Pharmacological Characterization of the Receptor Mediating the Anorexigenic Action of the Octadecaneuropeptide: Evidence for an Endozepinergic Tone Regulating Food Intake

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Peptides of the endozepine family, including diazepam-binding inhibitor, the triakontatetrapeptide, and the octadecaneuropeptide (ODN), act through three types of receptors, that is, central-type benzodiazepine receptors (CBR), peripheral-type (mitochondrial) benzodiazepine receptors (PBR) and a metabotropic receptor positively coupled to phospholipase C via a pertussis toxin-sensitive G protein. We have previously reported that ODN exerts a potent anorexigenic effect in rat and we have found that the action of ODN is not affected by the mixed CBR/PBR agonist diazepam. In the present report, we have tested the possible involvement of the metabotropic receptor in the anorexigenic activity of ODN. Intracerebroventricular administration of the C-terminal octapeptide (OP) and its head-to-tail cyclic analog cyclo₁₋₈OP (cOP) at a dose of 100 ng mimicked the inhibitory effect of ODN on food intake in food-deprived mice. The specific CBR antagonist flumazenil and the PBR antagonist PK11195 did not prevent the effect of ODN, OP, and cOP on food consumption. In contrast, the selective metabotropic endozepine receptor antagonist cyclo₁₋₈[DLeu⁵]OP (100–1000 ng; cDLOP) suppressed the anorexigenic effect of ODN, OP, and cOP. At the highest concentration tested (1000 ng), cDLOP provoked by itself a significant increase in food intake. Taken together, the present results indicate that the anorexigenic effect of ODN and OP is mediated through activation of the metabotropic receptor recently characterized in astrocytes. The data also suggest that endogenous ODN, acting via this receptor, exerts an inhibitory tone on feeding behavior.

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INTRODUCTION

The term endozepines designates a family of regulatory neuropeptides that have been originally isolated from rat brain extracts on the basis of their ability to displace benzodiazepines from their binding sites (Guidotti *et al*, 1983; Tonon *et al*, 2006). All endozepines characterized so far derive from diazepam-binding inhibitor (DBI), an 86 amino-acid polypeptide precursor which has the potential to generate several biologically active fragments including the triakontatetrapeptide DBI_{17–50} (TTN) (Slobodynsky *et al*, 1989) and the octadecaneuropeptide DBI_{33–50}

(ODN) (Ferrero *et al*, 1986). The DBI gene is widely expressed in the central nervous system (Alho *et al*, 1988; Tong *et al*, 1991; Yanase *et al*, 2002). In particular, high concentrations of endozepines have been found in brain areas which play a major role in the control of feeding behavior such as the arcuate nucleus, the dorso-medial and ventro-medial hypothalamic nuclei, and the lateral hypothalamic area (Alho *et al*, 1985; Malagon *et al*, 1993; Tonon *et al*, 1990).

Intracerebroventricular (i.c.v.) injection of low doses of ODN causes a marked reduction of food consumption in both food deprived and normally fed rodents (Garcia de Mateos-Verchere *et al*, 2001). The anorexigenic effect of ODN is long lasting, does not give rise to rapid tolerance, and is associated with a substantial loss of weight (Garcia de Mateos-Verchere *et al*, 2001). In rats deprived of food for 12 h, i.c.v. administration of ODN reduces the expression of neuropeptide Y (NPY), an orexigenic neuropeptide, and activates the expression of proopiomelanocortin, the precursor of the anorexigenic peptide α -melanocyte-stimulating hormone (Compère *et al*, 2003, 2005), suggesting that

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NPY- and POMC-producing neurons of the arcuate nucleus are involved in the anorexigenic effect of ODN.

The biological effects of endozepines are mediated through three types of receptors. DBI and ODN act as inverse agonists of central-type benzodiazepine receptors (CBR) associated with the GABA_A receptor complex (Guidotti *et al*, 1983; Tonon *et al*, 1989; Bormann, 1991; Louiset *et al*, 1993). TTN acts preferentially through peripheral-type benzodiazepine receptors (PBR) located either at the outer mitochondrial membrane or at the plasma membrane level (Guidotti *et al*, 1989; Berkovich *et al*, 1990; Gandolfo *et al*, 2001). Finally, ODN can also activate a metabotropic receptor positively coupled to phospholipase C through a pertussis toxin-sensitive G-protein (Patte *et al*, 1995; Gandolfo *et al*, 1997). We have previously shown that the anorexigenic effect of ODN was not affected by the mixed CBR/PBR agonist diazepam (Garcia de Mateos-Verchere *et al*, 2001). The aim of the present study was to investigate the possible involvement of the metabotropic receptor in the action of endozepines on feeding behavior.

MATERIALS AND METHODS

Animals

Male Swiss albinos CD1 mice (Iffa-Credo/Charles River, Saint-Germain sur l'Arbresle, France), weighing 22–25 g, were housed 20 in Makrolon cages (L: 40 cm, W: 25 cm, and H: 18 cm), with free access to standard semisynthetic laboratory diet (U.A.R., Villemoisson-sur-Orge, France) and tap water. The animals were kept in a ventilated room at a temperature of $22 \pm 1^\circ\text{C}$ under a 12-h light/12-h dark cycle (light on between 0700 and 1900).

All the experiments were carried out between 0900 and 1800 in testing rooms adjacent to the animal rooms. Animal manipulations were performed according to the European Community Council Directive of 24 November 1986 (86/609/EEC), were approved by the local Ethical Committee (authorization numbers: N/10-04-04-12 and N/13-04-04-15) and were conducted by authorized investigators. Each animal was used once and then immediately killed.

Drugs and Solutions

The metabotropic endozepine receptor agonists ODN (Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys), octapeptide (OP) (Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys) and cyclo₁₋₈OP (Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys; cOP), and the metabotropic endozepine receptor antagonist cyclo₁₋₈[DLeu⁵]OP (Arg-Pro-Gly-Leu-DLeu-Asp-Leu-Lys; cDLOP) were synthesized by solid phase methodology as described previously (Leprince *et al*, 1998, 2001). The peptides were dissolved in saline (0.9% NaCl). Flumazenil (Ro 15-1788) and PK11195 (Sigma Aldrich, Saint-Quentin Fallavier, France) were dissolved in 2.5% dimethylsulfoxide in saline. For PK11195, 0.5% Tween-80 was added to facilitate solubilization. All solutions were prepared just before injections.

I.c.v. Injection

Free-hand i.c.v. injections (10 μl /mouse) of saline, peptides and/or drugs were made in the left ventricle according to

the procedure of Haley and McCormick (1957), using a microsyringe (50 μl ; Hamilton, Bonaduz, Switzerland) connected to a needle (diameter 0.5 mm), of which the bevel protruded only 3.5 mm from a guard limiting its penetration into the brain. The injection in immobilized mice lasted approximately 5 s. I.c.v. injections were performed by an experienced investigator, who frequently controlled the regularity and success of the injections, using methylene blue dye and who observed (after killing and frontal brain sectioning) that the injection was successful in more than 95% of the trials. The i.c.v. injection method was approved by the Regional Ethical Committee for Animal Experimentation (Normandy; no. N/10-04-04-12).

Food Consumption Experiments in Food-Deprived Mice or Food-Restricted Mice

Two days before the experiments, mice were isolated in individual cages (L: 24 cm, W: 10, and H: 7 cm) with free access to water and the pellets of food laid down the floor of the cages, in order to make the animals accustomed to the test conditions. For experiments with food-deprived mice (fasted mice) (in order to evaluate an anorexigenic effect), 18 h before testing (1500–0900 h), animals were totally deprived of food and had access to tap water *ad libitum*. For experiments with food-restricted mice (in order to evaluate an orexigenic effect), 18 h before testing (1500–0900 h), animals had access to only 3 g of food (that represented approximately half of their daily consumption) and water *ad libitum*. In all groups, mice had access to a weighed food pellet (5 g) deposited on the floor of the cage 10 min after i.c.v. administration of saline, peptides, and/or drugs. Thereafter, the pellet was briefly (<20 s) removed with forceps and weighed every 30 min, for 3 h. The food consumption method was approved by the Regional Ethical Committee for Animal Experimentation (Normandy; no. N/13-04-04-15).

Measurement of Locomotor Activity

Ten min after i.c.v. administration of saline or peptides, the animals were placed individually in $20 \times 20 \times 30$ cm compartments, in a lighted and quiet room. Locomotor activity was assessed automatically in a Digiscan actimeter (Omni-tech Electronics Inc, Columbus, OH), which monitored the horizontal displacements and vertical movements, including rearing, leaning, and jumping. The responses were expressed as the total number of beams crossed by mice during four consecutive 15-min periods.

Statistical Analysis

Data are expressed as means $\pm$ SEM. Differences between groups were assessed by one-way analysis of variance (ANOVA) followed by a *post hoc* multiple comparison Student–Newman–Keuls test. Antagonistic effects were analyzed using two-way ANOVA, and a *post hoc* multiple comparison Student–Newman–Keuls test was used for multiple comparisons between groups. A probability level of 0.05 or lower was considered as statistically significant.

Figure 1 Time course of the effect of ODN, OP, or cOP on food intake in food-deprived mice. Mice deprived of food for 18 h were injected i.c.v. (10 μ l) with saline, ODN (100 ng/mouse), OP (100 ng/mouse), or cOP (100 ng/mouse). Ten min after i.c.v. injection, each animal had access to a weighed food pellet. Cumulative food intake was measured during 3 h at the time indicated. Mean $\pm$ SEM represents data from 14 mice per group. Student–Newman–Keuls *post hoc* test: * p < 0.05, ** p < 0.01, *** p < 0.001 vs saline-injected mice.

Figure 2 Effect of ODN, OP, or cOP on locomotor activity. Mice deprived of food for 18 h were injected i.c.v. (10 μ l) with saline, ODN (100 ng/mouse), OP (100 ng/mouse), or cOP (100 ng/mouse). Ten min after i.c.v. injection, animals were introduced into the actimeters. The horizontal (a) and vertical (b) components of motor activity were measured during four consecutive periods of 10 min. Mean $\pm$ SEM represents data from 12 mice per group.

RESULTS

Effects of ODN, OP, and cOP on Food Intake in Food-Deprived Mice

In food-deprived mice, i.c.v. injection of 100 ng ODN, OP, or cOP significantly reduced (p < 0.001) food intake during

Figure 3 Effect of flumazenil and PK11195 on ODN-induced inhibition of food intake in food-deprived mice. (a) Mice deprived of food for 18 h were injected i.p. with saline, flumazenil (2 mg/kg), or PK11195 (2 mg/kg), 30 min before i.c.v. injection (10 μ l) of saline, or ODN (100 ng/mouse). (b) Mice deprived of food for 18 h were injected i.c.v. (10 μ l) with saline, ODN (100 ng/mouse), flumazenil (2 μ g/mouse), PK11195 (2 μ g/mouse), or with ODN plus flumazenil or PK11195. Ten min after i.c.v. injection, each animal had access to a weighed food pellet. Cumulative food intake was determined during 3 h at the time indicated. Mean $\pm$ SEM represents data from 14 mice per group. Student–Newman–Keuls *post hoc* test: * p < 0.05, ** p < 0.01, *** p < 0.001 vs saline-injected mice.

the first 30-min period of testing. The mean $\pm$ SEM percentage of food intake inhibition induced by these peptides is: $55.3 \pm 6.8\%$ for ODN, $68.9 \pm 8.1\%$ for OP, and $51.5 \pm 7.4\%$ for cOP. Thereafter, the anorexic effect of all three peptides gradually vanished but the cumulative food consumption remained significantly reduced during the whole 3-h test period (Figure 1).

Effects of ODN, OP, and cOP on Locomotor Activity

The influence of i.c.v. administration of ODN, OP, or cOP on locomotor activity was measured over four consecutive periods of 15 min each (Figure 2). The peptides did not significantly modify horizontal (Figure 2a) and vertical (Figure 2b) locomotor activities at any time of the test period.

Figure 4 Effect of graded doses of cdLOP on ODN-induced inhibition of food intake in food-deprived mice. Mice deprived of food for 18 h, were injected i.c.v. (10 μ l) with saline, or ODN (100 ng/mouse) in the absence or presence of increasing doses of cyclo₁₋₈[DLeu⁵]OP (cdLOP; a, 100 ng/mouse; b, 500 ng/mouse; c, 1000 ng/mouse). Ten min after i.c.v. injection, each animal had access to a weighed food pellet. Cumulative food intake was measured during 3 h at the time indicated. Mean $\pm$ SEM represents data from 10 to 24 mice per group. Student–Newman–Keuls test: * p < 0.05, ** p < 0.01, *** p < 0.001 vs saline-injected mice. A two-way ANOVA revealed a significant interaction: between cdLOP (500 ng/mouse) and ODN at 30 min $F(1,36) = 14.6$, $^c p$ < 0.001, at 60 min $F(1,36) = 22.3$, $^c p$ < 0.001, at 120 min $F(1,36) = 11.9$, $^b p$ < 0.01, and at 180 min $F(1,36) = 7.2$, $^a p$ < 0.05; and between cdLOP (1000 ng/mouse) and ODN at 60 min after food presentation $F(1,52) = 4.8$, $^a p$ < 0.05.

Characterization of the Receptor Involved in the Anorexigenic Effects of ODN, OP, and cOP

To characterize the pharmacological profile of the receptor involved in the anorexigenic effect of ODN, food-deprived mice were treated with the CBR antagonist flumazenil, the

Figure 5 Effect of graded doses of cdLOP on OP-induced inhibition of food intake in food-deprived mice. Mice deprived of food for 18 h, were injected i.c.v. (10 μ l) with saline, or OP (100 ng/mouse) in the absence or presence of increasing doses of cyclo₁₋₈[DLeu⁵]OP (cdLOP; a, 100 ng/mouse; b, 500 ng/mouse; c, 1000 ng/mouse). Ten min after i.c.v. injection, each animal had access to a weighed food pellet. Cumulative food intake was measured during 3 h at the time indicated. Mean $\pm$ SEM represents data from 10 to 24 mice per group. Student–Newman–Keuls test: * p < 0.05, ** p < 0.01, *** p < 0.001 vs saline-injected mice. A two-way ANOVA revealed a significant interaction between cdLOP (500 ng/mouse) and OP at 30 min $F(1,44) = 23.3$, $^c p$ < 0.001, at 60 min $F(1,44) = 10.9$, $^b p$ < 0.01, at 120 min $F(1,44) = 4.4$, $^a p$ < 0.05, and at 180 min $F(1,44) = 4.2$, $^a p$ < 0.05.

PBR antagonist PK11195, or the metabotropic receptor antagonist cdLOP. Intraperitoneal (i.p.) and i.c.v. administration of flumazenil or PK11195 alone did not modify spontaneous food consumption (Figure 3a and b). Similarly, i.p. injection of flumazenil or PK11195, 30 min

Figure 6 Effect of graded doses of cdLOP on cOP-induced inhibition of food intake in food-deprived mice. Mice deprived of food for 18 h, were injected i.c.v. (10 μ l) with saline or cOP (100 ng/mouse) in the absence or presence of increasing doses of cyclo₁₋₈[DLeu⁵]OP (cdLOP; a, 100 ng/mouse; b, 500 ng/mouse; c, 1000 ng/mouse). Ten min after i.c.v. injection, each animal had access to a weighed food pellet. Cumulative food intake was determined during 3 h at the time indicated. Mean $\pm$ SEM represents data from 10 to 24 mice per group. Student–Newman–Keuls test * p < 0.05, ** p < 0.01, *** p < 0.001 vs saline-injected mice. A two-way ANOVA revealed a significant interaction: between cdLOP (500 ng/mouse) and cOP at 30 min $F(1,44) = 19.5$, * p < 0.001, at 60 min $F(1,44) = 10.2$, * p < 0.01, at 120 min $F(1,44) = 4.1$, * p < 0.05, and at 180 min $F(1,44) = 4.1$, * p < 0.05 after food presentation; and between cdLOP (1000 ng/mouse) and ODN at 30 and 60 min after food presentation $F(1,36) = 7.4$, * p < 0.05 and $F(1,36) = 5.5$, * p < 0.05, respectively.

before i.c.v. administration of ODN, had no effect on ODN-induced inhibition of food intake during the 3-h test period (Figure 3a). The inhibitory effect of

Figure 7 Effect of graded doses of cdLOP on food intake in food-restricted mice. Mice were food restricted (half of their daily food consumption) for 18 h and then injected i.c.v. (10 μ l) with saline or increasing doses of cyclo₁₋₈[DLeu⁵]OP (cdLOP; 100–2000 ng/mouse). Ten min after i.c.v. injection, each animal had access to a weighed food pellet. Cumulative food intake was determined during 3 h at the time indicated. Mean $\pm$ SEM represents data from 10 mice per group. Student–Newman–Keuls test: ** p < 0.01, *** p < 0.001 vs saline-injected mice.

ODN was not affected either by i.c.v. co-administration of flumazenil or PK11195 (Figure 3b). At the lowest dose tested (100 ng/mouse, i.c.v.), cdLOP did not impair the anorexic effect of ODN (Figure 4a), OP (Figure 5a) and cOP (Figure 6a) in food-deprived mice. At higher doses (500 and 1000 ng/mouse, i.c.v.) cdLOP totally suppressed the inhibitory effect of ODN (Figure 4b and c), OP (Figure 5b and c) and cOP (Figure 6b and c) on food intake. At the highest dose tested (1000 ng/mouse, i.c.v.), cdLOP significantly increased food consumption in food-deprived mice, throughout the 3-h observation period (Figures 4c, 5c and 6c).

Effect of cdLOP on Food Intake in Food-Restricted Mice

In mice that received approximately half of their daily consumption of food for 18 h (1500–0900), i.c.v. administration of graded doses of cdLOP (100–2000 ng/mouse) provoked a dose-related increase of cumulative food intake reaching statistical significance at a dose of 500 ng/mouse (Figure 7). For doses ranging from 500 to 2000 ng/mouse, cdLOP induced a significant orexigenic effect during the whole duration of the test with an increase in cumulative food consumption of $98.1 \pm 13.8\%$ for the 500 ng dose, $155.1 \pm 25.6\%$ for the 1000 ng, and $209.2 \pm 14.6\%$ for the 2000 ng dose, at the end of the 3-h test period (Figure 7).

Effects of Flumazenil, PK11195, and ODN on cdLOP-Induced Orexigenic Effect in Food-Restricted Mice

I.c.v. administration of flumazenil (2 μ g/mouse) or PK11195 (2 μ g/mouse) in food-restricted mice had no effect on food

Figure 8 Effect of flumazenil or PK11195 on cDLOP-induced increase of food intake in food-restricted mice. Mice were food restricted (half of their daily food consumption) for 18 h and then injected i.c.v. (10 µl) with saline, cyclo-[³H]OP (cDLOP; 500 ng/mouse), flumazenil (2 µg/mouse), PK11195 (2 µg/mouse) or with cDLOP plus flumazenil or PK11195. Ten min after i.c.v. injection, each animal had access to a weighed food pellet. Cumulative food intake was measured during 3 h at the time indicated. Mean ± SEM represents data from 12 mice per group. Student–Newman–Keuls test: *** $p < 0.001$ vs saline-injected mice.

consumption and did not significantly alter the orexigenic effect of cDLOP (500 ng/mouse), during the whole 3-h test period (Figure 8). In contrast, i.c.v. co-administration of ODN (10, 100, 200, or 300 ng/mouse) with cDLOP (500 ng/mouse) reversed the orexigenic effect of cDLOP and dose dependently restored its own anorexigenic effect (Figure 9). When injected alone, increasing doses of ODN (100, 200, and 300 ng/mouse) dose dependently and significantly decreased ($p < 0.05$ – 0.001) the cumulative food intake in food-restricted mice (Figure 9).

DISCUSSION

It has previously been reported that very low doses of ODN inhibit food intake in rat and mice (Garcia de Mateos-Verchere *et al.*, 2001) but the pharmacological profile of the receptor involved in the effect of ODN had not been characterized. In the present study, we show that the anorexigenic action of ODN is mimicked by the C-terminal fragment of ODN (OP) and its head-to-tail cyclic analog (cOP). Indeed, the C-terminal domain of ODN is capable of inducing several of the effects of ODN such as the anxiogenic activity (Garcia de Mateos-Verchere *et al.*, 1998a), the inhibition of apomorphine-induced yawning (Garcia de Mateos-Verchere *et al.*, 1998b) and the inhibition of pentylenetetrazol-induced convulsions (Garcia de Mateos-Verchere *et al.*, 1999). Interestingly, in the present study, we did not observe any effect of ODN, OP, or cOP on motor activity, indicating that the inhibitory effect of ODN and related peptides on food consumption could not be ascribed to a sedation or psychostimulant activity of these compounds.

It is now clearly established that ODN can interact with CBR associated with the GABA_A receptor (Ferrero *et al.*, 1986; Tonon *et al.*, 1989; Slobodyansky *et al.*, 1990). In addition, it has been shown that several of the effects induced by ODN are mediated via a G-protein-coupled receptor. In particular, *in vitro* studies have shown that ODN increases intracellular calcium concentration in cultured rat astrocytes through activation of a metabotropic receptor positively coupled to phospholipase C (Patte *et al.*, 1995; Gandolfo *et al.*, 1997). We have previously observed that the inhibitory effect of ODN on feeding behavior is not affected by diazepam, a mixed CBR and PBR agonist (Garcia de Mateos-Verchere *et al.*, 2001). In order to further characterize the type of receptor mediating the anorexigenic action of ODN and its analogs, in the present study we have investigated the effect of selective endozepine receptor antagonists on ODN, OP, and cOP-induced food consumption. The specific CBR antagonist flumazenil (Bonetti *et al.*, 1982) and the specific PBR antagonist PK11195 (Le Fur *et al.*, 1983) did not prevent the inhibitory effect of ODN, OP, and cOP on food intake. Conversely, cDLOP, which acts as a metabotropic endozepine receptor antagonist (Leprince *et al.*, 2001), suppressed the anorexigenic effect of ODN, OP, and cOP. These data indicate that the effects of ODN and its analogs on feeding behavior are mediated through activation of the recently characterized metabotropic receptor. Moreover, at the highest dose tested (1000 ng/mouse), cDLOP not only suppressed the inhibitory effect of ODN on food intake, but significantly increased by its own food consumption, indicating that endogenous ligands of the metabotropic endozepine receptor, including ODN, exert a tonic inhibitory effect on feeding behavior. Of note, cDLOP has already been used successfully to decipher physiological actions of endozepines in the brain. Thus, it has been recently shown that cDLOP not only prevented the inhibitory effects of ODN on gonadotropin-releasing hormone (GnRH) and NPY gene expression but also increased by itself GnRH and NPY mRNA levels (Compère *et al.*, 2004, 2005). Consistent with an endozepinergic tonus controlling feeding, in the present study, we found that the stimulatory action of cDLOP on food consumption was completely abolished by ODN. These data indicate that the orexigenic effect of cDLOP and the anorexigenic effect of ODN are mediated through activation of the same receptor. This latter observation provides strong evidence for the involvement of endozepine metabotropic receptors in the control of feeding.

In conclusion, the present study provides direct evidence that, in mice, the anorexigenic effect of ODN involves receptors that are distinct from the classical CBR and PBR. The pharmacological profile of these receptors corresponds to that of the metabotropic endozepine receptor positively coupled to phospholipase C. Our results also suggest the existence of an endozepinergic tonus that appears to play an important role in the regulation of feeding behavior.

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Figure 9 Effect of graded doses of ODN on cdLOP-induced increase of food intake in food-restricted mice. Mice were food restricted (half of their daily food consumption) for 18 h and then injected i.c.v. (10 μ l) with saline or cyclo₁₋₈[DLeu⁵]OP (cdLOP; 500 ng/mouse) in the absence or presence of increasing doses of ODN (a, 10 ng/mouse; b, 100 ng/mouse; c, 200 ng/mouse; d, 300 ng/mouse). Ten min after i.c.v. injection, each animal had access to a weighed food pellet. Cumulative food intake was measured during 3 h at the time indicated. Mean $\pm$ SEM represents data from 10–20 mice per group. Student–Newman–Keuls test post hoc: * p < 0.05, ** p < 0.01, *** p < 0.001 vs saline-injected mice; ## p < 0.01, ### p < 0.001 vs cdLOP-injected mice. A two-way ANOVA revealed a significant interaction between ODN and cdLOP: at 30 min $F(4,110) = 4.01$, p < 0.01, at 60 min $F(4,110) = 5.2$, p < 0.001, at 120 min $F(4,110) = 8.75$, p < 0.001, and at 180 min $F(4,110) = 8.13$, p < 0.001.

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REFERENCES

- Alho H, Costa E, Ferrero P, Fujimoto M, Cosenza-Murphy D, Guidotti A (1985). Diazepam-binding inhibitor: a neuropeptide located in selected neuronal populations of rat brain. *Science* **229**: 179–182.
- Alho H, Freneau Jr RT, Tiedge H, Wilcox J, Bovolin P, Roberts JL et al (1988). Diazepam binding inhibitor gene expression: location in brain and peripheral tissues of rat. *Proc Natl Acad Sci USA* **85**: 7018–7022.
- Berkovich A, McPhie P, Campagnone M, Guidotti A, Hensley P (1990). A natural processing product of rat diazepam binding inhibitor, triakontatetrapeptide (diazepam binding inhibitor 17–50) contains an alpha-helix, which allows discrimination between benzodiazepine binding site subtypes. *Mol Pharmacol* **37**: 164–172.
- Bonetti EP, Pieri L, Cumin R, Schaffner R, Pieri M, Gamzu ER et al (1982). Benzodiazepine antagonist RO 15–1788: neurological and behavioral effects. *Psychopharmacology* **78**: 8–18.
- Bormann J (1991). Electrophysiological characterization of diazepam binding inhibitor (DBI) on GABA_A receptors. *Neuropharmacology* **30**: 1387–1389.
- Compère V, Li S, Leprince J, Tonon MC, Vaudry H, Pelletier G (2003). Effect of intracerebroventricular administration of the octadecaneuropeptide on the expression of pro-opiomelanocortin, neuropeptide Y and corticotropin-releasing hormone mRNAs in rat hypothalamus. *J Neuroendocrinol* **15**: 197–203.
- Compère V, Li S, Leprince J, Tonon MC, Vaudry H, Pelletier G (2004). *In vivo* action of a new octadecaneuropeptide (ODN) antagonist on gonadotropin-releasing hormone gene expression in the male rat brain. *Neuroscience* **125**: 411–415.
- Compère V, Li S, Leprince J, Tonon MC, Vaudry H, Pelletier G (2005). *In vivo* action of a new octadecaneuropeptide antagonist on neuropeptide Y and corticotropin-releasing hormone mRNA levels in rat. *Mol Brain Res* **141**: 156–160.
- Ferrero P, Santi MR, Conti-Tronconi B, Costa E, Guidotti A (1986). Study of an octadecaneuropeptide derived from diazepam binding inhibitor (DBI): biological activity and presence in rat brain. *Proc Natl Acad Sci USA* **83**: 827–831.
- Gandolfo P, Louiset E, Patte C, Leprince J, Masmoudi O, Malagon M et al (2001). The triakontatetrapeptide TTN increases $[Ca^{2+}]_i$ in rat astrocytes through activation of peripheral-type benzodiazepine receptors. *Glia* **35**: 90–100.
- Gandolfo P, Patte C, Leprince J, Thoulas JL, Vaudry H, Tonon MC (1997). The stimulatory effect of the octadecaneuropeptide (ODN) on cytosolic Ca^{2+} in rat astrocytes is not mediated through classical benzodiazepine receptors. *Eur J Pharmacol* **322**: 275–281.
- Garcia de Mateos-Verchere J, Leprince J, Tonon MC, Vaudry H, Costentin J (1998a). The octadecaneuropeptide ODN induces anxiety in rodents: possible involvement of a shorter biologically active fragment. *Peptides* **19**: 841–848.

- Garcia de Mateos-Verchere J, Leprince J, Tonon MC, Vaudry H, Costentin J (1998b). The octadecaneuropeptide ODN inhibits apomorphine-induced yawning in rats. *Eur J Pharmacol* 357: 121–126.
- Garcia de Mateos-Verchere J, Leprince J, Tonon MC, Vaudry H, Costentin J (1999). Reduction of pentylentetrazol-induced convulsions by the octadecaneuropeptide ODN. *Peptides* 20: 1431–1436.
- Garcia de Mateos-Verchere J, Leprince J, Tonon MC, Vaudry H, Costentin J (2001). The octadecaneuropeptide (diazepam-binding inhibitor (33–50)) exerts potent anorexigenic effects in rodents. *Eur J Pharmacol* 414: 225–231.
- Guidotti A, Alho H, Berkovich A, Cox DH, Ferrarese C, Slobodyansky E et al (1989). DBI processing: allosteric modulation at different GABA/benzodiazepine receptor subtypes. In: Barnard EA, Costa E (eds). *Allosteric Modulation of Amino Acid Receptors: Therapeutic Implications*. Raven Press, Ltd: New York. pp 109–123.
- Guidotti A, Forchetti CM, Corda MG, Konkel D, Bennett CD, Costa E (1983). Isolation, characterization and purification to homogeneity of an endogenous polypeptide with agonistic action on benzodiazepine receptors. *Proc Natl Acad Sci USA* 80: 3531–3535.
- Haley TJ, McCormick WG (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br J Pharmacol* 12: 12–15.
- Le Fur G, Guilloux F, Rufat P, Uzan A, Renault C, Dubroeuq MC et al (1983). Peripheral benzodiazepine binding sites: effects of PK11195, 1-(2-chloro-phenyl)-N-(1-methylpropyl)-3-isoquinolinecarboxamide. II. *In vivo* studies. *Life Sci* 32: 1849–1856.
- Leprince J, Gandolfo P, Thoumas JL, Patte C, Fauchère JL, Vaudry H et al (1998). Structure-activity relationships of a series of analogues of the octadecaneuropeptide ODN on calcium mobilization in rat astrocytes. *J Med Chem* 41: 4433–4438.
- Leprince J, Oulyadi H, Vaudry D, Masmoudi O, Gandolfo P, Patte C et al (2001). Synthesis, conformational analysis and biological activity of cyclic analogs of the octadecaneuropeptide ODN. *Eur J Biochem* 268: 6045–6057.
- Louiset E, Vaudry H, Cazin L (1993). Allosteric modulation of the GABA-induced chloride current in frog melanotrophs. *Ann NY Acad Sci* 680: 564–566.
- Malagon M, Vaudry H, Van Strien F, Pelletier G, Gracia-Navarro F, Tonon MC (1993). Ontogeny of diazepam-binding inhibitor-related peptides (endozepines) in the rat brain. *Neuroscience* 57: 777–786.
- Patte C, Vaudry H, Desrues L, Gandolfo P, Strijdvén I, Lamacz M et al (1995). The endozepine ODN stimulates polyphosphoinositide metabolism in rat astrocytes. *FEBS Lett* 362: 106–110.
- Slobodyansky E, Berkovich A, Bovolín P, Wambebe C (1990). The endogenous allosteric modulation of GABA_A receptor subtypes: a role for the neuronal posttranslational processing products of rat brain DBI. In: Biggio G, Costa E (eds). *GABA and Benzodiazepine Receptor Subtypes*. Raven Press: New York. pp 51–61.
- Slobodyansky E, Guidotti A, Wambebe C, Berkovich A, Costa E (1989). Isolation and characterization of a rat brain triakontatetrapeptide, a posttranslational product of diazepam binding inhibitor: specific action at the RO 5-4864 recognition site. *J Neurochem* 53: 1276–1284.
- Tong Y, Toranzo D, Pelletier G (1991). Localization of diazepam-binding inhibitor (DBI) mRNA in the rat brain by high resolution *in situ* hybridisation. *Neuropeptides* 20: 33–40.
- Tonon MC, Adjeroud S, Lamacz M, Louiset E, Danger JM, Desrues L et al (1989). Central-type benzodiazepines and the octadecaneuropeptide modulate the effects of GABA on the release of alpha-melanocyte-stimulating hormone from frog neurointermediate lobe *in vitro*. *Neuroscience* 31: 485–493.
- Tonon MC, Désy L, Nicolas P, Vaudry H, Pelletier G (1990). Immunocytochemical localization of the endogenous benzodiazepine ligand octadecaneuropeptide (ODN) in the rat brain. *Neuropeptides* 15: 17–24.
- Tonon MC, Leprince J, Gandolfo P, Compère V, Pelletier G, Malagon M et al (2006). Endozepines. In: Kastin AJ (ed). *Handbook of Biologically Active Peptides*. Elsevier, Chapter 10, pp 813–819.
- Yanase H, Shimizu H, Yamada K, Iwanaga T (2002). Cellular localization of the diazepam binding inhibitor in glia cells with special reference to its coexistence with brain-type fatty acid binding protein. *Arch Histol Cytol* 65: 27–36.