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Dopamine D_3 receptors contribute to methamphetamine-induced alterations in dopaminergic neuronal function: Role of hyperthermia

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Abstract

Methamphetamine administration causes long-term deficits to dopaminergic systems that, in humans, are thought to be associated with motor slowing and memory impairment. Methamphetamine interacts with the dopamine transporter (DAT) and increases extracellular concentrations of dopamine that, in turn, binds to a number of dopamine receptor subtypes. Although the relative contribution of each receptor subtype to the effects of methamphetamine is not fully known, non-selective dopamine D_2/D_3 receptor antagonists can attenuate methamphetamine-induced changes to dopamine systems. The present study extended these findings by testing the role of the dopamine D_3 receptor subtype in mediating the long-term dopaminergic, and for comparison serotonergic, deficits caused by methamphetamine. Results indicate that the dopamine D_3 receptor selective antagonist, PG01037, attenuated methamphetamine-induced decreases in striatal DAT, but not hippocampal serotonin (5HT) transporter (SERT), function, as assessed 7 days after treatment. However, PG01037 also attenuated methamphetamine-induced hyperthermia. When methamphetamine-induced hyperthermia was maintained by treating rats in a warm ambient environment, PG01037 failed to attenuate the effects of methamphetamine on DAT uptake. Furthermore, PG01037 did not attenuate methamphetamine-induced decreases in dopamine and 5HT content. Taken together, the present study demonstrates that dopamine D3 receptors mediate, in part, the long-term deficits in DAT function caused by methamphetamine, and that this effect likely involves an attenuation of methamphetamine-induced hyperthermia.

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Keywords

Methamphetamine; dopamine D_3 receptors; D_3 antagonist; PG01037; dopamine transporter; serotonin transporter

1. Introduction

Methamphetamine use and dependence is a serious public health concern with a significant societal impact, including a burden on psychiatric and medical resources. Although several neurotransmitter systems are presumably involved in the reinforcing (Achat-Mendes et al., 2012; Munzar et al., 1999) and neurochemical (Eisch et al., 1996; Stephans and Yamamoto, 1994) effects of methamphetamine, there has been a large focus on dopamine systems (Koob and Volkow, 2010). Methamphetamine use can produce both acute and long-term changes in dopaminergic neurons, as demonstrated in rodents (Hadlock et al., 2010; Metzger et al., 2000), non-human primates (Melega et al., 1997), and humans (McCann et al., 1998; Wilson et al., 1996). In humans, these effects of methamphetamine are associated with motor slowing and memory impairment (Volkow et al., 2001) and may be related to psychiatric symptoms (Sekine et al., 2001).

Methamphetamine interacts with the dopamine transporter (DAT) and facilitates the release of dopamine (Kahlig et al., 2005; Raiteri et al., 1979). In turn, this enhances extracellular concentrations of dopamine (Kuczenski et al., 1995; O'Dell et al., 1991) that binds to a number of dopamine receptor subtypes. Preclinical studies indicate that high-dose methamphetamine administration causes long-term changes to dopaminergic systems, including long-term decreases in DAT activity, tyrosine hydroxylase activity, and striatal dopamine content (Kogan et al., 1976; Nakayama et al., 1993; Seiden et al., 1976; Wagner et al., 1980). Although the mechanisms underlying these long-term deficits are not completely understood, several dopamine receptor subtypes have been implicated in mediating the effects of methamphetamine. For example, nonselective dopamine D_2/D_3 receptor antagonists can attenuate methamphetamine-induced dopamine overflow (O'Dell et al., 1993), decreases in DAT activity (Gross et al., 2011; Hadlock et al., 2010), and decreases in dopamine content (Broening et al., 2005; Sonsalla et al., 1986). However, the ability of these antagonists to attenuate the effects of methamphetamine has primarily been attributed, at least in part, to antagonism of methamphetamine-induced hyperthermia (Albers and Sonsalla, 1995). A large body of literature suggests that dopamine D_2 receptors mediate changes in body temperature induced by direct-acting dopamine receptor agonists (Chaperon et al., 2003; Collins et al., 2007); thus, and to the extent that methamphetamine-induced hyperthermia is mediated by dopamine D_2, and not D_3, receptors, selective dopamine D_3 receptor ligands might be useful to investigate the association between methamphetamine-induced hyperthermia and subsequent long-term deficits.

Dopamine D_3 receptors have been suggested as targets for medication development to treat substance use disorders and for its purported role in mediating abuse-related effects of drugs, including methamphetamine (Heidbreder et al., 2005; Higley et al., 2011; Newman et al., 2005); however, it is unclear whether these receptors are involved in other effects of methamphetamine, such as long-term deficits. Compounds that selectively target dopamine

D_3 receptors have been developed, including PG01037 (functions as a selective dopamine D_3 receptor antagonist *in vivo;* Baladi et al., 2010; Collins et al., 2005). The present study utilized PG01037 to investigate the role of dopamine D_3 receptors in mediating the long-term dopaminergic, and for comparison, serotonergic deficits caused by methamphetamine.

2. Material and Methods

2.1. Animals

Male Sprague-Dawley rats (250–300 g upon arrival; Charles River Laboratories) were housed in an environmentally controlled room under a 14/10 light/dark cycle, with food and water provided ad libitum. On the day of the experiment, rats were housed in plastic cages (n = 4 rats/cage) and were maintained in an ambient temperature of ~24°C. Rats received PG01037 (4 × 32 mg/kg/injection, s.c.) or vehicle (4 × 1 ml/kg/injection, s.c.) 30 min before each administration of methamphetamine (4 × 7.5 mg/kg/injection, s.c.; 2-h intervals) or vehicle (4 × 1 ml/kg/injection, s.c.; 2-h intervals). Core (rectal) body temperatures were recorded using a digital rectal thermometer (Physitemp Instruments, Clifton, NJ). Rectal temperatures were recorded immediately before the first injection (i.e. PG01037 or vehicle) and then 30 min before and after every injection of either methamphetamine or vehicle, depending on the group. For experiments where body temperature was manipulated, some cages in which rats received methamphetamine were placed in a warm ambient environment to enhance drug-induced hyperthermia. All experiments were approved by the University of Utah Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2. Body Temperature

In studies designed to investigate the impact of PG01037 pretreatment on methamphetamine-induced alterations in core (rectal) body temperature, temperatures were measured by inserting a thermal probe into the rectum. Animals were adapted to the experimental procedure by measuring body temperature before studies with drug commenced. Cumulative dose-response curves were generated for methamphetamine alone (1–32 mg/kg s.c.) with increasing doses administered every 15 min and in combination with the D_3 receptor-selective antagonist PG01037 (32 and 56 mg/kg s.c.). Beginning 10 min after each injection, body temperature was recorded. The antagonist was given 30 min before administration of the first dose of methamphetamine. Experimental sessions were separated by at least 7 days.

2.3. Synaptosomal [^{3}H]dopamine and [^{3}H]5HT Uptake

Synaptosomal uptake of [^{3}H]dopamine through DAT and [^{3}H]5HT through the serotonin transporter (SERT) was determined 7 days after the last methamphetamine administration and according to methods reported previously (Fleckenstein et al., 1997; Hadlock et al., 2011). In brief, freshly dissected striatal (for DAT uptake) or hippocampal (for SERT) tissue was homogenized in ice-cold 0.32 M sucrose and centrifuged (800*g* for 12 min; 4°C). The supernatant (S1) was then centrifuged (22,000*g* for 15 min; 4°C), and the resulting pellet (P2) was resuspended in ice-cold 0.32 M sucrose. Assays were conducted in modified Krebs' buffer (126 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl2, 16 mM sodium phosphate, 1.4

mM MgSO4, 11 mM dextrose, 1 mM ascorbic acid; pH 7.4). Each assay tube contained synaptosomal tissue (i.e., resuspended P2 obtained from 1.5 mg of original wet weight striatal tissue) and 1 mM pargyline. Nonspecific values were determined in the presence of 1 mM cocaine (for DAT) or 10 mM fluoxetine (for SERT). After preincubation of assay tubes for 10 min at 37°C, assays were initiated by the addition of [^{3}H]dopamine or [^{3}H]5HT (0.5 nM final concentration). Samples were incubated at 37°C for 3 min, then filtered through Whatman GF/B filters soaked previously in 0.05% polyethylenimine. Filters were washed rapidly 3 times with 3 ml of ice-cold 0.32 M sucrose using a Brandel filtering manifold. Radioactivity trapped in filters was counted using a liquid scintillation counter. Remaining resuspended P2 samples were assayed for protein concentrations according to the previous methods (Lowry et al., 1951).

2.4. Dopamine and 5HT Content Determination

Seven days after drug treatment, animals were decapitated, and striatal tissue was immediately removed and frozen on aluminum foil placed over dry ice. Tissue was obtained from the striatum contralateral to that used for synaptosomal [^{3}H]dopamine uptake. Samples were stored at −80°C until assayed. Monoamine levels were determined in tissue homogenates using HPLC, with electrochemical detection (Chapin et al., 1986). Briefly, on the day of the assay, tissue samples (approximately 10 mg of striatal tissue) were thawed in 500 ml of ice-cold tissue buffer [0.1 M phosphate-citrate buffer (pH 2.5) containing 15% methanol], sonicated for 3 to 5 s, and then centrifuged (22,000*g* for 15 min at 4°C). Tissue pellets were retained and dissolved in 1 N NaOH, and protein content was determined according to the method of Lowry et al. (1951). The supernatant (S1) was then centrifuged (22,000*g* for 10 min at 4°C), and the resulting supernatant (S2) was injected onto an HPLC system equipped with a Partisphere C18 reverse-phase analytical column (5-mm spheres; 110 3 4.6 mm) and a reverse-phase guard column (Whatman Inc., Clifton, NJ). The mobile phase consisted of 0.05 M sodium phosphate, 0.03 M citrate buffer (pH 2.86) containing 0.1 M EDTA, 0.035% sodium octyl sulfate, and 25% methanol. Monoamines were detected with an electrochemical detector with the working electrode potential set at +0.70 V relative to an Ag+/AgCl reference electrode.

2.5. Drugs

(±)-Methamphetamine hydrochloride (Research Triangle Institute, Research Triangle Park, NC) was dissolved in 0.9% sterile saline, with the dose described as the free base form. PG01037 (N-{4 [4-(2,3-dichlorophenyl)-piperazin-1-yl]-trans-but-2-enyl}-4-pyridine-2-yl-benzamide HCl) was synthesized by Jianjing Cao (Medicinal Chemistry Section, National Institute on Drug Abuse, Baltimore, MD) using methods reported previously (Grundt et al., 2005) and dissolved in 10% β-cyclodextrin). The doses and pretreatment times for methamphetamine (Hadlock et al., 2010; McGuire et al., 2011) and PG01037 (Baladi et al., 2010; Collins et al., 2005) were selected based on previous publications. All drugs were administered s.c. in a volume of 1 ml/kg.

2.6. Data Analysis

Synaptosomal [^{3}H]dopamine and [^{3}H]5HT uptake data are expressed as average (± S.E.M.) fmol of radioligand per microgram (μg) protein and plotted as a function of treatment group. Values obtained were consistent with those reported previously (Howard et al., 2011; Metzger et al., 2000; Tata and Yamamoto, 2008). Comparisons were made within an experiment (versus across separate experiments) such that animals within a given experiment were treated concurrently, tissues were processed simultaneously and assay conditions to which samples were exposed were identical. Statistical analyses for uptake and monoamine content assays were conducted with an ANOVA with post hoc Bonferroni's for multiple comparisons.

Body temperature data are expressed as the average (± S.E.M.) temperature in degrees Celsius and plotted as a function of time or the average (± S.E.M.) temperature across experiment time for each individual rat (after the first methamphetamine injection) and plotted as a function of treatment group. Statistical analyses were conducted with an ANOVA with post hoc Bonferroni's for multiple comparisons. For the body temperature experiment using cumulative doses of methamphetamine, data are expressed as a change in degrees Celsius from baseline (i.e., body temperature determined after vehicle administration) averaged among six rats (± S.E.M.) and plotted as a function of dose. Differences between methamphetamine dose-response curves in the presence and absence of antagonist were analyzed by simultaneously fitting straight lines to the linear portion of the dose-response curves by means of GraphPad Prism (GraphPad Software Inc., San Diego, CA). The linear portion included doses that spanned the 50% level of effect and included not more than one dose with greater than 75% effect and not more than one dose with less than 25% effect. Differences between slopes and intercepts of the curves were analyzed with the *F* ratio test (GraphPad Prism), as detailed elsewhere (Koek et al., 2006). To calculate ED_{50} values for methamphetamine-induced hyperthermia in the absence and presence of antagonist, a common maximum effect was selected for individual rats. The 95% CLs were calculated from ED_{50} values averaged among rats. To evaluate changes in potency as a result of antagonist treatment, a dose ratio was calculated for each rat by dividing the ED_{50} obtained in the presence of antagonist by the ED_{50} obtained in the absence of antagonist. When the 95% CLs of the dose ratio did not include 1, the antagonist was considered to significantly change the potency of the drug relative to its potency in the absence of antagonist. For all tests, significance was set at $P<0.05$.

3. Results

Methamphetamine (4 × 7.5 mg/kg/injection, s.c., 2-h intervals) decreased striatal DAT uptake as assessed 7 days after the final injection (Fig. 1A; $P<0.05$; $F(3, 30)=7.44$, $P<.0009$). PG01037 (32 mg/kg/injection, s.c.), administered 30 min before each methamphetamine or vehicle injection, attenuated the effects of methamphetamine on DAT uptake. In contrast, PG01037 pretreatment did not attenuate the methamphetamine-induced deficits in hippocampal SERT function at this time point (0.51 ± 0.03, 0.25 ± 0.05*, 0.6 ± 0.07 and 0.36 ± 0.07* fmol/μg protein for vehicle/vehicle, vehicle/methamphetamine, PG01037/saline, PG01037/methamphetamine, respectively; *$P<0.05$; $F(3, 31)=7.01$, $P<0.001$).

PG01037 pretreatment likewise did not attenuate methamphetamine-induced decreases in striatal dopamine or hippocampal serotonin content (Table 1; $F(3, 29)=16.15$, $P<0.0001$, $F(3, 28)=7.940$, $P<0.001$, respectively) as assessed 7 days later. Administration of the antagonist alone did not impact DAT or SERT uptake or striatal monoamine content.

Methamphetamine dose-dependently increased body temperature and a single injection of PG01037 (32 and 56 mg/kg) did not attenuate the hyperthermic effects of methamphetamine (i.e., 95% CLs of dose ratios did include 1; Fig. 2). Similarly, results presented in Fig. 1B demonstrate that repeated methamphetamine injections increased core body temperature by approximately 2–3°C (Fig. 1B, $F(3, 31)=167.50$, $P<0.0001$). However, and in contrast to Figure 2, multiple administrations of PG01037 attenuated methamphetamine-induced increases in body temperature at certain time points throughout the course of treatment [$F(7, 31)=4.60$, $P<0.0001$]. For example, in methamphetamine-treated rats at the fourth temperature recording (i.e. 9:30 a.m.), the body temperature of rats pretreated with PG01037 was significantly lower than the body temperature of rats pretreated with vehicle (Fig. 1B; $P<0.0001$). Administration of the antagonist alone did not alter body temperature (Fig. 1B). When body temperature was plotted as the average temperature throughout the course of treatment for each individual rat, the group means were not different from each other; however, there was a trend for some rats that received PG01037 to have an overall lower temperature (Fig. 1B inset).

To examine whether the ability of PG01037 to attenuate the effects of multiple methamphetamine injections on DAT uptake was related to attenuation of methamphetamine-induced hyperthermia, in the second experiment, rats were treated in a warm ambient environment (a common practice used to promote methamphetamine-induced hyperthermia; see, for example Bowyer et al., 1993; Hadlock et al., 2010; McFadden et al., 2011; Myles et al., 2008). Results indicate that PG01037 failed to attenuate the methamphetamine-induced decreases in DAT (Fig. 3A, $F(3, 32)=31.24$, $P<0.0001$). Further, PG01037 did not attenuate the long-term deficits in SERT function (0.58 ± 0.10, 0.62 ± 0.11, 0.21 ± 0.06,* 0.22 ± 0.03* fmol/μg protein for vehicle/vehicle, vehicle/methamphetamine, PG01037/saline, PG01037/methamphetamine, respectively; *$P < 0.05$; $F(3, 30)=8.70$, $P<0.001$, $F(3, 30)=6.16$, $P<0.01$, respectively), nor the decreases in dopamine and 5HT content (Table 1), as assessed 7 days after drug administration. Methamphetamine increased core body temperature (Fig. 3B, $F(3, 31)=238.8$, $P<0.0001$) and body temperatures were not different between methamphetamine-treated groups pretreated with PG01037 or vehicle (Fig. 3B). Administration of the antagonist alone did not impact DAT or SERT uptake, striatal monoamine content, or body temperature.

4. Discussion

The current study examined the contribution of dopamine D_3 receptors to the long-term effects of methamphetamine on dopamine and 5HT systems. Results indicate that a selective dopamine D_3 receptor antagonist, PG01037, attenuates the long-term methamphetamine-induced decreases in DAT uptake. This effect of PG01037 was most likely due to D3 receptor-mediated antagonism of methamphetamine-induced hyperthermia, particularly during the latter time course of the repeated methamphetamine regimen. This is suggested

by findings that PG01037 did not acutely impact methamphetamine-induced hyperthermia, whether during the first 2 h of the repeated methamphetamine regimen (Fig. 1) or during the 90-min course of the cumulative-dose regimen (Fig. 2). Rather, the attenuation of methamphetamine-induced hyperthermia was observed at several time points beginning 2.5 h after the initiation of methamphetamine treatment (Fig. 1).

It is well established that hyperthermia contributes to the neuronal damage induced by various amphetamine-like compounds, including methamphetamine (Albers and Sonsalla, 1995; Bowyer et al., 1993; Bowyer et al., 1992; Farfel and Seiden, 1995; Metzger et al., 2000; Schmidt et al., 1990). Moreover, when methamphetamine-induced hyperthermia is prevented by manipulating ambient temperature or by pharmacological antagonism, long-term methamphetamine-induced deficits are attenuated (Albers and Sonsalla, 1995; Bowyer et al., 1993). Despite the importance of methamphetamine-induced hyperthermia to the neurotoxic effects of methamphetamine, the receptor mechanism(s) by which methamphetamine increases body temperature is unclear, and might involve dopamine (Bowyer et al., 1993), 5HT (Metzger et al., 2000), and NMDA systems (Bowyer et al., 2001). Furthermore, the receptor mechanism(s) mediating the effects of methamphetamine on body temperature might differ depending on not only the time period after initial drug exposure (as noted above), but also ambient temperature (see, for review Sabol et al., 2013). In support of this notion, in the current study, PG01037 attenuates methamphetamine-induced hyperthermia during the repeated methamphetamine treatment in a normal, but not a warmer, ambient environment. The complex interaction of these central, as well as peripheral mechanisms contributing to methamphetamine-induced hyperthermia (i.e., locomotor activity, metabolism) is an important future area of study (for review see Kiyatkin, 2013).

Findings that PG01037 attenuates the long-term methamphetamine-induced DAT deficits are similar to previous reports wherein nonselective dopamine D_1 and D_2/D_3 receptor antagonists attenuate long-term methamphetamine-induced dopaminergic deficits by reducing the hyperthermic effects of methamphetamine (Gross et al., 2011; Broening et al., 2005; Sonsalla et al., 1986). However, and in contrast to the effect of nonselective dopamine D_2/D_3 receptor antagonists (Broening et al., 2005; O'Dell et al., 1993), PG01037 failed to attenuate methamphetamine-induced decreases in striatal dopamine content. It is conceivable that a larger dose of PG01037 is necessary to attenuate methamphetamine-induced decreases in striatal dopamine content, although selectivity for D_3 receptors over other DA receptor subtypes might be lost. Alternatively, it might be possible that compensatory mechanisms occur that prevent methamphetamine-induced decreases in DAT function but not dopamine content or that the dopamine receptor subtype(s) that mediate the effects of methamphetamine on DAT function and DA content differ. Related to the latter point, evidence that dopamine receptor antagonists can differentially impact the effects of methamphetamine on markers of dopaminergic neuronal integrity is found throughout the literature. For example, pretreatment with the dopamine D_1 receptor antagonist SCH23390 differentially antagonizes long-term methamphetamine-induced decreases in tyrosine hydroxylase activity and dopamine content (Sonsalla et al., 1986). Furthermore, in dopamine D_2 receptor knockout mice, methamphetamine causes long-term deficits in tyrosine hydroxylase, but not DAT levels (Granado et al., 2011). Taken together with the current

data, these findings suggest that multiple dopamine (and perhaps non-dopamine) receptor subtypes may mediate the multiple aspects of methamphetamine-induced deficits.

PG01037 failed to attenuate the effects of methamphetamine on SERT uptake and 5HT content. This is consistent with other reports demonstrating that nonselective dopamine D_2/D_3 receptor antagonists fail to attenuate methamphetamine-induced decreases in SERT uptake and most likely reflects the paucity of dopaminergic neurons in the hippocampus (Haughey et al., 2000). In addition, and similar to other studies using nonselective dopamine D_2/D_3 receptor antagonists, PG01037 did not attenuate the effects of methamphetamine on striatal 5HT content (Broening et al., 2005; Sonsalla et al., 1986).

In conclusion, the present study has demonstrated that dopamine D_3 receptors mediate, in part, the long-term deficits in DAT function caused by methamphetamine, and that this effect likely involves an attenuation of methamphetamine-induced hyperthermia. However, other non-hyperthermic mechanisms remain to be elucidated. Investigating the receptor-mediated mechanisms contributing to methamphetamine-related changes to dopamine systems might enhance understanding of neurodegenerative disorders involving alterations in dopamine systems, such as Parkinson's disease.

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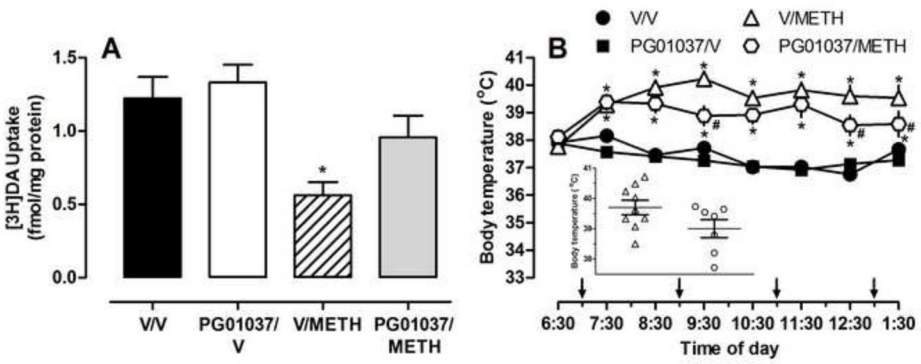

Figure 1.

The effects of PG01037 on methamphetamine-induced decreases in striatal DAT (A) and hyperthermia (B). Rats (n = 6–9/group) received PG010137 (4 × 32 mg/kg/injection, s.c.) or vehicle (4 × 1 ml/kg/injection, s.c.) 30 min before each administration of methamphetamine (4 × 7.5 mg/kg/injection, s.c., 2-h intervals) or vehicle (4 × 1 ml/kg/injection, s.c., 2-h intervals) and were sacrificed 7 days later. Ordinates: mean (± S.E.M.) fmol of [^{3}H]dopamine per μg protein or body temperature. Abscissa: treatment group; V = vehicle and METH = methamphetamine or time across experiment; arrows indicate methamphetamine administration. Inset figure: mean (± S.E.M.) body temperature across time after first methamphetamine injection for each individual rat. * Methamphetamine values significantly different from respective vehicle control group ($P<0.05$). # PG01037/ methamphetamine values significantly different from other methamphetamine group ($P<0.05$).

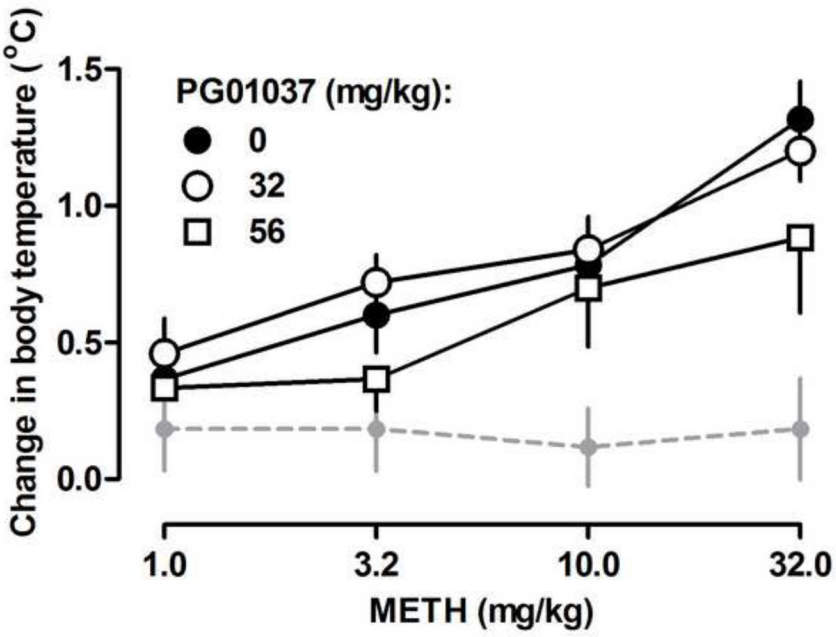

Figure 2.

Methamphetamine-induced hyperthermia when methamphetamine was administered alone and with different doses of PG01037 (n = 6). Abscissa, dose in milligrams per kilogram of body weight; dashed gray line indicate body temperature across time after saline administration. Ordinate, mean (± S.E.M.) change in body temperature.

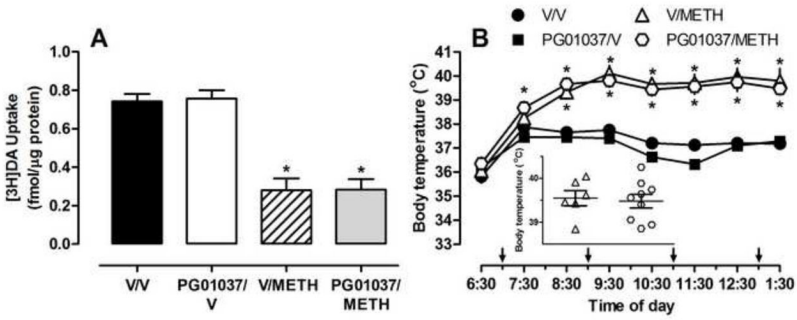

Figure 3.

The effects of PG01037 on methamphetamine-induced decreases in striatal DAT (A) and hyperthermia (B) when rats were treated in a warm ambient environment. Rats (n = 6–9/group) received PG010137 (4 × 32 mg/kg/injection, s.c.) or vehicle (4 × 1 ml/kg/injection, s.c.) 30 min before each administration of methamphetamine (4 × 7.5 mg/kg/injection, s.c., 2-h intervals) or vehicle (4 × 1 ml/kg/injection, s.c., 2-h intervals) and were sacrificed 7 days later. See figure 2 for other details.

Table 1

Effect of PG01037 on methamphetamine-induced decreases in dopamine and 5HT content

	Dopamine	5HT	Dopamine	5HT
Group			**Warm ambient environment**	
Vehicle/Vehicle	90.7 (±6.5)[a]	4.9 (±0.4)	115.5 (±17.9)	2.8 (±0.4)
PG01037/Vehicle	81.6 (±9.6)	4.2 (±0.6)	126.6 (±29.1)	3.5 (±0.6)
Vehicle/methamphetamine	27.1[b] (±5.1)	2.4[b] (±0.3)	40.4[b] (±12.1)	1.3[b] (±0.2)
PG010137/methamphetamine	38.1[b] (±9.2)	2.5[b] (±0.4)	30.2[b] (±7.3)	1.6[b] (±0.3)

[a] Average monoamine content (± S.E.M.) of 6–9 rats per group

[b] Methamphetamine values significantly different from vehicle control values (P<0.05)