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BRAIN RESEARCH

Research Report

Differential effects of mu-opioid receptor agonists in a hippocampal hypoxia/hypoglycemia model

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ABSTRACT

Background: In rat hippocampal slices, a short hypoxia/hypoglycemia causes immediate loss of evoked potentials (population spike amplitude) in the CA1 region and the extent of electrophysiological restoration during reperfusion can serve as a parameter for cell function. Previous experiments using this model revealed that exposure to morphine aggravates the neurotoxic effects of a subsequent hypoxia/hypoglycemia in a concentration-dependent manner. Therefore, the aim of the present study was to evaluate the effects of additional mu-opioid receptor (MOPr) agonists on the electrophysiological restoration after hypoxia/hypoglycemia. *Methods:* Rat hippocampal slices were exposed to either morphine (10 μM), pethidine (10 μM), fentanyl (100 nM/1 μM) or to the synthetic peptide [D-Ala^2, N-Me-Phe^4, Glycinol^5]-enkephalin (DAMGO, 10 μM) for 60 min; thereafter, slices underwent a brief hypoxic/hypoglycemic episode followed by reperfusion (drug-free) for 2.5 h. Electrophysiological recording consisted of determination of population spike amplitude in CA1 in response to constant stimulation of Schäffer's collaterals. *Results:* Exposure to morphine prior to hypoxia/hypoglycemia resulted in a significantly impaired electrophysiological recovery during reperfusion when compared to controls. Following exposure to pethidine, the electrophysiological recovery was slightly reduced, whereas fentanyl or DAMGO did not affect restoration of population spike amplitude during reperfusion. *Conclusions:* The results of the present study demonstrate that different MOPr agonists differentially influence the electrophysiological recovery of hippocampal slices following a brief hypoxia/hypoglycemia. It is speculated that known receptor-internalizing opioids such as fentanyl or DAMGO may have less neurotoxic effect in hypoxia/hypoglycemia than the non-internalizing drug morphine.

1. Introduction

Cerebral ischemia may result from a variety of causes that lead to deprivation of both, oxygen and glucose. Pathogenetic mechanisms include excitotoxicity, overproduction of free radicals, inflammation and apoptosis. On the other hand, autoprotective mechanisms are triggered, such as production of heat shock proteins, antiinflammatory cytokines and endogenous antioxidants (Leker and Shohami, 2002). Several drugs including anesthetics, potassium channel opener or adenosine receptor antagonists have been reported to modulate the neurotoxic effects of cerebral ischemia (Kehl et al., 2004; Nagy et al., 2004; Pugliese et al., 2003).

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To examine the influence of drugs on ischemia-induced neurotoxicity in vitro, an electrophysiological rat hippocampal model has been established (Rüthrich and Krug, 1999, 2001): In rat hippocampal slices, a short hypoxia/hypoglycemia causes immediate loss of evoked potentials (population spike amplitude) in the CA1 region and the extent of electrophysiological restoration during reperfusion can serve as a parameter for cell function.

Recent in vitro experiments using this model demonstrated that the opioid morphine may either increase the neurotoxic effects of hypoxia/hypoglycemia *per se* or may induce neuroprotection via preconditioning, depending on the time interval between drug exposure and subsequent hypoxia/hypoglycemia (Ammon-Treiber et al., 2005): If morphine exposure was followed by the hypoxic/hypoglycemic episode without a drug-free interval, a decreased electrophysiological recovery was observed; in contrast, if a drug-free interval (180 min) was placed between morphine exposure and hypoxia/hypoglycemia, the electrophysiological recovery was improved (preconditioning). Preconditioning effects of morphine have been reported also by other groups: Acute preconditioning (30-min drug-free interval) has been reported by Lim et al. (2004) examining morphological changes of Purkinje cells, delayed preconditioning (24-h drug-free interval) has been reported by Zhao et al. (2006) using a model of organotypic hippocampal slice cultures.

In the clinical setting, opioids are often used during surgery and anesthesia, conditions with a high risk of brain ischemia. The hippocampus is well suited for the study of opioid effects on ischemic injury in the brain because CA1 pyramidal cells are densely populated with opioid receptors and are extremely sensitive to ischemic injury. As described above, we have demonstrated that morphine aggravates the neurotoxic effects of hypoxia/hypoglycemia, if morphine exposure (1 μM, 10 μM) was followed by the hypoxic/hypoglycemic

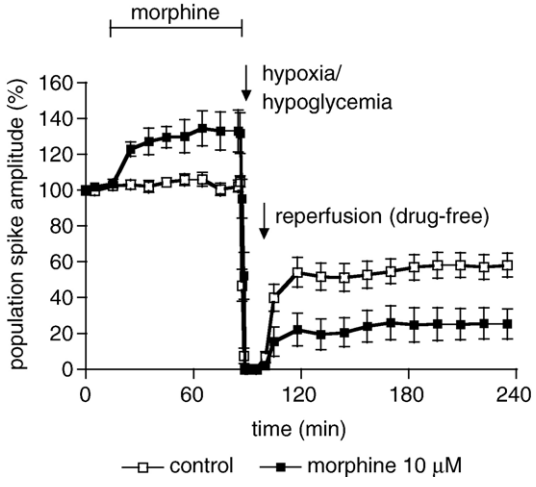

Fig. 1 – Effects of morphine. Time course of evoked field potentials (population spike amplitude in % of baseline, mean ± SEM) in hippocampal slices of controls ($n=14$) or during 60 min of perfusion with morphine 10 μM ($n=12$), followed by hypoxia/hypoglycemia (7 min) and reperfusion with drug-free medium until 240 min. Statistically significant differences obtained by two-way repeated measures ANOVA. Before hypoxia/hypoglycemia: interaction: $p<0.0001$; time points: $p<0.0001$; treatment: $p=0.0025$. During reperfusion: interaction: $p=0.0012$; time points: $p<0.0001$; treatment: $p=0.0083$.

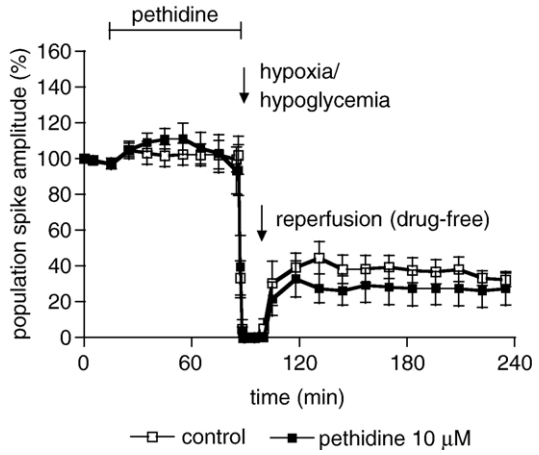

Fig. 2 – Effects of pethidine. Time course of evoked field potentials (population spike amplitude in % of baseline, mean ± SEM) in hippocampal slices of controls ($n=7$) or during 60 min of perfusion with pethidine 10 μM ($n=10$), followed by hypoxia/hypoglycemia (7 min) and reperfusion with drug-free medium until 240 min. Statistically significant differences obtained by two-way repeated measures ANOVA. Before hypoxia/hypoglycemia: interaction: $p>0.05$; time points: $p>0.05$; treatment: $p>0.05$. During reperfusion: interaction: $p>0.05$; time points: $p<0.0001$; treatment: $p>0.05$.

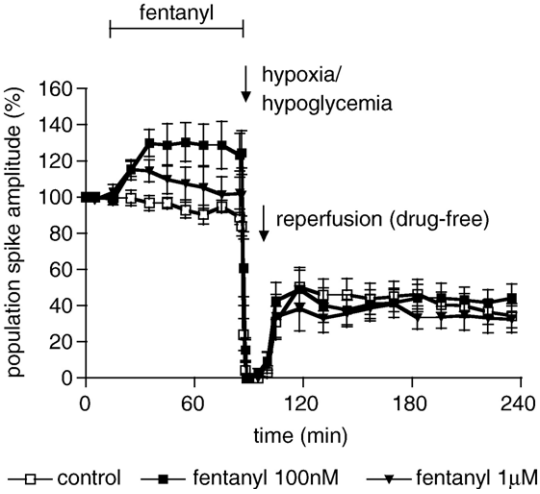

Fig. 3 – Effects of fentanyl. Time course of evoked field potentials (population spike amplitude in % of baseline, mean ± SEM) in hippocampal slices of controls ($n=10$) or during 60 min of perfusion with fentanyl 100 nM ($n=9$) or 1 μM ($n=9$), followed by hypoxia/hypoglycemia (7 min) and reperfusion with drug-free medium until 240 min. Statistically significant differences obtained by two-way repeated measures ANOVA. Before hypoxia/hypoglycemia: interaction: $p<0.0001$; time points: $p=0.0003$; treatment: $p=0.0198$. During reperfusion: interaction: $p>0.05$; time points: $p<0.0001$; treatment: $p>0.05$.