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Reinforcing effects of neurokinin substance P in the ventral pallidum: mediation by the tachykinin NK_1 receptor

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Abstract

The neurokinin substance P has reinforcing effects when administered into the nucleus basalis of the rat's ventral pallidum and these effects are encoded by its carboxy-terminal amino acid sequence. The present study examined the effect of prior treatment with the tachykinin NK_1 receptor antagonist WIN51,708 on the conditioned place preference produced by intrabasalis injection of substance P and its carboxy-terminal heptapeptide analog dimethyl-C7. Pretreatment with WIN51,708 (10 and 20 mg/kg, i.p.) dose-dependently reversed the place preference produced by intrabasalis substance P (0.74 pmol). The carboxy-terminal analog dimethyl-C7 (0.74 pmol) was also found to act as a reinforcer following injection into the nucleus basalis region, but unlike for substance P, the behavioral effects of dimethyl-C7 could not be completely antagonized by joint administration of the NK_1 antagonist. When injected alone, WIN51,708 did not influence the preference behavior. These findings suggest that the reinforcing effects of substance P in the nucleus basalis region might be mediated via NK_1 receptive sites. The failure of WIN51,708 to completely antagonize the behavioral effects of dimethyl-C7 is interesting in the light of evidence, indicating that the carboxy-terminal substance P analog shows higher affinity for the tachykinin NK_3 than for the NK_1 receptor subtype.

Keywords: Basal forebrain; Nucleus basalis magnocellularis; Dimethyl-C7; Tachykinin NK_1 receptor antagonist; WIN51,708; Reinforcement; Place conditioning

1. Introduction

The nucleus basalis magnocellularis is a region of the rodent basal forebrain consisting of parts of the capsula interna and ventromedial aspects of the globus pallidus, particularly at caudal levels (Alheid and Heimer, 1988). The nucleus basalis provides most of the cholinergic input to the neocortex and the amygdala (Bigl et al., 1982; Ingham et al., 1985). In the nucleus basalis, the cholinergic neurons are innervated by various transmitter systems, such as by cholinergic, serotonergic and noradrenergic afferents (Zaborszky et al., 1991) and by several neuropeptides, including the neurokinin substance P (Ljungdahl et al., 1978; Kiyama et al., 1993). The substance P input to the nucleus basalis probably originates from the nucleus accumbens (Napier et al., 1995) and within the nucleus basalis, these afferents contact cholinergic neurons which express mRNA encoding for substance P (NK_1) receptors (Bolam et al., 1986; Gerfen, 1991).

Previous studies revealed that local administration of substance P into the nucleus basalis region has memory-promoting (Kafetzopoulos et al., 1986; Nagel and Huston, 1988), reinforcing (Hasenöhrl et al., 1998a; Holzhäuer-Oitzl et al., 1988) and anxiolytic-like effects (Hasenöhrl et al., 1998b), pointing to a functional role for substance P-containing inputs to this area of the brain. In search for possible physiological mechanisms that may underlie these behavioral effects, it was shown that injection of substance P into the nucleus basalis can increase the level of dopamine in the nucleus accumbens (Boix et al., 1995) and acetylcholine in the frontal cortex (De Souza Silva et al., 1997). Recent experiments on the structure-activity relationship of substance P evidenced that the mnemonic and reinforcing effects of intrabasalis substance P are encoded by different amino acid sequences of the neurokinin, since the amino(N)-terminal substance P_{1-7} enhanced memory,

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whereas carboxy(C)-terminal hepta- and hexapeptide sequences proved to be reinforcing (Huston and Hasenöhrl, 1995; Huston et al., 1993). The sequence-specific effects of substance P and the minimal sequence overlap of the active substance P fragments raised the question on which type of tachykinin receptive site the neurokinin and its fragments exert their beneficial behavioral effects. Substance P acts preferentially at the tachykinin NK_1 receptor (Quirion and Dam, 1988). Since these receptors are expressed on cholinergic nucleus basalis neurons (Gerfen, 1991), it was held possible that the effects of intrabasalis substance P on reinforcement and memory are a consequence of its interaction with NK_1 receptors, either directly or through the release of an active fragment.

The intention of the present experiment was to gauge the possible involvement of the NK_1 receptor in the reinforcing action of intrabasalis substance P and its C-terminal sequence. This was accomplished by examining the effect of prior treatment with the tachykinin NK_1 receptor antagonist WIN51,708 on conditioned place preference produced by intrabasalis injection of substance P and its C-terminal heptapeptide analog dimethyl-C7. WIN51,708 (Venepalli et al., 1992) belongs to the family of nonpeptide tachykinin NK_1 receptor antagonists displaying a high affinity for the rat NK_1 receptive site (Appell et al., 1992; Sachais and Krause, 1994). Since it has been shown, for one, that intrabasalis injection of substance P and its C-terminal fragments can produce conditioned place preference (Huston and Hasenöhrl, 1995), and, secondly, that application of the NK_1 antagonist WIN51,708 can antagonize physiological effects of substance P and its C-terminus (Anderson et al., 1994; Khan et al., 1996, 1998), we supposed that prior treatment with WIN51,708 could block the reinforcing effects of both substance P as well as dimethyl-C7.

2. Materials and methods

2.1. Animals

The experiments were carried out in accordance with the German Law on the Protection of Animals and were approved by the state authority (Bezirksregierung Düsseldorf). Male Wistar rats ($n = 142$; TVA, University of Düsseldorf) weighing 200–300 g were used. They were housed under standard laboratory conditions with food and water continuously available. A light–dark schedule (12 L:12 D lights on 0700 h) was imposed and all behavioral testing was done during the rats' daylight period between 0900 and 1830 h.

2.2. Apparatus

The corral apparatus, which has been described in detail previously (Hasenöhrl et al., 1989), was used to measure conditioned place preference. The corral consisted of a circular open field, with a diameter of 83 cm and 43-cm high walls. Two intersecting lines in the floor divided the open field into four quadrants of equal size, identical floor and wall textures, and identical color. Spatial orientation was provided by distinct cues situated in the observation chamber around and above the apparatus. Each session was recorded by a video camera connected to a video recorder for later analysis.

2.3. Surgery and histology

Rats were anesthetized (0.9 ml/kg Ketavet; 0.4 ml/kg Rompun; i.p.) and implanted unilaterally with a guide cannula (22 G), aimed at the nucleus basalis (bregma coordinates: -1.3 anterior, ± 2.8 lat., 6.5 mm depth; Paxinos and Watson, 1986). The guide cannula, containing a stylet which extended 0.1 mm beyond the tip, was positioned 0.5 mm above the injection site. The cannula was attached to the skull by means of acrylic cement and stainless-steel screws. Postoperatively the animals were allowed to recover for eight days. During this interval, the rats were handled and weighed daily. At the beginning of the behavioral tests, all animals had recovered to at least 100% of their preoperative body weight. At the termination of behavioral testing, the animals were deeply anesthetized with Nembutal (3 ml/kg; i.p.) for perfusion and subsequent histological survey regarding the injection site. No animal had to be discarded on account of cannula placement outside the ventral pallidum/NBM complex.

2.4. Drug treatment

Peptides were purchased from Sigma (Germany). Substance P (molecular weight 1347.80) and dimethyl-C7 (molecular weight 880.13) were dissolved and diluted to the desired concentration with phosphate-buffered saline (PBS; pH 7.2). The dose of substance P and dimethyl-C7 used was 0.74 pmol, that is, 1 ng substance P and 0.65 ng dimethyl-C7. At this dosage both substance P as well as dimethyl-C7 have repeatedly been shown to serve as a reinforcer in place preference tasks after intrabasalis injection (Holzhäuer-Oitzl et al., 1988; Hasenöhrl et al., 1992). Rats of the control group received PBS. Intracerebral injections were made into hand restrained rats in a volume of 0.5 μl administered over 30 s with an injection cannula (28 G) inserted to a depth of 0.5 mm below the tip of the guide cannula. The hemisphere into which an injection was made was balanced within the treatment groups to control for possible lateralization effects. Upon completion of the injection, the injection cannula was left in place for an additional 30 s. After withdrawal of the injection cannula, another 0.5 μl of the solution was ejected to check for possible clogging. WIN51,708, from Biotrend Chemicals (Germany), was dissolved in PBS containing 0.3% dimethylsulfoxide (Sigma). The doses of WIN51,708 were

10 and 20 mg/kg, injected i.p. in a volume of 1 ml/kg; the same volume was used for injecting the vehicle (VEH; phosphate-buffered saline containing 0.3% dimethylsulfoxide).

2.5. Behavioral procedure

The place preference procedure was carried out on three consecutive days. Each daily trial lasted 15 min. During baseline (day 1), the rat was placed in the center of the open field and had free access to all parts of the apparatus ('open corral'). A rat was considered to be in one quadrant of the field when its head and the two forepaws were inside. After the baseline trial the treatment corral, that is, one of the four quadrants in which the animal had spent neither the most, nor the least time during the baseline session, was determined (As in previous studies using this corral method (Hasenöhrl et al., 1989, 1990, 1992) the rats did not develop a significant preference for a certain corral prior to drug treatment). During the conditioning session (day 2), the open field was divided by transparent Plexiglas barriers into four quadrants of equal size ('closed corral'). The rats were assigned to the following treatment groups: Part I (WIN51,708 in combination with substance P): VEH + PBS ($n = 19$), VEH + substance P ($n = 33$), WIN 10 mg/kg + substance P ($n = 16$), WIN 20 mg/kg + substance P ($n = 15$), WIN 10 mg/kg + PBS ($n = 16$) and WIN 20 mg/kg + PBS ($n = 8$); Part II (WIN51,708 in combination with dimethyl-C7): VEH + PBS ($n = 9$), VEH + dimethyl-C7 ($n = 14$) and WIN 20 mg/kg + dimethyl-C7 ($n = 12$). Thus, rats were injected i.p. with the different doses of WIN51,708 or VEH 20 min before being injected with substance P, dimethyl-C7 or PBS into the nucleus basalis region. Immediately thereafter, the rats were placed into one of the four restricted quadrants of the corral apparatus. On the test day (day 3) the Plexiglas barriers were removed and the rats had free access to the four quadrants ('open corral'). During the baseline and test session, time spent in each of the quadrants was scored and, as a measure of gross locomotor activity, the number of entries into the four quadrants was recorded.

2.6. Data analysis

The nonparametric Fisher–Pitman test (Krauth, 1988) was applied to test for between-group differences and exact P values are presented as a descriptive measure of effect.

3. Results

3.1. Conditioned corral preference

During the baseline trial, the rats spent a comparable amount of time in each of the four quadrants, with no preference for any compartment. A positive reinforcing

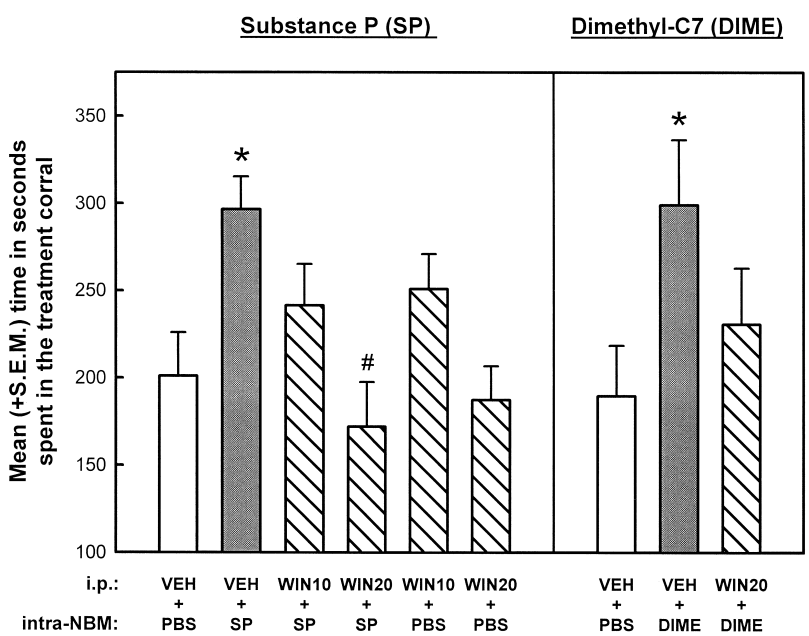

Fig. 1. Mean (+S.E.M.) time in seconds spent in the treatment corral on the day of testing. Rats were injected i.p. with different doses of the NK_1 receptor antagonist WIN51,708 (WIN 10 and WIN 20 mg/kg) or VEH (1 ml/kg) 20 min before being injected with 0.74 pmol (1 ng) substance P or with equimolar dosed dimethyl-C7 (0.65 ng) into the nucleus basalis magnocellularis region. During baseline, the different groups of animals spent a comparable amount of time in the treatment corral (about 225 s). $*P < 0.05$ vs. corresponding VEH + PBS treated controls; $\#P < 0.05$ vs. corresponding VEH pre-treated rats.

Table 1
(A) Number of entries into all quadrants, (B) entries into the treatment corral (TC) and (C) time in seconds spent per entry into the TC during baseline (BL) and test (TE) trials

	(A) Entries into all quadrants		(B) Entries into TC		(C) Time (s) spent per entry into TC	
	BL	TE	BL	TE	BL	TE
Substance P						
VEH + PBS	101.79 ± 7.04	92.42 ± 8.09	24.26 ± 1.76	23.11 ± 1.97	7.14 ± 0.35	9.11 ± 1.07
VEH + SP	101.97 ± 5.26	87.70 ± 4.93	24.18 ± 1.37	22.85 ± 1.29	8.56 ± 0.43	13.98 ± 1.04[a]
WIN10 + SP	106.75 ± 6.12	88.31 ± 6.44	26.12 ± 1.92	22.00 ± 1.67	7.83 ± 0.54	12.34 ± 1.89
WIN20 + SP	89.27 ± 5.77	75.53 ± 5.56	22.47 ± 1.45	18.27 ± 1.58	8.93 ± 1.06	9.34 ± 1.13
WIN10 + PBS	105.88 ± 5.28	103.44 ± 6.52	25.06 ± 1.57	26.56 ± 1.88	7.59 ± 0.38	10.11 ± 1.08
WIN20 + PBS	100.38 ± 8.64	89.38 ± 11.43	22.50 ± 2.67	21.00 ± 2.40	7.58 ± 0.53	9.32 ± 0.96
Dimethyl-C7						
VEH + PBS	102.78 ± 12.11	89.67 ± 5.97	24.67 ± 3.66	20.22 ± 1.48	7.92 ± 1.10	9.21 ± 1.23
VEH + DIME	89.58 ± 6.84	79.75 ± 4.83	21.17 ± 1.78	20.33 ± 1.28	10.13 ± 0.78	15.13 ± 1.93[a]
WIN20 + DIME	97.50 ± 5.54	75.43 ± 6.82	22.50 ± 1.31	18.93 ± 1.99	8.84 ± 0.64	14.95 ± 3.38

Values are means ± S.E.M.
[a] $P < 0.05$ vs. corresponding VEH-PBS controls.

effect of the drug treatment was defined by the occurrence of place preference behavior, as reflected by a significant increase in the amount of time spent in the treatment corral during test. Fig. 1 depicts the amount of time spent in the treatment corral for the different treatment groups. Rats treated with VEH in combination with 0.74 pmol substance P spent significantly more time in the drug-paired corral compared to VEH + PBS injected controls ($P = 0.002$). About 52% of the rats injected with substance P showed an absolute preference for the quadrant previously paired with the drug. WIN51,708, when given prior to substance P injection, was found to attenuate the corral preference produced by the neurokinin in a dose-dependent manner. Rats injected with substance P in combination with 10 mg/kg WIN51,708 spent less time in the treatment corral, while at the higher dose of 20 mg/kg the NK_1 antagonist completely blocked the substance P-induced corral preference (WIN20 mg/kg + substance P vs. VEH + substance P, $P < 0.001$). The pre-treatment with WIN51,708 without peptide had no significant effect on preference behavior on its own (WIN10 mg/kg + PBS vs. VEH + PBS, $P = 0.140$; WIN20 mg/kg + PBS vs. VEH + PBS, $P = 0.741$). Rats treated with VEH in combination with 0.74 pmol dimethyl-C7 spent significantly more time in the drug-paired corral compared to VEH + PBS injected controls ($P = 0.016$). About 42% of the rats injected with 0.74 pmol dimethyl-C7 showed an absolute preference for the quadrant previously paired with the drug. WIN51,708 was found to attenuate the corral preference induced by dimethyl-C7. Rats injected with dimethyl-C7 in combination with 20 mg/kg WIN51,708 did not display a conditioned corral preference (WIN20 mg/kg + dimethyl-C7 vs. VEH + PBS, $P = 0.193$); however, the NK_1 antagonist did not completely block the corral preference produced by the heptapeptide analog (WIN20 mg/kg + dimethyl-C7 vs. VEH + dimethyl-C7, $P = 0.159$).

3.2. Analysis of entry data

The analysis of entry data revealed that there were no treatment-related differences in the number of entries into the four quadrants of the open corral before (baseline) and after treatment (test) in the closed corral (Table 1A). In most cases, the animals were somewhat less active during the test session compared to baseline values. Further, the treatment conditions did not influence the number of entries into the treatment corral. During both baseline and test, about 25% of entries were into the treatment corral (Table 1B). However, rats treated with 0.74 pmol substance P or dimethyl-C7 in combination with VEH spent significantly more time per entry in the previously drug-paired corral compared to VEH + PBS controls (VEH + substance P, $P = 0.002$; VEH + dimethyl-C7, $P = 0.010$; Table 1C). These results suggest that the corral preference evident in substance P and dimethyl-C7 treated rats was due to a longer time spent in the treatment corral during each entry, rather than an increase in the number of entries into the treatment corral per se.

4. Discussion

The present results substantiate previous studies showing that substance P and its C-terminal heptapeptide analog dimethyl-C7 can exert positively reinforcing effects when administered into the nucleus basalis region (Holzhäuer-Oitzl et al., 1988; Hasenöhrl et al., 1992). As expected, a single injection of 0.74 pmol substance P and dimethyl-C7 into the nucleus basalis resulted in a preference for the corral which had previously been paired with the drug treatment. Novel is the finding that the reinforcing effects elicited by intrabasalis substance P could be blocked by peripheral administration of WIN51,708, whereas only a

partial reversal was observed for the reinforcing effect of dimethyl-C7. WIN51,708 is a competitive antagonist at the tachykinin NK_1 receptor (Venepalli et al., 1992) 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 substance P induced corral preference by WIN51,708 indicates that the reinforcing effects of the neurokinin in the nucleus basalis region were mediated via NK_1 receptive sites. Furthermore, the antagonistic effect of WIN51,708 on substance P-induced place preference appeared to be dose-dependent since rats injected with substance P in combination with 10 mg/kg WIN51,708 spent less time in the treatment corral, while at the higher dose of 20 mg/kg the antagonist completely blocked the substance P-induced corral preference. A similar dose-response relationship has recently been reported for the effects of systemically administered WIN51,708 on dopamine D_1 receptor-stimulated release of striatal acetylcholine (Anderson et al., 1994). Furthermore, the increase in striatal dopamine outflow caused by substance P and its C-terminus could be blocked by WIN51,708 (Khan et al., 1996, 1998), whereas only partial reversal was observed for its N-terminal sequence (Khan et al., 1995). With regard to the present findings, it is feasible that the behavioral (Huston and Hasenöhrl, 1995) as well as neurochemical effects of intrabasalis substance P revealed in previous experiments (Boix et al., 1995; De Souza Silva et al., 1997) were actually dependent on the action of the neurokinin on NK_1 receptive sites.

The limited sensitivity of dimethyl-C7-induced place preference to the action of WIN51,708 observed in the present study suggests, that the reinforcing effects of the C-terminal analog might be mediated, in part, through binding sites which are different from that activated by substance P. It has been reported that carboxy-terminal substance P analogs, including dimethyl-C7, display a greater affinity for the tachykinin NK_3 than for the tachykinin NK_1 receptor and show a higher NK_3/NK_1 receptor affinity ratio (for review see Regoli et al., 1994). Thus, the additional action of dimethyl-C7 at tachykinin NK_3 sites could explain why WIN51,708 only partially reversed its reinforcing effects. However, these findings raise the possibility that within the ventral pallidum both NK_1 as well as NK_3 receptors could be involved in the control of reinforcement processes. Congruent with this suggestion, the specific tachykinin NK_3 receptor agonist aminosenktide was found to exert reinforcing effects in a place preference task (Ciccocioppo et al., 1998) and to interfere negatively with the reinforcing effects of ethanol ingestion following injection into the nucleus basalis region (Ciccocioppo et al., 1997).

Alternative interpretations of the WIN51,708-produced attenuation of substance P and dimethyl-C7 induced corral preference other than in terms of pharmacological antagonism also have to be taken into account. WIN51,708 on its own neither significantly influenced the preference behavior, nor the entry parameters during the testing period. These results argue against the possibility that the antagonist might have attenuated the reinforcing effects of substance P and dimethyl-C7 as a result of having aversive effects. WIN51,708 at the dose of 10 mg/kg even showed a tendency to increase the time spent in the treatment corral, suggesting that in lower doses the compound can exert agonistic activity, which is possibly mediated via its binding to the Ser_{290} domain of the NK_1 receptor protein (Appell et al., 1992; Venepalli et al., 1992). Furthermore, it appears that substance P can have reinforcing as well as aversive effects, depending on the site of action in the brain (for review see Huston and Oitzl, 1989). Thus, it is also possible that the increase in sojourn time observed after systemic WIN51,708 injection could be related to the NK_1-antagonistic action of the compound at brain sites where substance P plays a role in the mediation of aversive states, such as the amygdala (Shaikh et al., 1993) and the periaqueductal gray (Aguiar and Brandao, 1994; De Araujo et al., 1998).

The substance P input to the nucleus basalis/ventral pallidum complex originates largely from the nucleus accumbens (Napier et al., 1995), which is known to be activated by a variety of reinforcing stimuli, including drugs of abuse (Bardo, 1998), and which is considered to function as an interface between motivational signals and motor or 'action systems' of the brain (Mogenson, 1987). The ventral pallidum itself sends fibers to several reward implicated regions such as the amygdala and the prefrontal cortex (Groenewegen and Berendse, 1993). Recent studies performed with in vivo microdialysis have evinced that substance P injections into the nucleus basalis can lead to increases of acetylcholine release in the frontal cortex (De Souza Silva et al., 1997). This finding suggests that substance P efferents from the nucleus accumbens may serve to relay to the neocortex, via pallidal cholinergic cells, processes related to reward, and thus, implicitly processes involved in memory consolidation and storage (Huston and Oitzl, 1989). In line with this premise it was recently reported that direct electrical activation of the ventral pallidum maintains self-stimulation (Panagis et al., 1995), which is accompanied by a frequency-dependent increase in c-*fos* expression in various reward-related brain regions, including the frontal cortex (Panagis et al., 1997). Furthermore, we recently found that intrapallidal injection of substance P can produce an increase of dopamine release in the nucleus accumbens which was linked to the reinforcing effects of the neurokinin (Boix et al., 1995). This indicates that there is also feedback to the nucleus accumbens in terms of substance P effects in the ventral pallidum, either directly from pallidal neurons, or, more likely, via the frontal cortex, which is known to innervate the nucleus accumbens (Groenewegen et al., 1991). Thus, the available information provides evidence for a potential

mechanism by which application of WIN51,708 could have antagonized the reinforcing effects of intrabasalis substance P, namely, that blockade of tachykinin NK_1 receptors within the nucleus accumbens-ventral pallidum circuitry interferes negatively with the activity of acetylcholine in frontal cortex and dopamine in nucleus accumbens, which, under normal conditions may determine the development of place preference and the 'strength' of conditioning.

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