0026-895X/90/020229-08$02.00/0

MOLECULAR PHARMACOLOGY, 38:229-236

Kainate Receptors Coupled to the Evoked Release of [^{3}H]-γ-Aminobutyric Acid from Striatal Neurons in Primary Culture: Potentiation by Lithium Ions

SAMUEL WEISS,* DOROTHY E. KEMP,* LORENZO BAUCE, and FREDERICK W. Y. TSE

Neuroscience Research Group, Departments of Medical Physiology (L.B., F.W.Y.T.), Pathology (S.W., D.E.K.), and Pharmacology and Therapeutics (S.W., D.E.K.), University of Calgary Faculty of Medicine, Calgary, Alberta, Canada T2N 4N1

Received January 18, 1990; Accepted May 21, 1990

SUMMARY

The pharmacological properties and modulation by lithium of the kainate (KA) receptor system coupled to the evoked release of [^{3}H]γ-aminobutyric acid ([^{3}H]GABA) from purified populations of striatal neurons in primary culture were examined. KA evoked a dose-dependent (EC_{50}, 100 μM) and saturable increase in [^{3}H]GABA release from striatal neurons that was unaffected by the removal of extracellular calcium and resistant to the actions of tetrodotoxin. The release of [^{3}H]GABA evoked by 100 μM KA was attenuated in a dose-dependent manner by the following excitatory amino acid antagonists (IC_{50}):6-cyano-2,3-dihydroxy-7-nitroquinoxaline (2 μM),2,3-dihydroxy-6,7-dinitroquinoxaline (2 μM), kynurenate (0.3 mM), and γ-D-glutamylglycine (2 mM). The antagonist properties of 6-cyano-2,3-dihydroxy-7-nitroquinoxaline, kynurenate, and γ-D-glutamylglycine were competitive in nature, inducing parallel rightward shifts of the KA dose-response curves. At concentrations at which it did not significantly increase basal levels of [^{3}H]GABA release, quisqualate attenuated in a dose-dependent manner (IC_{50}, *10* μM) the release due to 100 μM KA. The quisqualate receptor agonist α-amino-3-hydroxyisoxazolepropionic acid (AMPA), however, exerted a biphasic effect on 100 μM KA-evoked release of [^{3}H]GABA. At lower concentrations of AMPA (0.1–10 μM), the release due to 100 μM KA was potentiated 25–50%; at higher concentrations (>10 μM) AMPA induced a dose-dependent (IC_{50}, 100 μM) attenuation of KA-evoked release. The release of [^{3}H]GABA due to 100 μM KA was significantly potentiated by the replacement of sodium with lithium in the extracellular medium. A significant potentiation (20–30%) was detected with as little as 5–10 mM lithium, and maximal effects (100–110% increase) were obtained with 50–75 mM lithium. Replacement of sodium with choline or *N*-methyl-D-glucamine could not mimic the actions of lithium. Lithium (25 mM) also induced a 4-fold increase in the levels of endogenous GABA release due to 100 μM KA. Whole-cell voltage-clamp recordings of these striatal neurons indicated that the 100 μM KA-induced inward current was not significantly altered in the presence of 25 mM lithium. Lithium attenuated vasoactive intestinal polypeptide-stimulated cyclic AMP formation by 50%, with a dose dependence similar to that of its actions on KA-evoked release. The results of this study demonstrate a distinct pharmacological profile for the KA receptor system coupled to the evoked release of [^{3}H]GABA from striatal neurons. The selective ability of lithium to potentiate the release due to KA may be due to a modification of an intracellular process rather than a direct action on the KA-induced inward current.

The major excitatory input to the neostriatum arises from the cortico-striatal pathway that utilizes glutamate and/or aspartate as the principal neurotransmitters (1). It is widely accepted that the majority of EAA actions are transduced by at least three distinct receptor subtypes, termed NMDA, KA, and QA according to the electrophysiological and biochemical properties of these synthetic analogues (2, 3). In addition to mediating fast synaptic transmission in the mammalian CNS, the EAA receptor systems have received considerable attention recently, due to abundant evidence for their involvement in the pathogenesis of both acute and chronic neurodegenerative processes (4, 5). The development of novel functional assay techniques, in conjunction with the introduction of new synthetic agonists and antagonists, has provided tools and approaches for examining the roles for these receptor systems in both normal and pathological CNS physiology.

Cultured striatal neurons (6) have provided an ideal model system for the examination of postsynaptic EAA receptor systems of the neostriatum. Patch-clamp recording techniques

This work was supported by a grant (MA-10421) From the Medical Research Council of Canada and an establishment grant from the Alberta Heritage Foundation for Medical Research. S.W. is a Medical Research Council Scholar, F.W.Y.T. was an Alberta Heritage Foundation for Medical Research postdoctoral fellow.

ABBREVIATIONS: EAA, excitatory amino acid; KA, kainate; GABA, γ-aminobutyric acid; CNQX, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline; DNQX, 2,3-dihydroxy-6,7-dinitroquinoxaline; KYN, kynurenate; γ-DGG, γ-D-glutamylglycine; QA, quisqualate; AMPA, α-amino-3-hydroxyisoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; CNS, central nervous system; VIP, vasoactive intestinal polypeptide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; BSS, balanced salt solution.

have demonstrated that striatal neurons depolarize in response to NMDA, KA, or QA (7, 8). The activation of EAA receptor systems on striatal neurons results in a multitude of postsynaptic responses, including the regulation of phospholipase C (9–11) and A_2 (12) activities, intracellular calcium levels (13), and neurotransmitter release (14–18). Distinct EAA receptor systems appear to be associated with some of these responses. For example, a NMDA-preferring receptor system is exclusively associated with the activation of phospholipase A_2 (12), whereas a QA-preferring receptor system is coupled to the activation of phospholipase C (9). On the other hand, both NMDA- and KA-preferring receptor systems, distinguishable on the basis of their pharmacological and ionic properties, mediate the attenuation of carbachol-stimulated phospholipase C activity (10) and the evoked release of [^{3}H]GABA (15). Furthermore, we have found recently that two pharmacologically distinct QA-preferring receptor systems are present on striatal neurons and are involved in the regulation of inositol phosphate metabolism and modulation of evoked neurotransmitter release (16).

KA is the most effective of the EAAs in depolarizing striatal neurons and in evoking the release of endogenous or exogenously incorporated GABA (14, 15). We have recently found that KA selectively induces a significant release of endogenous glycine from striatal neurons (18), which may in turn activate both strychnine-sensitive and -insensitive glycine receptors in the striatum. In earlier studies (15), we found that most of the NMDA-preferring antagonists did not modify the release of [^{3}H]GABA due to KA. However, both γ-DGG and KYN appear to have blocking actions at both receptor systems. A distinguishing feature of the KA receptor system coupled to the evoked release of [^{3}H]GABA was the potentiation of its actions when lithium was substituted for sodium in the bathing medium (15). The pharmacological and regulatory properties of the major postsynaptic EAA receptor system on striatal neurons, the KA receptor, have not been reported. In the present study we have carried out a pharmacological characterization of the KA receptor system on striatal neurons, in order to examine the similarities or differences in the actions of antagonists at the NMDA and KA systems, and have carried out an examination of the nature and properties of lithium potentiation of KA-evoked [^{3}H]GABA release.

Materials and Methods

Primary culture of striatal neurons. Striata were removed from 15-day-old CD_1 albino mouse embryos (Charles River, St. Constant, Quebec, Canada) and mechanically dissociated with a fire-narrowed Pasteur pipette in serum-free medium. Cells were plated (1.0×10^6 cells/ml) in 6-well (2 ml/well) culture dishes (NUNC, Thousand Oaks, CA) previously coated successively with poly(L-ornithine) (15 μg/ml; M_r 40,000) and culture medium containing 10% fetal calf serum. After the last coating solution was withdrawn, cells were seeded in serum-free medium composed of a 1:1 mixture of Dulbecco's modified Eagle's medium and F-12 nutrient that included glucose (0.6%), glutamine (2 mM), sodium bicarbonate (3 mM), and HEPES buffer (5 mM). A defined hormone and salt mixture that included insulin (25 μg/ml), transferrin (100 μg/ml), progesterone (20 nM), putrescine (60 μM), and selenium chloride (30 nM) was used in place of serum. These cultures have been found to be predominantly (>93%) neuronal in nature by morphological studies with transmission electron microscopy, as well as by immunocytochemical studies with antibodies to neurofilament and glial fibrillary acidic proteins (19). Mature striatal neurons (6), maintained for 10 to 14 days *in vitro*, were used for all experiments.

Examination of [^{3}H]GABA release from striatal neurons. The release of [^{3}H]GABA was examined as previously described (15–17, 20). Briefly, the culture medium of 10–14 days *in vitro* striatal neurons grown on six-well culture dishes was aspirated and the cells were rinsed twice with 1 ml of BSS (composition in mM: NaCl, 137; KCl, 2.7; Na_2HPO_4, 8; KH_2PO_4, 1.5; $MgCl_2$, 0.5; $CaCl_2$, 0.9; and glucose, 5.5) and incubated with 0.02 μM [^{3}H]GABA (25 Ci/mmol) in BSS for 20 min at 37°. All experimental solutions contained 10 μM aminooxyacetic acid, an inhibitor of GABA transaminase. After this period, all subsequent changes were made at 3-min intervals, under atmospheric pressure at 37°. The BSS (1 ml) was replaced three times before the introduction of the depolarizing agent. For experiments with EAA antagonists, the agents were introduced during the two medium changes before, as well as during, the introduction of the EAA agonists. Monovalent cations were introduced in a similar fashion, with equimolar substitutions for sodium. The aliquots removed after each 3-min period were placed directly into scintillation vials and analyzed by liquid scintillation counting. At the end of the experiment, the neurons were solubilized with 1 ml of 0.1 N NaOH and counted. The evoked release of [^{3}H]GABA is defined as the [^{3}H]GABA released during a 3-min period due to the presence of a depolarizing agent and was quantified as follows:

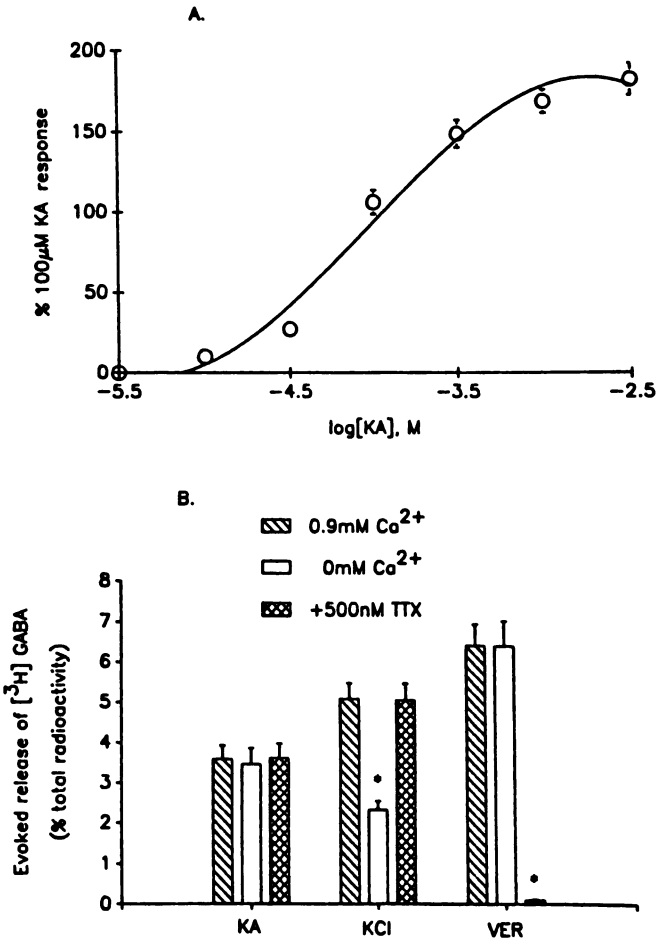


Fig. 1. Characteristics of KA-evoked release of [^{3}H]GABA from striatal neurons. A, The release of [^{3}H]GABA during a 3-min depolarization period was measured in the absence or presence of increasing concentrations of KA. B, [^{3}H]GABA released during a 3-min depolarizing period in the presence of KA (100 μM), KCl (56 mM), or veratrine (*VER*) (5 μg/ml) was examined in the presence of 0.9 mM calcium (control conditions) (▨), in the absence of added calcium (□), or in the presence of 0.9 mM calcium plus 500 nM tetrodotoxin (■). Data represent the mean ± standard error of three experiments on separate culture preparations, each performed in triplicate. *, Significantly different than control, $p < 0.01$.

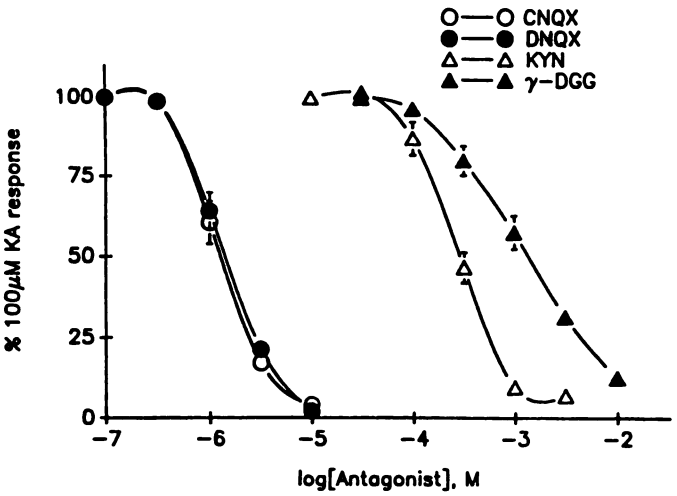


Fig. 2. Dose dependence of antagonist blockade of KA-evoked release of [^{3}H]GABA from striatal neurons. The release of [^{3}H]GABA due to 100 μM KA was measured in the presence of increasing concentrations of CNQX, DNQX, KYN, or γ-DGG. Data represent the mean ± standard error of three experiments on separate culture preparations, each performed in duplicate.

$$\text{Evoked release of [}^3\text{H]GABA (\% of total radioactivity)} = \frac{\text{GABA}_{ev}}{\text{GABA}_t} - \frac{\text{GABA}_b}{\text{GABA}_t} \times 100$$

where $GABA_{ev}$ was the amount of [^{3}H]GABA collected subsequent to a 3-min exposure to a depolarizing agent, $GABA_b$ (baseline release) was the mean of the two collections of [^{3}H]GABA (3 min each) before the depolarizing period, and $GABA_t$ was the total radioactivity incorporated during the 20-min prelabeling period.

The [^{3}H]GABA release due to presence of depolarizing agents was characterized as authentic GABA by thin layer chromatography (15).

Examination of endogenous GABA release from striatal neurons. The release of endogenous amino acids was performed as recently described (18). In brief, after 10–11 days *in vitro*, neuronal cultures were washed twice with 1 ml of prewarmed BSS (composition in mM: NaCl, 137; KCl, 2.7; Na_2HPO_4, 8; KH_2PO_4, 1.5; $MgCl_2$, 0.5; $CaCl_2$, 0.9; and glucose, 5.5) prepared in ultrapure, amino acid-free water. After standing for 3 min, the medium (1 ml) was renewed every 3 min. After five changes, the cultures were exposed to the stimulatory solution (56 mM KCl or EAAs) for 3 min. The media during the periods before, during, and after stimulation were collected in precooled glass tubes. The endogenous amino acid concentration in each sample was determined by high performance liquid chromatography with a Varian 5000 high performance liquid chromatograph with a model 401 data control system. Aliquots of 100 μl of sample were mixed with 50 μl of *o*-phthaldialdehyde/2-mercaptoethanol derivatizing solution and were injected 1 min later using a Waters WISP autosampler/injector. The *o*-phthaldialdehyde derivatives were then separated on a Beckman C18 Ultrasphere reverse phase column (4.5 × 45 mm; 5 μM particle diameter). Separation was obtained using a linear gradient of 25% to 80% methanol in 0.025 M imidazole phosphate (pH 7.0). Fluorescence was detected with a Varian Fluorochrome spectrofluorimeter. The area of each peak was automatically integrated and compared with those of external standards to determine the amino acid content. The majority of the data are expressed as picomol of GABA released during a 3-min depolarization period, from a 35-mm well in a six-well multi-well dish, above basal (defined as the mean of the two determinations from the medium changes immediately before the depolarization period.

Examination of electrophysiological responses of striatal neurons to KA with whole-cell voltage clamp. Neurons grown on individual coverslips were transferred to a recording chamber for whole-cell voltage clamp. All experiments were performed at room temperature (23°). The control solution that perfused the recording chamber was constantly bubbled with 95% O_2/5% CO_2 and contained (in mM): NaCl, 124; KCl, 5; $MgCl_2$, 1.3; $CaCl_2$, 2; $NaHCO_3$, 26; glucose, 10; and sucrose 30 (pH 7.35). The patch-type electrodes for whole-cell voltage clamp and internal dialysis were pulled from borosilicate capillaries (IB100F from WPI, New Haven, CT). The electrode filling solution contained (in mM): KF or CsF, 120; HEPES, 20; and EGTA, 10 (titrated with KOH or CsOH to pH 7.35). In the control solution, the electrode resistance was 5–8 MΩ. During whole-cell recording, the total series resistance was electronically compensated (at least 70%) with an Axopatch-A amplifier (Axon Instruments, Burlingame, CA). Signals from the amplifier were simultaneously displayed on an oscilloscope and a chart recorder and were stored on a video cassette recorder and a computerized data acquisition system (using PCLAMP software from Axon Instruments). Desired doses of KA were obtained by dilution of a concentrated stock in control solution. KA was applied to cells either slowly by bath perfusion or rapidly with pressure ejection (Picospritzer from General Valve, Fairfield, NJ) from a glass pipette (B150F from WPI) pulled to a bore diameter of approximately 5 μm.

Examination of cyclic AMP formation in striatal neurons. The formation of cyclic AMP in striatal neurons was determined as previously described (21), by measurement of the conversion of [^{3}H]adenine nucleotide precursors to [^{3}H]cAMP. Briefly, the culture medium of 10–14 days *in vitro* striatal neurons was aspirated and cells were rinsed twice with BSS and incubated with 4 μCi/ml [^{3}H]adenine in BSS. After 2 hr, the cultures were washed with BSS and incubated with 0.9 mM isobutylmethyl xanthine and test agents (all in BSS) for 5 min at 37°. The reaction was terminated by aspiration of BSS and addition of 1 ml of ice-cold trichloroacetic acid. The cells were scraped with the aid of a rubber policeman and to the mixture was added 100 μl of unlabeled 5 mM ATP and 5 mM cyclic AMP. Cellular protein was centrifuged at 5000 × *g* for 10 min and the supernatant was eluted through sequential chromatography on Dowex and alumina columns. Cyclic AMP formation was calculated as

$$\%\ \text{conversion} = [^3\text{H]cAMP} \,/\, ([^3\text{H]cAMP} + [^3\text{H]ATP}) \times 100.$$

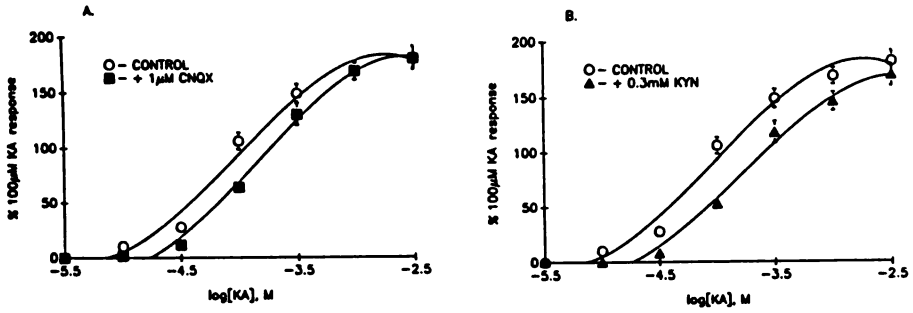
The data in Fig. 9 represent the formation of cyclic AMP due to the presence of 1 μM VIP, above basal. Lithium substitution was carried out as for the measurement of [^{3}H]GABA release.

Data analysis. To facilitate interexperimental comparisons, the majority of data are expressed as a percentage of the response due to 100 μM KA. In all cases, data represent the means of at least three separate experiments on independent cultures, with each experimental point carried out in duplicate. Dose-response curves were computer-analyzed as the best fit (least squares analysis) to a third degree polynomial with SIGMAPLOT software (Jandel Scientific, Corte Madera, CA). Statistical comparisons were made by the use of Student's *t* test.

Materials. [2,3-^{3}H(N)]GABA (25 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA) and [2,8-^{3}H]adenine (36 Ci/mmol) was purchased from ICN (Irvine, CA). Dulbecco's modified Eagle's medium, Ham's F-12 nutrient mixture, fetal calf serum, and glutamine were purchased from GIBCO Laboratories (Grand Island, NY). ATP, aminooxyacetic acid, choline chloride, cyclic AMP, L-glutamic acid, γ-DGG, isobutylmethyl xanthine, NMDA, *N*-methyl-D-glucamine, KA, KYN, HEPES, insulin, transferrin, progesterone, putrescine, and selenium chloride were purchased from Sigma Chemical Co. (St. Louis, MO). AMPA, CNQX, DNQX, and QA were purchased from Cambridge Research Biochemicals (Valley Stream, NY). VIP was purchased from Bachem Inc. (Torrance, CA). All other chemicals were of reagent grade.

Results

KA evoked a dose-dependent and saturable increase in the amount of [^{3}H]GABA released from striatal neurons (Fig. 1A); apparent half-maximal stimulation (EC_{50}) was obtained with



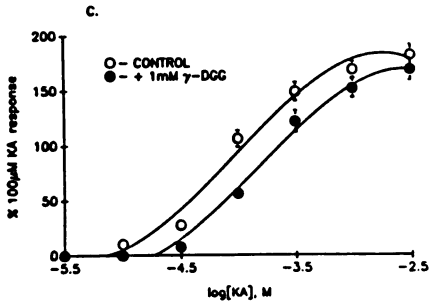


Fig. 3. CNQX, KYN, and γ-DGG effects on the dose dependence of KA-evoked release of [^{3}H]GABA from striatal neurons. The release of [^{3}H]GABA was measured with increasing concentrations of KA, in the absence or presence of the indicated concentrations of antagonists. A, CNQX; B, KYN; C, γ-DGG. Data represent the mean ± standard error of three independent experiments on separate culture preparations, each performed in duplicate.

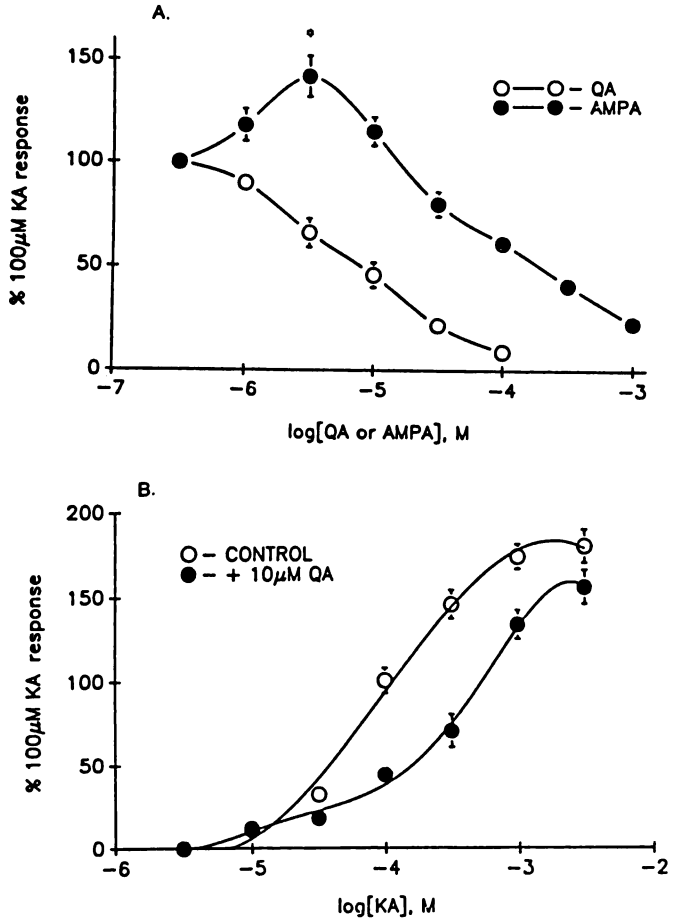


Fig. 4. Effects of QA and AMPA on KA-evoked release of [^{3}H]GABA from striatal neurons. A, The release of [^{3}H]GABA due to the presence of 100 μM KA was measured in the absence or presence of increasing concentrations of QA or AMPA. B, The release of [^{3}H]GABA was measured with increasing concentrations of KA, in the absence or presence of the indicated concentration of QA. Data represent the mean ± standard error of four experiments on separate culture preparations, each performed in duplicate. *, Significantly different than control, $p < 0.05$.

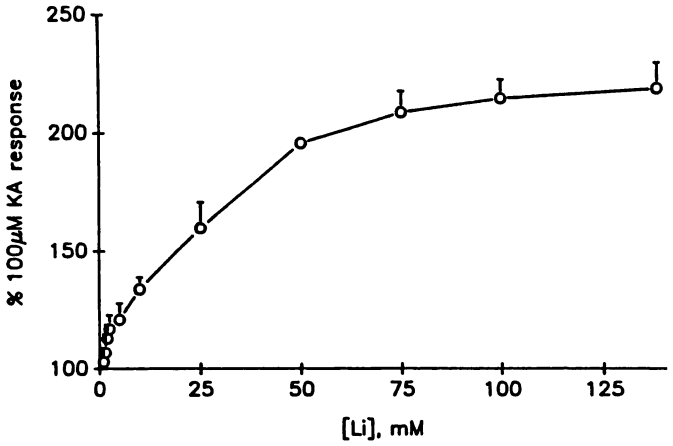


Fig. 5. Effects of increasing concentrations of lithium on KA-evoked release of [^{3}H]GABA. The effects of increasing concentrations of lithium on the evoked release of [^{3}H]GABA were measured in the presence of 100 μM KA. Data represent the mean ± standard error of three experiments on separate culture preparations, each performed in duplicate.

100 μM. The mechanism by which KA evoked the release of [^{3}H]GABA differed markedly from those mediating the action of KCl and veratrine (Fig. 1B). Whereas the release of [^{3}H]GABA evoked by 56 mM KCl and 5 μg/ml veratrine was dramatically attenuated by the omission of extracellular Ca^{2+} and addition of 0.5 μM tetrodotoxin, respectively, neither of these conditions was capable of modifying the release due to 100 μM KA.

The actions of a series of EAA receptor antagonists on the 100 μM KA-evoked release was next examined. All of the agents tested induced a dose-dependent and saturable attenuation of KA-evoked release (Fig. 2). Of those agents tested, CNQX and DNQX were by far the most potent antagonists; apparent half-maximal inhibition (IC_{50}) for both agents was achieved with 2

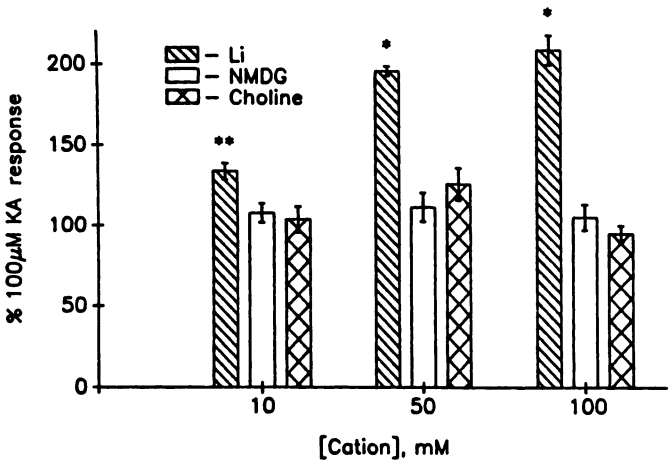


Fig. 6. Comparison of the effects of organic monovalent cations on KA-evoked release of [^{3}H]GABA from striatal neurons. The release of [^{3}H]GABA due to 100 μM KA was measured in the presence of the indicated concentrations of lithium (▨), *N*-methyl-D-glucamine (*NMDG*) (□), or choline (■). Data represent the mean ± standard error of four independent experiments on separate culture preparations, each performed in duplicate. *, Significantly different from that obtained in the absence of the monovalent cation, $p < 0.01$.

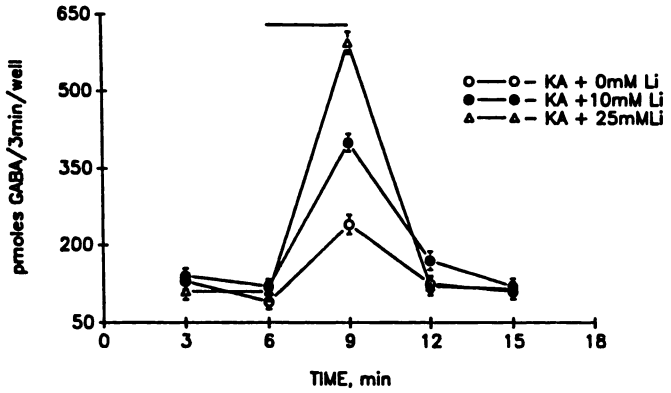


Fig. 7. Effects of increasing concentrations of lithium on the release of endogenous GABA from striatal neurons. The release of endogenous GABA due to 100 μM KA was measured in the absence or presence of 10 or 25 mM lithium. Data represent the mean ± standard error of three independent experiments on separate culture preparations, each performed in duplicate. *Bar*, period of exposure to KA.

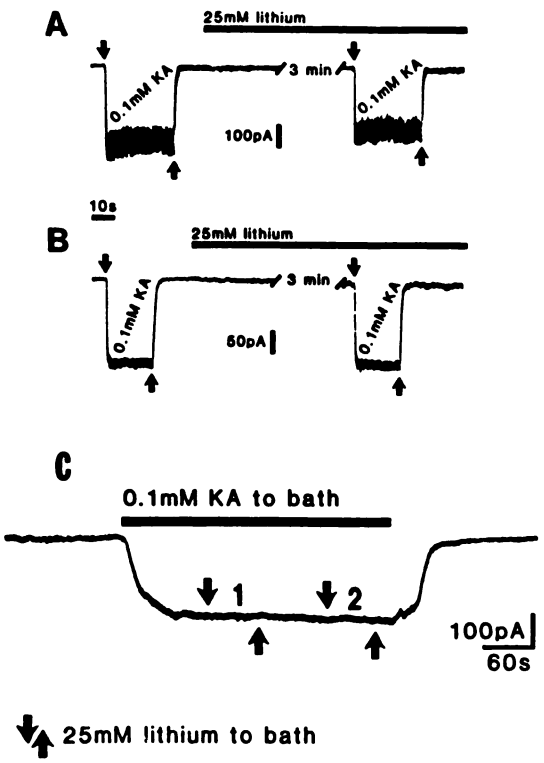


Fig. 8. Actions of lithium on the KA-induced inward current in striatal neurons. The inward current of whole-cell clamped striatal neurons induced by 0.1 mM KA was measured in the absence or presence of 25 mM lithium. A and B, Two examples of cells in which the inward current induced by pressure ejection of 0.1 mM KA was not potentiated when repeated in the presence of 25 mM lithium. *Arrows*, beginning and end of pressure ejection of KA. C, The inward current in neurons with bath application of 0.1 mM KA was similarly unchanged in the presence of 25 mM lithium.

μM. KYN (IC_{50}, 0.3 mM) was approximately 7-fold more potent as an antagonist than γ-DGG (IC_{50}, 2 mM). Examination of the KA dose-dependent curves in the presence of fixed concentrations of CNQX, KYN, or γ-DGG resulted in parallel rightward shifts (Fig. 3), indicative of a competitive type of inhibition. The calculated pA_2 values for CNQX, KYN, and γ-DGG were 5.77, 3.59, and 2.97, respectively.

In a variety of *in vitro* neuronal preparations, it has been demonstrated that QA may interact with KA receptors, resulting in an attenuation of KA-evoked responses (22–25). Increasing concentrations of QA (1–100 μM) resulted in a dose-dependent attenuation of 100 μM KA-evoked release of [^{3}H]GABA (Fig. 4A). Examination of the KA dose-response curve in the presence of 10 μM QA revealed a rightward shift that was parallel only at concentrations of KA of ≥100 μM (Fig. 4B), indicative of a complex interaction between the two EAA agonists. The complexity of this interaction was further underlined by the actions of the QA-selective agonist AMPA. When increasing concentrations of AMPA were coincubated with 100 μM KA, a biphasic curve was obtained (Fig. 4A). Between 1 and 10 μM, AMPA potentiated the release due to KA by 25–50%; at concentrations >10 μM, AMPA induced a dose-dependent inhibition of KA-evoked release.

In an earlier study, we found that the replacement of sodium with lithium induced a dramatic increase in the release of [^{3}H]GABA evoked by KA (15). In the following series of experiments, we sought to characterize the properties of this phenomenon. We first examined the effects of increasing concentrations of lithium being substituted for sodium in the extracellular medium on KA-evoked release of [^{3}H]GABA. With as little as 5–10 mM lithium, 100 μM KA-evoked release of [^{3}H]GABA was significantly potentiated by 20–35% (Fig. 5); this potentiation was unchanged whether lithium was added several rinses before or only during the depolarization period (data not shown). The maximal and saturating effects of lithium were achieved with 50–75 mM and yielded an increase of 95–110%. We compared the actions of lithium with those of two organic monovalent cation molecules, choline and *N*-methyl-D-glucamine. At concentrations of 10, 50, or 100 mM, neither choline nor *N*-methyl-D-glucamine significantly altered the release of [^{3}H]GABA due to KA (Fig. 6). As opposed to its ability to potentiate the

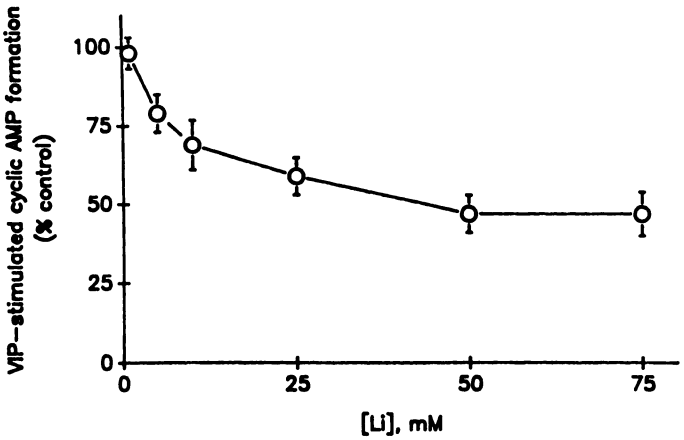


Fig. 9. Effects of increasing concentrations of lithium on VIP-stimulated [^{3}H]cAMP formation in striatal neurons. The formation of [^{3}H]cAMP from [^{3}H]adenine nucleotide precursors (percentage of conversion), induced by a 5-min exposure to 1 μM VIP was measured in the presence of increasing concentrations of lithium, as described in Materials and Methods. The results obtained in the presence of lithium are expressed as a percentage of the stimulation by VIP, over basal, measured in the absence of lithium. In the absence of lithium, basal and VIP-stimulated levels were 0.12 ± 0.02 and 0.85 ± 0.10%, respectively (three experiments). Data represent the mean ± standard error of three separate experiments on independent culture preparations, with each point performed in triplicate.

releasing actions of KA, lithium did not induce significant changes in the ability of NMDA or glutamate to evoke the release of [^{3}H]GABA (data not shown). In order to establish that the actions of lithium on KA-evoked release were not restricted to the pool labeled by [^{3}H]GABA, we examined whether the substitution of lithium for sodium would potentiate KA-evoked release of endogenous GABA. In the presence of 10 and 25 mM lithium, KA-evoked release of endogenous GABA was increased 2.3- and 4-fold, respectively; baseline levels were unaffected (Fig. 7).

To test the hypothesis that the potentiating actions of lithium were due to a direct action on the KA-induced depolarization, we examined KA actions with whole-cell voltage-clamp recording techniques. KA (100 μM) induced a large inward current when picospritzed or bath applied to whole-cell voltage-clamped striatal neurons. The application of the identical concentration of KA in extracellular medium containing 25 mM lithium did not significantly modify the inward current, no matter how the applications were made (Fig. 8). These findings suggest that the actions of lithium in potentiating the KA-induced release of endogenous or exogenously labeled GABA do not involve a direct action on the KA-induced inward current. Although we have confirmed in previous studies the requirement for low concentrations of lithium to measure the formation of inositol monophosphate, we had not previously examined the actions of lithium on cyclic AMP formation in striatal neurons. Cyclic AMP formation due to the presence of 1 μM VIP was attenuated by lithium in a dose-dependent fashion (Fig. 9). At 50–75 mM lithium, 50% of VIP-stimulated cyclic AMP formation was blocked.

Discussion

The objective of the present study was to examine the pharmacological properties of the KA receptor system coupled to the evoked release of [^{3}H]GABA from striatal neurons and to further characterize our previous observation of the selective potentiating actions of lithium ion substitution for sodium ions on this system (15). Our findings suggest that the distinct pharmacological properties of KA receptors on striatal neurons are clearly different from those we have reported for the NMDA receptors on these cells (17). In addition, our results suggest that lithium ions may act at intracellular loci to selectively potentiate the KA-coupled release process.

The release of [^{3}H]GABA evoked by KA was unaffected by the removal of extracellular calcium or by the addition of tetrodotoxin. We have previously reported that such calcium-independent processes underlie both NMDA- and KA-evoked release process (15–17). Our conclusions in those studies, confirmed in recent studies of both endogenous and exogenously labeled GABA release from striatal slices or cultured neurons (14, 26), is that a reversal of the GABA carrier may play a pivotal role in these processes. However, the KA-evoked release process differs from that evoked by glutamate or NMDA in two ways. First, KA-evoked release was unaffected by the removal of calcium, whereas that due to either glutamate or NMDA was significantly potentiated (15, 17). Second, whereas the actions of glutamate or NMDA were completely blocked when choline was substituted for sodium, KA-evoked release was unaffected by this substitution (discussed further below). A similar distinction, e.g., calcium dependence, has been reported for NMDA- and KA-evoked release of [^{3}H]GABA from hippocampal neurons (27); however, in this case the NMDA process was partially reduced when extracellular calcium was omitted, whereas that due to KA was not. On the other hand, Drejer and colleagues (28) reported that, in cultured cortical neurons, the release of of [^{3}H]GABA due to either NMDA or KA was absolutely dependent on extracellular calcium. Taken together, these finding suggest that the ionic mechanisms that underlie EAA-evoked release of GABA through distinct receptor subtypes may display a regional heterogeneity in the mammalian CNS.

In an earlier study, we reported that, of several antagonists tested, KYN and γ-DGG demonstrated blocking properties at both NMDA and KA receptor systems coupled to the release of [^{3}H]GABA (15). In fact, we have found that these antagonists are also blockers of the ionotropic QA receptor system on striatal neurons (16). Only the QA receptor system coupled to phospholipase C is resistant to blockade by these agents. In a recent pharmacological study of the NMDA receptor, we observed that the major difference between the actions of KYN and γ-DGG is that their blockades of the NMDA receptor system are of noncompetitive and competitive natures, respectively (17). The noncompetitive action of KYN is due principally to a blockade of the glycine allosteric site, which is saturated, under our culture conditions, by spontaneously released concentrations of this amino acid (18). KYN blockade of KA responses on striatal neurons, however, was competitive in nature. Such a competitive blockade was also observed for the newly described antagonists CNQX and DNQX, which, of those tested, were by far the most potent blockers of KA-evoked release of [^{3}H]GABA. These agents were first reported as relatively selective blockers of QA- and KA-evoked responses, with little if any action at NMDA receptors (29, 30). The results of recent studies, however, demonstrate that these agents are similar to KYN in that they antagonize NMDA-

evoked release of [^{3}H]GABA via a direct interaction with the glycine site on the NMDA receptor complex (31). These findings lead to an interesting hypothesis, that the pharmacological similarities between the NMDA and KA receptor systems are principally between the glycine and KA recognition sites on these systems.

In cerebellar granule cells (22), retinal horizontal cells (23, 24), and striatal neurons (25), it has been reported that, under specific conditions, coincubation of QA with KA results in an attenuation of KA-induced responses. Although the study of cerebellar granule cells favored two distinct KA and QA receptors, the findings in horizontal cells favored a common receptor-ion channel complex for the two agonists. Inasmuch as the former study involved examination of two neurochemical responses, e.g., neurotransmitter release and cyclic GMP formation, whereas the latter study examined agonist-induced inward current, it may be difficult to reconcile these differing observations. In cultured striatal neurons, Pin and colleagues (25) reported that the ability of QA, AMPA, and glutamate to attenuate the KA-evoked release of GABA was due to a competitive blockade of the KA recognition site. Although our data in this study confirm their finding to a large extent, a finding that they did not observe and/or report was the potentiation of KA-evoked release by low concentrations of AMPA. Gallo and colleagues (22) found a similar biphasic action of QA on KA-evoked release of [^{3}H]aspartate from cerebellar neurons but not with QA actions on KA-induced increases in cyclic GMP. Although the findings of this study do not allow us to distinguish between distinct QA and KA receptors or distinct QA and KA sites on the same receptor, we have recently carried out a study of these interactions with whole-cell clamp recordings of single striatal neurons (32). The findings of that study suggest that the complex interactions between KA/QA agonists are related to their differential activation and desensitization of KA/QA receptors, as revealed by clear differences in the efficacy of agonist action in evoking these two distinct processes.

A major finding of this study was that lithium ions selectively potentiate the actions of KA in evoking the release of endogenous or exogenously labeled GABA from striatal neurons. This action is not simply due to sodium replacement, because other monovalent cations, albeit organic in nature, did not mimic the actions of lithium. At these concentrations, lithium did not increase the release due to NMDA; thus, one may suggest a selective action at the KA receptor system. Examination of the KA-induced inward current showed no increase in amplitude in the presence of 25 mM lithium. In electrophysiological studies of hippocampal slices, lithium was found to have both inhibitory and excitatory actions that varied depending upon concentration, time of exposure, or pathway examined (33, 34). It is reasonable to suggest that lithium may act at an intracellular site to selectively potentiate the KA-induced release process. Activation of protein kinase C (35) and elevation of intracellular cyclic AMP (20) in striatal neurons result in a potentiation and attenuation, respectively, of the evoked release of [^{3}H]GABA from striatal neurons. Lithium interacts with the inositol phosphate signalling system by inhibiting the degradation of inositol monophosphate. Continual application of lithium has been reported to dampen receptor-stimulated inositol phosphate responses, presumably due to limitations in inositol recycling (36). It is unclear as to how such changes affect protein kinase C activity under basal conditions. Given the dose dependence of the lithium actions on KA-evoked release in relation to its reported affinity for acting on the inositol phosphate system (36), it is unlikely that this second messenger is involved in this process. At higher concentrations, however, lithium has also been demonstrated to inhibit adenylate cyclase (37). Thus, reduced levels of cyclic AMP would also favor increases in the evoked release of GABA from striatal neurons (20). This action of lithium was confirmed in this study, whereby an attenuation of VIP-stimulated cyclic AMP formation was observed. Remarkably, the dose dependence for attenuation of cyclic AMP formation was strikingly similar to that for potentiation of KA-evoked release.

The selective nature of lithium potentiation, e.g., only potentiating KA actions and not those of NMDA, is difficult to reconcile without a direct action on the KA-induced current rather than on the release process in general. Although we demonstrated that lithium did not modify the inward current due to KA, those experiments were performed with electrodes containing F^- and EGTA. Such analysis, where internal dialysis provides direct access for these agents to intracellular enzymes they have been reported to regulate, would interfere with actions due to either the inositol phosphate or cyclic AMP signalling systems. Nevertheless, we have found that KA-evoked release of [^{3}H]GABA is distinct, on the basis of pharmacology (15) and the actions of divalent cations,[1] from that evoked by NMDA and other depolarizing agents. Thus, it is reasonable to suggest that lithium may act through a second messenger system, such as the cyclic AMP-generating system, to potentiate the KA-induced release process. Further experimentation will be required to elucidate the precise site of action in the release process that is modified. Such strategies are warranted in light of the results of recent studies examining purified KA receptors from the chick cerebellum, which suggest that this protein is a substrate for the cyclic AMP-dependent protein kinase.[2] It is interesting to note that previous studies of chronic lithium exposure in rats found a decrease in GABA receptors in the striatum that was interpreted as evidence for overactivity of GABAergic neurotransmission (38). Because the KA receptor system is the primary system mediating cortico-striatal EAA neurotransmission (39); such overactivity might explain those observations.

In conclusion, the findings of these studies support the hypothesis that multiple receptor systems for EAAs, distinguished by their pharmacological and ionic properties, are present on striatal neurons. Further examination of these properties with biochemical and electrophysiological approaches may elucidate how the activities of these multiple receptor systems are integrated to transduce the normal and pathophysiological responses of striatal neurons to EAAs.

Acknowledgments

The authors would like to thank Dr. Q. J. Pittman for critical reading of an earlier version of this manuscript and Dr. B. A. MacVicar for providing the resources for electrophysiological analyses of striatal neurons.

References

1. McGeer, P. L., E. G. McGeer, U. Scherer, and K. Singh. A glutamatergic corticostriatal path. *Brain Res.* **128**:369–373 (1977).
2. Watkins, J. C., and R. H. Evans. Excitatory amino acid transmitters. *Annu. Rev. Pharmacol. Toxicol.* **21**:165–204 (1981).

[1] S. Weiss. Submitted for publication.
[2] D. Hampson, personal communication.

3. Monaghan, D. T., R. J. Bridges, and C. W. Cotman. The excitatory amino acid receptors. *Annu. Rev. Pharmacol. Toxicol.* **29**:365–402 (1989).
4. Choi, D. W. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* **1**:623–634 (1988).
5. Rothman, S. M., and J. W. Olney. Excitotoxicity and the NMDA receptor. *Trends Neurosci.* **10**:299–302 (1987).
6. Weiss, S., J. P. Pin, M. Sebben, D. E. Kemp, F. Sladeczek, J. Gabrion, and J. Bockaert. Synaptogenesis of cultured striatal neurons in serum-free medium: a morphological and biochemical study. *Proc. Natl. Acad. Sci. USA* **83**:2238–2242 (1986).
7. Ascher, P., and L. Nowak. Electrophysiological studies of NMDA receptors. *Trends Neurosci.* **10**:284–288 (1987).
8. Ascher, P., and L. Nowak. Quisqualate- and kainate-activated channels in mouse central neurones in culture. *J. Physiol. (Lond.)* **399**:227–245 (1988).
9. Sladeczek, F., J.-P. Pin, M. Recasens, J. Bockaert, and S. Weiss. Glutamate stimulates inositol phosphate formation in striatal neurons. *Nature (Lond.)* **317**:717–719 (1985).
10. Schmidt, B. H., S. Weiss, M. Sebben, D. E. Kemp, J. Bockaert, and F. Sladeczek. Dual action of excitatory amino acids on the metabolism of inositol phosphates in striatal neurons. *Mol. Pharmacol.* **32**:364–368 (1987).
11. Weiss, S., B. H. Schmidt, M. Sebben, D. E. Kemp, J. Bockaert, and F. Sladeczek. Neurotransmitter-induced inositol phosphate formation in neurons in primary culture. *J. Neurochem.* **50**:1425–1433 (1988).
12. Dumuis, A., M. Sebben, L. Haynes, J.-P. Pin, and J. Bockaert. NMDA receptors activate the arachidonic acid cascade system in striatal neurons. *Nature (Lond.)* **336**:68–70 (1988).
13. Murphy, S. N., S. A. Thayer, and R. J. Miller. The effects of excitatory amino acids on intracellular calcium in single mouse striatal neurons *in vitro*. *J. Neurosci.* **7**:4145–4158 (1987).
14. Pin, J.-P., and J. Bockaert. Two distinct mechanisms, differentially affected by excitatory amino acids, trigger GABA release from fetal mouse striatal neurons in primary culture. *J. Neurosci.* **9**:648–656 (1989).
15. Weiss, S. Excitatory amino acid-evoked release of γ-[^{3}H]aminobutyric acid from striatal neurons in primary culture. *J. Neurochem.* **51**:435–441 (1988).
16. Weiss, S. Two distinct quisqualate receptor systems are present on striatal neurons. *Brain Res.* **491**:189–193 (1989).
17. Weiss, S. Pharmacological properties of the NMDA receptor system coupled to the evoked release of [^{3}H]GABA from striatal neurons in primary culture. *J. Pharmacol. Exp. Ther.* **252**:380–386 (1990).
18. Weiss, S., D. E. Kemp, and L. Bauce. Kainate evokes the release of endogenous glycine from striatal neurons in primary culture. *Neurosci. Lett.* **107**:205–210 (1989).
19. Bockaert, J., J. Gabrion, F. Sladeczek, J.-P. Pin, M. Recasens, M. Sebben, D. Kemp, A. Dumuis, and S. Weiss. Primary culture of striatal neurons: a model of choice for pharmacological and biochemical studies of neurotransmitter receptors. *J. Physiol. (Paris)* **81**:219–227 (1986).
20. Weiss, S. Forskolin attenuates the evoked release of [^{3}H]GABA from striatal neurons in primary culture. *Brain Res.* **463**:182–186 (1988).
21. Weiss, S., D. E. Kemp, R. H. Lenox, and J. Ellis. α_2-Adrenergic receptors mediate inhibition of cyclic AMP production in neurons in primary culture. *Brain Res.* **414**:390–394 (1987).
22. Gallo, V., C. Giovannini, and G. Levi. Quisqualic acid modulates kainate responses in cultured cerebellar granule cells. *J. Neurochem.* **52**:10–16 (1989).
23. O'Dell, T. J. Pharmacological characterization of voltage-clamped catfish rod horizontol cell responses to kainic acid. *Brain Res.* **477**:118–125 (1989).
24. O'Dell, T. J., and B. N. Christensen. Horizontal cells isolated from catfish retina contain two types of excitatory amino acid receptors. *J. Neurophysiol.* **61**:1097–1109 (1989).
25. Pin, J.-P., B. J. Van Vliet, and J. Bockaert. Complex interaction between quisqualate and kainate receptors as revealed by measurement of GABA release from striatal neurons in primary culture. *Eur. J. Pharmacol.* **172**:81–91 (1989).
26. Bernath, S., and M. J. Zigmond. Characterization of [^{3}H]GABA release from striatal slices: evidence for a calcium-independent process via the GABA uptake system. *Neuroscience* **27**:563–570 (1988).
27. Harris, K. M., and R. J. Miller. Excitatory amino acid-evoked release of [^{3}H]GABA from hippocampal neurons in primary culture. *Brain Res.* **482**:23–33 (1989).
28. Drejer, J., T. Honore, and A. Schousboe. Excitatory amino acid-induced release of [^{3}H]GABA from cultured mouse cerebral cortex interneurons. *J. Neurosci.* **7**:2910–2916 (1987).
29. Blake, J. F., M. W. Brown, and G. L. Collingridge. CNQX blocks acidic amino acid-induced depolarization and synaptic components mediated by non-NMDA receptors in rat hippocampal slices. *Neurosci. Lett.* **89**:182–186 (1988).
30. Honore, T., S. N. Davies, J. Drejer, E. J. Fletcher, P. Jacobsen, D. Lodge, and F. E. Nielson. Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. *Science (Washington D. C.)* **241**:701–703 (1988).
31. Harris, K. M., and R. J. Miller. CNQX (6-cyano-7-nitroquinoxaline-2,3-dione)antagonizes NMDA-evoked [^{3}H]GABA release from cultured cortical neurons via an inhibitory action at the strychnine-insensitive glycine site. *Brain Res.* **489**:185–189 (1989).
32. Tse, F. W. Y., B. A. MacVicar, and S. Weiss. Interactions between quisqualate/kainate agonists at excitatory amino acid receptor on striatal neurons in primary culture. *Soc. Neurosci. Abstr.* **15**:461.2 (1989).
33. MacVicar, B. A., G. Weir, K. Riexinger, and F. E. Dudek. Paradoxical effects of lithium on field potentials of dentate granule cells in slices of rat hippocampus. *Neuropharmacology* **20**:489–496 (1981).
34. Rinaldi, P. C., M. D. Fairchild, and J. A. Kusske. Perfusion with lithium modifies neurophysiological responses in the CA1 region of the hippocampal slice preparation. *Brain Res.* **375**:302–312 (1986).
35. Weiss, S., J. Ellis, D. D. Hendley, and R. H. Lenox. Translocation and activation of protein kinase C in striatal neurons in primary culture: relationship to phorbol dibutyrate actions on the inositol phosphate generating system and neurotransmitter release. *J. Neurochem.* **52**:530–536 (1989).
36. Drummond, A. H. Lithium and inositol lipid-linked signalling mechanisms. *Trends Pharmacol. Sci.* **8**:129–133 (1987).
37. Newman, M., B. M. Ehud-Klein, M. Feinsod, and R. H. Belmaker. Lithium at therapeutic concentrations inhibits brain noradrenaline-sensitive cyclic AMP accumulation. *Brain Res.* **278**:380–381 (1983).
38. Bernasconi, R. The GABA hypothesis of effective illness: influence of clinically effective antimanic drugs on GABA turnover, in *Basic Mechanisms in the Action of Lithium* (H. M. Emrich, J. B. Aldenfoff, and H. D. Lux, eds.). Elsevier Science Publishers, Amsterdam, 183–191 (1981).
39. Herrling, P. L. Pharmacology of the corticocaudate excitatory postsynaptic potential in the cat: evidence for its mediation by quisqualate- or kainate-receptors. *Neuroscience* **14**:417–426 (1985).

Send reprint requests to: Dr. Samuel Weiss, Neuroscience Research Group, Department of Pathology, University of Calgary Faculty of Medicine, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1.