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The Reversal of Amphetamine-Induced Locomotor Activation by a Selective Neurotensin-1 Receptor Agonist Does Not Exhibit Tolerance

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Abstract

Neurotensin-1 (NT1) receptor agonists have been proposed as putative antipsychotic drugs. Recently brain penetrating NT analogues produced by stability enhancing modification of the smallest NT fragment, NT(8–13), have demonstrated antipsychotic-like efficacy after acute systemic injection in several preclinical animal tests predictive for antipsychotic efficacy. However, the evidence regarding the persistence versus tolerance of these effects after repeated administration is ambiguous. Previous studies have used compounds that non-selectively activated both NT1 and NT2 receptors, a factor which may have contributed to the ambiguity in findings regarding the emergence of tolerance. In this study, we investigated the effects of subchronic systemic administration of PD149163, a brain penetrating NT analogue with selectivity for the NT1 receptor, on amphetamine-induced locomotor activation, a classic preclinical test of antipsychotic-efficacy. Sprague Dawley rats were pretreated with eight consecutive daily subcutaneous (SC) injections of PD149163 or saline. On the ninth day rats received a pair of SC injections consisting of PD149163 or saline, followed by amphetamine (0.5mg/kg) or saline. Locomotor activity was then measured in photobeam equipped cages. The results indicated that repeated daily administration of PD149163 was able to antagonize amphetamine's locomotor activating effect comparable to that of the first dose, despite that repeated administration of PD149163 produced an increase in baseline locomotor activity not seen after the first dose. The results do not support development of tolerance for the acute antipsychotic-like effect of NT1 agonists and thus lend support to the contention that NT1 agonists are viable candidates as putative novel antipsychotic drugs.

Keywords

antipsychotic; neurotensin; PD148163; NT69L; locomotor activity; tolerance

INTRODUCTION

Neurotensin (NT) is a thirteen amino acid neuropeptide that appears to be able to regulate the mesolimbic dopamine and other neurotransmitter system implicated in psychosis (Boules et al. 2005; Boules et al. 2007; Kinkead et al. 1999; Kinkead and Nemeroff 2004). Administration of NT into the brain produces effects consistent with an antipsychotic drug (APD) (Feifel et al. 1997; Ervin and Nemeroff 1988). Due to these findings there has been considerable interest in developing NT agonists that, unlike NT itself, can resist enzymatic degradation in the periphery and penetrate the blood-brain-barrier after systemic administration. One approach

used has been to induce metabolically stabilizing modifications to the c-terminal hexapeptide, NT(8–13), which is the smallest fragment of the parent NT peptide to retain full biological activity (Boules et al. 2001). This approach has resulted in several small peptide NT analogues that produce central effects after systemic administration. One example is NT69L (N-methyl-Arg(8),L-Lys(9),L-neo-Trp(11),tert-Leu(12) (Boules et al. 2001). Acute systemic administration of NT69L produces many of the same effects produced by NT administered centrally, including hypothermia, antinociception and APD-like behavioral effects such as antagonism of dopamine agonist-induced hyperlocomotion, climbing and disruption of prepulse inhibition of the startle reflex (PPI) and catalepsy induced by haloperidol. (Boules et al. 2003; Boules et al. 2001).

Effective antipsychotic treatment requires chronic administration, thus it is important to investigate the effects of any putative APD after subchronic, in addition to acute administration. While the acute effects of NT and NT analogues have been extensively investigated, less research has been conducted on the effects of NT agonists after repeated administration. Boules et al. (Boules et al. 2003) studied the effects of NT69L administered systemically for up to five consecutive days and found that its ability to induce hypothermia, antinociception and block haloperidol-induced catalepsy exhibited rapid tolerance, whereas its ability to antagonize amphetamine- and cocaine-induced hyperlocomotion and apomorphine-induced climbing persisted. Similarly Hadden et al. (2005), testing a different modified NT(8–13) peptide, KK28, also found that it continued to block amphetamine-induced hyperlocomotion after administration for five consecutive days. In contrast, Hertel et al. (Hertel et al. 2001; Hertel et al. 2002), found that the APD-like reduction in spontaneous locomotor activity, antagonism of amphetamine-induced hyperlocomotion, and conditioned avoidance responding, produced by acute systemic administration of NT69L, exhibited tolerance after six consecutive, twice-daily systemic injections of this compound. Thus the evidence of whether there is tolerance to the acute APD-like effects of NT69L is ambiguous.

Modulation of dopamine function by NT and NT analogues plays an important role in their APD-like behavioral effects. For example, Wang et al. found that pretreatment with NT69L for five days produced tolerance to some aspects of the modulation of dopamine function produced by NT69L but not others (Wang et al. 2004). The authors suggested that distinct NT receptors may be involved in the effects exerted by NT69L and other NT analogues on dopamine function. The same group subsequently demonstrated that pretreatment with five consecutive days of NT69L administered systemically-induced down-regulation of NT1 but not NT2 receptors in the brain (Wang et al. 2005), supporting the possibility that distinct NT receptors types play different roles in the changes seen in the APD-like behavioral effects of NT and NT analogues with prolonged exposure to NT analogues. Thus the ambiguity regarding the question of tolerance resulting from studies using NT69L may be due to its non-selective affinity for both the NT1 and NT2 receptors (see Discussion, (Wang et al. 2005)).

PD149163 (Lys(CH₂NH)Lys-Pro-Trp-tLe-Leu-OET) is another NT analogue produced by chemical modification of NT(8–13) to enhance its stability after systemic administration (Wustrow et al. 1995). Unlike NT69L, PD149163 has selective affinity for the NT1 receptor (Petrie et al. 2004). The NT1 receptor is the NT receptor most strongly implicated in mediating the APD-like effects seen with acute administration of NT and NT analogues such as NT69L and PD149163. Acute systemic administration of PD149163 has produced robust APD-like effects in a number of preclinical predictive tests of APD efficacy including facilitation of PPI (Feifel et al. 2003a; b; 2004; Feifel et al. 1999). In a recent study, our laboratory found that the APD-like facilitation of the naturally low PPI in Brattleboro rats produced by acute administration of PD149163 persisted with no evidence of tolerance after consecutive daily subcutaneous administration of PD149163 for sixteen days (Feifel et al. 2007). To further investigate whether the APD-like effects of PD149163 exhibit tolerance, we examined the

effect of subchronic systemic injection of PD149163 on amphetamine-induced hyperlocomotion.

METHODS

Thirty-two male Sprague Dawley rats (250–300 g at testing, obtained from Harlan Laboratories, San Diego) were housed in pairs under a 12h:12h light:dark schedule (lights on at 7:00 AM). All testing occurred during the light phase of the rats' circadian illumination schedule. The test cages were standard cages (42 cm × 26 cm), surrounded by grids with eight pairs of infrared beams 4 cm above the bottom of each cage. A computer connected to these grids (San Diego Instruments) measured locomotor activity. Ambulations were defined as the number of times one animal disrupted two different beams in consecutive succession. Principles of animal care were followed and all procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

One day prior to drug testing, rats were placed in locomotor cages for 1 hour. Data from the last 30 minutes (after 30 minutes of acclimation to cages) was used to assign rats to one of four treatment groups of eight rats matched for locomotor activity. The subchronic pre-treatment phase began the next day and consisted of eight days of daily subcutaneous (SC) injections. Three groups were administered injections of saline for eight consecutive days. The other group of 8 rats received 1 mg/kg PD149163 for eight consecutive days. This dose was based upon the dose-response experiments we have previously performed with PD149163 showing that 1 mg/kg of this drug produced a robust reduction in amphetamine-induced behaviors such as PPI (Feifel et al. 1999) and amphetamine-induced locomotor activation (unpublished data). Twenty minutes before injections each rat was placed in the test cages to acclimate. After injections, they were put back in the test cages for a one hour period during which locomotor activity was measured.

Testing occurred on the ninth day. On this day all rats received two SC injections. After an initial 20 minute acclimation period in the locomotor cages, rats were given their first injection of either PD149163 or saline and then replaced in locomotor cages for another 20 minutes to allow absorption of the first injection. Then each rat received a second injection of either saline or 0.5 mg/kg of amphetamine, after which they were replaced in locomotor cages and locomotor activity was recorded.

Of the three groups that received saline during the subchronic treatment phase one group received saline/saline on day nine, another group received saline/amphetamine and another PD149163/amphetamine. The group that received PD149163 during the subchronic pretreatment phase received PD149163/amphetamine on day nine.

All drugs were dissolved in saline (0.9% NaCl), and injected subcutaneously (s.c.) in a volume of 1.0 ml/kg. Amphetamine was obtained from Sigma Chemicals, St. Louis, MO and PD149163 was generously made available by the NIMH Chemical Synthesis and Drug Supply Program, and RTI International, Research Triangle Park, NC, USA. Locomotor activity data for each rat was analyzed for 30 minutes following a 10-minute initial period (10 – 40 minutes after being returned to cages) to allow for drug absorption. Data from the twenty-four rats that received saline during the eight-day subchronic treatment period was aggregated. Locomotor data for the first eight days was analyzed using a two-way analysis of variance (ANOVA) with "Day" as the within-subject factor and "Drug Treatment" (saline or PD149163) as the between-subject factor. Planned independent samples t-tests using the Bonferroni method for multiple comparisons were performed comparing ambulations for both drug groups on each of the eight days. Locomotor data for day nine (amphetamine challenge) were analyzed using independent

samples t-tests with corrections for multiple comparisons. A P-value less than 0.05 was considered significant. All statistical analyses were performed using SPSS 11.0 software. Locomotor activity was measured as the number of times spatially adjacent beams pairs were interrupted in consecutive succession as this measure is indicative of ambulations rather than other causes of beam breaks (e.g. stereotypic movements). The number of times a beam was interrupted and not followed by adjacent beam interruption was also analyzed for day 9 (amphetamine challenge) as this measure is indicative of stereotypy.

RESULTS

Subchronic Pre-Treatment

An ANOVA performed on the ambulation data (consecutive beam pair interruption) first eight days of testing revealed no significant main effect of Day, or Drug Treatment but there was a significant Day X Group interaction, $F(7,210) = 2.76$, $p < 0.01$ (Figure 1). Visual inspection of the data comparing rats that received PD149163 to rats that received saline during the eight day pretreatment phase showed a tendency to produce fewer ambulations in rats treated with PD149163 on the first two days, whereas beginning on day 6, PD149163 treated rats exhibited more locomotor activity, and the difference between the two treatments increased until the end of the subchronic pretreatment phase (day 8). Independent samples t-tests corrected for multiple comparisons revealed that the PD149163 treated rats exhibited significantly more ambulations than the saline group on day 8, $t(30) = -7.08$, $p < 0.001$.

Acute Amphetamine Challenge

Rats pretreated with saline and given amphetamine plus saline on day 9 exhibited significantly increased ambulatory activity (adjacent beam pair interruption) compared to 1) saline-only treated rats [$t(14) = -7.44$, $p < 0.001$], 2) rats that received PD149163 for the first time on day 9 (acute PD149163) in conjunction with amphetamine [$t(14) = 3.99$, $p < 0.01$] and, 3) rats that received their ninth consecutive daily injection of PD149163 subchronic PD149163) in conjunction with amphetamine [$t(14) = 3.11$, $p < 0.05$] (Figure 2A). Furthermore, there was no significant difference in ambulatory activity between the acute or subchronic PD149163 groups. Rats pretreated with saline and given amphetamine plus saline on day 9 exhibited significantly increased non-ambulatory type beam interruptions (beam interruption not followed by adjacent beam interruption) compared to saline-only treated rats [$t(9.521) = 10.351$, $p < 0.001$]. Rats that received acute and chronic PD149163 in conjunction with amphetamine exhibited reduced non-ambulatory beam reductions compared to amphetamine/saline treated rats but in neither case did this reduction reach statistical significance (Figure 2B).

DISCUSSION

Our results showed that administration of a single dose as well as nine consecutive daily doses of PD149163 was able to significantly antagonize the locomotor activating effects of amphetamine. Since there was no significant difference between the effects of the acute and subchronic administration regimen, we can conclude that there was no tolerance exhibited by PD149163's anti-amphetamine effects. Reduction of amphetamine-induced hyperlocomotion is a classic preclinical test of APD potential. In order to distinguish between reduced locomotor activity due to enhanced amphetamine-induced-stereotypy versus inhibition of amphetamine-induced hyperlocomotion, we measured both the number of beam disruptions not followed consecutively by interruption of an adjacent beam and the number of consecutive disruptions of spatially adjacent beam pairs. The former is preferentially sensitive to stereotypic movements, whereas the latter is preferentially sensitive to ambulation. Acute and subchronic PD149163 were effectively able to antagonize the amphetamine-induced increase in the total number of ambulation-type, but not stereotypy-type beam interruptions, indicating that both

acute and subchronic PD149163 produced antagonism of amphetamine's locomotor effects rather than modulating any possible stereotypy-inducing effects of amphetamine. Another highly used preclinical test of APD efficacy is facilitation of PPI. The lack of tolerance exhibited by PD149163 on amphetamine-induced locomotor activation is consistent with previous findings from our laboratory, in which the acute APD-like facilitation of naturally low PPI in Brattleboro rats by acute PD149163 persisted after sixteen consecutive daily administrations of PD149163 (Feifel et al. 2007). Treatment of psychosis in most cases requires chronic daily use of APDs. Thus tolerance exhibited by a putative APD in preclinical tests of APD efficacy could be interpreted as weakening its viability to be developed as an APD. Interestingly, the predictive validity of chronic APD administration in preclinical tests is not clear. For example, although the acute effects of haloperidol on conditioned avoidance behavior persists with repeated administration (Hertel et al. 2002), haloperidol's ability to block amphetamine-induced PPI disruption does not persist (Andersen and Pouzet 2001). Most relevant to the current study, the acute reversal of amphetamine-induced hyperlocomotion by two established APDs, haloperidol and olanzapine, were maintained after chronic daily oral (24 consecutive days) administration (Andersen and Pouzet, 2001). Interestingly, another APD, sertindole tested at a single dose, did not show persistence of its acute antagonism of amphetamine-induced hyperlocomotion with chronic treatment (Andersen and Pouzet, 2001). Similarly, Samaha et al. (2008) demonstrated that haloperidol given subcutaneously daily for 7 or 12 consecutive days reversed amphetamine-induced hyperlocomotion. However, the same dose administered slowly over the entire day by osmotic minipump exhibited tolerance. In light of these data, the evidence that PD149163 produces robust APD-like effects that do not display tolerance supports the contention that NT1 agonists such as PD149163 are viable putative APDs.

Our findings are more consistent with previous findings that antagonism of amphetamine- and cocaine-induced locomotor activation, as well as apomorphine-induced climbing produced by acute administration of NT69L (Boules et al. 2003) and amphetamine-induced hyperlocomotion produced by KK28 (Hadden et al. 2005) persisted after five consecutive daily injections. Our findings stand in contrast to the findings of Hertel et al. (Hertel et al. 2001), in which it was found that acute effects produced by NT69L on amphetamine-induced hyperlocomotion and conditioned avoidance responding did not persist with repeated administration of that drug.

The reason for the differences between the findings of Hertel's group and the lack of tolerance found by Boules et al. despite that both groups studied NT69L is not clear. Differences in the dosing regimen may have contributed to the discrepant results. A detailed description of these dosing differences can be found in the Boules et al. (Boules et al. 2003). However, in general the studies conducted by Boules et al. used a single daily injection of NT69L, at a dose (1 mg/kg) that is in the well established optimal range for APD-like effects, whereas the Hertel et al. studies used twice daily administration of significantly lower doses of NT69L. These two dosing regimens may have produced a distinct activation pattern on NT1 and NT2 receptors since NT69L has relevant affinity for both those receptors. In this regard, PD149163 is selective for the NT1 receptor (Petrie et al. 2004) and therefore provides an optimal pharmacologic tool to examine the role of NT1 receptors and the potential of NT1 selective agonists as putative APDs.

The dosing regimen of PD149163 that we used in this study is more similar to the one used by Boules et al. Therefore, we can not rule out the possibility that the receptor selectivity of PD149163 plays no role in the persistence exhibited in the APD-like effects of PD149163, and that this is simply a result of the dosing regimen that we used in the current study which is similar to the one we used in our previous study examining its effects on PPI and the one used by Boules et al. However, our main goal was to investigate the APD potential of selective NT1

receptor agonists such as PD149163 and in this regard the single daily dosing regimen used in our studies (and by Boules et al.) is more traditional and more representative of the APD dosing regimen used in clinical practice.

Interestingly, while this paper was in the review process, a paper by Norman et al. (2008) was published with similar goals to this one, to examine the subchronic effects of PD149163 on amphetamine-induced hyperlocomotion. Norman et al. found that the ability of an acute infusion of ICV administered PD149163 to reverse amphetamine-induced hyperlocomotion dissipated after pretreatment with 7 or 21 days of continuous ICV infusion of PD149163. The seemingly contradictory findings of the Norman et al. study and the one presented here, is likely due to the differences in the manner in which PD149163 was administered in both studies. We administered it peripherally, whereas, Norman et al. administered this drug centrally. Furthermore, Norman et al. tested how the effects of a single dose of PD149163, centrally administered in a rapid one time infusion, was altered after pretreatment with that same daily dose of PD149163 administered continuously over 24 hours for 7 or 21 days by osmotic minipump. Thus in that study, the acute administration was different than the subchronic and chronic pretreatment, whereas in our study all administrations of PD149163 were done in a similar manner. Furthermore, it is not clear that the dose of PD149163 tested by Norman et al. delivered slowly over 24 hours produced antipsychotic like reversal of amphetamine-induced hyperlocomotion since this effect was never demonstrated in their study. There is evidence that chronic infusion of a drug dose continuously over 24 hours produces very different pharmacokinetic and pharmacodynamic effects compared to administration of that same dose in a rapid fashion. For example, a dose of haloperidol given peripherally over 24 hours continuously by osmotic minipump for 21 days lost its acute efficacy in two animal models of antipsychotic-like effects, i.e., amphetamine-induced hyperlocomotion and conditioned avoidance response; whereas when administered for the same period by daily subcutaneous injection it did not (Hertel et al. 2001; Hertel et al. 2002). Shamah et al. (2008), and Andersen and Poulet (2001) found a similar loss of the acute efficacy for several established APDs including haloperidol in another animal model of antipsychotic-like effects, disruption of PPI, when these antipsychotic drugs were administered continuously by osmotic minipump. Thus the findings of Norman et al. must be considered in the context of these findings. While there are differences between rats and humans in the pharmacokinetics of acute administration of APDs (Kapur 2003), nevertheless we believe that our methodology, daily rapid administration of doses peripherally is a better model of the common clinical administration of APDs in humans than the continuous central slow infusion, followed by the rapid central infusion method used by Norman et al. Compelling proof of this contention is that established APDs, whose therapeutic effects do not demonstrate tolerance with chronic use in humans, when delivered to rodents by the method of administration used by Norman et al, produce tolerance, whereas they do not do so when administered by the method that we employed.

An unexpected finding of this study was that subchronic administration of PD149163 resulted in stimulation of spontaneous locomotor activity, an effect not seen with acute administration. This difference between the effects of acute and subchronic PD149163 was the result of locomotor activity decreasing from the first to last day of testing in the saline-treated but not PD149163-treated animals. Therefore, the effect of PD149163 may have been to inhibit habituation to the locomotor cage over time rather than to stimulate locomotor activity. To address this possibility, we analyzed the locomotor data from the 20-minute period after rats were placed into locomotor cage each day for acclimation prior to receiving drug injections. Saline-treated rats decreased their spontaneous locomotor activity during this pre-treatment period by 58.3% from day 1 to day 8, which was comparable to the degree of habituation seen by PD-treated rats (65.3%) across this period. These data support the contention that it is unlikely that the subchronic PD149163-induced elevations in locomotor activity are due to an

inhibition of tolerance by this drug over time to the locomotor cage. Therefore, the effect of PD149163 seems to be a true stimulation of locomotor activity. The exact mechanism underlying this effect is not known but since NT1 receptors are known to regulate mesolimbic dopamine function, a neural system that mediates locomotor activity, this phenomenon may be produced via the action of this NT agonist on mesolimbic DA function. Acute administration of NT or NT receptor agonists are known to inhibit the effects of dopamine agonists, presumably via downregulation of D2 receptor affinity for dopamine (Fuxe et al. 1992). It is possible that with repeated activation of the NT1 receptor, mesolimbic dopamine function overcompensates, for example, by upregulating D2 receptor density or affinity. It is very interesting that the activation produced by subchronic administration of PD149163 on spontaneous locomotor activity did not preclude the ability of that treatment regimen to antagonize the hyperlocomotion induced by amphetamine. This result is analogous to the expected effects of partial D2 receptor agonists, which are able to increase transmission through that receptor under conditions of low ambient neurotransmission and decrease transmission through that receptor under abnormally high existing levels of activity such as occurs with amphetamine administration. This putative phenomenon warrants further investigation, for example, by examining possible differences in changes in dopamine release and D2 receptor density induced by acute versus subchronic administration of PD149163.

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Figure 1.

The effects of daily subcutaneous injection of saline or the NT1 receptor agonist PD149163 on spontaneous locomotor activity. Bars represent mean ($\pm$ S.E.M.) number of consecutive beam interruptions produced by rats treated with subcutaneous saline (SAL) or 1.0 mg/kg PD149163 during the thirty minutes beginning 10 minutes after drug administration. * represents significantly ($P < 0.01$) different from saline-treated group.

Figure 2.

The effects of a single subcutaneously administered dose of PD149163 (1.0 mg/kg) administered in drug naive rats and rats pre-treated with PD149163 (1.0 mg/kg) on the previous eight days on A) ambulation counts, and B) non-ambulatory beam breaks, induced by subcutaneously administered amphetamine (AMPH) (0.5 mg/kg). Bars represent mean ($\pm$ S.E.M.) number of A) consecutive beam interruptions and B) non-ambulatory beam breaks produced by rats during the thirty minutes beginning 10 minutes after drug administration. SAL = saline; * represents significantly ($P < 0.05$) different from saline/amphetamine treated group.