Research Report

Interaction of adenosine and naloxone on regional cerebral blood flow in morphine-dependent rats

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Abstract

The present research aimed at investigating the opioid–adenosine interaction on regional cerebral blood flow (rCBF). Therefore rCBF in the sensory cortex of morphine-naïve and -dependent rats was measured using the laser-Doppler flowmetry technique. The results showed that adenosine (10⁻⁵, 10⁻⁴, 10⁻³ M) significantly increased rCBF in morphine-dependent rats (MDR) (P < 0.01). This effect was inhibited by theophylline (5 × 10⁻⁵ M). Also systemic naloxone (0.5, 1.5 and 3 mg/kg, s.c.) significantly increased rCBF in MDR and it was accompanied by elevated blood pressure and heart rate. Local adenosine (10⁻⁴ M) significantly augmented naloxone (0.5 mg/kg)-induced increase in rCBF of MDR but had no significant effect on naloxone’s (1.5 and 3 mg/kg) increasing effect on rCBF. Theophylline also has no effect on naloxone increasing effect on rCBF. These data suggest that adenosine receptors responsiveness increase in sensory cortex of MDR. Naloxone also highly increased rCBF of MDR that probably not interfere with adenosine receptors. Also, it seems that adenosine acts as a modulator in rCBF regulation of morphine-dependent and morphine withdrawal rats.

Keywords: Cerebral blood flow Adenosine Naloxone Morphine-dependent rats

1. Introduction

The search for a better understanding of neurobiological mechanisms underlying the addictive actions of drugs of abuse should be given a high priority, as this should results in crucial advances in our ability to treat drug addiction (Nestler et al., 1992).

Many studies have demonstrated opioid–adenosine interaction at cellular and behavioral level (Peart and Gross, 2003; Poon and Sawynok, 1995), for example, adenosine receptors changes in morphine-dependent animals brains (Brundige and Williams, 2002; Kaplan et al., 1994; White et al., 1995). Adenosine analogue induces anti noiception similar to opiate and also inhibits the expression of morphine tolerance and withdrawal (Germany et al., 1990; Karlsten et al., 1991).

Opioids contribute to the regulation of cerebral blood flow. Opioid receptor binding has been demonstrated on cerebral micro vessels (Peroutka et al., 1980). Heterogeneous increase in regional cerebral blood flow (rCBF) 3 min after a single, acute dose of heroin or naloxone has been reported in the conscious rat (Fuller and Stein, 1991). It has been reported that morphine administration reduced rCBF in the control group and morphine-dependent rats (MDR), but the depressant effect of morphine in dependent rats was less than that in the control group. While naloxone had no considerable effect on rCBF in control group, it increased rCBF in MDR (Zamani et al., 2000).

Adenosine has many of the characteristics of a CBF regulator. It is a potent cerebral vasodilator and has been proposed as a metabolic regulator of CBF (Berne et al., 1974). It is an important neuromodulator that has been implicated in...
mediating both acute and chronic opiate effects (Kaplan and Leite-Morris, 1997).

In view of adenosine–morphine interaction, changes in adenosine receptors in morphine dependency and profound effect of naloxone and adenosine on rCBF in morphine-dependent and naive animals, the interaction of adenosine and naloxone on rCBF regulation of MDR was investigated in this study.

2. Results

2.1. Test of dependence on morphine

Withdrawal syndrome was precipitated by naloxone administration (3 mg/kg, s.c.). Defecation, diarrhea, chewing, weight loss, teeth chattering, writhing, sensitization and wet-dog shake were common among all the morphine-treated rats (n = 6). The control rats (n = 5) did not show any defined withdrawal signs.

2.2. Effects of adenosine and theophylline on rCBF

Adenosine was applied at three different concentrations (10\(^{-5}\), 10\(^{-4}\), 10\(^{-3}\) M). Adenosine increased rCBF in hind limb area of sensory cortex in all the three groups (n = 7 × 3). The percentage of increase in MDR was higher than in the control and sham-operated groups. It was significant at 10\(^{-4}\) and 10\(^{-3}\) M (Fig. 1). The effect of adenosine (10\(^{-4}\) M) on rCBF was inhibited significantly by an adenosine receptor antagonist theophylline (5 × 10\(^{-5}\) M), while it alone had no effect on rCBF.

2.3. Effects of naloxone (3 mg/kg), adenosine and theophylline on rCBF

Naloxone (3 mg/kg, s.c.) significantly increased rCBF (43.6%) in the MDR group (n = 7). Co-application of adenosine (10\(^{-4}\) M) and theophylline (5 × 10\(^{-5}\) M) had no effect on the naloxone-induced increase in rCBF in the MDR group, but in the control and sham-operated groups (n = 7 × 2), adenosine significantly increased rCBF while theophylline had no effect (Fig. 2).
2.4. Effects of naloxone (0.5, 1.5 and 3 mg/kg) alone and together with adenosine on rCBF

Naloxone, with the doses of 0.5, 1.5 and 3 mg/kg, s.c. increased rCBF of MDR \((n = 5 \times 3)\) by: 41.68%, 39.14% and 46.2%, respectively, without significant differences among doses. Co-application of naloxone (0.5, 1.5 and 3 mg/kg, s.c.) and adenosine \((10^{-4} \text{ M})\) also increased rCBF of MDR \((n = 5 \times 3)\) by: 64.33%, 60.3% and 50.22%, respectively. There was also no significant difference among doses. So naloxone in the presence of adenosine was more effective on rCBF than naloxone alone and the difference was statistically significant at the dose of 0.5 mg/kg (Fig. 3).

2.5. Effects of naloxone (0.5, 1.5 and 3 mg/kg) on blood pressure and heart rate

Local application of adenosine and theophylline on cortical surface had no effect on blood pressure and heart rate in the control, sham-operated and morphine-dependent rats. But naloxone administration (0.5, 1.5 and 3 mg/kg, s.c.) changed blood pressure and heart rate significantly in the MDR group. Naloxone administration primarily decreased blood pressure and heart rate (0–5 min after the naloxone administration), then returned to base line and following increase significantly (Fig. 4a and b).

2.6. Physiological parameter

Physiological variables of all the three groups were recorded as follows: heart rate: 330 ± 15 beat/min, systolic blood pressure: 102 ± 3 mm Hg, diastolic blood pressure: 76 ± 2.5 mm Hg, percent of arterial O2 saturation: 94.9 ± 0.75. The mean arterial blood gases and pH were: \(\text{Pa}_2\) 78 ± 3.5 mm Hg, \(\text{Pa}_2\) 37.5 ± 2.5 mm Hg and pH 7.39 ± 0.15.

3. Discussion

The findings of the present research suggested that morphine dependency induced some adaptive changes in rCBF. They also indicated that the vasodilative effect of adenosine on rCBF was augmented in MDR group, and the greater vasodilatation evoked in MDR was indicative of an increased adenosine responsiveness. This increase in adenosine responsiveness may be in adenosine receptors itself or through...
intracellular signal transduction pathways. Inhibition of this effect by theophylline, confirms that the responses are mediated by adenosine receptors.

Adenosine is an endogenous vasodilator considered to be involved in the local blood flow regulation of various tissues. Adenosine-induced vasodilation in the rats pial artery is mediated via activation of adenosine A2a and A2b receptors with different mechanisms (Shin et al., 2000; Ukena et al., 1987). On the other hand, adenosine is an inhibitory neurotransmitter in the central nervous system. Acute stimulation of adenosine A1 receptor results in decrease of Ca$^{2+}$ influx and suppressed release of neurotransmitter. As a result of these effects, neural excitability and firing rate are reduced causing a substantial reduction of brain metabolic demands (Ralevic and Burnstock, 1998). Neural activation and CBF are tightly coupled in both the resting and stimulated brain (Dirmagl et al., 1994). It is expected that adenosine to reduce rCBF by reducing the neural activity and metabolism. But our results showed increase in rCBF, confirming that adenosine A1 receptors have no effects on rCBF functionally or that dominant effects of adenosine A2 receptors on rCBF cover its effects. So the increase in adenosine responsiveness seems to be due to an increase in adenosine A2-receptors responsiveness, although this fact does not exclude the changes in adenosine A1 receptors, as many researches have indicated such a changes in this receptor (Kaplan et al., 1994; White et al., 1995). Any way, it is necessary to do radio ligand binding assay of adenosine receptors for any crucial statement.

The changes of adenosine A2-receptors in morphine dependency have been reported in different regions of the brain (Brundege and Williams, 2002; Kaplan et al., 1994; White et al., 1995). The increase in cortical adenosine receptors responsiveness in our work may be due to the decreased rCBF in morphine dependency that leads to a compensatory mechanism that returns rCBF to normal level. In agreement with this idea it has been reported that morphine decreased rCBF in the control and morphine-dependent rats, but the depressant effect of morphine in MDR group was less than in the control animals. On the other hand, while naloxone had no considerable effect on rCBF of the control animals, it increased rCBF of MDR (Zamani et al., 2000). It seems that morphine reduces absolute amount of CBF in MDR and naloxone reverses this effect. But it is necessary to evaluate this hypothesis by absolute CBF measurement and comparison in morphine-naive and morphine-dependent rats.

Systemic naloxone induced withdrawal signs in conscious MDR. In anesthetized animals, it increased rCBF, blood pressure and heart rate in MDR and had no effect on the control and sham-operated rats. The increase in rCBF of MDR is in agreement with our previous report. An increase in rCBF elicited by naloxone in MDR, seems to be due to different reasons. Some researchers suggest naloxone selective vasodilatory action on vessels, which inhibits opioid vasoconstrictive effect (Koskinen, 1991). It also could be attributed to naloxone vasodilative effect due to an increase in the brain activity and metabolism. Most neurons respond to either systemic or local opiate application with a decrease in firing rate and metabolism (Fuller and Stein, 1991). Therefore, in the withdrawal syndrome precipitated by naloxone, there is an increase in neuronal activity and metabolism due to the opioid absence that augments brain blood flow, as lidocaine inhibits naloxone increasing effect on rCBF (Zamani et al., 2000).

Other possibility seems to be related to the changes of adenosine level in the withdrawal rat brains. Chronic opiate exposure caused up-regulation of the cAMP pathway in many neurons of the brain. Upon removal of the opiate, the up-regulated cAMP pathway would become fully functional and contribute to the features of dependence and withdrawal (Nestler and Aghajanian, 1997). Increase in cAMP as a precursor of adenosine affects extracellular adenosine concentration as reported by Hong, when the cortical surface is suffused with cAMP, the release of adenosine is increased in the aCSF (Hong et al., 1999). So, in view of the increase of adenosine responsiveness in MDR group and the possibility of augmenting in brain cAMP and adenosine levels in withdrawal syndrome, it seems likely that naloxone increases rCBF through changes in these substances. Therefore, following the systemic naloxone (3 mg/kg) administration, adenosine (10$^{-4}$ M) and theophylline (5 × 10$^{-5}$ M) were applied locally in the sensory cortex. In MDR, adenosine and theophylline had no significant effect on naloxone that induced an increase in rCBF, while in the control group, adenosine increased rCBF and theophylline had no effect. It seems that naloxone increased rCBF independent of adenosine receptors.

Systemic naloxone increased significantly blood pressure and heart rate in MDR, too. It is likely that naloxone increased rCBF maximally and adenosine, therefore, it could not elicit any additive response. To reduce naloxone's effect on blood pressure, the following experiments were done: Naloxone (0.5 and 1.5 mg/kg, s.c.) alone and together with adenosine (10$^{-4}$ M) also increased rCBF in MDR. The results indicated that co-application of naloxone (0.5 and 1.5 mg/kg) and adenosine increased rCBF, 50% more than that of naloxone (0.5 and 1.5 mg/kg) alone, while in naloxone (3 mg/kg) plus adenosine, this response was reduced to less than 10%. Naloxone (3 mg/kg), might have stronger effect on neuronal activity and reactive hyperemia and adenosine as a neuromodulator, could modulate naloxone actions. In agreement with this idea, it has been reported that local lidocaine application in cortical surface, blunted naloxone induced increase in rCBF of MDR (Zamani et al., 2000).

As mentioned previously, naloxone has profound effect on blood pressure and heart rate. Regardless of the naloxone doses as a whole, its administration in MDR caused a transient hypotension and bradycardia (0–5 min after injection). The cause of this change is not clear. Naloxone injection in the control group had also no effect on this parameter. After this initial fall, the blood pressure and heart rate returned to baseline and then increased significantly (5–20 min after drug injection). A slight decrease in blood pressure is observed while heart rate continues to increase over the 20 min after naloxone injection, perhaps it is a heart failure for maintenance of blood pressure.

Adenosine level increased in the brain by moderate hypotension, and its increment is related to regulation of
CBF (Winn et al., 1985). It is possible that this phenomenon caused naloxone- induce increase in rCBF in the present work. But hypotension was not seen in all of the subjects and theophylline could not affect this response.

Naloxone-induced changes in rCBF and blood pressure may result from an increase in locus coeruleus (LC) activity in withdrawal syndrome. Some researches have shown that low frequency stimulation of the LC in spinalized cat, reduced rCBF in the cortex and basal ganglia, while no changes were seen in any brain areas with high frequency stimulation (Goadsby and Duckworth, 1989). Systemic or microinjected naloxone into the LC of MDR caused widespread stimulation of regional cerebral metabolic rate for glucose. So, LC is a major substrate of opioid withdrawal in the brain and plays an important role in the changing regional cerebral metabolic rate for glucose during the opioid withdrawal (Kimes et al., 1998).

Accordingly, adenosine agonists, with central and peripheral actions (modulation of neuronal activity and reduction of blood pressure), may be effective in attenuating some of the withdrawal syndrome signs.

In summary, our results indicated that the vasodilative effect of adenosine on rCBF was augmented in MDR and the greater vasodilatation evoked in MDR suggest an increased responsiveness of adenosine A2-receptor. Naloxone also highly increased rCBF of MDR that probably not interfere with adenosine receptors, although it seems that adenosine plays a modulatory role on rCBF regulation of morphine-dependent and morphine withdrawal rats.

4. **Experimental procedures**

4.1. **Animal preparation**

Adult male Sprague-Dawley rats (250–350 g) were used in all the experiments. The animals were housed in groups of 3–5 and maintained on pellets and water ad libitum. Animal (more than 100 rats) divided to 3 main groups and 10 subgroups. The first group (sham-operated) received tap water, the second group (control) received 3% sucrose in tap water and the third group (dependent group) received morphine sulfate and 3% sucrose in tap water. The rats were made morphine dependent by chronic administration of morphine sulfate at the doses of 0.1, 0.2 and 0.3 mg/ml each for 48 h and 0.4 mg/ml/day for up to 21 days, in their drinking water (Badavi and Evans, 1982). The withdrawal syndrome signs precipitated by naloxone (3 mg/kg, s.c.) were used and recorded for an hour, as indicators of the development of morphine dependency (Zamani et al., 2000).

Animal subgroups were as following: (1) Behavioral groups, (2) Adenosine groups, (3) Naloxone (3 mg/kg) groups, (4) Naloxone (3 mg/kg) + adenosine groups, (5) Naloxone (3 mg/kg) + theophylline groups, (6) Naloxone (0.5 mg/kg) groups, (7) Naloxone (0.5 mg/kg) + adenosine groups, (8) Naloxone (1.5 mg/kg) groups, (9) Naloxone (1.5 mg/kg) + adenosine groups. The animals were anesthetized with urethane (1.5 g/kg, i.p. initially, then 0.3 g/kg, if required, every 30 min) and placed on a temperature control unit (Narco Bio-system) to maintain a constant rectal temperature (37 ± 0.5 °C). After a tracheotomy, the rats were allowed to breathe spontaneously. The arterial oxygen saturation percent was monitored continuously with pulse oximeter (Radiometer-Copenhagen). Catheters were placed in the femoral artery for the measurement of systemic arterial blood pressure and heart rate (P=1000B pressure transducer, Narco Bio-system) and in the carotid artery for withdrawing and sampling of the arterial blood. The blood was collected periodically for blood gas and pH determination (Blood Gas Analyzer, AVL-993).

4.2. **Measurement of regional cerebral blood flow**

Laser Doppler Flowmeter (MBF3D, Moor instrument, Axminster, UK) was used for recording of rCBF. The animals were mounted in a stereotaxic frame and a 2-mm diameter hole was drilled in the skull above the parietal cortex, 2 mm caudal to bregma, and 2.7 mm lateral to midline. This point lies over the hind limb area of the sensory cortex (Paxinos and Watson, 1986). The dura matter was resected with caution and prewarmed (37 °C) artificial cerebrospinal fluid (aCSF) was suffused over the cortical surface. The composition (in mmol/l) of the aCSF was as follows: 131.9 NaCl, 2.95 KCl, 1.25 CaCl2, 0.665 MgCl2, 24.6 NaHCO3, 6.7 Urea and 3.7 D-glucose (pH = 7.4). The 1-mm diameter needle probe of Laser-Doppler flowmeter was positioned with a micromanipulator perpendicular to the surface, avoiding large vessels. Recordings were allowed to stabilize for at least 30 min to obtain baseline blood flow levels.

In order to determine the responses of the resting pial micro vessels to adenosine (10−5, 10−4, 10−3 M), theophylline (5 × 10−5 M) and naloxone (0.5, 1.5 and 3 mg/kg, s.c.), the cortical surface was suffused with aCSF containing adenosine or theophylline alone or in combination with each other and also systemic naloxone alone or together with adenosine or theophylline. Different doses of adenosine were applied in additive method and every dose for 5 min, after 15-min drug wash out (and aCSF wash in) from cortex surface. Theophylline was applied 30 min before and during the suffusion of adenosine. Every animal received only one dose of naloxone and rCBF recorded for 30 min. At the end of the experiment, the animals were sacrificed with saturated solution of KCl injected intravenously and the biological zero values were measured. The biological zero values were subtracted from the flow values before the calculation of percentage changes in blood flow.

4.3. **Drugs**

Adenosine, theophylline and naloxone hydrochloride were purchased from Sigma Chemical Co, and morphine sulfate from Temad-Iran. Sucrose and urethane were obtained from Merck.

4.4. **Statistical analysis**

The data were subjected to Student’s paired t test or one- and two-way analysis of variance (ANOVA) followed by protected Tukey’s test for multiple comparisons, as needed. The values were expressed as means ± SEM and a P < 0.05 was accepted as statistically significant.
REFERENCES


