The Transfer of Rats from a Familiar to a Novel Environment Prolongs the Increase of Extracellular Dopamine Efflux Induced by CCK8 in the Posterior Nucleus Accumbens

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The effects of cholecystokinin octapeptide (CCK8) on extracellular dopamine (DA) and its metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, were measured in the nucleus accumbens (N. Acc.). The experiments were carried out using *in vivo* microdialysis in awake rats submitted, or not, to a novel environment, the four-hole box. The exploratory behavior of the animals was studied concurrently in these boxes and in the elevated plus maze. Without CCK8 treatment, the transfer of animals from their home cages to the four-hole box induced a transient increase in DA efflux and also tended to increase its metabolites in the posterior N. Acc. In rats placed in a familiar environment, the administration of 25 pmol of CCK8 in this region immediately enhanced DA release, with levels rapidly returning to normal at the end of the perfusion. In contrast, this treatment produced a longer effect in rats transferred to the four-hole box, since the DA efflux was still increased 80 min after the removal of CCK8. The intra-accumbal administration of CCK8 induced a hypoexploration in the four-hole box. Moreover, an anxiogenic-like effect of CCK8 was found in the elevated plus maze, only in rats submitted to a novel environment (four-hole box). These data show that (1) the postero-accumbens DA neurons can be activated by environmental changes and (2) that the intensity of the CCK8 effects on extracellular DA levels and on anxiety-like responses seems to depend on the activity of these neurons previous to CCK8 treatment. The prolonged DA release induced by CCK8 in animals placed in a new situation could correspond to a biochemical anticipation preparing them to react when faced by another stimulus.

[Key words: dopamine release, cholecystokinin, environmental change, nucleus accumbens, microdialysis, freely moving rats]

The discovery of the carboxy-terminal sulfated octapeptide of cholecystokinin (CCK8) in mammalian brain (Dockray, 1976) has generated widespread interest on the role of this peptide in

the regulation of various CNS functions. In 1980, Hökfelt et al. reported that a subpopulation of mesencephalic dopamine (DA) neurons also contains CCK. In rodents, cats, primates, and in humans, these mixed cells were shown to be located primarily in the ventral tegmental area (VTA) and in the substantia nigra pars compacta (Hökfelt et al., 1980; Schalling et al., 1990). Their projections terminate in the limbic region, predominantly in the medial and caudal portions of the nucleus accumbens (N. Acc.) and olfactory tubercles (Studler et al., 1981; Hökfelt et al., 1985, 1986). In the last decade, the functional division of the N. Acc. into anterior and posterior parts has been shown by anatomical, biochemical, and behavioral data. The anterior part receives CCK fibers from cortical regions (pyriform cortex, medial prefrontal cortex, etc.) and from limbic structures such as amygdala (Fallon and Seroogy, 1985; Zaborsky et al., 1985), while the innervation of the postero-medial part arises from CCK neurons of the nucleus tractus solitarius (Wang et al., 1992) or the substantia nigra pars compacta and mainly from the mixed CCK-DA neurons of the VTA (Hökfelt et al. 1985). The distinction between these two regions has also been shown by release experiments where CCK8 differentially modulated DA release from the rostral and the caudal DA terminals of the N. Acc.: a decrease and an increase in DA release have, respectively, been found by different techniques in these two regions (Vickroy and Bianchi, 1989; Marshall et al., 1991; Ladurelle et al., 1993). However, it should be noted that some other groups have not found these effects (Hamilton et al., 1984; Voigt and Wang, 1984; Voigt et al., 1985, 1986; Lane et al., 1987; Ruggeri et al., 1987). These discrepancies probably depend on whether the basal or the K⁺-evoked DA release was measured by push-pull cannula, microdialysis in awake or anesthetized animals, or *in vitro* techniques. CCK8 also modifies several rat behaviors, depending on its injection site in the N. Acc. For instance, a rostral injection of CCK8 has been shown to elicit a decrease in intracranial self-stimulation (De Witte et al., 1987), to attenuate locomotor activation induced by amphetamine or apomorphine (Weiss et al., 1988; Vaccarino and Rankin, 1989) and to reduce the spontaneous alternation behavior of rats (Daugé et al., 1992). On the other hand, most of the studies have shown that CCK8 injected into the postero-median N. Acc. was unable to modify the spontaneous locomotor activity of rats but potentiated the DA- or amphetamine-induced hyperlocomotion (Crawley et al., 1985; Vaccarino and Rankin, 1989). Finally, some studies in rats have shown that some effects of CCK8 are revealed in paradigms generally conceptualized as producing anxiety-like internal states and/or tendencies to explore a novel environment.

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Thus, Daugé et al. (1989a) have reported that, injected in the postero-median N. Acc., CCK8 decreased locomotion and rearings measured in the open field only in rats unaccustomed to this environment. Furthermore, in these animals, CCK8 has been shown to decrease the exploration in the four-hole box and to induce an anxiogenic-like effect in the elevated plus-maze through CCK-A receptor stimulation (Daugé et al., 1989b). Interestingly, these latter effects were suppressed by a 6-hydroxy-dopamine lesion of the DAergic meso-accumbens pathway and by selective D2 but not D1 antagonists (Derrien et al., 1993).

It is now clearly established that the activity of the DA system, notably in the N. Acc., depends on motivational states, strongly suggesting that, in the previous experiments, the behavioral effects of CCK8 were observed only because the DA system was activated by the new situation (fear and/or curiosity induced by the novelty). In a previous study performed in awake rats habituated to their environment, CCK8 perfused in the posterior N. Acc. was shown to increase the DA efflux measured by microdialysis but had no effect on global motor activity (Ladurelle et al., 1993). Therefore, the goal of this study was to investigate the influence of a novel environment on DA release and on behavioral responses in rats before and after CCK8 treatment. With this aim, the release of DA in the posterior N. Acc. was measured by microdialysis in awake rats placed in a novel environment (the four-hole box) and in rats housed in a familiar one (the box in which they were placed 24 hr before experiments). The influence of CCK8, locally perfused in this region, was then studied on both animal behavior and extracellular levels of DA and of its two catabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The two doses of the peptide used in this study (1 fmol and 25 pmol) were based on previous studies, showing behavioral and biochemical modifications after injection of CCK8 into the postero-median N. Acc. (Daugé et al., 1989b; Ladurelle et al., 1993). In both cases, the locally injected doses corresponded to the quantities of CCK8 that were calculated to diffuse across the membrane of the microdialysis probes (~4%).

Materials and Methods

Animals

The subjects used throughout this study were male Wistar rats (Dépre, France) weighing 200–220 gm at the time of implantation. They were housed in groups of four in a well-ventilated environment under controlled temperature ($22 \pm 1^\circ\text{C}$) and humidity ($50 \pm 5\%$), with food and water made available *ad libitum*.

Surgery

A week before use, the rats were stereotactically implanted under chloral hydrate anesthesia (400 mg/kg, *i.p.*). For microdialysis experiments, an unilateral 20-gauge (0.9 mm in external diameter) stainless steel cannula guide was implanted 1.5 mm above the left side posterior N. Acc. using coordinates +1.2 mm anterior, 1.2 mm lateral, and –8 mm ventral, relative to bregma and skull surface (Atlas of Paxinos and Watson, 1986). For the intra-accumbens injections, rats were bilaterally implanted with stainless steel cannula guides of 25 gauge (0.6 mm in diameter) 1.5 mm above the N. Acc. (anterior, +1.2 mm; lateral, ± 1.2 mm; ventral, –7.0 mm). After the operation, animals received an intramuscular antibiotic injection to prevent infection (Extencilline, 0.3 MU/kg).

Injection procedure

Intra-accumbal injections were made using 30.5 gauge stainless steel needles connected to microsyringes (Hamilton) by polyethylene tubing. CCK8 or vehicle were administered by an infusion pump (Precinorm) under a constant volume (0.2 $\mu\text{l/side}$) in 2 min. The needles were left in place for 30 sec after the end of injection to allow diffusion of the

liquid. After the injection, rats were replaced in their home cages to wait for behavioral testing.

Brain dialysis procedure

The dialysis probes, constructed according to Robinson and Whislaw (1988), consisted of a 3 mm long semipermeable cellulose membrane (molecular cut-off 6000 Da, outer diameter 0.25 mm; Spectrum, Medical Industries, Inc.) connected to a perfusion system (previously described in Ladurelle et al., 1993). The probes were inserted into the chronically implanted cannula guides until the active membrane was positioned in the N. Acc. and were maintained by a locking screw. This placement was carried out 18–20 hr prior to the start of the experiments and animals were put into individual black boxes ($40 \times 40 \times 40$ cm). This latency period was required to minimize behavioral and biochemical disturbances due to the probe implantation.

The brain microdialysis procedure consisted of a constant probe perfusion with dialysis buffer (120 mM NaCl; 5 mM KCl; 1.8 mM CaCl_2 ; 1.2 mM MgCl_2 ; 0.2 mM phosphate-buffered saline; pH 7.4) at a flow rate of 2 $\mu\text{l/min}$. In order to reach a steady state of the dialysis around the probe membrane, perfusion was carried out for 2 hr before starting the experiments. For the basal efflux determination, three dialysis samples (10 min each) were collected in small tubes containing 40 μl of an antioxidant mixture (10 μl of EDTA 0.04% in 0.1 M perchloric acid plus 30 μl of HPLC mobile phase). The animals were then treated for 10 min by carefully replacing the perfusion medium by a CCK8 solution, avoiding the introduction of bubbles in the system. Two 15 min samples (to take the dead volume of the catheter and the duration of the behavioral test into account) were collected, after which the 10 min sample collection was continued for 140 min.

Drug and perfusion

Intra-accumbens dialysis administrations of CCK8 were made by perfusing the probes for 10 min with dialysis buffer (for composition, see above) containing sulfated CCK8 at a flow rate of 2 $\mu\text{l/min}$. Sulfated CCK8 (Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe- NH_2) was synthesized in the laboratory. For microdialysis experiments, two doses of CCK8 were administered: 25 pmol and 1 fmol (in 20 μl of perfusion medium). The global amounts of CCK8 reaching the N. Acc. were estimated by taking into account the passage of the peptide throughout the dialysis membrane. This recovery was determined *in vitro* at body temperature (37°C) by perfusing probes placed in a beaker of microdialysis buffer added to CCK8 radiolabeled ^3H . The recovery of probes was estimated by the following ratio: radioactivity found in the perfusate/radioactivity in the beaker and was about 3–4% (for more details in the procedure see Ladurelle et al., 1993).

For tests without microdialysis, CCK8 was dissolved in saline (NaCl 0.9%) and bilaterally injected at a dose of 25 pmol/side (0.2 $\mu\text{l/side}$ in 2 min).

Behavioral stimulation and monitoring

Procedure associated with the microdialysis collection. Fifteen minutes after the beginning of the intra-accumbens CCK8 perfusion, the animals, housed in a familiar environment (the individual black boxes mentioned above), were carefully transferred into four-hole boxes (Taghzouti et al., 1985). The length of the perfusion tubing allowed this transfer to be carried out without removing the probes and, therefore, did not perturb the dialysis. The four-hole box consisted of a black square box ($45 \times 45 \times 24$ cm) illuminated from the top (200 lux). Each corner of the box was extended by a small corridor, at the end of which was drilled a hole (diameter 3.5 cm, 4 cm above floor) extended by a metallic tube (length 8 cm) containing a photoelectric detector. During the test, the rats, initially placed in the middle of the arena, cut the photoelectric beams when they visited the holes. The number and the duration of these beam “interruptions” served as a measure of exploratory behavior and were registered on a computer for the first 15 min following the entrance of the animal in the box. To be sure that the effects observed in the groups transferred to the four-hole box were not an artifact from the stress of handling, a microdialysis experiment was done with animal handled or not, transferred or not, and treated or not by CCK8.

Procedure without microdialysis. Fifteen minutes after the intra-accumbens injection, rats were transferred from their home cage to the four-hole box and the same parameters as those cited above were measured for 15 min. Immediately after this test the animals were submitted

Figure 1. Representation of the histological control of several implantation sites of the dialysis probes. Continuous lines show right positions of the active membrane and broken track lines represent probe positions eliminated from data analysis. The number on each section refers to millimeters rostral to the bregma according to the atlas of Paxinos and Watson (1986).

to another situation: the elevated plus maze. This wooden apparatus consisted of four arms (50×10 cm) forming a cross with an open square at the intersection of two opposite "closed" arms (C) with walls of 40 cm height and two opposite "open" arms (O). The maze was elevated 50 cm above floor and illuminated (300 lux) from the top. At the beginning of the test, the rats were placed facing an open arm in the intersection square. An arm visit was counted when the animals placed all four paws into an arm. The number of entries and the cumulative time spent in the open and the closed arms were measured by an experimenter during a 5 min test session. Another group of animals were identically tested in the elevated plus maze 30 min after the intracerebral injection but without the previous passage in the four-hole box. The results were expressed as the total (O+C) number and time spent in the arms and the percentage of the total number or duration of open arms entries over the total visits (O/O+C number and O/O+C duration) were calculated.

HPLC assays

The levels of DA and its two metabolites, DOPAC and HVA, in the dialysates were determined by electrochemical detection after HPLC separation on a reverse-phase C18 column (100×2.3 mm; $3 \mu\text{m}$; Catecholamines, Europhor, France). The mobile phase (0.015 M sodium

acetate; 0.015 M citric acid, 1.2–1.7 mM sodium octane sulfonate, 0.2 mM EDTA, and 12.5% methanol, pH 3.8), pumped at a flow rate of 0.9 ml/min, was preoxidized by a guard electrode (5020, ESA) set at 400 mV in order to minimize background noise, and the three monoamines were assayed by a coulometric detector (Coulchem 5100A, ESA) after oxidation (at +350 mV) and reduction (at -230 mV) by a dual analytical cell (5011, ESA). The chromatograms were recorded by a data processor (CR5A, Shimadzu). Peak identification and quantification were carried out by comparison with artificial profiles obtained with standard samples containing from 0 to 2000 pg of DA, DOPAC, and HVA.

Histological control

At the end of the experiments, an overdose of hydrate chloral was administered to the animals. The brains were removed, frozen, and cut on a microtome and the slices ($50 \mu\text{m}$) were stained with cresyl violet. The position of the probes or needles was determined according to the atlas of Paxinos and Watson (1986). Figure 1 shows the location of some probes implanted in the posterior N. Acc. Probes that traversed more than 70% of the N. Acc. were considered to be correctly placed. The anterior and lateral positions of the probe were estimated from the island of Calleja and the anterior commissure, respectively. Approximately 7% of the rats were not correctly implanted and then eliminated from the data calculations. Ninety-three rats were correctly implanted.

Data analysis

For each rat, the extracellular levels of DA, DOPAC, or HVA were first calculated in picograms released per minute and in nanomolar concentrations, and they were then expressed as the percentage of change from the basal efflux, determined by the average values of the three first samples collected. Then, for each group, the mean and the SEM were calculated. The dialysis time-course data were analyzed using a two-way repeated measure (treatment and time) analysis of variance (ANOVA). If significant effects were observed, a one-way analysis of variance followed by a Dunnett's *t* test was used to determine the significance at each time point. Moreover, for each experiment, the extracellular levels were also expressed as the percentage of the levels in the control animals before being compared by the same statistical tests. The behavioral data were analyzed by a one-way analysis of variance (ANOVA) followed by a Dunnett's *t* test for comparison with controls or a Newman-Keuls test for multiple comparisons. The level chosen for statistical significance was 5%.

Results

Modification of the DA transmission in the posterior N. Acc.

Before CCK8 treatment and/or behavioral stimulation, there was no difference in the basal extracellular outflow of DA, DOPAC, and HVA in the posterior N. Acc. of rats between the treated and the saline groups. These levels were about 2.5, 70.0, and 43.0 pg/min respectively (for more details see Table 1).

Table 1. Average basal extracellular levels, uncorrected for recovery, of DA, DOPAC, and HVA in the posterior N. Acc. of rats

	DA	DOPAC	HVA
Experiment 1			
Saline ($n = 9$)	2.5 ± 0.3 (6.6 ± 0.8)	69.3 ± 7.2 (206.1 ± 21.4)	44.1 ± 4.8 (121.0 ± 13.2)
CCK8, 1 fmol ($n = 8$)	2.6 ± 0.3 (6.9 ± 0.8)	71.9 ± 8.6 (213.9 ± 25.6)	42.1 ± 6.2 (115.5 ± 17.0)
CCK8, 25 pmol ($n = 10$)	2.1 ± 0.3 (5.5 ± 0.8)	69.2 ± 6.9 (205.8 ± 20.5)	42.9 ± 7.2 (117.7 ± 19.8)
Experiment 2			
Saline ($n = 8$)	2.6 ± 0.4 (6.9 ± 1.1)	73.0 ± 7.8 (217.1 ± 23.2)	42.7 ± 4.9 (117.2 ± 13.4)
CCK8, 1 fmol ($N = 6$)	2.3 ± 0.3 (6.1 ± 0.8)	71.7 ± 8.1 (213.3 ± 24.1)	44.1 ± 5.2 (121.0 ± 14.3)
CCK8, 25 pmol ($N = 7$)	2.6 ± 0.4 (6.6 ± 1.1)	72.5 ± 8.1 (215.6 ± 24.1)	42.6 ± 4.5 (116.9 ± 12.3)

Experiment 1 corresponds to the study in acclimated rats, and experiment 2, in rats transferred to the four-hole box. Data are given in pg/min and in nanomolar concentrations (in parentheses) and expressed as means $\pm$ SEM of the three basal microdialysis samples taken before drug treatment and change of environment. One-way ANOVA: Experiment 1: DA: $F_{2,18} = 0.056$, $p = 0.9455$, NS; DOPAC: $F_{2,18} = 0.006$, $p = 0.9942$, NS; HVA: $F_{2,18} = 0.279$, $p = 0.7599$, NS. Experiment 2: DA: $F_{2,24} = 0.789$, $p = 0.4657$, NS; DOPAC: $F_{2,24} = 0.040$, $p = 0.9611$, NS; HVA: $F_{2,24} = 0.024$, $p = 0.9765$, NS.

Effects of a novel environment on extracellular DA levels

Without any treatment, the transfer of the animals from their black boxes to the four-hole boxes induced an increase in extracellular DA levels ($114.3 \pm 6.1\%$ of basal). This effect started as soon as the environment was changed, was maximum for approximately 30 min following the transfer, and then gradually decreased to basal level even though the rats were still in the box (Fig. 2). Although the metabolite levels also appeared to be modified, the increase in their levels was not significant.

Effects of CCK8 perfusion on rats accustomed to their environment

Two-way ANOVA showed a significant effect of the CCK8 treatment on extracellular DA levels, which occurred with 25 pmol but not with 1 fmol of the peptide (Fig. 3). Thus, the perfusion of CCK8 in the posterior N. Acc. induced an immediate increase to $145.1 \pm 4.7\%$ of basal DA levels. After removal of CCK8 from the perfusion medium, the extracellular DA levels returned rapidly to basal values. Despite a lack of significance, the catabolite levels, especially for DOPAC, were also increased by the treatment and returned more slowly to basal levels.

Effects of CCK8 perfusion on rats placed in a novel environment

As in previous experiment, the data analysis revealed a significant increase in DA outflow after administration of 25 pmol of CCK8 (Fig. 4). The intensity of this effect was equivalent to that obtained without changing the rats' environment ($155.5 \pm 5.8\%$ of basal) but its time-course profile was different since the increase persisted for a longer period. However, this prolongation in the effect of CCK8 could only be due to the increase in DA efflux triggered by the novel environment. Therefore, in order to eliminate this parameter, the increase of the extracellular DA levels was expressed in both experiments as the percentage of change compared with the control group (Table 2). This statistical comparison of the effects of CCK8 (25 pmol) showed a significant difference between the animals housed in their boxes and those transferred in the four-hole boxes. For both experiments, the DA increase measured immediately after CCK8 perfusion was of the same order, but from 50 to 140 min it was higher in animals submitted to the novel environment. Concerning metabolites, DOPAC levels were slightly increased by 25 pmol of CCK8, and HVA also appeared to be increased, but the analysis was not statistically significant.

Figure 2. Effect of the transfer of nontreated rats in the four-hole boxes on extracellular DA (A), DOPAC (B), and HVA (C) levels, uncorrected for recovery, in the posterior N. Acc. The 100% value was calculated by the mean of three baseline samples taken just before the first time (10 min) of the curves. The results are expressed as the percentage of this mean (the means are mentioned in Table 1), and bars are SEM. Black squares with thick lines represent the effects on animals ($n = 8$) submitted to the four-hole box; open squares with thin lines show the responses of rats housed in black boxes for 24 hr ($n = 9$). *, $p < 0.05$ versus control group; Dunnett's t test. Two-way ANOVA: DA: treatment, $F(1,15) = 0.311$, $p = 0.5856$; time, $F(10,150) = 1.913$, $p = 0.0474$; interaction, $F(10,150) = 4.249$, $p = 0.0001$. DOPAC: treatment, $F(1,15) = 2.705$, $p = 0.1208$; time, $F(10,150) = 1.034$, $p = 0.4179$; interaction, $F(10,150) = 1.038$, $p = 0.4148$. HVA: treatment, $F(1,15) = 3.260$, $p = 0.0911$; time, $F(10,150) = 0.556$, $p = 0.8472$; interaction, $F(10,150) = 1.232$, $p = 0.2752$.

Effect of handling on the DA release induced by CCK8

In order to determine if the prolongation of the DAergic response to CCK8 in the rats placed in the novel environment really resulted from their exposure to the four-hole box and not from the handling occurring during the transfer, an experiment was done with six groups of animals: saline not handled, saline handled but not transferred, saline transferred, CCK8 (25 pmol) not handled, CCK8 handled but not transferred, and CCK8 transferred. In the groups of rats treated with CCK8, the statistical analysis of the release of DA in the posterior N. Acc. revealed that the rats not handled and those handled but not transferred to the novel environment had the same profile of responses (respective areas under the curves, 14701 ± 205 and 14452 ± 659) that were both different from that of rats transferred [area: 18433 ± 652 , $F(5,39) = 23.753$, $p = 0.0001$]. Finally, it should be noted that, even if it was not significant, the handling of control rats induced a very slight increase of the extracellular DA levels as shown by the following areas under the curves: control rats not handled, 11751 ± 280 ; control rats handled, 12035 ± 531 ; and control rats handled and transferred, 12403 ± 425 .

Behavioral measurements

Effects of CCK8 perfusion on the rat exploratory activity in the four-hole box. The analysis of variance revealed the presence of behavioral modifications in the rats treated by perfusion of CCK8 during the microdialysis experiment (Fig. 5). Both doses (1 fmol and 25 pmol) produced a hypoexploration, measurable during the 15 min of the test by a reduction in the number of hole visits [$F(2,18) = 6.058$, $p = 0.0097$]. In contrast, the total duration of these visits was not significantly reduced, although it was close to the level of significance [$F(2,18) = 2.814$, $p = 0.0864$].

Effects of CCK8 injection on the rat exploratory activity in the four-hole box (data not shown). One-way ANOVA showed a significant reduction in the numbers of hole visits and a clear tendency of a decrease in visit durations measured over 15 min in animals locally injected with 25 pmol/side of CCK8. The number of hole visits passed from 32.3 ± 4.8 for saline to 16.5 ± 3.8 for treated rats [$F(2,18) = 6.674$, $p = 0.0187$] and the duration of visits from 34.3 ± 5.0 to 22.0 ± 5.7 , respectively [$F(2,18) = 2.636$, $p = 0.1219$].

Effects of CCK8 in rats submitted to the elevated plus maze. In saline-treated animals, the passage in the four-hole box before the elevated plus maze test did not modify behavior compared

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Figure 3. Time-course effects of a 10 min (between 20 and 30 min of the kinetic study) local perfusion of CCK8 on the extracellular outflow of DA (A), DOPAC (B), and HVA (C), uncorrected for recovery, in the posterior N. Acc. of rats accustomed to their environment. The period of CCK8 infusion in the N. Acc. is shown in the figures by gray areas. For each group of animals, the 100% value corresponds to the mean value of the first three baseline samples (these means are mentioned in Table 1, experiment 1) and the results are expressed as the percentage of these mean values; bars are SEM. Squares are saline ($n = 9$), circles are CCK8 1 fmol ($n = 8$), and triangles are CCK8 25 pmol ($n = 10$). **, $p < 0.01$ versus control group; Dunnett's t test. Two-way ANOVA: DA: treatment, $F(2,24) = 3.403$, $p = 0.0500$; time, $F(10,240) = 6.014$, $p = 0.0001$; interaction, $F(10,240) = 4.226$, $p = 0.0001$. DOPAC: treatment, $F(2,24) = 0.752$, $p = 0.4824$; time, $F(10,240) = 1.253$, $p = 0.2583$; interaction, $F(10,240) = 0.967$, $p = 0.5033$. HVA: treatment, $F(2,24) = 1.457$, $p = 0.2528$; time, $F(10,240) = 0.620$, $p = 0.7959$; interaction, $F(10,240) = 0.527$, $p = 0.9536$.

Figure 4. Time-course effects of a 10 min (between 20 and 30 min of the kinetic study) local perfusion of CCK8 on the extracellular outflow of DA (A), DOPAC (B), and HVA (C), uncorrected for recovery, in the posterior N. Acc. of rats newly placed in four-hole boxes. The period of CCK8 infusion in the N. Acc. is shown in the figures by dark gray areas and the time spent in the four-hole boxes is represented by

to rats directly tested in the maze. In treated animals, CCK8 (25 pmol/side) appeared anxiogenic-like only in rats previously submitted to the four-hole box in which it decreased the percentage of the duration of visits in the open parts [$F(3,35) = 4.093$, $p = 0.00137$] (Fig. 6). This effect was not the consequence of a general decrease in motor activity since the total number of arm visits (~ 8) was not significantly changed after CCK8 treatment [$F(3,35) = 1.010$, $p = 0.3998$]. However, it is interesting to note that the total duration of arm visits in the treated rats previously placed in the four-hole box was longer than in others animals [$F(3,35) = 4.422$, $p = 0.0097$, data not shown]. The statistical analysis revealed that this effect resulted from an increase in the time spent in the closed arms [$F(3,35) = 4.097$, $p = 0.0136$] (Fig. 6).

Discussion

The purpose of this microdialysis study was to examine concurrently the effects of intra-accumbens administrations of CCK8 on two parameters assumed to be related: behavior and the extracellular DA, DOPAC, and HVA levels in the posterior N. Acc. of awake rats submitted or not to a novel environment. As discussed in the introduction, the two doses (1 fmol and 25 pmol) of CCK8 used throughout this study were chosen according to previous studies (Daugé et al., 1989b; Ladurelle et al., 1993).

Before testing the CCK8 effects, the first step was to analyze the influence of an environmental change on DA neurotransmission in control rats. The results showed that the transfer of rats to the four-hole boxes slightly increased extracellular DA levels in the posterior N. Acc. (+14 % of basal) and tended also to increase catabolite levels. The modification of the DA release in transferred animals suggests that the novelty has stimulated DA functions. In agreement with this, an increase in DA turnover has previously been shown to occur following the placement of rats in an open field (Tassin et al., 1980). Furthermore, several studies using 6-hydroxydopamine lesions have emphasized the importance of the DA innervation of the N. Acc. in spontaneous and drug-induced exploratory and locomotor behaviors (Fink and Smith, 1980; Taghzouti et al., 1985; Calenco-Choukroun et al., 1991; Derrien et al., 1993). Systemic or intra-accumbens administrations of psychostimulant drugs that increase DA release, such as amphetamine, also modify these behaviors (Pijnenburg et al., 1976; Kelley et al., 1986). Taken together, these findings are in good agreement with the proposed functional role of the N. Acc. as an interface between motivation and action (Simon and Le Moal, 1984). Other studies using more stressful situations, such as electroconvulsive shocks (Nomikos et al., 1991) or electric tail shocks (Abercrombie et al., 1989),

pale gray areas. For each group of animals, the 100% value corresponds to the mean value of the first three baseline samples (these means are mentioned in Table 1, experiment 2), and the results are expressed as the percentage of these mean values; bars are SEM. Squares are saline ($n = 8$), circles are CCK8 1 fmol ($n = 6$), and triangles are CCK8 25 pmol ($n = 7$). *, $p < 0.05$; **, $p < 0.01$ versus control group; Dunnett's t test. Two-way ANOVA: DA: treatment, $F(2,18) = 7.612$, $p = 0.0040$; time, $F(10,180) = 23.661$, $p = 0.0001$; interaction, $F(20,180) = 5.520$, $p = 0.0001$. DOPAC: treatment, $F(2,18) = 3.463$, $p = 0.0534$; time, $F(10,180) = 10.356$, $p = 0.0001$; interaction, $F(20,180) = 0.9770$, $p = 0.4921$. HVA: treatment, $F(2,18) = 2.243$, $p = 0.1349$; time, $F(10,180) = 14.007$, $p = 0.0001$; interaction, $F(20,180) = 1.467$, $p = 0.0977$.

Table 2. Comparison of DA increase (percentage of controls) induced by 25 pmol of CCK8 perfused in the posterior N. Acc. of animals housed in a familiar environment (first line) or transferred in the four-hole box (second line)

Time (min)	10	20	35	50	60	70	80	90	100	120	140
Familiar environment	95.5 ± 3.5	96.2 ± 5.0	150.5 ± 4.9	113.9 ± 4.5	108.2 ± 4.5	104.3 ± 4.6	102.1 ± 5.4	94.6 ± 4.4	95.6 ± 4.7	104.4 ± 3.5	101.0 ± 2.9
4 hole box	102.4 ± 7.4	105.0 ± 2.3	163.5 ± 6.1	132.3 ± 4.9 ^a	126.8 ± 7.0 ^a	116.1 ± 5.8	116.2 ± 8.4	117.1 ± 5.3 ^b	115.8 ± 5.4 ^a	114.2 ± 5.1	114.4 ± 3.9 ^a

Results are expressed as the means ± SEM of the percentage of the control animals (without CCK8 treatment) respectively placed in the same conditions. Two-way ANOVA: treatment, $F(1,15) = 20.185$, $p = 0.0004$; time, $F(10,150) = 22.071$, $p = 0.0001$; interaction, $F(10,150) = 0.531$, $p = 0.8661$.

^a $p < 0.05$; ^b $p < 0.01$; Dunnett's t test.

Figure 5. Exploratory behavior over 15 min in the four-hole box. Upper figure shows the number (means ± SEM) of hole visits, lower part the duration (means ± SEM) of these visits. Saline, $n = 8$; CCK8 1 fmol, $n = 6$; CCK8 25 pmol, $n = 7$. *, $p < 0.05$; **, $p < 0.01$ versus control group; Dunnett's t test.

have also shown an increase of extracellular DA efflux in the N. Acc. Interestingly, in these studies, the intensity of the DA increase was two- to threefold higher than that obtained after transfer into the four-hole box. This suggests a direct relationship between the severity of the stress and the strength of the DA response and/or the existence of distinct DA networks differently recruited as function of the experimental situation, as suggested by the results of recent anatomical and pharmacological studies (Zahm, 1989; Heimer et al., 1991; Deutch and Cameron, 1992).

The local perfusion of 25 pmol, but not of 1 fmol, of CCK8 in the posterior N. Acc. of rats induced a large and immediate increase in DA outflow in both animals housed in a familiar environment and those transferred to the four-hole boxes. Interestingly, in both cases, these effects were maximal and of the same order of magnitude (+50 % and +63 % relative to the saline controls, respectively) in the first sample posttreatment, but they differed in their kinetics. In the first case, the extracellular DA levels returned rapidly to basal levels after interruption of the CCK8 perfusion (Fig. 3A), as previously reported (Ladurelle et al., 1993), while in animals placed in the four-hole boxes, a higher DA efflux was maintained for at least 80 min (Fig. 4A). This extended effect was not the consequence of the

Figure 6. Effect of 25 pmol/side of CCK8 injected in the posterior N. Acc. of rats in the elevated plus maze. Animals were previously placed or not in the four-hole box. *Left panel* shows the percentage of the duration of visits in the open parts of the maze and *right side* the total duration of closed arm visits. Results are expressed in seconds (means $\pm$ SEM). *, $p < 0.05$; Newman-Keuls test.

DA increase due to the change of environment (i.e., additive effect) since, when the results were expressed relative to the respective controls (see Table 2), the time-course effects remained significantly higher in the treated animals submitted to the four-hole box. Although there was an increase in catabolites levels in all cases, this was not significant (except for DOPAC in the third sample following the perfusion of 25 pmol of CCK8 in animals submitted to the novel environment). This prolongation of the effect of CCK8 in the transferred animals was not due to the handling of rats occurring during the passage from the familiar box to the four-hole box since in rats handled but not transferred, the increase of the DA release induced by CCK8 had the same duration as in rats not handled. The behavioral analysis showed hypoexploratory effects of both doses of CCK8, illustrated by a reduction in the number of hole visits measured over the first 15 min, following placement of the rats in the four-hole boxes. Even if the experimental conditions for injection and perfusion procedures cannot be exactly the same (25 pmol of CCK8 in 10 min for microdialysis perfusions and 25 pmol in 2 min for injections), a similar hypoexploration was found after local injection of 25 pmol/side of CCK8 in the posterior N. Acc. of chronically implanted rats showing that, as expected, the experimental conditions of microdialysis did not significantly perturb the rats' behavior. Considering the data reported here, the first interesting observation is the lack of correlation between the doses of CCK8 administered, the induced behavioral effects, and the changes in DA release. Indeed, the hypoexploration obtained with 25 pmol of CCK8 and with the 25,000-fold lower dose were not significantly different, whereas only the highest dose of CCK8 was associated with a prolonged DA release. This appears to exclude a direct relationship between the biochemical changes and the behavioral responses, despite the fact that the hypoexploration induced by the low dose of CCK8 (1 fmol) has been found to be dependent on the integrity of the DA pathway projecting to the posterior N. Acc. and is blocked by the local administration of a D2 receptor antagonist (Derrien et al., 1993). One reason for these apparent discrepancies could be that modification in DA release when the dose of 1 fmol of CCK8 was used was below the sensitivity of the method. Thus, the peptide

might have modified extracellular DA levels issuing from a restricted population of neurons, which were not detectable in our experimental conditions, but were sufficient to produce the maximal hypoexploratory effect. Furthermore, in the group of transferred animals, a low DA increase following CCK8 treatment might have been masked by the enhanced DA outflow caused by the environmental change. In contrast, the marked biochemical effect produced by the high dose of CCK8 could result from a strong stimulation of these limited number of neurons and/or from the recruitment of a larger population of neurons. The hypothesis of a specific DA network implied in the exploratory effects studied here seems to be reinforced by the ceiling effect (i.e., the maximal effect), already observed in rats by Daugé et al. (1989a), after injection of 1 fmol to 100 pmol of CCK8 in the posterior N. Acc. A third possibility could be that the behavioral effects obtained with the 1 fmol dose of CCK8 correspond to a modification of the synaptic turnover that is not measurable by microdialysis. Indeed, in 1991, Parsons et al. have shown that a modification of the total amount of DA and its metabolites in the nucleus accumbens can occur without a change of the extracellular levels of DA.

Finally, the most interesting result coming from the present experimental data was the prolonged DA release induced by CCK8 in animals stimulated by a novel environmental situation. It appears that the novelty has made the DA neurons more sensitive to CCK8 stimulation. Interestingly, this prolonged DA release resembles the profile of responses obtained with inhibitors of DA reuptake, such as cocaine or nomifensine (Hurd and Ungerstedt, 1989). Thus, one possible mechanism of action of CCK8 could include an effect at the level of the DAergic terminals. Nevertheless, CCK8 can also indirectly modify the DAergic transmission by an interaction on GABAergic, cholinergic, glutamatergic, or serotonergic neurons or terminals (review in Harro et al., 1993). From a functional point of view, the extended release of DA induced by CCK8 in rats placed in a novel environment could be related to modifications in DA neurons occurring in memory processes. Indeed, an animal confronted with an unknown territory takes some landmarks during the exploratory phase; the memorizing process could be linked

to the potentialized DA increase. From a behavioral point of view, this prolonged DA release, only observed when the DA neurons have been previously stimulated, could be related to the well-known increase in self-stimulation produced by local administration of CCK8 into the rat posterior N. Acc. (De Witte et al., 1987). The maintenance of increased DA activity might also enable a better adaptation to novel stimuli after the behavioral testing period. Alternatively, it could correspond to a reactional preparation of the neurons in the anticipation of a new situation. This supposition has notably been proposed by Louilot et al. (1986), who showed a maintenance of an increased DOPAC level occurring in a social interaction test (contact with an aggressive intruder), which did not depend on the duration of this behavioral test. This preadaptation, induced by increased DA neurotransmission, could then be favored by CCK8. Thus, the anxiogenic-like potential displayed by CCK8 after local injection into the N. Acc. in some stressful tests such as elevated plus maze or open-field tests (Daugé et al., 1989a,b) could occur and/or be reinforced exclusively, or at least preferentially, following an activation of DA neurons. In order to test this hypothesis, the anxiogenic-like effects of CCK8 have been measured in the elevated plus maze after injection of the peptide (25 pmol/side) in the posterior N. Acc. of rats previously submitted or not to the four-hole box. The experiments showed clearly that the rats "stimulated" by this environment spent less time in the open arms than those coming from their home cages (Fig. 6), supporting the proposition that the anxiogenic-like capacity of CCK8 is, in fact, increased by the change of environment. This is in line with the observation that certain experimental conditions are critical to reveal CCK8 effects (Daugé et al., 1989a) such as preinjection stress conditions in African green monkeys and rodents (review in Harro et al., 1993) or novel environment (Lavigne et al., 1992).

In conclusion, the results presented in this study show that (1) the meso-accumbens DA neurons are activated by novel environmental stimuli and (2) that the effects of CCK8 administered in the posterior N. Acc. on the increased extracellular DA levels seem to depend on the activity of these neurons. The prolonged activation of the mesoaccumbens pathways by CCK8 that occurs in rats facing novel conditions could correspond to reinforced effects and/or preparative actions (a kind of biochemical anticipation). Such a mechanism of action could imply that a disfunctioning of the CCKergic activity in this pathway could lead to some mental disorders such as pathological anxiety, panic disorders, and even drug addiction.

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