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Neuropeptide Y Y_5 receptor antagonist CGP71683A: the effects on food intake and anxiety-related behavior in the rat

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Abstract

The effects of neuropeptide Y Y_5 receptor antagonist (*trans*-naphtalene-1-sulphonic acid {4-[(4-amino-quinazolin-2-ylamino)-methyl]-cyclohexylmethyl}-amide hydrochloride; CGP71683A), on food intake, anxiety and locomotor activity were studied. CGP71683A (1–10 mg/kg, i.p.) dose-dependently decreased nocturnal and fasting-induced food intake. CGP71683A did not have an anxiogenic-like effect in the rat social interaction test. In the elevated plus-maze test, where novel neuropeptide Y Y_1 receptor antagonist (2*R*)-5-([amino(imino)methyl]amino)-2-[(2.2-diphenylacetyl)-amino]-*N*-[(1*R*)-1-(4-hydroxyphenyl)ethyl-pentanamide (H 409/22) had anxiogenic-like effect, CGP71683A was inactive. In the open-field test, carried out immediately after the elevated plus-maze test, CGP71683A inhibited horizontal and vertical activity. CGP71683A did modify the habituation of locomotor response in novel environment. These data show that the inhibition of food intake induced by CGP71683A could not be explained by increased fearfulness, a state that is induced by neuropeptide Y Y_1 receptor antagonists. Thus, our data, obtained with first neuropeptide Y Y_5 receptor antagonist CGP71683A, suggest that in contrast to the neuropeptide Y Y_1 receptor, Y_5 receptor is not involved in tonic neuropeptide Y-induced anxiolysis. © 2001 Published by Elsevier Science B.V.

Keywords: Neuropeptide Y; Neuropeptide Y Y_1 and Y_5 receptor; Neuropeptide Y Y_5 receptor; Food intake; Anxiety; Locomotor activity; Specificity; Side effect

1. Introduction

Neuropeptide tyrosine (Y) has a number of physiological effects (Dumont et al., 1992, 2000a), including the stimulation of food intake (Stanley, 1993; Inui 1999; Kalra et al., 1999). Six neuropeptide Y receptor subtypes (Y_1 – y_6) have been discovered in mammals (Michel et al., 1998), each of them having unique distribution in the brain (Dumont et al., 2000a). The neuropeptide Y Y_1 receptor was first cloned as an orphan receptor (Eva et al., 1992) and it is widely distributed in the rat brain. Soon thereafter, the human neuropeptide Y Y_2 receptor was identified (Rose et al., 1995), followed by the cloning of the rat homologue (St-Pierre et al., 1998). The so-called neuropeptide Y Y_3 receptor has not yet been cloned but its existence has been suggested on the basis of weaker potency of peptide YY compared to neuropeptide Y in the bioassays (Dumont et al., 1993). Recent data however, indicate that so-called neuropeptide Y Y_3 receptor-like profile could be explained with the combination of pharmacological properties of the neuropeptide Y Y_2 and Y_4 receptors (Pheng et al., 1999). A unique feature of the neuropeptide Y Y_4 receptor is that this protein prefer[Aentially binds pancreatic polypeptide, whereas neuropeptide Y is less potent and peptide YY is virtually inactive (Lundell et al., 1995). Neuropeptide Y Y_4 receptor has limited distribution in the brain (Dumont et al., 2000a). The neuropeptide Y Y_5 receptor has been cloned in human, rat, mouse (Nakamura et al., 1997) and dog (Borowsky et al., 1998). The neuropeptide Y Y_5 receptor and its mouse analogue (y_6 receptor) are newest members of the neuropeptide Y receptor family (Hu et al., 1996; Gerald et al., 1996). The neuropeptide Y Y_5 receptor was initially reported to have a very limited distribution (Gerald et al., 1996). However, later, the neuropeptide Y Y_5 receptor and

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its mRNA has been found in number of other brain regions (Nichol et al., 1999; Parker and Herzog, 1999; Dumont et al., 2000a), including those that have important role in the regulation of emotionality (hypothalamic nuclei, lateral septum, locus coeruleus and amygdala). Most recently, a new human neuropeptide Y receptor subtype (Y_7?), showing high homology to the neuropeptide Y Y_2 receptor was cloned (Parker et al., 2000b).

In the early nineties, the first non-peptide low molecular weight substances, selective for neuropeptide Y Y_1 receptor, were developed. Their use has confirmed the role of neuropeptide Y Y_1 receptors in the regulation of feeding (Kask et al., 1998a; Wieland et al., 1998), emotional behaviour (Kask et al., 1996, 1998b), cardiovascular regulation (Malmström, 2000) and numerous other physiological functions.

Recently, selective antagonists for other neuropeptide Y receptor subtypes have been discovered. One of them, CGP71683A (*trans*-naphtalene-1-sulphonic acid {4-[(4-amino-quinazolin-2-ylamino)-methyl]-cyclohexylmethyl}-amide hydrochloride), is considered to be the best tool for characterizing the function of the neuropeptide Y Y_5 receptor (Dumont et al., 2000b). CGP71683A has been shown to inhibit food and water intake in healthy rats and genetically obese animals and to block NPY-stimulated feeding indicating also that compound passes blood–brain barrier (Criscione et al., 1998; Parker et al., 2000a). Two other recent studies with CGP71683A have questioned the exclusive role of the neuropeptide Y Y_5 receptor in regulation of food intake (Duhault et al., 2000; Polidori et al., 2000). Although many studies have suggested that, apart from appetite, the neuropeptide Y Y_1 and Y_5 receptors may be involved in the regulation several other physiological functions, very few studies have considered the possibility that the effects of neuropeptide Y receptor antagonists on food intake may be secondary or unspecific. We have shown that neuropeptide Y Y_1 receptor antagonists potently inhibit food intake and they also increase experimental anxiety (Kask et al., 1996, 1998b; Kask and Harro, 2000). Several groups have shown that neuropeptide Y Y_5 receptor inhibit either spontaneous feeding or food intake stimulated by food deprivation or i.c.v. injection of neuropeptide Y. However, the effects of neuropeptide Y Y_5 receptor antagonists on anxiety-related and exploratory behaviour have not been studied.

The aim of this study was twofold. Firstly, we wished to confirm the hypothesis that neuropeptide Y Y_5 receptor blockade inhibits feeding in rats. Although the Y_5R antagonist CGP71683A has already been shown to inhibit feeding (Criscione et al., 1998), its effects on locomotor activity and emotional reactivity have not been studied. A high dose of CGP71683A has been shown to induce conditioned taste aversion (Criscione et al., 1998), suggesting that neuropeptide Y Y_5 receptor antagonism may cause anxiety or sickness behaviour. First possibility should be considered because pharmacological studies with subtype selective agonists and antagonists suggest that anxiolytic effects of neuropeptide Y are mediated via more than one receptor subtype (Kask et al., 1998b). Specifically, one of the prevailing hypotheses states that neuropeptide Y-induced anxiolysis is mediated via the neuropeptide Y Y_1 receptors in the amygdala (Heilig et al., 1993). We not able to confirm exclusive role of neuropeptide Y Y_1 receptors in amygdala in the regulation of anxiety by using intra-amygdala microinjections of BIBP 3226, a selective neuropeptide Y Y_1 receptor antagonist. This negative finding suggested that other subtypes may mediate anxiolytic effects of neuropeptide Y (Kask et al., 1998b). Indeed, unilateral injections of neuropeptide Y into the vicinity of locus coeruleus, a brainstem nucleus where neuropeptide Y is co-localized with noradrenaline, reduce anxiety (Kask et al., 1998c) and this effect is not mediated via neuropeptide Y Y_1 receptor since [Leu^{31},Pro^{34}]neuropeptide Y, the neuropeptide Y Y_1/Y_5 receptor selective agonist, being equally potent with neuropeptide Y, failed to cause anxiolysis, whereas neuropeptide Y_{13-36}, neuropeptide Y Y_2/Y_5 receptor selective agonist, mimicked the effect of neuropeptide Y suggesting a role for the neuropeptide Y Y_2/Y_5 receptor (Kask et al., 1998c). Collectively, these data suggest that in anxiolytic-like effect of neuropeptide Y is not mediated via the single receptor subtype and other neuropeptide Y receptor subtypes (Y_2 and/or Y_5) may be involved. This encouraged us to pursue the second aim: to test the hypothesis that neuropeptide Y Y_5 receptor blockade increases anxiety-like behaviour. To investigate this possibility, we studied the effects of CGP71683A in experimental models of anxiety that are based either on exploration of unfamiliar surroundings, such as open field or elevated plus-maze, or investigation of unfamiliar rat in novel environment. The effects of (2*R*)-5-([amino-(imino)methyl}amino)-2-[(2.2-diphenylacetyl)-amino]-*N*-[(1*R*)-1-(4-hydroxyphenyl)ethyl-pentanamide (H 409/22, see Malmström, 2000), a novel neuropeptide Y Y_1 receptor antagonist, were also studied for comparison in the elevated plus-maze test.

2. Experimental procedures

2.1. Animals and surgery

Male Wistar rats (280–350 g) were obtained from Kuopio Animal Research Center (Kuopio, Finland). They were acclimatized to the animal facility for at least 1 week before being subjected to any experimental procedure. During this period, animals were housed (in groups of three and four) in transparent macrolone cages with aspen shavings and fed R70 diet (Lactamin , Stockholm, Sweden) ad libitum. Three days before experiments, animals were adapted to single-housing conditions in macrolone cages. In food-deprivation experiment, separate group of

experimentally naive rats was housed in groups, and transferred to wire mesh cages just before refeeding with novel diet (R35) of similar hardness and energetic value (1255 and 1254 kJ for 100 g of diet for R34 and R70, respectively). The composition of these two diets is as follows (expressed as a percentage): protein—16.5/14.5, fat—4.0/4.5, linoleic acid—1.0/1.0, fibres—3.5/4.9, ash—6.0/5.0, water—<12/<11. The switch to different diet was due to practical reasons—pellets of R70 diet are too small for wire mesh baskets. A separate group of rats were implanted with custom-made cannula just above the left lateral ventricle (A: −0.8; L ± 1.4; V − 3.2, with tooth bar at +3.0) under chloral hydrate anaesthesia. After surgery, animals were housed singly and experiment started 1 week after surgery. All procedures were approved by Animal Care Committee of University of Tartu and they followed Estonian and European legislative documents concerning the experimentation on laboratory animals.

2.2. Drugs

Chloral hydrate was obtained from Oriola (Espoo, Finland). CGP71683A (*trans*-naphtalene-1-sulphonic acid {4-[(4-amino-quinazolin-2-ylamino)-methyl]-cyclohexylmethyl}-amide hydrochloride) was synthesized by traditional methods of organic chemistry and dissolved in dimethylsulphoxide (DMSO) after brief sonification. Initially, we determined the purity of CGP71683A by applying a series of potentials (+50 to +850 mV in 100 mV increment) to a solution containing 2 ng of CGP71683A that was injected into a high pressure liquid chromatography (HPLC) system coupled to series of coulometric electrodes (CoulArray ESA, Chelmsford, MA). Injectate eluted as a single peak at 7 min and was detected with an electrochemical cell at +650 mV potential. Purity of the synthesis product was verified also by HPLC coupled to mass-spectrometer. Both methods confirmed that the synthesis product was free from the by-products of the synthesis. The selectivity of the synthesis product was also tested in three assays of NPY Y_5 receptor binding- antagonist assay in rat and guinea pig brain homogenates and human embryonic kidney cells (HEK293) transfected with the rat neuropeptide Y Y_5 receptor cDNA. In all three assays, the affinity of CGP71683A was between 2 and 5 nM (Dumont, personal communication), those values being identical with these reported in previous studies (Criscione et al., 1998; Dumont et al., 2000b). Neuropeptide Y Y_1 receptor antagonist H 409/22 {(2*R*)-5-([amino(imino)methyl}amino)-2-[(2.2-diphenylacetyl)-amino]-*N*-[(1*R*)-1-(4-hydroxyphenyl)-ethyl-pentanamide}, a generous gift from Astra Zeneca (Gothenburg, Sweden), was dissolved in saline after brief sonification. Doses of H 409/22 were selected on the basis of previous studies with structurally similar compound BIBP3226 (Kask et al., 1996). As BIBP3226, H 409/22 does not cross blood–brain barrier.

2.3. Food intake measurements

Food intake was measured in home cages in order to minimize non-specific stress effects on food intake resulting from changes in housing conditions, unless stated otherwise. In the first food intake experiment, food and water was available ad libitum. Immediately before lights went off (8 PM), rats were randomized into four groups according to their body weight [280–305 g, ANOVA for pretreatment body weight $F(3.28) = 0.31$, NS] and injected intraperitoneally either with vehicle (10% DMSO) or CGP71683A (1.0, 3.0 or 10.0 mg/kg). Thereafter, rats were returned to home cages and food left on top covers was measured using Mettler PB 3002PB balance to the nearest 0.1 g. Remaining food was measured next morning at 8 AM. In the second experiment, a separate group of rats (315–350 g) was housed in plastic cages and transferred to individual wire mesh cages where they were deprived of food for 12 h overnight. Next morning, rats were injected either with vehicle (10% DMSO) or CGP71683A (1 and 10 mg/kg). Thereafter, rats were returned to home cage where diet R35 was presented in hanging wire mesh baskets. Food remaining in baskets and spillage beneath the cages was weighed 1, 2 and 4 h after CGP71683A administration.

2.4. Social interaction test

Social interaction test was carried according to general principles (File, 1980) with some modifications. The testing apparatus differed from the apparatus previously used in our laboratory (Kask et al., 1998d, 2000) and locomotor activity was not measured. Briefly, two singly housed unfamiliar rats (no close contact with the test partner after arrival to animal house) were placed in Plexiglas cage ($35 \times 35 \times 55$ cm) that was designed to measure aggressive behaviour in apomorphine-treated rats (Kask and Harro, 2000). Aspen bedding covered the floor of the apparatus, and it was changed before each testing session. Active social behaviour (sniffing, grooming, following and crawling under and over) and not passive contact were recorded by a trained person unaware of treatment conditions. The social interaction score is expressed as time spent in active social behaviour for each individual rat.

2.5. Elevated plus-maze test

Elevated plus-maze test, first described by Handley and Mithani (1984), was used after pharmacological validation in our laboratory conditions as described earlier (Kask et al., 1996, 1998b,c). The walls of the maze were made of pale brown plastic and floor from black plastic. The maze was located in dimly lit room and was elevated 60 cm from the floor. For the testing, rat was placed on the central platform, with its head facing a closed arm. The

behaviour of the rats was measured during 4-min test. Measures recorded by an observer blind to treatment conditions were: number of closed and open arm entries; time spent on open arms; line crossings in open part and approaches (rat is looking out from closed part but does not enter open part within 3 secs, so called peeking).

2.6. Exploration of novel open arena ("open field")

The open-field test was carried out immediately after the elevated plus-maze test. The square arena ($100 \times 100 \times 40$ cm) was made of wood and it was painted dark green. Black stripes divided the floor into 16 equal sections. The rat was placed in the center of the field and following parameters were measured over a 4-min testing period by an observer blind to treatment conditions: (1) squares visited; (2) rearing—full unprotected stretch and (3) the number of fecal boli deposited during session. Latter measure correlates with emotionality (Hall, 1934) and is increased after treatment with neuropeptide Y Y_1 receptor antagonist (Kask and Harro, 2000).

2.7. Long-term monitoring of locomotor activity (1 h)

Locomotor activity (distance traveled, time moving, rearing) was measured using ActiMot system (TSE Instruments, Bad Homburg, Germany). Rats were placed in transparent boxes ($0.45 \times 0.45 \times 0.45$ m) for 1 h. Selection of this time period is based on our earlier study in identical experimental setting (Kask and Harro, 2000), showing that rats spend the first 15–20 min exploring a novel environment. Thereafter, exploratory activity of control animals dramatically decreases, and short periods of immobility are interrupted by infrequent increases in locomotor activity. We reasoned that if initial exploratory phase is decreased due to novelty-induced fear, rats will still explore new environment later, whereas in case of general suppression of activity, they will remain less active for the whole duration of the test. Alternatively, CGP71683A may have stimulant-like effect that could explain anorexigenic activity of the compound.

2.8. Statistical analysis

All data are expressed as means ± S.E.M. Data were analysed either by factorial analysis of variance (ANOVA), followed by Scheffe's (food intake) of Fisher's protected least statistical difference (PLSD) test (other behavioural experiments) when appropriate using StatView 4.5 program for Macintosh. Data of long-term monitoring of locomotor activity were analysed with ANOVA for repeated measures.

3. Results

3.1. Food intake

In the first experiment, we administered CGP71683A to rats immediately before the animal house lights went off. The amount of food eaten during the dark cycle (12 h) is shown in Fig. 1. One-way ANOVA indicated that food intake (Fig. 1A) was significantly suppressed by CGP71683A [$F(3,28) = 5.223$, $P < 0.05$]. Body weight (Fig. 1B) was decreased in rats treated with CGP71683A [$F(3,28) = 3.657$, $P < 0.05$]. Consequently, metabolic efficiency, expressed as food/body weight conversion ratio, was significantly decreased in CGP71683A-treated rats (Fig. 1C). In the second experiment, where the rats were injected with after an overnight fast and then presented with novel food (diet R35), there was a significant effect on food intake [$F(2,15) = 3.987$; $P < 0.05$]. Post-hoc Scheffe's test indicated that CGP71683A (10 mg/kg, i.p.) significantly inhibited food deprivation-induced feeding (Fig. 2).

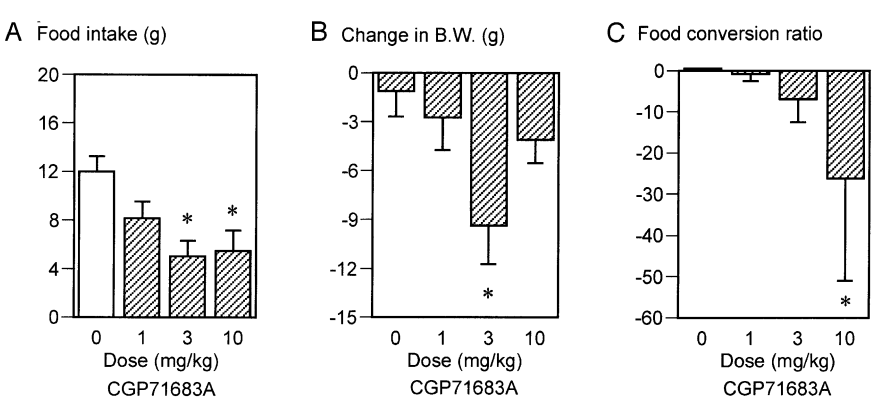

Fig. 1. The effect of CGP71683A on nocturnal feeding in male Wistar rats. CGP71683A (0, 1, 3, 10 mg/kg, $n = 8$ in each group) was administered i.p., before the lights went off. This treatment resulted in significant reduction of (A) food intake, (B) loss of body weight and (C) decreased food conversion ratio (amount of food ingested divided by change in body weight). All data are expressed as means ± S.E.M. $^{*}P < 0.05$ after significant ANOVA, Scheffe's test.

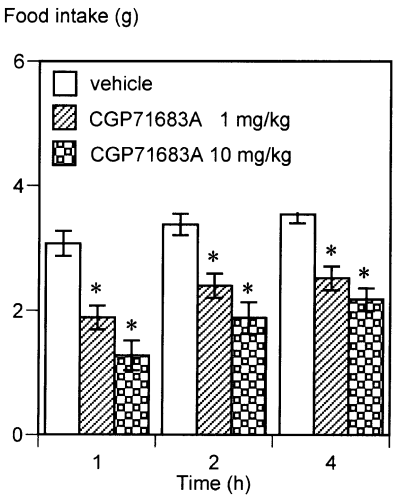

Fig. 2. The effect of CGP71683A (0, 1 and 10 mg/kg) on food intake stimulated by overnight fast. Both doses of CGP71683A significantly decreased food intake. Data are expressed as means ± S.E.M. There were six rats in each treatment group. * $P < 0.05$ after significant ANOVA, Scheffe's test.

3.2. Social interaction test

Pairs of experimentally naive, unfamiliar rats, housed individually for 2 weeks, were tested in a novel environment. CGP71683A (1, 3 and 10 mg/kg), injected i.p. 30 min before the test, did not decrease social interaction time [$F(3,28) = 0.84$; $P < 0.48$, Fig. 3A].

3.3. Elevated plus-maze test followed by open-field test

In general the rats treated with i.p. injection of either vehicle or three doses of CGP71683A did not show signs of sedation or robust behavioural disturbances. However, immediately after intraperitoneal injections of the drug, all rats developed short-lasting stretching and lordosis, these effects being attributable to DMSO used to dissolve the compound. The latency for first open part entry was not significantly changed [$F(3,28) = 0.11$, NS]. Neither the number of open nor the number of closed arm entries was changed by the treatment: $F(3,28) = 0.88$; $P < 0.46$ and $F(3,28) = 1.02$; $P < 0.40$, respectively. The number of line crossings in the open part, an indirect measure of locomotor activity was not changed either $F(3,28) = 0.98$; $P < 0.41$. The percentage of open arm entries relative to total arm entries most widely reported parameter of the elevated plus-maze test, was not different between the four treatment groups—$F(3.28) = 0.16$; 0.91, as shown in Fig. 3C. The defecation score in the elevated plus-maze was significantly decreased by CGP71683A [$F(3,28) = 3.6$; $P < 0.05$]. The open-field test, which was carried out immediately after elevated plus-maze test, revealed that CGP71683A significantly inhibited horizontal activity [$F(3,28) = 2.96$; $P < 0.05$, Fig. 3E]. There was a tendency towards inhibition of vertical activity (Fig. 3F) but these changes did not reach statistical significance due to within group variability [$F(3,28) = 2.26$; $p = 0.10$]. The lowest dose of CGP71683A (1 mg/kg) tended to increase the number of fecal boli deposited during open-field test but

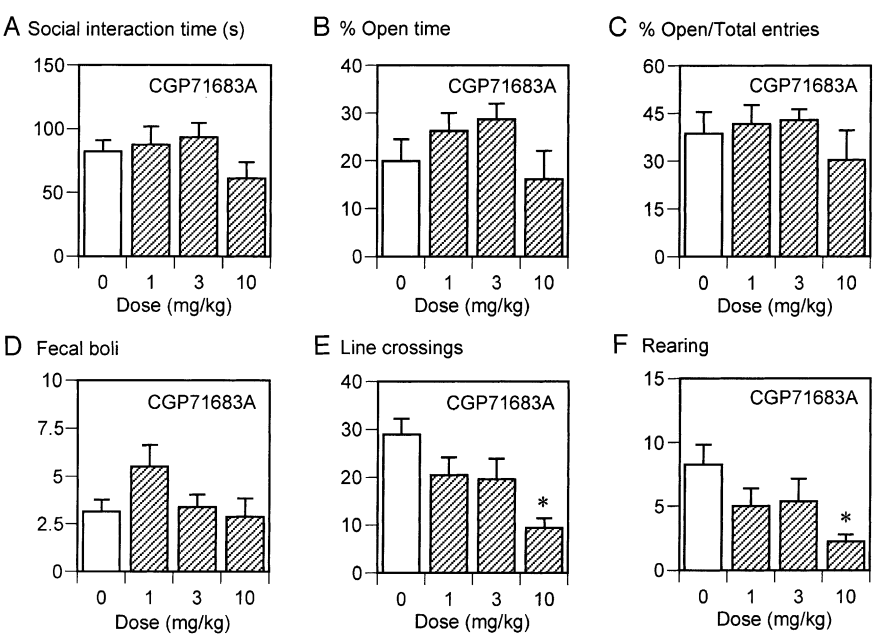

Fig. 3. The effects of CGP71683A (0, 1, 3, 10 mg/kg, i.p.) on the behaviour of the rats in the social interaction test (A), elevated plus-maze test (B, C) and open-field test (D–F). No anxiogenic-like behaviour was observed in elevated plus-maze and social interaction test, whereas in the open-field test CGP71683A significantly decreased horizontal and vertical activity, findings indicative of increased anxiety. All data are expressed as means ± S.E.M. There were eight rats in each treatment group. * $P < 0.05$ after significant ANOVA, Fisher's PLSD test.

Table 1
The effect of the neuropeptide Y Y_1 receptor antagonist H 409/22 injected intracerebroventricularly, on behaviour of the rats in the elevated plus-maze test

Dose (μg)	Latency of first open part entry	Open arm entries	Closed arm entries	Percentage of open/ total arm entries	Percentage of time in open arms	Lines crossed in open part	Approaches towards open part
0.0	20.5 ± 1.9	2.7 ± 0.4	4.4 ± 0.8	38.6 ± 5.5	18.8 ± 3.4	7.8 ± 1.8	0.3 ± 0.2
0.5	23.3 ± 2.9	1.6 ± 0.3	3.3 ± 0.5	30.1 ± 4.0	12.2 ± 2.5	4.3 ± 1.2	0.5 ± 0.2
5.0	86.7 ± 33.4*	0.8 ± .4*	3.0 ± 0.6	13.1 ± 5.5*	3.5 ± 1.8*	1.7 ± 0.9	1.2 ± 0.2*

All data are means ± S.E.M.
*$P < 0.05$ significantly different compared to vehicle-treated rats, Fisher's PLSD after significant ANOVA. $n = 8–10$ in each treatment group. H 409/22 was administered 20 min before the test.

ANOVA was not significant [$F(3,28) = 2.32$; $p = 0.09$, Fig. 3F]. A positive control in the elevated plus-maze test was novel neuropeptide Y Y_1 antagonist H 409/22. As shown in Table 1, intracerebroventricular administration of 5 μg of H 409/22 increased the latency of open part entry [$F(2,25) = 3.45$, $p < 0.05$]. H 409/22 in 5.0 μg significantly decreased the number of open arm entries [$F(2,25) = 5.63$, $p < 0.01$], time spent on open arms [$F(2,25) = 8.22$, $p < 0.01$], percentage of time spent on open arms [$F(2,25) = 6.27$, $p < 0.01$] and increased the number of approaches towards open part [$F(2,25) = 6.38$, $p < 0.01$]. The number of closed arm entries was not changed [$F(2,25) = 1.26$, NS] ruling out the possibility that anxiogenic-like changes resulted from non-specific effects of H 409/22 on locomotor activity (Table 1).

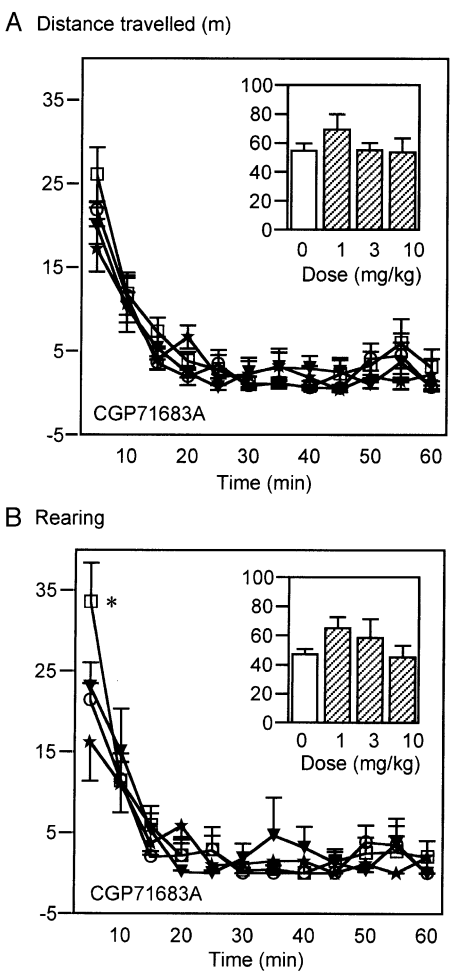

Fig. 4. The effect of CGP71683A on locomotor activity [distance travelled (A) and number of rears (B)] during 60-min test. Rats were tested immediately after i.p. administration of CGP71683A (0–10 mg/kg, i.p.). Vehicle—○; CGP71683A : 1.0—□; 3.0—★; 10.0—▼. There were six rats in each treatment group. Inserts with bars show the total number of events during testing session (60 min) whereas, lines refer to the data for each 5-min period. Data are expressed as means ± S.E.M. There were no significant differences between treatment groups for 60-min period (ANOVA for repeated measures), but during the first 5-min lowest dose of CGP71683A (1.0 mg/kg) significantly increased vertical activity. *$P < 0.05$ after significant factorial ANOVA, Fisher's PLSD test.

3.4. Long-term monitoring of locomotor activity

The habituation of locomotor response to novelty was studied in transparent boxes where rats were monitored for 1 h immediately following the injection of CGP71683A or vehicle. ANOVA for repeated measures indicated that neither horizontal [$F(3,220) = 0.86$, NS for distance travelled] nor vertical activity [$F(3,220) = 1.22$, NS for rears] was inhibited by CGP71683A (Fig. 4) suggesting that inhibitory effects of CGP71683A on food intake observed in previous experiments could not result from general inhibition of all ongoing behaviour or psychomotor stimulant-like activity of CGP71683A. When data were analysed for each 5 min separately, it was found that the lowest dose of CGP71683A stimulated locomotor activity during first 5 min of the test (Fig. 4).

4. Discussion

The main aim of this study was to investigate the involvement of the neuropeptide Y Y_5 receptor in the regulation of emotional behaviour. CGP71683A has been shown to induce conditioned taste aversion (Criscione et al., 1998). Therefore, we hypothesized that administration of CGP71683A, the neuropeptide Y Y_5 receptor selective antagonist either (1) elicits fear response by itself and renders rats more sensitive to anxiety-provoking stimuli

associated with novel testing environment or (2) causes changes (increase or decrease) in locomotor activity. In all these cases, the decrease in food intake would be unspecific, not related to appetite reduction.

4.1. Neuropeptide Y_5 receptors and food intake

Our data confirm the results of Criscione et al. (1998) showing that i.p. administration inhibits spontaneous and fasting-induced food intake in rats, adding further support to the notion that neuropeptide Y Y_5 receptor regulates feeding behaviour. The modulation of food intake by the family of neuropeptide Y-related peptides is complex. Neuropeptide Y is released from catecholaminergic nerve endings in the periphery after a meal (Rudnicki et al., 1990). Peptide YY and pancreatic polypeptide are synthesized in gastrointestinal tract and they are released postprandially. These three peptides may access the brain (Kastin and Akerström, 1999) and contrary to what is expected, act as peripheral signals activating satiety mechanisms in selected regions of the central nervous system (CNS). Overexpression of pancreatic polypeptide has been shown to reduce growth and cause leanness in mice, probably due to modest reduction in food intake resulting from delayed gastric emptying (Ueno et al., 1999). In the CNS, all three peptides, peptide YY, neuropeptide Y and pancreatic polypeptide increase food intake, although in the case of the pancreatic polypeptide, the effect is species specific (Gerald et al., 1996). The receptor subtype(s) mediating the effects of neuropeptide Y on food intake are not firmly established. Differential ability of modified analogues and fragments of neuropeptide Y led Stanley (1993) to propose that neuropeptide Y-induced feeding is mediated via the Y_1-like receptor. Soon thereafter, two groups cloned the neuropeptide Y Y_5 receptor (Gerald et al., 1996; Hu et al., 1996). Since then, there has been an intense debate, which neuropeptide Y receptor is pivotal for food intake—is it neuropeptide Y Y_1, Y_5 or Y_1-like (but still different from Y_5) receptor (see Kalra et al., 1999 for review)? Most studies, aiming to clarify this puzzle, have been carried out during the daytime when rats, active during nocturnal period, eat very little. Therefore, it is not surprising that these studies have failed to demonstrate significant inhibition of food intake in daytime after neuropeptide Y Y_1 receptor antagonists (Kask et al., 1998a; Yiengar et al., 1999). Neuropeptide Y Y_1 receptor antagonists effectively reduce fasting-induced and NPY-induced feeding. The relative importance of the neuropeptide Y Y_1 and Y_5 receptors in fine-tuning of feeding behaviour is still a matter of debate (Kask et al., 1998a; Wieland et al., 1998; Yiengar et al., 1999). The late-onset obesity in neuropeptide Y Y_5 receptor knockout mice (Marsh et al., 1998) suggests that this receptor subtype may be more intimately involved in the regulation of metabolism than food intake itself. In a recent study, it was shown that two synthetic neuropeptide Y Y_5 receptor selective agonists increased food intake (Cabrele et al., 2000) further strengthening the position that both neuropeptide Y receptor subtypes contribute to the regulation of energy intake. Neuropeptide Y may promote the weight gain also by modulating peripheral components of energy homeostasis by decreasing energy expenditure. Neuropeptide Y has been shown to inhibit insulin secretion, and there is evidence that insulin could act as a peripheral satiety factor also in the CNS (Porte et al., 1998; Brüning et al., 2000). Thus it could be hypothesized that the effects of CGP71683A could also be related to the changes of insulin levels. However, CGP71683A did not increase insulin levels but lowered them (Criscione et al., 1998). Moreover, the effect of the neuropeptide Y on glucose-stimulated insulin secretion is apparently not mediated via the neuropeptide Y Y_5 receptor (Morgan et al., 1998). Duhault et al. (2000) have recently demonstrated combined administration of neuropeptide Y Y_1 and Y_5 receptor antagonists is more efficient intervention to inhibit food intake than the usage of neuropeptide Y Y_1 or Y_5 receptor selective antagonists alone. Unfortunately, studies focusing on long-term efficacy and safety of neuropeptide Y receptor antagonists in terms of side effects on behaviour are lacking. It is not known if anxiogenesis induced by neuropeptide Y Y_1 antagonists wanes off with time and if there is a tolerance to anorectic effect. The expression of expected phenotype in neuropeptide Y Y_1 and Y_5 receptor knockout mouse was weak (Marsh et al., 1998; Pedrazzini et al., 1998) and neuropeptide Y knockout mouse did not have overt behavioural abnormalities (Bannon et al., 2000). Therefore, experiments addressing these issues are certainly warranted in order to explore the feasibility of using neuropeptide Y receptors as targets for anti-obesity drugs.

4.2. Neuropeptide Y_5 receptors and locomotion

One of the earliest behavioural effects of neuropeptide Y described was the suppression of home cage and open-field activity (Heilig et al., 1988). Lower doses of neuropeptide Y_{13-36}, a neuropeptide Y $Y_{2/5}$ receptor agonist, act in opposite way, they facilitate locomotor activity (Heilig et al., 1988). Injections of neuropeptide Y into frontal cortex increased locomotor activity in rats (Smialowski et al., 1992). Stimulatory effects of neuropeptide Y on locomotion appear to depend on intact noradrenergic system, as neuropeptide Y, despite its ability to restore deficits in social interaction, failed to reverse the decrease in locomotor activity induced by the selective noradrenergic denervation (Kask et al., 2000). The expression of neuropeptide Y Y_5 receptors not only in brain regions that regulate food intake but also in areas that are involved in generation and maintenance of motor activity (e.g. caudate–putamen) prompted us to study the effects of a neuropeptide Y_5 receptor antagonist on exploratory behaviour in detail. In 4-min open-field test, carried out after the elevated plus-maze test, CGP71683A decreased the

number of line crossings and rearing. The lowest dose of antagonist also tended to increase the number of defecations, being indicative of an anxiogenic-like profile of the drug. Results of detailed long-term monitoring of locomotor activity suggested that CGP71683A did not affect the habituation to novel environment, i.e. it did not cause sustained inhibition or stimulation of locomotor activity that could have lead to false positive result in feeding tests. These data clearly indicate that the suppression of food intake after CGP71683A administration occurs without any robust change in the locomotor activity of the rats.

4.3. Neuropeptide Y Y_5 receptors and emotionality

Neuropeptide Y has robust anxiolytic-like effects in various tests such as the elevated plus-maze (Heilig et al., 1989; Broqua et al., 1995), light–dark compartment (Pich et al., 1993), punished drinking (Heilig et al., 1993), fear-potentiated acoustic startle (Broqua et al., 1995) and social interaction test (Sajdyk et al., 1999). Accumulating evidence suggests that these effects of neuropeptide Y are mediated via the Y_1 receptor subtype. This possibility has also been confirmed with the first selective neuropeptide Y Y_1 receptor antagonist BIBP3226 that had anxiogenic-like effect in the elevated plus-maze test (Kask et al., 1996). Here, we find that novel, structurally similar neuropeptide Y Y_1 receptor antagonist H 409/22 has also anxiogenic-like effect in elevated plus-maze test, confirming previous data obtained with BIBP 3226 (Kask et al., 1996, 1998b,c). The main objective of our study was to examine the effects of neuropeptide Y_5 receptor selective antagonist CGP71683A in experimental models of anxiety. The presence of the neuropeptide Y Y_5 receptor binding sites in the brain regions that are thought to mediate anxiety (amygdala, hippocampus and locus coeruleus) suggests that this receptor subtype may be involved in the regulation of emotional response to stress. CGP71683A did not decrease exploratory activity of the rats in the elevated plus-maze test and it did not decrease social interaction under testing conditions that were appropriate to detect anxiogenic-like effect of the drug. Only when rats were stressed by previous exposure to elevated plus-maze that there was some evidence for increased anxiety in the open-field test. The reasons why the evidence for anxiogenic-like profile of CGP71683A could be detected only after previous stress are unknown. It could be hypothesized that neuropeptide Y Y_1 receptors mediate tonic neuropeptide Y-induced anxiolysis, whereas neuropeptide Y Y_5 receptor contribute to adaptive changes that occur after stress exposure. Further experiments are needed to explore this possibility. Our data suggest that reduction of spontaneous and food deprivation-induced food intake after intraperitoneal injection of neuropeptide Y Y_5 receptor antagonist CGP71683A are not due to unspecific effects of the drug. These findings suggest that neuropeptide Y Y_5 receptor may be a useful target for anti-obesity drug development. In contrast to the pharmacological inactivation of neuropeptide Y Y_1 receptor, blockade of neuropeptide Y Y_5 receptor subtype results in behaviourally specific reduction of food intake, i.e. CGP71683A-induced anorexia is not accompanied with undesirable side-effects such as increased anxiety or decreased locomotor activity. It cannot be excluded though, that acute effects of neuropeptide Y receptor antagonists may differ from those that may occur after chronic treatment. Therefore, long-term studies with both neuropeptide Y Y_1 and Y_5 receptor antagonists, focusing simultaneously on both on feeding and emotional behaviour would be illuminating, in order to evaluate the usefulness of NPY Y_5 receptor antagonists as anti-obesity drugs. It is also important to compare therapeutic prospects of the neuropeptide Y Y_5 receptor tools with ligands acting at other promising drug targets, such as melanocortin (MC_4) receptors (Wikberg, 1999).

5. Note added in proof

Most recently, CGP71683A was found to display nanomolar affinity for muscarinic receptors and serotonin uptake sites (Della Zuana et al., 2001). While this finding emphasizes the need for further improvement of currently available neuropeptide Y Y_5 receptor tools, it does not affect the main conclusion of the present study — CGP71683A-induced inhibition of food intake is behaviourally specific.

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