Available online at www.sciencedirect.com

SCIENCE DIRECT

Pharmacology, Biochemistry and Behavior 79 (2004) 109–117

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Cholecystokinin tetrapeptide improves water maze performance of neonatally 6-hydroxydopamine-lesioned young rats

André Rex*, Heidrun Fink

Institute of Pharmacology and Toxicology, School of Veterinary Medicine, Freie Universität Berlin, Koserstr. 20, D-14195 Berlin, Germany

Received 10 February 2004; received in revised form 17 June 2004; accepted 23 June 2004
Available online 11 August 2004

Abstract

This study addressed the proposed memory-modulating effect of the cholecystokinin (CCK) 2 agonist Boc-CCK-4 in rats using a Morris water maze. In the brain, CCK is colocalized and interacts with dopamine, respectively. To impair dopaminergic neurotransmission, and consequently, dopamine-mediated learning and memory, rat pups received the neurotoxin 6-hydroxydopamine (6-OHDA) into the left [Day 5 postnatal (p.n.)] and right (Day 8 p.n.) ventricles (50 μg/5 μl each). After 6-OHDA treatment, dopamine brain levels were reduced by 60% on Day 50 p.n. Lesioned rats had a lower body weight but normal swimming abilities. In the acquisition phase of the water maze (Day 50 p.n.), sham-lesioned rats learned quickly, compared to lesioned rats. Treatment with Boc-CCK-4 (40 μg/kg ip) did not affect performance in sham-lesioned rats but restored the learning curve in lesioned rats without increasing swimming speed indicating a better spatial learning in the dopamine-depleted rats. In summary, these findings demonstrate that stimulation of CCK2 receptors may counteract cognitive deficits of dopamine-depleted rats.

Keywords: Boc-CCK-4; Dopamine depletion; Learning; Lesion; Memory; Rat; Water maze

1. Introduction

Cholecystokinin (CCK) plays a complex role in the central nervous system by modulating many integrated functions (Crawley and Corwin, 1994; Fink et al., 1998; Beinfeld 2001). In addition to effects on anxiety-related (Rodgers and Johnson, 1995) and feeding behaviours, there is evidence for an influence of CCK in learning and memory.

Studies of the mnemotropic effects of CCK have resulted in remarkably varied findings (Gerhardt et al., 1994; Itoh and Lal, 1990; Yamaguchi et al., 1998; Voits et al., 2001). Both receptor subtypes, the CCK1 receptor (Li et al., 2002; Nomoto et al., 1999) and the CCK2 receptor (Dauge and Lena, 1998), play a role in the memory-modulating effects of CCK. However, there are inconsistent effects of CCK2 receptor ligands on memory and learning, i.e., a cognitive-enhancing effect of CCK2 agonists (Taghzouti et al., 1999), but also negative effects in an inhibitory avoidance task induced by the CCK2 agonist CCK-4 (Itoh et al., 1988). A recent study revealed an impaired spatial memory in mice lacking the CCK2 receptor (Dauge et al., 2001).

CCK has been shown to coexist and interact with dopamine in the regulation of behaviour (Beinfeld, 2003). In these experiments, the mixed CCK1/CCK2 agonists CCK-8 and caerulein were used (Beinfeld, 2001). It was shown that CCK-8 intensified dopamine-induced hyperlocomotion and apomorphine-induced stereotypy (Crawley, 1992). Results of these behavioural studies indicated that CCK-8 given locally into the nucleus accumbens could both antagonise and potentiate D1- and D2-receptor-mediated effects on exploratory and motor activity (Fink et al., 1991; Crawley, 1992) in the mesolimbic system, respectively.

Using compounds acting at the CCK2 receptor revealed that an activation of CCK2 receptors facilitates dopaminergic activity (Hommer et al., 1985). Other studies have shown that the CCK2 agonists BC 197 and BC 264 stimulate

* Corresponding author. Tel.: +49 30 83853512; fax: +49 30 83853112.
E-mail address: andrerex@zedat.fu-berlin.de (A. Rex).

0091-3057/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.06.008

dopamine release in the nucleus accumbens in vitro (Lena et al., 1997), whereas the CCK2 antagonist LY262691 decreased the activity of dopamine neurons (Rasmussen et al., 1991). Additionally, the CCK2 receptor agonists Boc-CCK-4 (Reum et al., 1997) and BC-264 (Ladurelle et al., 1997), given into the ventral tegmental area, increase dopamine release in the nucleus accumbens in vivo.

Several studies suggest that dopamine also plays a role in memory formation. Dopamine depletion by the neurotoxin 6-hydroxydopamine (6-OHDA) in neonatal rats leads to decreased acquisition of operant responses (Moy, 1995) and impaired performance in the radial arm test (Pearson et al., 1984) and in the T-maze escape test (Raskin et al., 1983). Diminished dopamine function, caused by a lack of dopamine receptors, leads to an impaired performance in the Morris water maze which is not associated with a swimming disability in transgenic mice (Smith et al., 1988).

The dopamine antagonist haloperidol also impairs learning (Ploeger et al., 1994), while administration of the dopamine D1 receptor agonists SKF 38393 and SKF 81297 enhanced performance in memory-impaired aged rats (Hersi et al., 1995).

A clinical microdialysis study demonstrated a sustained activation of the mesolimbic dopaminergic system with an increased release of dopamine during performance of cognitive tasks (Fried et al., 2001).

The Morris water maze is one of the most commonly used animal tests in behavioural neuroscience to investigate spatial learning in laboratory animals (D'Hooge and De Deyn, 2001). Compared with other spatial learning paradigms, such as the radial arm maze, and passive avoidance procedures, the water maze test has advantages because it purports to dissociate deficits in memory from deficits in sensory, motor and motivational processes (McNamara and Skelton, 1993) and needs neither appetitive stimuli nor additional punishment which may interfere with memory processes, although the animal's behaviour is partly aversively motivated (Lipp and Wolfer, 1998).

The aim of the present study was the assessment of the influence of the CCK2 receptor agonist CCK-4 on learning and memory in dopamine-impaired rats caused by neonatal 6-OHDA lesion.

2. Materials and methods

Timed mating was performed in Wistar rats [Mol: Wist (Shoe)], 7–10 months old (280±30 g). After birth, the litter size was adjusted to eight pups, housed one litter per cage (45×60×25 cm), kept under a 12-h light–dark schedule (lights on at 0600 h) with food (Altromin 1326) and water ad libitum. Following weaning, the rats were group housed (5 rats per cage) and kept under identical conditions. All experiments were carried out between 0900 and 1500 h. Animals were weighed on Days 3 and 5 postnatal (p.n.), every fifth day until weaning (Day 25 p.n.) and on Days 30, 40 and 50 p.n.

All procedures and experiments performed were approved by the Animal Protection Board of the State of Berlin.

2.1. Drugs

Boc-CCK-4 (butyl-oxycarbonyl-Trp-Met-Asp-Phe-NH_2) with stronger enzyme-resistance than CCK-4 was synthesized at the Institute of Pharmacology and Toxicology, Humboldt-Universität zu Berlin. Boc-CCK-4 (40 μg/kg) was suspended in vehicle (0.9% NaCl solution+1 vol.% Cremophor EL) and given intraperitoneally 5 min before the first (pretrial) or 5 min after the last training session (posttrial). Injection volume was 1 ml/kg body weight. In all experiments, the observers were blind to the treatment.

2.2. Neonatal dopamine depletion

Rat pups, anaesthetised with diethylether, were randomly divided into two groups, marked with a waterproof felt pen and given 6-OHDA (RBI, USA, 100 μg free base in 5 μl 0.1% ascorbic acid) or 5 μl 0.1% ascorbic acid (sham-lesioned animals) on Day 5 p.n. over a 30-s period using a 10-μl Hamilton syringe with a 30-gauge needle into the left ventricle (1.0 mm lateral, 2.5 mm ventral from bregma). After the treatment, the pups were warmed on a heating pad and returned to their home cage. To protect catecholaminergic neurons, all animals were pretreated with desipramine (AWD, Germany, 25 mg/kg sc, 30 min prior to lesion). Three days later (Day 8 p.n.), the animals received a second administration of the same dose of 6-OHDA or saline into the right ventricle also preceded by desipramine.

2.3. Test procedures

To determine the motor abilities of the rat pups, behavioural tests for motor skills were performed until 3 weeks prior to the Morris water maze test.

2.4. Swimming capability test

The test determines the animals' ability to swim by determining if they swim in a straight line, how much of the rat pups head is above the water while swimming and how they use their limbs while swimming (Adams et al., 1981; see Table 1).

On Days 12, 15 and 20 p.n., the rat pups were individually placed in a small tank (20×30 cm, 20 cm deep) filled with warm water (25 °C) for 20 s. The movement of the rat pups was recorded and ranked.

2.5. Voluntary climbing test

To investigate the voluntary climbing, a test for motor abilities was adapted (Costall et al., 1978; Drinkenburg et al., 2000). Wire cages (15 cm, 15×15 cm) were used. The test started by placing the rat pups at Days 18 and 25 p.n.

Table 1
Ranking list of swimming capability as description of different stages in the motor development (adapted from Adams et al., 1981)

Rank	Swimming behaviour
0	Head is below the surface
1	Head reaches the surface, nostrils are below the surface, leg movement is uncoordinated
2	Nostrils are above the surface, leg movement partly coordinated
3	Leg movement is coordinated, the animals swim in circles
4	Directed swimming by coordinated movement of all four legs
5	Less activity of the forelimbs
6	Adult swimming, propelling only by hindlimbs, forelimbs for change of direction only

individually in the wire cages. The time taken for each rat to climb the inside of the wire cage was recorded.

2.6. Basal motor activity

General and nonstimulated home cage motility was determined using an automated motameter (Animex, LKB, Sweden) at Day 25 p.n.

2.7. Apomorphine-induced hyperlocomotion

To assess the functional efficacy of the dopaminergic lesion, the animals were treated with the dopamine agonist apomorphine (AWD, 1.0 mg/kg ip, Day 60 p.n.). Twenty minutes later, the rats were individually placed in an open field (100×100×40 cm) for 5 min and the locomotion was determined by infrared beam breaks (10 equally spaced beams, 3 cm above the floor). Two consecutive beams had to be broken simultaneously to register locomotion, avoiding measuring and compromising stereotype and grooming behaviours.

2.8. Morris water maze test

The apparatus consisted of a square plastic tank (100×100 cm, 80 cm high) filled to a height of 50 cm with opaque water and cleaned daily. It was located in a small test room surrounded by several extra-maze cues at the walls of the pool and the room to provide both proximal and distal visual cues. The pool was illuminated by indirect light (180 lx). A submerged transparent escape platform (triangular with 7.6-cm-long edges, approximately 25 cm^2, 1.5 cm below the water level) was set at a definite place approximately in the middle of one of four imaginary maze quadrants in the tank. A video camera suspended over the tank was used for observation and data recording with tracking software (Videomot, TSE-Systems, Germany). For each session, rats were gently lowered into the tank. Start position was chosen in a random order but was kept constant for the individual rat throughout the experiment. Rats had to learn to escape to the submerged platform, utilising extra-maze spatial cues. After the training sessions (acquisition phase, three trials within 30 min on Day 50 p.n.), the rats were tested 24 h (Day 51 p.n.) and 7 days (Day 58 p.n.) later in a one-test session (memory phase) with an identical platform position. During the acquisition phase, the rat was allowed to swim until it found the platform. Animals, which did not find the platform during 120 s, were manually placed on the platform. In each case, the rats were allowed to remain on the platform for 30 s. Escape latencies (swim time) and distance travelled (Table 3) were registered and the swimming-speed was calculated.

2.9. Determination of dopamine tissue levels

Central dopamine levels were determined 24 h after the first memory session (Day 52 p.n.). The animals were sedated with isoflurane (Forene, Abbott, Germany), decapitated and the brains were removed. The brain, stored in a plastic vial, was snap frozen in liquid nitrogen for subsequent dissection of the nucleus accumbens which was removed using a tissue punch (i.d. 1 mm).

Samples were immersed in ice-chilled 0.1-M perchloric acid (600 μl) and homogenised. A Lowry assay was used to determine protein concentration (200 μl, Lowry et al., 1951). The remaining 400 μl was centrifuged for 10 min at 25,000×*g* at 5 °C. The supernatant was removed to determine dopamine levels by HPLC with electrochemical detection using a 12.5-cm GROM-Symbasic C18 column (GROM, Germany; 5 μm, i.d. 4 mm) at a flow of 1 ml/min. The mobile phase consisted of a sodium phosphate buffer containing 0.15 M NaH_2PO_4, 1.0 mM EDTA-disodium salt, 1.6 mM octane sulphane acid sodium salt (pH=3.7) and 3% isopropanol. For the analysis of the chromatograms, a data system BDS (BDS Data, Israel) was used. The concentrations of dopamine were displayed as picomole per milligram of protein.

2.10. Statistical analysis

The water maze data were analysed using a three-way ANOVA with the following factors: lesion, treatment and trial followed by the Holm–Sidak method. All other data were analysed using a one-way ANOVA followed by Dunnett's test. Differences of the means $P<.05$ were considered statistically significant. The data are displayed as mean±S.E.M. The group size was n=9–12.

3. Results

3.1. Development and motor function

3.1.1. Body weight

6-OHDA-lesioned animals gained less weight compared to the sham-lesioned rat pups and untreated animals, starting at Day 20 p.n. (F=4.816, df=2,27; P=.0163; Table 2).

Table 2
Behaviour of 6-OHDA-lesioned rat pups in tests for motor skills compared to sham-lesioned and untreated controls (n=9–11)

		Untreated animals	Sham-lesioned animals	Lesioned animals
Body weight (g)	Day 10 p.n.	19.1±0.9	19.3±0.8	17.6±0.3
	Day 15 p.n.	29.6±1.2	29.5±0.5	27.1±0.6
	Day 20 p.n.	38.9±1.5	39.5±0.7	35.3±0.8*
	Day 25 p.n.	61.7±2.6	65.0±1.5	47.1±1.8*
	Day 30 p.n.	91.1±3.7	94.1±2.6	60.7±4.5*
	Day 40 p.n.	161.6±4.3	167.8±7.7	98.9±9.7*
	Day 50 p.n.	259.3±6.4	243.5±14.7	147.9±14.7*
Swim-capability test [scores]	Day 12 p.n.	2.2±0.10	2.4±0.11	2.1±0.09
	Day 15 p.n.	3.4±0.12	3.5±0.08	3.1±0.07
	Day 20 p.n.	5.2±0.06	5.0±0.09	5.3±0.45
Climbing test (s)	Day 20 p.n.	32.4±3.7	36.4±3.9	48.8±3.5*
	Day 25 p.n.	17.5±6.6	16.0±3.4	44.2±4.7*
General activity (counts/min)	Day 25 p.n.	58.1±4.3	49.3±3.6	48.6±2.9
Apomorphine-induced locomotion (squares/5 min)	Day 60 p.n.	71.1±6.2	81.5±12.2	136.2±17.5*

Data presented as means±S.E.M.
*P<.05.

3.1.2. Swimming capability test

The development of motor skills in the lesioned rats was not significantly influenced by the 6-OHDA lesion. Lesioned rat pups did not differ from the sham-lesioned controls in the swimming skills, measured at Days 12, 15 and 20 p.n. (Table 2).

3.1.3. Voluntary climbing test

In the voluntary climbing test, the 6-OHDA-lesioned rats needed significantly more time to climb the wall of the wire cage compared to the sham-lesioned or untreated controls on both testing days (Day 20: F=5.586, df=2,25; P=.0109, F=10.352, Day 25: df=2,25; P=.007; Table 2).

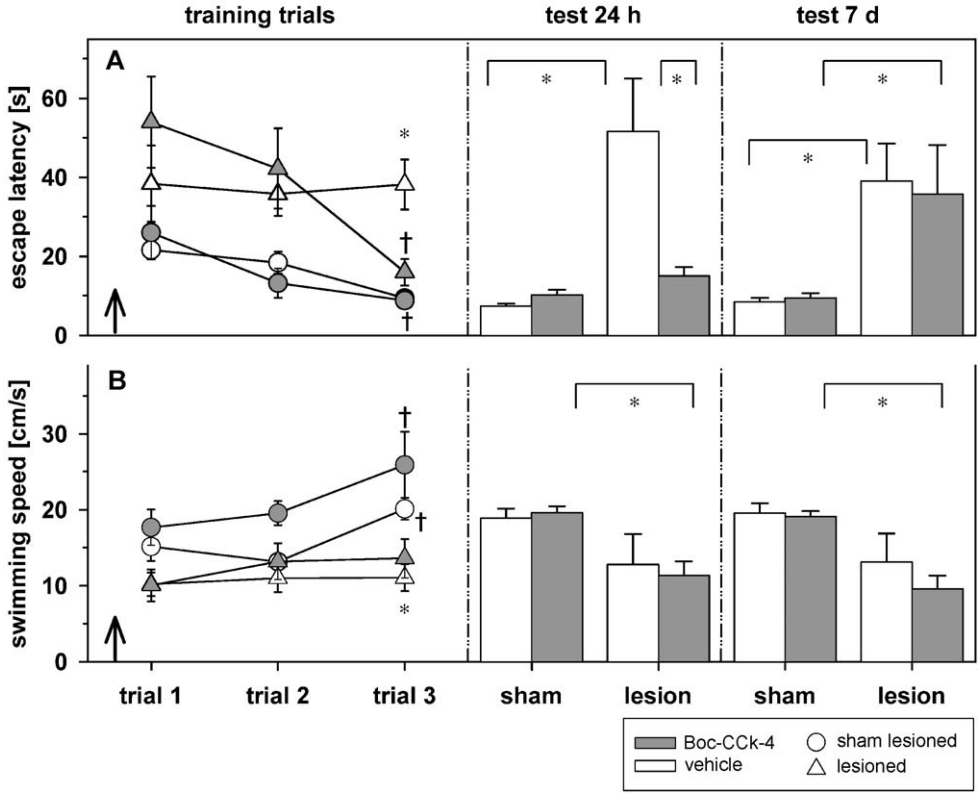

Fig. 1. Inferior performance of 6-OHDA-lesioned rats (△, n=12) in the Morris water maze compared to sham-lesioned rats (○, n=11) measured by the escape latencies (A) and the corresponding swimming speed (B). Boc-CCK-4 (40 μg/kg) given 5 min (↑) before the first trial restores the escape latencies in the 6-OHDA-lesioned rats (grey triangle, grey bars, n=11) to the level of sham-lesioned rats, but had no effect in the sham-lesioned rats (grey circle, grey bars, n=10). Data are presented as mean±S.E.M. ($^{\dagger}P$<.05 vs. Trial 1, *P<.05 vs. vehicle, lesioned vs. sham lesioned).

3.1.4. Basal motor activity

The spontaneous motor activity in the lesioned rats did not differ from the activity of the controls at Day 25 p.n. (Table 2).

3.1.5. Apomorphine-induced hyperlocomotion

The 6-OHDA-lesioned rats showed hyperactivity in the open field following apomorphine treatment at Day 60 p.n. (F=7.471, df=2,27; P=.003; Table 2).

3.1.6. Water maze test

In the acquisition phase (training trials) of the water maze test, the performance of lesioned rats, measured by escape latencies, was impaired compared to the sham-lesioned rats (F=32.245, df=1,108; P<.001; Figs. 1A and 2A).

The ability of sham-lesioned rats to find the submerged platform improved rapidly compared to lesioned animals. Additionally, three lesioned rats (of 11 rats) but none of the sham-lesioned rats failed to find the platform during the first 120 s and had to be placed on the platform manually.

Differences between the groups were obvious from the second training session onward (P<.05, t test, two-tailed), demonstrating a learning deficit of 6-OHDA-lesioned rats.

Administration of Boc-CCK-4 (40 μg/kg) had no effect on escape latencies in the sham-lesioned rats, either when given pretrial (Fig. 1A) or when given posttrial (Fig. 2A). However, Boc-CCK-4, given to lesioned rats pretrial, restored a learning curve to a higher level and decreased the escape latency compared to the first session (F=3.200, df=2,54; P=.045; Fig. 1A).

In the memory phase (test sessions), 1 and 7 days after the training sessions, the sham-lesioned rats showed similar escape latencies to the submerged platform as determined during the last training trial, indicating a memory effect, whereas the lesioned rats took significantly longer to find the platform (F=12.794, df=1,36; P<.001; Figs. 1A and 2A). Treatment with Boc-CCK-4 before the training increased the performance of the lesioned rats in the memory test in 24 h (F=8.272, df=1,36; P=.007, Fig. 1A).

To assess motor disturbances induced by the lesion or Boc-CCK-4 treatment, the distance travelled was measured (Table 3) and the resulting swimming speed was calculated.

During the first training trial, there was no difference in the distance travelled between the sham-lesioned rats and lesioned rats (Table 3). In sham-lesioned rats treated with vehicle or Boc-CCK-4, the distance travelled was decreased in the last training trial (F=3.278, df=1,108; P=.008) and in the memory tests (F=6.728, df=1,36; P=.017), compared to the first trial. If lesioned rats received a Boc-CCK-4 treatment before the swimming test, the distance travelled was decreased in the last training trial (F=3.278, df=5,64;

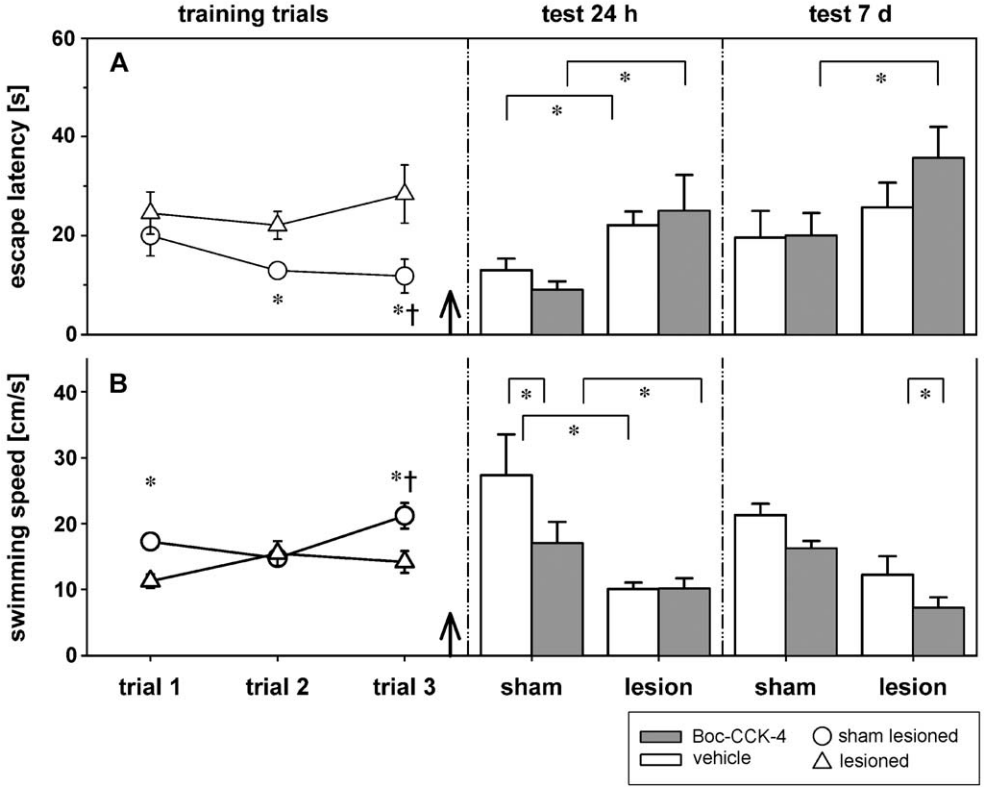

Fig. 2. Inferior performance of 6-OHDA-lesioned rats (△, n=20) in the Morris water maze compared to sham-lesioned rats (○, n=20) measured by the escape latencies (A) and the corresponding swimming speed (B). No behavioural effect of Boc-CCK-4 (40 μg/kg, grey bars) given 5 min after the third training trial (↑) on the performance in the test 24 h and 7 days later, compared to saline-treated rats (white bars). Data are presented as mean±S.E.M. ($^{\dagger}P$<.05 vs. Trial 1, *P<.05 vs. vehicle, lesioned vs. sham lesioned).

Table 3
Swim distance of 6-OHDA-lesioned and sham-lesioned rats in the Morris water maze treated with Boc-CCK-4 (grey fields) or vehicle (white fields) either before the first trial in the water maze (pretrial) or following the last training trial (posttrial)

	Treatment	Trial 1 [cm]	Trial 2 [cm]	Trial 3 [cm]	Memory 24 h [cm]	Memory 7 d [cm]
Post trial	sham/vehicle	325±36	183±41	164±27†	330±89	231±30
	sham/Boc-CCK-4				174±24*	315±69
	lesion/vehicle	241±40	257±41	351±95	206±21	202±17
	lesion/Boc-CCK-4				309±115	202±21
Pre trial	sham/vehicle	263±23	287±27	184±18†	133±12	155±14
	sham/Boc-CCK-4	341±57	216±44	171±23†	195±23*	175±21*
	lesion/vehicle	261±24	311±53	300±47	223±46	210±29
	lesion/Boc-CCK-4	394±29	428±66	186±16†	211±33*	199±22*

Data are presented as mean±S.E.M. (†$P<.05$ vs. Trial 1, *$P<.05$ vs. vehicle).

P=.008) and in the memory tests (F=3.278, df=5,64; P=.008), compared to the first trial (Table 3).

During the training sessions, sham-lesioned rats showed an increase in swim speed (F=3.278, df=5,64; P=.008). In the lesioned animals, the swim speed did not increase and was generally lower than in the sham-lesioned rats (F=3.278, df=5,64; P=.008; Figs. 1B and 2B). Treatment with Boc-CCK-4 did not affect swim speed neither in the sham-lesioned nor in the lesioned rats significantly, indicating no sole improvement of motor abilities by Boc-CCK-4 (Figs. 1B and 2B).

3.1.7. Dopamine tissue levels

The content of dopamine in the brain was substantially lower in the 6-OHDA-lesioned animals (28±94.3 pmol/mg protein) than in the sham-lesioned rats (62.7±13.2 pmol/mg protein) or untreated animals (65±19.4 pmol/mg protein; F=4.370, df=2,27; P=.023).

4. Discussion

The dopamine neurotransmission system plays a major role in cognitive processes (Kulisevsky, 2000). It seems that impaired dopamine function is associated with deficits in learning. Dopamine depletion by administration of 6-OHDA into the ventricle is an established technique (Archer et al., 2003). It is known, that the effects of an intraventricular administration of 6-OHDA in the early postnatal period range from general retardation (Smith et al., 1973), simple motor effects, to deficits in cognition and attention (Raskin et al., 1983; Archer et al., 1988).

In our study, neonatal intraventricular lesioning with 6-OHDA led to a sustained impairment of the dopaminergic system, demonstrated by diminished brain dopamine levels.

Lesioned rats showed a reduced body weight and slower climbing behaviour, but were capable of normal motor activity, as revealed by swimming and home cage activity. It could be argued that the tests for motor skills were performed in a hyperactivity phase, because activity levels in normal developing rat pups increase rapidly between 15 and 22 days but decline at maturity. However, it is known from the literature that activity in 6-OHDA-treated animals in this period should increase to an even greater degree (Shaywitz et al., 1977). An explanation for the mainly intact and indifferent motor behaviour could lie in the magnitude of the dopamine depletion to about 45% of the controls. The decrease in dopamine levels in the nucleus accumbens is smaller than described mostly in the literature (<20% of controls), which may allow a nearly normal motor function (Archer et al., 1988; Teicher et al., 1998). It has been shown that the magnitude of motor hyperactivity in rats is proportional to the extent of dopamine depletion (Miller et al., 1981; Fobes and Olds, 1981). However, our results are comparable with the impairment of spatial learning following neonatal dopamine depletion by about 40% of controls (Pappas et al., 1992).

The water maze task, introduced by Morris (1984), is often used to study changes in memory and the effects of pharmacological intervention in rats and mice (Andrews, 1996; Van der Staay, 2000).

In the first training trial, the escape latency of dopamine-depleted animals did not differ from the sham-lesioned rats. But in contrast to sham-lesioned rats which learned rapidly over three training sessions to find the platform, there was no learning curve visible in the dopamine-depleted rats. Thus, in the memory tests (1 and 7 days later) the lesioned rats, which did not learn the task at first, took longer to find the platform than the sham-lesioned rats, which still remembered the test.

During the first trail, the swim distance and swimming speed showed that the lesioned rats were able to swim almost as fast as the sham-lesioned rats and that they swam a similar distance until they found the escape platform, indicating no motor disability in the lesioned rats.

The sham-lesioned rats mastered the task in the second and the third training session more quickly, swimming on a shorter and more direct path to the platform, indicating a learning effect (Frick et al., 2000). In fact, in a preceding study in nonlesioned rats, swim speed even progressively decreased with task duration due to a more effective performance in the test (Rex et al., in press).

In contrast, in the lesioned rats, the swim distance did not decrease over the three training sessions. The constant swim distance of the lesioned rats indicates no optimised swimming path and hence no learning during the repeated exposure to the water. These results are in accordance with other findings where dopamine depletion caused marked deficits in learning and memory (Stancheva et al., 1993; Feeser and Raskin, 1987) accompanied partly by motor dysfunction, whereas activation of the dopaminergic system enhanced learning (Hersi et al., 1995). Rats with a 6-OHDA-induced lesion of the mesohippocampal dopaminergic system showed decreased performance in the water maze task (Gasbarri et al., 1996) and in the active and passive avoidance learning paradigms (Takasuna and Iwasaki, 1996). In the sham-lesioned rats, Boc-CCK-4 did not change the behaviour of the rats placed in the water maze, independent of the time point of application, indicating no improvement or impairment in cognitive function in healthy animals.

However, in the lesioned rats, Boc-CCK-4 given prior to the first exposure to the water maze led to a learning curve with shorter escape latencies in the second and third training sessions in those rats showing a learning effect, compared to the vehicle-treated lesioned rats. While the swimming speed of the animals was constant, the swim distance was decreased in the second and third training sessions because of the better knowledge about the surrounding acquired by learning and memory. The shorter escape latencies caused by an optimised swimming path suggests that the improvements were caused by better spatial orientation and not by improved motor abilities, which would mainly increase the speed of movement in the water maze. The restored learning also caused a memory effect in the lesioned rats. However, during the acquisition phase of the water maze, the lesioned saline-treated rats failed to reach the level of achievement seen in the other rats. A direct comparison of the subsequent level of retention at 24 h and 7days later requires similar performance in the acquisition phase. One could argue that with several further days of training, all groups could reach a common level but our own experience shows that rats with severe impairments do not reach the performance of young and healthy animals (Rex et al., 2004). Therefore, the ability of Boc-CCK-4 to reverse the 6-OHDA-induced deficit in learning is clear and valid, but it is difficult to interpret the apparent sustained improvement produced 24 h later, whether the improvement is caused by better learning or enhanced retention of the learned. Not surprisingly, Boc-CCK-4 given after the three training sessions had no effect in the consolidation phase of learning because no learning occurred in the 6-OHDA-lesioned rats in the first place.

Our results are comparable with facilitatory effects of the CCK2 agonists CCK-8 unsulphated or BC 264 on memory (Winnicka and Wisniewski, 2000; Dauge and Lena, 1998; Taghzouti et al., 1999).

The results of the present study show that the CCK2 agonist Boc-CCK-4 influenced the performance of 6-OHDA-lesioned rats but not of sham-lesioned animals in the acquisition phase (training sessions) of the Morris water maze test. It is well known that beside classical neurotransmitters, peptides, such as CCK, are involved in the process of learning and memory (Kovács and de Wied, 1994; Yamaguchi et al., 1998). Effects of CCK on learning and memory were found when given centrally as well as peripherally. However, the results are often ambiguous or contradictory (Huston et al., 1998; Voits et al., 2001; Itoh et al., 1988; Takashima and Itoh, 1989). Possible reasons for these differences could be variations in the animal models, as well as the doses, routes of administration, bioactivity and selectivity of the peptides used. Additionally, it has to be considered that cognitive processes are complex and the various animal models may only reflect distinct types of memory, which are mediated by different neuroanatomical and neurochemical correlates. It is also known that other drugs, e.g., nootropics, alter learning and memory in some procedures but may be without activity in many others (Heise, 1984). Therefore, it is possible that CCK is effective only in certain tests of learning.

It is difficult to validate an improvement in learning and/or memory in young and even older healthy rats, leading to the use of experimentally impaired animals.

There are many studies regarding the interaction of CCK and dopamine based on the colocalization of the two transmitters in the mesolimbic and mesocortical systems. Dopamine and CCK are able to alter each other's release, receptor binding and pharmacological effects. However, the interactions between CCK and dopamine are not simple and straightforward (Beinfeld, 2001). Reum et al. (1997) found a CCK2-receptor-mediated increase in extracellular dopamine in the anterior nucleus accumbens after application of CCK-4 into the ventral tegmental area or a decrease in midbrain dopamine unit activity by the selective CCK2 antagonist LY288513 (Helton et al., 1996), whereas a decrease in dopamine following CCK-4 treatment was described (Lodge et al., 2000).

It is known that CCK is able to affect learning and memory, even if the known effects are not uniform (see Introduction). From our results, it could be suggested that CCK-4, under pathophysiological conditions, functionally compensates the impaired learning caused by neonatal dopamine depletion. This can be concluded from the mnemotropic effect of Boc-CCK-4 given pretrial (learning), but not posttrial (memory) in the lesioned animals and by the lack of effect in the sham-lesioned rats.

In conclusion, we could show that neonatal treatment of rats with 6-OHDA causing dopamine depletion impairs acquisition learning in the Morris water maze in young adult rats. Acute treatment with Boc-CCK-4 (40 μg/kg ip) pretrial reversed the cognitive impairment, implying an interactive role of dopamine with the CCK system in cognitive behaviour, through the stimulation of CCK2 brain receptors.

Acknowledgements

The work was initiated by Prof. Helga Rex. The authors thank Dr. S. Beckett for his invaluable help during the final preparation of the manuscript. The work was supported by the DFG (Grant FI 491/2–3).

References

Adams PM, Fabricant JD, Legator MS. Cyclophosphamide-induced spermatogenic effects detected in the F1 generation by behavioral testing. Science 1981;211;80–2.

Andrews JS. Possible confounding influence of strain, age and gender on cognitive performance in rats. Cogn Brain Res 1996;3;251–67.

Archer T, Danysz W, Frederiksson A, Jonsson G, Luthman I, Sundström E, et al. Neonatal 6-hydroxydopamine-induced dopamine-depletions: motoractivity and performance in maze learning. Pharmacol Biochem Behav 1988;31;357–64.

Archer T, Palomo T, McArthur R, Fredriksson A. Effects of acute administration of DA agonists on locomotor activity: MPTP versus neonatal intracerebroventricular 6-OHDA treatment. Neurotox Res 2003;5;95–110.

Beinfeld MC. An introduction to neuronal cholecystokinin. Peptides 2001;22;1197–200.

Beinfeld MC. What we know and what we need to know about the role of endogenous CCK in psychostimulant sensitization. Life Sci 2003;73; 643–654.

Costall B, Naylor RJ, Nohria V. Climbing behaviour induced by apomorphine in mice: a potential model for the detection of neuroleptic activity. Eur J Pharmacol 1978;50;39–50.

Crawley JN. Subtype-selective cholecystokinin receptor antagonists block cholecystokinin modulation of dopamine-mediated behaviors in the rat mesolimbic pathway. J Neurosci 1992;12;3380–91.

Crawley JN, Corwin RL. Biological actions of cholecystokinin. Peptides 1994;15;731–55.

Dauge V, Lena I. CCK in anxiety and cognitive processes. Neurosci Biobehav Rev 1998;22;815–25.

Dauge V, Beslot F, Matsui T, Roques BP. Mutant mice lacking the cholecystokinin2 receptor show a dopamine-dependent hyperactivity and a behavioral sensitization to morphine. Neurosci Lett 2001;306; 41–44.

D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Rev 2001;36;60–90.

Drinkenburg WH, Vijn P, Martorana MG, Dickson M, van Schaijk W. A novel automated apomorphine climbing test. Proceeding of Measuring Behavior 2000, 3rd International Congress on Methods and Techniques in Behavioral Research, Nijmegen; 2000.

Feeser HR, Raskin LA. Effects of neonatal dopamine depletion on spatial ability during ontogeny. Behav Neurosci 1987;101;812–8.

Fink H, Morgenstern R, Ott T. CCK-8 modulates D2 receptor agonist-induced hypermotility in the nucleus accumbens. Brain Res Bull 1991;26;437–40.

Fink H, Rex A, Voits M, Voigt JP. Major biological actions of CCK—a critical evaluation of research findings. Exp Brain Res 1998;123; 77–83.

Fobes JL, Olds ME. Effects of neonatal 6-hydroxydopamine treatment on catecholamine levels and behavior during development and adulthood. Psychopharmacology 1981;73;27–30.

Frick KM, Stillner ET, Berger-Sweeney J. Mice are not little rats: species differences in a one-day water maze task. NeuroReport 2000; 11;3461–5.

Fried I, Wilson CL, Morrow JW, Cameron KA, Behnke ED, Ackerson LC, et al. Increased dopamine release in the human amygdala during performance of cognitive tasks. Nat Neurosci 2001;4;201–6.

Gasbarri A, Sulli A, Innocenzi R, Pacitti C, Brioni JD. Spatial memory impairment induced by lesion of the mesohippocampal dopaminergic system in the rat. Neuroscience 1996;74;1037–44.

Gerhardt P, Voits M, Fink H, Huston JP. Evidence for mnemotropic action of cholecystokinin fragments Boc-CCK-4 and CCK-8S. Peptides 1994;15;689–97.

Heise GA. Behavioral methods for measuring effects of drugs on learning and memory in animals. Med Res Rev 1984;4;535–58.

Helton DR, Berger JE, Czachura JF, Rasmussen K, Kallman MJ. Central nervous system characterization of the new Cholecystokinin B antagonist LY288513. Pharmacol Biochem Behav 1996;53;493–502.

Hersi AI, Rowe W, Gaudreau P, Quirion R. Dopamine D1 receptor ligands modulate cognitive performance and hippocampal acetylcholine release in memory-impaired aged rats. Neuroscience 1995;69;1067–74.

Hommer DW, Palkovits M, Crawley JN, Paul SM, Skirboll LR. Cholecystokinin-induced excitation in the substantia nigra: evidence for peripheral and central components. J Neurosci 1985;6;1387–92.

Huston JP, Schildein S, Gerhardt P, Privou C, Fink H, Hasenöhrl RU. Modulation of memory, reinforcement and anxiety parameters by intra-amygdala injection of cholecystokinin-fragments Boc-CCK-4 and CCK-8s. Peptides 1998;19;27–37.

Itoh S, Lal H. Influences of cholecystokinin and analogues on memory processes. Drug Dev Res 1990;21;257–76.

Itoh S, Takashima A, Katsuura G. Preventive effect of cholecystokinin octapeptide on scopolamine-induced memory impairment in the rat. Drug Dev Res 1988;12;63–70.

Kovács GL, de Wied D. Peptidergic modulation of learning and memory processes. Pharmacol Rev 1994;46;269–91.

Kulisevsky J. Role of dopamine in learning and memory: implications for the treatment of cognitive dysfunction in patients with Parkinson's disease. Drugs Aging 2000;16;365–79.

Ladurelle N, Keller G, Blommaert A, Roques BP, Dauge V. The CCK-B agonist, BC264, increases dopamine in the nucleus accumbens and facilitates motivation and attention after intraperitoneal injection in rats. Eur J Neurosci 1997;9;1804–14.

Lena I, Roques BP, Durieux C. Dual modulation of dopamine release from anterior nucleus accumbens through cholecystokinin-B receptor subsites. J Neurochem 1997;68;162–8.

Li XL, Aou S, Hori T, Oomura Y. Spatial memory deficit and emotional abnormality in OLETF rats. Physiol Behav 2002;75;15–23.

Lipp HP, Wolfer DP. Genetically modified mice and cognition. Curr Opin Neurobiol 1998;8;272–80.

Lodge DJ, Short JL, Mercer LD, Beart PM, Lawrence AJ. CCK/dopamine interactions in Fawn-Hooded and Wistar-Kyoto rat brain. Peptides 2000;21;379–86.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193;265–75.

McNamara RK, Skelton RW. The neuropharmacological and neurochemical basis of place learning in the Morris water maze. Brain Res Rev 1993;18;33–49.

Miller FE, Heffner TG, Kotake C, Seiden LS. Magnitude and duration of hyperactivity following neonatal 6-hydroxydopamine is related to the extent of brain dopamine depletion. Brain Res 1981;229;123–32.

Morris R. Development of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984;11;47–60.

Moy SS. Impaired acquisition and operant responding after neonatal dopamine depletion in rats. Pharmacol Biochem Behav 1995;52;433–41.

Nomoto S, Miyake M, Ohta M, Funakoshi A, Miyasaka K. Impaired learning and memory in OLETF rats without cholecystokinin (CCK)-A receptor. Physiol Behav 1999;66;869–72.

Pappas BA, Murtha SJ, Park GA, Condon KT, Szirtes RM, Laventure SI, et al. Neonatal brain dopamine depletion and the cortical and behavioral consequences of enriched postweaning environment. Pharmacol Biochem Behav 1992;42;741–8.

Pearson DE, Raskin LA, Shaywitz BA, Anderson GM, Cohen DJ. Radial arm maze performance in rats following neonatal dopamine depletion. Dev Psychobiol 1984;17;505–17.

Ploeger GE, Spruijt BM, Cools AR. Spatial localization in the Morris water maze in rats: acquisition is affected by intra-accumbens injections of the dopaminergic antagonist haloperidol. Behav Neurosci 1994;108; 927–934.

Raskin LA, Shaywitz BA, Anderson GM, Cohen DJ, Teicher MH, Linakis J. Differential effects of selective dopamine, norepinephrine or catecholamine depletion on activity and learning in the developing rat. Pharmacol Biochem Behav 1983;19;743–9.

Rasmussen K, Stockton ME, Czachura JF, Howbert JJ. Cholecystokinin (CCK) and schizophrenia: the selective CCKB antagonist LY262691 decreases midbrain dopamine unit activity. Eur J Pharmacol 1991; 209;135–8.

Reum T, Schäfer U, Marsden CA, Fink H, Morgenstern R. Cholecystokinin increases extracellular dopamine overflow in the anterior nucleus accumbens via CCK(B) receptors in the VTA assessed by in vivo voltammetry. Neuropeptides 1997;31;82–8.

Rex A, Spychala M, Fink H. Treatment with reduced nicotinamide adenide dinucleotide (NADH) improves water maze performance in old Wistar rats. Behav Brain Res 2004 [in press].

Rodgers RJ, Johnson NJ. Cholecystokinin and anxiety: promises and pitfalls. Crit Rev Neurobiol 1995;9;345–69.

Shaywitz BA, Gordon JW, Klopper JH, Zelterman DA. The effect of 6-hydroxydopamine on habituation of activity in the developing rat pup. Pharmacol Biochem Behav 1977;6;391–6.

Smith RD, Cooper BR, Breese GR. Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine: evidence for involvement of brain dopamine. J Pharmacol Exp Ther 1973;185;609–19.

Smith DR, Striplin CD, Geller AM, Mailman RB, Drago J, Lawler CP, et al. Behavioural assessment of mice lacking D1A dopamine receptors. Neuroscience 1988;86;135–46.

Stancheva S, Papazova M, Alova L, Lazarova-Bakarova M. Impairment of learning and memory in shuttle box-trained rats neonatally injected with 6-hydroxydopamine. Effects of nootropic drugs. Acta Physiol Pharmacol Bulg 1993;19;77–82.

Taghzouti K, Léna I, Dellu F, Roques BP, Daudé V, Simon H. Cognitive enhancing effects in young and old rats of pBC264, a selective CCKB receptor agonist. Psychopharmacology 1999;143;141–9.

Takashima A, Itoh S. Memory impairment induced by CCK-8 antagonists in passive avoidance response of rat. Drug Dev Res 1989;18;95–100.

Takasuna M, Iwasaki T. Active and passive avoidance learning in rats neonatally treated with intraventricular 6-hydroxydopamine. Behav Brain Res 1996;74;119–26.

Teicher MH, Andersen SL, Campbell A, Gelbard HA, Baldessarini RJ. Progressive accumbens degeneration after neonatal striatal 6-hydroxydopamine in rats. Neurosci Lett 1998;247;99–102.

Van der Staay FJ. Age-related changes in learning and memory in rats, assessed in the Morris water escape task. The study of behavioral dysfunctions: an evaluation of selected animal models. Groningen: BCN; 2000. p. 15–80.

Voits M, Hasenöhrl RU, Huston JP, Fink H. Repeated treatment with cholecystokinin octapeptide improves maze performance in aged Fischer 344 rats. Peptides 2001;22;1325–30.

Winnicka MM, Wisniewski K. Bilateral 6-OHDA lesions to the hippocampus attenuate the facilitatory effect of CCK-8 us and caerulein on memory in rats. Pharmacol Res 2000;41;347–53.

Yamaguchi Y, Kawashima S, Kobayashi H. Peptides in processes of learning and memory. Neuro Endocrinol Lett 1998;18;103–10.