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Effects of the $GABA_B$ receptor agonist baclofen administered orally on normal food intake and intraperitoneally on fat intake in non-deprived rats.

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ABSTRACT

It has been previously reported that the $GABA_B$ receptor agonist baclofen decreases food intake after oral administration and fat intake after intraperitoneal administration. The aim of the study was to investigate the effects of baclofen (1–4 mg/ kg) administered orally (Experiment 1) on food intake in non-deprived rats ($n=6$) and intraperitoneally (Experiment 2) on fat intake in non-deprived rats ($n=8$) that were naïve to baclofen (1st set of trials) and in the same group of rats after they were sub-chronically exposed to baclofen (2nd set of trials). The results from Experiment 1 show that baclofen had no effects on food intake during the 1st set of trials, but the 2 and 4 mg/kg doses significantly increased food consumption during the 2nd set of trials. Baclofen produced sedation during the 1st set of trials, but tolerance occurred to this effect and was not apparent during the 2nd set of trials. These observations suggest that the motor effects may have competed with the hyperphagic effects of baclofen during the 1st set of trials. The data from Experiment 2 show that baclofen had no effects on fat intake during either the 1st or 2nd set of trials. The results of the study thus indicate that orally administrated baclofen increases food intake and intraperitoneal administration has no effect on fat intake in non-deprived rats under the conditions used in this study. These findings may have important implications for research on the use of baclofen in studies concerned with ingestive behaviours.

1. Introduction

Experiments undertaken in this laboratory and elsewhere have shown that subcutaneous (s.c.) or intraperitoneal (i.p.) administration of the $GABA_B$ receptor agonist baclofen increases food intake in non-deprived rat (Ebenezer and Pringle, 1992; Ebenezer, 1996; Ebenezer and Patel, 2004., Higgs and Barber, 2004; Edwards and Freeman, 2005; Buda-Levin et al., 2005; Patel and Ebenezer, 2008a,b; 2010) and mouse (Ebenezer and Prabhaker, 2007). Intracerebroventricular administration of baclofen has also been found to increase food intake in non-deprived rats (Ebenezer, 1990). Baclofen 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.

The experiments carried out in the present study were conducted to examine two reported effects of baclofen on food intake. The first was that oral administration of baclofen decreases food intake in rats (Perdona et al., 2011). The second was that when non-deprived rats are presented simultaneously with normal rat chow and pure fat or emulsions with high fat content, baclofen decreases fat intake but generally increases normal food consumption (Buda-Levin et al., 2005; Rao et al., 2008; Wang et al., 2011). The experiments in these studies were carried out in animals that were naïve to baclofen or had very little previous exposure to the drug (Perdona et al., 2011; Buda-Levin et al., 2005; Rao et al., 2008; Wang et al., 2011). However, acute administration of baclofen has been reported to produce sedation, muscle relaxation, and ataxia in rat, mouse and pig (Ebenezer, 1990; Ebenezer and Pringle, 1992; Ebenezer and Prabhaker, 2007; Patel and Ebenezer, 2008a,b, 2010; Perdona et al., 2011) and it has been suggested that these behaviours may compete with the effect of the drug on food consumption (Patel and Ebenezer, 2008b, 2010). While tolerance does not develop to the hyperphagic effects of baclofen with repeated daily administration, it rapidly develops to the motor and sedative effects (Patel and Ebenezer, 2008b, 2010). The present study was therefore undertaken to investigate the dose-related effects of baclofen before and following sub-chronic exposure on (i) food consumption after oral administration, and (ii) fat consumption after i.p. administration.

2. Material and methods

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

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2.1. Animals and housing

Adult male Wister rats ($n=14$; starting body weights: 320–380 g) were housed in cages in groups of 3–4 where they had free access to 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: 3.6 kcal/g) and water at all times. The animals were kept on a 12 h light/dark cycle with light on at 08.30 h and lights off at 20.30 h. The animals were handled regularly prior to training and experimental sessions.

2.2. Experiment 1: Effects of orally administered baclofen on food intake in non-deprived rats

Rats ($n=6$) were given 4 training sessions in experimental cages measuring $32 \times 25 \times 10\ cm^3$ where they were given an oral preload of 0.75 g a palatable wet mash in a shallow cylindrical cups. (The wet mash was made up as follows: 5 g porridge oats [Mornflake Medium Oatmeal, Mornflake, Crew, UK], 20 ml water and 1 tablet of a low calorie sweetener (Sainsbury's Slenda Sweet, Sainsbury PLC, London, UK). The mixture was heated for 1 min in a microwave oven to partially solidify it into a wet mash). Ten minutes later, the wet-mash container were removed and replaced with ones containing their normal pelleted food (see above for details of composition) and cumulative food intake measured manually at 30, 60, 90 and 120 min by weighing the containers at these time points. Water was available at all times. During experimental sessions, the rats were dosed orally by mixing distilled water (Control, 1 ml/ kg) or solutions of baclofen (1, 2 or 4 mg/kg) into the palatable wet mash and the procedure described above for the training session was followed. Each rat received vehicle and all doses of baclofen in a Latin square repeated measures design with, at least, two days separating successive trials. Following the 1st set of trials, the rats received daily 10 min sessions over a period of 4 days in the experimental cages when they were given a 4 mg/kg dose of baclofen orally in the palatable wet mash. Two days later the experimental procedure as described for the 1st set of trials was repeated. The animals were observed at regular intervals during each of the experimental session for signs of ataxia, sedation and other abnormal behaviours, as described previously (Ebenezer, 2012).

2.3. Experiment 2: Effects of intraperitoneal administration of baclofen on fat intake in non-deprived rats

Rats ($n=8$) were given 4 training sessions lasting 120 min when they were allowed free access to a single block of 100% animal (pig) fat (Kerrymaid Lard^R; Kerry Food Ltd., Egham, Surrey, UK). During experimental sessions, each rat was injected with either saline or baclofen (1, 2 or 4 mg/ kg) and placed separately into experimental cages with free access to food (lard) and water. Cumulative food consumption measured at 30, 60, 90 and 120 min, as described for Experiment 1. A Latin square repeated measures design was used with each rat receiving all 4 treatments; at least 3 days separated successive drug trials. Following the 1st set of trials, the rats were injected daily over a period of 4 days with baclofen (4 mg/kg; i.p.).Two days later the experimental procedure as described for the 1st set of trials was repeated. The rats were observed at regular interval during each of the experimental session for signs of ataxia, sedation and other abnormal behaviours, as described previously (Ebenezer, 2012).

2.4. Drugs

(±) Baclofen was purchased from Sigma Biochemicals, Dorset, UK. The drug was dissolved in distilled water (Experimental 1) or physiological saline solution (0.9 w/v%, NaCl; Experiment 2) to give an injection volume of 0.1 ml/100 g body weight. Distilled water (Experiment 1) or physiological saline solution (Experiment 2) was used as the control for baclofen.

2.5. Statistics

The cumulative food intake data from Experiments 1 and 2, 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).

3. Results

3.1. Experiment 1: Effects of orally administered baclofen on food intake in non-deprived rats

All rats ate the wet mash preload with or without baclofen within 30–60 s after presentation. Fig. 1A shows the effects of oral administration of baclofen (1–4 mg/kg) on food intake. Analysis of the data showed that none of the doses produced significant effects on cumulative food intake at any of the measurement intervals. By contrast, as illustrated in Fig. 1B, following repeated exposure to the 4 mg/kg dose of baclofen once daily for 4 days, statistical analysis of the data revealed significant effects of drug treatment on cumulative food intake at 60 min ($F_{(3,15)}=3.438$, $P<0.05$), 90 min ($F_{(3,15)}=5.066$, $P<0.02$) and 120 min ($F_{(3,15)}=5.276$, $P<0.02$). Post-hoc tests showed that the 1 mg/kg doses was without effect, but that cumulative food intake was significantly increased ($P<0.05$ in each case) by oral administration of the 2 mg/kg dose at 120 min and by the 4 mg/kg dose at 90 and 120 min (see Fig. 1B).

Two days after the end of the 2nd set of trials, we gave the rats 8 mg/kg of baclofen orally and this dose significantly increased food intake at all measurement intervals compared with the control data. [Mean cumulative food intake (g) ± s.e.m.: 30 min: vehicle = 2.8 ± 0.3 g, baclofen (8 mg/kg) = 6.5 ± 0.9 g ($P<0.02$); 60 min: vehicle = 3.6 ± 0.3 g, baclofen (8 mg/kg) = 7.4 ± 1.1 g ($P<0.05$); 90 min: vehicle = 3.9 ± 0.3 g, baclofen (8 mg/kg) = 7.5 ± 1.1 g ($P<0.02$); 120 min: vehicle = 3.9 ± 0.2 g, baclofen = (8 mg/kg) 7.8 ± 1.1 g ($P<0.02$)].

During the 1st set of trials, the 1 and 2 mg/ kg doses of baclofen did not produce any overt behavioural effects compared to control treatment in the animals. However, the 4 mg/ kg dose of baclofen had a sedative effect on the animals which was most apparent in the last 60 mins of the trial. During the 2nd set of trials, there were no overt behavioural changes in the animals with any of the doses of baclofen compared with control treatment.

3.2. Experiment 2: Effects of intraperitoneal administration of baclofen on fat intake in non-deprived rats

Under control conditions, the rats consumed most of the fat (lard) during the 1st 30 min. Fig. 2A shows the effects of baclofen (1–4 mg/kg; i.p.) on fat intake in the non-deprived rats. Statistical analysis of the results revealed no significant effects of cumulative fat intake at 30 min ($F_{(3,21)}=0.4395$, ns), 60 min ($F_{(3,21)}=0.4777$, ns), 90 min ($F_{(3,21)}=0.3962$, ns), and 120 min ($F_{(3,21)}=0.5966$, ns) during the 1st set of trials. Fig. 2B illustrates the results obtained during the 2nd set of trials following repeated exposure to baclofen