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ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2018, 7(6): 63-67

Effects of Adipokinetic Hormone/Red Pigment-Concentrating Hormone (AKH/RPCH) Family of Peptides on Uterine Contraction

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ABSTRACT

Objective: *Many processes in insects, including metabolic, behavioral, developmental, and reproductive processes, are altered by neuropeptides. Adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family of peptides include adipokinetic and hypertrehalosemic peptides.* ***Methods:*** *The present study investigated the effects of Anax imperator AKH (Ani-AKH), Libellula auripennis AKH (Lia-AKH), Phormia terraenovae hypertrehalosemic hormone (Pht-HrTH) and oxytocin on uterine contractility in human uterus strips. Contraction formed by Ani-AKH, Lia-AKH, Pht-HrTH and oxytocin were obtained from percentages of the maximal contraction of KCl (80 mM).* ***Results:*** *No difference detected between contractions of Ani-AKH, Lia-AKH, or Pht-HrTH and oxytocin at doses of 10^{-8} M in human uterus strips. Lia-AKH (10^{-7}-10^{-4} M) significantly increased uterine contractions compared to oxytocin (10^{-7}-10^{-4} M) in human uterus strips. Both Ani-AKH (10^{-4} M) and Lia-AKH (10^{-4} M) significantly increased uterine contractions compared to oxytocin (10^{-4} M) in human uterus strips.* ***Conclusion:*** *These results show that the AKHs examined can be used to induce human uterine contraction.*

Keywords: Adipokinetic hormone, Uterus, Contraction

INTRODUCTION

Peptides in insects involved in metabolism, spontaneous muscle contractions, growth, and reproduction. A great variety of processes in insects, including metabolic, behavioral, developmental, and reproductive functions, are known to be influenced or regulated by neuropeptides [1]. Peptides of adipokinetic and hypertrehalosemic are major neuropeptides in insects belonging to adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family. An adipokinetic or hyperlipemic effect means an increase in the hemolymph lipid concentration. A hypertrehalosemic effect means an increase in hemolymph carbohydrates. Adipokinetic and hypertrehalosemic peptides alter fat and carbohydrate metabolism. Approximately 30 peptides in this family are known, making it a largest family of peptides. These peptides consist from 8-10 amino acids and there is a pyroglutamate residue at N-terminal while there is an amide at the C-terminal [1].

Oxytocin is a neuropeptide structured hormone. Oxytocin causes uterus contraction, initiates labor, or enhances labor speed and stops delivery bleeding. Oxytocin consists of nine amino acids. Its systematic name is cysteine-tyrosine-isoleucine-glutamine-asparagine-cysteine-proline-leucine-glycine-amide (cys-tyr-ile-gln-asn-cys-pro-leu-gly-NH_2, or CYIQNCPLG-NH_2). The cysteine residues form a sulfur bridge [2]. Oxytocin exerts both peripheral and central actions. Oxytocin receptors play important role in the action mechanism of oxytocin. Oxytocin has a G-protein-coupled receptor. Oxytocin affects social recognition, anxiety, maternal behaviors, and various other behaviors [3].

Adipokinetic hormone has a similar compound structure and amino acid sequence compared to oxytocin. It possesses central behavioral effects [4,5]. Based on these studies, we investigated the peripheral effects of adipokinetic hormone on uterus myometrium contractions compared to oxytocin, which has both central and peripheral effects.

MATERIAL AND METHODS

Human Uterus Strips

Uterus strips removed from 21 women during C-section were used in this study. Ethical approval was granted by the Sakarya University Clinical Trial Ethics Committee (Date: 22.01.2015, Number 050.01.04.17, Sakarya/Turkey), the study has been carried out in accordance with the Declaration of Helsinki and also informed consent from the patients was obtained.

Drugs

Potassium chloride and oxytocin were obtained from Sigma (St Louis, Mo, USA). Anax imperator AKH (Ani-AKH), Libellula auripennis AKH (Lia-AKH) and Phormia terraenovae hypertrehalosemic hormone (Pht-HrTH) were purchased from TRC (Toronto/Canada). Potassium chloride, oxytocin, and AKHs were dissolved in 0.9% physiological saline. The drugs were freshly prepared each experiment day. There were 7 uterus strips in each experimental group.

Experimental Design

The uterus divided into strips of 1-2 cm, and then each strip was put in 20 mL organ bath containing uterus solution (6.78 g NaCl; 0.4 g KCl; 0.28 g $CaCl_2$; 0.23 g NaH_2PO_4; 1.86 g $NaHCO_3$; 2 g glucose in 1 l distilled water) equilibrated with 95% O_2/5% CO_2 at 37°C. Isometric force was measured by a isometric force transducer (FDT 10 A Commat Iletisim, Ankara, Turkey) and recorded on a computer via a four-channel transducer data acquisition system (MP150 Biopac Systems Inc. Goleta) using software (ACQ4.0 Biopac Systems Inc. Goleta). Each strip was equilibrated with a basal tension of 1 g for 1 h, with the uterus solution replaced every 15 min with fresh solution. Then the strips were depolarized with 80 mM KCl in Krebs solution and allowed to equilibrate for 30 min. After equilibration, the concentration-response curves of 7 uterus strips to Ani-AKH (Anax imperator AKH; 10^{-8}-10^{-4} M), Lia-AKH (Libellula auripennis AKH; 10^{-8}-10^{-4} M), and Pht-HrTH (Phormia terraenovae hypertrehalosemic hormone; 10^{-8}-10^{-4} M) were taken cumulatively. Lastly, concentration-response of 7 uterus strips to oxytocin (10^{-8}-10^{-4} M) were taken cumulatively. Following the ending of each drug concentration-response curve, the tissues were again washed for 30 min.

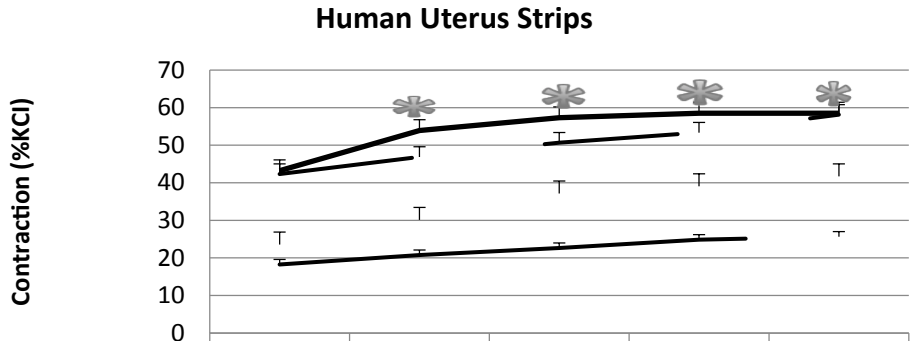
Statistical Evaluation

Results are calculated as mean ± SEM. Contraction of Ani-AKH, Lia-AKH, Pht-HrTH and oxytocin were calculated as percentage of the maximal contraction of KCl (80 mM). A non-parametric Kruskal-Wallis post hoc Dunn's test was performed as statistical analysis. $P<0.05$ was considered as significantly different.

RESULTS

Contraction of KCl was used as reference response of myometrium muscle. Contractile responses to Ani-AKH (10^{-8}-10^{-4} M), Lia-AKH (10^{-8}-10^{-4} M), Pht-HrTH (10^{-8}-10^{-4} M) and oxytocin (10^{-8}-10^{-4} M) were calculated as percentage of the maximal contraction caused by KCl (80 mM).

There was no significant difference between the contractions of Ani-AKH, Lia-AKH, or Pht-HrTH and those of oxytocin at 10^{-8} M doses of the drugs ($H=6.71$, $p=0.08$, Figure 1). There was significant difference between contractions of Ani-AKH, Lia-AKH, and Pht-HrTH and that of oxytocin at 10^{-7}, 10^{-6}, and 10^{-5} M doses of the drugs (respectively, $H=8.33$, $p=0.03$; $H=8.04$, $p=0.04$; $H=8.18$, $p=0.04$; Figure 1). Lia-AKH (10^{-7}-10^{-4} M) ($p<0.05$) significantly increased uterine contractions compared to oxytocin (10^{-7}-10^{-4} M) in human uterus strips.



	10-8 M	10-7 M	10-6 M	10-5 M	10-4 M
Ani-AKH	42.33	46.88	50.68	53.36	58.12
Lia-AKH	43.22	53.91	57.31	58.54	58.54
Pht-HrTH	23.57	30.13	37.17	39.07	41.73
Oxytocin	18.26	20.75	22.65	24.85	25.66

Figure 1 Contraction of human uterus strips after administration of Ani-AKH (10^{-8}-10^{-4} M), Lia-AKH (10^{-8}-10^{-4} M), Pht-HrTH (10^{-8}-10^{-4} M) and oxytocin (10^{-8}-10^{-4} M), which were calculated as percentages of the maximal contraction of KCl (80 mM). A Kruskal-Wallis test with a post hoc Dunn's test was used for statistical analysis. Data are indicated as the mean ± standard error of the mean (*p<0.05 compared to the oxytocin group)

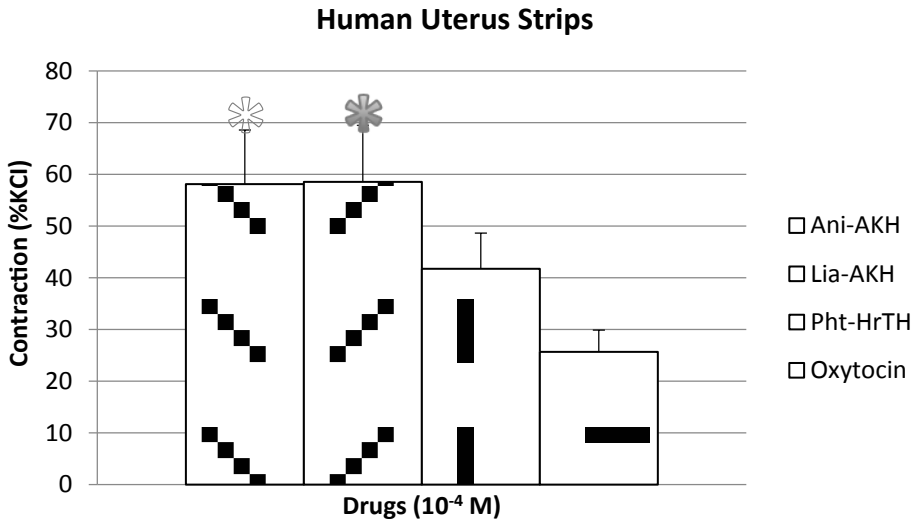


Figure 2 Contraction of human uterus strips after administration of Ani-AKH (10^{-4} M), Lia-AKH (10^{-4} M), Pht-HrTH (10^{-4} M) and oxytocin (10^{-4} M), which were calculated as percentages of the maximal contraction of KCl (80 mM). A Kruskal-Wallis test with a post hoc Dunn's test was used for statistical analysis. Data are indicated as the mean ± standard error of the mean (*p<0.05 compared to the oxytocin group)

There was a significant difference between the contractions of Ani-AKH, Lia-AKH, and Pht-HrTH and those of oxytocin at 10^{-4} M doses of the drugs (H=9.58, p=0.02; Figure 2). Both Ani-AKH (10^{-4} M) ($p<0.05$) and Lia-AKH (10^{-4} M) ($p<0.05$) significantly increased uterine contractions compared to oxytocin (10^{-4} M) in human uterus strips.

DISCUSSION

In this study, contractions of Ani-AKH, Lia-AKH and Pht-HrTH showed significant difference. AKHs elicited more contraction than oxytocin. These results show that the AKHs examined can be used to elicit uterus contraction in humans.

In recent studies, it was shown that oxytocin neurons may play a role in suppressing appetite and oxytocin neurons may control feeding and obesity [6]. Oxytocin also reduces fear by inhibiting amygdala [7] and exerts antidepressant

effects in animal models [8] and humans [9]. Adipokinetic hormone has a similar compound structure and amino acid sequence to oxytocin. Adipokinetic and hypertrehalosemic hormones regulate levels of energy metabolites, like trehalose (disaccharide) and diacylglycerol in the hemolymph [10]. These peptide hormones are secreted from corpora cardiaca which is the neuroendocrine gland attached to brain. Corpora cardiaca is similar to that of the hypothalamus and neurohypophysis of the vertebrate endocrine system [1,11]. Using a heterologous (in locusts and cockroaches) and a homologous bioassay, the neuropeptide pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-NH_2 was isolated from extracts of corpora cardiaca of the emperor dragonfly, Anax imperator [12]. In a recent study, we showed that the AKH/RPCH peptide family can be used in treatment of depression, anxiety, pain and locomotor disorders, and this peptide family exerts neuroprotective effects [4,5]. AKH seems to exert the same central and peripheral effects as oxytocin. It was also suggested adipokinetic hormone may induce weight loss by modulating lipid mobilization in human [13].

Oxytocin binds to G-proteins on the surface of myocytes and it forms diacylglycerol (DAG) and 1,4,5 inositol trisphosphate (IP_3) by phospholipase C. Then increased intracellular calcium binds to calmodulin and activate light chain myosin kinase and causes contraction of uterus smooth muscle [14]. AKH uses same pathways in the metabolization of carbohydrate and lipids. Adipokinetic and hypertrehalosaemic peptides control fat, carbohydrate, and protein metabolism in insects [1]. During the metabolization of lipids and carbohydrates by AKH, the endogenous AKHs bind to a G_q-protein-coupled receptor, activate phospholipase C and the resulting inositol trisphosphate releases Ca^{2+} from internal stores. In some of the insects adenylate cyclase and G_s protein-coupled receptor is also activated [15]. The effect mechanism of AKH on uterus contraction may be related to these second messenger pathways. Further studies should be done to explain the effect mechanisms of AKH on uterus contraction.

Birth injury and brain damage may be caused during birthing process as a result of difficult labor [16]. Cerebral palsy is one example of brain damage incurred before or during delivery. There is a continuous need for the development of new agents useful for uterine contraction to prevent complications that arise due to difficult delivery. The present study suggests the application of the adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family of peptides as alternative and useful agents for protection against birth injury and pregnancy complications.

CONCLUSION

In conclusion, AKH/RPCH family peptides may be used for uterine contraction to aid in aborting the fetus in cases of incomplete abortion or miscarriage, producing contractions during difficult labor, controlling bleeding after childbirth, and protecting against illness caused due to birth injury.

DECLARATIONS

Conflict of Interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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