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Noradrenergic Role in the Self-Administration of Morphine or Amphetamine[1]

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DAVIS, W. M., S. G. SMITH AND J. H. KHALSA. *Noradrenergic role in the self-administration of morphine or amphetamine.* PHARMAC. BIOCHEM. BEHAV. 3(4) 477–484, 1975. – The role of brain noradrenergic neurons in mediating the reinforcing properties of small intravenous doses of morphine and d-amphetamine was investigated by pretreatment of rats with the norepinephrine-depleting agents diethyldithiocarbamate and U-14,624, inhibitors of dopamine-β-hydroxylase (DBH). Such treatment prevented the reacquisition of a self-administration response (bar-press) for morphine (32 μg/kg/injection) or d-amphetamine (15 μg/kg/injection) made available on a CRF schedule. Pretreatment with a DBH inhibitor also prevented the development of a secondary (conditioned) reinforcer based on primary reinforcement associated with either drug. Observations indicating that the orienting reflex was intact are taken as evidence that depressant effects of the DBH inhibitors were not severe enough to disrupt the associative process. Therefore, any effect on learning does not seem sufficient to explain the present results. Thus, it is inferred that the mechanisms mediating reinforcement for both morphine and amphetamine were disrupted by the inhibition of central noradrenergic functions.

Drug Reinforcers Self-administration d-Amphetamine Morphine Dopamine-β-hydroxylase inhibitors Diethyldithiocarbamate U-14,624 Primary reinforcement Conditioned reinforcement Noradrenergic brain mechanisms Dopaminergic brain mechanisms

STUDIES of the neurochemistry of reinforcement (reward) systems of the mammlian brain have been based predominantly upon the reinforcement associated with intracranial self-stimulation (ICS). Numerous reports of the suppression of ICS after inhibition of catecholamine (CA) synthesis [1, 4, 5, 9, 21, 39, 57] or destruction of CA-producing neurons [6, 24, 50] suggest an essential role for central catecholaminergic neurons in the reinforcement associated with ICS. While some workers conclude that the neurons involved in this reinforcement mechanisms are noradrenergic [50, 55, 56], others [10–12, 31, 36] hold that activation of either noradrenergic or dopaminergic mechanisms can support ICS.

Similar investigations of the neurochemical basis for reinforcement arising from opiate and stimulant drugs have yielded data supporting the hypothesis that a catecholaminergic brain mechanism is responsible for the reinforcing properties of both morphine [13, 15, 16, 40] and d-amphetamine [16,17]. These studies, like the many with ICS cited above, utilized α-methyltyrosine (AMT) as a tool to demonstrate that pharmacological reinforcement depended on intact stores of brain CAs. Brain levels of both norepinephrine (NE) and dopamine (DA) are depleted by AMT [41] through inhibition of tyrosine hydroxylase.

Inhibitors of CA biosynthesis which selectively deplete NE but not DA were used in the present study to test whether reinforcement produced by morphine and d-amphetamine could be abolished by depleting only NE. Employing methods based on the reacquisition of self-administration behavior [13, 15–17] and on the establishment of a conditioned reinforcer [17,44], the functional state of the reinforcing properties of morphine and amphetamine was tested after pretreatment with diethyldithiocarbamate (DDC) or 1-phenyl-3-(2-thiazolyl)-2-thiourea (U-14,624), agents which deplete NE via dopamine-β-hydroxylase (DBH) inhibition [8, 26, 27, 32].

METHOD

Animals

One-hundred and twenty-four adult male Holtzman rats weighing 350–400 g were implanted with jugular catheters for studies of drug self-administration behavior and of drug-based conditioned reinforcement. All animals were experimentally naive.

Apparatus

For experiments the animals were placed in 8 operant

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test chambers which provided food, water and a response lever. Each lever response activated a pump to deliver in 0.2 second an intravenous (IV) dose of 0.018 ml into the precava near the right atrium via a leash and an external harness (saddle) assembly connected to the jugular cannula. The catheter, which resembles that of Weeks [51], the external saddle and the remainder of the infusion system are described in detail elsewhere [48]. The test chambers consisted of Plexiglas cylinders (24 cm in height and 25 cm in dia.) containing a removable response lever 3.5 cm above the floor. Each chamber was lighted and enclosed within a sound-attenuating enclosure outside of which is placed a screw-type, syringe-driver infusion pump. The infusion circuit passed into the enclosure to a swivel, which allowed free rotation by the animal and which connected to the leash, a 26 cm length of 18 ga. needle tubing that could be attached via an alligator clamp to the external saddle. The jugular cannula was connected to the injection circuit via the saddle during experimental sessions and disconnected and capped between sessions when the rat occupied an individual housing cage in another room.

Procedure

To determine the effects of DBH inhibitors on morphine self-administration (Experiment 1), we used a reacquisition design which is described more fully elsewhere [13, 15, 16–20]. All experimental sessions lasted 6 hr. On Day 1 of the experiment, a baseline level of lever-presses for each rat was recorded while every response caused only the infusion of 0.9 percent saline plus a 0.2 sec superimposed buzzer presentation. An acquisition session run at the same time on the next day included the same contingencies except that 32 μg/kg of morphine sulfate, a dose previously found to maintain a lever-press response at rates significantly higher than control levels [13, 16, 52], was substituted for saline. On Day 3 the contingencies of Day 1 were reinstated during an extinction session in which the lever-response was extinguished for all animals before the end of the session. On Day 4 two IP injections consisting of either saline (Group A1) or 670 mg/kg of DDC (Group A2) were given at a 3.5 hr interval to two groups (8 rats each). Two further groups at a later time received single peroral doses of 1 percent methylcellulose suspension in 0.9 percent saline (Group B1) or 600 mg/kg of U-14,624 suspended in 1 percent methylcellulose (Group B2). A session for reacquisition of the self-administration response (conditions as on Day 2) began at the end of a 2 hr delay after the last injection for Groups A1 and A2 or at 4 hr after treatment for Groups B1 and B2. These delays provided not only for development of CA depletion, but also for dissipation of transient local irritation from DDC injection.

The acquisition-reacquisition design was employed because: (1) it allows the experimenter to determine whether the morphine dosage of 32 μg/kg injection will be self-administered by the animal prior to testing with the DBH inhibitor. Some animals may not self-administer this dosage (although all did in the present work), and such rats would properly be dropped from the study before the reacquisition test; and (2) ambiguities can arise out of assessment of a chemical agent on an established self-administration baseline. The test agent (DBH inhibitor) might antagonize a mechanism which usually limits the amount of drug self-administered. The result could be an initial increase in drug-taking behavior, followed by a reduction in self-administration as the effectiveness of a test agent is decreasing. If the duration of the self-administered drug exceeded that of the test agent, the overall result could be a reduction of self-administration. The initial increase followed by a decrease in responding could be viewed as an extinction effect, i.e., that the test agent had antagonized the reinforcing effectiveness of morphine. This ambiguity cannot occur in the present design since the rat is extinguished prior to testing for reacquisition.

Experiment 2 was to ascertain whether DDC and U-14,624 suppressed reacquisition by affecting reinforcement or by producing a non-specific motor impairment or other toxicity. For this purpose a Pavlovian pairing procedure was used which permits development of a secondary (conditioned) reinforcer, based on a primary pharmacological reinforcer, independently of motor behavior [17]. Day 1 conditions were identical to those in Experiment 1. On the second day Groups D1 and E1 (vehicle-treated) and Groups D2 and E2 (treated with DDC and U-14,624, respectively) were injected as per the schedule of Experiment 1, but after the injections and the delay interval, each rat was placed in a chamber with the response-lever removed. The 50 non-contingent buzzer-morphine (32 μg/kg) pairings were presented during about 100 minutes. On a randomized program (the same for all animals) from 1 to 5 buzzer-morphine pairings occurred during successive 6-minute intervals. The rats were tested for conditioned reinforcement 4 days after the pairings with Day 1 contingencies reinstated. Elevation of lever-pressing above the initial baseline level showed the function of the buzzer as a conditioned reinforcer.

In Experiments 3 and 4, NE involvement in reinforcement associated with d-amphetamine was examined using the same experimental procedures as in Experiments 1 and 2 except that injections of 15 μg/kg of d-amphetamine were used instead of morphine, and only U-14,624 was used to deplete brain NE.

Drugs can produce increases in locomotor activity which may mimic reinforcing effects [38]. Under other conditions of dosage and route, not only amphetamine [37], but also morphine [2,14] is known to produce increased locomotor activity in rats. While dosages as low as those employed in the present study have not been tested, the possibility of activity effects cannot be ignored. Therefore, Experiment 5 was run to explore possible confounding by generalized activity effects through adding a second (control) lever to the acquisition–reacquisition design employed in Experiments 1 and 3. The extra lever had no scheduled consequences and was inserted into the chamber for the purpose of recording activity. Four rats were assigned to the right lever as the reinforced level, another four to the left lever. If general increases in activity were responsible for the difference between the baseline (no-drug) data and acquisition or reacquisition data (drug conditions), then responding on both levers should be nearly equal.

A number of investigators (e.g., [25, 38, 42]) have shown that pharmacologic effects of drugs can be elicited by stimuli associated (paired) with the drug injection. Therefore, to control for possible generalized conditioned activity effects during Experiments 2 and 4, Experiment 6 was conducted. The procedure was identical to that of Experiment 2 except that two levers (as in Experiment 5) were employed. Six animals were randomly assigned to either the right or left lever as the reinforcing lever. Statistical analysis of the data of Experiments 5 and 6 was by

TABLE 1

EFFECTS OF DOPAMINE-β-HYDROXYLASE INHIBITORS ON REACQUISITION OF A SELF-ADMINISTRATION RESPONSE FOR INTRAVENOUS MORPHINE SULFATE (MS) OR D-AMPHETAMINE SULFATE (AS)

	Group and Pretreatment*	Drug Reinforcer†	Day 1 Baseline Level Responses‡	Day 2 Responses in Acquisition‡	Day 4 Responses in Reacquisition‡
A1	Saline	MS	19 ± 4	104 ± 12	170 ± 19
A2	Diethyldithiocarbamate	MS	16 ± 3	130 ± 20	7 ± 2§
B1	Methylcellulose Vehicle	MS	36 ± 6	105 ± 14	138 ± 14
B2	U-14,624	MS	38 ± 7	114 ± 16	30 ± 4§
C1	Methylcellulose Vehicle	AS	60 ± 9	174 ± 27	308 ± 71
C2	U-14,624	AS	50 ± 8	172 ± 22	39 ± 8§

*Diethyldithiocarbamate (670 mg/kg × 2, IP), U-14,624 (600 mg/kg, PO) or corresponding vehicles were given before the test session on Day 4.

†Intravenous doses of either morphine sulfate (MS), 32 μg/kg/injection, or d-amphetamine sulfate (AS), 15 μg/kg/injection, were given for each lever response on Days 2 and 4. Responses on Days 1 and 3 were non-reinforced, i.e., caused injection of only 0.9 percent sodium chloride solution concurrently with a buzzer.

‡The tabulated values represent total lever-press responses (mean ± S. E.) for a 6-hr test session on a continuous reinforcement schedule.

§Differs from vehicle group in reacquisition at $p<0.01$; does not differ ($p>0.05$) from its own initial baseline level of responding.

Ryan's modification of the Mann-Whitney U Test to permit multiple comparisons [30].

Brain NE and DA levels were assayed in Experiment 7 using another 60 rats after treatment with the same dosages and schedules of drug or vehicle treatments as were used in the behavioral studies. Sodium diethyldithiocarbamate (J. T. Baker Chemical Co.) was given in 2 intraperitoneal (IP) doses of 670 mg/kg each at an interval of 3.5 hr. U-14,624 (Aldrich Chemical Co.) was given as one oral dose of 600 mg/kg. The appropriate vehicle control solutions were given in equal volumes and at the same times. Rats were decapitated for brain amine assays initially at 2 hr after the second dose of DDC (= zero time, corresponding to onset of sessions in the behavioral studies) and at 6, 24, 48 and 96 hr later. In the case of U-14,624 samples were taken initially at 4 hr after the U-14,624 (= zero time) and at 2, 4, 24 and 96 hr later. Thirty rats, 3 drugged and 3 control at each time, were used for each DβH inhibitor. Brains were frozen on dry ice immediately upon removal. Whole brains (minus cerebellum) were assayed for DA and NE by the method of Neff *et al.* [35].

Probability statments for Experiments 1–4 and 7 were based on statistical comparisons via the two-tailed Student *t* test for independent samples.

RESULTS

Data of Experiment 1 (Table 1) indicate that the rats treated with either DDC or U-14,624 failed to show reacquisition of morphine self-administration, while each vehicle control group showed excellent reacquisition. The differences between each drug group and corresponding control group were significant at $p<0.001$. Higher totals were seen in reacquisition than during initial acquisition for vehicle groups because the rats began to respond earlier in the test session. Extinction data for Day 3 (not tabulated) showed that levels of responding were comparable across groups, and that responding during the last two hours of the session had returned to baseline levels.

Data of Experiment 2 (Table 2) show that rats given buzzer-morphine pairings after DDC or U-14,624 failed completely to show development of the conditioned reinforcer, whereas the vehicle-treated groups clearly showed conditioned reinforcement by lever-pressing which was quite elevated relative to their initial baseline level ($p<0.01$). Differences between each drug and its respective control on the test of conditioned reinforcement were clearly significant ($p<0.01$). Furthermore, the absence of significant difference ($p>0.05$) between the initial baseline level and responses in the conditioned reinforcement test for the DDC and U-14,624 groups fails to give evidence of any residual inhibition of general activity for these drug groups during this test.

The data of Experiment 3 (Table 1) show that reacquisition of d-amphetamine self-administration also was blocked by pretreatment with U-14,624 as the U-14,624-treated group (C2) and the corresponding vehicle control group (C1) differed significantly ($p<0.001$). In the conditioned reinforcement paradigm (Experiment 4) U-14,624 also prevented the reinforcing action of d-amphetamine (Table 2), the U-14,624-treated group (F2) and vehicle control group (F1) differing markedly ($p<0.001$). That motor activity was not inhibited at the time of testing can be seen by comparing the responses in the conditioned reinforcement

TABLE 2

EFFECTS OF DOPAMINE-β-HYDROXYLASE INHIBITORS ON THE DEVELOPMENT OF CONDITIONED REINFORCEMENT BASED ON THE ACTION OF INTRAVENOUS INJECTIONS OF MORPHINE SULFATE (MS) OR D-AMPHETAMINE SULFATE (AS) AS PRIMARY REINFORCERS

	Group and Pretreatment*	Drug Reinforcer†	Day 1 Baseline Level Responses‡	Day 6 Responses for Conditioned Reinforcer‡
D1	Saline	MS	28 ± 7	111 ± 12
D2	Diethyldithiocarbamate	MS	34 ± 7	24 ± 5§
E1	Methylcellulose Vehicle	MS	51 ± 10	139 ± 27
E2	U-14,624	MS	55 ± 9	49 ± 10§
F1	Methylcellulose Vehicle	AS	28 ± 6	102 ± 11
F2	U-14,624	AS	33 ± 7	20 ± 3§

*Diethyldithiocarbamate (670 mg/kg × 2, IP), U-14,624 (600 mg/kg, PO) or corresponding vehicles were given before the test session on Day 2.

†Fifty intravenous doses of either morphine sulfate (MS), 32 μg/kg/injection, or d-amphetamine sulfate (AS), 15 μg/kg/injection, paired with a buzzer stimulus were given on Day 2 with no lever in the test chamber. Lever responses on Days 1 and 6 caused injection of only 0.9 percent sodium chloride concurrently with the buzzer stimulus.

‡The tabulated values represent total lever-press responses (mean ± S.E.) for a 6-hr test session on a continuous reinforcement schedule.

§Differs from vehicle group at $p<0.01$; does not differ ($p>0.05$) from its own initial baseline level of responding.

test for the U-14,624 group to their initial baseline response level ($p>0.05$).

Gross behavioral observations taken at the start of the reacquisition sessions indicated that injection of DDC has resulted in marked sedation and motor impairment. This observation is supported by data shown in Table 1 for this group (A2). Reacquisition responding is reduced to one-half that emitted during baseline. Behavioral impairment was not observed with the two groups (B2 and C2) that received the U-14,624. This observation is also substantiated by the data of Table 1 for these groups.

Observation of the animals during buzzer-drug pairing for the development of conditioned reinforcers corresponded to that observed during the reacquisition tests, i.e., motor impairment for the DDC group but not for the U-14,624 groups. A noteworthy observation during pairings was that subjects with DDC, and especially with U-14,624 were quite able to make orienting reactions, i.e., rats turned their heads towards the buzzer source, pricked their ears and sniffed, at the sound of the buzzer. A few rats given U-14,624 and only a morphine or amphetamine infusion, i.e., no buzzer, did not display these behavior parameters. This observation suggests that the dopamine-β-hydroxylase inhibitors did not interfere with the normal attentional mechanisms (which included the ability to discriminate direction) of these rats. This is important as the attentional mechanism is basic to the associative (learning process [33].

The results for Experiment 5 (morphine) are shown in Table 3. These data indicate no significant differences for levels of responding during baseline on the reinforced and activity (non-reinforced) levers (p required, 0.0125; p obtained, 0.360). No significance was observed between baseline and acquisition values (p required, 0.006; p obtained, 0.253) or baseline and reacquisition values (p required, 0.002; p obtained, 0.019) for responding on the activity lever. Significant differences were obtained between baseline and acquisition values (p required, 0.004; p obtained, 0.0015) and between baseline and reacquisition values (p required, 0.002; p obtained, 0.000) for the reinforced lever. Levels of responding also differed significantly between the reinforced lever and the activity lever during acquisition (p required, 0.0025; p obtained, 0.000) and reacquisition (p required, 0.0125; p obtained, 0.000). Clearly the only experimental effects observed gave evidence of the reinforcing effects of morphine.

Similar data for amphetamine are also shown in Table 3. There was no significant difference in level of responding on the reinforced lever and activity lever during the baseline period (p required, 0.0125; p obtained, 0.339). No difference was observed between the baseline activity-lever responding and activity-lever responding during acquisition (p required, 0.006; p obtained, 0.041) or during reacquisition (p required, 0.002; p obtained, 0.041). A significant difference was observed between baseline reinforced-lever responding and responding in acquisition (p required, 0.004; p obtained, 0.000) and in reacquisition (p required, 0.002; p obtained, 0.000). Significance was also obtained between

TABLE 3

TWO-LEVER ACTIVITY CONTROL DATA FOR SELF-ADMINISTRATION OF MORPHINE OR AMPHETAMINE

Group	Drug Reinforcer	Day 1 Baseline Level Responses		Day 2 Responses in Acquisition		Day 4 Responses in Reacquisition	
		Reinf.*	Act.*	Reinf.	Act.	Reinf.	Act.
G1	MS	32 ± 11	35 ± 8	111 ± 9	46 ± 10	161 ± 23	14 ± 6
G2	AS	35 ± 8	42 ± 9	155 ± 21	69 ± 18	294 ± 61	25 ± 7

*Reinf. refers to responses on lever associated with buzzer-infusion contingency. Act. refers to responses on a second lever having no associated contingencies, but serving only to provide an index of random activity.

TABLE 4

TWO-LEVER ACTIVITY CONTROL DATA FOR CONDITIONED REINFORCEMENT BASED ON MORPHINE OR AMPHETAMINE

Group	Drug Reinforcer	Day 1 Baseline Level Responses		Day 6 Responses for Conditioned Reinforcer	
		Reinf.*	Act.*	Reinf.	Act.
H1	MS	40 ± 13	38 ± 12	137 ± 23	53 ± 10
H2	AS	23 ± 7	30 ± 8	148 ± 20	17 ± 3

*Reinf. refers to responses on lever associated with buzzer-infusion contingency. Act. refers to responses on a second lever having no associated contingencies, but serving only to provide an index of random activity.

reinforced-lever and activity-lever responding in acquisition (p required, 0.0125; p obtained, 0.003) and in reacquisition (p required, 0.0125; p obtained, 0.000).

Thus, the two-lever data for amphetamine also showed significant effects only for the reinforced lever. Therefore, it seems doubtful that a generalized activity effect could account for primary reinforcement effects observed for morphine or amphetamine.

The results of Experiment 6 are shown in Table 4. The data for morphine indicate that the difference between the baseline levels of responding for the reinforced lever and the activity lever was not significant (p required, 0.025; p obtained, 0.439). No difference was observed between the baseline and conditioned reinforcement test for response levels on the activity lever (p required, 0.0125; p obtained, 0.155). A significant difference was observed between the baseline and conditioned reinforcement test for the reinforced lever (p required, 0.0125; p obtained, 0.004) and between the reinforced lever and activity lever during the conditioned reinforcement test (p required, 0.025; p obtained, 0.008). Thus, no significant activity effects were observed.

Data for amphetamine-based conditioned reinforcement are also shown in Table 4. The results reveal no significant difference between response levels on the two levers during the baseline period (p required, 0.025; p obtained, 0.343). No difference was found between the baseline and conditioned reinforcement test for the activity lever (p required, 0.025; p obtained, 0.136). However, a significant difference was observed between baseline and conditioned reinforcement for the reinforced lever (p required, 0.025; p obtained, 0.014) and between the reinforced lever and activity lever during the conditioned reinforcement test (p required, 0.025; p obtained, 0.014). Thus, reinforcement rather than activity effects were demonstrated also for amphetamine; therefore, a conditioned activity effect may not account for the effects of either morphine or amphetamine.

Brain CA assays of Experiment 7 confirmed that NE levels after both DDC and U-14,624, were markedly lowered ($p<0.001$) to 20 and 30 percent of controls, respectively, during the period corresponding to the course of the behavioral session on Day 2 (Fig. 1). Simultaneously, DA levels were unchanged after DDC or elevated considerably ($p<0.01$) after U-14,624. By 4 days after treatment with

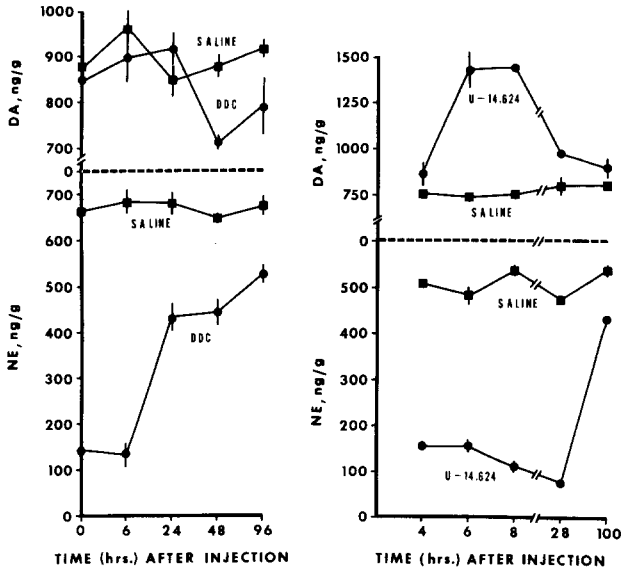

FIG. 1. Brain levels of dopamine (DA) and norepinephrine (NE) following treatment with the dopamine-β-hydroxylase inhibitors sodium diethyldithiocarbamate (DDC-left figure), U-14,624 (right figure) or with respective vehicle solutions. DDC was given in 2 IP doses of 670 mg/kg each at a 3.5 hr interval; U-14,624 was given as a single oral dose of 600 mg/kg; vehicle control groups were treated in a corresponding fashion. Each point and associated bar represents the mean ± S. E. for 3 rats. Absence of bar indicates that symbol height exceeded S. E. bar.

DDC or U-14,624, i.e., at the time of the tests for conditioned reinforcement, changes in brain CAs were largely reversed, i.e., NE concentration had returned to 80 percent or more of control levels.

DISCUSSION

The experiments on reacquisition of the self-administration response for solutions of morphine and d-amphetamine yielded results suggesting that pretreatment with DBH inhibitors block the drug-associated reinforcement. However, these data alone do not confirm that possibility, as it might be postulated that the drug pretreatments caused either a generalized disruption of behavior or an inhibition of schedule-controlled operant behaviors by a mechanism not related directly to positive reinforcement. These arguments were obviated by our additional use of the conditioned reinforcement paradigm. We have previously shown that durg treatment causing severe behavioral inhibition and/or disruption (i.e., extreme doses of haloperidol, up to 30 mg/kg) was not able to hinder the operation of morphine as a primary reinforcer [44]. That is the establishment of a conditioned reinforcer, via non-contingent buzzer-morphine pairings given while the animals were under such haloperidol treatment, was not affected. Thus, data from the two experimental procedures together support the conclusion that the DBH inhibitors were altering behavior through their interference with the action of morphine and d-amphetamine as primary reinforcers.

While the conclusion is drawn that DBH inhibitors produced their effects by suppressing the reinforcing effectiveness of morphine and amphetamine, it is equally plausible that the non-specific action of DBH inhibition affected the subjects' associative processes, i.e., their learning or discriminative capabilities. This hypothesis has some credence in the case of DDC for general behavioral observations cited above and those from other laboratories [8] indicate that DDC produces marked sedation and motor impairment. Although we have demonstrated that marked behavioral effects with extreme doses of haloperidol do not inhibit the reinforcing effectiveness of morphine this does not preclude the possibility that DDC might affect the associative process.

While an associative deficit may occur with DDC, gross behavioral observations suggest that this was not the case for U-14,624. In fact, by such observations it was difficult to distinguish the U-14,624-treated animals from their vehicle controls. Furthermore, observations during the Pavlovian pairings (i.e., buzzer-drug) indicated that normal attentional processes, as inferred from the orienting reaction, were not affected. The orienting reaction is an important index to an organism's discriminative and learning capabilities; i.e., a good orienting reaction is related to intact cortical functioning and good learning, while inhibited function is related to poor learning [33]. Recently apomorphine was shown to be effective in maintaining self-administration behavior [3,19]. In a further study (unpublished) from our laboratory, self-administration of apomorphine was not blocked by an identical U-14,624 dose regimen to that employed in the present research. If some non-specific action of U-14,624 were interfering with learning, it should affect self-administration of apomorphine in a similar fashion. Thus, there is a basis for ruling out an interpretation of the present findings as an interruption of the associative process.

Research with AMT, a depleter of DA and NE through inhibition of tyrosine hydroxylase, indicates that doses of 140 mg/kg for the monkey and 2.5 and 3.0 g/24 hr for the human do not affect the associative process (F. W. L. Kerr, 1974, personal communication). While AMT does not selectively inhibit NE alone, it does reduce NE levels significantly just as do the DBH inhibitors. Thus, there is some parity between the present question concerning associative processes as to the effects of DBH inhibitors and the reported observations for AMT.

Comments on the enzyme specificity of our DBH inhibitor and on the possible contribution of peripheral NE reduction to behavior deficits are in order. In this regard, U-14,624 is regarded to be selective for the inhibition of DBH and the depletion of NE [27,34]. As is shown here (i.e., Fig. 1, U-14,624) and elsewhere (e.g., [34]), levels of dopamine are increased at higher doses of DBH inhibitors. However, the increased availability of dopamine should not impair function of the dopaminergic system. Unpublished data from our laboratory indicates that serotonin levels are not effected by the DBH inhibitors employed here.

Regarding a reduction of peripheral NE levels and the present observations, it should be noted that the initial depletion of NE is more pronounced in the brain than in the peripheral nervous system due to the more rapid turnover of NE in the brain. Thus, as Goldstein [23] has indicated, the rate of NE depletion will depend on the rate of NE utilization and resynthesis, and NE will be depleted to a greater extent from a store showing rapid turnover (i.e., brain) than from one showing slow turnover (i.e., periphery). For such reasons the present authors and others (K. E. Moore, personal communication) assume little or no periph-

eral involvement in results of present or similar research. In a somewhat parallel research [20] on the role of central cholinergic mechanisms in morphine or amphetamine self-administration, only central cholinergic blockade was observed to have any effect (i.e., atropine was active, methylatropine was inactive).

Therefore, results of the present studies support the hypothesis that depletion of brain NE can eliminate the reinforcing actions of morphine and d-amphetamine. They seem to imply that the reinforcement mechanism associated with each of these drugs includes critical noradrenergic links in the neural circuits or pathways subserving reinforcement. Our selection of a dosage of U-14,624 sufficient not only to deplete NE, but also to elevate DA, has provided evidence that even an excess of DA can not compensate for or replace the depleted brain NE. At lower dosages U-14,624 lowers brain NE without altering DA; a similar capacity to block reinforcement as with the higher dosage was observed with a 400 mg/kg dose of U-14,624 (unpublished data).

These results seem to negate the possibility of an all-important role for dopaminergic activity in reinforcement. However, the data do not exclude the further possibility that dopaminergic and noradrenergic neurons may be required to function in concert for the mediation of positive reinforcement [31]. A test of dopaminergic involvement, separately from noradrenergic, has been made by means of the DA-receptor blocker, haloperidol. While no hindrance toward morphine based reinforcement was exerted by haloperidol [44], we have found it to block d-amphetamine based reinforcement [19]. The d-amphetamine findings are consistent with a model for the mechanism of reinforcement in which the joint function of both noradrenergic and dopaminergic system is required. However, the morphine results point to a dependence on NE and apparent independence of DA as mediator of reinforcement. Thus, although the two drugs both are acting to produce positive reinforcement via quite similar catecholamine-dependent functions of the brain, their actions appear not to be identical.

Assuming that the basis for the reinforcement associated with morphine or d-amphetamine self-administration is equivalent in rats and in human beings, then pretreatment of human drug users with a NE-depleter acting like AMT, DDC or U-14,624 should also prevent the primary pharmacological reinforcing actions which provide strong motivation toward abuse of these agents. Consequently, such agents might be applicable for therapeutic procedures designed to extinguish the conditioned responses underlying drug abuse behaviors [47, 53, 54]. That effective inhibition of morphine- and d-amphetamine-based reinforcement could result from CA depletion in human subjects is suggested in the blocking by AMT of euphoria upon IV injection of amphetamine in man [28,29]. The doses of AMT used (2.0–4.0 g/day) were apparently well tolerated. Another study reported that the same dose range produced only minimal sedation and no hindrance of eating or other activities [7]. Furthermore, extinction procedures conducted in the natural drug-use environment while under treatment with CA-depleters could meet the additional need for extinguishing the behavioral control exerted by conditioned reinforcers [22, 43, 45–47]. Thus, drugs which prevent the function of CAs in reinforcement seem to be a worthy subject of investigation for the clinical treatment of opiate and stimulant drug abuse.

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