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Naloxone-Precipitated Changes in Biogenic Amines and Their Metabolites in Various Brain Regions of Butorphanol-Dependent Rats

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TOKUYAMA, S., H. WAKABAYASHI, B. HOSKINS AND I. K. HO. *Naloxone-precipitated changes in biogenic amines and their metabolites in various brain regions of butorphanol-dependent rats.* PHARMACOL BIOCHEM BEHAV **54**(2) 461-468, 1996. – Influence of a naloxone (an opioid receptor antagonist) challenge (5 mg/kg, IP) on levels of biogenic amines and their metabolites in various brain regions of rats infused continuously with butorphanol (a $\mu/\delta/\kappa$ mixed opioid receptor agonist; 26 nmol/μl/h) or morphine (a μ-opioid receptor agonist; 26 nmol/μl/h) was investigated using high-performance liquid chromatography with electrochemical detection (HPLC-ED). Naloxone precipitated a withdrawal syndrome and decreased the levels of: dopamine (DA) in the cortex and striatum, 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum, homovanilic acid (HVA) in the striatum, limbic, midbrain, and pons/medulla regions in butorphanol-dependent rats. However, the levels of norepinephrine (NE), serotonin (5-hydroxytryptamine; 5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) in the regions studied were not affected by naloxone-precipitated withdrawal. In addition, naloxone increased the HVA/DA ratio in the cortex, while this ratio was reduced in the limbic, midbrain, and pons/medulla. The reduction of 5-HIAA/5-HT ratio was also detected in the limbic area. In the animals rendered dependent on morphine, the results obtained were similar to those of butorphanol-dependent rats except for changes of 5-HIAA levels in some brain regions. These results suggest that an alteration of dopaminergic neuron activity following a reduction of DA and its metabolites in specific brain regions (e.g., striatum, limbic, midbrain, and pons/medulla) play an important role in the expression of the opioid withdrawal syndrome.

Butorphanol Morphine Dependence Biogenic amines

BUTORPHANOL, an opioid receptor agonist/antagonist, has been introduced as a potent analgesic for moderate to severe pain (49). Physical dependence on this analgesic agent in humans has been evidenced by the appearance of withdrawal signs such as rhinorrhea, gastric distress, vomitting, dysphoria, emotional lability, and irritability (11,51). Butorphanol dependence has also been produced in rats by continuous intracerebroventricular (ICV) infusion of the drug for 3 days utilizing osmotic minipumps (34,36). It has been reported that butorphanol acts on μ-, δ-, and κ-opioid receptors (30) and is mediated the development of dependence on butorphanol through these opioid receptors (31,34,36,45). Butorphanol also exhibits pharmacological and biochemical differences as well as similarities to the prototype of μ-opioid receptor agonist, morphine (30,61,62).

It is well known that monoaminergic neurons containing biogenic amines such as norepinephrine (NE), dopamine (DA), and 5-hydroxytryptamine (5-HT), and opioidergic neurons are closely linked in several brain regions (42). Turnover of these neurotransmitters has been reported to be altered by both acute and chronic administration of morphine and other opioids, suggesting that these amines are closely involved in the development of tolerance to and dependence on opioids (22). Furthermore, it has been shown that morphine and other opioids alter the activity of dopaminergic neurons in the substantia nigra and the ventral tegmental area (33,64) in addition to that of NE-containing neurons in the nucleus locus coeruleus (38).

Little information is available concerning the changes in biogenic amines in butorphanol-dependent animals, although

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numerous reports are available for morphine dependence (6,28,37,60,69). As most studies of opioid dependence have focused on morphine, the use of butorphanol, which possesses multiple actions on the opioid receptor system (30), should be valuable in studies to evaluate the mechanism of the development of dependence on opioids.

The present study was designed to determine whether changes in levels of biogenic amines and their metabolites are precipitated by naloxone in several brain regions of butorphanol-dependent rats and if so, to compare the changes with those produced in morphine-dependent animals treated in a similar manner.

METHOD

Animals

Male Sprague-Dawley rats weighing 230 to 250 g (Charles River, Wilmington, MA) were purchased and housed in groups of three or four animals to a cage. They were kept in a room maintained at 21 ± 2°C and with a 12 L : 12 D cycle. They had free access to food and tap water. After reaching 280 to 300 g, they were used for experiments.

Surgical Procedures

Rats were anesthetized with Equithensin (4.25 g chloral hydrate, 2.23 g $MgSO_4 \cdot 7H_2O$, 0.972 g sodium pentobarbital, 44.4 ml propylene glycol, 10 ml 95% ethanol, and distilled water to make a final volume of 100 ml), 0.3 ml/100 g b.wt., IP, and then placed in a stereotaxic instrument. An indwelling stainless steel guide cannula (26 gauge, 10 mm long) was implanted into the right lateral cerebral ventricle (AP: −0.5 mm, LAT: +1.3 mm and DV: −4.5 mm) with the bregma chosen as the stereotaxic reference point (48). Dental acrylic cement (Lang Dental MFG Co., Wheeling, IL) was applied to the surface of the skull, and a protective cap was placed around the cannula. After the acrylic had hardened, the animal was removed from the stereotaxic frame. A stylet (32 gauge stainless steel tubing) was placed into the guide cannula to maintain patency. The presence of cerebrospinal fluid in the guide cannula was examined to assure proper placement. After surgery, rats were given 300,000 units of procaine penicillin G (Pfizerpen-AS, Pfizer Corp., New York), SC, to prevent infection and were allowed at least a week to recover before commencing the infusion of butorphanol-tartrate (17-cyclobutylmethyl-3,14-dihydroxy morphinan; a generous gift from Bristol-Myers-Squibb Corp., Evansville, IN) or morphine-HCl (Sigma Chemical Corp., St. Louis, MO).

Administration Schedule and Induction of Butorphanol and Morphine Dependence

Animals were infused ICV continuously with butorphanol (26 nmol/μl/h) or morphine (26 nmol/μl/h) for 3 days through osmotic minipumps (Alzet 2001, Alza Corp., Palo Alto, CA). This infusion period and dose paradigm were determined to be optimal from our previous experiments (34–36). Under ether anesthesia, animals were implanted SC with minipumps between the scapulae. A 4 cm piece of tygon tubing (0.38 mm inner diameter, Cole-Palmer, Chicago, IL) was applied to connect the minipump to a piece of L-shaped stainless steel injector tubing (32 gauge, 30 mm long) with one end having the same length as the guide cannula. All drug solutions were passed through a 0.2 μm sterile Acrodisk filter (Gelman Sciences, Ann Arbor, MI) before being introduced into the pumps, and the delivery apparatus was assembled under sterile conditions. Minipumps were primed overnight at room temperature in normal saline so that an optimal flow rate (1 μl/h) was obtained. Rats were injected IP with saline (0.1 ml/100 g) as control or naloxone (5 mg/kg, Sigma Chemical Corp., St. Louis, MO) 2 h after the termination of opioid infusion.

Measurement of Behavioral Signs During Butorphanol and Morphine Withdrawal

Ten distinct behaviors (escape behavior, wet dog shakes, teeth chattering, rearing, locomotion, stretching, scratching, salivation, penis-licking, and ptosis) were scored during a 15-min period following the naloxone injection as behavioral signs of withdrawal. The reactions of each animal were evaluated by an independent observer who did not have prior knowledge of the nature of the treatment received.

Extraction of Biogenic Amines and Their Metabolites

Immediately after the 15-min observation period for withdrawal behavior, rats were sacrificed by decapitation, and their brains were rapidly removed and rinsed with ice-cold saline. Six brain regions (cortex, striatum, limbic area, midbrain, pons/medulla, and cerebellum) were separated according to the method of Glowinski and Iversen (23). The brain regions were immediately frozen in liquid nitrogen and stored at −80°C until extraction. Frozen tissues were weighed and transferred to a 1.5 ml polypropylene tube, and each tissue was homogenized in 0.5 ml of 0.1 mM perchloric acid containing a predetermined quantity of isoproterenol as an internal standard. The homogenates were centrifuged at 14,000 rpm for 15 min at 4°C and supernatants were filtered through a Nalgene syringe filter (0.45 mm). A 5–20 ml aliquot of the clear filtrate was injected into the HPLC-ED system.

Measurement of Biogenic Amines and Their Metabolites

Measurments were conducted using an HPLC-ED system that consists of a model PM-80 pump (Bioanalytical Systems; BAS, West Lafayette, IN) and a Rheodyne 7125 injection valve (Cotati, CA) with a 100 μl sample loop. A separation column of Eicompak MA-50 ODS (150 × 4.6 mm i.d., Eicom, Kyoto, Japan) and a guard column (10 × 4.6 mm i.d., ODS, Eicom, Kyoto, Japan) were operated at ambient temperature. The electrochemical detector was a Model LC-4C (BAS, West Lafayette, IN) with a glassy carbon working electrode (Teflon cell gasket; 0.002) and an Ag/AgCl reference electrode. The applied oxidation potential was set at +0.75 V. A mixture of 0.1 M citrate buffer (pH 3.5)–methanol (85:15, v/v) containing 1.0 mM sodium octyl sulfate and 0.02 mM EDTA was used as the mobile phase at a flow rate of 1.0 ml/min. The mobile phase was passed through a 0.45 μm membrane filter and degassed under a vacuum. Data were recorded and evaluated using a Hewlett Packard Model 3390 A integrator (Avondale, PA). The amounts of biogenic amines (NE, DA, and 5-HT) and their metabolites (DOPAC, HVA, and 5-HIAA) were calculated from the ratios of the peak heights of these compounds relative to the internal standard (isoproterenol).

Statistics

Quantal (all or none) data from the behavioral studies on the experimental groups and saline controls were compared by the chi-square test. In the case of the measurement of biogenic amines and their metabolites, the data were presented as the

mean ± SEM. Statistical analysis of the data was carried out by the Student's t-test. A difference was considered significant at $p < 0.05$.

RESULTS

Table 1 shows that abrupt withdrawal from butorphanol and morphine, manifested as withdrawal signs (escape behavior, wet dog shakes, teeth chattering, rearing, locomotion, stretching, scratching, salivation, penis-licking, and ptosis) was precipitated by naloxone, 5 mg/kg, IP, 2 h after the termination of ICV infusion of butorphanol (26 nmol/μl/h) or morphine (26 nmol/μl/h) for 3 days. In animals that received saline instead of the naloxone challenge, no withdrawal responses were observed in either butorphanol- or morphine-dependent rats. Naloxone injection induced significant wet dog shakes ($p < 0.05$), teeth chattering ($p < 0.01$), locomotion ($p < 0.05$), stretching ($p < 0.01$), and penis licking ($p < 0.05$) in butorphanol-dependent animals ($n = 7$) as compared with the saline challenged control group ($n = 7$). In morphine-infused animals ($n = 7$), wet dog shakes [$p < 0.01$ over the control group ($n = 7$)], teeth chattering ($p < 0.01$), rearing ($p < 0.01$), locomotion ($p < 0.05$), stretching ($p < 0.01$), and scratching ($p < 0.05$) were also significantly induced by naloxone challenge.

In animals rendered dependent on butorphanol ($n = 7$), naloxone challenge significantly decreased DA levels in the cortex [−51.8% of the saline-treated control level ($n = 7$), $p < 0.01$] and striatum (−81.2%, $p < 0.05$). However, the levels of NE and 5-HT were unchanged in every region measured. Furthermore, the levels of DOPAC were decreased in the striatum (−66.6%, $p < 0.01$) and levels of HVA were decreased in the striatum (−79.0%, $p < 0.05$), limbic (−54.6%, $p < 0.01$), midbrain (−36.8%, $p < 0.05$), and pons/medulla (−45.8%, $p < 0.01$). On the other hand, a significant decrease of 5-HIAA, a metabolite of 5-HT, was detected only in the limbic area (−79.7%, $p < 0.05$) (Table 2). In the same groups, naloxone increased the HVA/DA ratio in the cortex (84.6%, $p < 0.05$), while this ratio was reduced in the limbic area (−53.8%, $p < 0.05$), midbrain (−53.8%, $p < 0.05$), and pons/medulla (−54.5%, $p < 0.05$). The reduction of 5-HIAA/5-HT ratio was also detected in the limbic area (−50.7%, $p < 0.05$) (Table 4).

In the morphine-dependent animals ($n = 7$), DA were decreased in the cortex [−60.5% of the saline treated control level ($n = 7$), $p < 0.01$] and striatum (−82.2%, $p < 0.05$), DOPAC levels were decreased in the cortex (−80.0%, $p < 0.05$), striatum (−51.0%, $p < 0.01$), and limbic area (−70.9%, $p < 0.01$) and HVA levels were decreased in the striatum (−58.1%, $p < 0.01$), limbic area (−47.5%, $p < 0.01$), midbrain (−42.9%, $p < 0.05$), pons/medulla (−60.9%, $p < 0.05$), and cerebellum (−45.5%, $p < 0.05$) following naloxone challenge. Although there were no significant changes in NE and 5-HT levels, 5-HIAA was decreased in the striatum (−66.0%, $p < 0.05$), limbic area (−66.6%, $p < 0.05$), and midbrain (−73.5%, $p < 0.05$) after naloxone injection (Table 3). On the other hand, in the same animals, the ratio of DOPAC/DA in the limbic area (−39.1%, $p < 0.05$), HVA/DA in the limbic (−58.3%, $p < 0.05$), midbrain (−53.8%, $p < 0.05$), pons/medulla (36.6%, $p < 0.05$), and cerebellum (−60.2%, $p < 0.05$), and 5-HIAA/5-HT in the striatum (−43.5%, $p < 0.05$), limbic area (−43.7%, $p < 0.05$), and midbrain (−39.3%, $p < 0.05$) were decreased by naloxone injection, while naloxone increased the HVA/DA ratio in the cortex.

Meanwhile, naloxone itself induced no changes of behavior and amines in saline-treated control animals instead of opioids (data not shown).

DISCUSSION

In accordance with our previous reports (31,34,36), IP injection of naloxone (an opioid receptor antagonist) precipitated withdrawal signs in butorphanol (a $\mu/\delta/\kappa$ mixed opioid receptor agonist)- and morphine (a μ-opioid receptor agonist)-dependent rats. The injection of naloxone also induced de-

TABLE 1

WITHDRAWAL SIGNS ELICITED BY IP INJECTION OF NALOXONE IN BUTORPHANOL- OR MORPHINE-DEPENDENT RATS

	Butorphanol		Morphine	
Withdrawal Signs	SAL	NAL	SAL	NAL
Escape behavior	0/7*	3/7	0/7	4/7
Wet dog shakes	1/7	6/7†	0/7	7/7‡
Teeth chattering	0/7	5/7‡	0/7	6/7‡
Rearing	0/7	4/7	0/7	6/7‡
Locomotion	0/7	5/7†	0/7	5/7†
Stretching	0/7	6/7‡	0/7	6/7‡
Scratching	1/7	4/7	1/7	6/7†
Salivation	0/7	2/7	0/7	3/7
Penis-licking	0/7	5/7†	0/7	4/7
Ptosis	0/7	3/7	1/7	3/7

Rats were treated with ICV infusion of butorphanol (26 nmol/μl/h) or morphine (26 nmol/μl/h) for 3 days and challenged with saline (SAL; 0.1 ml/100 g) or naloxone (NAL; 5 mg/kg) 2 h after the termination of drug infusion.

*Numbers denote the number of rats showing positive signs over the total number of rats tested.

†$p < 0.05$, ‡$p < 0.01$, values are significantly higher than control values as determined by the chi-square test.

TABLE 2

CHANGES INDUCED BY IP INJECTION OF NALOXONE IN LEVELS OF BIOGENIC AMINES AND THEIR METABOLITES IN VARIOUS BRAIN REGIONS OF BUTORPHANOL-DEPENDENT RATS

		Biogenic Amines (ng/g Wet Tissue)			Metabolites (ng/g Wet Tissue)		
Brain Regions		NE	DA	5-HT	DOPAC	HVA	5-HIAA
Cortex	SAL	247 ± 8	504 ± 69	190 ± 13	184 ± 23	82 ± 11	206 ± 21
	NAL	240 ± 15	261 ± 28*	195 ± 11	155 ± 17	62 ± 7	204 ± 13
Striatum	SAL	329 ± 26	12330 ± 385	245 ± 36	3030 ± 390	675 ± 69	365 ± 45
	NAL	301 ± 31	10012 ± 398†	304 ± 14	2017 ± 108*	533 ± 21†	302 ± 28
Limbic area	SAL	689 ± 45	2800 ± 240	629 ± 23	1097 ± 72	335 ± 21	418 ± 28
	NAL	621 ± 32	2798 ± 108	574 ± 21	952 ± 47	183 ± 14*	333 ± 15†
Midbrain	SAL	530 ± 34	533 ± 59	380 ± 28	161 ± 29	57 ± 11	413 ± 23
	NAL	501 ± 26	375 ± 28	458 ± 24	176 ± 11	21 ± 5†	362 ± 18
Pons/medulla	SAL	522 ± 26	57 ± 4	372 ± 23	38 ± 4	24 ± 2	352 ± 27
	NAL	489 ± 18	52 ± 3	437 ± 31	35 ± 3	11 ± 2*	390 ± 24
Cerebellum	SAL	169 ± 17	6 ± 1	22 ± 2	7 ± 1	7 ± 1	28 ± 3
	NAL	237 ± 15	7 ± 1	38 ± 7	8 ± 1	6 ± 1	36 ± 4

Rats were treated with ICV infusion of butorphanol (26 nmol/μl/h) for 3 days and challenged with saline (SAL; 0.1 ml/100 g) or naloxone (NAL; 5 mg/kg) 2 h after the termination of drug fusion.

Values are the means ± SEM of the data from seven animals.

*$p < 0.01$, †$p < 0.05$, values are significantly lower than control values as determined by the student's t-test.

creases in DA in the cortex and striatum and its metabolites (DOPAC and HVA) in the striatum, limbic, midbrain, and pons/medulla regions of butorphanol-dependent animals. Furthermore, naloxone increased the HVA/DA ratio in the cortex, while this ratio was reduced in the limbic area, midbrain, and pons/medulla, indicating that DA metabolism also decreased in response to naloxone in some brain regions except cortex. The increase in metabolism of DA observed only in the cortex might depend on compensatory action against the lowering of dopaminergic function in other regions. In

TABLE 3

CHANGES INDUCED BY IP INJECTION OF NALOXONE IN LEVELS OF BIOGENIC AMINES AND THEIR METABOLITES IN VARIOUS BRAIN REGIONS OF MORPHINE-DEPENDENT RATS

		Biogenic Amines (ng/g Wet Tissue)			Metabolites (ng/g Wet Tissue)		
Brain Regions		NE	DA	5-HT	DOPAC	HVA	5-HIAA
Cortex	SAL	231 ± 19	514 ± 21	212 ± 9	160 ± 14	73 ± 12	189 ± 16
	NAL	245 ± 19	311 ± 18*	223 ± 18	128 ± 4†	60 ± 7	201 ± 11
Striatum	SAL	407 ± 53	13385 ± 486	294 ± 40	4470 ± 603	915 ± 81	509 ± 61
	NAL	291 ± 27	11005 ± 46†	270 ± 23	2281 ± 213*	532 ± 30*	336 ± 24†
Limbic area	SAL	770 ± 77	3070 ± 114	653 ± 69	1324 ± 114	336 ± 19	467 ± 45
	NAL	607 ± 29	3104 ± 217	518 ± 31	939 ± 61*	174 ± 26*	311 ± 24†
Midbrain	SAL	712 ± 34	498 ± 50	443 ± 35	180 ± 21	63 ± 12	460 ± 32†
	NAL	753 ± 36	412 ± 28	354 ± 26	149 ± 14	27 ± 4†	338 ± 27†
Pons/medulla	SAL	480 ± 11	55 ± 4	371 ± 29	38 ± 3	23 ± 2	365 ± 12
	NAL	507 ± 23	47 ± 4	396 ± 27	32 ± 4	14 ± 2†	414 ± 35
Cerebellum	SAL	175 ± 11	5 ± 1	25 ± 2	8 ± 1	11 ± 1	29 ± 1
	NAL	181 ± 14	6 ± 1	30 ± 7	7 ± 1	5 ± 1†	31 ± 3

Rats were treated with ICV infusion of morphine (26 nmol/μl/h) for 3 days and challenged with saline (SAL; 0.1 ml/100 g) or naloxone (NAL; 5 mg/kg) 2 h after the termination of drug infusion.

Values are the means ± SEM of the data from seven animals.

*$p < 0.01$, †$p < 0.05$, values are significantly lower than control values are determined by the student's t-test.

TABLE 4

CHANGES INDUCED BY IP INJECTION OF NALOXONE IN METABOLITE/TRANSMITTER RATIO IN VARIOUS BRAIN REGIONS OF BUTORPHANOL- OR MORPHINE-DEPENDENT RATS

		Butorphanol			Morphine		
		DOPAC	HVA	5-HIAA	DOPAC	HVA	5-HIAA
Brain Regions		DA	DA	5-HT	DA	DA	5-HT
Cortex	SAL	0.44 ± 0.04	0.13 ± 0.02	1.09 ± 0.20	0.28 ± 0.03	0.13 ± 0.02	0.90 ± 0.19
	NAL	0.60 ± 0.10	0.24 ± 0.03*	1.06 ± 0.16	0.32 ± 0.06	0.26 ± 0.03*	0.88 ± 0.18
Striatum	SAL	0.27 ± 0.03	0.06 ± 0.01	1.32 ± 0.23	0.35 ± 0.06	0.09 ± 0.01	1.75 ± 0.32
	NAL	0.20 ± 0.03	0.05 ± 0.01	1.06 ± 0.19	0.28 ± 0.04	0.06 ± 0.01	1.01 ± 0.11*
Limbic area	SAL	0.36 ± 0.04	0.13 ± 0.01	0.69 ± 0.11	0.46 ± 0.07	0.12 ± 0.01	0.87 ± 0.15
	NAL	0.35 ± 0.06	0.06 ± 0.01*	0.34 ± 0.08*	0.28 ± 0.04	0.05 ± 0.01*	0.49 ± 0.10*
Midbrain	SAL	0.34 ± 0.07	0.13 ± 0.02	1.01 ± 0.22	0.38 ± 0.08	0.13 ± 0.02	1.17 ± 0.21
	NAL	0.41 ± 0.08	0.06 ± 0.01*	0.86 ± 0.17	0.36 ± 0.07	0.06 ± 0.01*	0.71 ± 0.13*
Pons/medulla	SAL	0.64 ± 0.13	0.44 ± 0.08	0.94 ± 0.23	0.66 ± 0.13	0.41 ± 0.06	0.98 ± 0.19
	NAL	0.65 ± 0.09	0.20 ± 0.04*	0.91 ± 0.15	0.68 ± 0.11	0.26 ± 0.03*	1.02 ± 0.20
Cerebellum	SAL	1.20 ± 0.28	1.11 ± 0.31	1.17 ± 0.22	1.55 ± 0.31	2.16 ± 0.35	1.14 ± 0.20
	NAL	1.15 ± 0.30	0.90 ± 0.20	0.98 ± 0.19	1.20 ± 0.23	0.86 ± 0.17*	1.05 ± 0.21

Values are the means ± SEM of the data from seven animals.
*$p < 0.05$, values are significantly higher or lower than control values as determined by the student's t-test.

animals rendered dependent on morphine, the changes in levels of DA, DOPAC, and HVA were similar to those of butorphanol-dependent rats. We also detected inconsistent changes of DA, DOPAC, and HVA in different brain regions. Although it is difficult to substantiate based on these phenomena, enzymes that are involved in metabolic pathway of DA during opioid withdrawal might be different in various brain regions. On the other hand, our recent report (65) has shown that a single dose of butorphanol (26 nmol/5 μl; ICV) or morphine (26 nmol/5 μl; ICV) increased levels of DOPAC in the striatum and limbic area and HVA in the cortex, striatum, and limbic regions. Region-specific changes in biogenic amines and their metabolites were noted in acutely treated (65) and naloxone-precipitated withdrawal rats (present study). Furthermore, levels of these amines were also decreased during natural withdrawal (65). The findings are the same as those of withdrawal precipitated by naloxone. Accordingly, it appears that decreases in levels of DA and its metabolites are closely related to the expression of the withdrawal syndrome from opioids. The interpretation of these results is discussed in the following sections.

It has been widely accepted that cerebral monoamines are linked to reinforcing and rewarding behaviors (14,57). The appearance of a reinforcing effect is known to involve dopaminergic neurons of the ventral tegmental reward system, which is located in the midbrain, striatum, and limbic areas (9,46,47,59,64). It has also been reported that brain nuclei, such as locus coeruleus (LC), central gray, and dorsal thalamus, are closely associated with the expression of withdrawal signs from opioids (2,10,24,52,66). Other evidence that the dopaminergic system may also be involved is that naloxone injection in morphine-dependent animals reduces extracellular DA levels in the ventral tegmental area and nucleus accumbens (50,55). Furthermore, systemic treatment with clonidine has been demonstrated to prevent opioid withdrawal and the withdrawal-associated decrease in accumbal DA levels (50). Recently, Harris and Aston-Jones (27) have shown that alterations in mesolimbic dopamine function contribute to the opioid withdrawal signs.

It has been reported that DA and its metabolites increase during exposure to opioids (4,5,21), while evidence also indicates that withdrawal from repeated morphine treatment decreases the synthesis and release of cerebral DA (3,7,54). In parallel, morphine-tolerant, -dependent, and -abstinent rats have been shown to exhibit differential changes in the central DA receptors. In nonabstinent morphine-dependent rats, DA receptors are unaltered in brain regions and spinal cord when labeled with [^{3}H]SCH 23390. In the abstinent rats, the binding (B_{max} value) of [^{3}H]SCH 23390 is increased in the hypothalamus, corpus striatum, and spinal cord (8), suggesting that DA receptors become supersensitive when there is a lack of DA in the synaptic cleft. In fact, rats withdrawn from morphine are hypersensitive to the behavioral effects of apomorphine (12), and the specific binding of neuroleptic compounds to striatal membranes of such rats is slightly enhanced (53). It seems that the decrease in DA and its metabolites following naloxone-precipitated withdrawal in butorphanol-dependent rats might lead to change of dopaminergic neurons.

Presently, opioid receptors can be classified into at least three different types, for instance, μ, δ, and κ receptors (25, 41). Previous studies from our laboratory have demonstrated that β-funaltrexamine (a μ-opioid receptor selective antagonist) failed to precipitate withdrawal in butorphanol-dependent rats (35), while both naltrindole (a δ-opioid receptor selective antagonsit) and nor-binaltorphimine (a κ-opioid receptor selective antagonsit) have been shown to precipitate withdrawal signs similar to those precipitated by naloxone (34,36). These data have suggested that κ- and/or δ-opioid receptors are more involved than μ-receptors in the expression of behavioral signs of withdrawal from butorphanol. Further-

more, it has been generally accepted that depolarization-induced NE release is inhibited selectively by μ-opioid receptor agonists; whereas, DA release is under the inhibitory control of κ-opioid receptors throughout the rat brain (29,44,56,67). Accordingly, the changes in DA and its metabolites produced by naloxone-precipitated withdrawal in butorphanol-dependent rats might be dependent upon the κ-receptor mediated system, although butorphanol has multiple actions on opioid receptors. On the other hand, similar results were obtained in animals rendered dependent on morphine. Taking into account the receptor selectivity of morphine (acting mainly on the μ-opioid receptor system), it appears that a decrease in DA and its metabolites in morphine-dependent animals is mediated through μ-receptors. Indeed, it has also been found that peripheral administration as well as local injection of μ-selective agonists into the substantia nigra, ventral tegmental area, or striatum resulted in increased firing of dopaminergic neurons and increased release of DA in the striatum (17-19,58). On the other hand, Wood et al. (68) have reported that the involvement of κ-receptors is negligible in the regulation of nigrostriatal dopaminergic function. This discrepancy could be best explained by the findings obtained from our laboratory (34). We proposed that there may be μ/κ allosterically interacting receptor complexes in addition to putatively distinct noncomplexed μ-, δ-, and κ-opioid receptors. Thus, similar changes of the dopaminergic neuron by naloxone challenge in rats rendered dependent on butorphanol and morphine might occur by way of these postulated μ/κ interacting receptor complexes.

It has been reported that naloxone-precipitated withdrawal from butorphanol (20) and morphine (1,70) is associated with increased extracellular levels of glutamate within the LC, which is located in the pons/medulla region. Furthermore, we have shown that direct ICV injection of glutamate is able to precipitate the expression of a withdrawal syndrome from opioids, suggesting that the rapid release of glutamate from a central specific area under an opioid-dependent state may be a trigger or key factor for the expression of the opioid withdrawal signs (63). Interestingly, changes in the HVA levels in the pons/medulla region were detected in rats infused continuously with butorphanol or morphine after naloxone challenge in this study. However, there may be no direct relation between glutamate and DA release in the LC region because LC is a brain stem nucleus, which largely contains clusters of adrenergic neurons (16). Furthermore, although excitatory amino acids, such as glutamate and NMDA, appear to stimulate DA release in the striatum, substantia nigra, and ventral tegmental area (32,39,43), there is no reports regarding the release of DA from the LC region. We have also shown that the duration of withdrawal from butorphanol induced by ICV injection of glutamate was shorter than that precipitated by naloxone. In addition, some withdrawal signs such as salivation and body weight loss were not observed in glutamate-challenged groups, while ICV injection of naloxone precipitated all behavioral signs tested. Presumably, the changes in dopaminergic neuron activity in the striatum, limbic, midbrain, and pons/medulla regions, independent of glutamate release in the LC region, might contribute to the maintenance of the withdrawal state and/or the induction of severe withdrawal signs precipitated by naloxone in butorphanol-dependent rats.

The NE level in every region measured was not altered by naloxone injection in butorphanol- or morphine-dependent animals. Considerable evidence suggests that the opioid withdrawal signs may involve changes in adrenergic activity in the central nervous system (15,26). In our recent study (65), NE levels in some brain regions of morphine- but not butorphanol-dependent animals that were treated in the same manner were gradually increased during abrupt withdrawal without naloxone injection. Accordingly, the data concerning changes in NE levels from opioid withdrawal seem to be less apparent as compared with other amines. Unfortunately, it was difficult to measure the principal metabolite of NE, 3-methoxy-4-hydroxy-phenylethylenglycol (MHPG), in the present study, because we had measured these biogenic amines and their metabolites simultaneously. Indeed, nalorphine- or naloxone-precipitated withdrawal has been reported to be accompanied by increased turnover of cerebral NA and increased concentrations of MHPG (40). Furthermore, hyperadrenergic activity in methadone-dependent human subjects given naltrexone has been observed simultaneously with increases in plasma MHPG levels (13). A definitive conclusion concerning the involvement of adrenergic neurons in the expression of withdrawal from butorphanol has not yet been obtained.

There are many conflicting reports regarding the level of 5-HT and 5-HIAA (decrease or increase) during withdrawal from sustained morphine treatment (3,6,28,60). In our study, prominent changes in serotonergic activity was not shown in animals rendered dependent on butorphanol. Meanwhile, in morphine-dependent rats, 5-HIAA was decreased in the striatum, limbic area, and midbrain by naloxone challenge. These results indicate that the involvement of serotonergic neurons in expressing withdrawal signs seems to be weaker than that of dopaminergic neurons for butorphanol but not for morphine.

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