Immunoprecipitation of $A_1$ Adenosine Receptor-GTP-Binding Protein Complexes In Ciliary Epithelial Cells

Martin B. Wax and Rajkumar V. Patil

**Purpose.** To examine the interaction of $\alpha$ subunits of guanine nucleotide binding proteins with $A_1$ adenosine receptors from the SV40-transformed bovine-derived pigmented (PE) and human-derived nonpigmented (NPE) ciliary epithelial cell lines using an immunoprecipitation approach and $[^3H]DPCPX$, a selective radioligand to adenosine receptor.

**Methods.** Solubilized preparations of adenosine receptors from PE and NPE cell lines were immunoprecipitated with G protein specific antisera 8730 (anti-Gia), 3646 (anti-Gal), 1521 (anti-Gia2), and 1518 (anti-Gia3), and of adenosine receptor-G protein complexes were detected by the binding of radioactive $[^3H]DPCPX$.

**Results.** Data indicate that $[^3H]DPCPX$ forms high-affinity complex with membrane-bound and solubilized forms of adenosine receptors from PE and NPE cells. Peptide-directed antisera against various G protein $\alpha$ subunits indicate that the $A_1$ adenosine receptors from these cells are specifically coupled to $G_{ia}$ complexes. The results further indicate that the $A_1$ adenosine receptors are predominantly associated to $G_{ia3}$.

**Conclusion.** The findings document a selective interaction between the $\alpha$ subunits of the inhibitory guanine nucleotide binding protein ($G_i$) and $A_1$ adenosine receptors in ocular ciliary epithelial cells in culture. The results suggest that adenosine receptors coupled to $G_{ia3}$ may provide a site at which modulation of aqueous humor production in the ciliary epithelium occurs via the G protein–adenylyl cyclase pathway. Invest Ophthalmol Vis Sci. 1994;35:3057-3063.

Guanine nucleotide binding proteins (G proteins) represent a family of heterotrimeric proteins that serve a critical biologic role by coupling membrane-bound receptors for neurotransmitters, hormones, and autacoids to cellular effector systems. In the ocular ciliary epithelium, G proteins couple membrane surface receptors to the effector systems cAMP and phosphatidylinositol (PI) hydrolysis. The molecular changes that affect the production of cAMP by adenylyl cyclase are clearly central to the regulation of aqueous humor flow, and it is likely that the calcium signaling PI hydrolysis pathway in the ciliary epithelium is also important for the stimulus–secretory activity of this issue.

Although the direct causal relationship of adenylyl cyclase activity and the production of aqueous humor is by no means clear, it is well-known that receptor-mediated alteration of the $\beta$-adrenergic receptor/adenylyl cyclase ($\beta$AR/AC) messenger pathway may result in the clinically desirable reduction of intraocular pressure in the treatment of glaucoma. For example, the $\beta$AR antagonist, timolol maleate, is thought to lower intraocular pressure by inhibiting aqueous humor production by the ciliary epithelium. Similarly, adenosine agonists that couple negatively to adenylyl cyclase, thus attenuating enzyme activity in the anterior segment of the eye, have been the subject of recent studies suggesting that these drugs may facilitate a hypotensive intraocular pressure response after topical application. Unfortunately, efforts to characterize the receptors in the rabbit anterior uvea that may mediate these effects have been difficult and incomplete, largely due to the unavailability of highly selec-
tive compounds only recently made available for study. However, the characterization of purinergic receptors in SV40-transformed bovine pigmented ciliary epithelial cells and human nonpigmented ciliary epithelial cells have identified the presence of functional receptors that activate G protein-mediated adenyl cyclase activity and phosphoinositide hydrolysis in both cell lines.\textsuperscript{10}

Although there is structural diversity of G protein \( \alpha \) subunits, demonstrated by the identification of several cDNA clones by separate genes for these proteins, the specificity of \( \alpha \) subunits is such that they receive incoming information from a limited subset of hormone receptors and convey that information to a specific subset of effectors.\textsuperscript{11,12} In this report, we show that the selective adenosine receptor agonist radioligand \([\text{3H}]\text{DPCPX}\) is capable of forming high-affinity interactions with membrane-bound as well as solubilized preparations of the purinergic \( \alpha_1 \) adenosine receptors from the SV40-transformed, bovine-derived pigmented (PE) and human-derived nonpigmented (NPE) ciliary epithelial cell lines. Furthermore, using an immunoprecipitation approach with antibodies raised against peptides corresponding to the unique internal sequences of G protein subtypes, we have investigated which G protein \( \alpha \)-subunits couple with solubilized \( \alpha_1 \) receptors in ciliary epithelial cells. The present study indicates the coupling of all three subtypes of \( G_{\alpha} \) to solubilized \( \alpha_1 \) receptors in PE and NPE cells; however, solubilized \( \alpha_1 \) receptors from PE and NPE cells predominantly associate with the \( G_{\alpha 3} \) subtype. These results demonstrate for the first time that selective interaction of a G protein \( \alpha \) subunit occurs in association with a family of receptors coupled to the inhibitory adenyl cyclase pathway in ciliary epithelial cells.

MATERIALS AND METHODS

Materials

8-Cyclopentyl-1,3-\([\text{3H}]\)dipropylxanthine (\([\text{3H}]\text{DPCPX}\) (108 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). Xanthine amine congener (XAC) was purchased from Research Biochemicals Inc. (Natick, MA). Protein A sepharose CL-4B beads, aprotinin, pepstatin, and antipain were from Sigma Chemical (St. Louis, MO). All other chemicals were of the highest quality commercially available.

Tissue Culture

A clone (ODM\(_2\)) of human NPE transformed with SV40 and of SV40-transformed bovine PE, as previously described,\textsuperscript{5–7,13} were grown in Dulbecco’s modified Eagle’s medium with 5% fetal calf serum and 5% each of penicillin and streptomycin. Cells were grown to single-layer confluency in a 90% air and 10% CO\(_2\) humidified atmosphere after plating at a density of 3000 to 5000 cells cm\(^2\).

Membrane Preparation

Membranes were prepared from SV40-transformed PE and NPE cells at confluency by aspirating the media and washing the plated cells with ice-cold phosphate-buffered saline, followed by incubation in ice-cold lysis buffer (2 mM Hepes, 2 mM EDTA, 0.5 mM EGTA, pH 7.4). Cell lysates were then removed from tissue culture flasks by scraping with a rubber policeman. The cells were further lysed with 40 strokes in a tight fitting glass–glass homogenizer. Intact cells and nuclear fragments were removed by low-speed centrifugation (5 minutes at 1000g). Membrane fragments were then sedimented by high-speed centrifugation (20 minutes at 40,000g), resuspended in tissue buffer (10 mM Tris, 1 mM EDTA, 0.5 mM EGTA, pH 7.4), sedimented a second time, and resuspended in the same tissue buffer. Membranes were stored in tissue buffer (2.5 mg/ml) at −80°C until use.

Membrane Solubilization

Approximately 10 × 10\(^6\) PE and NPE cells were used for membrane preparation and receptor solubilization for a single immunoprecipitation experiment performed in duplicate. The membranes were thawed immediately before preparation of solubilized \( \alpha_1 \) adenosine receptors–G protein complex. The membranes were centrifuged at 38,000g for 20 minutes at 4°C, and the supernatant was discarded. The pellet was resuspended in 10 ml of a buffer containing 50 mM Tris-HCl (pH 7.2), 25 mM CHAPS, 5 mM MgCl\(_2\), 1 mM EDTA, 100 mM NaCl, 1 mM DTT, 1 mM PMSF, 1 \( \mu \)g/ml each of aprotinin, pepstatin, antipain, and 1% sodium cholate in 20% glycerol. The membranes were homogenized with a Brinkmann (Westbury, NY) polytron (setting 2 for 15 seconds) and placed on ice for 1 hour with constant stirring. The sample was then centrifuged at 100,000g for 1 hour at 4°C, and the supernatant containing solubilized receptors was removed and stored at −20°C.

Radioligand Binding

\([\text{3H}]\text{DPCPX}\) binding assays were performed for 2 hours at room temperature in a total volume of 0.25 ml buffer containing 50 mM Tris-HCl (pH 7.2), 80 mM NaCl, 4 mM MgCl\(_2\), and 1 mM EDTA. Nonspecific binding was determined by the addition of 1 and 10 \( \mu \)M XAC for the adenosine receptor from PE and NPE cells, respectively. \([\text{3H}]\text{DPCPX}\) specific binding was determined by subtracting the nonspecific binding in the presence of XAC from the total binding in the absence of XAC. The binding reaction was terminated by the addition of cold 10 ml of 50 mM Tris-HCl.
(pH 7.2) buffer containing 1 mM EDTA. The samples were vacuum filtered over Whatman (Hillsboro, OR) GF/B glass fiber filters. The filters were washed two times with 10 ml cold Tris-HCl buffer (50 mM, pH 7.2) containing 1 mM EDTA. The filters were dried, and radioactivity was measured in 4 ml Ready-Solv (Beckman, Fullerton, CA) in a Searle betacounter (Searle Analytic, Des Plains, IL) at an efficacy of 60%. The Whatman filter #50 was used for the membrane suspension, and the Whatman filter #32 pretreated with 0.5% polyethyleneamine was used for the solubilized suspension of receptor. Saturation curves were analyzed by computer programs Cricket-Graph (Cricket Software, Malvern, PA) and Canvas (Deneba Systems, Miami, FL), and Scatchard plots were obtained using computer program Microsoft Excel (Microsoft, Redmond, WA).

**G Protein Antisera**

The G protein antisera used were obtained after injection of synthetic peptides corresponding to specific regions of $\alpha$ and were the generous gift of Dr. D. R. Manning, University of Pennsylvania (Philadelphia, Pennsylvania). Antisera 8730 (anti-$\alpha_1$), 3646 (anti-$\alpha_2$), 1521 (anti-$\alpha_3$), and 1518 (anti-$\alpha_4$) or preimmune sera were used to immunoprecipitate $\alpha_1$ adenosine receptors. The selectivity of these antisera to detect specifically the corresponding recombinant forms of $\alpha_1$, $\alpha_2$, and $\alpha_3$, and the efficiency of each antiserum to immunoprecipitate the $\alpha_1$ to a similar extent, have been shown.14-16

**Immunoprecipitation of Adenosine Receptor-G Protein Complexes**

To immunoprecipitate $\alpha_1$ adenosine receptor-G protein complexes, an aliquot of solubilized preparation of the receptor-G protein complexes (400 μl) was incubated with a sample of G protein-specific antiserum for 6 hours at room temperature on rotator. One hundred microliters of 50% (wt/vol) protein A sephrose beads, washed three times and diluted in 50 mM Tris-HCl buffer, pH 7.2, containing 1 mM EDTA, 5 mM MgCl₂, and 1 μg/ml each of aprotinin, pepstatin, and antipain (buffer A), was added to the sample and incubated overnight on rotator at 4°C. Another aliquot of antiserum was subsequently added, bringing the total antiserum dilution to 1:50, which is the optimal concentration of antiserum needed to immunoprecipitate G protein and $\alpha_1$ receptor coupled to G protein. The samples were incubated for 4 more hours and then centrifuged at 12000 rpm for 5 minutes in an Eppendorf (Brinkmann) microcentrifuge. The supernatant was removed, and adenosine receptors were detected using the $^3$H]DPCPX binding assay described above. The immunoprecipitate was resuspended in buffer A, and adenosine receptors were detected using the $^3$H]DPCPX binding assay.

**RESULTS**

**Characterization of Binding of $^3$H]DPCPX to $\alpha_1$ Adenosine Receptors**

The binding of the radioligand $^3$H]DPCPX to the $\alpha_1$ adenosine receptors associated with the membranes or in a solubilized state was determined as described in Materials and Methods. The binding of $^3$H]DPCPX to the receptors from PE cells was specific and saturable. The saturating concentration for the $^3$H]DPCPX was 2 nM for both membrane-bound (Fig. 1) and solubilized $\alpha_1$ adenosine receptors from PE cells. The binding of $^3$H]DPCPX to the receptors from NPE cells was saturable at 4 nM (Fig. 2). The nonspecific binding of the $^3$H]DPCPX in the presence of 1 μM XAC, a selective $\alpha_1$ antagonist, comprised approximately 10% of the total binding for the receptor from PE cells (Fig. 1). However, the nonspecific binding of the $^3$H]DPCPX to the receptor from NPE cells in the presence of 10 μM XAC comprised approximately 85% of the total binding (Fig. 2). Transformation of the saturation data to a linear Scatchard plot revealed that the equilibrium constant ($K_a$) of $^3$H]DPCPX was 0.7 ± 0.2 nM and 0.9 ± 0.2 nM to membrane-bound and solubilized forms of receptor from PE cells, respectively. The equilibrium constant ($K_a$) of $^3$H]DPCPX for membrane-bound and solubilized forms of receptor from NPE cells was 2.4 ± 0.3 nM and 1.3 ± 0.2 nM, respectively. A representative Scatchard plot of the $^3$H]DPCPX saturation binding data for membranes from bovine pigmented and human nonpigmented cili-
 FIGURE 2. [3H]DPCPX saturation curves in human nonpigmented ciliary epithelial membranes. The data represent a typical experiment performed in duplicate.

FIGURE 3. Representative Scatchard plots of the [3H]DPCPX saturation binding data for membranes from bovine pigmented ciliary epithelium (A) and human nonpigmented ciliary epithelium (B) is shown.

FIGURE 4. A$_1$ adenosine receptors from bovine pigmented ciliary epithelium were solubilized and immunoprecipitated with G$_{ia}$ subtype-selective antisera. The presence of A$_1$ adenosine receptor in the immunoprecipitate was measured using the [3H]DPCPX binding assay.

FIGURE 5. A$_1$ adenosine receptors from human nonpigmented ciliary epithelium were solubilized and immunoprecipitated with G$_{ia}$ subtype-selective antisera. The presence of A$_1$ adenosine receptor in the immunoprecipitate was measured using the [3H]DPCPX binding assay.

Selectivity of Adenosine Receptor With G$_{ia}$ Subunit

Antiserum 8730 recognizes a consensus sequence unique to the G$_a$ family of a subunits, which is common to G$_{ia}$ 1, 2, and 3. Antiserum 8730 immunoprecipitated approximately 50% of [3H]DPCPX binding activity in the solubilized A$_1$ receptors of PE and NPE cell lines. In a typical experiment, immunoprecipitation of the receptor–G protein complex assessed by [3H]DPCPX binding in the presence of XAC comprised less than 10% of the total [3H]DPCPX binding in the pellet. The inhibition of [3H]DPCPX binding activity by the addition of XAC, an antagonist highly selective to the A$_1$ receptor, thus indicates that the immunoprecipitation is specific. Preimmune 8730 antiserum failed to immunoprecipitate any [3H]DPCPX binding activity (Figs. 4, 5). In a typical experiment, preimmune control sera immunoprecipitated 20 ± 10, and 30 ± 10 cpm of specific [3H]DPCPX binding, and 1600 ± 100 and 1850 ± 100 cpm of specific [3H]DPCPX binding remained in the supernatants of solubilized preparations of receptors from PE and NPE cells, respectively. By contrast, antiserum 8730 immunoprecipitated 800 ± 100 and 850 ± 100 cpm of specific [3H]DPCPX binding sites, and 900 ± 100 and 1000 ± 100 cpm of specific [3H]DPCPX binding sites
remained in the supernatants of solubilized preparations of receptors from PE and NPE cells, respectively. These results are presented in the form of column graphs in Figures 4 and 5.

To identify further the subspecies of $G_{ia}$ coupled to the $A_1$ adenosine receptor, peptide-generated antisera directed against $G_{ia1}$, $G_{ia2}$, and $G_{ia3}$ were tested for their ability to immunoprecipitate solubilized adenosine receptor—$G$ protein complexes from PE and NPE cells. We observed that antisera to $G_{ia}$ subunits immunoprecipitated various percentages of solubilized $A_1$ receptor—$G$ protein complexes, as indicated by the loss of specific $[^3H]DPCPX$ binding in the supernatants of antisera immunoprecipitates. Results presented in Figures 4 and 5 indicated that antisera 3646, 1521, and 1518 immunoprecipitated adenosine receptor complexes from PE and NPE cells. The presence of $A_1$ adenosine receptors in the immunoprecipitate was detected by the binding of radioligand $[^3H]DPCPX$, as described in Materials and Methods.

The $A_1$ adenosine receptor from PE and NPE cells predominantly couple to $G_{ia3}$ as assessed by the ability of $G_{ia3}$ specific antisera to immunoprecipitate a greater percentage of receptor—$G$ protein complex in comparison with antisera directed against $G_{ia1}$ or $G_{ia2}$. The preimmune 3646, 1521, and 1518 antisera failed to precipitate any $[^3H]DPCPX$ radioligand binding. In a typical experiment, antisera 1518 immunoprecipitated $900 \pm 100$ cpm and $800 \pm 100$ cpm of specific $[^3H]DPCPX$ radioligand binding from the solubilized preparations of $A_1$ adenosine receptors in PE and NPE cells, respectively. Antiserum 1521 immunoprecipitated $450 \pm 30$ and $400 \pm 20$ cpm of specific $[^3H]DPCPX$ binding in PE and NPE cells, respectively. Antiserum 3646 immunoprecipitated $250 \pm 30$ and $500 \pm 40$ cpm of specific $[^3H]DPCPX$ binding in PE and NPE cells, respectively.

Therefore, antisera to $G_{ia3}$ immunoprecipitated approximately 50% of $[^3H]DPCPX$ binding activity from the solubilized receptor in PE cells, whereas antisera to $G_{ia1}$ and $G_{ia2}$ immunoprecipitated approximately 16% and 28%, respectively. In NPE cells, antisera to $G_{ia3}$ similarly immunoprecipitated approximately 43% of $[^3H]DPCPX$ binding activity, whereas antisera to $G_{ia1}$ and $G_{ia2}$ immunoprecipitated 27% and 21% of $[^3H]DPCPX$ binding activity. Thus, the order of the preference for the solubilized $A_1$ adenosine receptor to predominantly associate with $G_{ia}$ subunits is $G_{ia3} > G_{ia2} > G_{ia1}$ (Fig. 4) and $G_{ia3} > G_{ia3} > G_{ia2}$ (Fig. 5) from PE and NPE cells, respectively.

**DISCUSSION**

The results of this study indicate that the antagonist $[^3H]DPCPX$ binds with high affinity to the membrane and solubilized preparations of the $A_1$ adenosine receptors from PE and NPE cells of the ciliary epithelium (Figs. 1, 2). The properties of the antagonist DPCPX that make it an excellent choice as a radioligand in these studies include its nanomolar potency at the $A_1$ adenosine receptor and its greater selectivity for $A_1$ as opposed to $A_2$ adenosine receptors than other available selective antagonists such as XAC. Competition binding for radioligand binding to rat brain preparations reveals that the $K_i$ ratios ($A_1/A_2$) for DPCPX and XAC are 740 and 31, respectively; thus, DPCPX offers approximately 24-fold greater selectivity for the $A_1$ adenosine receptor than XAC. 

Several studies that use $[^3H]DPCPX$ to identify stable, solubilized preparations of partially purified $A_1$ receptors confirm its use as an excellent radioligand for these studies. 

In the solubilized receptor preparations, increased binding sites with little change of the $K_i$ for $[^3H]DPCPX$ may be due to a binding enhancement modulation by the addition of Mg$^{2+}$ during the solubilization procedure. The density of $A_1$ receptors in ciliary epithelial cell lines is comparable to that found in bovine retinal membranes (25 to 50 fmol/mg) but significantly less than the density of these receptors in crude membrane homogenates from brain (100 to 700 fmol/mg).

Coupling between the $A_1$ adenosine receptor and $G$ proteins appears to be very tight in membranes and after solubilization under denaturing conditions. Furthermore, studies suggest that the partially purified $A_1$ receptor displays low-affinity agonist and high-affinity antagonist binding. Thus, using an approach in which the solubilized receptor—$G$ protein complex is immunoprecipitated with peptide-directed antisera raised against the unique peptide sequences of the $\alpha$ subunits to $G_{ia1}$, $G_{ia2}$, or $G_{ia3}$, we have been able to measure receptor—$G$ protein interactions in the absence of agonist. After the solubilized preparation of the receptors is immunoprecipitated with $G$ protein selective antisera, the receptor—$G$ protein complex is identified by the $[^3H]DPCPX$ binding to the immunoprecipitate. This approach has recently been shown to be effective in identifying the selective association of $G_{ia}$ subunits to solubilized somatostatin receptors and to $\alpha_2$ adrenergic receptors. The antisera raised against the specific $G$ proteins have also been successfully used to identify by immunoblotting
all the subtypes of G_{w1-3} in the identical clones of ocular ciliary epithelial cells used in this study.28

The results indicate that the A_1 adenosine receptors from these cells can couple with all three subtypes of G_{w1}. The results further indicate that the A_1 adenosine receptors are efficiently coupled to G_{w3}, but association with G_{w2} and G_{w1} is also demonstrated (Figs. 4, 5). The antiseras 1518, 1521, and 3646, specific to G_{w3}, G_{w2}, and G_{w1}, respectively, immunoprecipitated approximately 50%, 28%, and 16% of the solubilized receptor–G protein complex from the PE cells and 43%, 21%, and 27% of the solubilized receptor–G protein complex from the NPE cells. Antiserum 8730 (raised against the C-terminal peptide sequence common to the G_{w1}, G_{w2}, and G_{w3} subtype) immunoprecipitated approximately 50% of the solubilized receptor–G protein complexes from PE and NPE cells. The unexpected lower efficiency of antiserum 8730 to immunoprecipitate the receptor bound G protein complex may be explained by the conformational change in the G protein after the receptor binding and the inaccessibility of the antiseras to their C-terminal epitopes. The immunoprecipitation of the adenosine receptor–G protein complex is specific because the binding of [3H]DPCPX is not detected in the precipitate in the presence of XAC.

Information concerning the relative amounts of the various G_i proteins in ciliary epithelial cells, in addition to the affinities of the G_i members for the A_1 receptor, would be helpful in quantitatively assessing the preferential association of the adenosine receptor for G_i proteins using immunoprecipitation. There is no information in the literature suggesting that members of the G_i family of proteins have different affinities for any family of receptors, nor is there a reliable method to quantitate the various G_i members in ciliary epithelial cells by immunoblotting. Without this information, we can not draw quantitative conclusions about the relative selectivity of G_i proteins for the A_1 receptor. However, data presented here do indicate that in this cellular context, adenosine receptors are predominantly associated with G_{w3} in ocular ciliary epithelial cells.

The observation that the A_1-adenosine receptor in ocular epithelia discriminates selectively between the different α subunits of G_i is in good agreement with the observations of Freissmuth et al.,29 who observed a preference for recombinant G_{w1} to reconstitute high-affinity agonist binding to purified brain A_1 adenosine receptors. Because the specificity of G protein coupling to receptors and effectors is becoming increasingly well documented,11,30 individual proteins that govern specific transmembrane signaling pathways are being identified with greater precision. The findings of the present study may be helpful in this context as we strive to examine the specific components of adenylyl cyclase activity in the ciliary epithelium and its role in regulating aqueous humor secretion. Although these experiments have been performed on SV40-transformed cell lines derived from the ciliary epithelium, the findings suggest a plausible basis for the efficacy of adenosine agonists when used in vivo and are consistent with preliminary experiments that have documented the presence of adenosine receptors in the rabbit iris–ciliary body by radioligand binding.8 The specificity of coupling of A_1 receptors to G proteins in the native human ciliary epithelium may not be identical to the findings reported here with clonal cell lines. Nevertheless, these cell lines have been successfully used in several studies that demonstrate they retain many properties of native ciliary epithelium.

Whether other receptor pathways that couple to the inhibition of adenylyl cyclase in the ciliary epithelium, such as α_2 adrenergic receptors, are selectively coupled to G protein α subunits in this tissue remains unknown. Equally important however, is the possibility that other regulatory subunits, such as βγ, may be subtype specific in the receptor–G protein–AC pathway in ciliary epithelia. These findings document a selective interaction between the α subunits of the inhibitory guanine nucleotide binding protein (G_i) and A_1 adenosine receptors in ocular ciliary epithelial cells in culture. The results suggest that adenosine receptors coupled to G_{w3} may provide a site at which modulation of aqueous humor production in the ciliary epithelium occurs via the G protein–adenylyl cyclase pathway. These findings may, therefore, be potentially useful in highlighting a specific interaction, or target, for manipulation of the G protein–AC pathways (in this case, coupled to A_1 adenosine receptors) in the ciliary epithelium in an effort to develop new therapy to lower intraocular pressure in patients with glaucoma.

Key Words
ocular ciliary epithelium, G protein antisera, G_{w3}, A_1 adenosine receptors, [3H]DPCPX

References


4. Sears ML. Regulation of aqueous flow by the adenylyl-
Immunoprecipitation of A1 Adenosine Receptors

3063


