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Short communication

The 5-HT_{1B} receptor mediates the effect of *d*-fenfluramine on eating caused by intra-hypothalamic injection of neuropeptide Y

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Abstract

d-Fenfluramine (0.63 mg/kg i.p.), a serotonin (5-hydroxytryptamine, 5-HT) releaser and re-uptake inhibitor, reduced the eating caused by neuropeptide Y (235 pmol) injected into the paraventricular nucleus of the hypothalamus. The 5-HT_1 and 5-HT_2 receptor antagonist metergoline (1.0 and 2.0 mg/kg i.p.) and the 5-HT_{1A} and 5-HT_{1B} receptor antagonist (±)-cyanopindolol (3.0 and 8.0 mg/kg s.c.) significantly antagonized the effect of *d*-fenfluramine. The 5-HT_{2A} and 5-HT_{2C} receptor antagonist mesulergine (0.1 and 0.3 mg/kg s.c.) and the 5-HT_{2A} receptor antagonist ketanserin (2.5 and 5.0 mg/kg i.p.) did not significantly modify the effect, nor did the 5-HT_{1A} and 5-HT_{1B} receptor antagonist (−)-propranolol (20–40 nmol), injected bilaterally into the paraventricular nucleus of the hypothalamus. The results suggest that *d*-fenfluramine reduces neuropeptide Y's hyperphagia by indirectly stimulating 5-HT_{1B} receptors outside the paraventricular nucleus of the hypothalamus.

Keywords: Hyperphagia; Neuropeptide Y; *d*-Fenfluramine; (±)-Cyanopindolol; Paraventricular nucleus of the hypothalamus; 5-HT receptor

1. Introduction

Bendotti et al. (1987) showed that *d*-fenfluramine, a serotonin (5-hydroxytryptamine, 5-HT) releaser and re-uptake inhibitor, was particularly potent in reducing eating caused by neuropeptide Y injected into the paraventricular nucleus of the hypothalamus of rats. It has been suggested that neuropeptide Y is involved in the pathogenesis of clinical hyperphagias, including bulimia (Morley et al., 1985), and fenfluramine did in fact reduce eating in patients with bulimia nervosa (Robinson et al., 1986). It was therefore important to clarify which 5-HT receptor subtype is involved in the ability of *d*-fenfluramine to reduce neuropeptide Y-induced hyperphagia.

5-HT_1 receptors, particularly the 5-HT_{1B} subtype, appear to play an important role in the hypophagic effect of *d*-fenfluramine in various feeding paradigms in rodents (Samanin and Garattini, 1993). Other authors have suggested a role of 5-HT_{2A} (Hewson et al., 1988a) or 5-HT_{2C} (Gibson et al., 1993) receptors in the feeding-suppressing effect of fenfluramine.

There is evidence that 5-HT stimulates the secretion of corticotropin releasing factor by explanted rat hypothalami through a mechanism which seems to involve 5-HT_{2A} receptors (Calogero et al., 1989). In view of the modulation of neuropeptide Y-induced eating by corticotropin releasing factor in the paraventricular nucleus of the hypothalamus (Heinrichs et al., 1993) hypothalamic 5-HT_{2A} receptors may be involved in the effect of *d*-fenfluramine on neuropeptide Y hyperphagia.

In order to clarify which 5-HT receptor subtype is involved in the effect of *d*-fenfluramine on eating induced by neuropeptide Y, we pretreated rats with antagonists having different affinities for various 5-HT receptor subtypes. In view of the fact that 5-HT_{1B} receptor agonists injected into the paraventricular nucleus of the hypothalamus have been found to reduce eating (Hutson et al., 1988), we also examined whether the 5-HT_{1A} and 5-HT_{1B} receptor antagonist (−)-propranolol (Hoyer, 1988) bilaterally injected into the paraventricular nucleus of the hypothalamus reduced

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the effect of *d*-fenfluramine on neuropeptide Y-induced eating.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (CD-COBS, Charles River, Italy), weighing 220–250 g were housed in groups of four and kept in a room at 21 ± 1°C and 60% relative humidity, with a 12 h light/12 h dark cycle (light off at 19.00 h) and water and food (Altromin pellets) ad libitum. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., Suppl. 40, February 18, 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, December 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

2.2. Experimental procedure

Under equithesin anesthesia (9.7 mg/ml sodium pentobarbital in saline + 42.6 mg/ml chloral hydrate in propylene glycol + 21.2 mg/ml Mg_2SO_4 in ethanol; 3.5 mg/kg i.p.) rats were stereotaxically implanted with chronic monolateral or bilateral guide cannulas constructed from 23 gauge stainless steel tubing, placed with the tip 2 mm above the target area (paraventricular hypothalamus). Stainless steel 30 gauge stylets kept the guide patent until the animals were given intracerebral injections 7–10 days later. On the day of the test the stylets were withdrawn and replaced by bilateral injection units terminating 2 mm below the tip of the guides. The coordinates (from the interaural line) were as follows (König and Klippel, 1963): A = 5660, H = +2.0, L = +0.2 for the monolateral injections of neuropeptide Y and A = 5660, H = +2.0, L = ±0.2 for the bilateral injections of (−)-propranolol.

The animals were accustomed to handling before testing and on the day of the experiment they were allowed to become habituated to the test cages for 1 h (Perspex cages with a grid and blotting paper on the floor), with food freely available on the cage floor.

Metergoline (1.0 and 2.0 mg/kg), ketanserin (2.5 and 5.0 mg/kg) and *d*-fenfluramine (0.63 mg/kg) were injected intraperitoneally (i.p.) respectively 3 h, 45 min and 15 min before the test. The long pretreatment time for metergoline was that reported to antagonize the effect of *d*-fenfluramine in various feeding experiments (see Samanin and Garattini, 1993 for references). (±)-Cyanopindolol (3.0 and 8.0 mg/kg) and mesulergine (0.1 and 0.3 mg/kg) were injected subcutaneously (s.c.) 45 min before testing.

(−)-Propranolol (20–40 nmol/0.5 μl) was injected bilaterally into the paraventricular nucleus of the hypothalamus 20 min before the test. Neuropeptide Y (235 pmol/0.5 μl) was injected into the right paraventricular nucleus of the hypothalamus immediately before testing.

After neuropeptide Y injection, the rats were placed in the test cages with a weighed amount of food pellets on the floor and food intake corrected for spillage was measured for 1 h and expressed as g/100 g of body weight. On completion of the experiments, the animals were anesthetized with equithesin and killed by decapitation; the location of the cannulas was determined histologically. Only data from rats in which the cannulas were accurately located within the paraventricular nucleus of the hypothalamus were included in the results.

2.3. Drugs

d-Fenfluramine hydrochloride (Servier, Neuilly-sur-Seine, France), ketanserin tartrate (R.B.I., Wayland, MA, USA), and (−)-propranolol (Sigma Chemical, St. Louis, MO, USA) were dissolved in saline. Porcine neuropeptide Y (Novabiochem, Laufelfingen, Switzerland) was dissolved in sterile distilled water.

(±)-Cyanopindolol and mesulergine (Sandoz, Basel, Switzerland) were dissolved in 100–200 μl 10% acetic acid, made up to almost the required volume with saline and brought to pH 6.5 before injecting. Metergoline base (Farmitalia Carlo Erba, Milan, Italy) was dissolved in 1% ascorbic acid.

2.4. Statistical analysis

Two-way analysis of variance was followed by Tukey's test and probabilities (P) less than 0.05 were considered statistically significant.

3. Results

Preliminary experiments using different doses of *d*-fenfluramine confirmed (Bendotti et al., 1987) that 0.63 mg/kg i.p. markedly reduced the hyperphagic effect of neuropeptide Y administered into the paraventricular nucleus of the hypothalamus. A higher dose of *d*-fenfluramine did not reduce food intake further and a lower dose (0.30 mg/kg) produced no effect. Food intake (g/100 g ± S.E.M.) for the various groups was: saline + saline = 0.08 ± 0.03[a]; saline + neuropeptide Y = 1.63 ± 0.14; *d*-fenfluramine (0.30 mg/kg) + neuropeptide Y = 1.08 ± 0.4; *d*-fenfluramine (0.625 mg/kg) + neuropeptide Y = 0.44 ± 0.11[a]; *d*-fenfluramine (1.25 mg/kg) + neuropeptide Y = 0.63 ±

Table 1
Effect of various 5-HT receptor antagonists on *d*-fenfluramine-induced inhibition of neuropeptide Y hyperphagia in rats

Treatment	Dose (mg/kg)	Food eaten (g/100 g)		Fint
		Saline	*d*-Fenfluramine (0.63 mg/kg)	
Vehicle		1.44 ± 0.20	0.35 ± 0.12[a]	4.4[d]
Metergoline	1.0	1.18 ± 0.16	0.98 ± 0.26	
Metergoline	2.0	1.19 ± 0.14	0.94 ± 0.04	
Vehicle		1.12 ± 0.11	0.32 ± 0.14[a]	4.8[d]
(±)-Cyanopindolol	3.0	0.78 ± 0.22	0.19 ± 0.07[b]	
(±)-Cyanopindolol	8.0	1.08 ± 0.13	1.14 ± 0.08[c]	
Vehicle		1.18 ± 0.15	0.34 ± 0.12[a]	0.3
Mesulergine	0.1	1.31 ± 0.24	0.74 ± 0.15	
Mesulergine	0.3	1.30 ± 0.17	0.64 ± 0.16	
Vehicle		1.32 ± 0.18	0.47 ± 0.13[a]	2.0
Ketanserin	2.5	0.98 ± 0.14	0.78 ± 0.24	
Ketanserin	5.0	1.06 ± 0.10	0.49 ± 0.16	

All the animals received 235 pmol neuropeptide Y in 0.5 μl of distilled water in the right paraventricular nucleus of the hypothalamus. Food intake of vehicle-treated rats was: 0.08 ± 0.05 g/100 g. See Materials and methods for details of pretreatment times. Values are the mean ± S.E.M. of at least five rats per group. [a]$P < 0.01$, [b]$P < 0.05$ vs. respective controls. [c]$P < 0.01$ vs. vehicle + *d*-fenfluramine-treated animals. Tukey's test. [d]$P < 0.05$, F interaction. Two-way analysis of variance (ANOVA).

0.16[a] ([a]$P < 0.01$ vs. saline + neuropeptide Y, Dunnett's test).

Metergoline, at the doses of 1.0 and 2.0 mg/kg, did not modify the amount of food eaten by neuropeptide Y-injected rats, but significantly antagonized the effect of *d*-fenfluramine (Fint(2,32) = 4.4; $P < 0.05$) on this measure (Table 1). A significant interaction was found between (±)-cyanopindolol and *d*-fenfluramine (Fint(2,32) = 4.8; $P < 0.05$). Further analysis showed that only the dose of 8.0 mg/kg antagonized the effect of *d*-fenfluramine on neuropeptide Y-induced eating ($P < 0.05$, Tukey's test).

Mesulergine did not modify the effect of *d*-fenfluramine on eating and ketanserin tended to reduce it though not significantly (Fint(2,41) = 0.3; $P > 0.05$ and Fint(2,42) = 2.0; $P > 0.05$ respectively for mesulergine and ketanserin). The effect of a higher dose of *d*-fenfluramine (2.5 mg/kg) on neuropeptide Y-induced eating was also not modified by these two drugs (data not shown).

As shown in Table 2, bilateral injection of two doses of (−)-propranolol into the paraventricular nucleus of the hypothalamus did not modify the amount of food eaten by neuropeptide Y-injected rats and did not antagonize the effect of *d*-fenfluramine on neuropeptide Y-induced eating (Fint(2,58) = 0.2; $P > 0.05$).

4. Discussion

The present study confirms previous findings that *d*-fenfluramine markedly reduces eating caused by injecting neuropeptide Y into the paraventricular nucleus of the hypothalamus at a dose (0.63 mg/kg i.p.) having little or no effect on laboratory chow (Bendotti et al., 1987). It also shows that metergoline, a non-

Table 2
Effect of (−)-propranolol on *d*-fenfluramine-induced inhibition of neuropeptide Y hyperphagia in rats

	Dose (nmol)	Food eaten (g/100 g)	
		Saline	*d*-Fenfluramine (0.63 mg/kg)
Saline		1.42 ± 0.21	0.51 ± 0.11[a]
(−)-Propranolol	20	1.68 ± 0.17	0.90 ± 0.15[a]
(−)-Propranolol	40	1.46 ± 0.11	0.40 ± 0.13[a]

All the animals received 235 pmol neuropeptide Y in 0.5 μl of distilled water in the right paraventricular nucleus of the hypothalamus immediately before the test. Food intake of vehicle-treated rats was: 0.08 ± 0.01 g/100 g. Values are the mean ± S.E.M. of at least nine rats per group. (−)-Propranolol (20 and 40 nmol/0.5 μl) was dissolved in saline and injected bilaterally into the paraventricular nucleus of the hypothalamus 20 min before the test. *d*-Fenfluramine (0.63 mg/kg) was dissolved in saline and injected intraperitoneally 15 min before the test. [a]$P < 0.01$ vs. respective controls. Tukey's test. Fint(2,58) = 0.2, $P > 0.05$. Two-way analysis of variance (ANOVA).

selective antagonist at 5-HT receptors (Hoyer, 1988), completely antagonized the effect of *d*-fenfluramine, suggesting that 5-HT is involved. Mesulergine, an antagonist with high affinity for 5-HT_{2A} and 5-HT_{2C} receptors, and ketanserin, which has much higher affinity for 5-HT_{2A} than for 5-HT_{2C} receptors (Hoyer, 1988), did not modify the ability of *d*-fenfluramine to reduce neuropeptide Y-induced eating whereas (±)-cyanopindolol, an antagonist at 5-HT_{1A} and 5-HT_{1B} receptors (Hoyer, 1988), antagonized it.

The lack of effect of mesulergine could not be attributed to its inability to block central 5-HT_{2A}/5-HT_{2C} receptors since doses lower than 0.3 mg/kg (the highest dose used in the present study) blocked various effects of the 5-HT_{2C} receptor agonist 1-(3-chlorophenyl)piperazine (Kennett and Curzon, 1988). The same holds for ketanserin which reduced the hypophagic effect of quipazine, a 5-HT_{2A} receptor agonist, at a dose lower than that used in the present study (Hewson et al., 1988b).

The results with (±)-cyanopindolol suggest that the 5-HT_{1B} receptor is involved in *d*-fenfluramine's effect on neuropeptide Y overeating. In fact, studies with 5-HT_{1A} and 5-HT_{1B} receptor agonists suggest that stimulation of the latter causes anorexia in the rat while stimulation of the former increases food intake (Bendotti and Samanin, 1987). Two doses of the 5-HT_{1A} and 5-HT_{1B} antagonist (−)-propranolol (Hoyer, 1988), bilaterally injected into the paraventricular nucleus of the hypothalamus, did not modify the effect of *d*-fenfluramine on neuropeptide Y-induced eating, suggesting that 5-HT_{1B} receptors outside the paraventricular nucleus of the hypothalamus mediate this effect of *d*-fenfluramine. This is strengthened by the fact that 20 nmol/0.5 μl (−)-propranolol injected in the paraventricular nucleus of the hypothalamus antagonized the hypophagic effect of 7 nmol/0.5 μl RU-24969 (unpublished results), a 5-HT_{1A}/5-HT_{1B} receptor agonist that administered in the paraventricular nucleus of the hypothalamus has been reported to reduce eating in food-deprived rats (Hutson et al., 1988). In the light of recent findings that bilateral radiofrequency lesion of the paraventricular nucleus of the hypothalamus did not modify the effect of *d*-fenfluramine on chow intake (Fletcher et al., 1993), it is likely that the 5-HT_{1B} receptor involved in the hypophagic effect of *d*-fenfluramine in other eating paradigms (Samanin and Garattini, 1993) is also located outside the paraventricular nucleus of the hypothalamus.

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