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The effects of intraperitoneal and intracerebroventricular administration of the $GABA_B$ receptor antagonist CGP 35348 on food intake in rats

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Abstract

In order to test the hypothesis that endogenous gamma-aminobutyric acid (GABA), acting at central $GABA_B$ receptors, plays a physiological role in the control of feeding behaviour, it was reasoned that blocking these receptors with a centrally active $GABA_B$ receptor antagonist should reduce food intake in hungry rats. In the present study, experiments were carried out to test this possibility using the $GABA_B$ receptor antagonist 3-aminopropyl-diethoxy-methyl-phosphinic acid (CGP 35348), which is water-soluble and can penetrate the blood–brain barrier from the systemic circulation. CGP 35348 (50 and 100 mg/kg, i.p.) had no effect on food intake in 22-h fasted rats, but a higher dose (i.e. 500 mg/kg., i.p.) significantly reduced cumulative food consumption. These findings are consistent with previous observations that high systemic doses of CGP 35348 are needed to block central $GABA_B$ receptors. However, to eliminate the possibility that the 500 mg/kg dose of CGP 35348 decreased food intake by a peripheral, rather than a central mode of action, further experiments were undertaken where the drug was given directly into the brain by the intracerebroventricular (i.c.v.) route. I.c.v. administration of CGP 35348 (5 and 10 μg) significantly decreased cumulative food intake food intake in rats that had been fasted for 22 h. By contrast, i.c.v. administration of CGP 35348 (10 μg) had no effect on water intake in 16-h water-deprived rats. The results indicate that CGP 35348 reduces food consumption in hungry rats by blocking central $GABA_B$ receptors in a behaviourally specific manner. These findings suggest that endogenous GABA acting at central $GABA_B$ receptors plays a physiological role in the regulation of feeding behaviour.

Keywords: CGP35348; $GABA_B$ receptor antagonist; Food intake; Baclofen; $GABA_B$

1. Introduction

Two studies published in 1990 reported that intracerebroventricular (i.c.v.) administration of the gamma-aminobutyric acid $_B$ ($GABA_B$) receptor agonist baclofen increases food consumption in satiated pigs (Ebenezer and Baldwin, 1990) and non-deprived rats (Ebenezer, 1990). A subsequent study by Ebenezer and Pringle (1992) showed that subcutaneous (s.c.) administration of baclofen also increases feeding in non-deprived rats in a dose-related manner. Baclofen readily crosses the blood–brain barrier (Faigle and Keberle, 1972) from the systemic circulation, and evidence has been provided that systemically administered baclofen increases food consumption by acting centrally (Ebenezer and Patel, 2004).

The demonstration that the hyperphagic response to i.c.v. administration of baclofen in experimental rats and pigs can be antagonised by i.c.v. administration of $GABA_B$ receptor antagonists (Ebenezer and Baldwin, 1990; Ebenezer, 1990) suggests that stimulation of central $GABA_B$ receptors is involved in the feeding response elicited by baclofen. More recently, direct microinjection of baclofen into the dorsal raphe nucleus (Wirtshafter et al., 1993) and nucleus accumbens (Stratford and Kelley, 1997; Ward et al., 2000) have elicited hyperphagia in rats and it is likely that these

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brain nuclei may be the target areas for the $GABA_B$ receptor agonist. However, it is not clear whether the hyperphagic action of baclofen has physiological significance or is merely a pharmacological effect.

More than a quarter of a century ago, Kimura and Kuiriyama (1975) reported that GABA levels in the brain are elevated in response to food deprivation. Similar results were obtained by Kasser et al. (1985). These findings indicate the possibility that endogenous central GABA may play a physiological role in the regulation of feeding. The observation that baclofen increases food consumption in experimental animals (Ebenezer, 1990, 1995, 1996; Ebenezer and Baldwin, 1990) suggests a role for central $GABA_B$ receptors in the control of food intake. In order to test this possibility, it was argued that if endogenous GABA acting on central $GABA_B$ receptors has a tonic physiological role in the regulation of food intake, then blocking these receptors with a selective $GABA_B$ receptor antagonist should reduce feeding in hungry animals. In the present study, experiments were carried out to test this possibility using the $GABA_B$ receptor antagonist 3-aminopropyl-diethoxy-methyl-phosphinic acid (CGP 35348), which is water-soluble and can penetrate the blood–brain barrier from the systemic circulation (Bittiger et al., 1990; Ople et al., 1990).

2. Materials and methods

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

2.1. Experiment 1: Effects of intraperitoneal CGP 35348 (50–100 mg/kg) on food intake

Male Wistar rats (body weight: 310–390 g) were housed in cages in groups of four and deprived of food for 22 h prior to each training and experimental session. Water was available ad libitum in the home cages. The animals were given four training sessions when they were allowed free access to food and water in experimental cages measuring 32×25×10 cm for 120 min. The food was presented to rats in shallow cylindrical cups, as described previously (Ebenezer, 1990). During the experimental sessions that followed, the rats were injected intraperitoneally (i.p.) with physiological saline or CGP 3548 (50 or 100 mg/kg). Food was presented 15 min after injection. The amount of food consumed was measured over 120 min. A repeated-measures design was used in which each rat received all treatments; 4 days separated successive trials.

2.2. Experiment 2: Effects of intraperitoneal CGP 35348 (500 mg/kg) on food intake

Male Wistar rats (n=8; 290–310 g) were used. A similar protocol to that described above for Experiment 1 was used, except that the rats received physiological saline or CGP 3548 (500 mg/kg) i.p. Each rat was given both treatments in a crossover design and 4 days separated successive trials.

2.3. Experiment 3: Effects of i.c.v. administration of CGP 35348 on food intake

Male Wistar rats (n=5; body weight 350–400 g) were chronically implanted, under Equithesin anaesthesia (Lumb, 1960), with guide cannulae directed towards the left lateral ventricle for subsequent i.c.v. injection, as described previously (Ebenezer, 2002). Seven days were allowed for recovery from surgery before experiments began. The rats were housed singly and were deprived of food in their home cages for 22 h prior to each training or experimental session. The rats received three training sessions when they were placed for 120 min in experimental cages that contained a weighed amount of food. Water was available ad libitum. During experimental sessions, the rats were injected i.c.v. with either physiological saline or CGP 35348 (5 μg) and placed into the experimental cages following injection for 120 min. The volume of the i.c.v. injection was 5 μl. The i.c.v. injection was administered slowly over 2 min. The amount of food eaten at the end of the 120-min period was measured. A crossover design was used in which each rat received both treatments; 4 days separated successive trials.

After a 7-day washout period, an additional experiment was carried out on these rats (body weight: 400–440 g), using the same protocol as described above, except that the animals were injected i.c.v. with a 10 μg dose of CGP 35348 instead of a 5 μg dose.

At the end of the experiment, the rats were deeply anaesthetised and injected i.c.v. with 5 μl of Black India Ink. The brains were removed and dissected to confirm that the ink filled the ventricular system, as described previously (Ebenezer, 1990).

2.4. Experiment 4: Effects of i.c.v. administration of CGP 35348 on water intake

Male Wistar rats (n=6; body weight 320–380 g) were chronically implanted, under Equithesin anaesthesia (Lumb, 1960), with guide cannulae directed towards the left lateral ventricle for subsequent i.c.v. injections. Seven days were allowed for recovery from surgery before experiments began. The rats were housed singly and were deprived of water in their home cages for 16 h prior to each training or experimental session. The rats received three training sessions when they were placed for 60 min in experimental cages and presented with water in graduated water bottles, as described previously (Ebenezer, 1995). During experimental sessions, the rats were injected i.c.v., as described for Experiment 3, with either physio-

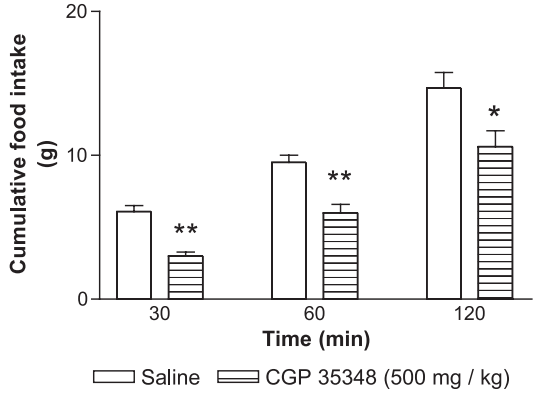

Fig. 1. Effects of i.p. administration of GCP 35348 (500 mg/kg) on cumulative food intake in rats that had been fasted for 22 h. Vertical lines represent +S.E.M. $^{**}P<0.01$, $^{*}P<0.05$.

logical saline or CGP 35348 (10 μg) and placed into the experimental cages 5 min later. Water intake was measured at 10-min intervals over a 60-min period. A repeated-measures design was used with each rat receiving all treatments; 4 days separated successive trials. At the end of each training or experimental session, the rats were returned to their home cages where they were given further access to water.

2.5. Drugs

The $GABA_B$ receptor antagonist GCP 35348 was purchased from Sigma Biochemicals, Dorset, UK. The drug was dissolved in physiological saline solution (0.9% w/v NaCl). Physiological saline solution was used in control experiments.

2.6. Statistical analysis

The cumulative food intake data for Experiment 1 were analysed at each time point by analysis of variance for repeated measures. The cumulative food intake data for Experiments 2, 3, and 4 were analysed at each time point by the paired *t*-test.

3. Results

3.1. Experiment 1: Effects of intraperitoneal CGP 35348 (50–100 mg/kg) on food intake

CGP 35348 (50 and 100 mg/kg, i.p.) did not significantly affect cumulative food intake at 30, 60 and 120 min after administration in rats that had been fasted for 22 h. Thus, for example, the mean food intake (g)±S.E.M. at 30 min was as follows: saline 8.8±0.4 g; CGP 35348 (50 mg/kg) 9.2±1.1 g; CGP 35348 (100 mg/kg) 8.4±0.3 g. These doses of CGP 35348 did not produce any overt abnormal behavioural changes in the rats.

3.2. Experiment 2: Effects of intraperitoneal CGP 35348 (500 mg/kg) on food intake

The effects of CGP 35348 (500 mg/kg; i.p.) on food intake in rats that had been fasted for 22 h are shown in Fig. 1. CGP 35348 (500 mg/kg; i.p.) produced a significant reduction in food consumption. Statistical analysis of the data showed that CGP 35348 (500 mg/kg; i.p.) caused significant suppression of cumulative food intake at 30 min ($P<0.01$), 60 min ($P<0.01$) and 120 min ($P<0.01$). This dose did not produce any overt abnormal behavioural effects in the animals.

3.3. Experiment 3: Effects of i.c.v. administration of CGP 35348 on food intake

The effects of i.c.v. administration of CGP 35348 (5 μg) on food intake in 22-h food-deprived rats are shown in Fig. 3A. Statistical analysis of the data showed that the cumulative food intake was significantly reduced at 15 min ($P<0.01$), 30 min ($P<0.01$), 60 min ($P<0.05$) and 120 min ($P<0.05$). Under control conditions, the rats generally spent a brief time exploring the experimental cages and food containers before they began to eat. The latency to eat was usually in the range 60–90 s. By contrast, when the rats received CGP 35348 (5 μg), they generally spend a longer

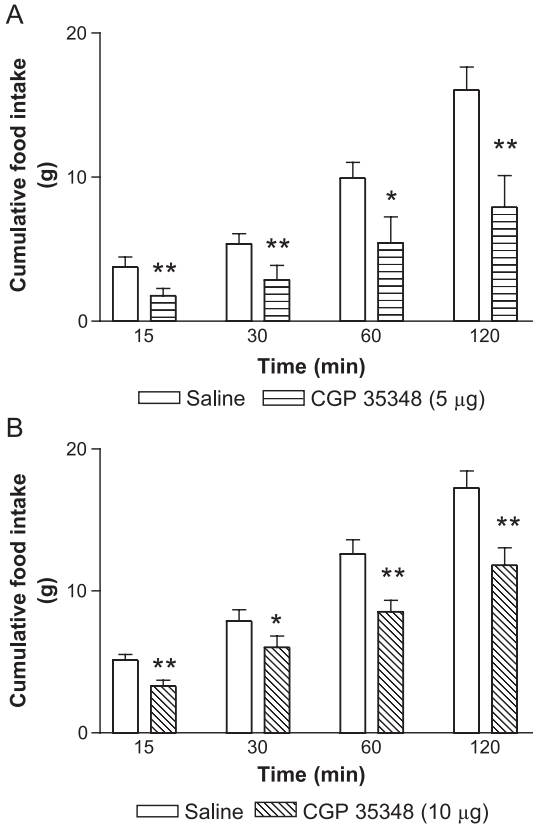

Fig. 2. Effects of i.c.v. administration of (A) GCP 35348 (5 μg/kg) and (B) GCP 35348 (10 μg/kg) on cumulative food intake in rats that had been fasted for 22 h. Vertical lines represent +S.E.M. $^{**}P<0.01$, $^{*}P<0.05$.

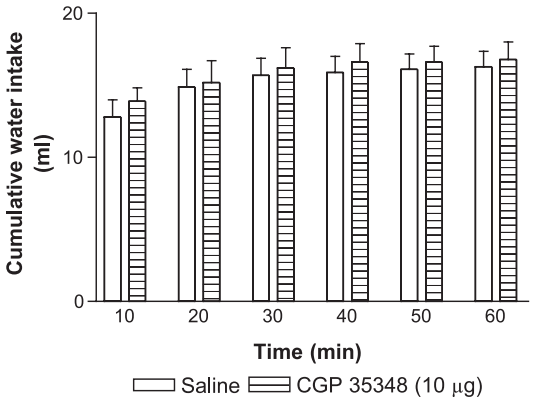

Fig. 3. Effect of i.c.v. administration of CGP 35348 (10 μg/kg) on cumulative water intake in 16-h water-deprived rats. Vertical lines represent +S.E.M.

time sniffing around the food containers before they commenced eating. The latency was in the range 60–180 s for four of the animals. One rat did not consume any food during the first 15 min. It was noteworthy that the rat did approach the food container on a number of occasions during the first 15 min but did not eat. Beside the decrease in food consumption, i.c.v. administration of CGP 35348 did not produce any overt abnormal behavioural effects in the animals.

After a 7-day dryout period, the experiment was repeated using a higher dose (i.e. 10 μg) of CGP35348. The results are shown in Fig. 2B. Similar results were obtained. Interestingly, the suppressant effect of the 10 μg dose on feeding was not greater than that of the 5 μg dose. Latencies for feeding after injection were similar as for the lower dose of CGP 35348. The rat that did not eat during the first 15 min when injected with the 5 μg dose (see above), ate 2 g of food after i.c.v. administration of the 10 μg dose.

3.4. Experiment 4: Effects of i.c.v. administration of CGP 35348 on water intake

The effects of i.c.v. administration CGP 35348 (1 and 5 μg) on water intake in 16-h water-deprived rats are shown in Fig. 3. Under control conditions, the animals readily approached the waterspouts in the experimental cages and started to drink. The latency to drink was in the range 30–60 s. The animal drank most of their water within the first 15 min after presentation. When the rats were given i.c.v. injections of CGP 35348, they behaved in a similar manner to the control animals and consumed most of their water within the first 15 min. There were no significant differences in water intake at any of the measurement intervals.

4. Discussion

The principle aim of this study was to investigate whether endogenous GABA, acting at $GABA_B$ receptors, plays a role in the regulation of food intake. It was reasoned that if endogenous GABA acting at $GABA_B$ receptors has a tonic role in the control of feeding, then a selective $GABA_B$ receptor agonist would be expected to reduce food intake in hungry animals. The selective $GABA_B$ receptor antagonist CGP 35348 was used in this study. CGP 35348 is a water-soluble compound that can penetrate the brain from the systemic circulation (Ople et al., 1990). However, a number of studies have indicated that systemic administration of high doses of CGP 35348 are required to significantly affect central $GABA_B$ receptors (Jackson and Nutt, 1991; Mathe et al., 2002). Thus, for example, it has been found that 400 mg/kg of CGP 35348, administered intravenously, is the minimum dose required to increase burst firing of midbrain dopamine neurones in the rats (Mathe et al., 2002). The results obtained in Experiments 1 show that CGP 35348 (50 or 100 mg/kg; i.p.) had no effects on food intake in the fasted rats. It is possible that the lack of effect of these doses was because they were too low to effectively block central $GABA_B$ receptors. By contrast, a dose of 500 mg/kg produced significant reductions in food consumption (Experiment 2; Fig. 1). Although this dose is very high, it did not produce any overt behavioural abnormalities in the animals. Moreover, this dose is within the range of doses that have previously been found to alter central $GABA_B$ function following systemic administration (see Mathe et al., 2002). These results therefore tentatively suggest that endogenous GABA, acting at central $GABA_B$ receptors, plays a tonic role in the regulation of food intake. However, it is possible that the 500 mg/kg dose of CGP 35348 decreased food intake by a peripheral, rather than central, mode of action. For example, it may have affected gut motility, which, in turn, may have affected feeding behaviour.

In an attempt to allay such criticism, CGP 35348 was administered directly into the central nervous system (CNS) by the i.c.v. route. The results obtained in Experiment 3 show that very low doses of CGP 35348 (i.e. 5 and 10 μg) produce significant and fairly long-lasting (at least 120 min) decreases in cumulative food intake in rats that had been fasted for 22 h. Interestingly, both doses produced almost similar decreases in food consumption, suggesting that these doses are probably producing maximal reductions in feeding. These doses were chosen on the basis of another report that showed that CGP35348 (10 and 30 μg, i.c.v.) did not alter locomotor activity in rats (Zarrindast et al., 2001). In order to eliminate the possibility that the hypophagia observed after i.c.v. administration of CGP 35348 is not behaviourally specific for food but due to a non-specific effect on consummatory behaviours, the effects of i.c.v. administration of CGP 35348 (10 μg) were investigated on water intake in 16-h water-deprived rats. The results from Experiment 4 show that CGP 35348 (10 μg) had no effect on water intake in water-deprived rats. These data thus indicate that the effects of i.c.v. administration of CGP 35348 on food intake is behaviourally specific and not due

to non-specific effects on consummatory behaviours. Furthermore, these results reinforce the conclusion (see above) that endogenous GABA acting at central $GABA_B$ receptors has a tonic role in the control of food intake. The central locus (or loci) for this action remains to be determined. Reports that baclofen produces hyperphagia in rats when microinjected into the dorsal raphe nucleus (Wirtshafter et al., 1993) and nucleus accumbens (Stratford and Kelley, 1997; Ward et al., 2000) would tend to implicate these brain areas as possible target sites for GABA.

In conclusion, the results of this study suggest that endogenous GABA regulates feeding behaviour by an action at central $GABA_B$ receptors. These finding have important implications for our understanding of the central mechanisms involved in the control of feeding behaviour.

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