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PHARMACOLOGY | RESEARCH ARTICLE

Catechins from green tea modulate neurotransmitter transporter activity in Xenopus oocytes

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Abstract: The GABAergic and glutamatergic systems play key roles in controlling activity of the central nervous system. Important membrane proteins in the mammalian central nervous system transporting extracellular GABA and glutamate are the GABA transporter GAT1 and the glutamate transporter EAAC1. We investigated the effect of catechins of green tea (*Camellia sinensis*) on the activity of GAT1 and EAAC1 by detecting the respective electrogenic transporter-mediated current under voltage clamp. Epigallocatechin-3-gallate inhibited GAT1-mediated current to 50% at about 100 µM. The EAAC1-mediated current could be stimulated up to 80% by (-)-epicatechin; 50% of maximum stimulation was achieved by about 5 µM. Inhibition of GAT1 and stimulation of EAAC1 will counteract hyperexcitability.

Subjects: Cell Biology; Neurobiology; Biophysics

Keywords: green tea; catechins; neurotransmitter transporter; voltage clamp

1. Introduction

The dominating inhibitory neurotransmitter in adult mammalian brain is γ-aminobutyric acid (GABA), and the dominating excitatory neurotransmitter is glutamate (Kandel, Schwartz, & Jessell, 2000). The GABAergic and glutamatergic systems play key roles in the occurrence of pathological conditions like epilepsy. To control synaptic transmission and concentration of extracellular neurotransmitter highly potent Na^+ gradient-driven transporters mediate the removal of respective extracellular neurotransmitter by uptake into presynaptic neurons or surrounding glial cells (Zhou & Danbolt, 2013). Transporters controlling extracellular concentrations of GABA and glutamate in the mammalian central nervous system are the GABA transporter 1 (GAT1) and the glutamate transporter EAAC1 (also named as EAAT3) (Bjørn-Yoshimoto & Underhill, 2016; Borden et al., 1994; Jensen, Chiu, Sokolova, Lester, & Mody, 2003; Lane et al., 2014). In addition to transporting glutamate, EAAC1

ABOUT THE AUTHORS

The focus of our research activities is the investigation of cellular events that might be involved in the effects of treatments by traditional Chinese medicine. This includes mechanisms of acupuncture in the periphery (acupuncture points) as well as in the central nervous system. In addition to acupuncture, effects of tradition Chinese herbal chemicals are tested. We investigate the modulation of activity of ion channels and carrier proteins by electrophysiological methods.

PUBLIC INTEREST STATEMENT

Chemicals extracted from plants used in traditional Chinese medicine have become an important source for effective drugs used in Western medicine. An example is the anti-malaria drug artemisinin extracted from *Artemisia annua* (woodworm), which was honoured by Nobel Prize to Youyou Tu in 2015 and formed the basis of modern anti-malaria drugs. In our work, we screened a number of chemicals extracted from herbs that are used in Chinese medicine to treat neuronal diseases such as epilepsy. We found that catechines from green tea have some potency to modulate neurotransmitter transporters that are essential in controlling brain activity.

mediates also neuronal uptake of cysteine and exerts critical neuroprotective function by interference with neuronal glutathione metabolism (Aoyama & Nakaki, 2013).

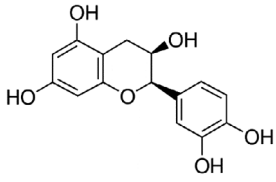
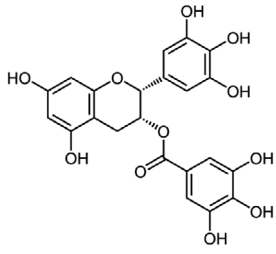
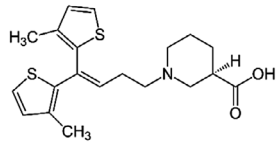
GAT1 utilizes the inward movement of 2 Na^+ ions and cotransport of 1 Cl^- ion to transport 1 GABA molecule into the cell. Therefore, the activity can be monitored by recording the electrogenic currents. Also, EAAC1 is an electrogenic transporter by coupling the uptake of 1 glutamate molecule to the inward movement of 3 Na^+ ions and 1 H^+ and the counter-transport of 1 K^+ ion.

For treatment of pathological conditions drugs have been designed to specifically modulate the function of a neurotransmitter transporter. For example, for treatment of epilepsy, drugs that increase the inhibitory synaptic activity by elevation of the concentration or dwell time of GABA in the synaptic cleft (see Löscher, 1998) became potent anti-epileptica. Tiagabine (TGB), a highly selective inhibitor of GAT1 that can inhibit the re-uptake of GABA, is one of these drugs (Borden et al., 1994; Schachter, 2001).

In analogy to inhibition of GAT1 and the associated elevation of the extracellular inhibitory GABA, stimulation of EAAC1 results in a reduction of the synaptically excitatory glutamate (see, e.g. Schwarz & Gu, 2003). Hence, drugs that inhibit GAT1 and stimulate EAAC1 will counteract hyperexcitability. It has been demonstrated that, e.g. stimulation of δ-opioid receptor inhibits GAT1-mediated current (Pu et al., 2012) and stimulates EAAC1-mediated current (Xia, Pei, & Schwarz, 2006), a mechanism that might contribute to pain suppression (Pu, Xu, & Schwarz, 2015; Schwarz & Gu, 2003; Yang et al., 2008).

Chinese medicine has become a promising source for the development of new drugs. This strategy has recently been recognized by Nobel Prize to Youyou Tu in 2015 (see Tu, 2011) for her discovery of artemisinin from *Artemisia annua* as an effective anti-malarial drug. In Asia, green tea consumption has a long history not only due to its flavour, it is also assumed to have potential health care benefits. Extracts of green tea (*Camellia sinensis*) have been used in Chinese medicine to prevent or treat various diseases (Cabrera, Artacho, & Giménez, 2006; Chen et al., 2011; Mereles & Hunstein, 2011), such as prostate cancer (Bettuzzi et al., 2006) and epilepsy (D'avila, Esteves Lopez, Patriarcha, & Araujo Restini, 2011; Noor, Mohammed, Khadrawy, Aboul Ezz, & Radwan, 2015; Xie et al., 2012); the effects had been attributed to the antioxidant effects of catechins. The most effective and most abundant component in the tea leaves is (-)-epigallocatechin-3-gallate (EGCG) (see Table 1). Although the effectiveness is controversially discussed (see e.g. Mereles & Hunstein, 2011; Mähler et al., 2013), interference of catechins from green tea with the GABAergic (Adachi, Tomonaga, Tachibana, Denbow, & Furuse, 2006) and glutamatergic system (Chou, Huang, Tien, & Wang, 2007)

Table 1. Relative effect of 40 or 50 μM (-)-epicatechin and EGCG on GAT1- and EAAC1-mediated current, respectively, and of 1 μM TGB on GAT1-mediated current at −100 mV with respect to the current in the absence of drug. Data represent averages ± SEM of *N* experiments

Current mediated by	(-)-Epicatechin	EGCG	Tiagabine
GAT1	1.00 ± 0.05	0.66 ± 0.08	0.34 ± 0.08
	(*N* = 3)	(*N* = 5)	(*N* = 3)
EAAC1	1.79 ± 0.10	1.06 ± 0.05	Ineffective
	(*N* = 5)	(*N* = 10)	
Structure			

could be demonstrated. Direct modulation of GABAA receptor as well as modulation of signalling pathways (e.g. (Hossain, Hamamoto, Aoshima, & Hara, 2002; Mandel, Weinreb, Amit, & Youdim, 2004) were described. In our investigation, we evaluated whether catechins might interfere with the activity of the neurotransmitter transporters, and hence could be involved in the neuroprotective effects of green tea extracts.

2. Materials and methods

2.1. Xenopus oocytes preparation and microinjection

Xenopus oocytes were used as expression system for the GABA transporter GAT1 and the glutamate transporter EAAC1 and as a model system to test the effects of drugs extracted from green tea. The procedure of oocyte preparation and microinjection of the respective cRNA were as described previously (Gu et al., 2010; Pu et al., 2012). For expression of GAT1 or EAAC1 14 ng of the respective cRNA was microinjected per oocyte. After incubation for 24–48 h, the oocytes were ready for the voltage-clamp experiments. Un-injected oocytes served as controls. In all our experiments we never could detect any GABA- or glutamate-induced responses in control oocytes. We like to point out that such tests are needed because occasionally batches of oocytes may show endogenous contributions (see, e.g. Steffgen et al., 1991).

2.2. Electrophysiological recording

To investigate the function of the neurotransmitter transporters, membrane currents were measured by conventional TEVC using Turbo TEC-03 with CellWorks software (NPI electronic, Tamm, Germany). Glass microelectrodes were filled with 3 M KCl, and balanced in ORi solution (see Solutions) for at least 30 min before recording. Steady-state current–voltage dependencies were determined by averaging membrane currents during the last 20 ms of 200 ms rectangular voltage pulses from −150 to +10 mV in 10 mV increments that were applied from a holding potential of −60 mV. Figure 1 shows original current traces in response to voltage pulses in the absence and presence of GABA (Figure 1(A)) and glutamate (Figure 1(B)), and the corresponding steady-state current–voltage dependencies. Transporter-dependent current was determined as the difference of total membrane current (see the lower traces in Figure 1) in the presence (middle traces) and absence (upper traces) of the respective neurotransmitter. The data were collected after analogue filtering at 100 or 300 kHz and analysed by Origin software (OriginLab Corp., USA). All experiments were performed at room temperature (about 25°C).

Figure 2 illustrates the protocol of a typical experiment for an oocyte with expressed GAT1. The holding current at −60 mV (Figure 2(A)) shows that perfusion of the oocyte chamber with solution containing different concentrations of GABA led to concentration-dependent increases of inward-directed currents. The difference of steady-state current in the presence and absence of GABA represents the GAT1-mediated current. To correct for possible drifts of current with time, the current in the absence of GABA was determined as the average of current in pure ORi before and after the application of GABA. The respective current–voltage dependencies of this experiment are shown in Figure 2(B). Corresponding protocols were also applied to measuring EAAC1-mediated currents using glutamate as activator.

2.3. Solutions and drugs

The composition of the standard bath solution (ORi) was (in mM): 90 NaCl, 2 KCl, 2 $CaCl_2$ and 5 MOPS (adjusted to pH 7.4 with Tris). All standard chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). EGCG (CAS 989-51-5, purity ≥98%) and (-)-epicatechin (CAS 490-46-0, purity ≥98%) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) or Seebio Biotech Co. Ltd. (Shanghai, China), respectively, and tiagabine (TGB, CAS 145821-59-6) from Biotrend Chemicals (Zurich, Switzerland). These chemicals were dissolved in dimethyl sulfoxide (DMSO) to prepare 10 mM stock solution, and diluted before the experiment to the desired concentration with the respective test solution. The final concentration of DMSO was always below 1%. The range of concentrations used in the experiments was based on preliminary screening to cover the range for 50% effects.

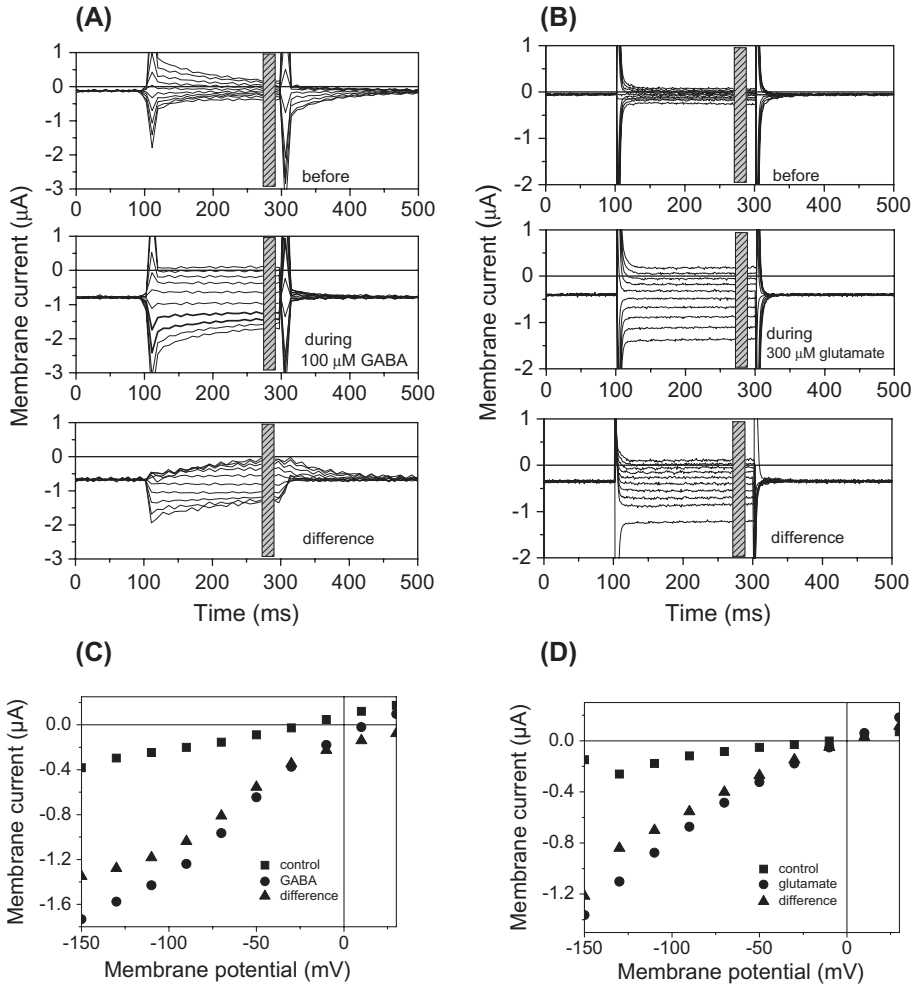


Figure 1. (A, B) Original current traces in response to rectangular voltage pulses. Pulses were applied from −150 to +30 mV in 20 mV increments. Top traces are in the absence, middle traces in the presence of 100 µM GABA (A) or 300 µM glutamate (B). Lower traces show the differences of currents in the presence and absence of drug, respectively. The hatched bars indicate the time period of 20 ms used to determine mean steady-state currents. (C, D) Steady-state current–voltage dependencies determined from the above traces.

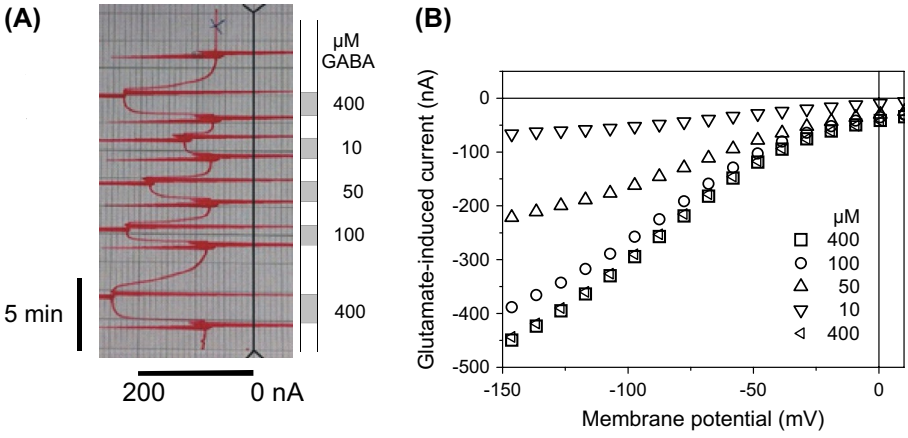


Figure 2. Protocol of a typical experiment. (A) Chart record of holding current at −60 mV illustrating the inward-directed current responses to different concentrations of GABA (deflections to the left). (B) Current–voltage dependencies of the respective GABA-induced steady-state currents determined as the difference of the currents in the presence and absence of GABA.

2.4. Data analysis

To estimate the concentration $K_{1/2}$ that is needed to obtain a 50% effect, we approximated the concentration dependence by a sigmoidal dependency using a Hill equation:

$$Y = Y_{max}\frac{[X]^n}{[X]^n + K_{1/2}^n} \quad (1)$$

with [*X*] representing the concentration of activator GABA or glutamate, or the concentration of the tested chemical, $K_{1/2}$ represents the concentration where *X* produces half-maximum effect, *n* is the Hill coefficient describing cooperativity of binding of the respective substrate. Averaged data are presented as means ± SEM and were considered as significantly different by Student's *t* test on the basis of $p < 0.05$.

3. Results

3.1. The effects of catechins on GABA transporter GAT1

As illustrated in Figures 1(A), (C) and 2, application of GABA induces inwardly directed current in oocytes with expressed GAT1. The dependency of current on GABA concentration at −100 mV (Figure 3) can be described by Equation (1); for 50% stimulation a $K_{1/2}$ value of 57.6 µM was obtained. Analysis of the concentration dependence at −60 and −150 mV revealed no significant difference in $K_{1/2}$ values. To test the effect of drugs on GAT1-mediated current an intermediate concentration of 50 µM close to the $K_{1/2}$ value was used in all experiments described below.

Since green tea extracts have been used in Chinese medicine for treatment of epilepsy (Noor et al., 2015; Xie et al., 2012), we tested in particular the dominating EGCG and (-)-epicatechin (see Table 1) with respect to their effects on the GAT1-mediated current. While 40 µM (-)-epicatechin exhibited no significant effect (Table 1), and even 500 µM was ineffective, 40 µM EGCG showed about 35% inhibition (Table 1, and Figure 4(A)). A more detailed analysis of the concentration dependency revealed 50% inhibition at about 100 µM EGCG at −100 mV (Figure 4(B)).

To compare the efficacy of EGCG with another potent GAT1 inhibitor that is successfully applied as antiepileptic drug, we determined the efficacy of TGB in inhibiting the GAT1-mediated current (Figure 4(C)), a $K_{1/2}$ value of 2.3 ± 0.8 µM was obtained (Figure 4(D)). These measurements confirm our earlier results from Eckstein-Ludwig, Fei and Schwarz (1999). TGB is by an order of magnitude more efficient in inhibiting GAT1 than EGCG, but nevertheless, this catechin may serve as a basis for the development of new antiepileptic drugs.

3.2. The effects of catechins on glutamate transporter EAAC1

Similar experiments as described above for the effects of the catechins on the GAT1-mediated currents were also performed for EAAC1-mediated currents, which were determined as glutamate-activated currents in oocytes with expressed EAAC1. The dependence of EAAC1-mediated current on glutamate concentration at −100 mV (Figure 5) can be described by Equation (1) with a $K_{1/2}$ value of 82 µM. Similar to the activation of GAT1 by GABA, analysis of glutamate dependency of EAAC1-mediated current at −60 and −150 mV revealed no significant difference in $K_{1/2}$ values. For analysing drug effects, EAAC1-mediated current was determined as current activated by 100 µM glutamate.

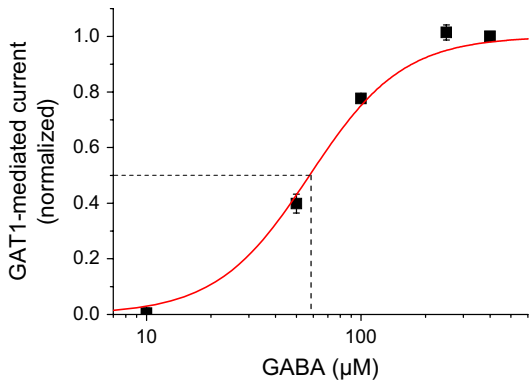


Figure 3. Dependence of GAT1-mediated current at −100 mV on GABA concentration. One corresponds to −415.7 ± 41.2 nA. Data point represent averages ± SEM from *N* = 5–11 oocytes. The line represents a fit of Equation (1) to the data with $K_{1/2}$ = 57.6 ± 3.8 µM (*n* = 2).

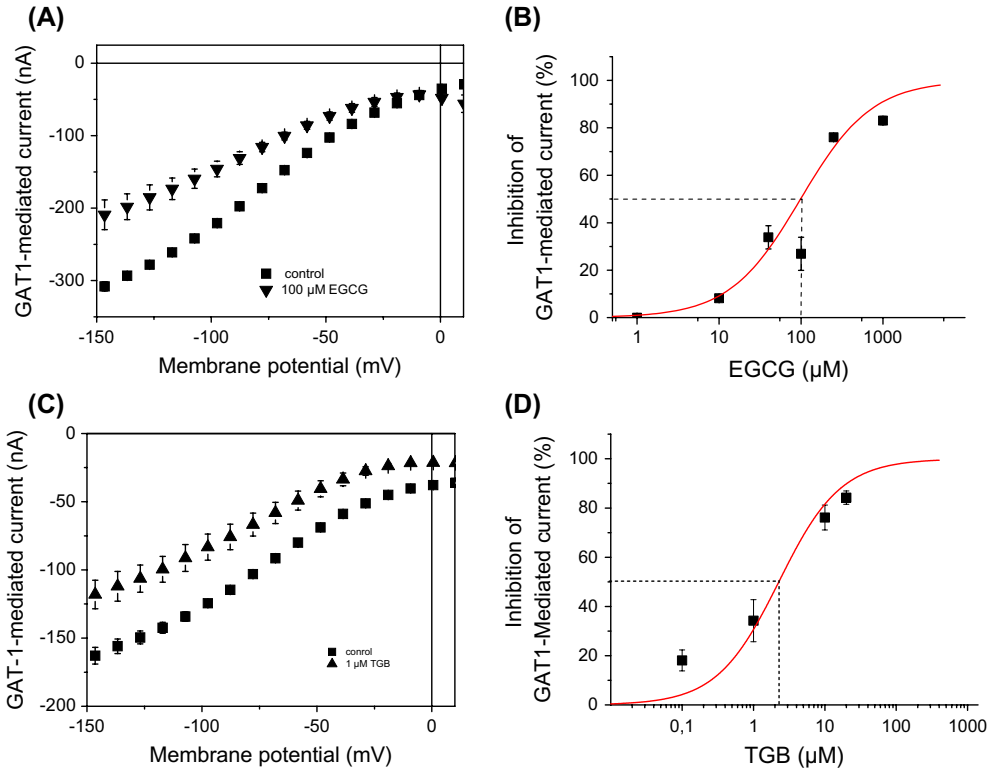


Figure 4. Effect of EGCG (A, B) and of TGB (C, D) on GAT1-mediated current (A, C) Voltage dependence of GAT1-mediated current (activated by 50 µM GABA) in the absence and presence of 100 µM EGCG (A: *N* = 3) and 1 µM TGB (B: *N* = 5). Dependence of the GAT1-mediated, steady-state current at −100 mV on EGCG (B) and TGB (D) concentration. 100% corresponds to −220 ± 11 nA (B) and −148 ± 17 (D). Data point represent averages ± SEM from *N* = 3 (B) and *N* = 5 (D) oocytes. The solid line represents a fit of Equation (1) to the data with $K_{1/2}$ values of 99 ± 14 µM (*n* = 1) (B) and 2.3 ± 0.8 µM (*n* = 1) (D).

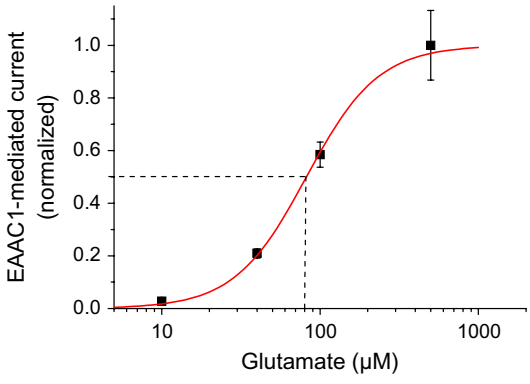


Figure 5. Dependence of EAAC1-mediated current at −100 mV on glutamate concentration. One corresponds to 570 ± 75 nA. Data point represent averages ± SEM from *N* = 7 oocytes. The line represents a fit of Equation (1) to the data with $K_{1/2}$ = 82.0 ± 2.9 µM (*n* = 1.9).

The effects of the two catechins on EAAC1 were opposite to those on GAT1. EGCG did not exhibit any effect on the EAAC1-mediated current, while (-)-epicatechin clearly affected the transporter. In fact, EAAC1-mediated current at −100 mV even became stimulated by about 80% by 50 µM (-)-epicatechin (see Figure 5(A) and compare values in Table 1). A more detailed analysis of the concentration dependency revealed 50% of maximum stimulation at about 5 µM of the (-)-epicatechin (Figure 5(B)). The stimulatory effects seemed to saturate at about 80%.

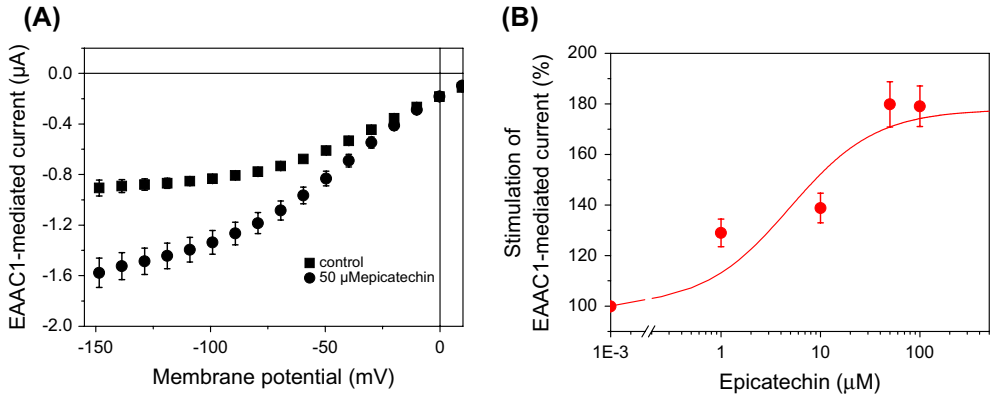


Figure 6. Effect of epicatechin on EAAC1-mediated current (A) Voltage dependence of EAAC1-mediated current (activated by 100 µM glutamate) in the absence and presence of 50 µM epicatechin. (B) Dependence of the EAAC1-mediated current at −100 mV on (-)-epicatechin concentration. 100% corresponds to 407 ± 92 nA. Data point represent averages ± SEM from *N* = 5 oocytes. The solid line represents a fit of Equation (1) to the data with $K_{1/2}$ = 4.9 + 2.6 µM and maximum stimulation of 79 + 9% (*n* = 1).

4. Discussion

To investigate modulations of activity of GABA transporter GAT1 and glutamate transporter EAAC1, we determined the currents induced by 50 µM GABA and 100 µM glutamate, respectively. These concentrations were close to the $K_{1/2}$ values of 57.6 µM for GAT1 and 82 µM for EAAC1.

Our results have shown that catechins extracted from green tea may indeed contribute to reported anti-epileptic effects (D'avila et al., 2011; Noor et al., 2015; Xie et al., 2012) by interfering with neurotransmitter transporters. The established anti-epileptic drug TGB exerts it effect as a specific inhibitor of GAT1. The catechin EGCG also inhibits the GABA-uptake activity of GAT1, which would favour inhibitory synaptic activity. Epicatechin, on the other hand, stimulates glutamate-uptake activity of EAAC1, which would reduce excitatory synaptic activity via reducing glutamate concentration in the synaptic cleft. Since EAAC1 also mediates uptake of cysteine, the stimulation may also promote neuroprotection via glutathione metabolism (Aoyama & Nakaki, 2013).

To achieve micromolar concentrations of EGCG or epicatechin in the human plasma, which are necessary for inhibition of GAT1 or EAAC1, respectively, a consumption of 50–60 cups of green tea would be necessary (Chow et al., 2005). Although it is not realistic to treat neuronal disorder like epilepsy by drinking tea, concentrated extracts may have stabilizing potency via the inhibition of GAT1 and stimulation of EAAC1. In addition, bioavailability of catechins is restricted due to metabolism (Yong Feng, 2006), and catechins may therefore form the basis of drug with higher bioavailability for modulating neurotransmitter transporters.

5. Conclusion

Our data demonstrate that catechins extracted from green tea can interfere with the activity of neurotransmitter transporters, EGCG inhibits GAT1 and (-)-epicatechin stimulates EAAC1, which may form the basis for the development of new drugs that exert their effects through interference with the neurotransmitter transporters.

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Competing Interests
The authors declare no competing interest.

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