European Journal of Pharmacology 815 (2017) 49–55

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Tranexamic acid impairs hippocampal synaptic transmission mediated by gamma aminobutyric acid receptor type A☆

Hedwig Irl[1], Stephan Kratzer[1], Stefan Schwerin, Eberhard Kochs, Manfred Blobner, Gerhard Schneider, Gerhard Rammes, Rainer Haseneder*

Department of Anesthesiology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany

ARTICLE INFO

Keywords:
Tranexamic acid
Seizures
Hippocampus
GABA

ABSTRACT

High-dose application of tranexamic acid (TXA), a widely used antifibrinolytic drug, can cause seizures in patients undergoing surgery. Mechanistically, seizures are considered to arise from an imbalance between inhibitory and excitatory synaptic transmission, whose main transmitters are gamma-aminobutyric acid (GABA) and glutamate. In the present study, we investigated the effects of TXA on neuronal excitability and synaptic transmission in the hippocampus, a structure that plays a pivotal role in human epilepsy.

In acute slices of the murine hippocampus, fast depolarization-mediated imaging signals (FDSs) and postsynaptic currents (PSCs) were recorded using voltage-sensitive dye imaging and whole-cell patch clamp technique, respectively. FDSs and PSCs were evoked upon stimulation of the dentate gyrus and Schaffer collateral/associational commissural pathway, respectively. $GABA_A$, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and *N*-methyl-D-aspartate (NMDA) receptor-mediated postsynaptic currents were isolated pharmacologically.

Application of TXA enhanced FDS propagation in the hippocampus. Neither the resting membrane potential of the investigated neurones nor synaptic transmission mediated by AMPA or NMDA receptors was changed by the application of 1 mM TXA. In contrast, TXA dose-dependently reduced $GABA_A$ receptor-mediated synaptic transmission.

TXA induced the inhibition of $GABA_A$ receptor-mediated synaptic transmission in the hippocampus with a potency similar to that of its antagonistic properties against $GABA_A$ receptors in the basolateral amygdala (Kratzer et al., 2014). Since impairment of GABAergic transmission is a major cause of epileptic seizures, the observed effect might contribute to the proconvulsive properties of TXA.

1. Introduction

In recent years, tranexamic acid (TXA), a lysine analogue inhibiting fibrinolysis, has gained increasing interest as medication to reduce bleeding and improve outcome in a variety of clinical settings. The use of TXA has been shown to reduce mortality in patients with traumatic bleeding (Ker et al., 2015) and blood loss and need for transfusions in both cardiac (Adler et al., 2011) and non-cardiac (Kagoma et al., 2009; Wei and Liu, 2015) surgical patients. However, TXA is not used only in surgical or trauma patients. Recent studies have shown positive effects in the treatment of hypermenorrhea (Leminen and Hurskainen, 2012), postpartum haemorrhage (Novikova et al., 2015; Sentilhes et al., 2015) and gastrointestinal bleeding (Manno et al., 2014). Therefore, even broader use of TXA in the future seems likely.

Although the use of TXA is considered to be safe with few adverse effects, numerous studies provide evidence that TXA increases the risk of seizures in patients undergoing major cardiac surgery (Kalavrouziotis et al., 2012; Manji et al., 2012; Murkin et al., 2010). These postoperative seizures are a serious side effect increasing both the length of hospital stay and the mortality (Koster et al., 2013; Sharma et al., 2014).

Both a TXA-induced reduction of inhibitory gamma-aminobutyric acid type A ($GABA_A$) and glycine receptor-mediated synaptic transmission are discussed as neuronal correlates for the seizure-promoting action of TXA (Lecker et al., 2016). We recently showed that TXA concentration-dependently inhibits $GABA_A$ receptor-mediated synaptic transmission in the mouse amygdala (Kratzer et al., 2014), a central nervous system (CNS) structure with fundamental importance for the

☆ This work should be attributed to the Department of Anesthesiology, Klinikum rechts der Isar, Technische Universität München, Germany.

* Correspondence to: Department of Anesthesiology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Straße 22, 81675 Munich, Germany.
E-mail address: rainer.haseneder@tum.de (R. Haseneder).

[1] Both authors contributed equally to this work, alphabetical order.

http://dx.doi.org/10.1016/j.ejphar.2017.10.001
Received 15 January 2017; Received in revised form 17 September 2017; Accepted 2 October 2017
Available online 05 October 2017
0014-2999/

initiation and propagation of seizures (Aroniadou-Anderjaska et al., 2008). In the present study, we wanted to determine whether TXA shows similar properties in another seizure-prone CNS structure, the hippocampus. Like the amygdala, the hippocampus is fundamentally involved in the pathophysiology of temporal lobe epilepsy (Chatzikonstantinou, 2014), the most common form of epilepsy in adults (Engel, 1989). Moreover, the hippocampus has been suggested to be pivotally involved in the generation of drug-induced seizures, e.g., for seizures induced by quinolone antibacterials (Ito et al., 1999).

2. Materials and methods

2.1. Brain slice preparation and staining

All experimental protocols were approved by the Ethical Committee on Animal Care and Use of the Government of Bavaria, Germany. Brains of male C57Bl6 mice (21–56 d), anesthetized with isoflurane and killed by decapitation, were rapidly cut in sagittal slices (350 μm) with a microtome (HM 650 V, Microm International, Walldorf, Germany). Preparation and subsequent recovery (45 min, 34 °C) of the slices for the voltage-sensitive dye imaging (VSDI) experiments was conducted in carbogen-saturated (95% O_2 / 5% CO_2) sucrose-based artificial cerebrospinal fluid (aCSF), containing the following (in mM): NaCl 87, KCl 2.5, $NaHCO_3$ 25, NaH_2PO_4 1.25, $CaCl_2$ 0.5, $MgCl_2$ 7, glucose 25 and sucrose 75 (pH 7.4). After recovery, the slices were stained for 15 min in carbogen-saturated standard aCSF (in mM: NaCl 125, KCl 2.5, $NaHCO_3$ 25, NaH2PO4 1.25, $CaCl_2$ 2, $MgCl_2$ 1, and glucose 25 (pH 7.4)) additionally containing the voltage-sensitive dye Di-4-ANEPPS (7.5 mg/ml; < 0.1% dimethyl sulphoxide). Before the start of the experiment, the slices were again incubated for at least 30 min in standard aCSF. Preparation and recovery (45 min, 34 °C) of the slices for the patch clamp experiments was carried out in carbogenated standard aCSF.

2.2. VSDI experiments

In the recording chamber, slices were fixed using a platinum ring with nylon filaments and continuously superfused with carbogen-saturated standard aCSF (flow rate 5 ml/min, room temperature (20–22 °C)). Electrical stimulation, performed using square-pulse stimuli (200 μs, 10–25 V), was applied to the dentate gyrus via a bipolar concentric tungsten electrode. Regions of interest (ROIs; 3 × 3 pixels) were established in the CA1 region of the hippocampus. As a parameter of neuronal activity, we used ROI-extracted, fast depolarization-mediated imaging signals (FDSs), which correlate with action potentials and excitatory postsynaptic potentials (EPSP; (Stepan et al., 2012)). VSDI recordings were performed using an Olympus BX51WI fluorescence microscope (Olympus, Hamburg, Germany) equipped with a MiCAM02-HR camera (BrainVision, Tokyo, Japan), an XLFluor4X/340 objective (NA 0.28; Olympus), a 530-nm excitation filter, and a 600-nm emission filter. The MiCAM02 software package (BrainVision) was used for further processing and analysis of the signals. Acquisition settings were 88 × 60-pixel frame size, 36.4 × 40.0 μm pixel size and 2.2 ms sampling time. Eight sequential acquisitions recorded at 15 s intervals were averaged to optimize the signal-to-noise ratio. From the recorded signals, the fractional change in fluorescence (ΔF/F) was calculated and smoothed using a 3 × 3-pixel spatial average filter and a temporal filter 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. The VSDI signals presented in the images were smoothed using a 5 × 5 (spatial) × 3 (temporal) average filter.

2.3. Patch clamp experiments

For the patch clamp experiments, slices were transferred to the recording chamber, fixed using a platinum ring with nylon filaments, and continuously perfused with carbogen-saturated standard aCSF (5 ml/min, room temperature (20–22 °C)). CA1 pyramidal neurones were visualized by using infrared videomicroscopy (Zeiss, Oberkochen, Germany). Postsynaptic currents were recorded using standard whole-cell patch clamp technique. The intracellular solution contained the following (in mM): K-D gluconate 130, KCl 5, EGTA 0.5, $MgCl_2$ 2, HEPES 10, D-glucose 5 and Na-phosphocreatine 20, leading to an open-tip resistance of 4–6 MΩ when filled in the pipette. Lidocaine-N-ethyl-chloride (5 mM) was added to the intracellular solution in most of the experiments, except from the experiments, were action potential characteristics were investigated (Fig. 2). A discontinuous voltage clamp/current clamp amplifier (SEC 10L, NPI Electronic, Tamm, Germany) with switching frequencies of 60–80 kHz (25% duty cycle) was used for whole-cell discontinuous voltage clamp (dVC) recordings.

A bipolar tungsten electrode placed on the Schaffer collateral/associational commissural pathway delivered square-pulse stimuli (6–50 V, 50–200 μs; interstimulus interval 15 s) to evoke compound (PSCs), excitatory (EPSCs) and inhibitory postsynaptic currents (IPSCs). Before each stimulation pulse, neuronal input resistance was determined using a hyperpolarizing voltage step (−10 mV for 200 ms).

Postsynaptic currents (PSCs) of basal synaptic transmission were recorded at a holding potential of −70 mV without the addition of any specific receptor antagonist. To analyse the current-voltage relationship in current clamp mode, voltage was recorded after injection of current pulses (500 ms length) into the neurone (stepwise increased from −90 to +130 pA; increments of 10 pA).

For recordings of *N*-methyl-D-aspartate (NMDA) receptor-mediated EPSCs (NMDA-EPSCs), 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*] quinoxaline-7-sulfonamide (NBQX; 5 μM), 3-amino-propyl(diethoxymethyl)phosphinic acid (CGP35348, CGP; 200 μM; Novartis Laboratories, Basel, Switzerland) and bicuculline methiodide (20 μM) were added to the aCSF, and the holding potential was set to −40 mV. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated currents (AMPA-EPSCs) were recorded in the presence of D(−)-2-amino-5-phosphonopentanoic acid (AP5; 50 μM), CGP (200 μM) and bicuculline methiodide (20 μM) at a holding potential of −70 mV. $GABA_A$ receptor-mediated currents were recorded using an intracellular solution with a high chloride concentration (in mM: $CsCH_3SO_3$ 100, CsCl 60, HEPES 10, $MgCl_2$ 1, EGTA 0.2 and creatine phosphate 20; reversal potential for Cl^-: −19.7 mV) in the presence of the specific receptor antagonists NBQX (5 μM), CGP (200 μM) and AP5 (50 μM) in the aCSF. In this manuscript, these currents are termed inhibitory postsynaptic currents ($GABA_A$-IPSCs), although activation of $GABA_A$ receptors in this experimental setting (holding potential: −70 mV) causes an efflux of chloride ions, that ultimatively results in excitatory currents. 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).

If not stated otherwise, all of the salts and chemicals were obtained from Sigma Aldrich (Steinheim, Germany).

2.4. Statistical analysis

SPSS Statistics version 20 (SPSS®, Inc., Chicago, IL, USA) and GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA) were used for statistical evaluation. Regarding VSDI data, repeated measurement analysis of variance and a pairwise *t*-test with Dunnett´s adjustment were used to test for differences between the groups. Because of multiple pairwise comparisons, adjusted P-values, i.e., weighted P-values obtained from the *t*-tests, were used.

For evaluation of the patch clamp experiments, we first partitioned the recording time into equal subintervals of five minutes and then determined the averaged relative amplitude and decay in each subinterval. Data were then tested for normal distribution using the