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Effects of intraperitoneal administration of the $GABA_B$ receptor positive allosteric modulator 2,6-di tert-butyl-4-(2-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) on food intake in non-deprived rats

Ivor S. Ebenezer [a,b,*]

[a] Neuropharmacology Research Group, School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth PO 1 2DT, United Kingdom
[b] Institute of Biomedical and Bimolecular Sciences, University of Portsmouth, Portsmouth, United Kingdom

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ABSTRACT

γ-Aminobutyric acid-$_B$ ($GABA_B$) receptor positive allosteric modulators (PAMs) act on an allosteric site on the $GABA_B$ receptor to potentiate the effects of GABA and $GABA_B$ receptor agonists. It has previously been demonstrated that the $GABA_B$ receptor agonist baclofen increases food intake in non-deprived rats. The aim of this study was to investigate whether the $GABA_B$ receptor PAM 2,6-di tert-butyl-4-(2-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) would (i) increase food intake, and (ii) potentiate the hyperphagic effects of baclofen in rats. In Experiment 1, the effects of intraperitoneal (i.p.) administration of CGP7930 (1, 6 and 12 mg/kg) was investigated on food intake in non-deprived male Wistar rats. The 12 mg/kg dose of CGP7930 significantly increased cumulative food intake 30, 60 and 120 min ($P<0.05$, in each case) after administration. The 1 and 6 mg/kg doses were without effect. In Experiment 2, the effects of pretreatment with CGP7930 (6 mg/kg; i.p.) 5 min prior to administration of baclofen (2 mg/kg, i.p.) was investigated on 30 min cumulative food intake in non-deprived male Wistar rats. Baclofen (2 mg/kg) significantly increased food intake compared with vehicle treatment ($P<0.01$). CGP7930 (6 mg/kg) had no effect on feeding. However, pretreatment with CGP7930 (6 mg/kg) significantly potentiated the hyperphagic effects of baclofen (2 mg/kg) ($P<0.01$). These findings show that CGP7930 increases food intake and enhances the hyperphagic effects of baclofen, and are consistent with in vitro studies that suggest that it potentiates the effects of endogenous GABA and $GABA_B$ receptor agonists by allosteric modulation of the $GABA_B$ receptor.

1. Introduction

The γ-aminobutyric acid-$_B$ ($GABA_B$) receptor is a heterodimer that consists of 2 subunits, namely the $GABA_{B1}$ and $GABA_{B2}$ subunits (Bettler et al., 2004). Each subunit has 7 transmembrane spanning domains, which, when coupled intracellularly, are inserted into the cell membrane to provide normal receptor function (Bettler et al., 2004; Pin et al., 2004). The $GABA_{B1}$ subunit contains the binding site for GABA and $GABA_B$ receptor agonists, such as baclofen (Bettler et al., 2004; Pin et al., 2004). The $GABA_{B2}$ subunit is responsible for G-protein coupling and also possesses an allosteric modulatory site (Bettler et al., 2004; Pin et al., 2004; Binet et al., 2004). Positive allosteric modulators (PAMs), such as 2,6-di tert-butyl-4-(2-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930; Urwyler et al., 2001) and N, N′-dicyclopentyl-2-methysulfanyl-5 nitropyridine-4,6-diamine (GS39783; Urwyler et al., 2003), which have no apparent intrinsic activity of their own, act on an allosteric site on the $GABA_{B2}$ subunit to potentiate the effects of GABA or $GABA_B$ receptor agonists in vitro (Urwyler et al., 2001, 2003, 2005) and in vivo (Carai et al., 2004; Liang et al., 2006; Filip and Frankowska, 2007; Patterson et al., 2008) experiments.

It has been previously demonstrated that the subcutaneous (s.c.) or intraperitoneal (i.p.) administration of the $GABA_B$ receptor agonist baclofen increases food intake in non-deprived rats (Ebenezer and Pringle, 1992; Ebenezer, 1996; Ebenezer and Patel, 2011; Higgs and Barber, 2004; Edwards and Freeman, 2005; Buda-Levin et al., 2005; Patel and Ebenezer, 2008a,b, 2010). Baclofen readily crosses the blood–brain barrier (Faigle and Keberle, 1972) and Ebenezer and Patel (2004) have provided evidence that the hyperphagia produced by systemic administration of the drug is mediated by a central action of the $GABA_B$ receptor agonist. However, there have been a paucity of studies that have examined the effects of positive allosteric modulators of the $GABA_B$ receptor on food intake. The results of a recent study

* Corresponding author at: Neuropharmacology Research Group, School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth PO 1 2DT, United Kingdom.
E-mail address: ivor.ebenezer@port.ac.uk

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by Perdona et al. (2011) have shown that oral administration of baclofen or positive allosteric modulators of the $GABA_B$ receptor given 1 h prior to access to food decreased 4 h food consumption in mildly fasted rats. The results of this study are not consistent with results obtained after i.p. and s.c. administration baclofen in non-deprived animals (see Ebenezer and Pringle, 1992; Ebenezer and Patel, 2011; Buda-Levin et al., 2005; Patel and Ebenezer, 2008a,b, 2010) and could be due to differences in methodology used (see Discussion). The present study was therefore undertaken to (i) investigate the dose-related effects of i.p. administration of the $GABA_B$ receptor PAM CGP7930 on food intake in non-deprived rats, and (ii) determine whether CGP7930 modulated the hyperphagic effects of baclofen

2. Material and methods

The protocols used in this study were approved by the Ethical Review Committee at the University of Portsmouth, U.K.

2.1. Experiment 1. Effects of CGP7930 on food intake in non-deprived rats

Adult male Wister rats ($n=6$; starting body weights: 320–380 g), that were bred and supplied by the Bioresources Unit at the University of Portsmouth, England, were housed in cages with wood shaving bedding on the floor in groups of 3 where they had free access to food and water at all times. The rats were kept on a 12 h light/dark cycle with light on at 08.30 h and lights off at 20.30 h. All training and experimental sessions were carried out between 13.50 h and 16.00 h. The rats were given 4 training sessions when they were allowed free access to their normal pelleted food (801304 Rodent Diet, Special Diets Service, Witham, Essex, England). Food composition: (a) percentage mass: protein 20%, oil 4.5%, carbohydrate 60%, fibre 5%, ash, 7%+traces of vitamins and metals, (b) percentage energy: protein 27.3%, oil 11.48% and carbohydrate 61.2%, (c) energy density: 3.6 kcal/g) and water in experimental cages measuring $32 \times 25 \times 10\ cm^3$. The food was presented to the rats in shallow cylindrical cups, as described previously (Ebenezer, 1990). During experimental sessions that followed, the rats in both groups were injected i.p. with either vehicle solution or CGP7930 (1, 6 or 12 mg/kg) just prior to 14.00 h and placed separately into experimental cages with free access to food and water and cumulative food consumption measured at 30 min intervals for 120 min. Each rat received all doses of saline and CGP7930 in a random fashion with, at least, two days separating successive trials. Each rat was visually assessed during the experimental sessions for signs of ataxia, sedation and other abnormal behaviours at 10 min intervals during the first 30 min and then at 30 min intervals.

2.2. Experiment 2. Effects of CGP7930 on baclofen-induced hyperphagia in non-deprived rats.

Adult male Wister rats ($n=6$; body weights: 335–400 g) were housed and given training sessions in experimental cages as described for Experiment 1. During experimental sessions, each rat was injected with either vehicle followed by saline, vehicle followed by baclofen (2 mg/kg), CGP7930 (6 mg/kg) followed by saline or CGP7930 (6 mg/kg) followed by baclofen (2 mg/kg). All injections were given i.p. A period of 5 min separated the two injections. Immediately after the second injection, the rats were placed separately into experimental cages with free access to food and water, and cumulative food consumption measured for 30 min. A repeated measures design was used with each rat receiving all 4 treatments in a random fashion. At least 3 days separated successive drug trials. Each rat was observed during the experimental sessions for signs of ataxia, sedation and other abnormal behaviours at 10 min intervals.

2.3. Drugs

(±) Baclofen was purchased from Sigma Biochemicals, Dorset, UK. The drug was dissolved in physiological saline solution (0.9% $^{w}/_{v}$, NaCl) to give an injection volume of 0.1 ml/100 g body weight. Physiological saline solution was used as the control for baclofen. CGP7930 (2,6-di tert-butyl-4-[2-hydroxy-2,2-dimethyl-propyl]-phenol) was purchased from Tocris Biosciences, Bristol, UK. The drug was prepared as follows: 30 mg was dissolved in 50 µl of DMSO (dimethyl sulfoxide) and made up to 0.6 ml in a solution of 80% propylene glycol (PEG) and 20% physiological saline. The drug was further diluted in a solution of 80% propylene glycol (PEG) and 20% physiological saline to give an injection volume of 0.1 ml/100 g body weight.

2.4. Statistics

The cumulative food intake data from Experiments 1 at each measurement interval were analysed by one way analysis of variance (ANOVA) with repeated measures and by the *post-hoc* Student Newman–Keuls test (Winer, 1971). The data from Experiment 2 were analysed by two way ANOVA with repeated measures and by the *post-hoc* Student Newman–Keuls test (Winer, 1971).

3. Results

3.1. Experiment 1. Effects of CGP7930 on food intake in non-deprived rats

Fig. 1 shows the effects of CGP7930 (1–12 mg/kg) on cumulative food recorded at 30, 60, 90 and 120 min following administration. Statistical analysis of the data (ANOVA) indicated that CGP7930 (1–12 mg/kg) significantly increased cumulative food intake at each of the measurement intervals ($F_{(3,15)}=6.423$, $P<0.01$ at 30 min; $F_{(3,15)}=3.596$, $P<0.05$ at 60 min; $F_{(3,15)}=6.266$, $P<0.01$ at 90 min; $F_{(3,15)}=7.762$, $P<0.01$ at 120 min). Post-hoc tests revealed that the 12 mg/kg dose produced significant increase in cumulative food intake at 30 min ($P<0.05$),

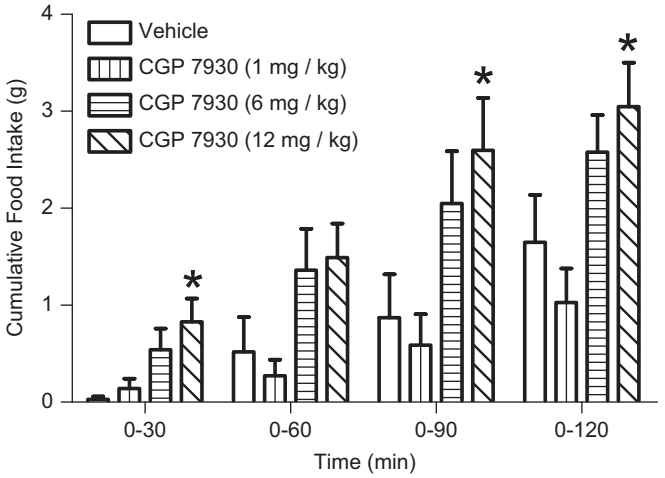

Fig. 1. Effects of CGP7930 (1–12 mg/kg) on cumulative food intake in non-deprived rats. See text for experimental details. Vertical lines represent+S.E.M. *$P<0.05$ vs. Vehicle.

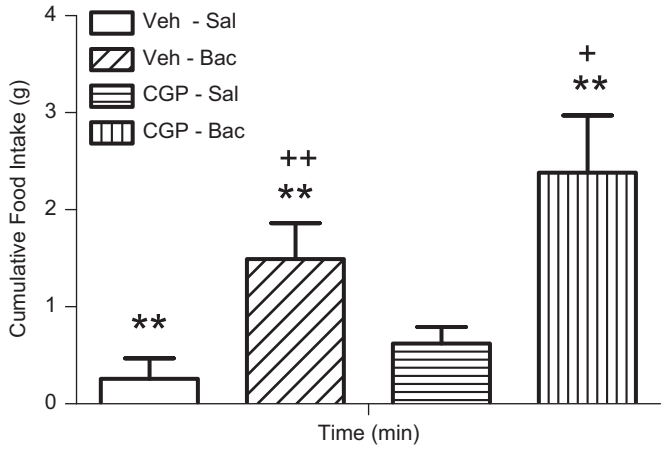

Fig. 2. Effects of CGP7930 (6 mg/kg) on baclofen-induced hyperphagia in non-deprived rats. See text for experimental details. Vertical line represent+S.E.M. $^{*}P<0.05$, $^{**}P<0.01$ vs. Veh–Sal, $^{+}P<0.05$, $^{++}P<0.01$ vs. Veh–Bac. Abbreviations: Sal=saline, Veh=vehicle, Bac=baclofen, CGP=CGP7930.

90 min ($P<0.05$) and 120 min ($P<0.05$). The 1 and 6 mg/kg doses of CGP7930 had no significant effects on cumulative food intake at any of the measurement times. Interestingly, post-hoc tests also indicated that the mean cumulative food intake for the 6 mg/kg dose of CGP 7930 was not significantly different from that of the 12 mg/kg dose at any of the measurement intervals.

None of the doses of CGP7930 used in this experiment produced any overt signs of ataxia, sedation or other abnormal behavioural changes in the animals compared with vehicle treatment

3.2. Experiment 2. Effects of CGP7930 on baclofen-induced hyperphagia in non-deprived rats.

The results obtained are illustrated in Fig. 2. Statistical analysis of the cumulative data (two-way ANOVA with repeated measures) obtained at 30 min (see Fig. 2) showed that there was a significant interaction between the 1st and 2nd injections ($F_{(1,5)}=6.812$, $P<0.05$) on cumulative food intake at 30 min indicating that the stimulant effect of baclofen (2 mg/kg) on feeding was potentiated by pretreatment with CGP7930. Post-hoc tests confirmed that baclofen (2 mg/kg) significantly increased cumulative food intake ($P<0.01$) and that the hyperphagic effect of the $GABA_B$ receptor agonist was significantly increased by CGP 7930 (6 mg/kg) pretreatment ($P<0.01$). CGP7930 (6 mg/kg) had no significant effects on food consumption on its own compared with control intake at 30 min.

None of the treatments produced any overt signs of ataxia, sedation or other abnormal behavioural changes in the rats compared with vehicle treatment.

4. Discussion

It has been previously reported that systemic administration of the $GABA_B$ receptor agonist baclofen produces hyperphagia in rodents (Ebenezer, 1995, 1996; Ebenezer and Patel, 2004, 2011; Patel and Ebenezer, 2008a,b). Experiment 1 was undertaken to extend these observations and investigate the effects of the $GABA_B$ receptor PAM CGP7930 (1, 6 and12 mg/kg; i.p.) on food intake in non-deprived rats. The results show that while the 1 and 6 mg doses of CGP7930 were without effect, the 12 mg/kg dose significantly increased cumulative food intake during the 2 h measurement period after administration (see Fig. 1). The most parsimonious explanation for the results is that CGP7930 acts on the allosteric site of the $GABA_{B2}$ subunit of the $GABA_B$ receptor to potentiate the effects of endogenous GABA on the $GABA_B$ receptor (see Introduction) and thus increases food intake. The observation that the 1 and 6 mg/kg doses of CGP7930 did not significantly increase food consumption suggests that they were below the threshold necessary to sufficiently potentiate the effects of endogenous GABA on the $GABA_B$ receptor to effect feeding. These results are consistent with the hyperphagia observed after stimulation of $GABA_B$ receptor by i.p. administration of baclofen (Ebenezer, 1995, 1996; Ebenezer and Patel, 2004, 2011; Patel and Ebenezer, 2008a,b). We have previously demonstrated that systemic administration of baclofen stimulates feeding by a central mode of action (Ebenezer and Patel, 2004) and, as behavioural studies have suggested that CGP7930 enters the brain from the systemic circulation (see Brusberg et al., 2009), it is likely that its effects on food intake are centrally mediated.

By contrast to the results reported for Experiment 1, Perdona et al. (2011) have recently reported that oral administration of baclofen or the $GABA_B$ receptor PAMs, GS39783 and CMPPE (2-{1-[2-(4-chlorophenyl)-5-methylpyrazolol [1,5-a]pyrimidin-7-yl]-2-piperidinyl}ethanol), decrease food intake in rats. The reasons for the differences in results observed for baclofen and CGP7930 in this study and those obtained by Perdona et al. (2011) are not known. However, the most likely explanation is that there were marked differences in the methodology used in these studies. It has been previously reported that s.c. or i.p. administration of baclofen to non-deprived rats increases short-term food consumption during the light phase of the light/dark cycle (Ebenezer and Pringle, 1992; Ebenezer, 1996; Higgs and Barber, 2004; Patel and Ebenezer, 2008a,b, 2010). By contrast, Perdona et al. (2011) (i) maintained the rats in individual cages for 3 weeks prior to the start of the experiment, (ii) fasted the animals for 2 h prior to access to food, (iii) gave the drugs by the oral route 1 h prior to the test session, (iv) presented the animals with food during the test session at the beginning of the dark phase of the light/dark cycle, and (v) recorded cumulative food intake 4 h after presenting the animals with food and 5 h after injection of the drugs. It is likely that any one or a combination of these factors may have contributed to the results obtained. For example, it has been reported that the hyperphagic effects of baclofen are attenuated if the experiment commences at the beginning of the dark phase of the light/dark cycle when rats are most motivated to eat (Ebenezer and Patel, 2011). It has also been found that baclofen does not increase food intake in rats that have been food deprived for 22 h (Ebenezer, 1996; Ebenezer and Patel, 2011) and it is therefore conceivable that mild fasting may also attenuate food intake (see Ebenezer and Tite, 2003). Recently, Collares and Vinagre (2010) have demonstrated that systemic administration of baclofen can inhibit gastric emptying of solid food in rats and it is possible that oral administration of these agents may have direct effects on the gut to suppress gastric emptying of solid food and thus decrease food consumption. In addition, keeping the rats isolated in individual cages for 3 weeks prior to the start of the experiment may have been stressful to the animals (see Nakhate et al., 2011) which could have also affected the results that were obtained on food intake with baclofen and the $GABA_B$ receptor PAMs. Moreover, as $GABA_B$ receptor PAMs facilitate the actions of endogenous GABA by allosteric modulation of the $GABA_B$ receptor (Bettler et al., 2004), the effects of these agents on food intake would be dependent on the levels of endogenous GABA in "relevant" areas of the brain. This is generally low in non-deprived animals (see Patel and Ebenezer, 2004) but may vary depending on internal and external factors, such as stress levels in the animals, time of day, and prior acclimatisation to the experimental procedure. Consequently,

there may be variations in the sensitivity of similar doses of $GABA_B$ receptor PAMs to stimulate feeding in non-deprived rats under different experimental conditions. Nevertheless, the results of the Perdona et al. (2011) study suggest that further work should be undertaken to investigate the effects of oral administration of baclofen and other $GABA_B$ receptor agents on short- and long-term food intake in rats to establish the mechanism(s) involved in the hypophagia reported by these authors.

Experiment 2 was conducted to determine whether pretreatment with CGP7930 (6 mg/kg) would potentiate the hyperphagic effects of baclofen (2 mg/kg) on food intake in non-deprived rats. In this study, a 2 mg/kg dose of baclofen was chosen because it has previously been found to increase food intake in rats with minimal overt behavioural adverse effects (see Ebenezer and Patel., 2011). The results show that, in agreement with previous observations, baclofen significantly increased cumulative food intake measured 30 min after administration (e.g. Ebenezer and Patel, 2011). On the other hand CGP7930 (6 mg/kg) had no significant effect on food consumption compared with control data. However, pretreatment with CGP7930 (6 mg/kg) significantly potentiated the hyperphagic effects of baclofen (see Fig. 2). These results are consistent with the hypothesis that CGP7930 acts on the allosteric site of the $GABA_{B2}$ subunit of the $GABA_B$ receptor (Urwyler et al., 2001; Pin et al., 2004) to enhance the effect of baclofen on food intake. Interestingly, it was found that pretreatment with CGP7930 (1 mg/kg; i.p.) was without effect on baclofen (2 mg/kg)-induced hyperphagia (Ebenezer, unpublished results). Thus, it is likely that the 1 mg/kg dose of GP7930 falls below the threshold i.p. dose that is required to significantly modulate the $GABA_B$ receptor to potentiate the effects of baclofen on food intake.

In summary, the results of this study have shown that i.p. administration of the $GABA_B$ receptor PAM CGP7930 (i) increases food intake in non-deprived rats and (ii) potentiates the hyperphagic effects of the $GABA_B$ receptor baclofen. These findings extend previous observations with $GABA_B$ receptor PAMs on behaviour (Carai et al., 2004; Liang et al., 2006; Patterson et al., 2008) and show that CGP7930 facilitates the effects of endogenous GABA and $GABA_B$ receptor agonist baclofen on food intake in non-deprived rats. These findings may have useful implications for both basic and clinical studies related to food intake.

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