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Quipazine reduces food intake in the rat by activation of 5-HT_2-receptors

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1 To determine which subtype(s) of 5-hydroxytryptamine (5-HT) receptor are involved in the anorectic action of quipazine, the ability of selective antagonists at 5-HT_2- and 5-HT_3-receptors, and an antagonist at 5-HT_1-like receptors, to block this response were investigated in non-deprived rats, trained to eat a palatable diet.

2 Quipazine (0.5–8 mg kg^{-1}, i.p.) produced a dose-related reduction in the intake of palatable diet.

3 The anorectic effect of 4 mg kg^{-1} quipazine was antagonized by the nonselective 5-HT-receptor antagonist methysergide (5 mg kg^{-1}, i.p.) and by the selective 5-HT_2-receptor antagonists ketanserin (1 mg kg^{-1} and 2.5 mg kg^{-1}, i.p.) and ritanserin (0.5 mg kg^{-1} and 1 mg kg^{-1}, i.p.). The selective 5-HT_3-receptor antagonist GR38032F (1 mg kg^{-1}, i.p.) and (−)-pindolol (4 mg kg^{-1}, i.p.), which blocks some of the effects mediated at 5-HT_1-like receptors, did not block the reduction in food intake produced by this dose of quipazine.

4 None of the 5-HT-receptor antagonists had any effect on food intake when they were administered alone, suggesting that endogenous 5-HT is not involved in the tonic control of food intake under the conditions of these experiments.

5 It is concluded that the anorectic action of quipazine is mediated, at least in part, by activation of 5-HT_2-receptors.

Introduction

There is considerable evidence supporting a role of 5-hydroxytryptamine (5-HT) in the control of food intake (Blundell, 1984; Garattini *et al.*, 1986). For example, the 5-HT releaser/reuptake inhibitor fenfluramine produces a reduction in food intake in both animals (Duhault *et al.*, 1975) and man (Silverstone & Goodall, 1986). In addition, drugs that are considered to act primarily by a direct action at 5-HT receptors, such as quipazine or *m*-chlorophenylpiperazine (mCPP), are capable of reducing food intake (Samanin *et al.*, 1980; Kennett *et al.*, 1987).

The role of multiple 5-HT-receptor subtypes, as defined by radioligand binding studies (Peroutka & Snyder, 1979; Pedigo *et al.*, 1981; Hoyer *et al.*, 1985; Pazos *et al.*, 1985; Kilpatrick *et al.*, 1987), in the control of food intake is less well understood. Studies using selective agonists at the putative 5-HT_{1A}-receptor, such as 8-hydroxy-2-(di-n-propylamino)tetralin (DPAT), have shown that these compounds increase food intake (Dourish *et al.*, 1985). Conversely, agonists with some selectivity for the putative 5-HT_{1B}-receptor, such as 5-methoxy-3(1, 2,3, 6-tetrahydropyridin-4-yl)1H indole (RU24969), reduce food intake (Kennett *et al.*, 1987). It is, however, undesirable to characterize effects mediated at a specific receptor on the basis of the action of an agonist alone. Bradley *et al.* (1986) have defined three functional subtypes of 5-HT receptor, namely 5-HT_1-like, 5-HT_2 and 5-HT_3, and proposed criteria for characterization of an action at a specific subtype of 5-HT receptor. For example, the response produced by a drug acting via 5-HT_2-receptors should be antagonized by a selective 5-HT_2-receptor antagonist, such as ketanserin (Leysen *et al.*, 1981a), and methysergide to preclude any involvement of

α_1-adrenoceptors, whereas antagonist compounds showing high selectivity for other 5-HT-receptor subtypes should not block the response.

Quipazine is known to interact directly with 5-HT$_1$-like (Engel *et al.*, 1986), 5-HT$_2$- (Leysen *et al.*, 1981b) and 5-HT$_3$-receptors (Kilpatrick *et al.*, 1987). The aim of the present study was to determine which subtype(s) of 5-HT receptor was involved in the anorectic action of quipazine using the criteria proposed by Bradley *et al.* (1987). Thus, the ability of the non-selective 5-HT-receptor antagonist methysergide, the selective 5-HT$_2$-receptor antagonists ketanserin and ritanserin (Leysen *et al.*, 1985), and the selective 5-HT$_3$-receptor antagonist GR38032F (Brittain *et al.*, 1987) to antagonize the anorectic effects of quipazine were investigated in non-deprived rats. Although no selective antagonists are yet available for 5-HT$_1$-like receptors, the (−)-isomer of pindolol has been shown to block stereoselectively some of the behavioural effects mediated at these receptors, whereas the (+)-isomer was inactive (Tricklebank *et al.*, 1985; 1986). The isomers of pindolol were therefore used in this study to determine the involvement of 5-HT$_1$-like receptors in the effects of quipazine on food intake. The results obtained suggest that the anorectic action of quipazine was mediated, at least in part, by 5-HT$_2$-receptors.

Methods

Animals

Experiments were performed on Hooded Lister rats (Olac) which had a starting weight of 175–200 g. They were housed individually and maintained on a 12 h light–12 h dark cycle (lights on 07 h 00 min) and a room temperature of 21 ± 1°C.

Experimental procedure

Rats were familiarized with a palatable diet (ingredients and proportions: 50 ml Nestlés sweetened condensed milk; 150 ml tap water; 200 g CRM powdered food (Labsure)) over a five day period by use of a method similar to that described by Cooper *et al.* (1985). Briefly, each rat was presented with 20–30 g of the diet in a Perspex petri-dish for 30 min each day in the home cage. Water and CRM pellet food were available *ad libitum* apart from this 30 min period. Intake of the diet was measured by successive weighings of the food container to an accuracy of 0.1 g on a top-loading electronic balance (Sartorius). Before experiments started rats were thoroughly accustomed to being handled and were familiarized with the injection procedure. All experiments were performed between 13 h 00 min and 16 h 30 min.

To determine the dose to use in subsequent interaction studies, the effects of several doses of quipazine on food intake were evaluated. Quipazine (0.5–8 mg kg^{-1}, i.p.), or vehicle, was administered to groups of 10 rats per dose level, 10 min before presentation of the palatable diet and 30 min intake measured.

Drug-interaction experiments were conducted using either a 2 × 2 or a 3 × 2 factorial design. Each rat received 2 injections before the 30 min feeding test. The antagonist under investigation, or its vehicle, was administered 30 min before, and quipazine, or its vehicle, administered 10 min before presentation of the palatable diet. Food intake was then measured over a 30 min period. Rats were used in a maximum of 2 drug-interaction experiments with at least 3 days between experiments in the same animals. They were assigned to treatment groups such that each animal received quipazine in one experiment only.

Drugs

Quipazine maleate (Research Biochemicals Inc.), methysergide maleate (Sandoz), ketanserin tartrate (Janssen) and GR38032F (1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one HCl · 2H$_2$O, synthesized by Parke-Davis) were dissolved in distilled water. Ritanserin (Janssen) was dissolved in 0.01 N tartaric acid (final pH of solution 4). The (+)- and (−)-isomers of pindolol (Sandoz) were dissolved in distilled water to which a few drops of glacial acetic acid had been added (final pH of solution 4). All drugs were administered by the intraperitoneal route in a dose volume of 1 ml kg^{-1}. Drug doses refer to the weight of base.

Statistics

Dose-response data for quipazine were analysed by one-way ANOVA for independent groups, followed by Dunnett's t test. Results of the drug-interaction studies were analysed by two-way ANOVA for independent groups and comparisons between treatment groups made using the Student Newman-Kuels test. Statistical significance was accepted at $P < 0.05$.

Results

Quipazine produced a dose-related reduction in the intake of the palatable diet (Figure 1). A dose of

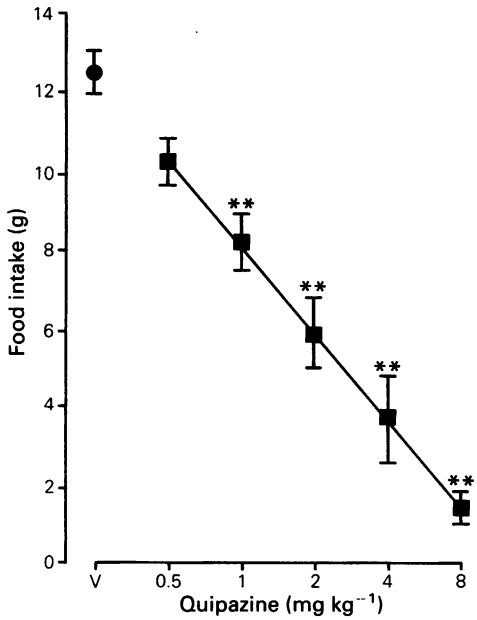

Figure 1 Effect of quipazine on the intake of a palatable diet. Quipazine (0.5–8 mg kg^{-1}, i.p., $n = 10$ rats per dose level) was administered to rats 10 min before presentation of the palatable diet. A dose-related reduction in the 30 min intake of the diet was produced. ** $P < 0.01$ Dunnett's t test following a significant difference between groups by one-way ANOVA [$F(5,54) = 27.33$, $P < 0.001$].

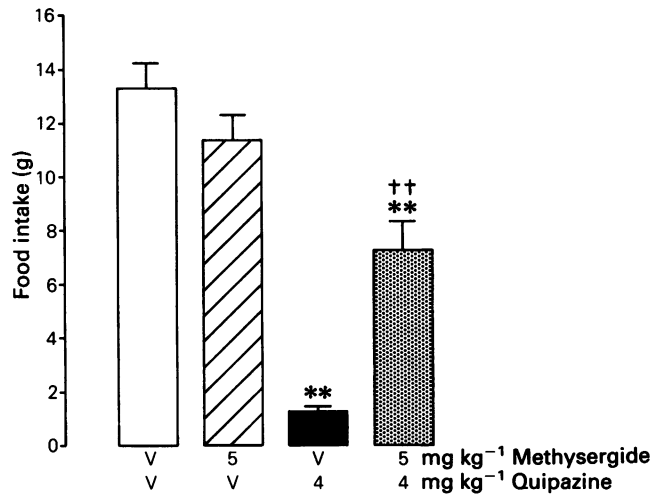

Figure 2 Antagonism by methysergide of the anorectic effect of quipazine. Methysergide (5 mg kg^{-1}, i.p.) or its vehicle (V) were administered 30 min before, and quipazine (4 mg kg^{-1}, i.p.) or its vehicle (V) administered 10 min before presentation of the palatable diet. Pretreatment with methysergide antagonized the reduction in 30 min food intake produced by quipazine. ** $P < 0.01$, significantly different from control group; †† $P < 0.01$ significantly different from V + quipazine group, Student Newman-Kuels test. $n = 10$ rats per treatment group.

4 mg kg^{-1} quipazine was selected for the drug-interaction studies since it produced a large, but not supramaximal, reduction in the intake of the palatable diet.

Methysergide versus quipazine

Methysergide (5 mg kg^{-1}) had no effect on the intake of the diet when given alone, but significantly attenuated the anorectic effect of quipazine [drug-interaction term $F(1,36) = 22.57$, $P < 0.001$] (Figure 2). The anorectic effect of quipazine was not blocked completely by this dose of methysergide, however, and a significant reduction in food intake compared to the control group was still observed.

Ketanserin and ritanserin versus quipazine

The selective 5-HT$_2$-receptor antagonist ketanserin at doses of 1 mg kg^{-1} and 2.5 mg kg^{-1} antagonized the reduction in food intake produced by quipazine [drug-interaction term $F(2,53) = 6.95$, $P < 0.005$] (Figure 3a). The response to quipazine was attenuated to a similar degree by both the low and the high dose of the antagonist. Food intake was still significantly reduced by quipazine in the antagonist pretreated groups compared to the control group.

Ritanserin (0.5 mg kg^{-1} and 1 mg kg^{-1}) also attenuated the anorectic effect of quipazine [drug-interaction term $F(2,54) = 8.88$, $P < 0.005$] (Figure 3b).

Neither of the 5-HT$_2$-receptor antagonists increased the intake of palatable diet when administered in combination with quipazine vehicle (Figure 3).

GR38032F/(+)- and (−)-isomers of pindolol versus quipazine

Figure 4a shows that the selective 5-HT$_3$-receptor antagonist GR38032F (1 mg kg^{-1}) did not block the anorectic effect of quipazine [drug-interaction term $F(1,35) = 0.69$, NS]. Neither the (−)-isomer of pindolol (4 mg kg^{-1}), nor its (+)-isomer, antagonized the reduction in food intake produced by quipazine [drug-interaction term $F(2,53) = 0.11$, NS] (Figure 4b). In addition, neither GR38032F nor the isomers of pindolol affected food intake when given alone (Figure 4).

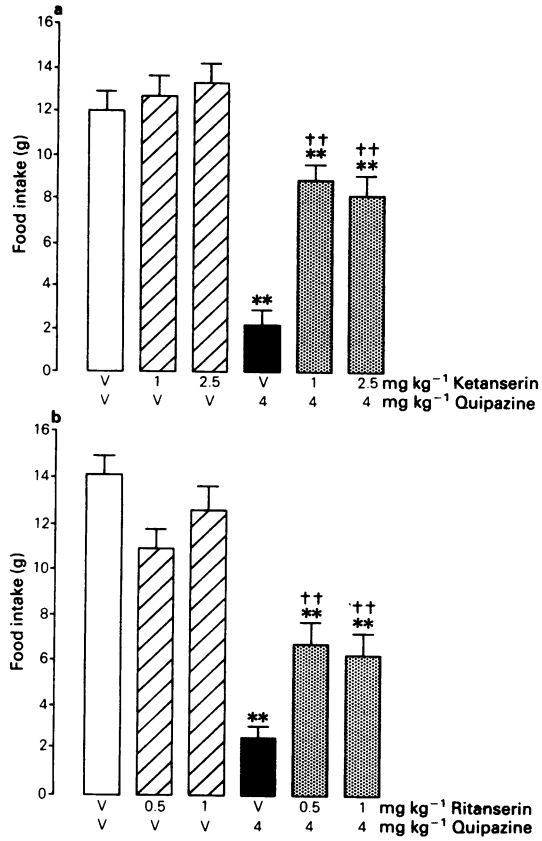

Figure 3 Antagonism by (a) ketanserin and (b) ritanserin of the anorectic effect of quipazine. The antagonists or their vehicles (V) were administered i.p. 30 min before, and quipazine or its vehicle (V) administered i.p. 10 min before presentation of palatable diet at the doses indicated. Ketanserin (1 mg kg^{-1} and 2.5 mg kg^{-1}) and ritanserin (0.5 mg kg^{-1} and 1 mg kg^{-1}) antagonized the reduction in 30 min food intake produced by quipazine. ** $P < 0.01$, significantly different from control groups; †† $P < 0.01$, significantly different from V + quipazine groups, Student Newman-Kuels test. $n = 10$ rats per treatment group, except ketanserin (1 mg kg^{-1}) + V group where $n = 9$.

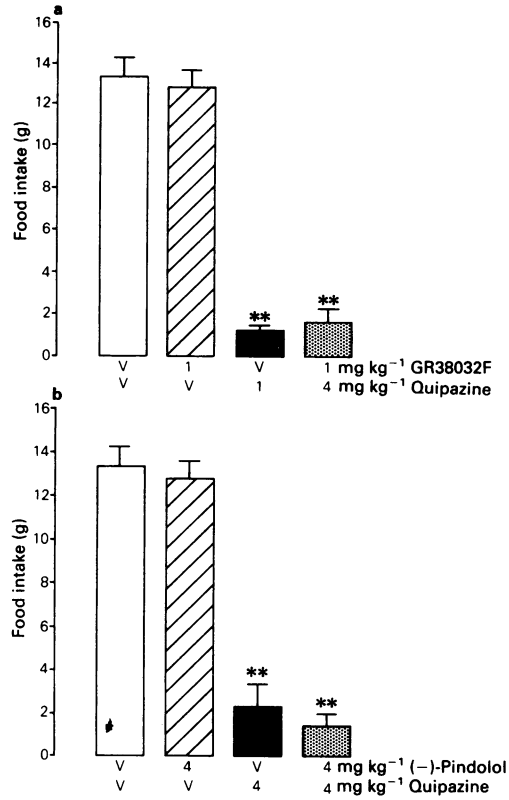

Figure 4 Failure of (a) GR38032F and (b) (−)-pindolol to antagonize the anorectic effect of quipazine. The antagonists or their vehicles (V) were administered i.p. 30 min before, and quipazine or its vehicle administered i.p. 10 min before presentation of the palatable diet. Neither GR38032F (1 mg kg^{-1}) nor (−)-pindolol (4 mg kg^{-1}) antagonized the reduction in food intake produced by quipazine. ** $P < 0.01$, significantly different from control groups, Student Newman-Kuels test. $n = 10$ rats per treatment group, except in (a) GR38032F + V group and in (b) V + quipazine group where $n = 9$.

Discussion

Quipazine produced a dose-related reduction in the intake of palatable diet in this study, confirming the anorectic effects reported in previous studies carried out in fasted rats (Samanin *et al.*, 1980; Rowland *et al.*, 1985), and extending their observations to include non-deprived animals. The primary purpose of the dose-response study, however, was to determine the optimum dose of quipazine to use in the subsequent drug-interaction studies. A dose of 4 mg kg^{-1} quipazine was selected on the basis that it produced a large, but not supramaximal, reduction in food intake on which any blockade of the response would be clearly seen. The design of the drug-interaction studies was such that although each animal was used in 2 experiments, they received quipazine on only one occasion. This ensured that there was no possibility that rapid tolerance to the anorectic effects of quipazine, such as that reported by Rowland *et al.* (1982), would influence the results.

Quipazine-induced anorexia was antagonized by ketanserin suggesting that this effect was mediated by 5-HT$_2$-receptors. At the doses used in this study, however, ketanserin can also block effects at

α_1-adrenoceptors (Fozard, 1982). The observations that ritanserin, another selective 5-HT_2-receptor antagonist, and the nonselective 5-HT-receptor antagonist methysergide, both of which have negligible affinity for α_1-adrenoceptors (Leysen *et al.*, 1985) also attenuate this response clearly demonstrates that the 5-HT_2-receptor blockade produced by ketanserin was responsible for the antagonism.

The involvement of 5-HT_2-receptors in the anorectic action of quipazine suggests that these receptors may possibly mediate the anorectic actions of other compounds which act through a 5-HT-related mechanism. In support of such a hypothesis, the anorectic effect of DL-fenfluramine was found to be antagonized by ketanserin (Hewson *et al.*, 1988a). Also, the reduction in food intake produced by peripheral administration of 5-HT itself is antagonized by ritanserin and ketanserin (Massi & Marini, 1987; Simansky *et al.*, 1987). The results from other studies have, however, suggested a role for 5-HT_1-like receptors in the anorectic actions of compounds acting through the 5-HT system. Thus, the anorectic action of D-fenfluramine was resistant to antagonism by ritanserin but was blocked by the nonselective 5-HT-receptor antagonist metergoline (Garattini *et al.*, 1987). Furthermore, Kennett *et al.* (1987) have shown that the anorectic effects of RU24969 were blocked by metergoline, but not ketanserin. Thus, it appears that activation of either 5-HT_2- or 5-HT_1-like receptors can result in anorexia in the rat.

Ketanserin, ritanserin and methysergide did not completely reverse the anorectic effect of 4 mg kg^{-1} quipazine. It is possible that the remaining significant reduction in food intake was due to an action at other subtypes of 5-HT-receptor. The failure of the potent, selective 5-HT_3-receptor antagonist GR38032F to block the anorectic effect of quipazine is consistent with the known pharmacology of quipazine in relation to 5-HT_3-receptors. Thus, quipazine itself is a potent antagonist at 5-HT_3-receptors on the vagus nerve (Ireland & Tyers, 1987) and its anorectic effect would therefore not be expected to be mediated by an agonist action at this receptor. Furthermore, the observation that GR38032F did not reduce food intake demonstrates that an antagonist action at 5-HT_3-receptors was not responsible for the anorectic action of quipazine.

The failure of (−)-pindolol to antagonize the anorectic effect of quipazine argues against the involvement of 5-HT_1-like receptors in the anorectic action of quipazine. Although (−)-pindolol does not antagonize all of the effects considered to be mediated by 5-HT_1-like receptors (Feniuk *et al.*, 1983; Charlton *et al.*, 1986), it does bind with high affinity to the putative 5-HT_{1A}- and 5-HT_{1B}-receptors and antagonizes functional responses thought to be mediated at these receptors (Middlemiss, 1984; Engel *et al.*, 1986; Tricklebank *et al.*, 1985; 1986). Also, of greater relevance to the present study, Kennett *et al.* (1987) have shown that (−)-pindolol and (±)-cyanopindolol antagonized the anorectic effects of the putative 5-HT_{1B}-receptor agonist, RU24969. One other line of evidence which argues against the involvement of 5-HT_{1B}-receptors in quipazine-induced anorexia is that quipazine is an antagonist at this receptor (Engel *et al.*, 1986). Nevertheless, because of the incomplete blockade of the quipazine response by methysergide, ketanserin and ritanserin, coupled with the lack of truly selective antagonists at 5-HT_1-like receptors, it must remain a possibility that this effect could be due in part to activation of another subtype of 5-HT receptor.

The results of the present study demonstrating an involvement of 5-HT_2-receptors in the anorectic action of quipazine are consistent with the reported agonist actions of quipazine at 5-HT_2-receptors in other functional systems. Thus, quipazine is a partial agonist at the 5-HT_2-receptor on vascular smooth muscle (Cohen *et al.*, 1981) and induces head twitches in mice, which is indicative of 5-HT_2-receptor activation (Malick *et al.*, 1977). In addition, Conn & Sanders-Bush (1987) have reported that quipazine stimulated phosphoinositol hydrolysis in the cerebral cortex and that this effect was antagonized by ketanserin.

The observation that none of the 5-HT-receptor antagonists significantly affected the intake of the palatable diet when given alone suggests that endogenous 5-HT does not play a major role in the regulation of food intake in this model. The palatable diet test has been shown to be sensitive to the hyperphagic effects of κ-receptor agonists (Cooper *et al.*, 1985) and benzodiazepines (Cooper & Yerbury, 1986), as well as those of the CCK-receptor antagonist L364,718 (Hewson *et al.*, 1988b). The lack of effect of the 5-HT-receptor antagonists cannot, therefore, be explained on the basis of the use of an insensitive test. The results obtained in the present study are in agreement with other studies which showed that 5-HT-receptor antagonists failed to affect food intake. Thus, Massi & Marini (1987) failed to observe an increase in food intake with ritanserin at doses that blocked the anorectic effect of 5-HT. Similarly, Kennett *et al.* (1987) reported that metergoline, (−)-pindolol and (±)-cyanopindolol did not increase food intake at doses that clearly antagonized the anorectic effect of RU24969.

It is concluded that quipazine produces a reduction of food intake, at least in part, by activation of 5-HT_2-receptors.

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