The Journal of Neuroscience, May 15, 1999, 19(10):4123–4131

Opposing Role of Dopamine D1 and D2 Receptors in Modulation of Rat Nucleus Accumbens Noradrenaline Release

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The role of dopamine receptors in the modulation of nucleus accumbens noradrenaline release was investigated in superfused rat brain slices. At concentrations of ≤1 μM, dopamine enhanced, whereas at higher concentrations dopamine inhibited electrically evoked [^{3}H]noradrenaline release. The D1 receptor agonist SKF-38393 increased, whereas the D2 agonist quinpirole inhibited evoked [^{3}H]noradrenaline release. These effects were attenuated by the D1 antagonist SCH-23390 and the D2 antagonist (−)-sulpiride, respectively, indicating that accumbens noradrenaline release is regulated by stimulatory D1 and inhibitory D2 receptors. Whereas (−)-sulpiride enhanced, SCH-23390 did not reduce evoked accumbens [^{3}H]noradrenaline release, indicating a tonic activation of D2 receptors only. Given the similar apparent affinity of dopamine for D1 and D2 receptors in striatal slices, the lack of tonic D1 receptor activation suggests that D1, unlike D2, receptors are extrasynaptically localized. No dopaminergic modulation of noradrenaline release was observed in rat medial prefrontal cortex or amygdala slices. To examine the regulation of accumbens noradrenaline release under conditions of increased dopaminergic activity, measurements were made using slices of amphetamine-pretreated rats. In these slices, the electrically evoked release of [^{3}H]dopamine and [^{3}H]noradrenaline was enhanced. The increasing effect of (−)-sulpiride on noradrenaline release was augmented, and SCH-23390 almost completely reversed this enhancement of [^{3}H]noradrenaline release. These data suggest that whereas although under a moderate dopaminergic tone, accumbens noradrenaline release is mainly regulated by inhibitory D2 receptors, under circumstances of increased dopaminergic activity, recruitment of extrasynaptic stimulatory D1 receptors contributes to enhancement of noradrenaline release.

Key words: noradrenaline release; nucleus accumbens; dopamine release; dopamine D1 receptor; dopamine D2 receptor; amphetamine

Because of extensive and reciprocal connections with limbic and motor systems, the nucleus accumbens (NAcc) is thought to be important for the generation of motor responses to emotionally relevant environmental stimuli (Mogenson, 1987; Kalivas et al., 1993). The dopaminergic projection from the ventral tegmental area to the NAcc, part of the so-called mesolimbic dopamine (DA) system, has received particular attention in this respect. For instance, NAcc DA neurotransmission has been shown to be involved in exploratory behavior, in the psychomotor and reinforcing effects of drugs of abuse, and in appetitive and preparatory behaviors. This has led to the general assumption that the mesolimbic DA system plays a key role in goal-directed and motivational behavior (Le Moal and Simon, 1991; Phillips et al., 1991; Koob, 1992; Amalric and Koob, 1993; Salamone, 1994; Schultz et al., 1997).

Interactions between the various inputs into the NAcc can be expected to serve to optimize information flow necessary for the generation of adaptive motor responses. In this respect, it has been shown recently that the shell portion of the NAcc receives a dense noradrenaline (NA)-containing projection, originating primarily in the nucleus tractus solitarius (NTS) (Berridge et al., 1997; Delfs et al., 1998). Because there is very little information on the possible interaction between NAcc NA and DA systems (Nurse et al., 1984; Yavich et al., 1997), we investigated here the role of DA receptor stimulation on electrically evoked NA release from rat NAcc slices *in vitro*.

Extracellular concentrations of NAcc DA and NA are enhanced by systemically and locally applied psychostimulant drugs, such as amphetamine and cocaine (Di Chiara and Imperato, 1988; Seiden et al., 1993; McKittrick and Abercrombie, 1997; Reith et al., 1997), and psychostimulant-induced locomotion is known to rely on increases in NAcc DA neurotransmission (Kelly et al., 1975; Pijnenburg et al., 1975; Delfs et al., 1990; Amalric and Koob, 1993). In addition, involvement of NA in the psychomotor effects of amphetamine and cocaine has also been demonstrated (Snoddy and Tessel, 1985; Dickinson et al., 1988; Harris et al., 1996). With regard to repeated exposure to psychostimulants, there is ample evidence that this causes NAcc DA nerve terminals to become hypersensitive (Kalivas and Stewart, 1991; Nestby et al., 1997; Pierce and Kalivas, 1997). If NAcc NA neurotransmission is modulated by DA, this regulation might be altered as a result of psychostimulant-induced increase in DA tone. Therefore, we also investigated the effects of DA receptor activation on NA release in NAcc slices of rats repeatedly treated with amphetamine. This is of particular interest given the notion that the neuroadaptations occurring after repeated psychostimulant expo-

Received Nov. 23, 1998; revised Feb. 22, 1999; accepted March 2, 1999.

This work was supported by the Netherlands Organization for Scientific Research (NWO) Grant 903–42-007.

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sure are involved in drug-induced addiction and psychosis (Robinson and Becker, 1986; Robinson and Berridge, 1993).

MATERIALS AND METHODS

Animals and drug pretreatments. All experiments were approved by the Animal Care Committee of the Free University of Amsterdam. Male Wistar rats (Harlan CPB, Zeist, The Netherlands), weighing 180–200 gm at the time of arrival in the laboratory, were housed two per cage in Macrolon cages under controlled conditions (lights on from 7:00 A.M. to 7:00 P.M.) for 1 week before use. Food and water were available *ad libitum*. Animals receiving drug pretreatment were briefly handled on the 2 d preceding the beginning of treatment. Pretreatment consisted of intraperitoneal injections with 2.5 mg/kg (+)-amphetamine or saline, administered once daily on 5 consecutive days. Three days after the last injection, the animals were killed, and neurotransmitter release was determined as described below.

Determination of neurotransmitter release. Rats were decapitated, their brains were rapidly removed, and NAcc (including core and shell), medial prefrontal cortex, or amygdala were dissected from a 1-mm-thick coronal slice using the atlas of Paxinos and Watson (1986). Slices (0.3 × 0.3 × 1 mm) were prepared using a McIlwain tissue chopper and incubated and superfused as described previously (Schoffelmeer et al., 1994). Briefly, slices were washed twice with Krebs'–Ringer's bicarbonate medium containing (in mM) 121 NaCl, 1.87 KCl, 1.17 KH_2PO_4, 1.17 $MgSO_4$, 1.22 $CaCl_2$, 25 $NaHCO_3$, and 10 D-(+)-glucose and subsequently incubated for 15 min in this medium under a constant atmosphere of 95% O_2–5% CO_2 at 37°C. After preincubation, the slices were rapidly washed and incubated for 15 min in 2.5 ml of medium containing 5 μCi of [^{3}H]NA or, in one set of experiments, 5 μCi of [^{3}H]DA under an atmosphere of 95% O_2–5% CO_2 at 37°C. Because the brain areas investigated have both dense dopaminergic and noradrenergic innervations, 1 μM GBR-12909 was added to the medium during incubation to prevent accumulation of [^{3}H]NA in DA nerve terminals, or 3 μM desipramine was added during incubation to prevent accumulation of [^{3}H]DA in NA nerve terminals. After labeling, the slices were rapidly washed and transferred to each of 24 chambers of a superfusion apparatus (~4 mg of tissue in 0.2 ml of volume) and superfused (0.20 ml/min) with medium gassed with 95% O_2–5% CO_2 at 37°C. The superfusate was collected as 10 min samples after 40 min of superfusion (t = 40 min). Ca^{2+}-dependent neurotransmitter release was induced during superfusion by exposing the slices to electrical biphasic block pulses (1 Hz, 10 mA, 2 msec pulses to evoke release of [^{3}H]NA, and 1 Hz, 30 mA, 2 msec pulses to evoke release of [^{3}H]DA) for 10 min at t = 50 min (electrical field stimulation). (−)-Sulpiride or SCH-23390 were added 30 min before, and DA, SKF-38393, quinpirole, or N^6-cyclopentyladenosine (CPA) were added 20 min before electrical field stimulation. In the experiments investigating the effects of DA on [^{3}H]NA release, 3 μM desipramine was present during superfusion to prevent uptake of DA into noradrenergic nerve terminals. Drugs remained present until the end of the experiment. In each experiment, quadruplicate observations were made.

Calculation of release data. The radioactivity remaining at the end of the experiment was extracted from the tissue with 0.1N HCl. The radioactivity in superfusion fractions and tissue extracts was determined by liquid scintillation counting. The efflux of radioactivity during each collection period was expressed as a percentage of the amount of radioactivity in the slices at the beginning of the respective collection period. The electrically evoked release of neurotransmitter was calculated by subtracting the spontaneous efflux of radioactivity from the total overflow of radioactivity during stimulation and the next 10 min. A linear decline from the 10 min interval before to the 20–30 min after the start of stimulation was assumed for calculation of the spontaneous efflux of radioactivity. The release evoked was expressed as percentage of the content of radioactivity of the slices at the start of the stimulation period. Effects of drugs were calculated as percentages of control and analyzed using one-way ANOVA and, where appropriate, followed by Student–Newman–Keuls tests. Curve fitting was done by nonlinear regression analysis.

Radiochemicals and drugs. [^{3}H]Noradrenaline (39 Ci/mmol) and [^{3}H]dopamine (47 Ci/mmol) were purchased from the Radiochemical Center (Amersham, Buckinghamshire, UK). DA and (−)-sulpiride were purchased from Sigma (St. Louis, MO), and SKF-38393, SCH-23390, quinpirole, GBR-12909, and CPA were purchased from Research Biochemicals (Natick, MA). Desipramine was a gift from Ciba-Geigy (Basel, Switzerland). (+)-Amphetamine-sulfate was purchased from O.P.G. (Utrecht, The Netherlands) and dissolved in sterile saline.

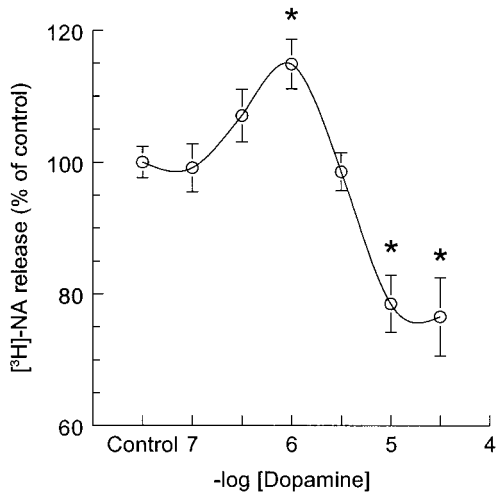

Figure 1. Effect of DA on the electrically evoked release of [^{3}H]NA from superfused slices of rat NAcc. The slices were superfused in the presence of 3 μM desipramine to prevent uptake of DA into noradrenergic nerve terminals and were stimulated electrically at t = 50 min for 10 min. DA was added to the superfusion medium 20 min before depolarization. Control [^{3}H]NA release, in the presence of 3 μM desipramine, amounted to 5.8 ± 0.4%. The data, expressed as percent of control release, represent means ± SEM of 24 observations. $^*p < 0.05$ compared with control values (Student–Newman–Keuls test).

RESULTS

DA modulates NAcc NA release through stimulatory D1 and inhibitory D2 receptors

DA had a biphasic effect on the electrically evoked [^{3}H]NA release ($F_{(6,142)} = 10.78$; $p < 0.001$). A concentration of 0.3 μM slightly increased and 1 μM significantly increased the evoked release of [^{3}H]NA from superfused rat NAcc slices by ~15%. At a concentration of 3 μM, DA had no effect on [^{3}H]NA release, and at 10 and 30 μM, DA appeared to suppress the electrically evoked release of [^{3}H]NA by 20–25% (Fig. 1).

To investigate the contribution of D1 and D2 receptors to the effects of DA on [^{3}H]NA release, selective D1 and D2 agonists and antagonists were applied. The DA D1 agonist SKF-38393 dose-dependently increased electrically evoked [^{3}H]NA release ($F_{(5,46)} = 7.06$; $p < 0.0001$). The maximal effective concentration of SKF-38393 (1 μM) caused an increase of [^{3}H]NA release of ~35% above control (Fig. 2*A*). In contrast, the evoked release of [^{3}H]NA was dose-dependently inhibited by the D2 agonist quinpirole ($F_{(5,63)} = 14.52$; $p < 0.0001$); a 40% inhibition was observed at a concentration of 1 μM quinpirole (Fig. 2*B*). When the effects of the D1 and D2 antagonists SCH-23390 and (−)-sulpiride, respectively, were tested, it appeared that 0.3 μM SCH-23390 tended to increase [^{3}H]NA release by ~10% ($F_{(1,53)} = 3.74$; $p = 0.06$) (Fig. 2*C*). (−)-Sulpiride potently increased evoked [^{3}H]NA release, with a 65% increase above control values observed with 1 μM (−)-sulpiride ($F_{(1,39)} = 109.00$; $p < 0.0001$) (Fig. 2*C*).

Consistent with the previous experiment, 1 μM SKF-38393 increased electrically evoked [^{3}H]NA release by 33%, whereas 1 μM quinpirole suppressed it by 32% (Fig. 3, *left*). In the presence of 0.3 μM SCH-23390, the increasing effect of SKF-38393 on [^{3}H]NA release was significantly attenuated, from 33% in the absence of to 10% in the presence of respective control values of

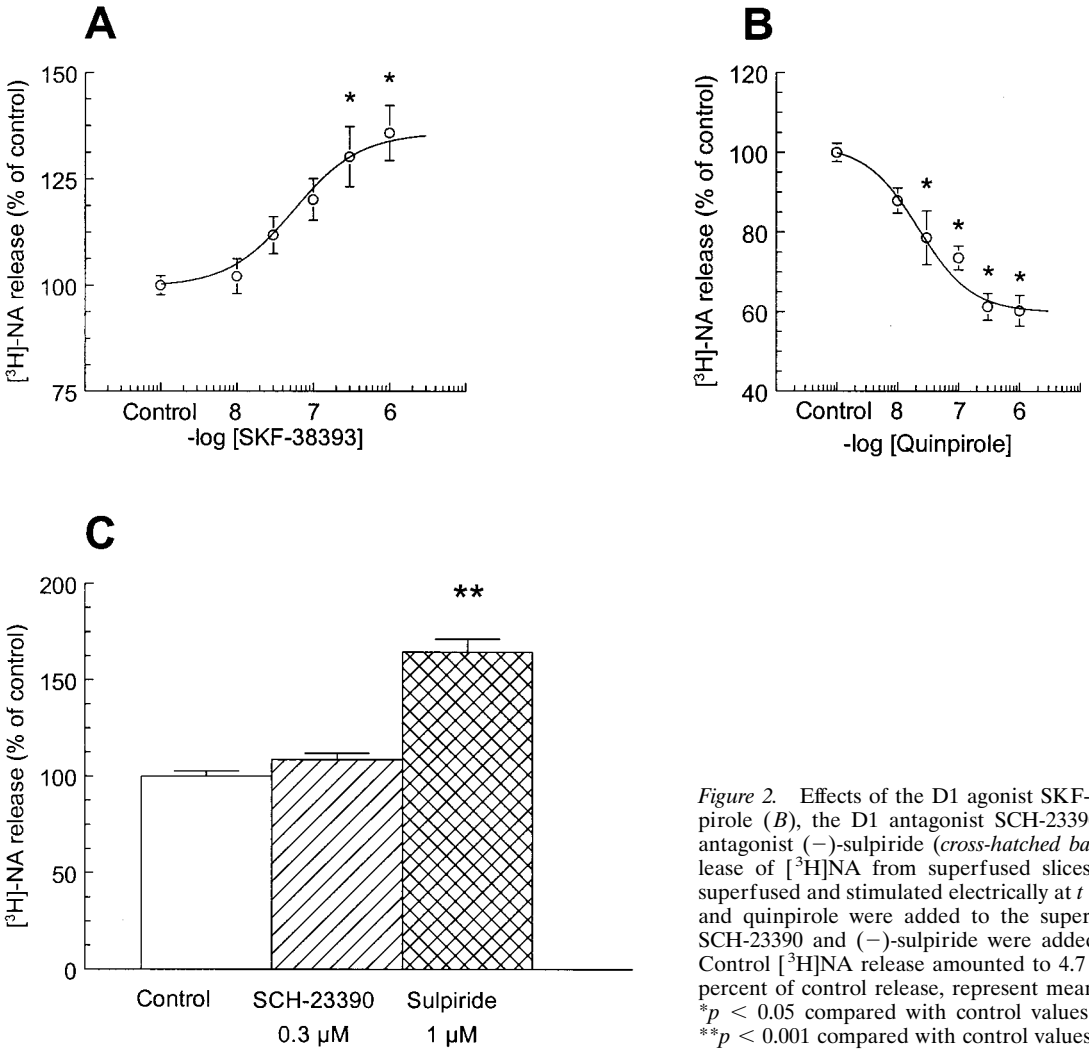

Figure 2. Effects of the D1 agonist SKF-38393 (*A*), the D2 agonist quinpirole (*B*), the D1 antagonist SCH-23390 (*C*; *hatched bar*), and the D2 antagonist (−)-sulpiride (*cross-hatched bar*) on the electrically evoked release of [^{3}H]NA from superfused slices of rat NAcc. The slices were superfused and stimulated electrically at $t = 50$ min for 10 min. SKF-38393 and quinpirole were added to the superfusion medium at 20 min, and SCH-23390 and (−)-sulpiride were added 30 min before depolarization. Control [^{3}H]NA release amounted to 4.7 ± 0.3%. The data, expressed as percent of control release, represent means ± SEM of 8–28 observations. $^*p < 0.05$ compared with control values (Student–Newman–Keuls test); $^{**}p < 0.001$ compared with control values (ANOVA).

SCH-23390 ($F_{(1,37)} = 15.53$; $p < 0.001$) (Fig. 3, *middle*). In contrast, 0.3 μM SCH-23390 did not at all affect the effect of quinpirole on electrically evoked [^{3}H]NA release; in both the absence and presence of SCH-23390, 1 μM quinpirole inhibited [^{3}H]NA release by 32% ($F_{(1,35)} = 0.02$; NS) (Fig. 3, *middle*). Exactly the opposite effect was found with (−)-sulpiride. In a concentration of 1 μM, (−)-sulpiride significantly antagonized the inhibitory effect of quinpirole on evoked [^{3}H]NA release; the decrease in [^{3}H]NA release induced by quinpirole was 32% in the absence of and 11% in the presence of (−)-sulpiride ($F_{(1,26)} = 11.65$; $p < 0.01$) (Fig. 3, *right*). In contrast, the increase in [^{3}H]NA release induced by SKF-38393 was not affected by (−)-sulpiride ($F_{(1,27)} = 0.28$; NS) (Fig. 3, *right*).

In the presence of 1 μM (−)-sulpiride, DA potently increased electrically evoked [^{3}H]NA release ($F_{(5,91)} = 7.85$; $p < 0.0001$). The dose–effect curve of DA was shifted leftward, as apparent from the finding that the lowest concentration of DA to significantly increase NA release was decreased from 1 μM to 30 nM. In addition, the dose–response curve of DA was also shifted upward, because the maximal effect of DA was increased from 15% in the absence of to 35% in the presence of (−)-sulpiride (Fig. 4). It should be noted that the increase in [^{3}H]NA release induced by DA in the presence of (−)-sulpiride was of a similar magnitude as that induced by SKF-38393 (compare Figs. 2*A*, 4). Experiments on the effects of DA in the presence of SCH-23390 yielded inconsistent results, probably because of the fact that the selectivity of SCH-23390 for D1 over D2 receptors in brain slices is less than 10-fold (Plantjé et al., 1984). Indeed, concentrations of SCH-23390 >0.3 μM caused a marked enhancement of [^{3}H]NA release (data not shown), as observed with (−)-sulpiride.

The effects of D1 receptor stimulation on NAcc NA release are not secondary to extracellular conversion of cAMP to adenosine

Stimulation of D1 receptors enhances adenylate cyclase activity (Stoof and Kebabian, 1984). It has been described recently that certain effects of D1 receptor stimulation are the consequence of extracellular conversion of cAMP to adenosine, which, through stimulation of adenosine A1 receptors, alters neuronal activity (Bonci and Williams, 1996; Harvey and Lacey, 1997). To investigate whether the effect of D1 receptor activation on NAcc [^{3}H]NA release was caused by such a mechanism, the effect of the adenosine A1 agonist CPA was investigated. CPA did not mimic the effect of SKF-38393. On the contrary, CPA appeared to suppress the electrically evoked [^{3}H]NA release by 13% at a concentration of 0.1 μM and by 19% at a concentration of 1 μM ($F_{(2,33)} = 5.67$; $p < 0.01$; data not shown).

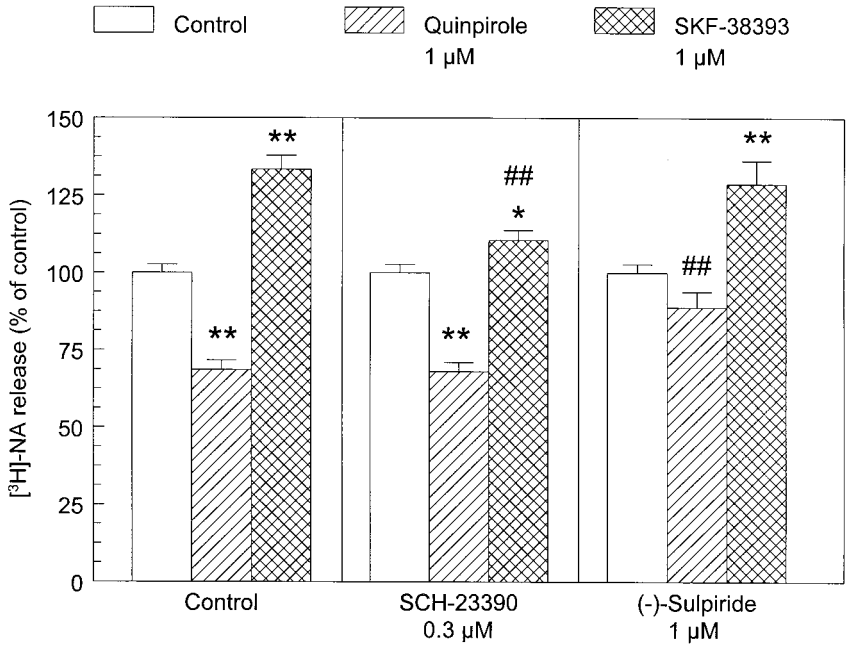

Figure 3. Effect of 0.3 μM SCH-23390 (*middle*) and 1 μM (−)-sulpiride (*right*) on the quinpirole-induced decrease and the SKF-38393-induced increase of electrically evoked [^{3}H]NA release in superfused rat NAcc slices. The slices were superfused and stimulated electrically at t = 50 min for 10 min. SCH-23390 or (−)-sulpiride were added 30 min before depolarization, and SKF-38393 or quinpirole were added to the superfusion medium at 20 min before depolarization. Control [^{3}H]NA release amounted to 4.7 ± 0.3% of total tissue radioactivity in the absence of antagonists, 5.1 ± 0.3% in the presence of SCH-23390, and 7.7 ± 0.5% in the presence of (−)-sulpiride, respectively. Data, expressed as percent of respective control release, represent means ± SEM of 24 observations. *Open bars* represent [^{3}H]NA release under control conditions, *hatched bars* represent release in the presence of 1 μM quinpirole, and *cross-hatched bars* represent release in the presence of 1 μM SKF-38393. *$p < 0.05$; **$p < 0.001$ compared with control values; ##$p < 0.001$ compared with same condition without antagonist (ANOVA).

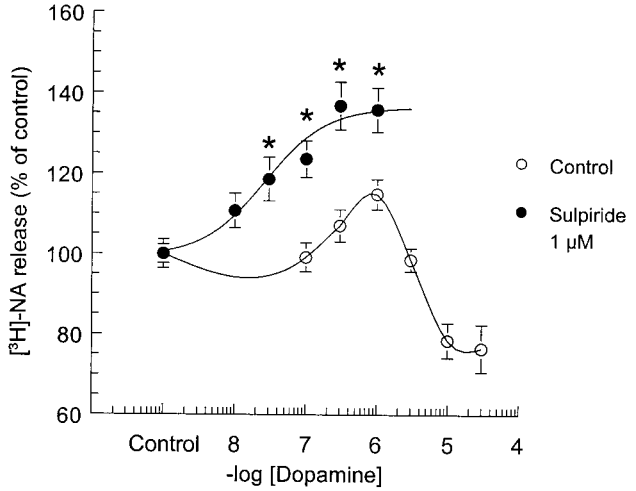

Figure 4. Effect of DA in the absence (compare with Fig. 1; *open circles*) and in the presence of 1 μM (−)-sulpiride (*closed circles*) on the electrically evoked [^{3}H]NA release of superfused rat NAcc slices. The slices were superfused in the presence of 3 μM desipramine to prevent uptake of DA into noradrenergic nerve terminals and were stimulated electrically at t = 50 min for 10 min. (−)-Sulpiride was added to the superfusion medium at 30 min before depolarization, and DA was added at 20 min before depolarization. Control [^{3}H]NA release amounted to 5.8 ± 0.4% of total tissue radioactivity in the absence of and 7.2 ± 0.5% in the presence of (−)-sulpiride, respectively. Data, expressed as percent of control release, represent means ± SEM of 24 observations. *$p < 0.05$ compared with control values in the presence of (−)-sulpiride (Student–Newman–Keuls test).

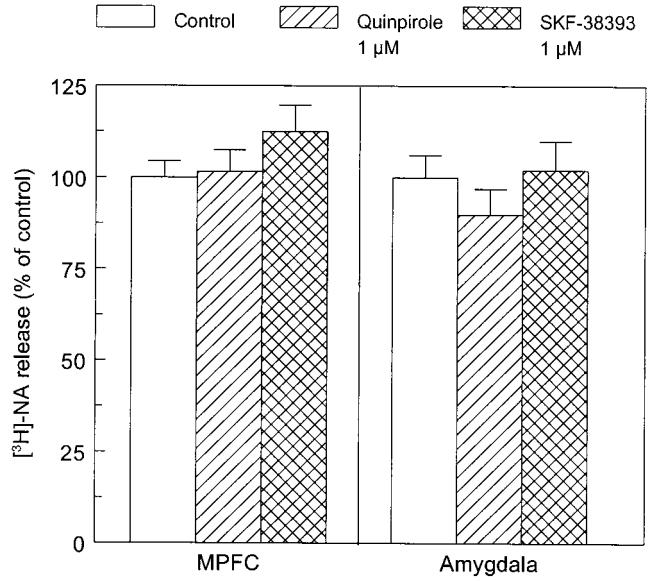

Figure 5. Effects of SKF-38393 (1 μM) and quinpirole (1 μM) on the electrically evoked release of [^{3}H]NA from superfused slices of rat medial prefrontal cortex (*MPFC*; *left*) or amygdala (*right*). The slices were superfused and stimulated electrically at t = 50 min for 10 min. SKF-38393 and quinpirole were added to the superfusion medium at 20 min before depolarization. Control [^{3}H]NA release amounted to 4.1 ± 0.3% in medial prefrontal cortex slices and 3.0 ± 0.2% in amygdala slices. Data, expressed as percent of control release, represent means ± SEM of 11–12 observations. *Open bars* represent [^{3}H]NA release under control conditions, *hatched bars* represent release in the presence of 1 μM quinpirole, and *cross-hatched* bars represent release in the presence of 1 μM SKF-38393.

DAergic regulation of NA release does not occur within medial prefrontal cortex and amygdala

Regulation by DA of NA release has been reported previously to occur in the hypothalamus (Misu et al., 1985) and hippocampus (Jackisch et al., 1985), but, in those areas only a D2-mediated inhibition of NA release was found. To investigate whether the opposite regulation of NA release by D1 and D2 receptors also occurred in other limbic areas, we studied the effects of SKF-38393 and quinpirole on NA release in slices of medial prefrontal cortex and amygdala. In slices of medial prefrontal cortex, 1 μM SKF-38393 slightly, but not significantly (12% above control), increased electrically evoked [^{3}H]NA release ($F_{(1,23)} = 2.17$; NS). Quinpirole, at a concentration of 1 μM, did not affect medial prefrontal cortex [^{3}H]NA release ($F_{(1,23)} = 0.05$; NS) (Fig. 5, *left*). In rat amygdala slices, SKF-38393 (1 μM) did not at all affect electrically evoked [^{3}H]NA release ($F_{(1,23)} = 0.04$; NS), whereas

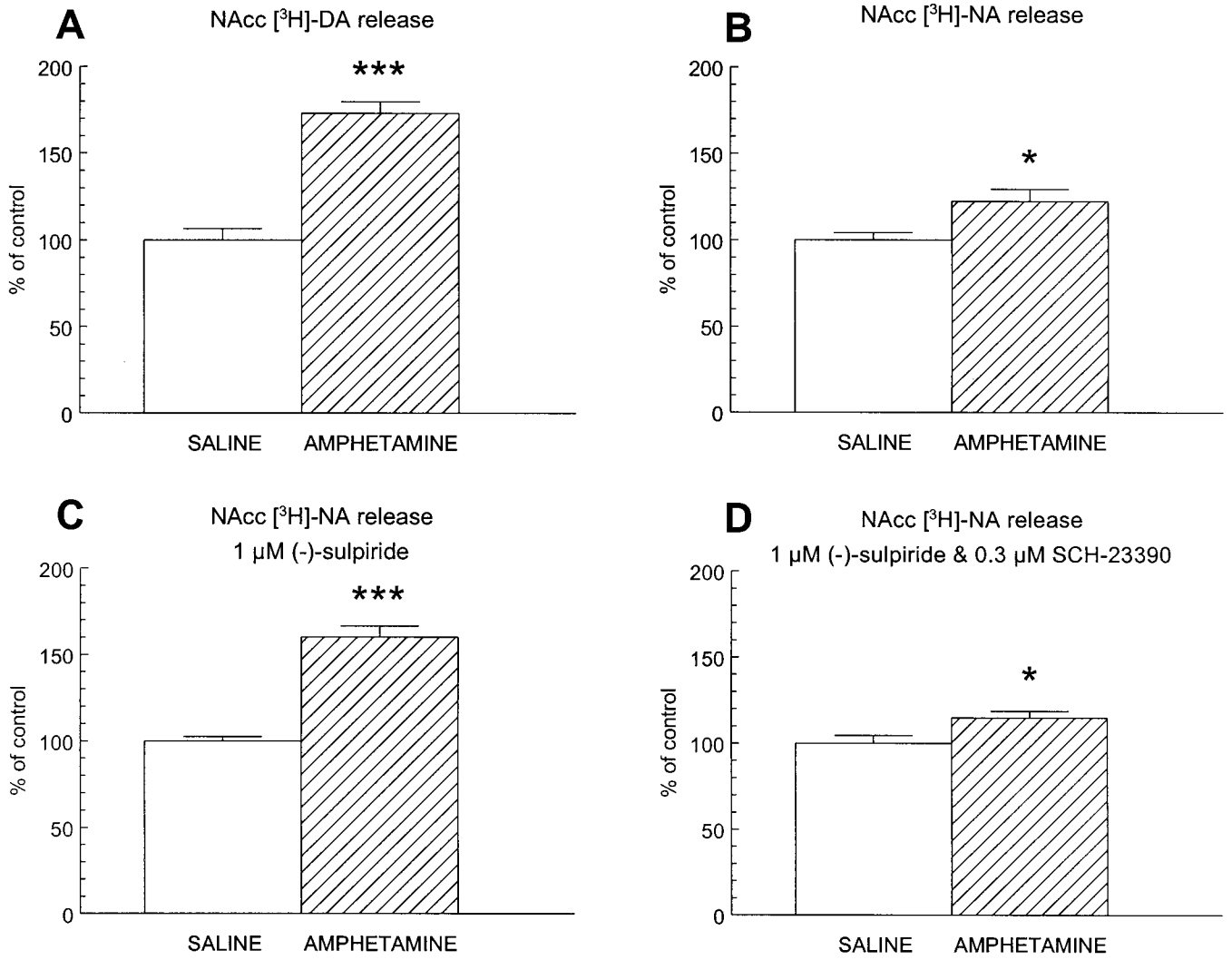

Figure 6. *A*, Electrically evoked release of [^{3}H]DA from superfused NAcc slices of rats pretreated with amphetamine (5 × 2.5 mg/kg, i.p.; *hatched bar*) or saline (*open bar*) 3 d after treatment. [^{3}H]DA release amounted to 1.0 ± 0.1% in slices of saline-pretreated rats. *B*, Electrically evoked release of [^{3}H]NA from superfused NAcc slices of rats pretreated with amphetamine (5 × 2.5 mg/kg, i.p.; *hatched bar*) or saline (*open bar*) 3 d after treatment. [^{3}H]NA release amounted to 4.1 ± 0.2% in slices of saline-pretreated rats. *C*, Electrically evoked release of [^{3}H]NA from superfused NAcc slices of rats pretreated with amphetamine (5 × 2.5 mg/kg, i.p.; *hatched bar*) or saline (*open bar*) in the presence of 1 μM (−)-sulpiride 3 d after treatment. [^{3}H]NA release amounted to 6.0 ± 0.3% in slices of saline-pretreated rats. *D*, Electrically evoked release of [^{3}H]NA from superfused NAcc slices of rats pretreated with amphetamine (5 × 2.5 mg/kg, i.p.; *hatched bar*) or saline (*open bar*) in the presence of 1 μM (−)-sulpiride and 0.3 μM SCH-23390 3 d after treatment. [^{3}H]NA release amounted to 7.8 ± 0.4% in slices of saline-pretreated rats. NAcc slices were superfused and stimulated electrically at $t = 50$ min for 10 min. (−)-Sulpiride and SCH-23390 were added to the superfusion medium at 30 min before depolarization. Note that the data are expressed as percent of respective control release in slices of saline-pretreated rats. The basic effects of (−)-sulpiride and SCH-23390 (Fig. 2*C*) are therefore not shown. Data represent means ± SEM of 8–23 observations. $^*p < 0.05$; $^{***}p < 0.0001$ compared with saline pretreatment (ANOVA).

quinpirole (1 μM) caused a slight (12%) nonsignificant inhibition of evoked [^{3}H]NA release ($F_{(1,22)} = 1.19$; NS) (Fig. 5, *right*).

Altered modulation of NAcc NA release by DA in slices of amphetamine-pretreated rats

In NAcc slices of amphetamine-pretreated animals, the electrically evoked release of [^{3}H]DA was augmented by 73% ($F_{(1,15)} = 61.25$; $p < 0.0001$) (Fig. 6*A*), and the electrically evoked [^{3}H]NA release was increased by 22% ($F_{(1,23)} = 7.34$; $p < 0.05$) (Fig. 6*B*). Whereas in slices of saline-pretreated rats 1 μM (−)-sulpiride caused a 46% increase in evoked NAcc [^{3}H]NA release (data not shown, but see Fig. 2*C*), in slices of amphetamine-pretreated rats, 1 μM (−)-sulpiride enhanced evoked [^{3}H]NA release by 92%. Thus, in the presence of 1 μM (−)-sulpiride, the relative enhancement of evoked [^{3}H]NA release in slices of amphetamine-pretreated rats was 60% ($F_{(1,45)} = 74.37$; $p < 0.0001$) (Fig. 6*C*) compared with 22% in the absence of sulpiride (Fig. 6*B*), indicating enhanced D2 receptor activation in slices of amphetamine-pretreated rats. SCH-23390 (0.3 μM) slightly enhanced evoked [^{3}H]NA release in NAcc slices of saline-pretreated animals, but in slices of amphetamine-pretreated rats, SCH-23390 suppressed [^{3}H]NA release by 20% (data not shown). However, these data cannot be interpreted unambiguously because 0.3 μM SCH-23390 may be expected to partially block D2 receptors (Plantjé et al., 1984). Therefore, the effect of SCH-23390 was investigated in the presence of 1 μM (−)-sulpiride. Interestingly, under circumstances of D2 receptor blockade, 0.3 μM SCH-23390 appeared to

diminish the increase in NAcc [^{3}H]NA release after previous amphetamine treatment to 15% ($F_{(1,22)} = 6.20$; $p < 0.05$) (Fig. 6*D*). Thus, SCH-23390 almost abolished the increase in electrically evoked [^{3}H]NA release observed in slices of rats preexposed to amphetamine.

DISCUSSION

The present data demonstrate that NA release in the rat NAcc is under the opposing influence of stimulatory DA D1 and inhibitory DA D2 receptors. These NA release-modulatory DA receptors are presumably localized on nerve terminals of NA neurons originating in the NTS (Delfs et al., 1998). Although occurrence of presynaptic receptors on central nerve terminals has indeed been demonstrated (Fisher et al., 1994; Sesack et al., 1994; Hersch et al., 1995), the involvement of indirect or transsynaptic regulation of neurotransmitter release cannot be excluded, even in superfused brain slices. It is therefore possible that DA indirectly affects NAcc NA release through modulation of excitatory or inhibitory neurotransmission. In this respect, electrophysiological experiments have shown that, in the NAcc, stimulation of presynaptic D1 receptors depresses both inhibitory and excitatory transmission (Pennartz et al., 1992; Harvey and Lacey, 1996; Nicola and Malenka, 1997, 1998), whereas activation of presynaptic D2 receptors suppresses excitatory transmission (O'Donnell and Grace, 1994). Microdialysis studies have shown that D1 receptor stimulation actually enhances NAcc GABA release, whereas D2 receptor stimulation appears to inhibit glutamate release in the NAcc (Kalivas and Duffy, 1997). Thus, some of these data seem to fit with the present observations of stimulatory effects of D1 receptors and inhibitory effects of D2 receptor stimulation, but others do not. The present data can therefore not be explained solely on the basis of DA effects on excitatory and inhibitory inputs into the NAcc rather than direct DA effects on NA varicosities. Neurotransmission-modulatory effects of D1 receptor stimulation may also be indirectly mediated by the release of adenosine (Bonci and Williams, 1996; Harvey and Lacey, 1997), but the selective adenosine A1 receptor agonist CPA appeared not to mimic the stimulatory effects of D1 receptor stimulation but even slightly decreased NAcc [^{3}H]NA release. This suggests that, although release-inhibitory adenosine A1 receptors may be present on NAcc NA nerve terminals, the stimulatory effect of D1 receptor activation is not mediated indirectly through activation of adenosine receptors. Together, although possible indirect effects of D1 and D2 receptor stimulation cannot be ruled out, it is most likely that the release-modulatory DA receptors are located on NAcc NA varicosities.

With regard to the tonic activation of these DA receptors, the D2 antagonist (−)-sulpiride strongly increased NAcc NA release, whereas the D1 antagonist SCH-23390 did not reduce NA release. Thus, released endogenous DA tonically inhibits NAcc NA release through stimulation of D2 receptors, whereas the stimulatory D1 receptors are not activated under the present *in vitro* conditions. Because one of our previous studies showed that, in superfused rat striatal slices, exogenous and endogenous DA displays an identical apparent affinity to D1 and D2 receptors (Schoffelmeer et al., 1994), differences in apparent affinity for DA cannot account for these findings. A more likely explanation is that D1 and D2 receptors are differentially located on or near NA nerve terminals. We hypothesize that D2 receptors are located near active zones formed by DA and NA nerve terminals, whereas D1 receptors are located more distal from the site of DA release (Fig. 7, *top*). Indeed, such a differential localization of D1 and D2 receptors is supported by ultrastructural studies indicating that NAcc D1 receptors are mainly localized extrasynaptically (Smiley et al., 1994; Hersch et al., 1995; Caillé et al., 1996), whereas D2 receptors can be found near DAergic nerve terminals (Fisher et al., 1994; Sesack et al., 1994; Hersch et al., 1995; Delle Donne et al., 1996). Interestingly, voltammetric measurements of synaptic DA efflux showed that extrasynaptic DA neurotransmission occurs in the NAcc (Garris et al., 1994) and that excitatory signals can be conveyed by extrasynaptic D1 receptors activated by released DA, diffusing up to 12 μM away from release sites (Gonon, 1997). In the case of DA modulation of NAcc NA release, this would imply that DA released from mesolimbic neurons preferentially interacts with D2 receptors, located in the vicinity of the site of release. D1 receptors, located further away, might be stimulated in case of higher rates of release and/or during later phases of neurotransmission by DA that has diffused away from the synapse (Fig. 7, *bottom*). The biphasic effects of exogenously applied DA, activating both D1 and D2 receptors (Schoffelmeer et al., 1994), could be the consequence of such a different role of D1 and D2 receptors. For instance, when low concentrations of exogenous DA are applied, the possible inhibitory effect of this exogenous DA could be masked by the tonic D2 receptor-mediated inhibition of NA release, causing the D1 receptor-mediated increasing effect to prevail (Fig. 1). It is also worth noting that, in the presence of (−)-sulpiride when DA will only stimulate D1 receptors, the dose–response curve of DA was shifted upward, as well as leftward, closely resembling the dose–response curve of SKF-38393 (compare Figs. 4, 2*A*).

Both the medial prefrontal cortex and the amygdala represent limbic brain areas that, similar to the NAcc, receive dense DA and NA innervations (Ungerstedt, 1971; Moore and Bloom, 1978, 1979; Le Moal and Simon, 1991). However, [^{3}H]NA release in slices of these areas does not seem to be modulated by DA. A possible explanation for this difference is that the NA projection to the NAcc originates mainly in the NTS (Delfs et al., 1998), whereas medial prefrontal cortex and amygdala receive a NA innervation from the locus ceruleus (Ungerstedt, 1971; Moore and Bloom, 1979). Similar phenomena have been observed with regard to the modulation of NA release by opioid receptors, which also seems to differ between different regions of origin (Heijna et al., 1991). The present data add to a growing body of evidence that NAcc NA release may be modulated in a unique manner. For instance, we have shown recently that, unlike in most other brain areas receiving NA input, NAcc NA release is not under the inhibitory influence of α_2-autoreceptors (Schoffelmeer et al., 1998).

The physiological relevance of the opposite regulation of NAcc NA release by D1 and D2 receptors remains to be elucidated. Coordinated activity of NAcc NA and DA neurotransmission may be necessary for adequate processing of motivational, visceral, and autonomic stimuli into behavioral responses (Le Moal and Simon, 1991; Phillips et al., 1991; Salamone, 1994; Schultz et al., 1997; Delfs et al., 1998). The subtle interregulation of NAcc NA and DA release therefore suggests the existence of a catecholaminergic fine-tuning mechanism modulating the generation of adaptive behavioral responses. In this respect, it is of interest to note that recent electrophysiological experiments have shown that, while in the NAcc DA, via D1 receptors, inhibits both excitatory and inhibitory transmission; NA, via α-receptors only inhibited excitatory, but not inhibitory, transmission (Nicola and

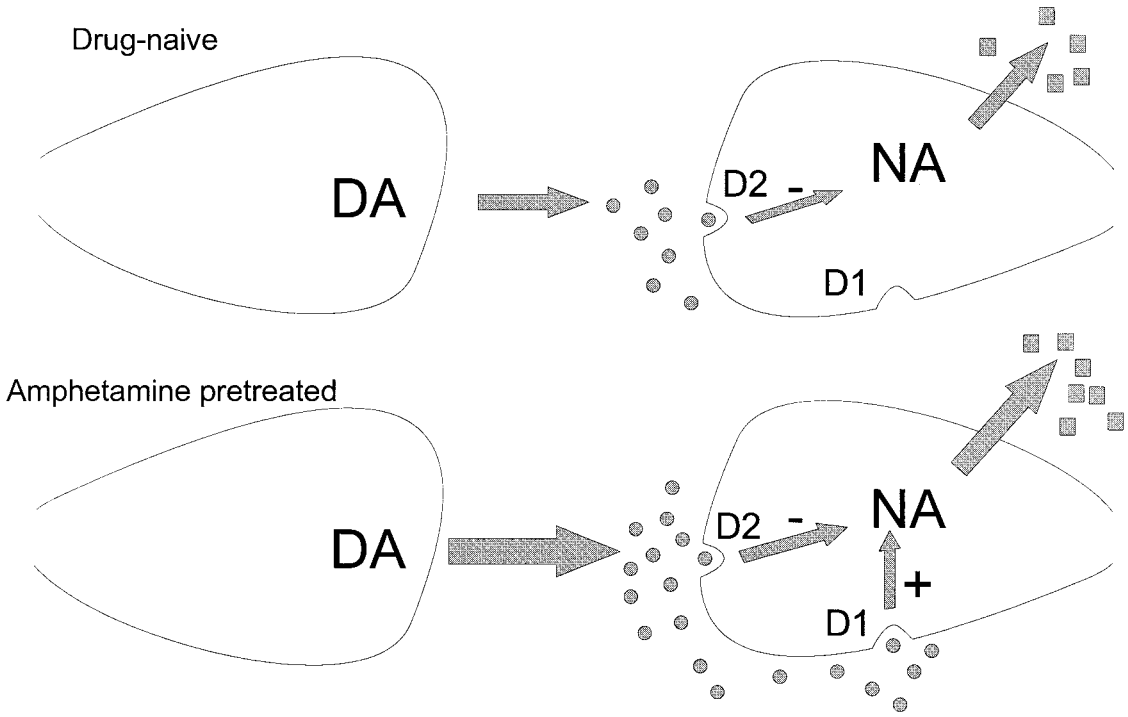

Figure 7. Hypothetical model of the modulation of NAcc NA release by DA and alterations therein after repeated exposure to amphetamine. DA (●), released from mesolimbic projections, is able to modulate NA (■) release in two directions: stimulation through D1 receptors and inhibition through D2 receptors. In drug-naive animals, released DA will tonically inhibit NA release via stimulation of inhibitory D2 receptors, whereas D1 receptors do not seem to be involved in the tonic DAergic regulation of NA release. We suggest that this is because of differential localization of D1 and D2 receptors on or near NA varicosities (*top*). In the case of enhanced DA overflow (e.g., caused by repeated exposure to amphetamine *in vivo*), the D2 receptor-mediated suppression of NA release will increase. In addition, excess DA will diffuse further away from the site of release and stimulate D1 receptors as well, causing NA release to become enhanced (*bottom*).

Malenka, 1998). This suggests that the balance of DA and NA neurotransmission in the NAcc might determine whether excitatory or inhibitory input into NAcc neurons will prevail. In addition, the entwinement of NAcc DA and NA systems could be involved in certain phenomena associated with drug abuse, such as psychostimulant sensitization and opiate withdrawal (Harris and Aston-Jones, 1994). In parallel to the effects on NAcc NA release described here, administration of D2 agonists into the NAcc shell has been shown to inhibit, and administration of a D1 agonist has been shown to enhance naloxone-evoked opiate withdrawal effects, whereas an intra-NAcc D2 antagonist appeared to evoke opiate withdrawal phenomena (Harris and Aston-Jones, 1994). Because increased NA activity accompanies opiate withdrawal (Akaoka and Aston-Jones, 1991; De Vries et al., 1993), it is likely that the effects of intra-NAcc-applied DAergic drugs on opiate withdrawal involve modulation of NAcc NA release.

In NAcc slices of amphetamine-pretreated rats, the electrically evoked release of both [^{3}H]NA and [^{3}H]DA was enhanced. Moreover, the increase in [^{3}H]NA release induced by D2 receptor blockade with (−)-sulpiride was enhanced, indicating an increase in the tonic D2-mediated suppression of NA release. Remarkably, SCH-23390 primarily antagonized this augmentation of NAcc NA release induced by amphetamine preexposure. These data indicate that, under conditions of different dopaminergic tone in the accumbens, NA release is differentially regulated by DA receptors. In addition, they are consistent with our hypothesis that D1 receptors represent extrasynaptic receptors, particularly stimulated under conditions of increased DA release. Thus, under circumstances of moderate DA tone, released DA, stimulating D2 receptors, tonically suppresses NAcc NA release, whereas extrasynaptically located D1 receptors play a less prominent role in the regulation of NA release (Fig. 7, *top*). When DA tone is increased, such as in amphetamine-pretreated rats, enhanced DA release from mesolimbic terminals increases the tonic D2-receptor-mediated suppression of NA release. In addition, augmented DA release will also stimulate extrasynaptic D1 receptors, resulting in a net increase in NAcc NA release (Fig. 7, *bottom*). Assuming that the balance between NAcc DA and NA activity is relevant for the formation of adequate adaptive behavioral responses, this amphetamine-induced disbalance in catecholamine neurotransmission could represent a substrate for the distorted motivational and affective behaviors characteristic for drug-induced addiction and psychosis.

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