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Opposite effects of substance P fragments C (anxiogenic) and N (anxiolytic) injected into dorsal periaqueductal gray

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Abstract

Recent findings implicating neurokinins in the expression of anxiety-like behaviors have stimulated interest in the participation of these neuropeptides in the dorsal periaqueductal gray matter (dPAG), one of the main output regions of the brainstem for the expression of defense reaction. Studies on the behavior of rats submitted to the elevated plus-maze test in this laboratory have shown that microinjections of substance P into the dorsal periaqueductal gray produce anxiogenic-like effects. Now, we analyze what portion of the molecule of substance P is responsible for these effects through the examination of the action of its C- and N-terminus fragments (6–11 and 1–7) in the elevated plus-maze. We also investigated whether these effects are influenced by prior treatment with the tachykinin NK_1 receptor antagonist 17-β-hydroxy-17-α-ethynyl-5α-androstanol[3,2-*b*]pyrimido[1,2-*a*]benzimidazole (WIN51,708). To this end, rats were implanted with a cannula in the dorsal periaqueductal gray and injected 1 week later with equimolar doses (17.5 and 35 pmol) of either C- or N-fragments of substance P and tested in the elevated plus-maze. The results show that the C-terminal fragment has an anxiogenic profile of effects, including reduction in the number of entries and time spent in the open arms of the maze, plus increases in scanning, stretched-attend posture, head dipping and flat-back approach. On the other hand, the N-terminal fragment produced opposite effects, namely, an increase in the number of entries and time spent in the open arms of the maze accompanied by an increase in end-arm activity, rearing and head dipping. The tachykinin NK_1 receptor antagonist WIN51,708 (20 mg/kg, i.p.) inhibited the effects of the carboxy-terminal of substance P while it did not change the effects of the N-terminal fragment. Microinjection of WIN51,708 (20 mg/kg, i.p.), by its own, did not produce any significant effects. Therefore, the results indicate that the anxiogenic effects of substance P injected into the dorsal periaqueductal gray are encoded by its carboxy-terminal sequence and due to its action on tachykinin NK_1 receptors.

Keywords: Substance P, N- and C-fragment; Elevated plus-maze; Dorsal periaqueductal gray

1. Introduction

Electrical stimulation of the dorsal periaqueductal gray matter (dPAG) has aversive properties (Brandão et al., 1982, 1994, 1999; Schmitt et al., 1986; Graeff, 1990). The behavioral and autonomic reactions induced by such stimulation are similar to those observed following acute pain stimulation (Brandão et al., 1990). A tonic inhibitory control seems to be exerted by $GABA_A$- and histamine (H_2)-receptor mediated mechanisms since injections of the $GABA_A$- and H_2-receptor antagonists, bicuculline and ranitidine, respectively, into the midbrain tectum mimic, and GABA and histamine themselves inhibit the expressions of such defensive reactions (Santos et al., 2001). Phasic control over the neural circuits of aversion in the dorsal periaqueductal gray has been found to be exerted by serotonergic and opioid mechanisms (Brandão et al., 1994; Schmitt et al., 1985; Graeff et al., 1986; Graeff, 1990; Motta and Brandão, 1993; Motta et al., 1995). On the other hand, it has been speculated that excitatory amino acids and neuropeptides seem to be primarily implicated in the generation and elaboration of defense reactions. Substance P, a member of the tachykinin family, has received consid-

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erable attention among the neuropeptides (for a review, see Brandão et al., 1999).

Significant concentrations of substance P have been found in the ventral and dorsal parts of the PAG (Brownstein et al., 1976; Cuello and Kanazawa, 1978; Ljungdahl et al., 1978; Hall et al., 1987; Nieuwenhuys, 1985; Li et al., 1990). A recent study using polyclonal antibodies has shown dense immunoreactivity to substance P localized mostly to cell bodies and dendrites of the dorsal subdivision of the periaqueductal gray (Barbaresi, 1998). A number of studies have linked substance P in midbrain regions to processes underlying aversive states. We have previously shown that injections of substance P into the dorsal periaqueductal gray produce aversion in a place conditioning paradigm, suggesting that these aversive states might involve the neural substrates of defensive behavior in this region (Aguiar and Brandão, 1994). Furthermore, such injections, including a C-terminal fragment, induce a behavioral activation with defensive characteristics (Aguiar and Brandão, 1994, 1996; De Araújo et al., 1998, 1999). Substance P has also been implicated in the mediation of defensive behavior in regions of the brain aversion system, such as the amygdala and medial hypothalamus (Shaikh et al., 1993; Kramer et al., 1998; Smith et al., 1999).

It has been suggested that the effects of exogenously administered substance P within the central nervous may be due to its enzymatic breakdown, which produces amino N- and C-terminal active fragments (Stewart et al., 1982; Sakurada et al., 1985; Hall et al., 1989; Igwe et al., 1990). Studies dealing with the structure–activity relationship of substance P have revealed that N- and C-terminal fragments can exert many of the actions of the whole substance P molecule (Hall et al.,1989; Huston and Hasenöhrl, 1995). N- and C-terminal substance P fragments were demonstrated in rat brain and spinal cord (Sakurada et al., 1985; Nievenhuys, 1985). Furthermore, there is an evidence for an active uptake mechanism for the C-terminal heptapeptide (Nakata et al., 1981).

In the present study, we examine the effects of N- and C-terminal fragments of substance P injected into the dorsal periaqueductal gray on the behavior of rats submitted to the elevated plus-maze test. As substance P acts preferentially at the tachykinin NK_1 receptor (Quirion and Dam, 1988), and these receptors are expressed on dorsal periaqueductal gray neurons, we supposed that prior treatment with the tachykinin NK_1 receptor antagonist 17-β-hydroxy-17-α-ethynyl-5α-androstanol[3,2-*b*]pyrimido[1,2-*a*]benzimidazole (WIN51,708) could block the aversive effects of the C-terminus in the dorsal periaqueductal gray. WIN51,708 displays a high affinity for the rat tachykinin NK_1 receptive site (Appell et al., 1992; Sachais and Krause, 1994). Consistent reports have shown that this nonpeptide tachykinin NK_1 receptor antagonist can inhibit the physiological effects of substance P and its C-terminus (Anderson et al., 1994; Khan et al., 1998; Nikolaus et al., 1999). The rationale of this study is further strengthened in view of the possibility of the clinical use of neurokinin receptor antagonists as anxiolytic agents (Kramer et al., 1998).

2. Material and methods

2.1. Animals and surgery

Wistar male rats weighing 230–280 g were used. The animals were housed in pairs in Plexiglas cages in a colony room with food and water ad libitum. They were maintained on a 12-h light/12-h dark cycle (lights on at 7 a.m.) at 23 ± 1 °C and tested during the light phase of the cycle. Each rat was implanted with a stainless-steel guide cannula (0.6 mm o.d., 0.4 mm i.d.) under tribromoethanol anesthesia (50 mg/kg, i.p.). The cannula was directed to the PAG at the following coordinates (using lambda as the reference for each plane): 0.5 mm anterior, 1.2 mm lateral, 4.5 mm ventral (Paxinos and Watson, 1997). Each cannula was fixed with polyacrylic cement anchored to the skull with three stainless-steel screws and was plugged with stainless-steel stylets. The experiments started after a 1-week postoperative delay.

2.2. Apparatus

The plus-maze consisted of two open arms, 50 × 10 cm, and two enclosed arms 50 × 10 × 50 cm, with an open roof, arranged such that the two arms of each type were opposite each other. The maze was elevated to a height of 50 cm. The walls of the closed arms were made of sheets of transparent Plexiglas. The level of illumination was 75 lx on the floor level of the walled arms.

2.3. Procedure

The rats were placed individually into the center of the maze facing a closed arm and allowed 5 min of free exploration. The behaviour of the animals was recorded by a videocamera positioned beside the maze and monitored in another room via a closed circuit TV camera. The maze was thoroughly cleaned after each test with a 20% ethanol solution and dried. Each rat was tested only once.

All the experiments were carried out between 08:00 and 12:00 h. Videotapes were subsequently scored blind by an observer using ethological analysis software (Observer) developed by Noldus (Netherlands). Using separate location and behaviour keys, this software allows the real-time scoring of any behavior by direct keyboard entry to a PC. Behaviours scored from videotape included conventional measures (number of entries into and time spent on the open and enclosed arms) and novel ethological parameters of the elevated plus-maze.

The behaviour of each animal in the maze was analyzed taking into account the frequency of the behavioral cate-

gories recorded in each section of the maze (closed and open arms, central platform) (Pellow et al., 1985). The items recorded were grooming, rearing, peeping-out, stretched-attend posture, flat-back approach, scanning, head dipping, end-arm activity and immobility. These categories were defined following work with rats (Blanchard et al., 1991; Anseloni and Brandão, 1997; De Araújo et al., 1999) and mice (Rodgers and Johnson, 1995): (a) grooming: species-typical sequences beginning with the snout, progressing to the ears, and ending with whole-body grooming; (b) rearing: partial or total rising onto the hind limbs; (c) scanning: olfactory exploration of maze floor and walls, including sniffing; (d) head dipping: exploratory movement of head/shoulders over sides of the maze and down towards the floor; (e) end-arm activity: number of times the rat reached the end of an open arm; (f) peeping-out: stretching the head/shoulders from the closed arms to the central platform; (g) stretched-attend posture: when the animal stretches to its full length and turns back to the anterior position; (h) flat-back approach: locomotion when the animal stretches to its full length and cautiously moves forward; and (i) immobility: animal still, without any movement over 6 s.

2.4. Intracranial injection procedure

The animals were gently wrapped in a cloth, and a thin dental needle (o.d. 0.3 mm) was introduced through the guide cannula until its lower end was 2.5 mm below this cannula. The injection needle was linked to a 5-μl Hamilton syringe by means of polyethylene tubing. A volume of 0.2 μl was injected over 20 s with the aid of an infusion pump (Harvard Apparatus, USA) and the needle was held in place for an additional 10 s. The displacement of an air bubble inside the polyethylene (PE-10) catheter connecting the syringe needle to the intracerebral needle was used to monitor the injection. Each rat received only one injection of either saline (control), substance P or its C- and N-fragments, in equimolar doses (35–70 pmol).

2.5. Drugs

The substance P fragments, N-terminal (1–7) and C-terminal (6–11) (Sigma, USA), and WIN51,708 (Sigma) were used. Precautions recommended for the use of peptides were taken (Stewart, 1983). Independent groups of rats were tested with only one treatment. C- and N-terminals of substance P were each injected at doses of 17.5 and 35 pmol. At equimolar doses for substance P, the C-terminal has been shown to have aversive effects after injection in the dorsal periaqueductal gray of rats submitted to the elevated plus-maze test (Aguiar and Brandão, 1996; De Araújo et al., 1999). The C- and N-fragments were each dissolved in phosphate-buffered saline (PBS, pH = 7.0) shortly before use. PBS served as vehicle control for the fragments. WIN51,708 (Sigma) was dissolved in PBS containing 0.3% dimethylsulfoxide (DMSO, Sigma). WIN51,708 was injected in a volume of 1 ml/kg (i.p.). The same volume was used for injecting the vehicle (Vehicle; phosphate-buffered saline containing 0.3% dimethylsulfoxide).

In the first experiment ($N = 12$ for each group), the effects of Win51,708 (10 and 20 mg/kg) were studied in the following groups: (a) PBS alone; (b) Vehicle + PBS; (c) Win10 + PBS; (d) Win20 + PBS. The second experiment ($N = 12$ for each group) was divided in two parts. Part I (Win51,708 in combination with the C-fragment): (a) Vehicle + PBS (control); (b) Vehicle + C-fragment, 17.5 pmol; (c) Vehicle + C-fragment, 35 pmol; (d) Win51,708 + PBS; (e) Win51,708 + C-fragment. Part II (Win51,708 in combination with the N-fragment): (a) Vehicle + PBS (control); (b) Vehicle + N-fragment, 17.5 pmol; (c) Vehicle + N-fragment, 35 pmol; (d) Win51,708 + PBS; (e) WIN51,708 + N-fragment. The groups received two microinjections; WIN51,708 or its vehicle followed 20 min later by a microinjection of the fragment (C or N) or phosphate-buffered saline (PBS) and tested in the elevated plus-maze immediately afterwards. Win51,708 (10 or 20 mg/kg) was always tested against the dose of the fragment that produced the greatest effect (17.5 pmol for C- and 35 pmol for N-fragment).

2.6. Histology

Upon completion of the experiments, the animals were deeply anesthetized with urethane and perfused intracardially with saline followed by formalin solution (10%). Three days later, the brains were removed and maintained in formalin solution for seven days. Serial 60-μm brain

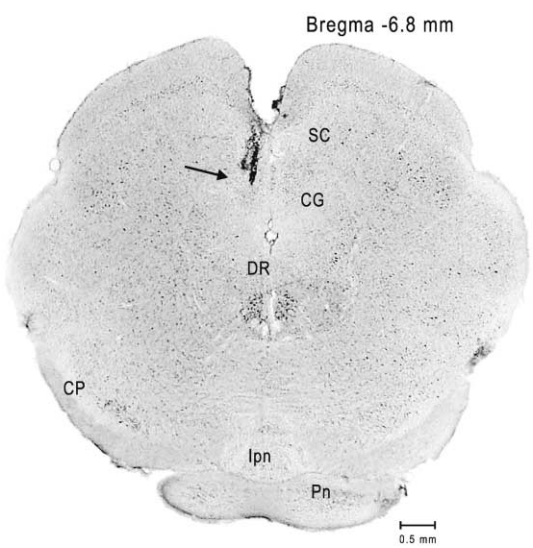

Fig. 1. Photomicrograph showing a typical example of a microinjection site into the dorsal periaqueductal gray. SC = superior colliculus; CG = dorsal periaqueductal gray; DR = dorsal raphe nucleus; CP = cerebral peduncle; Ipn = interpeduncular nucleus; Pn = pontine nuclei. Bar = 500 μm.

sections were cut using a microtome and stained with methylene blue in order to localize the positions of the microinjection sites according to the Paxinos and Watson (1997) atlas.

2.7. Analysis of results

The results are reported as means ± S.E.M. Data obtained for each of the traditional measures (entries and time spent in the arms of the maze) and for each recorded ethological categories (end-arm activity, scanning, head dipping, rearing, peeping-out, grooming, stretched-attend postures, flat-back approach and immobility) were analyzed by means of one-way analysis of variance (ANOVA). Newman–Keuls post-hoc comparisons were carried out if significant overall F values were obtained. Significance level was set at $P < 0.05$.

3. Results

The cannula placements of the animals used in this study were inside the dorsal periaqueductal gray matter. A representative slide with the cannula placement for microinjection can be seen in Fig. 1.

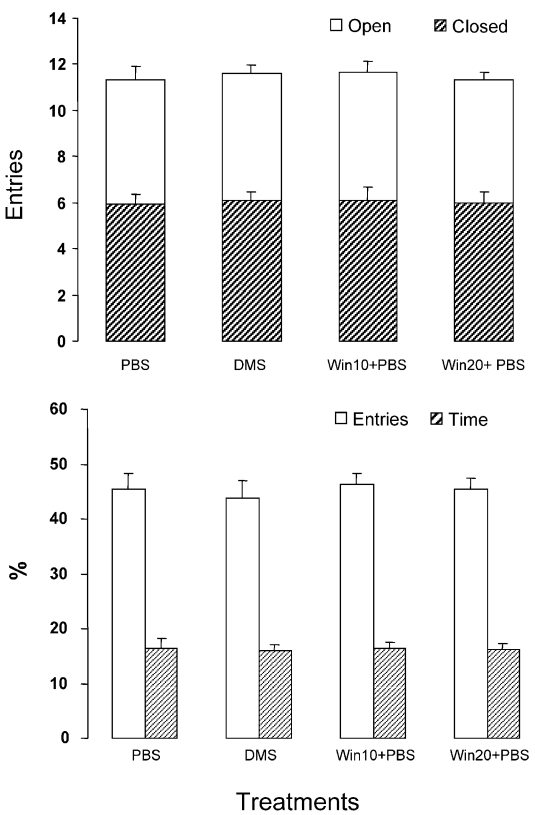

Fig. 2. Effects of Win51,708 (20 mg/kg, i.p.) on exploratory behavior of rats (mean ± S.E.M.) in the elevated plus-maze. Top: number of entries into both types of arms. Bottom: percent of entries and time spent in the open arms in relation to totals. PBS = phosphate-buffered saline, DMS = dimethylsulfoxide (0.3%) in PBS. $N = 12$ for each group.

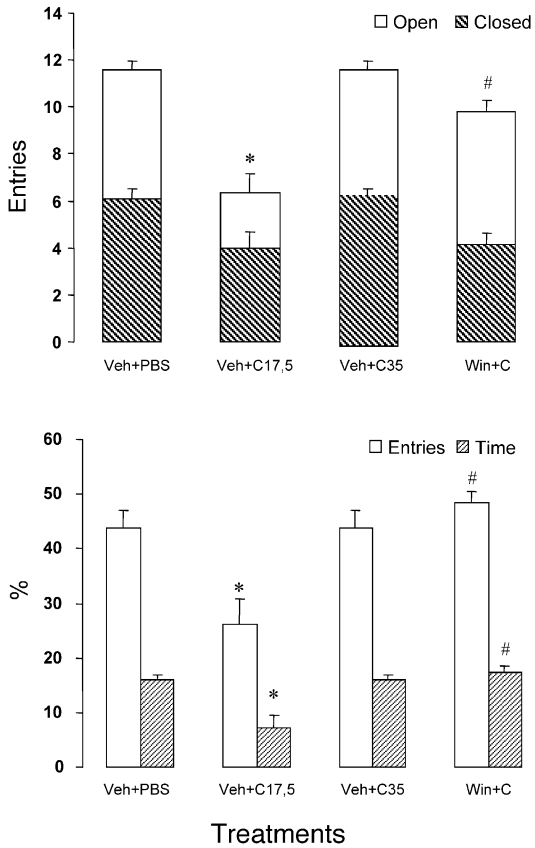

Fig. 3. Effects of Win51,708 (20 mg/kg, i.p.) on the effects of C-fragment of substance P (17.5 nmol) microinjections into the dorsal periaqueductal gray on exploratory behavior of rats (mean ± S.E.M.) in the elevated plus-maze. Each animal was injected twice; Win51,708 or its vehicle (dimethylsulphoxide 0.3%) followed by dorsal periaqueductal gray C-fragment or PBS (0.2 μl). The interval between the first and second injections was 20 min. Top: number of entries into both types of arms. Bottom: percent of entries and time spent in the open arms in relation to totals. $N = 12$ for each group. *: Different from entries and time spent in that type of arm in the control group and #: different from the Win + C group ($P < 0.05$, Newmann–Keuls test). PBS = phosphate-buffered saline. Veh = PBS containing 0.3% dimethylsulfoxide. C = C-fragment.

3.1. Effects of Win51,708

ANOVA showed no significant effect of Win51,708 injections ($df = 3,44$) upon the open arm entries ($F = 0.05$; $P > 0.05$), closed arm entries ($F = 0.03$; $P > 0.05$) and percent of entries in the open arms in relation of the total entries ($F = 0.15$; $P > 0.05$) or percent of time spent in the open arms in relation to the total time of the test ($F = 0.02$; $P < 0.05$).

The administration of Win51,708 ($df = 3,44$) did not affect end-arm activity ($F = 0.02$; $P > 0.05$), rearing ($F = 0.06$; $P > 0.05$); scanning ($F = 0.05$; $P > 0.05$), stretched-attend postures ($F = 0.6$; $P > 0.05$), flat-back approach ($F = 0.80$; $P > 0.05$), peeping-out ($F = 0.40$; $P > 0.05$), head-dipping ($F = 0.59$; $P > 0.05$), immobility ($F = 0.97$; $P > 0.05$) and grooming ($F = 0.84$; $P > 0.05$). These effects are depicted in Fig. 2. Thereafter, only the

effects obtained with the dose of 20 mg/kg Win51,708 are shown since the dose of 10 mg/kg of Win51,708 did not cause any significant changes on the effects of C- or N-fragments of substance P .

3.2. Effects of C-terminal fragment

ANOVA indicated a significant reduction of the C-fragment of substance P injections into the dorsal periaqueductal gray ($df = 4,55$) upon number of entries into the open arms ($F = 5.66$; $P < 0.01$) and time spent ($F = 14.40$; $P < 0.001$) on the open arms of the maze. The number in the closed arms was also reduced by the treatments ($F = 4.98$, $P = 0.005$) so that the percentage of entries open/closed arms did not change significantly ($F = 0.96$; $P > 0.05$). Post-hoc analysis showed that these effects were due mainly to the dose of 17.5 pmol of the C-terminal fragment (Fig. 3). The effects of this dose of the C-fragment were significantly reduced by previous treatment with Win51,708. These effects are illustrated in Fig. 3.

Fig. 4A illustrates the effects of C-terminal fragment injections into the dorsal periaqueductal gray. ANOVA ($df = 4,55$) detected significant effects on scanning ($F = 10.20$; $P < 0.001$), stretched-attend postures ($F = 9.10$; $P < 0.001$), flat-back approach ($F = 28.27$; $P < 0.001$). Dunnett's post-hoc analysis ($P < 0.05$) showed that C-terminal fragment increased scanning, stretched-attend postures and flat-back-approach at both doses used (17.5 and 35 pmol). The remaining behaviors were not affected by C-terminal fragment of substance P; end-arm activity ($F = 0.93$; $P > 0.05$), rearing ($F = 1.85$; $P > 0.05$), peeping-out ($F = 1.71$; $P > 0.05$), head-dipping ($F = 0.15$; $P > 0.05$), grooming ($F = 0.91$; $P > 0.05$) and immobility ($F = 1.03$; $P > 0.05$). Win51,708 (20 mg/kg) in combination with C-fragment (17.5 pmol) blocked the anxiogenic effects of C-fragment on the risk assessment behaviors. Win51,708 without the fragment had no significant effect on these measures. These effects are depicted in Fig. 4A.

3.3. N-terminal effects

ANOVA ($df = 4,55$) performed on the drug effects on conventional measures revealed a significant increase produced by the N-fragment of substance P injections into the dorsal periaqueductal gray upon number of entries into the open arms ($F = 9.25$; $P < 0.001$), in the closed arms ($F = 3.10$; $P > 0.05$) and time spent ($F = 15.02$; $P < 0.001$) on the open arms of the maze. Post-hoc analysis showed that these effects were due to the dose of 35 pmol of the N-terminal fragment (Fig. 5). Win51,708 (20 mg/kg) in combination with N-fragment (35 pmol) had no

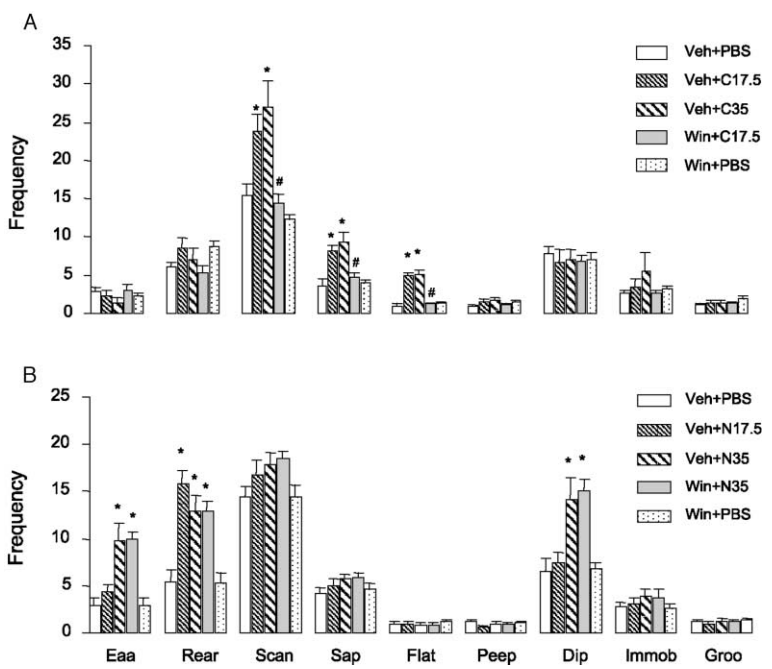

Fig. 4. (A) Antagonism by Win51,708 of the effects of injections of C-terminal fragment of substance P (17.5 and 30 pmol) into the dorsal periaqueductal gray on the behavior of rats on the elevated plus-maze. Win51,708 was tested against 17.5 pmol of C-fragment. (B) Effects of injections of N-terminal fragment of substance P (17.5 and 35 pmol) into the dPAG on the behavior of rats on the elevated plus-maze. Win51,708 was tested against 35 pmol of N-fragment. Eaa = end-arm activity; Rear = rearing; Scan = scanning; Sap = stretched-attend posture; Flat = flat-back approach; Peep = peeping-out; Immo = immobility; Groo = grooming. PBS = phosphate-buffered saline. Veh = PBS containing 0.3% dimethylsulfoxide. C and N = fragments C and N. Data are presented as mean ± SEM. $N = 12$ for each group. *: Different from the respective control group (PBS)and #: different from the Win + C group ($P < 0.05$, Newmann–Keuls test).

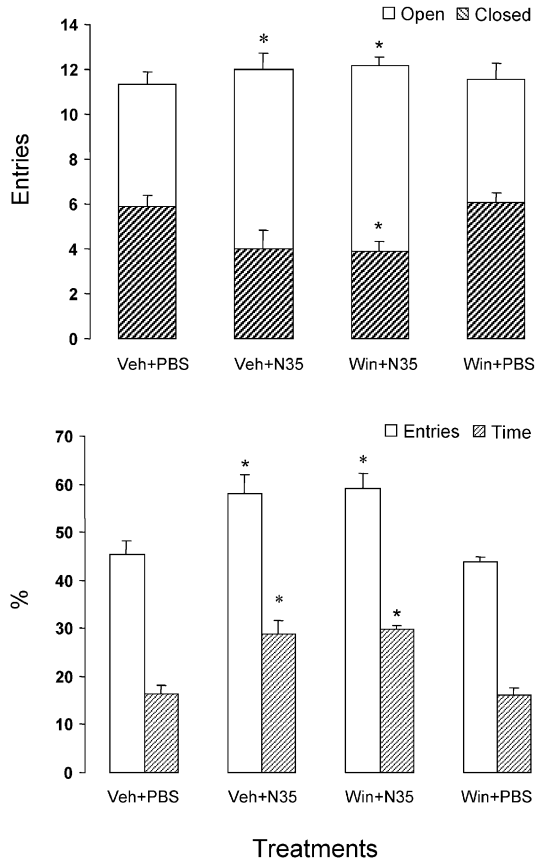

Fig. 5. Effects of Win51,708 (20 mg/kg, i.p.) on the effects of N-fragment of substance P (35 pmol) microinjections into the dorsal periaqueductal gray on exploratory behavior of rats (mean ± S.E.M.) in the elevated plus-maze. Each animal was injected twice; Win51,708 or its vehicle (dimethylsulphoxide 0.3%) followed by dorsal periaqueductal gray N-fragment or PBS (0.2 μl). The interval between the first and second injections was 20 min. Top: number of entries into both types of arms. Bottom: percent of entries and time spent in the open arms in relation to totals. $N = 12$ for each group. *: Different from entries and time spent in the control group ($P < 0.05$, Newmann–Keuls test). PBS = phosphate-buffered saline. Veh = PBS containing 0.3% dimethylsulfoxide. N = N-fragment.

influence on the effects of this fragment on the conventional measures. The pre-treatment with Win51,708 without the fragment had no significant effect on these measures.

Fig. 4B illustrates the effects of N-terminal fragment injections into the dorsal periaqueductal gray. ANOVA ($df = 4,55$) detected significant effects on end-arm activity ($F = 11.31$; $P < 0.001$), rearing ($F = 12.58$; $P < 0.001$) and head dippings ($F = 8.64$; $P < 0.001$). Newmann–Keuls post-hoc analysis ($P < 0.05$) showed that N-terminal fragment increased end-arm activity and head dippings only at the dose of 35 pmol whereas it increased rearing at both doses used (17.5 and 35 pmol). The remaining behaviors were not affected significantly by this fragment. Here, also Win51,708 (20 mg/kg) in combination with N-fragment (35 pmol) had no influence on the effects of this fragment on the ethological measures.

4. Discussion

In the present study, the C-fragment of substance P produced clear aversive effects, as measured by the elevated plus-maze test, when injected directly into the dorsal periaqueductal gray. This C-terminus peptide produced a clear increase of unconditional fear, as measured by the decrease in the number of entries into and the relative time spent on the open arms of the elevated plus maze. These data support previous reports showing that this fragment produces defensive behaviors when injected at this level of the brainstem of rats submitted to the open field test (De Araújo et al., 1998). Although the C-fragment of substance P also caused a reduction in the number of closed arm entries, these effects are not compatible with changes in motor activity since the same injections, at similar doses, of this neuropeptide into the dorsal periaqueductal gray caused a behavioral activation of rats submitted to an open field test (De Araújo et al., 1999). The effects of the C-fragment seem to be particularly linked to defensive reactions, as measured by the increase of anxiogenic-like responses, such as stretched-attend postures, scanning and flat-back approach (Blanchard et al., 1991; Rodgers and Johnson, 1995; Anseloni and Brandão, 1997). It should be mentioned here that injections of substance P and its C-terminus fragment into structures where they usually produce reinforcing effects, such as nucleus basalis magnocellularis, tend to produce anxiolytic-like effects (Nikolaus et al., 1999). In the present work, microinjections of C-fragment of substance P caused a clear anxiogenic-like effect, similar to the effects produced by the whole molecule. Thus, it is possible that the C-terminus of the substance P-molecule may also encode the aversive effects of substance P injection into the dorsal periaqueductal gray.

A number of studies have implicated substance P in processes of learning, memory, and reinforcement (Huston et al., 1993; Huston and Hasenöhrl, 1995; Holzhauer-Oitzl et al., 1988). Aversive properties have also been reported depending on the dose of substance P used and the site of action (Culman and Unger, 1995; De Araújo et al., 1998, 1999; Brandão et al., 1999). However, while medial forebrain bundle, medial septum and the nucleus basalis magnocellularis seem to be the preferential regions for the reinforcing effects of substance P (Huston et al., 1993; Huston and Hasenöhrl, 1995), the aversive effects have been associated to mesencephalic structures. In fact, previous papers have already shown the ability of i.c.v. administration (Elliot and Iversen, 1986; Elliot, 1988) and dorsal periaqueductal gray microinjections of substance P to cause place aversion (Aguiar and Brandão, 1994). Injections of substance P into the ventricles, the ventral mesencephalon and into the dorsal periaqueductal gray have been found to cause increased locomotion, grooming and scratching (Aguiar and Brandão, 1994; Elliot and Iversen, 1986; Elliot, 1988). Furthermore, it has been found that injec-

tions of substance P into the dorsal periaqueductal gray increase mean arterial blood pressure and tachycardia, autonomic effects characteristic of the defense reaction (Ku et al., 1998). As observed in previous work from this laboratory with substance P, the aversive effect of its C-terminus seem also to be dose-dependent since only 17.5 pmol was effective and the high dose used (35 pmol) was ineffective (De Araújo et al., 1998, 1999). Apart from differences in the range of doses of these latter studies (17.5–70 pmol), which might simply reflect the interaction of different batches of rats with the test conditions, this particular inverted U-shaped dose–effect function has also been reported in memory and reinforcement studies as well as in other works with animal models of anxiety, in which the effects of substance P occur over a narrow range of doses (Aguiar and Brandão, 1996; Huston et al., 1993, Hasenöhrl et al., 2000).

Tachykinin NK_1 receptors seem to be the type of tachykinin receptors involved in the aversive effects produced by substance P and its carboxi-terminal fragment, as peripheral administration of WIN51,708 clearly blocked these effects. WIN51,708 is a competitive antagonist at the tachykinin NK_1 receptor (Venepalli et al., 1992; Regoli et al., 1994) and has shown species-selective interaction with this receptive site, being more potent in rat than in guinea pig or human tissues in binding to and blocking of the tachykinin NK_1 receptor (Appell et al., 1992; Sachais and Krause, 1994). Hence, the attenuation of the effects of the C-terminus of substance P-induced aversion indicates that the aversive effects of the neurokinin in the dorsal periaqueductal gray were mediated via tachykinin NK_1 receptive sites. The mediation of the aversive effects of substance P by these kind of receptors are further supported by the fact that dorsal periaqueductal gray injections of agonists of these receptors caused freezing behavior (Mongeau et al., 1998). Furthermore, injection of agonists of these receptors into the ventricles produced anxiogenic-like effects and its antagonists produced anxiolytic-like effects in mice submitted to the elevated plus-maze test (Teixeira et al., 1996). Substance P has also been implicated in the mediation of defensive behavior in other regions of the brain aversive system, besides the midbrain tectum, such as the amygdala and medial hypothalamus (Shaikh et al., 1993; Smith et al., 1999). These studies support the hypothesis of the involvement of substance P in the mediation of central stress responses and may have relevance to the possibility of the clinical use of neurokinin receptor antagonists as anxiolytic or antidepressant agents (Kramer et al., 1998, Santarelli et al., 2001).

Injections of the N-terminal fragment of substance P into the dorsal periaqueductal gray produced a behavioral profile quite different from its C-terminal fragment. This fragment produced anxiolytic-like effects measured by the conventional and ethological behavioral categories recorded in this study. In line with these results are the studies of motor behavior following i.c.v. injections of substance P or fragments, showing that N-terminal fragments increased rearing while C-fragments did not (Hall et al., 1987). Furthermore, N-terminus reduced whereas C-terminus elevated monoaminergic activity (Hall and Stewart, 1992). Similarly, isolation-induced fighting is reduced by N-terminus, but elevated by C-terminus of the substance P molecule (Hall and Stewart, 1984). Several other reports have also suggested that this fragment has a functional role distinct from the C-terminal (Huston et al., 1993; Huston and Hasenöhrl, 1995; Holzhauer-Oitzl et al., 1998; Hasenöhrl et al., 1998).

Taken together, the present work shows that the C-terminal fragment of substance P seems to be related to the known aversive effects produced by the whole substance P molecule (1–11) in the dorsal periaqueductal gray matter. Injections of the N-terminal fragment into the dorsal periaqueductal gray produced opposite effects, confirming previous studies in other tests that indicate distinct functional roles for the N- and C-carboxi-terminals of substance P in brain.

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