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# Screening of Receptor Antagonists Using Agonist-Activated Patch Clamp Detection in Chemical Separations

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**We present a capillary electrophoresis−patch clamp detection system optimized for screening of antagonists and inhibitors of ligand-gated ion channels. In this system, highly selective receptor agonists are delivered through the electrophoresis capillary to the cell surface where they continuously activate a receptor, resulting in increased steady-state transmembrane currents. Thus, receptor selection and biosensor functionality is simply achieved by selection of an appropriate agonist. The antagonists are fractionated in the same electrophoresis capillary and inhibit the agonist-evoked response, resulting in transiently decreased steady-state transmembrane currents. Specifically, a mixture containing 6-cyano-7-nitroquinoxaline-2,3-dione, that reversibly blocks α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate and kainate receptors, and 6,7-dichloro-3-hydroxy-2-quinoxaline-carboxylate, a broad-spectrum glutamate receptor antagonist, were separated and detected by kainate-activated patch-clamped interneurons freshly dissociated from rat brain olfactory bulb. In addition, $Mg^{2+}$ that reversibly blocks the *N*-methyl-D-aspartate receptor in a voltage-dependent way was detected using the same cell detector system when activated by *N*-methyl-D-aspartate and the co-agonist glycine. The presented method offers new possibilities for drug screening and for identifying endogenous receptor antagonists and to determine their mode of action on any ionotropic receptor system of interest.**

It becomes increasingly evident that an abundance of unknown biologically active molecules exist in our bodies. The number of cloned orphan receptors is high, and many more will surface as a result of the human genome sequencing project.[1] Attempts to identify the ligands, including agonists, antagonists, and modulatory agents acting on these receptors as well as on more well-characterized receptor systems, such as the *N*-methyl-D-aspartate (NMDA) receptor, are set back by the difficulties in detecting them using conventional methods.

To overcome these difficulties, several screening systems based on biological sensors that measures the transduced response from ligand−receptor interactions have evolved over the past few years.[2−5] The receptors confer selectivity, and through their coupling to intracellular cascade systems or ion channels, they also offer amplification of the ligand-binding step, which can markedly improve their sensitivity.

Our approach has been to use cell-based sensors as on-line detectors in capillary electrophoresis (CE), a miniaturized chemical separation format. Here, plasma membrane receptor proteins serve the purpose of recognizing an electrophoretically separated target analyte, for example a neurotransmitter, in a chemically complex sample environment. The use of a chemical separation step prior to detection is of special importance because it offers a means to separate complex mixtures of agonists or antagonists that act on the same receptor system. In previous studies we have shown detection of receptor agonists fractionated by CE using fluorescence microscopy (e.g., acetylcholine and bradykinin)

(1) Herz, J. M.; Thomsen, W. J.; Yarbrough, G. G. *J. Recept. Signal Transduct. Res.* **1997**, *17*, 671−676.
(2) Rechnitz, G. A.; Ho, M. Y. *J. Biotechnol.* **1990**, *15*, 201−217.
(3) McConell, H. M.; Owicki, J. C.; Parce, J. W.; Miller, D. L.; Baxter, G. T.; Wada, H. G.; Pitchford, S. *Science* **1992**, *257*, 1906−1912.
(4) Copenhagen, D. R.; Jahr, C. E. *Nature* **1983**, *341*, 536−539.
(5) Leal, W. S ; Shi, X.; Liang, D.; Schal, C.; Meinwald, J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 1033−1037.



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and patch clamp (PC) recording [e.g., $\gamma$-aminobutyrate (GABA), glutamate, and NMDA].[6−10] These systems are developed for screening agonists of orphan receptors, endogenous and exogenous receptors, and cannot be used for antagonist-screening purposes. The main difference between fluorescence microscopy-based detection and PC detection is the amount of information that can be extracted from the detector responses. Patch clamp detection offers a broader range of information, which includes ion-channel gating kinetics, current-to-voltage ($I-V$) relationships, and ion-channel conductances and can be quantitative. The use of fluorescence detection from cell sensors has so far been used in a qualitative format where the presence of an analyte above a certain threshold concentration is signaled. Fluorescence microscopy detection can, however, be made quantitative by using, for example, ratiometric $Ca^{2+}$-chelating dyes and ratioimaging microscopy.

We report here an extension of the capillary electrophoresis-patch clamp (CE−PC) detection format to include separation and detection of natural and synthetic receptor antagonists, species that inhibit receptor/ion-channel functions and effectively decrease agonist-evoked membrane currents. The key to this advancement is that the receptor of choice can be selectively activated through delivery of a suitable agonist by the same CE capillary that fractionates the antagonists. In some respects this detection scheme is similar to, for example, indirect fluorescence detection in CE, where nonfluorescent analytes displace a fluorescent species added to the running buffer.[11] Antagonists have been used in previous accounts of using cell-based biosensors in CE, but solely to abolish agonist-induced receptor responses.[6,10] Therefore, the present report is the first demonstrating CE separation and detection of receptor antagonists. An advantage of this system is that the state of the biosensor, that is, degree of receptor activation, is known through its continuous activation by an agonist.

Because many drugs are designed to block specific receptor systems, the presented technique could be useful not only for discovering endogenous receptor antagonists but also for pharmacological screening purposes.

## EXPERIMENTAL SECTION

**Isolation of Neurons.** Interneurons from rat olfactory bulb were acutely isolated using proteases from *Aspergillus oryzae* as described previously.[12]

**CE Apparatus.** CE separations were performed in 30−35 cm long, 50 $\mu$m i.d. fused silica capillaries (Polymicro Tech., Phoenix, AZ). Electrophoresis was performed by applying a positive potential of 12 kV to the inlet end of the capillary with a high-voltage supply (LKB, Bromma, Sweden). The capillary was fractured and grounded 5 cm above the outlet.[7] A HEPES-saline buffer was used as an electrolyte and contained 10 mM HEPES, 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1mM $MgCl_2$, and 10 mM D-glucose (the pH was adjusted to 7.4 with NaOH). For detection of $Mg^{2+}$ (2mM in the injected sample), a $Mg^{2+}$-free HEPES-saline solution containing 200 $\mu$M NMDA and 20 $\mu$M glycine was used as the electrolyte. Responses evoked by NMDA and glycine gave a mean single channel conductance of 44.2 ± 11.9 pS and corner frequencies of 16 ± 3 Hz and 72 ± 13 Hz (mean ± SEM, $N = 5$) which is in close agreement with data previously reported.[12] In the case of detecting 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (250−500 $\mu$M) ($N = 5$) and 6,7-dichloro-3-hydroxy-2-quinoxaline-carboxylate (DiCl-HQC) (250−500 $\mu$M) ($N = 3$), a kainate (KA)-supplemented (100 $\mu$M) HEPES−saline buffer was used instead.

**Patch Clamp Detection.** Patch clamp detection was made in the whole-cell configuration.[13] The tip of the PC electrode was positioned ~5 $\mu$m from the center of the CE capillary outlet using a high-graduation micropositioner, as described previously.[7] The recording electrode contained 100 mM KF, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA, and 10 mM HEPES, at pH 7.2 (KOH). For the production of electropherograms, the signals from the videotape were digitized at 7 Hz. Mean current amplitudes ($A$) are presented as mean ± SEM. Since the power spectrum is the frequency distribution of the signal variance, the variance can be calculated from the integral of the spectrum.

$I-V$ relationships were obtained from current responses evoked by continuous superfusion of NMDA (200 $\mu$M) and glycine (20 $\mu$M), and from blocked responses activated by electrophoretically separated $Mg^{2+}$. The holding potential was changed by using a voltage ramp (−70 to +20 mV, duration 3−7 s, pClamp software, Axon Instruments, Foster City, CA). For elimination of responses evoked by voltage-dependent ion channels, the $I-V$ curve obtained between the responses was subtracted from the ramp obtained during the NMDA−glycine or $Mg^{2+}$-inhibited responses.

## THEORY

**Optimization of CE−PC Detection of Receptor Antagonists.** To optimize detection of antagonists using agonist-activated CE−PC detection, several instrumental and experimental parameters need attention. In principle, the optimal level of receptor activation that results in the highest signal-to-noise (S/N) ratio in detecting receptor antagonists is sought. Factors affecting the extent of receptor activation evoked by agonists include geometrical considerations of the CE and PC systems, receptor density at the cell surface, holding potential (in V), ion-permeability properties of ion-channels, ligand concentration, and agonist-efficacy. Also, the degree of receptor desensitization following ligand-binding to the receptor is of importance. During continuous agonist administration, the current response from receptors that undergo facile desensitization is partially shut off following an initial and short-lived activation period. Following this, a small-amplitude steady-state current is, generally, obtained (see Figure 1) which results in a small S/N ratio in CE−PC detection of antagonists. This is in contrast to nondesensitizing receptors that generally provide a large-amplitude current response. In some cases it is possible to overcome receptor desensitization by choosing a specific agonist, or by modulation of the receptor proteins. For example, desensitization of glutamate receptors can


(6) Orwar, O.; Jardemark, K.; Jacobson, I.; Moscho, A.; Fishman, H. A.; Scheller, R. H.; Zare, R. N. *Science* **1996**, *272*, 1779−1782.
(7) Jardemark, K.; Orwar, O.; Jacobson, I.; Moscho, A.; Zare, R. N. *Anal. Chem.* **1997**, *69*, 3427−3434.
(8) Orwar, O.; Jardemark, K.; Farre, C.; Jacobson, I.; Moscho, A.; Shear, J. B.; Fishman, H. A.; Lillard, S.; Zare, R. N. *Methods Enzymol.*, in press.
(9) Shear, J. B.; Fishman, H. A.; Allbritton, N. L.; Garigan, D.; Zare, R. N.; Scheller, R. H. *Science* **1995**, *267*, 74−77.
(10) Fishman, H. A.; Orwar, O.; Scheller, R. H.; Zare, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7877−7881.
(11) Kuhr, W. G.; Yeung, E. S. *Anal. Chem.* **1988**, *60*, 2642−2646.
(12) Jacobson, I.; Li, X. *Neurosci. Res. Commun.* **1992**, *10*, 177−185.
(13) Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. *Pflügers Arch.* **1981**, *391*, 85−100.

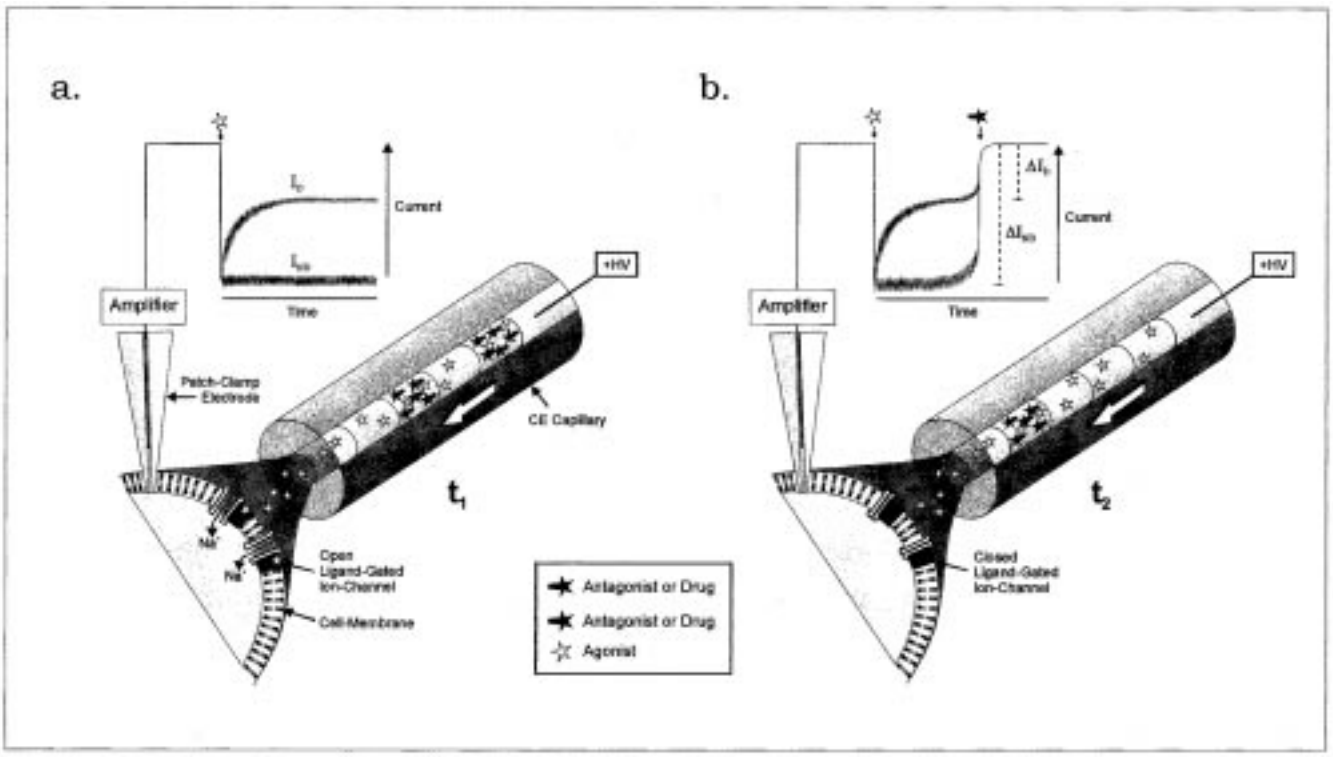


Figure 1. Capillary electrophoresis−patch clamp detection system for fractionation and detection of receptor antagonists. Example with competitive antagonists. (a) At time $t_1$ after sample injection, receptors expressed by a patch-clamped cell, which is placed at the outlet of a capillary, are constantly activated by an electrophoretically delivered agonist (yellow stars). The saturating concentration of agonists at the cell surface evoke either a receptor-mediated desensitizing ($I_D$) or a nondesensitizing ($I_{ND}$) current as indicated in the diagram at the top. Two injected competitive antagonists (red and green stars), are separated in the capillary and are migrating toward the receptor surface. (b) At time $t_2$, the receptors at the cell surface are blocked by one of the separated competitive antagonists (red stars). Because the antagonists bind to the receptor without eliciting a receptor response, this is registered as an attenuation of the agonist-evoked current. The maximal response amplitude obtained for a constantly desensitizing ($\Delta I_D$) and a nondesensitizing ($\Delta I_{ND}$) current is shown in the diagram at the top. The figure is a schematic representation of the experimental setup. It is not drawn to scale, and some instrumental details have been omitted for clarity.

be blocked by lectins.[14,15] Optimization of CE−PC detection of antagonists is, however, eligible to the same general factors irrespective if they act on nondesensitizing or desensitizing receptors.

To obtain an efficient and low-dispersive transfer of fractionated analytes from the CE outlet to the cell surface, at least two geometrical requirements need to be fulfilled. First, the diameter of the capillary outlet should ideally extend the diameter of the cell, and second, the patch-clamped cell needs to be placed adjacent to the CE outlet. Here, this was achieved by using 50 $\mu$m i.d. capillaries in combination with ∼10 $\mu$m o.d. cells placed approximately 5 $\mu$m from the center of the CE outlet. Displacement of the cell and capillary can be avoided by the use of rigid micromanipulators and vibration-isolated tables.

A high receptor expression at the cell surface is required to obtain large-amplitude agonist-evoked current responses. An estimate of the number of receptors present on the cell surface can be obtained from

$$N = I_m^2/(i\, I_m - \sigma_s^2) \qquad (1)$$

where $N$ is the number of ion channels expressed at the cell surface, $I_m$ is the mean current (A), $i$ (A) is the current passing one channel, and $\sigma_s^2$ is the current variance ($A^2$) of the agonist-evoked response. By knowing the number of receptors and the stoichiometry of the ligand−receptor binding, it is in principle possible to calibrate the biosensor. The maximum current amplitude ($I_{max}$) is then given by

$$I_{max} = Ni \qquad (2)$$

The S/N ratio can be increased by increasing the receptor expression. A high expression of a desired receptor type is best obtained by transfection of cells with a specific type of receptor, or by using receptor m-RNA-injected *Xenopus* oocytes. Also by using receptor expression systems, the dynamic range, which is dictated by receptor protein properties, can be dramatically extended by incorporation of multiple receptor clones with different $EC_{50}$-values for a selected ligand.

Another important factor affecting the S/N ratio in these systems is the holding potential which determines the electrical driving force across the cell-sensor membrane. According to the Nernst equation, it is possible to increase the agonist-evoked current response of this detector system by increasing the difference between the reversal potential and the holding potential. According to Nernst's equation, the equilibrium potential of an ionic species $V_{eq}$ can be written as

(14) Mayer, M. L.; Vyklicky, L., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1411−1415.
(15) Thio, L. L.; Clifford, D. B.; Zorumski, C. F. *Neuroscience* **1993**, *52*, 35−44.

$$V_{eq} = \frac{RT}{zF} \ln([X]_o/[X]_i) \quad (3)$$

where $R$ is the gas constant (8.314 J/mol K), $T$ is the temperature (K), $z$ is the valence of the ion, $F$ is Faraday's constant ($9.6487 \times 10^4$ As/mol), and $[X]_o$ and $[X]_i$ are the extracellular and intracellular concentrations, respectively, of the ionic species. At 20 °C, $RT/zF$ is about 25 mV for a monovalent cation. For simplicity, external and internal ion activities are assumed to be equal and thus cancel out. With knowledge about the reversal potential, estimated from $I-V$ relationships, of a specific ion-channel system and the membrane (holding) potential $V_m$ which can be controlled by a command voltage in the patch clamp amplifier, the ionic current $i$ through an open channel can be calculated from

$$i = \gamma(V_m - V_{eq}) \quad (4)$$

where $\gamma$ (S) is the conductance of the ion channel. Thus, by knowledge of the ion channel selectivity, selection of internal and external ionic compositions, together with the control over the membrane potential, it is possible to optimize the current flow across the cell membrane for a given ionic species and also to choose its direction, that is, inward or outward currents.

In the present experiments, the cells were held at a membrane potential of −70 mV, which is close to the resting potential of the olfactory neurons used. For both the NMDA channel (reversal potential about 0 mV) and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) channel (reversal potential about +7 mV), the inward current increases almost linearly with applied potential. Thus, a larger current response can be obtained by choosing more negative holding potentials.

The sensitivity of these detector systems can further be tuned by selection of a specific agonist and the concentration of the agonist. The relationship giving the mean current amplitude at different agonist concentrations is in most cases given by

$$I_m = I_{max}/(1+(EC_{50}/C)^n) \quad (5)$$

where $EC_{50}$ is the agonist concentration causing a half-maximal current response, $C$ is the agonist concentration, and $n$ is the Hill coefficient, which depends on the stoichiometry of the ligand−receptor interaction. The signal amplitude increases exponentially with increasing concentration of agonist and reaches a maximum when the receptor becomes saturated. As most frequently represented, however, in a lin−log plot, the dose−response curve is sigmoid.

The S/N ratio in patch clamp detection can be obtained by dividing $I_m$ with the RMS current noise which equals the square root of $\sigma_s^2$, which can be calculated from power spectra or from digitized records. It is also possible to estimate a S/N ratio based on the relationship between the current variance of the agonist-induced signal $\sigma_s^2$ and the current variance of the inhibited response $\sigma_N^2$ at the level of the antagonist peak maximum, provided that the peak duration is long enough. The S/N ratio is then equal to $\sigma_s^2/\sigma_N^2$.

In the present work, where competitive antagonists (CNQX and DiCl-HQC) acting on a nondesensitizing ligand−receptor system (KA on AMPA receptors), and a receptor inhibitor ($Mg^{2+}$) acting on a desensitizing ligand−receptor system (NMDA on NMDA receptors) were employed as model systems, we used the lowest concentration of the respective receptor agonist reaching half-maximal to maximal current response. In some cases, however, the combination of the antagonist's potency and concentration can be insufficient to modulate or inhibit a whole-cell agonist-induced response at any agonist concentration employed. The detection sensitivity can then be improved by using, for example, outside-out patch clamp recording which can produce measurable responses when activated by ultralow concentrations of agonists.[13] In contrast to the whole-cell recording mode where current averages from populations of ion channels are measured, the outside-out configuration is used to resolve unitary ion-channel events and has previously been used in CE to detect neuroreceptor agonists.[6]

Studies are needed to optimize these systems in further detail, and the present work only serves to give a first demonstration of the concept of detecting antagonists using CE−PC detection. An important aspect of the optimization issue, however, is that tailor-made detectors with respect to sensitivity, selectivity, and dynamic range can be created employing various receptor expression systems in cultured cell lines.

**CE−PC Detection of Competitive and Noncompetitive Antagonists.** In this approach of CE−PC detection, the separated antagonists act pharmadynamically in that they interfere with one or more steps in the cell activation pathway triggered by agonists. At the level of the receptor, the antagonist acts either competitively or noncompetetively, which is important to consider when optimizing the agonist concentration for a maximal antagonist-evoked response.

In competitive antagonism, elevation of the agonist concentration can overcome the effect of the antagonist. Thus, for truly competitive antagonists, increased agonist concentrations simply shift the dose−response curve in parallel to the right so that $EC_{50}$ values increase, without a change in maximum response. This relationship is often referred to as the "Schild" equation[16]

$$K_B = B_t/[(A_1/A_0) - 1] \quad (6)$$

where $K_B$ is the dissociation constant for the competitive antagonist, $B_t$ is the total concentration of antagonist, and $A_1/A_0$ is the "dose ratio", that is, the relative concentration of agonist in the presence or absence of antagonist that is required to yield the same number of agonist−receptor complexes. From this equation, it is possible to calculate the antagonist dissociation constant if one can determine the concentration of antagonist for which one must double the concentration of agonist to achieve the same response.

Noncompetitive antagonists in a system with a low degree of receptor reserve, on the other hand, do not shift the dose−response curve. Thus, the $EC_{50}$ value does not change, whereas the maximum effect caused by an agonist is reduced. Therefore, by studying shifts in dose−response relationships of the agonist-evoked response it is possible to determine binding characteristics of the antagonist to the receptor.

To be able to perform quantitative and routine analysis by CE−PC detection of antagonists, it is necessary to have a stable seal

between the cell membrane and the tip of the patch clamp pipet during the course of the separation procedure. Factors affecting the mechanical stability of the seal include type of cells used, firepolishing and chemical modification of the pipet tip, choice of electrode material, choice of electrode internal solution, stability of micromanipulators, and vibration isolation. These are all known properties of the patch clamp technique, and do not limit its applicability for electrophysiological experiments since they typically require only a few minutes of recording time. In the present CE−PC system, we managed to voltage-clamp a cell for about 10−30 min. Occasionally, we have succeeded in keeping a cell for 1−1.5 h. In order for CE−PC detection to be useful for routine screening purposes, however, improved mechanical stability of the seal between the cell and pipet needs to be obtained. Alternatively, it should be combined with ultrafast CE separations.

A highly stabile recording configuration is two-electrode voltage-clamp of *Xenopus* oocytes.[9] With this technique, recordings can be made for several hours. *Xenopus* oocytes which are about 1 mm in diameter (stage VI oocytes) are penetrated by one voltage-recording electrode and one current-passing electrode, both of which are of a small inner diameter (about 5 $\mu$m). Because of the large size of the oocytes, large capacitive currents and slow response functions are obtained, generally resulting in a system of lower sensitivity and poorer time resolution than patch clamp recordings.

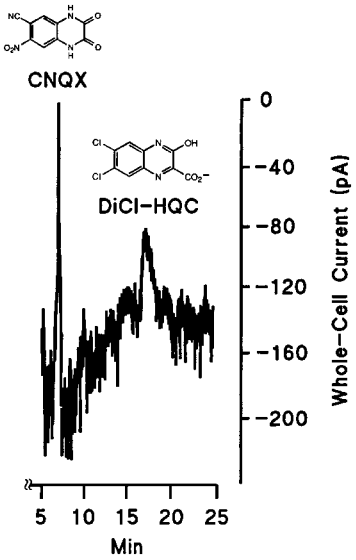


Figure 2. CE−PC detection of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 250 $\mu$M) and 6,7-dichloro-3-hydroxy-2-quinoxaline-carboxylate (DiCl-HQC, 250 $\mu$M). The antagonists were detected using endogenously expressed α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate (KA) receptors in patch-clamped (holding potential, −70 mV) rat olfactory bulb interneurons that were held under constant activation by electrophoretically administered kainate (100 $\mu$M). The capillary length was 35 cm and the applied voltage +12 kV.

## RESULTS AND DISCUSSION

**CE−PC Detection of Receptor Antagonists.** To detect a receptor antagonist with CE−PC detection, it is necessary to activate continuously the targeted receptor type during the time course of the electrophoretic separation. In the present configuration, continuous receptor activation was achieved by adding the specific agonist to the electrolyte solution contained in the electrophoresis capillary and inlet vial. In this way, the agonist is delivered, depending on its net charge, either by electroosmosis or electrophoresis or by a combination of both to the receptor surface of the patch-clamped cell. As shown in Figure 1a, binding of the agonist to ionotropic receptors results in activation of either desensitizing or nondesensitizing agonist−receptor systems. Antagonists injected into the electrophoresis capillary will migrate to the cell surface and inhibit the agonist-evoked response, thereby causing a measurable current response as illustrated in Figure 1b. Thus, the degree of receptor activation and dynamic range for each individual biosensor is known during the course of the experiment. This is in contrast to the CE-cell sensor agonist-screening systems described previously which, unless pretested for an agonist response, does not give information on whether the cell-sensor contains an appropriate number of receptors for a measurable response.

Here, in two separate assay systems, native NMDA and AMPA receptors expressed in periglomerular/granular interneurons from rat olfactory bulb were used as detectors.[6−8] NMDA and AMPA receptors, respectively, were held under constant activation through electrophoretic delivery of NMDA (200 $\mu$M)/glycine (20 $\mu$M) and KA (100 $\mu$M) through the CE capillary. The $EC_{50}$ values for NMDA and KA on NMDA, respectively, AMPA receptors are 15−30 and 80−130 $\mu$M. The NMDA receptor is rapidly desensitized when activated by glutamate and NMDA in the presence of glycine.[17] It is inactivated by MK-801 (dizocilpine) and $Mg^{2+}$ and modulated by such compounds as D-serine, thiols, disulfides, and polyamines, which enable a chemically graded tuning of the receptor response for a multifaceted repertoire of physiological functions.[17] The AMPA receptor can be blocked by several antagonists such as CNQX[18] and DiCl-HQC,[19] and is not desensitized when made active by KA.[20]

**Examples of CE−PC Detection of Antagonists Operating on Nondesensitizing Agonist−Receptor Systems.** Figure 2 shows CE−PC detection of the competitive antagonists CNQX and DiCl-HQC, using KA as the agonist. The KA-evoked (100 $\mu$M) mean current amplitudes which were attenuated by CNQX (250 $\mu$M) and DiCl-HQC (250 $\mu$M) were 90 ± 24 pA ($N = 5$ cells) and 79 ± 14 pA ($N = 3$), respectively. This gives a mean S/N (defined by $I_m/\sigma_s$ of 40 (CNQX) and 35 (DiCl-HQC), respectively. The response evoked by 100 $\mu$M KA, but not by 200 $\mu$M KA, was completely blocked by 250 $\mu$M CNQX in all separations. CNQX at 500 $\mu$M, however, completely attenuated the response from 200 $\mu$M KA, indicating a dose dependence of CNQX. The S/N ratio based on $\sigma_s^2/\sigma_N^2$ was estimated to 3 ± 1 ($N = 3$) for CNQX (250 $\mu$M).

CNQX is a highly potent and selective competitive non-NMDA receptor antagonist.[18] This inhibitor, however, does not display any selectivity for specific glutamate receptor subunits.[21,22] DiCl-HQC is a broad-spectrum glutamate receptor antagonist, which

(16) Kenakin, T. P. *Can. J. Physiol. Pharmacol.* **1982**, *60*, 249−265.
(17) McBain, C. J.; Mayer, M. L. *Phys. Rev.* **1994**, *74*, 723−760.
(18) Honore, T.; Davies, S. N.; Drejer, J.; Fletcher, E. J.; Jacobsen, P.; Lodge, D.; Nielsen, F. E. *Science*, **1988**, *241*, 701−703.
(19) Frey, P.; Berney, D.; Herrling, P. L.; Mueller, W.; Urwyler, S. *Neurosci. Lett.* **1988**, *91*, 194−198.
(20) Hollmann, M.; O'Shea-Greenfield, A.; Rogers, S. W.; Heinemann, S. *Nature* **1989**, *342*, 643−648.

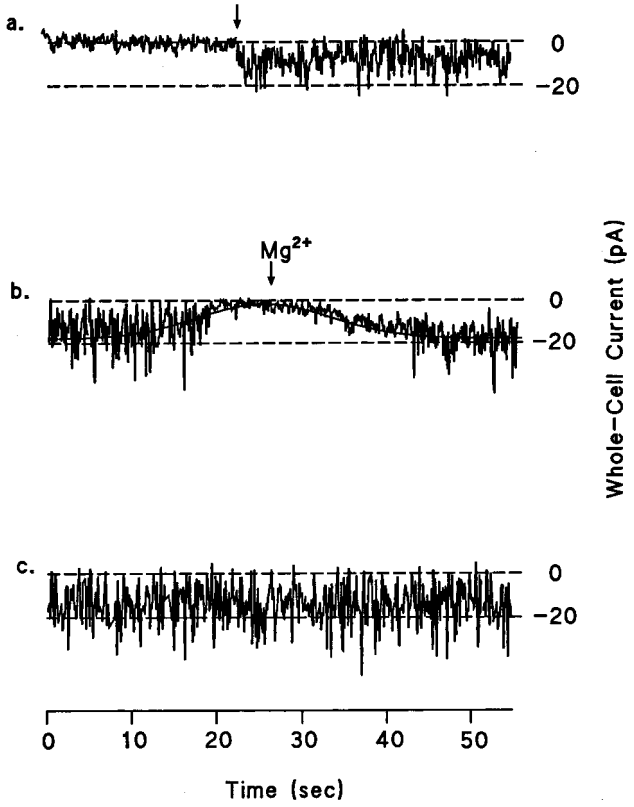


Figure 3. Electropherograms showing controls and detection of $Mg^{2+}$. (a) A control trace showing activation of NMDA receptors by NMDA (200 $\mu$M) and glycine (20 $\mu$M), which are delivered to the cell through the CE capillary by electrophoresis. As seen, the agonist-evoked response occurs immediately following start of the electrophoresis which is indicated by an arrow. (b) An electropherogram showing a blocked response of NMDA receptor-mediated inward currents by separated $Mg^{2+}$ migrating at approximately 25 s. The response has been fitted to a Gaussian function. (c) A control trace presenting the unaffected NMDA receptor response after injection of HEPES-saline containing 200 $\mu$M NMDA and 20 $\mu$M glycine. The holding potential was −70 mV. The length of the capillary and the applied high voltage were 30 cm and 12 kV, respectively.

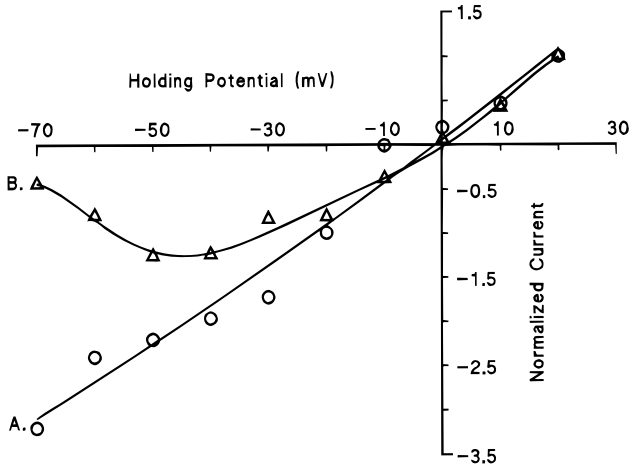


Figure 4. Examples of current−voltage ($I-V$) relationships obtained during constant receptor activation by electrophoretically delivered NMDA (200 $\mu$M) and glycine (20 $\mu$M) (○) and the attenuation of the response by separated $Mg^{2+}$ (2 mM) (△). The current responses at the different holding potentials were normalized against the current response at +20 mV. The normalized current values at the different potentials are mean values from three separate measurements. The length of the capillary and the applied voltage were 30 cm and 12 kV, respectively.

blocks the NMDA type and the AMPA/KA type in the glutamate receptor superfamily.[19] This antagonist is 25 times more potent than unsubstituted HQC. DiCl-HQC has also been tested in receptor binding assays, employing [$^3$H]KA and [$^3$H]AMPA as ligands.[23,24] [$^3$H] KA was displaced by DiCl-HQC with an $IC_{50}$ of 5 $\mu$M. Previous data have indicated that DiCl-HQC may be a competitive antagonist of AMPA/KA receptors. The low solubility of this antagonist, however, makes tests over a wide enough concentration range to evaluate its true competitiveness difficult.[19] The migration times of CNQX and DiCl-HQC obtained with CE−PC detection were verified in a CE system employing absorbance detection at 300 nm (not shown).

**Example of CE−PC Detection of an Antagonist Operating on Desensitizing Agonist−Receptor Systems.** Figure 3 shows detection of $Mg^{2+}$ using NMDA-activated whole-cell patched neurons. The NMDA/glycine (200 $\mu$M/ 20 $\mu$M) evoked mean current amplitudes attenuated by $Mg^{2+}$ (2 mM) was 19 ± 4 pA ($N = 5$ cells), which gives a mean S/N of 1.7. The $\sigma_s^2/\sigma_N^2$ ratio was estimated to 12 ± 3 [$N = 7$ (2mM $Mg^{2+}$)]. The blockade of NMDA receptors by $Mg^{2+}$ is voltage dependent, which means that the NMDA receptor is inhibited when nerve cells are at resting potential, even if glutamate binds to the receptor.[25] The blockade is then released when the cell membrane is depolarized by the opening of voltage-dependent ion channels or ligand-gated ion channels, such as the AMPA receptor, in the postsynaptic cell membrane. Thus, by analyzing the $I-V$ relationship of the attenuated response induced by an antagonist, it is possible to gain information about the characteristics of the receptor blockade which will refer to a certain class of inhibitors or antagonists. $I-V$ relationships obtained on-the-fly both during conditions of NMDA receptor activation and blockade by separated $Mg^{2+}$ (Figure 4) displayed the characteristic voltage dependence of this receptor system.[25]

Thus, because KA does not desensitize AMPA receptors, we observed a larger current response for this system than for the desensitizing NMDA receptor-based detector. Indeed, ligand-activated nondesensitizing currents do not need to have a larger amplitude than desensitized currents.

## CONCLUSIONS

The experiments reported here demonstrate that it is possible to use the CE−PC configuration for determination of substances that inhibit ligand-gated receptor/ion-channel systems. As shown, this system is applicable both for desensitizing and nondesensitizing receptors and should be well-suited for fractionation and detection of complex solutions of drugs and their metabolites,

(21) Lambolez, B.; Curuchet, P.; Stinnakre, J.; Bregestovski, P.; Rossier, J.; Prado de Carvalho, L. *Neurosci. Lett.* **1991**, *123*, 69−72.
(22) Stein, E.; Cox, J. A.; Seeburg, P. H.; Verdoorn, T. A. *Mol. Pharmacol.* **1992**, *42*, 864−871.
(23) Honore, T.; Lauridsen, J.; Krogsgaard-Larsen P. *J. Neurochem.* **1982**, *38*, 173−178.
(24) Simon, J. R.; Contrera, J. F.; Kuhar, M. *J. Neurochem.* **1976**, *26*, 141−147.
(25) Nowak, L.; Bregestovski, P.; Ascher, P.; Herbert, A.; Prochiantz, A. *Nature* **1984**, *307*, 462−465.

including combinatorial libraries and physiological samples. The experimental setup can be optimized for a certain class of receptor antagonists by using recombinant receptors with tailor-made selectivities and response characteristics and by affecting the separation selectivity in CE, for example, by using secondary equilibrium such as micellar electrokinetic chromatography or electrochromatography. Because CE handles samples in the attoliter range[26] and can be interfaced with molecular structure-sensitive techniques such as NMR and mass spectrometry, powerful drug-screening and drug-discovery protocols can be built around this basic concept.

Because both the competitive and noncompetitive actions, as well as dissociation constants of different antagonists, can be recognized from analyses of dose−response relationships, development of patch clamp electrodes with improved mechanical stability and longer lifetimes allowing such analyses would greatly improve the technique.

(26) Chiu, D. T.; Hsiao, A.; Gaggar, A.; Garza-Lopez, R. A.; Orwar, O.; Zare, R. N. *Anal. Chem.* **1997**, *69*, 1801−1807.


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