Tranexamic Acid Impairs γ-Aminobutyric Acid Receptor Type A–mediated Synaptic Transmission in the Murine Amygdala

A Potential Mechanism for Drug-induced Seizures?

Stephan Kratzer, M.D., Hedwig Irl, M.S., Corinna Mattusch, Ph.D., Martina Bürge, M.D., Jörg Kurz, M.D., Eberhard Kochs, M.D., Matthias Eder, Ph.D., Gerhard Rammes, Ph.D., Rainer Haseneder, M.D.

ABSTRACT

Background: Tranexamic acid (TXA) is commonly used to reduce blood loss in cardiac surgery and in trauma patients. High-dose application of TXA is associated with an increased risk of postoperative seizures. The neuronal mechanisms underlying this proconvulsant action of TXA are not fully understood. In this study, the authors investigated the effects of TXA on neuronal excitability and synaptic transmission in the basolateral amygdala.

Methods: Patch clamp recordings and voltage-sensitive dye imaging were performed in acute murine brain slices. Currents through *N*-methyl-D-aspartate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and γ-aminobutyric acid receptor type A ($GABA_A$) receptors were recorded. $GABA_A$ receptor–mediated currents were evoked upon electrical stimulation or upon photolysis of caged GABA. TXA was applied at different concentrations.

Results: Voltage-sensitive dye imaging demonstrates that TXA (1 mM) reversibly enhances propagation of neuronal excitation (mean ± SEM, 129 ± 6% of control; n = 5). TXA at concentrations of 0.1, 0.3, 1, 5, or 10 mM led to a dose-dependent reduction of $GABA_A$ receptor–mediated currents in patch clamp recordings. There was no difference in the half-maximal inhibitory concentration for electrically (0.76 mM) and photolytically (0.84 mM) evoked currents (n = 5 to 9 for each concentration), and TXA did not affect the paired-pulse ratio of $GABA_A$ receptor–mediated currents. TXA did not impact glutamatergic synaptic transmission.

Conclusions: This study clearly demonstrates that TXA enhances neuronal excitation by antagonizing inhibitory GABAergic neurotransmission. The results provide evidence that this effect is mediated *via* postsynaptic mechanisms. Because $GABA_A$ receptor antagonists are known to promote epileptiform activity, this effect might explain the proconvulsant action of TXA.
(ANESTHESIOLOGY 2014; 120:639-49)

TRANEXAMIC acid (TXA) is a synthetic lysine analogue that acts as an antifibrinolytic agent by binding to plasminogen and blocking the interaction of plasmin with fibrin, thereby preventing dissolution of the fibrin clot.[1] TXA is widely used, particularly in cardiac surgery to reduce blood loss and has been proven to reduce mortality in bleeding trauma patients.[2] Recent studies indicate that the use of TXA is associated with an increase in adverse events, particularly the occurrence of seizures.[3] Postoperative seizures after cardiac surgery lead to an increased rate of postoperative neurological complications, increased intensive care unit length of stay, and increased intensive care unit mortality.[4]

Epileptic seizures arise from aberrant electrical activity of a large population of neurons and occur as an episode of neurologic dysfunction.[5] Clinical symptoms include changes in motor control, sensory perception, and behavior and/or autonomic function. In the adult brain, γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter playing a pivotal role in the generation of electrical activity patterns and the prevention of seizure-like activity.[6] Application of antagonists

What We Already Know about This Topic

- Tranexamic acid is used clinically as an antifibrinolytic to reduce microvascular bleeding, but has been associated with seizures
- Proconvulsants are known to alter the balance between excitatory and inhibitory neuronal activity

What This Article Tells Us That Is New

- Tranexamic acid enhanced neuronal excitation at concentrations relevant to its clinical use by impairing neuronal inhibition in the mouse amygdala
- This was due to postsynaptic blockade of receptors for the inhibitory neurotransmitter γ-aminobutyric acid, with no effect on excitatory glutamate receptors

Submitted for publication January 26, 2013. Accepted for publication November 7, 2013. From the Department of Anesthesiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany (S.K., H.I., C.M., M.B., E.K., G.R., and R.H.); Department of Anesthesiology, Deutsches Herzzentrum München, Klinik an der Technischen Universität München, Munich, Germany (J.K.); and RG Neuronal Network Dynamics, Max Planck Institut of Psychiatry, Munich, Germany (M.E.).

 Anesthesiology 2014; 120:639-49

against the GABA receptor type A ($GABA_A$-receptor) leads to epileptiform activity *in vitro*[7] and seizures *in vivo*.[8]

The amygdala, a temporal lobe structure which is a part of the limbic system, is well known for its central role in emotions and emotional behavior.[9] The neuronal excitability of the basolateral nucleus of the amygdala (BLA) plays a substantial role in the pathophysiology of temporal lobe epilepsy.[10] The amygdala seems to be highly susceptible to generating epileptiform activity, as kindling, the development of spontaneous recurrent epileptiform activity after repeated electrical stimulation, occurs very quickly after stimulation of the amygdala.[10] Moreover, seizures induced by the local anesthetic lidocaine invariably originate in the amygdala.[11]

We hypothesized that TXA enhances neuronal signal propagation, which might underlie its proconvulsant side effects. To clarify the involved neuronal mechanisms, we investigated the effects of TXA on inhibitory and excitatory synaptic transmission and intrinsic neuronal excitability in the BLA.

Materials and Methods

The experimental protocols were approved by the Ethical Committee on Animal Care and Use of the Government of Bavaria, Munich, Germany. Male C57Bl6 mice (25 to 42 days) were killed by cervical dislocation and the brains were rapidly removed into ice-cold artificial cerebrospinal fluid (aCSF), containing 125 mM of NaCl, 2.5 mM of KCl, 25 mM of $NaHCO_3$, 2 mM of $CaCl_2$, 1 mM of $MgCl_2$, 25 mM of D-glucose, and 1.25 mM of NaH_2PO_4. Saturation with a mixture of 95% O_2–5% CO_2 (carbogen gas) led to a pH of 7.4. Coronal slices (350 μm thick) were prepared using a microtome (HM 650 V; Microm International, Walldorf, Germany).

For voltage-sensitive dye imaging (VSDI) experiments, slices were allowed to recover (34°C) for 45 min in carbogen-saturated, sucrose-based aCSF, containing 87 mM of NaCl, 2.5 mM of KCl, 25 mM of $NaHCO_3$, 1.25 mM of NaH_2PO_4, 0.5 mM of $CaCl_2$, 7 mM of $MgCl_2$, 25 mM of glucose, and 75 mM of sucrose. For staining, slices were stored for 15 min in carbogenated standard aCSF containing Pyridinium, 4-(2-(6-(dibutylamino)-2-naphthalenyl)et henyl)-1-(3-sulfopropyl)-,hydroxide, inner salt (Di-4-ANEPPS; 7.5 mg/ml; <0.1% dimethyl sulfoxide).[12] Afterwards, the slices were stored for at least 30 min in standard aCSF. In the recording chamber, slices were continuously superfused with carbogenated standard aCSF (at a flow rate of 2 to 3 ml/min). Electrical stimulation (10 to 30 V; 0.1 ms) was applied to the lateral amygdala *via* a bipolar concentric electrode, and circular regions of interest (3 × 3 pixels) were set according to anatomical landmarks in the BLA. As VSDI measure of neuronal activity, we used region of interest–extracted fast, depolarization–mediated imaging signals (FDSs).[12] VSDI and data analysis were performed using the MiCAM02 hardware and software package (BrainVision, Tokyo, Japan). The Olympus BX51WI fluorescence microscope (Olympus, Hamburg, Germany) was equipped with a MiCAM02-HR camera, an XLFluor4X/340 objective (NA 0.28; Olympus), a 530-nm excitation filter, and a 600-nm emission filter. Acquisition settings were 88 × 60-pixel frame size, 36.4 × 40.0-μm pixel size, and 2.2-ms sampling time. To improve the signal-to-noise ratio, eight acquisitions recorded at subsequent intervals of 15 s were averaged. Neuronal activity was evoked by square-pulse electrical stimuli (200 μs, 10 to 25 V) applied to the lateral nucleus of the amygdala *via* a bipolar tungsten electrode. From the recorded signals, the fractional change in fluorescence (ΔF/F) was calculated. For all measurements, the ΔF/F values were spatially smoothed using a 3 × 3-pixel average filter. In addition, a temporal filter was applied by calculating the fluorescence (F) of a pixel at the frame number (t) using the equation $F(t) = (F(t - 1) + F(t) + F(t + 1))/3$. VSDI signals presented in images were smoothed with a 5 × 5 (spatial) × 3 (temporal) average filter. Image pixelation was reduced using the interpolation function of the MiCAM02 software.

For patch clamp experiments, slices were allowed to recover at 34°C for 45 min in standard aCSF before they were transferred to the recording chamber. A platinum ring with nylon filaments was used to fix the slices on the bottom of the recording chamber, which was continuously perfused (5 ml/min) with aCSF. Infrared videomicroscopy[13] (Zeiss, Oberkochen, Germany) was used to visualize BLA pyramidal neurons, from which excitatory postsynaptic currents (EPSCs) were recorded using the standard whole-cell patch clamp technique. Pipettes had an open-tip resistance of 4 to 6 MΩ when filled with a solution containing 130 mM of K-D-gluconate, 5 mM of KCl, 0.5 mM of EGTA, 2 mM of $MgCl_2$, 10 mM of HEPES, 5 mM of D-glucose, and 20 mM of Na_2-phosphocreatine. In some experiments, lidocaine-*N*-ethyl-chloride (5 mM) was added to the intracellular solution to prevent neurons from generating action potentials (APs). Whole-cell discontinuous voltage clamp recordings were performed with a discontinuous voltage clamp/current clamp amplifier (SEC 10L; NPI Electronic, Tamm, Germany) with switching frequencies of 60 to 80 kHz (25% duty cycle). Once electrodes were in aCSF, we balanced capacitance compensation, series resistance, and adjusted zero direct current. To verify the correct setting of the switched mode, the amplifier was set to the continuous bridge mode. After balancing the bridge circuit, no differences between measurements made in bridge and switched current clamp modes could be observed. After obtaining a stable whole-cell configuration, the capacitance compensation was always readjusted. Under these conditions, switching to discontinuous voltage clamp mode was possible without artifacts. The discontinuous voltage clamp parameters were set using a pulse protocol as described.[14] Correct compensation of input capacitance and complete settling of the current injection artifact before sampling of the voltage signal in switched mode were controlled by continuously monitoring unsampled electrode potentials on an analogue oscilloscope triggered by the switching frequency.[15]

Excitatory postsynaptic currents and inhibitory postsynaptic currents (IPSCs) were elicited by square-pulse stimuli (6 to 50 V, 50 to 200 μs; interstimulus interval 15 s) delivered *via* a bipolar tungsten electrode that was placed on the external capsule. Before each tissue stimulation pulse, neuronal input resistance was determined with a hyperpolarizing voltage step (−10 mV for 200 ms). $GABA_A$ receptor–mediated currents were recorded (holding potential: −70 mV) using an intracellular solution with a high chloride concentration (100 mM of cesium methane sulfonate, 60 mM of CsCl, 10 mM of HEPES, 1 mM of $MgCl_2$, 0.2 mM of EGTA, and 20 mM of creatine phosphate; reversal potential for Cl^-: −19.7 mV). Under these experimental conditions, activation of $GABA_A$ receptors leads to an efflux of chloride ions that ultimatively results in excitatory currents. Nevertheless, $GABA_A$ receptor–mediated currents are referred to as IPSCs ($GABA_A$-IPSCs) for better understanding throughout the article. $GABA_A$-IPSCs were recorded in the presence of 1,2,3,4- tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline- 7-sulphonamide (NBQX, 5 μM), 3-amino-propyl(diethoxymethyl)phosphinic acid (CGP35348, CGP; 200 μM; Novartis Laboratories, Basel, Switzerland), and D(−)-2-amino-5-phosphonopentanoic acid (50 μM). Photolytically evoked $GABA_A$ receptor–mediated currents ($GABA_A$-pCs) were induced upon focal photolysis of caged *O*-(α-carboxy-2-nitrobenzyl caged) GABA. Thereby, the beam of an ultraviolet laser (355-nm wavelength, frequency-tripled Nd:YVO4, 100-kHz pulse repetition rate; DPSS Lasers, San Jose, CA) was focused by the objective (60×, 0.9 numerical aperture; Olympus) on a small spot (25 μM in diameter) positioned on a dendrite approximately 10 to 20 μm from the soma. Laser stimulation alternating with electrical stimulation was delivered in intervals of 15 s. Once a stable whole-cell recording had been obtained, caged-GABA was added to the recirculating perfusate at a concentration of 0.1 mM. GABA was released by Q-switching brief laser pulses (100 ns; intensity 25 to 50 mW), which were applied at regular intervals of 30 s throughout the experiment. Photolytically released GABA directly activates $GABA_A$ receptors and $GABA_A$-pCs can be recorded. Under these conditions, receptor activation does not depend on presynaptic release mechanisms and can be held constant throughout the experiment. Figure 1, A and B depicts schemes of the stimulation and recording conditions.

In a subset of experiments, paired-pulse stimulation was performed by delivering the same stimuli at 50- and 150-ms interstimulus intervals. When a synapse is stimulated in rapid succession, the second postsynaptic response is larger than the first (paired-pulse facilitation). The paired-pulse ratio was determined by dividing the amplitude of the second current response by the amplitude of the first response.

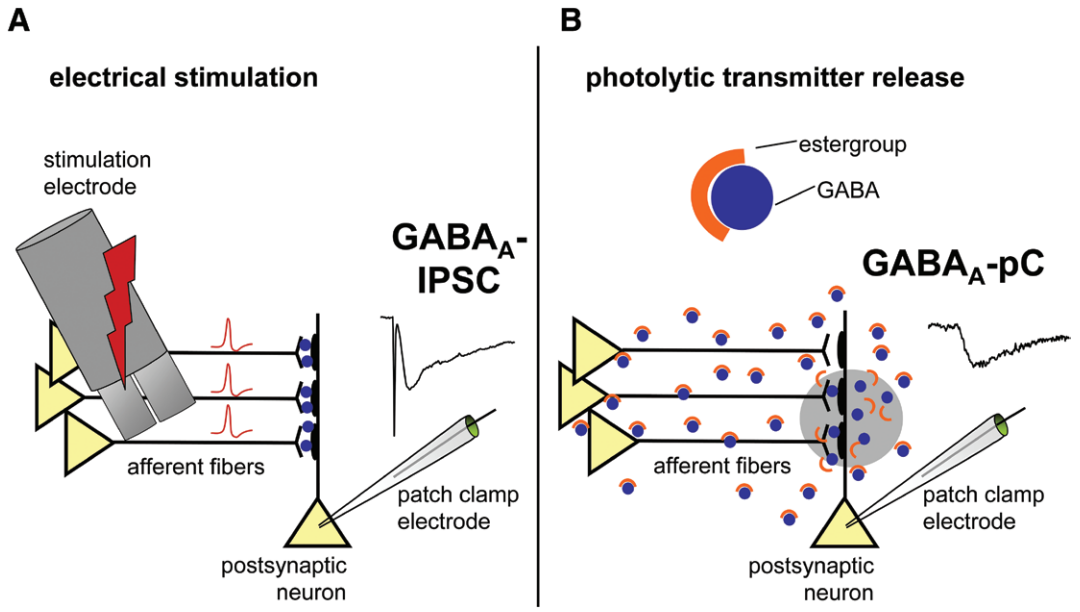

Fig. 1. Schematic of the stimulation and recording conditions. Patch clamp experiments were performed in the basolateral amygdala. The current responses were elicited upon either electrical stimulation of afferent fibers using an ultrafine bipolar tungsten electrode or focal photolysis of caged γ-aminobutyric acid (GABA). (*A*) The electrical stimulation leads to action potentials that propagate along the afferent fibers and consequently evoke transmitter release from the presynaptic terminals. The neurotransmitter GABA binds to receptors of the recorded neuron and an electrically evoked GABA receptor type A–mediated inhibitory postsynaptic current ($GABA_A$-IPSC) can be recorded. (*B*) The caged amino acid neurotransmitter *O*-(α-carboxy-2-nitrobenzyl caged) GABA is biologically inactive before photolysis. Photolysis of the caging group with ultraviolet illumination results in a localized release of the free neurotransmitter. The released neurotransmitter binds to GABA receptors, and a photolytically evoked GABA receptor type A–mediated current ($GABA_A$-pC) can be recorded.

A change of the paired-pulse ratio induced by a substance would point to a presynaptic mechanism of action.

For the measurement of *N*-methyl-D-aspartate (NMDA) receptor–mediated EPSCs (NMDA-EPSCs), neurons were held at a holding potential of −40 mV to release the magnesium block of NMDA receptors. NMDA-EPSCs were recorded in the presence of 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulphonamide (5 μM), CGP (200 μM), and bicuculline methiodide (20 μM). The current decay of NMDA-EPSCs was fitted biexponentially. For the measurement of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor–mediated currents (AMPA-EPSCs), cells were held at a membrane potential of −70 mV, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulphonamide was omitted from the aCSF, and D(−)-2-amino-5-phosphonopentanoic acid (50 μM) was added instead.

All current responses were amplified, low-pass filtered (3 kHz), digitized (ITC-16 Computer Interface; Instrutech Corp., Port Washington, NY) with a sampling frequency of 9 kHz, and stored on a hard drive (Power Macintosh G3 computer, data acquisition software Pulse v. 8.5; HEKA Electronic GmbH, Lambrecht, Germany).

All of the experiments were performed at room temperature (20° to 22°C). All of the salts and chemicals were obtained from Sigma–Aldrich (Steinheim, Germany), except CGP (Novartis Laboratories) and caged *O*-(α-carboxy-2-nitrobenzyl caged) GABA (Life technologies, Darmstadt, Germany).

Statistical Analysis

For statistical evaluation, SPSS Statistics version 16 (SPSS GmbH Software, Munich, Germany) was used. A repeated measurement ANOVA followed by a pairwise *t* test with Bonferroni adjustment was performed to test for differences between the groups of the VSDI data. Because multiple pairwise comparisons were performed, the *P* values obtained from the *t* tests were multiplied by the number of pairwise comparisons (adjusted *P* value). Due to reviewer concerns, the sample size of the VSDI data was increased after initial submission of the article. No attempts were made to further adjust the analyses for the additional multiple comparisons with the increased sample size.

For evaluation of the patch clamp experiments, we first partitioned the recording time in equidistant subintervals that were each 5 min in length and then determined the averaged relative amplitude, slope, and decay in each subinterval. Data were then tested for normal distribution using the Kolmogorov–Smirnov test. Statistical significance was determined using paired *t* tests for normally distributed samples and the Wilcoxon signed-rank test for samples that were not normally distributed.

For comparison of dose–response curves, we used a two-way ANOVA model that included the dose of TXA, type of current response, and their interaction as independent factors and relative amplitude as a dependent factor. The curve for the concentration–response relationship of $GABA_A$-IPSCs and $GABA_A$-pCs was calculated according to the Hill equation.

All statistical tests were performed on a two-sided level of significance of 5%. Numerical data are presented as the means ± SEM with the number of experiments (slices) indicated, if not stated otherwise. If error bars are not visible in graphs, the bars are smaller than the symbol size. Given the nature of the performed experiments, the experimenter was not blinded to the experimental conditions.

Results

In a first set of experiments, we determined the effect of TXA on neuronal excitation in the amygdala with the use of VSDI recordings (fig. 2A). Bath application of TXA (1 mM) increased peak amplitudes of FDSs to 129 ± 6% of the control (n = 5; adjusted *P* = 0.036; fig. 2, B and C). When the concentration of TXA was increased to 3 mM, FDSs were enhanced to 220 ± 26% (n = 5; adjusted *P* = 0.026; fig. 2, B and C) of the control. The effects were reversible upon washout of TXA. In the presence of 1 mM TXA, stimulation of the external capsule led to pronounced depolarization of the cortex (fig. 2D). This effect disappeared after washout (not shown).

In a subset of experiments, the effect of TXA was investigated in the presence of a $GABA_A$ receptor antagonist (bicuculline methiodide; 20 μM), a $GABA_B$ receptor antagonist (CGP; 200 μM), or a glycine receptor antagonist (strychnine; 1 μM). Under conditions where $GABA_A$ receptors were blocked, TXA did not increase FDS amplitudes (104 ± 4% of control; n = 7; adjusted *P* = 1.000; fig. 3). In contrast, the presence of either CGP or strychnine did not modulate the TXA-mediated effect on FDS amplitudes (fig. 3).

In the next step, we performed patch clamp recordings from principal neurons in the BLA to investigate whether increased intrinsic neuronal excitability might be responsible for the enhanced depolarization in the presence of TXA. Current clamp recordings revealed that application of 1 mM TXA did not impact resting membrane potential (57.9 ± 3.8 mV *vs.* 61.0 ± 1.6 mV; n = 7; *P* = 0.109; fig. 4A). The current–voltage relationship determined by current injection into the recorded cells was not affected by TXA (fig. 4B). Input resistance was 267 ± 40 MΩ under control conditions and 321 ± 64 MΩ in the presence of 1 mM TXA (n = 7 each; *t* test: *P* = 0.144 for control *vs.* TXA). Under control conditions, a current injection of +90 pA led to APs with a mean frequency of 14.3 ± 0.5 Hz and a mean amplitude of 55.5 ± 3.1 mV. These depolarizing responses remained unaffected by TXA: application of 1 mM TXA did not change the AP frequency (15.2 ± 1.3 Hz; n = 9; signed-rank test: *P* = 1.000) or the AP amplitude (55.2 ± 3.7 mV; n = 9; *t* test: *P* = 0.860; fig. 4C). APs occurred when cells were depolarized to −41.3 ± 0.1 mV under control conditions. TXA

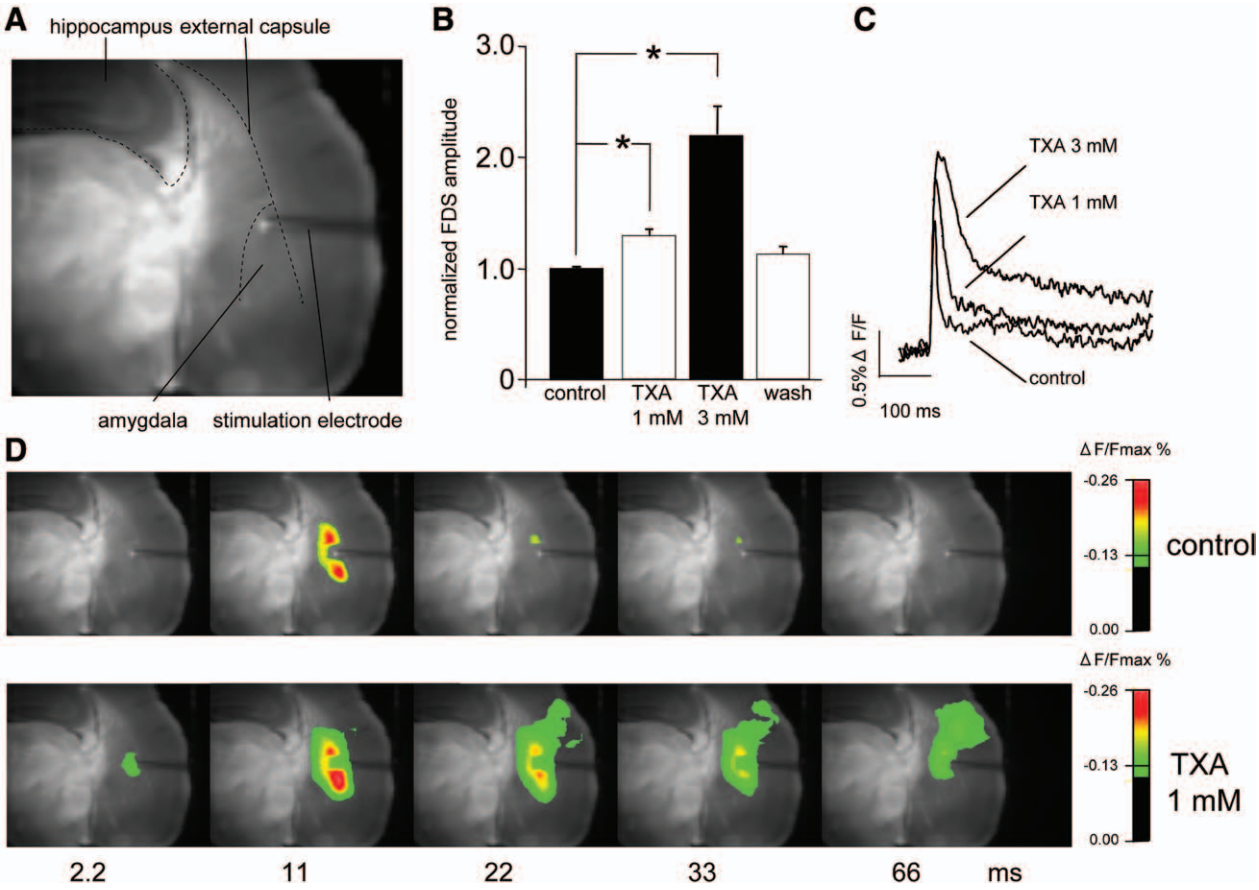

Fig. 2. Tranexamic acid (TXA) increases neuronal excitation propagation in the basolateral amygdala. Voltage-sensitive dye imaging recordings were performed in the basolateral amygdala. Slices were stained with the voltage-sensitive dye Pyridinium, 4-(2-(6-(dibutylamino)-2-naphthalenyl)ethenyl)-1-(3-sulfopropyl)-,hydroxide, inner salt (Di-4-ANEPPS). Images were recorded with a sampling rate of 2.2 ms. Signals were evoked by electrical stimulation of the lateral nucleus of the amygdala, and the region of interest was set to the basolateral amygdala. Signal fractional change in fluorescence (ΔF/F) and amplitude of fast, depolarization–mediated signals (FDS) were analyzed. (*A*) Overview of topography in coronal slices; (*B*) TXA (1 mM) increased FDS amplitude to 129 ± 6% of control (n = 5; adjusted $P = 0.036$). When the concentration of TXA was increased to 3 mM, FDS were enhanced to 220 ± 26% (n = 5; adjusted $P = 0.026$) of control. FDS amplitudes recovered to control levels upon TXA washout. (*C*) Representative recording trace of one experiment. (*D*) Representative filmstrip of one experiment. TXA led to an enhanced propagation of neuronal excitation and to pronounced depolarization in the cortex after electrical stimulation of the lateral amygdala. *$P < 0.05$.

did not affect this threshold (–41.4 ± 0.1 mV; n = 7; *t* test $P = 0.911$; fig. 4D). Hence, TXA is unlikely to affect membrane biophysics and consequently does not alter intrinsic neuronal excitability. Therefore, enhanced propagation of depolarization rather arises from a TXA-mediated modulation of synaptic transmission.

The effect of TXA on basal synaptic transmission in the BLA was examined. Compound postsynaptic currents evoked by electrical stimulation (cePSCs) of the external capsule were recorded from principal neurons in the absence of any receptor blockers. Peak amplitudes of cePSCs remained unchanged in the presence of 1 mM TXA (92 ± 7% of control; n = 6; $P = 0.481$; fig. 5A). In contrast, TXA (1 mM) significantly prolonged current decay from 18.4 ± 2.8 ms under control conditions to 23.6 ± 3.6 ms in the presence of 1 mM TXA (n = 6; $P = 0.032$; fig. 5B). Consequently, charge transfer, which was assessed as the area under the curve of the trace, was increased to 119 ± 5% of control (n = 6; $P = 0.023$; fig. 5C).

To further elucidate the receptors involved in the TXA-mediated prolongation of cePSCs, the effects of TXA on NMDA and AMPA receptors were investigated. TXA (1 mM) did not affect the amplitude of AMPA-EPSCs (97 ± 5% of control; n = 5; $P = 0.511$; fig. 6A) and did not have an impact on the current decay or charge transfer of AMPA-EPSCs (table 1).

Application of TXA (1 mM) did not result in a change of NMDA-EPSC peak amplitude (103 ± 9% of control; n = 5; $P = 0.706$; fig. 6B). Both time constants of NMDA-EPSCs, τ_{decay} fast, and τ_{decay} slow remained unchanged under 1 mM TXA and charge transfer was likewise not affected by TXA (table 1). TXA affected neither NMDA-EPSCs nor AMPA-EPSCs when it was bath-applied at a higher concentration of 5 mM (n = 3 for each receptor).

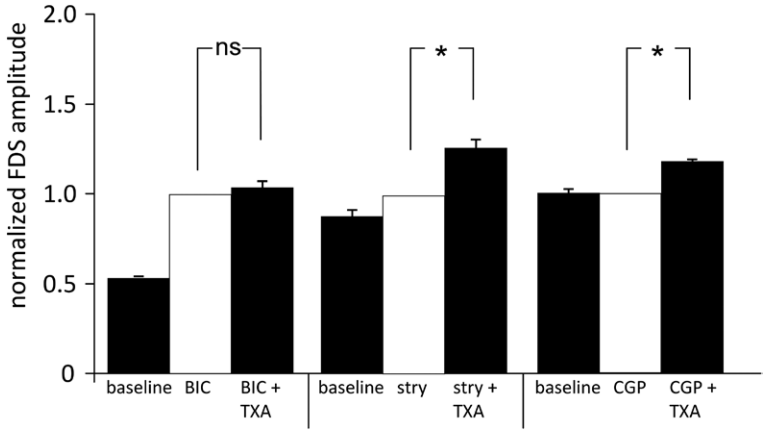

Fig. 3. The effect of tranexamic acid (TXA) on neuronal excitation propagation is dependent on γ-aminobutyric acid receptor type A and is independent of glycine and γ-aminobutyric acid type B receptors. The effect of TXA on fast, depolarization–mediated signals (FDS) in the basolateral amygdala was investigated in the presence of either bicuculline methiodide (BIC; 20 μM) to block γ-aminobutyric acid receptor type A receptors or stry (1 μM) to block glycine receptors. The application of BIC led to a strong enhancement of FDS amplitudes *versus* baseline levels. Under these conditions, TXA did not further augment FDS amplitudes (104 ± 4% of control; n = 7; adjusted P = 1.000). However, in the presence of stry, the application of TXA led to a significant increase in FDS amplitudes to 126 ± 5% of control (n = 5; adjusted P = 0.017). Under the influence of the γ-aminobutyric acid type B receptor antagonist 3-amino-propyl(diethoxymethyl)phosphinic acid (CGP35348) (CGP; 200 μM), TXA significantly enhanced FDS amplitudes to 119 ± 1% of control (n = 5; adjusted P = 0.001). Data were normalized to the FDS amplitudes recorded in the presence of the respective antagonists. *Adjusted P < 0.05. ns = not significant; stry = strychnine.

In a further step, the effect of TXA on $GABA_A$ receptor–mediated synaptic transmission was investigated. TXA (1 mM) reversibly diminished peak amplitudes of $GABA_A$-IPSCs evoked by endogenous synaptic GABA release upon electrical stimulation to 36 ± 3% (n = 9; P < 0.001; fig. 7A) of the control and reduced charge transfer (40 ± 8%; n = 9; P = 0.008; table 2) without affecting current decay (table 2). Amplitudes of $GABA_A$-pCs obtained by photolytic uncaging of exogenous GABA were decreased to 41 ± 5% of the control (n = 5; P < 0.001; fig. 7A). There was no

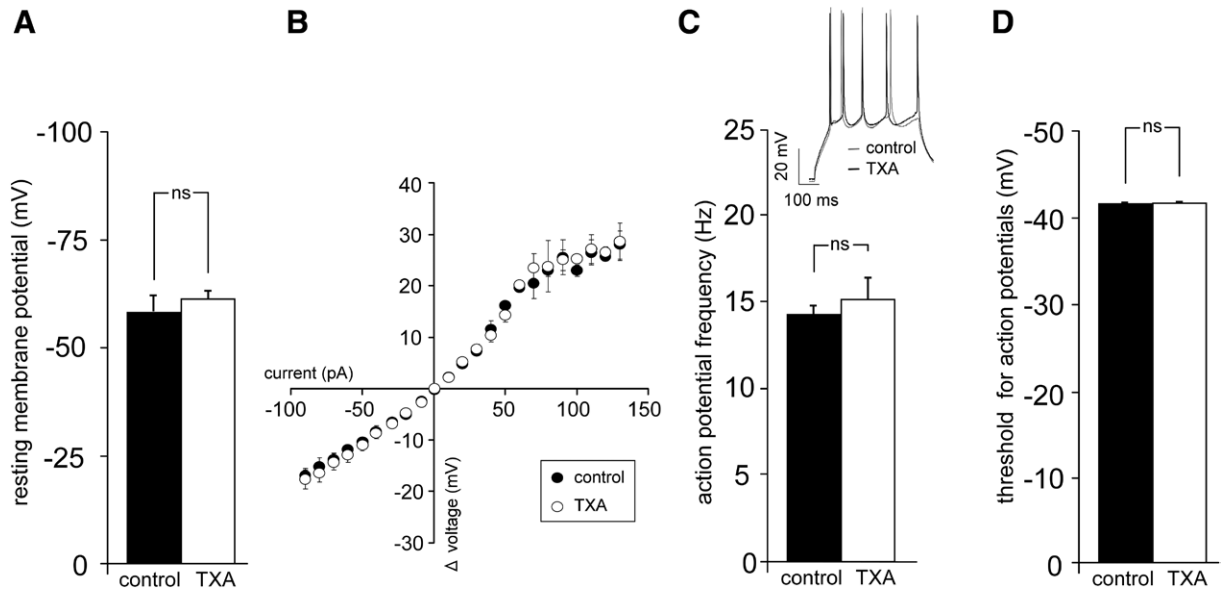

Fig. 4. Tranexamic acid (TXA) does not affect the resting membrane potential or the current–voltage relationship of the recorded neurons. Patch clamp recordings from principal neurons in the basolateral amygdala were performed in the current clamp mode. (*A*) Under control conditions, neurons had a resting membrane potential of 57.9 ± 3.8 mV. Application of TXA (1 mM) did not affect the resting membrane potential (61.0 ± 1.6 mV; n = 7; signed-rank test: P = 0.109). (*B*) Current (ranging from −90 to +90 pA in 10 pA increments) was injected into the neurons, and the resulting voltage was measured over the membrane. TXA (1 mM) did not impact the current–voltage relationship. (*C*) TXA did not affect the frequency of action potentials induced by depolarizing current injections. (*D*) The threshold for action potential generation remained unchanged in the presence of TXA. ns = not significant (P > 0.05).

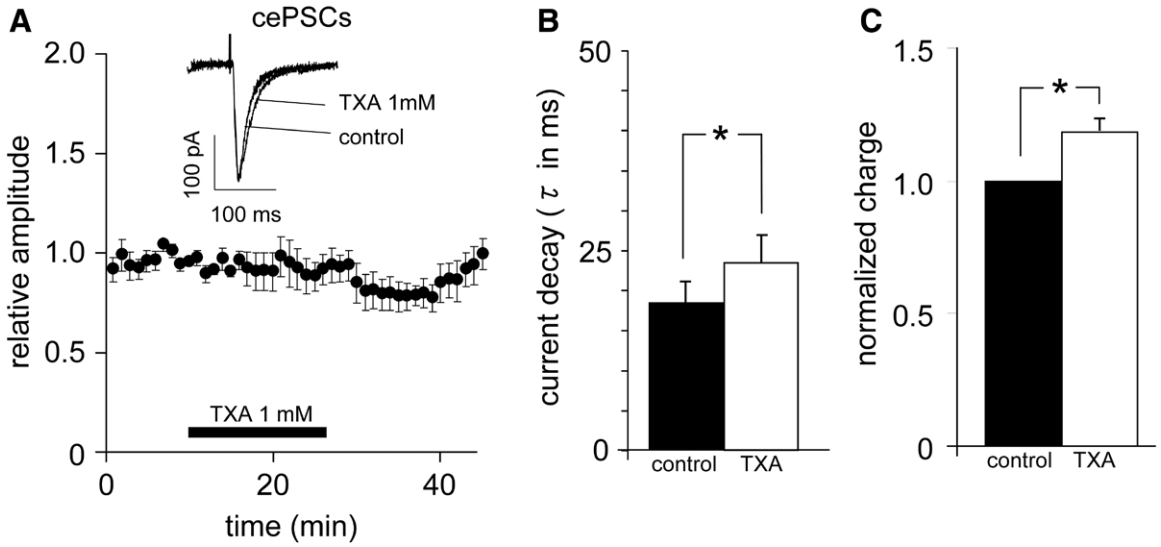

Fig. 5. Tranexamic acid (TXA) had no impact on the amplitude of basal synaptic transmission but prolonged current decay and thereby increased charge transfer. Principal neurons of the basolateral amygdala were held at a holding potential of −70 mV. Compound postsynaptic currents evoked by electrical stimulation (cePSCs) of the external capsule were recorded. (*A*) Peak amplitudes of cePSCs remained unaffected by TXA (92 ± 7% of control; n = 6; *t* test: *P* = 0.481). *Inset* shows representative recording traces. (*B*) Current decay was fitted exponentially and was prolonged from 18.4 ± 2.8 ms under control conditions to 23.6 ± 3.6 ms in the presence of 1 mM TXA (n = 6; *t* test: *P* = 0.032). (*C*) Charge transfer (area under the curve of the recording trace) was increased to 119 ± 5% (n = 6; signed rank test: *P* = 0.023). **P* < 0.05.

significant difference in the TXA-mediated degree of reduction between $GABA_A$-IPSCs and $GABA_A$-pCs, which is in favor of postsynaptic mechanisms. As paired-pulse ratio is a well-established tool to investigate presynaptic actions of neuroactive substances, the effect of TXA (1 mM) on the paired-pulse ratio of $GABA_A$-IPSCs was investigated. The paired-pulse ratios at 50 and 150 ms interstimulus intervals did not change when 1 mM TXA was applied (n = 5 each; *t* test: *P* = 0.295 for 50-ms interstimulus interval and *P* = 0.517 for 150-ms interstimulus interval; fig. 7B). The dose–response relationship of the TXA-mediated reduction of both $GABA_A$-IPSCs and $GABA_A$-pCs was investigated by

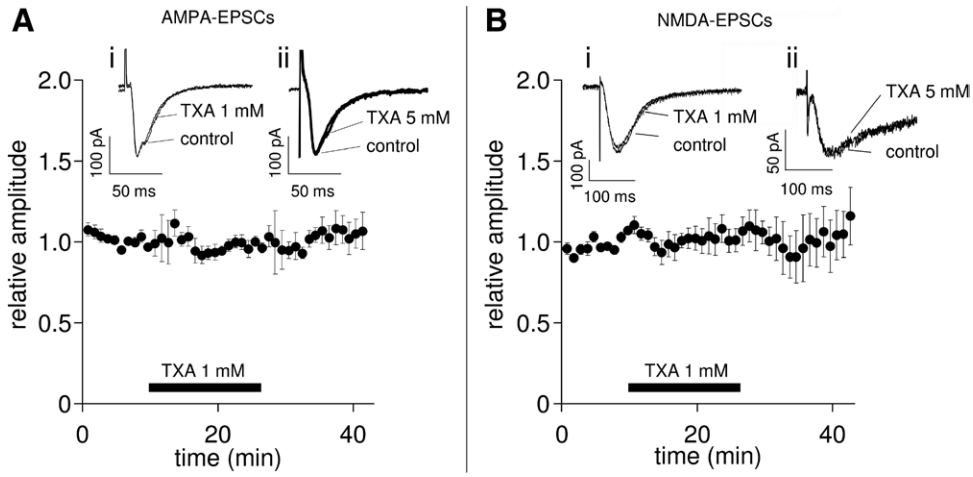

Fig. 6. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptor–mediated currents remained unaffected by tranexamic acid (TXA). (*A*) AMPA receptor–mediated excitatory postsynaptic currents (AMPA-EPSCs) were recorded in the presence of 3-amino-propyl(diethoxymethyl)phosphinic acid (CGP35348; 200 μM), bicuculline methiodide (20 μM), and D(−)-2-amino-5-phosphonopentanoic acid (AP5; 50 μM). The peak amplitudes of AMPA-EPSCs did not change (97 ± 5% of control; n = 5; *t* test: *P* = 0.511) when TXA (1 mM) was bath-applied. (*Ai*) Shows representative recording traces. (*Aii*) TXA did not affect AMPA-EPSCs when it was applied at a concentration of 5 mM. (*B*) NMDA-EPSCs were recorded at a holding potential of −40 mV in the presence of CGP35348 (200 μM), bicuculline methiodide (20 μM), and 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulphonamide (NBQX; 5 μM). The application of 1 mM TXA did not result in changed peak amplitudes of NMDA-EPSCs (103 ± 9% of control; n = 5; *t* test: *P* = 0.706). (*Bi*) Shows representative recording traces. (*Bii*) TXA did not affect NMDA-EPSCs when it was applied at a concentration of 5 mM.

Table 1. Deactivation Kinetics and Charge Transfer of Glutamatergic Currents

	Control	TXA 1 mM
AMPA-EPSCs		
τ_{decay} (ms)	19.7 ± 1.0	19.0 ± 1.4 (ns)
Relative charge transfer (%)	100	103.7 ± 15.2 (ns)
NMDA-EPSCs		
τ_{decay} fast (ms)	63.1 ± 4.2	63.6 ± 3.9 (ns)
τ_{decay} slow (ms)	74.1 ± 6.9	66.7 ± 3.6 (ns)
Relative charge transfer (%)	100	114.6 ± 15.2 (ns)

TXA did not affect current deactivation kinetics or charge transfer of AMPA-EPSCs and NMDA-EPSCs. Values are means ± SEM; n = 5–6.

AMPA-EPSCs = electrically evoked α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor–mediated excitatory postsynaptic currents; NMDA-EPSCs = *N*-methyl-D-aspartate receptor–mediated excitatory postsynaptic currents; ns = not significant *vs.* control; TXA = tranexamic acid.

the application of 0.1, 0.3, 1, 5 or 10 mM TXA (n = 5 to 9 for each data point). $GABA_A$-IPSCs were reduced by TXA at a half-maximal inhibitory concentration (IC_{50}) of 0.76 mM (Hill, –0.8; fig. 7C). TXA led to a dose-dependent decrease of $GABA_A$-pCs with an IC_{50} of 0.84 mM (Hill, –1.0; fig. 7C). Two-way ANOVA revealed a strong significant effect of dose ($P < 0.001$), but no significant effect of type of current response ($P = 0.760$) and no significant interaction effect between dose and type of current response ($P = 0.602$).

Discussion

In this study, we could clearly demonstrate that the application of TXA enhanced neuronal excitation in the BLA in acute murine brain slices. TXA did not affect passive electric neuronal membrane properties or excitatory synaptic transmission, but it dose-dependently reduced $GABA_A$ receptor–mediated inhibitory synaptic transmission.

The reduction of inhibitory GABAergic synaptic transmission occurred with a half-maximal inhibitory concentration that is clinically relevant. High doses of up to 100 mg/kg TXA have been shown to reduce blood loss and transfusion requirement after cardiac surgery with mild systemic hypothermia.[16] A positive correlation between TXA dosing and the incidence of postoperative seizures has been reported.[17] TXA administration at doses in the vicinity of 100 mg/kg has been shown to be associated with an increased risk of postoperative seizures.[3,18,19] After the administration of 100 mg/kg TXA, plasma levels exceed 4 mM[20] which results

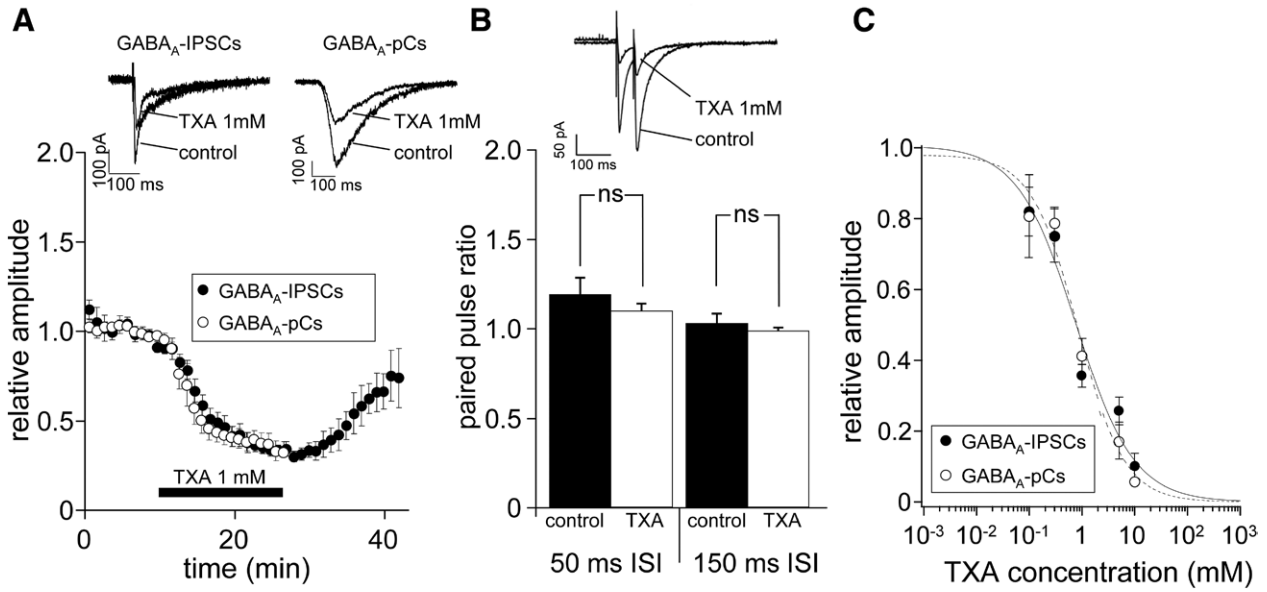

Fig. 7. Inhibitory synaptic transmission was dose-dependently reduced in the presence of tranexamic acid (TXA). γ-Aminobutyric acid receptor type A ($GABA_A$)–mediated inhibitory postsynaptic currents ($GABA_A$-IPSCs) were recorded in the presence of 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulphonamide (NBQX; 5 μM), 3-amino-propyl(diethoxymethyl)phosphinic acid (CGP35348) (CGP; 200 μM), and D(–)-2-amino-5-phosphonopentanoic acid (AP5; 50 μM). The currents were evoked upon either electrical stimulation of the external capsule ($GABA_A$-IPSCs) or focal photolytic release of $GABA_A$-pCs. (*A*) The application of TXA (1 mM) led to a reduction of $GABA_A$-IPSCs to 36 ± 3% of control (n = 9; *t* test: $P < 0.001$). In the presence of TXA (1 mM), photolytically evoked $GABA_A$-pCs were reduced to 41 ± 5% of control (n = 9; *t* test: $P < 0.001$). There was no difference in the TXA-mediated degree of reduction between $GABA_A$-IPSCs and $GABA_A$-pCs. *Insets* show representative recording traces. (*B*) Two consecutive $GABA_A$-IPSCs were evoked with interstimulus intervals (ISIs) of 50 and 150 ms, respectively. The paired-pulse ratio was evaluated as the ratio of the amplitudes of the first and second $GABA_A$-IPSC. Application of 1 mM TXA did not affect the paired-pulse ratio of $GABA_A$-IPSCs. *Inset* shows representative recording trace. (*C*) TXA was applied at concentrations of 0.1, 0.3, 1, 5 or 10 mM to investigate the dose-dependency of the TXA-mediated inhibition of $GABA_A$ receptor–mediated currents. $GABA_A$-IPSCs and $GABA_A$-pCs were reduced by TXA at half maximal inhibitory concentrations of 0.76 mM (Hill, –0.8) and 0.84 mM (Hill, –1.0), respectively. ns = not significant ($P > 0.05$), pCs = photolytically evoked currents.

Table 2. Deactivation Kinetics and Charge Transfer of γ-Aminobutyric Acid Receptor Type A–mediated Postsynaptic Currents

$GABA_A$-IPSC	Control	TXA 1 mM
τ_{decay} fast (ms)	21.9 ± 2.6	29.7 ± 7.8 (ns)
τ_{decay} slow (ms)	73.3 ± 8.1	79.4 ± 10.4 (ns)
Relative charge transfer (%)	100	40 ± 8*

TXA (1 mM) did not change the time constants τ_{decay} fast or τ_{decay} slow of $GABA_A$-IPSC (n = 9) but significantly reduced charge transfer. Values are means ± SEM.

*Significant *vs.* control.

$GABA_A$-IPSC = electrically evoked γ-aminobutyric acid receptor type A–mediated inhibitory postsynaptic current; ns = not significant *vs.* control; TXA = tranexamic acid.

in TXA concentrations of 0.62 to 1.24 mM[3] in the CSF when extrapolated from animal studies[21] and from CSF concentrations in patients with intracranial bleeding.[22] A recent study showed that peak CSF concentrations after intravenous administration of TXA in patients undergoing repair of thoraco-abdominal pathologies are approximately 0.2 mM.[23] Given that only 3% of TXA is bound to protein,[1] the observed IC_{50}s (0.76 and 0.84 mM) of TXA against $GABA_A$ receptors are in a clinically relevant range.

The VSDI results suggest that increased neuronal excitation propagation upon application of TXA is independent of $GABA_B$ or glycine receptors and dependent on $GABA_A$ receptors. Optical imaging with voltage-sensitive dyes permits the monitoring of a neuronal population with a high spatiotemporal resolution, providing an insight into spatiotemporal propagation patterns of neuronal excitation within a network.[12] In our study, excitation propagation in the BLA was strongly enhanced by TXA, which even resulted in a pronounced depolarization of the cortex.

Intrinsic neuronal excitability, that is, the possibility that the neurons react to changes in membrane potential with APs, is dynamically regulated by distinct classes of ion channels. Pharmacologic modulation of the activity of these channels can result in increased neuronal excitability and susceptibility for epileptiform activity. When background potassium channels are antagonized, neurons depolarize; thus, neuronal excitability increases.[24] The potassium channel blocker 4-aminopyridine is known to elicit epileptiform activity when applied to the BLA *in vitro*.[25,26] A common mechanism of 4-aminopyridine and TXA seems rather unlikely, as both AP frequency and AP amplitude increase in the presence of 4-aminopyridine[27] but not in the presence of TXA. In addition, the resting membrane potential maintained stable upon TXA application. Therefore, it seems rather unlikely that TXA promotes epileptiform activity by increasing intrinsic neuronal excitability.

Altered excitatory neurotransmission, primarily mediated *via* glutamate, is a major cause for the imbalance of excitation and inhibition that characterizes epileptic seizures.[28] Antagonists against NMDA and AMPA receptors have been described to be powerful anticonvulsants in many animal models of epilepsy.[29,30] Previous work has shown that the activation of NMDA receptors induces epileptiform activity in brain slices.[31] In the current study, TXA did not produce any alterations in NMDA or AMPA receptor–mediated synaptic transmission, which is, at least for the NMDA component, in accordance with a binding study showing that TXA does not interact with the NMDA receptor complex.[32] To our knowledge, the action of TXA on AMPA receptors has not yet been investigated. Despite the insensitivity of AMPA and NMDA receptors to TXA, we observed an increased charge transfer of cePSCs in the presence of TXA. Because the width of these currents is also limited by $GABA_A$ receptors,[33] a TXA-induced blockade of GABAergic inhibition could explain the observed delay in time course and the increase of charge transfer of cePSCs.

γ-Aminobutyric acid is the major central inhibitory neurotransmitter, and impaired synaptic inhibition leads to an increased neuronal excitation. Therefore, it is not surprising that GABAergic synaptic transmission is of fundamental importance for seizure pathophysiology. Epileptiform discharges occur more readily when inhibition is compromised by GABAergic blockade.[34–36] A proconvulsant action has been described for the $GABA_A$ receptor antagonist bicuculine.[8] In our experiments, TXA dose-dependently diminished GABAergic synaptic transmission. This observation is entirely consistent with the study by Furtmüller *et al.*[32] that describes a competitive, dose-dependent inhibition of $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors expressed in human embryonic kidney cells with an IC_{50} of 7.1 mM. The discrepancy with our results that concerns the IC_{50} might result from different subunit compositions of the GABA receptors: It has been shown that changes, for example, in the α-subunit composition profoundly affect the action of neuroactive substances on GABA receptors.[37,38] The predominating subunit composition in the amygdala is $\alpha_2\beta_n\gamma_1$.[39] Fast GABAergic postsynaptic currents in BLA principal cells primarily arise from α_2-containing $GABA_A$ receptors and to a lesser extent from α_1-containing $GABA_A$ receptors.[37] Thus the $GABA_A$ receptor–mediated currents recorded in our study are more likely to arise from α_2-containing $GABA_A$ receptors, and these receptors might be more sensitive to TXA than the α_1-containing receptors that were used by Furtmüller *et al.*

$GABA_A$-pCs are independent of presynaptic activation and were reduced to the same extent as $GABA_A$-IPSCs. These data suggest a pure postsynaptic mechanism of action on inhibitory GABAergic synaptic transmission that is additionally supported by paired-pulse experiments. The paired-pulse ratio depends on the probability of vesicular release; therefore, a modulation of paired-pulse ratio would point to a presynaptic mechanism of action.[40] As TXA did not affect the paired-pulse ratio, a presynaptic action of TXA on GABA-ergic signaling receptors seems rather unlikely.

Interestingly, in the intensive care unit, TXA-associated postoperative seizures mostly occur long after the cessation of TXA administration. Plausible explanations for these observations could be an accumulation of TXA during surgery

or, that proconvulsant effects of TXA are counteracted by the administered anesthetics during surgery, most of which positively modulate GABAergic signaling.[17]

In summary, we showed that TXA applied at clinically relevant concentrations increases the propagation of neuronal excitation in the BLA. The observed enhancement of neuronal excitation arises from reduced synaptic inhibition rather than from increased neuronal excitability. TXA impairs neuronal inhibition by a postsynaptic antagonism against $GABA_A$ receptors. In contrast, TXA does not affect excitatory glutamatergic synaptic transmission. Because inhibitors of $GABA_A$ receptors are known to act in a proconvulsant manner, this mechanism of action might explain the increased incidence of seizures in patients treated with TXA.

Acknowledgments

The authors thank Bernhard Haller, M.Sc., (Institute of Medical Statistics and Epidemiology, Technische Universität München, Munich, Germany), for statistical advice.

Support was provided solely from institutional and/or departmental sources.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Haseneder: Department of Anesthesiology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Straße 22, 81675 Munich, Germany. r.hasender@lrz.tu-muenchen.de. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. Anesthesiology's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

1. McCormack PL: Tranexamic acid: A review of its use in the treatment of hyperfibrinolysis. Drugs 2012; 72:585–617
2. Roberts I, Shakur H, Ker K, Coats T: Antifibrinolytic drugs for acute traumatic injury. Cochrane Database Syst Rev 2011:CD004896
3. Murkin JM, Falter F, Granton J, Young B, Burt C, Chu M: High-dose tranexamic Acid is associated with nonischemic clinical seizures in cardiac surgical patients. Anesth Analg 2010; 110:350–3
4. Manji RA, Grocott HP, Leake J, Ariano RE, Manji JS, Menkis AH, Jacobsohn E: Seizures following cardiac surgery: The impact of tranexamic acid and other risk factors. Can J Anaesth 2012; 59:6–13
5. McCormick DA, Contreras D: On the cellular and network bases of epileptic seizures. Annu Rev Physiol 2001; 63:815–46
6. Freund TF, Buzsáki G: Interneurons of the hippocampus. Hippocampus 1996; 6:347–470
7. Tschuluun N, Jürgen Wenzel H, Doisy ET, Schwartzkroin PA: Initiation of epileptiform activity in a rat model of periventricular nodular heterotopia. Epilepsia 2011; 52:2304–14
8. Schneider PG, Rodríguez de Lores Arnaiz G: Convulsant bicuculline modifies CNS muscarinic receptor affinity. BMC Neurosci 2006; 7:32
9. Killcross S, Robbins TW, Everitt BJ: Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. Nature 1997; 388:377–80
10. Aroniadou-Anderjaska V, Qashu F, Braga MF: Mechanisms regulating GABAergic inhibitory transmission in the basolateral amygdala: Implications for epilepsy and anxiety disorders. Amino Acids 2007; 32:305–15
11. DeToledo JC: Lidocaine and seizures. Ther Drug Monit 2000; 22:320–2
12. Stepan J, Dine J, Fenzl T, Polta SA, von Wolff G, Wotjak CT, Eder M: Entorhinal theta-frequency input to the dentate gyrus trisynaptically evokes hippocampal CA1 LTP. Front Neural Circuits 2012; 6:64
13. Dodt HU, Zieglgänsberger W: Visualizing unstained neurons in living brain slices by infrared DIC-videomicroscopy. Brain Res 1990; 537:333–6
14. Sutor B, Zieglgänsberger W: A low-voltage activated, transient calcium current is responsible for the time-dependent depolarizing inward rectification of rat neocortical neurons *in vitro*. Pflugers Arch 1987; 410:102–11
15. Polder HR, Swandulla D: The use of control theory for the design of voltage clamp systems: A simple and standardized procedure for evaluating system parameters. J Neurosci Methods 2001; 109:97–109
16. Karski JM, Dowd NP, Joiner R, Carroll J, Peniston C, Bailey K, Glynn MF, Teasdale SJ, Cheng DC: The effect of three different doses of tranexamic acid on blood loss after cardiac surgery with mild systemic hypothermia (32 degrees C). J Cardiothorac Vasc Anesth 1998; 12:642–6
17. Lecker I, Orser BA, Mazer CD: "Seizing" the opportunity to understand antifibrinolytic drugs. Can J Anaesth 2012; 59:1–5
18. Kalavrouziotis D, Voisine P, Mohammadi S, Dionne S, Dagenais F: High-dose tranexamic acid is an independent predictor of early seizure after cardiopulmonary bypass. Ann Thorac Surg 2012; 93:148–54
19. Keyl C, Uhl R, Beyersdorf F, Stampf S, Lehane C, Wiesenack C, Trenk D: High-dose tranexamic acid is related to increased risk of generalized seizures after aortic valve replacement. Eur J Cardiothorac Surg 2011; 39:e114–21
20. Dowd NP, Karski JM, Cheng DC, Carroll JA, Lin Y, James RL, Butterworth J: Pharmacokinetics of tranexamic acid during cardiopulmonary bypass. Anesthesiology 2002; 97:390–9
21. Yamaura A, Nakamura T, Makino H, Hagihara Y: Cerebral complication of antifibrinolytic therapy in the treatment of ruptured intracranial aneurysm. Animal experiment and a review of literature. Eur Neurol 1980; 19:77–84
22. Fodstad H, Pilbrant A, Schannong M, Strömberg S: Determination of tranexamic acid (AMCA) and fibrin/fibrinogen degradation products in cerebrospinal fluid after aneurysmal subarachnoid haemorrhage. Acta Neurochir (Wien) 1981; 58:1–13
23. Lecker I, Wang DS, Romaschin AD, Peterson M, Mazer CD, Orser BA: Tranexamic acid concentrations associated with human seizures inhibit glycine receptors. J Clin Invest 2012; 122:4654–66
24. Lesage F: Pharmacology of neuronal background potassium channels. Neuropharmacology 2003; 44:1–7
25. Panuccio G, D'Antuono M, de Guzman P, De Lannoy L, Biagini G, Avoli M: *In vitro* ictogenesis and parahippocampal networks in a rodent model of temporal lobe epilepsy. Neurobiol Dis 2010; 39:372–80
26. Gean PW: The epileptiform activity induced by 4-aminopyridine in rat amygdala slices: Antagonism by non-*N*-methyl-D-aspartate receptor antagonists. Brain Res 1990; 530:251–6
27. Gean PW, Shinnick-Gallagher P: The transient potassium current, the A-current, is involved in spike frequency adaptation in rat amygdala neurons. Brain Res 1989; 480:160–9

28. Raol YH, Lynch DR, Brooks-Kayal AR: Role of excitatory amino acids in developmental epilepsies. Ment Retard Dev Disabil Res Rev 2001; 7:254–60
29. Chapman AG: Glutamate receptors in epilepsy. Prog Brain Res 1998; 116:371–83
30. Rogawski MA: Revisiting AMPA receptors as an antiepileptic drug target. Epilepsy Curr 2011; 11:56–63
31. Mody I, Lambert JD, Heinemann U: Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. J Neurophysiol 1987; 57:869–88
32. Furtmüller R, Schlag MG, Berger M, Hopf R, Huck S, Sieghart W, Redl H: Tranexamic acid, a widely used antifibrinolytic agent, causes convulsions by a gamma-aminobutyric acid(A) receptor antagonistic effect. J Pharmacol Exp Ther 2002; 301:168–73
33. Ranft A, Kurz J, Deuringer M, Haseneder R, Dodt HU, Zieglgänsberger W, Kochs E, Eder M, Hapfelmeier G: Isoflurane modulates glutamatergic and GABAergic neurotransmission in the amygdala. Eur J Neurosci 2004; 20:1276–80
34. Connors BW: Initiation of synchronized neuronal bursting in neocortex. Nature 1984; 310:685–7
35. Trevelyan AJ, Schevon CA: How inhibition influences seizure propagation. Neuropharmacology 2013; 69:45–54
36. Curtis DR, Duggan AW, Felix D, Johnston GA: GABA, bicuculline and central inhibition. Nature 1970; 226:1222–4
37. Marowsky A, Fritschy JM, Vogt KE: Functional mapping of GABA A receptor subtypes in the amygdala. Eur J Neurosci 2004; 20:1281–9
38. Wieland HA, Lüddens H, Seeburg PH: A single histidine in $GABA_A$ receptors is essential for benzodiazepine agonist binding. J Biol Chem 1992; 267:1426–9
39. McKernan RM, Whiting PJ: Which $GABA_A$-receptor subtypes really occur in the brain? Trends Neurosci 1996; 19:139–43
40. Manita S, Suzuki T, Inoue M, Kudo Y, Miyakawa H: Paired-pulse ratio of synaptically induced transporter currents at hippocampal CA1 synapses is not related to release probability. Brain Res 2007; 1154:71–9