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BRAIN RESEARCH

Research Report

Dexamethasone enhances calcium-activated potassium channel expression in blood-brain tumor barrier in a rat brain tumor model

Yan-ting Gu[1], *Yi-xue Xue*[*,1], *Ping Wang*, *Hua Zhang*, *Li-juan Qin*, *Li-bo Liu*

Department of Neurobiology, College Basic of medicine, China medical University, Shenyang, 110001, Liaoning Province, PR China

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ABSTRACT

This study was performed to determine whether dexamethasone (DEX) had an effect on calcium-activated potassium channels (K_{Ca} channels) in blood-brain tumor barrier (BTB). Using a rat brain glioma model, we found that the expression of K_{Ca} channels protein was significantly increased in brain tumor tissue. And bradykinin-induced increase of K_{Ca} channels protein was further enhanced after DEX pretreatment for 3 days. In addition, DEX pretreatment enhanced bradykinin-mediated up-regulation of the density of I_{KCa} in the rat brain C6 cells in vitro BTB. Bradykinin markedly increased BTB permeability independent of DEX pretreatment. All of these results strongly suggest that DEX could regulate the target in the transcellular pathway of BTB-K_{Ca} channels.

1. Introduction

Increasing evidence suggests that there is an obvious decrease in both surgical morbidity and mortality following dexamethasone (DEX) treatment (Kaup et al., 2001), which is routinely used in the management of patients with brain tumors and peritumoral edema (Andersen, 1998). Brain scans in human glioma patients have demonstrated that DEX decreases contrast enhancement and uptake of radioisotopes into brain tumors (Yeung et al., 1993), but pretreatment with DEX did not prevent the opening of the BBB induced by bradykinin either at the low or high dose (Dean et al., 1999; Schurer et al., 1989). The detailed mechanisms have not yet been clarified. Thus, the pathophysiological mechanisms of DEX-mediated action on the BTB permeability should be further investigated.

There are two pathways of drug delivery into the tumor cells through the BTB: paracellular pathway and transcellular pathway. The transcellular transportation of drug is determined by their physicochemical properties; however, the paracellular pathway is usually accepted as the main way for absorption of hydrophilic drugs (proteins, peptides, etc.) (Salama et al., 2006). Our previous study has shown that bradykinin might selectively increase malignant glioma permeability through transcellular pathway (Liang et al., 2006; Ningaraj et al., 2003) and paracellular pathway (data not shown). Investigations have showed that DEX could be involved in modulating paracellular pathway in brain tumor capillary, which was correlation with regulating tight junction protein (Guan et al., 2004; Romero et al., 2003) and increasing transendothelial electrical resistance (TEER) (Cucullo et al., 2004). However, bradykinin markedly increased the BTB

* *Corresponding author.*
E-mail address: xueyixue999@yahoo.com.cn (Y. Xue).
[1] The two authors contribute equally to this work.

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permeability independent of DEX pretreatment. Therefore, we hypothesize that DEX could have a positive effect on the transcellular delivery in BTB.

Ningaraj et al. demonstrated that large conductance K_{Ca} channels serve as convergence points in the transcellular pathway regulation of BTB permeability, which were effective targets for inducing accelerated formation of transport vesicles in both brain tumor capillary endothelium and tumor cells (Hu et al., 2007; Ningaraj et al., 2003). Increasing evidence suggest that potassium channels on vessel endothelial cells are important targets for glucocorticoids regulation (Huang et al., 2006; Lin et al., 2006). Recently, we found that DEX could upregulate the expression of ATP-sensitive potassium channel (Gu et al., 2007). In pituitary CH3 and AtT-20 cells, DEX increased K_{Ca} channels activity and the increase in I_{KCa} density was suppressed by paxilline (Lovell et al., 2004). In addition, Wang and colleagues reported that DEX pretreatment increases Nitric Oxide (NO) and 3′, 5′-cyclic guanosine monophosphate (cGMP) content in hypoxia-induced pulmonary hypertensive rats (Murata et al., 2004; Wang et al., 1999). And NO donor and soluble guanylate cyclase (sGC) activator could increase the up-regulation of K_{Ca} channels expression (Prieto et al., 2006; Lee and Kang, 2001). Hence, it seems more reasonable to hypothesize that DEX could contribute to the regulation of K_{Ca} channel activity and/or to the synthesis of a mediator that could regulate the channel in brain tumor capillary endothelium. Based on the above-mentioned results, we deduce the hypothesis that DEX could mediate K_{Ca} channels activity and have effects on the ability of bradykinin to enhance the transcellular delivery through regulating K_{Ca} channels. This possibility is the main concern of the current paper.

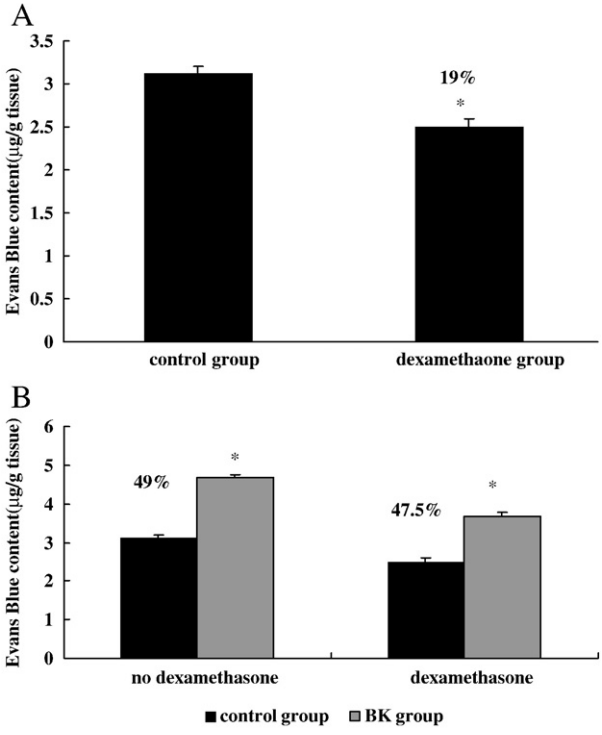

Fig. 1 – Effects of bradykinin on BTB permeability increase with or without dexamethasone. Three days of dexamethasone pretreatment (1.5 mg/kg/day) decreased levels of Evans blue in brain tumor by 19% (A). Intracarotid infusion of bradykinin (10 μg/kg/min) for 10 min enhanced Evans blue levels in the tumor by nearly the same percentage regardless of whether the animals were pretreated with dexamethasone or not (B). *P<0.05 vs. control group.

To test the hypothesis, we used a rat C6 glioma model and investigated the effects of DEX on potential interaction with the ability of BK to enhance penetration of Evans blue. Moreover, we studied whether DEX had an effect on the expression of α-subunit of K_{Ca} channels in rat brain tumor and I_{KCa} modulated by bradykinin with or without DEX pretreatment in rat brainC6 cells were also studied using the patch-clamp technique in a whole-cell configuration.

2. Results

2.1. *Effect on BBB permeability for EB extravasation*

The brain tissue of hemisphere with tumor was stained in blue, while non-tumoral hemisphere with no visible staining. EB content and scope of blue staining in the hemisphere with tumor were significantly decreased in DEX group compared with control group. There was a markedly increase in BK group compared with control group. The modest decrease in the ability of EB to enter the tumor caused by DEX did not impact the ability of bradykinin to increase levels of EB in tumor (Fig. 1). Statistical analysis revealed that BK increased EB levels in both the DEX-treated (47.5%) and non-DEX-treated animals (49%) and that the difference in the effect of bradykinin in the two conditions was comparable ($P>0.10$).

2.2. *DEX-induced over-expression of K_{Ca} channel proteins in rat brain glioma model*

In rat brain tumor tissues infused with sterile saline (control group), α-subunit of K_{Ca} channels was slightly expressed. Compared with control group, the expression of α-subunit of K_{Ca} channels protein in DEX group was markedly increased; the integrated density value (IDV) of α-subunit of K_{Ca} channels was 1.22-fold higher than the control group. In addition, DEX could greatly enhance the up-regulation of α-subunit of K_{Ca} channels induced by bradykinin. Bradykinin greatly increases α-subunit of K_{Ca} channels expression after 10 min infusion (Figs. 2A and B). The IDV of α-subunit of K_{Ca} channels with β-actin in control group, DEX group, BK group, BK+DEX group were 0.215±0.037, 0.458±0.058, 0.786±0.065, 1.259±0.055, respectively ($^{*}P<0.05$, $^{**}P<0.01$, $^{\#*}P<0.01$). Our study demonstrated that DEX has a direct effect on expression of K_{Ca} channels protein in rat brain tumor.

2.3. *BK infusion with DEX pretreatment further increases the conductance of K_{Ca} channels*

When the holding potential was at −40 mV, the command potential was from −60 to +60 mV. An outward potassium current was recorded, which could be inhibited by the K_{Ca} channel antagonist iberiotoxin (IBTX, 10 μM). This implies that the outward potassium current was I_{Kca}. During the 30 min of observation, the current was kept at a stable level. Fig. 3 shows