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Sequential and opposing alterations of 5-HT$_{1A}$ receptor function during withdrawal from chronic morphine

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Abstract

Addiction is a brain chronic relapsing disorder associated with emotional distress. The serotonergic system and especially the 5-HT$_{1A}$ receptor crucially regulate emotional behaviors both in humans and rodents. Using [^{35}S]GTPγS autoradiography in mice, we show that 5-HT$_{1A}$ receptor function is enhanced by chronic morphine treatment in the medial prefrontal cortex, and decreased in dorsal raphe nucleus one week later, two regions involved in emotional processing. These molecular adaptations could contribute to the development of emotional disorders experienced by former opiate addicts.

Keywords

addiction; 5-HT$_{1A}$ receptor; autoradiography; mouse; dorsal raphe nucleus; medial prefrontal cortex

1. Introduction

Serotonergic neurons, mainly located in the dorsal raphe nucleus (DRN), send axonal projections throughout the brain where serotonin (5-HT) release activates several 5-HT receptors. The 5-HT$_{1A}$ receptor (5-HT$_{1A}$R), a seven transmembrane domain receptor coupled to $G_{i/o}$ proteins (Pucadyil et al., 2005), has been identified as a key player in emotions. Functional imaging reveals its implication in the pathophysiology of depression in humans (Parsey et al., 2006), and buspirone, a classical 5-HT$_{1A}$R agonist, is used as an anxiolytic (Goodman, 2004). This receptor is strongly expressed both in the DRN and the medial prefrontal cortex (mPFC), two limbic regions implicated in emotional responses. Selective serotonin reuptake inhibitors (SSRI) are recognized as first-line antidepressants, and their effects have been correlated with 5-HT$_{1A}$R desensitization in DRN (Hensler, 2003; Savitz et al., 2009). In mice, 5-HT$_{1A}$R gene knock-out (Ramboz et al., 1998) or reduced 5-HT$_{1A}$R expression in the DRN (Richardson-Jones et al., 2010) decrease behavioural despair.

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Opiate addiction is associated with emotional distress. Acute withdrawal, defined when access to the drug is prevented, produces physical symptoms and a negative affect (Koob and Volkow, 2010). After prolonged periods of withdrawal, opiate addicts further show increased prevalence of anxiety and major depressive episodes (Grant et al., 2004). In rodents, SSRI treatment alleviates acute withdrawal symptoms following chronic exposure to morphine, a prototypical opiate (Gray, 2002). Further, SSRI prevent both heightened anxiety (Harris and Aston-Jones, 2001) and despair (Goeldner et al., 2011) during protracted withdrawal from chronic morphine.

Together therefore, data from both human and rodent studies suggest that the serotonergic regulation of emotional behaviors is altered during morphine withdrawal, and could implicate 5-HT_{1A}R dysfunction. In this study, we investigated the kinetics of 5-HT_{1A}R function following chronic morphine exposure. We treated mice using a morphine regimen known to induce dependence (Matthes et al., 1996) and focused on three time points (2 hours, 1 and 4 weeks after the last morphine injection) to match our previous study on behavioral adaptations to chronic morphine (Goeldner et al., 2011). We then performed [^{35}S]GTPγS autoradiography, stimulated by the specific 5-HT_{1A}R agonist 8-OH-DPAT (Meneses and Terron, 2001), to evaluate functional coupling of the receptor to G-proteins in DRN and mPFC. Our data show regional-specific and sequential modifications of 5-HT_{1A}R function.

2. Experimental Procedures

2. 1. Animals

Eight-week-old male C57Bl/6J mice (Charles River Laboratories, St-Germain-sur-l'Arbresle, France) were housed 4/cage (12h light/dark cycle, food and water ad libitum). All experiments followed ethical guidelines (European Community Guidelines 86/609/EEC) and were approved by the local ethical committee (CREMEAS, 2003-10-08-[1]-58).

2. 2. General Procedure

Experiment 1—4 naïve mice were used to determine the potency (defined as -logEC_{50}) and maximal effect (Emax) of (R)-(+)-8-Hydroxy-2-dipropylamino-tetralin hydrobromide (8-OHDPAT, Sigma) for stimulating [^{35}S]GTPγS binding across 4 different brain regions: DRN, mPFC, dorsal and ventral hippocampus (dHIPP, vHIPP, respectively).

Experiment 2—48 mice (consisting in 2 equivalent cohorts processed independently) were injected intraperitoneally with daily escalating doses of morphine sulfate (20, 40, 60, 80, 100 mg/kg; Francopia, Gentilly, France) or saline (0.9% sodium chloride) as control, twice daily for five days and received a single 100 mg/kg injection on day 6. Mice were then sacrificed (i) 2 hours after the last injection (chronic treatment; saline and morphine, n=8/group), (ii) 1 week after the last injection (1-week withdrawal; saline and morphine, n=8/group), (iii) 4 weeks after the last injection (4-week withdrawal; saline, n=8; morphine, n=7).

2. 3. Tissue preparation

Mice were cervically dislocated, brains rapidly removed and frozen in 2-methylpentane. Coronal sections (20 μm) were obtained at −20°C using a cryostat microtome (Leica CM 3050) at the level of the mPFC (+2.0 to +2.4 mm from bregma), dHIPP (−2.0 to −2.4 mm), vHIPP (−3.1 to −3.5) and DRN (−4.4 to −4.8 mm) according to the mouse brain atlas (Paxinos and Franklin, 1997). Sections were thaw-mounted onto gelatin-coated slides, air-dried (room temperature, 10 minutes) and stored at −80°C.

2. 4. [^{35}S]GTPγS autoradiography

Sections were thawed at room temperature and rehydrated for 20 minutes in plastic slide mailers in assay buffer containing 50 mM Tris-HCl, 5 mM MgCl2-6H_2O, 100 mM NaCl, and 10 mM EDTA (pH 7.4). Sections were preincubated for 1 hour with assay buffer plus 2 mM GDP (Sigma) and 1 μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, Tocris bioscience). The sections were then incubated for 1.5 hours with preincubation buffer plus 1 mM dithiothreitol (DTT) and 80 pM guanosine 5'(γ-^{35}S-thio)-triphosphate ([^{35}S]GTPγS; 1,250 Ci/mmol, Perkin Elmer). Mailers were allocated to three incubation conditions: basal (no agonist present), agonist-stimulated and nonspecific (no agonist and 1 μM unlabeled GTPγS, Sigma) bindings. We used 5 (0.001, 0.01, 0.1, 1, 10 μM) and 2 (0.1 and 10 μM) increasing concentrations of 8-OH-DPAT in experiments 1 and 2, respectively. Sections were then rinsed and exposed to Kodak Biomax MR films for 24 hours to generate autoradiograms.

2. 5. Image analysis

Autoradiograms were analyzed with an image processor (MCID Elite 7.0 software Imaging Research Inc., St Catharines, ON, Canada). Regions of interest (ROI, see figure 1) were drawn on images from basal binding sections and reported on images from non-specific and agonist-stimulated binding sections. Densitometric values in ROI were transformed into relative radioactive counts by calibration with simultaneously exposed [^{14}C] standards (ARC-146; American Radiochemicals) of known tissue equivalent values (nCi/g) (Miller, 1991). Non-specific binding was subtracted from both basal and agonist-stimulated binding.

2. 6. Data analysis

All data are expressed as mean±sem. For experiment 1, pEC_{50} and Emax of 8-OH-DPAT-stimulated [^{35}S]GTPγS binding were calculated for each mouse by non-linear regression analysis. One-way analysis of variance was then used to compare Emax and pEC_{50} across 4 brain regions. For experiment 2, we used two-way analysis of variance with treatment and duration of withdrawal as factors, followed by Fischer's post-hoc analysis when relevant.

3. Results

In experiment 1 we quantified [^{35}S]GTPγS binding stimulated by 8-OH-DPAT in 4 brain regions of naïve mice. Fig. 1 shows dose-response activation curves for 5 agonist concentrations and images at 3 representative concentrations. pEC_{50} and Emax values for each region are shown in Table 1. Analysis of variance indicates that 8-OH-DPAT is equally potent in stimulating [^{35}S]GTPγS binding in the 4 brain areas [$F(3,12)=0.52$, $p=0.67$]. In contrast and as expected, the Emax greatly varied [$F(3,12)=34.7$, $p<0.0001$], ranging from 69.2% in the DRN to 523% in the dHIPP, reflecting variations across brain regions in both 5-HT_{1A}R density and basal G-protein coupled receptors activity. Post-hoc comparisons of Emax values between regions were all significant ($p<0.001$), except for vHIPP and mPFC ($p=0.82$). This pilot experiment indicates that 8-OH-DPAT potency is comparable across brain areas, as was previously shown in rat [^{35}S]GTPγS autoradiography experiments (Meller et al., 2000).

In experiment 2, we analyzed the effects of escalating doses of morphine on 5-HT_{1A}R function. Because of the large sample number required for quantification, we did not perform full dose-response experiments. Based on experiment 1, we selected two critical agonist concentrations that reliably reflect modifications of agonist potency and efficacy in the two brain regions, i.e. a low (0.1 μM) and a high (10 μM) concentrations corresponding to EC_{50} value and maximal activation in naïve mice. In a first animal cohort, we found significant effects of morphine exposure on [^{35}S]GTPγS binding in mPFC and DRN, but not

in vHIPP or dHIPP (data not shown). Thus only mPFC and DRN were analyzed in a second cohort and data pooled with those of the first cohort. Basal binding was not significantly modified by chronic injections or duration of abstinence in any of the brain regions examined (Table 2). Binding values were analyzed separately for each 8-OH-DPAT concentration and brain region and results are shown in Fig. 2.

In the mPFC (Fig. 2A–C) and for 10 μM of 8-OH-DPAT, two-way analysis of variance revealed a main significant effect of morphine treatment [$F(1,41)=4.64$, $p=0.037$]. In the chronic group, post-hoc analysis showed a significant increase of [^{35}S]GTPγS binding in morphine-treated mice as compared to saline controls ($p=0.017$, Fig. 2A). At this concentration, the duration of withdrawal had no effect [$F(2,41)=1.90$, $p=0.16$] and there was no interaction between factors [$F(2,41)=1.19$, $p=0.31$]. For 0.1 μM 8-OH-DPAT, there was no effect of treatment [$F(1,41)=1.518$, $p=0.22$] or duration of withdrawal [$F(2,41)=0.077$, $p=0.93$] and no interaction between factors [$F(2,41)=1.96$, $p=0.15$]. Hence, response to 8-OH-DPAT is increased in mPFC at the end of the chronic treatment, and this effect is no more detected after 1- and 4-week withdrawal. Chronic morphine thus transiently sensitizes 5-HT$_{1A}$R, specifically at the level of mPFC.

In the DRN (Fig. 2D–F) and for 0.1 μM 8-OH-DPAT, there was a significant main effect of treatment [$F(1,42)=6.22$, $p=0.0167$]. In the 1-week withdrawal group, post-hoc comparison revealed a significant decreased agonist-stimulated binding in morphine-treated mice ($p=0.0091$, Fig. 2E). There was no effect of withdrawal duration [$F(2,42)=1.25$, $p=0.30$] and no interaction between factors [$F(2,42)=1.27$, $p=0.30$]. At 10 μM 8-OH-DPAT, we observed no effect of treatment [$F(1,42)=2.66$, $p=0.11$] or time [$F(2,42)=0.936$, $p=0.40$] and no interaction [$F(2,42)=0.57$, $p=0.57$]. Therefore, 8-OH-DPAT response is decreased in the DRN after 1-week withdrawal, an effect that is not detectable either at the end of the chronic regimen or after 4 weeks. Chronic morphine leads to delayed and transient 5-HT$_{1A}$R desensitization, specifically in the DRN.

4. Discussion

Our data show that 5-HT$_{1A}$R function is enhanced in mPFC immediately after repeated morphine exposure, and decreased in DRN after 1-week withdrawal. These successive and opposing adaptations of 5-HT$_{1A}$R coupling to G-proteins, which develop either at the level of receptor density or receptor/G-protein interaction (Sovago et al., 2001), do not persist since receptor coupling is restored in both brain areas 4 weeks after treatment.

After chronic morphine, 5-HT$_{1A}$R function is enhanced in mPFC, but not in DRN. While acute morphine disinhibits serotonergic neurons and activates 5-HT$_{1A}$Rs in both DRN and projection areas (Tao and Auerbach, 1995; Fadda et al., 2005), chronic morphine was shown to decrease 5-HT neurons firing rate (Jolas et al., 2000) and sensitize 5-HT$_{1A}$R responsivity to systemic 8-OH-DPAT (Sastre-Coll et al., 2002). Negative feedback mechanisms may operate at local (DRN) or distant (mPFC) sites (Stamford et al., 2000; Celada et al., 2001). Our result suggests that chronic morphine sensitizes 5-HT$_{1A}$R-mediated control over serotonergic neurons mainly at the cortical level.

Most 5-HT$_{1A}$R studies have focused on immediate effects of pharmacological treatment or behavioral manipulation. Here we examined delayed consequences of morphine exposure, and show for the first time that reduced 5-HT$_{1A}$R function is detected only after a drug-free period. Notably, this 5-HT$_{1A}$R desensitization is detected in DRN, as was found in previous rodent studies using chronic mild stress-induced models of depression (Lanfumey et al., 1999; Froger et al., 2004; Bambico et al., 2009), indicating that DRN-specific adaptations also occur in response to chronic opiates. The time course of DRN 5-HT$_{1A}$R desensitization

is intriguing. We recently showed (Goeldner et al., 2011) that emotional-like deficits following chronic morphine are undetectable at 1-week but significant at 4-week withdrawal. Despair behavior may in fact be related to the 5-HT_{1A}R dysfunction observed here. Chronic morphine could disturb 5-HT_{1A}Rs activity transiently, and this primary event may trigger further adaptations within 5-HT circuits which may ultimately contribute to the incubation of behavioral deficits expressed at a later time point.

In conclusion our data reveal dynamic modifications of 5-HT_{1A}Rs, which may contribute to homeostatic dysregulation of 5-HT system in morphine abstinence and have implications towards understanding emotional distress in former opiate addicts.

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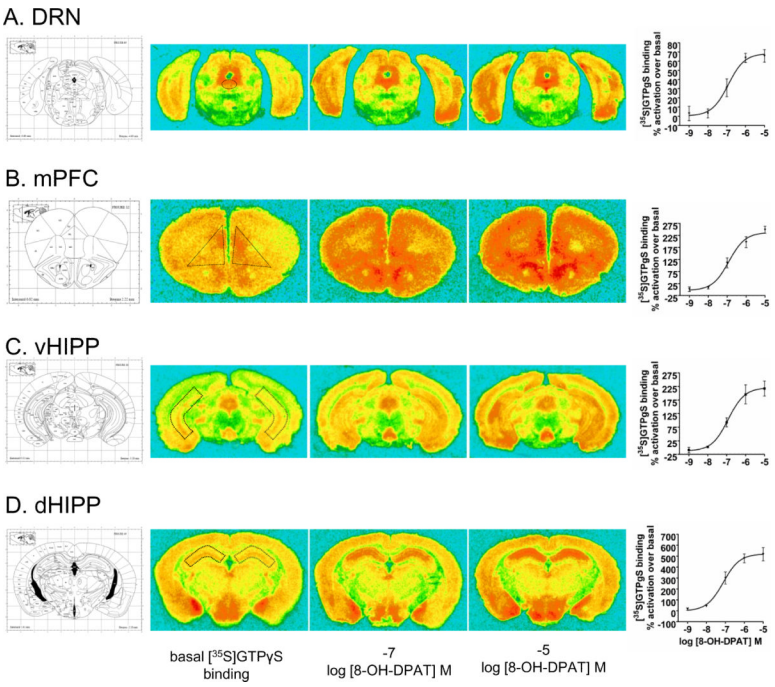

Figure 1.

Representative images (artificial colors, middle) and dose-response curves (right) for 8-OH-DPAT-stimulated [^{35}S]GTPγS binding in dorsal raphe nucleus (DRN) (A), medial prefrontal cortex (mPFC) (B), dorsal (dHIPP) (C) and ventral hippocampus (vHIPP) (D) of naive mice. Dashed lines, regions of interest for densitometry measurements according to the mouse brain atlas. Data are mean ± s.e.m. n=4 mice.

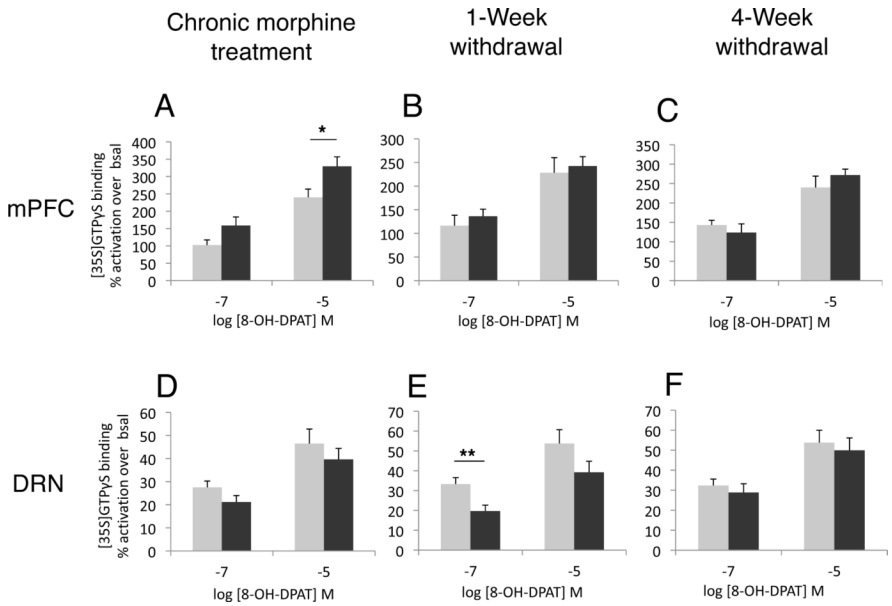

Figure 2.

5-HT_{1A} receptor function, measured by 8-OH-DPAT-stimulated [^{35}S]GTPγS binding, is increased after chronic morphine treatment (A, D) in mPFC, decreased after 1-week withdrawal (B, E) in DRN and unchanged after 4 weeks (C, F). Data are mean ± s.e.m. n=7–8 mice for each treatment at each time point. *p<0.05, **p<0.01, saline versus morphine-treated mice, two-way ANOVA with Fischer's post-hoc analysis.

Table 1

Maximal effect (Emax) and potency (pEC_{50}) of [^{35}S]GTPγS binding stimulated with 5 increasing concentrations of the 5-HT_{1A} receptor agonist 8-OH-DPAT, in dorsal raphe nuclei (DRN), medial prefrontal cortex (mPFC), dorsal (dHIPP) and ventral (vHIPP) hippocampus of naïve mice (n=4). Data are mean ± s.e.m.

Region	pEC_{50}	Emax (% over basal binding)
DRN	6.92±0.22	68.4±5.8
mPFC	6.90±0.15	238±15
dHIPP	7.11±0.17	521±32
vHIPP	6.91±0.20	221±18

Table 2

Basal binding (nCi/g) of [^{35}S]GTPγS in DRN and mPFC is not modified either by chronic injections or duration of withdrawal. Data are mean ± s.e.m. n=7–8 mice for each treatment at each time point.

	Chronic		1-Week		4-Week	
Region	**Saline**	**Morphine**	**Saline**	**Morphine**	**Saline**	**Morphine**
DRN	2356±148	2302±96	2348±77	2495±71	2378±55	2371±51
mPFC	483±31	413±30	414±40	428±19	437±41	334±61