 99), the benzoic acid derivative (−)AZ-DF 265 (100), the urea derivative U-37883A (101), and the pyridine derivative, U 56324 (102, 103; see Figure 2).

Marked differences between the potencies of the various hypoglycemic sulfonylureas have led to the suggestion that the most potent of the sulfonylureas, glyburide (104), which possesses both benzoic acid and sulfonylurea groupings, is capable of inhibiting K_{ATP} by an interaction with a site additional to that which recognizes the sulfonylurea moiety (105, 106; see Figure 3). Structure-activity studies have supported this view with the demonstration that the benzoic acid derivative meglitinide, which is similar

to glyburide or gliquidone but which lacks the sulfonylurea group, is capable of inhibiting β-cell K_{ATP} channels and of stimulating insulin secretion from islet cells (34, 106).

BINDING SITES Glyburide binds to high and low affinity sites in pancreatic β-cells, insulinoma cells, cardiac myocytes, smooth muscle cells, and rat cerebral cortex (62, 107–111). This property has been used to determine the characteristics of the binding site for the hypoglycemic and other agents in membrane fractions and isolated cells and, together with autoradiography, to indicate the possible cellular location of K_{ATP} channels in a variety of tissues.

In both cardiac membranes and rat insulinoma membranes, specific glyburide binding is enhanced by the presence of divalent cations or by a reduction in pH (108). The binding of this agent is insensitive to apamin (a selective blocker of a small conductance calcium-sensitive K-channel; 112) or to a variety of relatively nonselective K-channel blockers, including tetraethylammonium, quinine, quinidine, and 4-aminopyridine (110, 113, 114). Concentrations of quinine (20–500 μM), chlorpromazine (10–100 μM) or thiopentone (100–200 μM), which almost completely inhibit the opening of K_{ATP} in insulin-secreting cells (115–117) are without significant effect on [^{3}H]-glyburide binding under phosphorylating or dephosphorylating conditions, suggesting that these agents inhibit K_{ATP} by interacting at a site different from that of glyburide (113, 118).

In contrast, hypoglycemic agents based on either the sulfonylurea or benzoic acid moieties are potent displacers of [^{3}H]-glyburide from its binding sites in a variety of tissues (68, 100, 104, 110, 119, 120). A good correlation exists between the concentration of a range of hypoglycemic agents required to displace [^{3}H]-glyburide from insulinoma cell membranes and the therapeutic dose of these agents in humans (104). In membranes of rat brain cortex, several K-channel openers of different chemical classes are capable of displacing [^{3}H]-glyburide (114, 120). The benzoic acid derivative (−)-AZ-DF 265 stimulates insulin release and inhibits ^{86}Rb efflux from mouse pancreatic β-cells with a potency similar to that of glyburide (105). Like glyburide, (−)-AZ-DF 265 inhibits the mechano-inhibitory effects of cromakalim and diazoxide in rat aorta in a competitive-like manner and those of minoxidil sulfate noncompetitively (121). Both (−)-AZ-DF 265 and another benzoic acid derivative, meglitinide, displace [^{3}H]-glyburide from rat β-cell membranes, but with different characteristics. Furthermore, benzoic acid derivatives that are structurally similar to AZ-DF 265 (AG-EE 388, AG-EE 86, AG-EE 319 and AG-EE 436) differ in their ability to displace [^{3}H]-glyburide but not to displace the sulfonylurea, [^{3}H]-gliquidone (122). These data collectively suggest that the sulfonylurea moiety is recognized by a site different from that with which the benzoic acid derivatives interact but that

both sites are allosterically coupled. Complexities within the sulfonylurea domain may account for the observed differences between glyburide and gliquidone (see Figure 3).

The reported failure of K-channel openers to inhibit glyburide binding to the membranes of neuronal or muscle cells (110, 113) may be due to nonideal experimental conditions. Recently, Niki & Ashcroft (123) and Schwanstecher and coworkers (118, 124, 125) have shown that the presence of MgATP enhances displacement of [^{3}H]-glyburide binding by the K-channel openers in mouse pancreatic β-cells. Using whole segments of rat aorta, Bray & Quast (126) found a good correlation between the ability of a range of K-channel openers to stimulate ^{86}Rb efflux and to inhibit binding of the radiolabeled pinacidil derivative, [^{3}H]-P1075. In addition, the K-channel blockers, glyburide, glipizide, and AZ-DF 265, were also effective inhibitors of [^{3}H]-P1075 binding. The significance of these experiments in whole segments of aorta rather than in isolated membranes from this tissue is unclear. Nevertheless, on the basis of these and other experiments, Bray & Quast (126) and Schwanstecher and coworkers (118) each concluded that the K-channel openers and the hypoglycemic agents bound to different sites on the K_{ATP} channel that were coupled in a negatively allosteric manner.

Adenosine diphosphate (ADP) inhibits [^{3}H]-glyburide binding in an insulinoma cell line (HIT T15) (127, 128) and in rat brain cortex (110). Other nucleotides, including ATP and GTP and their nonhydrolysable analogs, ATPγS and GTPγS inhibit the binding of [^{3}H]-glyburide and [^{3}H]-tolbutamide to microsomes from pancreatic β-cells in the presence of magnesium (125, 130). In pancreatic islets, inhibition of sulfonylurea binding was also seen with ADP, GDP, or GDPβS, but was not observed in the additional presence of hexokinase (which would keep the concentrations of GTP and ATP low). Thus, prior conversion of these diphosphates to their equivalent triphosphates may be necessary for inhibition of binding (125). The nonhydrolysable triphosphate analogs of ATP and GTP (AMP-PNP and GMP-PNP) are also without effect on sulphonylurea binding, whereas AMP-PNP *is* able to inhibit K_{ATP} (see Figure 3, Site 2). These data collectively suggest that phosphorylation of Site 1 interferes with sulfonylurea binding whereas occupancy of Site 2 by ATP is without effect on this phenomenon (125, 130).

The ability of guanine nucleotides (potency ratio : Gpp[NH]p = GTPγS>GTP) to inhibit [^{3}H]-glyburide binding to its low affinity binding site in cardiac membranes, cerebral cortex, and rat insulinoma cells may indicate that G-protein activation by these nucleotides induces conformational changes that are less favorable for glyburide binding in the vicinity of the K_{ATP} channel (107, 108, 110, 131). Such inhibition does not occur after cell treatment with trypsin, which may destroy a protein component involved in the supposed G-protein/glyburide binding interaction (108).

INVOLVEMENT OF THE GLYCOLYTIC PATHWAY In pancreatic β-cells, the ability of (−)-AZ-DF 265 to stimulate insulin-secretion or to inhibit ^{86}Rb efflux is partially reversible (105) in contrast to the effects of glyburide, which are essentially irreversible (132). Such long-lasting effects could be explained by an interaction with both the benzoic acid and sulfonylurea binding sites within the lipid phase of the membrane (133, 134; see earlier). However, glyburide inhibits at least one enzyme indirectly involved in the glycolytic pathway and such an effect could account for some of the long-lasting effects of this agent.

Glyburide inhibits the A-kinase that regulates the tandem enzyme fructose-6-phosphate-2-kinase/frucose-2,6-bisphosphatase (135) and such an effect would shift the glycolytic pathway in the direction of pyruvate production. The subsequent net synthesis of ATP at the step involving phosphoglycerate kinase could result in inhibition of K_{ATP} and account for the effects of this agent on K_{ATP}. Such an explanation is usually rejected on the grounds that glycolytic enzymes are associated with cytosolic fractions rather than with plasma membranes (see 136), whereas inhibition of K_{ATP} by glyburide can be observed in isolated membrane patches. However, much "textbook" biochemistry concerning the location of glycolytic enzymes has been derived from studies in liver and yeast cells and in cardiac muscle, glycolytic enzymes are associated with the plasmalemma (137). This would be consistent with the view expressed by Petersen et al (138) that K_{ATP} in the pancreatic cell could perhaps be modulated by plasmamembrane-bound glycolytic enzymes. The recent finding in vascular smooth muscle (139) that an entire complement of these enzymes is associated with the plasmalemmal fraction lends further support to this possibility. Thus, ATP could be generated by glyburide-induced stimulation of glycolysis in isolated patches and such an action could at least contribute to the observed inhibition of K_{ATP}. This suggestion is supported by the finding that sulfonylurea-induced inhibition of K_{ATP} in the β-cell is greater in the presence of glucose (see 140) and that in the heart glycolytically derived ATP is more important for the inhibition of K_{ATP} than that derived from oxidative phosphorylation (141). In addition, glyburide inhibition of K_{ATP} continues to develop after removal of the drug (132), consistent with an indirect metabolic effect. Further studies to test the role of glycolytic enzymes in the actions of glyburide and other hypoglycemic agents are clearly required.

SITES OF ACTION IN THE CENTRAL NERVOUS SYSTEM Specific binding sites for sulfonylureas in the brain were first demonstrated by Kaubisch and coworkers (142) using [^{3}H]-gliquidone. Subsequently, using [^{125}I]-iodo- or [^{3}H]-glyburide, sulfonylurea binding sites have been found throughout the brain, with a high density in cells of the substantia nigra, CA3 region of the

hippocampus, globus pallidus, and caudate-putamen (59, 60, 62, 143). The site itself may be a lipoprotein since it is susceptible to proteolytic and lipolytic enzymes (144). Sulfonylureas are relatively selective inhibitors of K_{ATP} in pancreatic β-cells and exert no effect on nucleotide-sensitive nonselective cation channels (32), Ca-activated K-channels (32, 33), or voltage-sensitive channels (27) in this tissue. Thus, sulfonylurea binding is widely held to indicate the presence of K_{ATP} channels in the brain. This view is supported by the finding that purified sulfonylurea-binding sites extracted from brain or pancreatic β-cells have similar molecular weights (140 kd and 150 kd, respectively) (145). Although some sulfonylureas devoid of insulin-secreting properties are capable of displacing [^{3}H]-glipizide (144), a good correlation generally exists between the ability of hypoglycemic agents to inhibit both [^{3}H]-glyburide binding and ^{86}Rb efflux (104).

However, the extent to which binding of labeled hypoglycemic agents indicates the presence of K_{ATP} is uncertain in view of the recent finding that glyburide, tolbutamide, and gliquidone also inhibit a slowly inactivating K-current, I_D, in rat CA3 hippocampal neurones (146). This current differs from $I_{K(ATP)}$ since it is both voltage-dependent and sensitive to inhibition by a low concentration of 4-aminopyridine (30–40 μM) (146, 147). Glyburide inhibits a voltage-dependent, calcium- and ATP-independent K-current in a human neuroblastoma cell line (SH-SY5Y) (148).

Adrenoceptor Antagonists

Insulin secretion in the pancreas is reduced by $\alpha_2$adrenoceptor agonists (149) and thus the ability of phentolamine to stimulate insulin secretion was initially attributed to antagonism of this effect (150). However, the effectiveness of phentolamine in the absence of α-adrenoceptor agonists (151–153) led to a further investigation of the action of phentolamine (154). Using patch-clamp and ^{86}Rb efflux techniques, Plant & Henquin (154) found that phentolamine (20–100μM) inhibited both basal and diazoxide (100 μM)-stimulated ^{86}Rb efflux in mouse pancreatic β-cells. Under whole cell-clamp conditions, the effect of phentolamine on K_{ATP} was relatively slow in onset and was irreversible.

In smooth muscle, phentolamine inhibits the effects of the K-channel openers, pinacidil, nicorandil, levcromakalim, and cromakalim (155–160). Although the antagonism of the response to pinacidil was competitive-like (156), phentolamine produced a noncompetitive antagonism of the relaxant effect of levcromakalim and cromakalim (156, 157). The concentration of phentolamine required to inhibit the K-channel-opening effect (1–100 μM: 154, 155, 157, 158, 160) was 100–1000 times higher than that required to block α-adrenoceptors in vascular smooth muscle (161, 162). In addition, the ability of phentolamine to antagonize the effect of cromakalim was not shared

by rauwolscine (10–100 μM), phenoxybenzamine (1 μM), or prazosin (10 μM) (157, 160), each antagonists at α-adrenoceptors, thus suggesting that the inhibitory effect was associated with an effect at a different receptor site. However, several compounds structurally similar to phentolamine are capable of inhibiting the effects of the K-channel openers in smooth muscle or cardiac muscle (157, 163, 164). These agents differ in their actions at α-adrenoceptors, being antagonists (phentolamine, tolazoline and efaroxan) (163, 165), partial agonists (ST-91, tramazoline and naphazoline) (165), or inactive (alinidine) (166). Furthermore, cibenzoline, a 2-substituted imidazoline currently prescribed as an antiarrythmic agent, produces hypoglycemia in some patients (167) and is capable of stimulating insulin release from rat isolated islets (168).

In general, it appears that the 2-substituted imidazoline structure confers the ability to inhibit K_{ATP} and to stimulate insulin-secreting activity (154, 169–171). This is supported by the finding that not only do the imidazoline-based $\alpha_2$adrenoceptor antagonists efaroxan and idazoxan inhibit K_{ATP} in insulinoma cells (163), but antazoline, an imidazoline H_1 antagonist, also stimulates insulin secretion (171).

THE NONADRENERGIC IDAZOXAN BINDING SITE/IMIDAZOLINE-GUANIDINE RECEPTOR SITE Although not confirmed experimentally, we speculate that the site of action of the imidazolines is the location known variously as the imidazoline-guanidine receptor site (IGRS) or the nonadrenergic idazoxan binding site (NAIBS) (172). This is distinct from variants of the α-adrenoceptor and recognizes agents with either guanidino or imidazoline moieties. Thus, K_{ATP} channels may be inhibited not only by imidazoline derivatives but also by guanidino compounds. In smooth muscle, the adrenergic neurone-blocking drugs debrisoquine and guanethidine, both guanidine derivatives, exhibit in vitro properties suggestive of inhibition of K_{ATP}. Like glyburide, these agents do not modify spontaneous tone in the guinea-pig isolated trachealis and have no effect on basal ^{86}Rb efflux from preloaded tissues, but are capable of inhibiting the effects of the K-channel openers levcromakalim, RP 52891, and pinacidil in these systems (173, 174). The possible coupling between the NAIBS/IGRS in the plasmalemma of other tissues and intracellular effectors has been discussed (172).

Antiarrhythmic and Cardioactive Agents

Indirect evidence exists that the antiarrhythmic and bradycardic agent tedisamil (KC-8857) inhibits K_{ATP}. In rabbit isolated aorta, this agent inhibits the ^{86}Rb efflux stimulated by cromakalim but not the background efflux (175). Tedisamil is, however, not a selective inhibitor of K_{ATP} since it also inhibits

a calcium-dependent K-channel in hippocampal neurones (176, 177), and also a transient K-current and a delayed rectifier K-current in cardiac cells (178, 179). In cells isolated from the guinea-pig portal vein, tedisamil inhibits a transient outward current and reduces the open probability of the large conductance Ca-sensitive K-channel (BK_{Ca}) (180). The inhibition of the effects of K-channel openers in smooth muscle by another bradycardic agent, alinidine (157), also implies inhibition of K_{ATP}.

Cibenzoline is another antiarrhythmic agent that probably inhibits K_{ATP}. Like tedisamil, this compound is also somewhat nonselective and blocks sodium and calcium channels as well as K-channels in the heart (see 181 for review). Although it has not yet been shown to inhibit K_{ATP} per se, such an action is suggested since it produces hypoglycemia in some patients (167) and stimulates insulin secretion from rat islets (168). Cibenzoline is a 2-substituted imidazoline, and probably exerts its effects on insulin-secreting cells via the NAIB site (see above).

The cardiotonic agent AWD 122-60 is structurally very similar to milrinone but, in contrast to this drug, it exerts a negative chronotropic effect in guinea pig atria (182). AWD 122-60 inhibits K_{ATP} in skeletal muscle (183), suggesting that the observed reduction in heart rate results from inhibition of cardiac K_{ATP} channels (183). Since glyburide may be potentially useful in the treatment of cardiac arrhythmias (184), the additional cardiotonic properties of AWD 122-60 may prove to be especially beneficial in this condition (183). Interestingly, the terminal diethylamino group in AWD 122-60 is similar to that found in the pinacidil-like K-channel inhibitors described by Atwal (185) and this structural feature is also present in the class III anti-arrhythmic agent sematilide and in TMB-8, the recently described inhibitor of K_{ATP} (186; see Figure 3). Several phenothiazines have also been reported to block K_{ATP}. These include chlorpromazine and trifluoperazine (117), molecules each possessing a dimethylamino substituent. Such a grouping may be analogous to the diethylamino substituent on TMB-8 and AW 122-60 mentioned above.

In guinea-pig ventricular myocytes, shortening of the action potential by inhibition of glycolysis (using iodoacetate) could be reversed by 5-hydroxydecanoate (187). This compound was also found to block K_{ATP} in this tissue selectively (187). In ischemic rat hearts, the cardioprotective effects of pinacidil or cromakalim are inhibited by either glyburide or 5-hydroxydecanoate. However, under nonischemic conditions, only glyburide is capable of reversing the effects of these K-channel openers (188). Thus, 5-hydroxydecanoate appears to be effective only under conditions of ischemia (188). Although the reason for this efficacy remains to be established, this compound may prove useful in determining the mechanisms by which K_{ATP} is modulated by drugs.

Calmodulin Antagonists

Recent experiments in *Xenopus* oocytes have shown that calmodulin inhibitors can inhibit a cromakalim-induced current (189) which is sensitive to glyburide (189, 190). One possible explanation of this interaction would be inhibition of the phosphodiesterase responsible for the breakdown of the pool of cAMP, which activates protein kinase A. However, inhibition here would effectively activate protein kinase A, enhancing channel phosphorylation and thus priming the channel for opening. As discussed by Sakuta et al (189), this action of calmodulin inhibitors is thus presumably exerted on a calmodulin-like structure associated with the channel itself, although further studies are required to confirm this.

Miscellaneous Agents

In addition to the hypoglycemic sulfonylureas and benzoic acid derivatives, which appear to be relatively selective blockers of K_{ATP}, the ATP-sensitive K-channel is inhibited by less-selective K-channel blockers. These include millimolar concentrations of 4-aminopyridine (191–193) and intracellular tetraethylammonium (TEA; K_d in ventricular cells < 1 mM; K_d 1.4 mM in skeletal muscle) (193, 194). In addition, the antiarrhythmic agents amiodarone, quinidine and verapamil, as well as the antipsychotic drug haloperidol, inhibit K_{ATP} in the heart and pancreatic β-cell in concentrations ranging from 2 μM to 30 μM (115, 192, 195, 196). Intracellular cations also cause some inhibition of K_{ATP} and are thought to contribute to the inward rectifying properties of the channel. Extracellular barium (0.1–1 mM) may also inhibit the channel in cardiac, smooth and skeletal muscle (53, 197).

Ciclazindol is an anorectic agent (198) with monoamine uptake inhibitory properties (199), which has recently been shown to inhibit the mechano-inhibitory effects of levcromakalim in smooth muscle (200). Patch-clamp studies have demonstrated its ability to inhibit the channel opened by levcromakalim in cells isolated from rat portal veins, although it also inhibited a delayed rectifier current (201). The inability of ciclazindol to displace [^{3}H]-glyburide from porcine or rat brain membrane fragments (114, 201) suggests that ciclazindol and glyburide may each inhibit channel opening by acting at different sites in smooth muscle. However, mazindol, an anorectic agent structurally similar to ciclazindol (202), is capable of inhibiting [^{3}H]-glyburide binding (114). A simple hypothesis for the in vivo anorectic properties of ciclazindol would be that it is capable of blocking K_{ATP} in the ventromedial hypothalamus, thus mimicking an effect proposed for glucose (see 73) and inducing satiety. However, using cells isolated from rat ventromedial hypothalamus, Ashford and coworkers (personal communication) have failed to

demonstrate inhibition of the ATP-sensitive K-channel in the VMH by ciclazindol.

THE K_{ATP} CHANNEL: A WORKING PHARMACOLOGICAL MODEL

Clearly, K_{ATP} exists in a wide variety of cells. However, not all variants of this channel which can be broadly classed as "Type 1 are identical. Based on agonist potency ratios, the smooth muscle variant seems significantly different from its relatives in cardiac and skeletal muscle and in the pancreatic β-cell. The situation in neurones is currently unclear. Nevertheless, a general working model of K_{ATP} based on the available pharmacological evidence can

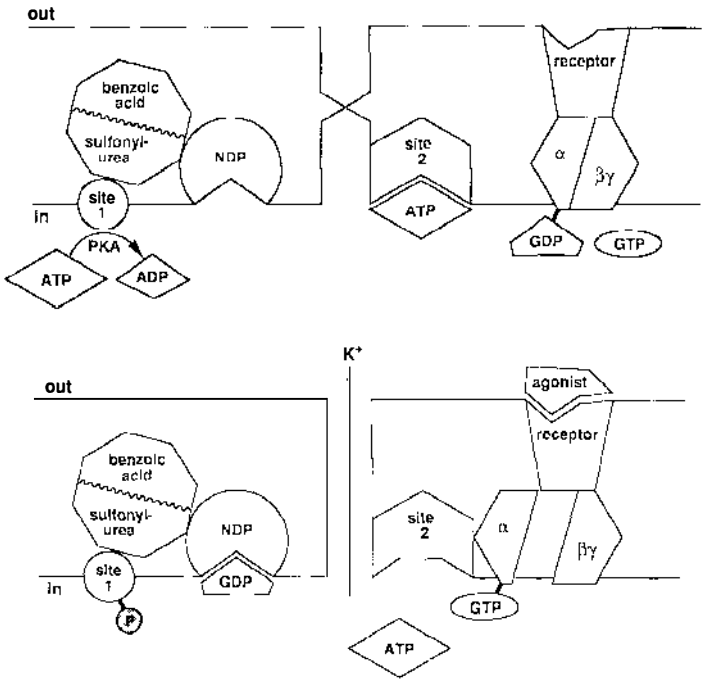

Figure 3 Model of the Type 1 ATP-sensitive K-channel and its regulatory complex showing: (*a*) Site 1: the phosphorylation site; Site 2: the regulatory site. Experimental evidence suggests that the benzoic acid/sulphonylurea domain and the nucleotide diphosphate site are closely associated with Site 1. (*b*) Ligand-receptor–G_i protein interactions probably reduce the affinity of Site 2 for ATP. GDP liberated after G_i protein activation may interact with the nucleotide diphosphate (NDP) site to enhance channel opening. K-channel openers probably influence Sites 1 and 2. At the latter, displacement of or a reduced affinity for ATP produces channel opening. At Site 1, a reduction in phosphorylation may produce anomalous channel closure by mimicking run-down (PKA, protein kinase A). For further details, see text.

be constructed and is shown in Figure 3. Much of the data on which this is based have been derived from insulinoma cells with contributions from cardiac muscle and to a lesser extent from skeletal musculature. Equivalent information from other cell types is sparse or nonexistent.

ATP-modulated sites

SITE 1: THE PHOSPHORYLATION SITE K_{ATP} channels require MgATP to prime the channel for opening. In the absence of ATP, and thus under the conditions often employed for isolated patch voltage-clamp recordings, "run-down" of the channel occurs (203). Typically, both magnesium and ATP are minimal requirements for the prevention or reversal of this phenomenon. Such channel phosphorylation plays an important role in the regulation of several ion channels (204) and since nonhydrolysable analogs of ATP are unable to substitute for ATP, even in the presence of magnesium, it seems likely that phosphorylation of the channel (Figure 3, Site 1) is a prerequisite for K_{ATP} channel opening (47, 205, 206). Thus, run-down, which is enhanced by calcium, is probably caused by dephosphorylation of the channel.

SITE 2: THE ATP REGULATORY SITE In a variety of tissues, inhibition of the opening of K_{ATP} is produced not only by ATP, but also, equieffectively, by its nonhydrolysable analogs (5, 207–209). This implies that ATP or its analogs inhibit channel opening by binding to, but not phosphorylating, a site (Figure 3, Site 2) in the vicinity of the channel. The detailed characteristics of this inhibitory site appear to exhibit tissue variation. Thus, although free ATP (ATP^{4-} or $ATPH^{3-}$) is required for inhibition of K_{ATP} in the pancreatic β-cell (207, 210), the bound form ($MgATP^{2-}$) is more effective in skeletal or cardiac muscle (211–213). The mechanism by which ATP induces channel closure is not known.

In insulin-secreting cells and ventricular myocytes the $[ATP]_i$:$[ADP]_i$ ratio rather than the $[ATP]_i$ alone seems responsible for modulation of K_{ATP}. In both tissue types, ADP reduces the sensitivity of the channel to ATP, such that higher concentrations of ATP are required to produce closure of the channel in the presence than in the absence of ADP (193, 205, 211, 214, 215). The stimulatory effect of ADP is not mimicked by the nonhydrolysable analog ADP-β-S, indicating that phosphorylation may be involved in this mechanism (214). However, ADP does not reactivate K_{ATP} after complete run-down (211). Thus, the requirement for magnesium in the stimulation of channel opening by ADP in rat ventricular myocytes may indicate some selectivity of the binding site for the MgADP complex rather than imply a role for phosphorylation in this action (211). Spruce et al (5) found that 0.1 mM ADP had no effect on the inhibition of channel opening by 1 mM ATP

in frog skeletal muscle. However, the omission of magnesium from the solution bathing the cytoplasmic surface of the skeletal cell membrane may have been responsible for the apparent lack of effect of ADP in these experiments.

Although ADP enhances channel opening in the presence of ATP, this nucleotide diphosphate generally inhibits channel opening in the absence of ATP (210, 211, 216). A possible explanation for these findings is that ADP is capable of binding to and activating the ATP regulatory site (Figure 3, Site 2), although with lower affinity and efficacy than ATP. Thus, acting competitively, ADP would reduce ATP-induced channel inhibition. However, in the complete absence of, or in the presence of, a low concentration of ATP such that K_{ATP} would be maximally open, binding by ADP would be inhibitory (210, 205, 217).

It is uncertain to what extent ADP might contribute to the open probability of K_{ATP} in vivo since it is normally present in a very low concentration in the β-cell (205). Nevertheless, in insulin-secreting cells (RINm5F) the mean intracellular concentrations of ATP and ADP are approximately 5.8 mM and 2.0 mM, respectively (210). Although such a concentration of ATP alone fully inhibits K_{ATP} opening in this cell line, the addition of ADP in a concentration normally present in RINm5F cells allows channel opening. Such a dual regulation of channel opening by ATP and ADP may account for the sensitivity of the channel to changes in ATP, since small changes in the concentration of ATP are magnified by similar changes (in the opposite direction) of ADP (210).

The Nucleotide Diphosphate Site

Tung & Kurachi (216) examined the effects of a range of nucleosides and nucleotide phosphates on K_{ATP} channels in ventricular myocytes. Although none of the nucleosides, nucleotide monophosphates, or nucleotide diphosphates affected channel opening, several nucleotide diphosphates (uridine, inosine, cytidine, and guanosine), in the presence but not the absence of magnesium, stimulated channel opening after channel run-down. Tung & Kurachi (216) thus concluded that K_{ATP} channels probably possess nucleotide diphosphate binding sites, which are capable of inducing channel opening after dephosphorylation of site 1 (Figure 3). Interestingly, ATP was capable of inhibiting K_{ATP} after nucleotide diphosphate-induced recovery from run-down. A low concentration of ADP (100 μM) stimulated some channel opening after run-down, but higher concentrations (1–10 mM) were inhibitory, suggesting that ADP is a poor ligand at the proposed diphosphate site. Bokvist et al (218) also found some evidence for a stimulatory effect of ADP on K_{ATP} via a site distinct from the ATP-regulatory site (Site 2). Insulinoma cells also possess a nucleotide diphosphate stimulatory site (219).

Other factors, for example the ratio of pyridine nucleotides (NAD(P):NAD(P)H) may also influence K_{ATP} opening (see 220)

The Hypoglycemic Domain

Two chemical classes of agent, the benzoic acids and the sulfonylureas, inhibit K_{ATP} and members of each class are able to displace the other (100). Although one interpretation would be that both classes share a common site, sufficient evidence exists to suggest that there are recognition sites for each class. These are separate yet closely associated in the lipid phase of the membrane (100, 122).

High and low affinity sites for [^{3}H]-glyburide are present in a variety of membranes and positive correlations exist between displacement of this ligand from either site and the physiological/therapeutic effects of the sulfonylureas. In the pancreatic β-cell, it appears that these agents inhibit K_{ATP} via an effect at the high affinity site (104), whereas the relative insensitivity of other tissues to glyburide (68, 120, 221) suggests that, in these, the low affinity site may be the more important.

Modification of several components in the K_{ATP} regulatory complex inhibits binding to the hypoglycemic domain. Thus, phosphorylation of Site 1 (130), G protein activation (107, 108, 110), and perhaps interaction of nucleotide diphosphates at the NDP site (127) inhibit ligand binding. In contrast, the interaction of nucleotides with Site 2 has no effect on glyburide binding (126, 130). (For further details on the interaction between hypoglycemic agents and their binding domain, see above.)

The K-channel Opener Site

Several attempts have been made to uncover the site of action of the K-channel openers. In some tissues, these agents displace [^{3}H]-glyburide (110, 113, 125, 126, 129), suggesting perhaps that they produce a conformational change in or near the hypoglycemic domain such that the opening of K_{ATP} is enhanced. Many studies with radioactively labeled openers have been performed using isolated membrane fractions in an attempt to detect a specific binding site, but without success. Recently, Bray & Quast (126) detected a binding site in *whole segments* of aorta using the ligand P-1075. They concluded that the binding sites for both the K-channel openers were not identical, but closely and negatively allosterically coupled.

In contrast, electrophysiological studies suggest that the K-channel openers directly or indirectly reduce the affinity of Site 2—the ATP-regulatory site—for ATP, which would result in channel opening (42, 48). Noack and co-workers recently drew a similar conclusion, following studies in single smooth muscle cells (93) that showed that the K-channels opened by levcromakalim or by ATP-depletion were identical and that no additional

effect of this agent could be detected during the development of the K-current induced by ATP-depletion. Furthermore, these authors (38, 93) also found evidence suggesting that the anomalous inhibition of K_{ATP} by K-channel openers reported in a variety of tissues (36, 39) could be due to the induction of a state of run-down by prevention of the phosphorylation of Site 1 by the openers. Both these effects at Sites 1 and 2 could be simply explained by a single process involving a reduction in the availability of ATP but further studies are required to test this possibility.

Ligand–Receptor–G protein Interactions

Evidence is growing that K_{ATP} is modulated by a variety of endogenous ligands. For example, the opening of this channel is believed to underlie some of the effects of adenosine in both cardiac (222) and smooth muscle (223). Furthermore, an important role for K_{ATP} in the vasodilator actions of acetylcholine, vasoactive intestinal polypeptide (VIP), and calcitonin gene-related peptide (CGRP) has been proposed by one group (78, 224, 225, 226). In neurones, K_{ATP} channels, linked presynaptically to a variety of receptor systems, probably serve to reduce transmitter release (227, 228), while in the pancreatic β-cell, adrenaline (149, 151) and possibly somatostatin and galanin (see later) open K_{ATP} and reduce insulin secretion.

ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR The discovery that acetylcholine and histamine could each release a hyperpolarizing agent from the vascular endothelium has been reviewed (229, 230). The substance, designated endothelium-derived hyperpolarizing factor (EDHF) (231) produces a marked hyperpolarization and associated increase in $^{86}Rb/^{42}K$ efflux from a variety of blood vessels, although these effects are relatively transient (231–236).

The K-channel opened by EDHF has not been unequivocally identified. Early studies reported that both the relaxation and associated hyperpolarization produced by EDHF in rabbit middle cerebral artery were glyburide-sensitive and it was concluded that the smooth muscle variant of K_{ATP} was involved (78, 224, 226). More recent studies in rabbit skeletal muscle and mesenteric resistance vessels have shown that adenosine diphosphate (ADP) produces mechanical relaxation and hyperpolarization, actions that were endothelium-dependent and glyburide-sensitive (237). These effects are also consistent with the release of EDHF and the subsequent opening of K_{ATP} channels. However, in a variety of other vessels, studies from several different laboratories have shown that the various effects of EDHF are not antagonized by glyburide (231, 233, 236, 238, 239).

These data may indicate that more than one hyperpolarizing factor can be liberated from the vascular endothelium, but there is little support for the

general view (226, 240) that the effects of EDHF in most vessels are mediated by the opening of K_{ATP}.

CALCITONIN GENE-RELATED PEPTIDE CGRP is a polypeptide located in neurones that form a close association with both central and peripheral blood vessels. In some, such as pig splenic arteries (241) and in bovine and porcine coronary vessels (242, 243), CGRP-induced relaxations are endothelium-independent. However, in others, including rat coronaries (244) and rat aorta (245, 246), the effects of CGRP are endothelium-dependent. In rat aorta, no CGRP-induced increase in cGMP occurs (246). However, relaxations are indomethacin-resistant but attenuated by L-NMMA (247) suggesting the involvement of the L-arginine/NO pathway at least in this tissue.

In rabbit mesenteric resistance arteries, CGRP was reported to hyperpolarize the vascular smooth muscle cells close to E_K and this electrical effect, together with part of the mechanical relaxation, was glyburide-sensitive (225). Whether these changes in whole blood vessels were endothelium-dependent was not reported. Using enzymatically separated cells in the same series of experiments, CGRP opened a K-channel of approximately 20 pS unitary conductance (quasi-physiological conditions), an action that was also glyburide-sensitive, although the ATP-dependency of the channel was not determined. These data, which are consistent with the involvement of the smooth muscle variant of K_{ATP}, contrast with those of other workers. Thus, a maximal relaxant concentration of CGRP only hyperpolarized feline cerebral anterior connecting arteries by a few millivolts (248) and no hyperpolarizing effect of CGRP was detected in pig coronary arteries (249). Furthermore, in rat coronary blood vessels and in the human mammary artery, the dilator effects of CGRP were not antagonized by glyburide (244, 250).

Collectively, these results suggest that a K-channel may be involved with a component of the CGRP-induced relaxation in certain blood vessels. Further data are clearly required to establish whether any such channel is K_{ATP} and the extent to which endothelium-derived factors such as EDHF (see above) play a part in the responses to this peptide.

VASOACTIVE INTESTINAL POLYPEPTIDE Vasoactive intestinal polypeptide (VIP), originally isolated from porcine intestine (251), relaxes a variety of smooth muscle systems. In certain blood vessels, such as cat cerebral artery and bovine coronary artery, the effects of VIP are endothelium-independent (252, 253) whereas in others, such as rat aorta and bovine intrapulmonary artery, little relaxation is seen in the absence of the endothelium (254, 255) from which VIP can release EDRF (256).

In rabbit middle cerebral artery, a brief microelectrode study showed that VIP hyperpolarized the cells and that this effect was reversed by glyburide

(78). Although the endothelium-dependence of the phenomenon was not established and no parallel mechanical or more detailed single-cell studies were performed, it was concluded that the effects of VIP were produced via the opening of K_{ATP} (78, 226). There is, however, little general support for this view. In a systematic study in rabbit mesenteric artery, Hattori et al (257) showed that glyburide had no effect on either the endothelium-dependent or -independent relaxant effects of VIP. In nonvascular tissues, such as canine colon (258), opossum esophagus, and canine intestine (259), the hyperpolarizing effects of VIP were relatively small or absent. In rat stomach, the VIP-induced hyperpolarization was associated with increases in both cAMP and cGMP (260) consistent with an earlier study in vascular smooth muscle (261).

The significance of the reported glyburide-sensitive effects of VIP in rabbit middle cerebral artery (78) requires further study. Furthermore, the general view (226, 240) that the effects of VIP are likely to be caused by the opening of K_{ATP} is not broadly substantiated by published data.

ADENOSINE Adenosine exerts widespread cardiovascular actions ranging from vasodilation to a reduction in heart rate via the A_1 and A_2 receptor systems (see 262). The detailed effects of adenosine in rat cultured ventricular myocytes have been studied by Kirsch et al (222) who showed that adenosine-induced activation of a G_i protein produced direct opening of cardiac K_{ATP} channels, an effect mediated via A_1 receptors (262). In vascular smooth muscle, no clear picture of the vasorelaxant effects of adenosine has emerged. In human pulmonary artery, adenosine-induced relaxation via A_2 receptors correlates well with an increase in cAMP levels (263). In contrast, in guinea-pig coronary arteries, the dilator effects of adenosine, together with those of cromakalim and following hypoxia, were inhibited by glyburide (264). Furthermore, Merkel et al (223) recently showed that the stable A_1 receptor-selective analog of adenosine, CPA, relaxed porcine coronary artery by a glyburide-sensitive mechanism whereas the stable A_2 agonist, DPMA, induced relaxations that were unaffected by this sulfonylurea.

Although further studies are clearly required, these recent observations suggest that the vascular A_1 adenosine receptor could be coupled to a K_{ATP} channel as in cardiac muscle.

GALANIN Galanin is a neuropeptide that, on release from nerves innervating pancreatic islets (265), hyperpolarizes β-cells and inhibits insulin release (265–269). Patch-clamp studies have demonstrated the ability of galanin to open K_{ATP} in insulinoma cells (RINm5F) and in mouse pancreatic β-cells (3, 267, 270). Since the effects of galanin are inhibited by pertussis toxin, K_{ATP} channel activation probably involves a G protein intermediate (270). Some

evidence indicates that galanin may also be released from neurons within the hippocampus (271). Although an effect by galanin on K_{ATP} in this tissue remains to be demonstrated, galanin was found to block anoxia-induced depolarization of hippocampal CA3 neurones and to inhibit glutamate release, effects consistent with K-channel opening. Since both of these effects of galanin were inhibited by glyburide, the involvement of K_{ATP} is suggested (272–274).

SOMATOSTATIN Like galanin, somatostatin is a hyperglycemic neuropeptide that inhibits insulin secretion from islet cells (275). In the presence of both ATP and GTP, somatostatin increases the open probability of K_{ATP} in insulinoma (RINm5F) cells (276). The inability of somatostatin to stimulate opening of K_{ATP} in the absence of GTP may indicate G protein coupling between the channel and the receptor (276). This is further supported by the finding that pertussis toxin prevents stimulation of ^{86}Rb efflux from insulinoma cells (227). Nevertheless, despite direct evidence for an effect of somatostatin on K_{ATP}, this agent is also capable of modulating the opening of other K-channel types (11, 278). In HIT insulinoma cells somatostatin does not stimulate K^+ efflux and it appears that in this cell type, inhibition of insulin secretion results from a reduction in calcium influx through voltage-dependent Ca^{2+} channels (279). Thus, the degree to which regulation of K_{ATP} opening contributes to the physiological effects of endogenous somatostatin remains to be established.

ENDOTHELIN In cultured porcine coronary artery cells, a single report (280) has shown that the open probability of the K_{ATP} channels described in this tissue (90, 91) was inhibited by endothelin. Such an observation, together with the finding that levcromakalim inhibits the binding of [^{125}I]-endothelin-1 to cardiac membranes could indicate that both agents interact at closely related sites on the K_{ATP} channel.

MISCELLANEOUS Indirect evidence suggests that several neurotransmitters may modulate K_{ATP}. Subsequent to the report that opioid μ and δ receptors may be linked to K-channels in the central nervous system (281), Ocana and coworkers (282) found that glyburide was capable of antagonizing morphine analgesia. These findings were confirmed and extended by Wild et al (283) with the report that, in mice, the antinociceptive effects of intracerebroventricular (i.c.v.) injections of morphine (a μ receptor agonist) and [D-Pen^2,D-Pen^5]enkephalin (a selective δ receptor agonist), but not U69,593 (kappa receptor agonist) were antagonized by glyburide (i.c.v.). Furthermore, pinacidil, which stimulates the opening of K_{ATP} (see section on K-channel openers) is capable of augmenting morphine analgesia (284). The inability of

glyburide to antagonize the effects of a second opioid receptor agonist, selective for δ receptors, [D-Ala^2]deltorphin II, (285) is thought to be due to the existence of δ receptor agonist subtypes (283).

Sulpiride (a D_2 receptor antagonist, 100 μM) was reported to produce a 90% inhibition of [^{3}H]-glyburide binding in the rat brain cortex (114). This compound is a sulfonamide, and its structural similarity to sulfonylureas might account for the inhibition of binding. Nevertheless, the inhibition of the response to the D_2 receptor agonist, quinpirole, by tolbutamide has been taken as evidence that dopamine may open K_{ATP} (286). Similarly, the $GABA_B$ agonist baclofen inhibited spontaneous firing in neurones freshly isolated from guinea-pig substantia nigra. Again, although tolbutamide had no effect alone, it inhibited the response to baclofen (286). In addition, adenosine is capable of stimulating the opening of K_{ATP} in cardiac tissue (222). A common feature of dopamine D_2, μ opioid, adenosine A_1 and $GABA_B$ receptors is that they are all linked to the opening of neuronal K-channels via G proteins (222, 287, 288). Although the available data are far from definitive, evidence is mounting that the above receptor systems are linked to K_{ATP}. When this is located presynaptically, the opening of this channel serves to reduce transmitter release (228) and may prove to be one of the most important presynaptic modulatory systems in the central nervous system.

ROLE OF G-PROTEINS GTP and GDP stimulate channel opening in β-cells by a mechanism that does not seem to involve phosphorylation (219). Run-down, a characteristic of K_{ATP}, may in part be due to loss of GTP or GDP (219). G protein activation is obligatory for the opening of some ion channels, for example the atrial muscarinic K-channel, but is thought to be modulatory in the case of K_{ATP} (289, 290). In skeletal muscle, K_{ATP} appears to be opened by a G protein-stimulated mechanism (291).

The G protein complex consists of α, β, and γ subunits, with the α-subunit associated with bound GDP (see 292). Currently 4 β- and 4 γ-subunits are known and there may be up to 20 different α-subunits. Different combinations of these subunits form distinct G protein types (see 289). Occupation of receptors linked to the G protein releases the GDP and promotes the binding of GTP, which stimulates the separation of the α-GTP complex from the βγ-subunit. It has been suggested that binding of the α-GTP complex to K_{ATP} produces a structural change that reduces the ability of ATP to interact with the inhibitory site and thus stimulates K_{ATP} opening (289; Figure 3). The α-subunits associated with the opening of K_{ATP} have been identified as of the α_i-type (cardiac myocytes; 222) and α_o (skeletal muscle; 291). The α-subunit possesses intrinsic phosphorylase activity that hydrolyses the GTP, terminating the interaction with the channel and allowing a reassociation of the α-GDP with the βγ subunit. Activation of channel opening via G proteins is obviously

dependent upon the presence of intracellular GTP. Since hydrolysis terminates the effect of the the α-GTP complex, K_{ATP} is irreversibly activated by the nonhydrolysable analog, GTPγS, although the inhibitory effect of ATP is still evident (222). In addition, GTPγS apparently displaces GDP from its binding site on the α-subunit of the G protein, allowing channel activation in the absence of receptor occupancy, although prolonged activation of K_{ATP} by α-GTPγS requires magnesium to stabilize the α-GTPγS complex (291).

It is interesting to postulate that not only might the α-GTP complex stimulate channel opening, but also that by interacting with the nucleotide diphosphate binding site described by Tung & Kurachi (216), GDP liberated on receptor occupancy might itself enhance the opening of K_{ATP}, thus amplifying the response. Under conditions in which a reduction in $[ATP]_i$ occurs, the intracellular concentration of other nucleotide diphosphates should also increase and this may additionally contribute towards an enhanced opening of K_{ATP}.

Protein Kinase C

Some evidence suggests that K_{ATP} is under the regulatory control of protein kinase C. On exposure to kinase C stimulators such as 4β-12-phorbolmyristate-13-acetate (PMA) or 1,2-didecanoylglycerol (DC_{10}), K_{ATP} channels in RINm5F cells initially close (294). However, exposure to these agents for periods greater than 5 min produces a marked increase in opening of K_{ATP} (276, 293) and prevents the inhibitory effect of ATP on channel opening (276). The significance of these observations has not been determined.

CONCLUSIONS

The Type 1 K_{ATP} channel clearly exists in a variety of tissues and its complex pharmacology is reviewed in this paper. No attempt has been made to extrapolate the detailed pharmacology of the channel itself to whole tissues or organisms. In particular, K_{ATP} may prove to be important in pathological conditions associated with hypoxia (for reviews, see 295–297).

K_{ATP} inhibitors have been successfully employed as hypoglycemic agents for many years. Therapeutic applications already exist for K-channel openers, or are in the process of clinical testing. Possibilities currently under evaluation include their use in hypertension, asthma, or the irritable bladder syndrome (298, 299). In addition, they are potentially useful as cardio- or neuro-protectants under ischemic or anoxic conditions (see 297, 300). A deeper understanding of the relative importance and mechanisms of action of the numerous modulators of K_{ATP} in different tissues may allow the future development of tissue-selective K_{ATP} regulators of enormous clinical benefit.

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