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GABA Uptake Inhibition Reduces In Vivo Extraction Fraction in the Ventral Tegmental Area of Long Evans Rats Measured by Quantitative Microdialysis Under Transient Conditions

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Abstract

Inhibitory signaling in the ventral tegmental area (VTA) is involved in the mechanism of action for many drugs of abuse. Although drugs of abuse have been shown to alter extracellular γ -aminobutyric acid (GABA) concentration in the VTA, knowledge on how uptake mechanisms are regulated in vivo is limited. Quantitative (no-net-flux) microdialysis is commonly used to examine the extracellular concentration and clearance of monoamine neurotransmitters, however it is unclear whether this method is sensitive to changes in clearance for amino acid neurotransmitters such as GABA. The purpose of this study was to determine whether changes in GABA uptake are reflected by in vivo extraction fraction within the VTA. Using quantitative (no-net-flux) microdialysis adapted for transient conditions, we examined the effects of local perfusion with the GABA uptake inhibitor, nipecotic acid, in the VTA of Long Evans rats. Basal extracellular GABA concentration and in vivo extraction fraction were 44.4 ± 1.9 nM (x-intercepts from 4 baseline regressions using a total of 24 rats) and 0.19 ± 0.01 (slopes from 4 baseline regressions using a total of 24 rats), respectively. Nipecotic acid (50 μ M) significantly increased extracellular GABA concentration to 170 ± 4 nM and reduced in vivo extraction fraction to 0.112 ± 0.003 . Extraction fraction returned to baseline following removal of nipecotic acid from the perfusate. Conventional microdialysis substantially underestimated the increase of extracellular GABA concentration due to nipecotic acid perfusion compared with that obtained from the quantitative analysis. Together, these results show that inhibiting GABA uptake mechanisms within the VTA alters in vivo extraction fraction measured using microdialysis and that in vivo extraction fraction may be an indirect measure of GABA clearance.

Keywords

GABA; Microdialysis; Nipecotic acid; No-net-flux; Uptake; Ventral tegmental area

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Compliance with Ethical Standards

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Conflict of interest The authors declare that they have no conflict of interest.

Introduction

The ventral tegmental area (VTA) is a brain region involved in reward processing, aversion and the development of some drug-dependent behaviors [1–3]. Inhibitory signaling in the VTA is altered by many drugs of abuse and γ -aminobutyric acid (GABA) neurons have an emerging role in regulating reward and aversive learning [4–6]. Despite the importance of inhibitory signaling in the VTA for drug-related behaviors, little is known about the regulation of extracellular GABA concentration in the VTA in vivo.

γ -Aminobutyric acid transporters (GATs) on neurons and astrocytes are critical in the regulation of GABA in the extracellular space [7, 8]. Within the central nervous system, GAT-1 is the primary neuronal isoform and GAT-3 is strongly expressed in astrocytes [9]. Inhibition of GATs reduces GABA uptake from the synaptic cleft and is a useful drug target for the treatment of epilepsy, pain and anxiety [10]. Tiagabine, a highly selective GAT-1 inhibitor, is currently approved as an adjunctive treatment for partial epilepsy. There is also clinical evidence that tiagabine may be effective in decreasing drug taking behavior, craving and withdrawal signs [11–13]. More recently, animal studies have shown that GATs can contribute to changes in GABAergic signaling during drug withdrawal [14], and midbrain dopamine neurons also express GATs to facilitate co-release of GABA [15]. Together, these results suggest the maintenance of drug-related behaviors could involve changes in the regulation of extracellular GABA concentration in certain brain regions.

Microdialysis is one technique used to study the regulation of GABA concentration in the extracellular space in vivo. Dialysate GABA concentrations are interpreted as an index of volume transmission from the interaction between neurons and astrocyte networks rather than a fast measure of synaptic release [16, 17]. However, the mechanisms of regulation of extracellular GABA in the VTA in vivo are not clear, in part due to low reported basal concentrations and specific chromatographic conditions required for accurate quantification [18, 19]. Despite these challenges, several studies have measured changes in dialysate GABA concentration within the VTA in response to acute or chronic treatment with drugs of abuse [20–26]. However, dialysate measurements may not accurately reflect changes in extracellular concentration due to the independent influence of in vivo extraction fraction, which can be altered by neurotransmitter clearance [27]. The high efficiency of GABA clearance by GATs maintains low extracellular concentrations and may interfere with accurate determination of effects on neuronal release following drug administration. Therefore, while previous work implicates changes in extracellular VTA GABA concentration in the mechanism of many drugs of abuse, it is critical to examine the influence of uptake when interpreting changes in dialysate GABA levels.

In contrast to conventional microdialysis, quantitative (no-net-flux) microdialysis is a technique that involves including different concentrations of the analyte sequentially within the perfusate in order to determine the point of “no-net-flux” across the probe membrane, indicating the actual extracellular concentration in the brain at steady state conditions [28, 29]. The in vivo extraction fraction, or relative recovery of the probe, is the slope of the regression line determined from the experiment and has been shown to be sensitive to changes in neurotransmitter clearance for monoamines and acetylcholine [30–34]. It is less

clear whether in vivo extraction fraction is altered by changes in clearance for amino acid neurotransmitters, which is regulated by both neurons and astrocytes. This is critical to examine as the contribution of transporters to overall clearance can change significantly between neurotransmitters and between brain regions. Indeed, the role of GAT-1 and GAT-3 in regulating extracellular GABA concentration has been shown to differ within the brain [7, 35]. In the VTA, reduced in vivo extraction fraction when measuring glutamate coincided with lower protein expression of excitatory amino acid transporter 1 (EAAT1) [36]. However, it is currently unknown whether changes in GABA uptake in the VTA are reflected by in vivo extraction fraction during microdialysis.

Removal of GABA from the extracellular space is primarily mediated through uptake by transporters, therefore we hypothesized that inhibiting GATs would significantly reduce the in vivo extraction fraction measured using microdialysis. We used a modification of quantitative microdialysis under transient conditions [37] to measure the direct effects of nipectic acid, a GAT inhibitor, on extracellular GABA concentration and in vivo extraction fraction over time in the VTA of Long Evans rats.

Methods

Animals

Adult, male Long Evans rats (n = 32; Harlan, Indianapolis, IN) were maintained in a humidity and temperature controlled room with a 12-hour light/dark cycle (lights on at 0700). Animals were pair housed and handled daily for at least 1 week to acclimate to the facility prior to surgery. Food and water were available ad libitum. All animal procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (8th Ed., 2011) and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

Materials

Nipectic acid, GABA, α -phthalaldehyde (OPA), sodium dihydrogen phosphate dihydrate, sodium sulfite and sodium tetraborate decahydrate were purchased from Sigma (Sigma-Aldrich, Saint Louis, MO). Methanol from Fisher Scientific (Fisher Scientific, Fair Lawn, NJ) and absolute ethanol from AAPER (AAPER Alcohol and Chemical Co., Shelbyville, KY) were used. All solutions were made with deionized water from a Milli-Q system (Millipore, Billerica, MA) and filtered using 0.2 μ m nylon filters (Pall Corp., Ann Arbor, MI). Gentamicin (APP Pharmaceuticals, Schaumburg, IL) and carprofen (Pfizer, New York, NY) were used in surgery. Nipectic acid and GABA were dissolved in artificial cerebrospinal fluid (ACSF) for microdialysis experiments.

Microdialysis Surgery

Rats were anesthetized with isoflurane (5% induction, 2.5% maintenance) and guide cannulae (21 gauge, Plastics One, Roanoke, VA) were stereotactically implanted above the posterior VTA (mm relative to bregma and skull surface: anteroposterior – 5.8, mediolateral + 2.1, ventral – 4.6 at 10° angle toward midline) according to atlas of Paxinos and Watson [38]. Stereotaxic coordinates were determined for the posterior VTA based on previous

anatomical and behavioral experiments [39, 40]. A catheter was also inserted into the jugular vein in rats in Experiment 1 (n = 8) as previously described [41] to accommodate additional pilot experiments performed following the completion of experiments described here. Probes extended 4.0 mm below the end of cannula when implanted for experiments. An obturator was placed in the guide cannula until probe implantation. Carprofen (5 mg/kg, subcutaneous) was given during surgery to minimize post-operative pain and discomfort. After surgery, rats were housed individually and underwent a recovery period of at least 5 days prior to microdialysis experiments.

Microdialysis Procedures

Microdialysis probes (1 mm active area, 13 kDa molecular weight cutoff cellulose membrane) were constructed similar to Pettit and Justice [42], and experimental conditions were described previously [43, 44]. Rats (328–495 g on experiment day) were lightly anesthetized and probes inserted into cannulae 12–18 h prior to experiments. Probes were perfused with ACSF (149 mM NaCl, 2.8 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 5.4 mM D-glucose) at 0.2 µl/min overnight, and the flow rate was increased to 1.0 µl/min at least 2 h prior to the start of microdialysis experiments. Following this equilibration period, samples were collected every 20 min, and all experiments occurred between 0700 and 1700 h, during the light phase of the cycle.

Experiment 1—We first conducted an experiment to determine the concentration of the GABA uptake inhibitor nipecotic acid to perfuse through the probe for the quantitative microdialysis experiment. Following a baseline period, the syringe containing probe perfusate (ACSF) was manually changed to one containing nipecotic acid in ACSF (30, 100, 300 µM) in sequential order. Each nipecotic acid concentration was perfused for 60 min. After the final nipecotic acid concentration, the perfusate was changed back to ACSF for three additional samples. In control animals, the syringe was changed to a different syringe containing only ACSF with no inhibitor at each time point. The range of concentrations tested for nipecotic acid was chosen based on previous reports [19, 26].

Experiment 2—A modification of the quantitative microdialysis (no-net-flux) adapted for transient conditions was used to determine the effects of local uptake inhibition on extracellular GABA concentration and in vivo extraction fraction [29, 37]. Probes were perfused with ACSF overnight as described above. The morning of the experiment, the perfusate was changed to ACSF including one of four GABA concentrations (0, 15, 45, 90 nM) and the flow rate increased to 1.0 µl/min for at least two hours. These GABA concentrations were chosen based on preliminary no-net-flux experiments (data not shown). After four baseline samples, the perfusate was manually changed to ACSF containing the same GABA concentration and nipecotic acid (50 µM) for five samples, then returned to the baseline ACSF solution for four samples. Dialysis samples were immediately frozen on dry ice and stored at – 80 °C until analysis for both experiments.

GABA Analysis

All samples underwent precolumn derivatization prior to analysis. The derivatization solution was based on previous reported methods [45, 46]. The working solution was made

by dissolving 11 mg OPA in 250 μ l absolute ethanol, 250 μ l sodium sulfite (1 M) and 4.5 ml sodium tetraborate decahydrate (0.1 M, adjusted to pH 10.4 with 5 M NaOH; borate). The working solution was stored in a covered vial at 4 °C. The derivatization procedure consisted of combining 9 μ l standard or sample with 2 μ l borate then adding 0.5 μ l OPA working solution, which was manually mixed and incubated in darkness at room temperature for 10 min. Stock GABA standards (1 μ M) were prepared in deionized water and stored at 4 °C for up to 1 month. Serial dilutions were prepared fresh daily.

The GABA concentration in samples was determined using high performance liquid chromatography (HPLC) with electrochemical detection. For the quantitative microdialysis experiment, GABA concentration in the ACSF perfusate for each rat was determined using external standards. Samples were run using a 9725i manual injector (Rheodyne, Cotati, CA), Luna C18(2) column (150 $\times$ 2.0 mm, 3 μ m; Phenomenex, Torrance, CA), Decade controller (column temperature 40–45 °C; Antec Leyden, Zoeterwoude, Netherlands) and 2 mm glassy carbon working electrode (SenCell or VT-03 with salt bridge Ag/AgCl reference; Antec) at + 700 mV potential. Aqueous mobile phase consisted of 0.1 M sodium dihydrogen phosphate dihydrate and 0.5 mM EDTA adjusted to pH 4.50 with 1 M phosphoric acid. Methanol was added (27% v/v) to the mobile phase, and it was sparged with helium prior to being pumped at a flow rate of 0.14–0.25 ml/min through the system. Following precolumn derivatization, 5–10 μ l of the reaction mixture was manually injected into the HPLC. Chromeleon 6.8 Chromatography Data System Software (Thermo Fisher Scientific, Waltham, MA) was used for chromatogram acquisition and peak integration. Chromatographic peaks were required to have a signal to background noise ratio of at least 2:1 in Experiment 1 and 3:1 in Experiment 2 to be included in the analyses. The limit of detection using our parameters was approximately 2.5 nM calculated using noise of the baseline from OPA blank injections.

Histological Analysis

After microdialysis experiments, animals were administered a lethal dose of sodium pentobarbital (150 mg/kg, intraperitoneal) under isoflurane anesthesia. Brains were extracted and stored in 10% formalin. Coronal sections (120 μ m) were stained with cresyl violet for probe location verification [38]. The probe active area was required to be located in the VTA and adjacent midbrain for animals to be included in the study.

Statistical Analysis

Experiment 1—Microdialysis data were expressed as percent of baseline from the average of the first three samples and analyzed using repeated measures ANOVA with perfusate treatment as a between-subjects factor and time as a repeated measure. Bonferroni corrections were used for post hoc analyses.

Experiment 2—Quantitative microdialysis data were analyzed according to procedures described in Yim and Gonzales [47]. There were four subgroups (each subgroup, $n = 5$ –7) based on the concentration of GABA (0, 15, 45, 90 nM) in the perfusate during the experiment. Data from subgroups were used to create a linear regression for each time point by plotting $[GABA]_{in} - [GABA]_{out}$, the difference between the concentration perfused

through the probe ($[GABA]_{in}$) and the concentration in the sample ($[GABA]_{out}$) compared with $[GABA]_{in}$ [37]. Linear regression was performed for each time point to calculate the extracellular GABA concentration (x-intercept) and in vivo extraction fraction (slope). The asymptotic standard errors for regression parameters were determined from the linear regression. The method of extra sum of squares was used to test if there were differences between regression lines [48]. If significant differences between regression lines were observed, additional F tests were carried out to determine whether changes in the x-intercept, slope or both parameters contributed to the difference.

Values are reported as mean $\pm$ sem and results with $p < 0.05$ were assigned significance. Data analysis was performed using GraphPad Prism (version 6; La Jolla, CA) or SPSS software (version 22; IBM, Armonk, NY).

Results

Histological Analysis

The probe locations were verified as shown in Fig. 1, and ranged from approximately 5.28–6.36 mm posterior from bregma according to Paxinos and Watson [38]. In the quantitative microdialysis experiment (Fig. 1b), 87% of probe placements were located in the posterior VTA (defined as – 5.6 to – 6.3 mm from bregma) [40].

Experiment 1: Dose Dependent Effects of Local Uptake Inhibition on GABA Release Using Conventional Microdialysis—In order to determine the concentration of nipecotic acid to use for the quantitative microdialysis experiment, we first tested the effects of three different concentrations (30, 100, 300 μ M) of nipecotic acid, a GABA uptake inhibitor, on VTA GABA dialysate concentration using local application through the microdialysis probe. A control group of animals received only ACSF throughout the experiment but manual syringe changes occurred at same times as the group that received increasing concentrations of nipecotic acid in the perfusate. Dialysate GABA concentration during the baseline period was 4.1 ± 0.4 nM in the nipecotic acid group and 4.5 ± 0.2 nM in the control group. The overall ANOVA revealed a significant interaction between treatment group and time ($F_{14,84} = 40.7$, $p < 0.05$). There was a significant difference over time in VTA GABA concentration as a percent of baseline in the group that received local nipecotic acid application (Fig. 2; $F_{14,42} = 47.2$, $p < 0.05$; $n = 4$). Post hoc analyses revealed GABA concentration significantly increased due to nipecotic acid treatment between 140 and 240 min compared to controls (all $p < 0.05$). Additionally, the GABA concentration in the first ACSF sample following the final nipecotic acid concentration (300 μ M) was significantly elevated compared to controls (260 min, $p < 0.05$). In contrast, GABA concentration did not significantly change over time in control animals as a result of manual ACSF syringe changes ($F_{14,42} = 0.69$, $p = 0.77$; $n = 4$).

Experiment 2: Effect of Local Uptake Inhibition on Extracellular GABA and In Vivo Extraction Fraction Using Quantitative Microdialysis Under Transient Conditions—The results of Experiment 1 indicated a concentration higher than 30 μ M nipecotic acid was necessary to significantly increase VTA GABA over baseline. Therefore, we tested the effect of 50 μ M nipecotic acid delivered through the probe on extracellular

GABA and in vivo extraction fraction in the VTA using quantitative microdialysis under transient conditions [37]. A separate group of animals was used for each GABA concentration in the ACSF perfusate (GABA (nM): 0, n = 6; 15, n = 5; 45, n = 7; 90, n = 6).

The extracellular concentration of GABA and in vivo extraction fraction were calculated using linear regression, as illustrated by selected time points in Fig. 3. A positive value on the y-axis indicates net diffusion of GABA into the brain and a negative number indicates net GABA diffusion from the brain into the perfusate [30]. The time course of extracellular GABA concentrations and in vivo extraction fraction are presented in Fig. 4. Analysis of the regression parameters between the four baseline time points revealed no significant differences (Fig. 3A; $F_{6,8} = 0.88$, $p = 0.55$). The average extracellular GABA concentration in the VTA and in vivo extraction fraction during the baseline were 44.4 ± 1.9 nM (mean $\pm$ sem from x-intercepts from 4 baseline regressions using a total of 24 rats) and 0.19 ± 0.01 (mean $\pm$ sem from slopes from 4 baseline regressions using a total of 24 rats), respectively.

During the nipecotic acid perfusion, there was a significant difference observed in the regression lines from 100 to 180 min compared to baseline regressions (all $p < 0.05$). Local nipecotic acid application significantly increased the extracellular GABA concentration (x-intercept) over baseline samples (Fig. 4; all $p < 0.05$). In addition to the significant increase in extracellular GABA, nipecotic acid also significantly reduced the in vivo extraction fraction (slope) in most samples during the perfusion (Fig. 4; 100, 140–180 min, $p < 0.05$). Both effects were maximal following 100 min of nipecotic acid perfusion. At this time point (180 min), extracellular GABA concentration increased to 170 ± 4 nM and in vivo extraction fraction had fallen by approximately 40% (0.112 ± 0.003).

After 100 min of nipecotic acid perfusion, the syringes were changed back to the original ACSF solution. There was a significant difference between the baseline regressions and the regression at the first time point following the nipecotic acid perfusion (200 min, $F_{8,10} = 9.73$, $p < 0.05$). At this time point (200 min), the extracellular GABA concentration (x-intercept) was still significantly elevated ($F_{4,10} = 19.1$; $p < 0.05$) but the in vivo recovery (slope) was not significantly different from baseline ($F_{4,10} = 0.72$; $p = 0.60$). There were no significant differences between the four baseline regressions and the last 60 min of the experiment (Fig. 4; 220 min, $F_{8,20} = 0.91$, $p = 0.55$; 240 min, $F_{8,20} = 0.84$, $p = 0.59$; 260 min, $F_{8,20} = 0.59$; $p = 0.77$). The average extracellular GABA concentration (44.4 ± 1.9 nM) and in vivo extraction fraction (0.19 ± 0.01) did not differ between baseline periods before and after the nipecotic acid perfusion [x-intercept: $t(5) = 1.21$, $p = 0.28$; slope: $t(5) = 0.39$, $p = 0.71$].

Figure 5 compares the time course of nipecotic acid perfusion on $[GABA]_{out}$ from the group that received 0 nM GABA in the perfusate to the extracellular GABA concentration determined from quantitative microdialysis as percent of baseline from the average of the four baseline time points. After 100 min of nipecotic acid perfusion, $[GABA]_{out}$ increased to 230% of baseline while the extracellular concentration increased to almost 400% of baseline. Therefore, our results indicate conventional microdialysis underestimated the percent increase in extracellular GABA concentration measured following uptake inhibition due to the decrease in extraction fraction.

Discussion

The present results provide the first neurochemical evidence that inhibiting GATs significantly reduces in vivo extraction fraction for GABA using quantitative microdialysis. Perfusion of nipecotic acid through the microdialysis probe significantly increased extracellular GABA concentration in the VTA while reducing in vivo extraction fraction. As a result of reduced in vivo extraction fraction, conventional microdialysis underestimated the percent increase in extracellular GABA concentration observed following GAT inhibition. Consistent with measurements for monoamines, these results suggest in vivo extraction fraction reflects changes in extracellular GABA clearance, and such changes in GABA clearance may influence the interpretation of the effects of drugs on dialysate GABA concentration within the VTA.

The principal finding of this study is that in vivo extraction fraction is sensitive to changes in GABA uptake in the VTA using quantitative microdialysis. Quantitative microdialysis has been applied extensively to determine actual extracellular concentrations of neurotransmitters during acute or following chronic drug administration. It is well established that in vivo extraction fraction is altered by changes in extracellular clearance for monoamines and acetylcholine [27, 30–33]. Regarding the relationship between uptake of amino acids and in vivo extraction fraction, previous work has shown reducing glutamate uptake by local application of an inhibitor through the microdialysis probe significantly reduced in vivo extraction fraction in the hippocampus [49], nucleus accumbens [50], and striatum [51]. However, there are mixed results on whether in vivo extraction fraction in quantitative microdialysis studies corresponds to changes in protein expression of glutamate transporters. Based on the relationship that has been established between in vivo extraction fraction and transporter function with monoamines, it would be expected a decrease in glutamate transporter expression should be reflected by reduced in vivo extraction fraction. When examining the excitatory amino acid transporter 2 (GLT-1), responsible for over 90% of glutamate uptake in the brain [52], some studies show changes in GLT-1 expression and in vivo extraction fraction in the same direction [50, 51]. However, other studies have reported reduced in vivo extraction fraction with no corresponding changes in GLT-1 expression [36, 53, 54]. Furthermore, Shen et al. [55] reported in vivo extraction fraction was significantly increased in the nucleus accumbens of rats with a history heroin self-administration, however this was opposite to their findings that both in vitro GLT-1 expression and in vivo glutamate uptake were significantly reduced. Thus, the evidence for a mechanistic link between in vivo extraction fraction and extracellular clearance of amino acids is not conclusive.

Consistent with previous reports, our results show nipecotic acid perfusion caused a dose-dependent increase in dialysate GABA concentration [19, 26, 56, 57]. It is possible a portion of the extracellular GABA measured is due to mechanisms other than inhibiting uptake as nipecotic acid has previously been reported to release GABA stores through heteroexchange at higher concentrations [58–60]. However, only a fraction of the concentration of nipecotic acid used in our study (50 μ M in the perfusate) crosses the probe membrane. Therefore, effects from heteroexchange are likely minimal. Additional quantitative experiments are

needed to more accurately estimate the concentration of nipecotic acid that crosses the probe membrane.

Nipecotic acid potently inhibits both GAT-1 and GAT-3 on neurons and astrocytes [61, 62]. Both GAT-1 and GAT-3 isoforms are expressed in the VTA, although little is known about the distribution and regulation of extracellular GABA in this region [9, 63]. The contribution of GAT-1 and GAT-3 to maintain extracellular GABA concentration differs between brain regions [7, 35], therefore additional studies using selective inhibitors are needed to determine the role of individual GAT isoforms in maintaining extracellular GABA levels in the VTA.

A limitation of our study is the nipecotic acid perfusion increased extracellular GABA concentrations that exceeded the highest concentration (90 nM) used in the quantitative microdialysis experiment. Therefore, the extracellular GABA concentration and extraction fraction at some time points may not reflect the most accurate measurement as the values were outside the concentration bracket and extrapolation had to be used to determine the point of no-net-flux. We cannot rule out the possibility that points higher than 90 nM may not continue to show linearity. However, this limitation does not significantly alter our conclusion that the slope significantly changed along with the point of no-net-flux during the nipecotic acid perfusion.

Our findings are the first report using quantitative microdialysis to measure *in vivo* extracellular GABA over time. To our knowledge, only one other report of quantitative microdialysis measuring GABA has been published [64]. In this study, the authors measured extracellular GABA within the nucleus accumbens at steady state in rats with a history of cocaine or saline treatments. However, the reported extraction fractions (slopes) were greater than 1, indicating a potential error or significant effects of ultrafiltration may have occurred.

Our results are also the first to report the extracellular GABA concentration in the VTA as measured by quantitative microdialysis. Currently, the range of reported basal dialysate GABA concentrations in the VTA measured using conventional microdialysis varies significantly. For example, a recent meta-analysis of microdialysis publications found basal dialysate GABA concentration reported in the VTA varied from 8 to 43 nM in rats [18]. Using quantitative microdialysis, the extracellular VTA GABA concentration in our study was approximately 45 nM. The majority of the probes in the quantitative microdialysis experiment were located in the posterior VTA. The posterior VTA was targeted due to its proposed role in the rewarding effects of many drugs of abuse [40]. However, due to the heterogeneity in GABAergic inputs to and local interneurons within the VTA [3], it is possible the extracellular GABA concentration may differ between subregions within the VTA. For example, a recent study used low-flow push-pull perfusion to measure the extracellular concentration of multiple neurotransmitters with high spatial resolution [65]. These authors determined the GABA concentration in the VTA to be 160 ± 50 nM, and noted significant differences between their results and basal dialysate concentrations previously reported for amino acid neurotransmitters in other brain regions. It is possible that probe placement or greater tissue damage from the microdialysis probe could contribute to the difference in extracellular concentration compared to our current results.

The significance of dialysate GABA measurements is often debated due to the contribution of non-neuronal sources to the extracellular concentration. Basal extracellular GABA concentrations are derived from both neuronal and non-neuronal sources that do not meet some classical criteria for exocytotic release [16, 66, 67]. In addition to the current results using nipecotic acid perfusion, our preliminary experiments demonstrated a potassium-evoked increase and a partial reduction following baclofen perfusion on dialysate GABA concentration in the VTA (data shown in Supplementary Material). We did not test the sensitivity of extracellular GABA to calcium omission or tetrodotoxin in the current experiment, therefore we are unable to determine the portion of extracellular concentration derived from neuronal sources. However, a previous study found dialysate GABA concentration stimulated by nipecotic acid perfusion in the dorsal striatum was reduced by approximately 50% following co-administration with tetrodotoxin [26].

The activity of GATs to regulate synaptic transmission and prevent neuronal spillover is thought to be critical to maintain extracellular GABA concentration. Inhibition of GABA uptake prior to drug administration or experimental manipulation may provide a better index for changes in neuronal GABA release by measuring synaptic spillover. Based on the current results, the direct effects of GAT inhibitors on in vivo extraction fraction would underestimate changes in dialysate concentration that may be observed following experimental manipulations. Additionally, there may also be effects on GABA clearance mechanisms from drug administration itself within experiments that could influence the in vivo extraction fraction and therefore interpretation of microdialysis results.

In summary, the present results are the first to demonstrate inhibiting GABA uptake significantly reduces in vivo extraction fraction as measured by quantitative microdialysis in the VTA. Our results indicate that in vivo extraction fraction may be a critical factor when interpreting microdialysis data examining the mechanism of action of drugs on extracellular GABA concentration. Future studies also measuring GAT function are needed to directly determine whether in vivo extraction fraction can be used to estimate changes in GABA clearance. Using quantitative microdialysis, examining in vivo extraction fraction over time may be a valuable tool to investigate how GABA uptake mechanisms are regulated and better define the role of extracellular GABA in the VTA in mediating the effects of drugs that alter inhibitory neurotransmission such as drugs of abuse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Microdialysis probe placements within the ventral tegmental area. Coronal sections showing representative probe placements for Experiment 1 (a) and Experiment 2 (b). Lines represent 1 mm active dialysis area. Numbers to the right indicate distance relative to bregma (mm). Histology figure adapted from Paxinos and Watson [38]

Fig. 2.

Effect of local uptake inhibition by nipecotic acid in microdialysis perfusate on GABA concentration in the VTA (Experiment 1). Local application of 100 and 300 μ M nipecotic acid significantly increased GABA concentration compared to control rats (asterisk indicates $p < 0.05$ compared to control). Each concentration of nipecotic acid (30, 100, 300 μ M) was administered in sequential order for 60 min ($n = 4$). In control rats, manual syringe changes occurred at the same times as the nipecotic acid group but syringes only contained artificial cerebrospinal fluid (ACSF; $n = 4$). Data are presented as mean $\pm$ sem, sem smaller than symbol size for some points

Fig. 3.

Regression lines showing extracellular concentration and extraction fraction for samples collected during baseline (**a**), during nipecotic acid perfusion (**b**, **c**) and return to baseline (**d**) (Experiment 2). The effect of four GABA concentrations (0, 15, 45, 90 nM) in the perfusate ($[GABA]_{in}$) was tested in four separate groups. The difference between $[GABA]_{in}$ and concentration in sample ($[GABA]_{out}$) was plotted against $[GABA]_{in}$ for the four groups at each time interval. Each panel illustrates the data and regression lines for four basal samples (open circles, dashed lines) with one regression obtained during or after local nipecotic acid (50 μ M) perfusion ($n = 24$ for combined data). **a** Comparison of the four regressions during baseline period. **b** The regression line for the first sample following 20 min perfusion with nipecotic acid compared to baseline. **c** The regression line for the last sample after 100 min perfusion with nipecotic acid compared to baseline. **d** The regression line for the sample 80 min following removal of nipecotic acid in perfusate compared to baseline. The r^2 values for the basal regressions are 0.99. The r^2 values for the time points during and after the nipecotic acid perfusion are 0.91–1.00. Data are presented as mean $\pm$ sem for each GABA concentration

Fig. 4.

Time course of extracellular GABA (**a**; filled circles) and in vivo extraction fraction (**b**; open squares) during local nipecotic acid perfusion (shaded area) in the ventral tegmental area (Experiment 2). Following four baseline samples, the artificial cerebrospinal fluid (ACSF) perfusate was changed to include nipecotic acid (50 μ M) for 100 min and then back to ACSF for the last four samples. The slopes and x-intercepts of the linear regression lines over the course of the experiment are presented. Error bars represent the asymptotic standard errors at each time point obtained from regression statistics. **a** Nipecotic acid perfusion significantly increased the extracellular concentration of GABA compared to baseline samples. **b** Additionally, nipecotic acid significantly reduced in vivo extraction fraction compared to baseline samples. The r^2 values for the individual regressions were 0.91–1.00, asterisk indicates $p < 0.05$ compared to baseline

Fig. 5.

Time course of the percent baseline change of $[GABA]_{out}$ for the group receiving 0 nM GABA (open circles; $n = 6$) and extracellular GABA calculated from quantitative microdialysis (filled circles; $n = 24$) during local nipecotic acid perfusion (50 μ M; shaded area) in the ventral tegmental area (Experiment 2). The percent baseline of x-intercept from the linear regression lines over the course of the experiment are presented for extracellular GABA ($[GABA]_{ext}$). The percent baseline of group receiving 0 nM GABA (0 nM)_{in} in the perfusate are presented as mean $\pm$ sem