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Effects of morphine withdrawal on μ-opioid receptor-stimulated guanylyl 5′-[γ-[^{35}S]thio]-triphosphate autoradiography in rat brain

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Abstract

Abstinence from chronic morphine results in characteristic withdrawal symptoms in humans and experimental animals. Despite a large number of studies, the cellular mechanisms underlying opiate withdrawal symptoms are not clearly understood, in particular, the regulation of μ-opioid receptor function during this process. The present study investigated the μ-opioid receptor-stimulated G-protein activity using guanylyl 5′-[γ-[^{35}S]thio]-triphosphate ([^{35}S]-GTPγS) autoradiography. [^{35}S]-GTPγS binding was performed using coronal rat brain sections (20 μm) in the presence or absence of the μ-opioid selective agonist [D-Ala^2,*N*-MePhe^{4}Gly^5-ol] enkephalin (DAMGO). In experiment 1, rats (male, Sprague–Dawley) were injected every 12 h with increasing doses of morphine (5–100 mg/kg, s.c.) for 12 days; a separate group of rats which received saline injections served as control. Opiate withdrawal was induced by abstinence from morphine. Thirty-six hours after the last morphine injection, spontaneous withdrawal symptoms were assessed. Rats were then decapitated and brains rapidly removed. In experiment 2, withdrawal symptoms were precipitated with the opioid receptor antagonist naloxone (1 mg/kg). Brains were taken at 5, 10, 20 and 60 min after naloxone injection. In experiment 3, morphine dependence was induced by implantation of three morphine pellets (75 mg per pellet). After 7 days, withdrawal symptoms were precipitated by naloxone (1 mg/kg) and brains were removed 30 min after naloxone injection. [^{35}S]-GTPγS binding was measured in the locus coeruleus, nucleus parabrachialis, nucleus accumbens and central nucleus of amygdala. Although clear withdrawal symptoms were observed in all morphine-withdrawn rats, no significant changes in [^{35}S]-GTPγS binding were detected in animals undergoing withdrawal. The present lack of differences between morphine-withdrawn and control rats indicates that μ-opioid receptor-stimulated G-protein activity is not modulated by chronic morphine administration and is not involved in the expression of opiate withdrawal.

Keywords: Opiate withdrawal; Morphine; Opioid receptor; G-protein coupling; [^{35}S]GTPγS-binding

1. Introduction

Chronic administration of morphine leads to tolerance and dependence. Tolerance and dependence are considered to be neuroadaptive intracellular changes to an altered pharmacological state. Abstinence from chronic opiate exposure results in a variety of physiological and psychological withdrawal symptoms based on these adaptions of the neuronal system. The withdrawal symptoms in humans and experimental animals are widely the same: lacrimation, yawning, rhinorrhea, elevations of temperature and blood pressure, alterations of pulse rates, restlessness, diarrhea, weight loss, anxiety, depression and craving (Bläsig et al., 1973; Jaffe, 1990; Spanagel et al., 1998). The molecular mechanisms involved in the expression of opiate withdrawal are not understood in detail, but most of the physiological aspects of opiate withdrawal are based upon overexcitability of the noradrenergic system. The locus coeruleus is the major noradrenergic nucleus in the brain and is thought to be involved in physical dependence (Nestler et al., 1999).

Changes in affinity and/or density of opioid receptors do not have a significant role in opiate dependence and withdrawal responses (for review, see Fleming and Taylor, 1995). Thus, the neuronal basis of opiate dependence and withdrawal may involve other intracellular mechanisms. In general, chronic opiate exposure results in an up-regulation of the cAMP system (Nestler et al., 1999). This up-regulated or ''hypertrophied'' cAMP system in the locus coeruleus and other brain stem nuclei can be viewed as a compensa-

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tory, homeostatic response to the inhibition raising from chronic opiate treatment. cAMP up-regulation results in the activation of the transcription factor cAMP Response Binding Element (CREB). Following chronic opiate exposure, CREB is selectively up-regulated in the locus coeruleus (Guitart et al., 1992; Nestler, 2001). The up-regulated cAMP system has been shown to contribute to the increase in the electrical excitability of locus coeruleus neurones associated with opiate withdrawal (Nestler et al., 1999) and transgenic mice deficient in CREB exhibit attenuated withdrawal signs compared to wild-type mice (Maldonado et al., 1996a,b). The mechanism by which chronic morphine administration up-regulates components of the cAMP pathway remains unknown. However, the activation of receptor coupled G-proteins by opiates is seen as the first step in regulating intracellular second messenger pathways suggesting that changes in G-protein activity might occur after chronic opiate administration and during opiate withdrawal.

Neuroanatomical localization of receptor-activated G-proteins in brain sections can be performed by in vitro autoradiography of guanylyl 5′-[γ-[^{35}S]thio]-triphosphate (GTP[γ-^{35}S]) binding in the rat brain (Sim et al., 1995) and in the human brain (Platzer et al., 2000). Using this technique in combination with the μ-opioid-selective peptide [D-Ala^2,*N*-MePhe^{4}Gly^5-ol] enkephalin (DAMGO), Sim et al. (1996) have recently shown that chronic administration of morphine results in a decrease in G-protein activity in several brainstem nuclei including the locus coeruleus and lateral/medial parabrachial nuclei. No significant changes were observed in the nucleus accumbens, amygdala (central nucleus of amygdala), thalamus and substantia nigra (Sim et al., 1996). This study may indicate that specific

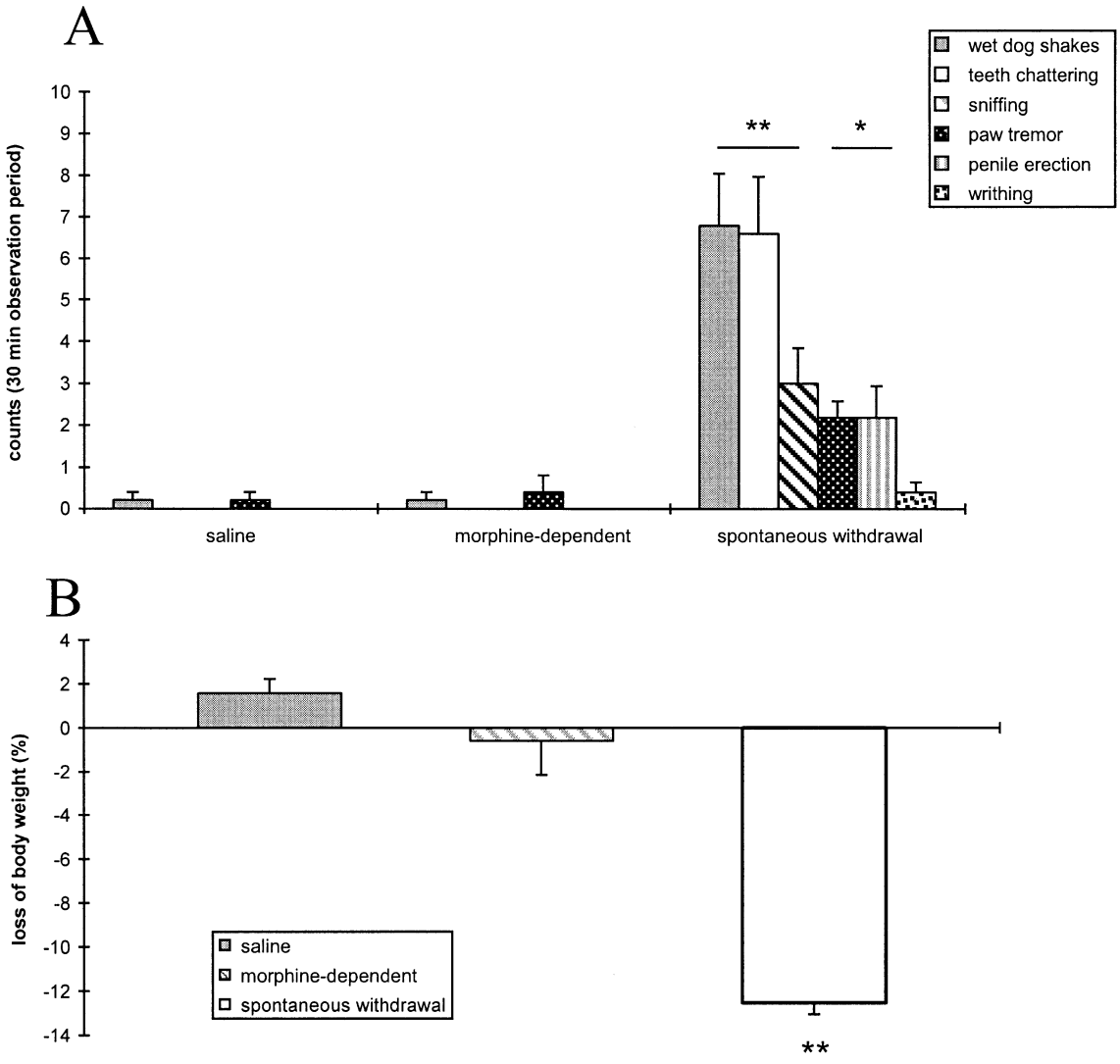

Fig. 1. (A) Withdrawal symptoms during 30 min observation period in control rats, morphine-dependent rats and rats suffering from spontaneous withdrawal for 36 h. (B) Loss of body weight in saline control rats, morphine-dependent rats and rats suffering from spontaneous withdrawal for 36 h. Data expressed as mean ± SEM. * $P<0.05$ and ** $P<0.01$.

brainstem nuclei participate in the regulation of physiological homeostasis and autonomic function and that these nuclei are involved in the development of physical dependence as well as in the expression of physical withdrawal symptoms. The present study was done in order to get new insights in the regulation of μ-opioid receptor coupled G-proteins during opiate withdrawal by using μ-opioid receptor-stimulated [^{35}S]-GTPγS autoradiography.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (200–220 g) were obtained from Charles River (Sulzfeld, Germany). At the beginning of the experiments, the rats were 2–3 months old. All animals were housed individually in standard (type 3) hanging rodent cages with food and water ad libitum. Artificial light was provided daily from 6:00 a.m. until 6:00 p.m. and room temperature and humidity were kept constant (temperature: 22 ± 1 °C; humidity: 55 ± 5%). The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body and carried out following the German Law on the Protection of Animals.

2.2. Morphine treatment and experimental design

For experiment 1 and 2 rats, were injected every 12 h with increasing doses of morphine–HCl (5–100 mg/kg, s.c.) (for more details see Spanagel et al., 1998) for 12 days. Separate groups of rats received saline injections and served as controls ($n = 5$ per group). The morphine-dependent rats were assigned to the following groups: morphine-dependent group (MOR) ($n = 8$), morphine withdrawal group undergoing spontaneous withdrawal for 36 h after the last morphine injection (SPWD) ($n = 8$) and precipitated withdrawal group (PRWD) ($n = 8$). In the PRWD group, withdrawal was precipitated with the opioid antagonist naloxone (1 mg/kg, s.c.). Rats in group MOR were maintained on 100 mg/kg morphine–HCl throughout the experiment. Thirty-six hours after the last morphine injection, withdrawal signs in the SPWD group were assessed for 30 min by a blind observer. The rats were then decapitated and brains rapidly removed and frozen on dry ice. Rats in group PRWD were decapitated at 5, 10, 20 and 60 min after naloxone injection (experiment 2). The effect of naloxone in control rats was also investigated. Animals were killed 5 min after naloxone injection (1 mg/kg). In the third experiment, rats were implanted with morphine pellets (three pellets, 75 mg each; morphine pellets were self-made according to the protocol of Gibbson and Tingstad, 1970; plasma concentrations of morphine following pellet implantation were determined by a radioimmunoassay at the forensic medicine of the University of Munich). In these rats, withdrawal was precipitated by naloxone (1 mg/kg) 7 days after pellet implantation. Withdrawal signs were assessed for 30 min by a blind observer. The rats were then decapitated and brains rapidly removed and frozen on dry ice.

2.3. Agonist-stimulated [^{35}S]-GTPγS binding autoradiography

Coronal sections (20 μm) were cut on a cryostat at −20 °C and mounted onto polylysine-coated slides. Sections were dried and stored at −80 °C until use. According to the protocol of Sim et al. (1995), sections were pre-incubated in assay buffer (50 mM Tris–HCl, pH 7.4, 3 mM $MgCl_2 \cdot 6\,H_2O$, 0.2 mM EGTA, 100 mM NaCl) at 25 °C for 10 min. Sections were then incubated in GDP-assay (guanylyl-diphosphate) buffer (2 mM GDP) at 25 °C for 15 min. Agonist-stimulated activity was determined by incubating the sections in [^{35}S]-GTPγS (0.04 nM) with the selective μ-opioid receptor agonist DAMGO (10 μM) and GDP-assay buffer at 25 °C for 2 h. Basal activity was assessed with GDP in the absence of the agonist and nonspecific binding was assessed in the presence of 10 μM unlabeled GTPγS. After incubation, slides were rinsed twice with ice cold assay buffer, fixed for 5 min with 0.5% formaldehyde solution at room temperature and dehydrated with alcohol. Sections were dried overnight and

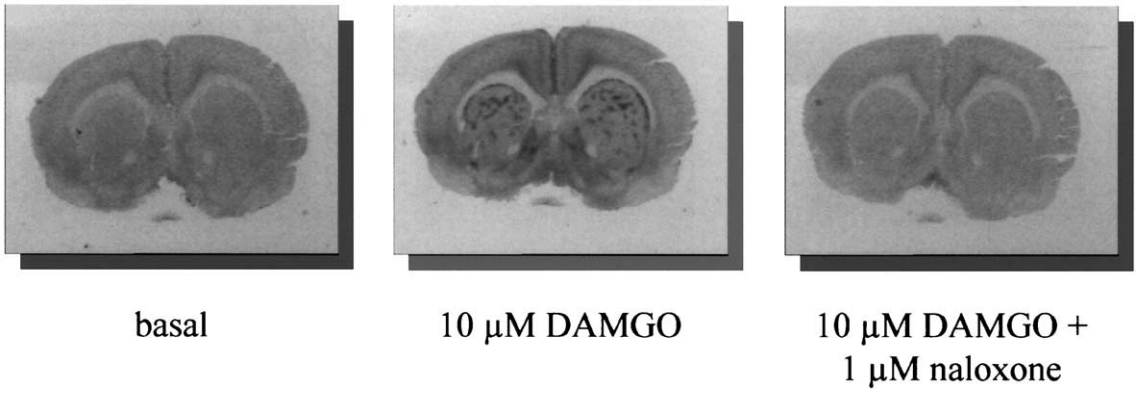

Fig. 2. Autoradiogram of [^{35}S]GTPγS binding in rat brain sections. Sections were incubated with 2 mM GDP for 15 min and then with [^{35}S]GTPγS (0.04 nM) and 2 mM GDP for 2 h under basal conditions, in the presence of 10 μM DAMGO and in the presence of 10 μM DAMGO/1 μM naloxone.

exposed to Hyperfilm-βmax for 40 h. Films were digitized with a Sony XC-77 video camera and analysed computer-assisted (Optimas, BioScan). Images were quantitated by densitometric analysis with ^{14}C-standards. Labelled sections were stripped from slides and ^{35}S radioactivity was determined by liquid scintillation spectrophotometry. All radiograms were performed in duplicate and repeated at least three times. Data are presented as mean values ± SEM from five to eight animals. Analysis of variance (ANOVA) was performed to test for statistically significant differences between groups. This was followed by Newman–Keul's test for post-hoc comparisons ($P < 0.05$).

[^{35}S]-GTPγS (1250 Ci/mmol) was purchased from NEN Life Sciences (Köln, Germany). DAMGO, GTPγS, GDP, naloxone and all other chemicals were obtained from Sigma (Germany). Autoradiography film (Hyperfilm-βmax) was purchased from Amersham (England).

3. Results

3.1. Experiment 1

Behavioural assessment was done in order to quantify the severity of opiate withdrawal. After 36 h of withdrawal, rats from the group SPWD were observed for 30 min for behavioural signs of withdrawal. Rats in group SPWD exhibited almost all characteristic withdrawal symptoms which can be observed in rats undergoing opiate withdrawal (Bläsig et al., 1973). Fig. 1A shows the most important withdrawal symp-

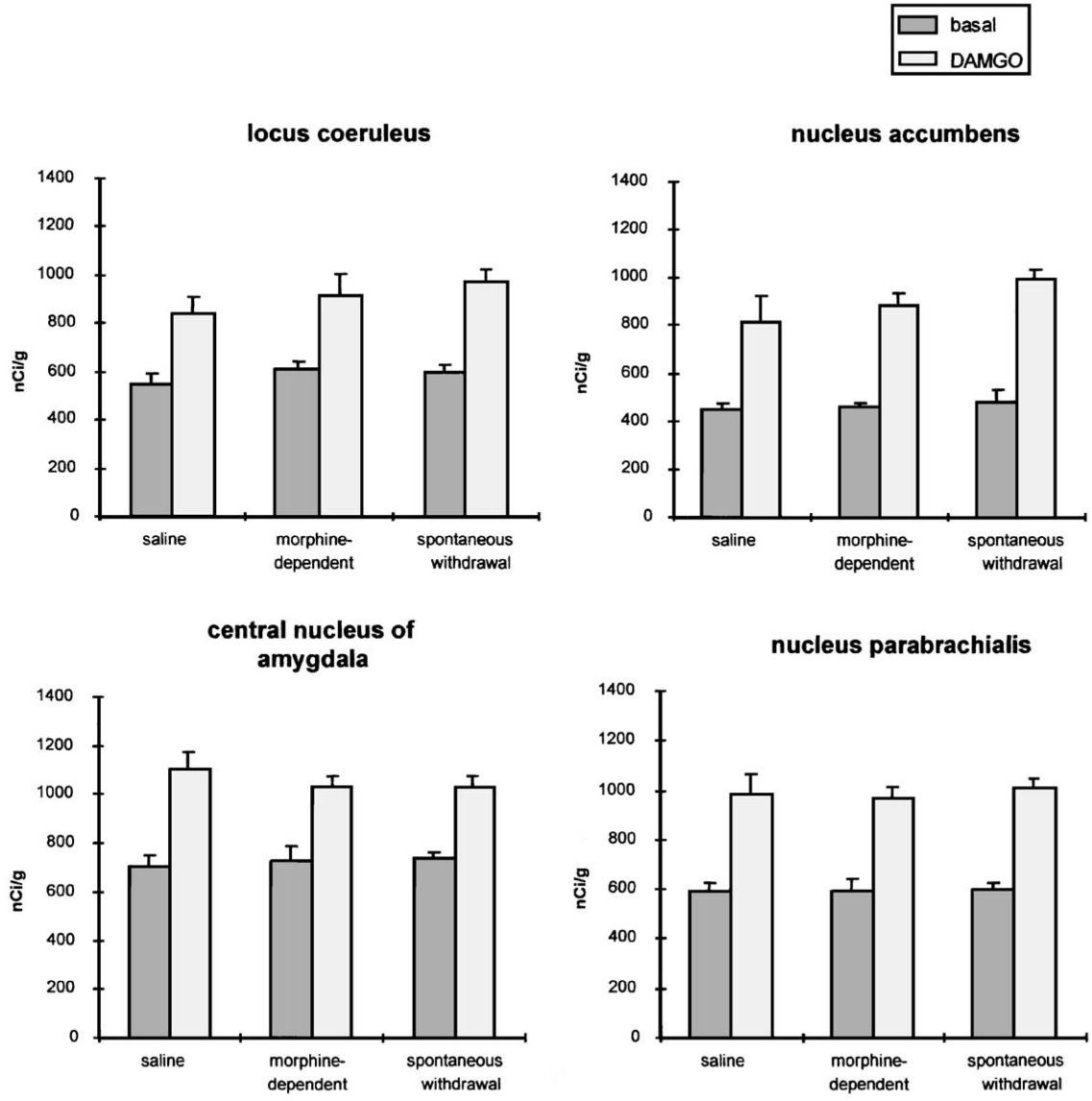

Fig. 3. Basal and DAMGO-stimulated [^{35}S]GTPγS binding in different brain regions in control rats, morphine-dependent animals and rats suffering from spontaneous withdrawal for 36 h. Sections were incubated with 2 mM GDP and then with [^{35}S]GTPγS (0.04 nM) and 2 mM GDP in the presence and absence of 10 μM DAMGO. [^{35}S]GTPγS binding is expressed as mean nanocurie/gram ± SEM from triplicate sections of five to eight animals.

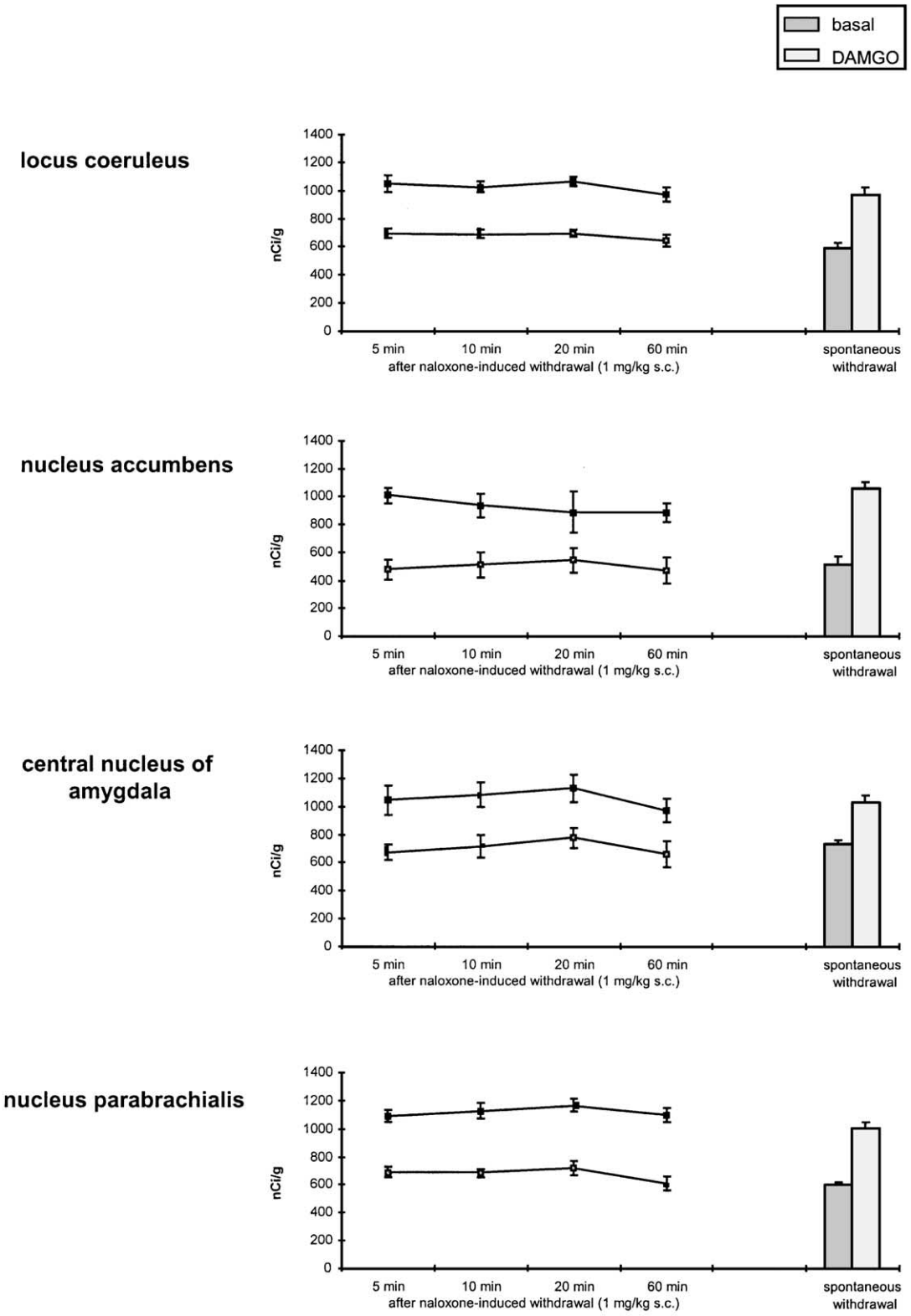

Fig. 4. Time course of basal and DAMGO-stimulated [^{35}S]GTPγS binding in different brain regions after naloxone-induced withdrawal (1 mg/kg) in morphine-dependent rats. [^{35}S]GTPγS binding is expressed as mean nanocurie/gram ± SEM from triplicate sections of five to eight animals. In comparison, data from spontaneous withdrawal are shown on the right side of the panel.

toms: 6.6 ± 1.2 episodes of wet dog shakes ($F[2,12] = 23.4$, $P < 0.0001$), 6.8 ± 1.4 teeth chattering ($F[2,12] = 26.8$, $P < 0.0001$), 3 ± 0.8 sniffing ($F[2,12] = 12.8$, $P = 0.001$), 2.2 ± 0.4 episodes of paw tremor ($F[2,12] = 10.8$, $P = 0.021$), 2.2 ± 0.7 penile erection ($F[2,12] = 8.9$, $P = 0.042$), 0.4 ± 0.2 episodes of writhing ($F[2,12] = 2.67$, $P = 0.11$). Fig. 1B depicts the loss of body weight: group SPWD lost during 36 h of withdrawal 12.5 ± 0.6% of initial body weight ($F[2,12] = 54.74$, $P < 0.0001$).

Agonist-stimulated [^{35}S]-GTPγS binding autoradiography was measured by incubating sections with the μ-opioid specific agonist DAMGO in the presence of [^{35}S]-GTPγS and GDP. The receptor specifity of agonist-stimulated [^{35}S]-GTPγS binding was verified by incubation sections with DAMGO in the presence of naloxone. The levels of [^{35}S]-GTPγS binding in the presence of agonist and antagonist were comparable to basal activitiy (in the absence of DAMGO) (Fig. 2). Evaluation of autoradiographic films and calculation of grey values to nCi/g tissue showed that in all brain areas investigated (central nucleus of amygdala, nucleus accumbens, locus coeruleus, nucleus parabrachialis), no significant changes in [^{35}S]-GTPγS binding between treatment groups occurred, although rats in group SPWD had severe withdrawal signs and rats in group MOR were maintained on high morphine levels. Fig. 3 shows the mean values of [^{35}S]-GTPγS binding under basal and stimulated conditions in the following brain areas: central nucleus of amygdala, nucleus accumbens, locus coeruleus, nucleus parabrachialis. Statistical analysis revealed high significance regarding DAMGO-stimulation vs. basal levels, but no differences in the factor treatment or interaction (locus coeruleus: stimulation $F[1,33] = 52.9$, $P < 0.0001$; treatment $F[2,33] = 1.47$, $P = 0.24$; interaction $F[2,33] = 0.34$, $P = 0.71$; nucleus accumbens: stimulation $F[1,29] = 83.6$, $P < 0.0001$; treatment $F[2,29] = 1.62$, $P = 0.21$; interaction $F[2,29] = 1,62$, $P = 0,21$; central nucleus of amygdala: stimulation $F[1,25] = 64.5$, $P < 0.0001$; treatment $F[2,25] = 0.15$, $P = 0.85$; interaction $F[2,25] = 0.15$, $P = 0.85$; nucleus parabrachialis: stimulation $F[1,33] = 93.6$, $P < 0.0001$; treatment $F[3.31] = 0.18$, $P = 0.83$; interaction $F[2,31] = 0.18$, $P = 0.83$).

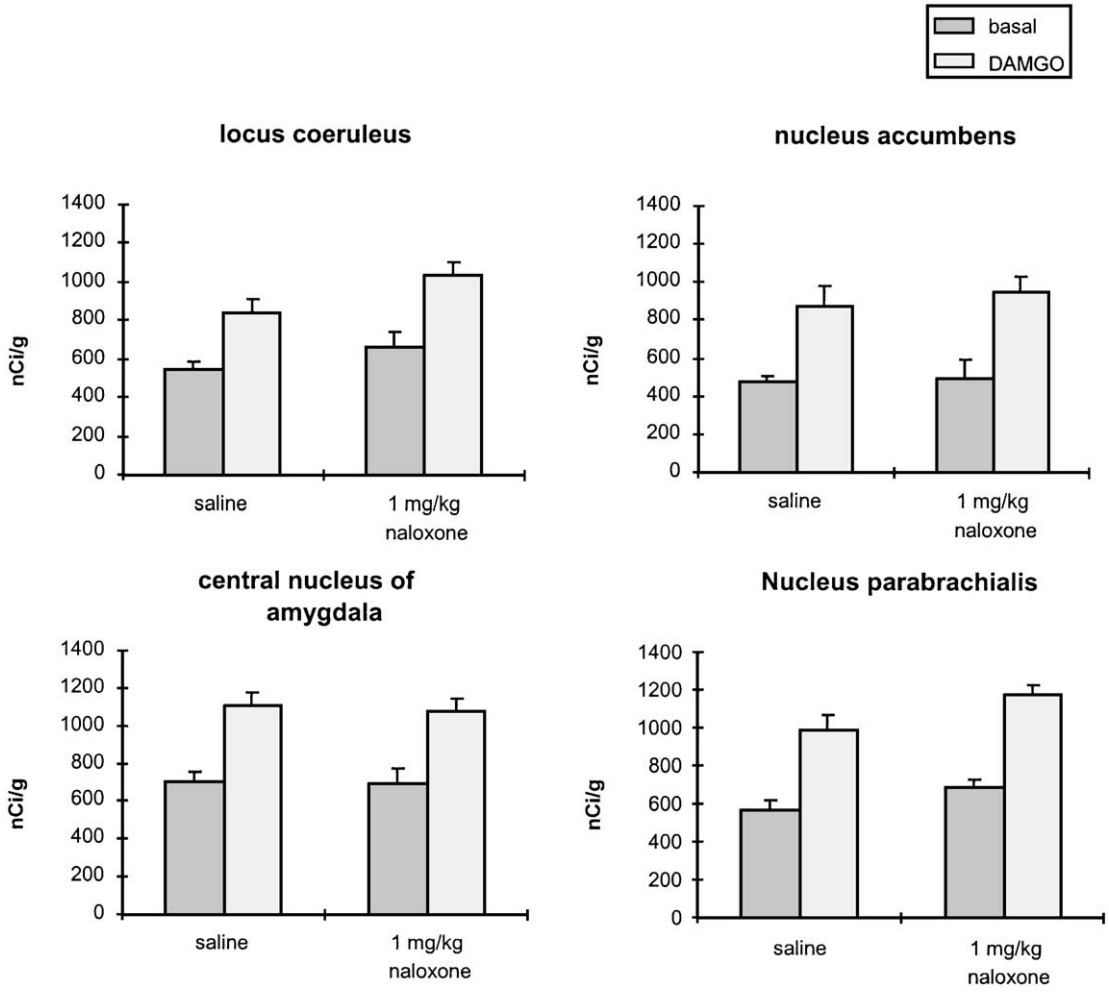

Fig. 5. Basal and DAMGO-stimulated [^{35}S]GTPγS binding in different brain regions in saline controls and rats injected with naloxone (1 mg/kg). [^{35}S]GTPγS binding is expressed as mean nanocurie/gram ± SEM from triplicate sections of five to eight animals.

3.2. Experiment 2

Due to the experimental design, behavioural data for the group PRWD could not be assessed in the same way as in experiment 1. Although not quantified, severe withdrawal symptons were observed. Fig. 4 depicts the G-protein activity at 5, 10, 20 and 60 min after naloxone-precipitated withdrawal in morphine-dependent rats. ANOVA for repeated measures revealed high statistical significance in DAMGO-stimulation vs. basal levels but no significant changes of [^{35}S]-GTPγS binding in any of the investigated brain areas at any time of naloxone-precipitated withdrawal (locus coeruleus: stimulation $F[1,9]=216$, $P<0.0001$; time $F[3,24]=1.11$, $P=0.36$; interaction $F[3,24]=0.11$, $P=0.95$; nucleus accumbens: stimulation $F[1,9]=19.3$, $P=0.0023$; time $F[3,24]=1.54$, $P=0.22$; interaction $F[3,24]=0.32$, $P=0.80$; central nucleus of amygdala: stimulation $F[1,9]=22.2$, $P=0.0015$; time $F[3,24]=0.14$, $P=0.93$; interaction $F[3,24]=0.15$, $P=0.85$; nucleus parabrachialis: stimulation $F[1,9]=100$, $P<0.0001$; time $F[3,24]=1.80$, $P=0.17$; interaction $F[3,24]=0.44$, $P=0.72$). The effect of naloxone on μ-opioid receptor coupled [^{35}S]-GTPγS binding in saline-pretreated rats was also investigated. Fig. 5 depicts the results of [^{35}S]-GTPγS binding autoradiography 5 min after naloxone injection (1 mg/kg) in saline-pretreated rats and control animals. In all investigated brain areas, DAMGO-stimulated μ-opioid receptor coupled G-protein activity vs. basal activity was statistically significant (locus coeruleus $F[1,20]=20.8$, $P=0.0002$; nucleus accumbens $F[1,16]=22.7$, $P=0.0002$; central nucleus of amygdala $F[1,14]=32.6$, $P<0.0001$; nucleus parabrachialis $F[1,16]=62.4$, $P<0.0001$) but no differences in the factor treatment or interaction was detected (statistics not shown). These results indicate that naloxone has no influence on the activity of μ-opioid receptor coupled G-proteins.

3.3. Experiment 3

Morphine dependence was induced by implantation of morphine pellets (three pellets, 75 mg each). Seven days following morphine pellet implantation, plasma concentrations of 396 ng/ml morphine were measured. Opiate withdrawal was precipitated by naloxone injections (1 mg/kg). Rats were observed 5 min after injection of naloxone for 30 min. These rats had 30% more intense withdrawal symptoms compared to the rats undergoing spontaneous withdrawal (data not shown; see also our previous data, Spanagel et al., 1998). Agonist-stimulated [^{35}S]-GTPγS binding autoradiography was only performed in the locus coeruleus (Fig. 6). Analysis of data revealed statistically significant differences in stimulation (DAMGO vs. basal), but no differences in the factor treatment or interaction (locus coeruleus: stimulation $F[1,23]=58.3$, $P<0.0001$; treatment $F[3,23]=0.69$, $P=0.56$; interaction $F[3,23]=0.58$, $P=0.63$).

In all experiments, values of μ-opioid receptor-coupled [^{35}S]-GTPγS binding during opiate withdrawal were not affected. Thus, no significant changes in [^{35}S]-GTPγS binding occurred. Additional μ-opioid receptor binding studies revealed also no significant changes in receptor density (data not shown).

4. Discussion

Our study investigated the involvement of μ-opioid receptor-activated G proteins in rat brain during opiate withdrawal. Rats were made morphine-dependent by injections of increasing doses of morphine or by implantation of morphine pellets. Opiate withdrawal was induced by abstinence from chronic morphine administration (spontaneous

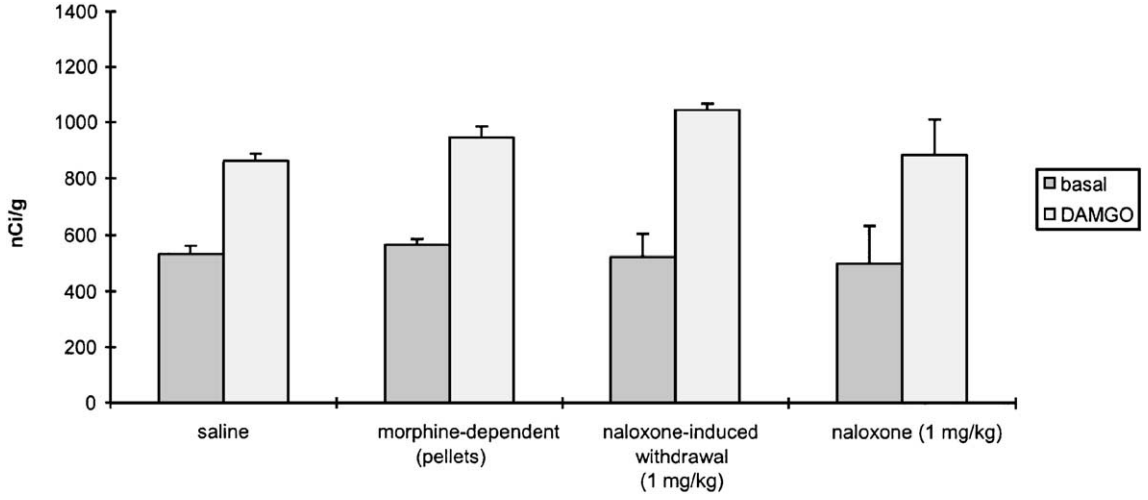

Fig. 6. Basal and DAMGO-stimulated [^{35}S]GTPγS binding in locus coeruleus sections of control rats, morphine-dependent rats (implantation of three morphine-pellets, 75 mg morphine-base each), naloxone-precipitated withdrawal and saline-pretreated rats injected with naloxone (1 mg/kg). [^{35}S]GTPγS binding is expressed as mean nanocurie/gram ± SEM from triplicate sections of five to eight animals.

withdrawal) or by precipitation with the opioid receptor antagonist naloxone. [^{35}S]-GTPγS binding was determinated by incubating slides with [^{35}S]-GTPγS and GDP in the presence and absence of the μ-opioid receptor selective agonist DAMGO. Although all rats suffered from severe withdrawal symptoms after 36 h of spontaneous withdrawal, no changes in [^{35}S]-GTPγS binding in comparison to control animals were detected. Naloxone-precipitated withdrawal was investigated at 5, 10, 20 and 60 min after naloxone injection. At no time point of naloxone-precipitated opiate withdrawal were any changes in μ-opioid receptor coupled [^{35}S]-GTPγS binding detected. The investigated time course of opiate withdrawal in our study guarantees that during the whole time course of the withdrawal period (5 min up to 36 h), G-protein activity was assessed and it is very unlikely that at any other time, points changes in [^{35}S]-GTPγS binding occur. Also, a different induction of morphine dependence with pellets had no influence on [^{35}S]-GTPγS binding in rats undergoing withdrawal. In summary, these findings indicate that altered G-protein receptor function is not involved in the expression of opiate withdrawal and other mechanisms in signal transduction, rather than interactions of μ-opioid receptors and their corresponding G-proteins, are responsible for the up-regulation of the cAMP system in the brainstem following chronic morphine exposure.

Decreased levels of DAMGO-stimulated [^{35}S]-GTPγS binding in several brainstem nuclei were reported by Sim et al. (1996) following chronic morphine administration. In particular, in the locus coerleus and the lateral/medial parabrachial nucleus, decreased levels of DAMGO-stimulated [^{35}S]-GTPγS binding were detected. In another study, enhanced [^{35}S]-GTPγS binding by DAMGO was attenuated in spinal cord membranes obtained from mice that were daily treated intrathecally with DAMGO for 5 and 7 days (Narita et al., 2001). Together, both studies indicate that chronic activation of μ-opioid receptors decreases levels of DAMGO-stimulated [^{35}S]-GTPγS binding in several sites of the central nervous system. In the present study, however, no decreased levels of DAMGO-stimulated [^{35}S]-GTPγS binding following chronic morphine administration were observed. These different results may be explained by the extraordinary high levels of morphine used in the study by Sim et al. (320 mg/kg/day given intravenously). In addition, morphine sulfate was used in this study which has more bound morphine in comparison to morphine–HCl, which we used in our study. Two different models of induction of morphine dependence were used in the present study: subcutaneous morphine injections every 12 h with increasing doses up to 100 mg/kg and chronic application by implantation of morphine pellets. These regimens of induction of morphine dependence are well established by many studies and provide stable and sufficient physical dependence (Bläsig et al., 1973; Cicero and Meyer, 1973; Maldonado et al., 1996b; Spanagel et al., 1998). Neuronal systems overloaded with external agonists may require additional neuronal circuits and intracellular mechanisms to protect them from damage and to maintain homeostasis under difficult conditions.

The present finding of unchanged [^{35}S]-GTPγS binding in morphine-dependent and withdrawn rats raises the central question of responsibility of the massive alterations of cAMP-levels in brainstem nuclei during opiate dependence and withdrawal. [^{35}S]-GTPγS autoradiography of other subtypes of opioid receptors like δ- and κ-receptors was not done in this experiment, so we do not know if [^{35}S]-GTPγS binding of these receptors is changed and if so, to what extent. The existence of interactions between opioid receptors and their role in opiate dependence is well documented. Repeated exposure to exogenous opiates results in adaptive changes mainly mediated by μ-opioid receptors, but there is also evidence for an involvement of δ- and κ-opioid receptors (Cowan et al., 1988; Maldonado et al., 1992; Spanagel et al., 1994). A direct cross-talk between μ- and δ-opioid receptors has been described, e.g. electron microscopy revealed that chronic morphine exposure leads to a marked increase in the density of δ-opioid receptors (Cahill et al., 2001) and immunohistochemical studies have demonstrated colocalization of these opioid receptors in some neurons (Ji et al., 1995). Furthermore, several studies have suggested the existence of a μ-/δ-opioid receptor complex (Schoffelmer et al., 1990; Spanagel et al., 1990; Cvejic and Devi, 1997), opening the possibility for physical interactions between these receptors. Therefore, there is increasing evidence that opioid receptors do not necessarily act independently from each other. It should be noted, however, that Narita et al. (2001) demonstrated no change in levels of [^{35}S]-GTPγS binding induced by either a δ-opioid receptor agonist or a κ-opioid receptor agonist in spinal cord membranes obtained from mice that were treated chronically with DAMGO showing the specificity evoked by chronic μ-opioid receptor activation.

In addition to the traditional coupling of opioid receptors to inhibitory G-proteins, there is growing evidence that opioid receptors are also coupled to excitatory G_s-proteins. G_s-coupled opioid receptors have been extensively characterised by Crain and Shen (1996), and have been proposed to play a role in tolerance and dependence. One reason for our finding of unchanged [^{35}S]-GTPγS binding may be based on the assay itself: [^{35}S]-GTPγS autoradiography detects overall G-protein activity and changes in the activity of one subtype of G-protein within the overall population may not be detected. The ratio between $G_{i/o}$ and G_s may change dramatically during chronic morphine administration and opiate withdrawal, but remains undetected by this assay.

Changes in the coupling of opioid receptors and their G-protein complexes are not necessarily important for maintaining homeostasis of a neuron. As mentioned before, many intracellular mechanisms are involved in opiate dependence and withdrawal, e.g. up-regulation of the cAMP/CREB cascade and as consequence increased transcription

of several target genes. These compensatory intracellular mechanisms seem to be sufficient for an adaptive response to chronic morphine exposure.

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