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Short communication

Morphine causes a delayed increase in glutamate receptor functioning in the nucleus accumbens core

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Abstract

Enhanced excitatory neurotransmission in the mesocorticolimbic system may contribute to the persistence of addiction behaviour. Here, we demonstrated that glutamate-, *N*-methyl-D-aspartate (NMDA)- and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-induced [^{3}H]-γ-aminobutyric acid (GABA) release from superfused rat nucleus accumbens core slices is profoundly enhanced 3 weeks, but not 3 days, after a single s.c. morphine injection. This delayed increase in glutamate receptor functioning is associated with enhanced gene transcript levels of ionotropic NMDA and AMPA/kainate receptor subunits. These data reveal that morphine may progressively enhance glutamate neurotransmission within the nucleus accumbens core subsequent to drug exposure.

Keywords: Ionotropic glutamate receptor; Gene expression; GABA release; Nucleus accumbens core

1. Introduction

Exposure to psychostimulants and opiates causes time-dependent neuroadaptive changes within the mesocorticolimbic system that may underlie certain behavioural characteristics of drug addiction such as sensitization and stimulus-induced drug-seeking (Vanderschuren and Kalivas, 2000; Nestler, 2001; Robinson and Berridge, 2003). The nucleus accumbens, a point of convergence integrating dopamine and glutamate neurotransmission, is of particular importance in this respect. Dopamine and glutamate afferents coordinate the activity of nucleus accumbens neurones that, for the major part, consist of γ-aminobutyric acid (GABA) producing medium-sized spiny neurones (Pierce and Kalivas, 1997).

Whereas most neurochemical studies thus far addressed the role of dopamine in addiction behaviour, recent evidence indicates that long-lasting expression of major features of addiction behaviour, such as sensitization and propensity to relapse, may be due to changes in pre- and postsynaptic glutamate neurotransmission particularly within the core region of the nucleus accumbens. This long-term neuroplasticity may strengthen the excitatory control of glutamate afferents onto nucleus accumbens neurones (Tzschentke and Schmidt, 2003; Kalivas, 2004). Therefore, we examined the occurrence of time-dependent alterations in the functioning of ionotropic glutamate receptors in nucleus accumbens core slices following a single exposure of rats to morphine, i.e., representing the context-independent effect of the opiate.

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2. Materials and methods

2.1. Drug administration

In their home cage, male Wistar rats (180–200 g) received a single s.c. injection with saline (1 ml/kg) or morphine–HCl (30 mg/kg, dissolved in saline), inducing long-lasting behavioural and neurochemical sensitization (Vanderschuren et al., 2001). Three days or 3 weeks after pretreatment, the animals were decapitated and the nucleus accumbens core was dissected from a coronal slice (bregma +1.2 to +2.2 mm), used for neurochemical experiments or stored at −80 °C for real-time quantitative polymerase chain reaction (PCR) analysis.

2.2. Neurotransmitter release

Neurotransmitter release was measured as previously described (Schoffelmeer et al., 2000). In short, nucleus accumbens core slices (0.3×0.3×1 mm) were washed twice with 5 ml Krebs–Ringer's bicarbonate medium (containing 121 mM NaCl, 1.87 mM KCl, 1.17 mM KH_2PO_4, 25 mM $NaHCO_3$, 0.1 mM $CaCl_2$, 10 mM D-(+)-glucose and 10 μM aminooxyacetic acid, pH 7.4), incubated for 15 min in this medium containing 0.1 μM [^{3}H]-GABA under an atmosphere of 95% O_2/5% CO_2 at 37 °C. After labelling, the slices were washed and transferred to each of 24 chambers of a superfusion apparatus (approximately 3 mg tissue in 0.2 ml volume per chamber) and superfused (0.25 ml/min) with medium gassed with 95% O_2/5% CO_2 at 37 °C. The superfusate was collected as 10-min samples after 40 min of superfusion (t=40 min). [^{3}H]-GABA release was induced during superfusion by exposing the slices to medium containing a maximally effective concentration of glutamate (100 μM), *N*-methyl-D-aspartate (NMDA, 100 μM), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA, 30 μM) or to KCl (10 mM) (NaCl was reduced accordingly to maintain isomolarity) for 10 min at t=50 min. Radioactivity remaining at the end of the experiment was extracted from the tissue with 0.1 N HCl. Radioactivity in superfusion fractions and tissue extracts was determined by liquid scintillation counting. The efflux of radioactivity during each collection period was expressed as a percentage of the amount of radioactivity in the slices at the beginning of the respective collection period. Evoked neurotransmitter release was calculated by subtracting the spontaneous efflux of radioactivity from the total overflow of radioactivity during stimulation and the following 10 min. Because the neurotransmitter release was returned to basal levels during the next 10-min period, a linear decline from the 10-min interval before that to 20–30 min after the start of stimulation was assumed for calculation of the spontaneous efflux of radioactivity. The evoked release was expressed as percentage of the content of radioactivity of the slices at the start of the stimulation period. Statistical significance of differences in evoked neurotransmitter release between experimental groups was evaluated using Student–Newman–Keuls tests.

2.3. Real-time quantitative PCR

Total RNA of each treatment group was pooled, divided into six equal quantities, treated with DNAse I (20 U/μg RNA), random primed with 50 pmol hexanucleotides and reverse transcribed using 200 U MMLV H^- reverse transcriptase. Quantitative PCR was performed in triplicate on each plate and repeated three to six times in separate experiments on an ABI PRISM 7700 Sequence detector as described previously (Jacobs et al., 2002). PCR conditions and SYBR green reagents were used in a reaction of 20 μl using transcript-specific primers (0.3 μM) and 0.7 μl cDNA according to guidelines of the manufacturer with the exception of 45 cycles and elongation at 59 °C. Transcript-specific primers were generated with Primer Express software based on GenBank sequence information, verified by NCBI BLAST search and custom synthesized. Primers corresponded to nucleotides (nt) 4091–4114 and 4173–4195 of NMDA NR1 (GenBank accession number X63255.1), nt 6050–6066 and 6117–6135 of NR2A (AF001423), nt 3531–3553 and 3586–3606 of NR2B (M91562.1), nt 3667–3687 and 3723–3745 of NR2C (M91563.1), nt 3211–3231 and 3259–3281 of NR2D (L31612.1), nt 3809–3828 and 3857–3879 of NR3 (AF073379.1), nt 2866–2886 and 2919–2941 of GluR1 (X17184.1), nt 2995–3015 and 3057–3077 of GluR2 (M85035.1), nt 2877–2897 and 2948–2969 of GluR3 (M85036.2), nt 2831–2850 and 2918–2937 of GluR4 (M85037.1), nt 2933–2955 and 2977–2993 of GluR5 (M83560.1), nt 2938–2958 and 3014–3033 of GluR6 (Z11548.1), nt 2710–2730 and 2799–2817 of GluR7 (M83552.1), nt 3001–3024 and 3081–3101 of kainate (KA) KA1 (U08257.1), nt 3392–3408 and 3450–3470 of KA2 (Z11581.1) and nt 267–286 and 322–343 of hypoxanthine phosphoribosyltransferase (HPRT, M63983.1) rat mRNA sequences. Primer sets were tested on nucleus accumbens cDNA by PCR and gel electrophoresis in addition to melting curve analysis using Dissociation Curves 1.0 software for the absence of primer–dimer artefacts and multiple products. Amplification efficiency was determined by quantitative PCR using repetitive dilution series of nucleus accumbens cDNA; an efficiency of ~2 was accepted. The cycle of threshold (C_t) was determined as the value 3 cycles above noise. For each primer set, a no template control was performed. C_t values with a difference smaller than 6 cycles were discarded. C_t values were used to calculate the relative level of expression normalized to the transcript for HPRT (Spijker et al., 2004). Student–Newman–Keuls analysis was performed on relative