Level of the nuclear factor c-Fos in human aldosteroma cells after potassium treatment

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Abstract. Potassium ions play an important role in the regulation of steroidogenesis, water-salt balance, apoptosis, proliferation. However, the nuclear phase of the agonist signaling in adrenocorticocytes remains poorly understood. The aim of the work was to elucidate the effect of potassium on the level of nuclear transcription factor c-Fos in human tumor adrenocortical tissue (aldosteroma). Methods. Expression of c-Fos transcription factor in aldosteromas was detected using Western-blot analysis. Results. The increase of c-Fos level in human aldosteroma cells at potassium physiological concentration of 3.5 mmol/l compared to medium without potassium was observed. The level of cFos did not change after 8.5 mmol/l K⁺ administration. The possible participation of transcription factor c-Fos in steroidogenic effects of K⁺ is being discussed. Conclusions. The results of study suggest that K⁺ can have a direct effect on the expression level of nuclear transcription factor c-Fos in tissues from human aldosteromas.

Keywords: K⁺, human adrenocorticocytes, aldosteroma, transcription factors.
The aim of this work was to elucidate the effect of potassium in the human tumor adrenocortical tissue on the level of MAPK-dependent transcription factor cFos.

**Materials and methods**

**Material.** All the salts were provided by Merck (Germany); Tween 20 - by Ferak (Germany); HEPES, BSA (V fraction, content of lipids less than 0.1%) - by Serva (Germany). Lysis buffer, primary and secondary antibodies for c-Fos, β-actin by Cell Signaling Technology and Sigma (USA). The rest of reagents were obtained from local suppliers.

**Tissue.** The study was approved by the Institute's Ethics Committee. Effects of potassium ion were studied in tumor tissue (aldosteromas) of the human adrenal cortex.

**Conditions of incubation.** The adrenal tumor tissue was placed on ice, trimmed from fat and connecting tissue and cut into slices. Slices were incubated during 30 min at 37 °C in 1 ml of Krebs-Ringer phosphate buffer (pH 7.6) containing 2 mmol/l CaCl₂, 20 mmol/l HEPES, 2 mg/ml BSA and 0-8.5 mmol/l KCl. At the end of incubation tubes were cooled.

**Western blotting.** The tissue was homogenized in 200 µl of the lysis buffer containing a cocktail of protease and phosphatase inhibitors, homogenate were centrifuged for 15 min at 15,000 g and stored at -60 °C until use. Protein concentration was determined according to Bradford [11]. Protein samples were boiled in the sample buffer (100 mmol/l Tris-HCl, 4% sodium dodecyl sulfate, 0.2% bromophenol blue, 20% glycerol, 10% dithiothreitol) and separated by SDS-PAGE 9% gels [12]. 30 µg of protein were applied per each lane. Proteins were transferred onto nitrocellulose membranes Hybond-C (Amersham Life Science, UK) by semidry blotting. Membranes were blocked with Tris-buffered saline/0.1% Tween 20 containing 5% nonfat dry milk and incubated with primary antibodies for 1 h at RT. After washing three times with Tris-buffered saline/0.1% Tween 20, the blots were incubated with horseradish peroxidase-conjugated species-specific secondary antibody for 1 h at room temperature and then were again washed three times. Complexes were visualized using the ECL reagents (Amersham Life Science, UK). As loading control β-actin was used. X-ray films were photographed by a digital video camera, scanned using «Gel Pro Analyzer» v. 4.0 software.

**Statistics.** All data were expressed as a mean ±M. Differences between groups were examined for statistical significance using Student’s t-test. P<0.05 denoted the presence of a statistically significant difference.

**Results and discussion**

Activity of MAPK and its downstream transcription factors was associated with apoptotic or proliferative processes [13]. In addition it was suggested that this cascade kinases participate in the gluco- and mineralocorticoids biosynthesis regulation [14, 15]. c-Fos is one of the main transcription factor activated by MAPK. To study the effect of K⁺ on transcription factor c-Fos expression and activation, Western blotting of the proteins from tissue of the human adrenal cortex tumor (aldosteromas) incubated at different potassium concentrations was performed. The decrease of K⁺ concentration in incubation medium from basal, physiological 3.5 mmol/l to 0 causes 1.3-fold decrease of c-Fos quantity (Fig. 1, 2). Rise of potassium content to 8.5 mmol/l - the concentration that stimulates steroidogenesis under normal conditions - did not change the c-Fos level after 30 min of incubation (Fig. 1, 2).

There are no reports in the literature on changes in the level of the nuclear transcription factors under the influence of potassium ions on the adrenocortical tissue. However, the participation of the c-Jun factor in the potassium effect implementation was shown on cerebral neurons. Potassium increased the level of c-Jun protein in the cells and also accelerated the formation of the transcription factor AP-1 as a homodimer com-

**Fig. 1.** Representative Western-blot analysis of c-Fos, β-actin in adrenocortical tumors after K⁺ administration. One case of 3 studied is exemplified. 1 — medium without KCl, 2-3.5 mmol/l KCl, 3-8.5 mmol/l KCl.
plex c-Jun, or heterodimer c-Jun/JunB. The level of two AP-1 transcriptional targets (Bim and FasL) was also increased [16].

Using the Western blotting method a translocation of protein kinase C (PKC)α from cytosol to membranes was showed after adrenal tissue preincubation in the medium with increased K+ content (8.5 mmol/l). The translocation means an activation of the enzyme. After incubation of slices in a medium without potassium, the activity at the physiological concentration of potassium ions (3.5 mmol/l). [1]. On the other hand, PKC is a regulator of transcription factor АР-1 which play the role on steroidogenesis.

Thus, a decrease in the expression of transcription factor c-Fos in the potassium ions free incubation medium may indicate the involvement of c-Fos in the inhibitory mechanisms of aldosterone synthesis in adrenocorticoctyes with potassium concentrations below physiological parameters.

It was shown that the rise of K+ concentration to 5.5-11 mmol/l caused intensification of DNA laddering in conventionally normal human adrenal tissue. On the contrary, the high K+ concentration (elevated) decreases the intensity of DNA laddering in tumor tissues [1]. We can conclude that MAPK/c-Fos activation does not represent the mechanisms responsible for such difference.

The results presented here demonstrate that K+ can have a direct effect on the expression level of nuclear transcription factor c-Fos in the human aldosteroma tissues. This is evidence for involving MAPK to mediate K+ effects in adrenocorticoctyes. However, the mechanisms of c-Fos-cellular signaling remain unclear. Therefore, further studies should be performed to identify c-Fos target genes in the human adrenal cortex.

Conclusions

The results of study suggest that K+ can have a direct effect on the expression level of nuclear transcription factor c-Fos in tissues from human aldosteromas.

References

1. Пушкарьов ВМ. Біохімічні механізми регуляції стероїдогенезу в корі надниркових залоз іонами калію. Автореф. дис. ... доктора біол. наук: Київ, 2005:34 с. (PushkarevVM. Biochemical mechanisms of steroidogenesis regulation in the adrenal cortex by potassium ions. Avtored. dys. … doktora biol. nauk: Kyiv, 2005:34 p.).
2. Zhu J, Zang S, Chen X, Jiang L, Gu A, Cheng J, et al. Involvement of the delayed rectifier outward potassium channel Kv2.1 in methamphetamine-induced neuronal apoptosis via the p38 mitogen-activated protein kinase signaling pathway. J Appl Toxicol. 2018 May;38(5):696-704.
3. Ali BH, Za’abi MA, Karaca T, Suleimani YA, Balushi KA, Manoj P, et al. Potassium bromate-induced kidney damage in rats and the effect of gum acacia thereon. Am J Transl Res. 2018 Jan;10(1):126-37.
4. Hyatt PJ, Tait JF, Tait SA. The mechanism of the effect of K+ on the steroidogenesis of rat zona glomerulosa cells of the adrenal cortex: role of cyclic AMP. Proc R Soc Lond B Biol Sci. 1986 Feb;227(1246):21-42.
5. Betancourt-Calle S, Jung EM, White S, Ray S, Zheng X, Calle RA, et al. Elevated Kt(+) induces myristoylated alanine-rich C-kinase substrate phosphorylation and phospholipase D activation in glomerulosa cells. Mol Cell Endocrinol. 2001 Nov;184(1-2):65-76.
6. Pushkarev VM, Mikosha AS. The participation of cAMP and protein kinase C in the regulation of aldosterone biosynthesis by potassium. Biomed Sci. 1991 Feb;2(2):135-9.
7. Ganguly A, Chiu S, Fineberg NS, Davis JS. Greater importance of Ca(2+)-calmodulin in maintenance of ang I- and Kt(+)-mediated aldosterone secretion: lesser role of protein kinase C. Biochem Physiol Res Commun. 1992 Jan;182(1):254-61.
8. Jiang KW, Yu ZS, Shui QX, Xia ZZ. Activation of ATP-sensitive potassium channels prevents the cleavage of cytosolic mu-calpain and abrogates the elevation of nuclear c-Fos and c-Jun expressions after hypoxic-ischemia in neonatal rat brain. Brain Res Mol Brain Res. 2005 Jan;133(1):87-94.
9. Panguluri SK, Tur J, Chapalamadugu KC, Katnik C, Cuevas J, Tippuraju SM. MicroRNA-301a mediated regulation of Kv4.2 in diabetes: identification of key modulators. PLoS One. 2013 Apr;8(4):e60545.
10. Kwak Y, Han J, Ryhu MR, Nam TS, Leem JW, Lee BH. Different spatial expressions of c-Fos in the nucleus of the solitary tract following taste stimulation with sodium, potassium, and ammonium ions in rats. J Neurosci Res. 2015 Feb;93(2):340-9.
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976 May;72:248-54.
12. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970 Aug;227:680-5.
Уровень ядерного фактора c-Fos в клетках альдостеромы человека под влиянием калия

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Резюме. Іони калію відіграють важливу роль в регуляції стероїдогенезу, водно-сольового балансу, апоптозу, проліферации. Проте ядерний етап перенесення сигналу агоніста в адренокортикоцитах залишається мало вивченим. Метою роботи було визначення дії іонів калію на рівень ядерного чинника транскрипції c-Fos у пухлинах кори надниркових залоз (альдостеромах) людини. Методи. Експресію фактора транскрипції c-Fos визначали в альдостеромах за допомогою вестерн-блот аналізу. Результати. При фізіологічній концентрації іонів калію 3,5 ммоль/л набувалося підвищення рівня чинника c-Fos у клітинах альдостеромах людини порівняно з таковим в інкубуванні без калію. При впорядкованій 8,5 ммоль/л іонів калію рівень c-Fos не змінювався. Обговорюється можливе участь транскрипціонного фактора c-Fos в стероїдогенних ефектах K+.

Висновок. Показано, що K+ може непосередньо впливати на рівень експресії ядерного транскрипційного фактора c-Fos у тканині альдостером людини.

Ключові слова: іони калію, адренокортикоцити людини, альдостерома, чинники транскрипції.