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Unexpected Responses of the Obese "Cafeteria" Rat to the Peptide FMRF-Amide

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ROBERT, J. J., M. OROSCO, C. ROUCH, C. JACQUOT AND Y. COHEN. *Unexpected responses of the obese "cafeteria" rat to the peptide FMRF-amide*. PHARMACOL BIOCHEM BEHAV **34**(2) 341–344, 1989.—The relationship between the acute effects of FMRF-amide on central monoamines and feeding effects were investigated simultaneously in normophagic and "cafeteria" rats. This tetrapeptide is considered as being representative of an endogenous related peptides family with antagonistic properties on opioid-induced behavioural effects. In normophagic rats, no feeding effect was observed, but there was a decrease in serotonergic metabolism similar to that induced by the classical antagonist, naltrexone. However, in the "cafeteria" rats, FMRF-amide enhanced food intake and increased serotonergic metabolism, exhibiting the classical effects of opiate agonists. Since the effects of FMRF-amide differ according to the ponderal and/or nutritional status, this peptide would appear to act rather as a modulator than a true opiate antagonist on food intake. This raises the question as to the exact role of the recently-discovered endogenous FMRF-amide related family in obesity and/or stimulated feeding patterns.

FMRF-amide Food Intake "Cafeteria" rat Brain areas Dopamine Serotonin Monoamine metabolites

THE obese "cafeteria" rat displays a disturbed response to opiates, presumably due to modifications in its endogenous opioid systems. In effect, this model shows an intensified response to naloxone (1), and other studies suggest a dependence syndrome to opiates (24).

In a previous study (submitted data), we observed an altered response to classical opioid agonists in the "cafeteria" rat, both in terms of feeding effects and of variations in brain monoamines.

A new tool for investigating further the particularities of the "cafeteria" rat is the tetrapeptide Phe-Met-Arg-Phe-NH_2 (FMRF-amide). This neuropeptide of clam origin (22) has been shown to be involved in numerous functions in molluscs (8). A large family of related peptides (23) have been discovered in the central nervous system of various vertebrate species using radioimmunoassay and immunochemistry (4, 5, 16). The related peptides possess a common C-terminal Arg-Phe-NH_2 sequence, essential to their activity,and are extended at their N-terminal by sequences immunochemically distinct from other known peptides (19).

Behavioural experiments showed that FMRF-amide appeared to interact with the opioid system. Sequenced FMRF-amide-like peptides have been found to attenuate morphine-induced analgesia (28,30) and have been described as putative endogenous opioid antagonists (14). Although devoid of effects on feeding behaviour by itself, FMRF-amide inhibits feeding stimulated by morphine and deprivation (13), κ agonists (12) and stress (14).

In addition, FMRF-amide is active on neural tissues (7). FMRF-amide immunoreactivity has been found in hypothalamic nuclei involved in feeding regulation (2,29) and containing the monoamines also involved in these mechanisms. The likely interaction between these systems was investigated here by the assay of monoamines and their metabolites after FMRF-amide administration to normophagic and obese "cafeteria" rats.

METHOD

Animals

Female 8-week-old Sprague-Dawley rats were divided into two groups. The first (normophagic) group received only ordinary lab chow ad lib, while the second ("cafeteria") group received, in addition, four palatable foods per day, rotated every three days, e.g., cheese, ham, salami, bread, rusks, crackers, etc. After eight weeks, the "cafeteria" rats had developed significant obesity (17,25), with a weight gain 80–100% greater than that of the normophagic rats.

Surgical Procedure

A polyethylene cannula was implanted into the lateral ventricle under light ether anesthesia in order to perform intracerebroventricular (ICV) administration of FMRF-amide (SERVA) (0.5 μg/5 μl) or saline. After surgery, the animals were housed in individual cages and maintained for one week for recovery in a temperature-controlled room with a 12-12 hr light-dark cycle (light on at 0600 hr).

Food Intake

At 1700 hr (one hour before the onset of the dark cycle) the animals received either the FMRF-amide ICV or an equal volume

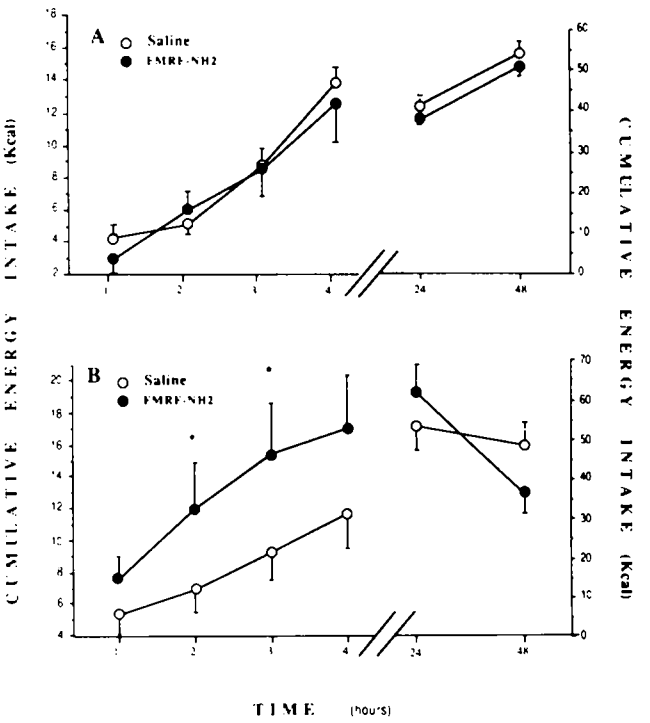

FIG. 1. Effects of FMRF-NH_2 on cumulative energy intake in normophagic rats (A) and "cafeteria" rats (B). Mean (kcal) ± SEM; n (number of measures) = 12. *$p<0.05$ vs. saline.

of saline (5 μl) as follows. Four rats were randomly selected from each dietary group of 8 to receive the FMRF-amide ICV followed 48 hr later by saline ICV. The remaining 4 rats in each group received the same treatments in reverse order. The above schedule was then repeated so that each rat received 4 administrations and 16 values were obtained for each treatment.

After each injection, the animals were immediately provided with a known quantity of their respective diet. The amount consumed was then measured after 1, 2, 3, 4, 24 and 48 hours. Each item in the "cafeteria" diet was weighed and total energy intake was calculated for each macronutrient (carbohydrates, lipids, proteins) using the manufacturers' values. Food consumption at 48 hr was used as a recovery index before proceeding with the subsequent injections. Data were analysed using Wilcoxon test for paired values.

Monoamine and Metabolite Determination

In another experiment, naive animals from both dietary groups were randomized to receive FMRF-amide (0.5 μg/5 μl) or saline ICV. The animals were sacrificed by decapitation 1 hour after administration. The brains were removed and dissected on a chilled plate in order to separate the hypothalamus, hippocampus, striatum and cortex which were stored at −80°C until use. The brain samples were homogenized in 0.4 N perchloric acid containing 0.1% EDTA, $Na_2S_2O_5$ and cystein and then centrifuged. The supernatant was analyzed by liquid chromatography with electrochemical detection as previously described (20) for monoamine and metabolite assays. Dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxy-tyramine (3-MT), 5-hydroxytryptamine (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) were separated and quantified.

Results are the mean of 6–7 determinations. Data were analysed using Student's *t*-test for each dietary group.

RESULTS

Food Intake

In the normophagic rats, FMRF-amide had no effect on food intake. In the "cafeteria" rats, FMRF-amide increased total energy intake during the first four hours of the study (Fig. 1).

Monoamine and Metabolite Assays

Serotonergic system (Table 1). FMRF-amide reduced 5-HIAA levels ($p<0.05$) in the hippocampus of normophagic rats, inducing

TABLE 1

ACTION OF FMRF-AMIDE (0.5 μg ICV) ON THE SEROTONERGIC SYSTEM IN NORMOPHAGIC AND "CAFETERIA" RATS

	N		CAF		N		CAF	
	C	FMRF	C	FMRF	C	FMRF	C	FMRF
	HT				HC			
5HIAA	668.8 ±40.6	603.9 ±55.6	643.2 ±48.0	755.3 ±34.9	360.2 ± 8.4	331.8 ± 9.4*	376.2 ±24.1	451.2 ±18.5*
5-HT	584.1 ±19.2	562.7 ±36.4	434.7 ±27.3	496.0 ±35.8	257.1 ± 4.9	267.9 ±16.0	232.9 ± 4.4	247.4 ±15.1
5HIAA / 5-HT	1.15 ± 0.10	1.04 ± 0.12	1.47 ± 0.15	1.56 ± 0.11	1.40 ± 0.04	1.19 ± 0.06**	1.61 ±0.08	1.88 ± 0.19
	ST				CX			
5HIAA	576.5 ±22.1	542.6 ±29.1	457.2 ±38.3	480.4 ±18.8	253.2 ± 8.9	239.5 ±15.9	218.6 ±11.8	251.5 ±16.7
5-HT	404.9 ±18.5	388.9 ±15.8	367.3 ± 6.9	404.6 ± 7.3†	305.5 ±16.5	318.8 ±23.0	377.9 ±19.5	349.2 ±29.3
5HIAA / 5-HT	1.44 ± 0.07	1.40 ± 0.07	1.24 ± 0.09	1.19 ± 0.03	0.84 ± 0.05	0.77 ± 0.12	0.59 ± 0.05	0.72 ± 0.05

Values are expressed as mean (ng/g) ± SEM. n = 6–7. *$p<0.05$, †$p<0.01$ (Student's *t*-test).
N: normophagic rats; CAF: "cafeteria" rats. C: control saline; FMRF: FMRF-amide (0.5 μg ICV).
HT: hypothalamus; HC: hippocampus; ST: striatum; CX: cortex.

TABLE 2

ACTION OF FMRF-AMIDE (0.5 μg ICV) ON THE DOPAMINERGIC SYSTEM IN NORMOPHAGIC AND "CAFETERIA" RATS

	Cortex			
	N		CAF	
	C	FMRF	C	FMRF
DOPAC	89.0 ± 10.0	94.7 ± 5.1	100.0 ± 4.4	84.9 ± 6.7
DA	184.3 ± 16.4	240.1 ± 18.9*	260.5 ± 19.8	225.4 ± 19.5
DOPAC / DA	0.483 ± 0.031	0.403 ± 0.026	0.392 ± 0.026	0.380 ± 0.014

Values are expressed as mean (ng/g) ± SEM. n = 6–7.
*$p<0.05$ (Student's t-test).
N: normophagic rats; CAF: "cafeteria" rats.
C: control saline; FMRF: FMRF-amide (0.5 μg ICV).

a reduction in the 5-HIAA/5-HT ratio ($p<0.01$), whereas in the "cafeteria" rats, an increase in 5-HIAA levels ($p<0.05$) in the hippocampus and an increase in striatal 5-HT levels ($p<0.01$) were noted.

In the other brain areas, although the changes were not statistically significant, the same tendency was observed, i.e., increases in 5-HT and 5-HIAA levels in "cafeteria" rats and corresponding decreases in normophagic rats.

Dopaminergic system (Table 2). The only effect of FMRF-amide on the dopaminergic system occurred in the cortex of normophagic rats, with an increase in DA levels ($p<0.05$) and a concomitant decrease in the DOPAC/DA ratio.

DISCUSSION

The dose of FMRF-amide used in this study (0.5 μg ICV) appeared to be sufficiently potent to produce consistent effects. In effect, FMRF-amide had been described to act in a dose-dependent way on opiate-induced feeding (15). Furthermore, in a preliminary study, we found that 1 μg ICV produced undesirable side-effects in some animals although other experiments have been reported with doses of 10 and 50 μg ICV in mice (11).

FMRF-amide had no effect on normophagic rats' feeding behaviour. This is consistent with a previous study on mice (12). However, an increase in food intake was observed in "cafeteria" rats for four hours following FMRF-amide administration. This result is surprising since FMRF-amide was expected to reduce food intake in the light of data showing antagonism to opiate-induced increased food intake (12, 13, 15).

It is well known that opiate antagonists such as naloxone decrease feeding and generally more intensively in situations where food intake is stimulated (1,3). It is interesting to note that FMRF-amide, although inducing the inverse effect, acted only in this kind of situation.

For the study of monoaminergic variations, the animals were sacrificed one hour after FMRF-amide administration, the time at which increased feeding behaviour commenced in the "cafeteria" rats. The enhancement of serotonergic metabolism coupled with an increased food intake in the "cafeteria" rats recalls the effects of opiate agonists in normophagic rats (submitted data). Furthermore, the decrease in 5-HIAA levels and 5-HIAA/5-HT ratio observed here in the normophagic rats, reflecting a decrease in 5-HT turnover (6), is similar to the response to naltrexone observed in previous work (submitted data). Overall, these results suggest an involvement of the serotonergic system in the action of FMRF-amide.

FMRF-amide-related peptides generally show inhibitory effects on opiate-induced behaviour (15) and have been considered as physiological opiate antagonists (15). However, some behavioural studies with FMRF-amide alone have shown opiate agonist-like effects. A decrease in colonic propulsion has been described in mice (11), this effect being similar and additive to that of morphine and able to be blocked by naloxone. An increase in latency in the hot plate test has been observed with FMRF-amide at 0.15 and 15 but not 1.5 μg (10). In our study, both "agonist and antagonist" responses were observed according to the dietary model. FMRF-amide seems to act differently according to the tone of the opioid system which is known to be disturbed in genetically (18,26) or "cafeteria" [unpublished data, (9)] obese rats. FMRF-amide appears to be a modulator rather than a true inhibitor of opioid systems since, although it can inhibit the binding of μ and κ agonists, its own affinity for opiate receptors is very low (31).

These results, while again evoking the particular role of the opioid system, also suggest the possible involvement of FMRF-amide-like peptides in obesity. Despite its close links with the opioid system, the recent discovery of gene encoding-related peptides (27) suggest that the extended family of FMRF-like peptides based on a common C terminal Arg-Phe-NH_2 sequence is an independent system. Furthermore, the unexpected response of the "cafeteria" model might be related either to the obesity or the highly palatable diet, or both. Further investigations are necessary to elucidate the physiological roles of FMRF-amide-related peptides, their relationships with other neurotransmitter or neuromodulator systems and its effects on other obesity models. FMRF-amide might prove a useful tool in the study and comparison of various models of obesity.

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