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Glibenclamide Opens ATP-Sensitive Potassium Channels in *Xenopus* Oocyte Follicular Cells during Metabolic Stress

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SUMMARY

Follicular cells from *Xenopus* oocytes offer a particularly interesting system to study ATP-sensitive K^+ channels (K_{ATP} channels). In these cells, as in many other cell types, glibenclamide is a classical blocker of K_{ATP} channels. Metabolic inhibition with dinitrophenol (DNP) converts this inhibitory effect into an activation. Follicular cells treated with DNP keep their sensitivity to the K_{ATP} channel opener P1060, but this opening effect becomes insensitive to glibenclamide inhibition. Glibenclamide activation of K_{ATP} channels in DNP-treated follicular cells occurs with an EC_{50} of 3 μM. Glibenclamide activation is antagonized by blockers of K_{ATP} channels that do not belong to the sulfonylurea family, such as U-37883A, tedisamil, and LH 35. Other sulfonylureas display the same activating behavior as does glibenclamide in DNP-treated cells. Two of the properties of K_{ATP} channels in follicular cells are activation by cAMP through protein kinase A and inhibition by muscarinic effectors through protein kinase C activation. The stimulating effects of cAMP and glibenclamide in DNP-treated cells seem to be synergistic, as are the cAMP and P1060 effects in control follicular cells. Glibenclamide-activated K_{ATP} channels in DNP-treated cells (conductance of 15 pS) are also inhibited by acetylcholine and by phorbol esters. The internal acidosis produced by metabolic exhaustion with DNP appears to be the key element in the conversion of glibenclamide from a blocker to an activator of K_{ATP} channels.

K_{ATP} channels in *Xenopus* follicular cells and in smooth muscle cells share common biophysical, pharmacological, and regulation properties (1–9). They have a low unitary conductance (19 pS in a physiological K^+ gradient) (5), they are blocked by relatively high concentrations of glibenclamide (IC_{50} of about 200 nM) (4), and they are opened by synthetic vasorelaxing factors, including the pinacidil derivative P1060, cromakalim, and RP49356 (4). They are opened by endogenous hyperpolarizing vasorelaxing factors such as calcitonin gene-related peptide, vasoactive intestinal peptide, and prostaglandin E_2 (3) via cAMP-dependent protein kinase A phosphorylation (5). They are blocked by protein kinase C phosphorylation (4). Previous work from this laboratory has shown that pituitary gonadotropins, which act on specific receptors, open K_{ATP} channels (by cAMP-dependent protein kinase A phosphorylation) in follicular cells attached to the oocyte, hyperpolarize the oocyte because of the electrical coupling through gap junctions, and thereby promote oocyte maturation by sensitizing the oocyte to the effect of progesterone (10).

A general property of K_{ATP} channels is to open when intracellular concentrations of ATP are decreased (11–13). Another general property of K_{ATP} channels in different tissues is that they are blocked by glibenclamide and related antidiabetic sulfonylureas (14–18).

This work describes the properties of K_{ATP} channels in follicular cells under various metabolic conditions and reports that, surprisingly, during metabolic inhibition K_{ATP} channels in follicular cells are activated instead of being inhibited by glibenclamide and some other sulfonylureas.

Materials and Methods

***Xenopus* oocyte isolation procedure.** *Xenopus laevis* were purchased from the Centre de Recherche de Biochimie Macromoléculaire CNRS (Montpellier, France). Frogs were anesthetized with ice, and pieces of the ovary were surgically removed. Individual oocytes were dissected away in a saline solution (ND96) containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, at pH 7.4 (adjusted with NaOH). Stage V–VI oocytes were then kept for 1–3 days at 4° in saline solution (ND96). For complete removal of follicular cells, oocytes were treated with 1 mg/ml collagenase (type IA; Sigma) for 2 hr and then the surrounding follicular cell layer was manually removed with fine forceps (5).

Electrophysiological experiments. Experiments were performed at room temperature (20–22°). Oocytes were impaled with two glass microelectrodes filled with 3 M KCl (1–2-MΩ resistance)

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ABBREVIATIONS: K_{ATP} channel, ATP-sensitive K^+ channel; DMSO, dimethylsulfoxide; DNP, dinitrophenol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; PMA, phorbol myristate acetate; $[K^+]_{ext}$, external K^+ concentration.

and were voltage clamped using a Dagan TEV-200 voltage-clamp amplifier. The virtual current monitor/bath-clamp mode was used (i) to record the total bath current at ground potential and (ii) to clamp the bath to ground. To achieve both of the aforementioned functions, one pellet reference electrode that senses bath voltage and a silver chloride wire that passes current were both connected to the virtual current monitor headstage of the amplifier. A holding potential of −20 mV, which is the predicted value for the Cl^- equilibrium potential, was routinely used (unless indicated). This protocol minimized possible influences of chloride on the recorded ionic currents. In some experiments, drugs were applied into the experimental chamber within 1 sec by a puffer pipette (250 μl). The chamber was perfused using a peristaltic pump at a flow rate of 300 μl/min. The standard saline ND96 solution was used in all experiments. A CO_2-enriched solution was prepared by bubbling 100% CO_2 into the standard ND96 solution for 5 min. An acetate solution was made by replacing 96 mM NaCl with 96 mM sodium acetate. For experiments with acidic external solution, HEPES was replaced with MES and the pH was adjusted to 5.3 with NaOH. All chemicals used were from Sigma unless otherwise stated. Stimulations, data acquisition, and data analysis were performed with the software pClamp (Axon Instruments). The variability of the results is expressed as the standard error, with *n* indicating the number of cells contributing to the mean.

The patch-clamp technique was used with isolated follicular cells to record single-channel currents (5). Pipettes were coated with Sylgard resin to reduce pipette capacitance and current noise. An RK300 patch-clamp amplifier (Bio-Logic, Claix, France) equipped with a 10-GΩ feedback headstage was used for single-channel recordings. Currents were stored in digitized form on digital audio tapes, using a Bio-Logic DTR1201 recorder, for further analysis. Currents flowing out of the pipette in the outside-out configuration are represented as positive. A 1-kHz filter was used and the sampling frequency was 10 kHz. The cut-off frequency of the chart recorder (BD 100; Kipp and Zonen, Delft, Holland) was 5 Hz.

Current analysis was done with the software Biopatch (Bio-Logic). For each experimental condition, NP_o (number of channels × mean open probability of the single channels) was calculated over 60 sec by dividing the mean patch current (*I*) by the single-channel current amplitude (*i*).

The internal Ca^{2+}-free medium, pH 7.2 (adjusted with KOH), contained 115 mM KCl, 1 mM $MgCl_2$, 5 mM EGTA, and 10 mM HEPES. The external solution was ND96. P1060 stock solution was prepared in DMSO at a concentration of 100 mM. Stock solutions of glibenclamide and derivatives were prepared in DMSO at a concentration of 10 mM. Parallel control experiments were performed with DMSO to establish the lack of effect of the solvent at the concentrations used.

Results

Fig. 1A shows that a puff application of the K^+ channel opener P1060 (10 μM), a pinacidil derivative, induced a large outward current (335 ± 21 nA, n = 10) in a follicular oocyte voltage-clamped at −20 mV. This response has previously been attributed to the opening of glibenclamide-sensitive K_{ATP} channels in follicular cells (5). A puff application of glibenclamide (10 μM) under the same experimental conditions did not induce any current (Fig. 1B), although it completely blocked the P1060 response under control conditions (2). Addition of the metabolic inhibitor DNP (1 mM) to the superfusing medium induced, after a delay of 2 ± 1 min (n = 20), a small transient outward current (102 ± 11 nA, n = 20) (Fig. 1, A and B). The size of the DNP response was quite variable among different oocytes from different frogs.

In the continuous presence of DNP, a puff application of glibenclamide (10 μM) elicited a substantial outward current

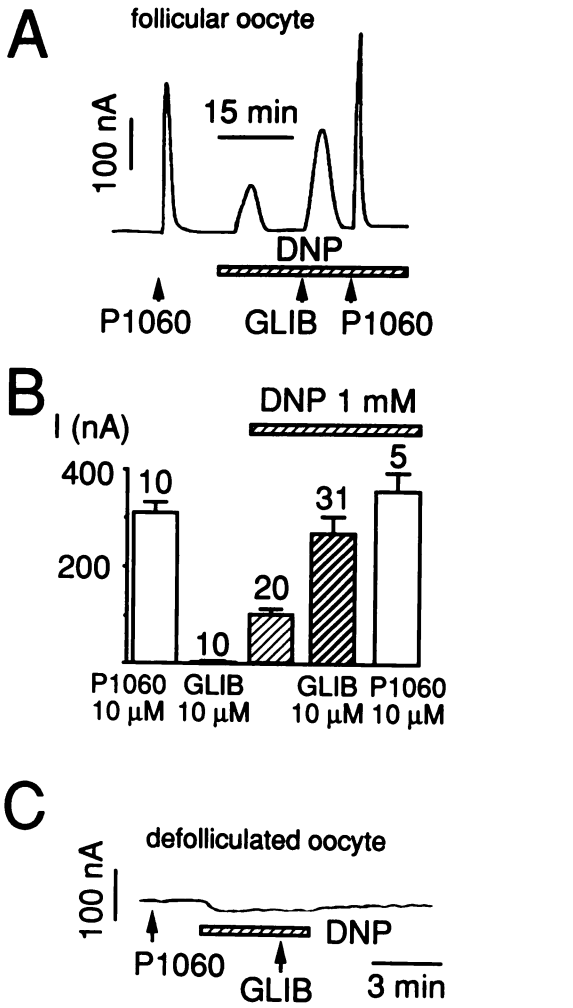

Fig. 1. A, A puff application of P1060 (10 μM) induced a large transient outward current in a follicular oocyte voltage-clamped at −20 mV. In the same follicular oocyte, superfusion of 1 mM DNP activated a small and transient outward current. In the continuous presence of DNP (1 mM), puff applications of glibenclamide (*GLIB*) (10 μM) and then P1060 (10 μM) activated outward currents. *Arrows*, puff applications; *striped horizontal bar*, DNP superfusion. B, An amplitude histogram shows the effects of DNP (1 mM) on glibenclamide (10 μM) and P1060 (10 μM) responses in follicular oocytes. *Numbers above the bars*, numbers of oocytes. *Striped horizontal bar*, presence of DNP. C, The effects of P1060 (10 μM), DNP (1 mM), and glibenclamide (10 μM) on a defolliculated oocyte are shown. *Arrows*, puff applications; *striped horizontal bars*, superfusions.

(274 ± 27 nA, n = 31) (Fig. 1A). The histogram shown in Fig. 1B compares current amplitudes induced by P1060 and glibenclamide both under control conditions and during metabolic inhibition with DNP (1 mM). In the presence of DNP, the amplitude of the glibenclamide response was not significantly different from the P1060 response (Fig. 1B). Addition of 1 mM cyanide and 2 mM iodoacetate, either in the presence or in the absence of glibenclamide (10 μM), did not induce any current (n = 5) (data not shown).

Follicular cells were removed by using a combination of collagenase treatment and manual dissection (4, 5). Defolliculated oocytes (n = 12) did not show any response to either P1060 or glibenclamide, either in the absence or in the presence of DNP (Fig. 1C).

Fig. 2A shows the dose-effect relationship of the DNP-induced outward current in follicular oocytes (in the absence

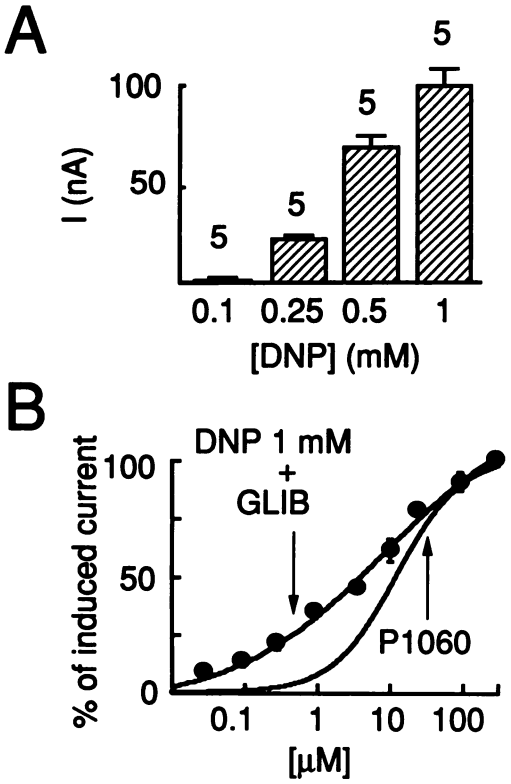

Fig. 2. A, Dose-response histogram for DNP in follicular oocytes. *Numbers above the bars*, numbers of oocytes. B, Dose-response curve for the glibenclamide-activated current (n = 13) in the presence of DNP (1 mM). The EC_{50} was 3 μM. For comparison, the dose-response curve for P1060 under control conditions is presented. The EC_{50} was 13 μM (2). *GLIB*, glibenclamide.

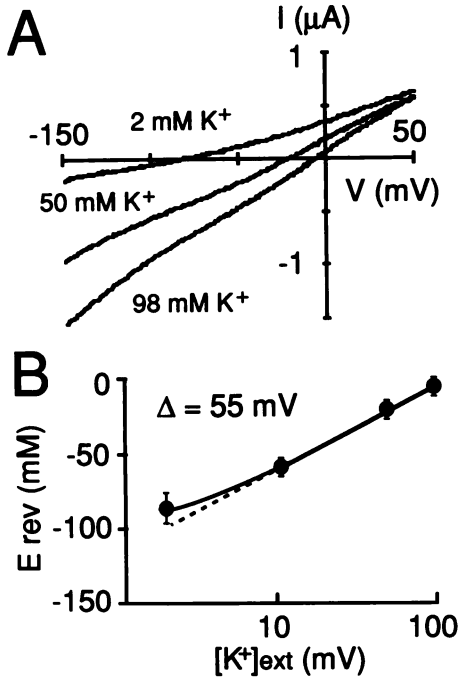

Fig. 3. A, Current-voltage curves for the glibenclamide-activated currents (with 1 mM DNP) recorded in the same follicular oocyte in the presence of 2 mM K^+, 50 mM K^+, and 98 mM K^+ in the external medium. Current-voltage curves were measured during 500-msec voltage ramps from a holding potential of −150 mV. B, Relationship between the reversal potential (n = 3) and the logarithm of the extracellular K^+ concentration (*E rev*). A linear relationship was extrapolated from the experimental data (*dashed line*) measured in K^+-rich solutions. The reversal potential changed by 55 mV/decade.

of P1060 and glibenclamide). The threshold concentration was 100 μM and the amplitude of the current increased in a dose-dependent manner. Fig. 2B shows the dose-response curve for current activation by glibenclamide in the presence of 1 mM DNP. The EC_{50} was 3 μM. The dose-effect curve for P1060 under control conditions is shown for comparison (2).

Current-voltage relationships for the glibenclamide-induced current (in the presence of DNP) are presented in Fig. 3A. Current-voltage curves were measured with a voltage ramp protocol in the presence of increasing K^+ concentrations in the same follicular oocyte. The reversal potential was shifted to positive values when $[K^+]_{ext}$ was increased. Fig. 3B shows that the reversal potential was close to E_K. Its dependence on $[K^+]_{ext}$ is consistent with a channel that is selective for K^+ over Na^+, with a permeability ratio P_{Na}/P_K of 0.02. The slope conductance calculated from the current-voltage curves presented in Fig. 3A increased as $[K^+]_{ext}$ was raised. The slope conductances measured at the reversal potential were 3.1 μS, 8.1 μS, and 11.5 μS at $[K^+]_{ext}$ of 2 mM K^+, 50 mM K^+, and 98 mM K^+, respectively.

Fig. 4A shows that, under conditions of metabolic inhibition, glibenclamide lost its inhibitory effect on the P1060 response. In the presence of DNP, instead of having antagonistic effects glibenclamide and P1060 induced additive currents (Fig. 4A). Outward currents activated by either P1060 or glibenclamide (in the presence of DNP) were fully inhibited by the class III antiarrhythmic agent tedisamil (10 μM) (Fig. 4B), which is also a blocker of K_{ATP} channels in smooth muscle (15, 17, 19). Fig. 4C shows that the glibenclamide-activated current was resistant to charybdotoxin (50 nM), a specific blocker of large-conductance, Ca^{2+}-dependent, K^+ channels (20). The glibenclamide-induced K^+ current was fully inhibited by U-37883A, a specific blocker of K_{ATP} in smooth muscle and follicular cells (2).

Fig. 4D shows that, in the presence of DNP, LH 35, a glibenclamide-like molecule that lacks the sulfonylurea group (Fig. 5), blocked both P1060- and glibenclamide-induced responses. We thus compared the effects of various sulfonylurea derivatives, at a concentration of 10 μM, on follicular oocytes in the presence of 1 mM DNP (Fig. 5). Glibenclamide, LH 4, LH 25, LH 26, and LH 29 activated outward currents during metabolic inhibition. All of these compounds (activators and inhibitors) completely reversed channel opening induced by P1060 (30 μM) under control conditions.

DNP is a metabolic inhibitor that uncouples the H^+ gradient in mitochondria and thereby decreases ATP biosynthesis. DNP has been reported to induce internal acidosis in various cells (for review, see Ref. 21). Therefore, the following experiments were carried out to analyze the possible contribution of this acidosis to the change of properties of glibenclamide (activation instead of inhibition) in the presence of DNP. A classical way to acidify the internal medium is to use the ammonia prepulse technique (22). When cells are incubated in the presence of NH_4Cl, NH_3 rapidly enters the cytoplasm, associates with intracellular H^+, and produces rapid cell alkalinization. The internal pH then slowly returns to normal values as a consequence of the operation of internal pH-regulating systems and the transmembrane movements

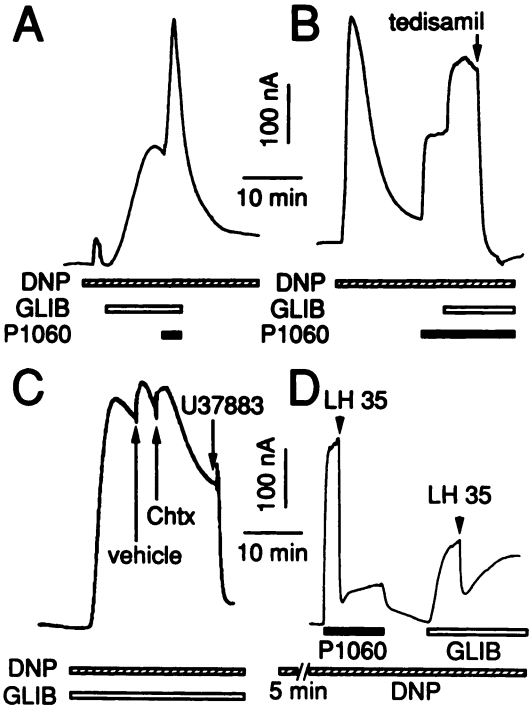

Fig. 4. A, P1060 (10 μM) and glibenclamide (*GLIB*) (10 μM) produced additive outward currents in the continuous presence of DNP (1 mM). B, Tedisamil (10 μM) inhibited both glibenclamide (10 μM)- and P1060-activated currents in the presence of DNP (1 mM). C, The glibenclamide (10 μM)-activated outward current in the presence of 1 mM DNP was insensitive to charybdotoxin (*Chtx*) (50 nM), although it was fully inhibited by a puff application of U-37883 (10 μM). The control ND96 solution puff application (vehicle) was applied before K^+ channel blockers. D, In the presence of DNP (1 mM), puff applications of LH 35 (10 μM) blocked both P1060 (10 μM) and glibenclamide (10 μM) responses.

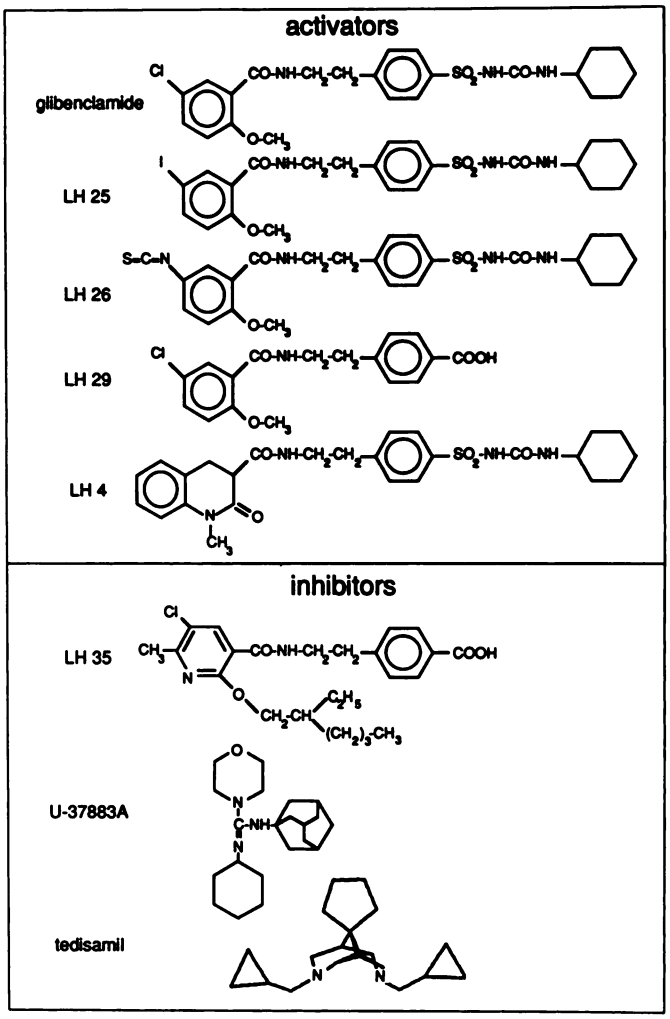

Fig. 5. K_{ATP} channel effectors. The effects of various K^+ channel effectors were tested at a concentration of 10 μM, with follicular oocytes clamped at −20 mV, in the presence of DNP (1 mM). Activators are compounds that activate a K^+ current in the presence of DNP (1 mM). Inhibitors are compounds that inhibit the glibenclamide (10 μM)-activated current observed in the presence of DNP (1 mM). All of these compounds (activators and inhibitors) blocked the P1060 (30 μM) response in the absence of DNP.

of NH_4^+. When external ammonia is removed it causes the rapid efflux of intracellular ammonia in the uncharged form (NH_3), hence loading the cells with H^+. During the NH_4^+ (20 mM) superfusion (i.e., internal alkalinization), glibenclamide did not induce any current (Fig. 6A). During the subsequent wash-out of the NH_4^+ solution (i.e., during internal acidosis), a small outward current was recorded and the addition of glibenclamide potentiated this current (Fig. 6A). Similarly, glibenclamide induced a large outward current in oocytes treated with acetate, which is known to produce internal acidosis because it readily enters the cell in the neutral form and subsequently dissociates to produce intracellular H^+ (Fig. 6B). A CO_2-bubbled solution has also been used to induce intracellular acidosis. Because the membrane permeability to CO_2 is high, carbonic anhydrase produces both H^+ and HCO_3^- intracellularly (22). The bicarbonate transport system then acts as a cell-acidifying mechanism because of the extrusion of HCO_3^-. Fig. 6C shows that during superfusion of a CO_2-enriched solution the addition of glibenclamide induced a large outward current. Fig. 6D shows that in the presence of an acidic external solution (external pH of 5.3) no effect of glibenclamide could be detected, although the K^+ channel opener P1060 could still induce an outward current.

It was previously shown that K^+ channels that were activated by P1060 in follicular cells could be blocked by muscarinic stimulation and this effect was mimicked by protein kinase C activation with the phorbol ester PMA (3, 4). Fig. 7A shows that three consecutive puff applications of glibenclamide produced repetitive responses. The third glibenclamide response was fully inhibited in the presence of acetylcholine (10 μM) or PMA (60 nM) (Fig. 7, B and C). We also previously demonstrated that chlorophenylthio-cAMP, a permeant analogue of cAMP, induced a glibenclamide-sensitive K^+ current comparable in size to the P1060-activated K^+ current in follicular oocytes and that there was synergy between the cAMP-dependent activation and the P1060 activation of K_{ATP} channels (4). Fig. 7D shows that, in the presence of DNP, glibenclamide potentiated the activating effect of chlorophenylthio-cAMP instead of inhibiting it.

The tight-seal patch-clamp technique was used to identify, at the single-channel level, K^+ channels opened by glibenclamide in isolated follicular cells. In the cell-attached configuration, superfusion of P1060 (10 μM) opened 15-pS K_{ATP} channels in a physiological K^+ gradient (Fig. 8A). Under these control conditions, addition of glibenclamide to the external medium did not induce channel opening (Fig. 8A). In the cell-attached configuration, DNP superfusion induced

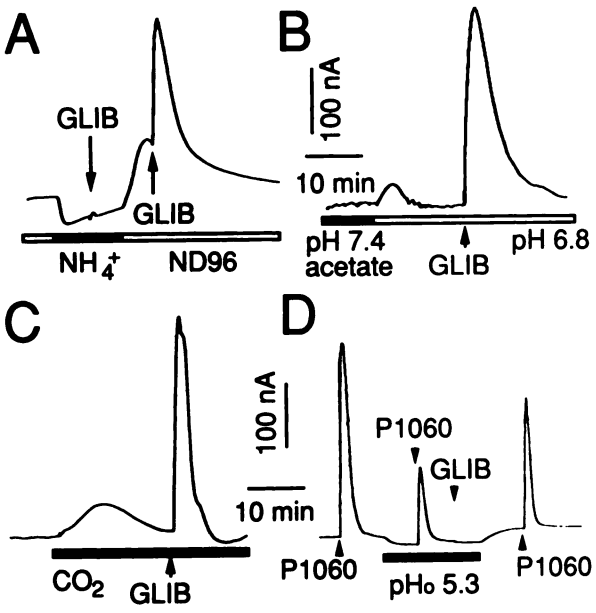

Fig. 6. A, Effects of a NH_4^+ (20 mM) superfusion pulse on the glibenclamide (*GLIB*) (10 μM) response. B, Effects of 96 mM sodium acetate, at an external pH of 7.4 (*black line*) or an external pH of 6.8 (*white line*), on the glibenclamide (10 μM) response. C, Effects of the addition of a CO_2-enriched solution on the glibenclamide (10 μM) response. D, Effect of external pH on P1060 (10 μM) and glibenclamide (10 μM) responses. *Arrows*, puff applications; *horizontal bars*, superfusions.

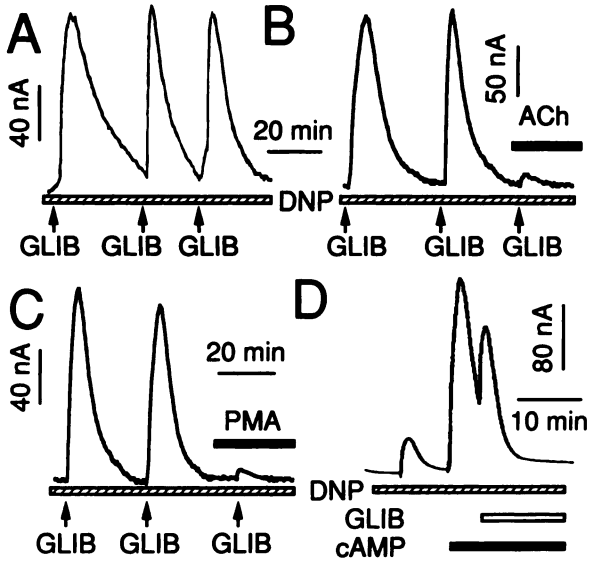

Fig. 7. A, Three successive puff applications of glibenclamide (*GLIB*) (10 μM) produced similar responses in a follicular oocyte in the presence of 1 mM DNP. B, Effects of the addition of 10 μM acetylcholine on the glibenclamide (10 μM) response in the presence of 1 mM DNP are shown. C, Effects of PMA (60 nM) on the glibenclamide (10 μM) response in the presence of 1 mM DNP are shown. D, In the presence of DNP (1 mM), chlorophenylthio-cAMP (*cAMP*) produced a transient activation (300 μM), which was increased by glibenclamide (10 μM). *Arrows*, puff applications; *horizontal bars*, superfusions.

channel opening after a delay of about 1 min (Fig. 8B). The channels opened by DNP and P1060 had similar single-channel current amplitudes (Fig. 8B). Addition of glibenclamide in the presence of DNP enhanced channel opening (Fig. 8B). The mean NP_o values calculated in the presence of P1060, DNP, and DNP with glibenclamide were 0.21 ± 0.02 (n = 5), 0.19 ± 0.07 (n = 5), and 0.35 ± 0.08 (n = 5), respectively (Fig. 8C). Addition of DNP (1 mM) to the cytosolic face of an inside-out patch (with or without 200 μM ATP), either in the absence or in the presence of glibenclamide (10 μM), did not open K_{ATP} channels (n = 5) (data not shown). The experiment shown in Fig. 8D started in the cell-attached configuration in the presence of DNP and glibenclamide. When channel activity reached its maximum, the patch was excised in the inside-out configuration (Fig. 8D). Channel activity was maintained in the inside-out patch, and the addition of 1 mM ATP to the internal medium reversibly blocked channel activity (Fig. 8D). The mean NP_o decreased from 0.21 ± 0.02 to 0.02 ± 0.01 (n = 3) when 1 mM ATP was added to the internal medium. Fig. 8E shows the current-voltage curve for channels opened by the addition of DNP and glibenclamide in the cell-attached configuration. Fig. 8E, *inset*, shows a current trace recorded at a membrane potential of −30 mV. The slope conductance of the current-voltage curve between −70 mV and +20 mV was 15 ± 2 pS (n = 3), and the extrapolated reversal potential was −82 mV. Fig. 8F shows that superfusion of a CO_2-enriched external solution mimicked the effect of DNP in the cell-attached configuration. Furthermore, in the presence of CO_2, the addition of glibenclamide enhanced channel opening (Fig. 8F). The mean NP_o values calculated in the presence of P1060, CO_2, and CO_2 with glibenclamide were 0.30 ± 0.04 (n = 4), 0.10 ± 0.01 (n = 4), and 0.20 ± 0.06 (n = 4), respectively. In the inside-out configuration (with or without internal 200 μM ATP), lowering of the internal pH (pH 6.0), either in the absence or in the presence of glibenclamide (n = 5), did not induce channel opening (data not shown).

Discussion

DNP treatment, which produces metabolic exhaustion (decrease of the internal ATP concentration and increase of the internal ADP concentration), activates K_{ATP} channels in follicular cells, as in many other cellular types in which K_{ATP} channels are present (23, 24). This activation is not (or is not easily) observed with cyanide treatment, which blocks the terminal cytochrome oxidase reaction of oxidative phosphorylation. This paper shows that, under conditions of metabolic blockade of follicular cells by DNP, glibenclamide, a classical inhibitor of K_{ATP} channels (2, 4, 10), becomes a K^+ channel activator.

The properties of the channel activated by glibenclamide in DNP-treated follicular cells are as follows. (i) Activation by glibenclamide (10 μM) is nearly as great as activation by the classical K^+ channel opener P1060, a pinacidil derivative. (ii) The half-maximal effect of glibenclamide is observed at 3 μM. (iii) The conductance of the glibenclamide-activated K^+ channel in DNP-treated cells is 15 ± 2 pS, very similar (if not identical) to the conductance of follicular K_{ATP} channels activated by ATP depletion or by activation by classical K_{ATP} channel openers (5). (iv) The glibenclamide-activated K^+ channel is inhibited by ATP applied to the intracellular face of the membrane. (v) P1060 and glibenclamide produce additive outward currents during metabolic inhibition, suggesting the existence of two separate sites of action for the two different drugs. (vi) The glibenclamide-activated K_{ATP} channel is blocked by a series of compounds such as tedisamil and U-37883A, which are structurally unrelated to antidiabetic

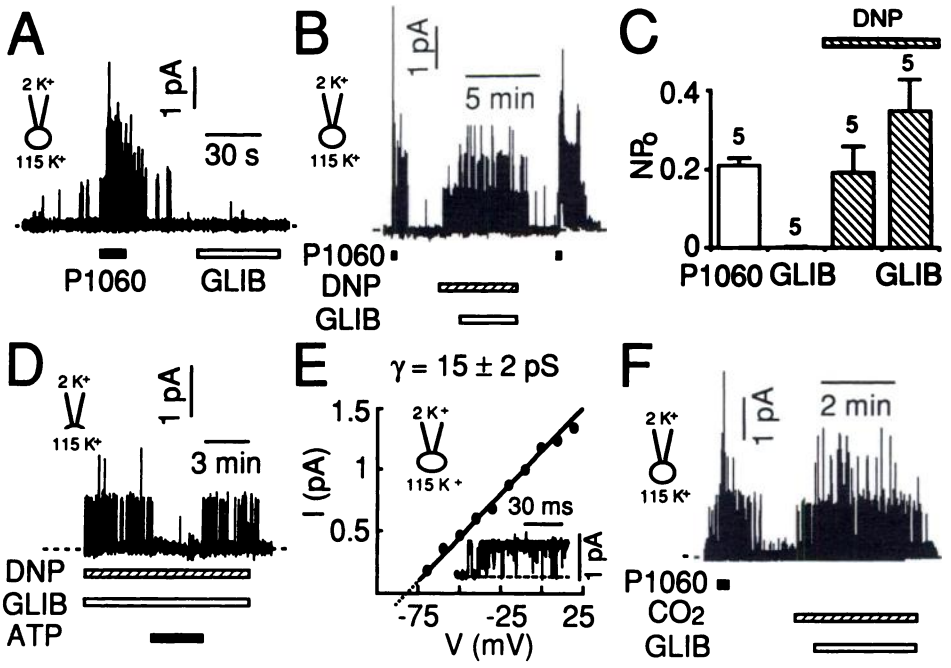

Fig. 8. A, Cell-attached patch single-channel recording on a follicular cell under control conditions. The pipette medium contained the ND96 solution (2 mM K^+) and the bath solution was the internal medium (115 mM K^+). *Horizontal bars*, P1060 (30 μM) and glibenclamide (*GLIB*) (10 μM) superfusions. B, Same as in A but in the presence of 1 mM DNP. C, Mean NP_o recorded in the cell-attached configuration (n = 5) under various experimental conditions, as indicated. *Horizontal striped bar*, DNP (1 mM) addition. D, Effects of internal ATP (1 mM) on channel activity recorded in a patch excised in the inside-out configuration during metabolic inhibition with DNP (1 mM) and in the presence of 10 μM glibenclamide. E, Current-voltage curve for the channels activated by glibenclamide (10 μM) in the presence of DNP (1 mM) in the cell-attached configuration. The slope conductance determined by a linear regression was 15 ± 2 pS (n = 3). *Inset*, current trace recorded at −30 mV. F, Same as in B but in the presence of an external CO_2-enriched solution instead of DNP.

sulfonylureas and which block K_{ATP} channels activated by ATP depletion or by classical K^+ channel openers (P1060) (2, 17, 19).

A number of other sulfonylureas, such as LH 4, LH 25, and LH 26, produce the same activation of K_{ATP} channels as does glibenclamide (Fig. 5). LH 29, which acts on K_{ATP} channels like sulfonylureas but which is not a sulfonylurea, behaves like glibenclamide in DNP-treated cells (Fig. 5). The glibenclamide derivative LH 35, which is not a sulfonylurea (Fig. 5), inhibits the activating effects of glibenclamide in DNP-treated cells.

DNP is a protonophore that acts by dissipating the protonmotive force across the inner mitochondrial membrane (21). DNP, by uncoupling oxidative phosphorylation, produces a decrease in intracellular ATP levels, an increase in intracellular ADP levels, and an internal acidification. The most obvious candidate to produce the changes of glibenclamide properties during DNP treatment is internal pH. Activation of K_{ATP} channels by glibenclamide was also observed in situations in which the intracellular medium was acidified by NH_4^+ treatment, by acetate treatment, or by exposure to a CO_2-enriched solution.

The change of K_{ATP} channel pharmacology with respect to glibenclamide requires the integrity of the follicular cell. The agonist properties of this sulfonylurea have been observed after whole-cell treatment with DNP or with agents that produce internal acidification. DNP treatment or application of an acidic solution to inside-out patches did not activate K_{ATP} channels in the absence or in the presence of glibenclamide. A possible explanation of these results would be that metabolic exhaustion with DNP and/or intracellular acidification leads to the release of a component that drastically modifies the pharmacological behavior of glibenclamide. The recent isolation of a clone producing glibenclamide-insensitive K_{ATP} channel activity suggests that the sulfonylurea receptor is an auxiliary subunit of the channel (25). Therefore, it may be that internal acidification modifies interactions between the channel subunit and the sulfonylurea receptor subunit.

It remains to be seen how the results obtained with follicular cells can be extrapolated to other cellular systems. It has been observed previously that metabolic inhibition in various cell types leads to the opening of glibenclamide-sensitive K_{ATP} channels. It has also been observed that K_{ATP} channels become resistant to glibenclamide during prolonged metabolic inhibition in both cardiac and skeletal muscle cells (23, 24).

It also remains to be seen how these observations apply to disease states. Anoxia, ischemia, and fatigue can lead to intracellular acidification in many cell types containing K_{ATP} channels. One particularly interesting cellular type in relation to observations made in this paper is smooth muscle, and particularly vascular smooth muscle, because K_{ATP} channels in vascular smooth muscle seem to have properties very similar to those described for follicular cells (for a review, see Ref. 16).

Acknowledgments

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