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The effects of acute multiple intraperitoneal injections of the $GABA_B$ receptor agonist baclofen on food intake in rats

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ABSTRACT

This study was undertaken to examine the effects of acute repeated administration of the $GABA_B$ receptor agonist baclofen on food intake in rats. In Experiment 1, the effects of repeated intraperitoneal (i.p.) injections of the $GABA_B$ receptor agonist baclofen (1 and 2 mg/kg) at 2 h intervals were investigated on food intake in non-deprived male Wistar rats. Both doses of baclofen significantly increased food intake after the 1st injection ($P<0.05$), but had no effects on intake following the 2nd and 3rd injections. By contrast, in Experiment 2, diazepam (1 and 2 mg/kg, i.p.) significantly increased food intake (at least, $P<0.05$) after each of 3 injection separated by 2 h in non-deprived rats. These data show that tolerance occurs to the hyperphagic effects of baclofen with acute multiple injections, and may have important implications for future studies investigating the effects of $GABA_B$ receptor agonists on food intake and energy homeostasis.

1. Introduction

Experimental evidence gathered over the last 3 decades has suggested that the inhibitory neurotransmitter gamma-amino butyric acid (GABA), acting at both central $GABA_A$ and $GABA_B$ receptors, plays an important role in the regulation of food intake (Kelley et al., 1979; Patel and Ebenezer, 2004a). Central administration of $GABA_A$ receptor agonists, such as muscimol, and central or systemic administration of drugs, such as the benzodiazepines that enhance the effects of GABA at $GABA_A$ receptors (Ticku, 1977), stimulate feeding in experimental animals (Kelley et al., 1979; Baldwin et al., 1990; Naruse, 1994; Cooper and Higgs, 1996, and others). Similarly, both central and systemic administration of the $GABA_B$ receptor agonist baclofen increases food intake in a variety of animal species, including rat, mouse and pig (Ebenezer, 1990, 1995; Ebenezer and Baldwin, 1990; Ebenezer and Pringle, 1992; Wirtshafter et al., 1993; Ward et al., 2000; Patel and Ebenezer, 2004a; Ebenezer and Prabhaker, 2007).

Most of reports published on the effects of $GABA_A$ and $GABA_B$ receptor agonists on food intake have examined the effects of single doses of these drugs on short-term feeding responses (Ebenezer, 1990, 1995; Ebenezer and Pringle, 1992; Ebenezer and Patel, 2004; Ebenezer and Prabhaker, 2007; Ward et al., 2000; Kelley et al., 1979; Cooper and Higgs, 1996; Baldwin et al., 1990). However, there is a paucity of information on the effects of acute repeated administration of drugs that stimulate GABA receptors on food intake. In an early study, Naruse (1994) demonstrated that intravenous (i.v.) injection of the benzodiazepine diazepam in rats every 3 h during the light cycle produced short-term hyperphagia following each injection. Diazepam is an agonist at specific benzodiazepine receptors associated with the $GABA_A$ receptor complex and acts to potentiate the inhibitory effects of GABA on post-synaptic membranes in the CNS by modulating $GABA_A$ receptor mediated transmission (Ticku, 1977). Thus, the hyperphagia produced by diazepam appears to be due to an enhanced response to GABA at central $GABA_A$ receptors (Cooper and Higgs, 1996). Research in our laboratory has focused on the effects of $GABA_B$ receptor agonists and antagonists on food intake. As mentioned above, we have shown that intracerebroventricular (icv) injections of baclofen elicits feeding in satiated pigs and non-deprived rats by an action at central $GABA_B$ receptors (Ebenezer, 1990; Ebenezer and Baldwin, 1990) and we have also demonstrated that systemic administration of the $GABA_B$ receptor agonist increases food intake in non-deprived rodents (Ebenezer and Pringle, 1992; Ebenezer, 1996; Ebenezer and Prabhaker, 2007) by a central action (Ebenezer and Patel, 2004). We have additionally provided evidence that suggest that endogenous GABA, acting at central $GABA_B$ receptors, may play a physiological role in the regulation of feeding behaviour (Patel and Ebenezer,

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2004a,b). It was therefore of interest to examine the effects of acute repeated administration of baclofen on food intake as this would extend previous observations and provide more information as to the nature of the stimulant effects of $GABA_B$ receptor agonists on food intake. Thus, the present study was undertaken to investigate the effects of acute multiple intraperitoneal injections of the $GABA_B$ agonist baclofen on food intake in non-deprived rats. For comparison, we also examined the effects of acute multiple intraperitoneal injections of diazepam on food intake in non-deprived rats.

2. Material and methods

The protocols used in this study were approved by the Ethical Review Committee at the University of Portsmouth, U.K. and carried out under licence granted by the United Kingdom Home Office.

2.1. Experiment 1. Effects of acute repeated administration of baclofen on short-term food intake in rats

Adult male Wister rats (n=7; body weights: 350–420 g) were housed in cages in groups of 3 and where they had free access to food and water at all times. The animals were maintained on a 12 h light/dark cycle (lights on at 830 h and lights off at 2030 h). The rats were given four 6 h training sessions when they were allowed free access to their normal pelleted food (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: 3.600 kcal/g) and water in experimental cages measuring 32×25×10 cm. The food was presented to the rats in shallow cylindrical cups, as described previously (Ebenezer, 1990). During experimental sessions that followed, the rats were injected i.p. (1st injection) with either saline or baclofen (1 or 2 mg/kg) at 1100 h and placed separately into the experimental cages immediately after injection with free access to food and water. Cumulative food intake was measured at 60 and 120 min. At 120 min, the rats were injected again (2nd injection) with their respective treatments and cumulative food intake measured in 60 min time bins for 120 min. At the end of this period, the animals received a 3rd injection of saline or baclofen and cumulative food intake measured for a further 120 min in 60 min time bins. A repeated measure design was used in which each animal received all treatments with 3–4 days separating successive trials.

2.2. Experiment 2. Effects of acute repeated administration of diazepam on short-term food intake in rats

A similar experimental design as described for Experiment 1 was used except that the male Wistar rats (n=8; b. wt. 330–420 g,) were injected with either vehicle (see Section 2.3 below for information about the vehicle) or diazepam (1 or 2 mg/kg).

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. Diazepam was obtained from Phoenix Pharmaceuticals, Gloucester, UK and dissolved in a 20% $^{v}/_{v}$ ethanol solution in physiological saline to give an injection volume of 0.1 ml/100 g body weight. A 20% ^{v}/v ethanol solution in physiological saline was used as the control vehicle.

2.4. Statistics

The cumulative food intake data at each measurement time point for both experiments were analysed by one way analysis of variance (ANOVA) with repeated measures on treatment followed by the Newman-Kuels *post-hoc* test (Winer, 1971).

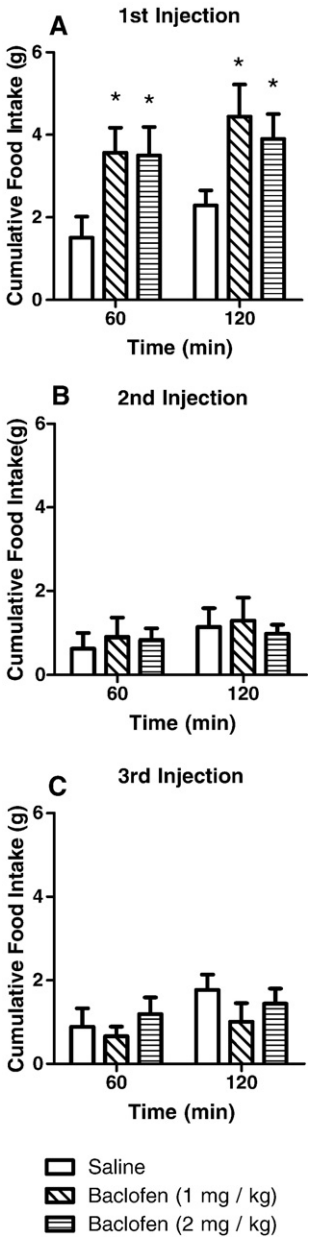

Fig. 1. Effects of repeated intraperitoneal injections of physiological saline or baclofen (1 and 2 mg/kg) administered at 120 min intervals on food intake in non-deprived rats (n=7). Cumulative food intake was measured 60 and120 min after (A) the 1st injection, (B) the 2nd injection, and (C) the 3rd injection of saline or baclofen. See text for further details. Vertical lines represent+S.E.M. *P<0.05.

3. Results

3.1. Experiment 1. Effects of acute repeated administration of baclofen on short-term food intake in rats

The results are illustrated in Fig. 1. Statistical analysis (ANOVA) of the results obtained following the 1st injection (see Fig. 1A) showed that baclofen significantly increased cumulative food intake at 60 min ($F_{(2,14)}$=4.969; P<0.05) and 120 min ($F_{(2,14)}$=4.326, P<0.05). Post-hoc tests revealed that both doses of baclofen (i.e. 1 and 2 mg/kg) produced significant increases in cumulative food intake at 60 and 120 min when compared with control data (P<0.05, in each case; Fig. 1A).