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BRAIN RESEARCH

Research Report

Dexamethasone enhances adenosine 5′-triphosphate-sensitive potassium channel expression in the blood–brain tumor barrier in a rat brain tumor model

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ABSTRACT

This study was performed to determine whether dexamethasone (DEX) had an effect on ATP-sensitive potassium channels (K_{ATP} channels) in blood–brain tumor barrier (BTB). Using a rat brain glioma model, we found that DEX could significantly increase the expression of K_{ATP} channels protein at tumor sites. And bradykinin-induced increase of K_{ATP} channels protein was further enhanced after DEX pretreatment for 3 consecutive days via Western blots and immunohistochemistry methods. In addition, DEX pretreatment enhanced bradykinin-mediated increase of the density of I_{KATP} in the cultured rat C6 glioma cells using the patch-clamp technique in a whole-cell configuration. DEX significantly decreased the BTB permeability, but it did not reduce bradykinin-mediated BTB permeability increase, which were significantly attenuated by the K_{ATP} channel antagonist glibenclamide. This led to the conclusion that DEX-mediated change in BTB permeability is, at least partly, due to accelerated formation of K_{ATP} channel, an important target in the biochemical regulation of this process.

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 was routinely used in the management of patients with brain tumors and peritumoral edema (Andersen, 1998). DEX can significantly decrease the permeability of the blood–brain tumor barrier (BTB), however, it cannot decrease bradykinin-mediated the delivery increase of agents into brain tumour (Dean et al., 1999; Matsukado et al., 1997). The detailed mechanisms of DEX-mediated change in BTB permeability are poorly understood.

There are two pathways of antineoplastic drug delivery into brain tumors through the BTB: paracellular pathway and transcellular pathway. Most drugs are transported transcellularly on their physicochemical properties. Moreover, the paracellular pathway is usually accepted as the main way for absorption of hydrophilic drugs (proteins, peptides, etc.) (Salama et al., 2006). Investigations have shown that DEX could be involved in modulating paracellular transport in brain tumor capillary, which was in correlation with regulating tight junction protein (Romero et al., 2003) and increasing transendothelial electrical resistance (TEER) (Cucullo et al., 2004). However, it is unclear whether DEX has also effects on the targets of transcellular pathway.

K_{ATP} channels serve as convergence points in the transcellular regulation of BTB permeability, which are effective targets for inducing accelerated formation of transport vesicles in both brain tumor capillary endothelium and tumor cells (Ningaraj et al., 2003). And K_{ATP} channels are heteromultimeric structures formed by a member of the sulfonylurea receptor (SUR) family and a member of the inwardly rectifying potassium channel

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family (Kir6.x) (Nichols and Lopatin, 1997). Studies suggest that potassium channels on vessel endothelial cells are important targets for glucocorticoids regulation (Huang et al., 2006; Lin et al., 2006). DEX regulated K_{ATP} channels activity in primary vascular smooth muscle cell culture (d'Emmanuele di Villa Bianca et al., 2003). 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). We found that NO donor and soluble guanylate cyclase (sGC) activator could increase the upregulation of K_{ATP} channels expression induced by BK in a rat brain glioma (C6) model (data not shown). Recently our studies demonstrated that bradykinin increased BTB permeability by regulating K_{ATP} channels activity (Zhang et al., 2007). Based on the abovementioned results, we hypothesize that DEX could have a positive effect on the BK-induced transcellular transportation across BTB by regulating K_{ATP} channels.

To test the hypothesis, we utilized 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 and [^{14}C]-aminoisobutyric acid ([^{14}C]-AIB) uptake. In addition, we studied whether DEX had an effect on the expression of Kir6.2 subunit in brain tumors at protein level and I_{KATP} modulated by DEX in cultured rat C6 glioma cells were also studied using the patch-clamp technique in a whole-cell configuration.

2. Results

2.1. *Effect on BBB permeability for Evans blue extravasation*

The brain tissue of hemisphere with tumor was stained in blue, while non-tumoral hemisphere with no visible staining.

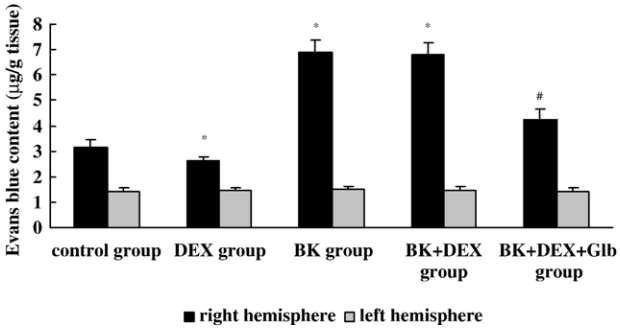

Fig. 1 – Effects of DEX on BTB permeability increase induced by BK. Control group: tumor-bearing rat brain infused with sterile saline; DEX group: DEX was injected (8 mg/kg/day, i.p, for 3 consecutive days) on the 14th day after implanting C6 cells; BK group: BK was pumped into glioma-rat brain for 10 min via the proximal end of external carotid artery; BK+DEX group: DEX was injected (8 mg/kg/day, i.p, for 3 consecutive days) and then BK was pumped into glioma-rat brain for 10 min via the proximal end of external carotid artery. BK+DEX+Glb group: DEX was injected (8 mg/kg/day, i.p., for 3 consecutive days) and then bradykinin (10 μg/kg/min, i.c.) was co-infused with the K_{ATP} channel antagonist glibenclamide (5 μg/kg/min, i.c.) for 10 min. Data present means ± SD (n=8, each). $^{*}P<0.01$ vs. control group; $^{\#}P<0.01$ vs. BK+DEX group.

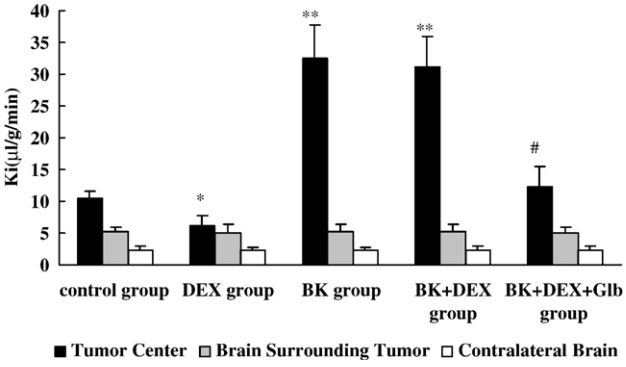

Fig. 2 – Effect of bradykinin and DEX on [^{14}C]-AIB uptake. The mean K_i for [^{14}C]-AIB significantly increased after intracarotid infusion of bradykinin (n=8) compared with control group (n=8) and could not be attenuated by pretreatment of DEX (n=8). $^{*}P<0.05$, $^{**}P<0.01$ vs. control group; $^{\#}P<0.05$ vs. BK+DEX group.

Coronal slides of the brain were shown stained in tumor sites of caudate nucleus. Evans blue (EB) content and scope of blue staining in the hemisphere with tumor were significantly decreased in DEX group compared with control group (2.595 ± 0.416 and 3.126 ± 0.622 μg/g, respectively, $^{*}P<0.05$). There was a marked increase in BK group compared with control group (6.895 ± 0.416 and 3.126 ± 0.622 μg/g, respectively, $^{*}P<0.05$). And between DEX+BK group and BK group, there was no significant difference (6.776 ± 0.114 and 6.895 ± 0.416 μg/g, respectively, $P>0.05$). EB content in BK+DEX group was significantly attenuated by glybenclamide at a dose of 5 μg/kg/min for 10 min (6.776 ± 0.114 and 4.25 ± 0.524 μg/g, respectively, $^{\#}P<0.05$, Fig. 1). Our results demonstrated that DEX could not stop the BK-induced BTB permeability increase, which could be in correlation with K_{ATP} channel.

2.2. *Effect on BTB permeability for [^{14}C]-AIB uptake*

BTB permeability, K_i (μl/g/min), was measured by quantitative autoradiographic (QAR) of cryosections obtained from C6 tumor bearing rat brains after the injection of a [^{14}C]-labeled tracer, 10 min after i.c. BK infusion. To determine whether DEX attenuates the permeability increases induced by BK, K_i was determined for radiotracer [^{14}C]-AIB in the tumor core, tumor-adjacent brain tissue, and contralateral brain tissue. After pretreatment with DEX, K_i was significantly decreased in the tumor center (6.5 ± 1.2 μl/g/min, $^{**}P<0.01$) compared with control group (10.5 ± 1.5 μl/g/min). However, BK-induced K_i increase (32.5 ± 6.5 μl/g/min) was not blocked by DEX pretreatment (31.2 ± 5.6 μl/g/min, Fig. 2), which was significantly inhibited (12.25 ± 3.1 μl/g/min, $^{\#\#}P<0.01$) by coadministration with glybenclamide at a dose of 5 μg/kg/min for 10 min (Fig. 2), indicating a K_{ATP} channel-specific effect.

2.3. *DEX increased expression of K_{ATP} channels on brain microvessels*

In tumor tissues treated with sterile saline, Kir6.2 subunits were slightly expressed (Fig. 3A), mainly on the tumor capillaries and tumor cells. After DEX administration, Kir6.2-