Isolation And Characterization Of Fluorescent Derivatives Towards The Development Of A New Method For The Determination Of Hypothalamic Hormones

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The human endocrine system is an internal system that maintains the body's homeostasis by secreting chemical messengers known as hormones. This system consists mainly of endocrine glands, with the hypothalamus playing a central role. It is closely linked to the pituitary gland, and in addition to producing neurohormones that affect physiological functions, the hypothalamus produces two hormones of its own: oxytocin and vasopressin. These hormones are stored and secreted by the posterior lobe of the pituitary gland.

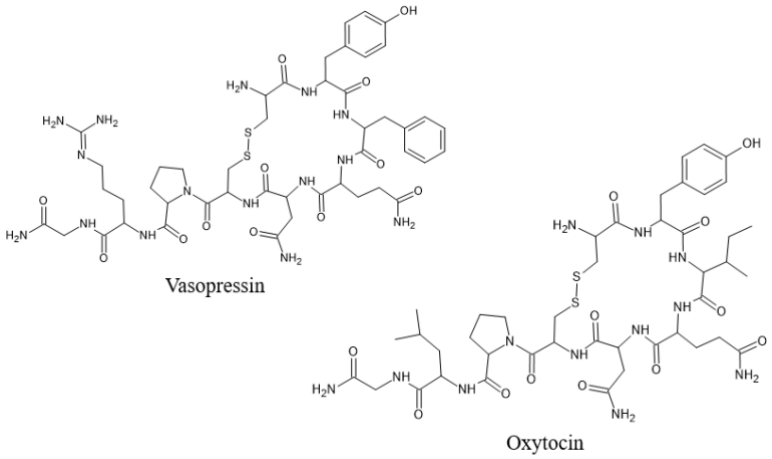

Figure 1 – Structural formulas of oxytocin and vasopressin.

Oxytocin plays a major role in regulating reproductive, perinatal, and maternal behaviors. It is secreted in response to stimulation of receptors in the cervix, vagina, and nipples and is responsible for contracting the uterus and the blood vessels within it during labor, as well as their constriction after labor.[1] In addition, reduced levels of this hormone are of great significance in patients with symptoms of depressive disorders.[2] Due to its properties, oxytocin is primarily used in medicine as one of the pharmacological methods for inducing labor. It is administered intravenously until effective uterine contractions are achieved.

Vasopressin is the primary hormone responsible for water retention in the body in all mammals. It is released when blood osmotic pressure rises, in order to stimulate the renal tubules to reabsorb more water and sodium ions from the urine. As a result, urine becomes more concentrated, and blood pressure and peripheral blood volume increase. In addition, vasopressin plays a role in electrolyte balance by increasing the reabsorption of sodium and potassium ions and causing vasoconstriction. A deficiency of vasopressin leads to so-called diabetes insipidus (excessive urine output), while an excess leads to SIADH (water retention).[3][4]

The concentrations of these hormones in the blood are very low, in the pg/ml range. Vasopressin concentrations range from 1 to 5 pg/ml, and in healthy, well-hydrated individuals, they may be undetectable.[5] Oxytocin levels in non-pregnant individuals range from 1 to 10 pg/ml, although values reported in the literature can vary by as much as 100 to 1,000 times.[6]

The use of these hormones in medicine necessitates the development of analytical techniques for their determination in biological fluids or medications. Combining chromatographic techniques with the conversion of the target hormones into fluorescent derivatives will increase the sensitivity and selectivity of the assays.

During the course of the study, screening experiments were conducted using cystine to evaluate the effects of various reducing agents and identify the most suitable disulfide bond reducer under the given reaction conditions. In the next step, new fluorescent derivatives of the target hormones were synthesized. Some of the compounds were successfully isolated from the mixture using preparative chromatography, and their fluorescent properties were confirmed using UV-VIS spectroscopy and a spectrofluorometer. The quantum yield was also calculated, and NMR analyses are planned for the next stage to precisely determine the structure.

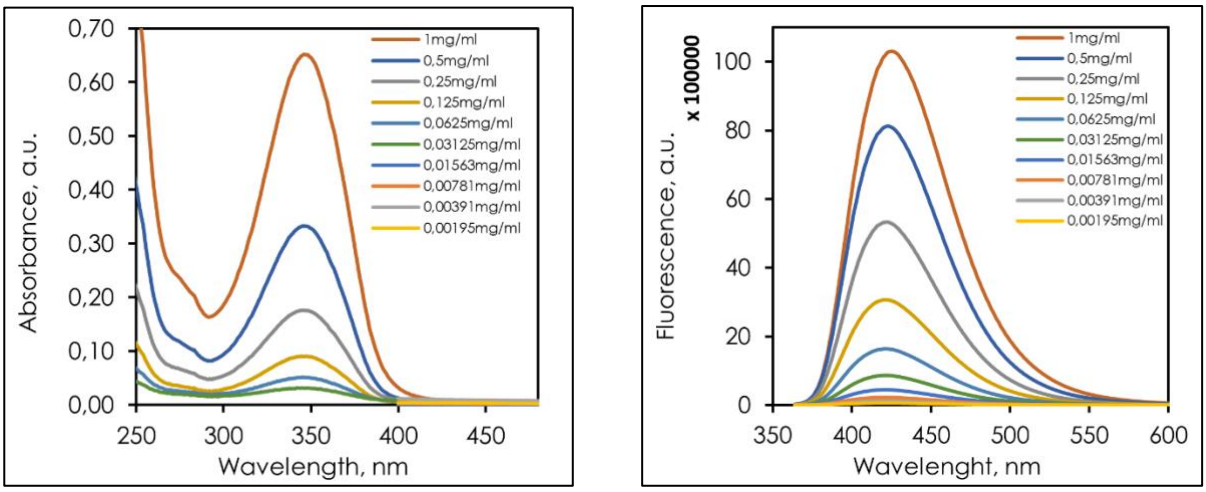

Figure 2 – Absorbance and fluorescence spectra of one of the fractions obtained from the reaction with oxytocin.

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