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Post-Amphetamine Depression of Self-Stimulation Responding from the Substantia Nigra: Reversal by Tricyclic Antidepressants

LARRY KOKKINIDIS,[1] ROBERT M. ZACHARKO AND PATRICK A. PREDY

University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

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KOKKINIDIS, L., R. M. ZACHARKO AND P. A. PREDY. *Post-amphetamine depression of self-stimulation responding from the substantia nigra: Reversal by tricyclic antidepressants.* PHARMAC. BIOCHEM. BEHAV. 13(3) 379–383, 1980.—The effects of long-term amphetamine treatment were examined on self-stimulation responding from the substantia nigra. Rates of self-stimulation responding were substantially depressed among rats chronically treated with amphetamine and tested in the absence of the drug. When rats were subsequently retested after a two day hiatus in which they received imipramine or amitriptyline, the post-amphetamine depression of rates of self-stimulation responding was mitigated. The efficacy of imipramine and amitriptyline in reversing the post-amphetamine depression of self-stimulation responding was also evident during a continuation of the drug (imipramine or amitriptyline)/test sequence, for seven test sessions. The results of the present investigation were related to changes in dopamine and acetylcholine neurotransmission following long-term amphetamine treatment.

Self-stimulation Chronic amphetamine treatment Imipramine Amitriptyline Response depression

TO date, there exist several animal models by which the pharmacology of depression has been evaluated. First, reserpine-induced depression in animals has received considerable attention following the observation that humans treated with reserpine for hypertension developed depressions that were similar in many respects to clinically observed endogenous depressions [6, 8, 20]. Among infrahuman subjects, reserpine also induces certain symptoms that are characteristic of depression [6], and neurochemically, it appears that drug-induced depletion of monoamines may be responsible for the observed behavioral depression [8,20]. Consistent with this notion, tricyclic antidepressants that are efficacious in the treatment of endogenous depressions [6,14], and among other things, block the reuptake of monoamines [8,20], alleviate the reserpine-induced depression in animals [6,24]. In addition to their effects on monoamine activity, tricyclic antidepressants are also potent anticholinergic agents [7]. For example, pretreatment with tricyclic antidepressants was found to potentiate apomorphine-induced stereotypic behaviors [21]. Given the well defined balance between dopamine and acetylcholine in the nigro-neostriatal pathway [12], it was suggested that the anticholinergic actions of the tricyclic antidepressants potentiated the dopamine effects of apomorphine [21]. Although it is well documented that tricyclic antidepressants have anticholinergic effects there is a relative paucity of data implicating the anticholinergic effects of tricyclic antidepressant agents to the antidepressant properties of these drugs.

A second model of depression that has received considerable attention is the development of "learned helplessness" in animals following exposure to inescapable shock [1, 19, 23]. In particular, when animals are exposed to a stressor such as a footshock, and the organism has little or no control over its response to the shock, then in a subsequent escape task animals display pronounced deficits in escape performance [19]. The appearance of the escape deficits following inescapable shock appear to be related to a stress-induced decrease in catecholamine activity [1,26], and are mitigated by pretreatment with tricyclic antidepressants [23]. Somewhat related to the stress-induced interference of escape performance is the behavioral despair model of depression [4]. In this paradigm, rats or mice that are forced to swim in a cylinder with little chance of escape become immobile and assume a floating posture. As is the case with the "learned helplessness" phenomena, treatment with tricyclic

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antidepressants produced a marked improvement in the stress-induced immobility [4].

Finally, a third model involves the effects of chronic amphetamine treatment on self-stimulation behavior. It has been demonstrated that chronic exposure to amphetamine results in a pronounced depression in rates of self-stimulation responding for intracranial brain stimulation [15]. More specifically, if animals receive long-term amphetamine treatment and are subsequently tested for intracranial self-stimulation following a saline injection, performance is substantially depressed relative to that observed among animals that were not exposed to the drug [15]. Moreover, the post-amphetamine depression of self-stimulation responding is evident when electrode placements are either in the lateral hypothalamus [17], or the substantia nigra [15]. Although the neurochemical effects of chronic amphetamine treatment are not well understood, long-term amphetamine administration produces a depletion of norepinephrine [3], a decrease in dopamine synthesis [22] and neurotoxic effects on dopamine terminals [10]. Thus, it is likely that hypoactive catecholamine activity induced by repeated amphetamine treatment may be involved in the observed post-amphetamine depression of self-stimulation responding [15].

The present study was designed to evaluate the effects of tricyclic antidepressants on the post-amphetamine depression of intracranial self-stimulation. Since the neurochemical consequences of chronic amphetamine treatment parallel those observed after reserpine treatment or exposure to shock (i.e., decreased catecholamine activity), then it is expected that administration of a tricyclic antidepressant will mitigate the detrimental effects of long-term amphetamine administration on self-stimulation responding. Accordingly, the effects of the tricyclic antidepressants, imipramine and amitriptyline on the depression of self-stimulation responding following chronic amphetamine treatment were evaluated in two experiments.

METHOD

Subjects

In Experiment 1a twenty-four naive male rats (Charles River CD, outbred albino) procured from the Canadian Breeding Farms and Laboratories, Quebec, Canada served as subjects. Sixteen naive rats served as subjects in Experiment 1b. Rats weighed approximately 300–350 g at the initiation of the experiment. Subjects were housed individually and permitted free access to food and water throughout the duration of the experiment. Rats were maintained on a regular 12 hr light/dark cycle and behavioral testing was carried out during the light portion of the cycle.

Apparatus

The apparatus was similar to that described by Kokkinidis and Zacharko [15] and consisted of two identical Plexiglas boxes 60 cm in length, 20 cm in width and 30 cm in height. In each box, two photobeam units were mounted 2.5 cm above the grid floor and 6.0 cm from each end of the box. When the photobeams were interrupted by either head or body movements, electrical brain stimulation was initiated for a duration of 0.3 sec. Brain stimulation was delivered from an Ortec Dual Channel Stimulator with a standard current intensity of 30 μA (biphasic square wave), pulse frequency of 100 Hz and pulse width of 0.1 msec.

Procedure (Experiment 1a)

Subjects were anesthetized with sodium pentobarbital (45 mg/kg) and were stereotaxically implanted in the substantia nigra with an insulated bipolar nichrome electrode (Plastic Products) that had 0.5 mm of the tips separated and scraped. The co-ordinates for electrode placement were anterior-posterior, −4.5 mm from bregma, lateral, −2.5 mm from the midline suture, and vertical, −8.5 mm from a horizontal skull surface.

Self-Stimulation Training

Following a seven day post-operative period, rats were tested for self-stimulation for a ten min session daily for eight consecutive days. During the first three days of training the duration of the reinforcement was made contingent upon the initiation and termination of the animal's response. During the final five days of self-stimulation training the duration of the electrical brain stimulation remained constant at 0.3 sec per response. Only the rates of responding during the last five days of self-stimulation training were considered in the average baseline score for each animal. We have found that this method of training yields reliable and stable rates of self-stimulation responding.

Chronic Drug Treatment

Following baseline testing, rats were assigned to one of three groups (N=8 per cell). Animals assigned to the three groups were selected to equalize average baseline response rates among the three groups. After baseline testing, two groups of animals received two intraperitoneal (IP) injections of 7.5 mg/kg of d-amphetamine sulfate daily (10:00 a.m. and 4:00 p.m.) for ten consecutive days, whereas the remaining group of animals received chronic saline treatment in the same manner. On Day 11, all animals were tested for self-stimulation during a ten min session, 30 min after a saline injection (Test Session 1).

Imipramine Treatment

The day following the first test session animals in one of the chronic amphetamine groups and subjects in the chronic saline group received an injection of imipramine hydrochloride (10 mg/kg). A second injection was administered 24 hr after the first. The remaining chronic amphetamine group received two saline injections in the same manner. Behavioral testing was not conducted during the two days of drug administration. Twenty-four hours following the second drug injection all animals were tested for self-stimulation for a 10 min session, 30 min after a saline injection (Test Session 2). This sequence (imipramine, imipramine, test or saline, saline test) was continued for a total of seven test sessions (a total of fourteen drug injections).

Procedure (Experiment 1b)

Following surgical implantation of electrodes in the substantia nigra and self-stimulation training, rats were assigned to one of two groups (N=8/cell) based on equalized average baseline scores. During the chronic phase of Experiment 1b, animals in both groups were treated with two IP injections of d-amphetamine sulfate (7.5 mg/kg) daily for ten consecutive days. Twenty-four hours following the last amphetamine injection, animals in both groups were tested for self-stimulation for a ten min session, 30 min after a saline injection (Test Session 1). The day following the first test session, rats in one of the experimental groups received an IP injection of amitriptyline hydrochloride (10 mg/kg), followed by a sec-

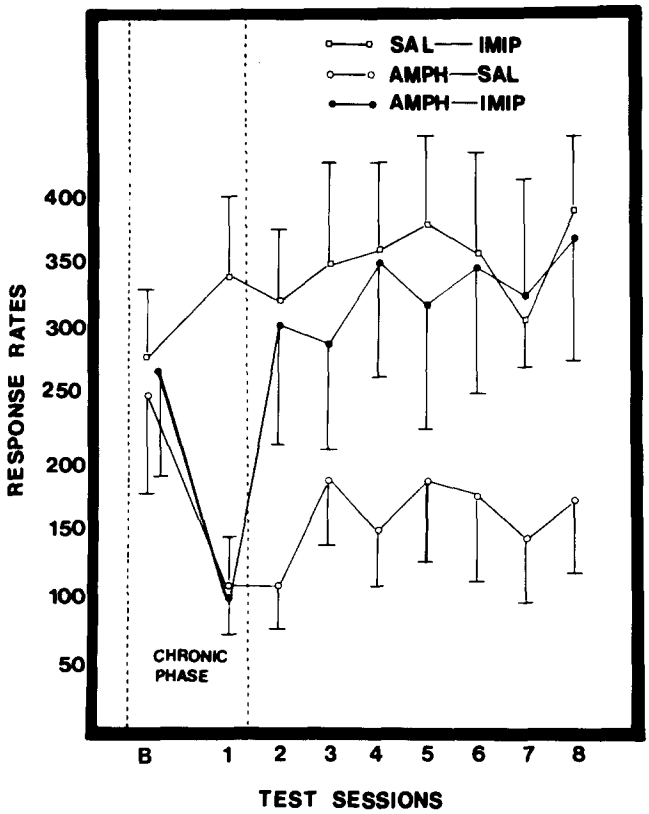

FIG. 1. Mean (±SEM) rate of self-stimulation responding as a function of chronic drug treatment (saline or two injections of 7.5 mg/kg of d-amphetamine daily for ten consecutive days) and drug treatment (saline or 10 mg/kg of imipramine) two days prior to each of the last seven test sessions. B, on the abscissa represents average baseline rates of the three groups. Test sessions were 10 min in duration. Test session 1 represents performance of animals exposed to drug treatment during the chronic phase and saline on test day.

ond injection 24 hr later. The remaining group received two injections of saline in the same manner. Twenty-four hours following the second drug injection, rats in both groups were tested for self-stimulation for a ten min session following a saline injection (Test Session 2). This sequence (amitriptyline, amitriptyline, test or saline, saline test) was continued for seven test sessions.

At the termination of the experiment rats were sacrificed under chloroform anesthesia, perfused intracardially with 0.9% physiological saline followed by 10% Formalin and the brains were removed for histological verification of electrode placements.

RESULTS

Microscopic examination of electrode tract loci revealed that all placements were situated in the substantia nigra between sections A2180–A2580 of König and Klippel [16]. The majority of the electrode placements were located in the lateral portion of the pars compacta. Five tracts were found to be in the medial portion of the pars compacta. In eight animals electrode tips were also verified within the zona reticulata. In all cases reliable and stable rates of self-stimulation responding were noted.

Analysis of variance of the self-stimulation scores in Experiment 1a yielded a significant Group×Test Session interaction $F(16,168)=2.35$, $p<0.01$. Subsequent Newman Keuls multiple comparisons ($\alpha=0.05$) of the simple main effects involved in the interaction revealed that long-term exposure to amphetamine had pronounced effects on self-stimulation responding (see Fig. 1). Both groups of rats that were exposed to the chronic drug regimen and then tested with saline on the first test session, showed a significant depression of self-stimulation responding relative to baseline performance rates. A significant depression of self-stimulation responding was also evident when performance of animals in the two chronic amphetamine groups were compared to that of animals that were chronically treated with saline and tested with saline on the first test session. As can be seen in Fig. 1, treatment with imipramine for two days prior to each test session had a small facilitative effect on rates of responding among animals treated with saline during the chronic phase. However, the increased response rates were not significantly different from baseline performance. Although imipramine had no effect on self-stimulation responding among control animals, administration of imipramine for two days prior to each test session to animals chronically exposed to amphetamine had substantial effects on self-stimulation performance. In particular, rats treated with amphetamine during the chronic phase and imipramine for two days prior to each test session showed significantly higher response rates relative to animals in the amphetamine-saline group. The difference between the two chronic amphetamine groups was evident on test sessions 2–8. Indeed, on test sessions 2–8 performance of rats treated with amphetamine during the chronic phase and imipramine two days prior to each test session, exhibited rates of responding that were comparable to that evidenced among animals that were administered saline during the chronic phase, and treated with imipramine two days prior to each test session.

The mean rates of self-stimulation responding in Experiment 1b as a function of amphetamine treatment during the chronic phase and drug administration two days prior to each of the last seven test sessions is depicted in Fig. 2. Analysis of variance of these data yielded a significant Group×Test Session interaction $F(8,112)=7.10$, $p<0.001$. Newman Keuls multiple comparisons ($\alpha=0.05$) of the simple main effects involved in this interaction revealed that repeated injections of amphetamine during the chronic phase, produced depressed rates of self-stimulation responding on the first test session relative to baseline performance in both groups of animals. As can be seen in Fig. 2, rats in the amphetamine-saline group showed a larger depression of self-stimulation responding relative to the remaining group, however, this difference was not statistically significant ($p>0.1$). Although the depression of self-stimulation responding observed in the amphetamine-saline group recovered somewhat, performance on all test sessions was significantly lower than baseline rates. Rates of responding of animals in the amphetamine-amitriptyline group were significantly lower than baseline rates on the second and third test sessions, despite the fact that animals were treated with amitriptyline two days prior to each test session. However, self-stimulation responding of these animals on test sessions 4–8 significantly improved following treatment with amitriptyline two days prior to each test session and performance did not significantly differ from baseline rates of responding. Response rates of animals treated with amphetamine during the chronic phase and amitriptyline two days prior to each test session were significantly higher on test sessions 2–8 relative to performance of animals in the amphetamine-saline group.

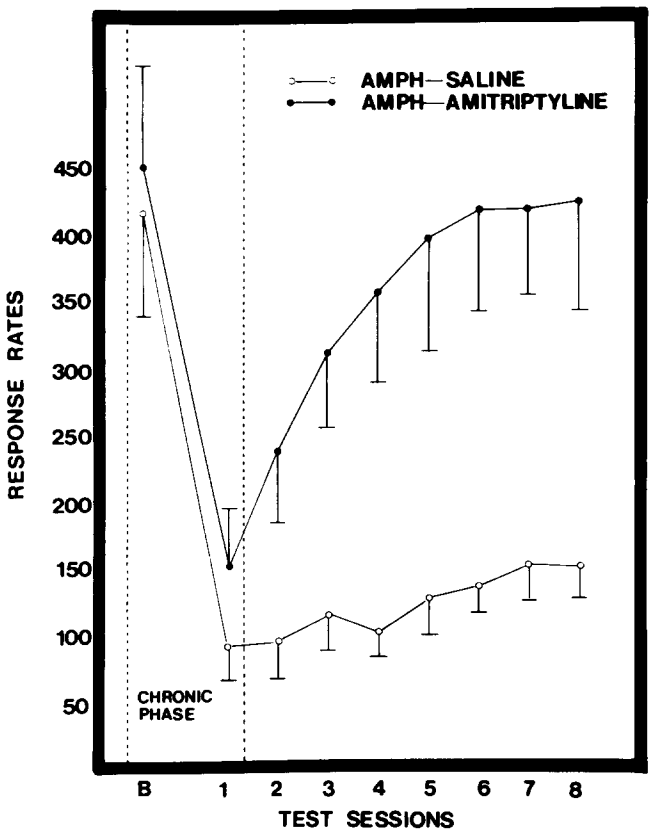

FIG. 2. Mean (±SEM) rates of self-stimulation responding as a function of chronic drug treatment (two injections of 7.5 mg/kg of d-amphetamine daily for ten consecutive days) and drug treatment (saline or 10 mg/kg of amitriptyline) two days prior to each of the last seven test sessions. B, on the abscissa represents average baseline rates of the two groups. Test session 1 represents performance of animals following chronic amphetamine treatment.

DISCUSSION

Consistent with previous reports [15,17], long-term amphetamine treatment produced pronounced effects on intracranial self-stimulation. In particular, rats with electrodes situated in the substantia nigra demonstrated stable and reliable rates of self-stimulation responding. Following long-term amphetamine treatment, however, self-stimulation responding was substantially depressed relative to baseline rates when animals were tested in the absence of the drug.

The observed post-amphetamine depression of self-stimulation responding was modified by treatment with tricyclic antidepressants. Specifically, imipramine injections were effective in reversing the depressed rates of self-stimulation responding induced by repeated amphetamine administration. This was the case despite the fact that imipramine administration to rats that were exposed to saline during the chronic phase of Experiment 1a had no effect on self-stimulation responding, even after fourteen days of exposure to the drug. This finding is consistent with reports from other laboratories, showing that imipramine has little or no effect on self-stimulation behavior [25]. The results of the present investigation also revealed that the reversal of the post-amphetamine depression of self-stimulation responding by imipramine was not specific to this compound. Treatment with amitriptyline was found to be effective in alleviating the depressing effects of repeated amphetamine administration on self-stimulation responding as well.

Although treatment with both imipramine and amitriptyline antagonized the post-amphetamine depression of self-stimulation responding, the mechanism of action of these compounds is not entirely clear. Clinically, it is thought that the antidepressant action of these agents involves the inhibition of the reuptake of monoamines at the synapse [6, 8, 20, 24]. Both imipramine and amitriptyline are effective in blocking the reuptake of serotonin [8], but imipramine, in contrast to amitriptyline strongly blocks the reuptake of norepinephrine, as well [6,24]. The noradrenergic effect of imipramine is presumably the result of an active metabolite, desemethylimipramine [6]. The general consensus is that tricyclic antidepressants have little or no effect on dopamine reuptake mechanisms [7], although there are some studies to the contrary [5].

Thus, several possibilities exist that may account for the effects of imipramine and amitriptyline on the post-amphetamine depression of self-stimulation responding from the substantia nigra. Since chronic amphetamine treatment produces a substantial depletion of brain norepinephrine [3], it might be the case that decreased noradrenergic activity is responsible for the post-amphetamine depression of self-stimulation behavior. Although self-stimulation was supported from the substantia nigra, an area containing primarily dopamine cell bodies, it is well documented that noradrenergic activity is an important substrate for self-stimulation responding from this area [2]. Incongruous with such a position, however, is the fact that amitriptyline, which has weak effects on norepinephrine reuptake mechanisms [8], was found to be effective in mitigating the post-amphetamine depression of self-stimulation responding from the substantia nigra, suggesting a role for serotonin, in this respect. The available data concerning the effects of long-term amphetamine treatment on serotonergic activity, however, are somewhat enigmatic. Prolonged exposure to amphetamine has been reported to result in either no change [11], an increase [9], or a decrease [18], of brain serotonin levels. Given this state of affairs, the question of whether the antidepressant effects of imipramine and amitriptyline on the post-amphetamine depression of self-stimulation responding from the substantia nigra, involves the inhibition of reuptake of norepinephrine or serotonin, remains an open one.

An alternative possibility deals with the effects of tricyclic antidepressants on cholinergic activity. Specifically, tricyclic antidepressants are known to be potent anticholinergic agents [7]. Clinically, treatment with imipramine and amitriptyline may result in undesired side effects, such as weakness and fatigue, that are attributable to the central cholinergic actions of these agents [7]. The anticholinergic effects of tricyclic antidepressants have also been well documented in animals. For example, Pederson [21] found that imipramine and amitriptyline potentiated the stereotypic response to apomorphine in mice. It was suggested that these effects were due to the anticholinergic actions of the antidepressant agents, resulting in a concomitant enhancement of dopaminergic activity [21]. More specifically, it is well documented that there exists a reciprocal balance between acetylcholine and dopamine in the nigro-neostriatal pathway. Thus, it might well be the case that the anticholinergic properties of imipramine and amitriptyline are responsible for the reversal of the observed post-amphetamine depression of self-stimulation responding from the substantia nigra. Consistent with this position, chronic amphetamine

treatment has been shown to produce increased choline acetylase activity implying enhanced cholinergic activity at nerve terminals [13]. Further to this point, chronic exposure to amphetamine also results in a decreased synthesis of dopamine [22]. Taken together then, these findings suggest that the post-amphetamine depression of self-stimulation responding may reflect the effects of long-term amphetamine treatment on the dopamine/acetylcholine balance in the nigro-neostriatal pathway. Thus, a speculative suggestion is that chronic amphetamine administration disrupts the dopamine/acetylcholine balance in the nigro-neostriatal pathway resulting in a depression of self-stimulation responding from the substantia nigra. The efficacy of imipramine and amitriptyline in reversing the behavioral depression may be related to a restoration of the dopamine/acetylcholine balance as a result of the anticholinergic properties of these drugs.

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