ORIGINAL INVESTIGATION

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Sigma₁ receptor upregulation after chronic methamphetamine self-administration in rats: a study with yoked controls

Abstract *Rationale:* Sigma₁ receptors (Sig-1R) are implicated in behavioral sensitization, conditioned place preference, and cellular restructuring induced by psychostimulants. We previously reported that rats which actively self-administered methamphetamine for 5 weeks and were then withdrawn from methamphetamine for 24 h showed downregulation of dopamine D_2 autoreceptors (approximately 30%) in the midbrain and this was not seen in rats that passively received injections of methamphetamine or saline at the same time (yoked controls). Involvement of Sig-1R in the self-administration of psychostimulants, however, has never been reported. *Objectives:* This study examined neuroadaptive changes in Sig-1R in the brains of rats self-administering methamphetamine. *Methods:* Three groups of rats were tested simultaneously 5 days per week, for 5 weeks (25 daily sessions). Two groups served as yoked controls and passively received an injection of either 0.1 mg/kg methamphetamine or saline (not contingent on responding) each time a response-contingent injection of 0.1 mg/kg methamphetamine was actively self-administered by the first group of rats. Protein and mRNA levels of Sig-1R were then measured by Western and Northern blottings, respectively. *Results:* There was a marked upregulation of Sig-1R proteins (50%) in the midbrain and altered levels of Sig-1R mRNA in the frontal cortex and hippocampus of rats that learned to actively self-administer methamphetamine, but not in yoked methamphetamine- or saline-control rats. *Conclusions:* Neuroadaptive increases in Sig-1R seen in this study may contribute to the reinforcing effects of methamphetamine. This upregulation of Sig-1R may be mediated by increased protein kinase A activity due to downregulation of dopamine D_2 autoreceptors.

Keywords Sigma · Receptor · Methamphetamine · Drug self-administration · Yoked control · Midbrain · D · Autoreceptor

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Introduction

Neuroadaptation plays an important role in addictive processes (White and Kalivas 1998; Everitt and Wolf 2002). In midbrain neurons, in particular, an altered neuroplasticity has been shown to be critical in addictive behavior (Ungless et al. 2001; Everitt and Wolf 2002; Saal et al. 2003). What may cause the neural plasticity leading to drug addiction, however, is unknown. We previously found that methamphetamine self-administration in rats caused downregulation of dopamine D_2 receptors in the midbrain, specifically in the ventral tegmental area (34%) and the dorsal part of the substantia nigra zona compacta (21%; Stefanski et al. 1999). However, this change in dopamine D_2 receptors was seen only in rats that were actively self-administering methamphetamine and not in

rats that received non-contingent injections of methamphetamine or saline at the same time (yoked controls).

Sigma$_1$ receptors (Sig-1R) in the brain have been shown to bind dextrorotatory benzomorphans, neurosteroids, and psychostimulants, such as cocaine and methamphetamine, with high to moderate affinities (Sharkey et al. 1988; Quirion et al. 1992; Maurice et al. 2002; Matsumoto, personal communication). Sig-1R also play important roles in certain actions of cocaine and methamphetamine. For example, cocaine- or methamphetamine-induced locomotor stimulation and behavioral sensitization to these effects with repeated injections were blocked by Sig-1R antagonists (Ujike et al. 1996; Matsumoto et al. 2002). In addition, development of cocaine-induced conditioned place preference was attenuated by Sig-1R antagonists, as well as by antisense oligodeoxynucleotides directed against cloned Sig-1R (Romieu et al. 2000, 2002). Further, Sig-1R appear to be involved in cellular restructuring by translocating cholesterol and cytoskeletal proteins from endoplasmic reticulum to the plasma membrane and nucleus (Hayashi and Su 2001, 2003a, 2003b). Since Sig-1R are involved in these psychostimulant-induced behaviors and since Sig-1R are implicated in neuronal restructuring, which are both intimately related to addictive processes, one would expect that Sig-1R may also be involved in the neuroplastic changes associated with the development and subsequent maintenance of psychostimulant self-administration behavior. To date, however, no involvement of Sig-1R in the self-administration of psychostimulants has been reported.

The purpose of this study was to investigate the potential involvement of Sig-1R in the self-administration of methamphetamine by examining protein and mRNA levels of Sig-1R in brain regions of rats which had actively self-administered methamphetamine and from rats that passively received injections of methamphetamine or saline at the same time (yoked controls). As we found previously with dopamine D$_2$ autoreceptors, Sig-1R were altered only in rats actively self-administering methamphetamine, and not in the "yoked-methamphetamine" or "yoked-saline" rats. These findings suggest that neuroplasticity involving Sig-1R may be involved in the development of drug self-administration behavior and that the alteration of Sig-1R may be related to down-regulation at dopamine D$_2$ receptors and a resulting increase in protein kinase A (PKA) activity. In order to provide supporting evidence for this hypothesis, we subjected NG-108 cells to cAMP treatment, which is known to activate PKA, and then measured changes in Sig-1R protein levels.

Material and methods

Yoked methamphetamine self-administration

Subjects

Male Sprague-Dawley rats (Charles River, Wilmington, Mass., USA) weighing approximately 300–330 g at the start of experiment were individually housed in a temperature- and humidity-controlled environment under a 12-h light/dark cycle (lights on at 7:00 a.m.) and food and water were available ad libitum in the home cage. Rats were tested in the light phase. They were experimentally and drug naive at the start of this study.

Rats were implanted with intravenous (IV) catheters, as described previously (Shoaib et al. 1997; Munzar et al. 1999). Under Equithesin (9.72 mg/ml pentobarbital and 44.4 mg/ml chloral hydrate, 3 ml/kg intraperitoneally) anesthesia, a silastic catheter was implanted into the external jugular vein and exited the skin near the midscapular region. A nylon bolt (screw thread # 4-32) was embedded under the same anesthesia on the skull surface and fixed in place with dental acrylic to stainless steel screws imbedded in the skull. The nylon bolt served as a tether, preventing the catheter from being pulled out during self-administration sessions. Following surgery, the IV catheter was flushed daily during the first week with 0.2–0.3 ml sterile 0.9% saline containing heparin (1.25 IU/ml) and a cephalosporin antibiotic (100 mg/ml Cefazolin For Injection, USP; Bristol Mayer Squibb Company, N.J., USA) and then flushed after each daily session with heparin solution to maintain its patency.

Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animals (AAALAC) and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, NIH.

Apparatus

Eighteen standard operant-conditioning chambers (Coulbourn Instruments, Lehigh Valley, Pa., USA) were used. Each chamber contained a white house light and two holes containing nose-poke operanda. Each nose poke produced a brief feedback tone. One hole was defined as active (left in eight chambers, right in remaining eight) and the other hole as inactive. Methamphetamine or saline were delivered through Tygon tubing, protected by a metal spring and suspended through the ceiling of the experimental chamber from a single-channel fluid swivel. This tubing was attached to a syringe pump (Harvard Apparatus, South Natick, Mass., USA), which was programmed to deliver 2-s injections. The injected volume was adjusted for every animal to deliver a methamphetamine dose of 0.1 mg/kg per injection. Experimental events were controlled by microcomputers using Med Associates interfaces and software (Med Associates Inc., East Fairfield, Vt., USA).

Procedure

Eighteen naive rats were divided into three groups and tested simultaneously. Two groups served as yoked controls and passively received an injection of either 0.1 mg/kg methamphetamine or saline (which was not contingent on responding) each time a response-contingent injection of 0.1 mg/kg methamphetamine was actively self-administered by the first group of rats. Nose-poke responses by the yoked control rats were recorded, but had no programmed consequences. Two-hour sessions were conducted daily, Monday to Friday, between 1 and 5 p.m. At the start of each session, a white house light was turned on and a priming injection of 0.1 mg/kg methamphetamine (or saline for third group), sufficient to fill the "dead" space of the IV catheter, was automatically delivered. Each nose-poke response in the active hole produced an IV injection of

0.1 mg/kg methamphetamine followed by 30-s timeout period, during which the chamber was dark and responses in either hole had no programmed consequences. Nose-poke responses in the "inactive" hole were recorded but had no programmed consequences.

After responding by rats actively self-administering methamphetamine was initiated, the number of responses required to produce each injection was gradually increased over a period of 10–13 days from one to a final value of five (a 5-response fixed-ratio schedule; FR5). After 5 weeks, all rats were killed by decapitation 24 h after the last (25th) session and brains were quickly removed and dissected on an iced glass plate into six regions: cerebellum, frontal cortex, striatum, midbrain, brainstem, and hippocampus. Tissues were kept at −80°C until use.

Western blottings of Sig-1R in the brain and in NG-108 cells

Tissues from brain regions were homogenized in 1 ml of 2×sample buffer (100 mM Tris-HCl, pH 6.8; 20% glycerol; 4% SDS) with a Teflon homogenizer (setting at 11,000 rpm, 10 strokes; TRI-R Instruments, Model K41, Rockville Center, N.Y., USA). The homogenate was centrifuged at 10,000 rpm for 10 min, and the supernatant collected. The supernatant was incubated with trichloroacetic acid (final concentration at 13%) on ice for about 15 min and centrifuged at 10,000 rpm for 10 min at 4°C. The pellet was washed with 100% ethanol under sonication and then centrifuged at 10,000 rpm for 10 min at 4°C. The resultant pellet was dissolved in 2×sample buffer and used in Western blotting for Sig-1R as described elsewhere (Takebayashi et al. 2002).

Since rats that actively self-administer methamphetamine show a downregulation of dopamine D_2 receptors (Stefanski et al. 1999) as well as upregulation of Sig-1R (this study) in the midbrain, these two events might be related. We speculated that protein kinase A (PKA) activation resulting from the downregulation of dopamine D_2 autoreceptors might be involved in the upregulation of Sig-1R. In order to provide supporting evidence for this potential mechanism, we used NG-108 cells, which have been used extensively in our laboratory to examine functional roles of Sig-1R (e.g. Hayashi and Su 2001, 2003a, 2003b), and subjected them to cAMP treatment, which is known to activate PKA. In the experiments examining the effect of cAMP on Sig-1R, NG-108 cells were plated at 100,000 cells per well in 12-well plates and incubated with 0.5 mM of a stable cAMP analog dibutyryl cAMP. The medium was changed daily with the addition of freshly prepared dibutyryl cAMP. The NG-108 cells were treated with dibutyryl cAMP in this manner for 3 days before they were assayed for Sig-1R with Western blottings, as described previously (Hayashi and Su 2001). Briefly, cells were collected using ice-cold phosphate-buffer saline and then solubilized in the sample buffer [100 mM Tris-HCl (pH 6.8), 20% glycerol, 4% SDS]. After addition of β-mercaptoethanol, samples were boiled for 4 min. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transblotted to polyvinylidene difluoride membranes. The non-specific sites were blocked with 5% (w/v) non-fat milk for 5–6 h at 4°C. For immunodetection, the membrane was incubated at 4°C overnight with antibodies in Tris-buffered saline/Tween 20 containing 1% of NP-40. Protein bands were visualized by an enhanced chemiluminescence detection kit (Amersham Biosciences Inc., Piscataway, N.J., USA), scanned digitally, and densitometrically analyzed by a Macintosh computer-based analysis system with "NIH Image" software.

Northern blotting of Sig-1R

Tissues were homogenized with a Teflon homogenizer (dial 7, 10 strokes; TRI-R Instruments, Model K41, Rockville Center, N.Y., USA) in 4 M guanidine thiocyanate solution containing 0.6% 2-mercaptoethanol. Total RNA was extracted according to the acid guanidinium-thiocyanate-phenol-chloroform method by Chomczynski and Sacchi (1987). Homogenates were centrifuged in a mixture of sodium acetate, phenol, and chloroform-isoamylalcohol, and aqueous phases were collected. Total RNA was precipitated by the addition of isopropanol and collected after centrifugation. The rat Sig-1R gene was cloned from NG108-15 cells (Hayashi and Su 2001). Subcloned Sig-1R cDNA in pCR 3.1 vectors (Invitrogen) was digested by EcoR I and purified by agarose electrophoresis (Concert gel extraction system; Gibco). The resultant cDNA probe with a sequence complementary to bases 5–900 is radiolabeled with [^{32}P]dCTP using the Random Primed DNA Labeling Kit (Boehringer Mannheim Biochemical). RNA (10 μg) was electrophoresed on 1.0% agarose gels. MOPS buffer (×1) was used as the running
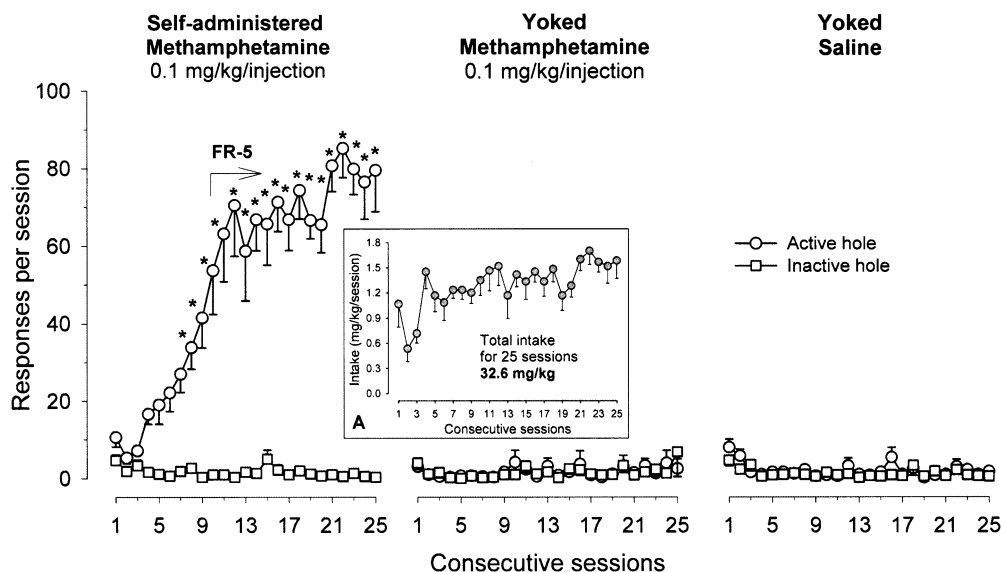

Fig. 1 The mean number (±SEM) of responses in the active and inactive holes for rats that were allowed to acquire self-administration of methamphetamine at a dose of 0.1 mg/kg per injection (*n*=6) and their yoked controls that received yoked infusions of methamphetamine (*n*=6) or saline (*n*=6) during each of the daily 2-h sessions. The *arrow* indicates the period when methamphetamine self-administration was maintained under the final FR5 schedule of reinforcement. *Asterisks* (*) denote significant differences $P<0.01$ between active and inactive nose-pokes. *Inset A*: the symbols (mean ±SEM) represent the total amount of actively self-administered (first group) or passively delivered (yoked methamphetamine group) methamphetamine during each of 25 experimental sessions

buffer. After electrophoresis, gels were soaked in autoclaved water and ×10 SSC solution, and then transblotted onto the membrane (Hybond N, Amersham) for 1.5 h. Transblotted RNA was cross-linked by UV (Stratagene). The radiolabeled cDNA probe (total counts/2 ml of hybridization solution=$2.0×10^6$, specific activity >10^8 cpm/μg) was hybridized with the RNA on the membrane in a hybridization solution overnight at 68°C (Quickhybri, Stratagene). After hybridization, the membranes were washed in 1×SSC containing 0.1% SDS for 20 min at room temperature, followed by a 60 min wash at 50°C. Membranes were then exposed to X-ray film (Hyperfilm MP, Amersham) with two intensifying screens for 6 days at −70°C. mRNA of glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was used as an internal control.

Drugs

S-(+)-Methylamphetamine hydrochloride was obtained from NIDA (Rockville, Md., USA) and dissolved in sterile 0.9% saline. Injection speed was adjusted daily according to the weight of each rat, in order to provide injections of 0.1 mg/kg methamphetamine in a volume of 250 μl/kg over a 2-s period. Doses refer to the salt form of the drug.

Data analysis

Behavioral results are expressed as total number of nose-poke responses in the active- and the inactive hole per 2-h session and each value represents a group mean (±SEM). Methamphetamine intake in mg/kg per 2-h session was also calculated (Fig. 1, inset A). Data were analyzed using multifactorial analysis of variance (ANOVA) for repeated measures and post-hoc Student's *t*-test comparisons were performed to locate differences between group means. For Western and Northern blottings, the NIH Image was used to digitize optical densities obtained on the film. One-way ANOVA followed by Fisher's PLSD post-hoc test were used to detect statistical difference with the significance level set at $P<0.05$.

Results

Yoked methamphetamine self-administration

Figure 1 shows the average number of active- and inactive-hole responses for rats actively self-administering methamphetamine and paired control rats receiving yoked injections of either methamphetamine or saline. In the methamphetamine self-administering group (left panel), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [$F_{(1,120)}=63.34$, P<0.01] over the 25 sessions [$F_{(24,120)}=15.56$, P<0.01]. In addition, there was an overall significant interaction between nose-poke responding and sessions [$F_{(24,120)}=24.04$, $P<0.01$]. Post-hoc analysis revealed that a significant preference for the active hole occurred on sessions 8–25 ($P<0.01$). In the yoked-methamphetamine control group (middle panel), a two-factor ANOVA for repeated measures did not indicate a significant effect of sessions. The difference between nose-poke responding in the active and inactive holes, as well as the interaction between nose-poke responding and sessions, also failed to reach significance in the yoked-methamphetamine group. In the yoked-saline control group (right panel), a two-factor ANOVA for repeated

measures indicated a significant effect of sessions [$F_{(24,120)}=2.28$, $P<0.01$]. The effect between active and inactive hole responding, as well as the interaction between nose-poke responding and sessions, failed to reach significance.

Protein and mRNA levels of Sig-1R

The highest levels of Sig-1R proteins were seen in the striatum of the male Sprague-Dawley rat brain, followed by frontal cortex and hippocampus (Fig. 2A). Intermediate levels were seen in the cerebellum and midbrain. The brainstem had the lowest level of Sig-1R, which is below the detection level in this study (Fig. 2A).

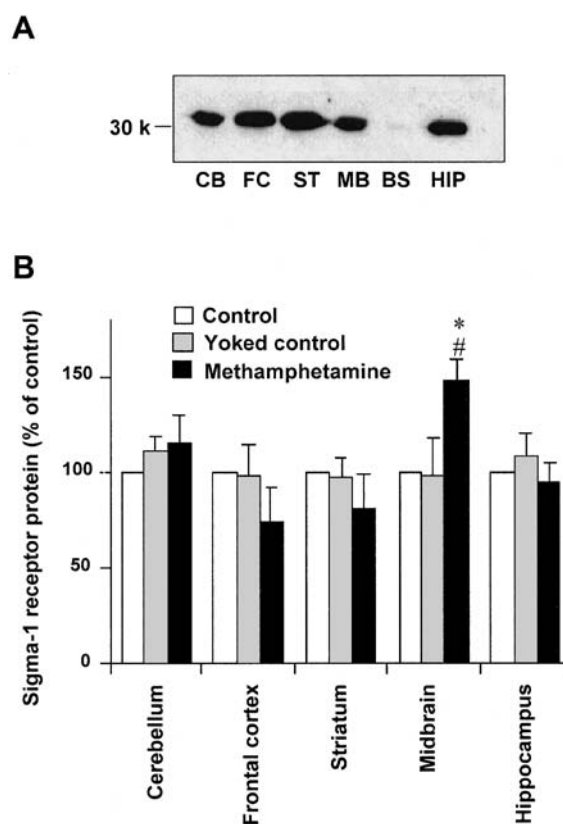There was a marked increase in Sig-1R density (about 50%) in the midbrain of rats actively seeking the self-

Fig. 2 Western blotting of sigma₁ receptors in different brain regions in rats actively self-administering methamphetamine, passively receiving methamphetamine (*yoked-methamphetamine*), or passively receiving saline (*yoked-saline*). Aliquots of 40 μg protein from each brain area were applied to each lane of the SDS/PAGE apparatus. Densitometric data were analyzed by ANOVA followed by Fisher's PLSD. **A** A representative Western blotting of sigma₁ receptors in different brain regions of the male Sprague-Dawley rat. *CB* cerebellum; *FC* frontal cortex; *ST* striatum; *MB* midbrain; *BS* brainstem; *HIP* hippocampus. **B** Histograms showing the results of sigma₁ receptor densities in different brain regions from the three groups of rats. Each brain region from five animals was assayed as an individual sample. Data were normalized to *yoked-saline* animals which serve as controls (100%). *$P<0.05$ compared with controls; #$P<0.05$ compared with *yoked-methamphetamine*

administration of methamphetamine, but not in yoked methamphetamine- or saline-control rats [one-way ANOVA, $F_{(2,12)}=4.52$, $P<0.05$; Fisher's PLSD, $P<0.05$ between control versus METH, $P<0.05$ between yoke versus METH; $n=5$ rats; Fig. 2B]. No changes in Sig-1R protein levels were found in any other brain region in the three groups of animals. There were no differences in Sig-1R densities in any brain region between the yoked-methamphetamine and the yoked-saline control rats (Fig. 2B).

In rats that had learned to actively self-administer methamphetamine, but not in yoked-methamphetamine or yoked-saline control rats, mRNA levels were lower in the frontal cortex [one-way ANOVA; $F_{(2,13)}=7.30$, $P<0.01$; Fisher's PLSD, $P<0.01$ for control versus METH; $n=5–6$ rats; Fig. 3] and higher in the hippocampus [one-way ANOVA; $F_{(2,18)}=4.27$; Fisher's PLSD, $P<0.05$ for control versus METH, $P<0.05$ for yoke versus METH; Fig. 3]. As with Sig-1R protein levels, there were no differences in Sig-1R mRNA levels in any brain region between the yoked-methamphetamine and the yoked-saline control rats (Fig. 3).

Dibutyryl cAMP treatment and Sig-1R in NG-108 cells

There was almost a 100% increase in Sig-1R protein level in NG-108 cells after a 3-day treatment with dibutyryl cAMP (0.5 mM) compared with treatment with vehicle (Fig. 4). This increase in Sig-1R was statistically significant according to a Student t-test ($P<0.01$; control versus cAMP-treated cells), which was used for analysis of data from four independent experiments.

Fig. 4 Effects of 3-day treatment with vehicle or dibutyryl cAMP on the protein level of Sig-1R in NG-108 cells. *Bars* represent means (±SEM) of densitometric quantification of Western blottings of Sig-1R from control cells (*Control*) and dibutyryl cAMP-treated cells (*cAMP*) from four independent experiments. A representative Western blotting is also shown. Densitometric data were analyzed by Student t-test; ** $P<0.01$

Discussion

Active self-administration of drug is an essential element of drug addiction and is the most direct means of preclinically assessing a drug's abuse liability. However, the determinants of self-administration behavior are com-

Fig. 3 Northern blotting of sigma₁ receptor mRNAs in different brain regions in rats actively self-administering methamphetamine, passively receiving methamphetamine (*yoked-methamphetamine*), or passively receiving saline (*yoked-saline*). Densitometric data were analyzed by ANOVA followed by Fisher's PLSD. **A** Representative Northern blotting of sigma, receptor mRNAs in hippocampus (*left panels*) and frontal cortex (*right panels*) with glyceraldehyde 3-phosphate dehydrogenase (*G3PDH*) as controls. *S* yoked-saline rats; *Y* yoked-methamphetamine rats; *M* methamphetamine self-administering rats. **B** Relative Sig-1R mRNA levels normalized to the yoked-saline group (*Control*). $n=5–7$ rats. *$P<0.05$, **$P<0.01$ compared to Control; #$P<0.05$ compared to yoked-methamphetamine group (*Yoked*)
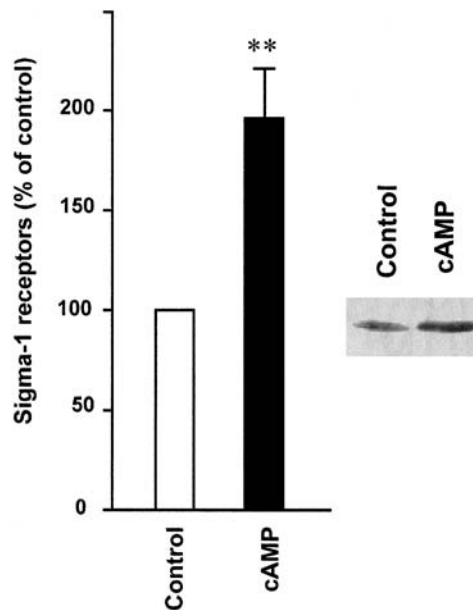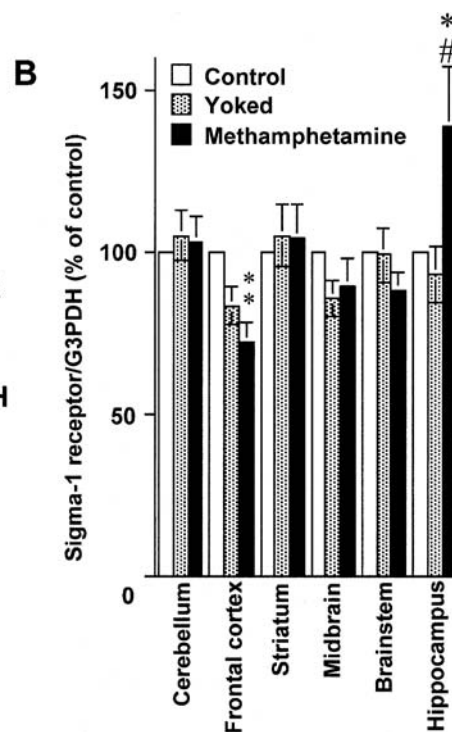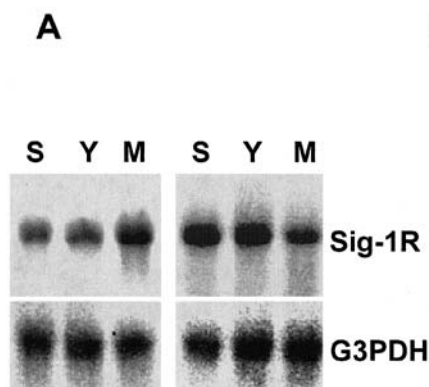
plicated and the biochemical mechanisms underlying the behavior are only recently being elucidated (see reviews by Robinson and Berridge 1993; Everitt et al. 1999; Shalev et al. 2002). By measuring neuroadaptive changes in animals actively self-administering methamphetamine and comparing them with changes in yoked controls, we recently reported an unusual finding: downregulation of dopamine D_2 receptors in the midbrain (the ventral tegmental area and the dorsal part of the substantia nigra zona compacta) of the rat, which occurred only in animals that learned to actively self-inject IV methamphetamine and not in yoked methamphetamine-control animals that passively received exactly the same amounts of methamphetamine at the same time (Stefanski et al. 1999). These findings suggest that neuroadaptive changes in dopamine D_2 receptors are related to the learned acquisition of methamphetamine self-administration behavior by as yet unknown mechanisms, and that these neuroadaptive changes are not simply due to the pharmacological actions of methamphetamine. Because Sig-1R appear to play a role in learning and memory and in neuronal restructuring, and because acquisition and subsequent maintenance of drug self-administration behavior obviously involves certain neuroplastic changes mediating mnemonic processes (e.g. White 1996), in this study we examined neuroadaptive changes in Sig-1R after the learned acquisition of methamphetamine self-administration behavior. Since exposure of rats to methamphetamine has been shown to increase Sig-1R in the frontal cortex and substantia nigra (Itzhak 1994), we postulated that animals that had learned to actively self-administer methamphetamine might show an increase in Sig-1R, which we have shown to cause cellular alterations by translocating cholesterol and cytoskeletal proteins from endoplasmic reticulum to different parts of neurons (Hayashi and Su 2001, 2003a, 2003b).

There was a marked increase in Sig-1R density (about 50%) in the midbrain of rats actively self-administering methamphetamine. Since the upregulation of midbrain Sig-1R in the present study and the downregulation of midbrain dopamine D_2 receptors in our previous study (Stefanski et al. 1999) were not seen in yoked animals that received identical amounts of methamphetamine passively, these changes appeared to be the result of neuroadaptations related to the acquisition (learning) of active drug-seeking behavior and not simply the result of direct pharmacological actions of IV methamphetamine injections. At present, however, we cannot completely rule out the possibility that the change in Sig-1R in the midbrain is related to a combination of drug exposure and increased movement related to the active self-administration behavior, rather than being a specific consequence of the cognitive demands during acquisition of methamphetamine self-administration.

Although there has been a previous report (Itzhak 1994) of direct effects of passively administered methamphetamine on Sig-1R levels in the rat (an upregulation in the frontal cortex and substantia nigra), the daily dose of methamphetamine in that study was much higher. In the study by Itzhak (1994), rats were given a single IP dose of methamphetamine (4 mg/kg) once a day for 10 days. In contrast, in the present study, rats either actively self-administered or passively received an average daily dose of 1.3 mg/kg methamphetamine, delivered as a series of IV injections distributed over a 2-h session for 25 consecutive daily sessions. However, it is noteworthy that the methamphetamine treatment paradigm employed by Itzhak (1993, 1994) caused behavioral sensitization in rats that included rearing and stereotypic head movement. It is known that expression of sensitization is usually related to changes in the striatum, while midbrain changes are necessary earlier in learning. Thus, it is tempting to speculate that the discrepancy between the two studies may be explained by the fact that the FR5 task in our present study is sufficiently more complicated than sensitization procedures, thus invoking changes in the midbrain region. As such, when taken together with dopamine D_2 receptor downregulation, our results might be in congruence with the hypothesis that VTA bursting and DA release serve as teaching or directing signals for certain learned behaviors (Ljungberg et al. 1992).

There were also altered levels of Sig-1R mRNA in rats actively self-administering methamphetamine. Levels of mRNA were significantly reduced in the frontal cortex and increased in the hippocampus. These changes in Sig-1R mRNA levels, when taken together with the changes in Sig-1R protein levels, suggest that self-administration may differentially affect Sig-1R protein turnover in different brain regions. For example, in the frontal cortex where Sig-1R mRNA levels decreased and in the midbrain where there was no change in Sig-1R mRNA levels (Fig. 3), there were correspondingly similar or higher Sig-1R protein levels (Fig. 2B), suggesting a lower turnover rate of Sig-1R proteins in these regions. Conversely, there appeared to be a higher turnover rate of Sig-1R protein in the hippocampus of rats actively self-administering methamphetamine, since Sig-1R mRNA levels rose significantly (Fig. 3) while protein levels of Sig-1R remained unchanged (Fig. 2B). Sig-1R mRNA and protein levels were altered only in rats that had learned to actively self-administer methamphetamine and not in yoked-methamphetamine or yoked-saline controls. Of particular note, the mRNA alterations seen in the self-administering rats were in brain regions (frontal cortex and hippocampus) where higher brain orders related to cognition and learning and memory are executed. The mismatch between changes in the protein and the mRNA level of a gene product is not unusual. Such mismatches have been reported for dopamine D_4 receptors (Defagot et al. 1997; De la Garza and Madras 2000) and for brain-derived neurotrophic factor (Tropea et al. 2001).

Our present results, together with those from our previous report (Stefanski et al. 1999), suggest a potential relationship between the downregulation of dopamine D_2 receptors and the upregulation of Sig-1R, specifically in the midbrain. One of the main signaling pathways for dopamine D_2 receptors is through inhibition of adenylate cyclase, and, consequently, inhibition of PKA activity.

Downregulation of dopamine D_2 receptors would increase PKA activity and this may lead to increases in Sig-1R. In order to provide supporting evidence for this hypothesis, we used NG-108 cells, which have been used extensively in our laboratory for examining the functional roles of Sig-1R (e.g. Hayashi and Su 2001, 2003a, 2003b), and subjected them to cAMP treatment that is known to activate PKA. The cAMP treatment caused an almost 100% increase in Sig-1R in NG-108 cells. Therefore, our results suggest that Sig-1R upregulation, induced by active methamphetamine self-administration behavior, might be mediated by increased PKA activity due to dopamine D_2 receptor downregulation. Further investigations are needed to test the hypothesis that dopamine D_2 receptor down-regulation may regulate the dynamics of Sig-1R and to demonstrate with immunohistochemical techniques that Sig-1R upregulation is, in fact, taking place in dopaminergic neurons in the midbrain.

Regardless of the exact mechanism whereby Sig-1R might be upregulated, the functional consequences of increased Sig-1R might involve morphological changes in midbrain neurons. We have shown that Sig-1R translocate in the cell (Hayashi and Su 2001, 2003b) and that the translocation of Sig-1R affects the dynamics of cytoskeletal proteins that are important for morphological alteration of neurons (Su and Hayashi 2001). Thus, upregulation of Sig-1R in the self-administering animals may cause morphological changes in dopaminergic neurons in the VTA. Although more studies are required to prove this hypothesis, results from our previous study provide some support. A 4-fold overexpression of Sig-1R in PC12 cells shifts the dose-response curve for nerve growth factor-induced neurite sprouting 2-fold to the left (Takebayashi et al. 2002).

In summary, the neuroadaptive changes in Sig-1R seen in this study may be related to the downregulation of dopamine D_2 autoreceptors and contribute to the reinforcing effects of methamphetamine, either by potentiating the physiological and subjective effects of methamphetamine directly or by facilitating development of learned associations between the physiological and subjective effects of methamphetamine injections, environmental stimuli predicting the availability of methamphetamine injections, and operant responses leading to injections.

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