Valproate prevents the induction of sensitization to methylphenidate (ritalin) in rats

Pamela Yang, Anitra Beasley, Kary Eckermann, Alan Swann, Nachum Dafny

Department of Psychiatry and Behavioral Sciences, The University of Texas Medical School at Houston, PO Box 20708, Houston, TX 77225, USA

Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, PO Box 20708, Houston, TX 77225, USA

Accepted 19 September 2000

Abstract

Repetitive exposure to methylphenidate (MPD) elicits sensitization to its locomotor effects. Drugs that affect the GABA system may modify adaptations to drug exposure. Therefore, we have examined the effect of sodium valproate, which enhances GABA function, on the development of sensitization to MPD using an automated, computerized animal activity monitoring system to record each rat’s motor activities for 15 consecutive days. Rats were recorded before and after saline injection (Days 1–2) to provide baseline activity. Animals were then randomly assigned to the following three groups that received: (1) 2.5 mg/kg MPD (s.c.) for six consecutive days (Days 3–8), (2) a single dose of valproate (50 mg/kg; i.p.) 1 h prior to the first (Day 3) of six daily doses of MPD (2.5 mg/kg; s.c.), or (3) five daily doses of valproate (50 mg/kg, i.p.) 1 h prior to MPD (2.5 mg/kg, s.c.) on Days 4–8. There was no drug treatment during the next 5 days (Days 9–13). All rats were then re-challenged with MPD (2.5 mg/kg, s.c.) on Day 14. Group 2 rats were also re-challenged with 50 mg/kg valproate followed by 2.5 mg/kg MPD 1 h later on Day 15. Administration of MPD alone produced a sensitized response. Multiple valproate injections prevented the induction of MPD-elicited sensitization in all four motor indices, while a single valproate injection prevented the induction of MPD-elicited sensitization in two of four motor indices studied. In conclusion, a single injection 50 mg/kg valproate given prior to any MPD treatment partially blocked the induction of MPD sensitization while repeated injections of valproate co-administered with MPD treatment completely prevented this effect.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Behavioral pharmacology

Keywords: Sensitization; Locomotor activity; Psychostimulants; GABA; Dopamine

1. Introduction

Repeated administrations of psychomotor stimulants such as amphetamine, cocaine [20,21], and methylphenidate [11] have been shown to cause behavioral sensitization. Behavioral sensitization is characterized by an increased locomotor and/or stereotypic behavior resulting from repetitive treatment over time to a given dose of a stimulant [27]. Systemic administration of GABA agonists reduces dopamine turnover in the mesolimbic system [2].

Increased locomotor activities and stereotypic movements incited by dopaminergic stimulation can be blocked by GABA agonists at doses where these agonists have no direct effects [7,28]. Clonazepam, a potent GABA-benzodiazepine agonist, was reported to prevent the development of stimulant-induced sensitization [16]. Sodium valproate, an anticonvulsant drug used in treating schizophrenia and mania [24], can enhance GABA activity and has effects similar to that of benzodiazepine derivatives in conditional avoidance response studies [26]. The present study was carried out to determine whether enhancement of GABA activity from a single pretreatment of valproate prior to any MPD administration or multiple doses of valproate given 1 h before MPD administration would prevent the induction of locomotor sensitization to MPD.

*Corresponding author. Tel.: +1-713-500-5616; fax: +1-713-500-0621.

E-mail address: Nachum.Dafny@uth.tmc.edu (N. Dafny).
2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (N=28; Harlan, Houston, TX), weighing from 180 to 230 g, were housed in groups of four in Plexiglas cages inside the experimental room on a 12-h light/dark schedule (light on at 07:00 h). The experimental room was maintained at an ambient temperature of 21±2°C and at a relative humidity of 37–42%. After a minimum of 48 h of habituation, each rat was randomly placed inside a test (home) cage where its locomotor activity recording began in all groups after 24 h of acclimation to the home cages and continued for 15 days. Rats were supplied with food pellets and water ad libitum.

2.2. Drugs

Both sodium valproate (VAL; Research Biochemicals International, Natick, MA) and methylphenidate hydrochloride (MPD; Sigma Chemicals, St Louis, MO) were dissolved in 0.9% saline. Valproate was injected intraperitoneally (i.p.), while MPD was injected subcutaneously (s.c.). All injections were of equal volume (0.8 ml) and given between 12:00 h and 14:00 h. The selected valproate dose was 50 mg/kg and MPD dose was 2.5 mg/kg. These dosages were selected based on previous dose–response studies [8,10,11]. It was shown in preliminary studies that the dose of valproate chosen had no motor effect of its own [8].

2.3. Apparatus

A computerized animal activity monitoring (CAAM, AccuScan Instruments, Inc., Columbus, OH) system was used to record locomotor activities continuously throughout the 15 experimental days [10]. The activity chambers consisted of clear acrylic open field boxes (40.5×40.5×31.5 cm) with two levels tiers, each consists of 16 infrared beams. The first and second levels of the tiers were placed 6 and 12.5 cm from the cage floor, respectively. The CAAM recorded any interruption of each of the beams at a frequency of 100 Hz. The interruption of any beam was counted. Total counts were compiled and downloaded every 10 min into the OASIS data collection program, which grouped them into several different locomotor indices. Animal activity was recorded continuously for 15 days. The following four locomotor indices were analyzed: (1) horizontal activity (HA), which measured the overall motor activity in the lowest tier of the testing cages and assessed the overall amount of motor activity, (2) total distance (TD), which recorded the specific motor behavior of forward movement, (3) vertical activity (VA) which counted the specific instances of rearing, and (4) number of stereotypes (NOS) which determined the stereotypic behaviors, measured by repeated interruption of the same beam [10].

2.4. Control and treatment groups

There were three experimental groups: Group 1 (N=12) — after baseline and saline, animals treated with 6 days of MPD followed by five washout days and MPD re-challenge on Day 14 (Table 1); Group 2 (N=8) — similar to Group 1 but, in addition, animals were given a single administration of valproate (50 mg/kg) on Day 3 and Day 15 1 h prior to MPD; and Group 3 (N=8) — multiple administrations of valproate (50 mg/kg) was given on Days 4–8 1 h prior to each dose of MPD (Table 1). The following is the general protocol for these groups: Day 1 — baseline activity; Day 2 — saline injection (0.9%, i.p.); Day 3 — Groups 1 and 3 were treated with MPD, Group 2 was given valproate 1 h prior to MPD; Days 4–8 — Groups 1 and 2 were treated with daily MPD, Group 3 was given valproate 1 h prior to daily MPD on Days 4–8; Days 9–13 — washout for all three groups; Day 14 — MPD was given to all three groups; and Day 15 — valproate 1 h prior to MPD was given to Group 2.

2.5. Data analysis

Variability in baseline activity among animals was observed; therefore, the best comparison for the drug effects is to have each animal served as its own control. The drug effect for each motor index was determined by subtracting the activity score of the 2-h after saline injection (Day 2) from that of the 2 h after MPD injection to eliminate any handling and injection effects [10–12]. Thus, the activity of saline injection served as baseline and the absolute change in activity from saline represented

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4–8</th>
<th>9–13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (N=12)</td>
<td>H</td>
<td>B</td>
<td>S</td>
<td>MPD @ 14:00</td>
<td>MPD @ 14:00</td>
<td>WO</td>
<td>MPD @ 14:00</td>
<td>WO</td>
</tr>
<tr>
<td>Group 2 (N=8)</td>
<td>H</td>
<td>B</td>
<td>S</td>
<td>VAL @ 13:00; MPD @ 14:00</td>
<td>S @ 13:00; MPD @ 14:00</td>
<td>WO</td>
<td>S @ 13:00</td>
<td>VAL @ 13:00</td>
</tr>
<tr>
<td>Group 3 (N=8)</td>
<td>H</td>
<td>B</td>
<td>S</td>
<td>S @ 13:00; MPD @ 14:00</td>
<td>VAL @ 13:00; MPD @ 14:00</td>
<td>WO</td>
<td>S @ 13:00</td>
<td>WO</td>
</tr>
</tbody>
</table>

H=habituation; B=baseline; S=0.9% saline (i.p.); MPD=2.5 mg/kg methylphenidate (s.c.); WO=washout; VAL=50 mg/kg sodium valproate (i.p.).
effects of the drugs. For Groups 1 and 3, the presence of sensitization in each treatment group was determined by comparing results of Days 4 to 8 and 14 with that of MPD challenge on Day 3 (first day of MPD administration in naive animals) within that group. For Group 2, the presence of sensitization was determined by comparing results of Days 5 to 8 and 14 to 15 with that of Day 4 (second day of MPD administration, without valproate). Results were subjected to a within-group repeated measure analysis of variance (ANOVA, two levels: treatment day and 10 min sample). The significant differences in individual 10-min samples were determined with post-hoc Fischer’s (LSD) method. Significance for all of the above comparisons was set at \( P < 0.05 \), \( *P < 0.01 \), or \( **P < 0.001 \). Values are presented as the mean ± S.E.M. for each 10 min after injection or for the sum of 2-h sample. In addition, the difference in the magnitude of motor responses was also determined by calculating the ratio of the absolute change in activity between MPD challenge (Day 3 for Groups 1 and 3; Day 4 for Group 2) and MPD re-challenge (Day 14). Results were analyzed using repeated measure analysis of variance (one-way ANOVA: treatment day), and any significant difference between the groups was determined with the Dunnett test. Significance for this comparison was set at \( *P < 0.05 \).

3. Results

3.1. Control

Time control (15 days) and saline injection (Days 2 to 8) on identical experimental protocols showed that the motor indices studied had remained similar over time as well as following multiple saline injections, and any deviation from the time control or saline treatment was the result of the drug effect(s) [6,10,12]. The effects of repeated MPD (2.5 mg/kg) treatment for HA, TD, VA, and NOS are summarized in Fig. 1. The bar graph shows the total activity score for the 2 h after MPD injection throughout the experimental protocol. The line graph represents the 10-min temporal response over the 2 h after MPD injection on Days 3 and 14. In general, MPD’s effects on motor activity differed significantly between Day 3 (the initial challenge day) and Days 4 to 8 (the induction days) and Day 14 (the re-challenge day). For HA, Days 6, 8, and 14 showed the greatest difference (\( P < 0.001 \)), but Days 4, 5, and 7 were also significantly different from Day 3 (\( P < 0.01 \) for Day 7; \( P < 0.05 \) for Days 4 and 5). For VA, only Days 6, 8, and 14 significantly differed from Day 3 (\( P < 0.01, P < 0.001, \) and \( P < 0.001 \), respectively). For TD, sensitization was exhibited by the fourth MPD injection on Day 6 (\( P < 0.001 \)), while stereotypic sensitization (NOS) had developed by the second MPD injection, i.e., Day 4 (\( P < 0.05 \)). In summary, by Day 6 (the fourth MPD injection) sensitization had developed in all four motor indices studied.

In the line graph (Fig. 1), each 10-min sample on Day 3 was compared to the same sample on Day 14 for the initial 120 min following MPD administration. With respect to HA and VA, the first seven samples (i.e., 70 min after MPD injection) significantly differed in Days 3 and 14 (\( P < 0.01 \)). For TD, five of the initial samples of Day 14 after MPD treatment differed significantly from Day 3. The differences in Day 3 and 14 were strongest in NOS with the first seven samples (70 min after injection; \( P < 0.001 \)). Thus, MPD induced activities were greatest within the first 70 min after drug injection.

3.2. Single administration of valproate

The effect of a single dose of valproate given 1 h prior to MPD injection on Day 3 is summarized in Fig. 2. A single injection of valproate given prior to any MPD treatment prevented the induction of MPD sensitization for TD and NOS as expressed in the first 2 h after MPD injection. However, there were significant differences on Day 7 (Fig. 2 bar graph) and 14 from Day 3 with respect to HA (\( P < 0.05 \)) and VA (\( P < 0.05 \) for Day 7; \( P < 0.01 \) for Day 14); thus, a single valproate injection did not prevent the induction of MPD-sensitization for these indices. The effects of the second injection of valproate 1 h prior to MPD (Day 15) also differed significantly from the effects of the first valproate injection 1 h prior to MPD (Day 3; \( P < 0.05 \)) for VA, while there were no significant differences for the other three indices. Therefore, a single injection of valproate prior to any MPD injection prevented the induction of sensitization to TD and NOS but not to HA and VA.

3.3. Multiple administration of valproate

The effect of multiple injections of valproate 1 h prior to MPD injections on Days 4 to 8 on the induction of MPD sensitization is summarized in Fig. 3. The total count of 2 h after MPD injection of Days 4 to 8 compared to Day 3 exhibited no significant differences in locomotor activity between any of the days with respect to HA, TD, VA, and NOS. Thus, concurrent administration of MPD and valproate prevented the development of MPD sensitization.

3.4. Magnitude of sensitization between groups

The motor activity response ratio of MPD re-challenge and challenge for Groups 1–3 and the statistical comparison between these groups is summarized in Fig. 4. Fig. 4a shows that multiple administration of valproate (Group 3) significantly (\( P < 0.05 \)) decreased the HA response ratio of MPD re-challenge and challenge compared to that of Group 1, which was treated with only MPD. However, there is no difference in the HA response ratio when Group 2, which received a single administration of valproate, was compared to Group 1. Fig. 4b shows the observation of the TD response which was similar to the HA response (Fig.
Fig. 1. The effects of multiple MPD (2.5 mg/kg, s.c.) treatment on locomotor activity. Temporal response in 10-min samples for 120 min after MPD injection (line graph) and 2-h total activity (bar graph) for the four motor indices studied: horizontal activity, vertical activity, total distance, and number of stereotypic movements following administration of MPD. Sensitization to locomotor activities resulted from daily injection of MPD was well established by the third MPD injection (Day 6) and was still expressed several days later (Day 14). Presence of sensitization to MPD was determined by comparing results obtained after the second to the sixth injection with that obtained at Day 3. Values are presented as the mean±S.E.M. for each 10-min or 2-h sample. *P<0.05; **P<0.01 and ***P<0.001 compared to Day 3.
Fig. 2. The effects of single valproate (50 mg/kg, i.p.) administration (‘—’ indicates day of valproate injection in bar graph) 1 h prior to the initial MPD injection on experimental Day 3 and Day 15. Temporal response (line graph) and 2-h total (bar graph) for horizontal activity, vertical activity, total distance, and number of stereotypic movements following administration of 2.5 mg/kg MPD (s.c.). All days are compared to the MPD challenge day (Day 4) in both line and bar graphs. A single dose of valproate given 1 h prior to the initial MPD treatment prevented significant increases in total distance and number of stereotypic movements but not in horizontal ($P<0.05$) and vertical activities ($P<0.01$). Values are presented as the mean±S.E.M. for each 10-min or 2-h sample. *$P<0.05$; **$P<0.01$ and ***$P<0.001$ as compared to Day 4.
Fig. 3. The effects of multiple valproate (50 mg/kg, i.p.) administration (*—* indicates days of valproate injection in bar graph) 1 h prior to MPD on Days 4 to 8. The total count over the 2-h (bar graph) and the temporal response (line graph) after MPD injection are shown for horizontal activity, vertical activity, total distance, and number of stereotypic movements. Multiple valproate administration prevented significant increases in MPD induced locomotor activities throughout the experimental phase in all four behavioral indices. Values are presented as the mean±S.E.M. for each 10-min or 2-h sample. *P<0.05 and **P<0.01 as compared to Day 3.
Fig. 4. This figure shows the motor activity response ratio of MPD re-challenge and challenge for Groups 1–3 and the statistical comparison between these groups. (a) shows that multiple administration of valproate (Group 3) significantly (P<0.05) decreased the horizontal activity (HA) response ratio of MPD re-challenge and challenge compared to that of Group 1; while there is no difference in the HA response ratio for Group 2, which received a single administration of valproate, when compared to Group 1. Similar results are shown in (b) involving total distance (TD) response.

4a). These observations indicate that multiple administration of valproate prevented MPD-elicited sensitization.

4. Discussion

Methylphenidate has the potential of abuse because it has pharmacological stimulant properties similar to amphetamine and cocaine [9,31]. Its use in the treatment of attention deficit/hyperactivity disorder (ADHD) has drastically increased over the years [15]. The chronic use of psychostimulants, such as amphetamines, has been reported to produce a progressive enhancement in paranoid behaviors that can result in psychosis [18,27] and in a reciprocal cross-sensitization between itself and other stimulants [4], which may be analogous to sensitization in rodents [27].

The results show that daily repeated administration of MPD (2.5 mg/kg) for six consecutive days produced sensitization to its effects on locomotor and stereotypic behavior from the second or fourth injection, which persisted when rats were re-challenged with MPD on Day 14. Similar behavioral sensitization has been reported following multiple treatment with MPD [11] and other psychostimulants such as amphetamine and cocaine [19,27]. The objective of the present study was to determine whether a single or multiple administration of valproate would prevent the development, or induction, of behavioral sensitization to the locomotor effect of repetitive treatment with MPD. The data suggests that single administration of valproate given 1 h prior to the initial MPD administration did not prevent the induction of MPD sensitization following repetitive MPD administration as expressed by horizontal and vertical activities, although single valproate injection prevented the induction of MPD sensitization in total distance and number of stereotypic movements. In contrast, repeated daily valproate administration prevented the induction of MPD-sensitization in all four motor indices throughout the 6 days of repeated MPD treatment and in the MPD re-challenge on Day 14. Interestingly, in a comparison of between groups involving the motor activity response ratio of MPD re-challenge and challenge, it was found that multiple administration of valproate (Group 3) prevented MPD-elicited sensitization compared to the administration of MPD alone (Group 1) as demonstrated in the HA and TD responses (Fig. 4a). However, single administration of valproate (Group 2) did not prevent MPD-elicited sensitization compared to the administration of MPD alone (Fig. 4b). These observations further support the efficacy of multiple treatments of valproate in blocking behavioral sensitization.

Behavioral sensitization consists of two phases: initiation/induction and expression. There is evidence suggesting that the induction and the expression of sensitization to psychostimulants involve different anatomical and physiological mechanisms. The initiation of sensitization consists of the immediate molecular and/or cellular effects that induce behavioral sensitization and are altered by drug actions in the somatodendritic regions of the A10/A9 dopamine neurons [18]. In contrast, changes in dopamine transmission within the nucleus accumbens seem to be responsible for the expression of sensitization, which refers to the long-term consequences of molecular and/or cellular effects that induce behavioral sensitization [18].

It has been hypothesized that dopamine plays a crucial part in the locomotor sensitization elicited by psychostimulants because these stimulants elevate extracellular dopamine in brain regions that mediate enhanced locomotion and stereotypic behaviors [5,14,17,22,23,30]. The significant role of dopamine in locomotor sensitization has also been supported by the observation that hyperactive behavior is a result of high concentrations of extracellular dopamine in the stratum of dopamine transporter knockout mice [14]. γ-Aminobutyric acid (GABA) participates in
the regulation of the activity of dopamine neurons by reducing dopamine release and inhibiting dopaminergic activity [1]. GABAergic neurons in the A10/A9 region play an important role in the modulation of the D1 receptors that are involved in the initiation of behavioral sensitization [18].

γ-Aminobutyric acid agonists have been shown to block the augmented locomotor activity and stereotyped behavior resulting from dopaminergic stimulation [1,7,28]. Baclophen, a specific GABA B agonist, was found to block the effect of amphetamine on discriminative learning in rats [3]. Locomotor stimulation in rodents induced by amphetamine or apomorphine was also inhibited by GABAergic drugs [2]. In the present study, multiple administration of valproate prevented the induction of MPD sensitization in all four motor indices studied. However, a single administration of valproate given 1 h before the initial MPD treatment prevented the induction of sensitization to MPD in total distance and number of stereotypic movements but not in horizontal and vertical activities. Valproate treatment enhanced GABA activity within the brain [25] by inhibiting its degradation, stimulating its synthesis and release, and directly enhancing its postsynaptic effect [24]. Therefore, valproate may have prevented sensitization to MPD by increasing GABAergic function. In addition to enhancing GABA activity, valproate has also been reported to enhance voltage-dependent inward currents involving Na+, Ca2+, and K+ in the neocortex [35]. This effect might represent another possible mechanism of action for valproate.

In similar experimental protocols, multiple injections of valproate given after the induction of MPD sensitization prevented the expression of behavioral sensitization (Days 9 to 13) as measured by both total distance and vertical activity but not horizontal activity or number of stereotypic [33]. However, a single administration of valproate given on Day 9 after MPD sensitization did not prevent the expression of behavioral sensitization to MPD in any of the four motor indices studied [33]. These observations suggest that the effects of valproate on the induction of MPD sensitization are different from those on the expression of MPD sensitization. The difference in the timing of valproate injection also supports the hypothesis that the induction and the expression of behavioral sensitization to MPD involve different mechanisms. Thus, the results of this study suggest that repeated co-administration injection of valproate with MPD prevented the induction of sensitization to MPD more effectively than the expression of sensitization, perhaps via increased GABA activity.

The above observation suggests that the induction and the expression of behavioral sensitization for stimulants may have different neuronal mechanisms. It was reported that lesions of the fornix, which resulted in interrupted projections from the hippocampus to nucleus accumbens, prevented the induction of locomotor sensitization, while the same lesion did not affect the expression of locomotor sensitization [34]. Moreover, there is evidence that excitatory amino acids, particularly N-methyl-D-aspartate (NMDA), may be essential for the development of a sensitized response [32]. Pretreatment with dizocilpine (MK-801), a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, prior to amphetamine [20] or methylphenidate injection [13,29] prevented the induction of behavioral sensitization produced by these drugs but not the expression of behavioral sensitization [32]. Therefore, it was suggested that the disruption of glutamatergic projections to dopamine terminal fields may affect the induction of sensitization [18] and that the induction and the expression of behavioral sensitization involve separate mechanisms [20]. Our results suggest that stimulation of GABA activity, like inhibition of NMDA receptors, prevented induction of behavioral sensitization.

Acknowledgements

The authors wish to thank Dr. Miguel Bedolla for his help with the statistics. This study was supported in part by the Pat Rutherford Chair in Psychiatry.

References


[19] PW. Kalivas, J.E. Alesdatter, Involvement of N-methyl-D-aspartate receptor stimulation in the ventral tegmental area and amygdala in behavioral sensitization to cocaine, J. Pharmacol. Exp. Ther. 267 (1993) 486–495.


