Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2046–2050, March 1992
Pharmacology

Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway

(microdialysis/nucleus accumbens/dopamine release and metabolism/opiate dependence)

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Communicated by Avram Goldstein, November 9, 1991 (received for review May 24, 1991)

ABSTRACT **The mesolimbic dopaminergic system has been implicated in mediating the motivational effects of opioids and other drugs of abuse. The site of action of opioids within this system and the role of endogenous opioid peptides in modulating dopamine activity therein remain unknown. Employing the technique of *in vivo* microdialysis and the administration of highly selective opioid ligands, the present study demonstrates the existence of tonically active and functionally opposing μ and κ opioid systems that regulate dopamine release in the nucleus accumbens, the major terminal area of A10 dopaminergic neurons. Thus, stimulation of μ-type receptors in the ventral tegmental area, the site of origin of A10 dopaminergic neurons, increases dopamine release whereas the selective blockade of this opioid receptor type results in a significant decrease in basal dopamine release. In contrast, stimulation of κ-type receptors within the nucleus accumbens decreases dopamine release whereas their selective blockade markedly increases basal dopamine release. These data show that tonic activation of μ and κ receptors is required for the maintenance of basal dopamine release in the nucleus accumbens. In view of the postulated role of the mesolimbic system in the mediation of drug-induced alterations in mood and affect, such findings may have implications for the treatment of opiate dependence and affective disorders.**

There is evidence that exogenous opioids can influence the activity of mesolimbic dopaminergic neurons and it has been postulated that such actions underlie the motivational (1, 2) and locomotor effects (3, 4) of these agents, as well as the development of various aspects of opiate dependence (5–7).

The behavioral effects of opioids differ depending on the opioid receptor type with which they interact. Thus, systemically applied μ-receptor agonists function as positive reinforcers and increase locomotor activity. In contrast, κ-receptor agonists have aversive and sedating effects (8, 9). Opposing effects of these agents are also observed at the neurochemical level within the mesolimbic dopaminergic system: μ agonists increase, whereas κ agonists decrease, dopamine release in the nucleus accumbens (10, 11), the major terminal projection site of mesolimbic dopaminergic neurons. The behavioral effects of opioids noted above are abolished following 6-hydroxydopamine lesions of the mesolimbic system or selective blockade of the dopamine receptors therein (2, 4, 9, 12, 13), suggesting that mesolimbic dopaminergic neurons are necessary for the expression of these actions. Specifically, it has been hypothesized that the opposing effects of μ and κ agonists on mesolimbic dopaminergic release underlie their different effects on motivation and motor behavior.

Neither the site of action of exogenous opioid agonists within this system in affecting dopamine release nor the role of endogenous opioid systems in regulating mesolimbic dopaminergic system activity is known. The latter issue has, until recently, been complicated by the lack of selective antagonists (14) for each of the receptor types.

The present *in vivo* microdialysis study sought to address these issues by monitoring dopaminergic transmission in the mesolimbic system following the administration of highly selective opioid ligands into either the nucleus accumbens or the ventral tegmental area (VTA).

MATERIALS AND METHODS

Animals and Surgery. Male Sprague–Dawley rats (Charles River WIGA, Sulzfeld, F.R.G.) weighing 250–270 g were housed individually in plastic cages in a climate-controlled colony room. Animals were maintained on a 12 hr light/dark cycle with food and water freely available.

Guide cannulae (Carnegie Medicine, Stockholm, Sweden) for microdialysis were implanted under anesthesia into the nucleus accumbens (relative to interaural: A, 9.9; L, ±1.4; V, −6.0) and for microinjections in the VTA (relative to interaural: A, 3.8; L, ±1.0; V, −7.5) (15).

Brain Dialysis and Opioid Administration. Perfusion experiments commenced 1 week after surgery. Animals were anesthetized with halothane and core body temperature was maintained at 37°C by using a thermoregulated heating pad in conjunction with a rectal probe. The microdialysis probe (Carnegie Medicine; 2-mm membrane length), which was connected to a Carnegie microliter syringe pump, was inserted through the guide cannula. The dialysis tube was perfused with Ringer solution [containing 147 mM Na^+, 2.25 mM Ca^{2+}, 4 mM K^+, and 155.6 mM Cl^- (pH 7.0)] at a constant flow rate of 2.4 μl/min and perfusates were collected every 20 min. Once monoamine levels in the perfusates had stabilized (80–120 min), four consecutive samples were collected for determination of basal levels of dopamine and its metabolites. Opioids were then dissolved in Ringer solution and either infused via the probe into the nucleus accumbens (20-min infusion) by means of a liquid switch (Carnegie Medicine) or injected in the VTA.

The microinjections into the VTA were made by inserting a 33-gauge injection cannula connected via polyethylene tubing to a microinfusion pump. The infusion volume (0.3 μl) was delivered over a 15-sec period and the injection needle was left in place for an additional 60 sec to ensure complete delivery.

For the VTA injections opioids were dissolved in both Ringer solution and sterile water. However, due to the effects of Ringer solution injected by itself (see *Results*), sterile water was used as the vehicle for all dose–response testing.

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Abbreviations: CTOP, D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; DAGO, [D-Ala^2,*N*-methyl-Phe^4,Gly^5-ol]enkephalin; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; nor-BNI, norbinaltorphimine; VTA, ventral tegmental area.

***In Vitro* Recovery Test.** The effective concentrations of the drugs applied during infusions of nucleus accumbens were estimated by a modified *in vitro* recovery test. To estimate the recovery of the tested opioids through the dialysis membrane, probes were perfused *in vitro* (2.4 μl/min) and placed in a vial containing 50 μl of Ringer solution at 37°C. The perfusion liquid contained the desired concentrations of opioids to be infused in the *in vivo* experiments. After 20 min of perfusion the external Ringer solution was injected into an HPLC system with UV detection. The HPLC system (Beckman; System Gold) was equipped with a stainless steel column (4 × 150 mm) packed with Sperisorb 5-μm ODS (Bischoff, Leonberg, F.R.G.) and opioids were separated by a linear gradient (solvent A, 0.05 M NaH_2PO_4; solvent B, 60% CH_3CN in A; 0–100% in 40 min; flow rate, 1.0 ml/min). The amount of opioid in the perfusion liquid was compared with the amount outside the dialysis probe and expressed as percent recovery (16).

Analytical Procedure. Samples collected from the probe were immediately run on a reverse-phase ion-pair HPLC system equipped with a stainless steel column (4 × 150 mm) packed with Sperisorb 5-μm ODS. Dopamine, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were then analyzed by two electrochemical detection systems (Gynkothek, München, F.R.G., and ESA, Leonberg, F.R.G.). The analytical procedure used has been described (17).

Statistical Analysis. Only data from animals with histologically correct cannula placements were used for subsequent statistical analysis. Data were transformed by the arc tangent transformation prior to analysis and are expressed as percentages of basal values (means ± SEM). Dose–response curves were analyzed by a one-way random-effects-model factorial analysis of variance. A one-way analysis of variance and the Newman–Keuls post-comparison test were used to compare monoamine levels in drug-treated and control (sterile water injection) groups.

Drugs. The μ-receptor antagonist was D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP; Peninsula Laboratories), where Pen is penicillamine and Orn is ornithine. The μ-receptor agonist was [D-Ala^2,*N*-methyl-Phe^4,Gly^5-ol]enkephalin (DAGO; Bachem). The κ-receptor antagonist was norbinaltorphimine (nor-BNI; synthesized and generously provided by A. W. Lipkowski, Minneapolis). The κ-receptor agonist was (5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)-dec-8-yl]benzeneacetamide; (U-69593; Upjohn). Drugs were dissolved in both sterile water and Ringer solution for VTA injections and in Ringer solution for nucleus accumbens infusions. When appropriate, the pH was adjusted to 7.

RESULTS

***In Vitro* Recovery.** *In vitro* recovery values were as follows: CTOP, 16 ± 2%; DAGO, 12 ± 2%; nor-BNI, 10 ± 1%; and U-69593, 16 ± 2%; *n* = 3 for each substance. The nucleus accumbens infusion concentrations given in the text have been corrected on the basis of recovery values estimated *in vitro*. The *in vitro* recovery values obtained are valid for *in vivo* conditions because the drugs diffuse from an aqueous solution to the extracellular fluid during infusion of the nucleus accumbens (18).

Effects of μ-Receptor Ligands on the Mesolimbic Dopaminergic System. In the VTA, 9 of 10 animals, and in the nucleus accumbens, all animals, showed histologically correct injection cannula placements. The control group, which received sterile water into the VTA, showed no changes in basal release of dopamine (see Fig. 3*A*). The mean basal level of dopamine in the nucleus accumbens was 0.19 ± 0.02 pmol per 20-min sample. The administration of the selective μ agonist DAGO into the VTA, the site of origin of A10 dopaminergic neurons, resulted in a significant enhancement of dopamine release in the nucleus accumbens (Fig. 1*A*). The magnitude of this effect was linearly related to dose [$F(2, 139) = 8.1$; $P < 0.001$] with significant stimulation of dopamine release occurring at doses of 0.2 and 1.0 nmol ($n = 6$; $P < 0.05$). The output of DOPAC and HVA was also dose-dependently enhanced. These effects were of later onset and longer duration than the effect on dopamine release (data not shown). However, infusions of DAGO (0.1–1 nmol) into the terminal field of the mesolimbic pathway, the nucleus accumbens, failed to affect dopamine release or metabolite levels. Thus, no dose of DAGO {maximal effect: 0.1 nmol, 107 ± 14%, *n* = 3; 0.2 nmol, 112 ± 16%, *n* = 3; 1.0 nmol, 110 ± 9%; *n* = 5 [$F(2, 82) = 0.39$; $P < 0.5$]} significantly increased dopamine release above basal levels.

Microinjections of the highly selective μ antagonist CTOP (19) into the VTA resulted in dose-dependent decreases in basal release of dopamine [$F(2, 147) = 5.4$; $P < 0.005$) (Fig. 1*B*). This effect was significant at doses of 1 and 3 nmol. The

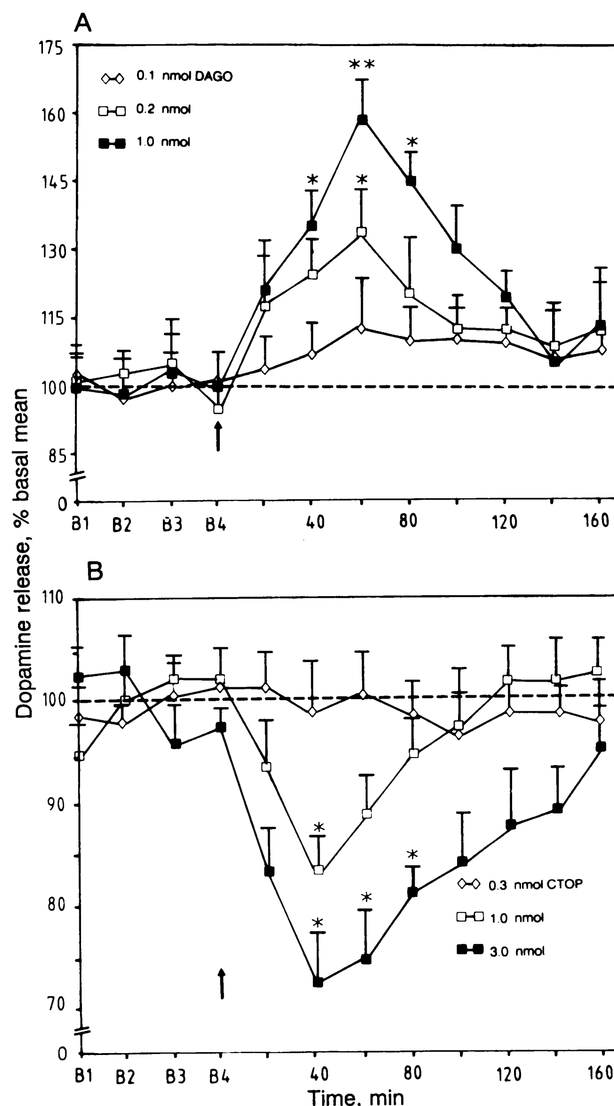

FIG. 1. Effect of VTA injections of the μ agonist DAGO (0.1, 0.2, and 1.0 nmol, dissolved in sterile water) (*A*) and the μ antagonist CTOP (0.3, 1.0, and 3.0 nmol) (*B*) on dopamine release in the nucleus accumbens. Data are expressed as percentages (mean ± SEM) of four predrug dialysates (B1–B4). Arrow indicates injection time. Significant difference from control values: *, $P < 0.05$; **, $P < 0.01$; $n = 5$–8.

maximal decrease in dopamine overflow (73 ± 5%, $n = 8$; $P < 0.05$) was observed 40 min after administration of 3.0 nmol of CTOP. CTOP produced slight but nonsignificant decreases in DOPAC and HVA. In contrast, infusions of CTOP (0.3–3.0 nmol) into the nucleus accumbens did not alter basal levels of dopamine or its metabolites {maximal effect on dopamine release: 0.3 nmol, 97 ± 7%, $n = 4$; 1.0 nmol, 87 ± 8%; $n = 4$; 3.0 nmol, 91 ± 9%; $n = 5$ [$F(2, 93) = 0.59$; $P < 0.5$]}.

Injection of Ringer solution into the VTA produced a marked increase in dopamine release compared with both basal release and that observed in response to sterile water injection (Fig. 2*A*). Under these conditions DAGO also increased dopamine release, and this effect was significantly greater than that produced by Ringer solution alone (Fig. 2*A*). In contrast, injection of CTOP resulted in a significant decrease in dopamine release compared with the Ringer control group (Fig. 2*A*).

Effect of κ-Receptor Ligands on the Mesolimbic Dopaminergic System. The κ-agonist U-69593 decreased dopamine overflow when administered via the microdialysis probe into the nucleus accumbens. A one-way random-effects-model analysis of variance revealed that this effect followed a biphasic function of dose [$F(1, 146) = 7.4$; $P < 0.001$] (Fig. 3*A*). Significant decreases were observed at doses of 2 and 10 nmol. In contrast, administration of 20 nmol of U-69593 did not significantly reduce dopamine release or metabolite levels. The maximal decrease in dopamine (52 ± 4%, $n = 6$; $P < 0.05$) occurred 40 min after the administration of 10 nmol of U-69593. Doses of 2 and 10 nmol produced significant decreases in DOPAC and HVA levels as well; however, these effects were less pronounced than the effect on dopamine (data not shown). In contrast to the nucleus accumbens, microinjections of U-69593 (2–20 nmol) into the VTA failed to affect dopamine release and metabolism {maximal effect on dopamine release: 2 nmol, 88 ± 11%, $n = 3$; 10 nmol, 89

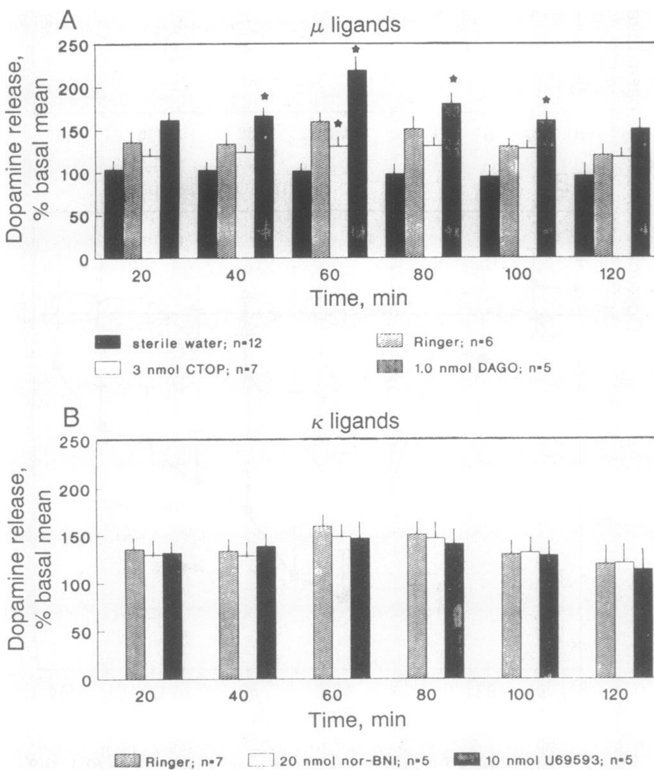

FIG. 2. Dopamine release in response to VTA injection of opioid agonists and antagonists. Bars represent percentage of basal mean levels ± SEM. In these experiments all drugs were dissolved in Ringer solution. Star denotes a significant difference of an opioid treatment relative to the Ringer control group.

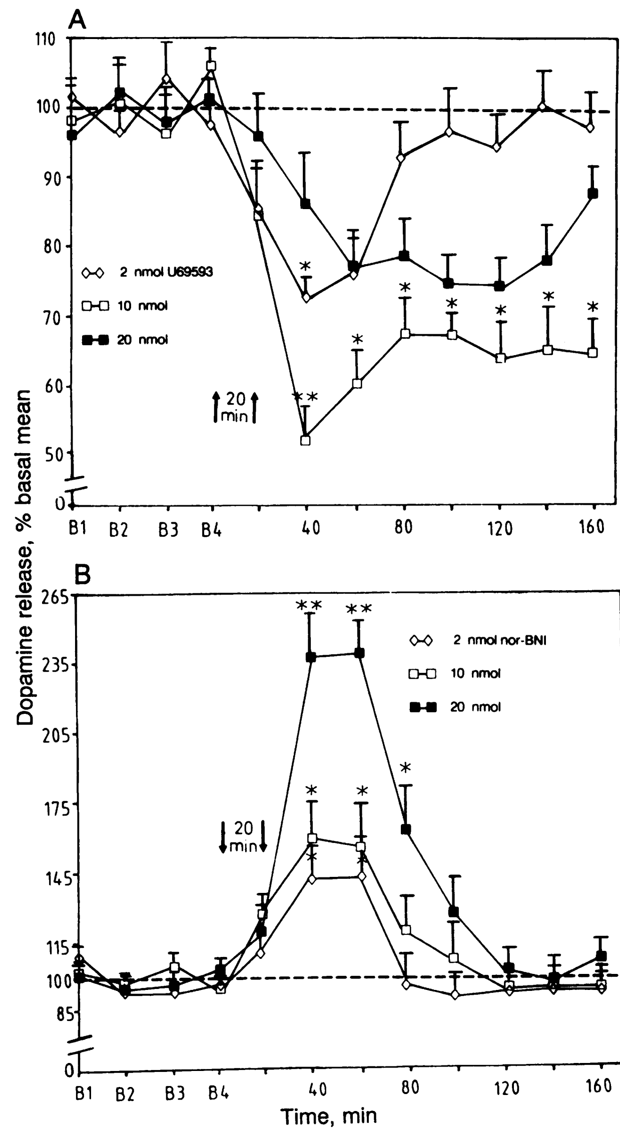

FIG. 3. Effect of nucleus accumbens infusions of the κ agonist U-69593 (2, 10, and 20 nmol) (*A*) and the κ antagonist nor-BNI (2, 10, and 20 nmol) (*B*) on dopamine release in the nucleus accumbens. Data are expressed as percentages (mean ± SEM) of four predrug dialysates (B1–B4). Significant difference from control values: *, $P < 0.05$; **, $P < 0.01$; $n = 5$–8.

± 16%, $n = 4$; 20 nmol, 95 ± 6%; $n = 4$ [$F(2, 81) = 0.87$; $P < 0.5$]}.

Similarly, when U-69593 was administered in Ringer solution, dopamine release did not differ from that observed in the Ringer control group (Fig. 2*B*).

Administration of nor-BNI, a selective κ antagonist (20), into the nucleus accumbens resulted in a dose-related increase in dopamine release [$F(2, 129) = 18.7$; $P < 0.0001$] (Fig. 3*B*). Significant increases in dopamine release were evident at doses of 2, 10, and 20 nmol and occurred 40–60 min after infusion of nor-BNI. The output of DOPAC and HVA was only slightly enhanced and these effects were of later onset than the effect on dopamine. Nor-BNI (2–20 nmol) did not modify dopamine release and metabolism when injected into the VTA {maximal effect on dopamine release: 2 nmol, 109 ± 7%, $n = 3$; 10 nmol, 116 ± 14%, $n = 4$; 20 nmol, 114 ± 11%, $n = 5$ [$F(2, 89) = 0.49$; $P < 0.5$]}.

Thus, regardless of whether the vehicle for this drug was water or Ringer, no significant alteration in dopamine release was seen (Fig. 2*B*).

DISCUSSION

The results show that the μ-opioid receptor agonist DAGO and the μ-receptor antagonist CTOP exert marked effects on mesolimbic dopamine release following their injection into the VTA. Thus, DAGO produced a significant dose-related increase in dopamine release in the nucleus accumbens, whereas CTOP produced a significant decrease in dopamine release. In contrast, administration of either ligand into the nucleus accumbens was without effect. In view of the receptor selectivity of these ligands, we conclude that the observed effects of these agents on dopamine release result from, respectively, the activation and inactivation of μ receptors.

The κ-opioid receptor ligands failed to modify dopamine release following their injection into the VTA. However, a marked inhibition of dopamine release was observed in response to infusion of the κ agonist U-69593 into the nucleus accumbens. The highest dose of U-69593 was less effective in modifying dopamine release than the lower doses. A similar dose–response curve was observed following the intracerebroventricular administration of other κ agonists (11). Interestingly, the aversive effects of U-69593 as well as other κ agonists follow a biphasic function of dose (8, 21) and doses producing this motivational effect are those which result in a significant decrease in dopamine release. Indeed, evidence that a central and mesolimbic dopamine-dependent action of κ agonists contributes to their aversive effects has been presented (9, 13, 21). In contrast to U-69593, the selective κ antagonist nor-BNI increased dopamine release. Thus, these data demonstrate opposing and anatomically distinct effects of opioids on dopamine release in the nucleus accumbens. Further, they provide neurochemical evidence for the existence of opposing tonically active μ and κ opioid systems that regulate mesolimbic dopamine release.

The postulated role of the VTA versus the nucleus accumbens in mediating the effects of μ and κ opioids, respectively, receives additional support from previous studies. Autoradiographic studies indicate dense binding of ^{125}I-DAGO in the VTA (22, 23). In contrast, only very low κ binding density can be detected within the VTA (22, 24), an area that receives little dynorphinergic innervation (25). Electrophysiological data provide some evidence for an action of μ agonists on A10 dopaminergic neurons within the VTA, whereas κ agonists are devoid of action in this region (26, 27). On the other hand, high κ binding is evident in the medial portion of the nucleus accumbens (22, 24), a region that is also densely innervated by dynorphin-containing fibers (25). The nucleus accumbens shows only moderate binding of ^{125}I-DAGO (22, 23), and it appears that μ receptors in the nucleus accumbens may be located postsynaptically, since neither 6-hydroxydopamine nor quinolinic acid lesions of the nucleus accumbens reduce binding of ^{125}I-DAGO (23). Furthermore, *in vitro* studies have demonstrated that κ agonists decrease the spontaneous release of newly synthesized [^{3}H]dopamine from slices of the nucleus accumbens, whereas μ agonists are inactive (28, 29).

The administration of CTOP (19), a highly selective μ antagonist, into the VTA decreased dopamine release in the nucleus accumbens. This suggests that there is a tonically active μ opioidergic system in the VTA that increases basal dopamine release. The blockade of VTA μ receptors would thus lead to an attenuation of dopamine release. The blockade of κ receptors in the nucleus accumbens by the highly selective κ antagonist nor-BNI (20, 30) leads to an enhancement of dopamine release, suggesting the existence of an inhibitory κ opioidergic system that is active in the nucleus accumbens.

The concept of tonically active endogenous opioid systems is in line with other data regarding the motivational effects of selective μ-receptor antagonists. In particular, the finding that these agents induce aversive effects in naive animals suggests that the blockade of a tonically active μ opioid system underlies such effects (2). Furthermore, destruction of the mediobasal arcuate nucleus (31), the primary site of β-endorphin synthesis in the brain (32), attenuates the aversive effects of naloxone, suggesting that this tonically active μ-opioid system may in fact be β-endorphinergic. Chronic naloxone treatment, which results in opioid-receptor upregulation (33), augments morphine-induced changes in monoamine metabolism in several limbic brain areas (34), again suggesting that endogenous opioid systems can modulate monoaminergic neurotransmission.

In summary, A10 dopaminergic neurons projecting to the nucleus accumbens are modulated by two opposing opioid systems that are differentially located within the mesolimbic system. Fig. 4 depicts a model derived from the present data. In this model, the activation of μ receptors located on inhibitory neurons that are presumably γ-aminobutyratergic (35) leads to a disinhibition of A10 dopaminergic neurons projecting to the nucleus accumbens and an increase in dopamine release. In contrast, the activation of κ receptors by endogenous ligands, probably dynorphin (24, 26), located presynaptically in the nucleus accumbens inhibits dopamine release. The concerted action of the two systems enables the maintenance of basal dopamine release in the nucleus accumbens. This model may provide a basic neurochemical framework for understanding the mechanisms of opiate dependence. A recent study demonstrated a long-lasting reduction of dopamine release in the mesolimbic system after morphine withdrawal (7). Thus, after prolonged μ-agonist administration, there may be a compensatory increase in the activity of the functionally opposing dynorphin system located within the nucleus accumbens and/or a hypoactive endogenous μ system. Either effect may play a role in the genesis of tolerance and/or dependence.

Finally, it is important to note that the dose–effect curves for intra-VTA-applied opioids were generated using sterile water as the vehicle, since the intra-VTA injection of Ringer solution resulted in a marked increase in basal dopamine release. Although an explanation for such an effect of Ringer solution by itself is lacking, it may be that the presence of Ca^{2+} and/or K^+ in this vehicle was sufficient to stimulate the firing of VTA dopaminergic neurons (36). However, when Ringer solution was used as the vehicle, a similar effect of opioids on dopamine release was observed. Thus, the intra-VTA application of DAGO significantly increased dopamine release as compared with the injection of Ringer solution, whereas CTOP decreased dopamine release. In contrast, κ ligands were ineffective. Therefore, the differential effects of μ and κ ligands in the VTA as compared with the nucleus accumbens cannot be attributed to differences in the injection

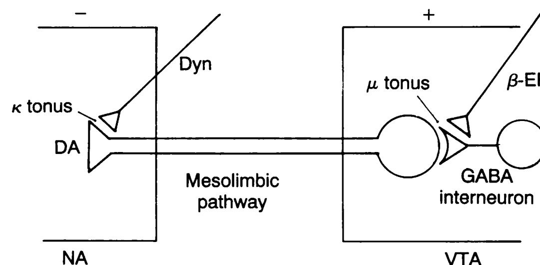

FIG. 4. Model for the modulation of A10 neurons by opposing tonically active endogenous opioid systems. In the VTA the A10 neurons are stimulated by a tonically active μ system (β-EP, β-endorphin) via disinhibition of a γ-aminobutyrate (GABA)-containing interneuron (35). In the nucleus accumbens (NA) the release of dopamine (DA) by A10 neurons is suppressed by a tonically inhibitory κ system (Dyn, dynorphin). The action of both opioid systems is necessary for the maintenance of basal dopamine release.

procedures. Rather, they demonstrate that with regard to both endogenous and exogenous opioids, two distinct sites of action underlie the effects of μ- and κ-receptor ligands. Although additional studies, employing different experimental conditions, will be necessary to determine the generality of such findings, it is interesting that over a decade ago, the suggestion was put forth that a deficiency in an endogenous opioid peptide such as β-endorphin may predispose opioid-seeking behavior in certain individuals (37). In view of the present results, it is tempting to speculate that individual differences in sensitivity to the reinforcing effects of opioids may result, at least in part, from either a decrease in the activity of an endogenous opioid reward pathway (e.g., β-endorphin) or an increased activity of κ opioidergic systems.

This research was supported by the Bundesgesundheitsamt Berlin, F.R.G.

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