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A heroin-, but not a cocaine-expecting, self-administration state preferentially alters endogenous brain peptides

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Abstract

The purpose of the current study was to assess neuropeptidergic alterations during a phase of the drug addiction cycle associated with drug craving as compared to a time period when the drug had been recently self-administered. Male Wistar rats were allowed to self-administer cocaine, heroin or saline for 6 h for 5 consecutive days. Immediately following the last self-administration session ('acute drug on board' state), and just before the next scheduled session ('drug expecting' state), the animals were decapitated and the levels of dynorphin A and B, [Met^5]- and [Leu^5]-enkephalin and substance P were measured in different brain areas. During the 'acute drug on board' state, peptide levels in animals that self-administered heroin or cocaine were not significantly changed. In contrast, during the 'drug expecting' state, heroin-treated animals had increased levels of dynorphin A, dynorphin B and [Met^5]-enkephalin in the caudal striatum as compared to the cocaine- and saline-treated animals, and the level of [Leu^5]-enkephalin was increased as compared to the cocaine-treated group. In the septum, an increase of [Met^5]-enkephalin and substance P was observed in the animals expecting heroin as compared to the saline- and/or cocaine-treated animals. In the caudal striatum, substance P levels were elevated in the heroin- and cocaine-expecting animals. In conclusion, heroin, as compared to cocaine, appears to have a more pronounced effect on dynorphin, enkephalin and substance P levels in the caudal striatum and septum, especially during periods when self-administration of the drug is expected.

Keywords: Craving; Dynorphin; Enkephalin; Self-administration; Substance P; Withdrawal

1. Introduction

Previous studies have demonstrated that the endogenous opioid neuropeptide systems may be involved in the neural processes of drug addiction, not only related to the intake of opiates, but also of stimulant drugs. Chronic infusion or repeated morphine injections elevate the concentration of prodynorphin peptides in the striatum, with the most pronounced effects observed in the dorsal (sensorimotor) areas, while the concentration of proenkephalin peptides levels is not changed (Bergström and Terenius, 1979; Trujillo et al., 1995). Following intracerebroventricular (i.c.v.) administration of morphine the dynorphin A-immunoreactivity in the hypothalamus, hippocampus and striatum is unchanged after the administration of both opioid receptor agonists and antagonists, but there is down-regulation of the prodynorphin gene expression (Romualdi et al., 1991, 1995). A single injection of cocaine has been shown to have little (Hurd and Herkenham, 1992) or no effect (Daunais and McGinty, 1994) on prodynorphin gene expression. Short-term (4 days) intermittent cocaine administration, in contrast, increases dynorphin and to a lesser extent enkephalin mRNA expression in the striatum (Steiner and Gerfen, 1993): then 1–24 h following the last injection of cocaine dynorphin-immunoreactivity is increased, but enkephalin- and substance P-immunoreactivity is unchanged (Sivam, 1989; Smiley et al., 1990). The self-administration of cocaine also in-

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creases striatal prodynorphin mRNA levels (Hurd et al., 1992; Daunais et al., 1993). Chronic 'binge' cocaine administration increases both μ-opioid receptor densities in a number of brain regions, such as the striatum, nucleus accumbens and amygdala (Unterwald et al., 1993), and prodynorphin mRNA expression in caudate–putamen (Daunais and McGinty, 1995). More recently, it was shown that acute 'binge' cocaine administration increases both prodynorphin and proenkephalin mRNA in the caudate–putamen, whereas μ-opioid receptor mRNA decreased in the substantia nigra (Spangler et al., 1997).

Although the effects of acute and chronic drug administration have been investigated, only a few studies have addressed the potential involvement of the endogenous opioids at a moment when the 'craving' for an addictive drug is expected to be enhanced. A study comparing the effects of heroin and cocaine self-administration on β-endorphin brain levels showed that animals awaiting their daily self-administration of cocaine or heroin have a marked decrease of β-endorphin-immunoreactivity in the anterior part of the limbic system, whereas immediately after a drug session this was normal in animals self-administering cocaine or heroin (Sweep et al., 1989). In the present study, the drug self-administration model was used to examine the effects of drugs of abuse on the levels of dynorphins, enkephalins and substance P in different regions of the rat brain. Heroin, an opiate inducing both psychological and physical dependence, and eliciting a withdrawal syndrome when its administration is discontinued, and cocaine, a stimulant inducing psychological but not physical dependence, were selected (Kalant, 1978). Naive animals were allowed to self-administer intravenously (i.v.) cocaine or heroin for five consecutive daily sessions lasting 6 h. Peptide levels were measured immediately following the last drug self-administration session ('acute drug on board' state) and just before the next scheduled self-administration session, i.e., 18 h following the last session ('drug expecting' state), when the 'craving' for the addictive drugs was expected to be enhanced.

2. Materials and methods

2.1. Animal experiments

2.1.1. Animals and housing conditions

Male Wistar rats (Cpb: Wu) weighing 250–300 g at the start of the experiments were used. Before surgery, the animals were group-housed, had water and food ad libitum and were maintained under a 12-h light/dark cycle (lights on 7:00 AM). Following the operation, the animals were housed individually. Three days before the start of the self-administration tests (i.e., 5–7 days following the operation) all animals were brought to the experimental rooms and were deprived of food in order to obtain a weight reduction (±20%), which facilitates the acquisition of self-administration (de Vry et al., 1989). A reversed 12-h light/dark cycle (lights on 7:00 PM) was maintained during the whole experiment. Standard diet and water (ad libitum) were available following each self-administration session in the home cages, which were kept in the experimental rooms.

2.1.2. Surgical procedure

Rats were anesthetized with Hypnorm® (0.10 ml kg^{-1}, i.m.) and a cannula was inserted into the jugular vein. The cannula was passed subcutaneously up to the skull and fixed to a curved metal tube, which was secured onto the skull with screws and dental acrylic cement as previously described (van Ree et al., 1978).

2.1.3. Self-administration procedure

Details of the procedure have been reported previously (Sweep et al., 1989). Briefly, testing was done in sound-attenuated conditioning cages for 6 h of the dark period of the illumination cycle. The test cages were equipped with two levers, one of which was marked by a light placed just above the lever. The cannula of each animal was connected to an infusion pump. Pressing the left lever (marked by a red light) resulted in the i.v. administration of 0.25 ml of fluid delivered in 13 s on a continuous reinforcement schedule. During the infusion, the stimulus light was turned off and pressing the same lever had no programmed consequences. Depression of the other lever (non-reinforcement lever) had no programmed consequences. The drug-naive animals were placed in the test cages and were allowed to self-administer a drug solution for 6 h a day (starting at 9:30 AM) or until a maximum of 60 self-infusions was reached. Testing took place on five consecutive daily sessions. The number of animals per group varied between 6 and 11.

2.1.4. Experimental groups

The animals were allowed to self-administer 0.25 ml saline, 0.16 mg kg^{-1} cocaine or heroin per infusion. The rats were decapitated immediately after the fifth self-administration session ('acute drug on board' state) or 18 h following the fifth session, just before the time of the next scheduled session ('drug expecting' state). Before decapitation, the observer checked the animals for visible withdrawal signs, e.g., teeth-chattering and wet-dog shakes (Cappendijk et al., 1994). Following decapitation, the brain was immediately removed and cut manually with a rodent brain matrix (Activational Systems, Warren, MI, USA). Prefrontal cortex, nucleus accumbens, rostral and caudal striatum, septum, hippocampus and amygdala were dissected (Gispen et al., 1972) and were immediately frozen on dry ice. The tissues were stored at −80°C until analysis of peptides. All experiments were conducted in accordance with the guidelines of the Experimental Animal Use Committee of Utrecht University.

2.1.5. Drugs

Hypnorm® was manufactured by Janssen Pharmaceutica (Tilburg, The Netherlands). Heroin (di-acetylmorphine–HCl) and cocaine (cocaine–HCl) were purchased from OPG (Utrecht, The Netherlands) and were dissolved in saline. The pH of the drug solutions and saline prepared for self-administration was adjusted to 7.30 ± 0.05.

2.1.6. Statistical analysis

The results are presented as means ± S.E. The data obtained during the self-administration sessions were analysed using two-way analysis of variance with repeated measurements (MANOVA). Treatment (drug vs. saline), time of terminating experiment ('acute drug on board' state or 'drug expecting' state) and time (five sessions) were grouping variables and the number of self-infusions, the dependent variable. Significance was set at $P \leq 0.05$.

2.2. Peptide analysis

Brain specimens from the 'acute drug on board' and the 'drug expecting' groups were processed simultaneously to minimize differences in the extraction, separation and radioimmunoassay procedures.

2.2.1. Extraction and separation of the peptides

Tissue extraction was performed with 1 M acetic acid. The samples were heated at 95°C for 5 min and, after cooling on ice, homogenized with sonication. The samples were reheated at 95°C for 5 min, cooled on ice, and then centrifuged in a Beckman centrifuge for 15 min. The supernatants were applied onto small (1 ml) ion exchange columns with SP-Sephadex C25 to concentrate and separate opioid peptides in the tissue extract. Four buffers with increasing concentrations of pyridine and formic acid were added sequentially and peptides were eluted in separate fractions according to charge (Bergström et al., 1983). The fractions were evaporated in a vacuum centrifuge.

2.2.2. Radioimmunoassay (RIA)

Peptides were measured with specific RIAs for dynorphin A and B, [Met^5]- and [Leu^5]-enkephalin and substance P; assay antisera were generated in rabbits, as described previously (Nylander and Terenius, 1987; Nylander et al., 1995a), with some modifications. The tracer peptides were labeled with ^{125}I according to the chloramine T method and were purified by reversed phase high performance liquid chromatography using a gradient of 15–40% acetonitrile in 0.04% trifluoroacetic acid. A 25-μl aliquot of the sample, dissolved in methanol: 0.1 M HCl (1:1, v/v) was incubated with 100 μl of antiserum and 100 μl of ^{125}I-labeled peptide, diluted in buffer D (dynorphin assay) or in gel buffer (enkephalin and substance P assay). Samples were incubated for 24 h with the antiserum and the tracer peptide. Following the incubation period, the dynorphin samples were incubated for 1 h with 100 μl of a sheep–antirabbit antiserum dilution, Pharmacia Decanting Suspension 3. After centrifugation (10 min) the pellet was counted in a γ-counter. To separate free and antibody-bound peptide in the enkephalin and substance P assays, 200 μl of a charcoal suspension was added and the samples were incubated for 10 min. The samples were centrifuged (1 min) and an aliquot (300 μl) of the supernatant was counted.

2.2.3. Chemicals

Dynorphin A and B, [Met^5]- and [Leu^5]-enkephalin were purchased from Bachem Feinchemikalien (Buben-

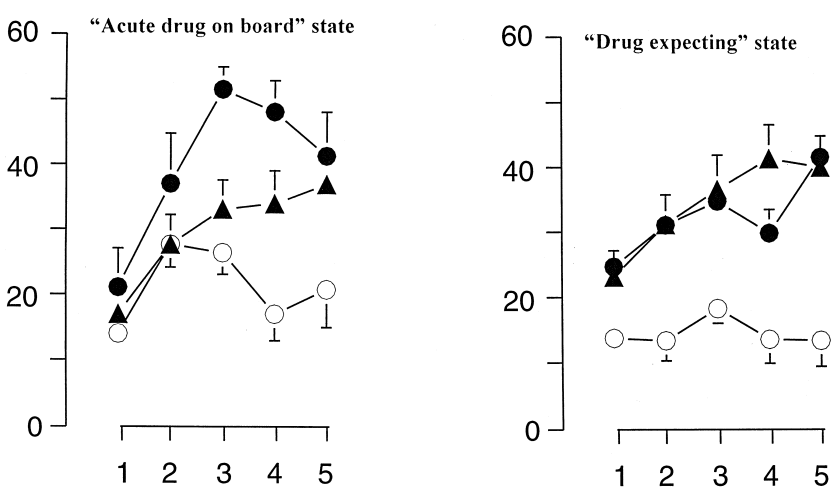

Fig. 1. Groups of naive animals were allowed to i.v. self-administer saline (-○-), or 0.16 mg kg^{-1} per infusion heroin (-▲-) or cocaine (-●-) for five consecutive daily sessions of 6 h under a continuous reinforcement schedule. The number of self-infusions per daily session (mean ± S.E.) is shown for each experimental group (6–11 animals per group). The animals were either decapitated immediately following the fifth self-administration session ('acute drug on board' state) or just before the next scheduled self-administration session ('drug expecting' state). For statistical analysis see text.

dorf, Switzerland) and substance P was obtained from Peninsula Laboratories (San Carlos, CA, USA). Sephadex C25 was purchased from Pharmacia LKB (Sollentuna, Sweden) and Pharmacia Decanting Suspension 3 was purchased from Pharmacia Diagnostics (Uppsala, Sweden). Buffer D (gelatin buffer) used for the dynorphin assays contained 0.15 M NaCl, 0.02% sodium azide, 0.1% gelatin, 0.1% Triton X-100 and 0.1% bovine serum albumin in a 0.05 M sodium phosphate buffer. The gel buffer used for the enkephalin and substance P assays contained 0.15 M NaCl, 0.025 M EDTA, 0.1% gelatin and 0.1% bovine serum albumin in 0.05 M sodium phosphate buffer. The charcoal suspension (250 mg) contained 25 mg dextran T-70 in 100 ml 0.05 M sodium phosphate buffer.

2.2.4. Statistics

The peptide levels in different brain structures are expressed as fmol/mg tissue. The data were analysed using a two-way analysis of variance, with treatment (drugs vs. saline) and time of the terminating experiment ('acute drug on board' state or 'drug expecting' state) as grouping variables. Since the analyses indicated differences with respect to time of terminating the experiment, the data from the 'acute drug on board' and the 'drug expecting' state were analysed using one-way analysis of variance, followed by the Student–Newman–Keuls test. Significance was set at $P \leq 0.05$. In addition, the results were considered as a trend with P-values $P \leq 0.10$.

3. Results

3.1. Self-administration

The number of self-infusions for the animals with saline, heroin or cocaine is shown in Fig. 1. Two-way analysis of variance revealed a significant interaction between treatment and time (five sessions) [$F(4,184) = 11.6$, $P < 0.001$] without a significant difference between animals studied during the 'acute drug on board' or the 'drug expecting' state [$F(1,46) = 1.5$, n.s.]. In addition, a significant main treatment effect (cocaine vs. saline) [$F(1,28) = 38.2$, $P < 0.001$] and treatment and time interaction [$F(4,112) = 2.8$, $P = 0.02$] were found, as well as a significant main treatment effect for heroin vs. saline [$F(1,35) = 24.2$, $P < 0.001$] and treatment and time interaction [$F(4,140) = 3.9$, $P < 0.01$]. Thus, the rats showed proper initiation of both heroin and cocaine self-administration, in that the intake (number of self-infusions) by animals offered heroin or cocaine was significantly higher than that by rats offered saline and also in that drug intake increased over time. Neither the heroin- nor the cocaine-treated animals showed obvious physical withdrawal symptoms (e.g., teeth-chattering, wet-dog shakes) during the 'drug expecting' state.

3.2. Peptide levels

3.2.1. 'Acute drug on board' state

In no brain area of the animals killed immediately at the end of the fifth self-administration session did the i.v. self-administration of heroin or cocaine significantly affect the levels of dynorphins, enkephalins and substance P as compared to those in the saline animals (Table 1). There was however, a trend for some brain areas. For example, in the nucleus accumbens of the heroin-treated animals, the level of substance P [$F(2,13) = 3.5$, $P = 0.07$] was higher than in either the saline ($P = 0.04$) or the cocaine-treated ($P = 0.02$): as in the caudal striatum of the heroin-treated animals the level of dynorphin B [$F(2,16) = 3.5$, $P = 0.06$] was higher than in that of the cocaine-treated animals ($P = 0.06$).

Table 1
The effects of heroin, cocaine or saline self-administration on dynorphin, enkephalin and substance P levels, measured immediately following the last self-administration session ('acute drug on board' state)

	Dynorphin A	Dynorphin B	[Met^5]-enkephalin	[Leu^5]-enkephalin	Substance P
Prefrontal cortex					
Saline	1.3±0.3	0.7±0.1	5.0±1.0	3.3±0.3	1.6±0.5
Heroin	1.8±0.4	0.5±0.1	3.6±0.3	3.9±1.3	0.8±0.2
Cocaine	1.2±0.2	0.6±0.1	13.3±5.2	4.1±0.5	0.9±0.1
Nucleus accumbens					
Saline	31.1±8.3	3.8±1.0	21.8±5.7	15.4±3.2	10.3±2.3
Heroin	42.6±10.1	3.1±1.1	50.6±16.6	21.0±7.5	3.7±1.1
Cocaine	25.8±5.9	2.5±0.5	30.8±7.4	17.0±2.6	11.7±2.7
Rostral striatum					
Saline	3.8±0.5	8.2±0.7	103.9±24.4	37.2±8.4	16.9±3.3
Heroin	3.5±0.7	7.6±1.4	125.4±37.7	33.0±8.5	16.7±4.1
Cocaine	4.1±0.8	5.8±0.9	132.3±18.5	31.7±6.6	16.4±3.0
Caudal striatum					
Saline	16.6±5.5	2.1±0.5	141.7±21.0	33.2±6.0	12.9±2.1
Heroin	22.4±3.7	3.0±0.5	113.3±27.5	28.3±5.2	12.6±3.5
Cocaine	27.5±4.3	2.0±0.2	112.8±20.3	27.7±4.4	12.2±1.0
Septum					
Saline	3.3±0.8	1.6±0.2	16.4±3.9	4.0±0.7	2.7±0.3
Heroin	2.2±0.7	1.3±0.4	8.7±1.0	6.8±3.9	1.5±0.6
Cocaine	2.2±0.5	1.4±0.3	14.2±2.2	3.4±0.7	4.7±0.9
Amygdala					
Saline	9.9±2.6	2.7±0.5	32.3±4.2	10.8±1.6	4.4±1.2
Heroin	5.8±1.2	1.8±0.3	31.9±4.5	10.2±0.8	3.6±0.5
Cocaine	8.5±2.4	1.9±0.3	30.1±6.1	11.4±1.8	4.0±0.7
Hippocampus					
Saline	18.9±3.1	4.4±0.4	16.3±3.2	6.2±0.8	1.0±0.2
Heroin	21.1±4.8	4.1±0.6	16.9±2.1	5.9±0.5	1.6±0.3
Cocaine	17.6±2.3	4.2±0.5	14.3±3.0	5.6±0.9	1.1±0.3

Legends: The effects of heroin, cocaine or saline self-administration on dynorphin A and B, [Met^5]- and [Leu^5]-enkephalin and substance P levels in different brain areas of rats. Values represent means±S.E. (6–11 animals per group) and are expressed as fmol/mg tissue. Data were analysed by One-way analysis of variance, followed by the Student–Newman–Keuls test. No significant differences were observed.

3.2.2. 'Drug expecting' state

In the animals killed just before the time of the next scheduled self-administration session, the most pronounced changes in peptide levels were observed in the caudal striatum and the septum of the heroin-treated animals (Table 2). In the caudal striatum of the heroin-treated animals, the levels of dynorphin A [$F(2,16) = 2.4$, $P = 0.05$], dynorphin B [$F(2,13) = 5.0$, $P = 0.03$] and [Met^5]-enkephalin [$F(2,17) = 4.6$, $P = 0.03$] were increased as compared to those in the cocaine- and saline-treated animals, and the level of [Leu^5]-enkephalin [$F(2,9) = 4.6$, $P = 0.05$] was increased as compared to that in the cocaine-treated group. In this area, the levels of substance P were higher in both heroin- and cocaine-treated animals than in saline-treated animals [$F(2,13) = 6.4$, $P = 0.01$]. In the septum, there was an increase of [Met^5]-enkephalin [$F(2,17) = 3.8$, $P = 0.04$] in the heroin-treated animals as compared to the saline-treated animals, and of substance P [$F(2,13) = 3.2$, $P < 0.01$] as compared with the saline- and cocaine-treated animals.

In addition, some brain areas showed significant trends since analysis of the data showed a P-value between 0.05 and 0.1. For example, in the prefrontal cortex, there was an increase of dynorphin B [$F(2,19) = 2.7$, $P = 0.09$] in the cocaine-treated animals as compared to the saline- and heroin-treated animals. In the rostral striatum of the heroin-treated animals, an increase of substance P [$F(2,13) = 2.7$, $P = 0.10$] was observed. A decrease of the [Leu^5]-enkephalin level [$F(2,17) = 2.7$, $P = 0.10$] was measured in the nucleus accumbens of the heroin-treated animals.

Table 2
The effects of heroin, cocaine or saline self-administration on dynorphin, enkephalin and substance P levels, measured just before the next scheduled self-administration session ('drug expecting' state)

	Dynorphin A	Dynorphin B	[Met^5]-enkephalin	[Leu^5]-enkephalin	Substance P
Prefrontal cortex					
Saline	3.1 ± 0.5	1.0 ± 0.1	10.6 ± 1.1	6.8 ± 0.7	1.6 ± 0.2
Heroin	2.9 ± 0.3	1.0 ± 0.1	13.9 ± 3.1	7.3 ± 0.6	2.6 ± 0.6
Cocaine	3.9 ± 0.6	1.3 ± 0.1	11.1 ± 1.9	6.5 ± 1.3	2.7 ± 0.3
Nucleus accumbens					
Saline	56.0 ± 13.9	5.7 ± 1.6	111.2 ± 35.9	26.0 ± 6.0	19.1 ± 4.9
Heroin	49.0 ± 14.5	5.2 ± 1.1	87.8 ± 19.9	20.0 ± 3.4	15.1 ± 3.0
Cocaine	69.6 ± 9.7	4.3 ± 0.8	128.2 ± 16.7	28.8 ± 5.2	18.2 ± 2.3
Rostral striatum					
Saline	6.3 ± 0.6	7.9 ± 1.6	152.5 ± 26.8	40.1 ± 6.2	20.7 ± 3.4
Heroin	8.4 ± 1.3	9.5 ± 1.2	171.4 ± 32.9	50.1 ± 5.9	30.4 ± 6.4
Cocaine	5.5 ± 0.5	8.2 ± 1.5	125.2 ± 30.4	34.2 ± 6.1	22.0 ± 1.2
Caudal striatum					
Saline	17.9 ± 5.8	1.8 ± 0.3	139.5 ± 30.1	40.4 ± 7.4	12.5 ± 2.1
Heroin	**36.9 ± 6.2**[b]	**4.1 ± 0.9**[b]	**206.3 ± 14.9**[b]	**55.6 ± 5.3**[a]	**21.0 ± 1.9**[c]
Cocaine	17.6 ± 3.9	1.9 ± 0.5	121.4 ± 10.3	37.6 ± 4.2	**19.8 ± 2.5**[c]
Septum					
Saline	4.5 ± 1.2	1.6 ± 0.3	34.5 ± 7.2	15.4 ± 4.6	5.2 ± 1.0
Heroin	5.1 ± 0.9	2.5 ± 0.4	**96.9 ± 20.4**[c]	17.4 ± 4.7	**11.9 ± 0.8**[b]
Cocaine	2.3 ± 0.4	1.8 ± 0.4	53.3 ± 17.7	13.7 ± 2.3	6.4 ± 1.6
Amygdala					
Saline	9.2 ± 1.8	3.2 ± 0.8	46.5 ± 3.2	12.7 ± 1.3	3.3 ± 0.8
Heroin	8.6 ± 1.4	2.8 ± 0.1	43.5 ± 2.6	15.8 ± 1.1	4.2 ± 0.4
Cocaine	6.7 ± 1.3	2.9 ± 0.5	39.8 ± 7.5	13.2 ± 2.2	4.2 ± 0.4
Hippocampus					
Saline	29.0 ± 6.3	5.3 ± 0.4	21.7 ± 3.8	8.2 ± 1.1	0.9 ± 0.2
Heroin	21.8 ± 3.5	6.2 ± 0.8	22.2 ± 3.6	8.5 ± 0.9	1.3 ± 0.2
Cocaine	36.2 ± 7.0	7.3 ± 0.8	23.6 ± 2.6	8.5 ± 1.0	1.2 ± 0.2

The effects of heroin, cocaine or saline self-administration on dynorphin A and B, [Met^5]- and [Leu^5]-enkephalin and substance P in different brain areas of rats. Values represent means ± S.E. (6–11 animals per group) and are expressed as fmol/mg tissue. Data were analysed by One-way analysis of variance, followed by the Student–Newman–Keuls test. [a]Significant vs. cocaine and [b]significant vs. saline and cocaine, [c]significant vs. saline ($P \leq 0.05$).

3.2.3. 'Acute drug on board' state vs. 'drug expecting' state

Comparing the peptide levels measured during the 'acute drug on board' state with those in the 'drug expecting' state revealed specific regional effects for certain peptides. During the 'heroin expecting' state, dynorphin A was increased in the prefrontal cortex and the rostral striatum, [$F(1,29) = 27.7$, $P < 0.01$ and $F(1,29) = 7.3$, $P < 0.04$, respectively] in comparison to the levels measured during the 'acute heroin on board' state. In addition, [Met^5]-enkephalin was increased in the septum [$F(1,26) = 5.8$, $P = 0.02$] and amygdala [$F(1,37) = 8.6$, $P < 0.01$], [Leu^5]-enkephalin was increased in the prefrontal cortex and septum [$F(1,29) = 7.6$, $P < 0.01$ and $F(1,21) = 5.2$, $P = 0.04$, respectively] and the level of substance P was increased in the nucleus accumbens [$F(1,19) = 6.4$, $P = 0.02$].

4. Discussion

Peptide levels were studied immediately following the last self-administration of heroin or cocaine (referred to as the 'acute drug on board' state) and just prior to the next scheduled self-administration session, when the 'craving' for drugs is expected to be enhanced (referred to as the 'drug expecting' state). Since the neurobiology underlying drug 'craving' is still unknown and the term is not clearly defined (Markou et al., 1993; Robinson and Berridge, 1993; Altman et al., 1996), we prefer to use the more descriptive term 'drug expecting' state, since the animals were in a state awaiting to be placed into the self-administration cage. Animals expecting heroin, but not those expecting cocaine, showed marked changes in the levels of dynorphin and enkephalin, specially in the caudal striatum.

During the 'acute drug on board' state, there were no significant changes in either the dynorphin, enkephalin or substance P levels of animals that had self-administered heroin or cocaine. However, there was a trend to a de-

crease of substance P in the nucleus accumbens and an increase of dynorphin B in the caudal striatum of heroin-treated animals. These results suggest that the endogenous peptide systems currently studied are not directly or strongly influenced by these drugs at this particular time. However, it is important to note that the peptide content is the result of several dynamic processes, including biosynthesis, processing, release and metabolism. Since an alteration in the peptide content may be produced by a change in any one or more of these processes it is of course possible that the apparently unchanged levels are the result of several processes balancing each other.

4.1. 'Acute drug on board' state

Neither heroin nor cocaine significantly affected the peptide levels in any brain region studied immediately following the self-administration session. The fact that the enkephalin system was not significantly changed during the 'acute drug on board' state is in accordance with results of several studies (Bergström and Terenius, 1979; Sivam, 1989; Smiley et al., 1990; Trujillo et al., 1995). Similarly, Sivam (1989) found no changes of substance P levels in the striatum following acute or subchronic cocaine administration with which our results are consistent. The finding that the dynorphin system was not significantly changed during either the 'heroin' or the 'cocaine on board' state was rather unexpected and in contrast with some previous studies. For example, an increased dynorphin-immunoreactivity was found after repeated exposure to cocaine (Sivam, 1989; Smiley et al., 1990) and following chronic cocaine self-administration an increased level of striatal prodynorphin mRNA was observed (Hurd et al., 1992; Daunais et al., 1993). Furthermore, chronic infusion or repeated morphine injections increased the concentration of prodynorphin peptides in the striatum (Trujillo et al., 1995). In the present study, there was only a trend to significance of the dynorphin B level (increase) in the caudal striatum of the heroin-treated animals. Numerous methodological differences among the studies may have contributed to the differences in findings, such as injection schedule, strain of rats and drug dose. For example, it has been shown that the injection schedule (intermittent, chronic), and the route of administration (passive, self-administration) can influence the development of dependence and tolerance in different ways (Nylander and Terenius, 1987; Engber et al., 1992). Nylander et al. (1995a,b) demonstrated that dynorphin and enkephalin peptide levels are differently affected by repeated injections of morphine, when measured in brain areas of Lewis, Fischer and Sprague–Dawley rat strains, whereas Wistar rats show no differences in dynorphin and enkephalin levels under a similar treatment schedule (Cappendijk and Terenius, unpublished). The relatively small dose of cocaine used in the present study (0.16 mg kg^{-1} per infusion) might also have contributed to the fact that no changes were observed in the levels of the endogenous opioid peptides of animals in the 'drug on board' state. As a comparison, Hurd et al. (1992) used a cocaine dose which gave the animals approximately 0.225 mg kg^{-1} per infusion, 7 days unlimited access, and Daunais et al. (1993) allowed animals to self-administer cocaine at a dose of 0.6 mg kg^{-1} per infusion for 2–4 weeks. Both these studies showed elevation of dynorphin mRNA expression in the striatum. On the other hand, in two other studies using the same 'low' cocaine dose as in the present study, animals awaiting their daily self-administration session showed changes in β-endorphin brain levels (Sweep et al., 1989) and decreased opioid receptor occupancy in distinct areas throughout the brain, including several terminal areas of the mesocorticolimbic dopaminergic system (Gerrits et al., personal communication). Based on results of these two prior studies, the low dose of cocaine seemed to be justified and the fact that the animals were self-administering cocaine throughout the experiment indicates that the dose used in this study was suitable for modulating neurobiological systems relevant for drug addiction in this rat strain.

4.2. 'Drug expecting' state

Since the mode of action and time course of heroin and cocaine dependence differ, it is difficult to draw a uniform conclusion concerning the effect of these drugs on endogenous peptide levels in the 'expecting state'. The fact that heroin had a more pronounced effect on the opioid peptide system than did cocaine suggests that these effects were due to the direct stimulation of opioid receptors. The greatest changes in peptide levels in animals expecting heroin were in the caudal striatum and the septum. Although the ventral, more limbic-related, region of the striatum has been particularly linked to drug reward (Koob et al., 1994), the caudal striatum could still contribute to drug craving; a role for the septum in drug craving has also been hypothesized (Latimer et al., 1987; Sweep et al., 1989). Though craving is a central component of drug withdrawal, the operational term 'drug expecting' state is preferred here since 'craving' and the general withdrawal phenomena are complex and incorporate a number of psychological conditions that would have been difficult to assess in the current study. Physical withdrawal is generally associated with heroin use. However, the peptidergic changes observed in the rats that self-administered heroin did not seem to be related to physical withdrawal since the animals failed to show any obvious physical withdrawal symptoms (e.g., teeth-chattering, wet-dog shakes). In addition, peptide levels, measured during spontaneous and naloxone-precipitated withdrawal, in male Wistar rats treated i.p. twice a day, for 8 days with an increasing dose of morphine (Cappendijk and Terenius, unpublished), did not show the same alteration of peptide levels as that

found in the present study. These results would indicate that the changes in peptide levels we now observed were not due to physical withdrawal.

4.3. 'Acute drug on board' state *vs.* 'drug expecting' state

In general, almost all peptide levels were higher in the various brain areas when measured during the 'drug expecting' state than during the 'acute drug on board' state, with the most pronounced changes in the levels of [Met^5]-enkephalin and substance P in the septum. An approximately 10 times higher level of peptides was measured, which might point to a rebound effect. Previous studies have shown that during states of hyperexcitability (for example, opioid withdrawal) enkephalin levels are particularly increased in the hippocampus (Hong et al., 1988). The fact that in the present study the enkephalin levels in hippocampus were not significantly different in the 'acute drug on board' state and in the 'drug expecting' state might further support the idea that the changes in peptide levels were not due to physical withdrawal.

Comparing the endogenous peptide levels of self-administering animals during the 'acute drug on board' state with those in the 'drug expecting' state, showed some significant changes in the prefrontal cortex (dynorphin A, [Leu^5]-enkephalin), amygdala ([Met^5]-enkephalin), septum ([Leu^5]-enkephalin) and rostral striatum (dynorphin A). These changes were also seen in saline self-administering animals. The reasons for these increases are not clear, but one might speculate that, e.g., food deprivation and stress might have played a role, since it is known that both factors can induce endogenous opioid activity (for review, Olson et al., 1993; Carr, 1996).

4.4. Conclusions

Although both heroin and cocaine at the doses studied affected endogenous peptides, heroin had a more pronounced effect on dynorphin, enkephalin and substance P, especially during periods when self-administration of the drug was expected. Further research is warranted to understand the precise role of endogenous opioid peptides and tachykinins during different periods of the 'drug expecting' (craving) state.

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