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Activity Responses to Morphine and Amphetamine in Rats with Elevated NE Levels in the Pons

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OLDS, M. E. AND J. L. FOBES. *Activity responses to morphine and amphetamine in rats with elevated NE levels in the pons.* PHARMAC. BIOCHEM. BEHAV. **15**(2) 167–171, 1981.—The catecholaminergic basis of the stimulant actions of amphetamine and morphine was investigated in adult rats treated neonatally with 6-hydroxydopamine (6-OHDA) to produce depletion of cortical catecholamines and marked elevation of norepinephrine in the pons. On days 3, 5, 7, and 9 after birth, rat pups were injected bilaterally in the lateral ventricles with 100, 200, or 400 μg of 6-OHDA, dissolved in artificial cerebrospinal fluid (CSF). A control group was injected with the CSF vehicle. The capacity of amphetamine (2 mg/kg) and morphine (1.25, 2.5, and 3.5 mg/kg) to produce behavioral stimulant effects was then subsequently tested in adults. The stimulant effect of amphetamine was attenuated in animals pretreated with 100 and 200 μg 6-OHDA and was blocked in those treated with 400 μg 6-OHDA. The stimulant effects obtained with morphine were blocked by all 6-OHDA doses (100 and 200 μg). For morphine, no tests were made in the 400 μg 6-OHDA group on the basis of results obtained in animals pretreated with the lower doses of 6-OHDA. These results are discussed in terms of differing roles played by the catecholamine systems in the production of behavioral stimulation.

Activity Amphetamine Catecholamines 6-Hydroxydopamine Morphine

IT is well established that the central catecholamine (CA) systems play a role in the behavioral stimulant effects of amphetamine and morphine [4,10]. But in what way the roles differ for these two compounds that belong to different classes and whether their effects are exerted at the same brain level are questions that as yet have no definite answers.

The work of several investigators [2, 3, 4, 5] shows that, for amphetamine, the responsible mechanism appears to lie in the forebrain, to involve the dopaminergic (DA) system, and to depend on synthesis of this transmitter substance. For morphine, less is known of sites mediating stimulant effects and of the role played by CA systems in producing them. For this compound, interest has focused on its analgesic-sedative action [20] and stimulant effects are of a less robust nature than those produced by amphetamine. There are also differences in modulation of stimulant effects produced by the two drugs when activity in the central CA systems is altered [11,17], but the significance of these data is not yet clear.

Investigations of the stimulant effects of amphetamine have relied on the use of drugs to effect alteration in CA systems. One of these drugs is the neurotoxin 6-OHDA whose action on CA neurons results in depletion of levels in regions receiving CA input. However, the action of this compound is long-lasting and complex [9, 12, 18] and therefore may influence subsequent drug effects depending on the time of testing in relation to administration of the neurotoxin. In recent years, this drug has been given neonatally to animals to effect a more drastic depletion of central CA levels and at the same time, when tested in adults, to allow enough time for the CA systems to become reorganized. One of the findings of these studies is that levels of norepinephrine (NE) in the pons become elevated in the adult animal [6, 13, 16, 19]. We have taken advantage of this preparation to obtain evidence of the role played by CA systems, when they are reorganized, in the behavioral stimulant effects of amphetamine and morphine on the assumption that such information may contribute to an understanding of the possible differential role played by forebrain and hindbrain CA regions in drug-induced hyperactivity.

METHOD

Subjects

Comparable numbers of male and female Holtzman-derived rats were used. These animals were housed in plastic cages in a room that was temperature- (23°C) and light- (12 hr light-dark cycle) controlled.

Neurotoxin Treatments

Pups were randomly assigned to the 6-OHDA and control groups and were injected according to the procedures described by Creese and Iversen [3]. Briefly, on days 3, 5, 7,

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and 9 they were given bilateral, intraventricular injections of 6-OHDA, in an artificial cerebrospinal fluid (CSF) vehicle, or the vehicle only [15]. Solutions injected in test and control animals contained ascorbic acid as an antioxidant (1 mg/ml). The skin over the skull was retracted under metafane anesthesia and a 5 μl Hamilton syringe was inserted over each lateral ventricle. The coordinates used were 0.2–0.3 mm posterior to bregma and 0.5 mm lateral to the midline; the depth of needle insertion varied with the subject's age. After the injection was completed, the needle was left in position for one minute to prevent the fluid backing up along the track of the needle.

Different groups of rats received different total doses of 6-OHDA. Three doses—100, 200, and 400 μg 6-OHDA (total dose given as free base)—were given. Each of these was divided into four equal amounts to be injected on the four treatment days. On each of these injection days, the amount to be injected was divided into two equal parts, one for each lateral ventricle, and injected in a volume of 5 μl per ventricle.

Drugs

Morphine sulfate was tested at doses of 1.25, 2.5, and 3.5 mg/kg and d-amphetamine sulfate at a dose of 2 mg/kg. The drugs were dissolved in physiological saline and injected IP, midway in the 180-minute sessions.

Activity Measurements

Activity was measured in a stabilimeter cage contained inside of a darkened and sound-deadening chamber. A 20×33 cm Plexiglas floor was suspended by a thin wire at each corner and this floor was capable of moving as much as 2.5 cm along its width and length. Attached to one side was a checkerboard of alternating, optically transparent and opaque squares through which passed a light beam to a photocell. Movements of the floor disrupted the beam with the photocell producing a voltage recorded as an activity count. The sensitivity of the apparatus was adjustable and, for the present experiments, was set to record shifts of body, rearing, grooming, as well as locomotion.

The behavioral stimulant effects of amphetamine sulfate (2 mg/kg IP) were tested at 30 (n=20) and 60 (n=20) days of age on rats neonatally treated with CSF or 100, 200, or 400 μg total dose of 6-OHDA. The behavioral stimulant effects of morphine were tested in 21 adult rats treated with CSF or 100 or 200 μg 6-OHDA. No tests were conducted with morphine in a group which received a 400 μg dose of 6-OHDA because the results obtained with those treated with the two lower doses indicated that such a test was not necessary.

Morphine was tested at different, low doses in view of the lower robustness of the stimulant effects of morphine usually reported. Amphetamine was tested at a dose of 2 mg/kg on the basis of information in the literature that this dose usually produces behavior stimulation in control subjects.

A test session lasted 180 min and the drug was injected after the first 90 min of testing. After the injection of amphetamine or morphine, the animal was immediately replaced in the test chamber.

Analysis of the Data

Scores for individual animals were normalized by an arc sin of the square root transformation to control for any minor changes in sensitivity of the apparatus. Group scores computed for 30-min segments, before and after midpoint drug administration, provided tests for drug effects; the values for time segment 3 (just before drug injection) were compared with those in segments 5 and 6 after drug injection. Statistical evaluation of changes in activity was based on a mixed design analysis of variance and Duncan Multiple Range Tests ($p<0.05$).

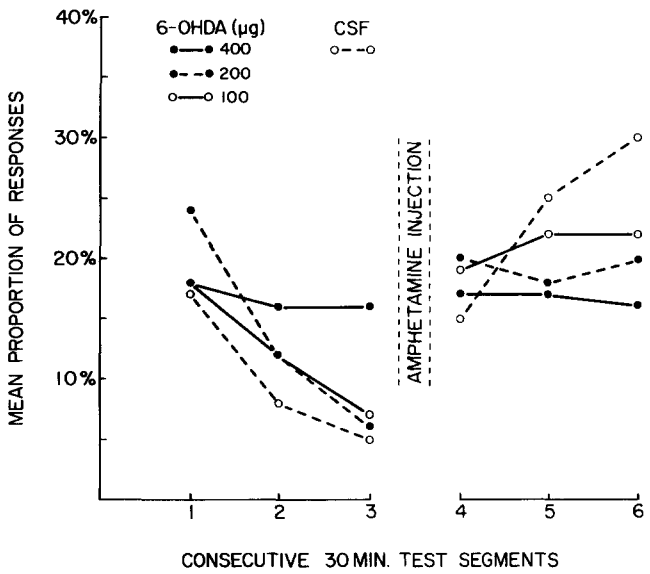

FIG. 1. Mean activity counts with amphetamine, expressed as percentages of the total number of responses during the entire 180 minute session, as a function of time (abscissa) for several treatments.

RESULTS

Activity Levels of Adult Rats Treated Neonatally with 6-OHDA

The pre-injection results are shown in Figs. 1 and 2, Segments 1–3, comprising the first 90 min of a test session. In Fig. 1 controls and subjects treated with 100 and 200 μg 6-OHDA became less active over time, $F(15,160)=6.73$, $p<0.001$. From proportions which ranged from approximately 24–17% during the first 30 min of the session, activity declined to 12–8% during the next 30 min, and then to levels of 7–5%. Thus, in the 90 min period before drug injection, controls and rats treated with 100 and 200 μg showed a similar reduction over time of motor activity, indicating a process of habituation to the test situation. The same effect is shown in Fig. 2 (Segments 1–3) prior to morphine injection, $F(10,180)=3.85$, $p<0.001$.

In contrast, animals treated with 6-OHDA at the 400 μg dose did not evidence habituation (Fig. 1). The activity during the first 30 min of the session was lower than that for the group treated with 200 μg, but it remained at the same level for the next two 30 min segments. For the group treated with 200 μg, activity in the second and third segments showed a sharp decline.

Effects of Amphetamine on Behavioral Activity Levels

An analysis of variance was used to evaluate the changes in activity accompanying amphetamine taking into account the animals' ages at testing (30 and 60 days), the neonatal treatment received (CSF or 100, 200, 400 μg 6-OHDA), and test segment in terms of consecutive 30 min intervals. The

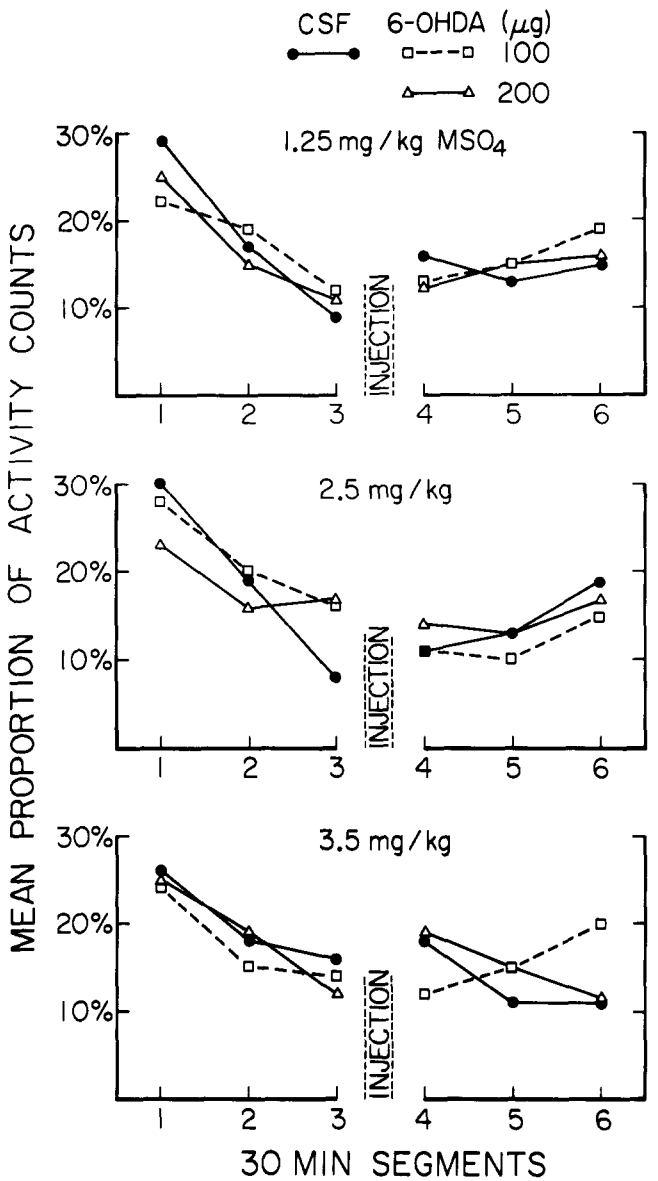

FIG. 2. Mean activity counts with morphine, expressed as the percentages of the total number of responses during the entire 180 minute session, as a function of time for several treatments.

type of pretreatment given and test segment were significant variables; the age of the animals was not. The neurotoxin by test segment interaction indicated that the amphetamine produced an increase in activity in controls (Fig. 1), $F(15,160)=6.73$, $p<0.001$. This effect resulted in the level of activity returning to the level shown in the first 30 min in the test chamber (segment 1) before the injection of amphetamine. From that level, activity continued to increase throughout the 90 min of the post-injection period (segments 4, 5, 6). Since the injection itself could have initially increased the activity level of the animals, the first segment after injection (segment 4) was not examined by the Duncan test. However, it should be noted that counts represented activity for 30 min and we found activity due to handling the controls to last only a few minutes.

The animals treated at birth with 100 and 200 μg doses of 6-OHDA also showed increases in activity after amphetamine (segment 3 versus 5 and 6) and these also lasted for the duration of the session (90 min) without evidencing the habituation pattern found during the first half of the session. However, in these two groups of animals, compared to controls, the stimulant effect was attenuated. During the first 30 min post injection, the level of activity of these animals was the same as in controls and the change effected by amphetamine was of the same magnitude as that produced in controls. However, while activity during the next two segments was greater than that during segment 3, further increases in activity did not take place in the two treated groups as it did in controls.

No increase in activity was produced by amphetamine in the group of animals treated with a dose of 400 μg of 6-OHDA. The level of activity throughout the 90 min period before the injection of amphetamine was maintained throughout the 90 min after injection, and there was no evidence that handling of the animals had any effect. Thus, treatment at birth with 6-OHDA altered the animals' responses to amphetamine. The lower doses of the toxin attenuated the stimulant behavioral effects, but the higher dose blocked it, although the base line activity of these animals was higher than in the other groups toward the end of the pre-injection period.

Effects of Morphine on Behavioral Activity Levels

An analysis of variance of the changes in activity accompanying the injection of morphine took into account the neonatal pretreatment (CSF, 100, 200 μg of 6-OHDA), the dose of morphine injected (1.25, 2.5, or 3.5 mg/kg) and the test segment in terms of 30 min intervals. The dose of morphine given and test segments were significant variables; neonatal pretreatment was not.

The analysis of the neonatal neurotoxin treatment by morphine doses and test segments interaction showed that, in control animals, morphine (1.25 and 2.5 mg/kg) increased motor activity by the sixth segment. That is, between 60 and 90 min after injection, $F(20,180)=1.82$, $p<0.01$, as depicted in segment 3 versus 5 and 6 in Fig 2. Morphine at the 3.5 mg/kg dose had no effect on the motor activity of control subjects (Fig. 2).

In the two groups of treated animals, morphine produced no significant changes in motor activity during the 90 min of postinjection observation. Thus, for the period analyzed, the stimulant effects obtained with morphine in controls, at doses of 1.25 and 2.5, were blocked in treated subjects.

DISCUSSION

The evidence of these experiments indicates that the central CA systems influence the stimulant action of morphine and amphetamine. Treatment with the neurotoxin shortly after birth produced in the adult animal elevated NE in the pons [6,16] that, for morphine, was not adequate to compensate for the loss of forebrain CA to produce stimulant effects. The stimulant effect of amphetamine was attenuated, but not blocked, in the groups treated with 100 and 200 μg 6-OHDA; it was blocked in the group of rats treated with 400 μg. This indicates a dose response effect suggesting that stimulant effects depend on the magnitude of the CA depletion in regions containing terminal fields. These findings can be interpreted to support the notion that the stimulant effects of both

compounds are mediated in the regions of CA terminal fields.

We have established that cortical and ventral diencephalic levels of CA are virtually depleted after 400 μg 6-OHDA compared to 100 μg and 200 μg [6] using identical procedures to inject 6-OHDA into rat pups. In the midbrain and the hindbrain, at 90 days of age the levels are significantly depleted only after the 400 μg dose. Thus, it appears that when enough axons remain functional, as indicated by a moderate to mild degree of depletion of CA levels, amphetamine produces its stimulant effects. Where depletion was drastic in the forebrain, and sufficient in midbrain and hindbrain, it blocked the stimulant effects. There was no compensatory effect produced by supersensitivity of the surviving axons. We conclude therefore that activity of the CA terminal fields which lie in cortex, ventral diencephalon, and possibly other regions in forebrain, mediated the stimulant effects of amphetamine. The elevation of NE in pons, which we reported after treatment with 100 and 200 μg doses, apparently was not effective in counteracting the effect of CA depletion in forebrain terminal fields.

The evidence obtained likewise supports the notion that the stimulant action of morphine is mediated in forebrain, not in midbrain or hindbrain. In the 100 and 200 μg treated rats, the delayed stimulant action of morphine (in 60–90 min period after injection) was blocked. It seemed apparent that the stimulant effect of morphine was less robust than that of amphetamine and, as a result, was more sensitive to interference than the amphetamine action. It could mean that for amphetamine, whose action is said to depend on the release of newly synthesized CA [1], the axons that remained released sufficient transmitter to activate the necessary number of target neurons. Whereas for morphine, whose action depends on binding to opiate receptors, injury to CA terminals might have resulted in an alteration of the interaction between the CA systems and opiate receptors resulting in a blockade of the stimulant effect of morphine. Thus, injury to CA terminal fields in the forebrain reduced or abolished the behavioral stimulation produced by amphetamine, owing probably to reduction in the amount of transmitter released, and blocked the stimulant effect of morphine probably through alteration of the anatomical relationship between CA terminals and opiate receptors in forebrain.

A number of studies have been concerned with the role played by the central CA systems in the stimulant actions of amphetamine and morphine [3, 7, 10, 17]. The evidence obtained suggests that different mechanisms underlie the two effects, although they may be mediated in the same structures by mechanisms involving the CA systems. For example, it was reported that inhibition of NE and DA synthesis, produced by chronic administration of methyl-tyrosine, blocked the stimulant effects of amphetamine, but only transiently affected the stimulant effects of morphine (produced with a dose of 5 mg/kg) [11]. In view of the dependence of amphetamine on newly synthesized CA for its stimulant action [3,4], but not of morphine, whose action depends directly on its binding to opiate receptors and only indirectly on CA release, these results are not surprising. Our experiments highlight a different aspect of the role played by CA in the behavioral stimulation of these two drugs. The opposite effect was shown, as morphine stimulant effects were abolished at doses that produced only an attenuation of the amphetamine stimulant effect. In our experiments, it may have been that synthesis of CA was reduced. However, we suggest that the primary effect involved inefficiency of the machinery, through a reduction of terminals which made the transmitter available in certain regions of the forebrain, that was correlated with the altered behavioral responses to amphetamine and morphine.

Additional evidence for a different role of CA systems in the stimulant effects of amphetamine and morphine was obtained by Teitelbaum and associates in mice [17]. They showed that electrolytic or 6-OHDA-induced DA lesions of the nucleus accumbens, a region receiving its DA input from the ventral tegmentum, are more effective in blocking the stimulant effects of amphetamine than of morphine. The absence of CA terminals in the nucleus accumbens may have produced a lack of post-synaptic action after amphetamine, while the absence of CA terminals may have led to a less efficacious binding of morphine to opiate receptors. Their study implicates the nucleus accumbens in the stimulant action of amphetamine and less so in the stimulant action of morphine. Our findings are consistent with these effects on the basis of the interpretation we have given our results. We conclude that the stimulant action of both drugs depends on activity of CA terminal fields in forebrain rather than on activity of such terminals in midbrain and hindbrain, since no compensatory action was observed here. Furthermore, the evidence suggests a different role for forebrain CA in mediating the stimulant action of both drugs.

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