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BRAIN RESEARCH

Research Report

Anxiolytic properties of green tea polyphenol (–)-epigallocatechin gallate (EGCG)

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ARTICLE INFO

Article history:
Accepted 19 June 2006
Available online 21 July 2006

Keywords:
$GABA_A$ receptor
Anxiolytic
Flavonoid
Electrophysiology
Behavior

ABSTRACT

Naturally occurring polyphenols are potent antioxidants. Some of these compounds are also ligands for the $GABA_A$ receptor benzodiazepine site. This feature endows them with sedative properties. Here, the anxiolytic activity of the green tea polyphenol (–)-epigallocatechin gallate (EGCG) was investigated after acute administration in mice, using behavioral tests (elevated plus-maze and passive avoidance tests) and by electrophysiology on cultured hippocampal neurons. Patch-clamp experiments revealed that EGCG (1–10 μM) had no effect on GABA currents. However, EGCG reversed $GABA_A$ receptor negative modulator methyl β-carboline-3-carboxylate (β-CCM) inhibition on GABA currents in a concentration dependent manner. This was also observed at the level of synaptic $GABA_A$ receptors by recording spontaneous inhibitory synaptic transmission. In addition, EGCG consistently inhibited spontaneous excitatory synaptic transmission. Behavioral tests indicated that EGCG exerted both anxiolytic and amnesic effects just like the benzodiazepine drug, chlordiazepoxide. Indeed, EGCG in a dose-dependent manner both increased the time spent in open arms of the plus-maze and decreased the step-down latency in the passive avoidance test. $GABA_A$ negative modulator β-CCM antagonized EGCG-induced amnesia. Finally, state-dependent learning was observable after chlordiazepoxide and EGCG administration using a modified passive avoidance procedure. Optimal retention was observed only when animals were trained and tested in the same state (veh–veh or drug–drug) and significant retrieval alteration was observed in different states (veh–drug or drug–veh). Moreover, EGCG and chlordiazepoxide fully generalized in substitution studies, indicating that they induced indistinguishable chemical states for the brain. Therefore, our data support that EGCG can induce anxiolytic activity which could result from an interaction with $GABA_A$ receptors.

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doi:10.1016/j.brainres.2006.06.062

1. Introduction

Green tea polyphenol, epigallocatechin-3-gallate (or EGCG), is one of the most potent dietary antioxidant. Indeed, its protective effect against oxygenated free radicals (ROS)-mediated cellular damages is now widely recognized. Its preventative activity against oxidative stress has made EGCG a popular human food additive in various preparations. In the central nervous system, the neuroprotective action of EGCG has been described both in vitro and in vivo. For instance, EGCG could limit neuronal loss mediated by oxidative stress in cellular and animal models of neurodegenerative diseases, such as Parkinson's disease (Levites et al., 2001; Mandel et al., 2004; Mercer et al., 2005). Bioavailability studies in mice indicated that EGCG crosses the blood–brain barrier (Suganuma et al., 1998). Indeed, it is of course a requirement for any antioxidant or its metabolites to reach neuronal cells in order to induce their protection against oxidative damages (Gilgun-Sherki et al., 2001). Therefore, EGCG and its related flavonoid congeners may represent potential therapeutic agents to slow-down or prevent the progression of neurodegenerative pathologies related to oxidative stress (Floyd and Hensley, 2002; Gilgun-Sherki et al., 2002). Along to its ROS scavenging activity, modulatory actions of EGCG on synaptic transmission have been described. Indeed, EGCG may increase presynaptic release of acetylcholine in myenteric neurons (Katayama et al., 2002). Moreover, EGCG has been reported to act as an antagonist of glutamate AMPA receptor-mediated responses (Bae et al., 2002). Therefore, the beneficial neuroprotective actions of EGCG could also be due to a limitation of glutamate-mediated excitation on neurons. Another way to limit excitation can be obtained by increasing GABA-mediated inhibition. In this respect, naturally occurring flavonoids related to EGCG, such as apigenin, quercetin or chrysin, have been reported as potential ligands of benzodiazepine $GABA_A$ receptor site (Medina et al., 1997; Marder and Paladini, 2002). However, the sedative activity of some of these compounds does not appear to be correlated with a positive allosteric modulation of $GABA_A$ receptor since these compounds rather have a negative modulatory action on $GABA_A$ receptor-associated responses (Goutman et al., 2003). By contrast, the flavone hispidulin displays a positive allosteric activity on $GABA_A$ receptor correlated with an anticonvulsant activity as recently reported (Johnston and Beart, 2004; Kavvadias et al., 2004). Therefore, the anxiolytic action of flavonoids could be due to different types of modulations of $GABA_A$ receptor activity. In this respect, it has been reported that low concentrations of EGCG could enhance the potentiating effect of benzodiazepine on $GABA_A$-receptor-mediated currents (Campbell et al., 2004). In addition, a recent report suggests that EGCG reduces stress-mediated effects via a modulation of the GABAergic system (Adachi et al., 2006). Taken together, these two reports suggest that EGCG could interact with $GABA_A$ receptors. Therefore, in the present study, we have evaluated whether by itself EGCG could have anxiolytic and amnesic properties using behavioral tests and whether these properties could account for a functional modulation of $GABA_A$ receptors by recording $GABA_A$ receptor-mediated whole-cell currents in cultured hippocampal neurons. The behavioral activity of EGCG was compared to that elicited by a 'conventional' benzodiazepine drug, chlordiazepoxide.

2. Results

2.1. $GABA_A$ currents

In an attempt to examine a possible interaction of EGCG with native $GABA_A$ receptors, $GABA_A$-receptor-mediated currents were recorded in cultured hippocampal neurons after 15 days in vitro. GABA elicited currents in a concentration-dependent fashion with an EC_{50} of 4.0±0.2 μM (n=3), obtained by fitting data with the equation $y=ymin+(ymax-ymin)/(1+(x/EC50)$ Hill slope) using Sigmaplot 9.0 software (Jandel Scientific). $GABA_A$ currents obtained by applying 10 μM GABA reversed at −64±2 mV (n=4). For further pharmacological experiments, $GABA_A$ currents were obtained by applying 10 μM GABA on neurons voltage-clamped at 0 mV. We verified that picrotoxin (25 μM) effectively antagonized (Fig. 1A) and benzodiazepine agonist chlordiazepoxide (10 μM) potentiated these currents (Fig. 1B). Under these experimental conditions, EGCG (10 μM) by itself did not elicit any current in neurons (Fig. 1C). When applied concomitantly to GABA, EGCG had no detectable effect on GABA currents (n=10). However, we observed that EGCG could reverse significantly in a concentration-dependent manner the inhibition of GABA currents induced by benzodiazepine negative modulator β-carboline (β-CCM) ($F(4,42)=42.14$; $P<0.0001$). In the presence of β-CCM (10 μM), GABA currents were significantly reduced to 75±2% of control GABA currents (n=12; $P<0.0001$) (Figs. 1C–E). It is interesting to note that this value was close to that obtained for another negative benzodiazepine agonist methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) when tested at 10 μM on acutely dissociated CA1 hippocampal neurons (Tietz et al., 1999). In the presence of EGCG (from 0.1 to 10 μM) a concentration-dependent reversal of the β-CCM-induced GABA currents inhibition could be observed. In these experiments, no effect of EGCG could be detected at 0.01 μM. On average, GABA+β-CCM elicited a current which amplitude was 85±3% (n=6), 86±2% (n=5) and 99±2% (n=10) of control GABA current in the presence of 0.1, 1 and 10 μM EGCG, respectively. No significant difference could be found between currents obtained by GABA application and those elicited by the application of GABA+β-CCM+EGCG 10 μM (P=0.7923). Chlordiazepoxide induced enhanced of GABA current to 168±11% of control (n=5). In the presence of 10 μM EGCG, this value was 167±10% of control (n=5), not significantly altered (P=0.7488).

2.2. Spontaneous postsynaptic currents

Next, we examined whether the antagonism of EGCG on β-CCM-induced inhibition was also observed at the level of synaptic $GABA_A$ receptors. For this purpose, spontaneous inhibitory currents (sIPSCs) were recorded in hippocampal neurons. By itself EGCG at 1–10 μM had no effect on sIPSC (data not shown). However, the reduction of sIPSC amplitude induced by β-CCM (10 μM) was greatly attenuated by the further application of EGCG (10 μM) (Fig. 2A). On average, sIPSC amplitude was reduced to 55±6% of control (n=3) and was 98±7% in the