

Homer1 proteins and AMPA receptors modulate cocaine-induced behavioural plasticity

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Keywords: antisense, glutamate, nucleus accumbens, rat, sensitization

Abstract

Homer proteins form functional assemblies in the excitatory postsynaptic density, and withdrawal from repeated cocaine administration reduces the expression of Homer1b/c in the nucleus accumbens. To determine if the reduction in Homer1b/c may be contributing to cocaine-induced behavioural sensitization, antisense oligonucleotides were infused over two weeks into the nucleus accumbens of rats to reduce Homer1 gene expression by approximately 35%. Infusion of antisense sequences (AS1 and AS2) caused a sensitization-like augmentation in the motor response to acute cocaine administration in naive rats. One of the sequences (AS1) also prevented the development of sensitization to repeated cocaine treatment, while AS2 was without effect. A panel of immunoblots for other proteins in the excitatory postsynaptic density revealed that AS1, but not AS2 reduced the level of the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunit GluR1 protein. This posed the possibility that altered AMPA signalling may mediate the inhibitory effect of AS1 on the development of sensitization. To examine this possibility, rats were pretreated in the accumbens with drugs to block AMPA/kainate, *N*-methyl-D-aspartate, group 1 metabotropic glutamate or dopamine receptors prior to each daily injection of cocaine. Only AMPA/kainate receptor blockade prevented the development of behavioural sensitization to cocaine. These data indicate that the expression of behavioural sensitization arises in part from a reduction in Homer1 gene products in the accumbens, while the development of sensitization requires stimulation of AMPA/kainate receptors.

Introduction

Locomotor sensitization is an enduring form of behavioural plasticity produced by the repeated administration of cocaine or amphetamine (Wolf, 1998; Nestler, 2001) and glutamatergic neurotransmission is intimately involved in this neuroplasticity (Wolf, 1998; Thomas *et al.*, 2001). Recent studies indicate that synaptic scaffolding proteins regulate glutamate receptor trafficking and intracellular signalling, and have implicated these proteins in establishing neuroplastic changes at glutamatergic synapses (Scannevin & Huganir, 2000). The Homer family of proteins contribute to the excitatory synaptic scaffold (Brakeman *et al.*, 1997; Kato *et al.*, 1998; Tu *et al.*, 1998; Tu *et al.*, 1999). Three genes encode the Homer protein family, which all contain an N-terminal EVH1 domain capable of binding complementary motifs found in group I metabotropic glutamate receptors (mGluR), inositol phosphate 3 receptors (IP3R), and shank (Kato *et al.*, 1998; Xiao *et al.*, 1998). The constitutively expressed long forms, Homer1b/c, 2, and 3 make multimers through a C-terminal coiled-coil domain and can thereby tether group I mGluR, IP3R, and ionotropic glutamate receptors by binding to the shank-GKAP-PSD95 network of postsynaptic scaffolding proteins (Naisbitt *et al.*, 1999; Tu *et al.*, 1999). The short form, Homer1a, lacks the coiled-coil domain and is significantly expressed only in response to an increase in synaptic activity, including an acute injection of cocaine (Brakeman *et al.*, 1997). Withdrawal from repeated exposure to cocaine reduces Homer1b/c expression selectively in the nucleus accumbens (Swanson

et al., 2001); an anatomical interface of reward and motor circuitry that is implicated in both the acute and enduring effects of psychostimulants (Everitt & Wolf, 2002; McFarland & Kalivas, 2003). Consistent with a scaffolding function in excitatory synapses, the reduction in Homer1b/c was associated with decreased behavioural and neurochemical effects of group I mGluR stimulation (Swanson *et al.*, 2001).

In the present study it was hypothesized that reduced Homer1b/c proteins in the nucleus accumbens produced by repeated cocaine administration contributes to the expression of locomotor sensitization. An antisense (AS) oligonucleotide strategy was employed to mimic the selective reduction of Homer1 gene products in the nucleus accumbens. Intra-accumbens infusion of two different AS sequences were used to produce a partial reduction in Homer1b/c that mimicked the reductions produced by repeated cocaine administration, and both AS sequences caused a sensitization-like augmentation in the acute motor stimulant effect of cocaine. One of the AS sequences not only reduced Homer1 expression, but also lowered the level of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunit GluR1 and was uniquely effective in preventing the development of sensitization. This posed a possible role for AMPA receptors in the development of sensitization, and intra-accumbens administration of antagonists selective for various glutamate receptor subtypes was used to evaluate this hypothesis.

Materials and methods

Animal housing

Male Sprague–Dawley rats were obtained from Harlan laboratories (Indianapolis, IN) and housed two per cage with water and food

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Received 19 March 2003, revised 20 June 2003, accepted 8 July 2003

available *ad libitum*. All housing and experimental procedures were conducted in AAALAC approved facilities according to guidelines established by the National Institutes of Health. A 12-h light:12-h dark cycle was used, with lights on at 07.00 h. Behavioural experiments and cocaine injections were performed during the light cycle, and animals were acclimated to the housing facility for one week before starting the experiments.

Stereotaxic surgery

Before surgery, rats weighing 275–300 g were anaesthetized with a mixture of ketamine:xylazine (100 mg/kg, i.p.: 1 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Guide cannula (26 gauge; Plastics One, Roanoke, VA) were bilaterally implanted in nucleus accumbens (A/P 1.2, L/M 2.0, D/V 7.3 mm from Bregma at $\theta = 8^\circ$) (Paxinos & Watson, 1986) and cemented in place by affixing dental acrylic to three stainless steel screws that were tapped into the skull. Each guide cannula was connected to a 14-day osmotic minipump (Alza Corp., Cupertino, CA) via PE-60 plastic tubing that was implanted in a subcutaneous pouch tunnelled between the animal's scapulae. The rats were permitted a minimum of 7 days after surgery prior to beginning the experiments to insure down-regulation of Homer1 gene products. The pumps were removed from rats at the end of the 14-day oligonucleotide delivery period. In some experiments bilateral guide cannulae for drug microinjection were implanted into the nucleus accumbens as described above for the osmotic minipump guide cannulae, except that the tip of the cannula was located 1 mm above the desired injection site.

Oligonucleotides

Oligonucleotide sequences (IDT Technologies, Coralville, IA) were dissolved in sterile saline, and the concentration was determined by optical density measurement. Both antisense and random oligonucleotide sequences were 25 bases and were 5' and 3' tailed with five phosphorothioated nucleic acids to protect from degradation by nucleases. The sequences for Homer1 antisense and random oligonucleotides were as follows: AS1 5'-GGTTGTTCCCCCATTTTGCC-CAATG-3'; AS2 5'-GGAAGACATGAGCTCGAGTGCTGAA-3'; RN 5'-TGACTAGGTCTCGTTACCTGTCTC-3'. All sequences were checked against the GenBank using the BLAST program for sequence homology and no sequence similarity was found to known genes. The oligonucleotide solutions were adjusted to 42 μM with saline for delivery of 500 pmol/24 h at a flow rate of 0.5 $\mu\text{L}/\text{h}$.

Microinjection of receptor antagonists

Drugs microinjected into the nucleus accumbens included: AMPA/kainate receptor antagonist 6-nitro-7-sulfamoylbenzo[f]quinolizine-2,3-dione (NBQX; 0.15 or 1.5 nmol/side), the *N*-methyl-D-aspartate (NMDA) receptor antagonist 3-(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid (CPP; 0.3 nmol/side), the D1/D2 dopamine receptor antagonist fluphenazine (30 nmol/side), a combination of 2-methyl-6(phenylethynyl)pyridine (MPEP; 0.5 nmol/side) and 7-(hydroxyamino)cyclopropa(b)chromen-1a-carboxylate (CPCCOEt; 5 nmol/side) to block both group I mGluR subtypes or sterile 0.9% saline vehicle. All drug doses were chosen from previous experience as behaviourally effective doses for intracranial administration (Kalivas & Alesdatter, 1993; Cornish & Kalivas, 2000; Swanson & Kalivas, 2000; McFarland & Kalivas, 2001). To microinject drug or saline vehicle, the obturators were removed from the top of the bilateral guide cannulae and replaced by injection needles (33 gauge stainless steel) that were extended 1 mm below the tip of the guide cannulae into the nucleus accumbens. Bilateral infusions were made over 60 s in a volume of 0.5 $\mu\text{L}/\text{side}$. One minute later, the injector was removed slowly and the obturator

replaced. Microinjections were made 5 min prior to injection of cocaine or saline in the behavioural sensitization treatment protocol (see below).

Cocaine sensitization paradigm

Behavioural activity was monitored using a photocell apparatus (AccuScan, Columbus, Ohio). This apparatus consisted of clear acrylic cages, which have photobeam sensors on X, Y and Z axes. Each beam break was monitored and counted. The day before the start of experiment animals were habituated to the photocell boxes for 2 h. On the first day of experimentation the rats were habituated to the photocell boxes for 1 h. After habituation, rats received either saline (1 mL/kg, i.p.) or cocaine (15 mg/kg, i.p.), were returned to the photocell boxes and motor activity was quantified for 2 h. Subjects then received saline (1 mL/kg, i.p.) or cocaine (30 mg/kg, i.p.) for the next 5 days in the home cage. The next day, rats were again habituated to the photocell boxes for 1 h, administered either saline (1 mL/kg, i.p.) or cocaine (15 mg/kg, i.p.) and returned to the photocell boxes for 2 h. All animals were withdrawn from drug treatment and remained in the home cage for three weeks. After three weeks, all animals were again habituated to the photocell boxes for an hour, subsequently administered either saline (1 mL/kg, i.p.) or cocaine (15 mg/kg, i.p.) and motor activity monitored for 2 h.

Infusion of oligonucleotides typically began 7 days prior to administering daily cocaine or saline injections for 7 days, and the pumps were removed 24 h after the last injection. In some experiments microinjections were made 5 min prior to each daily injection of cocaine or saline (days 1–7), and animals were given only cocaine or saline on day 28.

Western blot analysis

Immunoblotting methods are described in detail elsewhere (Toda *et al.*, 2002). Protein concentration was determined by the DC assay (Biorad) and the proteins in the sample were separated using a SDS/PAGE gel. Proteins were transferred from the gel to a PVDF membrane using a semidry transfer apparatus (Biorad). Proteins bound to the membrane were stained with Ponceau S solution (Sigma) to determine the quality of the transfer. Membranes were blocked for 1 h at room temperature and incubated with the primary antibody in antibody buffer containing Tween-20 (50 $\mu\text{L}/100\text{ mL}$) overnight at 4 $^\circ\text{C}$, washed with the antibody buffer and incubated with secondary antibody for 1 h at room temperature. Membranes were washed thoroughly and immunolabelling was visualized by enhanced chemiluminescence and the density of bands quantified by densitometry using NIH Image 6.2. Homer1b/c and Homer3 antibodies were gifts from Dr Paul Worley (Johns Hopkins University Medical Center). Other antibodies were from commercial sources.

Immunohistochemistry and histology

For experiments examining potential oligonucleotide toxicity, rats were implanted with guide cannulae and osmotic pumps as described above. Each rat was given AS1 and random oligonucleotide infusions in the contralateral nucleus accumbens. The experiment was designed so that nucleus accumbens on one side of the brain could be compared to the other side within one animal to reduce variability between subjects. In addition, one group of rats ($n=4$) was bilaterally implanted with infusion guide cannulae as described above but the cannulae were not administered an overdose of pentobarbital or connected to osmotic pumps. For histological evaluation animals were transcardially perfused with PBS and ice-cold 4% paraformaldehyde solution and brains were incubated in 2% paraformaldehyde for 4 h postfixation. Subsequently, coronal sections (50 μm thick) were taken

and processed for immunohistochemistry. Sections were blocked and incubated with primary antibody for GFAP (Sigma, 1:800 dilution) overnight at room temperature to stain for astroglia. The immunostaining was developed using reagents from Vector laboratories following the recommended procedure. In each experiment near adjacent sections were stained with cresyl violet to visualize microglia and neurons.

After the behavioural experiments, rats were given an overdose of pentobarbital (>100 mg/kg, i.p.) and perfused transcardially with PBS followed by 10% formalin. Brains were stored in 10% formalin for at least 1 week, blocked and coronal sections (100 µm thick) were taken at the level of the nucleus accumbens with a vibratome. The sections were mounted on gelatin-coated slides and stained with cresyl violet. Probe and cannulae placements were determined according to an individual unaware of the rat's behavioural response (Paxinos & Watson, 1986).

Statistical analysis

Statistical evaluation of behavioural responses involved one- or two-factor analysis of variance (ANOVA) with repeated measures over time when the full time course was examined. A least significance test for *posthoc* comparison was used (Milliken & Johnson, 1984). The effect of antisense treatment on protein levels was compared using a two-tailed paired Student's *t*-test because each rat was infused with random and antisense oligonucleotide in contralateral nucleus accumbens.

Results

Antisense oligonucleotides down-regulate Homer1b/c

Rats were infused for 7 days with an antisense oligonucleotide sequence that spanned the initiation codon (AS1) or a random sequence into the nucleus accumbens and the level of Homer1b/c protein measured. Figure 1 shows that the level of Homer1b/c protein was reduced by approximately 35% (see Table 1). In contrast, AS1 was without effect on the structurally related Homer3 protein, nor did it alter levels of other cytoskeletal proteins, including actin or GFAP. To determine if AS1 affected the level of any other proteins in the nucleus accumbens related to Homer1, the content of proteins that bind to the

TABLE 1. Effect of AS1 and AS2 on the level of proteins in the nucleus accumbens associated with Homer1

Protein	Normalized to random (% ± SEM)	<i>n</i>
H1b/c	68.9 ± 12.9*	16
H1b/c (AS2)	61.4 ± 9.5*	6
GluR1	58.9 ± 13.1*	16
GluR1 (AS2)	130 ± 17.3	8
Homer3	104.3 ± 6.4	11
mGluR1	89.7 ± 11.9	17
mGluR2/3	114.2 ± 18.7	11
mGluR5	95.8 ± 9.3	18
NMDAR1	81.1 ± 10.1	18
NMDAR2A	104 ± 20.8	17
NMDAR2B	113.7 ± 13.1	17
Shank1a	99 ± 8.8	7
GKAP	116.5 ± 20.4	8
PSD95	92 ± 17.7	9
IP3R1	109.8 ± 18.4	11
Actin	100 ± 13.5	11
GFAP	108 ± 15.1	11

Each rat was infused with random and antisense into contralateral accumbens for 7 days. Unless indicated all measurements correspond to effects of AS1. Data are shown as mean ± SEM per cent of random oligonucleotide OD value. **P* < 0.05, using a two-tailed paired Student's *t*-test comparing random with antisense oligonucleotide.

Homer EVH1 domain was also determined. No effect by AS1 was found on the levels of IP3R1, shank1a, or the group I mGluRs, mGluR1α or mGluR5. Homer1b/c has been shown to crosslink with NMDA receptors *via* a shank-GKAP-PSD95 postsynaptic complex (Naisbitt *et al.*, 1999; Tu *et al.*, 1999), and AS1 had no effect on the levels of GKAP, PSD95, NMDAR1, NMDAR2A or NMDAR2B in the nucleus accumbens. Surprisingly, AS1 lowered the level of GluR1 protein in the nucleus accumbens (Fig. 1 and Table 1). This finding was particularly unexpected as AS1 sequence has no homology with any portion of GluR1 mRNA and coimmunoprecipitation of Homer and GluR1 has not been demonstrated (Naisbitt *et al.*, 1999). This indicated a possible nonspecific effect (Braasch & Corey, 2002), and caused us to investigate a second, distinct AS sequence (AS2). Figure 1 and Table 1 show that AS2 produced a significant reduction in Homer1b/c protein without affecting the level of GluR1 indicating that the GluR1 protein reduction by AS1 was unrelated to the targeted reduction in Homer1.

The reduction in Homer1b/c and GluR1 proteins by AS1 were reversible. Three weeks after removing the minipumps the level of Homer1b/c (random, *n* = 6, 100 ± 11.9; AS1, *n* = 6, 105.3 ± 13.5; mean ± SEM change from random) and GluR1 (random, *n* = 6, 100 ± 9.3; AS1, *n* = 6, 93.1 ± 10.1) was equivalent between the AS1 and random treatment groups.

Figure 2 shows examples of cannula placement for animals that were either sham implanted with an infusion cannula, or infused with random or AS1 oligonucleotide for 2 weeks. Tissue sections were stained for Nissl substance to estimate neurotoxicity or GFAP to estimate astroglial infiltration. All three treatments resulted in approximately equivalent glial infiltration around the edge of the cannula track and at the infusion site. An adjacent Nissl stained section illustrates the presence of morphologically normal neurons and equivalent microglial infiltration at the infusion site of all three cannulae.

Antisense oligonucleotide causes sensitization to cocaine

At one week after beginning oligonucleotide infusion rats were injected with cocaine (15 mg/kg, i.p.). Figure 3A shows that the motor stimulant effect of cocaine was significantly greater for the two AS

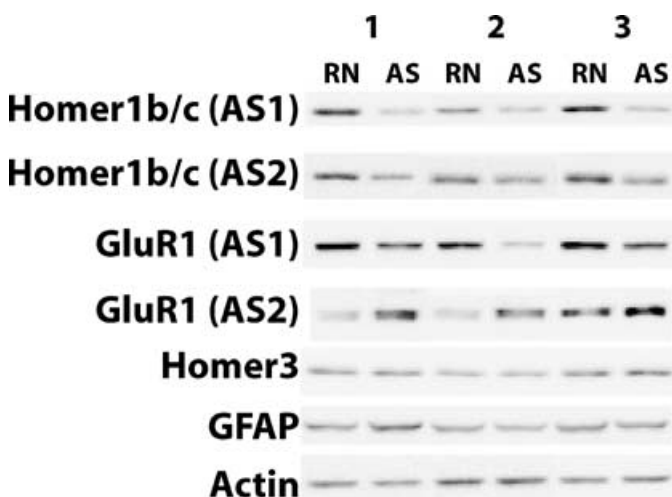


FIG. 1. Homer1b/c is reduced by one week of antisense oligonucleotide (AS1 and AS2) infusion into the nucleus accumbens compared to infusion with a random oligonucleotide sequence (RN). AS1 had no effect on Homer3 and other structural proteins, GFAP and actin, but reduced the level of GluR1. AS2 sequence did not have any effect on GluR1 protein levels.

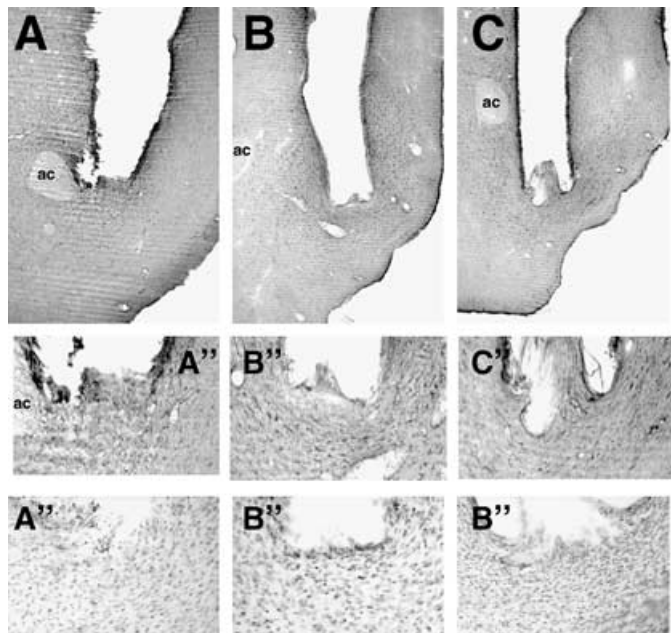


FIG. 2. Representative histology from animals infused for two weeks with nothing (A), AS1 (B) or random oligonucleotide (C). (A–C) Low power micrograph (20 \times) showing cannula track and immune-staining for GFAP to evaluate astroglial proliferation. (A'–C') High power micrograph (200 \times) illustrating the equivalent extent of glial proliferation following all three infusions. (A''–C'') Near adjacent Nissl stained section (200 \times) of the infusion site showing the presence of healthy neurons and equivalent microglial proliferation (densely stained cells) following all three treatments. ac, anterior commissure.

treatment groups than for the random oligonucleotide group. In order to determine if the down-regulation of Homer1b/c affected the development of behavioural sensitization to repeated cocaine administration, the animals shown in Fig. 3A were administered daily cocaine injections for one week in the presence of AS1, AS2 or random

oligonucleotide infusion into the accumbens. Figure 3B shows that on the last day of seven daily injections of cocaine, before the oligonucleotide minipumps were removed, all three groups showed a similar motor stimulant response to cocaine. Thus, the motor stimulant response in the random group increased (sensitized) between day 1 and 7 of injection, while the already augmented motor response in the AS groups remained elevated. On day 7 of injection the motor responses were equivalent between random and AS1, while the motor response to AS2 was significantly elevated over random at 50–80 min after cocaine administration. At three weeks after the last cocaine injection and removal of the oligonucleotide minipumps the random treatment group continued to show a sensitized motor response compared to day 1 of cocaine administration. The AS1 treatment group demonstrated significantly less motor activity than the random group on day 28, while the AS2 treatment group showed motor activity equivalent to the random group.

Inhibition of the development of sensitization and AMPA receptors

As indicated above, reducing Homer1b/c protein levels with AS1 infusion also reduced GluR1 content (Fig. 1). In contrast, reducing Homer1b/c with AS2 did not alter GluR1 (Fig. 1, Table 1). While both antisense sequences reduced Homer1b/c and caused a sensitized motor response to an acute injection of cocaine (Fig. 3A), only AS1 prevented the development of behavioural sensitization (Fig. 3C). To determine if changes in AMPA receptor subunit composition may mediate the capacity of AS1 to inhibit the development of behavioural sensitization to repeated cocaine treatment, rats were pretreated with the AMPA/kainate antagonist NBQX into the nucleus accumbens prior to each daily injection of cocaine. Figure 4A demonstrates that blockade of AMPA/kainate receptors with the highest dose of NBQX (1.5 nmol) potentiated the motor stimulant effect of acute cocaine, while the lower dose (0.15 nmol) was without effect. This higher dose also produced a significant motor stimulant response when administered with saline. However, both doses of NBQX administered prior to each daily injection of cocaine prevented the development of behavioural sensitization. Thus, when tested with cocaine alone on day 28 the beha-

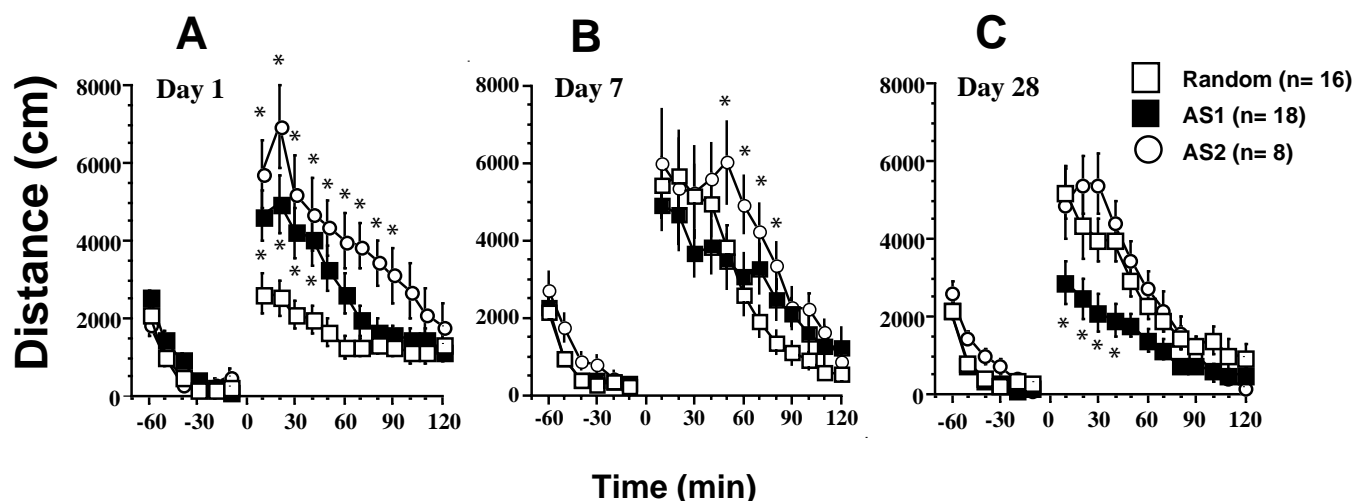


FIG. 3. Reduction in Homer1 expression sensitizes the motor stimulant response to cocaine (15 mg/kg, i.p.). (A) The motor response elicited by the first injection of cocaine is augmented by AS1 and AS2 infusion. (B) The motor response on the seventh day of daily cocaine administration. (C) The motor response to cocaine given three weeks after the last daily injection. Infusion pumps were removed after the last daily injection of cocaine. All the data were analysed using a 3-way ANOVA with repeated measures over time and day. Significant effects were found for day $F_{2,118} = 3.76$, $P = 0.026$; oligonucleotide $F_{2,118} = 9.26$, $P < 0.001$; time $F(17, 2006) = 123.35$, $P < 0.001$; time \times oligonucleotide $F_{34,2006} = 2.01$, $P < 0.001$; time \times day $F_{34,2006} = 2.98$, $P < 0.001$; time \times oligonucleotide \times day $F_{68,2006} = 1.90$, $P < 0.001$. * $P < 0.05$, comparing cocaine in the random group with the AS groups using a least significant difference *posthoc* analysis (Milliken & Johnson, 1984).

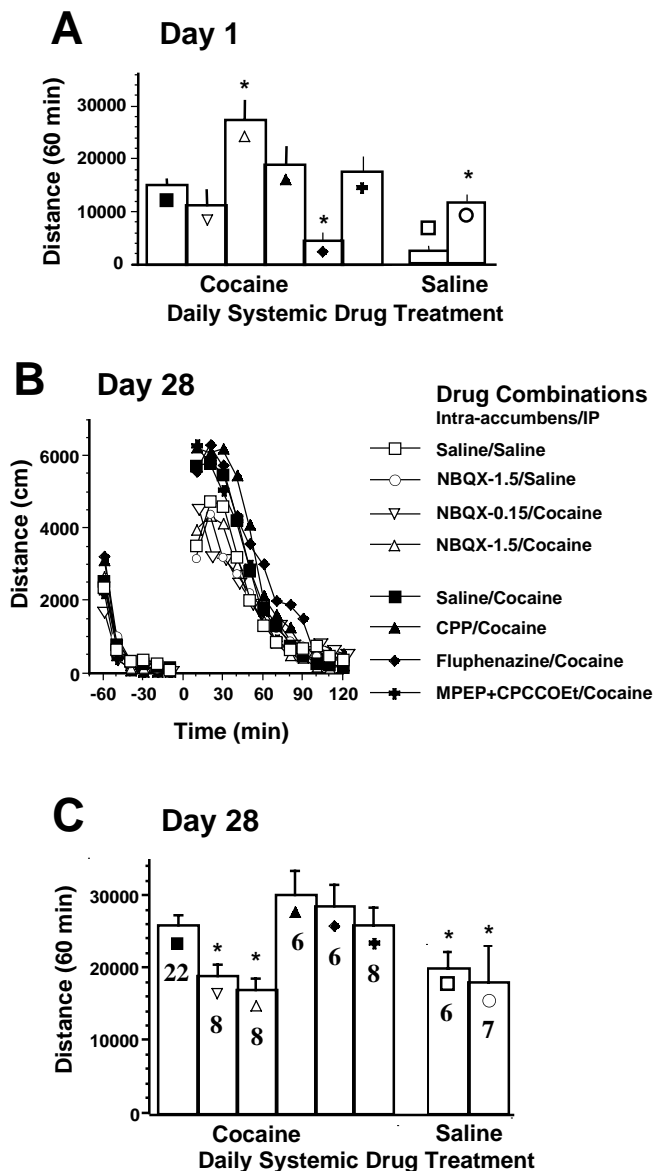


FIG. 4. Blockade of AMPA receptors in the nucleus accumbens with NBQX prevents the development of behavioural sensitization to repeated cocaine. Prior to each daily injection of cocaine or saline, rats were pretreated in the accumbens with a microinjection of saline, NBQX (0.15 or 1.5 nmol), CPP (0.3 nmol), fluphenazine (30 nmol) or a mixture of mGluR1 (CCOCPET, 5 nmol) and mGluR5 (MPEP, 0.5 nmol) antagonists. Three weeks after the last daily injection subjects were injected with cocaine alone (15 mg/kg, i.p.). (A) Effect of intra-accumbens drug pretreatment on the acute response to cocaine or saline. Data are shown as mean \pm SEM distance travelled (cm) over 60 min after injection. n for each group is shown in C. $F_{7,63} = 12.21$, $P < 0.001$. * $P < 0.05$ compared to rats microinjected with saline within the repeated saline or cocaine treatment groups using a least significant difference test (Milliken & Johnson, 1984). (B) Time course of the effect of cocaine challenge three weeks after the last daily injection. treatment $F_{7,63} = 3.20$, $P = 0.006$, time $F_{17,1071} = 191.28$, $P < 0.001$, interaction $F_{119,1071} = 2.22$, $P < 0.001$. (C) Effect of cocaine challenge over 60 min after injection, corresponding to the time course data shown in B. $F_{7,63} = 5.48$, $P < 0.001$. * $P < 0.05$ compared with the group microinjected with saline prior to systemic cocaine administration (saline/cocaine) using a least significant difference test (Milliken & Johnson, 1984).

vioural response in the NBQX groups was equivalent to the response in the daily saline, i.p., group and significantly lower than the daily cocaine treatment group that was pretreated with daily intra-accumbens saline (Fig. 4B and C). Moreover, the high dose of NBQX that

elicited motor activity when given without cocaine did not produce cross-sensitization to cocaine.

The antagonism of the development of sensitization was selective for AMPA/kainate receptors as blockade of neither NMDA receptors with the competitive antagonist CPP (0.3 nmol), group I mGluRs with a combination of mGluR1 (CPCCOEt; 5 nmol) and mGluR5 (MPEP; 0.5 nmol) antagonist, nor D1/D2 dopamine receptors with fluphenazine (30 nmol) prevented the development of behavioural sensitization (Fig. 4B and C). None of these drugs affected the acute motor response to cocaine (day 1) except fluphenazine, which significantly inhibited the cocaine-induced motor stimulation (Fig. 4A).

Discussion

The significance of cocaine-induced changes in Homer1 gene products in the expression of behavioural sensitization was assessed. Intracranial infusion of AS oligonucleotides was employed to mimic the reduction in Homer1b/c content in the nucleus accumbens produced by withdrawal from repeated cocaine (Swanson *et al.*, 2001). This experimental strategy revealed that reducing Homer1 expression causes a sensitization-like augmentation motor response to an acute cocaine administration, thereby mimicking the behavioural plasticity elicited by withdrawal from repeated cocaine administration (Post & Rose, 1976; Pierce *et al.*, 1996; Wolf, 1998). It was also found that one AS sequence (AS1) inhibited the development of sensitization by repeated cocaine administration. However, this may have resulted from an AS1-induced change in AMPA receptor subunits that accompanied the reduction in Homer1b/c as: (i) another AS sequence (AS2) that lowered Homer1b/c without altering GluR1 did not prevent the development of sensitization, and (ii) blocking AMPA/kainate receptors in the nucleus accumbens prevented the development of sensitization.

Antisense oligonucleotide specificity of action and technical considerations

The phosphorothioate-substituted oligonucleotide strategy for reducing *in vivo* protein synthesis has well characterized potential pitfalls (Hebb & Robertson, 1997; Ho & Hartig, 1999; Dean, 2001). One concern is that phosphorothioate-substituted nucleotides are neurotoxic. In order to reduce this possibility, concentrations at least 10-fold below those producing overt toxicity were employed, and only the five bases on the 3' and 5' ends were phosphorothioate-substituted (Hebb & Robertson, 1997). Moreover, histology revealed equivalent glial infiltration between oligonucleotide infused and sham infused neuropil. Another consideration is specificity for the targeted mRNA sequence using 25-mer oligonucleotides. To partly compensate this concern, two distinct AS sequences were shown to reduce Homer1b/c protein and to cause a sensitized behavioural response to cocaine. Also, the content of a number of proteins that are functionally related to Homer1b/c were unaltered by AS1 infusion, including proteins related by structural similarity (Homer3), by having a complementary EVH1 binding domain (mGluR1 α , mGluR5, IP3R1, shank1a), by being linked to Homer1b/c in the excitatory postsynaptic density (GKAP, PSD95, NMDAR1, NMDAR2A, NMDAR2B), or by serving a cytoskeletal function (actin, GFAP). Surprisingly, the content of GluR1 was reduced by AS1. Although it is possible that Homer proteins could contribute to AMPA receptor scaffolds *via* binding to shank, which binds to GKAP-SAP97, a physical association has not been demonstrated by immunoprecipitation (Tu *et al.*, 1999). The alteration in GluR1 does not appear to result from AS1 reduction in Homer1b/c as AS2 produced equivalent reductions in Homer1b/c, but did not significantly alter the level of GluR1. Also, withdrawal from repeated

cocaine administration reduces Homer1b/c without equivalent changes in GluR1 (Churchill *et al.*, 1999; Swanson *et al.*, 2001).

Homer1 and the expression of behavioural sensitization to cocaine

Homer proteins serve a variety of functions in modulating the integrity of the excitatory postsynaptic density, including scaffolding between mGluR, IP3R and NMDA receptors, and the trafficking and signalling of group I mGluRs (Tu *et al.*, 1998; Xiao *et al.*, 1998; Ango *et al.*, 2000; Ciruela *et al.*, 2000; Kammermeier *et al.*, 2000). Withdrawal from repeated cocaine reduces the content of Homer1b/c in the nucleus accumbens and decreases the ability of group I mGluRs to stimulate glutamate release (Swanson *et al.*, 2001). The present data indicate that the reduction in Homer1b/c may underlie the expression of behavioural sensitization as down-regulating the expression of Homer1 gene products with AS oligonucleotides augmented the motor stimulant effect elicited by an acute injection of cocaine. The change in GluR1 caused by AS1 likely did not contribute to the augmented response as the augmentation was also produced by infusion of AS2, which caused equivalent reductions in Homer1b/c without altering GluR1 content.

The participation of Homer1 gene products in the expression of behavioural sensitization is consistent with an emerging appreciation for the involvement of glutamate transmission in the accumbens in mediating the long-lasting effects of repeated cocaine administration, including behavioural sensitization and the reinstatement of drug-seeking behaviour (Wolf, 1998; Cornish & Kalivas, 2000; Di Ciano & Everitt, 2001; McFarland *et al.*, 2003). The mechanism by which lowered Homer1 in the accumbens may enhance the motor stimulant effect of cocaine is unknown. However, Homer2 knock-out mice also show a 'presensitized' motor response to cocaine that is associated with reduced capacity of group I mGluR stimulation to release glutamate (K. Szumlinski, unpublished observation), posing the binding of Homers to group I mGluRs as a potentially critical mechanism. In apparent contrast to this possibility is the recent finding that constitutive deletion of the gene encoding mGluR5 in mice markedly reduced behavioural responses to cocaine administration (Chiamulera *et al.*, 2001). However, the interaction between Homer and mGluR1 appears most critical for group I mGluR mediated locomotor activity and glutamate release in the nucleus accumbens (Swanson & Kalivas, 2000; Swanson *et al.*, 2001). Distinctions between mGluR5 and mGluR1 receptors have also been observed in anatomical distribution, cell signalling and electrophysiological actions, supporting the possibility that the effects of Homer1 AS knock down on mGluR1 and the consequences of mGluR5 gene deletion may interact differently with cocaine-induced behaviours (Balazs *et al.*, 1997; Tallaksen-Greene *et al.*, 1998; Awad *et al.*, 2000; Hubert *et al.*, 2001; Wittmann *et al.*, 2001; Poisik *et al.*, 2003).

While Homer1a could not be quantified due to a lack of antibody, it is possible that AS suppression of Homer1a expression may also be critical to the sensitized behavioural response produced by AS infusion. Homer1a lacks the coiled-coil binding domain that permits the Homers to multimerize and link other proteins in the excitatory synapse, and Homer1a mRNA synthesis is increased by an acute cocaine injection (Brakeman *et al.*, 1997; Ciruela *et al.*, 1999). This immediate early gene-like increase in synthesis permits Homer1a to act as a short-term dominant negative that displaces other Homers from the EVH1 binding site and disrupts signalling through group I mGluRs (Tu *et al.*, 1998). Thus, in this regard reduced Homer1a expression by AS infusion would be expected to produce opposite effects of reduced Homer1b/c. However, the Homer1 splice variants may have distinct effects on the trafficking and mGluR signalling that might produce

convergent influences on the motor stimulant effect of cocaine (Ango *et al.*, 2000; Kammermeier *et al.*, 2000).

AMPA receptors and the development of behavioural sensitization to cocaine

In addition to producing sensitization to the acute motor stimulant effect of cocaine, treatment with AS1 blocked the development of behavioural sensitization. The fact that an equivalent reduction in Homer1b/c by AS2 did not block the development of sensitization argues that distinct effects of the oligonucleotides may be involved. The alteration in GluR1 protein produced by AS1, but not by AS2, is one such distinction. Consistent with a role of AMPA receptors in the inhibition of the development of behavioural sensitization suggested by AS1 treatment, AMPA/kainate receptor blockade prevented the development of behavioural sensitization. Further indirect support for a role of GluR1 in sensitization is found in a study showing that the overexpression of GluR1 in the accumbens produces place aversion in a cocaine place conditioning paradigm (Kelz *et al.*, 1999). Although, blockade of the glycine site on NMDA receptors prevented the development of cocaine sensitization (Khan & Shoab, 1996), in the present study blockade of neither NMDA (Kalivas & Alesdatter, 1993), group I mGluRs nor D1/D2 dopamine receptors in the nucleus accumbens prevented the development of sensitization to repeated cocaine. While earlier studies found an important role for both glutamate and dopamine transmission in the ventral tegmental area in the development of behavioural sensitization to psychostimulants (Kalivas & Alesdatter, 1993; Vezina, 1996; Vezina & Queen, 2000), the present study clearly implicates a role for glutamate transmission in the nucleus accumbens. Exactly how the nucleus accumbens is involved is not clear. However, unlike the VTA (White & Kalivas, 1998), direct microinjection of psychostimulants into the nucleus accumbens does not induce behavioural sensitization, indicating that the role of excitatory transmission in the accumbens may be not reflect a direct action of cocaine, but rather involvement of circuitry distal to the anatomical site of action.

Conclusions

The present study indicates that a reduction of Homer1 gene products in the nucleus accumbens produces a sensitized behavioural response to cocaine. Therefore, the previously reported decrease in Homer1b/c elicited by withdrawal from repeated cocaine administration may underlie the expression of behavioural sensitization to cocaine. In contrast, while selectively reducing Homer1 expression in the nucleus accumbens did not prevent the development of sensitization to repeated cocaine administration, pretreatment of the nucleus accumbens with an AMPA/kainate receptor antagonist was effective. The dissociation between AMPA and Homer1 is consistent with the inability to show a physical association between AMPA receptor subunits and Homer1 gene products (Tu *et al.*, 1999). Thus, while reduced Homer1b/c protein levels in the accumbens is an important consequence of the cocaine-induced remodelling of excitatory transmission that mediates behavioural sensitization, the induction of cocaine-induced neuroplasticity is independent of reduced Homer1b/c and requires excitatory transmission through AMPA/kainate receptors.

Abbreviations

AMPA, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid; AS, antisense oligonucleotide; CPCCOEt, 7-(hydroxyamino)cyclopropa(b)chromen-1a-carboxylate; CPP, 3-(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid; GFAP, glial acidic fibrillary protein; GluR1, glutamate 1 receptor subunit; IP3R, inositol phosphate 3 receptor; mGluR, metabotropic glutamate receptors

(mGluR); MPEP, 2-methyl-6(phenylethynyl)pyridine; NBQX, 6-nitro-7-sulfamoylbenzo[f]quinolizine-2,3,dione; NMDA, *N*-methyl-D-aspartate; OD, optical density; PSD-95, postsynaptic density 95.

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