Pharmacological Reports
2012, 64, 351–359
ISSN 1734-1140

Antiarrhythmic effect of prolonged morphine exposure is accompanied by altered myocardial adenylyl cyclase signaling in rats

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Abstract:
Background: Morphine is often administered to patients for pain management, but it is also recommended to ameliorate some types of cardiovascular diseases. Nevertheless, there is a lack of information regarding the effect of prolonged morphine treatment on myocardial adenylyl cyclase (AC) signaling, which plays an important role in regulating heart function.
Method: The present work has investigated the consequences of 10-day administration of high morphine doses (10 mg/kg per day) to adult Wistar rats for functioning of the G-protein-mediated AC signaling system.
Results: Morphine treatment appreciably affected neither the number of myocardial β-adrenoceptors nor the content of selected subunits of trimeric G-proteins ($G_s\alpha$, $G_{i/o}\alpha$, $G_z\alpha$, $G_{q/11}\alpha$ and Gβ) but the amount of the dominant myocardial AC isoform V/VI almost doubled. These alterations were accompanied by a marked AC supersensitization: the enzyme activity stimulated by manganese, fluoride, forskolin or isoproterenol was considerably increased (by about 50–100%). In contrast, the ability of opioid agonists to inhibit forskolin-stimulated AC activity was slightly but significantly decreased in both groups. Besides that, morphine markedly decreased the incidence of ischemic ventricular arrhythmias induced by coronary artery occlusion, but did not significantly influence infarct size and arrhythmias occurring during reperfusion.
Conclusion: Overall, these results indicate that prolonged treatment of rats with high doses of morphine substantially alters the function of myocardial G-protein-regulated AC signaling. These alterations are accompanied by a reduced susceptibility to ischemia-induced ventricular arrhythmias.

Key words:
rat myocardium, morphine, adenylyl cyclase, G-proteins, arrhythmias

Introduction

Morphine is well known for its ability to exert significant cardiovascular effects. However, a number of controversial data have been published about its favorable or adverse effects on the heart. This drug can apparently induce increased parasympathetic activity and suppress cardiovascular functions [32, 45]. Prolonged morphine administration and subsequent withdrawal can affect catecholamine turnover and thus

myocardial adrenergic signaling and function [15, 44]. Morphine can block reuptake of norepinephrine resulting in cardiotoxic effects [4]. It might be important to differentiate between the consequences of short- and long-term morphine application. Markiewicz and colleagues showed that one-shot administration of morphine to rats prior to a permanent coronary artery occlusion produced a statistically significant increase in infarct size [25]. In contrast, morphine given by three 5-min infusions was able to mimick the beneficial effect of preconditioning in open-chest rats subjected to myocardial ischemia and reperfusion [50]. Since then, several studies have been published that confirm a role of opioid receptors in morphine-induced acute and delayed preconditioning [9, 11, 17, 19, 27, 39, 48, 55]. In addition, cardioprotective effect of chronic morphine exposure has been also observed in a mouse model of myocardial infarction [40, 41].

Morphine can induce changes at the receptor level as well as in some other proteins engaged in signaling pathways initiated by opioid receptors and regulated by their cognate trimeric G-proteins [10, 23]. Whereas acute opioid action is characterized by diminution of intracellular cAMP levels (through the inhibitory effect of $G_{i/o}$ proteins on adenylyl cyclase (AC)), sustained opioid treatment may increase AC activity [53]. Heterologous sensitization of the AC signaling cascade, termed AC supersensitivity, was first described in studies aiming to explain the development of tolerance and withdrawal syndrome, which can occur after chronic opioid exposure [21, 34]. The majority of studies addressing the effects of morphine on AC signaling were done on samples of brain tissue obtained from laboratory animals [5, 20, 47], but changes in AC activity (superactivation or superinhibition) caused by sustained morphine treatment were also observed in cell cultures [49, 51]. Surprisingly, only little attention has so far been paid to the possible interference of morphine with the myocardial AC signaling system. To the best of our knowledge, there is only one report concerning this issue. Napier and colleagues observed that chronic administration of morphine increased expression of $G_i\alpha$ and $G_s\alpha$ proteins in the dog heart, but they did not find any significant change in the enzyme activity of AC [32].

In the present work, we have studied the presumed effect on myocardial AC signaling of sustained administration of morphine in high doses to rats. Parallel experiments have been conducted to find out whether this morphine exposure may have some cardioprotective potential.

Materials and Methods

Materials

[^{3}H]CGP-12177 was purchased from Amersham Biosciences (Buckinghamshire, UK) and scintillation cocktail CytoScint from ICN Biomedicals (Irvine, CA, USA). Nitrocellulose membrane was purchased from Schleicher-Schuell (Erdmannhausen, Germany) and Whatman GF/C filters from Whatman Ltd. (Oxford, UK). Acrylamide and bis-acrylamide were from SERVA (Heidelberg, Germany). All other chemicals were from Sigma (St. Louis, MO, USA) and they were of the highest purity available.

Experimental model

Animal experiments were conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (National Academy Press, 1996) and they were approved by local institutional animal care and use committee. Male Wistar rats kept under standard laboratory conditions with free access to water and a standard pellet diet were given intramuscular morphine (10 mg/kg per day, ~300 μl) for 10 days. Control animals were injected with sterile normal saline (0.9% NaCl). The animals designated for biochemical analysis were killed by decapitation one day after the last morphine dose. The hearts were rapidly excised, divided into left and right ventricles, snap frozen in liquid nitrogen and stored at –80°C until use.

Myocardial membrane preparation

Frozen samples of left ventricular (LV) myocardium were placed into 10 volumes of ice-cold homogenization buffer (20 mM Tris, 3 mM $MgCl_2$, 1 mM EDTA and 0.25 M sucrose; pH 7.4) containing the protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany), cut in small pieces and homogenized in an Ultra-Turrax blender (15 s). The resulting suspension was further homogenized for 1 min in a glass homogenizer with a motor-driven Teflon pestle, and then centrifuged at 600 × g for 10 min at 4°C in order to remove large tissue debris and nuclear fragments. A portion of the resulting postnuclear supernatant (PNS) was centrifuged at 50,000 × g for 30 min in order to isolate crude membranes. The pellet containing