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Short communication

Chronic zolpidem treatment alters $GABA_A$ receptor mRNA levels in the rat cortex

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Abstract

The effect of chronic zolpidem treatment on the steady-state levels of γ-aminobutyric $acid_A$ α1–6, β1–3 and γ1–3 subunit mRNAs in rat cortex has been investigated. Male Sprague-Dawley rats were injected once daily, for 7 or 14 days, with 15 mg/kg of zolpidem in sesame oil vehicle. The levels of the α4 and β1 subunit mRNAs were significantly increased after 7 days of treatment and the level of α1 subunit mRNA was significantly decreased after 14 days of treatment, as determined by solution hybridization. These results are compared to the previously determined effects of an equivalent schedule of treatment with diazepam. © 1997 Elsevier Science B.V.

Keywords: Benzodiazepine tolerance; Gene expression; Coordinate gene regulation

1. Introduction

Zolpidem is a hypnotic drug which produces its overt effects by interaction with the benzodiazepine allosteric site on the γ-aminobutyric acid type A ($GABA_A$) receptor (Sieghart, 1995). Zolpidem and the classical benzodiazepines differ in their $GABA_A$ receptor subtype selectivity. Specifically, recombinant receptor studies have shown that while the classical benzodiazepines have a similar affinity for α1, α2, α3 or α5 subunit-containing $GABA_A$ receptors (Pritchett et al., 1989; Faure-Halley et al., 1993), zolpidem binds with highest affinity to α1 subunit-containing $GABA_A$ receptors and has very low affinity for α5 subunit-containing receptors (Pritchett and Seeburg, 1990; Faure-Halley et al., 1993; Hadingham et al., 1993).

On chronic treatment with the classical benzodiazepines tolerance develops to some of their therapeutic effects, though at different rates: the sedative effects are lost rapidly and the anticonvulsant effects are compromised on a longer time scale (File and Pellow, 1990). The molecular mechanisms which underlie the development of tolerance remain poorly understood, but a component of tolerance development may involve changes in $GABA_A$ receptor gene expression. Two lines of evidence, when taken together, support this hypothesis. Firstly, the degree to which recombinant $GABA_A$ receptors expressed in vitro are modulated by benzodiazepines differs depending on their specific subunit composition (Sieghart, 1995). Secondly, chronic treatment of rats with classical benzodiazepines, such as diazepam, changes the expression of several $GABA_A$ receptor subunit genes (Heninger et al., 1990; Wu et al., 1994; Holt et al., 1996; Impagnatiello et al., 1996).

Zolpidem, upon chronic treatment, shows reduced tolerance liability in comparison with the classical benzodiazepines (Perrault et al., 1992; Scharf et al., 1994) and this is the reason for undertaking the present study. We have measured the steady-state mRNA levels of $GABA_A$ receptor α1–6, β1–3 and γ1–3 subunit isoforms in rat cortex after 1 and 2 weeks of chronic zolpidem exposure to determine if the effects of this drug contrast with the previously investigated effects of diazepam.

2. Materials and methods

2.1. Drug treatment

Adult, male Sprague-Dawley rats were injected subcutaneously, once daily, for 7 or 14 days with 15 mg/kg zolpidem in 1 ml of sesame oil vehicle. Vehicle-treated animals were injected in the same manner with 1 ml of pure sesame oil. The results of the present study are

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directly comparable with the previously determined effects of 15 mg/kg/day of diazepam because in vivo binding studies have indicated that equivalent doses of zolpidem and diazepam give comparable levels of occupancy of the benzodiazepine binding site in rat cortex (Benavides et al., 1992). The 'slow release' sesame oil vehicle was used to account for the different kinetics of these drugs. Rats were killed 2 h after their last injection and cortex was isolated, frozen on liquid nitrogen, and stored at −80°C.

2.2. Quantification of drug levels

Cortical zolpidem concentrations were determined using reversed-phase HPLC (high pressure liquid chromatography). Briefly, 100 mg of cortex was taken from each animal treated with zolpidem, homogenized in five volumes of methanol and centrifuged 15 min at 12000 × *g*. A 50 μl aliquot of the supernatant was injected onto a 30 cm Spherisorb C_{18} 5 μm column (Alltech) and eluted isocratically with 70% (v/v) acetonitrile/tetrahydrofuran (10:1) and 30% (v/v) 0.013 M sodium phosphate buffer (pH 7) mobile phase at a flow rate of 1 ml/min. Peaks were detected using a fluorescence detector with an excitation wavelength of 254 nm and an emission wavelength of 390 nm. Harmane (4 ng per 50 μl injected) was used as an internal standard.

2.3. Solution hybridization

Quantification of $GABA_A$ receptor mRNA was performed using an S1 nuclease protection assay and autoradiography as previously described (Holt et al., 1996). The probe complimentary to the γ2 subunit transcript does not distinguish between the long and short splice variants. Data were compared using one-way analysis of variance.

3. Results

The mean concentration of zolpidem in rats killed 2 h after their last of 7 daily injections was 181 ± 12.6 (6) (mean ± S.E. (*n*)) ng per gram of cortex. In rats killed 2 h after their last of 14 daily injections, the concentration of zolpidem in cortex was 200.5 ± 19.6 (6) ng/g. These values are consistent with the concentrations of diazepam measured in the cortex of rats killed 2 h after equivalent diazepam dosing regimens.

No significant changes were observed in the levels of any of the $GABA_A$ receptor subunit mRNA species investigated after 7 or 14 days of exposure to vehicle alone (not shown). The amount of α6 subunit mRNA was not sufficient to allow quantification in any sample of cortex mRNA analyzed. The levels of α4 and β1 subunit mRNAs were significantly increased after 7 days of zolpidem treatment to 170.8 ± 10.3% (5) (mean ± S.E. (*n*)) and 149.1 ± 5.5% (5), respectively (Fig. 1A). After 2 weeks of

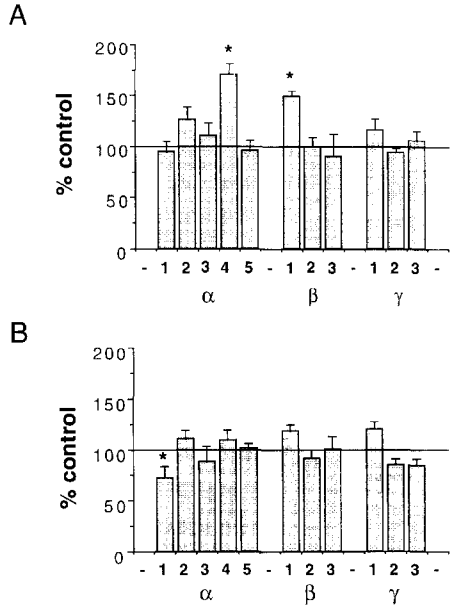

Fig. 1. The effect of 7 days (A) or 14 days (B) of zolpidem treatment on the steady-state levels of mRNAs encoding the various subunits of the $GABA_A$ receptor in rat cortex. Data are expressed as a percentage of the mean untreated control values. Error bars represent the standard error expressed as a percentage of the mean ($n = 4–6$). * $P < 0.05$.

treatment, a significant decrease in the level of α1 subunit mRNA to 72.6 ± 11.1% (6) of untreated control values was found (Fig. 1B). Neither 1 nor 2 weeks of zolpidem treatment produced any significant effect on the amount of any other $GABA_A$ receptor subunit mRNA in rat cortex (Fig. 1).

4. Discussion

We report changes in the steady-state levels of different $GABA_A$ receptor subunit mRNAs in response to chronic zolpidem treatment. Although there are no previous reports on the effect of chronic zolpidem treatment on $GABA_A$ receptor gene expression, several groups have shown changes in the levels of specific $GABA_A$ receptor subunit mRNAs in the cortex of rats chronically treated with diazepam (Heninger et al., 1990; Wu et al., 1994; Holt et al., 1996; Impagnatiello et al., 1996). We will compare the results of the present study with our own previous findings with diazepam (Holt et al., 1996) because identical treatment regimens and assay conditions were used in these two studies.

Exposure to either diazepam or zolpidem for 7 days caused significant increases in the levels of the α4 and β1 subunit mRNAs. In addition, 7 days of diazepam, but not zolpidem treatment, increased the level of γ3 subunit mRNA. After 14 days of treatment, the increase in the level of α4 and β1 subunit mRNA seen after 1 week of diazepam or zolpidem treatment was sustained for diazepam-treated rats only. At this timepoint the diazepam-

treated animals also showed significant increases in the levels of α3 and α5 and γ3 subunit and a significant decrease in the level of γ2 subunit mRNA. In contrast, the only zolpidem specific effect seen after 14 days of treatment was a significant decrease in the level of α1 subunit mRNA.

Interestingly, zolpidem does not bind $GABA_A$ receptors which contain the α4 subunit (Scholze et al., 1996), and yet the consequences of zolpidem treatment include an increase in the steady-state level of α4 subunit mRNA. In this case we must conclude that the changes we observe in $GABA_A$ receptor mRNA levels are not necessarily a result of direct drug-receptor interaction, but may occur in response to changes in GABAergic tone caused by interaction of the drug elsewhere in GABAergic pathways.

We have previously investigated the effects of chronic treatment with abecarnil, another subtype selective benzodiazepine site agonist, on $GABA_A$ receptor steady-state mRNA levels (Holt et al., 1996). Abecarnil, like zolpidem, shows selectivity for α1 subunit-containing $GABA_A$ receptors (Stephens et al., 1991) and chronic treatment with abecarnil has been shown not to produce diazepam-like tolerance or withdrawal effects in experimental animals (Steppuhn et al., 1993; Serra et al., 1994). Although the profile of significant changes in $GABA_A$ receptor subunit mRNA levels produced by chronic treatment with abecarnil or zolpidem are not identical (abecarnil treatment significantly decreases the levels of the γ2 and β2 $GABA_A$ receptor subunit mRNAs only) it is notable that the significant increases in the levels of α3, α5 and γ3 subunit mRNAs caused by diazepam do not occur with either zolpidem or abecarnil treatment. Impagnatiello et al. (1996) have compared steady-state $GABA_A$ receptor mRNA levels in selected neocortical regions of rats chronically treated with either diazepam or the benzodiazepine-site partial agonist imidazenil. In this study diazepam tolerant rats showed a significant decrease in the levels of α1, γ2S, γ2L and an increase in the level of α5 subunit mRNAs in the frontoparietal motor cortex. Imidazenil-treated animals, which failed to develop tolerance, did not show changes in the levels of any of the $GABA_A$ receptor mRNA species investigated. Of particular interest is the inability of the non-tolerance producing benzodiazepine site ligands (zolpidem, abecarnil and imidazenil) to increase the level of $GABA_A$ receptor α5 subunit mRNA in the same manner as diazepam.

We have previously discussed the possibility of a mechanism of coordinate control over the changes in $GABA_A$ receptor gene expression which occur in response to treatment with benzodiazepine site agonists (Holt et al., 1996). The data presented here are consistent with this premise. Briefly, chromosomal mapping studies have revealed that mammalian $GABA_A$ receptor subunit genes tend to be organized as α/β/γ clusters, i.e. on the human chromosomes 5q32–5q33 (α1, α6, β2, and γ2), 4p13–4q11 (α2, α4, β1 and γ1) and 15q11–15q13 (α5, β3 and γ3)

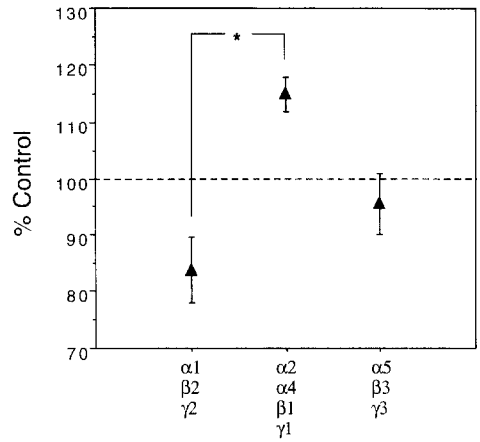

Fig. 2. $GABA_A$ receptor steady-state mRNA levels measured after 14 days of zolpidem treatment and grouped according to gene cluster. The relative mean mRNA level of each cluster with the appropriate standard error is shown. Statistical comparison was done using the Newman-Keuls test.

(McKernan and Whiting, 1996). Although no gene mapping studies have been done in the rat, there is evidence to suggest the presence of homologous clusters in this species (see Holt et al., 1996). The steady-state mRNA levels measured after the 14-day zolpidem treatment regimen have been grouped according to gene cluster and the mean mRNA level of each cluster, with the appropriate standard error, has been plotted (Fig. 2). The mean change in expression of the α1, β2 and γ2 subunit gene cluster is significantly different from that of the α2, α4, β1 and γ1 subunit gene cluster. Furthermore, the effect of diazepam on the α5, β3, γ3 subunit gene cluster (Holt et al., 1996) is significantly greater than the effect of zolpidem (not shown). To determine whether these effects are due to actual coordinate transcriptional activation or, alternatively, due to the activation of selected genes in different neuronal populations within the cortex, investigation using homogeneous cell systems will be required.

Benzodiazepine stimulation has been found to induce several compensatory changes in the brain, all of which may contribute, on different time-scales, to the development of tolerance to these drugs. In addition to altered steady-state mRNA levels, effects at the level of the protein have been reported. These include allosteric uncoupling of the GABA and benzodiazepine binding site (Primus et al., 1996) and down-regulation of specific subunit proteins (Brown and Bristow, 1996). Our data demonstrate changes in the steady-state levels of specific $GABA_A$ receptor subunit mRNAs in response to chronic treatment with zolpidem, a subtype selective benzodiazepine site ligand with low tolerance liability. These results, when compared with the effects of chronic diazepam on $GABA_A$ receptor mRNA levels, are consistent with the idea that $GABA_A$ receptor subunit isoform switching contributes to the development of benzodiazepine tolerance.

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