# Effect of Ions of Potassium and Lithium on NO Synthase Expression in the Human Adrenal Cortex

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The expression of endothelial and inducible NO synthase in the human adrenal glands was studied under a change in the concentration of $K^+$, which plays a regulatory role in aldosterone secretion. $K^+$ ions stimulated the expression of both isoforms of NO synthase in the human adrenal cortex. A stimulatory effect of $K^+$ on NO synthase is probably related to activation of the calmodulin system and potassium-induced translocation of protein kinase C. Lithium produced an inhibitory effect on both isoforms of NO synthase, which suggests that protein kinase C serves a major regulator of expression in the human adrenal glands.

**Key Words:** *adrenal cortex; endothelial and inducible NO synthase; potassium; lithium*

Mineralocorticoid function of the adrenal cortex is regulated by a complex mechanisms mainly involving angiotensin II, ACTH, and $K^+$ ions. These agonists activate aldosterone synthesis. By contrast, a decrease in $K^+$ concentration in the blood or incubation medium inhibits the production of this substance [9].

Studying the role of protein synthesis in the influence of $K^+$ on aldosterone secretion showed that addition of amanitin and canavanine in very low concentrations to the incubation medium is followed by a sharp increase in the production of this hormone. Aldosterone synthesis is suppressed at low concentrations of $K^+$, which probably results from the effect of a protein inhibitor. Addition of amanitin or α-canavanine to the medium with low concentrations of $K^+$ probably abolishes this inhibitory effect [1].

Various messenger systems, including NO and NO synthases, are involved in signal transduction of agonists. The majority of authors believe that these substances produce the inhibitory effect [2-4,8]. Endothelial NO synthase (eNOS) was identified in the adrenal cortex of rats, sheep, and monkeys. However, neuronal and inducible NO synthases (iNOS) were not found in sheep and monkeys [3,6]. The adrenal gland tissue in humans was not examined. It remains unclear which agonists modulate functional activity of the NO-ergic system. The role of this system in the physiological regulation of adrenal function is poorly understood.

Here we studied whether a change in the concentration of $K^+$ (important regulator of aldosterone secretion) has a modulatory effect on the expression of NO synthases in the human adrenal cortex.

## MATERIALS AND METHODS

The study was performed on conditionally normal tissues of the adrenal cortex from 5 women (31-75 years). The patients were operated on for hormonally inactive tumors of the adrenal cortex at the Surgical Department of the Institute. All experiments were approved by the Ethics Committee of the Institute. Sections of a conditionally normal cortex of the adrenal glands (localized at the boundary of tumor) were prepared on ice. The samples (20-40 mg) were put into 1 ml solution containing 130 mM NaCl, 1.27 mM $MgSO_4$, 2 mM $CaCl_2$, 10 mM $Na_2HPO_4$ (especially pure and pure for analysis; Merck), 20 mM HEPES

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(pH 7.4; Calbiochem), and 2 mg BSA (Serva). KCl (especially pure; Merck) in concentrations of 3.5, 5.5, and 8.5 mM was added to study samples. LiCl (pure for analysis; Merck) in a concentration of 10 mM was added to some samples (in addition to KCl at the same concentrations). All samples were incubated at 37°C and constant shaking for 1 h.

Incubation was stopped by placing the samples on ice. The incubation medium was cooled and removed. RNA was extracted from the tissue. A section of the adrenal cortex was homogenized in 1 ml TRIzol LA (Invitrogen). The homogenate was agitated with chloroform. The phases were separated by centrifugation at 12,000*g* and 4°C for 15 min. RNA was precipitated from an aqueous phase with isopropanol. The precipitate was collected by centrifugation at 12,000*g* and 4°C for 15 min, washed twice with 75% ethanol (7500*g*, 4°C, 5 min), dried, and dissolved in water without ribonucleases. The solution of RNA was heated at 60°C for 10 min. The concentration and purity of RNA were estimated on a Nanodrop spectrophotometer at 260 and 280 nm.

The reverse transcription reaction was conducted on a GeneTech amplifier (Stuart Scientific). The reaction mixture contained a standard buffer for PCR, 5 mM $MgCl_2$, 1 mM dNTP, RNAse inhibitor (1 U/µl), reverse transcriptase (2.5 U/µl; Sigma), mixture of random hexamers (2.5 µM), and 1 µg extracted RNA. Incubation was performed as follows: at 22°C for 10 min; at 42°C for 15 min; at 99°C for 5 min; and at 4°C for 5 min. PCR was conducted in the mixture containing a standard buffer for PCR, 2 mM $MgCl_2$, 1 mM dNTP, 5 U/µl Taq DNA polymerase (Sigma), eNOS primers (forward primer 5'-CTGTGTCCAACATGCTGCTAGAAATTG-5', reverse primer 5'-TAAAGGTCTTCTTCCTGGTGATGCC-3'), iNOS primers (forward primer 5'-CACGGAGAACAGCAGAGTTGG-3', reverse primer 5'-GGAACACAGTAATGGCCGACC-3'; 0.2 µM; Sigma), and 5 µl cDNA (obtained in the reverse transcription reaction). Incubation (eNOS and iNOS) was performed as follows: at 94°C for 1 min; at 55°C for 30 sec; and at 72°C for 1 min (35 cycles).

PCR products were analyzed in 1.7% agarose gel. This gel was prepared in the buffer containing 40 mM Tris-acetate (pH 8.5) and 2 mM EDTA (TAE buffer). Ethidium bromide in a concentration of 1 µg/ml was added to the gel. The samples were prepared by mixing with bromophenol blue and sucrose (0.025 and 45%, respectively) in TAE buffer. The total volume of each sample did not exceed 20 µl. A study was performed with DNA markers of 100-1000 b.p. Electrophoresis was conducted at 100 mV for 1 h. Gels were photographed in a transilluminator and scanned with a software for the analysis of electrophoretic images (GelPro). We compared the intensity of DNA bands that were obtained by amplification of complementary DNA.

The results were analyzed by Student's *t* test. The differences were significant at $p<0.05$.

## RESULTS

eNOS and iNOS were expressed in the human adrenal cortex (Figs. 1 and 2). The increase in $K^+$ concentration above the physiological level (3. 5 mM) was followed by activation of mRNA expression for eNOS and iNOS. The expression of eNOS and iNOS in the presence of $K^+$ at a concentration of 8.5 mM increased by 1.8 (Fig. 1) and 4.5 times (Fig. 2), respectively. A significant activation of aldosterone production by adrenal gland sections from guinea pigs was previously observed at the maximum concentration of $K^+$ (8.5 mM) [7].

It should be emphasized that the expression of iNOS mRNA increased in the absence of $K^+$ (Fig. 2). Aldosterone synthesis was reduced at low concentrations of $K^+$. It can be suggested that this effect is realized with the involvement of iNOS and NO.

Addition of lithium to the incubation medium prevented an increase in mRNA expression, which was observed at high concentrations of $K^+$ (Figs. 1 and 2). Our previous studies have demonstrated that lithium completely blocked translocation of protein kinase C in adrenocortical cells caused by elevated $K^+$ concentrations [7]. Protein kinase C was shown to be involved in eNOS expression in the vascular endothelium [5]. Lithium inhibits the expression of both isoforms of NO synthase, which suggests that protein kinase C serves as a major regulator of expression in the human adrenal glands.

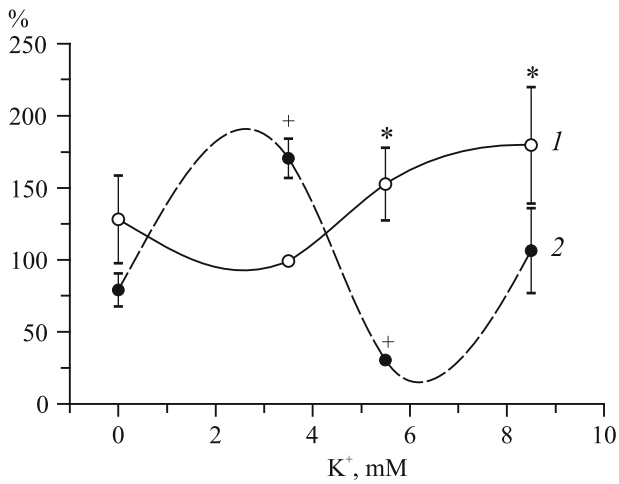


**Fig. 1.** eNOS mRNA expression at various concentrations of $K^+$ and in the presence of lithium. Here and in Fig. 2: KCl (*1*); KCl+10 mM LiCl (*2*). Values at the physiological concentration of $K^+$ are taken as 100%. $p<0.05$ compared to: *control (3.5 mM), $^+$*1*. Five samples.

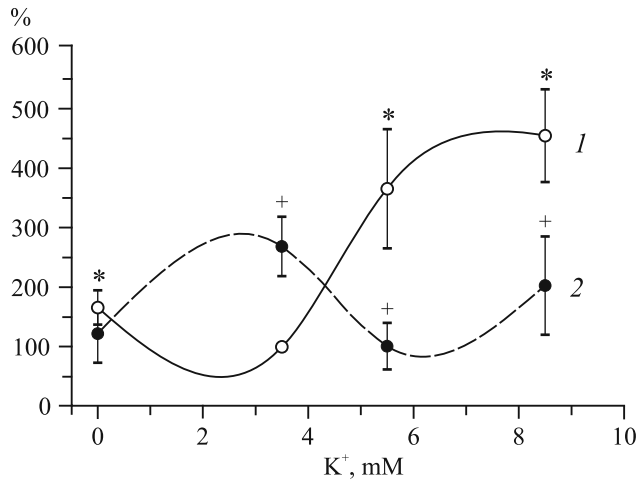


**Fig. 2.** iNOS mRNA expression at various concentrations of $K^+$ and in the presence of lithium.

Our results indicate that $K^+$ ions stimulate the expression of both isoforms of NO synthase in the human adrenal cortex. A stimulatory effect of $K^+$ is probably related to activation of the calmodulin synthesis. It can be suggested that NO is an autocrine/paracrine modulator of steroidogenesis, which provides a fine regulation of the cell response. This effect is probably associated with the influence of NO on activity or expression of steroidogenesis enzymes due to a change in functions of transcriptional factors.

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