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Rapid communication

Altered response to benzodiazepine anxiolytics in mice lacking $GABA_{B(1)}$ receptors

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Abstract

Recently, we demonstrated that mice lacking the $GABA_{B(1)}$ subunit were more anxious than wild-type animals in several behavioural paradigms, most notably in the light–dark test. In an attempt to assess the effects of classical benzodiazepine anxiolytics on anxiety-like behaviour observed in these mice, animals were administered either chlordiazepoxide (10 mg/kg, p.o.) or diazepam (7.5 mg/kg, p.o.) prior to testing in the light–dark box. Surprisingly, in contrast with the wild-type mice, neither benzodiazepines decreased anxiety-like behaviour in $GABA_{B(1)}^{-/-}$ mice. These data suggest that targeted deletion of $GABA_{B(1)}$ subunit alters $GABA_A$ receptor function in vivo.

Keywords: Anxiety; $GABA_B$; Anxiolytics

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system (CNS) and plays a key role in many physiological and psychological processes. There are two different classes of GABA receptors: ionotropic $GABA_A$ receptor and metabotropic $GABA_B$ receptor. The $GABA_B$ receptor is a heterodimer made up of two subunits, $GABA_{B(1)}$ and $GABA_{B(2)}$, both necessary for $GABA_B$ receptors to be functionally active. Although, it is well known that GABA is involved in the pathophysiology of anxiety, the role of the $GABA_B$ receptor in anxiety is still unclear. The recently generated $GABA_{B(1)}$ knockout mice offer a new and useful tool to elucidate the role of $GABA_B$ receptors in psychiatric disorders. We recently demonstrated that targeted deletion of $GABA_{B(1)}$ subunit increased anxiety in the light–dark box (decreased number of transitions). In an attempt to understand the processes involved in this hyperanxious phenotype, and to assess whether these anxious mice may represent a model to assess anxiolytic activity, we administered classical benzodiazepine anxiolytics, ligands of $GABA_A$, to wild-type and $GABA_{B(1)}^{-/-}$ mice (Fig. 1).

The $GABA_{B(1)}^{-/-}$ mice were generated on a BALB/c background as described previously (Schuler, 2001). Both male and female animals (8–12 weeks old) were used in this age- and sex-matched study (~equal in number). Mice were housed in groups of one to three at room temperature on a 12-h light–dark cycle (lights on 06:00) with food and pellets available ad libitum. The light–dark box test was carried out as described previously (Mombereau et al., 2004). Briefly, the apparatus consisted of a clear plexiglass cage (44×21×21 cm) separated into two compartments by a partition, which had a small opening (12×5 cm) at floor level. The open compartment was open topped made of transparent plexiglass and brightly illuminated by a 60-W desk lamp overhead (approximately 1000 Lux). The smaller compartment was 14 cm long and the cover on top all made from black plexiglass. Mice were injected with chlordiazepoxide (10 mg/kg, p.o.) or diazepam (7.5 mg/kg, p.o.) or methycellulose, 1 h prior to behavioural testing. Doses were selected from previous data showing robust anxiolytic effects at these doses in normal mice (Cryan, unpublished observations). Subsequently, mice were individually placed in the centre of the brightly lit

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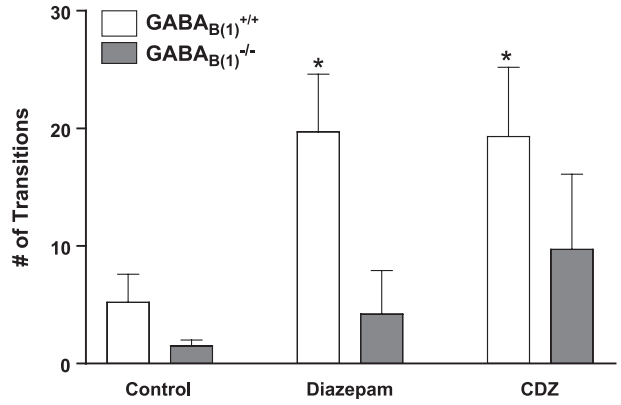

Fig. 1. Blunted anxiolytic effect of benzodiazepines in GABA$_{B(1)}^{-/-}$ mice. Acute administration of diazepam (7.5 mg/kg, p.o.) and chlordiazepoxide (CDZ, 10 mg/kg, p.o.) increased the number of transitions in GABA$_{B(1)}^{+/+}$ mice. n=10–11 per treatment group. These two treatments did not affect the number of transitions in GABA$_{B(1)}^{-/-}$ mice. n=10–11 per treatment group. All bars represent mean values with vertical lines indicating 1 S.E.M. *Groups that differed significantly to vehicle-treated GABA$_{B(1)}^{+/+}$ mice (P<0.05).

compartment, facing away from the partition and allowed to freely explore the apparatus for 10 min. The apparatus was cleaned thoroughly between subjects. The number of light–dark transitions, time spent in the light compartment, and latency to enter dark were recorded by a trained observer, transitions being the most reliable indicator of anxiety-like behavior in the test (Crawley and Davis, 1982; Holmes, 2001). The results were statistically evaluated using two-way Analysis of Variance (ANOVA), which was then followed by appropriate Fisher's post hoc test. As previously reported (Mombereau et al., 2004), many GABA$_{B(1)}^{-/-}$ mice froze upon being placed in the apparatus (30% in vehicle-treated animals), which was not decreased by drug treatment. In addition, as previously described, GABA$_{B(1)}^{-/-}$ mice displayed lower number of transitions than their wild-type counterparts, although this did not reach statistical significance on this occasion (P=0.16). On examination of the number of transitions, ANOVA revealed an effect of genotype [$F(1,58)$=6.623, P=0.013], treatment [$F(2,58)$=3.283, P=0.045], but no genotype×treatment interaction [$F(2,58)$=0.829, P=0.442]. Post hoc analysis indicated that diazepam or chlordiazepoxide increased significantly the number of transitions in wild-type mice, but this effect was markedly blunted in GABA$_{B(1)}^{-/-}$ mice.

This study demonstrated that the selective ablation of GABA$_{B(1)}$ subunit abolished the response to benzodiazepines in the light–dark box test. This suggests a functional relationship between both GABA$_B$ and GABA$_A$ receptors in vivo. Further studies are required to assess if the absence of anxiolytic effect are caused by alterations in benzodiazepine binding and/or GABA$_A$ subunit expression, as is the case with the 5-HT$_{1A}$ receptor knockout (Sibille et al., 2000). Furthermore, we cannot exclude that this lack of efficiency of chlordiazepoxide and diazepam is due to an interaction between loss of GABA$_B$ receptor function and the background strain employed (Lepicard et al., 2000). In summary, given the fact that classical anxiolytics are not able to reduce anxiety in GABA$_{B(1)}^{-/-}$ mice, this may not be a useful model to assess the anxiolytic potential of novel compounds or to assess the in vivo selectivity of novel anxiolytics acting at GABA$_B$ receptors such as GABA$_B$ receptor-positive modulators (Mombereau et al., 2004).

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