Involvement of Fibroblast Growth Factor in the Restoration of Arterial Baroreflex by Dietary Restriction

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Dietary restriction (DR) can prolong the lifespan of many organisms [1]. The protective effects of DR against age-related diseases are also been fully understood [2,3]. Recently, our data showed that DR can promote protection against ischemic cerebral injury [4]. We further concluded that the restoration of arterial baroreflex, one of the most important regulators of cardiovascular functions [5,6], involved in the protective effect of DR against stroke [4]. However, the mechanism of DR on the restoration of arterial baroreflex is unclear.

The fibroblast growth factor (FGF) signaling is very important in modulating brain function and other biological functions [7–9]. Many of these studies are focused on the central nervous system. As FGF can regulate the food intake [10], we speculate that FGF might be a mechanism for the regulation of DR on arterial baroreflex.

Male Sprague-Dawley (SD) rats (200–220 g) purchased from Sino-British SIPPR/BK Lab Animals (Shanghai, China) were used in this study. All the animals received humane care in compliance with institutional guidelines of the Second Military Medical University for health and care of experimental animals. And all experiments were approved by the Ethic Committee of the Second Military Medical University.

In the first set of study, the relationship between FGF and DR was investigated in SD rats. First, 30 rats were randomly divided into three groups. All animals were fasted for 2 days, and then received diet without restriction (AL, *ad libitum*), placed on dietary restriction (food intake at 60% of the amount consumed by AL group animals) or fasting. FGF level was measured in the medulla

Figure 1 Dietary restriction reduced the levels of FGF. (A) SD rats were fasted for 2 days, and then received control diet (AL) or placed on DR (60% food intake of AL). FGF levels were measured in the medulla oblongata (left) and in the serum (right) 1.5 h after food intake. (B) FGF levels were measured in the medulla oblongata (left) and in the serum (right) later after DR for 2 months. Data were analyzed by one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) *t*-test. Mean $\pm$ SD. * P < 0.05, ** P < 0.01, n.s., not significant; n = 10 in each group. Abbreviations are as follows: AL, *ad libitum*; DR, dietary restriction; FGF, fibroblast growth factor.

Figure 2 Fibroblast growth factor injection decreased the BRS value. The changes of SBP and BRS of SD rats treated by both systemic (20 µg/kg, i.v., **A**) and direct injection of FGF into the NTS (0.05 µg/side; **B**). Data are expressed by mean $\pm$ SD. Paired student's *t*-test. **P* < 0.05, ***P* < 0.01 versus before administration. *n* = 5–6 in each group. Abbreviations are as follows: AL, *ad libitum*; DR, dietary restriction; FGF, fibroblast growth factor; NTS, nucleus of solitary tract; BRS, baroreflex sensitivity; SBP, systolic blood pressure.

oblongata and in the serum 1.5 h after being placed on diet. The data were determined with an enzyme-linked immunosorbent assay (ELISA) using an automatic microplate reader (Infinite M200; Tecan Austria GmbH, Grödig, Austria). Compared with fasting state, feeding treatment significantly increased FGF levels both in the medulla oblongata (248 ± 21 vs. 183 ± 15 pg/ml in the fasting state, left panel of Figure 1A) and in the serum (730 ± 173 vs. 367 ± 92 pg/mL in the fasting state; right panel of Figure 1A). This alteration in FGF levels was not observed in the DR group. Compared with feeding AL animals, DR significantly decreased the level of FGF in both medulla oblongata and serum (Figure 1A). Second, 20 rats were randomly divided into two groups and placed under AL or DR for 2 months. Compared with AL group, DR significantly decreased FGF levels in medulla oblongata (203 ± 20 vs. 267 ± 41 pg/mL in AL group, Figure 1B left

panel), as well as in serum (490 ± 146 vs. 727 ± 160 pg/mL in AL group; Figure 1B right panel).

In the second set of study, we examined the effect of FGF injection on the baroreflex sensitivity in rats. SD rats were randomly divided into 2 groups. Systolic blood pressure (SBP) and baroreflex sensitivity were recorded as previously described [4,6] before and after FGF injection. First, animals were administered with FGF (20 µg/kg, Wenzhou Medical University, Wenzhou, China) or vehicle (0.9% normal saline) by intravenous (IV) injection (*n* = 6 in each group). Second, anesthetized rats (*n* = 5 in each group) were treated with FGF by nuclei tractus solitarii (NTS) injection. NTS (coordinating in mm with respect to calamus scriptorius: 0.4–0.5 rostral, 0.5–0.6 lateral, and 0.4–0.5 deep) was identified by injection of L-glutamate (1 nmol) and observing a depressor response of at least 25 mmHg. L-glutamate and FGF (0.05 µg/side) were dissolved in artificial cerebrospinal fluid (aCSF, NaCl 126 mM, KCl 5 mM, CaCl₂ 2 mM, HEPES 10 mM, MgSO₄ 2 mM, Glucose 10 mM). The time interval between bilateral injections was within 2 min. SBP had no significant change after FGF injection (Figure 2). Baroreflex sensitivity was reduced by both systemic administration of FGF (Figure 2A) and its direct injection into the NTS (Figure 2B).

We conducted a series of experiments to probe into the mechanisms by which DR increased baroreflex function. Consistent with a previous report [10], we observed that FGF level was increased in the brain upon regular AL diet; however, DR reduced the elevation of FGF level. More importantly, FGF administration, either by systemically or directly into the NTS, significantly decreased baroreflex sensitivity. These observations taken together suggest that FGF may be involved in the effect of DR on the regulation of arterial baroreflex function. These observations are worthy of further investigation.

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Conflict of Interest

The authors declare no conflict of interest.

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