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Behavioural evidence for cholecystokinin-dopamine D_1 receptor interactions in the rat

Jackalina Van Kampen, Hanna Frydryszak [1], A. Jon Stoessl *

Department of Clinical Neurological Sciences, University of Western Ontario and Robarts Research Institute, London, ON, Canada

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Abstract

The effects of cholecystokinin (CCK) on behavioural responses to the dopamine D_1 receptor agonist (±)SKF 38393 ((±)-2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine HCl) were studied in the rat. SKF 38393 (5 mg/kg s.c.) induced stereotyped grooming and vacuous chewing movements. Both responses were inhibited by CCK-8S (10–50 μg/kg i.p.), but the preferential CCK_B receptor agonist CCK-4 (20–100 μg/kg i.p.) attenuated SKF 38393-induced grooming only. Suppression of SKF 38393-induced grooming and vacuous chewing movements by CCK-8S was blocked by the selective CCK_A receptor antagonist MK-329 (also known as devazepide or L-364,718) (0.1, 0.3 mg/kg i.p.) but unaffected by the CCK_B receptor antagonist L-365,260 (0.1, 0.3 mg/kg i.p.). We conclude that CCK can modify dopamine-mediated behavioural responses, possibly reflecting an action post-synaptic to dopamine terminals. The effect on dopamine D_1 receptor agonist-induced vacuous chewing movements is probably mediated by CCK_A receptors, while the effect on grooming may reflect an interaction with the CCK_A receptor and/or a novel CCK_B receptor subtype.

Keywords: Cholecystokinin; Dopamine; Dopamine D_1 receptor; Grooming; (Rat); Vacuous chewing movement

1. Introduction

Tardive dyskinesia manifests as a complication of long-term neuroleptic use in approximately 20–30% of patients so treated and is characterized by repetitive, involuntary movements, typically of the orofacial region. While the precise mechanism by which neuroleptics produce these effects remains unclear, dopamine receptor supersensitivity has been thought to be a key factor, based on findings of increased dopamine receptor binding (Burt et al., 1977), alteration of dyskinetic behaviours in humans by dopaminergic agents (Kane and Smith, 1982), and changes in the behavioural responses to dopamine receptor agonists in rodents following chronic neuroleptics (Clow et al., 1979; Ellison et al., 1988). However, the poor temporal and spatial correlation between the development of dopamine receptor supersensitivity and tardive dyskinesia (Fibiger and Lloyd, 1984) suggests that this mechanism alone may not provide an adequate explanation and that other factors may be involved.

Recently, attention has turned towards the peptide, cholecystokinin (CCK). Originally defined as a gut hormone, CCK is now known to exist throughout the central nervous system (Savasta et al., 1990; Schiffmann and Vanderhaeghen, 1991) including the striatum and nucleus accumbens, which also contain a moderate to high concentration of CCK binding sites (Van Dijk et al., 1984; Zarbin et al., 1983). In these regions, CCK has been found to exist both independent of and colocalized with dopamine (Hökfelt et al., 1980; Seroogy et al., 1989). Biochemical and electrophysiological studies indicate a modulatory role for CCK on dopamine release (Altar et al., 1988; Markstein and Hökfelt, 1984; Marshall et al., 1991; Ruggeri et al., 1987), neuronal firing (Freeman and Chiodo, 1988; Skirboll et al., 1981; Yim and Mogenson, 1991; Wu and Wang, 1994), receptor binding (Fuxe et al., 1981; Murphy and Schuster, 1982), and dopamine-induced adenylate cyclase activity (Dourish et al., 1992; Snyder et al., 1993; Studler et al., 1986). In keeping with these effects, CCK modulates the behavioural response to dopaminergic stimulation (Crawley, 1994; Crawley and Corwin, 1994; Vaccarino and Rankin, 1989; Moroji and Hagino, 1986).

Peripheral administration of the sulphated octapeptide (CCK-8S) has been shown to effectively suppress chronic neuroleptic-induced vacuous chewing movements in a ro-

* Corresponding author. Clinical Neurological Sciences, University Hospital, 339 Windermere Road, London, ON, Canada, N6A 5A5. Tel.: (519) 663-3694; fax: (519) 663-3162; e-mail: ⟨jstoessl@julian.uwo.ca⟩.

[1] Deceased.

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dent model of tardive dyskinesia (Stoessl et al., 1989). These vacuous chewing movements additionally appear to be mediated by heightened activity at the dopamine D_1 receptor (Ellison et al., 1988; Stoessl et al., 1989).

The precise nature of this modulation remains unclear. One question which remains unanswered is at which CCK receptor subtype CCK has these effects. Pharmacological (Moran et al., 1986) and molecular evidence (Wank et al., 1992a, b) exists for two known receptor subtypes termed 'A' and 'B'. The majority of central CCK receptors are of the 'B' type (Wank et al., 1994). However, in previous work we found intracerebroventricular CCK-8S to be ineffective in suppressing neuroleptic-induced vacuous chewing movements, while intraperitoneal administration of CCK-8S was effective (Stoessl and Polanski, 1993). Additionally, some (but not all; see Kihara et al., 1992) of the behavioural and neurochemical effects of peripheral CCK are attenuated by vagotomy or lesions of the nucleus tractus solitarii (Crawley et al., 1981; Crawley and Schwaber, 1984; Hamamura et al., 1989), which contains CCK_A receptors (Hill et al., 1987). Furthermore, autoradiographic (Hill et al., 1987, 1990), neurochemical (Vickroy et al., 1988), and electrophysiological (Hommer et al., 1985) evidence suggests that CCK_A receptors may be of primary importance in dopamine cell body and terminal regions of the brain.

In addition to the uncertainty regarding the site (central vs peripheral) and receptor subtype (A vs. B) involved, it is also unclear whether the behavioural effects of CCK are mediated via modification of endogenous dopamine release, or whether CCK may regulate the effects of dopamine receptor stimulation at a post-synaptic level.

In the present studies we examined the effects of CCK on behavioural responses induced by the dopamine D_1 receptor agonist SKF 38393 ((±)-2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine HCl) including stereotypic grooming, as previously described by Molloy and Waddington (1987), and vacuous chewing movements. In order to determine the CCK receptor type involved, we also examined the effects of the preferential CCK_B receptor agonist, CCK-4, on SKF 38393-induced behaviours, as well as the effects of pretreatment with selective CCK_A and CCK_B receptor antagonists on the actions of CCK-8S.

2. Materials and methods

2.1. Effects of CCK-8S on SKF 38393-induced behaviours

2.1.1. Animals

Male Sprague-Dawley rats (Charles River, Montreal), weighing approximately 250 g at the start of the experiment were housed in a temperature-controlled environment with a 12 h light/dark cycle and ad libitum access to standard rat chow and water.

2.1.2. Behavioural testing

Animals ($n = 10$) were habituated to plexiglass boxes ($50 \times 50 \times 30$ cm) for at least 2 h prior to testing. Each animal was then injected subcutaneously with either (±)SKF 38393 (Research Biochemicals International, MA; 5 mg/kg) or its vehicle (0.9% saline). This dose was selected based on the dose-response curve of an earlier study. Thirty minutes later the animals were injected intraperitoneally with either CCK-8S (Bachem California; 10, 30, or 50 μg/kg) or its vehicle (0.9% saline). Due to its limited lifespan in solution, CCK-8S was prepared freshly each day. It was initially dissolved in distilled water with saline added only after the peptide was fully in solution. Immediately following administration of CCK-8S, the animals were observed continuously for 3 min out of every 6 min block, for a total of 10 blocks (60 min). Previous work in our laboratory has found that the dose of SKF 38393 employed (5 mg/kg) produces consistent response levels during this time. The frequency and duration of various behavioural responses were recorded using a microcomputer keyboard and custom-designed software (BEBOP: Dr. M.T. Martin-Iverson, University of Western Australia). The following behavioural responses were recorded: vacuous chewing movements, grooming, locomotion, sniffing, and rearing. *Vacuous chewing movements* were defined as all nondirected mouth movements including chewing and tongue protrusion but excluding directed movements such as licking, eating, grooming, and yawning. Jaw tremor was also excluded as it is thought to be distinct from vacuous chewing movements and not correlated with neuroleptic treatment (Glenthoj et al., 1990). *Grooming* was defined to include scratching, forepaw licking, body fur grooming, and face washing. Penile grooming is thought to reflect selective activation of high affinity dopamine D_2 receptors (Stoessl et al., 1987) rather than dopamine D_1 receptors and was thus excluded. *Sniffing* was marked by head bobbing and whisker movements. *Rearing* was defined as the time spent with both forepaws off the ground and the head elevated. *Locomotion* was defined as the time spent in forward movement involving all four limbs. Each animal received all five treatments [vehicle/vehicle, SKF/vehicle, SKF/CCK (10 μg/kg), SKF/CCK (30 μg/kg), SKF/CCK (50 μg/kg)] which were spaced a minimum of 48 h apart. The order of treatments was randomized using a Latin square design.

2.1.3. Statistical analysis

Data were analyzed using a one-way analysis of variance with repeated measures (TREATMENT). Where significant *F*-values were found, planned pairwise comparisons were made using Bonferroni-adjusted paired *t*-tests.

2.2. Effects of CCK-4 on SKF 38393-induced behaviours

2.2.1. Animals

Male Sprague-Dawley rats (Charles River, Montreal), weighing approximately 250 g at the start of the experi-

ment were housed in a temperature-controlled environment with a 12 h light/dark cycle and ad libitum access to standard rat chow and water.

2.2.2. Behavioural testing

Animals ($n = 10$) were habituated to plexiglass boxes ($50 \times 50 \times 30$ cm) at least 2 h prior to testing. Then, each animal was injected subcutaneously with either (±)SKF 38393 (Research Biochemicals International; 5 mg/kg) or its vehicle (0.9% saline). Thirty minutes later the animals were injected intraperitoneally with either CCK-4 (Bachem California; 20, 50, or 100 μg/kg) or its vehicle (0.9% saline). Immediately following administration of CCK-4, the animals were observed for 60 min as described above. Each animal ($n = 10$) received all five treatments [vehicle/vehicle, SKF/vehicle, SKF/CCK4 (20 μg/kg), SKF/CCK4 (50 μg/kg), SKF/CCK4 (100 μg/kg)] which were spaced a minimum of 48 h apart. The order of treatments was randomized using a Latin square design.

2.2.3. Statistical analysis

Data were analyzed using a one-way repeated measures analysis of variance. Where significant F-tests were found, planned pairwise comparisons were made using Bonferroni-adjusted paired t-tests.

2.3. Effects of CCK receptor antagonists on the action of CCK-8S

2.3.1. Animals

Male Sprague-Dawley rats (Charles River, Montreal), weighing approximately 250 g at the start of the experiment were housed in a temperature-controlled environment with a 12 h light/dark cycle and ad libitum access to standard rat chow and water.

2.3.2. Behavioural testing

Animals ($n = 20$) were habituated to plexiglass boxes ($50 \times 50 \times 30$ cm) at least 2 h prior to testing. Then, each animal was injected subcutaneously with either (±)SKF 38393 (Research Biochemicals International; 5 mg/kg) or its vehicle (0.9% saline) followed immediately by intraperitoneal administration of either the CCK_A receptor antagonist, MK-329 (also referred to as devazepide or L-364,718) ($n = 10$) (Merck Sharp and Dohme Research Laboratories, NJ; 0.1, 0.3 mg/kg), the CCK_B receptor antagonist, L-365,260 ($n = 10$) (Merck Sharp and Dohme Research Laboratories, NJ; 0.1, 0.3 mg/kg), or their vehicle (0.5% CMC). The antagonists were suspended in carboxymethylcellulose (CMC) by means of a sonicator. Thirty minutes later the animals were injected intraperitoneally with either CCK-8S (50 μg/kg) or its vehicle (0.9% saline). Immediately following the final injection, the animals were observed for 60 min as described above. The observer was blind to drug condition. Each animal received five treatments [vehicle/vehicle/vehicle, SKF/vehicle/vehicle, SKF/vehicle/CCK, SKF/ANTAG (0.1 mg/kg)/CCK, SKF/ANTAG (0.3 mg/kg)/CCK] spaced a minimum of 48 h apart. The order of treatments was randomized using a Latin square design.

2.3.3. Statistical analysis

Data were analyzed using a two-factor analysis of variance (TREATMENT × ANTAGONIST) with repeated measures on one factor (TREATMENT). Where significant F-tests were found, planned pairwise comparisons were made using Bonferroni-adjusted paired t-tests.

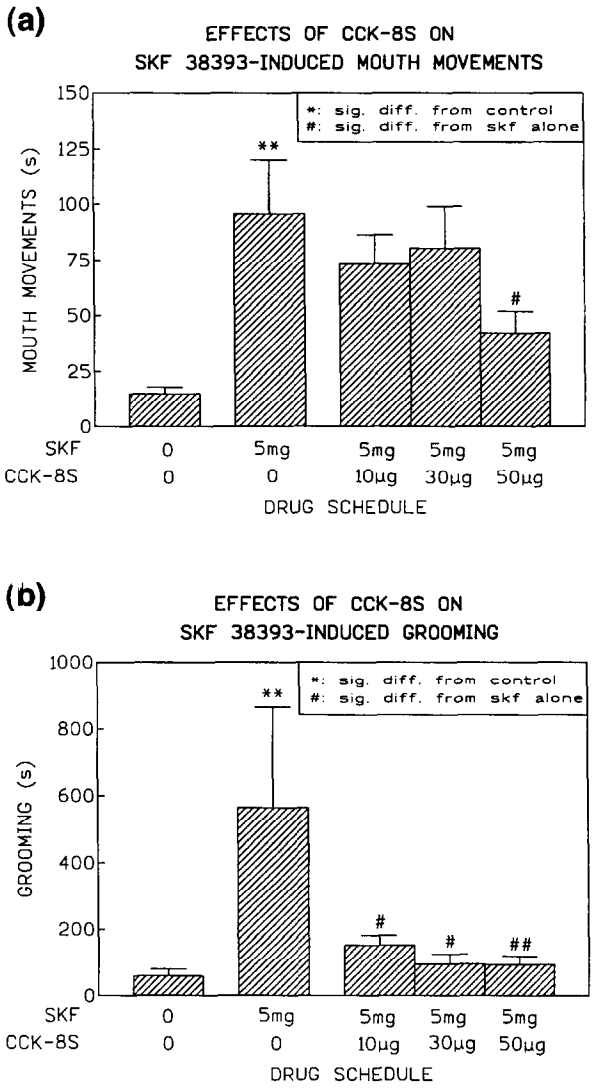

Fig. 1. (a) Vacuous chewing movements induced by SKF 38393 (5 mg/kg s.c.) are suppressed by CCK-8S (50 μg/kg i.p.). Each bar represents the mean (±S.E.M.) ($n = 10$) duration (s) scored over 10 blocks of 3 min. (b) Grooming induced by SKF 38393 (5 mg/kg s.c.) is suppressed by CCK-8S (10, 30, and 50 μg/kg i.p.). Each bar represents the mean (±S.E.M.) ($n = 10$) duration (s) scored over 10 blocks of 3 min. $^{*,\#}P < 0.05$, $^{**,\#\#}P < 0.01$.

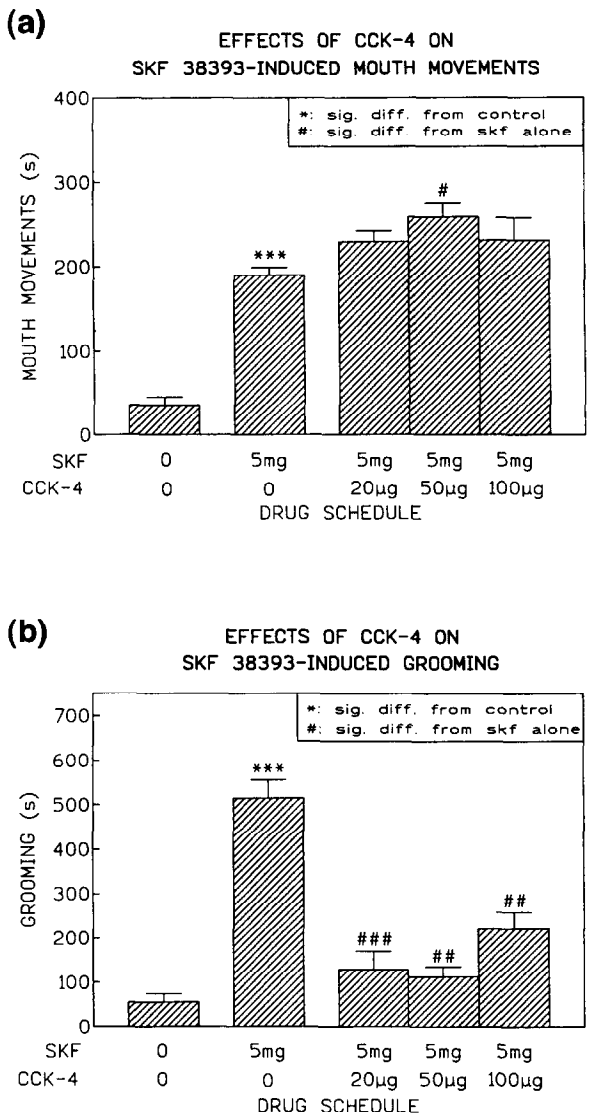

Fig. 2. (a) Vacuous chewing movements induced by SKF 38393 (5 mg/kg s.c.) are not suppressed by CCK-4. Each bar represents the mean (±S.E.M.) ($n = 10$) duration (s) scored over 10 blocks of 3 min. (b) Grooming induced by SKF 38393 (5 mg/kg s.c.) is suppressed by CCK-4 (20, 50, and 100 µg/kg i.p.). Each bar represents the mean (±S.E.M.) ($n = 10$) duration (s) scored over 10 blocks of 3 min. $^{*,\#}P < 0.05$, $^{***}P < 0.001$.

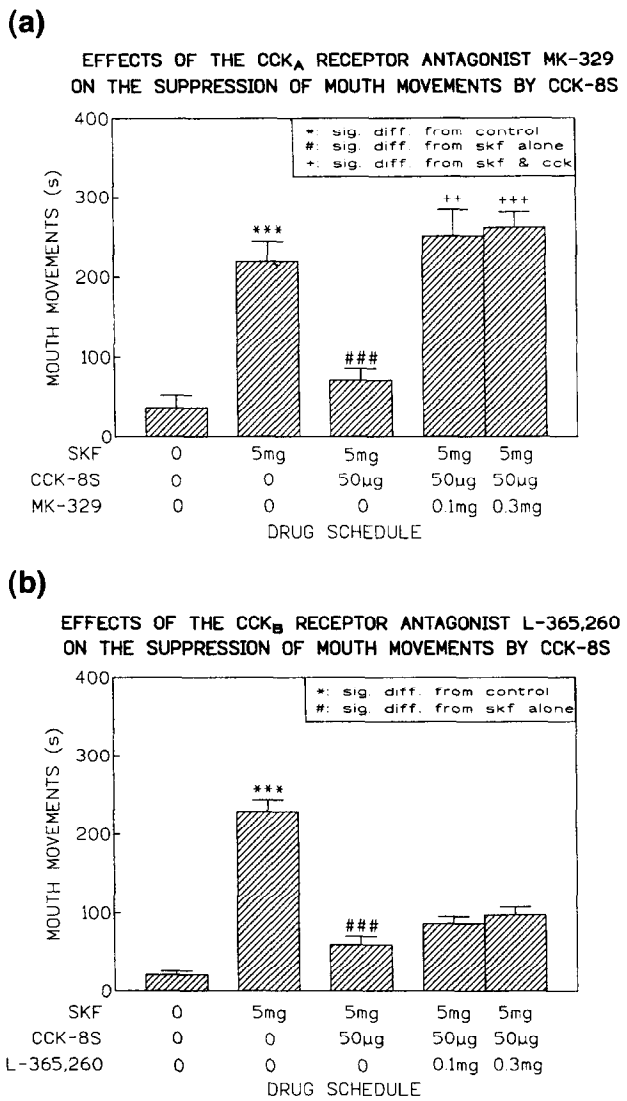

Fig. 3. (a)Vacuous chewing movements induced by SKF 38393 (5 mg/kg s.c.) are suppressed by CCK-8S (50 µg/kg i.p.). This suppression is blocked by the CCK$_A$ receptor antagonist, MK-329 (0.1 and 0.3 mg/kg i.p.). Each bar represents the mean (±S.E.M.) ($n = 10$) duration (s) scored over 10 blocks of 3 min. (b) Vacuous chewing movements induced by SKF 38393 (5 mg/kg s.c.) are suppressed by CCK-8S (50 µg/kg i.p.). This suppression is not blocked by the CCK$_B$ receptor antagonist L-365,260 (0.1 and 0.3 mg/kg). Each bar represents the mean (±S.E.M.) ($n = 10$) duration (s) scored over 10 blocks of 3 min. $^{**,\#\#,++}P < 0.01$, $^{***,\#\#\#,+++}P < 0.001$.

Table 1
The effects of CCK-8S (10, 30, and 50 µg/kg, i.p.) on total time engaged in sniffing, rearing, and locomotion following SKF 38393 (5 mg/kg, s.c.)

Treatment	Sniffing	Rearing	Locomotion
Vehicle/vehicle	63.44 ± 23.15	1.17 ± 0.91	1.76 ± 1.76
SKF/vehicle	81.26 ± 15.51	1.75 ± 0.97	0.91 ± 0.65
SKF/CCK-8S (10 µg)	60.27 ± 13.31	0.21 ± 0.21	0.00
SKF/CCK-8S (30 µg)	30.20 ± 12.11	0.23 ± 0.23	3.56 ± 2.94
SKF/CCK-8S (50 µg)	54.13 ± 21.72	3.56 ± 2.94	0.37 ± 0.37

Values represent the mean (±S.E.M.) duration (s) scored over 10 blocks of 3 min each.

3. Results

3.1. Effects of CCK-8S on SKF 38393-induced behaviours

3.1.1. Vacuous chewing movements

SKF 38393 (5 mg/kg) induced vacuous chewing movements which were attenuated by the highest dose of CCK-8S (50 μg/kg) ($F(4,36) = 5.56$, $P = 0.001$) (Fig. 1a).

3.1.2. Grooming

SKF 38393 (5 mg/kg) induced grooming which was attenuated to control levels by all three doses of CCK-8S (10, 30, and 50 μg/kg) ($F(4,36) = 4.52$, $P = 0.005$) (Fig. 1b).

3.1.3. Other behaviours

Sniffing, rearing, and locomotion were not affected by any of the drug treatments (Table 1).

3.2. Effects of CCK-4 on SKF 38393-induced behaviours

3.2.1. Vacuous chewing movements

SKF 38393 (5 mg/kg) induced a robust response which was unaffected by CCK-4 at any dose. A slight increase in VCMs, of questionable significance, was seen following 50 μg/kg CCK-4 ($F(4,36) = 37.21$, $P < 0.001$) (Fig. 2a).

3.2.2. Grooming

SKF 38393 (5 mg/kg) produced a 3-fold increase in grooming which was significantly attenuated by CCK-4 at all three doses (20, 50, and 100 μg/kg) ($F(4,36) = 29.07$, $P < 0.001$) (Fig. 2b).

3.2.3. Other behaviours

SKF 38393 (5 mg/kg) produced a significant increase in sniffing which was unaffected by CCK-4 at any dose ($F(4,36) = 4.89$, $P = 0.003$). Neither rearing nor locomotion was affected by any of the drug treatments (Table 2).

3.3. Effects of CCK receptor antagonists on the action of CCK-8S

3.3.1. Vacuous chewing movements

SKF 38393 (5 mg/kg)-induced vacuous chewing movements were significantly suppressed by CCK-8S (50 μg/kg) (Fig. 3). This effect of CCK was blocked by the CCK_A receptor antagonist, MK-329, (0.1 mg/kg and 0.3 mg/kg) (Fig. 3a) but not by the CCK_B receptor antagonist, L-365,260, (Fig. 3b) ($F(4,72) = 40.76$, $P < 0.001$, TREATMENT main effect; $F(1,18) = 62.76$, $P < 0.001$, ANTAGONIST main effect; $F(4,72) = 11.30$, $P < 0.001$, TREATMENT × ANTAGONIST interaction effect).

3.3.2. Grooming

SKF 38393 (5 mg/kg)-induced grooming was suppressed by CCK-8S (50 μg/kg) (Fig. 4), an effect which was reversed by the CCK_A receptor antagonist, MK-329 (0.1 mg/kg and 0.3 mg/kg) (Fig. 4a) but not the CCK_B receptor antagonist, L-365,260, (Fig. 4b) ($F(4,72) = 29.47$, $P < 0.001$, TREATMENT main effect; $F(1,18) = 10.35$,

Table 2
The effects of CCK-4 (20, 100, and 500 μg/kg, i.p.) on total time (s) engaged in sniffing, rearing, and locomotion following SKF 38393 (5 mg/kg, s.c.)

Treatment	Sniffing	Rearing	Locomotion
Vehicle/vehicle	123.66 ± 31.82	7.93 ± 3.69	18.67 ± 6.11
SKF/vehicle	399.80 ± 40.21 [c]	9.81 ± 4.33	10.51 ± 3.78
SKF/CCK-4 (20 μg)	365.06 ± 78.56	22.04 ± 14.04	19.06 ± 11.77
SKF/CCK-4 (100 μg)	303.65 ± 58.18	13.14 ± 7.90	11.75 ± 3.76
SKF/CCK-4 (500 μg)	365.22 ± 43.22	33.77 ± 14.51	12.92 ± 5.37

Values represent the mean (± S.E.M.) duration (s) scored over 10 blocks of 3 min each. [c] $P < 0.001$ as compared to all-vehicle control.

Table 3
The effects of CCK-8S (50 μg/kg, i.p.) and its antagonists, MK-329 (0.1 and 0.3 mg/kg, i.p.) and L-365,260 (0.1 and 0.3 mg/kg, i.p.), on total time (s) engaged in sniffing, rearing, and locomotion following SKF 38393 (5 mg/kg, s.c.)

Antagonist	Treatment	Sniffing	Rearing	Locomotion
MK-329	Vehicle/vehicle/vehicle	120.19 ± 31.86	4.66 ± 1.96	4.87 ± 3.13
	SKF/vehicle/vehicle	347.79 ± 61.45 [b]	21.39 ± 9.90	16.59 ± 6.62
	SKF/CCK-8S/vehicle	235.17 ± 35.46	8.30 ± 5.52	6.20 ± 2.68
	SKF/CCK-8S antagonist (0.1 mg)	295.55 ± 38.19	15.21 ± 5.01	21.09 ± 6.38
	SKF/CCK-8S antagonist (0.3 mg)	330.70 ± 54.67	11.14 + 5.09	11.56 ± 4.02
L-365,260	Vehicle/vehicle/vehicle	131.95 ± 35.90	8.32 ± 4.00	11.88 ± 5.89
	SKF/vehicle/vehicle	384.10 ± 53.20 [a]	29.73 ± 11.38	14.58 ± 6.42
	SKF/CCK-8S/vehicle	334.95 ± 55.23	7.19 ± 2.37	20.28 ± 6.43
	SKF/CCK-8S/antagonist (0.1 mg)	196.83 ± 45.66	2.86 ± 2.01	7.46 ± 3.61
	SKF/CCK-8S/antagonist (0.3 mg)	183.48 ± 33.25	5.12 ± 3.67	8.66 ± 2.84

Values represent the mean (± S.E.M.) duration (s) scored over 10 blocks of 3 min each. [a] $P < 0.05$, [b] $P < 0.01$ as compared to all-vehicle control.

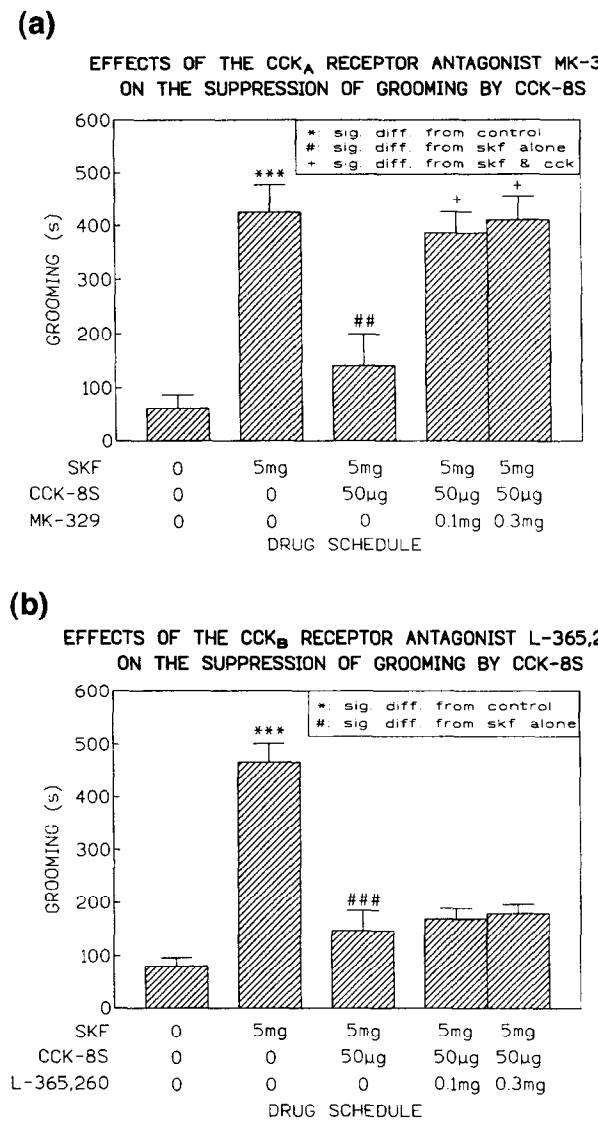

Fig. 4. (a) Grooming induced by SKF 38393 (5 mg/kg s.c.) is suppressed by CCK-8S (50 µg/kg i.p.). This suppression is blocked by the CCK$_A$ antagonist, MK-329 (0.1 and 0.3 mg/kg i.p.). Each bar represents the mean (±S.E.M.) ($n = 10$) duration (s) scored over 10 blocks of 3 min. (b)Grooming induced by SKF 38393 (5 mg/kg s.c.) is suppressed by CCK-8S (50 µg/kg i.p.). This suppression is not blocked by the CCK$_B$ antagonist, L-365,260 (0.1 and 0.3 mg/kg i.p.). Each bar represents the mean (±S.E.M.) ($n = 10$) duration (s) scored over 10 blocks of 3 min. $^{\#,+}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$, $^{***,\#\#\#}$ $P < 0.001$.

$P = 0.005$, ANTAGONIST main effect; $F(4,72) = 6.38$, $P < 0.001$, TREATMENT × ANTAGONIST interaction effect).

3.3.3. Other behaviours

SKF 38393 induced a significant sniffing response which was unaffected by CCK-8S or either of the two antagonists ($F(4,72) = 7.32$, $P < 0.001$, TREATMENT main effect). Although rearing displayed a significant TREATMENT main effect ($F(4,72) = 3.72$, $P = 0.008$, TREATMENT main effect), post-hoc tests revealed no significant differences. Locomotion was unaffected by any of the treatments (Table 3).

4. Discussion

Our findings demonstrate that the behavioural responses to a direct-acting dopamine D_1 receptor agonist in the rat are attenuated by peripherally administered CCK-8S, thereby substantiating earlier observations (Dourish et al., 1992; Masuda et al., 1992). While the effects of CCK-8S alone were not examined here, intraperitoneal CCK-8S has been shown to have no effect on mouth movements or grooming when administered alone (Dourish et al., 1992; Stoessl et al., 1989). If CCK is indeed capable of modulating dopaminergic activity to influence these behaviours, it may prove valuable as an antidyskinetic agent. In fact, evidence from preclinical (Boyce et al., 1990; Stoessl et al., 1989) and clinical (Nishikawa et al., 1986) trials found CCK and its analogue ceruletide to be of some benefit in patients with dyskinesia or Huntington's disease (Hashimoto and Yanagisawa, 1990).

There was considerable variability in the behavioural data *between* experiments in these studies. This likely reflects the time elapsed between different studies and consequent use of different batches of animals, as well as possible alterations in the ambient conditions of the testing environment and the use of two different observers. However, the use of a repeated measures design ensures that there is internal consistency *within* experiments and that group differences are accordingly likely to reflect a true drug effect.

One issue inherent to behavioural studies incorporating CCK involves the sedative effects shown to occur following i.p. administration (Mansbach and Lorenz, 1983). However, CCK-8S did not appear to produce behavioural sedation in these studies. Neither CCK-8S nor CCK-4 had any effect on behaviours other than VCMs or grooming, including sniffing, rearing and locomotion (Tables 1–3) which would conceivably be suppressed during a state of global sedation. Furthermore, other investigators have noted an *inverse* correlation between oral activity and gross motor behaviour (Levy et al., 1987) and VCMs are actually induced by sedating low doses of dopamine receptor agonists (Stoessl et al., 1987). Thus, suppression of SKF-induced VCMs and grooming by CCK cannot readily be attributed to sedation.

Interactions of CCK with dopamine in the CNS are varied. Thus, CCK has been demonstrated to both potentiate and inhibit dopaminergic activity via its effects on neuronal firing (Freeman and Chiodo, 1988; Yim and Mogenson, 1991), receptor binding (Murphy and Schuster, 1982), release (Marshall et al., 1991; Ruggeri et al., 1987), and cAMP activity (Dourish et al., 1992; Studler et al., 1986) depending on the site, dose, basal activity levels, and the receptor subtypes involved (Crawley et al., 1985;

Daugé et al., 1989; Hommer et al., 1986; Phillips et al., 1988; Studler et al., 1986). Such diversity necessitates further investigation in order to better understand the role CCK might play in dyskinesias and consider the possible use of a CCK analogue for therapeutic purposes.

One issue which has hitherto been unresolved is the CCK receptor subtype mediating these effects. In these studies, we have demonstrated that suppression of SKF 38393-induced vacuous chewing movements is likely to be mediated by the CCK_A receptor, as indicated by the failure of the preferential CCK_B receptor agonist, CCK-4, to suppress VCMs as well as the ability of the CCK_A receptor antagonist MK-329, but not the CCK_B receptor antagonist L-365,260, to block suppression by CCK-8S. The effects on grooming were less clear. Thus, while CCK-4 potently suppressed SKF 38393-induced grooming, the selective CCK_B receptor antagonist L-365,260 failed to significantly attenuate suppression by CCK-8S, whereas the CCK_A receptor antagonist MK-329 significantly blocked suppression of grooming by CCK-8S. These effects cannot be attributed to any independent action of the antagonists as neither antagonist influences these behaviours when administered alone, as is also the case for CCK-4 (data not shown). While seemingly disparate, these results are, in fact, consistent with recent findings of others based on studies using anxiety and analgesia paradigms (Derrien et al., 1994; O'Neill et al., 1992).

Systemically administered CCK has been demonstrated to modulate central dopaminergic activity (Altar and Boyar, 1989; Freeman and Chiodo, 1988; Hommer et al., 1985; Kariya et al., 1994; Murphy and Schuster, 1982), yet it is not thought to readily cross the blood brain barrier (Oldendorf, 1981). This suggests that peripherally administered CCK may exert the majority of its central effects via indirect actions at a more peripheral site. The vagal afferent system including the solitary nucleus, which contains a significant amount of CCK (Palkovits et al., 1982), has been implicated as the site of action. Projections from the vagus terminate in the solitary nucleus which, in turn, forms direct and indirect connections with midbrain dopaminergic neurons (Herbert, 1992; Leslie et al., 1982; Norgren, 1978; Ricardo and Koh, 1978; Wang et al., 1992). Lesions of vagal afferents as well as of the solitary nucleus have been demonstrated to disrupt the effects of CCK on dopaminergic activity (Hamamura et al., 1989; Hommer et al., 1985) and behaviour (Crawley et al., 1981; Crawley and Schwaber, 1984).

CCK receptors have been termed 'peripheral' (CCK_A) and 'brain' (CCK_B), with the majority of CCK receptors in the brain being of the 'B' type. However, CCK_A type receptors have been discovered in a number of discrete areas of the CNS, including the solitary nucleus (Hill et al., 1987; Moran et al., 1986). Hence, our findings might further support the suggestion that the effects of systemically administered CCK are peripherally or vagally mediated.

However, while peripheral or vagal effects of CCK might reasonably be expected to modify the release of endogenous dopamine, our data indicate that CCK blocks the behavioural responses to a direct-acting dopamine D_1 receptor agonist. This suggests that the effects we observed are mediated at a post-synaptic level, either via an interaction with striatal D_1 receptors or downstream to the D_1 receptor. These possibilities are in keeping with the observations that CCK blocks dopamine-stimulated adenylyl cyclase (Studler et al., 1986) and forskolin-stimulated phosphorylation of the phosphoprotein DARPP-32 (Snyder et al., 1993) in vitro as well as observations that peripherally administered CCK attenuates in vivo SKF 38393-stimulated cAMP efflux in the striatum (Dourish et al., 1992).

The concentrations of CCK required to alter behaviour may be substantially lower than those needed to modify biochemical indices such as cAMP efflux (Dourish et al., 1992). In addition, not all centrally mediated actions of systemically administered CCK are affected by vagotomy (Hommer et al., 1985; Kihara et al., 1992) and for those that are, attenuation is not always complete (Hommer et al., 1985). Together, these findings have led to the proposal of a dual-site model (Dourish, 1992; Hommer et al., 1985) incorporating both vagal/solitary nucleus actions as well as direct actions in the CNS possibly involving the small amount of peptide that may make its way across the blood-brain barrier (Kastin et al., 1979).

Collectively, our findings indicate that CCK can alter dopaminergic behavioural responses. The effects on SKF 38393-induced vacuous chewing movements appear to be mediated primarily by the CCK_A receptor while those on SKF 38393-induced grooming may be mediated by the CCK_A receptor and/or a novel CCK_B subtype. These effects possibly reflect an action post-synaptic to dopamine terminals in the striatum, either directly and/or indirectly via the vagal afferent system.

The finding that CCK can selectively modify certain behavioural responses to dopaminergic stimulation at a post-synaptic level lends further support to the potential role of CCK analogues in the treatment of dyskinesias and also suggests that abnormalities of CCK should be explored for their contribution to the pathogenesis of these disorders. Further studies on the anatomical pathways and mechanisms underlying these interactions are needed.

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