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Research Report

Abnormal intracellular calcium homeostasis associated with vulnerability in the nerve cells from heroin-dependent rat

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ARTICLE INFO

Article history:
Accepted 12 May 2014
Available online 20 May 2014

Keywords:
Heroin
Opiate
Intracellular calcium concentration
Cytotoxicity
Overdose

ABSTRACT

The cellular mechanisms by which opiate addiction develops with repetitive use remain largely unresolved. Intercellular calcium homeostasis is one of the most critical elements to determine neuroadaptive changes and neuronal fate. Heroin, one of the most addictive opiates, may induce neurotoxicity potentially inducing brain impairment, especially for those chronic users who get an overdose. Here we examined changes in intracellular calcium concentration ($[Ca^{2+}]_i$) after repeated exposure to heroin using cultured cerebral cortical neurons. Dynamic changes in $[Ca^{2+}]_i$ indicated by fluo-3-AM were monitored using confocal laser scan microscopy, followed by cytotoxicity assessments. It showed that the cells dissociated from heroin-dependent rats had a smaller depolarization-induced $[Ca^{2+}]_i$ responses, and a higher elevation in $[Ca^{2+}]_i$ when challenged with a high concentration of heroin (500 μM). The restoration ability to remove calcium after washout of these stimulants was impaired. Calcium channel blocker verapamil inhibited the heroin-induced $[Ca^{2+}]_i$ elevations as well as the heroin-induced cell damage. The relative $[Ca^{2+}]_i$ of the nerve cells closely correlated with the number of damaged cells induced by heroin. These results demonstrate that nerve cells from heroin-dependent rats manifest abnormal $[Ca^{2+}]_i$ homeostasis, as well as vulnerability to heroin overdose, suggesting involvement of $[Ca^{2+}]_i$ regulation mechanisms in heroin addiction and neurotoxicity.

1. Introduction

Opiates such as opium, heroin, and morphine are among the most powerful analgesics in clinical practice, but also among the most commonly abused drugs, posing a huge threat to global society, since they have a strong addictive potential including tolerance, withdrawal and a high rate of relapse (Hartnoll, 1994; Hoffmann, 1990; Ramsey and Van Ree, 1992). Opiate addiction has now been considered as a chronic relapsing disease, involving a central nervous system disorder caused by long-term opiate exposure (Leshner, 1997). Cumulative evidence has revealed that opiate addiction

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http://dx.doi.org/10.1016/j.brainres.2014.05.016
0006-8993/

is associated with changes in brain function (Eisch et al., 2000; Kelley et al., 2000; Nestler and Aghajanian, 1997), and neurodegenerative processes (Bredesen et al., 2004; Leshner, 2000). Chronic exposure with these opiates, even following detoxification and protracted abstinence, is often accompanied by several neurological impairments including psychomotor and cognitive performances, such as deficits in cognition, motivation, attention, memory and movement (Majewska, 1996; Pau et al., 2002).

Heroin (3, 6-diacetylmorphine) is probably the most notorious opiate, as it may rapidly develop chemical dependency in even first-time users, and the withdrawal from heroin is more uncomfortable, difficult and painful than other opiates (Hubner and Kornetsky, 1992; Oppenheimer et al., 1994). Heroin abusers present poorer performance in learning, cognition and memory (Papageorgiou et al., 2004; Verdejo et al., 2005). It is a depressant which slows down the activity of the central nervous system. Repeated use of heroin leads to the characteristic psychoactive effect and physical dependence (Gruber et al., 2007). A possible explanation for these symptoms may be associated with heroin-induced neurotoxicity (Bredesen et al., 2004). The organic damage of various cerebral structures and the non-specific ventricular and cortical volume loss was observed in heroin abusers during neuropathological investigations (Ersche et al., 2006; Li et al., 2005). Postmortem brain studies revealed changes in the signal molecules of death (apoptotic signaling) pathways in those addicted to opiates (Garcia-Fuster et al., 2008; Ramos-Miguel et al., 2009). In some cases, toxic leukoencephalopathies manifested by a progressive neurological decline might occur after heroin intake, especially for the long-term heroin abusers (Tormoehlen, 2011).

Opiate addiction, characterized by tolerance and physical dependence, has afflicted mankind for centuries, yet the mechanisms remained elusive. Neurological impairments observed in those addicted to heroin may reflect heroin-induced neuronal dysfunction and neurotoxicity. Free intracellular Ca^{2+} ($[Ca^{2+}]_i$) is a ubiquitous intracellular second messenger or signal transducer involved in the control of a large number of cellular and physiological processes including neuronal excitability, synaptic plasticity, neurotoxicity, and even drug addiction. Recent investigations suggest that disturbance of Ca^{2+} homeostasis in neurons is relevant to drug dependence (Vitcheva and Mitcheva, 2004). Our previous findings revealed that a high concentration of heroin might induce calcium overload in the cultured cardiomyocytes (Liu et al., 2007) and apoptosis in cerebellar granule neurons (Lai et al., 2011). It is intuitive to assume that the mechanism of heroin addiction may be associated with disturbance in calcium homeostasis and consequently activation of multiple intracellular cell-death signaling pathways. This hypothesis is attractive because excessive Ca^{2+} loading and alterations in the expression of calcium channels have been shown in many cell lines with exposure to opiates (Cunha-Oliveira et al., 2010; Jin et al., 1992; Spadoni et al., 2004), and consistently suggested to trigger neurodegeneration (Rodriguez et al., 2009). The calcium buffering ability may be related to heroin addiction, and heroin-induced neuronal vulnerability. If such relationship is better understood, we may find a common action mechanism for opiate addiction and opiates-induced brain damage, and identify novel strategies for prevention or reversal of opiate addiction. In this study, we applied the cortical cells that acutely dissociate from heroin-dependent rat brain to examine their Ca^{2+} homeostasis. By comparing the Ca^{2+} responses to different conditions, we attempted to approve the hypothesis that disturbance of intracellular calcium homeostasis might be involved in the development of heroin addiction and neurotoxicity.

2. Results

2.1. *Depolarization-induced $[Ca^{2+}]_i$ responses are less active in the nerve cells from heroin-dependent rat brain*

The two groups of acutely dissociated cells were placed onto the confocal laser-scanning microscope and the relative changes in $[Ca^{2+}]_i$ were dynamically measured for 5 min. We tested the effects of a short (1 min) depolarization with 30 mM KCl on Ca^{2+} responses. Perfusion with 30 mM KCl triggered Ca^{2+} elevations in the most cells for both groups, which were manifested as spikes in the fluorescence signal (Fig. 1a). Under depolarizing conditions, KCl perfusion in the group of heroin-dependent rats produced a slow and moderate rise in Ca^{2+}-dependent fluorescence. The peak elevations were achieved after around 50 s of KCl perfusion. We used two indexes, peak amplitude and time delay (latency to peak), to evaluate calcium rise pattern with response to depolarization. According to statistical analysis, the heroin-dependent rats manifested a lesser magnitude by 26% in average peak amplitude ($P<0.05$) and a longer delay to the peak ($P<0.05$) (Fig. 1c and d). Subsequent washout of KCl for 4 min could reverse the calcium elevation to a certain extent in both groups. In the vehicle controls, $[Ca^{2+}]_i$ of most cells declined to the resting level within 1 min after removal of depolarizing condition, whereas the cells from the heroin-dependent rats manifested a more consistent elevation in $[Ca^{2+}]_i$ after washout. We compared the relative calcium fluorescence value at the end of measurement (4 min after washout) between the two groups. It showed that the $[Ca^{2+}]_i$ levels in the treatment group were much higher than those in the vehicle controls ($P<0.01$), suggesting an impaired $[Ca^{2+}]_i$ restoration ability in the nerve cells of heroin-dependent rats (Fig. 1e).

2.2. *High concentration of heroin induces a sustained $[Ca^{2+}]_i$ elevation in the heroin-dependent nerve cells*

One of the biggest dangers for heroin abusers is acute overdose. The risk of brain injury caused by heroin overdose is especially increased in those addicted to heroin. Considering the implications of our previous reports that high concentration of heroin could induce an overload of intracellular calcium and cellular apoptosis, we here aimed to explore whether the calcium responses to heroin overdose were different between those addicted and normal individuals. The two groups of nerve cells, this time, were challenged with 500 μM heroin for 1 min. As expected, 500 μM heroin led to a slight but consistent elevation of $[Ca^{2+}]_i$ in vehicle control (Fig. 2). In contrast, the cells from heroin-dependent rats