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κ-Opioid Receptors Couple to Inwardly Rectifying Potassium Channels when Coexpressed by *Xenopus* Oocytes

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SUMMARY

Xenopus oocytes expressed κ-opioid specific binding sites after injection of cRNA prepared from a clone of the rat κ-opioid receptor. Coinjection of κ receptor cRNA with cRNA coding for a G protein-linked, inwardly rectifying, K^+ channel (GIRK1, or KGA) resulted in oocytes that responded to the κ agonist U-69593 by activating a large (1.0–1.5-μA) K^+ current. U-69593 exhibited an EC_{50} of 260 ± 50 nM and was blocked by the opioid antagonists norbinaltorphimine and naloxone. The κ agonist bremazocine was 200-fold more potent than U-69593 in eliciting K^+ current but exhibited a partial agonist profile in this expression system. The present results indicate that stimulation of inwardly rectifying K^+ channels may be a potential effector mechanism for κ-opioid receptors.

Opioid receptors are generally grouped into three major types, termed δ, κ, and μ (1, 2). Activation of the κ receptor results in analgesia (3) and opioid-induced dysphoria (4). The cellular basis of κ receptor action was shown to involve inhibition of the release of several neurotransmitters, including acetylcholine (5), glutamate (6–9), dopamine (10), and the endogenous κ agonist dynorphin (6).

The molecular basis of κ receptor-mediated inhibition of neurotransmitter release has been studied in several systems and a variety of effectors have been identified. Considerable evidence suggests that κ-opioid receptors are coupled to G proteins and can inhibit N-type calcium channels in preparations of cortical synaptosomes (11), dorsal root ganglion neurons (12, 13), and ileum myenteric plexus (5). In addition, a recent finding by Grudt and Williams (14) indicates that κ receptors increase K^+ conductance in substantia gelatinosa neurons within the guinea pig medulla oblongata. κ receptors can exert inhibitory effects on the adenylate cyclase transduction pathway in guinea pig brain (15) or when expressed in COS-1 cells (16).

The recent cloning of the κ-opioid receptor (16–19) offers an opportunity to study the coupling mechanisms of this molecule in a controlled environment. In the present study we used the *Xenopus* oocyte expression system to describe rat κ receptor-mediated activation of the GIRK1 channel (also called KGA) (20, 21).

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Materials and Methods

Cloning and characterization of rat brain κ-opioid receptor cDNA. Total RNA was prepared from freshly dissected rat striatum. Double-stranded cDNA was randomly primed and synthesized from 5 μg of total RNA using the Superscript system (GIBCO/BRL, Gaithersburg, MD). A pair of degenerate primers with unique restriction enzyme sites at their 5′ ends were designed based on conserved sequences present in putative TM-III and -VI of a wide variety of cloned G protein-coupled receptors, as follows: TM-III, 5′-GAGTCGACCTGTG(C/T)G(C/T)(C/G)AT(C/T)(AG)CIIT(G/T)GAC(C/A)G(C/G)TAC-3′ (where I is inosine); TM-VI, 5′-CAGAATTCAG(T/A)AGGGCAICCAGCAGAI(G/C)(G/A)(T/C)GAA-3′.

One hundred picomoles of both the sense (TM-III) and antisense (TM-VI) primers were mixed with 1 μg of thermally denatured, rat striatum cDNA at 45° for 2 min, in the presence of a thermostable DNA polymerase prepared from *Thermus flavus* (Epicenter, Madison, WI), 0.2 mM deoxynucleoside triphosphates, 50 mM Tris, pH 9.0, 20 mM ammonium sulfate, and 0.5 mM $MgCl_2$. After 35 cycles of PCR, the resulting DNA products were electrophoresed in an agarose gel and size-selected to enrich for molecules between 300 and 800 base pairs in length. These PCR products were digested with *Sal*I and *Eco*RI and subcloned into M13. Thirty plaques were picked and processed for dideoxy DNA sequencing (Sequenase; United States Biochemicals, Cleveland, OH). The resulting nucleotide sequence information was analyzed using software designed by Intelligenetics

ABBREVIATIONS: GIRK1 channel, G protein-linked, inwardly rectifying, K^+ channel; TM, transmembrane domain; PCR, polymerase chain reaction; hK buffer, high-K^+ buffer; nBNI, norbinaltorphimine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; 5-HT, 5-hydroxytryptamine.

(Mountain View, CA) and Genetics Computer Group (Madison, WI). Deduced peptide sequences were used to query molecular biology databases maintained by the National Center for Biotechnology Information, by using BLAST, a sequence similarity program available through the Internet (blast@ncbi.nlm.nih.gov), and the RETRIEVE server (retrieve@ncbi.nlm.nih.gov). The deduced peptide sequence of one PCR product, R21, was found to share approximately 70% identity with the mouse δ-opioid receptor (22, 23). To obtain a cDNA that encodes a complete protein, R21 was labeled with [α-^{32}P]dCTP (GIBCO/BRL) by nick translation, boiled, and used to probe duplicate nylon membranes (Colony/Plaque Screen; NEN, Wilmington, DE) lifted from cDNA libraries. Hybridization was performed in 50% formamide, 5× standard saline citrate, 1% sodium dodecyl sulfate, 5× Denhardt's solution, at 37° for 24–72 hr. Filters were washed twice at room temperature in 2× standard saline citrate, 0.1% sodium dodecyl sulfate, followed by several washes at 55°, and were exposed to Kodak XAR-5 film, with an intensifying screen, for 24–72 hr at −70°. A 1.3-kilobase rat brain cDNA, R21–1, that hybridized strongly with the R21 PCR probe was identified and partially sequenced. Analysis of this sequence revealed that 350 bases at the 5′ end and 175 bases at the 3′ end of this clone were identical to a published rat κ-opioid receptor (17). To confirm that the receptor encoded by R21–1 bound κ-opioid ligands, the the cDNA was subcloned into the expression vector pRc/RSV (Invitrogen, San Diego, CA) and expressed in both COS-7 and Chinese hamster ovary cells. Membranes prepared from wild-type cells exhibited no significant binding affinity for [^{3}H][D-Ala^2,*N*-MePhe^4,Gly-ol]-enkephalin, [^{3}H]diprenorphine, or [^{3}H][D-Pen^2,D-Pen^5]-enkephalin. In contrast, membranes prepared from either COS-7 or Chinese hamster ovary cells transfected with R21–1 bound [^{3}H]diprenorphine with high affinity (K_d = 0.45 nM). This binding was saturable and completely inhibited in the presence of 10 μM dynorphin A-(1–13) amide (data not shown). For the purpose of preparing RNA the R21–1 cDNA was subcloned into the *Sal*I site of pGEM^R-3 (Promega, Madison, WI).

Preparation of cRNAs. GIRK1 channel cRNA was prepared as described by Dascal *et al.* (20). The amount of κ receptor cDNA was first increased by utilization of Amplitaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) in a standard PCR, using oligonucleotides designed to add an SP6 promoter region and a 45-base poly(A)$^+$ tail. Purity and yield of the product were verified by gel electrophoresis and measurement of absorbance spectra. SP6 RNA polymerase (Ambion) was used to generate capped cRNA. The cRNA was then dissolved in RNase-free water and stored at −75° until injection.

Oocyte maintenance and injection. Healthy stage V and VI oocytes were harvested from mature anesthetized *Xenopus laevis* (Xenopus One, Ann Arbor, MI) and defolliculated enzymatically as described previously (24). The oocytes were maintained at 18° in standard ND96 medium (96 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.5) supplemented with 5% heat-inactivated horse serum, 2.5 mM sodium pyruvate, and 50 μg/ml gentamicin (Sigma Chemical Co.). One day after harvest, a Drummond 10-μl syringe fitted with a tapered glass pipette tip (15–20 μm in diameter) was used to inject 50 nl of cRNA into the vegetal pole of each oocyte. In G protein subunit coexpression studies, each subunit cRNA (1–2 ng) was coinjected with κ-opioid receptor and GIRK1 channel cRNA.

Radioligand binding assays. Oocytes were homogenized in sucrose buffer (320 mM sucrose, 1.0 mM EGTA, 10 mM sodium phosphate, 5 mM $MgCl_2$, 0.5 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A; Sigma) and centrifuged at low speed (1000 × *g* for 10 min at 4°) to remove high density debris. The supernatant was collected and the pellet was resuspended in buffer for a second identical centrifugation. The supernatants from the two centrifugations were pooled and ultracentrifuged at 100,000 × *g* for 30 min at 4°. For radioligand binding assays, the resulting pellet was resuspended in 50 mM Tris buffer, pH 7.8, containing EGTA, $MgCl_2$, and the protease inhibitors listed above. Membranes (15–20 μg of protein/tube) were incubated with 1 nM [^{3}H]U-69593 for 60 min at 22–24°, in a total volume of 1 ml for saturation experiments or 2 ml for homologous displacement studies. The amount of bound radioactivity remaining in the presence of 10 μM naloxone defined nonspecific binding. Binding assays were conducted in triplicate, and the reaction was terminated by vacuum filtration through Whatman GF/B filter paper saturated with 0.1% polyethylenimine. Each filter was washed three times with 4.0 ml of ice-cold 50 mM Tris, pH 7.8, and a liquid scintillation counter was used to determine bound radioactivity. Protein content was determined using a Bio-Rad assay. One- versus two-site fit analysis was conducted with the LIGAND program. IC_{50} values from displacement binding experiments were generated by log-logit transformation and linear regression, and K_i values were calculated using the Cheng-Prusoff equation (25).

Electrophysiological studies. An Axoclamp 2A amplifier was used for standard two-electrode voltage-clamp experiments. The pCLAMP program (Axon Instruments) was used for data acquisition and analysis. Oocytes were removed from incubation medium, placed in the recording chamber containing ND96 medium, and clamped at −80 mV. Because the conductance of the inward rectifier K^+ channel is dependent on both voltage and the ionic composition of the medium, recordings of ligand-gated activity were made in hK buffer (2 mM NaCl, 96 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.5) (20). The use of hK buffer obviated the need for strongly hyperpolarizing voltage-clamp conditions to observe responses to U-69593. The current observed in hK buffer is actually a combination of K^+ flux through the GIRK1 channel and a current endogenous to the oocytes (26). Because the GIRK1 channel is more sensitive to Ba^{2+} block than are endogenous channels, the current remaining during application of hK buffer plus 300 μM Ba^{2+} can be subtracted from the total hK buffer-induced current to yield GIRK1 current (26). Half-life values were generated by measuring the time required for GIRK1 current plus U-69593-gated current to decay to half of the peak response. For antagonism studies, each oocyte was exposed, under voltage-clamp conditions in the recording chamber, to nBNI or naloxone for 5 min in ND96 buffer and during introduction of hK buffer and agonist. Controls were perfused with ND96 buffer for 5 min under voltage-clamp conditions. Individual comparisons of drug effects or G protein subunit coinjections were conducted using oocytes from the same harvest and injection batch. Comparisons between G protein subunit-coinjected groups and controls were made by an analysis of variance, followed by Dunnett's test on individual points of the dose-response curve. EC_{50} values were calculated by log-logit transformation and linear regression of dose-response curves.

Results

Xenopus oocytes expressed κ-opioid specific binding sites 5–14 days after injection of κ cRNA. After injection of 2 ng of cRNA/oocyte, Scatchard analysis indicated a single-site fit, with a peak expression of 47 pmol of [^{3}H]U-69593 sites/mg of membrane protein and an estimated K_d of 68 nM (Fig. 1). Similar results were observed in two homologous displacement experiments with 1 nM [^{3}H]U-69593 (mean K_i = 61 nM, one-site fit favored). Although Fig. 1, *inset*, suggests that U-69593 may bind to two sites [two-site linear regression analysis of the data in Fig. 1 proposes high (K_d = 21 nM) and low (K_d = 109 nM) affinity subpopulations of κ receptors], statistical analysis with the LIGAND program indicated that a two-site fit was not significantly favored over a one-site fit [$F(2,17) = 0.482$, $p > 0.05$, not significant].

Coinjection of κ receptor cRNA with GIRK1 channel cRNA resulted in oocytes that displayed large, inwardly rectifying K^+ currents in response to 10 μM U-69593 (Fig. 2A). Fig. 2A illustrates the desensitization of the agonist-gated K^+ conductance after sustained exposure of the oocyte to U-69593; the observed $t_{½}$ for desensitization in these experiments was 10.5 ± 1.1 min (n = 3). Fig. 2B illustrates the current-voltage

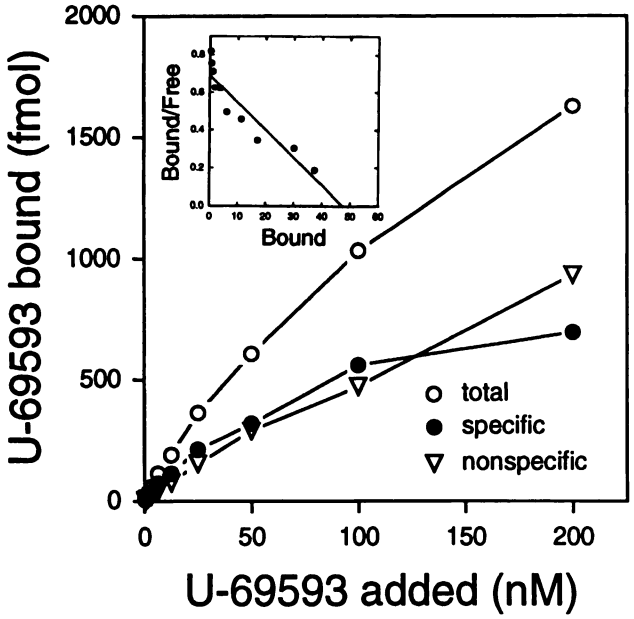

Fig. 1. Saturation binding curves from oocytes injected with 2 ng of κ-opioid receptor cRNA 7 days before testing. See Materials and Methods for details of the binding assay. *Inset*, Scatchard transformation of these data. The *x*- and *y*-axes are pmol/mg of protein and pmol/mg of protein/nM, respectively.

relations observed in the same oocyte, sampled at various times during the experiment shown in Fig. 2A. The reversal potential for K^+ (−22 mV) did not significantly change during desensitization to U-69593, suggesting that accumulation of K^+ within the oocyte was not responsible for the reduced response to the κ agonist.

The maximum amplitude of U-69593-gated current was dependent upon the amount of κ receptor cRNA previously injected into the oocyte (Fig. 3). The data show an apparent lack of spare receptors in this expression system. The κ-selective opioid antagonist nBNI (1 μM) inhibited the response to 100 nM U-69593 (>95% blockade, $n = 3$), as did 1 μM application of the nonspecific opioid antagonist naloxone (>95% blockade, $n = 3$). This agonist-gated K^+ conductance was not observed in oocytes injected only with cRNA for the κ receptor ($n = 3$) or the GIRK1 channel ($n = 3$) and was blocked by application of 300 μM Ba^{2+} (>95% blockade, $n = 4$).

When oocytes injected with 1 ng of κ receptor cRNA plus 1 ng of GIRK1 channel cRNA were compared for sensitivity to the κ_1 agonist U-69593 and the nonselective κ agonist bremazocine, bremazocine exhibited >200-fold higher potency (EC_{50} = 1.7 nM) than did U-69593 (EC_{50} = 370 nM) (Fig. 4). The results of six independent experiments conducted with six different batches of oocytes coinjected with the same amount of κ receptor and GIRK1 channel cRNA revealed an EC_{50} of 260 ± 50 nM for U-69593. Bremazocine exhibited a partial agonist profile, with a maximal response of 34% of the largest response elicited by U-69593 (Fig. 4). These findings were replicated in three independent experiments with identically injected oocytes from three different harvest batches.

Oocytes coinjected with cRNAs coding for various G protein subunits exhibited no significant shifts in EC_{50} values for U-69593. Only the group coinjected with 1 ng of $G_{i\alpha 2}$ cRNA exhibited a change in maximal U-69593-stimulated current that was significantly different from controls ($p < 0.05$, Dunnett's test) (Fig. 5). When oocytes expressing κ-opioid receptors and GIRK1 channels were pretreated with the κ-specific antagonist nBNI (200 pM), the dose-response curve was shifted to the right but was not parallel to the control dose-response curve, suggesting that the antagonism exhibited by

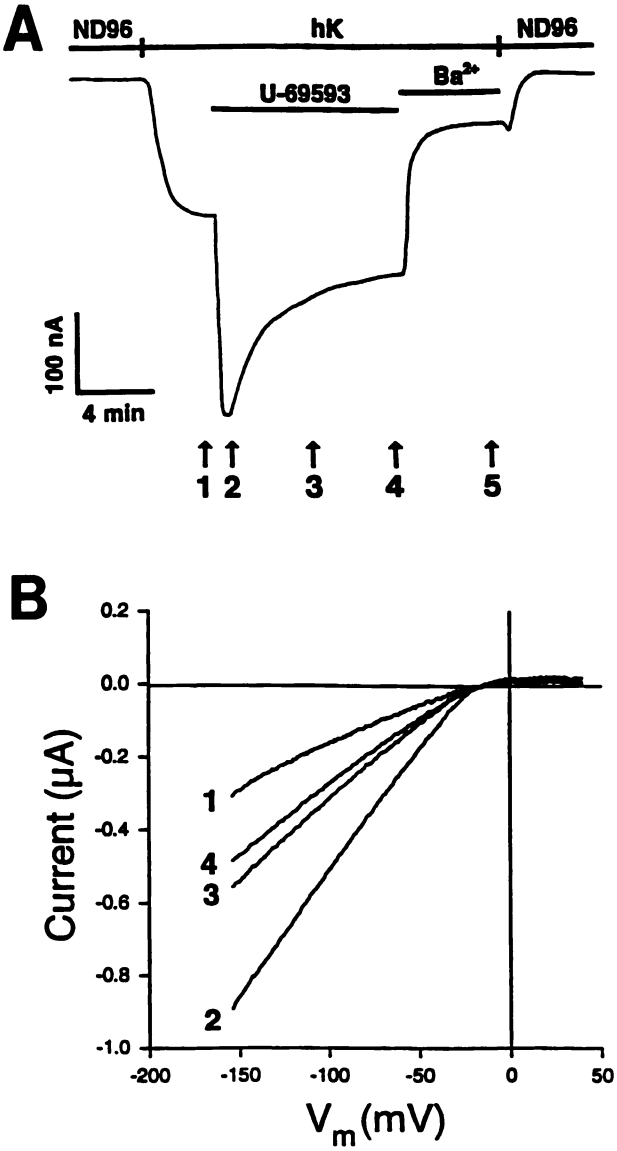

Fig. 2. Activation of an inward K^+ conductance by the κ agonist U-69593 in oocytes injected with κ receptor plus GIRK1 channel cRNA. A, Inward currents evoked by the exchange of ND96 buffer for hK buffer in a representative oocyte voltage-clamped at −80 mV (see Materials and Methods). Superfusion of the κ agonist U-69593 (10 μM) elicited large inward currents that decayed rapidly upon continuous application. Introduction of Ba^{2+} (300 μM in hK buffer) blocked the response to hK buffer and U-69593 and left a small, non-GIRK1 channel-mediated component of the inward current response to hK buffer that did not desensitize. Ba^{2+} and hK buffer were washed out simultaneously, resulting in a small inward current tail. *Numbered arrows*, times at which current-voltage relationships were sampled for the plots illustrated in B. B, Current-voltage relationships for the response of the oocyte illustrated in A to hK buffer and the κ agonist U-69593 upon prolonged stimulation. *Numbers*, sample times indicated in A. Note that the reversal potential for K^+ in hK buffer (−22 mV) did not significantly change during desensitization, indicating that the attenuated agonist response was not caused by accumulation of K^+ inside the oocyte. Residual currents persisting in the presence of 300 μM Ba^{2+} were subtracted from these plots (see A, *arrow 5*).

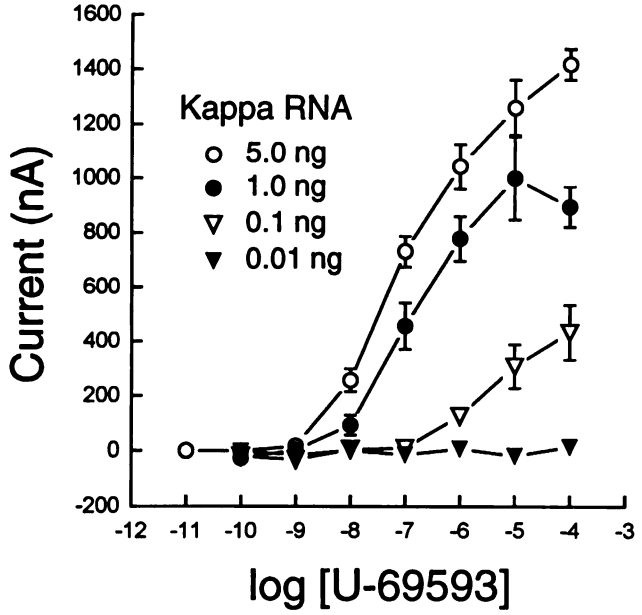

Fig. 3. Dose-response relationships for the κ agonist U-69593 in oocytes injected with 1 ng of GIRK1 channel cRNA plus 0.01–5.0 ng of κ receptor cRNA. Each oocyte was exposed only once to a given dose of U-69593. Each *point* represents the average ± standard error of the peak current response minus the basal hK buffer response of three to six oocytes.

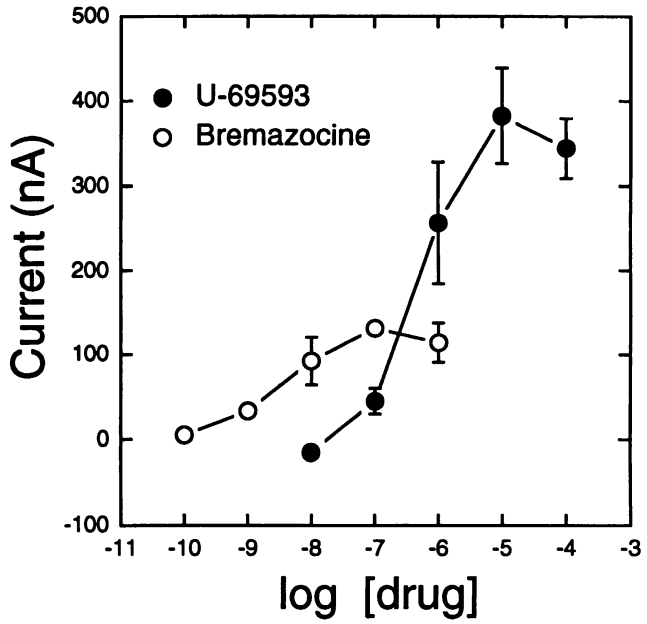

Fig. 4. Dose-response relationships for the κ agonists bremazocine and U-69,593 in oocytes (from the same harvest batch) injected with 1 ng of GIRK1 channel cRNA and κ-opioid receptor cRNA. Each oocyte was tested only once with one of the two compounds. Each *point* represents the average ± standard error of the peak current response minus the basal hK buffer response of three or four oocytes. These findings were replicated in three independent experiments with identically injected oocytes from three different harvest batches.

nBNI was not of a purely competitive nature (Fig. 6). Similar results were observed when this experiment was repeated with a second batch of identically injected oocytes.

Discussion

These results indicate that κ receptor stimulation can activate an inwardly rectifying K^+ channel. Although both δ-opioid (20) and μ-opioid (27–29) receptors have been reported to couple to inwardly rectifying K^+ channels, κ receptor activation has been most often associated with inhibition of N-type Ca^{2+} channels (12). A recent report indicated that κ receptors can stimulate K^+-mediated hyperpolarization in substantia gelatinosa neurons *in vitro* (14). In that study, a preliminary characterization of the biophysical properties of this conductance indicated only weak inward rectification, but a portion of the response may have been mediated by a GIRK1-like channel. The coupling between the κ receptor and the GIRK1 channel demonstrated in oocytes may reflect the process reported in substantia gelatinosa neurons.

The existence of multiple subtypes of the κ-opioid receptor has been proposed (30), and κ receptors have been assigned two (31) or more (32) subtypes, based upon binding displacement curves, autoradiography, and drug discrimination paradigms. The κ_1 subtype binds arylacetamide derivatives such as U-69593 with high affinity (K_d = 1–10 nM), whereas the κ_2 subtype exhibits a low affinity for these compounds (31). The benzomorphan bremazocine generally binds with high affinity to both sites (31). The existence of such subtypes has been debated (e.g., see Ref. 33) due to incomplete functional data supporting their existence.

The use of the *Xenopus* expression system enabled us to study the properties of a population of κ receptors expressed from a single clone. A direct comparison revealed that the κ receptor clone expressed in our studies exhibited an approximately 200-fold preference for bremazocine over U-69593, suggesting a κ_2 subtype. The low potency of U-69593 (EC_{50} = 260 ± 50 nM) was surprising, because several groups (17–19) reported a κ_1-like binding profile, exhibiting high affinity [^{3}H]U-69593 binding (K_d or K_i of 2–30 nM), when similar or identical rat clones were expressed in COS-1 or COS-7 cells. The results from our Scatchard analysis are also more suggestive of a κ_1 profile.

Compared with the data reported here, U-69593 is approximately 10-fold more potent in other bioassays. In substantia gelatinosa neurons, U-69593 stimulated K^+-mediated hyperpolarization with an EC_{50} of 23 nM (14), and in the hippocampus U-69593 exhibited an EC_{50} of 26 nM in inhibiting glutamate release (7). Because oocytes injected with 1 ng each of κ receptor and GIRK1 channel cRNA do not express spare κ receptors (Fig. 3), the EC_{50} measured should reflect the K_d of the active receptor conformation. The observed difference in potency may result from differences in the post-translational modifications of the receptor, differences in the G proteins expressed by the oocyte, or differences in the spare receptor fraction.

The 4-fold difference between the dissociation constant and the EC_{50} determined in voltage-clamp assays may reflect differences in the coupling state of the receptor (34). Although LIGAND analysis did not statistically favor a two-site fit over a one-site fit, a two-site linear regression analysis of the data in Fig. 1 suggests high affinity (K_d = 21 nM) and low affinity (K_d = 109 nM) subpopulations of κ receptors. If the assumption of two affinity states is valid, then the data suggest that in *Xenopus* oocytes κ receptor-GIRK1 channel coupling may be mediated by the low affinity κ state.

To test the possibility that *Xenopus* oocytes lacked a G protein required for high agonist potency, cRNAs coding for

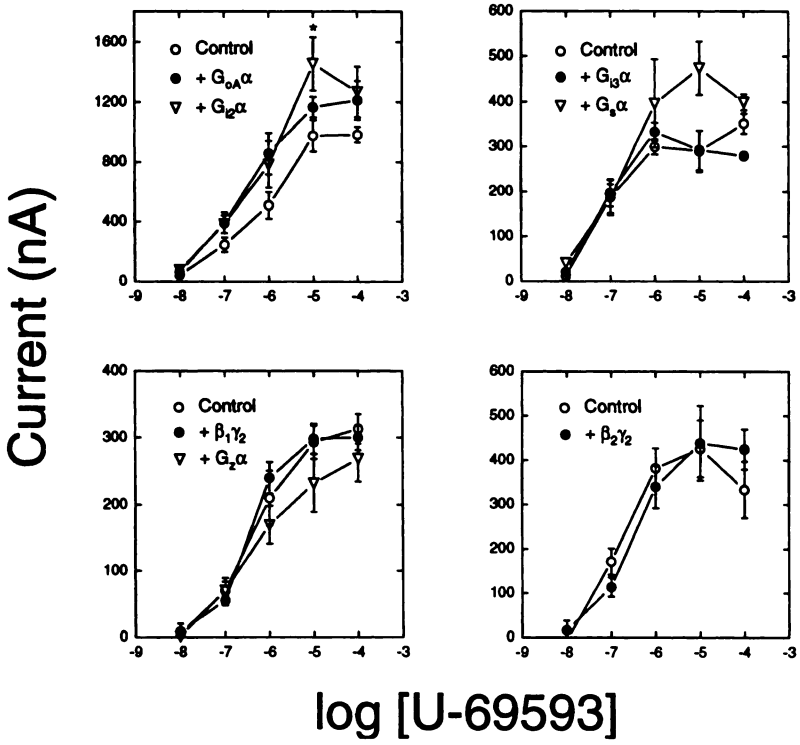

Fig. 5. Effects of G protein cRNA subunit coinjection on coupling between the κ-opioid receptor and GIRK1 channel. Dose-response relationships for the κ agonist U-69593 in oocytes (from four different harvest batches) injected with 1 ng of κ-opioid receptor cRNA and 1 ng of GIRK1 channel cRNA plus differing G protein subunit cRNAs, are shown. The β and γ subunit cRNAs were injected at 2 ng and 1 ng, respectively, whereas 1 ng of all other G protein α subunit cRNAs was injected. Comparisons were made only within each oocyte batch; note the varying maximal levels of U-69593-gated current expressed in controls from each batch. Each *point* represents the average ± standard error of the peak response minus the basal hK buffer response of three to six oocytes. *, $p < 0.05$, Dunnett's test.

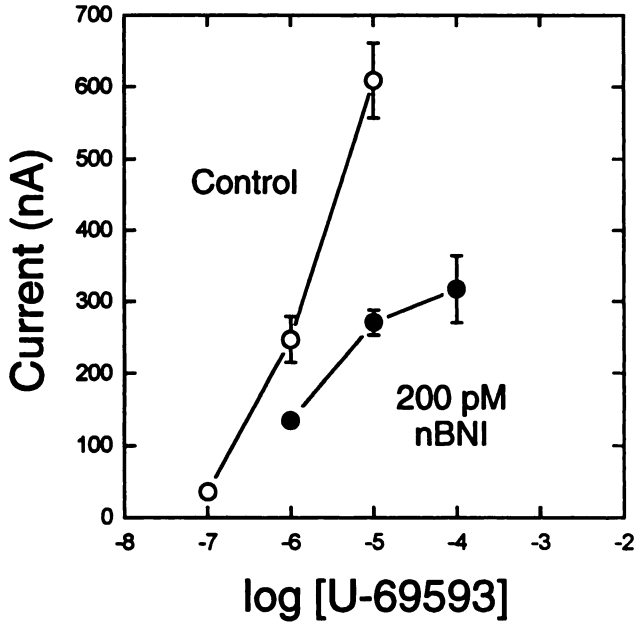

Fig. 6. Dose-response relationships for the κ agonist U-69593 alone or after a 5-min pretreatment with the κ-specific antagonist nBNI (200 pM). Oocytes from the same harvest batch were injected with 1 ng of κ-opioid receptor plus 1 ng of GIRK1 channel cRNA. Each *point* represents the average ± standard error of the peak response minus the basal hK buffer response of three or four oocytes. Similar results were observed when this experiment was repeated with a second batch of identically injected oocytes.

various G protein subunits were coinjected with κ receptor and GIRK1 channel cRNA. No coinjections produced significant shifts in the potency (as measured by EC_{50}) of U-69593, relative to controls, suggesting that none of the G protein subunits studied functions as a high affinity intermediate in κ-opioid receptor-GIRK1 channel coupling. Only oocytes coinjected with $G_{i\alpha2}$ cRNA exhibited a small but significant potentiation of the maximal responses to U-69593, indicating that this subunit can mediate coupling between κ receptors and GIRK1 channels. Similar results with $G_{i\alpha2}$ were observed for coupling between 5-HT_{1A} receptors and the GIRK1 channel (26).

Although bremazocine was more potent than U-69593 in stimulating the GIRK1 channel, it was considerably less efficacious, exhibiting a maximal effect approximately one third as large as the maximal current elicited by U-69593. This partial agonist behavior of bremazocine has previously been noted using preparations of vasa deferentia of mice (35) or rats (36). However, in many reports bremazocine displayed a full agonist profile (e.g., see Ref. 37). The ability of a partial agonist to mimic a full agonist is indicative of a large receptor reserve in the physiological assay system (35). Thus, our data indicate that oocytes injected with 1 ng each of κ receptor plus GIRK1 channel cRNA do not express a significant receptor reserve.

Pretreatment with nBNI shifted the U-69593 dose-response curve to the right but did not produce a parallel shift. This result reflects noncompetitive antagonism by nBNI, which has been reported in preparations of mouse vas deferens (38). The idea that nBNI may act as an apparent irreversible antagonist is supported by the persistent antagonistic effects (up to 28 days) of this compound *in vivo* (39). However, studies by others using a guinea pig ileum assay (40) supported a competitive antagonist profile for nBNI. Again, these differences are likely to arise from the relative lack of spare receptors in our assay system.

Originally cloned from rat atrium (20, 21), the GIRK1 channel is also expressed in brain, with the only difference being a 41-base pair insert in a 5′ noncoding segment of DNA (20). In addition to its interaction with κ receptors in the present studies, the GIRK1 channel can also be stimulated

by other neurotransmitter receptors coexpressed in *Xenopus* oocytes. These include the δ-opioid (20), μ-opioid (27, 29), 5-HT_{1A} (20, 26), and muscarinic (21, 26, 41) receptors. Because these receptors couple to G_i (and possibly G_o), these G proteins presumably mediate activation of GIRK1 channels. The precise molecular nature of κ receptor-GIRK1 channel coupling awaits further definition. The coupling of 5-HT_{1A} (26) and muscarinic (41) receptors to GIRK1 channels is blocked by guanosine-5′-*O*-(2-thio)diphosphate injection but is sometimes insensitive to pertussis toxin treatment. This variability occurs largely between various batches of oocytes, indicating that transduction may occur via a pertussis toxin-insensitive G protein endogenous to *Xenopus* oocytes (26). Our findings also suggest that transduction occurs via an endogenous intermediate, because κ receptor-GIRK1 channel coupling did not require addition of exogenous G protein cRNA. Indeed, recent results suggest that this cellular intermediate may be a βγ complex of an as yet undetermined G protein, because this complex activates inwardly rectifying K^+ channels in atrial myocytes (42) and stimulates the expressed GIRK1 channel in *Xenopus* oocytes (41). Studies with oocytes coexpressing opioid receptors and GIRK1 channels are currently underway to address this possibility. In addition, further studies will be required to determine whether κ-opioid receptors and GIRK1 channels coexist and interact in neurons within the central nervous system. Indeed, the study of Grudt and Williams (14) suggests that this may be the case. It is hoped that the use of oocytes coexpressing cloned κ-opioid receptors and GIRK1 channels will aid in a better understanding of the stoichiometry and regulation of opioid receptor-effector coupling.

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