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Research report

α1- and α2-containing $GABA_A$ receptor modulation is not necessary for benzodiazepine-induced hyperphagia

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ABSTRACT

Benzodiazepines increase food intake, an effect attributed to their ability to enhance palatability. We investigated which $GABA_A$ receptor subtypes may be involved in mediating benzodiazepine-induced hyperphagia. The role of the α2 subtype was investigated by observing the effects of midazolam, on the behavioural satiety sequence in mice with targeted deletion of the α2 gene (α2 knockout). Midazolam (0.125, 0.25 and 0.5 mg/kg) increased food intake and the amount of time spent feeding in α2 knockout mice, suggesting that BZ-induced hyperphagia does not involve α2-containing $GABA_A$ receptors. We further investigated the roles of α1- and α3-containing $GABA_A$ receptors in mediating BZ-induced hyperphagia. We treated α2(H101R) mice, in which α2-containing receptors are rendered benzodiazepine insensitive, with L-838417, a compound which acts as a partial agonist at α2-, α3- and α5-receptors but is inactive at α1-containing receptors. L-838417 (10 and 30 mg/kg) increased food intake and the time spent feeding in both wildtype and α2(H101R) mice, demonstrating that benzodiazepine-induced hyperphagia does not require α1- and α2-containing $GABA_A$ receptors. These observations, together with evidence against the involvement of α5-containing $GABA_A$ receptors, suggest that α3-containing receptors mediate BZ-induced hyperphagia in the mouse.

Introduction

Benzodiazepines (BZs) increase food consumption in a range of mammalian species, by enhancing the palatability of food (Berridge & Pecina, 1995; Berridge & Treit, 1986; Cooper & Yerbury, 1988). BZs bind to $GABA_A$ receptors (Mohler, 2006) which are pentameric protein structures, assembled from two α1, α2, α3 or α5 subunits or two different α subunit forms (Benke et al., 2004), in combination with a β variant and the γ2 subunit (Benke, Mertens, Trzeciak, Gillessen, & Mohler, 1991; Pritchett et al., 1989). α1-, α2-, α3- and α5-containing $GABA_A$ receptors are differentially expressed in brain regions, and consequently have different functional roles (Dias et al., 2005; Low et al., 2000; McKernan et al., 2000; Morris, Dawson, Reynolds, Atack, & Stephens, 2006; Rudolph et al., 1999). To date, it is unclear which $GABA_A$ subtypes mediate BZ-induced hyperphagia and so we investigated the roles of α1- and α2-containing receptors.

It is argued that, because BZ-induced hyperphagia is present in the absence of α1-mediated sedation, activation of the α1-containing $GABA_A$ receptor is not necessary for BZ-induced hyperphagia (Higgs & Cooper, 1996; Yerbury & Cooper, 1989). Consistent with this idea, zolpidem, an α1-preferring BZ agonist, does not induce hyperphagia in rodents (Davies et al., 1994; Perrault, Morel, Sanger, & Zivkovic, 1990; Yerbury & Cooper, 1989). However, the α1-preferring compounds, zolpidem and zaleplon significantly increased the intake of sucrose pellets in adult squirrel monkeys (Duke et al., 2006). This evidence suggests that the hyperphagic effects of zolpidem may be species dependent and may be due to the activation of α3- and/or α2-containing $GABA_A$ receptors, where zolpidem also acts as an agonist, despite having lower affinity for these subtypes compared to α1-containing receptors (Pritchett & Seeburg, 1990; Puia, Vicini, Seeburg, & Costa, 1991).

It is unlikely that α5-containing receptors are involved in BZ-induced hyperphagia. Although, α5 knockout mice showed reduced levels of operant responding for sucrose, the preferential α5 inverse agonist, α5IA-II, did not affect responding for sucrose under the same reinforcement schedule (Stephens, Pistovcakova, Worthing, Atack, & Dawson, 2005). Furthermore, the α5-preferring

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Abbreviations: BZ, benzodiazepine; PBN, parabrachial nucleus; PVN, paraventricular nucleus; α2 KO, α2 knockout; WT, wildtype.

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Table 1
Subtype selectivity of compounds which bind to the benzodiazepine site of $GABA_A$ receptors and the effects these drugs have on feeding behaviour.

Compound	Efficacy	$GABA_A$ subtype selectivity	Effects on feeding behaviour
Zolpidem	Agonist	Higher affinity (10-fold) at α1- than at α2- and α3- and no affinity at α5-containing receptors (Pritchett & Seeburg, 1990).	Increases the intake of sucrose pellets in adult squirrel monkeys (Duke et al., 2006), but does not increase food intake in rodents (Yerbury & Cooper, 1989).
α5IA-II	Inverse agonist	Greater efficacy at α5- than at α1-, α2- or α3-containing $GABA_A$ receptors (Stephens et al., 2005).	Did not affect operant responding for sucrose in rodents (Stephens et al., 2005) or squirrel monkeys (Duke et al., 2006).
CGS 17867A	Agonist	Greater efficacy at α2- and α3- than at α1-containing receptors (Mitchinson et al., 2004).	Increased consumption of palatable food in rats (Yerbury & Cooper, 1989).
Abecarnil	Agonist	Full agonist at α1- and α3- and partial agonist at α2- and α5-containing receptors (Knoflach et al., 1993).	Increases sucrose intake and palatability in rats (Cooper & Ridley, 2005).
Midazolam	Partial agonist	Partial agonist at α1-, α2-, α3- and α5-containing receptors.	Increases food intake in rats (Cooper & Yerbury, 1986; Higgs & Cooper, 1998).
Diazepam	Agonist	Full agonist at α1-, α2-, α3- and α5-containing receptors.	Increases food intake in mice (Dua & Sharma, 1995), rats (Cooper, 1980) and squirrel monkeys (Duke et al., 2006).
L-838417	Partial agonist	Partial agonist at α2-, α3- and α5-containing receptors. Antagonist at α1-containing receptors (McKernan et al., 2000).	Not known.

agonist, QH-ii-066 did not increase the intake of sucrose pellets in squirrel monkeys (Duke et al., 2006).

Cooper (2005) argued that the α2 and/or α3 $GABA_A$ receptor subtypes mediate BZ-induced hyperphagia. His view was based on the observation that CGS 17867A, a compound with significant efficacy at α2- and α3-containing subtypes and reduced efficacy at α1-receptors (Mitchinson et al., 2004), increased consumption of palatable food in non-deprived rats (Yerbury & Cooper, 1989). Furthermore, abecarnil, thought to act selectively as a full agonist at α1- and α3-containing $GABA_A$ receptors (Knoflach, Drescher, Scheurer, Malherbe, & Mohler, 1993; Pribilla et al., 1993), increases sucrose intake (Cooper & Ridley, 2005) providing further support for the involvement of the α3 and/or α1 subtype. Table 1 provides a summary of the subtype selective properties of the compounds discussed above and the effects they have on feeding behaviour.

The role of α1-, α2- and α3-containing $GABA_A$ receptors in mediating BZ-induced hyperphagia remains unclear. With the use of transgenic mice with BZ-insensitive α2-containing $GABA_A$ receptors and a subtype selective BZ which is inactive at α1-containing receptors we were able to investigate the role of α1- and α2-containing receptors. Firstly, to determine whether α2-containing $GABA_A$ receptors are involved in BZ-induced hyperphagia, we investigated whether the increase in food intake induced by the non-selective BZ midazolam (Cooper, Barber, Gilbert, & Moores, 1985; Cooper & Yerbury, 1986; Higgs & Cooper, 1996) was apparent in the absence of modulation of α2-containing receptors. We used α2 knockout (α2 KO) mice, in which the $GABA_A$ receptor α2 gene encoding $GABA_A$ α2 subunits is deleted (Dixon, Rosahl, & Stephens, 2008). Secondly, we investigated the role of α1-containing $GABA_A$ receptors using L-838417, a partial α2, α3 and α5 agonist and an antagonist at α1-containing receptors (McKernan et al., 2000), in wildtype (WT) mice.

As with non-selective BZs, L-838417 is thought to be inactive at α4- and α6-containing $GABA_A$ receptors, where it has substantially reduced affinity (McKernan et al., 2000). Whilst BZ-sensitive $GABA_A$ receptors have a histidine residue at the drug-binding domain, BZ-insensitive α4- and α6-receptors have an arginine residue in the corresponding position (Wieland, Luddens, & Seeburg, 1992). Replacement of a histidine with an arginine residue at the BZ-binding site of α2-containing $GABA_A$ receptors in α2(H101R) mice, renders α2-receptors BZ-insensitive (Benson, Low, Keist, Mohler, & Rudolph, 1998; Dias et al., 2005). Thus, it is likely that L-838417 will have a considerably lower affinity at mutated α2-receptors and so its behavioural effects in α2(H101R) mice may be attributed to partial agonism of α3- and α5-containing receptors. Since α5-containing receptors are unlikely to play a significant role in BZ-induced hyperphagia (Duke et al., 2006), an enhancement of food intake by L-838417 in α2(H101R) mice would also indicate a role for the α3-receptor.

Method

Animals

Twelve α2 KO (Dixon et al., 2008) and 12 WT female mice were used in experiment 1, and 12 α2(H101R) (Dias et al., 2005) and 12 WT, male and female mice, (6 of each sex) were used in experiment 2. All mice were bred from heterozygous parents supplied by Merck, Sharp and Dohme (Terlings Park, UK) on a mixed 50% C57BL6J–50% 129SvEv genetic background. Mice weighed between 23 and 34 g at the start of the experiment. The mice were singly housed in shoebox cages under a 12:12-h light/dark cycle (lights on at 7:00 AM). Room temperature was maintained at 19–21 °C and humidity was kept at 50 ± 10%. All mice had *ad libitum* access to food and water. Experiments were performed between 11:00 and 15:00. All experiments were authorised by the UK Animal (Scientific Procedures) Act 1986, following ethical review from the Local Ethical Review Committee at the University of Sussex.

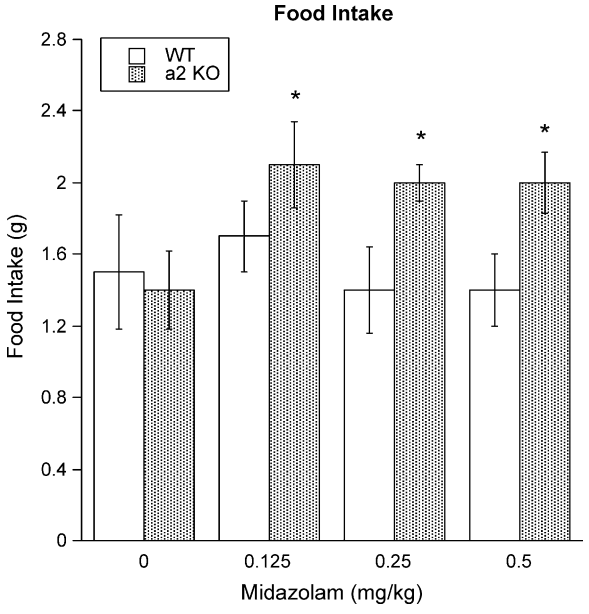

Fig. 1. The effect of midazolam (0.125, 0.25 and 0.5 mg/kg) on food intake in α2 KO and WT mice. All doses of midazolam increased food intake in α2 KO, but not WT mice ($p = 0.080$) $^*p < 0.05$.