The Effects of Hypokalaemia on the Hormone Exocytosis in Adenohypophysis and Prolactinoma Cell Culture Model Systems

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Key words
- adenohypophysis cell cultures
- extracellular ion milieu
- hypokalaemia
- prolactinoma

Abstract

The extracellular ion milieu determines the exocytosis mechanism that is coupled to spontaneous electrical activity. The K^+ ion plays crucial role in this mechanism: as the potassium current is associated with membrane hyperpolarization and hormone release through protein cascade activation. The primary aim of this study was to investigate the response mechanisms of normal adenohypophysis and adenohypophyseal prolactinoma cell populations at different extracellular K^+ levels with an otherwise isoionic milieu of all other essential ions. We focused on prolactin (PRL) and adrenocorticotrophic hormone (ACTH) release.

In our experimental study, female Wistar rats (n = 20) were treated with estrone-acetate (150 µg/kg b.w./week) for 6 months to induce prolactinomas in the adenohypophysis. Primary, monolayer cell cultures were prepared by enzymatic and mechanical digestion. PRL and ACTH hormone presence was measured by radioimmunoassay or immuno-chemiluminescence assay. Immunocytochemistry was used to assess the apoptotic cells.

Differences between the effects of hypokalaemia on normal adenohypophysis cultures and prolactinoma cell populations were investigated. Significant alteration ($p < 0.001$, n = 10) in hormone exocytosis was detected in K^+ treated adenohypophyseal and prolactinoma cell cultures compared to untreated groups. Immunocytochemistry showed that Bcl-2 expression was reduced under hypokalaemic conditions.

The decrease in hormone exocytosis was tightly correlated to the extracellular K^+ in both cell types, leading to the conclusion that external K^+ may be the major factor for the inhibition of hormone release. The significant increase in hormone content in supernatant media suggests that hypokalaemia may play important role in apoptosis.

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Introduction

There is a persistent and dynamic contact among the living structure and its environment, thus in this context, the cell and its extracellular milieu comprise an operational unit.

2 structurally and functionally distinct entities of hypophysis are the neurohypophysis and the adenohypophysis: both of which are under strict hypothalamic control. The present study is focused on the multiple cell types of the heterogeneous anterior pituitary gland that produce peptide hormones essential for reproduction, lactation, growth, development, response to stress, and metabolic homeostasis [1–4]. Prolactin (PRL) and adrenocorticotropic hormone (ACTH) are studied in this paper.

We intended to investigate response mechanisms in the function of normal, monolayer, primary adenohypophysis cell cultures (Adh) at low extracellular [K^+]. The novelty of our experimental method is that other essential ions were under homeostatic, isoionic conditions. It would be interesting to determine whether environmental stress, namely extracellular hypo [K^+] may modify the cells' rapid accommodation and basic regulatory functions. *In this paper 2 cell cultures were studied, an Adh cell culture and another one formed from a monolayer derived from estrone-acetate induced prolactinomas and their adjoining adenohypophsial cells (PRLoma).*

The effect of extracellular hypoionic conditions on cellular functions is intriguing and likely important factor in a number of pathologies. Several studies revealed that altered extracellular [K^+] plays a crucial role in endocrine-related diseases; for example in chronic kidney disease [5] and in cardio-renal decompensation syndrome

[6]. It is also known that hypo [K^+] is involved in cell proliferation [7] (e.g. in small-cell lung cancer [8]) by the activation of voltage-gated K^+-channel (K_v) pathways leading to plasmalemma hyperpolarization.

It is documented that hypokalaemia may induce cell aging and cell death through mitogen-activated protein kinases (MAPK), particularly the p38 and c-Jun N-terminal kinases (JNK) [9]. In connection with kinase activation, the generation of reactive oxygen species (ROS) is known to trigger apoptosis [10, 11] leading to apoptosis signal regulating kinase 1 (ASK1) activation [12]. Furthermore, the caspase cascade is activated by death signals such as the members of the Bcl-2 family, leading to degradation of cellular structures [13].

Although most pituitary neoplasms are benign, they are associated with high morbidity and mortality. Prolactinomas are the most common benign pituitary adenomas in the general population [14]. Hyperprolactinaemia can be caused by physiological processes [3, 15], pharmacological interventions [16], and pathological effects [17, 18]. One possible explanation for PRL oversecretion is fluctuation in membrane hyperpolarization that decreases the driving force for Ca^{2+} ions [19]. Steroid hormones, mainly estrogen, are known to potentiate the expression and synthesis of PRL in a manner, which is dependent on elevated intracellular Ca^{2+} concentration and Ca^{2+} influx [20, 21]. A rapid increase in the rate of PRL synthesis was detected both *in vivo* and *in vitro* with estradiol treatment [22, 23].

Methods

Experimental protocol

Female Wistar rats (Charles River, Isaszeg, Hungary, medically certified) from different litters (weighing 120–250 g, aged 4–6 weeks at the beginning of the research) were used for hypophysis cell culture model systems. The animal care and research protocols were in full accordance with the guidelines of University of Szeged, Hungary. During the research period, rats were kept under controlled relative air humidity of 55–65% and 22±2°C ambient temperature. Experimental animals lived under automated diurnal conditions (12 h dark and 12 h light system) in groups of 10 animals for 6 months. Standard pellet food and tap water were available ad libitum. Female Wistar rats (n=20) were treated subcutaneously with estrone-acetate (CAS registry number: 901-93-9, Sigma, Germany; 150 µg/kg b.w./week) for 6 months to induce adenohypophyseal prolactinomas.

After pentobarbital anaesthesia (4.5 mg/kg b.w. Nembutal, Abbott, USA) the animals were killed and decapitated. Tissues were separated under a preparative microscope. Primary, monolayer cell cultures were prepared by enzymatic and mechanical dissociation. The tissues were digested enzymatically (trypsin: 0.2%/Sigma, Germany/ for 30 min; collagenase/Sigma, Germany/: 30 µg/ml for 40 min; dispase/Sigma, Germany/: 50 µg/ml for 40 min in phosphate-buffered saline/PBS-A/; temperature: 37 °C). Mechanical dispersion was achieved with nylon blutex sieves (∅: 83 and 48 µm). Cultures were controlled for both viability (>95%; trypan blue exclusion) and function and the cell density was determined to be $2\times10^5/cm^3$. The dissociated cells were placed onto 24 well-plastic plates (5% collagen coated/ Nunc., Germany/; Dulbecco's Modified Essential Medium/ DMEM/+20% Fetal Calf Serum/FCS/+antibiotics/Penicillin+Streptomycin: 1.0 µg/cm^3). The cells were cultured at 37 °C in a CO_2 incubator that provided a humidified environment of 95% air and 5% CO_2. The medium was changed every 3 days. Primary cell cultures were standardized by immunohistochemical methods, marking for PRL and ACTH protein release. After functional standardization, the basal ACTH and PRL levels were determined in both normal Adh and PRLoma (Tyrode's medium/ Sigma, Germany/). In the medium, only the [K^+] was modified; all other essential anions and cations were under homeostatic (e.g. isoionic) conditions. The hormone release of primary cell cultures was detected under hypokalaemic conditions of varying degrees ([K^+]: 0; 0.5; 1.0; 1.5; 2.0 mM; n=10 in each group). Samples were taken at 10, 20, 30, 60 and 90 min after treatments to measure hormone kinetics.

The PRL and the ACTH content were detected in the supernatant media. From the supernatant media, 500 µl samples were removed by Gilson pipette at appropriate times and stored at −80 °C until peptide radioimmunoassay (RIA) [24, 25] and immuno-chemiluminescence assay (LIA) were performed.

A rat PRL RIA KIT (Institute of Isotopes Ltd., Budapest, Hungary) was used to determine the supernatant PRL content; all components were stored at 2–8 °C, where they were stable. Non-specific binding, defined as the proportion of tracer bound in the absence of antibody, was determined to be <5%. The sensitivity of the RIA procedure was 0.07 ng/tube. The intra-assay precision obtained was 0.92±0.03 ng, PRL data are given in ng PRL/mg protein.

The ACTH levels of supernatant media were measured by LIA with an Immulite 2000 apparatus (Siemens Healthcare Diagnostic, Deerfield, IL) and DPC kit (L2KAC-02; Euro/DPC Ltd, Glyn Rhonwy, UK). ACTH data are given in pg ACTH/mg protein.

A modified Lowry Method [26] and Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc., Rockford, USA) were used for the determination of total protein content.

Immunocytochemistry

At 90 min of the experiment, the 0 mM [K^+] manipulated monolayers and the controls were fixed by 4% paraformaldehyde and stored until staining. Immunostaining with anti Bcl-2 (Santa Cruz Biotechnology Inc., 1:25 dilution, N-19, sc-492) for 60 min was performed (samples were washed in Tris buffered saline/TBS, 0.05 M, pH 7.4 and 0.85% NaCl/ for 5 min before treatment) after incubating of monolayers with peroxidase blocking reagent for 5 min. After additional washes TBS (5 min) bound antibodies were visualized using 3,3'-diaminobenzidine tetra-hydrochloride (DAB, Sigma, Germany) for 2 min. Samples were then washed in TBS and image was captured using a phase contrast invertoscope (Zeiss) equipped with an Olympus camera (Olympus C-7070).

Statistical analysis

To compare various effects of treatment in Adh and PRLoma over time, two-way repeated measures ANOVA was used for each independent set of data: ACTH secretion of Adh or PRLoma, or PRL release of Adh or PRLoma. Treatment was considered as a between-subject factor and time (5 time points: 10, 20, 30, 60, 90 min) as a within-subject factor for the analysis. We compared the ACTH and PRL secretion of untreated Adh with that of the PRLoma group under hypokalaemia over time using repeated measures ANOVA. Not only group differences, but also individual within-subject variation in time can be modeled.

Significant interaction was found between the 2 investigated factors ($p<0.001$), thus both effects could not be reported independently. When Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was performed. Pairwise

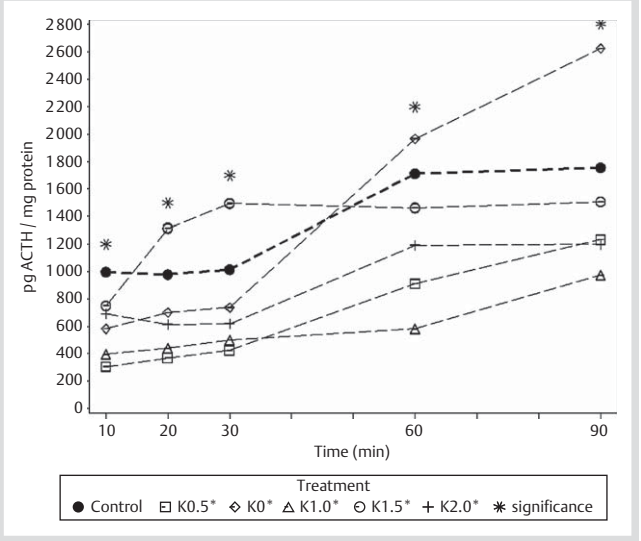

Fig. 1 The effects of different potassium concentrations on the release of ACTH in Adh. Asterisks indicate the significance between the hormone release of various treated groups vs. the ACTH release of normal Adh as the control group.

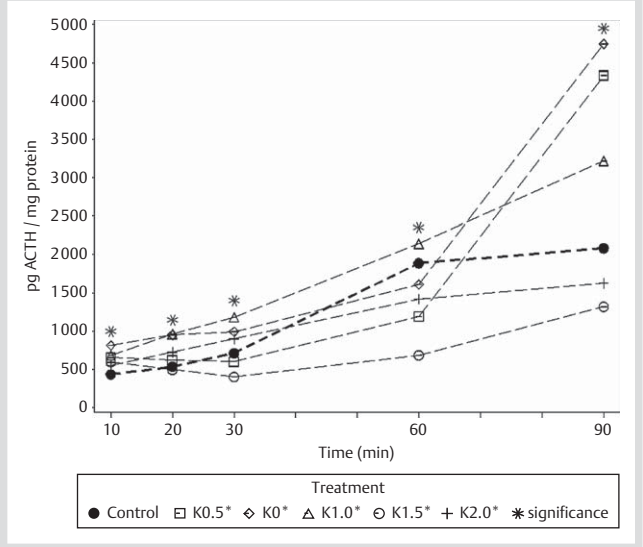

Fig. 2 The effects of different potassium concentrations on the release of ACTH in PRLoma. Asterisks indicate the significance between treated groups vs. the PRL release of untreated PRLoma as a control group.

comparisons of group means were performed, based on estimated marginal means with Sidak adjustment for multiple comparisons. Data are represented as means and S.E.M.

Statistical analyses were carried out using SPSS, version 17 (SPSS Inc., Chicago) software. All tests were two-tailed, and $p<0.05$ was considered to be statistically significant. All graphs were made with SAS 9.2 software (SAS Institute Inc., Cary, NC, USA).

Results

Cell culture standardization resulted that the ACTH positive cells were accounted for approximately 15.81% in Adh and 18.43% in the *in vivo* estron-acetate pretreated, than cultured adenohypophysis tissues (the monolayer contained prolactinoma cells and the adjoining adenohypophysial tissue). The percentage of PRL positive cells was 24.0% in normal Adh and 52.2% in PRLoma. Our *in vitro* experiments revealed statistical differences between treated and untreated groups. **Fig. 1, 2** show the ACTH release of both the control systems and the treated groups. The basal ACTH level of normal Adh and PRLoma is labelled as $CAdh_{ACTH}$ and $CPRLoma_{ACTH}$ respectively.

The basal PRL release of the control Adh ($CAdh_{PRL}$) and PRLoma ($CPRLoma_{PRL}$) systems and the treated primary cell cultures are represented in **Fig. 3, 4**.

In **Fig. 5, 6**, the PRL and ACTH release of CAdh and CPRLoma was compared with that of the cell cultures treated with 0 mM [K^+]. All p-values for within group and between group effects were determined to be statistically significant ($p<0.001$).

The ACTH release of induced prolactinoma and adjoining adenohypophysial cell cultures and normal adenohypophysis cultures by the effects of different potassium concentration

At 10, 20 and 30 min of the experiment (**Fig. 1**) the ACTH secretion of the groups treated with 0 mM [K^+] decreased appreciably (583.5±1.86, 701.4±1.65, 738.1±1.26 pg hormone/mg protein; means±S.E.M., $p<0.001$) compared with the $CAdh_{ACTH}$ (996.9±2.55, 978.4±1.23, 1014.5±1.89 pg hormone/mg protein; means±S.E.M.). In the supernatant media, the hormone content was increased significantly (1966.4±1.36, 2625.7±0.97 pg hormone/mg protein; means±S.E.M., $p<0.001$) at 60 and 90 min under hypokalaemic conditions compared with $CAdh_{ACTH}$ (1711.3±2.07, 1754.5±1.34 pg hormone/mg protein; means±S.E.M.).

As shown in **Fig. 1**, the ACTH release of Adh treated with 0.5 mM [K^+] diminished significantly ($p<0.001$) depending upon the duration of exposure (306.0±1.96, 369.8±1.26, 424.6±1.45, 911.2±1.17, 1235.4±1.25 pg hormone/mg protein; means±S.E.M.).

The hormone levels of cell cultures treated with 1.0 mM [K^+] (**Fig. 1**) were reduced significantly (396.7±1.82, 439.3±1.58, 499.6±1.10, 582.3±1.75, 973.5±1.48 pg hormone/mg protein; means±S.E.M., $p<0.001$) depending upon the duration of exposure, correlating with the $CAdh_{ACTH}$.

It was observed that the ACTH release of cell cultures treated with 1.5 mM [K^+] was decreased significantly (1461.8±3.61, 1506.8±1.48 pg hormone/mg protein; means±S.E.M., $p<0.001$) at 60 and 90 min correlating with the $CAdh_{ACTH}$. The secretion of ACTH was decreased significantly as a consequence of 2.0 mM [K^+] treatment depending upon the duration of exposure (691.2±1.58, 614.4±1.66, 618.7±1.57, 1192.9±1.75, 1199.3±1.46 pg hormone/mg protein; means±S.E.M., $p<0.001$).

In **Fig. 2**, the ACTH release of PRLoma was increased significantly (813.4±1.19, 951.9±3.03, 992.6±1.92, 1610.5±1.91, 4746.2±3.61 pg hormone/mg protein; means±S.E.M., $p<0.001$) in the 0 mM [K^+] group compared with that of $CPRLoma_{ACTH}$ (435.1±1.39, 536.3±3.10, 713.6±1.82, 1887.9±2.52, 2083.0±3.42 pg hormone/mg protein; means±S.E.M.).

The hormone release of ACTH treated with 0.5 mM [K^+] increased (4333.7±5.37 pg hormone/mg protein; means±S.E.M., $p<0.001$) at 90 min of the experiment compared with the $CPRLoma_{ACTH}$ group.

As depicted in **Fig. 2**, the hormone levels in the PRLoma group treated with 1.0 mM [K^+] were elevated significantly (685.4±1.53, 962.0±2.05; 1181.3±1.83, 2142.0±1.72, 3217.7±2.54 pg hormone/mg protein; means±S.E.M., $p<0.001$) compared to $CPRLoma_{ACTH}$.

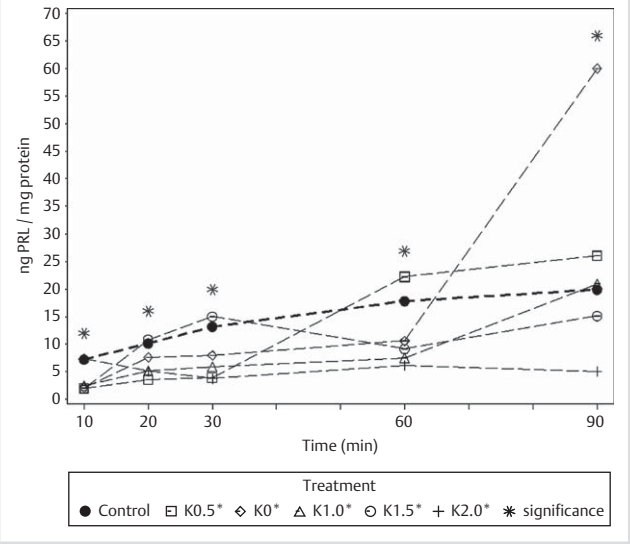

Fig. 3 The effects of different potassium concentrations on the release of PRL in Adh. Asterisks indicate the significance between treated groups vs. the PRL release of untreated Adh as control group.

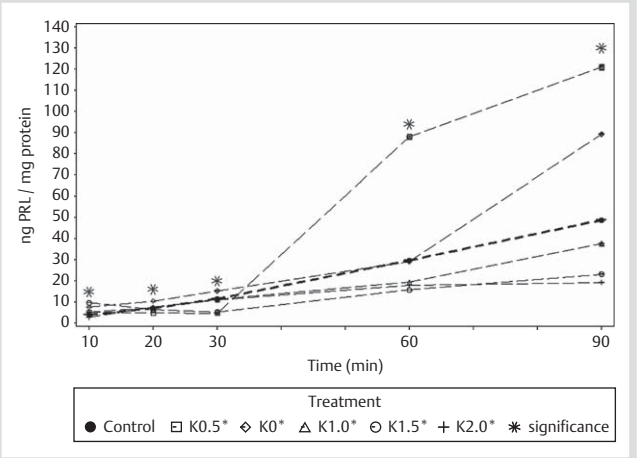

Fig. 4 The effects of different potassium concentrations on the release of PRL in PRLoma cell populations. Asterisks indicate the significance between treated groups vs. the PRL release of PRLoma cultures as control group.

In the 1.5 mM [K^+] manipulated groups the ACTH secretion was decreased (495.6±2.71, 403.1±2.18, 685.0±2.68, 1319.7±2.25 pg hormone/mg protein; means±S.E.M., $p<0.001$) depending upon the duration of exposure. The hormone levels of treated PRLoma under 2.0 mM [K^+] were decreased significantly (1418.9±2.13, 1626.1±2.24 pg hormone/mg protein; means±S.E.M., $p<0.001$) at 60 and 90 min of the experiment.

The effects of different potassium concentrations on the release of PRL in pretreated and normal adenohypophysis cultures

The PRL levels of the 0 mM [K^+] group decreased (**Fig. 3**; 2.11±0.01, 7.65±0.01, 7.95±0.01, 10.61±0.02 ng hormone/mg protein; means±S.E.M., $p<0.001$) at 10, 20, 30 and 60 min of experiment compared with $CAdh_{PRL}$ (7.24±0.01, 10.15±0.01, 13.18±0.02, 17.84±0.02 ng hormone/mg protein; means±S.E.M.). In contrast to this, the hormone secretion of treated cell cultures increased (60.04±0.02 ng hormone/mg protein; means±S.E.M., $p<0.001$) at 90 min, compared to the untreated groups (19.93±0.01 ng hormone/mg protein; means±S.E.M.).

Notable enhancement was detected in the PRL secretion of 0.5 mM [K^+] manipulated groups (22.25±0.05, 26.09±0.02 ng hormone/mg protein; means±S.E.M., $p<0.001$) at 60 and 90 min, in contrast to the $CAdh_{PRL}$.

As shown in **Fig. 3**, PRL release at 90 min was increased significantly (21.0±0.02 ng hormone/mg protein; means±S.E.M., $p<0.001$) by the effects of 1.0 mM [K^+] compared with the $CAdh_{PRL}$.

In **Fig. 3** significant decrease (1.97±0.01, 9.14±0.01, 15.14±0.01 ng hormone/mg protein; means±S.E.M., $p<0.001$) was noticed in the PRL release at 10, 60 and 90 min of the treatment by the application of 1.5 mM [K^+].

As shown in **Fig. 3**, the PRL secretion was reduced significantly (7.41±0.01, 5.11±0.01, 3.77±0.01, 6.13±0.01, 5.07±0.01 ng hormone/mg protein; means±S.E.M., $p<0.001$) depending upon the duration of exposure to 2.0 mM [K^+].

In **Fig. 4**, an increase in PRL release (7.64±0.01, 10.48±0.02, 15.32±0.05, 89.23±0.17 ng hormone/mg protein; means±S.E.M., $p<0.001$) was observed in the 0 mM [K^+] groups compared to $CPRLoma_{PRL}$ (3.86±0.01, 7.11±0.02, 11.52±0.01, 48.78±0.02, ng hormone/mg protein; means±S.E.M.) at 10, 20, 30 and 90 min of the experiment.

Fig. 4 shows that the levels of PRL were reduced significantly (4.82±0.02, 4.73±0.02 ng hormone/mg protein; means±S.E.M., $p<0.001$) at 20 and 30 min compared with the $CPRLoma_{PRL}$. Then the PRL level was increased significantly (88.07±0.25, 121.0±0.25 ng hormone/mg protein; means±S.E.M., $p<0.001$) by the effects of 0.5 mM [K^+] at 60 and 90 min of treatment.

The PRL secretion of the 1.0 mM [K^+] groups was reduced significantly (19.34±0.02, 37.67±0.21 ng hormone/mg protein; means±S.E.M., $p<0.001$) at 60 and 90 min, in contrast to $CPRLoma_{PRL}$.

Fig. 4 shows the PRL release of PRLoma was increased (9.63±0.02 ng hormone/mg protein; means±S.E.M., $p<0.001$) by 1.5 mM [K^+] treatment at 10 min; however, the PRL secretion in the cardinal points of the research protocol decreased significantly (6.28±0.04, 5.32±0.03, 15.77±0.02, 23.24±0.07 ng hormone/mg protein; means±S.E.M., $p<0.001$).

The hormone secretion was reduced significantly (10.94±0.03, 17.93±0.03, 19.29±0.03 ng hormone/mg protein; means±S.E.M., $p<0.001$) by the effects of 2.0 mM [K^+] depending upon the duration of exposure.

The effects of hypokalaemia on the ACTH and PRL release in adenohypophysis containing prolactinoma vs. normal adenohypophysis

We next examined the correlation between the normal and the altered endocrine regulation modified by hypokalaemia. As shown in **Fig. 5**, under hypokalaemia the ACTH release of PRLoma was increased significantly at 90 min of the experiment, in contrast to $CAdh_{ACTH}$. A similar interaction is depicted in **Fig. 6**: the PRL release increased significantly in PRLoma compared with $CAdh_{PRL}$.

Immunostaining

Immunocytochemical staining with anti-Bcl-2 was shown in **Fig. 7**. It was observed that the levels of Bcl-2 were higher in control groups than in 0 mM [K^+] manipulated groups.

Discussion and Conclusion

In this paper, *in vitro* model systems (primary monolayer cell cultures) were used and their cellular functions were standardized to investigate cellular phenomena. We decided to focus on the alteration of ACTH and PRL release in Adh and PRLoma under hypokalaemic conditions. Differences between the above mentioned primary cell cultures were investigated. The role of the extracellular ionic milieu in cell function can be defined by hormone exocytosis, sensitivity of intracellular receptors, or the discrete alteration of intracellular messenger molecules.

Inhibitory signals depend upon extracellular K^+ conductance. A reduction of the external [K^+] depolarizes the membrane [27], while an increase in external [K^+] hyperpolarizes the plasmalemma. In the anterior lobe of the pituitary gland, hormone exocytosis is associated with electrical and protein cascade signaling pathways. Recently published literature has revealed that in lactotrophs and corticotrophs the spontaneous electrical activity couples to hormone secretion [28].

The electrical properties of cell membrane activate cell surface receptors, which mediate variable cellular processes, including G-protein utilization and cyclic mononucleotide accumulation. Activation of the Gq/G_{11} protein induces membrane-bound phospholipase-C that catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to yield inositol 1,4,5-trisphosphate (IP_3) and diacylglicerol (DAG) [29]. In lactotrophs and corticotrophs, IP_3 is essential to mediate the mobilization of non-mitochondrial Ca^{2+}. DAG activates Ca^{2+} dependent protein kinase C and protein kinase B, which phosphorylates voltage-sensitive

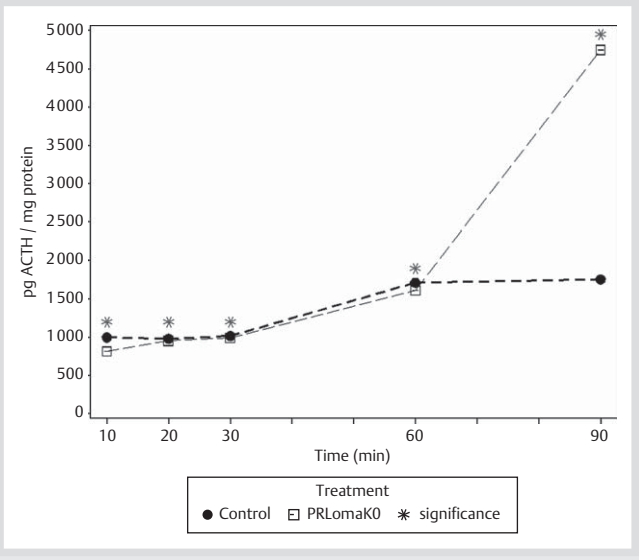

Fig. 5 The effects of hypokalaemia on the ACTH release of PRLoma vs. normal Adh hormone release. Asterisks indicate the significance among the hormone release of hypokalaemic PRLoma group (PRLomaK0) vs. normal Adh as control group, n = 10 in each group.

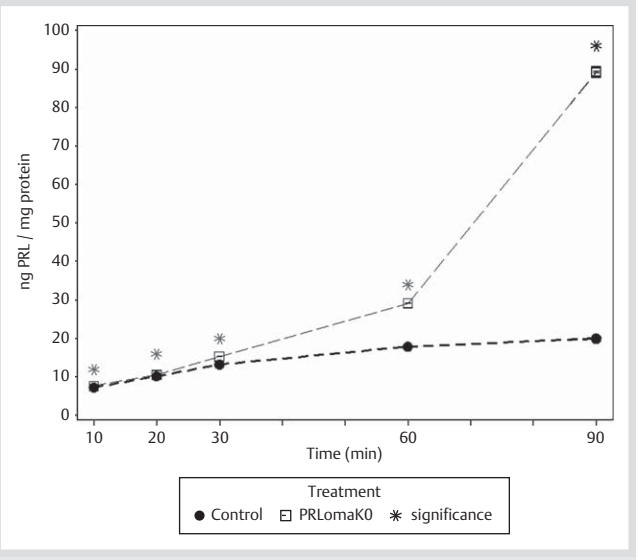

Fig. 6 The effects of hypokalaemia on the PRL release of PRLoma vs. normal Adh hormone release. Asterisks indicate the significance among the hormone release of hypokalaemic PRLoma group (PRLomaK0) vs. untreated Adh as control group, n = 10 in each group.

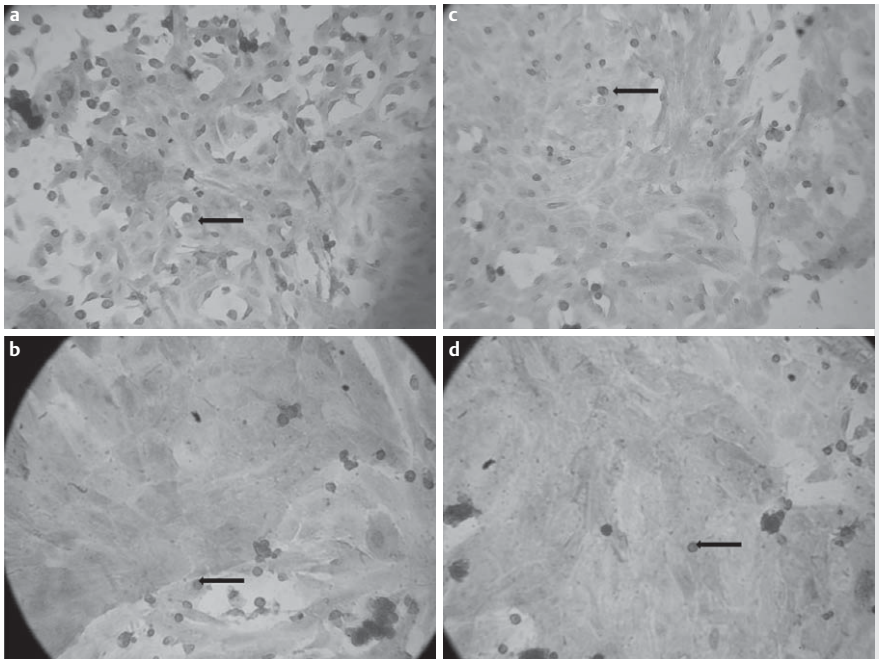

Fig. 7 Immunocytochemical analysis of Bcl-2 protein in rat adenohypophysis monolayer cultures. The cells were immunostained with antisera specific for Bcl-2. Antibodies were detected by DAB method; **a, b** control monolayer cultures; **c, d** monolayer cultures under hypokalaemic conditions. Arrows indicate Bcl-2.

Ca^{2+}-channels resulting in an increased Ca^{2+} influx [30]: enhancing hormone exocytosis through SNARE complex activation [31].

Since the extracellular environment is constantly fluctuating, the cell must adapt to it [32,33]. A reduced rate of hormone exocytosis was observed as a result of higher, but still hypoionic extracellular ion milieu. Interestingly, some reports suggest an evoked hormone release in hypophysis by the effects of higher extracellular [K^+] [34–36]. Under hypokalaemic conditions potassium channels, to maintain the equilibrium of K^+, may open and cause efflux of K^+ leading to cell membrane hyperpolarization [27,37,38]. According to our hypothesis hyperpolarization may block AC activity through $G_{i\text{-}3\alpha}$ activation that leads to a reduction of IP_3 metabolism and a decreased intracellular Ca^{2+} concentration. Diminution of Ca^{2+} influx inhibits the SNARE mediated fusion of ACTH and PRL containing vesicles to the plasma membrane. The discrete elevation of ACTH and PRL production by the effects of increased extracellular [K^+] suggested that hyperpolarization showed both time and cell type dependence.

The results showed that there was relation between the hormone release of PRLoma by K^+ depletion and the hormone exocytosis of untreated normal Adh. Under hypokalaemic conditions an increase in hormone content was observed in the supernatant media of PRLoma in the latest part of the experiment (at 90 min; occasionally at 60 min). The cell aging machinery may play role in this phenomenon. Apoptosis is known to be crucial in controlling cell number and proliferation [39]. Different extracellular stimuli activate tyrosine kinases through phosphorylation such as MAPK, which induces cell death. Recent studies suggest that specifically p38 and the stress kinase JNK [9] and ASK1 play key role in the apoptosis machinery mediated by hypoionic conditions. Under hypokalaemia ASK1 is activated, which then triggers JNK and p38 activation. These crucial events induce the pro-apoptotic protein Bad activation and Bcl-2 diminution. Activated Bad then triggers Bax-Bak oligomerization leading to cell aging, and cell death [40], which can be observed in the kinetics of ACTH and PRL release. The hormone content of the supernatant media increased significantly depending upon the duration of exposure. This phenomenon may be explained by membrane disintegration through caspase cascade signalling system [41], thus several types of caspases mainly the caspase-12 pathway recruits to dismantle the cellular structures [13] including the hormone containing vesicles.

Our results indicate that a strict association exists among certain biophysical properties, especially the extracellular K^+ milieu, hormone vesicle exocytosis and apoptosis. Understanding of hormone secretion and extracellular ion milieu has improved dramatically over the past few years: yet, there is much that remains to be explored. Considering this, it is important to improve our knowledge of the relationship between the hormone release of untreated cultures and PRLoma under K^+ depletion.

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Declaration of interest: The authors report no declarations of interest.

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