Abstract Rationale: The activation of NMDA receptors in the rat ventral tegmental area has been proposed to be necessary for the induction of locomotor sensitization by amphetamine, yet there has been no direct assessment of this view. Objective: The present study examined the ability of the competitive NMDA receptor antagonist D(-)-2-amino-5-phosphonopentanoic acid (AP-5) to block this effect when infused either into the ventral tegmental area or, for comparison, into the nucleus accumbens. These sites are known to be important for the induction and expression, respectively, of locomotor sensitization by amphetamine. Methods: Rats in different groups received four pairs of injections (one IC and one IP), one pair given every third day. The IC injection (0, 1 or 5 nmol/side AP-5) was administered immediately before the IP injection (saline or amphetamine, 1 mg/kg). Locomotor activity was measured following each pair of injections and again 2 weeks later when all rats were tested for sensitization following a challenge injection of amphetamine (1 mg/kg, IP). AP-5 was not administered on this test. Results: As expected, rats previously exposed to amphetamine alone showed higher levels of horizontal locomotion and rearing on the test for sensitization when compared to saline pre-exposed rats. Preceding the amphetamine pre-exposure injections with infusions of AP-5 into the ventral tegmental area, but not the nucleus accumbens, dose-dependently blocked the induction of this effect. Rats previously exposed to AP-5 alone in either site did not differ significantly from saline pre-exposed rats on the test for sensitization. Conclusion: The results indicate that NMDA receptor activation in the ventral tegmental area, but not the nucleus accumbens, is necessary for the induction of locomotor sensitization by amphetamine.

Keywords Locomotor sensitization · Amphetamine · Ventral tegmental area · Nucleus accumbens · NMDA receptor · AP-5 · Excitatory amino acids · Psychomotor stimulant · Locomotion

Introduction

Previous exposure to amphetamine is known to lead to sensitized locomotor responding to subsequent administrations of the drug (for review, see Kalivas and Stewart 1991). Midbrain dopamine neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) play a critical role in this effect. The results of several studies now indicate that amphetamine acts in the VTA to initiate the induction of locomotor sensitization and in the NAcc to promote its expression (Kalivas and Weber 1988; Vezina and Stewart 1990; Hooks et al. 1992; Perugini and Vezina 1994; Cador et al. 1995).

Both the VTA and the NAcc receive excitatory amino acid inputs from a number of sources including the medial prefrontal cortex (mPFC) and limbic structures such as the hippocampal formation and amygdala (Christie et al. 1987; Kalivas 1993; Meredith et al. 1993). Of these projections, those originating in the PFC and innervating the VTA have been most implicated in the locomotor sensitization produced by psychomotor stimulants (for review, see Wolf 1998). For example, bilateral lesions of the mPFC prevent the induction of locomotor sensitization by amphetamine and cocaine as well as neuroadaptations in the VTA reported to accompany this process (Wolf et al. 1995; Li et al. 1999). Conversely, electrical kindling of the mPFC has been reported to sensitize cocaine’s locomotor effects (Schenk and Snow 1994). In addition, induction of behavioral sensitization by amphetamine and cocaine is accompanied by transient increases in the VTA both in glutamate receptor subunit expression (Fitzgerald et al. 1996) and in the reactivity of dopamine neurons to glutamate (White et al. 1995; Zhang et al. 1997). Consistent with these findings suggesting a role for excitatory amino acids in locomotor...
sensitization by psychomotor stimulants, there have been many reports that NMDA receptor antagonists can prevent its induction when administered prior to sensitizing drug injections during pre-exposure (Karler et al. 1989; Wolf and Khansa 1991; Stewart and Druhan 1993; Wolf et al. 1995; for review, see Wolf 1998). Surprisingly, very few studies to date have assessed directly the effect of blocking excitatory amino acid receptors in the VTA on the development of locomotor sensitization to stimulant drugs. In one, for example, bilateral infusions of 3-(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid or dizocilpine (competitive and non-competitive NMDA receptor antagonists, respectively) into the VTA, but not the NAcc, blocked the induction of “one shot” locomotor sensitization by systemically administered cocaine (Kalivas and Ales-datter 1993). In another, it was found that co-injecting amphetamine with the selective metabotropic glutamate receptor antagonist (RS)-α-methyl-4-carboxyphenylglycine on four occasions into the VTA prevented the induction of locomotor sensitization normally produced by amphetamine alone (Kim and Vezina 1998). The present study assessed the ability of the competitive NMDA receptor antagonist d(-)-2-amino-5-phosphonopentanoic acid (AP-5) infused either into the VTA or, for comparison, into the NAcc to prevent the induction of locomotor sensitization by systemically administered amphetamine.

Materials and methods

Animals and surgery

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, Wisc., USA) weighing 250–275 g on arrival were used. They were individually housed with freely available food and water in a reverse cycle room (12-h light/12-h dark) for the duration of the experiment. All procedures were conducted during the dark period of the light cycle. Following 4–5 days of acclimation to housing conditions and daily handling, rats were anaesthetized with ketamine (100 mg/kg, IP) and xylazine (6.0 mg/kg, IP), mounted on a stereotaxic instrument with the incisor bar positioned 5.0 mm above the interaural line and implanted with bilateral guide cannulae (22 gauge; Plastics One, Roanoke, Va., USA) aimed either at the VTA (AP, −3.6; L, ±0.6; D/V, −8.9) or the NAcc (AP, +3.4, L, ±1.5; D/V, −7.5). Coordinates refer to distance from bregma and skull surface (Pellegrino et al. 1979). Cannulae were angled at 16° (VTA) or 10° (NAcc) to the vertical, secured with dental acrylic cement anchored to stainless steel screws fixed to the skull and positioned 1 mm above the final injection site. Following surgery, 28 gauge Plastics One obturators were inserted to a depth 1 mm below the guide cannula tips and rats were returned to their home cage of a 10-day recovery period. All procedures involving animals were conducted according to an approved Institutional Animal Care and Use Committee protocol.

Apparatus

A bank of 12 activity boxes was used to measure locomotor activity. Each box (22×43×33 cm) was constructed of opaque plastic (rear and two side walls) and a Plexiglas front-hinged door with a floor and ceiling consisting of evenly spaced stainless-steel rods. Two photobeams, positioned 3.5 cm above the floor and spaced evenly along and perpendicular to the long axis of each chamber, were used to estimate horizontal locomotion. Two additional photobeams, positioned 16.5 cm above the floor and spaced 5 cm from and parallel to the front or back walls, estimated rearing. Separate interruptions of photobeams were detected and recorded via an electrical interface by a computer situated in an adjacent room. The activity boxes were kept in a room lighted dimly with red light.

Drugs and intracranial microinjections

The amphetamine (d-amphetamine sulfate; Sigma-Aldrich, St Louis, Mo., USA) and the AP-5 [d(-)-2-amino-5-phosphonopentanoic acid; Tocris, Ballwin, Mo., USA] were dissolved in sterile 0.9% saline. Final solution pHs for AP-5 were 3.5–4.0, values well within the limits of rat brain buffering power (e.g., Chesler 1990). Amphetamine was administered IP in a volume of 1 ml/kg. AP-5 was administered IC. Bilateral intracranial microinjections into the VTA and the NAcc were made in the freely moving rat. Injection cannulae (Plastics One; 28 gauge) connected to 1-µl syringes (Hamilton, Reno, Nev., USA) via PE-20 tubing were inserted to a depth 1 mm below the guide cannula tips. Injections were made in a volume of 0.5 µl/side over 30 s. Sixty seconds later, the injection cannulae were withdrawn and the obturators replaced.

Design and procedures

The experiment consisted of two phases: drug pre-exposure and test for sensitization.

Pre-exposure

In this phase, rats in different groups were administered four pairs of injections (one IC and one IP), one pair given every third day. On each injection day, rats were first administered their respective IC injection (AP-5; 0, 1 or 5 nmol/0.5 µl per side) followed immediately by their respective IP injection (amphetamine; 0 or 1 mg/kg, salt). Individual rats received the same combination of injections on each of the 4 injection days. Immediately following the second injection, rats were placed in the activity boxes and their locomotor activity was recorded for 2 h.

Test for sensitization

Two weeks following the last pre-exposure injection, all rats were tested for locomotor sensitization. Following an injection of amphetamine (1.0 mg/kg, IP), their locomotor activity was recorded for 2 h. AP-5 was not administered on this test. During the 2 weeks prior to the test, rats were left undisturbed in their home cages.

Histology

After completion of the experiment, the rats were anaesthetized and perfused via intracardiac infusion of saline and 10% formalin. Brains were removed and postfixed further in 10% formalin for 5–7 days. Coronal sections (40 µm) were subsequently stained with cresyl violet for verification of cannula tip placements.

Data analyses

The data collected in the pre-exposure phase were analyzed with three-way between-within analyses of variance (ANOVA) with AP-5 dose (3: 0, 1 and 5 nmol/side) and amphetamine dose (2: 0 and 1 mg/kg) as the between factors and days (4) as the within factor. The data collected on the test for sensitization were analyzed with two-way between ANOVA with AP-5 dose (3) and amphetamine dose (2) as the factors. Post hoc Scheffé comparisons were made according to Kirk (1968).

185