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The octadecaneuropeptide ODN inhibits apomorphine-induced yawning in rats

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Abstract

High concentrations of diazepam-binding inhibitor (DBI) have been detected in brain areas containing dopaminergic cell bodies and nerve terminals. In the present study, we have investigated the effect of a proteolytic fragment of DBI, the octadecaneuropeptide ODN, on apomorphine-induced yawning in Sprague–Dawley rats. Injection of graded doses of ODN (12.5 to 100 ng i.c.v.) caused a dose-dependent inhibition of apomorphine-induced yawning and penile erections. At a dose of 100 ng, intracerebroventricularly administered ODN was able to inhibit, during more than 3 h, the apomorphine-evoked yawning. ODN also inhibited pilocarpine-induced yawning. Apomorphine induces a bell-shaped dose-dependent effect on yawning with a maximum response at the dose of 100 μg/kg and a much lower effect at a dose of 200 μg/kg. Injection (i.c.v.) of 100 ng ODN markedly attenuated the number of yawns induced by 100 μg/kg apomorphine but partially restored the yawning behavior in rats treated with a 200 μg/kg dose of apomorphine. At doses of 0.5 or 5 mg/kg s.c., diazepam did not modify the inhibitory effect of ODN on the apomorphine-induced yawning. Taken together, the present data suggest that ODN inhibits yawning downstream dopaminergic as well as cholinergic synapses involved in yawning. In addition, the effect of ODN cannot be ascribed to an inverse agonistic activity on central-type benzodiazepine receptors.

Keywords: ODN (Octadecaneuropeptide); Endozepine; Apomorphine; Yawning; Diazepam; Pilocarpine

1. Introduction

The term endozepine designates a family of regulatory neuropeptides that have been isolated from rat brain extracts on the basis of their ability to displace benzodiazepines from their binding sites (Guidotti et al., 1983). All endozepines characterized so far derive from diazepam-binding inhibitor (DBI), an 86-amino acid polypeptide which can generate, through proteolytic cleavage, several biologically active fragments including the triakontatetraneuropeptide DBI-(17–50) (TTN) (Slobodyansky et al., 1989) and the octadecaneuropeptide DBI-(33–50) (ODN) (Ferrero et al., 1986). Pharmacological studies have shown that ODN interacts predominantly with central-type benzodiazepine receptors (Ferrero et al., 1986; Slobodyansky et al., 1989; Berkovich et al., 1990) while TTN is a selective ligand for peripheral-type (i.e., mitochondrial) benzodiazepine receptors (Slobodyansky et al., 1989; Berkovich et al., 1990). Endozepines may also activate a membrane receptor positively coupled to phospholipase C through a pertussis toxin sensitive G-protein (Patte et al., 1995; Gandolfo et al., 1997). Endozepines are widely distributed in the central nervous system (Alho et al., 1989; Costa and Guidotti, 1991; Rouet-Smih et al., 1992; Malagon et al., 1993). In particular, high concentrations of endozepines have been found in brain areas sending or receiving dopaminergic projections, such as the cortex, the amygdala, the hypothalamus (Costa and Guidotti, 1991), the preoptic area (Malagon et al., 1992), the substantia nigra (Ball et al., 1986) and the striatum (Malagon et al., 1993). Concurrently, it has been shown

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that agonists and antagonists of the $GABA_A$–benzodiazepine receptor complex modify dopamine release in the striatum (Finlay et al., 1992; Gruen et al., 1992). However, the effect of endozepines on dopaminergic neurotransmission has never been investigated.

In order to explore possible interactions between endozepines and the dopaminergic systems, we have here investigated the effect of ODN on apomorphine-induced yawning. Yawning may be influenced by a variety of neuroamines and neuropeptides (for review see, Argiolas and Melis, 1998). In rat, the reference direct dopamine receptor agonist apomorphine modulates yawning in a biphasic manner (Protais et al., 1983): at low doses apomorphine increases dose-dependently yawning and penile erection whereas, at high doses, apomorphine reduces yawning frequency (Urbá-Holmgren et al., 1977; Holmgren and Urbá-Holmgren, 1980; Dubuc et al., 1982; Ushijima et al., 1984).The induction of yawning evoked by low doses of apomorphine has been ascribed to the activation of dopamine autoreceptors (Carlsson, 1975) and the subsequent decrease of the tonic dopaminergic transmission. Conversely, the disappearance of yawning provoked by high doses of apomorphine can be accounted for by direct activation of postsynaptic dopamine D_2 receptors which mimicks the effect of the dopaminergic tone. The present report provides evidence that the endozepine ODN is a potent modulator of apomorphine-induced yawning.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (180–220 g) were purchased from Charles River (Saint-Aubin lès Elbeuf, France). The animals were housed by five in Makrolon cages ($L = 40$ cm, $W = 25$ cm, $H = 18$ cm) kept in a ventilated room, at a temperature of $21 \pm 1°C$, under a 12-h light/12-h dark cycle (light on between 7:00 a.m. and 7:00 p.m.). The animals had free access to water and food (U.A.R., France). Experiments were carried out between 9:00 a.m. and 5:00 p.m. Animal manipulations were performed according to the recommendations of the French Ethical Committee and under the supervision of authorized investigators.

2.2. Intracerebroventricular injections

Rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and a small area of the skin (about 25 mm^2) was dissected out. An incomplete drilling of the skull was made with a dentary miller at the following coordinates: $L = 2$ mm posterior and 1.8 mm lateral to bregma, according to Albe-Fessard et al. (1966). An organo-mercurial antiseptic was applied for preventing infection. Forty eight hours later, an i.c.v. injection (20 μl) was made free-hand in the left ventricle, with a microsyringe (Hamilton 50 μl) connected to a needle (diameter 0.5 mm) of which the median part of the bevel protruded 5 mm from a guard limiting its penetration into the brain. Intracerebroventricular injections were performed by an experienced investigator. Pilot experiments which consisted in injecting a dye solution (diluted India ink) had shown after brain sectioning a ventricular localization in more than 95% of the trials.

2.3. Yawning

After i.c.v. injection, rats were placed before testing into individual Makrolon cages ($L = 23$ cm, $W = 9$ cm, $H = 8$ cm). Administration of apomorphine (25, 100, 200 or 400 μg/kg, s.c.), pilocarpine (2 mg/kg, i.p.) or saline was performed immediately before the animals were introduced into observation cages ($L = 25$ cm, $W = 18$ cm, $H = 30$ cm). Four observation cages, 10 cm distant from each other, were arranged on two floors 40 cm distant. They were equipped with vertical walls made of wire netting (0.8 cm^2) constructed with metal bars (1 mm in diameter). Cages were assigned to different drug treatments in randomized order. All experiments were performed blind, by the same observer. The number of yawns (number of wide-stretched opening of the mouth) was determined in rats which received an i.c.v. injection of saline or ODN 1 h before s.c. administration of apomorphine (25, 100, 200 or 400 μg/kg, s.c.) or pilocarpine (2 mg/kg, i.p.). Diazepam (0.5 or 5 mg/kg, s.c.) was administered 45 min after an i.c.v. injection of saline or ODN and apomorphine (100 μg/kg, s.c.) was administered 15 min after diazepam injection. The COOH-terminal octapeptide fragment of ODN (100 ng, i.c.v.) was administered simultaneously with apomorphine (100 μg/kg, s.c.). The number of yawns was determined between the 5th and the 65th min after apomorphine administration.

For establishing the time course of the effect of ODN on apomorphine-induced yawning, rats received an i.c.v. injection of saline or ODN (100 ng) 0, 1, 3 or 6 h before s.c. administration of apomorphine (100 μg/kg, s.c.). The number of yawns was determined between the 5th and the 65th min after apomorphine administration.

2.4. Penile erection

Rats received an i.c.v. injection of saline or ODN (100 ng) 1 h, before s.c. administration of apomorphine (100 μg/kg, s.c.). The number of penile erections (repeated pelvic trusts immediately followed by an upright position, an emerging engorged penis which the rat proceeds to lick), was determined between the 5th and the 65th min after apomorphine administration.

2.5. Sniffing

Rats received an i.c.v. injection of saline or ODN (100 ng) 1 h before s.c. administration of apomorphine (200 or

400 μg/kg) and tested the sniffing score. The sniffing score was established as follows. From the 5th min after the s.c. administration of apomorphine and during 1 h, we examined, during 10 s, every 2 min, whether the rat displayed a sniffing behaviour. Score 2 was attributed to the animals which sniffed in a sustained manner, score 1 when sniffing was irregular and score 0 when rat did not sniff. The sum of individual scores, attributed at each of the 30 observation periods, corresponded to the «sniffing score».

2.6. Drugs

Rat ODN (Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys) and the C-terminal octapeptide (Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys) were synthetized by the solid phase methodology, as previously described (Gandolfo et al., 1997). The peptides were dissolved in saline, in plastic tubes, just before i.c.v. injection. Chloral hydrate (Sigma, Saint-Quentin Fallavier, France) was dissolved in distilled water. Apomorphine HCl (La Cooper, Melun, France) was dissolved in saline containing 0.1% ascorbic acid to prevent oxidation. Pilocarpine HCl (Sigma) was dissolved in saline. Diazepam (injectable solution of Valium®; Roche, Neuilly-sur-Seine, France) was diluted in saline.

2.7. Statistics

The data are expressed as means ± S.E.M. The Student's *t*-test was used for comparison of the mean number of yawns between two groups, The Dunnet's *t*-test or two-way analysis of variance (ANOVA) was used for comparisons between multiple groups.

A Tukey's test was used for statistical analysis of the interaction between diazepam-ODN and apomorphine. The mean scores of sniffing or the number of penile erections were compared using Student's *t*-test.

3. Results

3.1. Effect of ODN or octapeptide on apomorphine-induced yawning

As expected, administration of apomorphine (100 μg/kg, s.c.) elicited frequent yawning. Injection of graded doses of ODN (12.5 to 100 ng, i.c.v.), 60 min before the administration of apomorphine, induced a dose-dependent reduction in the number of yawns (Table 1). At the dose of 100 ng, ODN virtually abolished the effect of apomorphine on yawning (Table 1).

The COOH-terminal octapeptide fragment of ODN (100 ng, i.c.v.), administered simultaneously with apomorphine (100 μg/kg, s.c.), significantly reduced the number of yawns during 1 h (Table 1).

Table 1
Effect of ODN and the octapeptide on apomorphine and pilocarpine-induced yawning

Treatment			Number of yawns in 1 h
A	saline, i.c.v.	saline, s.c.	1.2 ± 0.1
	ODN 100 ng, i.c.v.	saline, s.c.	1.3 ± 0.4
	saline, i.c.v	apomorphine (100 μg/kg, s.c.)	15.1 ± 0.8
	ODN 12.5 ng, i.c.v.	apomorphine (100 μg/kg, s.c.)	13 ± 2
	ODN 25 ng, i.c.v.	apomorphine (100 μg/kg, s.c.)	5 ± 1[a]
	ODN 100 ng, i.c.v.	apomorphine (100 μg/kg, s.c.)	1.2 ± 0.3[a]
B	saline, i.c.v	apomorphine (100 μg/kg, s.c.)	16.8 ± 0.7
	octapeptide 100 ng, i.c.v.	apomorphine (100 μg/kg, s.c.)	9.4 ± 0.4[b]
C	saline, i.c.v.	pilocarpine (2 mg/kg, i.p.)	10.7 ± 0.8
	ODN 100 ng i.c.v.	pilocarpine (2 mg/kg, i.p.)	2 ± 0.4[b]

A—Rats received an i.c.v. injection of saline or 12.5, 25 or 100 ng ODN; 60 min later the animals were injected s.c. with apomorphine (100 μg/kg).
B—Rats received an i.c.v. injection of saline or 100 ng octapeptide, immediately before the s.c. administration of apomorphine (100 μg/kg).
C—Rats received an i.c.v. injection of saline or 100 ng ODN; 60 min later the animals were injected i.p. with pilocarpine (2 mg/kg). The number of yawns was determined between the 5th and the 65th min after apomorphine administration.
Mean ± S.E.M. of six rats per group.
Dunnet's *t*-test: [a] $P < 0.01$ as compared to the apomorphine-saline group.
Student's *t*-test: [b] $P < 0.001$ as compared to the respective i.c.v.-saline group.

3.2. Effect of ODN on pilocarpine-induced yawning

Administration of pilocarpine (2 mg/kg, i.p.), 60 min after i.c.v. injection of saline, also induced yawning. The number of yawns was significantly reduced when pilocarpine was administered 60 min after i.c.v. injection of ODN at a dose of 100 ng (Table 1).

3.3. Effect of ODN on apomorphine-induced appearance and disappearance of yawning

As previously reported (Protais et al., 1983), graded doses of apomorphine induced a biphasic effect on yawning. The effect of apomorphine on yawning behavior appeared at a dose of 25 μg/kg, culminated at a dose of 100 μg/kg and vanished at doses of 200 and 400 μg/kg (Fig. 1). Injection of ODN (i.c.v.) at a dose of 100 ng virtually abolished the yawning induced by the 25 and the 100 μg/kg-doses of apomorphine. In contrast, ODN significantly increased the number of yawns induced by the 200 μg/kg-dose of apomorphine but did not reverse the

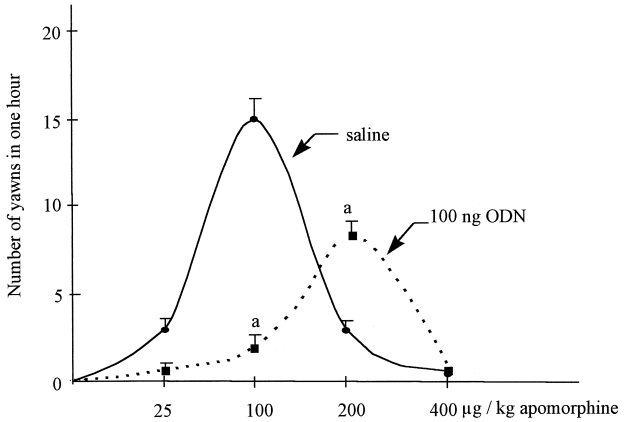

Fig. 1. Effect of ODN on the biphasic yawning response to various doses of apomorphine. Rats received an i.c.v. injection of saline (●) or 100 ng ODN (■), 60 min before s.c. administration of increasing doses of apomorphine (25, 100, 200 and 400 μg/kg, s.c.). The number of yawns was determined between the 5th and the 65th min after apomorphine administration. Mean ± S.E.M. of 12 rats (100 and 200 μg/kg apomorphine) or six rats (25 and 400 μg/kg apomorphine) per group. ANOVA: $F(9,50) = 54$, $^{a}P < 0.001$; as compared to the apomorphine-saline group.

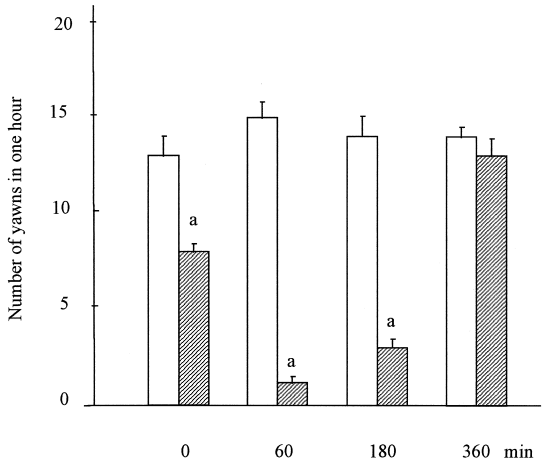

Fig. 2. Time-course of the inhibitory effect of ODN on apomorphine-induced yawning. Rats received an i.c.v. injection of saline (open bars) or ODN (100 ng; hatched bars) 0, 1, 3 or 6 h before s.c. administration of apomorphine (100 μg/kg, s.c.). The number of yawns was determined between the 5th and the 65th min after apomorphine administration. Mean ± S.E.M. of six rats per group. $^{a}P < 0.001$; Student's *t*-test, as compared to the apomorphine-saline group.

complete inhibition of yawning elicited by the 400 μg/kg-dose of apomorphine (Fig. 1).

Administration of diazepam (0.5 or 5 mg/kg, s.c.) had no effect on the apomorphine-induced yawning and did not reverse the inhibitory effect of ODN on yawning (Table 2).

3.4. Time course of the inhibitory effect of ODN on apomorphine-induced yawning

When administered concomitantly with apomorphine (100 μg/kg, s.c.), ODN (100 ng, i.c.v.) induced a modest reduction of the apomorphine-evoked yawning. The maximum inhibition of yawning episodes occurred when ODN was injected i.c.v. 60 min prior to the administration of apomorphine (Fig. 2). A marked inhibition of yawning was still observed when ODN was injected 3 h before apomorphine but completely disappeared after 6-h lag period (Fig. 2).

3.5. Effect of ODN on apomorphine-induced sniffing

The sniffing score in rats treated with 200 μg/kg apomorphine was significantly reduced by 100 ng of ODN, whereas the higher sniffing score elicited by 400 μg/kg apomorphine was not affected by ODN (Table 3).

Table 2
Interaction of diazepam with the inhibitory effect of ODN on the apomorphine-induced yawning

Treatment			Number of yawns in 1 h
ODN 100 ng, i.c.v. (+) or saline i.c.v. (−)	Diazepam (mg/kg, s.c.)	Apomorphine (100 μg/kg, s.c.)	
−	0	+	15.3 ± 1.7
+	0	+	0.8 ± 0.3[a]
−	0.5	+	9.8 ± 0.7
+	0.5	+	0.8 ± 0.5[b]
−	5	+	11.6 ± 1.9
+	5	+	1.2 ± 0.5[b]

Rats received an i.c.v. injection of saline or 100 ng ODN; 45 min later the animals were injected s.c. with diazepam (0.5 or 5 mg/kg); 60 min after the latter injection they were injected s.c. with apomorphine (100 μg/kg). The number of yawns was determined between the 5th and the 65th min after apomorphine administration.
Mean ± S.E.M. of six rats per group.
ANOVA: $F(5,30) = 28$, $P < 0{,}001$.
Post hoc comparisons: $^{a}P < 0.001$ as compared with the apomorphine-saline group; $^{b}P < 0.001$ as compared with the ODN-saline group.

Table 3
Effect of ODN on the apomorphine-induced sniffing

Treatment		Sniffing score in 1 h
Saline, i.c.v.	apomorphine (200 μg/kg, s.c.)	16 ± 1
ODN 100 ng, i.c.v.	apomorphine (200 μg/kg, s.c.)	7 ± 1[a]
Saline, i.c.v.	apomorphine (400 μg/kg, s.c.)	26 ± 3
ODN 100 ng, i.c.v.	apomorphine (400 μg/kg, s.c.)	25 ± 1

Rats received an i.c.v. injection of saline or 100 ng ODN; 60 min later they were injected s.c. with apomorphine (200 or 400 μg/kg).
The sniffing score was determined between the 5th and the 65th min after apomorphine administration.
Mean ± S.E.M. of six (400 μg/kg apomorphine) or 12 (200 μg/kg apomorphine) rats per group.
$^{a}P < 0.001$, Student's *t*-test, as compared to the group apomorphine-saline 200 μg/kg.

3.6. Effect of ODN on apomorphine-induced penile erection

Administration of apomorphine (100 μg/kg, s.c.) also induced penile erections with a mean frequency of 1.2 ± 0.1 erection episodes per hour. Intracerebroventricular injection of ODN dose-dependently reduced the number of apomorphine-evoked erections: 0.6 ± 0.2 erection episodes after a 25-ng dose of ODN ($P < 0.05$) and 0.2 ± 0.1 erection episodes after a 100-ng dose of ODN ($P < 0.001$), (mean ± S.E.M. of 30 rats per group, Student's *t*-test).

4. Discussion

Apomorphine is a reference direct agonist of dopamine receptors (Andén et al., 1967; Ernst, 1967) which stimulates all types of identified dopamine receptors, i.e., D_1, D_2, D_3, D_4 and D_5 (Sibley and Monsma, 1992). However, since apomorphine exhibits a higher affinity for autoreceptors which are associated with either the somatodentritic region or the axon terminals of dopamine neurons, low doses of apomorphine cause primarily a decrease in dopamine release (Carlsson, 1975). In rat, the apomorphine-induced depression of the tonic dopaminergic transmission is associated with the occurrence of a yawning behavior (Protais et al., 1983). It is assumed that the decrease in the dopaminergic tone evoked by apomorphine causes a direct or indirect stimulation of cholinergic neurons and thereby activates muscarinic receptors leading to the appearance of yawning (Holmgren and Urbá-Holmgren, 1980; Yamada and Furukawa, 1980; Dubuc et al., 1982; Ushijima et al., 1984; Gower, 1987). As a matter of fact, pilocarpine elicits yawning behavior in rats, whereas the cholinesterase inhibitor physostigmine potentiates the apomorphine-induced yawning response by preventing degradation of acetylcholine whose release is triggered by suppression of the dopaminergic tone (Holmgren and Urbá-Holmgren, 1980; Yamada and Furukawa, 1980; Dubuc et al., 1982; Ushijima et al., 1984; Gower, 1987). At higher doses than those stimulating dopamine autoreceptors, apomorphine also stimulates post-synaptic dopamine receptors and thus restores the dopaminergic tone. As a result, high doses of apomorphine repress the cholinergic tone, so that the yawning response disappears (Protais et al., 1983).

The present study has demonstrated that i.c.v. administration of ODN causes a dose-dependent inhibition of apomorphine-induced yawning. According to the functional pattern aforementioned, ODN might have inhibited yawning elicited by low doses of apomorphine, by increasing dopaminergic transmission, and or by inhibiting cholinergic transmission. Previous reports have shown that ODN acts as an inverse agonist of central-type benzodiazepine receptors (Alho et al., 1985; Ferrarese et al., 1987). Since benzodiazepines are known to depress the activity of discrete populations of dopaminergic neurons (Tam and Roth, 1990; Zetterstrom and Filleuz, 1990; Invernizzi et al., 1991; Finlay et al., 1992; Gruen et al., 1992), it was conceivable that ODN, as an inverse agonist of central-type benzodiazepine receptors, might have activated dopaminergic neurons involved in yawning. However, our data did not support this hypothesis, inasmuch as the effect of ODN on apomorphine-induced yawning was not reversed by diazepam. This observation indicates that the effect of ODN cannot be ascribed to its inverse agonistic activity on central-type benzodiazepine receptors. Consistent with this finding, it has been recently shown that the stimulatory effect of ODN on cultured rat astrocytes is mediated through activation of non-benzodiazepinic receptor (Gandolfo et al., 1997). In addition, it appeared that the inhibitory effect of ODN on yawning cannot be accounted for by a direct action of the peptide on dopaminergic neurones since ODN also inhibited the yawning response evoked by pilocarpine. These observations indicate that ODN likely operates downstream the cholinergic synapse.

The reappearance of yawning induced by ODN in rats treated with 200 μg/kg apomorphine was associated with an inhibition of the stereotyped sniffing. When the dose of apomorphine was increased to 400 μg/kg, the effect of 100 ng ODN on the sniffing score was not impaired while the yawning was completely abolished. This observation is consistent with a previous report which showed that yawning and sniffing are two mutually exclusive behaviors (Protais et al., 1983).

We have previously shown that the anxiogenic effect of ODN may require cleavage of the molecule to generate a biologically active fragment (Garcia de Mateos-Verchere et al., 1998). In the present study, we have found that the C-terminal octapeptide fragment of ODN, at a dose of 100 ng, mimicked the inhibitory effect of ODN on apomorphine-induced yawning. In addition, we have noticed that the effect of ODN on yawning only culminated after a time lag of 60 min whereas the octapeptide was more rapidly effective. These observations suggest that the action of ODN on the yawning behavior might also involve the formation of a biologically active proteolytic fragment.

The fact that i.c.v. administration of ODN increases anxiety in rat (Garcia de Mateos-Verchere et al., 1998) raised the question as to whether the inhibitory effect of the peptide could be related to its anxiogenic activity. The present data have shown that diazepam does not alter the effect of ODN on yawning, whereas the anxiogenic responses evoked by ODN are antagonized by diazepam (Garcia de Mateos-Verchere et al., 1998) indicating that the effect of ODN on apomorphine-induced yawning is not mediated by central-type benzodiazepine receptors and cannot be ascribed to the anxiogenic activity of the peptide. Whether the recently described ODN receptor coupled to phospholipase C (Patte et al., 1995) is involved in the effect of ODN on yawning deserves further investigations.

5. Conclusion

ODN and its C-terminal octapeptide appear to inhibit apomorphine-induced yawning. The effect of ODN cannot be accounted for by its inverse agonistic activity on the $GABA_A$–benzodiazepine receptor complex and appears to take place downstream the dopaminergic and cholinergic synapses involved in yawning.

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