THE ROLE OF THE SELECTIVE CRF RECEPTOR AGONISTS AND ANTAGONISTS IN NICOTINE ADDICTION

Ph.D. Thesis

Summary

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2019

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ABBREVIATIONS

[^{3}H] = tritium
ACTH = adrenocorticotropic hormone
AVP = arginine-vasopressin
BNST = bed nucleus of the stria terminalis
CeA = central nucleus of the amygdala
CNS = central nervous system
CRF = corticotropin-releasing factor
CRF-BP = corticotropin-releasing factor-binding protein
CRFR1 = corticotropin-releasing factor receptor type 1
CRFR2 = corticotropin-releasing factor receptor type 2
CRFRs = corticotropin-releasing factor receptors
CRH = corticotropin-releasing hormone
DSM-5 = Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
EWN = Edinger-Westphal nucleus
GABA = gamma-aminobutyric acid
GI = gastrointestinal
GPCR = G protein-coupled receptor
HPA = hypothalamic-pituitary-adrenal
IBD = inflammatory bowel diseases
IBS = irritable bowel syndrome
ICV = intracerebroventricular
IP = intraperitoneal
LC = locus coeruleus
NA = noradrenaline
NAcc = nucleus accumbens
nAChR = nicotinic acetylcholine receptor
PVN = paraventricular nucleus
SC = subcutaneous
SNS = sympathetic nervous system
UCN = urocortin
VTA = ventral tegmental area

1. INTRODUCTION

1.1. CRF and CRF receptors

Corticotropin-releasing hormone (CRH) or corticotropin-releasing factor (CRF) is a 41 amino acid neuropeptide. It is the major neurohormone that stimulates the release of adrenocorticotropic hormone (ACTH), along with the synergistic arginin-vasopressin (AVP), both being released from the paraventricular nucleus of the hypothalamus (PVN). CRF is also an important extrahypothalamic neurotransmitter released from the medulla oblongata and the central nucleus of the amygdala (CeA). Thus, CRF is expressed mainly in the central nervous system (CNS), but it is also found in the periphery: in the gastrointestinal (GI) tract, skin, and adrenal gland. The main role of CRF is to mediate the neuroendocrine, autonomic and behavioral stress responses. The neuroendocrine response is represented by the activation of the hypothalamic-pituitary adrenal (HPA) axis that is mediated by CRF released from the PVN in order to stimulate the release of ACTH from the anterior pituitary and the subsequent release of glucocorticoids from the adrenal cortex. The autonomic response is represented by the activation of the sympathetic nervous system (SNS) that is mediated by CRF released from the medulla oblongata which, in turn, stimulates the locus coeruleus (LC) noradrenaline (NA) system, and eventually the release of adrenaline from the adrenal medulla. The behavioral response is manifested by the increased locomotor activity, decreased food and water intake, decreased social and sexual activity which are mediated among others by amygdalar CRF.

The actions of CRF are mediated via two different CRF receptors (CRFRs), CRFR1 and CRFR2, which belong to the class B subtype of G protein–coupled receptor (GPCR) superfamily and they have several splice variants expressed on the surface of various tissues. CRFR1 has α and β isoforms in addition to subtypes designated c-h, which have been detected in rodent and human. CRFR2 is expressed in three functional subtypes, α, β, and γ. CRFR2α and CRFR2β have been detected in rodents, primates and humans, but CRFR2γ has only been reported in humans. CRFR1 is expressed more abundantly in the CNS, whereas CRFR2 is more dominant in the periphery. In the CNS CRFR1 is distributed throughout the cerebral cortex, cerebellum, olfactory bulb, medial septum, hippocampus, amygdala, and anterior pituitary. CRFR2 is limited centrally to

subcortical regions, such as the lateral septum, hippocampus, amygdala, hypothalamus, and posterior pituitary. Both CRFRs are expressed in the ventral and dorsal striatum. Classically, it was presumed that CRFR1 and CRFR2 promote antagonistic effects in the CNS, since activation of CRFR1 induced activation of the HPA axis, anxiety, depression, and locomotor hyperactivity, whereas activation of CRFR2 produced anxiolytic, antidepressant, and locomotor suppressive effects. Recently, it was proposed that the role of CRFR1 and CRFR2 in the stress response is not a matter of simple dualism, but it depends upon the brain regions and neuron populations being activated.

1.2. CRF receptor agonists

Since CRF has been isolated, a growing family of CRF-like peptides, termed urocortins were discovered. Today the mammalian family of CRF-related peptides consists of three ligands: urocortin I (UCN I), urocortin II (UCN II), and urocortin III (UCN III), two receptors: CRFR1 and CRFR2, and one binding protein: CRF-BP. The urocortins have similar chemical structures, but different anatomical distribution, pharmacological properties and physiological functions compared to CRF. CRF is expressed predominently in the PVN and the CeA, but it was also found in the periphery: the GI tract, skin, and adrenal gland. In contrast, UCN I is expressed prominently in the Edinger–Westphal nucleus (EWN), rostroventral midbrain, supraoptic nucleus of the hypothalamus and superior lateral olive. In the periphery UCN I was shown in the GI tract, testis, cardiac myocytes, thymus, skin, and spleen. UCN II is expressed in the PVN, arcuate nucleus of the hypothalamus and LC. In the periphery UCN II was found in the heart, blood cells, and adrenal gland. The third member of the urocortin family, UCN III is expressed in the forebrain regions, the preoptic nucleus and perifornical region of the hypothalamus and medial amygdala. While in the periphery UCN III was found in the GI tract and pancreas.

CRF has tenfold higher affinity for CRFR1 than for CRFR2, while UCN I show equal affinity for both CRFRs. In contrast, UCN II and UCN III have 1000 fold higher affinity for CRFR2, therefore they are considered selective agonists of CRFR2.

Central administration of CRF and UCN I induced activation of the HPA axis, anxiety-like and depression-like behavior, while central administration of UCN II and UCN III produced anxiolytic and antidepressant actions. Accordingly, activation of the CRFR1, expressed predominantly in the cerebral cortex and the anterior pituitary, is believed to initiate the endocrine, autonomic and behavioral reactions to stress, whereas activation of the CRFR2, expressed centrally in hypothalamus and the lateral septum, is thought to terminate these stress responses. Nevertheless, the exact role of CRFR1 and CRFR2 in the stress response and stress-related behavior is still under debate, because studies in mice and rats led to contradictory findings.

1.3. CRF receptor antagonists

In order to determine the role of CRF in the regulation of the neuroendocrine, autonomic and behavioral stress responses, non-selective CRFR antagonists were developed. The first CRF antagonist synthesized was α-helical CRF 9-41, the second was D-Phe CRF; both being peptidic and competitive antagonists of CRF. Both antagonists were derived from CRF and blocked efficiently the CRF- and stress-induced ACTH secretion and locomotor activation. The third CRFR antagonist synthesized, astressin was shown to be particularly potent at inhibiting the HPA axis. This antagonist was also proved to reduce the CRF- and stress-induced anxiogenic-like behavior, but it failed to reverse the CRF and stress-induced locomotor hyperactivity.

To investigate the exact role of CRFR1 and CRFR2 in the stress responses, selective CRFR antagonists were also developed. The first selective CRFR1 antagonists was CP-154,526 that was followed by antalarmin. These are non-peptidic and competitive antagonists, which were able to penetrate the blood-brain barrier and inhibit the stress-induced neuroendocrine and behavioral response. Therefore it was suggested that selective CRFR1 antagonists could be used as future therapy in stress-related psychiatric diseases, such as anxiety and depression. The first selective antagonists of CRFR2 were antisauvagine-30 and astressin2B, derived from the frog analogue sauvagine and the non-selective CRF antagonist astressin, respectively. These antagonists have peptidic structure, hence they are not able to penetrate the blood-brain barrier. Therefore, they were

administered preferentially in the periphery in order to elucidate the role of CRFR in colonic transit and gastric emyptying. The use of selective CRF antagonists concluded that CRFR1 increases colonic transit, whereas CRFR2 decreases gastric empyting, thus it was suggested that selective CRFR2 antagonists could be used as potential therapeutics in stress-related GI diseases, such as in inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS).

1.4. Nicotine and nicotine receptors

Besides the regulation of the stress responses, CRF has been implicated in nicotine addiction based on several lines of evidence. First, acute administration of nicotine induces a dose-dependent activation of the HPA axis that is initiated by hypothalamic CRF. Second, nicotine withdrawal syndrome resembles the behavioral stress response that is mediated by extrahypothalamic CRF. Third, exposure to stressors is one of the leading causes of nicotine relapse that implies the activation of the CRF systems. Finally, both CRF receptors participate in the acute, chronic and withdrawal actions of nicotine.

Nicotine is the main psychoactive component of tobacco that causes addiction. Nicotine addiction leads to the harmful habit of smoking that has high morbidity and mortality throughout the world. It is the most frequent type of substance dependence from all substances of abuse resulting in the loss of more than 7 million people per year worldwide. Nicotine is an alkaloid that is naturally extracted from tobacco; there is approximately 1 mg of nicotine in every cigarette produced from tobacco. During cigarette smoking nicotine enters the lungs, it is absorbed into the bloodstream and reaches the brain in about 8 seconds. Besides its psychostimulant effect, nicotine induces increase of the heart rate, arterial and venous constriction *via* release of adrenaline, but also contraction of the skeletal and smooth muscles *via* release of acetylcholine. The actions of nicotine are mediated by nicotinic acetylcholine receptors (nAchRs) that are considered ligand-gated ion channels composed of pentameric combinations of α and β subunits, since normally they respond to acetycholine. Binding to these pentametric ligand-gated ion channels, nicotine causes a rotation of the receptor that results in the

opening of the integral cation channel. Activation of nAChRs leads to increased permeability to both Na^+ and Ca^{2+} resulting in local depolarization, inducing the release of various neurotransmitters. Based on their primary sites of expression, nAchRs are classified into two subtypes: muscle-type nicotinic receptors found in neuromuscular junctions and neuronal-type nicotinic receptors found on neuronal bodies and nerve terminals. In the brain there are nine isoforms of the neuronal α-subunit (α2- α10) and three isoform of β-subunit (β2- β4). These are combinations of two α- and three β-, or five α7-subunits with different distinct pharmacological properties, as regards nicotine sensitivity and rate of desensitization. The most abundant neuronal nAchRs are α4β2, α3β4 and α7 located both pre- and postsynaptically where they can influence the release of other neurotransmitters, such as dopamine, glutamate and gamma-aminobutyric acid (GABA). A better understanding of the mechanisms of nicotine addiction has led to the development of new drugs, such as varenicline (a partial agonist of the α4β2 nAChR) and bupropion (an antagonist of nAChRs), which block the negative and positive reinforcement, respectively

2. AIM OF STUDY

Besides different types of nAChRs, CRFR1 and CRFR2 can also be potential targets in the therapy of nicotine addiction. Based on the initial concept that CRFR1 and CRFR2 mediate mainly antagonistic effects in the CNS, there are two potential ways to approach the therapy of nicotine addiction: to use selective antagonists of CRFR1, such as antalarmin or to use selective agonists of CRFR2, such as UCN II and UCN III.

Previous studies have already indicated that blocking CRFR1 would reduce some of the affective symptoms (the dysphoria and the reward deficit), whereas blocking CRFR2 would reverse some of the somatic symptoms (the excessive food intake and the increased body weight) of nicotine withdrawal syndrome. Other studies have also indicated that activating CRFR2 would reduce the anxiety- or depression-like behavior observed during alcohol withdrawal.

The aim of the present study was to investigate the potential therapeutical actions of selective CRFR1 and CRFR2 antagonists and selective CRFR2 agonists in rodents

exposed to chronic nicotine treatment and consequent acute nicotine withdrawal. On the one hand, the effects of antalarmin and astressin 2B on the alterations of the dorsal and ventral striatal dopamine release and the vertical and horizontal locomotor activity were examined in nicotine-treated rats. On the other hand, the impacts of UCN II and UCN III upon the anxiety- and depression-like behavior and hyperactivity of the HPA axis were determined in nicotine-treated mice.

3. MATERIALS AND METHODS

3.1. Materials

UCN II (Bachem, Switzerland), a selective CRFR2 agonist;

UCN III (Bachem, Switzerland), a selective CRFR2 agonist.

Antalarmin (Sigma-Aldrich, UK), a selective CRFR1 antagonist;

Astressin 2B (Sigma-Aldrich, UK), a selective CRFR2 antagonist.

[^{3}H]dopamine (Amersham, USA), a tritium labelled excitatory neurotransmitter;

Krebs solution: NaCl, KCl, $MgSO_4$, $NaHCO_3$, glucose, KH_2PO_4 and $CaCl_2$ (Reanal, Hungary);

Saline solution (NaCl inj. of 0.9 %, Biogal, Hungary);

Nicotine solution (1.4-2 mg/kg, Biogal, Hungary);

Ultima Gold (Perkin Elmer, USA), a scintillation fluid;

Mixture of 5 % CO_2 and 95 % O_2 for continuous gassing of the tissues;

Euthasol (Pentobarbital sodium, CEVA-Phylaxia, Hungary) for general anesthesia of the rats;

Ethyl alcohol, methylene chloride and sulfuric acid of analytical grade (Reanal, Hungary) for determination of the plasma corticosterone concentration;

Ethyl alcohol and sodium hypochlorite (Reanal, Hungary) for cleaning in between the experimental sessions.

3.2. Animals

First, male Wistar rats weighing 150-250 g upon arrival were used. The rats were housed together and kept in their home cages at a constant temperature on a standard

illumination schedule with 12-h light and 12-h dark periods (lights on from 6:00). Commercial food and tap water were available *ad libitum*. Second, male CFLP mice weighing 24-30 g were used. The animals were housed in their home cages at constant room temperature (23°C) on a standard illumination schedule, with 12-h light and 12-h dark periods (lights on from 6:00 a.m.). Commercial food and tap water were available *ad libitum.*

3.3. Surgery

The rats were implanted with a stainless steel Luer cannula (10 mm long), aimed at the right lateral cerebral ventricle under anesthesia with 60 mg/kg pentobarbital sodium. The stereotaxic coordinates were made according to the stereotaxic atlas of the rat brain. Cannulas were secured to the skull with dental cement and acrylate. The rats were allowed for 7 days to recover before experiments were started. After the experiments were concluded, 10 µl of methylene blue at 1 g/100 ml were injected into the lateral cerebral ventricle of the decapitated animals and the position of the cannula was inspected visually. Animals without the dye in the lateral cerebral ventricle were excluded from the final statistical analysis. The mice were implanted with a polyethylene Luer cannula (6 mm long) aimed at the right lateral cerebral ventricle under anesthesia with 60 mg/kg of pentobarbital sodium. The stereotaxic coordinates were 0.5 mm posterior and 0.5 mm lateral to the bregma, and 3 mm deep from the dural surface. Cannulas were secured to the skull with cyanoacrylate containing instant glue. The mice were allowed for 7 days to recover after the surgery. After the end of the experiments, 2 µl of methylene blue was injected *via* the cannula of decapitated animals to check the permeability and the right position.

3.4. Treatment

The rats were treated intraperitoneally (IP) with 1.4 mg/kg nicotine tartrate or 10 ml/kg of 0.9% saline solution for 7 days, two times per day (at 8:00 and at 20:00). Half of the rats were treated ICV with 0.1 µg/2µl antalarmin or 1 µg/2µl astressin 2B or 2 µl of 0.9% saline solution on the 8th day (after 12 hours following the last IP administration).

The other half of the animals were treated intracerebroventricularly (ICV) on the 9th day (after 24 hours following the last IP administration) based on the same treatment protocol. The mice were treated IP with 2 mg/kg nicotine tartrate or 10 ml/kg saline solution for control for 7 days, 4 times per day. Half of the mice were treated ICV on the 8th day, the other half on the 9th day with 2 μg/2μl UCN II, 2 μg/2μl UCN III or 2 μl of saline solution for control.

3.5. *In vivo* conducta studies

Thirty minutes after the ICV injection, the horizontal and vertical locomotor activities were recorded in an *in vivo* conducta system (MDE, Ltd, Germany), which is based on the principles of the open-field test and was described in previous studies. The horizontal activity, representing a measure of overall activity and arousal, and the vertical activity, representing a measure of exploratory and stereotype behavior, were monitorized for 30 minutes.

3.6. *In vitro* superfusion studies

After the decapitation of the rats the changes of dorsal and ventral striatal dopamine releases were determined by an *in vitro* superfusion system (MDE, Ltd, Germany) described in previous studies.

3.7. Elevated plus-maze test

Thirty minutes after the ICV treatment, the animals were evaluated in an elevated plus-maze test, validated by Lister and Rodgers to investigate anxiety-like behavior. For a 5 minutes period the following parameters were recorded by an observer sitting at 100 cm distance from the center of the plus-maze: a. the percentage of the number of entries into the open arms relative to the total number of entries, b. the percentage of the time spent in the open arms relative to the total time and c. the total number of entries into the open and the closed arms. Entry into an arm was defined as the entry of all four feet of the animal into that arm. The apparatus was cleaned up with sodium hypochlorite solution between the subjects.

3.8. Forced swim test

In parallel, the animals were evaluated in a forced swim test, invented by Porsolt et al. to investigate depression-like behavior. For a 5 minutes period the following parameters were recorded by an observer sitting at 100 cm distance from the table: a. the climbing activity (the time that animals spent with climbing the walls, in their attempt to escape the cylinder), b. the swimming activity (the time that animals spent with swimming in the water, in their attempt to remain at the surface) and c. the time of immobility (the time that animals spent in an upright position on the surface with its front paws together). A 5 second period was considered a time unit, thus the climbing and the swimming activities and the time of immobility were expressed in time units.

3.9. Chemo-fluorescent assay

After the decapitation of the mice, the trunk blood was collected for determination of plasma corticosterone concentration by a chemo-fluorescent assay that was described by Zenker and Bernstein and later modified by Purves and Sirett.

3.10. Statistical analysis

Statistical analysis of the results was performed by analysis of variance (Prism 7 Statistics, GraphPad Inc., USA). The differences between groups were determined by two-way ANOVA followed by Tukey's test for pairwise comparisons when prerequisites were fulfilled. A probability level of 0.05 or less was accepted as indicating a statistically significant difference.

4. RESULTS

4.1. After 12 hours of nicotine withdrawal

On the 8^{th} day, the horizontal and vertical locomotor activity increased significantly in nicotine-treated rats, compared with the saline-treated rats. In parallel, the dorsal and ventral striatal dopamine release increased significantly in nicotine-treated

group, compared with the saline-treated group. All the changes observed on the 8th day were reduced significantly after treatment with antalarmin, but not astressin 2B.

On the 8th day, the time spent in the open arms increased significantly, but the number of entries into the open arms and the total number of entries did not change significantly in nicotine-treated mice, compared to the saline-treated ones. The first two parameters increased further after treatment with UCN II or UCN III in the nicotine-treated groups, but only the time spent in the open arms was increased significantly. The swimming and the climbing activity decreased significantly in nicotine-treated mice, compared to the saline-treated ones, but the time spent immobile was unaltered. After treatment with UCN II and UCN III the swimming and the climbing activity decreased further in the nicotine-treated groups, both parameters decreasing significantly, but the time of immobilization was not altered. The plasma corticosterone concentration was elevated considerably, but insignificantly in the nicotine-treated group, compared to the saline-treated one, and this elevation of the plasma corticosterone level was reversed significantly after treatment with UCN II and UCN III.

4.2. After 24 hours of nicotine withdrawal

On the 9th day, significant increases of the horizontal locomotor activity and dorsal striatal dopamine release were observed in the nicotine-treated group, compared with the saline-treated group. In contrast, the vertical locomotor activity and the ventral striatal dopamine release were decreased significantly in the nicotine-treated rats, compared with the saline-treated rats. All the changes described on the 9th day were reversed completely after treatment with antalarmin, but not astressin 2B.

On the 9th day, the number of entries into the open arms/total number of entries and the time spent in the open arms decreased significantly in nicotine-treated mice, compared to the saline-treated ones, but the total number of entries was not affected significantly in the nicotine-treated group. The decreasing effects were reversed considerably after treatment with UCN II and UCN III, but only the number of entries was reversed significantly, and the total number of entries was not affected in the nicotine-treated groups. The swimming and the climbing activity decreased significantly, and the

time of immobilization increased significantly as well, in nicotine-treated mice, compared to the saline-treated ones. After treatment with UCN II or UCN III the time spent with climbing and swimming was enhanced significantly and the time spent immobile was reduced significantly in the nicotine-treated groups. The plasma corticosterone concentration was augmented remarkably and significantly in the nicotine-treated group, compared to the saline-treated one, but this augmentation of the plasma corticosterone level was abolished completely after treatment with UCN II and UCN III.

5. DISCUSSION

5.1. The effects of chronic nicotine treatment

In concordance with the features of the stage of binge/intoxication, in rats exposed to 7 days of nicotine treatment (after 12 hours following the last nicotine administration) we observed increases in horizontal and vertical locomotor activity along with increases in the dorsal and ventral striatal dopamine release. This finding is in line with previous studies which reported locomotor hyperactivity on the 4th and the 10th day of a chronic nicotine exposure. The authors of these studies suggested that nicotine-treated rats develop locomotor hyperactivity in response to nicotine, initially due to increases of both the density of dopamine receptors (D1 and D2) and dopamine concentration, and lately due to dopamine receptor supersensitivity in the striatum. The interpretation of the behavioral changes observed following chronic nicotine treatment is somewhat complicated by the observation that the impact of chronic nicotine exposure on locomotion depend upon sex, age, and housing conditions. Hence, the investigation of additional factors, such as sex, age and housing conditions is desirable, but would require more complex experimental design and statistical analysis.

In mice exposed to 7 days of nicotine treatment (after 12 hours following the last nicotine administration) we observed signs of anxiolysis, as mice treated with nicotine spent more time in the open arms of the elevated plus-maze than those treated with saline. This result is supported by a previous study which reported that subchronic administration of nicotine (0.1 mg/kg subcutaneously = SC for 6 days) produces anxiolytic effect in mice and it is opposed by other studies which referred that subchronic (0.3 mg/kg/day nicotine

SC for 4 days) or chronic administration (25 mg/kg/day nicotine *via* minipump for 14 days) of nicotine in higher doses than 0.1 mg/kg induces anxiogenic behavior in mice. Also, we observed signs of depression, as mice treated with nicotine spent less time with swimming and climbing in the water than those treated with saline. This result is underlined by previous studies according to which repeated IP nicotine treatment (0.3 mg/kg/day IP for 4 days) produces depression-like behavior. Although in our study despite of the remarkable decrease in the time spent with swimming and climbing, there was no significant difference in the time spent immobile - this being a more typical sign of depression in the forced swim test - in nicotine-treated animals, when compared to the saline-treated animals. Therefore, our results could be rather interpreted as a consequence of the locomotor suppressive effect exerted by nicotine, than an apparently coexisting anxiolytic and depressive behavior. In addition, the behavioral changes described in mice were not accompanied by significant elevation of the plasma corticosterone concentration.

5.2. The effects of acute nicotine withdrawal

In accordance with the features of the stage of withdrawal/negative affect, in rats exposed to 1 day of nicotine withdrawal (after 24 hours following the last nicotine administration) we expected a decrease of general locomotor activity and a decrease of global striatal dopamine release, which were actually assessed in a previous study following 14 days of nicotine exposure and 24 hours of nicotine withdrawal. Interestingly, in the present experiments only the vertical locomotor activity and the ventral striatal dopamine release were decreased, while the horizontal locomotor activity and the dorsal striatal dopamine release remained increased following acute nicotine withdrawal. The explanation of this finding might be offered by the authors of this previous study, who showed a reduction of the maximum number of D2 receptor sites in the nucleus accumbens, but found no alteration of the density and binding affinity of dopamine receptors (D1 and D2) in the putamen and nucleus caudatus. Additionally, the dose and the schedule of the nicotine exposure may also contribute to the difference between the previous and present results. Thus, while tolerance is more likely to be induced by continuous infusion of nicotine (performed in the previous study), behavioral sensitization

is frequently induced by intermittent injection of nicotine (performed in the present study). Nonetheless, continuous exposure to nicotine at doses that result in tolerance to the nicotine-induced sensitization, induces itself a sensitization that is demasked as the tolerance wears off. Hereby tolerance and sensitization must be regarded as two distinct adaptive changes that usually require different conditions, but may also occur following the same dose and schedule of chronic nicotine exposure. Consequently, during acute nicotine withdrawal these competing phenomena could be manifested differently between the two subdivisions of the striatum and accordingly, the two aspects of locomotor activity. The discrepancies between the behavioral and neurochemical parameters observed following acute nicotine withdrawal is underlined by the differential nicotinic regulation of the nigrostriatal and mesolimbic dopaminergic pathways. For example, there are clear differences in the distribution and function of various nAchR subtypes between the dorsal and ventral striatum that might explain the dopamine dysregulation assessed during acute nicotine withdrawal.

Mice exposed to 1 day of nicotine withdrawal (after 24 hours following the last nicotine administration) exhibited signs of anxiety, since the number of entries into the open arms and the time spent in the open arms of the plus-maze decreased in the nicotine-treated group, compared to the saline-treated one. This result is in agreement with previous studies, which showed that acute withdrawal following chronic administration of nicotine (1 day of withdrawal following 0.1 mg/kg/day IP treatment for 14 days or 12-24-48 mg/kg/day treatment *via* minipump for 14 days) precipitates anxiety-like behavior in mice tested in light-dark box or elevated plus-maze. Mice exposed to 1 day of nicotine withdrawal expressed signs of depression as well, since the time spent with swimming and climbing in the water increased in parallel with the time of immobilization in the nicotine-treated group, compared with the saline-treated one. This result coincides with that of a previous study using a similar treatment protocol (2 mg/kg nicotine IP, 4 times/day), following which signs of depression were indicated during acute and chronic nicotine withdrawal in mice investigated in forced swim test. In concordance with these behavioral changes, significant elevation of the plasma corticosterone concentration, reflecting the hyperactivity of the HPA axis, was observed on the 9^{th} day of our study. Indeed,

hyperactivity of the HPA axis is associated frequently with nicotine withdrawal syndrome and generally with states of anxiety and depression. ICV injection of UCN II or UCN III performed in the morning of the 9th day increases the open-arm activity that was previously decreased by acute nicotine withdrawal. Concomitently, ICV injection of UCN II or UCN III reverses the swimming and the climbing activity and the immobility of mice, which were increased and decreased respectively by acute nicotine withdrawal. As a matter of fact, the anxiolytic and the antidepressant effects of the urocortins validated in the present study have been already suggested by previous studies using the same methods. Additionally, a single administration of UCN II or UCN III attenuated the levels of the plasma corticosterone which were remarkably and significantly augmented on the 9th day, at least in the nicotine-treated animals.

6. CONCLUSION

Taken together, this study demonstrate that both selective CRFR1 antagonists, such as antalarmin and selective CRFR2 agonists, such as UCN II and UCN III could be promising candidates as potential therapy in nicotine addiction.

On the one hand, we demonstraded that antalarmin reversed the alterations of the dorsal and ventral striatal dopamine release and the vertical and horizontal locomotor activity which characterize the binge/intoxication phase of nicotine addiction. Actually, these experiments are the first to elucidate that both the rewarding, positive reinforcing effects of chronic nicotine treatment and the aversive, negative effects of acute nicotine withdrawal can be attenuated by administration of selective CRFR1 antagonists, such as antalarmin. On the other hand, we demonstrated that UCN II and UCN III reduce the anxiety- and depression-like behavior and hyperactivity of the HPA axis that arise during the withdrawal/negative affect phase of nicotine addiction and may persist during the preoccupation/anticipation phase as well. Consequently, these are the first experiments to demonstrate that administration of selective CRFR2 agonists, such as UCN II and UCN III ameliorate the anxiety- and depression-like signs developed following chronic nicotine treatment and the consequent acute nicotine withdrawal, and the hyperactivity of the HPA axis that is associated to them.

PUBLICATIONS

1. Original publications the present work is based on:

I. **Buzás A**, Bokor P, Balangó B, Pintér D, Palotai M, Simon B, Csabafi K, Telegdy G, Szabó G, Bagosi Z: Changes in striatal dopamine release and locomotor activity following acute withdrawal from chronic nicotine are mediated by CRF1, but not CRF2, receptors (Brain Research, 2019; 1706: 41–47.) **IF: 3.125**

II. Bagosi Z, Palotai M, Simon B, Bokor P, **Buzás A**, Balangó B, Pintér D, Jászberényi M, Csabafi K, Szabó G. Selective CRF2 receptor agonists ameliorate the anxiety- and depression-like state developed during chronic nicotine treatment and consequent acute withdrawal in mice (Brain Research, 2016; 1652:21-29.) **IF: 2.746**

2. Conference presentations related to the present work:

I. **Buzás A**, Bokor P, Bagosi Z, Palotai M, Jászberényi M, Csabafi K, Szabó G: The effects of selective CRF receptor antagonists in alcohol-treated rats (HMAA, 2013, Balatonfüred, Hungary)

II. Bokor P, **Buzás A**, Bagosi Z, Palotai M, Jászberényi M, Csabafi K, Szabó G: The effects of urocortin II and urocortin III on the anxiety- and depression-like symptoms in nicotine-treated mice (HMAA, 2013, Balatonfüred, Hungary)

3. Poster presentations related to the present work:

I. Bagosi Z, Palotai M, **Buzás A**, Bokor P, Csabafi K, Szabó G. The effects of selective CRF receptor antagonists in rats following chronic alcohol treatment and acute alcohol withdrawal (MITT, Budapest, Hungary, 2013)

II. Bagosi Z, Bokor P, **Buzás A**, Palotai M, Jászberényi M, Csabafi K, Szabó G. The effects of urocortin II and urocortin III on the anxiety- and depression-like symptoms in nicotine-treated mice (MÉT, Budapest, Hungary, 2013)

III. Bagosi Z, Palotai M, **Buzás A**, Bokor P, Jenei A, Csabafi K, Jászberényi M, Telegdy G, Szabó G. Role of the hypothalamic CRF and AVP in mediating the activation of the HPA axis in alcohol-treated and alcohol-deprived rats (FEPS, Budapest, Hungary, 2014)

IV. Bagosi Z, Palotai M, Simon B, Bokor P, **Buzás A**, Csabafi K, Szabó G. The effects of a selective CRFR1 antagonist in rats exposed to chronic nicotine treatment and consequent acute withdrawal (IBRO, Budapest, Hungary, 2016)

V. Bagosi Z, Bokor P, **Buzás A**, Balangó B, Pintér D, Csabafi K, Szabó G. The effects of the selective CRF2 receptor agonists in mice exposed to chronic nicotine treatment and consequent acute withdrawal (FAMÉ, Pécs, Hungary, 2016)

VI. Bagosi Z, Balangó B, Pintér D, Bokor P, **Buzás A**, Csabafi K, Szabó G. The effects of selective CRF receptor antagonists in rats exposed to chronic nicotine treatment and consequent acute withdrawal (FENS, Pécs, Hungary, 2017)

VII. Bagosi Z, Karasz G, **Buzás A**, Csabafi K, Telegdy G, Szabó G. The effects of selective CRF receptor antagonists on the affective signs of binge drinking (MÉT, Szeged, Hungary, 2018)

TOTAL IMPACT FACTOR: 5.871
TOTAL CITATION: 5 (WITHOUT SELF-CITATION: 4)

ACKNOWLEDGEMENT

Hereby I would like to seize the opportunity to express my utmost gratitude to my leaders and Professors, my colleagues, fellows and assistants without whom this thesis could not have been written.

First, I must express how indebted I am to Professor György Lázár, the head of the Department of Surgery, my clinical leader, for providing me with the opportunity to use my findings and experimental experience at the Department of Pathophysiology for completing my Ph.D. thesis.

I am also indebted to Professor Gyula Szabó for the opportunity to work under his chairmanship and for sharing his suggestions and meticulous way of thinking with me.

I must also thank for the kindness, help and invaluable efforts of Péter Bokor, Krisztina Csabafi, Júlia Szakács and Miklós Jászberényi along with Gusztáv Kiss, and Ágnes Pál for without their assistance these experiments could not have been performed.

One person stands out, though, from among the people contributing to my thesis, namely my supervisor, Zsolt Bagosi, with whom not only the experimental work, but all the scientific challenges that I have faced along the years have been spent in a delightful mood and positive environment. I am lucky to have had him as my practical teacher at the beginning and that he has kept on sharing his immense scientific knowledge with me along these years. I am convinced that the present work could never have been accomplished without his guidance, expertise, support and kind assistance.

Last, but not least, I am most grateful for my family: my parents, my twin sister Anna and my elder brothers Balázs and Péter; who gave me the possibility to study and become a doctor of medicine and have helped me through the tough periods of my life, when I needed their help, support, kindness and, most of all, their empathy.

The present experiments were supported by the following grants: TÁMOP 4.2.2-A-11/1/KONV-2012-0052, KTIA_13_NAP-A-III/8 and EFOP-3.6.2-16-2017-00006.