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Effects of Dexfenfluramine and Opioid Peptides, Alone or in Combination, on Food Intake and Brain Serotonin Turnover in Rats

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ROBERT, J. J., M. OROSCO, C. ROUCH, Y. COHEN AND C. JACQUOT. *Effects of dexfenfluramine and opioid peptides, alone or in combination, on food intake and brain serotonin turnover in rats.* PHARMACOL BIOCHEM BEHAV **38**(4) 775–780, 1991.—Dexfenfluramine (d-FF) and opiate agonists both act on food intake but in opposite ways. Serotonin is known to be involved in the pharmacological action of both d-FF and opiates, but not necessarily in the feeding effect of the latter. In order to test this hypothesis, the effects of three opioid agonists, β-endorphin, dynorphin and D-Ser^2-Leu-Enk-Thr^6 (DSLET) and of an antagonist, naltrexone, were investigated individually and in combination with d-FF on food intake and brain serotonin turnover. The opioid agonist-d-FF combinations generally produced a similar anorectic effect to that of d-FF alone, with the exception of DSLET which showed a reciprocal antagonism. The serotonergic effects varied according to the opioid tested, alone or in combination with d-FF. This does not allow to highlight a general pattern of serotonin involvement in the feeding effects of these peptides. However, all the treatments which decreased feeding (d-FF, naltrexone and the combinations dynorphin-d-FF and β-endorphin-d-FF) displayed similar trends in hypothalamic serotonergic variations. This study evidences a role of serotonin in the feeding effect of opiates, although not similar for all of them. The use of d-FF provides a tool for assessing this involvement.

Opioid peptides Naltrexone Dexfenfluramine Food intake Brain serotonin

DEXFENFLURAMINE (d-FF) is a well-known anorectic agent (5, 27, 39). Opioid agonists, in contrast, are generally reported to enhance feeding (3, 33, 36, 41). A direct relationship appears to exist between d-FF and opiates. In effect, essentially after chronic treatment, d-FF induces changes in brain and/or peripheral β-endorphin, dynorphin (19, 20, 29–31) and Leu-enkephalin levels (26). These peptides are suspected of being involved in behavioural tolerance to d-FF (18).

Dexfenfluramine induces a release of serotonin (5-hydroxytryptamine, 5-HT) and inhibits its reuptake (16,21); these effects are thought to be important in its anorectic activity (6, 9, 17, 32). The serotonergic system seems to be necessary for opioids to exert other pharmacological effects, such as analgesia, catatonia and hyperthermia (8,40). Furthermore, the administration of natural or synthetic opioids has been found to stimulate 5-HT synthesis and metabolism (1, 2, 8). Thus 5-HT is clearly involved in the action of both d-FF and opioid receptor agonists, although not surely in the feeding action of opioids. In a previous work, we did find opposing serotonergic effects of the antagonist, naltrexone, and of some agonists (37), but the lack of a common pattern in the effects induced by the agonists did not allow to draw general conclusions.

The use of d-FF, by the means of the known relationship between its anorectic and serotonergic effects, appeared of interest to study the interactions between 5-HT and opioids. The effects of combinations of d-FF and opioid peptides were thus investigated and compared to those of either d-FF alone or the opioid agonists/antagonist alone. The two variables measured were modifications in food intake and changes in brain serotonin levels.

METHOD

Surgical Procedure

A polyethylene cannula was implanted into the lateral ventricle of female Sprague-Dawley rats (240–260 g) under light ether anesthesia in order to perform intracerebroventricular (ICV) administration of opioid compounds or saline. After surgery, the animals were housed in individual cages and allowed to recover

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for one week in a temperature-controlled room with a 12–12-h light-dark cycle (light on at 06.00 h).

Drugs

The opioid agonists (Sigma, USA) were injected by the intracerebroventricular (ICV) route. The doses were 2 μg for β-endorphin, 5 μg for the Leu-enkephalin analogue, D-Ser^2-Leu-Enk-Thr^6 (DSLET) and 10 μg for dynorphin 1–13. The drugs were solubilized in saline and infused under a volume of 5 μl over 60 s. The doses were chosen on the basis of a previous study (37). Naltrexone (Dupont de Nemours, France) was injected by the intraperitoneal route (IP) at the dose of 10 mg/kg. Dexfenfluramine (Servier, France) was injected by the intraperitoneal route (IP) at the dose of 0.5 mg/kg.

Food Intake

Eight rats were used for each opiate agonist experiment and were divided into two groups to form a balanced cross-over Latin square design (four treatments: opiate, saline, d-FF/opiate and d-FF with a two-day wash-out between each treatment).

At 17:00 h (one hour before the onset of the dark cycle) the animals received either the opiate agonist ICV or an equal volume of saline. Dexfenfluramine or saline was administered one hour before β-endorphin and DSLET, and half an hour before dynorphin (1–13), on the basis of preliminary studies.

After each injection, the animals were immediately provided with a known weight of lab chow (UAR) The amount consumed was then measured after 1, 2, 3, 4, 24 and 48 hours. Food consumption at 48 h was used as a recovery index before proceeding with the subsequent injections. Data were analyzed using analysis of variance followed by the PLSD Fisher intertreatment test when results were significant.

Serotonin and Metabolite Assay

Fresh animals were randomized to receive one of the above treatments including naltrexone, alone or in combination with d-FF (half an hour before naltrexone). The animals were sacrified by decapitation 30 min after β-endorphin and DSLET, and 1 hour after dynorphin and naltrexone administration.

The brains were quickly removed and dissected on a chilled plate in order to separate the hypothalamus and striatum which were stored at −80°C until analysis. The brain areas were then homogenized in 0.4 N perchloric acid containing 0.1% EDTA, $Na_2S_2O_5$ and cysteine and centrifuged. The supernatant was analyzed using liquid chromatography with electrochemical detection as previously described (35) for 5-hydroxytryptamine (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) assays. Results are the mean of 6–8 determinations and data were analyzed using analysis of variance followed by the PLSD Fisher intertreatment test when results were significant.

RESULTS

Food Intake

As Fig. 1 shows, d-FF alone (0.5 mg/kg) decreased food intake from the 1st to the 4th hour. The opioid agonists generally increased feeding. Dynorphin and β-endorphin both increased feeding at the 4th hour, while d-FF completely reversed this effect, reducing food consumption to the same values as after d-FF alone. Feeding was significantly increased from the 3rd hour after DSLET alone and remained higher, although not significantly

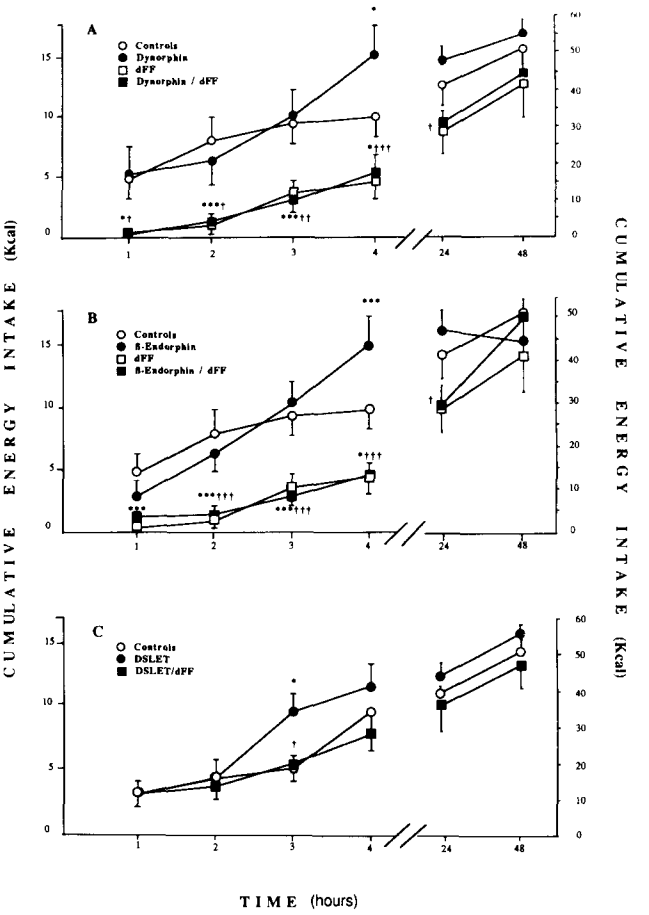

FIG. 1. Effects of dynorphin (A), β-endorphin (B), DSLET (C) and their respective association with d-fenfluramine (d-FF) on cumulative energy intake. Results are expressed as means (kcal) ± SEM. n = 8–9. *$p<0.05$ vs. Controls; ***$p<0.001$ vs. Controls; †$p<0.05$ vs. opiate; ††$p<0.01$ vs. opiate; †††$p<0.001$ vs. opiate. d-FF was always significantly different from Controls.

at the 4th hour. d-FF cancelled this effect, bringing food intake back to the control level.

Brain Serotonin

Dexfenfluramine alone, at the dose used here (0.5 mg/kg), produced few serotonergic effects which were decreases in 5-HIAA levels, and a decrease in the 5-HIAA/5-HT ratio. Although not always statistically significant, the same tendencies were found in the hypothalamus and the striatum for each experimental series.

DSLET alone produced no effect in the hypothalamus. The decrease in the 5-HIAA/5-HT ratio observed with d-FF alone was abolished by its association with DSLET. DSLET significantly increased 5-HIAA levels and the 5-HIAA/5-HT ratio in the striatum, while its association with d-FF produced no effect in this region (Table 1).

Dynorphin alone decreased hypothalamic 5-HT levels and showed a tendency to increase the 5-HIAA/5-HT ratio in this area. These effects were contrary of those of d-FF alone in the hypothalamus and striatum. The combination of both drugs gave results contrary to those obtained with dynorphin alone but similar to those with d-FF alone in the hypothalamus, while in the striatum, the values obtained were intermediate between all

TABLE 1

EFFECTS OF DSLET AND d-FENFLURAMINE (d-FF), ALONE AND IN COMBINATION, ON BRAIN SEROTONIN TURNOVER

		5-HIAA		5-HT		5-HIAA/5-HT	
HT	Saline	642.9 ± 31.1		530.5 ± 22.3		1.268 ± 0.077	F=4.48
	DSLET	673.9 ± 16.3		572.2 ± 20.7		1.177 ± 0.055	
	d-FF	568.4 ± 24.7		578.2 ± 18.1		0.913 ± 0.031†‡	
	DSLET/d-FF	625.3 ± 41.3		542 ± 23.4		1.159 ± 0.086#	
ST	Saline	557.3 ± 24.1	F=4.48	471.2 ± 4.8	F=3.04	1.182 ± 0.056	F=5.54
	DSLET	678.8 ± 29.7†		478 ± 16.2		1.435 ± 0.092†	
	d-FF	554.5 ± 26.2§		511.8 ± 14.4*		1.089 ± 0.057¶	
	DSLET/d-FF	596.7 ± 28.9‡		517.5 ± 16.8*		1.155 ± 0.05§	

Results are expressed as means (ng/g wet weight tissue) ± SEM. n=6–8.
F value is indicated when ANOVA is significant, *$p<0.05$ vs. saline, †$p<0.01$ vs. saline, ‡$p<0.05$ vs. DSLET, §$p<0.01$ vs. DSLET, ¶$p<0.001$ vs. DSLET, #$p<0.05$ vs. d-FF.
HT: hypothalamus; ST: striatum.

others (Table 2).

β-Endorphin alone induced no significant effect but in combination with d-FF, potentiated the tendency of d-FF alone to decrease hypothalamic 5-HIAA levels and the striatal 5-HIAA/5-HT ratio, and rendered them significant. The decrease in hypothalamic 5-HIAA/5-HT ratio after d-FF alone, although not significant, was also stronger after treatment with both drugs (Table 3).

The effects of naltrexone were similar to those of d-FF alone in the hypothalamus and striatum and were also observed with the combined drug treatment (Table 4).

DISCUSSION

As expected, the three opioid peptide agonists increased feeding. However, these effects occurred later here than in reports from other authors, but this latency has already been observed in one of our previous works under similar experimental conditions (37). The effect on food intake of the antagonist, naltrexone, was not assayed here since its effects are now well-recognized (3, 23–25). Despite the low dose used, d-FF significantly decreased food intake during the four hours of the study. Such a low dose has seldom been assayed; most studies involve 1.5 or 2.5 mg/kg (7,42), although lower doses have been shown to decrease feeding in particular situations such as stress-induced food intake (15,39), glucoprivic feeding (10) and feeding induced by norepinephrine injected into the paraventricular hypothalamus (28).

The combined treatments with d-FF and opioid agonists generally produced a feeding effect similar to that of d-FF alone, with the exception of DSLET, a delta agonist, which showed a reciprocal antagonism with d-FF.

d-FF is generally reported to decrease 5-HT levels one hour after injection and to decrease 5-HIAA levels, although to a lesser extent, 3 to 5 hours after injection. With much higher doses and longer times of action than those used in our study, the 5-HIAA/5-HT ratio is found to be elevated (11, 12, 15, 16, 34). In the present study, d-FF induced few serotonergic effects as compared to those generally described. However, this is not surprising with regard to the low dose used here, as it has been reported that doses under 2.5 mg/kg do not produce serotonergic effects although the anorectic properties are retained (13,21). It is interesting to note that the serotonergic effects found in our study occurred in the hypothalamus and striatum. Although it was not significant, the tendency was towards a decrease in the 5-HIAA/5-HT ratio due to lower 5-HIAA levels, indicating decreased 5-HT activity, despite a continuation in the anorectic effect. Similar findings have previously been reported, but in different experimental conditions (38), and thus can hardly be compared. The question of whether low doses of dexfenfluramine produce anorexia by decreasing brain serotonergic activity thus remains to be-

TABLE 2

EFFECTS OF DYNORPHIN (DYN) AND d-FENFLURAMINE (d-FF), ALONE AND IN COMBINATION, ON BRAIN SEROTONIN TURNOVER

		5-HIAA	5-HT		5-HIAA/5-HT	
HT	Saline	682.7 ± 22.4	591.4 ± 18.6	F=4.95	1.158 ± 0.053	F=3.57
	DYN	686.5 ± 13.3	482.6 ± 26.1*		1.364 ± 0.103	
	d-FF	607.8 ± 34.8	649.6 ± 43.8‡		0.965 ± 0.071‡	
	DYN/d-FF	660.3 ± 53.1	570.1 ± 22.9		1.095 ± 0.12†	
ST	Saline	611.4 ± 58.2	457.1 ± 35.8		1.336 ± 0.055	F=3.33
	DYN	600.6 ± 45.9	463.8 ± 34.6		1.301 ± 0.067	
	d-FF	536.6 ± 53.6	462.8 ± 25.9		1.114 ± 0.053*†	
	DYN/d-FF	578.3 ± 38.5	471.7 ± 19.1		1.23 ± 0.035	

Results are expressed as means (ng/g wet weight tissue) ± SEM. n=6–8.
F value is indicated when ANOVA is significant, *$p<0.05$ vs. saline, †$p<0.05$ vs. DYN, ‡$p<0.01$ vs. DYN.
HT: hypothalamus; ST: striatum.

TABLE 3

EFFECTS OF β-ENDORPHIN (β-END) AND d-FENFLURAMINE (d-FF), ALONE AND IN COMBINATION, ON BRAIN SEROTONIN TURNOVER

		5-HIAA		5-HT	5-HIAA/5-HT	
HT	Saline	475.7 ± 26.3	F=4.46	487.4 ± 17.7	0.987 ± 0.085	
	β-END	455 ± 26.3		438.4 ± 43.5	1.068 ± 0.086	
	d-FF	438.6 ± 13.0		499.5 ± 20.3	0.883 ± 0.038	
	β-END/d-FF	359.4 ± 27.1*†§		474.8 ± 39.2	0.864 ± 0.1	
ST	Saline	461.1 ± 8.2	F=4.58	619.3 ± 16.4	0.825 ± 0.009	F=4.35
	β-END	522.8 ± 13.8		614.8 ± 12.6	0.853 ± 0.034	
	d-FF	468.4 ± 18.9		588.3 ± 24.0	0.789 ± 0.025	
	β-END/d-FF	501.5 ± 10.0		641.3 ± 25.5	0.739 ± 0.019*‡	

Results are expressed as means (ng/g wet weight tissue) ± SEM. n=6–8.
F value is indicated when ANOVA is significant, *$p<0.05$ vs. saline, †$p<0.05$ vs. β-END, ‡$p<0.01$ vs. β-END, §$p<0.05$ vs. d-FF.
HT: hypothalamus; ST: striatum.

determined.

The stimulation of the serotonergic system by opiate agonists alone are in agreement with various neurochemical studies described in the literature (1, 2, 8), but differ according to the agonist studied, as previously observed (37).

When combined with d-FF, the different opioids showed various patterns of serotonergic effects. The antagonism between d-FF and DSLET, a delta but also partly mu-1 agonist (14,22), might involve a common site of action on the 5-HT system. The antagonism was especially evident in the striatum, while in the hypothalamus the effect of d-FF on the 5-HIAA/5-HT ratio was cancelled by DSLET. This antagonism was also seen in terms of the feeding effect.

Dynorphin 1–13, an endogenous kappa agonist, maintained the serotonergic effect of d-FF in the hypothalamus but cancelled it in the striatum. Since the feeding effect of d-FF was not antagonized by dynorphin 1–13, the serotonergic effect in the striatum would not appear to be specifically related to food intake.

The serotonergic system did not appear to be involved in the feeding effect of β-endorphin, but this agonist seemed to have a facilitative effect on d-FF since the behavioural effect of the latter was maintained and its serotonergic effects were enhanced. The β-endorphinergic system appeared to be the most sensitive to serotonin modifications. This is in agreement with the findings that d-FF increases hypothalamic β-endorphin levels, and not dynorphin levels, not only after acute high dose administration (29), but also after chronic treatment (20,31).

Several reports have indicated that experimental conditions associated with a modification of 5-HT levels also result in an alteration of brain opiate levels, especially in the hypothalamus and striatum (19, 20, 26, 29–31).

The hypothalamus is strongly involved in hunger and satiety and is thought to play a important role in d-FF-induced anorexia (4). In our study, a relationship was observed between the trends in hypothalamic serotonergic variations and changes in food intake. Indeed, the three treatments which decreased food intake (naltrexone, although not assayed in this study, β-endorphin/d-FF and dynorphin/d-FF) had similar serotonergic effect as d-FF alone. On the contrary, the DSLET/d-FF association, which had no effect on food intake, reversed the serotonergic effect of d-FF alone.

Although each peptide presents particular serotonergic effects, some general conclusions may be drawn. As usually observed, variations in serotonergic activity reflect changes in feeding behavior. The involvement of serotonin in the feeding effect of opiates is highly possible, although to different degrees. The use of a low dose of d-FF in this study allowed to highlight not only the particularities of the peptides, but also their interactions with the serotonergic system in relation to feeding.

TABLE 4

EFFECTS OF NALTREXONE (NTX) AND d-FENFLURAMINE (d-FF), ALONE AND IN COMBINATION, ON BRAIN SEROTONIN TURNOVER

		5-HIAA	5-HT	5-HIAA/5-HT	
HT	Saline	652.6 ± 24.4	698.6 ± 37.1	0.958 ± 0.081	
	NTX	580.6 ± 36.5	710.8 ± 10.9	0.834 ± 0.067	
	d-FF	576.6 ± 30.9	698.1 ± 30.2	0.838 ± 0.067	
	NTX/d-FF	591.1 ± 32.3	722.8 ± 38.3	0.833 ± 0.065	
ST	Saline	567.6 ± 26.4	454.2 ± 13.6	1.312 ± 0.066	F=4.01
	NTX	509 ± 35.1	482.5 ± 18.0	1.054 ± 0.064†	
	d-FF	515.5 ± 22.1	489.6 ± 12.0	1.054 ± 0.043†	
	NTX/d-FF	550.3 ± 37.3	496.9 ± 19.8	1.108 ± 0.063*	

Results are expressed as means (ng/g wet weight tissue) ± SEM. n=6–8.
F value is indicated when ANOVA is significant, *$p<0.05$ vs. saline, †$p<0.01$ vs. saline.
HT: hypothalamus; ST: striatum.

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