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Eosinophils play a key role in the pathogenesis of asthma and other allergic inflammatory diseases. We have previously shown that treatment of eosinophils with lidocaine preferentially inhibits IL-5-induced survival. This inhibition cannot be overcome by increasing concentrations of IL-5 and is not due to the blocking of Na\(^+\) channels by lidocaine. Here we report that one class of K\(^+\) channel blockers, the sulfonylureas, inhibits eosinophil survival in a manner similar to lidocaine. The sulfonylurea glyburide inhibits eosinophil survival even at high concentrations of IL-5. In contrast, increasing concentrations of IL-3 or granulocyte-macrophage CSF overcome glyburide inhibition. Glyburide also blocks cytokine-induced eosinophil superoxide production. Similar results were seen with the sulfonylureas tolbutamide and glipizide. Interestingly, the effects of glyburide are not antagonized by the ATP-sensitive K\(^+\) channel openers cromakalim, pinacidil, or diazoxide. Although Scatchard analysis of \(^{3}\text{H}\)glyburide binding to eosinophil membranes indicated that the high affinity sulfonylurea receptor (SUR1) is not present on eosinophils, human eosinophils do express mRNA homologous to the sulfonylurea receptor family, in keeping with the presence of a sulfonylurea receptor. Finally, coculture of eosinophils with combinations of glyburide, lidocaine, and dexamethasone resulted in synergistic inhibition of cytokine-mediated eosinophil survival and superoxide production. These results have intriguing clinical implications for the treatment of eosinophil-associated diseases.


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uman asthma is characterized by reversible bronchial hyperreactivity and infiltration of inflammatory cells, especially eosinophils, into the lung (1, 2). In recent years it has become obvious that treating the underlying inflammation is critical to maintaining good airway function (3). The most potent agents currently used for controlling inflammation are the glucocorticoids. However, the adverse effects of long term treatment with oral glucocorticoids have stimulated efforts to identify effective anti-inflammatory substitutes (4, 5). We have previously reported the clinical efficacy of the topical anesthetic lidocaine in the treatment of asthma in an open label trial (6). Treatment of glucocorticoid-dependent patients with nebulized lidocaine allowed for the complete elimination of steroid treatment in 13 of 20 patients, although exacerbations still required oral steroids. This study suggests that lidocaine functions as an anti-inflammatory agent and is thus able to replace long term oral steroid treatment.

Lidocaine, like the glucocorticoids, may partially exert anti-inflammatory effects by directly inhibiting eosinophil survival and activation in the lung. In the absence of exogenous cytokines, such as IL-5, IL-3, or GM-CSF, eosinophils undergo apoptosis (7–9). This mode of cell death with consequent engulfment by macrophages appears to be critical for preventing the release of toxic eosinophil granule proteins into sensitive tissues, such as the lung (7–11). If, however, cytokines are present (as they are in bronchial lavage fluid from patients with asthma (12)) eosinophil survival is prolonged, granule proteins are released (13), and eosinophil apoptosis is substantially delayed (12). We reported previously that lidocaine treatment of eosinophils in vitro ablates cytokine-induced survival in a manner similar to dexamethasone (14–16). Other topical anesthetics tested blocked eosinophil survival as well, but their potencies in the survival assay did not correlate with their potencies as anesthetics (17). Additionally, Na\(^+\) channel blockers that should mimic the anesthetic effect of lidocaine had no effect on cytokine-stimulated eosinophil survival in vitro (17, 18). These results support the hypothesis that the effect of lidocaine on eosinophil survival is a novel function of the drug.

Several reports in the literature indicate that lidocaine at high concentrations blocks K\(^+\) channels (19–21). Using three classes of K\(^+\) channel blockers, we investigated the effect of K\(^+\) channel regulation on eosinophil survival. Here we report that the sulfonylureas, one class of K\(^+\) channel blockers, inhibit eosinophil survival and activation in vitro.

Materials and Methods

Materials

HIT T15 cells were obtained from American Type Culture Collection (Rockville, MD). Human rIL-5 was a gift from Schering Plough (Kenilworth, NJ). IL-3 and GM-CSF were purchased from R&D Systems (Minneapolis, MN). Tolbutamide, glipizide, apamin, tetraethylammonium chloride (TEA), diazoxide, and pinacidil were purchased from Research Biochemicals International (Natick, MA). Unless otherwise indicated, all other materials were purchased from Sigma (St. Louis, MO).

Eosinophil purification

Eosinophils were purified from normal human subjects and patients, usually allergic, with slightly elevated numbers of eosinophils using a magnetic bead separation protocol previously described (22, 23). Briefly, blood was layered on Percoll (density = 1.085 g/ml) and centrifuged at 900 × g to remove PBMC and hypodense (activated) eosinophils. Erythrocytes
were lysed by osmotic shock, and neutrophils were removed from the eosinophils by separation using a steel wool column (type CS) and anti-CD16-conjugated magnetic beads (Miltenyi Biotec, Auburn, CA). Eosinophil purity exceeded 95% as determined by Randolph’s stain. The primary contaminating cells were neutrophils.

**Survival assays**

Purified eosinophils were washed with HybriCare medium (American Type Culture Collection) supplemented with 2 mM glutamine, 50 μg/ml gentamicin sulfate, and 2.5 to 10% α calf serum (HyClone, Logan, UT). Eosinophils were resuspended at 0.5 × 10^6 cells/ml in supplemented HybriCare and were incubated with drugs and cytokines in 96-well plates (Costar, Cambridge, MA) as indicated in the text. Drugs that were not directly soluble in media were first dissolved in DMSO and diluted to the appropriate concentrations in media. Unless indicated, the final DMSO concentration in the well never exceeded 0.5%, which was shown in control experiments to have no effect on eosinophil survival. The final concentration of eosinophils was 5 × 10^4/well in a total volume of 200 μl. Plates were incubated at 37°C in 5% CO₂ for 4 days. At that time, each well was transferred into 12- × 75-mm polystyrene tubes (Falcon, Becton Dickinson Labware, Lincoln Park, NJ), and 200 μl of propidium iodide (PI; final concentration, 0.5 μg/ml) were added to each tube to stain dead cells. Stained cells were analyzed using a FACS™ flow cytometer and PC LYSIS software (Becton Dickinson Immunocytometry Systems, San Jose, CA). Eosinophil survival is plotted as relative percent survival (percentage of the control) according to the following equation: relative eosinophil survival = (% survival/100 × survival at maximum cytokine concentration) × 100. Eosinophils cultured with IL-5 (1000 pg/ml; = 22 pM) usually showed >90% survival, whereas cells cultured in control medium usually had <30% spontaneous survival.

**Apoptosis assay**

Eosinophil apoptosis was assayed using flow cytometry and the DNA-binding fluorophores Hoechst 33342 (Molecular Probes, Eugene, OR) and PI as previously described (24, 25). Following 48 h of stimulation, eosinophils were stained with 10 μM Hoechst and 32 μM PI and analyzed on a Becton Dickinson FACS™ flow cytometer (San Jose, CA) with UV laser excitation at 357 nm. Dead cells fluoresced red and blue, live cells had low intensity blue fluorescence only, and apoptotic cells had high intensity blue fluorescence.

**Superoxide assay**

Ninety-six-well plates were blocked with 50 μl of 1% human serum albumin for 2 h at 37°C. Before adding cells, blocked wells were washed twice with 200 μl of saline. Purified eosinophils were washed with HBSS medium (10 mM HEPES, pH 7.4) and resuspended to 5 × 10^5 cells/ml in cytochrome c solution (2.4 mg/ml cytochrome c in HBSS medium). Eosinophils were plated at a final concentration of 5 × 10^5 cells/well in 200 μl and were pretreated with drugs for 30 min at 37°C before cytokines were added. Immediately after cytokines were added, the absorbance at 550 nm was analyzed in a Thermomax plate reader (Molecular Devices, Sunnyvale, CA) every 15 to 30 min for 2 h. Conversion of absorbance to nanomoles of cytochrome c reduced per 1 × 10^6 eosinophils used the following equation: 19.1(absoorbance – absorbance<sub>time</sub>/0.05) = nmol cytochrome c reduced/10^6 cells (23).

**Glubryde binding assay**

Cell membranes were prepared as previously described (26), and 20 μg of membrane protein was incubated in 0.1 ml of 50 mM 3-(N-morpholino)propanesulfonic acid, pH 7.4, with 0.05–50 nM [1H]glubryde (Amersham, Arlington Heights, IL) for 2 h at room temperature. Bound radioligand was separated from free using a Skatron cell harvester (Skatron Instruments, Sterling, VA) with double glass filters. The filters were dried and counted in 2 ml of scintillation fluid using a beta counter. Specific picomoles of [1H]Glubryde bound per milligram of protein were determined by subtracting nonspecific binding in the presence of excess unlabeled glubryde.

**RNase protection assay**

Biotinylated probes for human actin or the NBF2 region of human sulfonlurea receptor (SUR) (27) were made with the BrightStar BIOTINscript Kit (Ambion, Austin, TX). Guanidine thiocyanate lysates of purified human eosinophils were prepared and analyzed with the Direct Protect Lysate Ribonuclease Protection Assay Kit (Ambion). Briefly, the lysates were incubated with biotinylated probes and treated with RNase to degrade any remaining single-stranded probe. After the RNase was inactivated with sarcosyl and proteinase K, the RNase-protected fragments were precipitated, resuspended in loading buffer, and separated on a 10% polyacrylamide gel. The gel was transferred to a positively charged membrane (Ambion), and hybridized biotinylated probe was detected using the BrightStar BioDetect Kit (Ambion) and Kodak autodensitometric film (Eastman Kodak, Rochester, NY).

**Results**

**Effects of K⁺ channel blockers on human eosinophil survival**

To test the hypothesis that K⁺ channel blockers are involved in eosinophil survival, we compared the activities of three different K⁺ channel blockers (glubryde, TEA, and apamin) with the activity of lidocaine on IL-5-stimulated eosinophils. Glubryde is a blocker of ATP-sensitive K⁺ channels, TEA blocks K⁺ channels and is a nicotinic cholinergic receptor antagonist, and apamin blocks Ca²⁺-activated K⁺ channels (28, 29). As shown in Figure 1, TEA and apamin had no significant effect on IL-5-induced eosinophil survival. Only glubryde (1 × 10⁻⁴ M) inhibited IL-5-mediated eosinophil survival as effectively as lidocaine. To determine whether this was a dose-response effect, doses of glubryde between 10⁻⁵ and 10⁻⁴ M were tested. As shown in Figure 2, glubryde exhibited a dose-response effect, which was especially evident at lower IL-5 concentrations. However, only the inhibition mediated by 10⁻⁴ M glubryde was statistically significant.

One of the characteristics of lidocaine inhibition of eosinophil survival is a relative specificity for IL-5 (17). To determine whether glubryde exhibits a similar preference, we examined the ability of glubryde to inhibit eosinophil survival mediated by IL-3 and GM-CSF. As shown in Figure 3, eosinophil survival was inhibited by glubryde at low IL-3 and GM-CSF concentrations, but the inhibition was completely overcome by increased concentrations of these cytokines. This pattern of inhibition was essentially identical with that seen with lidocaine (compare left and right columns of Fig. 3). Glubryde and lidocaine both preferentially inhibit IL-5-induced survival compared with IL-3- or GM-CSF-mediated survival, and the inhibition could not be overcome by increased concentrations of IL-5 (up to 10,000 pg/ml; data not shown). Neither lidocaine nor glubryde mediated a general toxic effect on the cells, since eosinophils stimulated with IL-3 or GM-CSF survived in the presence of the drugs. Additionally, the decreased eosinophil survival seen in the glubryde-treated cells correlated with increased apoptosis, as detected by staining cells with Hoechst 33342 and PI (data not shown). This effect was statistically significant (p < 0.05) and dose dependent (r<sub>s</sub> = 1.0; p < 0.05). Additionally, the concentrations of glubryde required to increase apoptosis were essentially identical with the concentrations required to inhibit eosinophil survival.

Glubryde, also called glybenclamide, belongs to the sulfonlurea family of compounds. Sulfonylurea block ATP-sensitive K⁺ channels by binding to an accessory regulating receptor, the SUR (30). If glubryde inhibits eosinophil survival by blocking an ATP-sensitive K⁺ channel, then other sulfonylureas should have similar activities. To test this, we examined the effects of two other sulfonylureas: tolbutamide and glipizide. As shown in Figure 4, tolbutamide and glipizide inhibited eosinophil survival as well as did glubryde. High concentrations (1000 pg/ml) of IL-3 or GM-CSF...
overcame the inhibitory effects of the sulfonylureas, whereas eosinophils treated with 1000 pg/ml IL-5 in the presence of glyburide, tolbutamide, or glipizide showed a 20 to 30% average decrease in survival. Thus, glyburide is representative of the general effectiveness of sulfonylureas in inhibiting eosinophil survival.

**K**⁺ channel openers rescue stressed eosinophils

If blocking an ATP-sensitive K⁺ channel results in inhibition of eosinophil survival, then an ATP-sensitive K⁺ channel opener should block the effects of glyburide and/or potentiate survival (31). To test this, we examined the effects of the ATP-sensitive K⁺ channel opener cromakalim on IL-5-mediated eosinophil survival. We were unable to inhibit the effects of glyburide by cromakalim pretreatment despite repeated efforts (n = 8; data not shown). However, we observed the potentiation of eosinophil survival in the presence of IL-5. As shown in Figure 5, the IL-5-mediated survival in these experiments was markedly less than usual; eosinophil absolute survival is normally >90% after 4-day stimulation with 1000 pg/ml IL-5. The reason for the low survival in these experiments was a high (1%) final concentration of DMSO in the medium from preparation of the cromakalim stock. However, this decreased survival response to IL-5 in the presence of 1% DMSO allowed us to observe the phenomenon of cromakalim “rescue” of eosinophils. As shown in Figure 5, this rescue was dramatic and restored the survival of IL-5-treated eosinophils to normal. This rescue was also observed when eosinophils were treated with the K⁺ channel openers pinacidil and diazoxide and was a dose-dependent effect (data not shown).

**Glyburide inhibits human eosinophil superoxide production**

Eosinophil activation and degranulation, not their mere presence, are probably critical in initiating the tissue damage associated with eosinophilic inflammation (1). Activation of eosinophils in vitro can be detected by measuring superoxide production in response to stimuli. We have previously shown that lidocaine significantly inhibits cytokine-mediated eosinophil superoxide production (17). To determine whether glyburide also inhibits eosinophil superoxide production, we cultured eosinophils with IL-5, IL-3, or GM-CSF in the presence of glyburide. As shown in Figure 6, all three cytokines stimulated superoxide production by eosinophils. However, superoxide production initiated by the cytokines was dramatically inhibited by glyburide, and excess cytokine did not overcome this inhibition. Interestingly, no preferential inhibition of IL-5 stimulation was seen in the superoxide assay compared to that in the survival assay. Instead, glyburide inhibited superoxide generation induced by all cytokines, including that generated by platelet-activating factor (data not shown). As in the survival assay, pretreatment of eosinophils with cromakalim did not block the glyburide effect under normal (low DMSO) conditions (data not shown).
Lidocaine, glyburide, and dexamethasone synergize to inhibit eosinophil survival and activation

Lidocaine and glyburide directly inhibit cytokine-mediated eosinophil survival and activation, probably through mechanisms distinct from those of dexamethasone (16). This follows because glucocorticoid inhibition of IL-5-stimulated survival is overcome at a concentration of 1000 pg/ml IL-5, whereas this is not the case with lidocaine or glyburide. Thus, these drugs may have additive or even synergistic effects when used in combination. To test this, we incubated eosinophils with IL-5 in the presence of lidocaine, dexamethasone, glyburide, or combinations of these drugs. As shown in Table I, eosinophils treated with IL-5 in the presence of lidocaine, dexamethasone, glyburide, or combinations of these drugs. As shown in Table I, eosinophils treated with IL-5 in the presence of lidocaine, dexamethasone, glyburide, or combinations of these drugs. As shown in Table I, eosinophils treated with IL-5 in the presence of lidocaine, dexamethasone, glyburide, or combinations of these drugs. As shown in Figure 3, eosinophils preferentially bind IL-5 and have decreased survival at 1000 pg/ml IL-5. All combinations showed synergistic activity in the survival assay. Most strikingly, the combination of lidocaine and glyburide essentially abolished the delay of apoptosis induced by IL-5.

Eicosanophils express a SUR

The effects of the sulfonylureas suggest that a SUR is present on eosinophils. To detect message for a SUR family member in eosinophil lysates, we used a biotinylated RNA probe for the conserved NBF2 region of SUR1 (27) in a ribonuclease protection assay (RPA). The NBF2 region is conserved in both SUR1 and SUR2, and is presumably common to the entire family of SUR proteins (32). Binding of the SUR probe should result in a protected mRNA fragment of 80 bp. As shown in Figure 3, eosinophil lysates from three separate donors expressed mRNA that hybridized with the SUR probe (lanes 6, 8, and 10), indicating that the eosinophils are actively transcribing a gene that is homologous to the NBF2 region of human SUR1.

FIGURE 3. Glyburide preferentially inhibits IL-5-mediated eosinophil survival. Purified eosinophils were cultured for 4 days with various concentrations of IL-5, IL-3, or GM-CSF. Eosinophil survival was analyzed as described in Materials and Methods and is plotted as a percentage of eosinophil survival at maximal cytokine concentrations. Each panel shows the mean ± SD of at least four independent experiments using eosinophils from both normal and allergic patients. When not visible, the SD was smaller than the symbol. Eosinophils were cocultured with cytokines and medium control (○), 1 × 10⁻³ M lidocaine (■, left), or 1 × 10⁻⁴ M glyburide (▲; right). A single asterisk indicates p < 0.05; a double asterisk indicates p < 0.005 (compared with 1000 pg/ml cytokine alone).
Discussion

Apoptosis of lung eosinophils is correlated with the resolution of airway inflammation in asthma (10, 11, 34). Glucocorticoid therapy presumably is effective in part by directly inhibiting cytokine-mediated eosinophil survival, resulting in eosinophil apoptosis and clearing by macrophages (16). Here we report that sulfonylureas, including glyburide, tolbutamide, and glipizide, mimic glucocorticoids by inhibiting cytokine-mediated survival of eosinophils obtained from both normal and allergic patients. However, the sulfonylureas differ from the glucocorticoids because the effects of the sulfonylureas, unlike those of the glucocorticoids, cannot be overcome with high concentrations of IL-5, a key mediator of eosinophil survival and activation in vitro and in vivo (12, 35, 36). This preferential inhibition of IL-5-induced signals is also observed when eosinophils are treated with lidocaine (14, 17). The ability of glyburide and lidocaine to synergize with dexamethasone in the survival assay suggests that the actions of the drugs are complimentary and work through independent mechanisms. One can speculate that glyburide and lidocaine target a channel(s), whereas glucocorticoids work at the nuclear level to alter gene transcription (37).

To damage the airway epithelium and promote bronchial hyperreactivity, eosinophils must not only be present, but also activated (38, 39). Glyburide and lidocaine are effective at blocking cytokine-induced superoxide production. Interestingly, the drugs show no specificity for IL-5 stimulation (as they do in the survival assay), but inhibit equally well superoxide induced by IL-3 and GM-CSF. The combination of lidocaine and glyburide in the superoxide assay resulted in synergistic inhibition of eosinophil activation, suggesting that in this assay lidocaine and glyburide have unique mechanisms of action. It is interesting that glucocorticoids do not inhibit eosinophil superoxide production, suggesting that glyburide (and lidocaine) may be more effective in the prevention of eosinophil-mediated tissue damage.

Similar functions of glyburide and lidocaine suggest that the sulfonylureas and lidocaine may be working through similar mechanisms. Our proposed model is that lidocaine exerts its effects by directly blocking a K$_{+}$ channel, comparable to its blocking of Na$_{+}$ channels (18). Similarly, sulfonylureas block ATP-sensitive K$_{+}$ channels by binding to the associated SUR on the cell surface. Thus, both drugs may exert their effects by blocking K$_{+}$ channels, but through different mechanisms. This model is consistent with our results showing that lidocaine and glyburide have unique mechanisms of action. It is interesting that glucocorticoids do not inhibit eosinophil superoxide production, suggesting that glyburide (and lidocaine) may be more effective in the prevention of eosinophil-mediated tissue damage.
of survival, but not inhibition of superoxide generation. Although the receptors for GM-CSF, IL-3, and IL-5 share a common β-chain that is thought to be responsible for signal transduction, several reports have indicated a signaling role for the specific α-chains of the receptors (40–44). Thus, one could postulate that the signaling events responsible for prolonging eosinophil survival are influenced by the α-chain, whereas the signals required for superoxide production depend primarily on β-chain activation. This hypothesis could explain why IL-5-induced survival is more sensitive to glyburide (and lidocaine) than that induced by IL-3 or GM-CSF, and why superoxide production initiated by all three cytokines is equally well inhibited. Support for this view comes from a recent report describing the biologic activity of an IL-5 protein in which an important β-chain contact site contained a charge reversal, thus affecting β-chain activation and subsequent signal transduction (45). The mutant IL-5 still induced eosinophil survival, but did not induce activation (as measured by adhesion) and, in fact, functioned as a specific antagonist in that assay.

A SUR family has recently been identified, and members of the family associate with and regulate the inwardly rectifying K+ channel, Kir6.2 (32). The two identified members, SUR1 and SUR2, are structurally related but functionally distinct, in that the SUR2/Kir6.2 combination is less sensitive to glyburide than is the SUR1/Kir6.2 complex. Based on the concentration of glyburide necessary to inhibit eosinophil survival in our experiments (10−4 M), we speculate that glyburide is not acting through the classic SUR1/Kir6.2 complex. However, because SUR1 has a lower affinity for glyburide (and lidocaine) than the classic SUR1/Kir6.2 complex, we speculate that glyburide is not acting through the classic SUR1/Kir6.2 complex. However, because SUR1 has a lower affinity for glyburide (and lidocaine) than the classic SUR1/Kir6.2 complex, we speculate that glyburide is not acting through the classic SUR1/Kir6.2 complex. However, because SUR1 has a lower affinity for glyburide (and lidocaine) than the classic SUR1/Kir6.2 complex, we speculate that glyburide is not acting through the classic SUR1/Kir6.2 complex. However, because SUR1 has a lower affinity for glyburide (and lidocaine) than the classic SUR1/Kir6.2 complex, we speculate that glyburide is not acting through the classic SUR1/Kir6.2 complex. However, because SUR1 has a lower affinity for glyburide (and lidocaine) than the classic SUR1/Kir6.2 complex, we speculate that glyburide is not acting through the classic SUR1/Kir6.2 complex. However, because SUR1 has a lower affinity for glyburide (and lidocaine) than the classic SUR1/Kir6.2 complex, we speculate that glyburide is not acting through the classic SUR1/Kir6.2 complex.$^{a}$

$^{a}$ Expected survival values represent the predicted additive effect of the drugs based on their individual effects. Actual survival values are the experimental values actually obtained when drug combinations were used.

$^{b}$ $p < 0.05, n = 10$; $^{**} p < 0.01, n = 10$.  

$^{c}$ Eosinophil survival was assayed as described in Materials and Methods and is listed as a percentage of the eosinophil survival at 1000 pg/ml IL-5 in the absence of drug, defined as 100%. All treatments are in the presence of 1000 pg/ml IL-5.

$^{d}$ Eosinophil survival was assayed as described in Materials and Methods and is listed as a percentage of the eosinophil survival at 1000 pg/ml IL-5 in the absence of drug, defined as 100%. All treatments are in the presence of 1000 pg/ml IL-5.
FIGURE 8. Eosinophils express mRNA for a SUR family member. The presence of SUR and actin mRNA in the eosinophil lysates was determined by RPA. Samples were prepared as described in Materials and Methods. Hybridized biotinylated probe was detected by the BrightStar BioDetect Kit. Lanes 1 and 3 show the actin and SUR probes alone, respectively. Lanes 2 and 4 show that the probes are completely digested in the presence of RNase, indicating that there is no residual signal. Lanes 5, 7, and 9 show eosinophil lysates from three donors probed with the actin probe to demonstrate the integrity of the mRNA. Lanes 6, 8, and 10 are lysates from the same three donors probed with the SUR1 NBF2 probe. Lane 11 is blank. Biotinylated RNA size markers are shown in lane 12. Expected sizes of the protected fragments are 127 bp for actin and 80 bp for SUR and are indicated by the arrows. Shown is a representative experiment (n = 2).

much less than that of SUR1. This is consistent with the eosinophil glyburide target being SUR2-like, as the affinity of SUR2 for glyburide hampers detectable surface binding (L. Aguilar-Bryan, unpublished observations).

Although we could not detect SUR1 on eosinophil membranes, the presence of a SUR homologous transcript is worth emphasizing. In the absence of specific Abs to confirm the existence of a low affinity SUR protein, we rely on the response of the cell to sulfonylureas to determine whether the protein is expressed and functional. By definition, a cell responsive to sulfonylureas expresses a sulfonylurea receptor, although the affinity of that receptor may be low. We believe that this is the case for the putative SUR expressed on eosinophils. The family of SUR proteins may be quite large, as shown by the recent discovery that the ATP-binding cassette family of proteins (of which the SUR are members) comprises 5% of the *Escherichia coli* genome (46). This suggests that we have only scratched the surface in our identification of the human homologues. Additionally, even the two identified human SUR proteins have different ligand binding characteristics (27, 32), indicating that there may be a wide range of SUR specificities.

If glyburide is exerting its effects on eosinophil survival and activation by binding to a conventional SUR, then we should be able to reverse that inhibition with an ATP-sensitive K⁺ channel opener, such as cromakalim (31). Interestingly, the ATP-sensitive K⁺ channel opener cromakalim has been tested in clinical trials as an oral asthma drug (47–50). The proposed mechanism of cromakalim is relaxation of airway smooth muscle via the opening of plasmalemmal K⁺ channels (47). Cromakalim has no effect on eosinophilic inflammation (48) and causes a high incidence of side effects (50). Although we were unable to block the action of lidocaine or glyburide with 1 x 10⁻⁵ M cromakalim, pinacidil, or diazoxide pretreatment (data not shown), we were able to potentiate the IL-5-induced survival of eosinophils stressed by high concentrations of DMSO. The latter data suggest that there is a cromakalim-responsive K⁺ channel on human eosinophils. However, because we could not block the action of glyburide with the openers, this is probably distinct from the channel opened by glyburide. These data suggest that glyburide either is binding to a novel SUR that is unresponsive to the openers or, alternatively, is targeting a receptor other than a SUR. Other ion channels, such as the cystic fibrosis transmembrane regulator, are inhibited by the sulfonylureas (51). We are confident that the eosinophil SUR is not SUR1, based on both its lack of responsiveness to cromakalim (under low DMSO conditions) and the high (10⁻⁴ M) concentration of glyburide required to inhibit eosinophil survival. Thus, eosinophils may express SUR2 or a novel SUR family member with distinct characteristics.

The observations presented here have intriguing clinical implications for the treatment of asthma and other eosinophil-associated diseases. The results suggest that ion channels on eosinophils, especially ATP-sensitive K⁺ channels, may be targets for future drug development. Achieving a concentration of 10⁻⁴ M glyburide in an asthmatic lung is most likely possible, but may precipitate unwanted side effects. The synergy of the sulfonylureas with glucocorticoids and lidocaine could allow the use of lower concentrations if necessary. Also, the risk of adverse reactions resulting from sulfonylurea treatment would be minimized by local (rather than systemic) application. Because lidocaine has been shown to be effective in an open label clinical trial (6), the sulfonylureas may similarly represent a potential class of anti-inflammatory asthma drugs for the treatment of asthma and eosinophil-associated diseases.

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**References**


