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Effect of intracerebroventricular administration of the $GABA_B$-receptor agonist baclofen on operant feeding in satiated pigs

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1 The present study investigated the effects of intracerebroventricular (i.c.v.) administration of the $GABA_B$-receptor agonist baclofen on food and water intake in satiated pigs previously trained to make operant responses for food and water, which were available *ad libitum*.

2 Baclofen (25–100 nmol) i.c.v. produced a dose-related increase in food intake. Baclofen (50 nmol) increased feeding during the first 15 min after administration ($P < 0.01$), while the 100 nmol dose increased feeding during the first 30 min ($P < 0.01$). None of these doses of baclofen had any affect on the daily (24 h) food intake.

3 The effect of baclofen (50 nmol) on feeding was prevented by pretreating the animals with the $GABA_B$ antagonist phaclofen (500 nmol, i.c.v.).

4 Baclofen (25–100 nmol) i.c.v. had no significant effects on water intake.

5 Intravenous administration of baclofen (100 nmol) had no effect on food intake, thus eliminating the possibility that i.c.v. baclofen might have stimulated feeding by a peripheral mode of action.

6 These results show that baclofen increases food intake in satiated pigs, and that this effect is mediated by the drug acting at central $GABA_B$-receptors.

Introduction

It is now widely accepted that γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in mammalian brain (Cooper *et al.*, 1986), and in recent years considerable evidence has accumulated to support the view that a central GABAergic mechanism is involved in the control of food intake (Kelly *et al.*, 1979; Kelly & Grossman, 1980; Panksepp & Meeker, 1980). GABA exerts its effects by acting at two pharmacologically distinct receptor subtypes, namely $GABA_A$- and $GABA_B$-receptors (Bowery, 1989). Much of the work on food intake has focused on the $GABA_A$-receptor subtype, and it has been shown that intracerebroventricular (i.c.v.) injections of $GABA_A$-agonists, such as muscimol, increase meal size in a number of animal species, including the rat (Kelly *et al.*, 1979), the sheep (Seoane *et al.*, 1984), and the pig (Ebenezer & Baldwin, 1990). Moreover, microinjections of GABA and muscimol into the ventromedial nucleus or paraventricular nucleus of the hypothalamus also increase feeding (Kelly *et al.*, 1977; 1979; Grandison & Guiditti, 1977; Girard *et al.*, 1985), and these effects can be completely antagonized by $GABA_A$-receptor antagonists. The results of such studies thus indicate that a central $GABA_A$-receptor mechanism may be involved in the control of food intake. Further evidence to support this view comes from studies which show that drugs, such as the barbiturates and the benzodiazepines, which are believed to exert some of their pharmacological actions by acting on regulatory sites on the $GABA_A$-receptor to enhance the effects of endogenous GABA (Olsen, 1982; Turner & Whittle, 1983), also increase food intake.

Very little is known about the pharmacological and physiological actions of GABA that are mediated via $GABA_B$-receptors. We were therefore interested in finding out if pharmacological stimulation of central $GABA_B$-receptors would produce increased consumption of food and water. Thus, in the present study we investigated the effects of intracerebroventricular (i.c.v.) administration of the $GABA_B$-receptor agonist baclofen on food and water intake in pigs. The results show that baclofen causes a short-lasting dose-related increase in operant food intake, and that this effect can be antagonized by the competitive $GABA_B$-receptor antagonist phaclofen. Baclofen had no effect on fluid intake. A preliminary account of these results has been published in abstract form (Ebenezer & Baldwin, 1989).

Methods

Animals

Prepubertal large white pigs ($n = 18$; 10 male, 8 female) weighing between 30–60 kg, were used in these experiments. The pigs were housed individually in metabolism cages for the duration of the experiment. Each cage was equipped with two operant switch panels at the front end, which, when activated, delivered food (Right Panel) or water (Left Panel) into two bowls within the cage. The pigs were trained to press each panel with their snouts on a fixed ratio of 5 to obtain a single food reinforcement (10 g of pelleted pig food) or a single water reinforcement (10 ml of water).

Surgery

Each pig was implanted under halothane anaesthesia with an 18 gauge stainless steel guide tube aimed at the lateral cerebroventricle for subsequent i.c.v. injection of drugs. Details of the surgical procedures and the methods used for i.c.v. injections in the pig have been published previously (Baldwin & Thornton, 1986). In addition, two pigs were implanted under halothane anaesthesia with a catheter in the right external jugular vein for the intravenous (i.v.) administration of drugs. The catheter was exteriorized to the dorsal surface of the animal's neck.

Experimental procedure

Food and water were available *ad libitum*. Feeding and drinking were continously monitored for the duration of the experiment by means of a computer-based data logging system. In the first experiment we investigated the effects of baclofen on

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food and water intake. Injections of baclofen (25, 50 and 100 nmol) or vehicle (physiological saline solution) were administered i.c.v. between 10 h 00 min–11 h 00 min. The criterion for drug or saline administration was that pigs had not eaten for at least 30 min before injection. Drug sessions were usually conducted twice a week, and saline control sessions bracketed each drug session. The doses of baclofen were administered in random fashion. The logger recorded the amount of food (g) or water (ml) consumed by the pigs 0–15 min, 15–30 min, 30–60 min and 60–120 min after saline or baclofen. The total daily food and water intake was also recorded.

In the second experiment, we examined the effects of the $GABA_B$-antagonist phaclofen (50, 100 and 500 nmol i.c.v.) on food and water intake in the pigs. A similar protocol to that described for experiment 1 was used.

In the third experiment, the effects of pretreating the animals with phaclofen before the administration of baclofen were investigated. The pigs were injected with phaclofen (50, 100 or 500 nmol) or saline (control) i.c.v. 5 min before receiving a second i.c.v. injection of baclofen (50 nmol).

Statistical analysis

The effects of drug treatment on food and water intake for each pig was compared with the saline control values recorded on the day preceding the drug session by use of the two-tailed paired *t*-test. *P* values less than 0.05 were considered significant.

Drugs

The drugs used were (−)-baclofen (CIBA) and phaclofen (Cambridge Research Biochemicals). The drugs were dissolved in physiological saline solution (0.09% w/v). Physiological saline solution was used in control experiments.

Results

The pigs ate and drank *ad libitum*. They usually had a meal every 2–3 h, although considerable individual differences were noted among the animals as to the frequency and duration of their meals. The pigs normally drank water before they commenced eating, but also drank during and after the meal, and at various times in between bouts of eating. The metabolism cages were cleaned each morning between 9 h 00 min–9 h 30 min, and the pigs had a meal shortly thereafter. They were injected with saline or drug solutions about 30 min after their last meal, and were consequently not hungry at the time of injection. The animals were usually lying down in their cages before injection. Occasionally after i.c.v. administration of saline, the animals would get up and press the operant panels a few times for food and water, and this was thought to be due to the arousing effects of the injection procedure. Usually the pigs would continue to lie down in their metabolism cages after i.c.v. saline and not get up to eat or drink.

Effects of baclofen

Baclofen (25–100 nmol i.c.v.) caused a short-lasting dose-related increase in food intake (Figure 1). Baclofen (25 nmol) had no significant effect on feeding compared with control data. In contrast, baclofen (50 nmol) increased feeding ($P < 0.01$) during the first 15 min after injection, while the 100 nmol dose increased feeding ($P < 0.01$) during both the first and second 15 min periods after injection. The onset of eating usually began 2–5 min after administration. Administration of baclofen i.c.v. did not signficantly alter the total daily food intake of the animals, thus indicating that there was

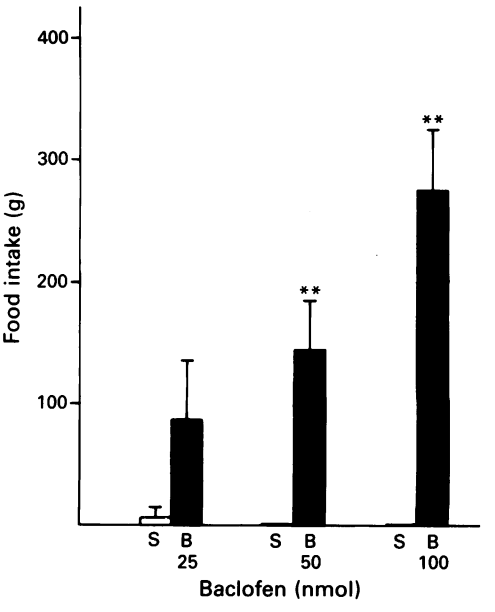

Figure 1 The dose-related effects of baclofen (25–100 nmol) on food intake (g) in pigs recorded in the first 15 min after i.c.v. administration. S = saline; B = baclofen. Vertical lines represent s.e.mean. ** $P < 0.01$. ($n = 9$ for 25 and 50 nmol doses; $n = 10$ for 100 nmol dose). Refer to text for further details.

no long-term effect of the drug on feeding (e.g. 2591 ± 322 g with saline controls and 2265 ± 340 g with 100 nmol baclofen treatment, $n = 10$). Baclofen had no significant effect on water intake compared with control data.

The 50 and 100 nmol doses of baclofen often caused the pigs to display signs of ataxia about 10–20 min after injection. The ataxia sometimes prevented the pigs pressing the food panel in a standing position, especially at the 100 nmol dose. However, they continued to eat and press the lever, sometimes from a semi-reclining position, for as long as they could. Eventually, the pigs succumbed to a state resembling deep sleep. They lay on their sides in the cages with their eyelids closed and were unresponsive to prodding in the ribs or to gentle shaking. These symptoms persisted in the majority of the pigs for between 2 and 3 h. After this time, the pigs displayed no signs of illness and began eating and drinking normally again.

Effects of phaclofen

The $GABA_B$-receptor antagonist phaclofen (50, 100 and 500 nmol i.c.v.) did not produce any significant short- or long-term effects on eating and drinking. Moreover, we did not observe any untoward behavioural side-effects with any of the doses of the drug.

Figure 2 shows the effects on food intake of pretreating pigs with phaclofen (50, 100 and 500 nmol i.c.v.) prior to administration of baclofen (50 nmol i.c.v.). Baclofen (50 nmol i.c.v.) given after saline caused a significant ($P < 0.05$) increase in food intake during the first 15 min after administration (Figure 2). Pretreatment with phaclofen (50 and 100 nmol) failed to reverse the effect of baclofen on feeding. In contrast, pretreating the animals with phaclofen (500 nmol i.c.v.) completely prevented the effects of baclofen (50 nmol) on food intake (Figure 2) and also abolished the motor and behavioural effects associated with i.c.v. administration of baclofen (50 nmol) (see above). The lower doses of phaclofen did not prevent or reduce the behavioural side-effects of baclofen. We observed no effects of phaclofen pretreatment on water intake.

Effects of intravenous administration of baclofen

In order to eliminate the possibility that i.c.v. baclofen produced its effects on food intake by a peripheral mode of action, the highest i.c.v. dose of the drug used in this study

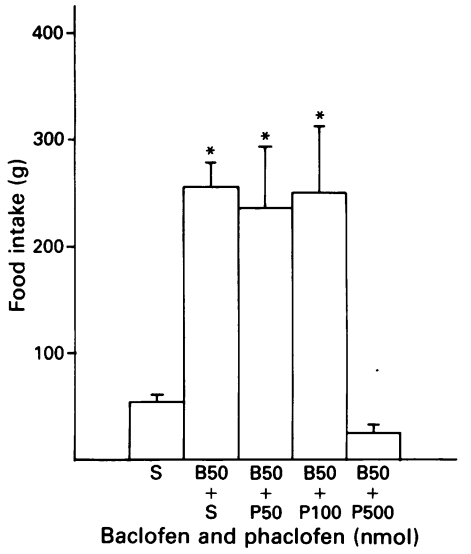

Figure 2 The effects of pretreating pigs with phaclofen (50, 100 and 500 nmol i.c.v.) on the feeding response elicited by baclofen (50 nmol, i.c.v.). S = saline; B = baclofen; P = phaclofen. Vertical lines represent s.e.mean. * $P < 0.05$, $n = 4$. Refer to text for further details.

was administered i.v. to 2 pigs. Systemic administration of baclofen (100 nmol) produced no increases in food or water intake, or any of the motor and behavioural side-effects associated with i.c.v. administration of this dose of the drug.

Discussion

The results of this study show that baclofen (25–100 nmol) i.c.v. causes a short-lasting dose-related increase in food intake in satiated pigs. Moreover, the observation that the highest dose of baclofen (i.e. 100 nmol) failed to elicit a feeding response when administered intravenously, suggests that this effect is centrally mediated. Operant methods were used in these experiments to eliminate the possibility that the increases in food intake were due to some non-specific effect, such as drug-induced chewing.

The mechanism(s) by which baclofen increases food intake is not known. However, it has been established that baclofen acts centrally on $GABA_B$-receptors to exert its pharmacological actions (Hill & Bowery, 1981; Bowery *et al.*, 1983). Thus, it is likely that baclofen enhances feeding by an action at the $GABA_B$-receptor subtype. This view is strengthened by the demonstration that the effect of baclofen on food intake is prevented by pretreating the pigs with the novel $GABA_B$-antagonist phaclofen (Kerr *et al.*, 1987). In this study a relatively high dose of phaclofen (i.e. 500 nmol, i.c.v.) was required to antagonize significantly the effects of baclofen (50 nmol) on feeding. However, these results are consistent with the observations of Kerr *et al.* (1987) who have demonstrated that phaclofen is a relatively weak antagonist at the $GABA_B$-receptor. In their experiments they found that the depression of guinea-pig ileal twitch responses produced by 8×10^{-6} M baclofen was only blocked by 2×10^{-4} M phaclofen.

Although the $GABA_A$-receptor is the most ubiquitious subtype in the CNS, $GABA_B$-receptors have been found throughout the CNS, in areas such as the cortex, hippocampus, cerebellum, hypothalamus, thalamus, striatum and spinal cord (Palacios *et al.*, 1981; Newberry & Nicoll, 1984; Bowery *et al.*, 1984; Gehlert *et al.*, 1985; Conzelmann *et al.*, 1986; Bonnano *et al.*, 1988). As there is strong evidence to support the view that the hypothalamus is involved in the control of food intake it is probable that i.c.v. baclofen acts in periventricular regions of this structure to stimulate feeding, although other brain areas may also be involved.

Recent experiments with GABA analogues and the $GABA_B$-antagonist phaclofen have suggested that $GABA_B$-receptors are not a homogeneous population (Scherer *et al.*, 1988), but there is more than one subset of $GABA_B$ receptors. The subtype of $GABA_B$-receptor on which baclofen acts to stimulate feeding in pigs has been termed 'phaclofen sensitive' (Kerr *et al.*, 1987). These 'phaclofen sensitive' receptors have been found at both pre- and post-synaptic sites (Pittaluga *et al.*, 1987; Dutar & Nicoll, 1988), and it is not possible at present to state whether baclofen is acting at a prejunctional, and/or postjunctional level to decrease eating.

Baclofen i.c.v. has no effect on water intake although normally, increases in food consumption in pigs are associated with increases in fluid intake (Bigelow & Houpt, 1988). The observation that there was no effect on the overall daily intake of food and water after baclofen (25–100 nmol) indicates that the animals accurately regulate their daily food and water consumption despite the acute actions of the drug.

Intracerebroventricular injections of the higher doses of baclofen (i.e. 50 and 100 nmol) caused the pigs to display signs of ataxia about 15–20 min after administration. However, despite the motor side-effects, the pigs continued to make operant responses for food, indicating a powerful drug-induced motivation to eat. A possible explanation of these motor disturbances is that they were due to the putative anaesthetic properties of baclofen. It has been demonstrated that both i.c.v. and systemic administration of baclofen can induce anaesthesia in rats and mice (Smith & Vestergaard, 1979; Sawynok & La Bella, 1981). Thus, it is possible that the i.c.v. doses of baclofen necessary to produce increases in feeding, eventually induce a state of anaesthesia. However, it is likely that the motor deficits and subsequent loss of consciousness are mediated by specific $GABA_B$-receptors as they were also abolished by phaclofen.

In conclusion, the results of this study indicate that stimulation of central $GABA_B$-receptors by baclofen will elicit feeding in satiated pigs. Further work is necessary to localize the site(s) of action of baclofen, and to establish whether a $GABA_B$-receptor-mediated mechanism plays a physiological role in the regulation of food intake.

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