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Involvement of Opioid μ_1-Receptors in Opioid-Induced Acceleration of Striatal and Limbic Dopaminergic Transmission

T. P. PIEPPONEN,[1] A. HONKANEN, T. KIVASTIK,[2] A. ZHARKOVSKY,[2] A. TURTIA, J. A. V. MIKKOLA AND L. AHTEE

Department of Pharmacy, Division of Pharmacology and Toxicology, P.O. Box 56, FIN-00014 University of Helsinki, Finland

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PIEPPONEN, T. P., A. HONKANEN, T. KIVASTIK, A. ZHARKOVSKY, A. TURTIA, J. A. V. MIKKOLA AND L. AHTEE. *Involvement of μ_1-opioid receptors in opioid-induced acceleration of striatal and limbic dopaminergic transmission.* PHARMACOL BIOCHEM BEHAV **63**(2) 245–252, 1999.—The role of μ_1-opioid receptors in the acceleration of cerebral dopaminergic transmission induced by morphine and the putative μ_1-opioid agonist, etonitazene, was studied in rats by measuring the tissue levels of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the dorsal striatum and nucleus accumbens. The striatal extracellular concentrations of DA and its metabolites in freely moving rats were estimated as well. Morphine (3 mg/kg) and etonitazene (2.5 μg/kg) increased the striatal and accumbal dopamine metabolism as measured by the tissue ratios of DOPAC/DA and HVA/DA. The μ_1-opioid receptor antagonist, naloxonazine (15 mg/kg), significantly antagonized these elevations except the morphine-induced elevation of striatal HVA/DA ratio. Both morphine (3 mg/kg) and etonitazene (1, 2.5, and 5 μg/kg) elevated the striatal extracellular DA, DOPAC, and HVA. Naloxonazine antagonized the effects of morphine and etonitazene on striatal extracellular DA concentration as well as etonitazene's effects on DOPAC and HVA, but not morphine's effects on DOPAC and HVA. As we previously showed concerning morphine, the conditioned place preference induced by etonitazene was inhibited by naloxonazine. These findings emphasize the role of μ_1-opioid receptors in opioid reward, in which the mesolimbic dopaminergic system is considered to be importantly involved. Our results clearly show that in addition to the mesolimbic dopaminergic system the μ_1-opioid receptors are also involved in the control of nigrostriatal DA release and metabolism. However, the effects of etonitazene on the striatal DA differ from those of morphine, suggesting that the opioid mechanisms regulating these two DA systems differ. © 1999 Elsevier Science Inc.

Dopamine release and metabolism Microdialysis Striatum Nucleus accumbens Morphine Etonitazene Naloxonazine Conditioned place preference

IT is well-known that morphine and related opioids increase both nigrostriatal and mesolimbic dopamine (DA) turnover, metabolism, and release. As morphine is relatively selective for the μ-opioid receptors (5), these receptors seem to be the most important ones mediating this effect. There is ample evidence indicating that mesolimbic DA is critically involved in the rewarding effects of various classes of drugs of abuse [for reviews, see (9,27)], whereas the activation of nigrostriatal dopaminergic systems is involved in the stereotyped behavior induced by opioids (3,14). There is, however, substantial evidence that opioid-induced reward may also involve DA-independent mechanisms (2,6,8,17,20). Although the role of mesolimbic DA in the rewarding effects of opioids is still controversial, it seems clear that the acceleration and particularly sensitization of mesolimbic dopaminergic transmission is crucial in the development of opioid addiction (21).

Binding studies have led to the hypothesis that there are two subtypes of μ-opioid receptors, one (μ_1) with high affinity to both μ-opioids and enkephalins, and the other one (μ_2) with lower affinity to opioids but binding morphine far more potently than enkephalins (28). The physiological roles of these subtypes have been characterized primarily with the μ_1-

[1]Requests for reprints should be addressed to T. P. Piepponen, Department of Pharmacy, Division of Pharmacology and Toxicology, P.O. Box 56 (Viikinkaari 5), FIN-00014 University of Helsinki, Finland.
[2]Present address: Department of Pharmacology, University of Tartu, EE2400 Tartu, Estonia.

selective antagonist, naloxonazine. Naloxonazine selectively antagonizes a variety of morphine's actions including analgesia without affecting a number of other ones such as respiratory depression [for a review, see (15)]. Clarification of the subtypes of μ-receptor has suffered from a lack of selective agonists for μ_1- and μ_2-receptors. Recently it was found that synthetic opioid, etonitazene, is relatively selective for μ_1-subtype of μ-receptors (13). Etonitazene is a potent analgesic (about 1000-fold more potent than morphine), and like morphine, it induces catalepsy in larger doses (22). Etonitazene also produces conditioned place preference (22), and it is orally self-administered by rats (4,26), indicating that etonitazene may have rewarding properties as well. The effects of etonitazene on cerebral DA transmission have not yet been studied.

The role of μ_1-opioid receptors in the development of opioid addiction as well as in the opioid-induced acceleration of cerebral DA turnover is still unclear. Early findings suggested that μ_1-receptors are not involved in the increase of striatal DA metabolism induced by morphine (29). This assumption was further supported by our previous findings that naloxonazine does not antagonize accelerated DA metabolism caused by a large dose of morphine in either the rat striatum or limbic forebrain (18). However, Latimer et al. (11) found that enhanced DA metabolism induced by centrally administered μ-receptor agonist, DAMGO, was partially antagonized by naloxonazine. In the same study it was found that DAMGO-induced stimulation of locomotor activity was partially antagonized by naloxonazine as well, indicating that naloxonazine may antagonize some of the rewarding effects of DAMGO. We recently showed that morphine-induced reward is antagonized by naloxonazine (19).

The present study was undertaken to further clarify the roles of μ-receptor subtypes in the control of nigrostriatal and mesolimbic DA systems of the rat brain by using the selective μ_1-receptor antagonist, naloxonazine. Furthermore, we wanted to clarify whether naloxonazine simultaneously when it antagonizes the rewarding effect produced by morphine antagonizes the morphine-induced acceleration of mesolimbic DA metabolism. In addition, our aim was to compare the effects of morphine and those of the putative μ_1-receptor agonist, etonitazene, on striatal and limbic DA release and metabolism. To further study the role of the μ_1-receptors in the rewarding effects of opioids we tested the effect of naloxonazine on etonitazene-induced conditioned place preference. We measured the tissue concentrations of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the dorsal striatum and nucleus accumbens. The DOPAC/DA ratio can be used as an indicator of the rate of DA turnover (synthesis and metabolism) when DA levels are not altered by the drug treatments (1), and the HVA/DA ratio can be used as an indicator of the sum of DA metabolism and release (25). Furthermore, we assessed striatal DA release and metabolism by measuring the extracellular concentrations of DA, DOPAC, and HVA in the dorsal striatum of freely moving rats.

METHOD

Animals

Male Wistar rats were housed in groups of four to six under a 12 L:12 D cycle (lights on at 0600 h) and at an ambient temperature of 22°C. The animals had free access to standard pellet diet and tap water ad lib. The animal experiments were approved by the local institutional animals care and use committee and the chief veterinarian of the county administrative board, and were conducted according to the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes."

Measurement of DA and Its Metabolites in Tissue Samples

Forty-two rats weighing 310–420 g were used. Naloxonazine (15 mg/kg, IP) was administered 12–14 h before the agonists. Morphine (3 mg/kg) and etonitazene (2.5 μg/kg) were given SC 60 min before the rats were killed by high-intensity microwave irradiation focused to the head using an NJE 6203-10kW microwave instrument (New Japan Radio Inc., Japan). The rats were then decapitated and brains were removed from the skull and placed in a stainless steel brain mold (RBM-4000C, ASI Instruments, USA). Brains were sectioned with razor blades, the caudate-putamen and the nucleus accumbens were dissected from a coronal slice [2.7 to −0.3 mm from the bregma; (16)] using needles with inner diameters of 3 and 2 mm, respectively. Brain samples were placed in 1.5-ml Eppendorf tubes, frozen on dry ice, and stored at −80°C until assay.

The brain samples were homogenized in 1.0 ml of 0.2 M perchloric acid, and KOH/HCOOH buffer was added to homogenates to set pH to 2.4. Samples were centrifuged at 5500 × *g* for 45 min at 4°C using Sorvall RC-5 (Dupont, DE) centrifuge. The amines were purified and isolated in Sephadex gel columns as described by Haikala (7). The concentrations of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed by HPLC using electrochemical detection. The system for determining DA consisted of a ESA Coulochem 5100A detector (ESA Inc., MA) equipped with a model 5014 analytical cell and a Pharmacia LKB model 2150 HPLC pump (Pharmacia LKB, Sweden) with a SSI model 20-0225 pulse damper (Scientific Systems Inc., PA). The separation and quantification were performed as described by Lagerqvist (10). The column (Nucleosil SA 5μ, 20 cm, i.d. 4 mm) was kept at 45°C with a Bio-Rad column heater. Mobile phase was a 15/85 (vol/vol) mixture of solutions A (300 mM citric acid, 700 mM NaOH) and B (75 mM citric acid, 175 mM NaOH, 30 vol/vol methanol) and contained 0.004% EDTA. The flow rate of the mobile phase was 0.6 ml/min.

The system for determining DOPAC and HVA consisted of ESA Coulochem II detector equipped with model 5014 analytical cell, Beckman model 110B pump (Beckman Instruments Inc., USA) with SSI model LP-21 pulse damper and Spherisorb 5 μ 25 cm (i.d. 4.6 mm) column. The mobile phase was prepared by mixing 0.1 M citric acid and 0.2 M Na_2HPO_4 to set the pH to 4.3. The mobile phase also contained 10% (vol/vol) methanol and 0.2 mM EDTA, and the flow rate was 1.2 ml/min.

Microdialysis

Thirty-nine rats weighing 250–360 g were used (excluding the rats with incorrect placement of the probe or with unstable baselines). An I-shaped microdialysis probe (23) was implanted into the rat striatum [A +1.0, L +3.0, D −6.0; (16)] under halothane (3–1.5%) anaesthesia. The dialysis tube was prepared from polyacrylonitrile/sodiummetasulfonate copolymer (Filtral 20; o.d./i.d. 310:220 μm; Hospal, France). The exposed tip of the dialysis membrane was 4 mm. After the surgery the rats were placed individually in test cages and allowed to recover for approximately 40 h. In the morning of the experiment day the probe was connected via polyethylene

tubing to a 1-ml microsyringe, and Ringer solution was infused through the microdialysis probe with a microinjection pump (2 μl/min). The samples (30 μl) were collected every 15 min. The basal output of DA and its metabolites was defined as an average of first four stable samples after a stabilization period of 1 h, and was defined as 100%.

The system used for determination of the extracellular concentrations of DA, DOPAC, and HVA consisted ESA Coulochem II detector (ESA Inc., MA) equipped with a model 5014A microdialysis cell and a Pharmacia LKB model 2248 HPLC pump (Pharmacia LKB, Sweden) with an SSI model LP-21 pulse damper (Scientific Systems Inc., PA). The column (Spherisorb ODS2, 4.6 × 100 mm) was kept at 40°C with a column heater (Croco-Cil, France). The mobile phase used consisted of 0.5 M NaH_2PO_4 buffer, pH 4.0 (adjusted with 1.0 mM citric acid), 0.1–0.2 mM octane sulfonic acid, 16 % methanol and 450 mg/l EDTA. The flow rate of the mobile phase was set to 1.0 ml/min. Twenty microliters of the dialysate sample were injected into the chromatographic system with a CMA/200 autoinjector (CMA, Stockholm, Sweden). DA was reduced with an amperometric detector (potential—80 mV) and DOPAC and HVA were oxidized with a coulometric detector (+300 mV). The chromatogram was processed with a Shimadzu C-R5A Cromatopac integrator (Shimadzu, Kyoto, Japan).

Conditioned Place Preference (CPP)

Thirty-six rats weighing 290–370 g were used. CPP was studied in an apparatus consisting of two square-base compartments (h 40 × 30 × 30 cm), one with white and the other with dark gray walls and floor. Compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling. The apparatus was placed in a dimly lit room with masking noise provided by a ventilation fan.

Before starting the experiments, the rats were acclimated to experimenter contact for 3 days by handling and weighing them. The procedure was similar to that described previously (8,19).

Each experiment consisted of three phases: 1) preconditioning—for 3 days (days 1, 2, and 3) rats were given free access to both compartments of the apparatus for 15 min (900 s) each day. On day 3, the time spent by the rats in each compartment was recorded and these values served as a baseline. 2) Conditioning was conducted for 4 days (days 4, 5, 6, and 7), and included two sessions each day. Rats were given SC etonitazene (2.5 μg/kg) or saline (controls) immediately before placing the rat in the nonpreferred compartment for 60 min. After an interval of 4 h all rats received saline and were placed in the preferred compartment for 60 min. The order of etonitazene and saline presentation paired with the given environment was balanced across treatment groups. In a separate experiment naloxonazine (15 mg/kg, IP) or its vehicle were given 12 h before etonitazene. 3) Postconditioning—on day 8, rats were allowed to freely choose their preferred compartment in the apparatus for 15 min (no drugs were administered), and the time spent in the white compartment was recorded.

Drugs

Naloxonazine (RBI, Natick, MO) was suspended in 2.5% Tween® 80 solution. Morphine HCl (Ph Eur. 2nd Ed.) and etonitazene HCl (a generous gift from Ciba-Geigy Limited, Basel, Switzerland) were dissolved in saline. Drugs were administered in a volume of 2 ml/kg, and doses refer to base forms. Naloxonazine was administered 12–14 h before morphine because it initially also reversibly blocks other types of opioid receptors in a naloxone-like manner, and only its long-lasting effects (perhaps irreversible effects) are μ_1-selective (12).

Statistics

The DOPAC/DA and HVA/DA ratios in tissue samples were analyzed by two-way analysis of variance (ANOVA), naloxonazine or vehicle as pretreatment, and morphine, etonitazene, or saline as treatment. When appropriate, multiple comparisons were made using Student–Newman–Keuls post hoc test. Microdialysis data was analyzed with either one-way (dose response of etonitazene) or two-way (Naloxonazine × opioid interaction) analysis of variance for repeated measurements. Multiple comparisons were conducted using the contrast analysis with Bonferroni levels. The data obtained in CPP experiments were subjected to one-way analysis of covariance (ANCOVA), baseline serving as covariate. Student–Newman–Keuls post hoc test was used for multiple comparisons.

RESULTS

Metabolism of Dopamine in Tissue Samples

The steady-state concentrations of DA are presented in the legend of Table 1. None of the drug treatments significantly altered the concentration of DA either in the dorsal striatum or in the nucleus accumbens.

Both morphine (3 mg/kg) and etonitazene (2.5 μg/kg) significantly increased DOPAC/DA ratios in the striatum compared with the control [Table 1; treatment effect, $F(1, 23) = 6.47$, $p = 0.0181$; $F(1, 23) = 7.86$, $p = 0.0101$; for morphine and etonitazene, respectively]. Naloxonazine significantly antagonized these effects [pretreatment × treatment interaction, $F(1, 23) = 5.55$, $p = 0.0273$; $F(1, 23) = 6.53$, $p = 0.0177$; for morphine and etonitazene, respectively]. Both opioids increased the striatal HVA/DA ratios as well [Table 1; treatment effect, $F(1, 23) = 31.51$, $p < 0.0001$; $F(1, 23) = 27.80$, $p < 0.0001$; for morphine and etonitazene, respectively]. Naloxonazine significantly antagonized the effect of etonitazene, but did not significantly antagonize the effect of morphine despite a strong tendency [pretreatment × treatment interaction, $F(1, 23) = 3.39$, $p = 0.0785$; $F(1, 23) = 4.70$, $p = 0.0408$ for morphine and etonitazene, respectively].

In the nucleus accumbens, morphine and etonitazene significantly increased DOPAC/DA ratios [Table 1; treatment effect, $F(1, 24) = 36.37$, $p < 0.0001$; $F(1, 23) = 36.40$, $p < 0.0001$, for morphine and etonitazene, respectively] and HVA/DA ratios, $F(1, 24) = 45.92$, $p < 0.0001$; $F(1,24) = 13.10$, $p = 0.0014$, for morphine and etonitazene, respectively]. Naloxonazine significantly antagonized these effects [DOPAC/DA: pretreatment × treatment interaction, $F(1, 24) = 7.55$, $p = 0.0112$; $F(1, 23) = 4.47$, $p = 0.0467$, for morphine and etonitazene, respectively; HVA/DA, $F(1, 24) = 13.82$, $p = 0.0011$; $F(1,24) = 5.14$, $p = 0.0326$ for morphine and etonitazene, respectively].

Effects of Etonitazene and Morphine on the Extracellular Concentrations of Dopamine, DOPAC, and HVA in the Dorsal Striatum

The baseline levels of DA, DOPAC, and HVA in the striatal dialysates were 76.3 ± 37.6 fmol, 6.2 ± 2.3 pmol, and 5.3 ± 2.8 pmol per 15 min sample (mean ± SD, $n = 39$), respectively. As there were no significant differences between the groups vehicle + saline and saline alone, these groups were combined. Similarly, as there was no difference between the

TABLE 1

EFFECTS OF MORPHINE AND ETONITAZENE ON DOPAMINE METABOLISM IN THE STRIATUM AND NUCLEUS ACCUMBENS OF RATS PRETREATED WITH NALOXONAZINE

Pretreatment/ Treatment	DOPAC/DA				HVA/DA			
	Vehicle	%	Naloxonazine	%	Vehicle	%	Naloxonazine	%
Dorsal striatum								
Saline	0.075 ± 0.004	100	0.092 ± 0.011	100	0.069 ± 0.001	100	0.077 ± 0.003	100
Morphine	0.124 ± 0.013*	165	0.093 ± 0.009‡	101	0.114 ± 0.008†	165	0.100 ± 0.007*	129
Etonitazene	0.117 ± 0.006†	156	0.093 ± 0.010‡	101	0.101 ± 0.004†	146	0.092 ± 0.008‡	119
Nucleus accumbens								
Saline	0.096 ± 0.007	100	0.099 ± 0.005	100	0.064 ± 0.005	100	0.078 ± 0.004	100
Morphine	0.170 ± 0.014†	177	0.127 ± 0.004‡	128	0.123 ± 0.008†	189	0.096 ± 0.007‡	123
Etonitazene	0.152 ± 0.012†	158	0.127 ± 0.004‡	128	0.112 ± 0.013*	124	0.089 ± 0.007‡	114

Saline, morphine (3mg/kg) or etonitazene (2.5 μg/kg) were given SC 1 h before the rats were killed by head-focused microwave irradiation. Naloxonazine (15 mg/kg) or vehicle were given IP 12–14 h before the opioids. Given are means ± SE of six to seven animals. The percentage changes (%) are given compared with corresponding control (vehicle + saline or naloxonazine + saline). The steady-state concentrations (μg/g) of DA were in the dorsal striatum and nucleus accumbens, respectively: vehicle + saline 11.9 ± 0.5, 14.5 ± 1.0; naloxonazine + saline 12.1 ± 0.8, 14.8 ± 0.8; vehicle + morphine 12.5 ± 0.9, 13.2 ± 0.9; naloxonazine + morphine 12.1 ± 1.5, 13.9 ± 0.6; vehicle + etonitazene 12.8 ± 1.0; 14.0 ± 0.9; naloxonazine + etonitazene 10.6 ± 1.1, 15.3 ± 0.7.

* $p < 0.05$, † $p < 0.01$ compared with corresponding control (vehicle + saline or naloxonazine + saline group).

‡ Indicates significant ($p < 0.05$) interaction between pretreatment (vehicle or naloxonazine) and treatment (saline, morphine, or etonitazene; two-way ANOVA).

groups vehicle + etonitazene (2.5 μg) and etonitazene alone, these groups were combined as well.

Etonitazene

Etonitazene (1, 2.5, and 5 μg/kg SC) increased the extracellular concentration of DA in the dorsal striatum [Fig. 1; treatment effect, $F(3, 19) = 11.3$, $p < 0.0002$; dose × time interaction, $F(3, 45) = 2.8$, $p < 0.0004$]. Multiple comparisons showed that the effects of all three doses on the extracellular concentration of DA were significant compared with the control during the first 120 min ($p < 0.05$, $p < 0.05$, $p < 0.0001$, for 1, 2.5, and 5 μg/kg of etonitazene, respectively). The smallest doses of etonitazene (1 and 2.5 μg/kg) increased the extracellular DA equally (by about 30%), whereas the effect of 5 μg/kg of etonitazene was considerably larger (increase by about 80%), and this effect lasted longer as well. The increase of extracellular dopamine induced by etonitazene (2.5 μg/kg) was abolished by pretreatment with naloxonazine (15 mg/kg) [Fig. 2, Pretreatment effect, $F(1, 18) = 3.4$, $p < 0.0835$; treatment effect, $F(1, 18) = 8.4$, $p < 0.0094$; pretreatment × treatment interaction, $F(1, 18) = 8.8$, $p < 0.0082$].

The concentrations of DOPAC and HVA in the dialysates were dose dependently increased by etonitazene [Fig. 1, treatment effect, $F(3, 19) = 20.4$, $p < 0.0001$; $F(3, 19) = 59.0$, $p < 0.0001$ for DOPAC and HVA, respectively; treatment × time interaction, $F(3, 45) = 7.3$, $p < 0.0001$; $F(3, 45) = 9.6$, $p < 0.0001$, for DOPAC and HVA, respectively]. The increases of extracellular concentrations DOPAC and HVA induced by 2.5 μg/kg of etonitazene were completely antagonized by pretreatment with naloxonazine [Fig. 2, pretreatment effect, $F(1, 18) = 23.0$, $p < 0.0001$; $F(1, 18) = 25.1$, $p < 0.0001$; treatment effect, $F(1, 18) = 27.7$, $p < 0.0001$; $F(1, 18) = 32.5$, $p < 0.0001$; pretreatment × treatment interaction, $F(1, 18) = 5.3$, $p < 0.0331$; $F(1, 18) = 13.7$, $p < 0.0016$ for DOPAC and HVA, respectively].

Morphine

Morphine (3 mg/kg) increased the extracellular concentration of DA in the dialysates maximally by about 65%. This increase peaked at about 90 min after administration of morphine. The morphine-induced increase of extracellular DA concentration was significantly antagonized by naloxonazine pretreatment [Fig. 2, pretreatment effect, $F(1, 18) = 2.8$, $p < 0.1089$; treatment effect, $F(1, 18) = 26.2$, $p < 0.0001$; pretreatment × treatment interaction, $F(1, 18) = 13.2$, $p < 0.0019$].

Morphine significantly increased the extracellular concentrations of DOPAC and HVA as well [Fig. 2, treatment effect, $F(1, 18) = 42.8$, $p < 0.0001$; $F(1, 18) = 66.7$, $p < 0.0001$ for DOPAC and HVA, respectively]. The increases of extracellular DOPAC and HVA were not significantly antagonized by naloxonazine [Fig. 2, pretreatment effect, $F(1, 18) = 4.7$, $p < 0.0436$; $F(1, 18) = 2.7$, $p < 0.1153$; pretreatment × treatment interaction, $F(1, 18) = 0.4$, $p < 0.5630$; $F(1, 18) = 0.7$, $p < 0.4062$, for DOPAC and HVA, respectively].

Conditioned Place Preference

Etonitazene induced significant place preference compared with saline-treated animals [Fig. 3; $F(1, 17 = 13.65$, $p = 0.0018$, one-way ANCOVA). Etonitazene-induced place preference was significantly antagonized by naloxonazine (vehicle + etonitazene vs. naloxonazine + etonitazene $p < 0.05$, Student–Newman–Keuls test). Naloxonazine at the dose and time point used was earlier shown not to alter the place preference (19).

DISCUSSION

In the present experiments etonitazene, similarly to morphine, increased the tissue ratios of DOPAC/DA and HVA/DA in the dorsal striatum and nucleus accumbens as well as the extracellular concentrations of DA, DOPAC, and HVA in the dorsal striatum, indicating that it increased DA release and metabolism. These effects of etonitazene were antago-

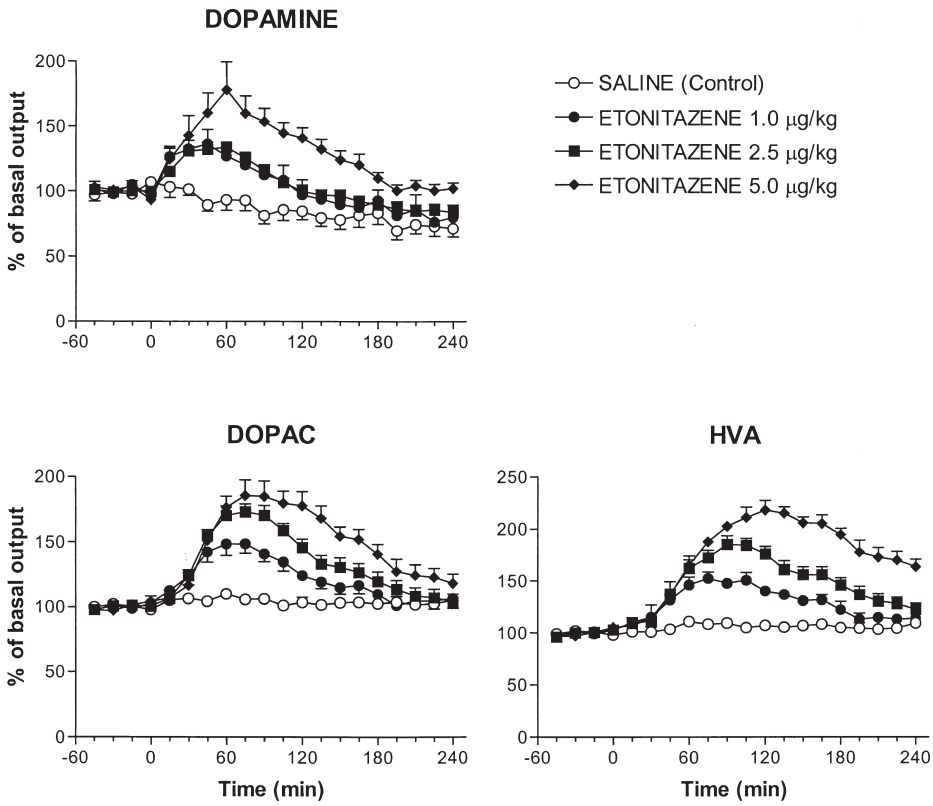

FIG. 1. Effect of etonitazene on the extracellular concentrations of dopamine, DOPAC, and HVA in the dorsal striatum of freely moving rats. Etonitazene (1, 2.5, and 5 μg/kg) or saline were administered SC at time point 0. All values are presented as mean percentages of the basal level ± SE (n = 5–6).

nized by the putative μ_1-opioid receptor antagonist, naloxonazine. Naloxonazine also antagonized the morphine-induced elevation of extracellular DA and the elevations of DOPAC/DA ratios in the dorsal striatum. Thus, our results suggest that μ_1-opioid receptors are involved in the increase of striatal DA release and metabolism induced by opioids. μ_1-Opioid receptors seem to regulate the mesolimbic dopaminergic transmission as well, because the elevation of DOPAC/DA and HVA/DA ratios induced by morphine and etonitazene in the nucleus accumbens were clearly antagonized by naloxonazine. The involvement of the μ_1-opioid receptors in the regulation of mesolimbic dopaminergic transmission is also supported by the fact that naloxonazine antagonizes the effect of heroin on DA release in the nucleus accumbens (24).

Moolten et al. (13) found that the binding of etonitazene to cerebral membranes is highly selective for μ_1-receptors compared with morphine. Thus, etonitazene's affinity for μ_1-receptor was found to be over 2600-fold higher than that of morphine, whereas etonitazene's affinity for μ_2-receptor was only 10-fold higher than that of morphine. Further, etonitazene has been found to be about 1000–2000 times more potent than morphine in behavioural tests (26). Therefore, theoretically the dose of 2.5 μg/kg of etonitazene should activate the μ_2-opioid receptors considerably less than the dose of 3 mg/kg of morphine. Indeed, we found that the selective μ_1-opioid antagonist, naloxonazine, was more effective in antagonizing the effects of etonitazene on DA metabolism and release than those of morphine. Regarding the DA metabolism in the dorsal striatum, this difference was especially striking. Thus, the elevations of the striatal extracellular concentrations of DOPAC and HVA induced by etonitazene were fully antagonized by naloxonazine, whereas the effects of morphine were not significantly antagonized. Therefore, it is tempting to speculate that both subtypes of the μ-opioid receptor are involved in the control of nigrostriatal DA transmission, and in addition to μ_1-receptors also the μ_2-opioid receptors are involved in the effect of morphine. In contrast, the effect of 2.5 μg/kg of etonitazene appears to be mediated rather selectively by the μ_1-opioid receptors. However, as there are currently no μ_2-receptor antagonists available, this hypothesis cannot be verified.

The previous studies investigating the role of μ_1-opioid receptors in the regulation of cerebral DA metabolism have provided controversial results. In accordance with our present results Latimer et al. (11) showed that naloxonazine (15 mg/kg IP 12 h before) partially antagonizes the increase of striatal and limbic DA metabolism induced by administration of the selective μ-agonist, DAMGO, in the ventral tegmental area of rats. However, Wood and Pasternak (29) and we (18) have reported that naloxonazine has no effect on the increase of DA metabolism induced by morphine. In these experiments naloxonazine was given 24 h before morphine. Therefore, it is possible that in these studies the effect of naloxonazine was weakened. Furthermore, in our previous study (18) the dose of morphine was relatively large (15 mg/kg SC), which induces maximal effects on striatal and limbic DA metabolism. It has been reported that the ability of naloxonazine to antagonize the effects of morphine decreases when the dose of morphine is increased (12).

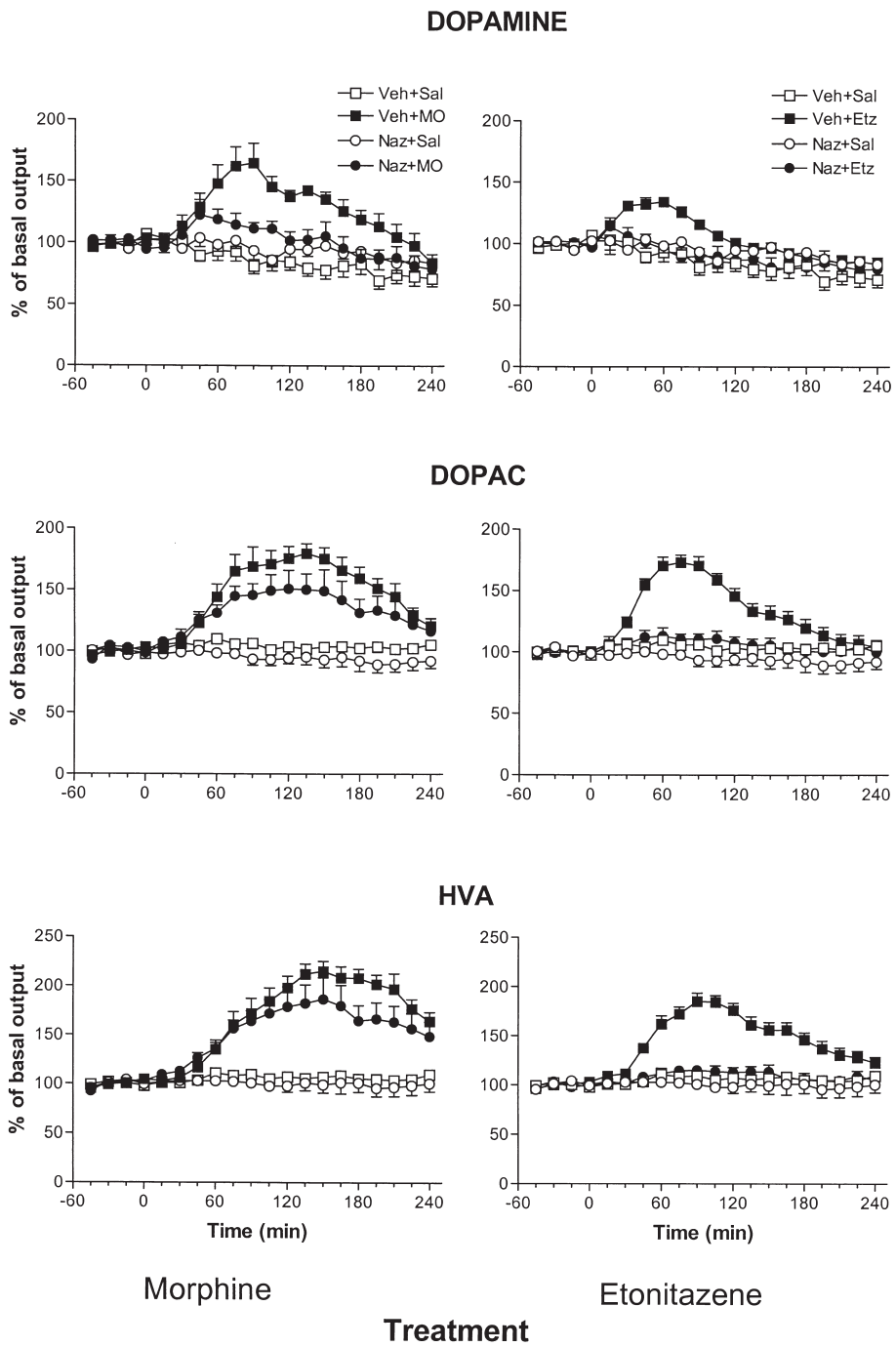

FIG. 2. Effects of morphine or etonitazene on the extracellular concentrations of dopamine, DOPAC, and HVA in the dorsal striatum of freely moving rats pretreated with naloxonazine. Morphine (MO, 3 mg/kg SC), etonitazene (ETZ, 2.5 μg/kg SC), or saline (SAL) were administered at time point 0. Naloxonazine (NAZ, 15 mg/kg) or vehicle (VEH) were administered IP 12–14 h before the agonists. All values are presented as mean percentages of the basal level ± SE (n = 6, except in the NAZ + SAL group n = 4).

Previously we showed that morphine-induced conditioned place preference is antagonized by naloxonazine, indicating that μ_1-opioid receptors have a crucial role in the rewarding properties of morphine (19). Now we found that naloxonazine completely abolishes the etonitazene-induced place preference, suggesting that rewarding properties of etonitazene are mediated by μ_1-opioid receptors as well. Thus, our results further emphasize the role of μ_1-opioid receptors in the mediation of opioid reward. As discussed in the introduction, mesolimbic DA has been proposed to be crucial in the mediation

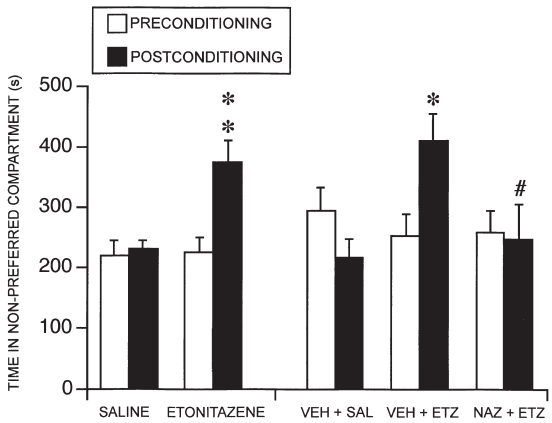

FIG. 3. The effect of pretreatment with naloxonazine (NAZ, 15 mg/kg IP 12 h) on the conditioned place preference induced by etonitazene (ETZ, 2.5 μg/kg, SC). Control rats received saline (SAL, SC) or vehicle (VEH, IP) at corresponding time points. The columns show the times (means ± SE) the rats (n = 5–10) spent in the initially non-preferred, white compartment during preconditioning (open columns) and postconditioning (filled columns). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. corresponding control group; $^{\#}p < 0.05$ vs vehicle + etonitazene group (Student–Newman–Keuls test).

of the rewarding properties of various classes of abused drugs, including opioids. The fact that naloxonazine simultaneously antagonizes the increase of mesolimbic DA transmission and the rewarding effects induced by opioids gives further support for the involvement of mesolimbic DA in the rewarding effects of opioids.

In conclusion, our results show that in addition to the mesolimbic dopaminergic system the μ_1-opioid receptors are involved in the control of nigrostriatal dopaminergic system. Etonitazene, at least in small doses, appears to be selective for the μ_1-opioid receptors. Furthermore, the effects of the etonitazene on striatal DA metabolism differ from those of morphine. In addition, our results further emphasize the role of μ_1-opioid receptors in the mediation of rewarding effects.

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