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European Journal of Pharmacology 523 (2005) 67–70
www.elsevier.com/locate/ejphar

Short communication

Differential G-protein coupling to $GABA_B$ receptor in limbic areas of alcohol-preferring and -nonpreferring rats

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Received 27 June 2005; received in revised form 6 September 2005; accepted 8 September 2005
Available online 13 October 2005

Abstract

The function of the γ-aminobutyric $acid_B$ ($GABA_B$) receptor, measured as baclofen-stimulated [^{35}S]GTPγS binding, was evaluated in some brain regions of Sardinian alcohol-preferring (sP) and -nonpreferring (sNP) rats. EC_{50} value of baclofen-stimulated [^{35}S]GTPγS in limbic areas was approximately 125% higher in alcohol-naive sP than sNP rats; voluntarily consumed alcohol reduced the EC_{50} value to a level similar to that of alcohol-naive sNP rats. These results suggest the presence of a genetically determined lower function of the $GABA_B$ receptor in limbic areas of sP than sNP rats; this differential functioning of the $GABA_B$ receptor may contribute to the opposite preference for alcohol in these rat lines.

Keywords: $GABA_B$ receptor; [^{35}S]GTPγS binding; voluntary alcohol consumption; (sP) Sardinian alcohol-preferring and (sNP) Sardinian alcohol-nonpreferring (Rat)

1. Introduction

In recent years, accumulating lines of evidence have suggested that γ-aminobutyric $acid_B$ ($GABA_B$) receptor agonists, including the prototype baclofen, effectively reduce alcohol consumption in selectively bred Sardinian alcohol-preferring (sP) rats. Specifically, the repeated administration of baclofen suppressed the acquisition and maintenance of alcohol drinking behavior in sP rats exposed to the "alcohol vs water" 2-bottle choice regimen. Further, acute baclofen suppressed (a) the transient increase in alcohol consumption in sP rats after a period of alcohol deprivation (a model of the alcohol relapse in humans), and (b) the motivational properties of alcohol, measured by the extinction responding procedure, in sP rats (see Colombo et al., 2004). Together, these data suggest a role for the $GABA_B$ transmission in the control of alcohol consumption and appetitive properties of alcohol in sP rats.

The apparent consistency of the suppressive effect of baclofen on different aspects of alcohol drinking behavior in sP rats suggests that these rats may constitute a proper animal model for further investigation of the possible relationship between excessive alcohol drinking and the $GABA_B$ receptor function. Accordingly, the present study was designed to investigate: (a) the possible presence of genetically determined differences in the $GABA_B$ receptor function between sP rats and their alcohol-nonpreferring counterpart [Sardinian alcohol-nonpreferring (sNP) rats]; (b) whether voluntarily consumed alcohol in sP rats altered the function of the $GABA_B$ receptor system.

To possibly achieve these aims, baclofen-stimulated guanosine 5′-*O*-(3-[^{35}S]thiotriphospate) ([^{35}S]GTPγS) binding—a measure of the function of the $GABA_B$ receptor—was assessed in cortex, hippocampus, and limbic areas of alcohol-naive sP, alcohol-naive sNP, and alcohol-experienced sP rats.

2. Materials and methods

2.1. Animals and alcohol drinking procedure

Male sP ($n=21$) and sNP ($n=10$) rats, from the 59th generation, were used. Rats were individually housed in an animal facility under standard environmental conditions. Standard rat chow was always available.

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0014-2999/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2005.09.011

At the age of 75 days, sP rats were divided into two groups (n=10–11). One rat group continued to have unlimited access (24 h/day) to water as the sole fluid available (alcohol-naive rats). Rats of the second group were continuously (24 h/day) offered 2 bottles containing alcohol (10% v/v, in water) and water, respectively (alcohol-experienced rats). Rats were maintained under the 2-bottle choice regimen for 28 consecutive days. Daily alcohol intake averaged approximately 6 g/kg. The alcohol bottle was removed 12 h before sacrifice. Rats of the sNP line were kept under the same regimen of alcohol-naive sP rats.

The experimental procedures employed in the present study were in accordance with the Italian Law on the "Protection of animals used for experimental and other scientific reasons".

2.2. [^{35}S]GTPγS binding assay

Cerebral cortices, hippocampus and limbic areas (specifically olfactory tubercles, nucleus accumbens, and septal nuclei) were dissected according to Glowinski and Iversen (1966) and processed as previously described (Castelli et al., 2003). The final membrane pellet was frozen and stored at −80°C until use. The Bradford protein assay (Bradford, 1976) was used for protein determination, according to the protocol of the supplier (Bio-Rad, Milan, Italy).

[^{35}S]GTPγS binding assay was performed as previously described (Castelli et al., 2003). Membranes were incubated on ice for 1 h and then centrifuged at 4 °C for 15 min at 20,000 ×g. The pellet was resuspended in GTPγS buffer (50 mM Tris–HCl pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 1.8 mM $CaCl_2$) to a final concentration of 5–10 μg protein. Membrane homogenates and drugs were preincubated in the presence of 30 μM GDP for 30 min at 30 °C. [^{35}S]GTPγS (1250 Ci/mmol) (0.2 nM; NEN, Boston, MA, USA) was added. After 40 min incubation, samples were filtered using a Packard Unifilter-GF/B, and radioactivity on the filters was counted by TopCount NXT (Packard, Meridien, CT, USA).

Non-specific binding was measured in the presence of unlabeled GTPγS (10 μM; Sigma/RBI, St. Louis, MO, USA) (Kushner and Unterwald, 2001; Galvez et al., 2000). Basal binding was assayed in the absence of agonist and in the presence of GDP. Concentration effect curves were determined by incubating membranes with increasing concentrations of baclofen (from 0.1 μM to 1.0 mM) in the presence of 0.2 nM [^{35}S]GTPγS and 30 μM GDP. The stimulation by agonist was defined as a percentage increase above basal levels.

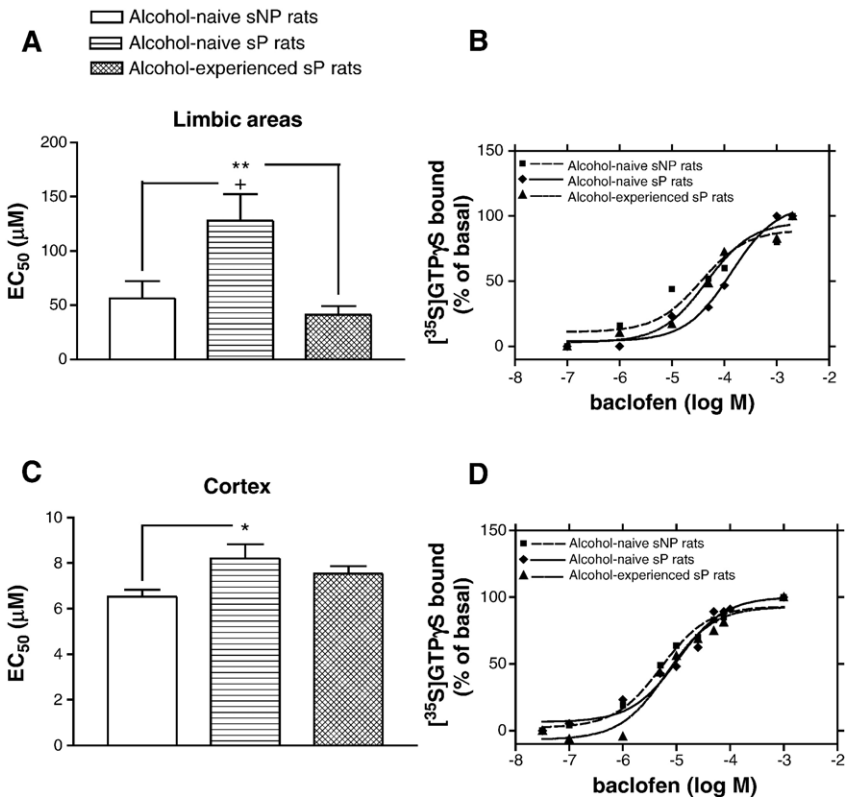

Fig. 1. EC_{50} value in limbic areas (A) and cortex (C) of alcohol-naive sNP, alcohol-naive sP, and alcohol-experienced sP rats. Data are the mean±S.E.M. of n=7 (limbic areas) and n=11 (cortex) assayed in triplicate. +: P<0.05; **: P<0.01 (Newman–Keuls test); *: P<0.05 (Newman–Keuls test). Baclofen concentration–effect curves for the $GABA_B$ receptor-mediated stimulation of [^{35}S]GTPγS binding to limbic areas (B) and cortex (D). Basal binding is defined as 100% on the y axis. The data represent a typical experiment out of three independent experiment from three different rats. (B) EC_{50}=36.5, 135 and 45.7 μM for alcohol-naive sNP, alcohol-naive sP, and alcohol-experienced sP rats, respectively. E_{max}=125%, 130%, 129% for alcohol-naive sNP, alcohol-naive sP, and alcohol-experienced sP rats, respectively. (D) EC_{50}=5.3, 10 and 6.6 μM for alcohol-naive sNP, alcohol-naive sP, and alcohol-experienced sP rats, respectively. E_{max}=147%, 156%, 148% for alcohol-naive sNP, alcohol-naive sP, and alcohol-experienced sP rats, respectively.

Non-linear regression analysis of concentration–response data was performed using Prism 3.0 software (GraphPad Prism Program, GraphPad, San Diego, CA, USA) to calculate E_{max} (maximal stimulation of baclofen over basal levels) and EC_{50} (concentration of baclofen necessary to obtain 50% of the maximal effect) value. Data are reported as mean±S.E.M. of $n=7–11$ experiments, performed in triplicate. Data were statistically evaluated by one-way analysis of variance followed by the Newman–Keuls test for multiple comparisons.

3. Results

Basal levels of [^{35}S]GTPγS binding did not significantly differ in any selected brain region of the three rat groups (data not shown). Baclofen stimulated [^{35}S]GTPγS binding in a concentration-dependent manner in cortex, hippocampus, and limbic areas in all rat groups. EC_{50} value of baclofen-induced stimulation of [^{35}S]GTPγS binding significantly differed among the three rat groups in limbic areas [$F(2,20)=7.04$, $P<0.01$] (Fig. 1, top panel) and cortex [$F(2,30)=3.51$, $P<0.05$] (Fig. 1, bottom panel). Specifically, EC_{50} value was significantly higher, by approximately 125% and 25%, in limbic areas and cortex, respectively, in alcohol-naive sP than sNP rats.

Voluntarily consumed alcohol produced a decrease, by approximately 70%, in the EC_{50} value in limbic areas (alcohol-experienced vs alcohol-naive sP rats) (Fig. 1, top panel). In cortex, alcohol-induced reduction of the EC_{50} value averaged approximately 10% and did not reach statistical significance (Fig. 1, bottom panel).

No difference was recorded in the EC_{50} value in hippocampus (alcohol-naive sNP, alcohol-naive sP, and alcohol-experienced sP rats: 94.7±12.3, 93.3±24.4, and 88.8±14.7 μM, respectively).

Finally, E_{max} value of baclofen-induced stimulation of [^{35}S]GTPγS binding did not differ among the three rat groups in either cortex, hippocampus, or limbic areas. Specifically, E_{max} value of baclofen-induced stimulation of [^{35}S]GTPγS binding were: (a) 154.5±3.4%, 154.4±2.7%, and 156.4±4.9% in cortex of alcohol-naive sNP, alcohol-naive sP, and alcohol-experienced sP rats; (b) 151.2±2.4%, 151.0±3.3%, and 149.8±3.1% in hippocampus of alcohol-naive sNP, alcohol-naive sP, and alcohol-experienced sP rats; (c) 128.7±2.9%, 139.9±6.6%, and 137.0±3.9% in limbic areas of alcohol-naive sNP, alcohol-naive sP, and alcohol-experienced sP rats.

4. Discussion

The results of the present study indicate that the function of the $GABA_B$ receptor differed between alcohol-naive sP and sNP rats in limbic areas—i.e. the brain regions primarily deputed to mediation of the rewarding properties of addictive drugs, including alcohol (see Weiss and Porrino, 2002)—and, to a much lower extent, in cortex. Specifically, EC_{50} value of baclofen-stimulated [^{35}S]GTPγS in limbic areas was more than two times higher in sP than sNP rats, suggesting a lower function of the $GABA_B$ receptor in alcohol-naive sP than sNP rats. This difference is genetically determined, since these rats were not exposed to alcohol before sacrifice.

The decreased potency of baclofen to stimulate [^{35}S]GTPγS in alcohol-naive sP rats might be due to a desensitization of $GABA_B$ receptors, which would reflect differences in G-protein activation; specifically, G-proteins might be reduced in their number or changed in their conformational structures, altering the ability of G-proteins to bind [^{35}S]GTPγS. Since in this initial work we did not investigate $GABA_B$ receptor density and affinity, we can not currently exclude that the observed differences in EC_{50} value of baclofen-stimulated [^{35}S]GTPγS might reflect a decreased receptor affinity.

These results also indicate that alcohol, voluntarily consumed by sP rats under the 2-bottle choice for 4 consecutive weeks, reduced the EC_{50} value of baclofen-stimulated [^{35}S]GTPγS to a level similar to that of alcohol-naive sNP rats. The repeated administration of the $GABA_B$ receptor agonists, baclofen and CGP 44532, has recently been found to suppress the acquisition of alcohol drinking behavior in alcohol-naive sP rats exposed to the 2-bottle choice regimen (see Colombo et al., 2004). Taking into account the results of the present study, the suppressing effect of baclofen and CGP 44532 on acquisition of alcohol drinking behavior in sP rats may be explained as the repeated stimulation of the $GABA_B$ receptor, exerted by the agonists, producing an effect on $GABA_B$ receptor function similar to that of voluntarily consumed alcohol. Should this hypothesis be correct, baclofen- and CGP 44532-induced changes on $GABA_B$ receptor function would substitute for those produced by voluntarily consumed alcohol, making alcohol consumption less urgent and, in turn, suppressing alcohol drinking.

Several lines of evidence suggest that $GABA_B$ receptors are involved in the neural substrate mediating anxiety-related behaviors (see Cryan and Kaupmann, 2005). Previous studies found that alcohol-naive sP rats displayed a higher degree of anxiety-related behaviors when compared to sNP rats (Colombo et al., 1995; Richter et al., 2000; Cagiano et al., 2002). Accordingly, it may be hypothesized that the observed lower function of the $GABA_B$ receptor in sP than sNP rats may contribute to the development of these anxiety-related behaviors in sP rats. Consistently, baclofen-induced suppression of alcohol drinking behavior in sP rats might be secondary to the substitution of its anxiolytic effect for that of voluntarily consumed alcohol.

Acknowledgements

The authors are grateful to Ms. Anne Farmer for language editing of the manuscript.

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