Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 7844–7848, August 1993
Immunology

Activation of mast cell K^+ channels through multiple G protein-linked receptors

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Communicated by Harden M. McConnell, May 11, 1993

ABSTRACT **The rat basophilic leukemia (RBL) mast cell line possesses cell surface receptors for adenosine whose ligation markedly potentiates antigen-driven Ca^{2+} influx and secretion. Here we show that engagement of these receptors and of separate P_2 purinergic receptors rapidly activates an outwardly rectifying K^+ conductance [$G_{K(OR)}$] in RBL cells. Activation of $G_{K(OR)}$ by the ligands 5′-(*N*-ethylcarboxamido)adenosine (NECA), ADP, and ATP was prevented by cytoplasmic guanosine 5′-[β-thio]diphosphate as well as by pretreatment of the cells with pertussis toxin, implicating mediation by a G protein. Multiple cycles of induction and decay of $G_{K(OR)}$ were produced upon application and removal of ligand. Induction of $G_{K(OR)}$ by either ligand was much faster than the induction caused by guanosine 5′-[γ-thio]triphosphate ($t_{1/2}$ < 10 sec vs. 210 sec). In control cells the maximal whole-cell conductance elicited by ADP (2.25 ± 0.30 nS) or ATP (2.50 ± 0.33 nS) was about twice as large as that induced by NECA (1.03 ± 0.11 nS), and similar to that previously reported for the guanosine 5′-[γ-thio]triphosphate-elicited $G_{K(OR)}$ in RBL cells (2.58 ± 1.59 nS). Treatment of RBL cells with dexamethasone upregulated Ca^{2+} responses to NECA, and it also nearly doubled the maximal conductance elicited by NECA without appreciable effect on responses to ADP or ATP. The failure of water-soluble second messengers to activate $G_{K(OR)}$ and the inability of 11 mM EGTA (<10 nM Ca^{2+}) to prevent activation by ADP suggest that the relevant pathway is membrane-delimited. Two ion-channel blockers inhibited antigen-stimulated secretion with IC_{50} values similar to those at which they blocked $G_{K(OR)}$, suggesting that activity of the outwardly rectifying K^+ channel may be important for stimulus-response coupling in these cells. Potentiation of the secretory response by NECA may reflect, in part, the activation of $G_{K(OR)}$, which serves to repolarize the membrane more effectively than does the constitutive mechanism, thereby enhancing antigen-driven Ca^{2+} influx. This channel and its functionally associated receptors may allow neighboring cells of the host to modulate the response of mast cells to exogenous antigen.**

Mast cells and basophils initiate the allergic response when their surface receptors for IgE ($Fc_\varepsilon RI$) are crosslinked by multivalent antigens. Inflammatory mediators such as histamine are then released in a process that requires the sustained influx of extracellular Ca^{2+}. In rat basophilic leukemia (RBL) mast cells and possibly in mast cells in general, Ca^{2+} influx requires a sufficiently negative membrane potential: depolarization of the plasma membrane with increased external K^+ (1, 2) or the protonophore carbonylcyanide 3-chlorophenylhydrazone (3) inhibits antigen-stimulated Ca^{2+} influx and secretion. In RBL cells antigenic stimulation causes a biphasic response of membrane potential: an initial depolarization is followed within 3–4 min by repolarization (4) associated with enhanced efflux of intracellular K^+ (5).

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Given the crucial role of membrane potential in Ca^{2+} influx, mast cell K^+ or Cl^- channels offer potential sites for regulation of the secretory response. Especially if the activity of such channels is modulated by cell surface receptors, the possibility exists for exogenous control of the intrinsic response of mast cells to antigens. Pharmacologic studies have suggested that repolarization of antigen-activated RBL cells is mediated by a K^+ efflux pathway distinct from the constitutively active inwardly rectifying K^+ (K^+_{IR}) channel (4, 5). Thus Ba^{2+} at a concentration sufficient to completely block the K^+_{IR} channel (0.5 mM) does not prevent K^+ efflux, repolarization, or secretion. The molecular nature of the permeability pathways that mediate depolarization and repolarization remains unknown.

Previous work identified a latent outwardly rectifying K^+ (K^+_{OR}) channel in RBL cells that was activated in a membrane-delimited manner by stimulation of pertussis toxin-sensitive G proteins with intracellular guanine nucleotides or AlF_4^- (6). It is possible that the K^+_{OR} channel contributes to the normal repolarizing response or that its stimulation in parallel with the IgE receptor would prevent membrane depolarization and enhance Ca^{2+} influx. To explore these possibilities it is necessary to understand the physiological mechanisms that regulate this channel.

We found that two cell surface receptors for purinergic ligands rapidly activated the K^+_{OR} channel in a GTP-dependent and pertussis toxin-sensitive manner. Stimulation of the K^+_{OR} channel thus appears to be G protein-mediated, and evidence suggests that the activation pathway is membrane-delimited. Two antagonists of the K^+_{OR} channel inhibited IgE-mediated secretion with dose–response relations similar to those for channel inhibition. Moreover, an agonist at one of these receptors, 5′-(*N*-ethylcarboxamido)adenosine (NECA), is known to potentiate markedly antigen-stimulated Ca^{2+} signals and secretion, without itself causing appreciable secretion. These results suggest that activity of the K^+_{OR} channel may be a critical element in stimulus–response coupling in RBL cells.

MATERIALS AND METHODS

Reagents. Nitrendipine was generously supplied by Alexander Scriabine (Miles, West Haven, CT). NECA was obtained from Sigma. Adenosine 5′-[β-thio]diphosphate (ADP[βS]), adenosine 5′-[β,γ-methylene]triphosphate (ATP[β,γCH_2]), GTP, guanosine 5′-[β-thio]diphosphate (GDP[βS]), and guanosine 5′-[γ-thio]triphosphate (GTP[γS]) were from Boehringer Mannheim. The H1 DNPε26.82 monoclonal anti-dinitrophenyl IgE (7) was purified from ascites by affinity chromatography on a column containing dinitrophe-

Abbreviations: ADP[βS], adenosine 5′-[β-thio]diphosphate; ATP[β,γCH_2], adenosine 5′-[β,γ-methylene]triphosphate; GDP[βS], guanosine 5′-[β-thio]diphosphate; GTP[γS], guanosine 5′-[γ-thio]triphosphate; NECA, 5′-(*N*-ethylcarboxamido)adenosine; $Fc_\varepsilon RI$, receptor(s) for Fc_ε portion of IgE; RBL, rat basophilic leukemia; G_K, K^+ conductance; subscript IR, inwardly rectifying; subscript OR, outwardly rectifying.

nyllysine. Trinitrophenylated bovine serum albumin was synthesized by reaction of trinitrobenzenesulfonic acid with the protein (6). Nitrendipine, quinidine, and NECA were dissolved in dimethyl sulfoxide and diluted 1000-fold into aqueous buffer just before use.

Cell Culture. RBL-2H3 cells were maintained in monolayer culture (8). For patch-clamp experiments they were trypsinized and seeded 12–24 hr before recording (8×10^4 per 12-mm-diameter coverslip). For secretion assays they were seeded 12–24 hr before the experiment in 10-cm dishes (6–8 $\times 10^6$ cells in 10 ml of medium containing anti-dinitrophenyl IgE at 3–10 μg/ml).

Electrophysiology. The procedures for patch clamping were essentially as described (6). Standard potassium aspartate pipette solution was (in mM) 160 potassium aspartate/1.1 Na_2EGTA/0.1 $CaCl_2$/2.0 $MgCl_2$/10 Hepes titrated to pH 7.2 with KOH ($[K^+] \approx 163$ mM). This yields an estimated free $[Ca^{2+}]$ of $\approx$15 nM. To this buffer was added, in some experiments, 0.3 GTP, 3.0 GDP[βS] plus 0.3 GTP, 0.5 MgATP plus 0.1 GTP[γS], or 10 EGTA plus 0.3 GTP. The bath solution was mammalian Ringer solution (in mM: 160 NaCl/4.5 KCl/2 $CaCl_2$/1 $MgCl_2$/5 Hepes titrated to pH 7.4 with NaOH). In K^+ Ringer solution the NaCl was replaced with KCl. The "instantaneous" whole-cell current–voltage (*I–V*) curves shown were recorded upon stimulation of the cells every 10 sec with voltage ramps of 250 msec (−80 mV holding potential between ramps). This method was justified in that during application of voltage steps the currents induced by ADP and GTP[γS] were maximal within the response time of the voltage clamp. For ADP dose–response experiments a saturating concentration of ADP (10 or 50 μM) was applied just long enough to allow maximal induction of K^+_{OR} conductance [$G_{K(OR)}$] and then rinsed away and ADP was reapplied, usually at a single subsaturating concentration (1, 2.5, or 5 μM). In experiments with antagonists, a single concentration of antagonist usually was tested per cell, and after 3–7 min reversibility was confirmed by washout of antagonist with Ringer solution.

Secretion Assays. Antigen-stimulated and spontaneous release of [^{3}H]serotonin was measured as described (9). RBL cells were grown overnight (12–24 hr) in medium containing IgE and [^{3}H]serotonin (1 μCi/ml; 1 Ci = 37 GBq). They were released from the culture dishes with phosphate-buffered saline containing EDTA (0.2 mg/ml), harvested, and suspended at 4×10^6 per ml in Hepes-buffered saline (in mM: 135 NaCl/5 KCl/1 $MgCl_2$/2 $CaCl_2$/5.6 glucose/10 Hepes, pH 7.4) containing 0.05% bovine serum albumin. To start the assay, 50 μl of cell suspension (kept on ice) was added to 500 μl of prewarmed Hepes-buffered saline containing the channel antagonist. After 10 min at 37°C, 10 μl of secretagogue (trinitrophenylated bovine serum albumin) was added and the reaction was allowed to proceed for 60 min at 37°C. Aliquots of the total cell suspension and the supernatant obtained after rapid pelleting of the cells in a microcentrifuge were assayed for radioactivity in a liquid scintillation spectrometer. Percent secretion refers to percent of total radioactivity present in the supernatant; optimal secretion varied from 33.5% to 63.9% above background.

RESULTS

Induction of K^+_{OR} Current by Extracellular Ligands. Fig. 1*a* shows whole-cell *I–V* curves obtained upon voltage ramp stimulation of an RBL cell at 10-sec intervals, before and after addition of 10 μM ADP to the bath. An outward conductance rapidly appeared while the constitutively active $G_{K(IR)}$ remained stable. This contrasts with the effect of GTP[γS]—a nonselective, irreversible activator of G proteins—which causes decay of the $G_{K(IR)}$ and the appearance of a $G_{K(OR)}$ (6). The whole-cell *I–V* relation for the ADP-induced current in normal Ringer solution was obtained by digital subtraction of traces acquired before application of ADP from those obtained after ADP addition (Fig. 1*b*). Note the strong outward rectification at membrane potentials above −80 mV.

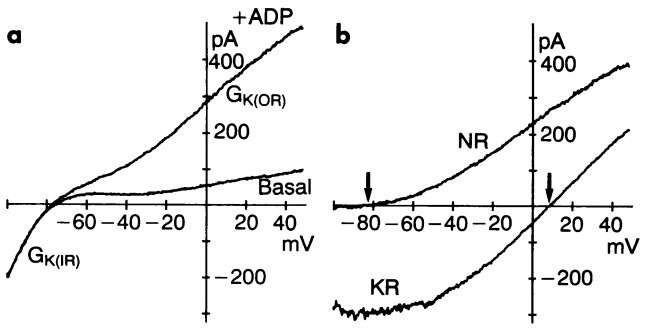

FIG. 1. ADP-induced $G_{K(OR)}$ in RBL cells. (*a*) Whole-cell *I–V* curves recorded before (basal) and 20 sec after addition of 10 μM ADP to the bath (normal Ringer solution). Note stability of inwardly rectifying conductance [$G_{K(IR)}$] to ADP. (*b*) *I–V* curves for ADP-induced current in normal Ringer solution (NR) and in K^+ Ringer solution plus 0.2 mM $BaCl_2$ (KR), obtained by digital subtraction of traces recorded before from those recorded after ADP addition. Data for NR are from the same cell as in *a* (C242), and data for KR pertain to another cell (C486). K^+ selectivity is indicated by the large positive shift in reversal potential (arrows) upon increase in external K^+ from 4.5 to 164.5 mM (internal K^+ was 163 mM).

The ionic selectivity of an ion channel can be determined from the behavior of its reversal potential upon ionic substitution. This was complicated by the presence of the K^+_{IR} channel, which is stable in the presence of cytoplasmic ATP or GTP (6); hence we recorded the whole-cell *I–V* curve in K^+ Ringer solution containing 0.2 mM Ba^{2+}, which produces a complete block of the K^+_{IR} channel in normal Ringer solution and a voltage-dependent block in K^+ Ringer solution. Outward current through the ADP- and GTP[γS]-induced pathway (6) is marginally affected by 0.2 mM Ba^{2+}. Fig. 1*b* also shows the *I–V* curve obtained by digital subtraction of averaged traces acquired before from those obtained after addition of 10 μM ADP to another cell bathed in K^+ Ringer containing 0.2 mM Ba^{2+}. Comparison of the two *I–V* curves in Fig. 1*b* shows a large positive shift in the reversal potential (arrows) for the ADP-induced current from −80 mV to near 0 mV when the ratio of intracellular to extracellular K^+ was changed from 163/4.5 to 163/164.5. The average reversal potential of the ADP-induced current in K^+ Ringer solution was 5.5 ± 0.7 mV (mean ± SE, $n = 13$), essentially the same as that of the GTP[γS]-induced current, 5.8 ± 0.9 mV ($n = 6$). This behavior is very close to that predicted by the Nernst equation for a K^+-selective conductance. The small deviation from theory is not due to a Ca^{2+} conductance (6), but it could reflect a Donnan potential between the pipette and cell interior or imprecise current balancing just prior to seal formation. Clearly, the induced current is K^+-selective.

There is striking similarity between the current induced by extracellular ADP and that elicited by intracellular GTP[γS] (figure 6*A* in ref. 6) in terms of reversal potential, maximal conductance (see below), and the shape of the *I–V* curve. In RBL cells the K^+_{IR} channel is inactivated upon whole-cell dialysis with GTP[γS]; hence the records are uncontaminated with currents through the K^+_{IR} channel even without external Ba^{2+}. Comparison of the *I–V* curves reveals similar noise at hyperpolarized potentials in K^+ Ringer solution, outward rectification, and K^+ selectivity for both currents. Thus, nonselective stimulation of G proteins with GTP[γS] can elicit a current remarkably similar if not identical to that induced by extracellular ADP, ATP, and NECA. Evidence

discussed below indicates that channel activation by these extracellular ligands also is mediated by G proteins.

Separate Receptors for NECA and ADP/ATP Activate the K^+_{OR} Channel. Adenosine receptors of novel pharmacology (10) and conventional P_2 purinergic receptors (11) have been identified in RBL cells. Stimulation of phospholipase C by NECA acting at the adenosine receptor is mediated by a pertussis toxin-sensitive G protein (10). The K^+_{OR} current was induced by NECA, presumably via the adenosine receptor, and by ADP or ATP acting through a separate P_2 receptor. Several lines of reasoning implicate the existence and involvement of both receptor types. First, the relative potency of different nucleotide agonists, ADP[βS] $\gg$ ADP, ATP > ATP[$\beta,\gamma CH_2$] $\gg$ AMP, is characteristic of P_{2y} receptors rather than adenosine receptors (12).

Ectoenzymes that convert adenine nucleotides to adenosine exist on many cell types, making it conceivable that the effects of ATP or ADP actually were due to adenosine acting at the adenosine receptor. Three findings indicate that this pathway for activation of $G_{K(OR)}$ was not operative. (*i*) The immediate precursor of adenosine, AMP, did not reproducibly elicit $G_{K(OR)}$. (*ii*) The poorly hydrolyzable analogs ADP[βS] and ATP[$\beta,\gamma CH_2$] both activated $G_{K(OR)}$. In fact, ADP[βS] was much more potent than ADP or ATP in this respect. (*iii*) The conductance elicited by ADP (2.25 ± 0.33 nS, n = 17) or ATP (2.50 ± 0.23 nS, n = 17) in control cells was at least twice that induced by NECA (1.03 ± 0.12 nS, n = 11), suggesting that a receptor distinct from that which binds NECA mediates the effects of adenine nucleotides.

Further experimental separation of the pathways for activation of $G_{K(OR)}$ by NECA and ADP derives from studies with the antiinflammatory steroid dexamethasone. Pretreatment of RBL cells with 0.1 μM dexamethasone upregulates the cellular response to NECA while inhibiting antigen-triggered signals (13). Treatment overnight with 0.1 μM dexamethasone increased the maximal conductance induced by NECA whereas the response of $G_{K(OR)}$ to ADP or ATP was minimally affected (Fig. 2). The selective effect of dexamethasone on the response to NECA suggests that separate receptors and/or G proteins mediate the effects of ADP and NECA on the K^+_{OR} channel.

Given that two pathways for elicitation of $G_{K(OR)}$ exist, the question of their possible interaction arises. The relevant data can be summarized as follows. When the K^+ conductance was elicited by ADP, no further increase followed the subsequent addition of NECA (plus ADP). However, when $G_{K(OR)}$ was induced first by NECA, subsequent addition of ADP (plus NECA) activated a conductance 1.7–1.9 times that

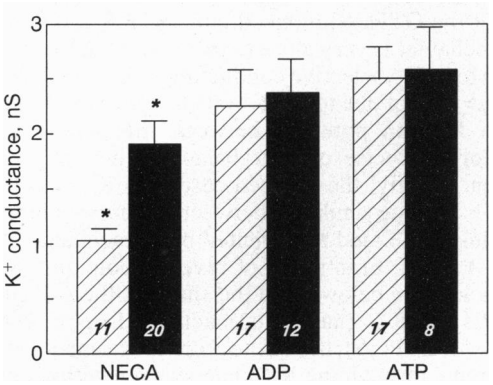

FIG. 2. Separation of pathways for activation of $G_{K(OR)}$ by NECA and adenine nucleotides. Treatment with 0.1 μM dexamethasone for 12–18 hr (solid bars) increased maximal value of $G_{K(OR)}$ induced by NECA, without affecting significantly the response to ADP or ATP. Numbers within bars give sample size, and error bars give SEM. Asterisk indicates statistical significance ($P < 0.01$).

elicited by NECA alone (1.93 ± 0.14 nS, n = 7, vs. 1.03 ± 0.12 nS, n = 11) and not significantly different ($P > 0.1$) from that elicited by ADP alone (2.25 ± 0.33 nS, n = 17). This is also similar to the maximal $G_{K(OR)}$ induced by intracellular GTP[γS] (2.58 ± 0.21 nS, n = 58) (6). Thus, the sum of $G_{K(OR)}$ induced by separate application of NECA and ADP was considerably larger than the actual $G_{K(OR)}$ induced by their simultaneous application. Clearly there was no synergism between ADP and NECA, and in fact, their effects were not even additive. This suggests that the signal transduction pathways from the NECA and ADP/ATP receptors converge in activation of the same K^+ channel.

Rate and Magnitude of $G_{K(OR)}$ Induction by Different Ligands. A linear least-squares fit to the *I–V* relation at +20 to +40 mV was performed on whole-cell records like that shown in Fig. 1, and the slope conductance was plotted vs. time since break-in to the whole-cell recording configuration. Representative induction curves for $G_{K(OR)}$ under different experimental protocols (Fig. 3*a*) show that induction by extracellular ADP was much faster than that due to intracellular GTP[γS]. This also was true for other adenine nucleotides and NECA. Previous work showed that induction of $G_{K(OR)}$ in RBL cells occurred with an average half-time of ≈210 sec (6), and similar results were obtained in the present study (Fig. 3*a* is representative). Because our method for exchange of the bathing solution was relatively slow, it permitted estimation only of the upper limit on the half-time for induction by extracellular ligands ($t_{1/2} \leq 10$ sec).

Interaction of ADP with its receptor was rapidly reversible. Removal of extracellular ADP was followed by rapid decay of the induced current (Fig. 3*b*). The current could be

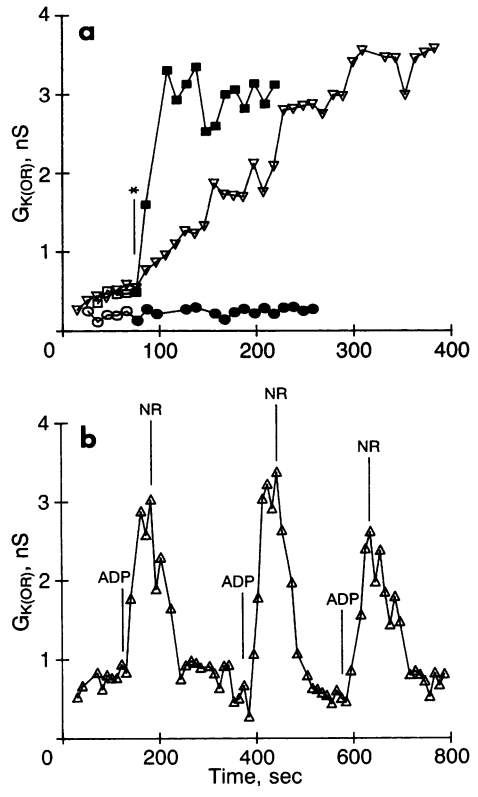

FIG. 3. Induction of $G_{K(OR)}$ by ADP is rapid and reversible. (*a*) Plots of slope conductance between +20 and +40 mV vs. time: squares, cell dialyzed with GTP and exposed to extracellular ADP (10 μM) at asterisk; circles, pertussis toxin-pretreated cell exposed to ADP at asterisk; triangles, cell dialyzed with 100 μM GTP[γS] in the absence of extracellular ADP. Filled symbols indicate presence of ADP. (*b*) Repeated rapid inductions and decays of $G_{K(OR)}$ upon addition of 10 μM ADP followed by normal Ringer solution (NR).

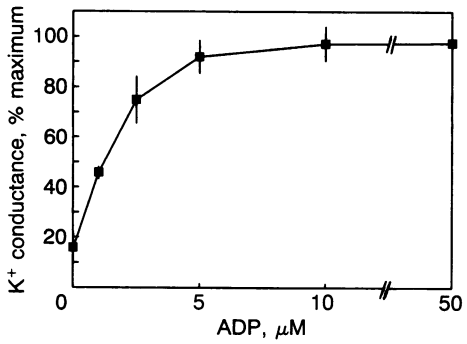

FIG. 4. Induction of $G_{K(OR)}$ by ADP is dose-dependent rather than all-or-none. Each point represents mean ± SEM of four to nine cells, where at least two concentrations of ADP were tested on the same cell as described in *Materials and Methods*.

reinduced multiple times by subsequent additions of ADP, at least under the conditions of whole-cell recording at room temperature. The ready reversibility of the process suggests that continued engagement of the ADP/ATP receptor is necessary for activation of $G_{K(OR)}$ and that subsequent steps are also readily reversible.

There was a graded, saturable response of $G_{K(OR)}$ to ADP (Fig. 4). Half-maximal induction occurred at ≈1 μM ADP and the response was saturated by ≈10 μM ADP.* This places an approximate lower limit on the K_D of interaction of ADP with its receptor, given the possible existence of "spare" receptors. In contrast to the graded response of $G_{K(OR)}$ to increased ADP, in RBL cells ATP induces a transient, all-or-nothing rise in intracellular Ca^{2+}, the peak being ≈1 μM (11). Given that even in single cells $G_{K(OR)}$ responds to [ADP] in a graded rather than all-or-none fashion, it does not appear that ADP or ATP activated $G_{K(OR)}$ via the elicitation of a Ca^{2+} transient. Further support of this argument is given below.

G Proteins Mediate Ligand-Induced K^+_{OR} Channel Activation. Inclusion of 0.3 mM GTP in the pipette solution invariably supported the induction of $G_{K(OR)}$ upon addition of ADP. This was true even when ADP was added 3 min or more after break-in to the whole-cell recording configuration (five out of five trials). To address the question of a GTP requirement, we dialyzed cells for 3 min with a pipette solution containing 0.3 mM GTP and 3 mM GDP[βS], a phosphorylation- and hydrolysis-resistant GDP derivative that competes effectively with GTP for binding to heterotrimeric G proteins (14). GDP[βS] prevented the induction of $G_{K(OR)}$ in five out of five trials [increase in conductance, 0.07 ± 0.03 nS (mean ± SEM)], suggesting that the binding of cytoplasmic GTP to some coupling factor is a necessary event in channel activation by extracellular ADP. Further evidence consistent with mediation of channel activation by a GTP-binding protein is that pretreatment of cells with pertussis toxin (100 ng/ml, 12–16 hr) essentially prevented the ability of ADP (Fig. 3*a*) and ATP to induce $G_{K(OR)}$, the increase in conductance following addition of 10 μM ATP was 0.14 ± 0.06 nS (mean ± SEM, n = 11), compared with 2.03 ± 0.20 (n = 17) in control cells. Cholera toxin (1 μg/ml, 2 hr) did not enhance the magnitude of $G_{K(OR)}$ induced by 10 μM ADP (2.30 ± 1.6 nS, n = 3). It thus appears that the ADP/ATP receptor in RBL cells is coupled to a pertussis toxin-sensitive G protein whose activation leads to induction of $G_{K(OR)}$.

Activation of the K^+_{OR} Channel Does Not Require Soluble Cytoplasmic Messengers. Cytoplasmic inositol 1,4,5-trisphosphate, inositol 1,3,4,5-tetrakisphosphate, ATP, GTP, and cAMP or cGMP (with or without ATP) do not induce $G_{K(OR)}$ in RBL cells (6). Our standard potassium aspartate pipette solution contained 1.1 mM EGTA and had an estimated free [Ca^{2+}] = 15 nM, and it should have blunted ligand-induced Ca^{2+} transients. Dialysis of cells with 11 mM EGTA (10 nM free Ca^{2+}) prior to addition of 10 μM ADP failed to alter the magnitude of induction of $G_{K(OR)}$. Strong buffering of Ca^{2+} with 11 mM EGTA prevents increases in intracellular Ca^{2+} by GTP[γS] or inositol 1,4,5-trisphosphate in rat peritoneal mast cells (15). The average conductances in cells dialyzed with 11 mM EGTA (10 nM Ca^{2+}) and 1.1 mM EGTA (15 nM Ca^{2+}) were 2.24 ± 0.41 nS (n = 7) and 2.25 ± 0.33 nS (n = 17), respectively. This difference is not significant even at $P < 0.10$. Hence it appears that ADP or NECA alone can activate the channel through a G protein-linked pathway at or below resting intracellular [Ca^{2+}].

Channel Pharmacology. Outward currents through the ligand-induced K^+_{OR} channels were blocked by bath-applied Ba^{2+} and nitrendipine (data not shown), as were currents through the GTP[γS]-activated channel. Quantitative data were obtained on the GTP[γS]-elicited current because it desensitizes less rapidly than the receptor-induced current. In normal Ringer solution, Ba^{2+} is a much more effective inhibitor of $G_{K(IR)}$ than it is of $G_{K(OR)}$ (6); the present data (Fig. 5*a*) show that 50% inhibition of $G_{K(OR)}$ by Ba^{2+} occurred at ≈1.2 mM, at room temperature and a holding potential of −80 mV. Inhibition of the K^+_{IR} channel is complete under the same conditions at <0.2 mM Ba^{2+}.

Nitrendipine is a high-affinity antagonist of L-type voltage-gated Ca^{2+} channels but also blocks, with lower affinity, the voltage-gated K^+ channels in human T cells (16). It blocked $G_{K(OR)}$ with an IC_{50} ≈ 1.3 μM, considerably above that observed for block of L-type Ca^{2+} channels. Three other findings indicate that antagonism of $G_{K(OR)}$ by nitrendipine

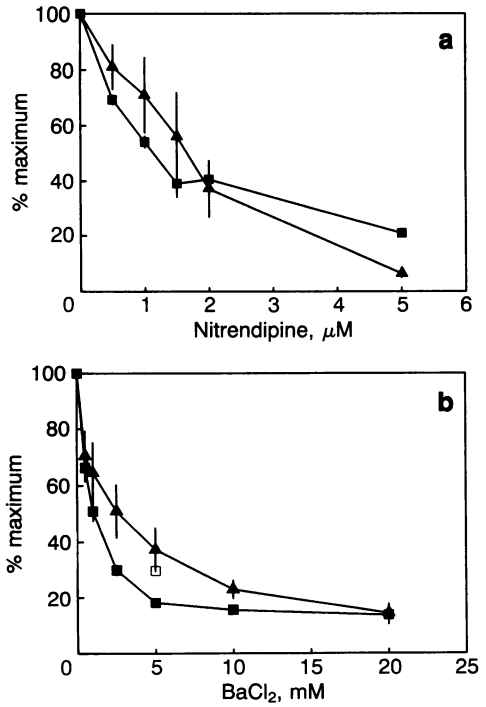

FIG. 5. Dose–response curves for inhibition of $G_{K(OR)}$ (squares) and antigen-triggered secretion (triangles) of [^{3}H]serotonin by nitrendipine (*a*) and Ba^{2+} (*b*). In *b*, the open square gives block of $G_{K(OR)}$ by 5 mM Ba^{2+} at holding potential of −25 mV. Each point on $G_{K(OR)}$ curves represents the mean ± SEM of four to seven cells, and points on secretion curves represent mean ± SEM of three to five assays on separate cultures, each conducted in triplicate.

*The smaller induction of $G_{K(OR)}$ by NECA than by ADP does not reflect a subsaturating concentration of agonist: $G_{K(OR)}$ elicited by 50 μM NECA was 92% of that induced by 200 μM NECA (n = 10), yet in the five cells tested for an ADP response, $G_{K(OR)}$ induced by 200 μM NECA was only 47.8% of that elicited by 10 μM ADP.

was not an indirect effect due to inhibition of voltage-gated Ca^{2+} channels. (*i*) Voltage-gated Ca^{2+} channels have not been detected electrophysiologically in mast cells. (*ii*) Ca^{2+} in mast cells is not increased upon depolarization of the plasma membrane with elevated extracellular K^+. (*iii*) As noted above, strongly buffering the intracellular [Ca^{2+}] at 10 nM with 11 mM EGTA did not prevent prolonged activation of $G_{K(OR)}$ by ADP.

Inhibition of Antigen-Induced Secretion by Antagonists at the K^+_{OR} Channel. The K^+ channel blockers Ba^{2+} and quinidine inhibit antigen-stimulated secretion from RBL cells at similar concentrations to those at which they block membrane repolarization (4). Ba^{2+} has almost no effect on secretion at concentrations sufficient to block completely the RBL K^+_{IR} channel, and quinidine has no effect on $G_{K(IR)}$ (6). Intriguingly, quinidine blocked $G_{K(OR)}$ with the same efficiency ($IC_{50} \approx 30$ μM, ref. 6) as that previously reported for inhibition of secretion (4). To explore further the possible contribution of the K^+_{OR} channel to the regulation of secretion, we directly tested the effects of two blockers of $G_{K(OR)}$ on antigen-stimulated secretion. The dose–response curves (Fig. 5) suggest that activity of the K^+_{OR} channel is required for an optimal secretory response of RBL cells to antigen.

DISCUSSION

Sustained influx of Ca^{2+} is necessary for IgE-mediated secretion by intact mast cells, and this influx is critically dependent upon maintenance of a sufficiently negative membrane potential. Particularly in rat mast cells of the mucosal phenotype—which secrete more slowly than those of the serosal phenotype (17)—the prolonged influx of Ca^{2+} is a potential site for regulation of the secretory response. From this perspective it is crucial to understand the control of membrane potential by ion channels in mast cells. Previous work in this area has focused on Cl^- channels in rat peritoneal mast cells, the prototype serosal mast cell (18).

It was shown using hydrophobic ion partitioning (19, 20) and the fluorescent probe bisoxonol (21) that crosslinkage of $Fc_\varepsilon RI$ on RBL cells depolarized the plasma membrane. Depolarization was followed within a few minutes by repolarization (4), and repolarization was associated with enhanced efflux of $^{86}Rb^+$ (5). It is thus possible that K^+ efflux mediates or contributes to repolarization and thereby supports continued Ca^{2+} influx. Some mechanism of repolarization independent of the K^+_{IR} channel seems likely, because once the membrane is depolarized to potentials substantially above the K^+ equilibrium potential the K^+_{IR} channel is inactive. In support of this argument, Ba^{2+} at a concentration (0.5 mM) that completely inhibits the K^+_{IR} channel in RBL cells did not block either the repolarizing response or the secretion (4). Activation of a K^+ or Cl^- channel that passes sufficient outward current at depolarized potentials would, however, counteract the depolarizing currents.

Ba^{2+} inhibited antigen-stimulated secretion at concentrations similar to those at which it blocked the K^+_{OR} channel (Fig. 5*a*). The agreement between dose–response curves for inhibition of secretion and $G_{K(OR)}$ is more striking for nitrendipine, which does not block currents through the K^+_{IR} channel. These observations support the idea that activity of the K^+_{OR} channel contributes to or is required for stimulus–secretion coupling in RBL cells. We hypothesize that progressive reduction of $G_{K(OR)}$ by the channel blockers reduces the relative contribution of the K^+ diffusion potential to the total membrane potential of antigen-stimulated cells.

Because pharmacologic studies are limited by the possibility of pleiotropic action of the antagonists, a key question becomes, is the activity of the K^+_{OR} channel regulated by $Fc_\varepsilon RI$ crosslinkage? Previous experiments failed to demonstrate direct coupling of the $Fc_\varepsilon RI$ to this channel (6), but the possibility of indirect coupling within intact cells was not eliminated. That pertussis toxin blocks 60% of antigen-stimulated Rb^+ efflux from RBL cells (5) hints that an indirect mechanism might mediate activation of G proteins and $G_{K(OR)}$ by the $Fc_\varepsilon RI$. Calculations indicate that autostimulation by ATP released from the mast cells themselves is not critical.

The present results suggest that exogenous sources of adenine nucleotides may potentiate the secretory response through activation of $G_{K(OR)}$. Adenosine and adenine nucleotides have been implicated in regulation of the inflammatory response by a variety of immune effector cells. These purines are released by activated platelets, endothelial cells, and injured tissue, and they have both stimulatory and inhibitory effects on immune cell function. For example, neutrophils possess two types of adenosine receptor, A_1 and A_2, which mediate enhancement of the chemotactic response and repression of superoxide production, respectively (22). Many cell types, including rat peritoneal mast cells (23) possess P_{2z} purinoceptors that bind ATP^{4-} and cause membrane permeabilization. The receptor which mediates the effects of ADP and ATP described here is clearly distinct from P_{2z} purinoceptors.

We hypothesize that the K^+_{OR} channel and its functionally associated receptors provide a regulatory mechanism for the host to control mast cell responses to exogenous antigen. Analogous indirect control of insulin secretion by ATP-dependent K^+ channels in pancreatic β cells provides precedent for the physiological importance of such systems. Future studies of the K^+_{OR}/K^+_{IR} channel system may reveal further details of how the antigen-driven Ca^{2+} signal is controlled in rat mucosal mast cells.

We are grateful to Dr. Fu-tong Liu of the Research Institute of Scripps Clinic (La Jolla, CA) for the ascites fluid containing anti-dinitrophenyl IgE. We appreciate valuable comments on the manuscript by Dr. Philip Haydon and Dr. Duane Enger of Iowa State University. This work was supported by Biotechnology and University Research Grants from Iowa State University.

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