EFFECT OF OXYTOCIN HORMONAL IMPRINTING AND K-203 ACETYLCHOLINESTERASE REACTIVATOR ON BIOGENIC AMINES IN THE RAT CENTRAL NERVOUS SYSTEM

Ph.D Thesis

Seyed Farzad Hashemi Dolatabadi

Doctoral School of Pharmaceutical Sciences

Semmelweis University

Supervisor: Kornélia Tekes, Pharm.D, Ph.D, D.Sc

Official reviewers: Dr. Juhász Béla, Ph.D
Dr. Dalmadi Kiss Borbála, Ph.D

Head of Final Examination Committee: Éva Lemberkovics, Ph.D

Members of Final Examination Committee: Mahmoud Al-Khrasani, Ph.D
István Zupkó, Ph.D

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1. Introduction

Oxytocin as a hormonal imprinter: Oxytocin is a mammalian neurohypophysial hormone, which was discovered by British pharmacologist Sir Henry Dale in 1906. Oxytocin is produced in the paraventricular nuclei of the hypothalamus and stored in the posterior part of the neurohypophysis, but also in neurons projecting from the paraventricular nucleus and surrounding structures to extra hypothalamic brain areas (i.e., the septum, the ventral tegmental area, the hippocampus, the amygdala, the medulla oblongata and the spinal cord). Oxytocin has role in various behaviors, including numerous central functions such as sexual, maternal behaviour, social recognition, anxiety, memory, learning, stress and social behaviors. Most of oxytocin's roles are due to the cooperation with biogenic amines in different brain region. It has emerged that disturbance in peripheral and central oxytocin levels have been detected in some patients with dopamine dependent disorders. Thus, oxytocin is proposed to be a key neural substrate that interacts with central dopamine systems. Stress induced increases in sympathoadrenal release of adrenaline and noradrenaline, which increases heart and respiratory rate. Oxytocin administered cause reduce the rate and force of cardiac cells intrinsic contractions causing them to beat more slowly and contract less forcefully therefore the connection between the catecholamines and oxytocin was always important. Serotonin effect on the mood is through its association with influence on the release of oxytocin. Stimulation of the hypothalamus by serotonin has been shown to lead to release of oxytocin.

Investigation of biogenic amines roles in the mechanism of drug action, their relation with oxytocin, their relations with the developing hormone receptors (hormonal imprinting), and their possible alteration in neuropsychiatric disorders all require an accurate determination of biogenic amines.

Hormonal imprinting is a basic biological phenomenon, which was first observed, named, and defined in 1980 by Csaba. The phenomenon means that in the developmentally critical periods, animals or their cells memorize normally or pathologically this first encounter with a given hormone or related structures, and this determines the receptor's later binding capacity as well as the reaction of the imprinted cell to the hormone for life.

The present study is concerned with the effect of oxytocin on the biogenic amine levels of the adult rat brain.

K-203 as an acetylcholinesterase reactivator: Organophosphates (OPs) are widely used all over the world in agriculture (pesticides, insecticides, acaricides) and in chemical industry (softeners, additives to lubricants). In the terrorist attack were used as an organophosphate warfare agents at Tokyo metro station and during the Iraq-Iran war, therefore organophosphate poisoning is a constant danger in the agriculture, giving hundred thousands of fatal cases in each year.

Organophosphates are esters, amides or thiol derivatives of phosphoric, phosphonic, phosphinic acids, and phosphorothioic or phosphonothioic acid. The phosphonic acids derivatives are more toxic than the phosphinic acids. They are very lipophilic agents and acute exposure results in acute cholinergic crisis at muscarinic and nicotinic acetylcholine receptors (AChR) both in the central and the peripheral nervous systems. Organophosphates cause irreversible inhibition of cholinesterases via a covalent reaction with the serine in the active center of the enzyme. The therapy is known by the acronym "AFLOP" (atropine, fluid, oxygen and pralidoxime).

Pyridinium aldoximes such as pralidoxime and obidoxime are the only clinically available acetylcholinesterase enzyme reactivators (AChERs) applied to organophoshates poisoned persons. In the Department of Toxicology at the Faculty of Military Health Sciences, Defence University, Hradec Kralove, Czech Republic more than 500 asymmetric pyridinium aldoxime compounds (K-compounds, kukoximes) were synthesized. Following preliminary toxicological and *in vitro* effectiveness studies some of them were show to be used as acetylcholinesterase reactivators for the treatment of intoxication following exposure to tabun, soman and certain organophosphate pesticides. Oximes are well known to reactivate the inhibited/phosphylated acetylcholinesterase but after so many years of the discovery, no oximes found to be a broad spectrum and effective against different groups of organophosphorus anticholinesterases therefore requesting more investigation. The present study is concerned with the effect of K-compounds on the biogenic amine levels of the adult rat brain but for the better understanding AChE, organophosphorus compounds and oximes are being discussed here.

2. Aims and objectives

2.1. Optimize a sensitive bioanalytical method for determination of the biogenic amines and their metabolites by High Performance Liquid Chromatography electrochemical detection (HPLC-EC)

2.2. Determine the possible hormonal imprinting effect of oxytocin on the biogenic amine and their metabolite levels of the adult rat brain

2.3. Determine the effect of K-203 (AChERs), as a potential antidote in OPs intoxication on the biogenic amine and their metabolite levels of the adult rat brain

3. Materials and Methodes

3.1. Materials

Oxytocin acetate salt hydrate, dopamine hydrochloride, DOPAC (3-4-dihydroxyphenylacetic acid), homovanillic acid (HVA), serotonin hydrochloride (5-HT), 5-hydroxy-3-indole acetic acid (5-HIAA), 5-hydroxytriptophol (5-HTOL), phosphoric acid (H_3PO_4), disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 12H_2O$), citric acid monohydrate, l-octane sulfonic acid sodium salt and Na_2EDTA were from Sigma-Aldrich (Steinheim, Germany). Acetonitrile was from Merck (Darmstadt, Germany) and perchloric acid 70% (PCA) was from Fluka (Buchs, Switzerland). K-203 (E-1-(4-carbamoylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide) was synthesized and kindly donated by K. Kuca (Department of Toxicology, Faculty of Military Health Sciences, Defence University, Hradec Kralove, Czech Republic).

Samples were analyzed by reversed phase high-performance liquid chromatography with amperometric/electrochemical detection (HPLC–EC) consisting from a Jasco pump (PU1580, Tokyo, Japan) equipped with a DG-2080-54 four-line degasser, an AS 2057 Plus Automatic injector and connected to an Intro digital amperometric (Antec, Leyden, Zoeterwoude, Netherlands) detector operated at E_{ox}=+0.65 V with a sensitivity of 1-10 nA/V with a time filter of 1.0 sec, and JMBS Hercule 2000 Chromatography Interface (Le Fontanil, France). Standard temperature of the column was 25±0.15 °C.

Chromatograms were electronically stored and evaluated using Borwin 1.50 Chromatography Software (JMBS, Le Fontanil, France). The separations were done using a Zorbax RX-C18 4.6x12.5 mm (5-μm) pre-column and a 4.6x250 mm, 5-μm Zorbax RX-C18 octadecyl silica column (Agilent Technologies, supplied by Kromat Kft, Budapest, Hungary). For the serial determinations of the biogenic amines the mobile phase contained 56.2 mmol/L Na_2HPO_4, 47.9 mmol/L citric acid, 0.027 mmol/L Na_2EDTA, 0.925 mmol/L octane sulfonic acid sodium and 75:925 mL acetonitrile/phosphate buffer for hormonal imprinting oxytocin treatment and 65:935 mL acetonitrile/phosphate buffer for K-203 treatment. The pH was adjusted to 3.7 with 85% phosphoric acid (H_3PO_4) (inoLab pH Level 2, WTW GmbH, Germany). The flow rate of the mobile phase was 1 mL/min. The limit of quantitation (LOQ) was determined at a signal to noise ratio of 10. The limit of detection (LOD) was determined at a signal to noise ratio of 3.

3.2. Experimental animals

During all the experiments, the guiding principles in the care of and use of laboratory animals have been observed. All experimental procedures conformed to 86/509/EEC regulation on the well-being of experimental animals, and the experimental protocol was approved by the local ethical committee (permission No: 1806/007/2004 ANTSZ, Budapest, Hungary).

3.3. Treatments

Treatments of rats with oxytocin for hormonal imrinting: Wistar rats of our (Charles River originated) closed breeding colony were housed at room temperature under normal light cycle and food and water were available *ad libitum*. Offsprings were treated subcutaneously (sc.) with a single dose of 5 mg/kg oxytocin (dissolved in saline) when they were one day old. When the off-spring were 4 months old, they were exsanguinated through the canthus under ether anesthesia and 8 regions of the brain (front cortex, FC; hypothalamus, HT; hippocampus, HC; striatum, ST; medulla oblongata, MO; cerebellum, CB; spinal cord, SC; and truncus cerebri, TC) were dissected on a 0 °C aluminum surface according to the method of Paxinos G. and Watson C. (1998) and Palkovits M. (2001). The samples were kept frozen at - 80 °C.

Treatments of rats with K-203: Rats were injected in 0.2 mL volume intramuscularly (i.m) with 50 μmol of K-203 freshly dissolved in distilled water. The control group received an equal volume (0.2 mL) of solvent treatment. Five rats were used for each data point. Rats were sacrificed by decapitation 15 or 60 min following treatment. Seven regions of the brain (FC, HT, HC, ST, MO, CB and SC) were dissected and immediately placed on an ice-cold aluminum surface according to the method of Paxinos G. and Watson C. (1998) and Palkovits M. (2001). All samples were kept frozen at -80 °C.

3.3. Sample preparation

Sample preparation in the oxytocin-experiment: The brain samples were homogenized in 5 volumes of 0.8 mol/L ice cold perchroric acid (PCA) and serum samples were homogenized in 10 volumes of 0.8 mol/L PCA by an Ultra Turrax T25 Janke&Kunkel homogenizer at 20,000 rpm for 20 seconds (IKA Labortechnik, Staufen, Germany), then the brain samples were centrifuged in an Eppendorf centrifuge (A. Hettich, Tuttlingen, Germany) at 14,000 rpm for 10 min at 4 °C and the serum samples for 20 min at 4 °C. The supernatants gained were used for HPLC analysis. The samples were injected in 50 μL into the HPLC. Samples were kept at -80 °C before their analysis.

Sample preparation in the K-203-experiment: Brain samples were homogenized in 4 volumes of 0.8 mol/L ice cold PCA by an Ultra Turrax T25 Janke&Kunkel homogenizer at 20,000 rpm for 20 seconds and after centrifuged in an Eppendorf centrifuge at 14,000 rpm for 10 min at 4 °C and the supernatants were used for HPLC analysis. The samples were injected in 50 μL into the HPLC. Samples were kept at -80 °C before their analysis.

4. Results

4.1. Oxytocin hormonal imprinting measurement

Norepinephrine (NA): There is a significant difference in norepinephrin tissue levels between the control males and females in case of the blood serum, hippocampus, and frontal cortex in favour of the females. Oxytocin-treated male samples contain higher norepinephrine neurotransmitter levels in the frontal cortex and cerebellum, while there is a decrease in the medulla oblongata.

Epinephrine: There is no significant difference in epinephrine tissue levels in the blood serum and different parts of brain region (hypothalamus; medulla oblongata; striatum) between the control and oxytocin-treated animals.

Dopamine (DA): There is a significant difference between the male and female blood serum dopamine content in favour of males. Treated males serum contains significantly less dopamine, then the control ones. However significantly elevated dopamine level is present in the frontal cortex in oxytocin-treated females.

DOPAC (3, 4-Dihydroxyphenyl acetic acid): There is a significant difference between the male and female DOPAC content in the hypothalamus in favor of males. Less DOPAC was found in male and female serum, hypothalamus, spinal cord, male striatum, and female truncus oxytocin-treated rats compared to the control ones

HVA (homovanillic acid): There is a significant difference in homovanillic acid tissue levels between the control and oxytocin-treated animal serum, male hippocampus, hypothalamus, striatum, and truncus. Control female truncus contains less homovanillic acid, than control male

5-HIAA (5-hydroxyindole acetic acid): There is a significant difference in 5-HIAA tissue levels between the male and female control sera in favor of females. Oxytocin-treated male 5-HIAA values are less in hippocampus, hypothalamus, spinal cord and striatum as well as in female serum, hypothalamus, and medulla oblongata. The female serum value is higher than male in the controls.

5-HTOL (5-hydroxytryptophol): There is a significant decrease in 5-HTOL, tissue level in the hypothalamus in both males and females in control and oxytocin-treated animals and a lower level 5-HTOL in the male oxytocin-treated in medulla oblongata

Serotonin (5-HT): There is a significant difference in the serotonin tissue levels between the oxytocin-treated male and female sera in favor of females. In the hypothalamus and the striatum of females, the serotonin tissue level was higher than the female control values.

Figure 1 show the representative chromatograms of biogenic amines (DA and 5-HT) and their metabolites (DOPAC, HVA and 5-HIAA) determinations from the oxytocin hormonal imprinted rat brain and serum.

Table 1, Summarized data on the effect of oxytocin hormonal imprinting on biogenic amines and their metabolites level:

Biogenic Amine		Serum	HC	HT	MO	SC	FC	CB	ST	TC
DA	m	↓	nda	ns	ns	ns	ns	nda	ns	ns
	f	ns	nda	ns	ns	ns	↑	nda	ns	↓
DOPAC	m	↓	ns	↓	ns	↓	ns	nda	↓	ns
	f	↓	ns	↓	ns	↓	ns	nda	ns	↓
HVA	m	↓	↓	↓	ns	ns	ns	ns	↓	↓
	f	↓	ns	ns	ns	ns	ns	ns	ns	ns
5-HIAA	m	↑	↓	↓	ns	↓	ns	ns	↓	ns
	f	↓	ns	↓	↓	ns	ns	ns	ns	ns
5-HTOL	m	nda	ns	↓	↓	ns	ns	nda	nda	ns
	f	nda	ns	↓	ns	ns	ns	nda	nda	ns
5-HT	m	ns	nda	ns	ns	ns	nda	nda	ns	ns
	f	↑	nda	↑	ns	ns	nda	nda	↑	ns
NA	m	ns	ns	ns	↓	ns	↑	↑	ns	ns
	f	ns	ns	ns	ns	ns	↑	ns	ns	ns

Table 1. Effect of oxytocin hormonal imprinting on biogenic amines and their metabolites level, Abbreviations: f - female, m - male, ↑- significant ($p < 0.05$) increase, ↓- significant ($p < 0.05$) decrease, ns – no significant change, nda – no data available. Hippocampus, HC; hypothalamus, HT; medulla oblongata, MO; spinal cord, SC; frontal cortex, FC; cerebellum, CB; striatum, ST; truncus cerebri, TC.

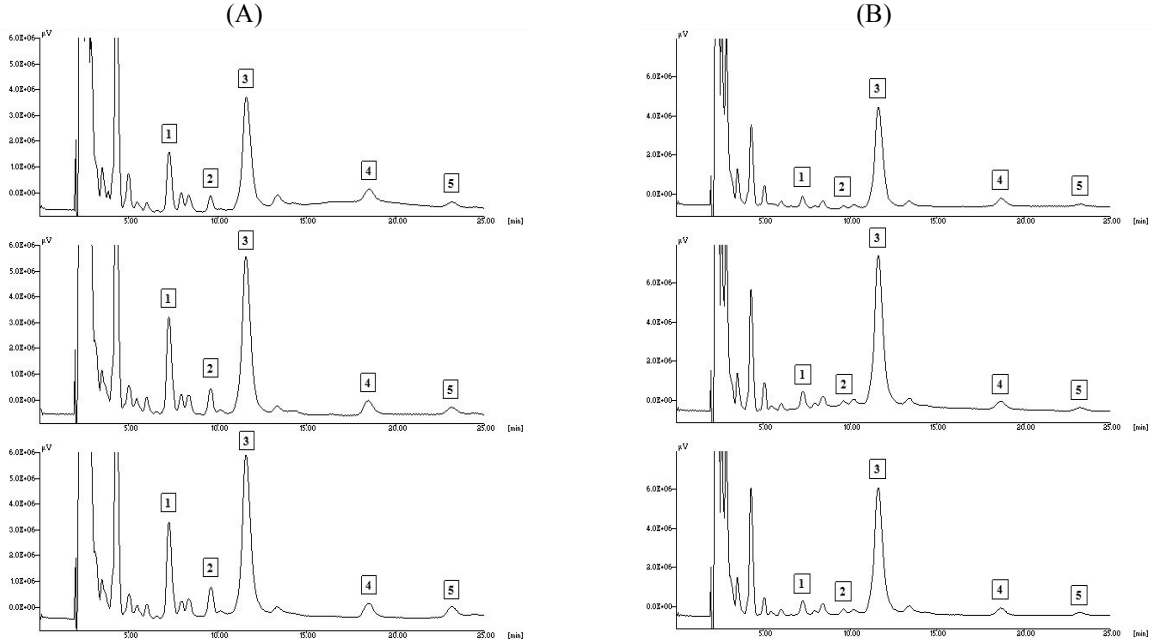

Figure 1. Representative chromatograms of biogenic amines and their metabolites (1: DOPAC; 2: dopamine; 3: 5-HIAA; 4: HVA and 5: 5-HT) tissue level from the rat brain (A) and serum (B) (upper chromatogram: control animal, on middle chromatogram: spiked and on bottom chromatogram: oxytocin treated rats).

4.2. K-203 treated rats

Figure 2 show the representative chromatograms of biogenic amines (DA and 5-HT) and their metabolites (DOPAC, HVA and 5-HIAA) tissue levels that were obtained from the K-203 treated rats brain, Cerebrospinal fluid (CSF) and serum.

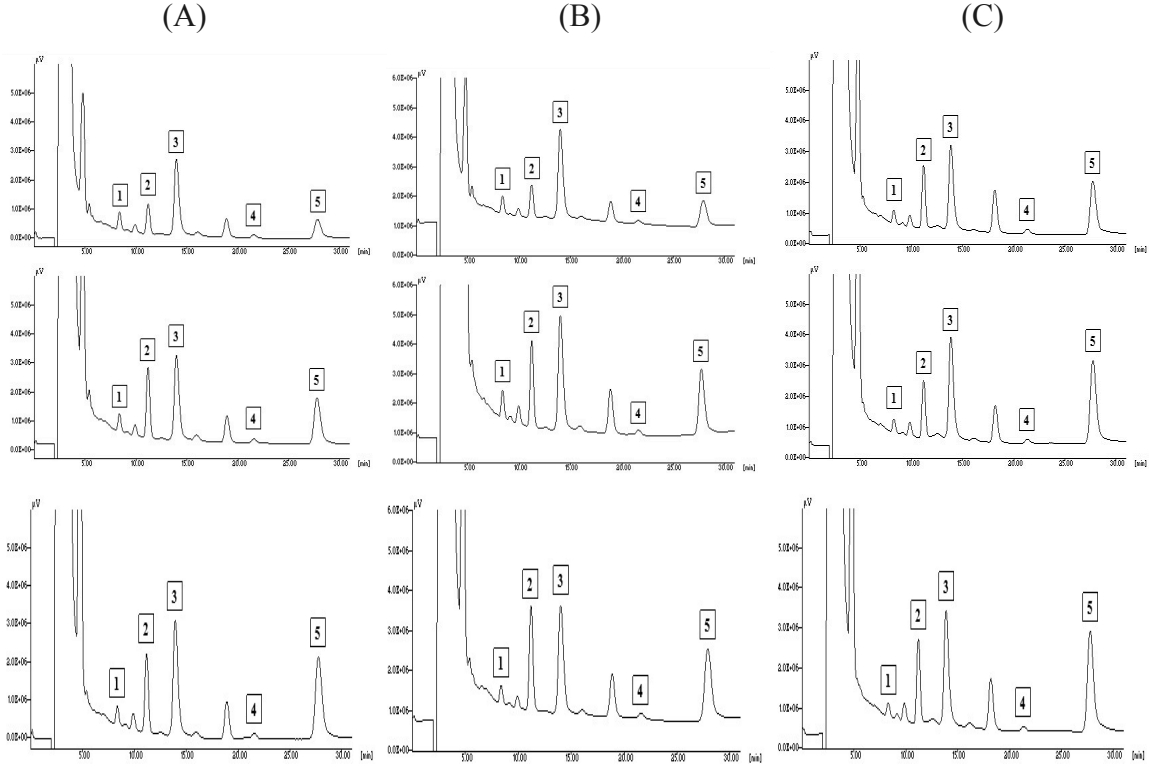

Figure 2. Representative chromatograms of biogenic amines and their metabolites (1: DOPAC; 2: dopamine; 3: 5-HIAA; 4: HVA and 5: 5-HT) tissue level from the rat brain (A), CSF (B) and serum (C) (upper chromatogram: control animal, on middle chromatogram: spiked and on bottom chromatogram: K-203 treated rats).

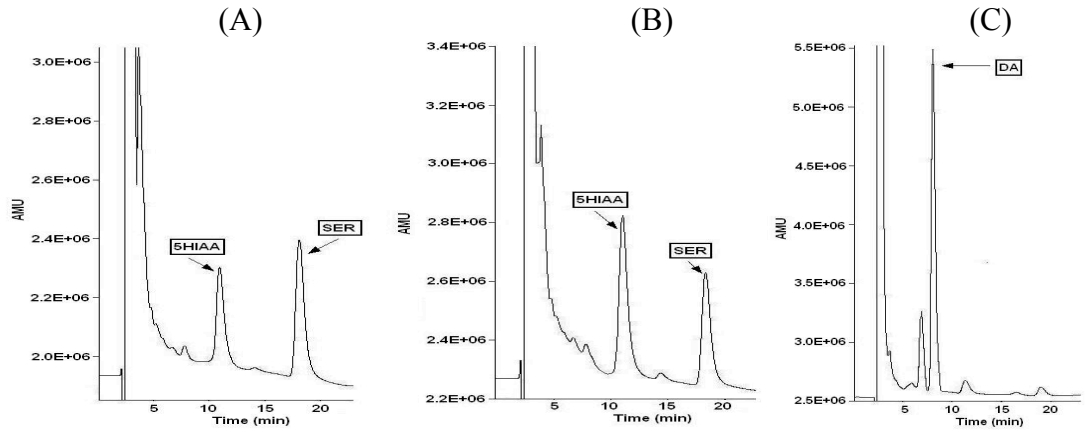

Figure 3. Representative chromatogram of serotonin (SER) and 5-HIAA levels in the spinal cord sample following 15 (A) and 60 min (B) and dopamine level in the striatum sample of rat following 60 min (C) of K-203 (50-µmol, i.m) treatment.

Table 2 summarizes biogenic amines (DA and 5-HT) and their metabolites (HVA and 5-HIAA) tissue levels in the different brain areas (FC, HT, HC, ST, MO, CB and SC) studied 15 and 60 min following 50-μmol intramuscular K-203 injections.

	Tissue level			
Rat brain area	**5-HT (ng/g ± SD)**	**5-HIAA (ng/g± SD)**	**DA (ng/g± SD)**	**HVA (ng/g± SD)**
Cerebellum				
Control	21.33±4.24	114.1±8.97	16.23±0.62	< LOQ(1ng/g)
K203				
15 min	42.82±11.0	125.1±11.1	17.43±1.09	
60 min	24.62±6.87	197.0±26.7	16.16±1.55	
Spinal cord				
Control	489.4±70.8	342.7±19.5	30.03±4.88	< LOQ(1ng/g)
K203				
15 min	620.9±31.4	358.1±24.4	36.07±4.28	
60 min	440.1±11.1	426.2±62.1	33.85±2.35	
Hippocampus				
Control	32.17±5.65	170.4±34.3	13.04±0.31	< LOQ(1ng/g)
K203				
15 min	52.08±8.55	177.1±10.8	14.07±0.82	
60 min	37.50±10.4	186.5±7.60	14.11±0.94	
Hypothalamus				
Control	108.2±10.3	263.0±64.1	65.24±8.52	< LOQ(1ng/g)
K203				
15 min	170.9±35.7	265.7±19.5	91.98±28.5	
60 min	132.8±37.4	260.0±50.6	63.34±26.7	
Striatum				
Control	61.64±23.2	173.3±39.3	1493±529	285.9±69.3
K203				
15 min	86.47±14.0	179.9±12.9	1844±136	220.0±17.0
60 min	70.79±27.4	193.6±9.77	1683±413	277.8±59.6
Medulla oblongata				
Control				
K203	145.5±22.3	201.8±53.42	22.25±2.56	< LOQ(1ng/g)
15 min				
60 min	167.1±29.5	194.6±29.4	24.68±4.36	
	121.0±35.1	184.2±38.7	21.76±3.15	
Frontal cortex				
Control	22.46±9.04	154.2±23.5	15.56±1.36	< LOQ(1ng/g)
K203				
15 min	51.71±15.6	146.4±5.57	16.83±1.83	
60 min	52.59±37.7	155.0±17.8	23.48±19.1	

Table 2. Effect of K-203 on the biogenic amine and their metabolites tissue levels in rat brain areas.
Each value represents data from five animals (n=5). (Hashemi et al. 2013)

As serotonin and 5-HIAA tissue levels showed a consistent changing tendency, we calculated the 5-HIAA/5-HT ratios (known as a measure for turnover) in all the brain areas (Table 3).

	5-HIAA/5-HT (mean ± SD)			
Rat brain area	**Control**	**15 min**	**60 min**	**P-value**
Cerebellum	11.26 ± 4.20	4.73 ± 1.73*	10.36 ± 4.23	0.015
Spinal cord	0.84 ± 0.22	0.70 ± 0.05	0.97 ± 0.26	0.206
Hippocampus	9.08 ± 3.73	4.90 ± 0.69*	8.37 ± 2.94	0.040
Hypothalamus	3.13 ± 0.74	2.05 ± 0.67*	2.60 ± 0.56	0.043
Striatum	4.39 ± 2.04	2.79 ± 0.43	4.39 ± 2.09	0.126
Medulla oblongata	1.78 ± 0.58	1.47 ± 0.16	2.44 ± 1.38	0.238
Frontal cortex	12.09 ± 6.16	3.57 ± 0.63*	4.41 ± 1.77+	* 0.036 + 0.031

Table 3. Serotonin turnover in rat brain areas following K-203 50-μmol i.m administration. The P< 0.05 was considered significantly different. *: compared to control; +: compared to 15 min value. Each value represents data from five animals (n=5). (Hashemi et al. 2013)

4.3. Calibration curve determination

The calibration sequences from stock solutions (100 μg/mL) were prepared at a concentration of at least 8 and measurements were replicated 3 times in the range of 2-200 ng/mL for oxytocin hormonal imprinting experiment and in the range of 10-200 ng/mL for K-203 experiment. Solvent used in the sample preparation was 0.8 M PCA solution and for spiked chromatograms the supernatant originating from the brain and serum samples gained from vehicle-treated controls were used. From all the areas of the peaks as a function of concentration the least squares method (Microsoft Excell 2003) was used. Calibration curves for all noradrenaline, adrenaline, DA, DOPAC, 5-HIAA, HVA, 5-HT, and 5-HTOL were linear.

5. Conclusion

The following conclusions may be drown from the present research work:

>> The developed and optimized RP-HPLC method using electrochemical (EC) detection is a valuable bioanalytical method to determine biogenic amines and their metabolites from different rat brain areas following neonatal oxytocin hormonal imprinting and a single dose of K-203 treatment.

>> The single dose of 5 mg/kg oxytocin treatment (hormonal imprinting) of neonates, after 4 months, strongly, permanently and in brain region specific manner influenced the adult level of biogenic amines (noradrenaline, adrenaline, dopamine and 5-HT) and their metabolites (DOPAC, 5-HIAA, HVA and 5-HTOL). The hypothalamus and striatum are the most sensitive to the effect of neonatal oxytocin imprinting.

>> Oxytocin is a strong imprinter, which interacts with the brain dopamine and serotonin systems influencing emotional and social behavior. Our data may indicate impact of oxytocin on the development of such diseases, as autism spectrum disorder. Considering the strong and late manifesting effect of hormonal imprinting, the single oxytocin treatment of neonates may explain the symptoms that are characteristic to diseases.

>> Studying the effect of K-203, a potential antidote in organophosphate poisoning on the biogenic amines and their metabolites levels in seven brain areas of rat in a dose (50-μmol) and by the proposed type of administration (i.m) it can be concluded, that K-203 is a safe potential antidote. Its effect on the 5-HT and dopamine metabolism is brain-area specific and transient. In line with literature data it can be summarized, that K-203 has much higher antidotal effectiveness than any other pyridinium aldoximes used hitherto and milder side effects than other pyridinium aldoximes.

6. Summary

1. Oxytocin is a mammalian neurohypophysial hormone and has role in various behaviors, including numerous central nervous system functions such as sexual, maternal behaviour, social recognition, anxiety, memory, learning, stress and social behaviors. Most of oxytocin's roles are due to the cooperation with biogenic amines in different brain regions.
Our results show that the single dose of 5mg/kg oxytocin treatment to rat neonates has a significant hormonal imprinting effect resulting in strongly and permanently influenced adult level of biogenic amines in different brain regions.
The hypothalamus is the most sensitive to imprinting followed by striatum.
Literature data unanimously show that there are diseases that are in close connection with the metabolism of dopamine and serotonin in the hypothalamus and striatum (Parkinson disease, autism, schizophrenia), and aggressive behavior is influenced by brain serotonin metabolism. It is supposed that oxytocin is a key molecule, which interacts with the brain dopamine and serotonin system, therefore it can be established that perinatal oxytocin treatment seems to be a serious factor.

2. As organophosphate poisoning is a constant danger and the therapeutic usefulness of currently available antidotes is low and is still a matter of controversy. There is a permenant need for more effective broad-spectrum antidotes. K-203 is a newly synthesized bispyridinium monoaldoxime type antidote with low toxicity and superior *in vitro* potency for use in organophosphate poisoning, especially in the case of tabun-poisoned.
Measuring the effect of K-203 on the biogenic amines and their metabolites in the different brain areas of the rat brain in a dose (50-μmol) and by the proposed type of administration (i.m) we can conclude that the effect of K-203 on the 5-HT and dopamine metabolism is brain-area specific and transient. The K-203 can be evaluated as an effective and safe antidote in organophosphate intoxication with effective acetylcholine-esterase reactivator activity.

6.1. Összefoglalás

1. Az oxytocin, mint a neurohipofízis nonapeptid hormonja emlősökben számos viselkedési forma (nemi funkciók, anyai gondoskodás, a fajtársak felismerése, félelmi reakciók, memória, tanulási képesség, stressz-válasz, társas kapcsolatok) központi idegrendszeri szabályozásában is fontos szerepet játszik.Ezek a sokrétű hatások döntően a biogénaminok anyagcseréjének befolyásolása útján valósulnak meg. Vizsgálatainkban kimutattuk, hogy újszülött patkányok egyszeri 5 mg/kg oxytocinnal történő kezelése jelentős hormonális „imprinting" hatású, mellyel tartós és jelentős valamint régiószelektív változást okoz a felnőttkori agyi biogénamin anyagcserében. A hormonális imprintig a nyolc vizsgált agyterület közül legerőteljesebben a hypothalamus és a striatum dopamin és szerotonin anyagcseréjét befolyásolja. Irodalmi adatok egyértelműen bizonyítják, hogy a hypothalamus és a striatum dopamin és szerotonin anyagcseréjének zavarával jellemezhetőek olyan betegségek, mint pl. a Parkinson kór, autizmus, skizofrénia valamint a szerotonin anyagcsere zavara fontos szereppel bír az aggresszív viselkedésforma létrejöttében. A kísérletes adatok tükrében az oxytocin kulcsszerepet játszik a dopamin és a szerotonin anyagcsere szabályozásában, ezért perinatális oxytocin-kezelés esetén súlyos következményekkel lehet számolni.

2. Az organofoszfátokkal történő véletlen és szándékos mérgezések súlyossága és gyakorisága valamint a klinikai gyakorlatban hozzáférhető antidótumok csekély terápiás értéke miatt az új, nagyhatékonyságú és az organofoszfátok széles skálájával szemben is alkalmazható acetilkolineszteráz- reaktivátor antidótumok kutatása nagy erőkkel folyik. A többszáz közelmúltban szintetizált vegyület közül a bispyridinium monoaldoxim szerkezetű K-203 kiemelkedő *in vitro* hatékonyságot mutat különösen tabun-mérgezéssel szemben és csekély önálló toxicitású. Kimutattuk, hogy a K-203 további előnyös tulajdonságai közé tartozik az is, hogy terápiásan hatékony mennyiségben bejut a központi idegrendszerbe is. Vizsgálatainkban a terápiás alkalmazást modellezve (50-μmol, i.m.) megállapítottuk, hogy a K-203 a központi idegrendszeri dopamin és szerotonin anyagcserére csak átmeneti és gyenge hatást fejt ki. A K-203 vizsgálataink tükrében egy igen hatékony és biztonságos acetilkolineszteráz reaktiváló antidótumnak minősül organofoszfátok okozta mérgezés esetén.

7. Publications

7.1. Publications related to the thesis

1. Kalasz H, Nurulain SM, Veress G, Antus S, Darvas F, Adeghate E, Adem A, **Hashemi F** and Tekes K. (2015) Mini review on blood-brain barrier penetration of pyridinium aldoximes. Journal of Applied Toxicology, 35(2): 116-123. **IF: 3.174**

2. **Hashemi F**, Laufer R, Szegi P, Csomor V, Kalasz H and Tekes K. (2014) HPLC determination of brain biogenic amines following treatment with bispyridinium aldoxime K203. Acta Physiologica Hungarica, 101(1): 40-46. **IF: 0.747**

3. **Hashemi F**, Tekes K, Laufer R, Szegi P, Tothfalusi L and Csaba G. (2013) Effect of a Single Neonatal Oxytocin Treatment (Hormonal Imprinting) on the Biogenic Amine Level of the Adult Rat Brain: Could Oxytocin-Induced Labor Cause Pervasive Developmental Diseases? Reproductive Sciences, 20(10): 1255-1263. **IF: 2.179**

4. Nurulain S, Kalász H, Szegi P, Kuca K, Adem A, Hasan M, **Hashemi F** and Tekes K. (2013) HPLC analysis in drug level monitoring of K027. Acta Chromatographica, 25(4): 703-710. **IF: 0.485**

7.2. Other publications

1. Tekes K, Tariq S, Adeghate E, Laufer R, **Hashemi F**, Siddiq A and Kalasz H. (2013) Nociceptinergic system as potential target in Parkinson's disease. Mini-Reviews in Medical Chemistry, 13(10): 1389-1397. **IF: 3.186**

2. Tekes K, Szegi P, **Hashemi F**, Laufer R, Kalasz H, Siddiq A and Ertsey C. (2013) Medicinal Chemistry of Antimigraine Drugs. Current Medicinal Chemistry, 20(26): 3300-3016. **IF: 3.715**

3. Ram N, Kalász H, Adeghate E, Darvas F, **Hashemi F** and Tekes K. (2012) Medicinal Chemistry of Drugs with Active Metabolites (N-, O-, and S-desalkylation and Some Specific Oxidative Alterations). Current Medical Chemistry, 19(33): 5683-5704. **IF: 4.070**

4. Csomor V, **Hashemi F**, Laufer R, Szegi P, Hantos M, Kalász H and Tekes K. (2012) Gyógyszerek okozta ízérzékelési zavarok. Gyogyszereszet, 56(11): 645-649.

5. Tekes K, **Hashemi F**, Szegi P, Sótonyi P, Laufer R and Kalász H. (2011) Prodrugs and active metabolites among antidepressive compounds. Neuropsychopharmacologia Hungarica, 13(2): 103-110.

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