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Articles

Patch Clamp Detection in Capillary Electrophoresis

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We describe a capillary electrophoresis-patch clamp (CE−PC) analysis of biomolecules that activate ligand-gated ion channels. CE−PC offers a powerful means for identifying receptor ligands based on the combination of the characteristic receptor responses they evoke and their differential electrophoretic migration rates. Corner frequencies, membrane reversal potentials, and mean and unitary single-channel receptor responses were calculated from currents recorded with patch clamp detection. This information was then combined with the electrophoretic mobility of the receptor ligand, which is proportional to the charge-to-frictional-drag ratio of that species. We applied CE−PC to separate and detect the endogenous receptor agonists γ-aminobutyrate and L-glutamate and the synthetic glutamate receptor agonists *N*-methyl-D-aspartate and kainic acid. We present dose−response data for electrophoretically separated kainic acid and discuss its implications for making the CE−PC detection system quantitative.

Recently, a novel peptide for an orphan receptor of the opiate family was discovered in porcine brain homogenates.[1] Without doubt many other neurotransmitters, neuromodulators, and neuropeptides remain to be discovered in the mammalian brain, and the role and localization of several known and putative neurotransmitters need to be established.

A limiting factor for successful neurotransmitter identification is the general detection problem. Because many fast-acting neurotransmitters and neurotransmitter mimetics are simple amino acids, amino acid derivatives, or small peptides, these molecules do not contain features for their sensitive detection with conventional techniques. Catecholamines and indolamines areexceptions, and they can be detected by using microelectrodes in situ (at the site of the sample origin) even down to the level of individual vesicles.[2] Detection of single serotonin-containing vesicles in RBL cells using confocal three-photon excitation fluorescence microscopy has also been demonstrated.[3] In order to detect and quantitate neurotransmitters lacking intrinsic detection qualities in neurobiologic samples, researchers have used in combination with liquid chromatography various labeling strategies, based primarily on molecular fluorescence. This procedure is of limited utility because many neurotransmitters, especially those lacking primary or secondary amine, or thiol groups, are extremely difficult to label in aqueous solution. Moreover, derivatization always involves loss of sample integrity and dilution of the original sample. Furthermore, fluorescence detection, like all other physical detection techniques, does not exclusively detect neurotransmitters and therefore lacks selectivity.

All fast-acting neurotransmitters, however, do activate membrane-bound ionotropic receptors. These receptors confer a high degree of selectivity by biomolecular recognition of a certain ligand or a group of ligands. They also provide a biological amplification system because binding of the ligand results in the opening of channels through which ∼10 000 permeable ions flow per millisecond.[4] This flow of ions can be measured with patch clamp (PC) recording techniques down to the level of a unitary receptor complex.[4−6] Patch clamp probes thus make an excellent and

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general strategy for detecting fast-acting neurotransmitters.

Receptor systems are often activated by multiple structurally related endogenous agonists that also can show neurotransmitter-like behavior.[7−9] When these ligand−receptor systems are studied, in situ patch clamp probes are difficult to implement unless they have absolute specificity for a ligand.[10,11] Thus, patch clamp detection must in most cases be coupled to a technique that enable us to discriminate between different receptor agonists.

As recently shown by us,[12] this discrimination can be achieved by coupling patch clamp detection on-line to capillary electrophoresis (CE), a miniaturized chemical separation technique. In this CE−PC system, receptor ligands are separated electrophoretically, delivered onto the surface of a patch-clamped cell, and detected by the cell at a characteristic migration time through the capillary. Thus, agonist identification is obtained from analysis of electrophoretic migration times, and the cellular response they evoke can be analyzed quantitatively (agonist potency) or qualitatively (receptor and agonist identification). This idea is conceptually similar to the use of single-cell biosensors for CE demonstrated by us,[13−15] and the use of electroantennographic detection in gas chromatography.[16] Compared to the fluorescence and two-electrode voltage clamp-based detection strategies used by us previously,[13−15] patch clamp is in general a more selective detection technique that provides detailed information about the activated receptors, which includes kinetics, rates of ion channel desensitization, distribution of conductance states, and ion−channel pharmacology.[4−6]

We believe that CE−PC detection is generally applicable as a fractionation and detection routine for neurobiological samples, such as tissue extracts and microdialysates, but is particularly useful in situations for which sample volumes are small and analyte concentrations are low.

In what follows, we discuss the CE−PC configuration on a more theoretical basis and present applications of this technique to the detection and partial quantitation of several important neuroactive ligands.

EXPERIMENTAL SECTION

Cell Isolation. Olfactory interneurons from new-born and adult Sprague Dawley rats of either sex (10−200 g) were acutely isolated as described previously.[17,18] In short; the olfactory bulbs were dissected from the rat brains, sliced, and incubated in a HEPES−saline buffer (140 mM NaCl, 5 mM KCl, 10 mM HEPES, 10 mM glucose, 2 mM $MgCl_2$, pH adjusted to 7.4 with NaOH), containing proteases from *Aspergillus oryzae* (2.5 mg/mL). Following the enzymatic treatment and washing, the slices were kept in a HEPES−saline buffer containing 1 mM Ca^{2+}, which was continuously bubbled with 95% O_2 and 5% CO_2. The tissue was then disintegrated using shear forces by gentle suction through a fire-polished Pasteur pipet. The resulting cell suspension was placed in a small Petri dish, diluted with buffer solution, and transferred to a microscope stage. Viable neurons could be harvested up to 6 h after the cessation of the enzymatic treatment. Chemicals and enzymes for tissue dissociation were obtained from Sigma (St. Louis, MO).

Capillary Electrophoresis−Patch Clamp Configuration. CE separations were performed in 20−50-cm-long, 50-μm i.d. fused-silica capillaries (Polymicro Tech., Phoenix, AZ) using HEPES−saline containing 1 mM Ca^{2+} as the electrolyte [in detection of *N*-methyl-D-aspartate (NMDA) receptor-mediated responses, a Mg^{2+}-free HEPES−saline containing 10 μM glycine was used]. Electrophoresis was performed by applying a positive potential of 12 kV to the inlet end of the capillary by a high-voltage power supply (LKB, Bromma, Sweden). Because the high voltages produce electrical field strengths of several hundred volts per centimeter, the CE capillaries were fractured and grounded 5 cm above the outlet.[19] Injections were made hydrodynamically by raising the inlet end of the capillary 10 s, 20 cm above the outlet.

Patch clamp detection was performed in the whole-cell and outside-out configurations.[4−6] The CE−PC detection experimental setup is shown in Figure 1A. The placement of the patch−clamped cell relative to the CE outlet is critical for the sensitivity of the system and warrants detailed investigation. In the present experiments, however, the tip of the patch clamp electrode was placed 5−25 μm from the center of the CE capillary outlet (Figure 1B) and resulted, in most cases, with the analyte concentrations used here, in a detectable response. In future studies aimed at optimizing the sensitivity of CE−PC detection systems, the geometrical configuration of the detector, receptor density, and receptor response characteristics together with postcapillary diffusion and convection must be taken into account. Patch pipets were fabricated from thick-walled borosilicate glass (code No. GC150-10, Clark Electromedical Instruments, Pangbourne, Reading, UK). The diameters and the resistances of the tips were about 2−5 μm and 5−15 MΩ, respectively. The estimated series resistance was always less than 50 MΩ. In outside-out patch clamp experiments, the electrode tips were fire polished in a butane flame, and the shanks were treated with Sigmacote (Sigma, St. Louis, MO). All experiments were performed at room temperature of 18−22 °C. The pipets (reference and patch electrodes) contained a solution of (in mM) 140 CsCl, 1 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, and 10 HEPES (adjusted to pH 7.4 with KOH), or 120 KF, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, and 10 HEPES (adjusted to pH 7.2 with KOH).

Procedures. To estimate the steady-state potential in the bath solution induced by the capillary electrophoresis, a naked patch clamp pipet was placed in front of the capillary outlet and the potential was measured in the current clamp mode.[4]

A superfusion system (RSC 100, Biologic Systems, Claix-Grenoble, France) was used for agonist delivery in order to evaluate whether the voltage drop over the capillary end piece

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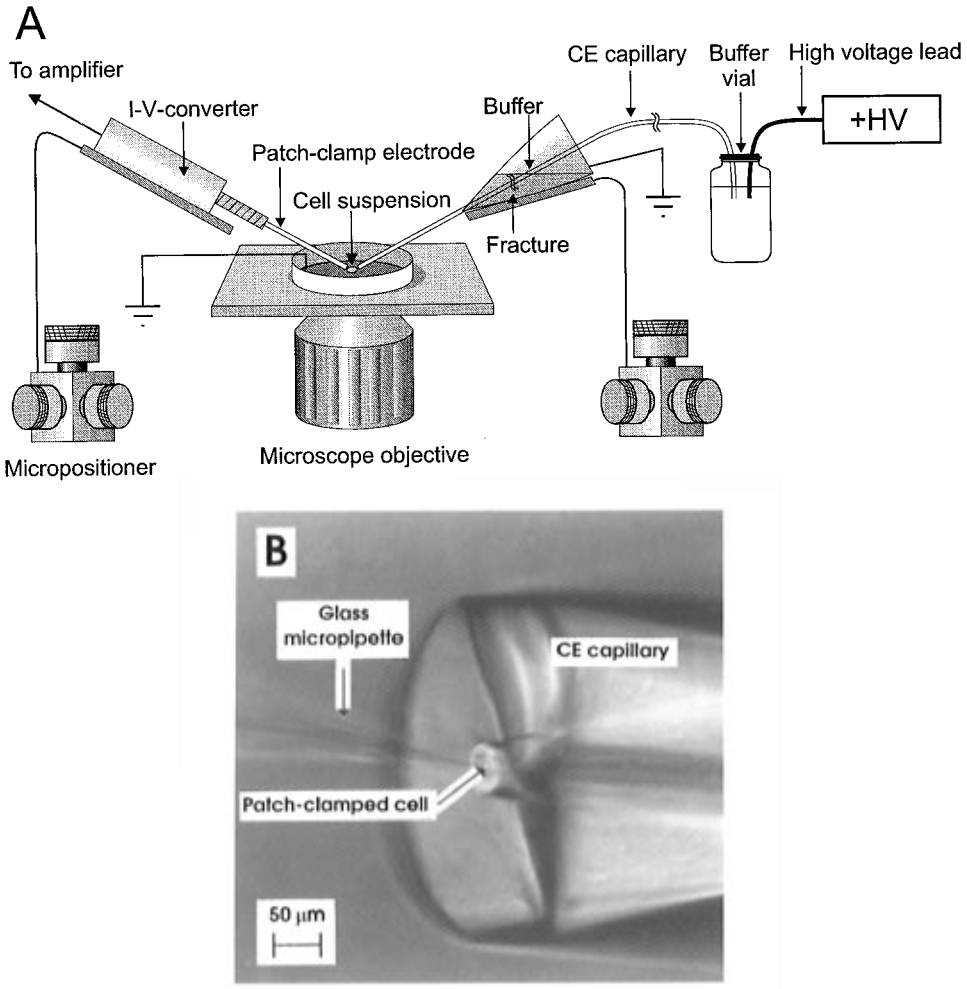

Figure 1. (A) Experimental setup for patch clamp detection in capillary electrophoresis. An inverted microscope is placed inside a Faraday cage that shields the patch clamp amplifier and other electronic apparatus from electrical and magnetic fields generated by the high-voltage power supply. The patch clamp electrode and the separation capillary are mounted on micromanipulators to facilitate the positioning of the patch-clamped cell relative the CE capillary outlet. The recordings are made in a small Petri dish containing the cell suspension. The CE capillary is fractured 5 cm above the outlet and connected to a high-voltage lead via a buffer vial. This buffer vial is housed in a polycarbonate holder (not shown) equipped with a safety interlock to prevent electric shock. (B) Photomicrograph of a patch-clamped cell positioned in front of the outlet of a separation capillary.

(from fracture to outlet) resulted in any significant changes to the amplitude of agonist-evoked responses recorded by the whole-cell patch clamp. In these experiments, the patch-clamped neurons were placed ~5 μm from the center of the capillary outlet and continuously superfused by a stream of Ca^{2+}-containing (1 mM) HEPES−saline solution. At certain intervals, the superfusion medium was changed to a HEPES−saline solution containing 100 μM kainic acid (KA), and recordings were made during conditions when the high voltage power supply was on or off. Data were assessed with the use of a paired *t*-test.

Experiments were performed to check the retention times obtained with CE−PC detection. Here, high concentrations of γ-aminobutyrate (GABA) and Glu (~50 mM of each) were injected into the CE system, and fractions were collected from the CE outlet every 45 s. The fractions were analyzed for Glu and GABA using HPLC and labeling with *o*-phthalaldehyde-β-mercaptoethanol.[20]

Data Analysis. Signals were recorded with a patch clamp amplifier (Model List L/M EPC-7, List-Electronic, Darmstadt, Germany), digitized (20 kHz, PCM 2 A/D VCR adapter, Medical Systems Corp., New York), and stored on videotape. For the production of electropherograms, the signals from the videotape were digitized at 2 Hz.

For spectral analysis of whole-cell currents, the signal from the videoadaptor was sent through an eight-pole Butterworth filter (bandwidth 1 kHz) and digitized at 2 kHz. Records were divided into 0.5 s blocks prior to calculation of the spectral density. The mean power spectrum was calculated by averaging all power spectra obtained from these blocks (at least 20). The KA-induced power spectra were subtracted from power spectra obtained during membrane resting conditions. The resulting power spectrum was fitted by a double Lorentzian function using a least-squares Levenberg−Marquardt algorithm with proportional weighting. The quality of the fitted curve was judged from *t*-ratios of fitted parameters, plots of residuals, and parameter correlations. The apparent single-channel conductances were estimated according to the equation $\gamma = \sigma^2/[(E - E_r)I_m]$ where σ^2 is the current variance, E the holding potential, E_r the reversal potential, and I_m the mean current.

Current-to-voltage ($I-V$) relationships were obtained from whole-cell responses evoked by electrophoretically separated KA

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(200 μM) at different holding voltages using a voltage ramp (−70 to +50 mV, duration 3 s, pClamp software, Axon Instruments, Foster City, CA). The ramp obtained between receptor activations was subtracted from that obtained during the response from separated KA. Then, any contribution of voltage-dependent ion channels to the overall current-to-voltage relationship was eliminated.

The single-channel current amplitudes obtained from outside-out patch clamp recordings of electrophoretically separated GABA and NMDA were displayed in so-called amplitude histograms. The histograms present the percentage of the total recorded time spent at each of a series of preset (up to 512 bins with 0.13 pA bin width) current levels in the total current input range. To improve the resolution, the amplitude histograms were compiled from the running average of a fixed series of adjacent sample points of the current signal.[21] The method also included a calculation of the variance of the preset sample point sequence (2−256), excluding measurements for which the variance exceeded a preset value. The single-channel amplitude analyses were performed using software developed and kindly provided by J. Dempster (University of Strathclyde, UK).

RESULTS AND DISCUSSION

CE−PC Configuration. In a patch clamp experiment, a cell is firmly attached by suction to the tip of a glass micropipet and manipulated to yield one of several desired configurations, i.e., outside-out, inside-out, or whole-cell recording modes.[4−6] In the outside-out and inside-out configurations, a small piece of the cell membrane is attached to the tip of the electrode, with the extracellular surface of the membrane exposed to the bath solution and interior of the patch electrode, respectively. For all these recording configurations, it is possible to control the concentrations of the ionic species on the respective sides of the cell membrane. The measuring principle of the patch clamp relies on the fact that the ionic flow across a cell membrane can be measured as an electrical current if the membrane potential is held constant, typically for neuronal cells in the range −30 to −90 mV. It is important to control accurately the holding potential because it determines the magnitude of the transmembrane electrical driving force exerted on a ionic species. Offset potentials created in interfaces between solutions of different ionic compositions (liquid junction potentials), for example, can cause the holding potential to deviate more than ±100 mV from the expected value. Consequently, large offset potentials must be controlled and compensated for.[4]

In this study, we used as a detector a patch-clamped cell placed outside the outlet of the capillary electrophoresis column. Even if the CE capillaries were fractured and grounded 5 cm above the outlet,[19] some residual current passes the capillary outlet and creates an offset potential (V_{cap}). Figure 2 shows an equivalent electrical circuit of a capillary electrophoresis−patch clamp setup with different contributions to the voltage offset.

The CE-induced offset potential ($V_{cap.tot}$) (the total exponential increase preceding the steady state) varied slightly with different capillaries but was generally in the range of 5−20 mV. To determine whether V_{cap}, after reaching a steady-state potential, was constant or variable during the course of a 15 min CE separation, we placed a naked recording pipet in front of the capillary outlet and measured the potential in the current clamp

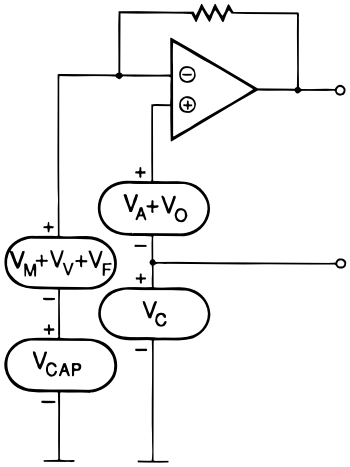

Figure 2. Electrical circuit equivalent to a patch clamp test measurement. The circuit includes two branches containing seven voltage sources, four in the external pathway and three in the internal (internal to the amplifier). V_M and V_c represent the membrane and command potential, respectively. In the external branch the electrode potentials represented as V_F (voltage drop at the silver/silver-chloride surface) are fixed during the course of the experiment. V_v is assumed to be a variable potential that is the sum of liquid potentials. In the branch internal to the amplifier, V_A is a fixed amplifier offset and V_o is a variable offset potential. V_{cap} is a potential induced by the capillary and adds to the circuit if the CE high power supply is on.

mode. It was found that the relative changes in potential induced by V_{cap} were smaller than ±5 mV and thus negligible for most CE−PC applications. Furthermore, CE-induced steady-state offset potentials measured with patch-clamped cells were only at a 1.4 ± 1.1 mV (mean ± SEM, n = 3) higher level than the offset potential measured with naked patch clamp pipets. Thus, if no other fluctuations of the potential occur in the bath solution during the course of the experiment, the command potential (V_c) will be identical to the membrane potential provided that the access resistance of the electrode is low.

In our experiments, we used an adjustable offset potential (V_o) in the patch clamp amplifier system to compensate for the offset potentials in the bath solution created by the liquid junction (V_v), the electrode (V_F), and the capillary (V_{cap}). This adjustment was made before the cell was attached to the micropipet. We tested this potential-nulling procedure by analyzing responses evoked by agonists delivered by a superfusion system during conditions when the high voltage was on and off. No significant difference between KA-evoked current amplitudes with the high voltage on or off were found (p > 0.3, paired t-test, n = 6, three cells) (Figure 3). Similarly, inside-out and cell-attached patch experiments with GABA (40−80 μM) in the patch electrode solution (CsCl), resulted in equally frequent receptor activations, with and without the capillary electrophoresis high voltage on (data not shown). Potential offset compensation can also be solved by introducing a virtual ground system,[22] which keeps the bath solution close to zero potential during the course of the experiment.

Separation and Detection of Endogenous and Synthetic Neuroactive Compounds in CE−PC. We used periglomerular/granular interneurons freshly dissociated from the rat olfactory bulb,[17,18,23] as biosensors in the patch clamp detection system.

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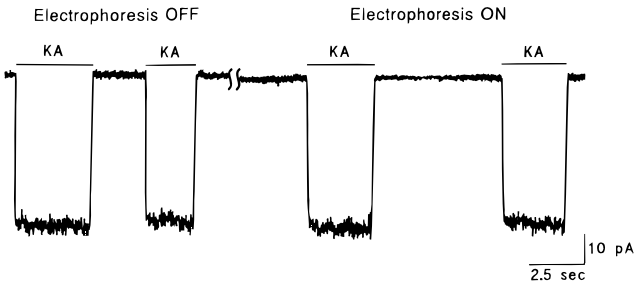

Figure 3. Effect of separation voltage on KA-evoked current responses. KA (100 μM) was applied by a superfusion system during conditions when the CE high-voltage supply was on or off. The capillary length was 50 cm, and the holding potential was −70 mV.

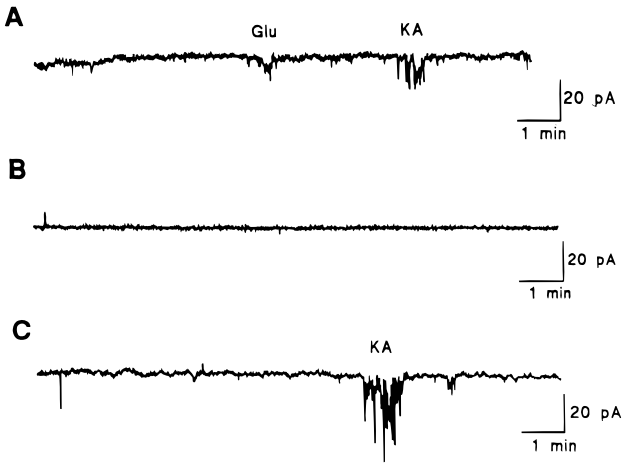

Figure 4. CE separation of Glu and KA, which induced inward currents mediated by glutamate receptors expressed by olfactory interneurons. (A) Separation of Glu (200 μM) and KA (200 μM) with outside-out patch clamp detection. (B) Separation of a HEPES−saline buffer on the same cell as in (A). (C) Separation of KA (2 mM) with detection by the outside-out patch clamp configuration. Current traces were sampled at 2 Hz. The capillary length was 20 cm, and the holding potential was −70 mV. The patch clamp pipet solution contained KF.

These cells are known to have $GABA_A$ receptors[24,25] and glutamate receptors of the AMPA[17,18,26] and NMDA type.[17,18] GABA, Glu, NMDA, and KA were used as analytes for identification in the CE−PC configuration. GABA and Glu are the major inhibitory and excitatory neurotransmitters active in synapses in the mammalian brain,[27,28] and NMDA and KA are synthetic agonists that activate NMDA receptors[29] and non-NMDA receptors,[30−32] respectively. KA is a convulsant and excitotoxic compound that produces patterns of limbic seizures and brain damage reminiscent of human temporal lobe epilepsy, when administered intraperitoneally or into the amygdala.[33,34]

We recently demonstrated CE−PC detection of GABA, Glu, and NMDA.[12] Here we present a separation of Glu and KA obtained under similar conditions except that the NMDA receptors were inactivated by using Gly-free and Mg^{2+}-containing (1 mM) cell and CE buffers (Figure 4). The identity and migration times of Glu and GABA using CE−PC detection was confirmed by HPLC analysis of CE fractions that were collected every 45 s (data not shown). The detector responses obtained from Glu and KA were of different character. Glu activated a low-amplitude current caused by a desensitization of the response, which under "normal" circumstances may modulate synaptic efficacy.[35−37] Glutamate receptor desensitization can readily be overcome by incubating neurons in a solution containing a specific lectin.[38,39]

KA, on the other hand, produces nondesensitized currents,[32−34] and yielded a larger amplitude response than Glu (Figure 4). The responses were only induced by ionotropic receptor−ion channel complexes, because the cell content is dialyzed in the whole-cell recording mode, which excludes involvement of intracellular cascade coupling systems. Metabotropic receptor systems remain functional, however, when pore-building substrates like nystatin are included in the electrode solution.[40−43] Nystatin treatment keeps the interior of the cell intact and makes it possible to use G-protein-coupled cascade processes including activation of ion channels.

Current-to-Voltage Relationships. When a cell that expresses a broad range of receptor systems is used, an important contribution to the identification of an analyte is the estimation of the ion permeability of the activated receptor−ion channel complex.

Concerning glutamate receptors, it is known that non-NMDA and NMDA receptors differ in respect to ion permeation profiles. In most regions of the central nervous system, non-NMDA receptors are regarded as impermeable to Ca^{2+} ions,[44−47] whereas the NMDA receptors show high Ca^{2+} ion permeability.[48] GABA-activated channels, on the other hand, are permeable to Cl^- ions and show different $I-V$ relationships.[49−51]

Estimation of ion permeability can be achieved by determining the reversal potential from current-to-voltage plots, which are based on mesurement of agonist-evoked currents at different

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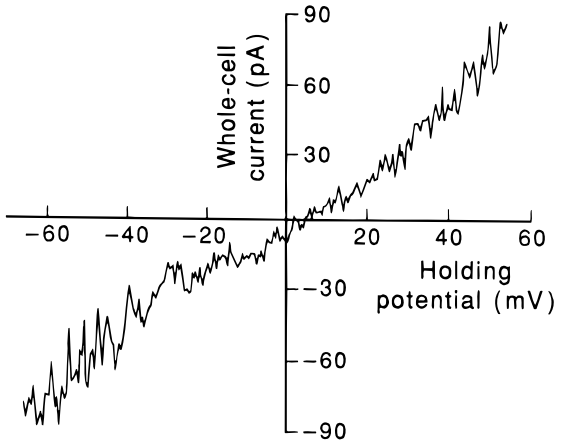

Figure 5. Typical example of a current−voltage (I−V) relationship obtained during electrophoretic application of 200 μM KA. The current−voltage curve was generated by a voltage ramp (3 sec.) where the holding potential was changed from −70 mV to +50 mV. The patch clamp pipet solution contained KF.

holding potentials. Together with knowledge of the ionic composition of the intra- and extracellular solutions, reversal potentials can be employed in different models, as the Eyring rate (2B1S) model[52,53] or the Goldman−Hodgkin−Katz constant field theory,[53−57] to determine the relative ion−channel permeability properties. Thus, by changing the intra- and extracellular solutions in current-to-voltage experiments, and determining reversal potentials, information concerning ion channel permeability will isolate the analyte to be a member of a certain group of ligands. Also, the rectification of the $I-V$ curve can be used to extract information of the ion−channel permeability. For example, AMPA receptors with high Ca^{2+} permeability give, with few exceptions,[58,59] an inwardly rectifying relationship,[60,61] in contrast to Ca^{2+}-impermeable AMPA receptors that yield outwardly rectifying or linear $I-V$ curves.[26]

In this study we demonstrate a current-to-voltage relationship generated by a voltage ramp during exposure to electrophoretically separated KA (Figure 5). The reversal potential of $+1 \pm 5$ mV (mean ± SEM, $n = 7$) is close to values reported for the AMPA type of glutamate receptors,[26] which were estimated in experiments under similar conditions.

Analysis of Power Spectra: Mean Single-Channel Conductances and Corner Frequencies. Analysis of ion−channel currents can be performed on both the ensemble- and single-channel level to reveal the identity of the agonist−receptor interaction. The Fourier transformation of the autocorrelation function for the total current obtained with whole-cell patch clamp

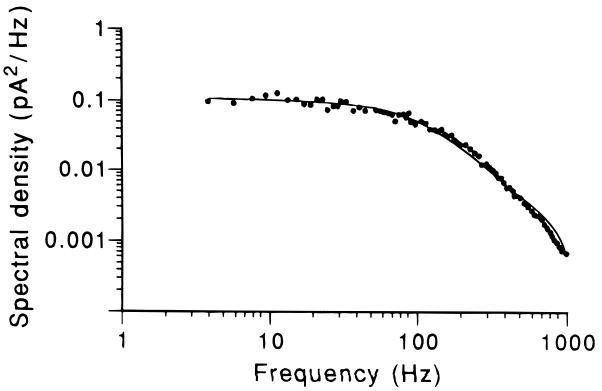

Figure 6. Spectral analysis of whole-cell patch clamp currents evoked by separated KA (200 μM) on freshly dissociated rat olfactory interneurons. The holding potential was −70 mV.

yields power spectra that can be fitted by single or double Lorentzian functions.[62] From these fits, mean single-channel conductances (γ) (in units of S), and kinetic properties through analysis of corner frequencies (f_{ci}) (in units of Hz) can be estimated.[62] Single-receptor systems must be activated, however, by single agonists, to avoid averaging of data. It is, therefore, important to control both the separation process and the expression of different receptor systems in the cell used for detection.

We used KA as an analyte to demonstrate the agonist−glutamate receptor specificity in rat olfactory bulb interneurons, which is a well-characterized system.[17,18,26] Spectral analysis (Figure 6) of whole-cell currents evoked by separated KA gave a mean single conductance of 4.4 ± 0.7 pS and corner frequencies of 14.9 ± 1.4 and 147.1 ± 28.5 Hz (mean ± SEM, $n = 3$), which is in close agreement with the data reported elsewhere.[26] We have previously demonstrated that GABA-, Glu-, and NMDA-evoked currents in the same cell system give mean single-channel currents of 22.4 ± 3.1, 10.3 ± 2.3 (response mediated by AMPA/KA receptors), and 39.2 ± 3.0 pS.[12] respectively. Yet another example is strychnine-sensitive glycine receptors that have high-conductance Cl^- channels, on the order of 100 pS. It is therefore feasible to discriminate between these different receptor systems by spectral analysis. Because mean single-channel conductances and corner frequencies for a given receptor system can vary somewhat for different cell types, it is recommended that these parameters be characterized for each detector cell system used.

Single-Channel Recordings: Single-Channel Conductances and Amplitude Histograms. Spectral analysis deals with averages, and does not reveal the distribution of subconductance levels of activated ion channels. Outside-out patch clamp is a recording configuration capable of resolving single-channel openings, and different conductance levels mediated by the same receptor−ion channel complex can thus be obtained. This measurement results in more intrinsic information about a particular ligand−receptor interaction. Also, by resolving channel openings with intervening channel shuttings, the distribution of open and shut times can be analyzed to yield information about channel kinetics. Nevertheless, problems concerning the statistical significance of the information gained from the analysis can occur with analyte-evoked receptor responses of a very short duration.

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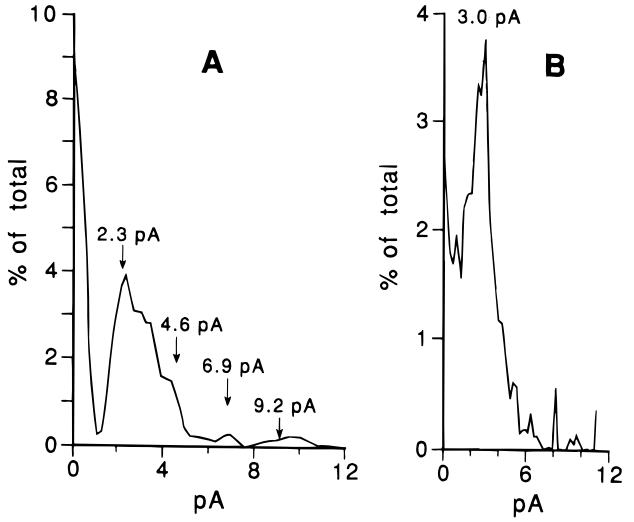

Figure 7. Amplitude histograms from outside-out responses evoked by electrophoretically separated GABA (250 μM) (A) and NMDA (250 μM) (B). The measurements were performed on two different interneurons of the rat olfactory bulb. The holding potential was −70 mV. The *Y*-axis represents the percentage of the total recorded time spent at each of a series of preset current levels in the total current input range. The *X*-axis represents different current amplitude levels.

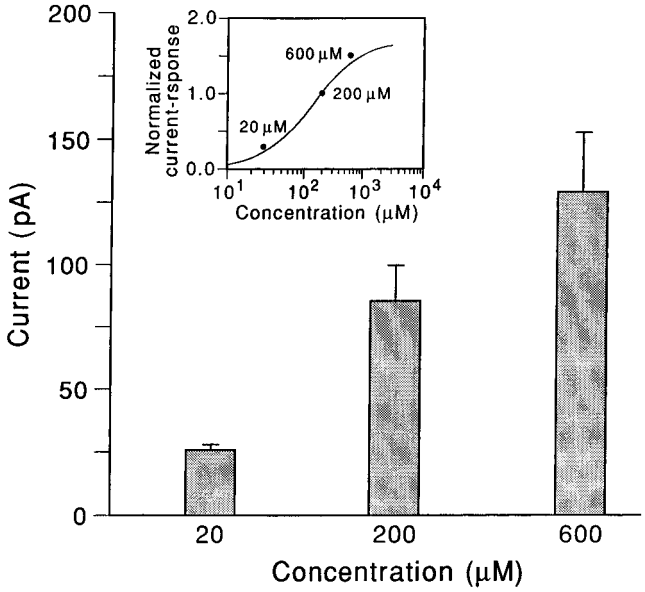

Figure 8. Dose−response relationship constructed from receptor responses evoked by KA at 20, 200, and 600 μM, respectively (concentration of the injected sample). The current responses, in pA, are means ± SEM (n = 3−4 cells for each concentration). The sigmoid curve represents a dose−response relationship created from current amplitudes evoked by tube superfused KA on interneurons from rat olfactory bulb,[28] i.e., the same cell system used in the CE−PC experiments. The different averaged current responses, both from the experiments using the tube superfusion system and the CE−PC measurements at the respective concentration levels, were normalized against the current amplitude produced by 200 μM KA. The dose−response relationship estimated from experiments using the tube superfusion system were fitted by an empirical equation as described in the text. The length of the capillary was 50 cm, and the holding potential was −70 mV. The patch clamp pipet contained CsCl in the CE−PC measurements.

The main single-channel conductance event for GABA-activated currents is represented in an amplitude histogram (Figure 7A). It is shown that the GABA-induced openings were mainly at the 2 pA level. This corresponds to a channel conductance of ∼30 pS, which agrees with values previously reported for $GABA_A$ receptors in various central neurons.[63−66] The NMDA-evoked currents yielded a unitary single-channel current of 3 pA (Figure 7B) which corresponds to a conductance of ∼43 pS (if we assume a reversal potential of 0 mV). This behavior agrees well with the high-conductance channels observed in several other studies.[67]

Dose−Response Relationships. Quantitation of the analyte concentration is often an important consideration. As an example, it is of interest to determine the differences in concentration and maintenance of the release of a transmitter when studying dysfunctional processes such as epilepsy or ischemia.[68,69] Release of glutamate, a potent neurotoxin,[70] is, for example, enhanced during episodes of cerebral ischemia in rat models.[68,69]

To test the ability of the CE−PC configuration to sense an agonist in a dose-dependent manner, we used KA, which activates nondesensitizing currents mediated by AMPA receptors. Figure 8 shows that the amplitudes of KA-evoked receptor responses increase with higher concentrations of KA in the injected sample. Without any calibration of the patch detector, however, it is difficult to know what the analyte concentration is at the membrane surface because some of the injected analyte molecules leak out through the crack in the separation capillary and are also subject to dilution by postcapillary diffusion and convection. In the dose−response experiments, one cell was used for detection at one analyte concentration level and for one blank only because of the limited lifetime of the patches (see below). Our results indicate, however, that the different cells used here displayed rather uniform response characteristics. Dose−response relationships can be fitted by an empirical equation, $I_{max}/(1+ (EC_{50}/C)^n)$, where I_{max} is the maximal current amplitude, EC_{50} is the concentration producing a half-maximal current response, C is the agonist concentration, and n is the Hill coefficient.[71] The Hill coefficient aids in discriminating between different analytes by giving a stoichiometric characterization of the ligand−receptor interaction, and the EC_{50} value determines the ligand potency. To determine whether the dose−response relationship measured in CE−PC tracks the empirical equation, we fitted the dose−response data to this equation in which rat olfactory bulb interneurons were exposed to different concentrations of KA by using a tube superfusion system.[26] We made the assumption that the response characteristics of the AMPA receptors to KA were identical in both experimental setups, i.e., the tube superfusion system and the CE−PC configuration. The inset in Figure 8 shows that the three concentrations of KA gave normalized responses that correlated well with currents evoked by superfused KA on the same cell system.

Nevertheless, for quantitative analysis using CE−PC detection, and for stringent estimations of Hill coefficients and EC_{50} values,

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it will be required that multiple doses can be electrophoretically delivered to the same cell in order to make possible normalization to a maximum response. This goal can be achieved by the use of shorter capillaries that allow faster separations and a correspondingly higher sample turnover number. Additional runs would also become possible if long-lived patches with higher mechanical stability could be developed through the use of new electrode materials or electrode solutions. In the present setup, patches could be maintained up to 2 h when KF was used as the electrode solution, but typically patches were functional for only 20−30 min. These short lifetimes of the present patch-clamped cells appear to be the major limitation to the use of this technique for quantitation.

CONCLUSIONS

We have characterized a capillary electrophoresis−patch clamp detection system that uses the membranes from cells for identification of biologically active analytes in complex mixtures. A broad spectrum of information, including response characteristics, current-to-voltage relationships, dose−response measurements, kinetics, and ion−channel conductance levels were extracted from the recorded currents evoked by electrophoretically separated receptor agonists. When this information is combined with the measurement of migration times, the biologically active analytes present can be uniquely identified. An important aspect of this technique is that detector cells with tailor-made selectivities and response characteristics can be designed by using receptor clones expressed by molecular biology techniques in receptor-deficient cell systems.[13]

Capillary electrophoresis embodies both a separation and a sampling system, in which the inlet end of the separation capillary can be used to sample intracellular and extracellular analytes[72] even down to the single-biomolecule level.[73] By combining CE−PC detection with such sampling schemes, an integrated system can be created which introduces minimal sample handling and dilution of the original sample and which is capable of analyzing subfemtoliter samples quantities. Thus, CE−PC detection should make possible the analysis of neurotransmitter dynamics on the single-cell or even single-synapse level. Although the identification power of the CE−PC technique seems to be superb, more efforts will be required to increase the lifetime of the patch-clamped cells if quantitation is to become a routine procedure.

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