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Monitoring Neurotransmitter Release from Isolated Retinas Using Online Microdialysis-Capillary Electrophoresis

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Release of neurotransmitters and other primary amine-containing analytes from intact, isolated larval salamander (*Ambystoma tigrinum*) retinas maintained in a 6.5-μL perfusion chamber was monitored using online microdialysis-capillary electrophoresis with laser-induced fluorescence detection (CE-LIF). Primary amines were derivatized online with *o*-phthaldialdehyde (OPA) and β-mercaptoethanol. With the use of overlapping injections, the perfusate was sampled every ∼10 s. Although separation conditions were optimized using 20 mM hydroxypropyl-γ-cyclodextrin (HP-γ-CD) for a number of important neuromessengers including D- and L-serine, D- and L-asparate, glutamate, GABA, serotonin, dopamine, norepinephrine, and taurine, only glutamate (0.48 ± 0.27 μM), GABA (0.25 ± 0.12 μM), taurine (5.5 ± 2.1 μM), and L-serine (2.8 ± 1.0 μM) were identified in the perfusate. Elevated levels of glutamate, GABA, and taurine were detected during stimulation with 60 mM K^+. This method is the first to directly sample multiple neurotransmitters from perfused, isolated retinas and to observe changes in efflux of these neurotransmitters as a result of pharmacological stimulation.

The retina does much more than simply transmit light information back to the brain. The vertebrate retina performs initial processing of visual images entering the eye before transmitting this information to the brain for further processing, leading to visual perception. Some of the major functions of the retina include transduction of light by photoreceptors, light and dark adaptation, and initial processing for color, contrast enhancement, and feature detection.[1] Since the brain and retina are both part of the central nervous system, the retina can be used as a model tissue to study aspects of neuronal function that have direct implications for the brain as well. The retina contains most, if not all, of the neurotransmitters found in the brain, including GABA, glutamate, glycine, and dopamine.[1] The retina has been referred to as "nature's brain slice" because the thin nature of the tissue (150−250 μm) makes it relatively simple to isolate and perfuse while retaining functional light responses.[2]

Retinal function can be studied ex vivo in eye-cup preparations where the front of the eye is removed and the retina remains intact in the eye-cup. Alternatively, intact or sliced retinas can be removed from the eye and placed in a perfusion chamber for study. When using avascular salamander and mudpuppy retinas, oxygen and nutrients can be provided through the perfusion buffer. With proper control of temperature and a supply of oxygen and nutrients, salamander retinas housed in perfusion chambers remain viable, maintaining light responses as evidenced by numerous electrophysiological experiments.[3−5] Pharmacological experiments using perfusion setups are easily performed by switching the perfusate from control Ringer's buffer to buffer containing the pharmacological agent. Unfortunately, these experiments often rely on indirect measures such as electrophysiology or imaging techniques to make inferences regarding neuromessenger release. Slaughter et al.[6] have used HPLC to assay extracellular levels of aspartate, glutamate, and serine from mudpuppy retinas incubated in 100-μL of Ringers solution for 1−3 h. We have previously measured the efflux of dopamine, GABA, taurine, L-serine, and glutamate from salmander retinas incubated in 50-μL of Ringers solution for 3 h using microdialysis CE-LIF.[7] However, chemical efflux from a continuously perfused retina, which would allow release dynamics to be studied, has never been measured before.

Microdialysis is a simple sampling technique for low molecular weight compounds that has been used extensively in vivo, most notably for pharmacokinetic and neurotransmission studies.[8−10] Perfusion buffer is pumped through a probe incorporating a small diameter dialysis membrane. Small molecules diffuse from the surrounding area, across the membrane, and into the probe. Conventionally, fractions on the order of several microliters are collected from the outlet of the probe for subsequent analysis.

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This fraction collection provides temporal information, allowing changes in analyte concentrations to be monitored over time. There are a number of advantages to the microdialysis approach. Larger molecules such as proteins are excluded from the sample stream, simplifying sample cleanup. No volume is removed from the tissue under study allowing many replicate measurements to be made while minimizing the effect on the sample tissue.[11]

The most commonly cited disadvantage to microdialysis-based techniques is poor temporal resolution. Small volume probes are limited to perfusion rates of several microliters per minute. Analyses that require several microliters for an analysis are therefore limited to temporal resolutions on the order of minutes. Even if more sensitive techniques are available, collecting, storing, possibly derivatizing, and analyzing dozens of submicroliter samples is challenging.

This limitation to temporal resolution has been overcome with the introduction of online microdialysis-capillary electrophoresis (CE). Online derivatization and sample injection eliminate the need for collecting submicroliter fractions. The high mass sensitivity of laser-induced fluorescence (LIF) or electrochemical CE detectors makes analysis of subnanoliter volumes feasible. Temporal resolutions on the order of seconds have become common using the online microdialysis-CE approach.[12−14] For example, in vivo analysis of analytes such as glutamate and dopamine by online microdialysis-CE-LIF has been demonstrated with sampling rates as fast as 5−10 s.[15−20]

Online microdialysis-CE assays developed for neurotransmitters have been most successful monitoring glutamate and aspartate to date.[13,18,19] This success arises from the fact that there are relatively few doubly charged analytes in the perfusate after derivatization with OPA or NDA. Improvements in detection limits and injection techniques have improved the peak capacity of online CE separations allowing singly charged labeled analytes, such as GABA, to be analyzed.[7,21] Enantiomeric separations are more difficult. Resolution of D-serine has been achieved in an online microdialysis CE assay by adding hydroxypropyl-γ-cyclodextrin (HP-γ-CD) to the separation buffer.[7] Glutamate, GABA, dopamine, norepinephrine, D-, L-aspartate, taurine, and serotonin were also resolved under these conditions. In the current paper we use these separation conditions to measure the efflux of several important analytes, including glutamate, GABA, taurine, and L-serine, from larval tiger salamander retinas housed in a microliter scale perfusion system.

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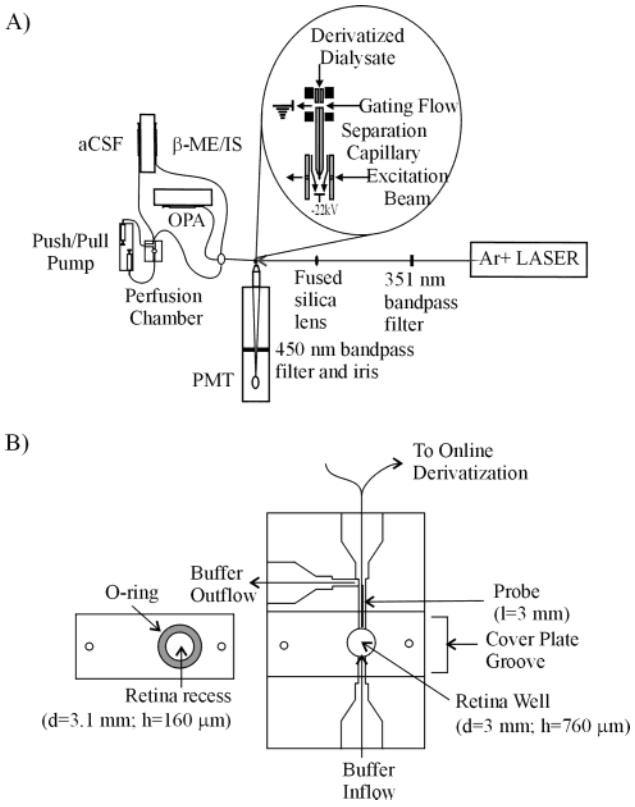

Figure 1. Schematic of the online microdialysis CE-LIF setup (A) and microliter perfusion chamber (B). Refer to the Experimental Section for further details.

EXPERIMENTAL SECTION

Reagents. Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich (St Louis, MO). Artificial cerebrospinal fluid (aCSF) contained the following: 145 mM NaCl, 2.7 mM KCl, 1.0 mM $MgSO_4$, and 1.2 mM $CaCl_2$ in deionized water. Amphibian Ringers solution contained: 111 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM HEPES, and 5 mM glucose, pH 7.4. High-K^+ amphibian Ringers solution contained: 51 mM NaCl, 62.5 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM HEPES, and 5 mM glucose, pH 7.4. All buffers and standard stock solutions were prepared in deionized water (Milli-Q, 18.2 MΩ, Millipore Corporation, Bedford, MA) and were filtered (0.20 μm) prior to use. CE separation buffer contained the following: 20 mM HP-γ-CD (Cerestar, Hammond, IN) and 50 mM or 100 mM borate, pH 10.5.

Microdialysis. A schematic of the online microdialysis-CE-LIF instrument is shown in Figure 1A. "Side-by-side" microdialysis probes were fabricated in-house.[7−9] Briefly, two capillaries (40-μm i.d./110-μm o.d.), staggered by 3 or 5 mm, were placed inside a regenerated cellulose microdialysis fiber (200-μm i.d./216-μm o.d., 18 000 MWCO, Spectrum Laboratories, Inc., Rancho Dominguez, CA) that was sealed at one end with polyimide resin (Alltech Associates Inc., Deerfield, IL). The volume of the 3-mm probe is ~66 nL and that of the 5-mm probe is ~110 nL. Perfusion fluid (aCSF) was pumped through the probe at 20 μL/h (0.333 μL/min) with a microsyringe pump (Harvard Apparatus Inc., Holliston, MA).

Derivatization. Primary amines were fluorescently derivatized using *o*-phthaldialdehyde (OPA) in the presence of β-mercapto-

ethanol.[22,23] The dialysate was mixed online with derivatization solution (40 mM OPA in 4.5/5.5 methanol/75 mM borate (pH = 10.5)) and an internal standard (5 μM 2-aminoadipic acid (IS), 115 mM β-mercaptomethanol in aCSF) in a 0.25-mm i.d. stainless steel cross (Valco Instruments Co. Inc., Houston, TX). The reaction took place in a 150-μm i.d./360-μm o.d. capillary connected to the fourth arm of the cross as the reagents flowed to the CE interface. The OPA solution flow rate was 10 μL/h; the internal standard/β-mercaptomethanol solution and dialysate flow rates were 20 μL/h. The length of the reaction capillary was 12 cm, giving a reaction time of 3 min.

CE-LIF. Injections were made using a flow-gated injection interface, as previously described.[7,14,18,19,21,24−27] The interface was machined out of polycarbonate (UMN Machine Shop). Electrokinetic injections (0.75−2 kV, 500−700 ms) were made 1−2 s after stopping the gating flow. CE separations were performed in an 8-cm long, 5-μm i.d./150-μm o.d. fused-silica capillary (Polymicro Technologies, Phoenix, AZ). The outlet end of the separation capillary was ground to a point using a Dremel tool. A separation voltage of −20 to −22 kV was applied to the outlet end of the separation capillary by a CZE1000R high-voltage power supply (Spellman High Voltage Co., Hauppauge, NY). LIF detection was performed off-capillary using a sheath flow detector cell.[28−31] The outlet end of the separation capillary was inserted into a square fused-silica cuvette with a 200-μm × 200-μm square inner bore (NSG Precision Cells, Inc., Farmingdale, NY). Sheath flow buffer (50 mM borate, pH 10.5) flowed through the cuvette and around the separation capillary to maintain the laminar flow profile of the sample stream as it exited the separation capillary. The 351-nm line (20-mW multiline UV) of an argon ion laser (Enterprise II, Coherent Laser Group, Santa Clara, CA) was passed through an interference filter (351 ± 2 nm, Melles Griot, Carlsbad, CA) and focused by a 1× fused-silica lens onto a spot immediately after the separation capillary for excitation. Fluorescence was collected at 90° by a microscope objective, passed through an interference filter (450 ± 25 nm, Melles Griot), and focused onto a side-on photomultiplier tube (PMT R1477, Hamamatsu Corporation, Bridgewater, NJ). Current from the photomultiplier tube was amplified (10^6 V/A) and filtered (10-ms rise time) using a current amplifier (Keithley Instruments, Inc., Cleveland, OH) and recorded on a data acquisition card (PCI-MIO-16XE-50, National Instruments Corp., Austin, TX). LabView (National Instruments Corp., Austin, TX) was used for data acquisition and instrument control.

In Vitro Characterization. The temporal resolution of the microdialysis CE-LIF setup was determined by introducing a step change in analyte concentration at the probe. All solutions were stirred and maintained at 16 °C. The probe was transferred manually between solutions of 0.25 μM and 1 μM GABA and glutamate. The smallest concentration change detectable by the microdialysis CE-LIF instrument was also determined in this manner. Recovery of the probes was measured in stirred solution and compared to recovery in the perfusion chamber.

Microliter Perfusion Chamber. The microliter perfusion chamber (Figure 1B) was custom-made by the University of Minnesota Physics Machine Shop. The chamber consists of two pieces: the chamber base and cover plate. The chamber base houses the connection ports for the buffer flow and microdialysis probe and the retina well (3.0 mm diameter, 760 μm deep). The cover plate fits into a groove on the chamber base. Retinas were positioned in a recess on the chamber cover plate (3.1-mm diameter, 160-μm deep), and the cover plate was secured to the base with two screws. An O-ring in the cover plate seals the well. A push−pull pump (Harvard Apparatus Inc., Holliston, MA) was connected to the chamber. The tip of the microdialysis probe was positioned at the exit of the retina well, thus sampling analytes released by the entire retina. All connections were made using PEEK ferrules and $^1/_{16}$-in. tubing (Valco Instruments Co. Inc., Houston, TX).

Retina Preparation. All animal experiments were performed in strict accordance with protocols approved by the Institutional Animal Care and Use Committee at the University of Minnesota, using methods described previously.[32] Larval tiger salamanders (*Ambystoma tigrinum*) were sacrificed by decapitation and immediate pithing. The eyes were rapidly removed, and retinas were dissected away from the pigment epithelium. The retinas were then placed in the perfusion chamber (Figure 1), ganglion cells facing up. The retina was continuously perfused with oxygenated Ringer's solution at 50 μL/h. Once assembled, the perfusion chamber was rinsed for 45 min to remove analytes released during the excision process and to establish baseline levels before beginning the first stimulation. Potassium stimulations were made using a four-way valve (Valco Instruments Co. Inc., Houston, TX) to switch the perfusion solution between control Ringer's and high-K^+ Ringer's. The retina was maintained at 16 °C by placing the chamber on a Peltier device.

RESULTS AND DISCUSSION

Isolated retinas are commonly studied in perfusion chambers using imaging and electrophysiological methods. We began with a chamber originally designed for microscopy and electrophysiological studies of isolated salamander retinas.[3] Several modifications of this design were necessary to enable direct monitoring of the perfusate by microdialysis CE-LIF. The volume of the original perfusion chamber was relatively large (255 μL) and the flow through the chamber relatively fast (~1 mL/min). At this flow rate, the chamber volume was cleared once every 15.3 s. Dilution of analytes released into this large, rapidly exchanged volume resulted in concentrations below the limits of detection. Assuming the rate of analyte release from the retina remains constant, the dilution factor is directly proportional to the flow rate of the perfusate through the chamber. Unfortunately, lowering the perfusion rate increases the clearance time of the chamber, consequently limiting the temporal resolution. To address this, the volume of the chamber must be decreased as the flow rate of the perfusate is reduced.

In our modified chamber, the dimensions of the retina determine the minimum volume of the retina well. Larval tiger

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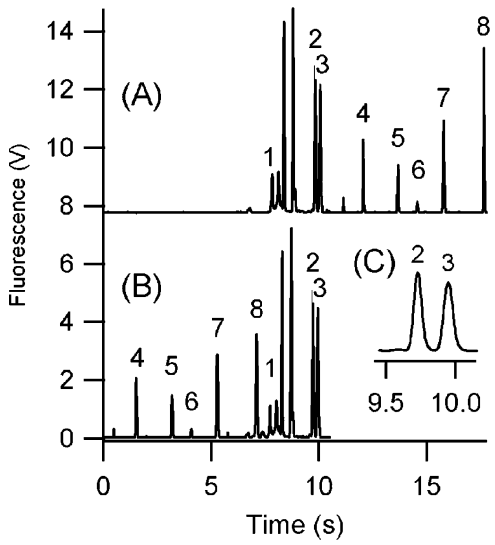

Figure 2. Electropherograms demonstrating overlapping injections. In (A), the separation time allows all analytes to migrate off the capillary, while in (B) an overlapping injection was made. In (B), peaks 4−8 remain on the column during a second injection and migrate at the beginning of the subsequent separation. Overlapping injections were made once every 15.2 s. Enantioseparation of D- and L-serine is achieved (C). Peak identifications: dopamine (1), D-serine (2), L-serine (3), phosphoethanolamine (4), glutathione (5), 2-aminoadipic acid (internal standard) (6), glutamate (7), L-aspartate (8). Flow-gated injections (700 ms, −2.5 kV) were performed after a 3 s delay. Separations were performed on a 7.8-cm capillary with a separation voltage of −22.0 kV using 20 mM HP-γ-CD, 100 mM borate, pH 10.5 separation buffer. Sheath flow buffer was 50 mM borate, pH 10.5.

salamander retinas are approximately 3 mm in diameter and 150 μm thick. The retina well in the modified chamber is 3 mm in diameter, and 760 μm deep. The retina is positioned in a cover plate recess that is 3 mm in diameter and 160 μm deep. Without the retina in place, the volume of the well is 6.5 μL, a 39-fold reduction from the original perfusion chamber. The actual volume is further reduced as a result of the volume occupied by the retina itself. The flow rate was decreased to 50 μL/h. Again, assuming that the rate of analyte release from the retina remains constant, decreasing the perfusion flow rate from 1 mL/min to 50 μL/h decreases dilution in the chamber by a factor of 1200. At 50 μL/h, without a retina in place, the retina well was cleared only once every 7.8 min, a 30-fold increase relative to the original chamber.

Flow of perfusate through the original chamber was driven by gravity flow. However, gravity flow proved unreliable at the lower flow rates and smaller volumes in the modified chamber. More reproducible flow was obtained using a push−pull pump. The push−pull pump also prevents significant back pressure in the chamber, which may damage the retina. The retina well was enclosed and sealed using an O-ring to prevent evaporation and ensure a constant volume in the modified chamber. Maintaining a constant volume in the chamber throughout the experiment is vital, as fluctuations in volume affect analyte concentrations measured in the perfusate.

Figure 2A shows an electropherogram of 10 primary amines expected to be released by the retina. A number of important neuromessengers are baseline resolved including glutamate, GABA, serotonin, dopamine, norepinephrine, taurine, D- and L-asparate, and D- and L-serine. The concentration of borate in the separation buffer was increased from previous studies[7] to 100 mM to improve the resolution of D-serine. Efficiencies of 350 000−400 000 theoretical plates are achieved in separations less than 17 s long. As demonstrated in Figure 2B, overlapping injections permit even shorter analysis times. No peaks are detected during the first 6 s of the separation. This time can be used more effectively by performing overlapping injections. After ∼8 s, the separation is stopped and a second injection is performed. Analytes that have not yet exited the capillary (i.e., migrating after 8 s) migrate past the detector before the first peak of the subsequent separation. Due to the high efficiency of the separations, broadening of the analyte zones left on the separation capillary during the second injection is minimal. The total time for analysis, including the time for injection, is ∼10 s. There is interday variation in migration times due to differences in separation voltages and separation capillary lengths, however, the relative separation profile remains constant allowing unambiguous peak identification. Migration times between separations performed on the same day vary less than 5% RSD.

The response of the online microdialysis CE-LIF instrument was examined in vitro. Figure 3A shows the temporal response of the instrument after the introduction of a step change in analyte concentration at the probe. It takes approximately 4.5 min for the analyte to flow through the online reaction connections to the CE interface. Once the concentration front reaches the interface, the signal increases from baseline to a plateau within 10−20 s. It is this rise time that determines the fastest change in concentration that can be monitored. Diffusional broadening in the probe, the reaction cross, and the connecting tubing also contribute to the temporal resolution since the time to reach the plateau is observed over 1−2 separations. The time required to return to baseline after the probe is returned to the original analyte solution is similar, indicating that the analytes do not adsorb onto the probe or the connecting tubing. The smallest detectable change was also determined in vitro (see Figure 3B). Concentration changes from 0.35 to 0.45 μM were detectable for glutamate and GABA ($p <$ 0.001, Student's t-test).

The relative recoveries of four microdialysis probes in bulk solution were compared to recoveries observed when the probe was placed in the exit channel of the perfusion chamber (see Table 1). "Bulk" recoveries were measured in stirred solutions to mimic the rapidly exchanging volume of the exit channel of the perfusion chamber. The observed recoveries are typical for probes of this type operated under these conditions.[7] Somewhat surprisingly, recoveries observed in the perfusion chamber were significantly lower than those observed in bulk solution. The volume of the perfusion chamber's exit channel is so small that the probe significantly depletes analyte concentration in the perfusate. It should be noted that since the probe is placed in the exit channel, this mass clearance is not expected to affect the function of the retina.

Figure 4 shows an online CE-LIF analysis of dialysate collected from an intact, light-adapted larval tiger salamander retina housed in the perfusion chamber. The perfusate was analyzed using online microdialysis CE-LIF as it exited the perfusion chamber. Microdialysis sampling excludes proteins released from the retina, eliminating the need for sample pretreatment. Direct analysis of the perfusate with the online CE-LIF instrument was attempted,

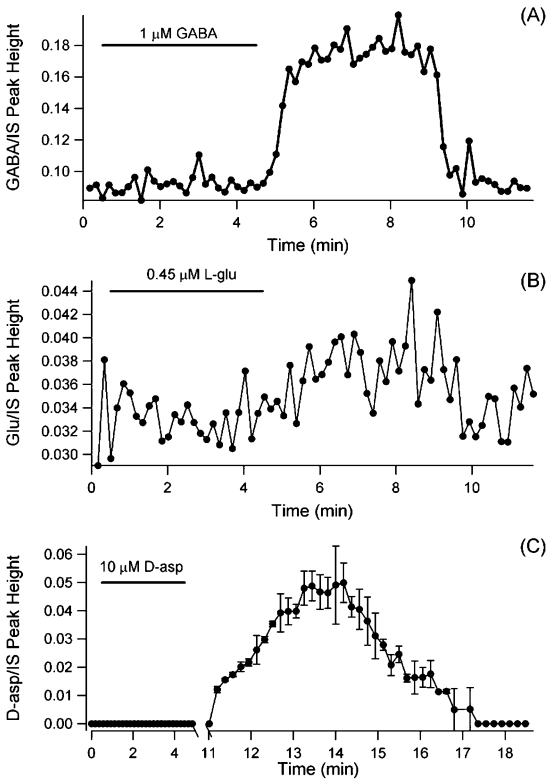

Figure 3. Temporal response of the chamber and online microdialysis CE-LIF system. The probe was moved from 0.25 to 1 μM GABA and then back (A) or from 0.35 to 0.45 μM glutamate and then back (B) after 4 min. The solid line marks the time that the probe was switched between solutions. The delay in response arises from the time for the dialysate to react online and reach the CE analysis. Solutions were stirred and maintained at 16 °C. Separations were performed once every 10.35 s. (C) A solution of 10 μM D-aspartate was applied from 0.5 to 4.5 min with a retina in the chamber to demonstrate the clearance profile of the chamber. The solid line marks the time that the valve was switched to the D-aspartate solution. The delay in response time is due to the time for the sample to reach the chamber from the valve, fill the retina well, and then reach the detector.

Table 1. Comparison of the Microdialysis Relative Recovery in a Stirred Bulk Solution with the Recovery Observed in the Perfusion Chamber (n = 4 Probes)[a]

	recovery in bulk solution (%, ± SE)	recovery in perfusion chamber (%, ± SE)
GABA	64 ± 5	51 ± 6
taurine	73 ± 5	60 ± 5
L-serine	48 ± 8	28 ± 7
L-glutamate	81 ± 16	59 ± 12

[a] Recovery is defined as the ratio of the analyte concentration inside the probe to the analyte concentration in solution in the absence of the probe.

but proteins and membrane fragments released by the retina quickly degraded the CE separation. Basal levels of glutamate, GABA, taurine, and L-serine were detected in the perfusate (see Table 2). Minimizing the dilution in the low-volume perfusion chamber combined with the high sensitivity of the LIF detection cell made detection of low-concentration analytes possible. Surprisingly, D-serine was not detected in the perfusate. D-Serine is a putative neuromessenger that has previously been detected in

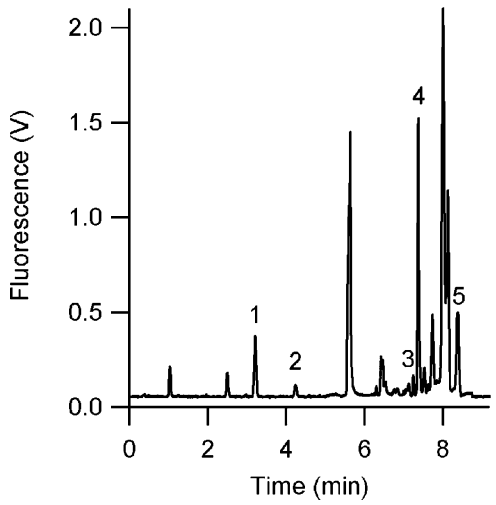

Figure 4. Electropherogram obtained from the perfusate of an isolated retina. Peak identifications: 2-aminoadipic acid (1), glutamate (2), GABA (3), taurine (4), and L-serine (5). Separations were performed on a 7.8-cm capillary with a separation voltage of −22.0 kV using 20 mM HP-γ-CD, 100 mM borate, pH 10.5 separation buffer. Sheath flow buffer was 50 mM borate, pH 10.5.

Table 2. Basal Concentrations of Various Neurotransmitters Detected in the Perfusate from Isolated Larval Salamander Retinas Housed in a 6.5-μL Perfusion Chamber

	concentration (μM ± SE, n = 4 retinas)[a]	limit of detection (μM; n = 3 days)
GABA	0.25 ± 0.12	0.11
taurine	5.5 ± 2.1	0.10
L-serine	2.8 ± 1.0	0.17
L-glutamate	0.48 ± 0.27	0.15

[a] Analyte concentrations have been adjusted to account for the relative recoveries listed in Table 1.

retinal homogenates using CE-LIF[7] and in intact retinas using immunocytochemistry.[3] Several strategies for improving the LOD, including improving separation conditions, increasing probe length, and decreasing dilution in the reaction cross were attempted but D-serine was still not detected. L-Aspartate and dopamine have also been measured in retinal homogenates [7] but were below the limit of detection in the perfusate.

Studying the retina in a perfusion chamber allows dynamic events to be measured. For example, pharmacological agents can be added to the perfusion buffer and delivered to the retina at variable concentrations for variable lengths of time. Drugs delivered to the surface of the retina readily enter the tissue as there are no occlusions in the extracellular tissue space that act as diffusion barriers.[2] Changes in efflux from the retina can be sampled every 10 s using the online microdialysis-CE-LIF instrument. The clearance profile of the chamber is an important consideration when performing these types of experiments since this will determine the time course that the retina will be exposed to the drug. A solution of 10 μM D-aspartate was perfused through the chamber for 4 min to study the clearance profile. The clearance profile of the chamber was examined with a retina in place since the retina occupies a significant volume of the chamber, affecting the clearance time. Since endogenous D-

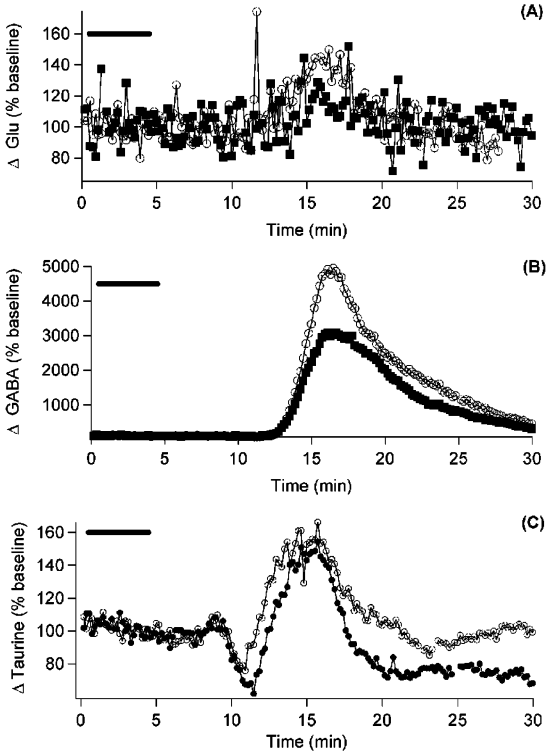

Figure 5. Perfusate levels of (A) glutamate, (B) GABA, and (C) taurine in response to potassium stimulations. Analyte peak heights have been ratioed to the internal standard peak height, and the change resulting from the stimulation is expressed as percent baseline vs time. (○) is the first stimulation, (■) is a second stimulation on the same retina. A solution of 60 mM K^+ Ringer's buffer was applied from 0.5 to 4.5 min (denoted by the solid line). The delay in response arises from the time for the dialysate to reach the CE interface. Separations were performed once every 11.1 s with 20 mM HP-γ-CD, 50 mM borate, pH 10.5 separation buffer.

aspartate has not been detected in retina perfusates or homogenates,[7] any D-aspartate detected in the perfusate can be considered as exogenous. Figure 3C shows the time course of D-aspartate through the chamber. The signal reaches detectable levels in the perfusate approximately 10.5 min after the start of the stimulation. This delay arises from the time it takes D-aspartate to travel from the valve to the chamber, fill the retina well volume, and then reach the detector. The concentration of D-aspartate in the chamber signal peaks at 1.2 μM, significantly below the 10 μM applied. A 4 min plug of D-aspartate was introduced into the chamber. At a flow rate of 50 μL/h though it takes 7.8 min to fill one full chamber volume. It takes an additional ~6 min for the concentration of D-aspartate to fall back below the limit of detection. Note that in the current experimental setup the temporal resolution of a pharmacological experiment will be limited by the chamber clearance time and not the microdialysis-CE-LIF analysis.

Potassium stimulations were performed to determine if the microdialysis-CE-LIF instrument could measure changes in analyte efflux. High extracellular potassium concentrations depolarize neurons, resulting in exocytotic release of neurotransmitters. Potassium stimulations resulted in increased efflux of the neurotransmitters GABA and glutamate, as well as other analytes including taurine and L-serine (see Figure 5). All analyte concentrations reached a peak value and then decreased back to baseline values as potassium washed out of the chamber. Two potassium stimulations were performed on each of four retinas, and the maximum increase was determined in terms of percent baseline (see Figure 6). Increases in GABA were largest (5200 ± 1700% first stimulation, 2400 ± 705% second stimulation, $n = 4$ retinas). Taurine (170 ± 10% first stimulation, 150 ± 9% second stimulation, $n = 4$ retinas), L-serine (200 ± 25% first stimulation, 170 ± 14% second stimulation, $n = 4$ retinas), and glutamate (170 ± 15% first stimulation, 160 ± 13% second stimulation, $n = 3$ retinas) exhibited smaller but still significant increases. The increases in glutamate, taurine, and L-serine release were not significantly different between the first and second stimulations. However, the second stimulation resulted in a significantly smaller increase in GABA concentration. This may be due to a depletion of GABA stores following the first stimulation or insufficient recovery time between stimulations.

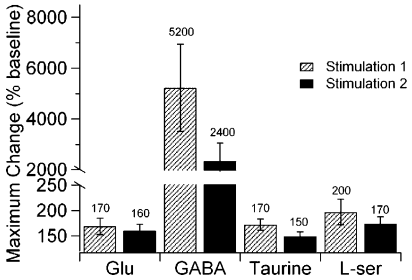

Figure 6. Average maximum responses during potassium stimulations. The maximum response of four analytes from four retinas was determined for two stimulations (60 mM K^+ Ringer's buffer, 4 min). Error bars are the standard error. An increase in glutamate concentration was detected in three of four retinas during the second stimulation.

CONCLUSIONS

Neurotransmitter efflux in the perfusate from isolated retinas housed in a 6.5-μL perfusion chamber has been measured using online microdialysis-CE-LIF. This method allows simultaneous, direct measurement of multiple neurotransmitters as well as other primary amine-containing analytes. The online nature of the analysis eliminates the need for sample collection, pretreatment, and manual derivatization. The small sample volume requirements and high-speed CE separations allow the perfusate to be sampled every 10 s. Basal levels of glutamate, GABA, taurine, and L-serine were detected. Potassium stimulations result in elevated levels of glutamate, GABA, taurine, and L-serine in the perfusate. This approach could also be applied to the study of other tissue types or other analytes by simply altering the separation conditions.

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