

5-HT_{1A} receptor blockade reverses GABA_A receptor α_3 subunit-mediated anxiolytic effects on stress-induced hyperthermia

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Abstract

Rationale Stress-related disorders are associated with dysfunction of both serotonergic and GABAergic pathways, and clinically effective anxiolytics act via both neurotransmitter systems. As there is evidence that the GABA_A and the serotonin receptor system interact, a serotonergic component in the anxiolytic actions of benzodiazepines could be present.

Objectives The main aim of the present study was to investigate whether the anxiolytic effects of (non-)selective α subunit GABA_A receptor agonists could be reversed with 5-HT_{1A} receptor blockade using the stress-induced hyperthermia (SIH) paradigm.

Results The 5-HT_{1A} receptor antagonist WAY-100635 (0.1–1 mg/kg) reversed the SIH-reducing effects of the non- α -subunit selective GABA_A receptor agonist diazepam (1–4 mg/kg) and the GABA_A receptor α_3 -subunit selective agonist TP003 (1 mg/kg), whereas WAY-100635 alone was without effect on the SIH response or basal body temperature. At the same time, co-administration of WAY-100635 with diazepam or TP003 reduced basal body temperature. WAY-100635 did not affect the SIH response when combined with the preferential α_1 -subunit GABA_A

receptor agonist zolpidem (10 mg/kg), although zolpidem markedly reduced basal body temperature.

Conclusions The present study suggests an interaction between GABA_A receptor α -subunits and 5-HT_{1A} receptor activation in the SIH response. Specifically, our data indicate that benzodiazepines affect serotonergic signaling via GABA_A receptor α_3 -subunits. Further understanding of the interactions between the GABA_A and serotonin system in reaction to stress may be valuable in the search for novel anxiolytic drugs.

Keywords Interaction · Zolpidem · TP003 · Benzodiazepine · Serotonin antagonist · Serotonergic · WAY-100635 · WAY100,635 · α_3 · α_3 · Stress · Anxiety

Introduction

Stress-related disorders are associated with dysfunction of both serotonergic and GABAergic pathways (Akimova et al. 2009; Kalueff and Nutt 2007; Nemeroff 2003). The clinical anxiolytic effects of selective serotonin reuptake inhibitors, 5-HT_{1A} receptor agonists and GABA_A receptor agonists, indicate that both the GABA_Aergic as well as the serotonergic system may be involved in the pathological basis underlying anxiety disorders (Nutt 2005; Zohar and Westenberg 2000). There is evidence that the GABA and the serotonergic system interact (Fernandez-Guasti and Lopez-Rubalcava 1998; Gao et al. 1993; Lista et al. 1989), although the evidence whether it plays a role in the stress response is inconsistent (Shephard et al. 1982; Thiebot 1986). Specifically, a serotonergic component in the anxiolytic actions of benzodiazepines has been suggested (Harandi et al. 1987; Stein et al. 1977; Thiebot et al. 1984). Hence, studying the interactions of the GABA_A and

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serotonin system in stress and anxiety could be valuable in the search for novel anxiolytic drugs.

Here, we investigate whether the anxiolytic effects of GABA_A receptor agonists are dependent on 5-HT_{1A} receptor activation using the stress-induced hyperthermia (SIH) paradigm. The SIH response is the transient rise in body temperature in response to acute stress that is mediated by the autonomic nervous system (Bouwknicht et al. 2007; Vinkers et al. 2008). Both classical benzodiazepines and 5-HT_{1A} receptor agonists consistently reduce the SIH response (as well as basal body temperature at higher doses), whereas dopaminergic and noradrenergic systems are generally ineffective (Olivier et al. 2003). Classical (non-subunit selective) benzodiazepines bind to GABA_A receptor α_1 -, α_2 -, α_3 -, or α_5 -subunits, and the various benzodiazepine effects are thought to be mediated through different GABA_A receptor subtypes (Rudolph and Mohler 2006). Interactions with the serotonergic system may thus depend on the GABA_A receptor composition. In the present study, we investigated whether the silent 5-HT_{1A} receptor antagonist WAY-100635 (WAY) could alter the SIH-reducing and hypothermic effects of the non-subunit selective GABA_A receptor agonist diazepam, the selective GABA_A receptor α_3 -subunit agonist TP003 (Dias et al. 2005), and the preferential GABA_A receptor α_1 -subunit agonist zolpidem.

Materials and methods

Animals

Eighty-four male NMRI mice (Charles River, The Netherlands) were housed in Macrolon type 3 cages enriched with bedding and nesting material under a 12-h light/12-h dark cycle (lights on from 0600 to 1800 hours) at controlled temperature (22±2°C) and relative humidity (40–60%) with free access to standard food pellets and tap water. Experiments were carried out with approval of the ethical committee on animal experiments of the Faculties of Sciences, Utrecht University, The Netherlands, and in accordance with the Declaration of Helsinki.

The stress-induced hyperthermia (SIH) procedure

The SIH tests were carried out one time per week according to standard procedures (Groenink et al. 2009). A between-subject design was used, and animals were randomly allocated to an experimental group. Cages were randomly and evenly allocated over daytimes (morning to afternoon). The temperature of mice was measured by rectally inserting a thermistor probe by a length of 2 cm. Digital temperature recordings were obtained with an accuracy of 0.1°C using a

Keithley 871A digital thermometer (NiCr–NiAl thermocouple). The probe, dipped into silicon oil before inserting, was held in the rectum until a stable rectal temperature had been obtained for 20 s. Animals were injected intraperitoneally with vehicle or WAY-100635 on the left flank and with vehicle, diazepam, zolpidem, or TP003 on the right flank. All drugs were injected 60 min before the first temperature measurement (T_1). This first temperature measurement, representing the basal body temperature, functioned as an adequate stressor as well. The temperature was again measured 10 min later (T_2), representing the stress-induced body temperature. The SIH response was calculated by subtracting T_1 from T_2 .

Drugs

Diazepam (base), zolpidem (tartaric acid), and WAY-100635 (maleate) (*N*-{2-[4-(2-methoxy)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide tri-chloride) were obtained from Sigma Aldrich. TP003 was synthesized according to published methods (Dias et al. 2005; Humphries et al. 2006). An injection volume of 10 ml/kg was used for intraperitoneal injections of all drugs. WAY-100635 was dissolved in saline. Diazepam, zolpidem, and TP003 were suspended in gelatin–mannitol 0.5%/5%. Fresh solutions and suspensions were prepared each testing day. Doses of TP003 and zolpidem were based on previous SIH studies (Dias et al. 2005; Olivier et al. 2002, 2003).

Data analysis

All experiments were carried out using a between-subject design. For each individual mouse, a basal temperature (T_1), an end temperature (T_2), and the difference (SIH response= T_2-T_1) were determined. Treatment effects were evaluated using a two-way analysis of variance with explanatory factors drug₁ (WAY-100635 or vehicle) and drug₂ (diazepam/zolpidem/TP003 or vehicle). In addition, a post-hoc analysis was carried out comparing diazepam/zolpidem/TP003 with vehicle under both WAY-100635 and vehicle conditions using a Tukey's Honestly Significant Difference (HSD) test. A probability level of $p<0.05$ was set as statistically significant.

Results

Effects on the stress-induced hyperthermia response

Diazepam 1 mg/kg and WAY-100635 (n = 8–9)

WAY-100635 (WAY) significantly reversed the diazepam effect on the SIH response at all three WAY doses tested (WAY 0.1 mg/kg, WAY × diazepam interaction, $F_{1,31}=5.02$,

$p < 0.05$; WAY 0.3 mg/kg, WAY \times diazepam interaction, $F_{1,31} = 4.71$, $p < 0.05$; WAY 1.0 mg/kg, WAY \times diazepam interaction, $F_{1,32} = 5.76$, $p < 0.05$; Fig. 1a). Post-hoc analysis showed that diazepam reduced the SIH response when it was co-administered with vehicle in one out of three experiments (veh-veh vs. diazepam-veh: WAY 0.1 mg/kg, $p < 0.05$; WAY 0.3 mg/kg, $p = 0.53$, NS; WAY 1.0 mg/kg, $p = 0.17$, NS), but that WAY altered the diazepam-induced reduction of the SIH response (diazepam-veh vs. diazepam-WAY: WAY 0.1 mg/kg, $p < 0.01$; WAY 0.3 mg/kg, $p < 0.05$; WAY 1.0 mg/kg, $p < 0.01$). In contrast, diazepam had no effects when it was combined with WAY (veh-WAY vs. diazepam-WAY: WAY 0.1 mg/kg, $p = 0.77$, NS; WAY 0.3 mg/kg, $p = 0.10$, NS; WAY 1.0 mg/kg, $p = 0.61$, NS).

Diazepam 4 mg/kg and WAY-100635 ($n = 8-10$)

WAY significantly reversed the diazepam effects on the SIH response at higher doses (WAY 0.3 mg/kg, WAY \times diazepam interaction, $F_{1,32} = 3.88$, $p = 0.05$; WAY 1.0 mg/kg, WAY \times diazepam interaction, $F_{1,36} = 4.74$, $p < 0.05$; Fig. 1b). In contrast, WAY did not alter the diazepam effects at the lowest dose (WAY 0.1 mg/kg, WAY \times diazepam interaction, $F_{1,35} = 1.20$, $p = 0.28$, NS). Post-hoc analysis showed that diazepam reduced the SIH response when co-administered with vehicle (veh-veh vs. diazepam-veh: WAY 0.1 mg/kg, $p < 0.01$; WAY 0.3 mg/kg, $p < 0.05$; WAY 1.0 mg/kg, $p < 0.01$), and that WAY altered the diazepam-induced reduction of the SIH response at the highest dose (diazepam-veh vs. diazepam-WAY: WAY

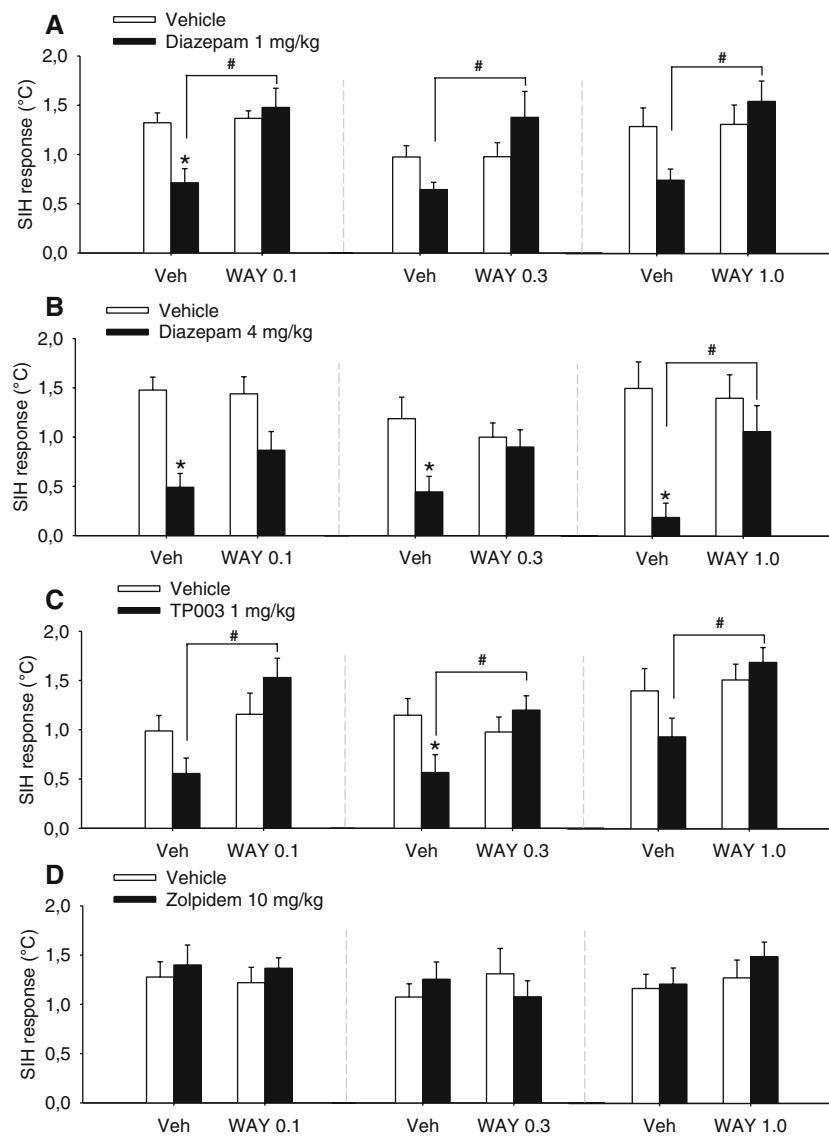


Fig. 1 Effects of (a, b) diazepam (1 and 4 mg/kg, IP, $n = 8-10$), (c) TP003 (1 mg/kg, IP, $n = 7-10$), and (d) zolpidem (10 mg/kg, IP, $n = 8-10$) administration in combination with WAY-100635 (0.1–1 mg/kg, IP) or vehicle on the stress-induced hyperthermia (SIH) response.

Asterisk (*), $p < 0.05$: drug effect compared to corresponding vehicle; number sign (#), $p < 0.05$: drug effect under vehicle vs. WAY conditions

0.1 mg/kg, $p=0.31$, NS; WAY 0.3 mg/kg, $p=0.28$, NS; WAY 1.0 mg/kg, $p<0.05$). Diazepam did not reduce the SIH response combined with WAY (*veh-WAY* vs. *diazepam-WAY*: WAY 0.1 mg/kg, $p=0.06$, NS; WAY 0.3 mg/kg, $p=0.98$, NS; WAY 1.0 mg/kg, $p=0.74$, NS).

TP003 (1 mg/kg) and WAY-100635 (n=7–10)

WAY influenced the TP003 effect on the SIH response at the lower WAY doses (WAY 0.1 mg/kg, WAY×TP003 interaction, $F_{1,30}=4.56$, $p<0.05$; WAY 0.3 mg/kg, WAY×TP003 interaction, $F_{1,30}=4.87$, $p<0.05$; WAY 1.0 mg/kg, WAY×TP003 interaction, $F_{1,31}=3.38$, $p=0.06$, NS; Fig. 1c). Post-hoc analysis showed that TP003 reduced the SIH response when co-administered with vehicle in one out of three experiments (*veh-veh* vs. *TP003-veh*: WAY 0.1 mg/kg, $p=0.11$, NS; WAY 0.3 mg/kg, $p<0.05$; WAY 1.0 mg/kg, $p=0.27$, NS). However, WAY altered the TP003 effects on the SIH response (*TP003-veh* vs. *TP003-WAY*: WAY 0.1 mg/kg, $p<0.01$; WAY 0.3 mg/kg, $p<0.05$; WAY 1.0 mg/kg, $p<0.05$). In contrast, TP003 did not alter the SIH response combined with WAY (*veh-WAY* vs. *TP003-WAY*: WAY 0.1 mg/kg, $p=0.52$, NS; WAY 0.3 mg/kg, $p=0.77$, NS; WAY 1.0 mg/kg, $p=0.47$, NS).

Zolpidem 10 mg/kg and WAY-100635 (n=8–10)

Zolpidem did not affect the SIH response in all three experiments (WAY 0.1 mg/kg, zolpidem effect, $F_{1,32}=0.77$, $p=0.39$, NS; WAY 0.3 mg/kg, zolpidem effect, $F_{1,31}=0.30$, $p=0.87$, NS; WAY 1 mg/kg, zolpidem effect, $F_{1,32}=0.72$, $p=0.40$, NS; Fig. 1d). WAY did not change the zolpidem effects at any dose (WAY 0.1 mg/kg, WAY×zolpidem interaction $F_{1,32}=0.001$, $p=0.94$, NS; WAY 0.3 mg/kg, WAY×zolpidem interaction, $F_{1,31}=1.63$, $p=0.21$, NS; WAY 1 mg/kg, WAY×zolpidem interaction, $F_{1,32}=0.29$, $p=0.59$, NS). Post-hoc analysis indicated that zolpidem did not reduce the SIH response, regardless whether it was co-administered with vehicle (*veh-veh* vs. *zolpidem-veh*: WAY 0.1 mg/kg, $p=0.95$, NS; WAY 0.3 mg/kg, $p=0.99$, NS; WAY 1.0 mg/kg, $p=0.97$, NS) or with WAY (*veh-WAY* vs. *zolpidem-WAY*: WAY 0.1 mg/kg, $p=0.90$, NS; WAY 0.3 mg/kg, $p=0.76$, NS; WAY 1.0 mg/kg, $p=0.72$, NS).

Effects on basal body temperature

Diazepam 1 mg/kg and WAY-100635 (n=8–9)

At higher doses, WAY appeared to enhance the temperature-reducing effects of diazepam (WAY 0.1 mg/kg, WAY×diazepam interaction, $F_{1,31}=0.23$, $p=0.63$, NS; WAY 0.3 mg/kg, WAY×diazepam interaction, $F_{1,31}=4.40$, $p<0.05$; WAY 1.0 mg/kg, WAY×diazepam interaction,

$F_{1,32}=3.43$, $p=0.07$; Fig. 2a). Post-hoc analysis confirmed that diazepam reduced basal body temperature combined with WAY at higher doses (*veh-WAY* vs. *diazepam-WAY*: WAY 0.1 mg/kg, $p=0.31$, NS; WAY 0.3 mg/kg, $p<0.01$; WAY 1.0 mg/kg, $p<0.01$) and that WAY enhanced the diazepam effects on basal body temperature at higher doses (*diazepam-veh* vs. *diazepam-WAY*: WAY 0.1 mg/kg, $p=0.80$, NS; WAY 0.3 mg/kg, $p<0.01$; WAY 1.0 mg/kg, $p<0.05$). In contrast, diazepam did not reduce basal body temperature when co-administered with vehicle (*veh-veh* vs. *diazepam-veh*: WAY 0.1 mg/kg, $p=0.73$, NS; WAY 0.3 mg/kg, $p=0.96$, NS; WAY 1.0 mg/kg, $p=0.12$, NS).

Diazepam 4 mg/kg and WAY-100635 (n=8–10)

WAY enhanced the effect of diazepam on basal body temperature at higher doses (WAY 0.1 mg/kg, WAY×diazepam interaction, $F_{1,35}=0.91$, $p=0.35$, NS; WAY 0.3 mg/kg, WAY×diazepam interaction, $F_{1,32}=4.86$, $p<0.05$; WAY 1 mg/kg, WAY×diazepam interaction, $F_{1,36}=18.47$, $p<0.001$; Fig. 2b). Post-hoc analysis showed that diazepam reduced basal body temperature when it was combined with WAY (*veh-WAY* vs. *diazepam-WAY*: WAY 0.1 mg/kg, $p<0.001$; WAY 0.3 mg/kg, $p<0.001$; WAY 1.0 mg/kg, $p<0.001$), and that WAY enhanced the diazepam effects on basal body temperature at the highest WAY dose (*diazepam-veh* vs. *diazepam-WAY*: WAY 0.1 mg/kg, $p=0.21$, NS; WAY 0.3 mg/kg, $p=0.20$, NS; WAY 1.0 mg/kg, $p<0.001$). In contrast, diazepam did not reduce basal body temperature when it was co-administered with vehicle in two out of three experiments (*veh-veh* vs. *diazepam-veh*: WAY 0.1 mg/kg, $p<0.01$; WAY 0.3 mg/kg, $p=0.37$, NS; WAY 1.0 mg/kg, $p=0.65$, NS).

TP003 (1 mg/kg) and WAY-100635 (n=7–10)

WAY altered the effects of TP003 on basal body temperature at all WAY doses tested (WAY 0.1 mg/kg, WAY×TP003 interaction, $F_{1,30}=4.54$, $p<0.05$; WAY 0.3 mg/kg, WAY×TP003 interaction, $F_{1,30}=9.01$, $p<0.01$; WAY 1 mg/kg, WAY×TP003 interaction, $F_{1,31}=4.28$, $p<0.05$; Fig. 2c). Post-hoc analysis showed that TP003 reduced basal body temperature when it was combined with WAY in two out of three experiments (*veh-WAY* vs. *TP003-WAY*: WAY 0.1 mg/kg, $p=0.13$, NS; WAY 0.3 mg/kg, $p<0.05$; WAY 1.0 mg/kg, $p<0.05$) and that the TP003-WAY combination reduced basal body temperature compared to the TP003-vehicle combination (*TP003-veh* vs. *TP003-WAY*: WAY 0.1 mg/kg, $p<0.01$; WAY 0.3 mg/kg, $p=0.06$, NS; WAY 1.0 mg/kg, $p<0.05$). In contrast, TP003 did not reduce basal body temperature when co-administered with vehicle (*veh-veh* vs. *TP003-veh*: WAY 0.1 mg/kg, $p=0.87$, NS; WAY 0.3 mg/kg, $p=0.47$, NS; WAY 1.0 mg/kg, $p=0.91$, NS).

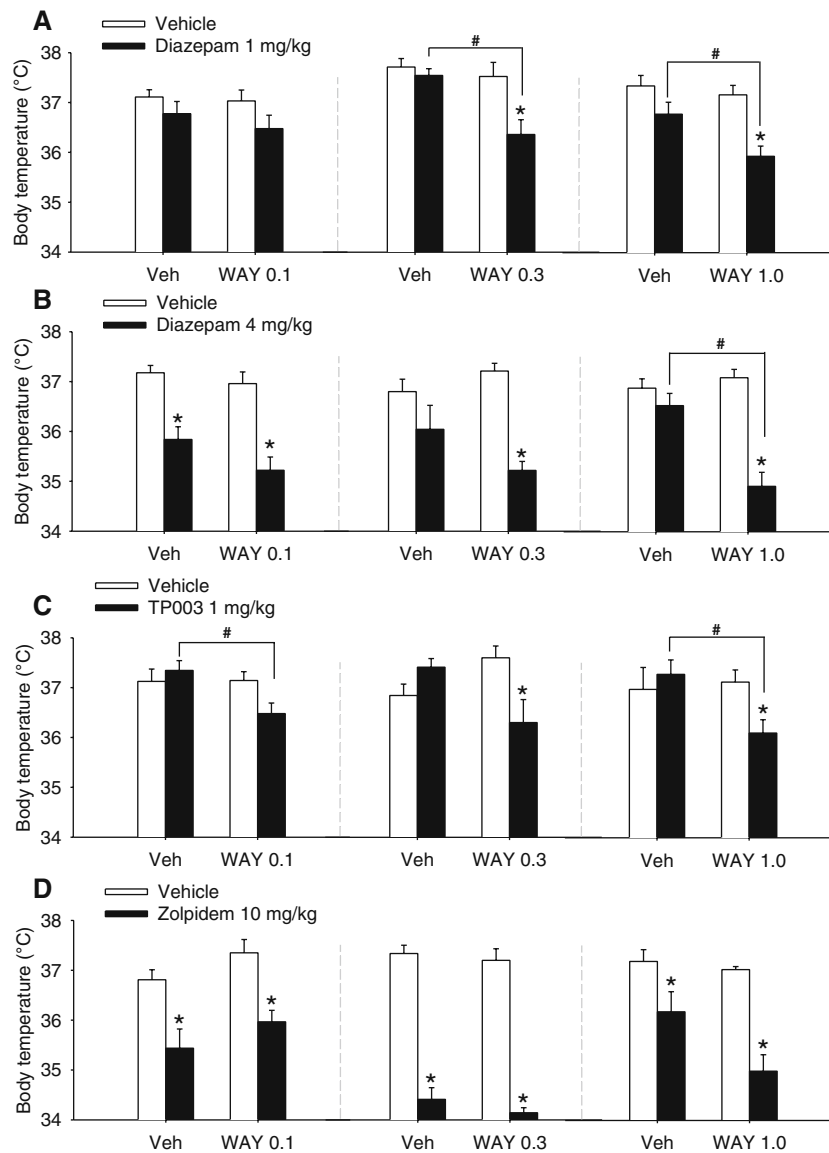


Fig. 2 Effects of (a, b) diazepam (1 and 4 mg/kg, IP, $n=8-10$), (c) TP003 (1 mg/kg, IP, $n=7-10$), and (d) zolpidem (10 mg/kg, IP, $n=8-10$) administration in combination with WAY-100635 (0.1–1 mg/kg,

IP) or vehicle on basal body temperature. Asterisk (*), $p<0.05$: drug effect compared to corresponding vehicle; number sign (#), $p<0.05$: drug effect under vehicle vs. WAY conditions

Zolpidem 10 mg/kg and WAY-100635 ($n=8-10$)

In all experiments, zolpidem reduced basal body temperature (WAY 0.1 mg/kg, zolpidem effect, $F_{1,32}=25.07$, $p<0.001$; WAY 0.3 mg/kg, zolpidem effect, $F_{1,31}=136.20$, $p<0.001$; WAY 1 mg/kg, zolpidem effect, $F_{1,32}=41.39$, $p<0.001$; Fig. 2d). WAY did not alter the zolpidem effects on body temperature (WAY 0.1 mg/kg, WAY \times zolpidem interaction, $F_{1,32}=0.01$, $p=0.98$, NS; WAY 0.3 mg/kg, WAY \times zolpidem interaction, $F_{1,31}=0.38$, $p=0.54$, NS; WAY 1 mg/kg, WAY \times zolpidem interaction, $F_{1,32}=2.28$, $p=0.14$, NS). Post-hoc analysis indicated that zolpidem reduced basal body temperature, regardless whether it was co-administered with vehicle (WAY 0.1 mg/kg, $p<0.01$;

WAY 0.3 mg/kg, $p<0.01$; WAY 1.0 mg/kg, $p<0.05$) or with WAY (WAY 0.1 mg/kg, $p<0.01$; WAY 0.3 mg/kg, $p<0.001$; WAY 1.0 mg/kg, $p<0.01$). Furthermore, WAY did not alter the zolpidem effect on basal body temperature (zolpidem-veh vs. zolpidem-WAY: WAY 0.1 mg/kg, $p=0.56$, NS; WAY 0.3 mg/kg, $p=0.43$, NS; WAY 1.0 mg/kg, $p=0.07$, NS).

Discussion

The present study investigated putative GABA-serotonin interactions using the SIH paradigm. Our main finding is that the non-selective GABA_A receptor agonist diazepam

and the α_3 -subunit selective GABA_A receptor agonist TP003 no longer reduced the SIH response and augmented hypothermia in the presence of the 5-HT_{1A} receptor antagonist WAY-100635, suggesting an interaction between the activation of the GABA_A receptor α_3 -subunits and 5-HT_{1A} receptors. In contrast, WAY-100635 did not have any effect when it was combined with the preferential α_1 -subunit GABA_A receptor agonist zolpidem. As WAY-100635 has no affinity for GABA_A receptors (Fletcher et al. 1996), our data suggest that in the SIH paradigm, anxiolytic effects of GABA_A receptor agonists may be mediated via the serotonin system. Thus, benzodiazepines may affect serotonergic signaling via α_3 -subunits on a distinct group of serotonergic neurons. In support, the vast majority of serotonergic neurons express GABA_A receptor α_3 -subunit immunoreactivity but not GABA_A receptor α_1 -subunit staining (Gao et al. 1993). This is remarkable as the α_1 -subunit is highly prevalent in the central nervous system.

The effects of the GABAergic drugs diazepam, TP003, and zolpidem on the SIH response and body temperature are generally in line with earlier SIH studies (Olivier et al. 2002; Vinkers et al. 2008, 2009). Diazepam effects on basal body temperature slightly varied over the experiments, which may be attributed to fluctuations in body temperature under vehicle conditions due to physiological variance, differences in environmental temperature, or the time of testing. Classical non-selective benzodiazepines enhance the inhibitory actions of GABA by binding to an allosteric site on GABA_A receptors that contain α_1 -, α_2 -, α_3 -, or α_5 -subunits in combination with a β and a γ_2 subunit (Rudolph and Mohler 2006). Zolpidem is approximately five- to tenfold more selective for α_1 -subunit-containing GABA_A receptors than α_2/α_3 -subunit-containing receptors (Petroski et al. 2006), whereas TP003 is α_3 -subunit selective with low modulation via α_1 -, α_2 -, and α_5 -containing subtypes (Dias et al. 2005). Recently, genetic and pharmacological evidence has indicated that α -subunits may differentially contribute to the various classical benzodiazepine-induced effects such as anxiolysis, dependence, anticonvulsant activity, sedation, and amnesia (Crestani et al. 2001; Rudolph et al. 1999). Here, we confirm and extend our earlier findings suggesting a role for the GABA_A receptor α_1 subunit in hypothermia and a role for the GABA_A receptor $\alpha_{2/3}$ subunit in reduction of the SIH response (Vinkers et al. 2009).

In the present study, WAY-100635 did not affect the SIH response in any of the experiments when it was administered alone which is in line with earlier studies (Olivier et al. 2003, 2008). The 5-HT_{1A} receptor antagonist WAY-100635 is generally assumed to act as silent antagonist but has also been reported to exert anxiolytic or even anxiogenic effects depending on the experimental design (Cao and Rodgers 1997; Fletcher et al. 1996; Forster et al.

1995; Griebel et al. 2000; Groenink et al. 1996; Joordens et al. 1998; Stanhope and Dourish 1996). WAY-100635 has also been shown to reverse the SIH-reducing effects of 5-HT_{1A} receptor agonists such as buspirone and flesinoxan, confirming that WAY-100635 targets 5-HT_{1A} receptors (Iijima et al. 2007; Olivier et al. 1998). Interestingly, WAY-100635 has also been able to reverse the SIH reduction caused by the mGluR_{2/3} receptor antagonist MGS0039, suggesting that the 5-HT_{1A} receptors may also be involved in the effects of glutamate receptor antagonists (Iijima et al. 2007).

WAY-100635 could reverse the α_3 -induced effects in the SIH paradigm by blocking presynaptic 5-HT_{1A} receptors that disinhibit serotonin release and turnover at synaptic levels (Wesolowska et al. 2003), which may then activate postsynaptic 5-HT receptors. Electrophysiological studies show that WAY-100635 increases serotonergic neuronal activity probably by blocking 5-HT_{1A} autoreceptors (Corradetti et al. 1996; Fornal et al. 1996; Munday et al. 1996). In support, serotonergic raphe nuclei receive a prominent GABAergic input via distant sources as well as interneurons (Bagdy et al. 2000; Gervasoni et al. 2000; Harandi et al. 1987; Varga et al. 2001). However, this can only provide a partial explanation as WAY-100635 also augmented the benzodiazepine-induced hypothermia, putatively via an activation of α_1 -subunits (Vinkers et al. 2009). Also, raphe lesions did not attenuate the anticonflict activity of peripherally administered benzodiazepines, suggesting that difficulties may exist in generalizing findings from one paradigm to another (Green and Hodges 1986). Furthermore, it is striking that WAY-100635 reverses the SIH response while it augments the hypothermia. It may be hypothesized that GABA_A–serotonin interactions relevant for thermoregulation exist in the preoptic area or dorsomedial hypothalamus (Dimicco and Zaretsky 2007) or, alternatively, that dorsal and median raphe projections differentially affect basal and stress-induced body temperature levels. In support, 5-HT was found to presynaptically inhibit GABA release in the thermoregulatory hypothalamic medial preoptic area, which could be blocked by a 5-HT_{1A} receptor antagonist (Lee et al. 2008). We also cannot exclude the possibility that downstream activation of hypothalamic 5-HT_{1A} receptors, subsequent to the direct activation of GABA_A receptors, is needed to maintain the core body temperature. Thus, detailed hypotheses on the potential interactive sites of GABA_A receptors and 5-HT_{1A} receptors and their functional relevance must await further experimental analysis. Some studies have found decreased serotonin activity and turnover after benzodiazepine administration (Chase et al. 1970; Pratt et al. 1979; Stein et al. 1977; Trulson et al. 1982; Wright et al. 1992), although others have not found such effects (Shephard et al. 1982; Thiebot 1986; Thiebot et al. 1984). The present study used

the SIH paradigm as an anxiolytic assay as it can repeatedly be used over the weeks without any habituation. However, the use of a single paradigm prevents a direct generalization of our results to other anxiolytic tests.

In conclusion, the present study shows that 5-HT_{1A} receptor blockade reversed the anxiolytic effects of the non-selective GABA_A receptor agonist diazepam and the α_3 -selective GABA_A receptor agonist TP003, whereas it enhanced benzodiazepine-induced hypothermia. In contrast, these effects were not present in combination with the preferential GABA_A receptor α_1 -subunit agonist zolpidem. Together, these data suggest that the GABA_A receptor α_3 -subunit functionally interacts with 5-HT_{1A} receptors of the serotonin system to exert its anxiolytic effects in the SIH paradigm.

Conflicts of interest The authors declare no financial disclosures or conflicts of interest.

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