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Short communication

Effects of morphine and methadone treatments on glutamatergic transmission in rat frontal cortex

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Abstract:
The effects of repeated administration of morphine and methadone, followed by a challenge dose of either morphine or methadone were examined *ex vivo* in rat frontal cortical slices that were prepared 1 h after final drug administration. Morphine challenge dose (5 mg/kg), administered 14 days after the end of repeated morphine pretreatment (10 mg/kg, administered 7 times) decreased both the AMPA/kainate and the NMDA components of field potentials that were evoked in cortical layer II/III by electrical stimulation. This effect did not occur either when a methadone challenge dose (2.5 mg/kg) was administered instead of morphine or after repeated morphine treatment. Moreover, after repeated methadone treatment (2.5 mg/kg, administered 7 times), neither morphine nor methadone challenge affected AMPA/kainate or NMDA components of the field potentials. These data indicate a specific effect of repeated morphine followed by morphine challenge on cortical glutamatergic transmission.

Key words:
field potential, cortical slice, AMPA receptors, NMDA receptors

Introduction

Repeated administration of opioids results in the development of sensitization, the phenomenon of an enhanced behavioral response to a given dose of an opioid [3]. In animals subjected to earlier repetitive morphine treatment, due to behavioral sensitization, a challenge dose of morphine may produce an enhanced locomotor response after periods of abstinence that last for months [15]. Sensitization is thought to play a role in the development of drug addiction and relapse [3, 7]. It has recently been shown that in addition to the effects on the profile of behavioral responses, morphine challenge given 14 days after the end of the pretreatment with morphine, induced an increase in Fos protein expression [6] in various rat brain structures [17, 18]. In particular, the induction of Fos protein in certain parts of the neocortex (including the frontal cortex) by a challenge dose of morphine was markedly enhanced by the repetitive administration of morphine.

Methadone, an agonist of μ opioid receptors, is used in substitution therapy of heroin addicts due to its potential to modify the reactions to opiates [10]. However, the cellular mechanisms of methadone action and its potential for modifying the sensitization that results from opioid treatment are not fully understood. Taracha et al. [17] have recently shown that, in contrast to morphine pretreatment, there was no en-

Tab. 1. Animal treatment schemes

Rat group (number of animals/slices)	Pretreatment[1]	Challenge[2]
control (8/25)	7 × saline	1 × saline
morphine 1 (8/27)	7 × saline	1 × morphine, 5 mg/kg
methadone 1 x (7/12)	7 × saline	1 × methadone, 2.5 mg/kg
morphine 7 (7/18)	7 × morphine, 10 mg/kg	1 × saline
methadone 7 (7/12)	7 × methadone, 2.5 mg/kg	1 × saline
morphine 7 morphine 1x (7/21)	7 × morphine, 10 mg/kg	1 × morphine, 5 mg/kg
morphine 7 methadone 1x (7/18)	7 × morphine, 10 mg/kg	1 × methadone, 2.5 mg/kg
methadone 7 methadone 1x (7/13)	7 × methadone, 2.5 mg/kg	1 × methadone, 2.5 mg/kg
methadone 7 morphine 1 (7/12)	7 × methadone, 2.5 mg/kg	1 × morphine, 5 mg/kg

[1] saline or drug administered over 7 days; [2] saline or drug administered once, 14 days after the end of the pretreatment

hancement of Fos protein expression in layer II/III of rat frontal cortex by a challenge dose of morphine after repetitive methadone administration. Furthermore, the locomotor responses of rats to the challenge were enhanced after morphine, but not methadone, treatment [18]. These findings may partly explain the beneficial effects of methadone in therapy for addiction.

It has been well established that Fos proteins (among several expression products of immediate early genes) play a role in stabilizing changes of synaptic efficacy in cortical circuits that result from synaptic plasticity processes [reviewed in: 8, 9]. Thus, it seemed conceivable that modifications in the level of c-Fos protein induction in cortical neurons by morphine treatment and reexposure to the drug [17, 18] might be reflected in functional alterations in the activity of cortical neural circuitry. Therefore, the aim of the present study was to determine the effects of single and repetitive administration of morphine and methadone on the AMPA and NMDA receptor-mediated components of field potentials that were evoked in *ex vivo* brain slices of the frontal cortex by electrical stimulation of glutamatergic pathways.

Materials and Methods

Animals

Experimental procedures were carried out in accordance with the European Community guidelines for the use of experimental animals, and national law and were approved by the local Animal Care and Use Committee. Male Wistar rats, weighing approx. 90–100 g at the beginning of the experiment, were housed in groups, subjected to a controlled light/dark cycle (light on: 7.00–19.00) and had free access to standard food and tap water.

Drugs

Morphine (Polfa, Poland) and methadone ((R,S)-methadone hydrochloride (Molteni Farmaceutici, Scandicci, Italy) were kindly provided by dr E. Taracha, Institute of Psychiatry and Neurology, Warszawa, Poland), and dissolved in water. The rats were randomized between experimental groups and were given *sc* injections of physiological saline, morphine and methadone according to the schedule and group count shown in Table 1. The experimental design and the choice of drug dosage were based on earlier reports [17, 18].

Slice preparation and recording

Brain slices were prepared 1 h after the final morphine, methadone or saline administration, as described previously [4, 5]. In brief, rats were decapitated under halothane anesthesia, their brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 130 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.25 KH_2PO_4, 26 $NaHCO_3$, 10 D-glucose, that was bubbled with a mixture of

95% O_2–5% CO_2. Frontal cortical slices (400 μm thick) were cut in the coronal plane using a vibrating microtome (Leica VT1000). Slices were superfused at 2.5 ml/min with warm (32 ± 0.5°C), modified ACSF containing 132 mM NaCl and 2 mM KCl.

A bipolar stimulating electrode (FHC, USA) was placed within cortical field M1 (motor cortex) approx. 3 mm lateral to the midline and approx. 1.5 mm below the pial surface (layer V) [4, 5]. Constant-voltage stimuli (0.2 ms, 4–15 V) were delivered at 0.1 Hz. Field potentials were recorded using glass micropipettes filled with 2 M NaCl (2–5 MΩ), which were placed approx. 0.2–0.3 mm below the cortical surface (layer II/III). Recordings were amplified (Axoprobe, Axon Instruments, USA), bandpass filtered (1–500 Hz), acquired using Micro 1401 interface (CED, UK) and analyzed off-line using Signal 2 software (CED, UK).

To isolate the AMPA/kainate component of the field potential, NMDA receptors were blocked by 2 μM (±)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849; Tocris, UK). To isolate the NMDA component of the field potential, the slices were incubated in ACSF devoid of Mg^{2+} and containing 5 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide (NBQX, Tocris, UK) to block AMPA/ kainate receptors. For each condition, after stable responses had been obtained, input-output curves were generated.

Statistical analysis

Results are expressed as means ± SEM. Statistical analysis was carried out using the two-tailed Student's *t*-test.

Results

Field potentials evoked in layer II/III of frontal cortical slices by stimulation of the underlying sites consisted mainly of single, negative-going waveforms, in agreement with earlier studies [4, 5]. Incubation of the slices in ACSF that contained 2 μM CGP 37849 allowed for pharmacological isolation of the AMPA/ kainate receptor-mediated component of the field potential (Fig. 1A). When NBQX (5 μM) was applied in ACSF that was devoid of Mg^{2+}ions, after the washout of CGP 37849, a longer-latency waveform was generated (Fig. 1B); this represented the NMDA receptor-mediated component of the field potential.

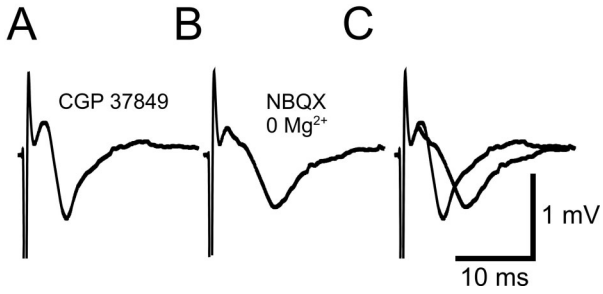

Fig. 1. Field potentials recorded in the frontal cortical slice during a representative experiment. Shown are examples of responses (averages of 4) evoked at the same stimulus intensity. (**A**) Responses recorded in ACSF containing CGP 37849 (the AMPA/kainate receptor-mediated component). (**B**) Responses recorded in ACSF devoid of Mg^{2+} ions and containing NBQX (the NMDA receptor-mediated component). (**C**) Superposition of waveforms illustrated in **A** and **B**

After repeated morphine administration and subsequent morphine challenge dose, marked decreases in the amplitudes of both the AMPA/kainate and the NMDA components were evident (Figs. 2A, 3A). In contrast, methadone challenge after chronic morphine treatment did not modify the field potentials (Figs. 2A, 3A). No significant effects on the recorded responses were evident after chronic methadone treatment combined with subsequent morphine or methadone challenge (Figs. 2B, 3B). Similarly, repeated administration of saline and a challenge dose of either morphine or methadone did not modify the recorded responses (Figs. 2C, 3C).

Discussion

The results of the present study demonstrated that when a challenge dose of morphine was administered 14 days after the end of pretreatment that consisted of 7 daily morphine injections, there was a reduction in both AMPA/kainate and NMDA receptor-mediated components of the field potentials that were evoked in M1/M2 of the frontal cortex. In contrast, a challenge dose of methadone did not induce such an effect. No change in the amplitude of recorded potentials occurred after a single administration of morphine to rats that were pretreated with saline. Thus, the data demonstrate a specific effect of repeated morphine followed by morphine challenge on glutamatergic transmission in rat frontal cortex.

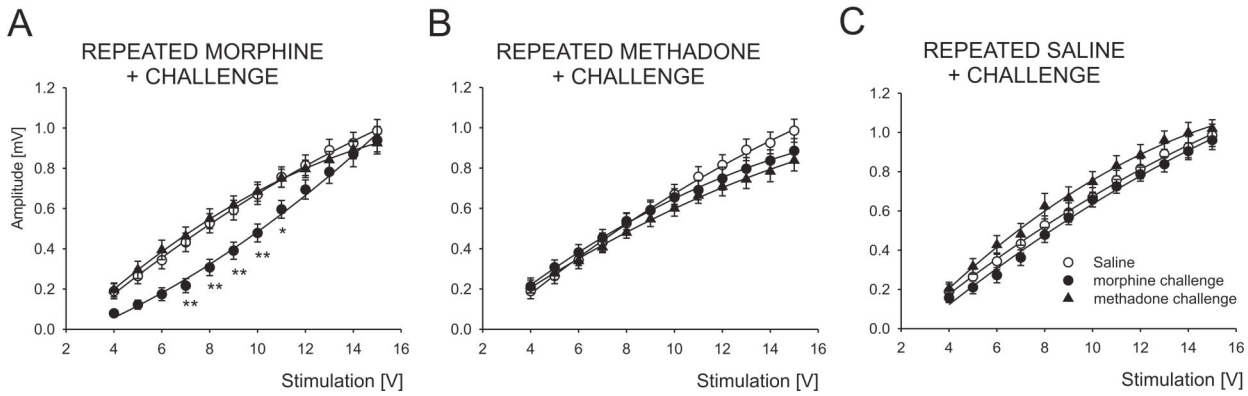

Fig. 2. The effects of various treatments on the relationship between stimulus intensity and the magnitude of isolated AMPA/kainate receptor-mediated field potentials. (**A**) Repeated morphine administration. In slices prepared from rats that received morphine after the withdrawal period (morphine challenge, filled circles), the magnitude of responses evoked by stimuli of intermediate intensity was lower than in slices prepared from animals that received methadone (methadone challenge, filled triangles) or saline (open circles) after the withdrawal. (**B**) Repeated methadone administration. Neither morphine (filled circles) nor methadone (filled triangles) challenge resulted in a reduction of the responses. (**C**) Repeated saline administration. Neither morphine (filled circles) nor methadone (filled triangles) challenge resulted in a reduction of the responses. * $p < 0.05$; ** $p < 0.01$

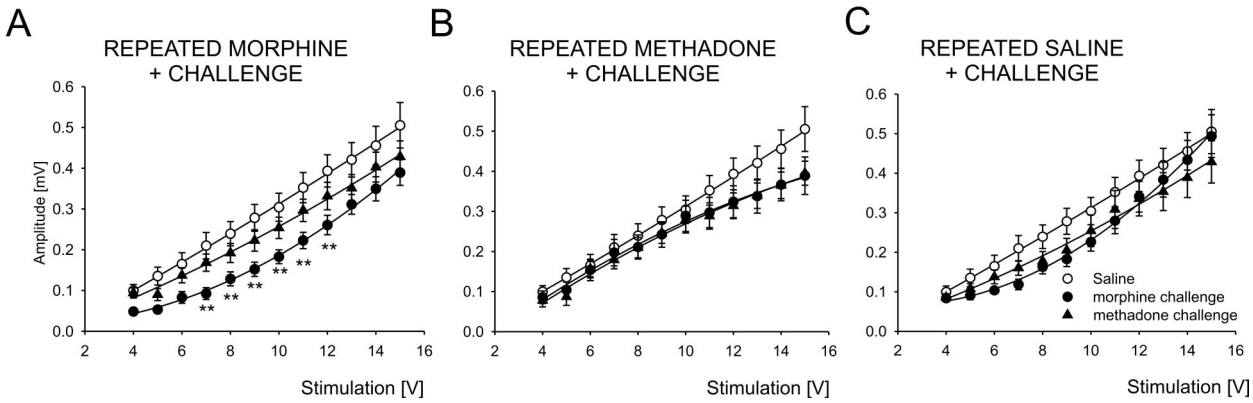

Fig. 3. The effects of treatment on the relationship between stimulus intensity and the magnitude of isolated NMDA receptor-mediated field potentials. (**A**) Repeated morphine administration. After the withdrawal period (morphine challenge, filled circles), the magnitude of responses evoked by stimuli of intermediate intensity was lower in slices prepared from rats that received morphine (morphine challenge, filled circles) than in slices prepared from animals that received either methadone (methadone challenge, filled triangles) or saline (open circles) after the withdrawal. (**B**) Repeated methadone administration. Neither morphine (filled circles) nor methadone (filled triangles) challenge resulted in a reduction of the responses. (**C**) Repeated saline administration. Neither morphine (filled circles) nor methadone (filled triangles) challenge resulted in a reduction of the responses. ** $p < 0.01$

To date, cortical glutamatergic transmission after reexposure to morphine has not been investigated. Immediately following chronic morphine treatment, rats exhibit markedly increased locomotor activity but this effect lasts for less than 48 h [e.g., 14]. Although the effects of chronic morphine administration on the functional properties and morphology of cortical neurons have not been thoroughly studied, the available data suggest that they depend on the cortical area. It has been shown that chronic morphine self-administration results in an expansion in the dendritic arborization of pyramidal cells in rat prefrontal cortex, whereas a reduction in the size and branching complexity of dendrites is apparent in the motor cortex; although the spine density in both areas is increased [2]. Other studies reported either cortical area-dependent chronic morphine-induced increases or reductions in cortical spine density [11, 16]. Electrophysiological studies have shown that chronic morphine treatment does not produce changes in basal neuronal activity within the somatosensory cortex, but it does induce a modest alteration of responses to afferent stimula-

tion [1]. At the cellular level, it has been shown that chronic treatment with morphine results in decreases in the frequency and amplitude of and a shortening of the waveform of miniature excitatory postsynaptic currents in cortical neurons [12]. These effects have consistently been attributed to the activation of μ opioid receptors. It has also been shown that reexposure to cues associated with heroin results in a depression of GluR2 subunit-containing AMPA receptor-mediated transmission in rat prefrontal cortex [19].

In the present study, synaptic transmission was not tested immediately after the end of the series of repetitive morphine injections; however, we obtained data that demonstrated that any potential modification of glutamatergic transmission lasted for a shorter period than two weeks. At the time of the administration of the challenge dose of drugs, the responses in slices obtained from rats that received morphine treatment were not different from those of slices prepared from animals that received saline. However, a challenge dose of morphine resulted in a decrease in the recorded responses. It has been shown that the effect of Fos protein induction by morphine administration could be markedly enhanced by an earlier treatment with morphine [17]. As increases in Fos protein levels may relate to a depression of synaptic transmission [13], it is likely that the observed decrease represents a form of synaptic depression. Alterations in the efficacy of motor cortical synaptic transmission, together with modifications occurring in other brain areas [17], might influence the motor output [20]. However, we note that the relationship between depressed synaptic transmission in the motor cortex that results from morphine challenge, and enhanced locomotor response to such challenge [18] requires further studies.

Interestingly, the administration of a morphine challenge dose 14 days after the end of the treatment with methadone, did not result in a decrease in the recorded responses in our study. This was consistent with the results of previous studies that discovered no changes in either Fos expression or the locomotor response resulting from a similar treatment [17]. A challenge dose of methadone, administered 14 days after the end of the treatment with either morphine or with methadone, also did not result in a decrease in the recorded responses. This result is also in keeping with the lack of change in Fos expression or the locomotor response that resulted from similar treatment with lower doses of these drugs in previous studies [17, 18]. Thus, our results are in agreement with those of previous studies, which demonstrated that in contrast to morphine, methadone does not induce long-lasting effects on glutamatergic synaptic transmission in the frontal cortex. Conceivably, this effect may be related to reduced behavioral sensitization in rats after methadone treatment.

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