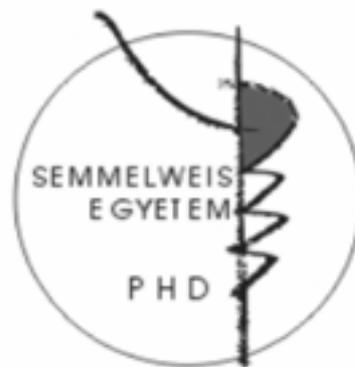


Non-conventional effects of antidepressants in the central and peripheral nervous system

Doctoral Thesis

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INTRODUCTION

At the present time, depression is one of the most common neuropsychiatric disorders. According to the study of World Health Organization published in 2007, the 3.2 % of investigated population suffered from depression alone, but an average between 9.3% and 23.0% of participants had one or more chronic physical disease beside the depression. During a multicentric european study it was found, that the prevalence of depression in european adult population in 2001 it was approximately 8.56%. In all countries the rate of depressed women's patients was higher than that of the number of depressed men's patients. The epidemiology data of depression in Hungary shows similar rates: in 1998 the lifetime rate for major depressive disorder was 15.1%, and for bipolar disorders 5.1%. The female-to-male ratio was 2.7 for major depression. Nevertheless, today more than 30 antidepressants, with favourable side-effects profile are in the clinical use. The rate of efficient treatments don't beyonds more than 50-60%, which means, that approximately 40% of the patients is not responder to the initial medication. The clinical efficacy of the latest antidepressants it isn't better than that of the substances used 40 years ago, only one significant difference could be discovered between them: the favorable side-effects profile. With regard to relatively high prevalence of depression, it is clear, that at the present, the antidepressants are one of the most important drugs acting at the central nervous system. Most of the presently used antidepressants (with only few exceptions) exert their effect by increasing the serotonergic and/or noradrenergic neurotransmission. But in the past few years, in the clinical practice appeared some new antidepressant substances, which didn't act in accordance with the monoamine theory of depression (one of them, is the serotonin reuptake enhancer tianeptine).

BACKGROUND

The monoamine theory of depression was initiated in the late sixties of the last century, and it supposes, that the depression is a consequence of insufficient function in the noradrenergic and serotonergic neurotransmission. But in the last 30 years several observations have been accumulated, which are not in accordance with the monoamine theory (the latency time of clinical effect of antidepressants is about 2-3 weeks; new antidepressants has been developed, that don't act at monoaminergic transmission or even they decrease the extracellular levels of monoamines).

Under these circumstances, several alternative depression theories appeared about the etiology of depression. One of the most modern of them is the „network“-hypothesis, which is able, to combine the other different theories, and it supposes, that the depression is the consequence of neuronal cell-degeneration and cell death caused by different harmful influences at the nervous system.

The effect of antidepressants on nACh receptors in the central nervous system

Our previous results showed, that the antidepressant substances have other targets in the central nervous system, than monoamine systems. During our experiments with the nicotinic agonist DMPP we found, that this drug acts both on nAChRs, causing exocytosis, and produce NA release by reversing the function of the NA transporter.

Later, we demonstrated, that the NA uptake inhibitors (desipramine, nioxetine, nomifensine, cocaine) and the selective 5-HT reuptake inhibitor type antidepressants (fluoxetine, citalopram) as well as the selective DA uptake inhibitor GBR-12909, besides to their specific transporter blocking effect, also inhibit very efficiently the nAChRs in the 1-10 μM concentration range. Thus, the monoamine reuptake blocker type substances, independently from their chemical structure and selectivity are able to inhibit the nAChRs in the central nervous system, with very similar efficacy to that of the channel-blocker type nicotine receptor antagonist mecamylamine.

Our results may have also clinical importance, because the plasma concentration of these substances during antidepressant therapy is between 1-2 μM , and in the brain they could have even higher concentration, than in the plasma, and at this higher concentration, they inhibit the function of neuronal nicotinic receptors.

Similarities between monoamine transporters and nAChRs

The neuronal nAChRs could be found in neuromuscular junction, in sympathetic and parasympathetic ganglia and widespreadly in the central nervous system. The neuronal nAChRs are ligand-gated ionotropic receptors, with pentameric structure, composed by five subunits, two subunits participate in their conformation: the α and the β subunit. They could be heteromeric or homomeric receptors, depending on the type of contained subunits. The nAChRs in the central nervous system play an important role in cognitive processes like learning and memory. The disturbances in the nicotinic cholinergic neurotransmission may play role in several pathological processes (e.g. Alzheimer-

disease). In the peripheral nervous system (for example in the neuromuscular junction), the presynaptically localized receptors stimulates the release of acetylcholine.

The ion-channel properties of monoamine transporters

The most important role of the monoamine (e.g. NA, DA and 5-HT) transporters is the removal of the transmitters from extracellular space. Also, the functional properties of different monoamine transporters are very similar. These uptake systems are members of the Na/Cl-dependent transporters family (beside the GABA, glycine, proline, betaine and the taurine transporters). The monoamine transporters shows high homology: the human DAT is identical in 67 % with the human NET, the SERT reveals more than 48% amino acid homology with NET. This homology results in the fact, that their structural conformation is also very similar.

Electrophysiological data indicates, that several membrane transporter proteins possess ion-channel like properties. Although, it has been described, that the NMDA receptor antagonist dizocilpine (MK-801) inhibits the monoamine transporters expressed in HEK cells, that also seems to support our hypothesis.

Our pharmacological observations fit quite well to the hypothesis, that channel-like structures may exist in the monoamine transporters.

According to our conception, the monoamine uptake blockers (e.g. the antidepressants) are able to efficiently inhibit the nAChRs, because the channels of these receptors share common pharmacological properties with the ion-channels in monoamine transporters.

Effect of monoamine uptake blocker type antidepressants on the NMDA receptors. Common properties of NMDA and nACh receptors.

The NMDA receptor is the ionotropic receptor of glutamate, its native forms have tetrameric structure, with two subunits in composition: NR1 and NR2, these subunits form a non-selective cation channel.

The NMDA receptor is unique, because -besides of the ligand-gated function- is able also for voltage-dependent function, and it is the only one ionotropic receptor, which needs two co-agonists for its function (glycine and glutamate). NMDA receptors play a critical role in the excitatory synaptic neurotransmission and plasticity. At the same time, through their unique channel-characteristics, they participate in a complex manner in the molecular mechanisms of learning and memory. Under pathological conditions, the chronic over-stimulation of these receptors leads to excitotoxicity and cell death.

Nevertheless, the ionotropic receptor-families possess different composition, but the nAChRs and the NMDA receptors share some common pharmacological properties, since their corresponding channel-blocker type antagonists, the mecamylamine and the MK-801, respectively shows “cross-inhibition”, which means that the nAChR antagonist mecamylamine inhibits the function of NMDA receptors, and the channel-blocker type NMDA receptor antagonist MK-801 is able to block the nAChRs. Based on previous findings, that the monoamine uptake blocker type antidepressants could antagonize the nAChRs with channel-blocker mechanism, similarly to mecamylamine, we supposed that the NMDA antagonism could be also a general feature of uptake blocker-type antidepressants. In this work, one of the aims was to investigate this hypothesis.

AIMS

Based on our previous results, we intended to make investigations in two main categories.

Category I. With what kind of manner and degree could influence the different type antidepressants acting on monoamine transporters (maprotiline, tianeptine, desipramine, fluoxetine) the function of ligand-gated ionotropic receptors in the central nervous system ?

Aim 1. Investigation of the effect of atypical antidepressants (the noradrenaline reuptake blocker maprotiline and the serotonin reuptake enhancer tianeptine) on the nACh receptors in the central nervous system.

Our team’s previous results demonstrated, that different monoamine uptake blocker substances (like nomifensine, cocaine, desipramine, fluoxetine etc) are able to inhibit the nAChRs in the central nervous system, independently from their chemical structure and selectivity.

Partially based on these results, a new theory of depression was developed (Shytle et al., 2002), which explains the formation of the symptoms with the over-stimulation of the nAChRs, and in turn, the effect of the antidepressants with the blockade of these receptors. In our experiments we intended to examine this new conception, therefore we examined the effect of an atypical antidepressant, the tianeptine (which acts unbecoming to the monoamine theory, because it increase the re-uptake of serotonin) to nAChRs.

Apart from this, we also studied the effect of another antidepressant, the NA uptake inhibitor maprotiline at the nicotine-evoked NA-release in rat hippocampal slices.

Aim 2. Investigation of the effect of monoamine uptake blocker type antidepressants on the NMDA receptors in the central nervous system.

Our previous results showed, that the monoamine uptake inhibitor antidepressants inhibits the nAChRs with a channel-blocker type mechanism, similar to that of mecamylamine. Since the nACh receptor's and the NMDAR's channel-blocker type antagonists shows „cross-inhibition” (Snell and Johnson, 1988; Ramoa et al., 1990), arise the question, if the antidepressants are also able to block the NMDA receptor's function, as well as the nAChRs function ? To clarify this, we examined the effect of the SSRI fluoxetine and the tricyclic desipramine on the NMDA-evoked NA-release in rat hippocampal slices.

Aim 3. Investigation of the effect of monoamine uptake blocker type antidepressants on the purinergic P2X receptors in the peripheral nervous system.

With regard to our previous results, which demonstrated, that the tricyclic desipramine and the SSRI type fluoxetine have nAChR-antagonistic properties, we wanted to extend the investigation of the effect of these antidepressants on other representants from ionotropic receptor's family.

In our experiments, we looked for the answer to our question if the nAChR-antagonist desipramine and fluoxetine also possess inhibitory properties on the ionotropic P2X receptors involved in the purinergic transmission ? Therefore, we investigated the effect of these antidepressants on the ATP-evoked NA-release in guinea-pig heart right atrium.

Category II. With what kind of manner and degree could influence the channel-blocker type ionotropic receptor antagonists (MK-801 and mecamylamine) the function of monoamine transporters in the central nervous system ?

Aim 4. Investigation of the effect of channel-blocker type ionotropic receptor antagonist substances on the striatal dopamine (DA) release.

Our results obtained during investigations in the first category revealed, that monoamine uptake blocker-type antidepressants inhibit the function of both the nAChRs and the NMDA receptors, and based on literature data, this inhibition is most likely by a channel-blocker mechanism. Since the results obtained in the last ten years show, that the monoamine transporters also possess channel-like properties, which plays important

role in the mechanism of transmitter re-uptake, we intended to study if the channel-blokker type antagonists of the nAChRs and the NMDA receptors could influence the function of the monoamine transporters.

Therefore we studied the effect of nAChR-antagonist mecamylamine and the NMDA receptor antagonist MK-801 (dizocilpine), respectively, on the extracellular DA-level in the striatum using *in vivo* microdialysis technique.

METHODS

1. *In vitro* slice perfusion technique

Measurement of [³H]NA release from rat hippocampal slices

The hippocampal slices (thickness: 400 μm) were loaded for 45 minutes, at 37 °C, in 1 ml, continuously gassed Krebs solution, containing 10 μCi [³H]NA. After the incubation period, the slices were transferred into a four-channel microvolume perfusion system and the chambers were superfused with Krebs solution continuously gassed with a mixture of 95% O₂ and 5% CO₂, at a rate of 0.6 ml/min for 60 minutes (preperfusion period). After this, 3-minute fractions were collected. The receptor agonist (nicotine or NMDA) was applied from the 6th fraction for six minutes, the antidepressants (maprotiline, tianeptine, desipramine or fluoxetine) were continuously present in the medium from the 3rd fraction. In the NMDA-experiments, magnesium-free Krebs solution was used. At the end of the experiments slices were homogenized in 500 μl of 10 % trichloroacetic acid. 2 ml of scintillation cocktail (Ultima Gold, Packard) was added to each collected sample and to homogenized tissue. The radioactivity of these mixtures was measured with a Packard 1900 TR liquid scintillation counter. Radioactivity was expressed in terms of disintegration per second per gram of tissue (Bq/g). The fractional release (FR) was expressed in terms of the percentage of tritium present in the tissue at the beginning of a sample collection period. FR data were normalized to decrease variance between subjects. The average of three fractions before treatment was taken as 100%, and all fractions were expressed relative to this value as normalized fractional release (nFR) data.

Electrical stimulation-evoked [³H]noradrenaline release

Slices were prepared as described above, after preperfusion period nineteen 3-min samples were collected. Electrical stimulation (60 V, 2 Hz, 1 ms, 240 impulses) was

applied during the third (S₁) and the thirteenth sample (S₂). Drugs (maprotiline, tianeptine, desipramine or fluoxetine) were administered from the 8th sample. The tritium content of the samples was determined as described above.

Measurement of [³H]NA release from guinea-pig heart right atrium

The right atria was removed from heart, and dissected into two equal parts. In every experiment 2 animals were used, the half-atrias of each animal were treated separately. The experimental protocol (application of ATP and electrical stimulation) and the manipulation of the collected samples was identical to that in the case of hippocampal experiments.

2. *In vivo* microdialysis technique on anesthetized rats

Operation and sample collection. The male Wistar rats were anesthetized with urethane (1.300mg/kg). Animals were placed in a stereotaxic frame and a home-made concentric dialysis probe (polyacrylonitrile/sodium methallyl sulphonate copolymer dialysis tube, i.d.: 0.22 mm, o.d.: 0.31 mm, active membrane length 5 mm) was implanted into the right striatum (coordinates with respect to bregma: A/P +2.0; M/L 3.0; D/V 8.0 according to the stereotaxic atlas of Paxinos and Watson). The probe was perfused with a modified Ringer's solution at a rate of 2.0 µl/min using a CMA 100 microdialysis pump. After a 60-min equilibrium period, 15-min samples were collected. After collection of four basal samples, different substances (MK-801, mecamlamine, nomifensine) were dissolved in perfusion medium and administered locally in the striatum via microdialysis probe.

At the end of the experiments the rats were over-anesthetized, the brain removed and after a 48h fixation in formaline (10%) the location of the probe was verified by stereomicroscopic examination. The results were used only in case of adequate placement of the probe.

Sample analysis. The dopamine (DA), DOPAC and HVA content of the samples was determined immediately after collection by an HPLC-ECD system consisting of a Waters M510 pump, a Waters M460 electrochemical detector with glassy carbon electrode set at +650 mV and the Maxima 820 chromatography software.

Separation was achieved on a reverse-phase Hypersil ODS column (Hewlett Packard, 5 µm, 100 x 2.1 mm), the flow rate of mobile phase on the column was 0.5 ml/min.

Under these conditions, the detection limit of dopamine was approx. 1 pg/ sample.

RESULTS

1. nAChR antagonistic properties of atypical antidepressants (maprotiline and tianeptine) acting on monoamine uptake systems

In spite of the fact, that depression is one of the most common neuropsychiatric disorder (its lifetime-prevalence is between 15-25%), the neurochemical background of this disease is still not precisely clarified. After the most accepted hypothesis, -the monoamine theory-, depression is the consequence of monoaminergic neurotransmission's deficiency. Nevertheless a lot of preclinical and clinical observations accumulated in the last decades cannot be explained by monoamine theory. Our team's previous results demonstrated, that monoamine uptake blocker type antidepressants (like desipramine, fluoxetine and citalopram), with different chemical structure and selectivity are able to inhibit in a dose-dependent manner the function of the nAChRs in the central nervous system. Since our publications, several independent researcher-teams, working with other methods than ours, described similar results, namely that different uptake-blocker type antidepressant acts as nicotinic antagonists.

Based on all of this results, a new hypothesis was formulated, which suppose, that the inhibition of neuronal nAChRs plays a determinant role in the formation of antidepressant effect, thus one important process during depression could be the cholinergic predominance mediated by the nicotinic receptors.

Our work's -one of the most important- question was whether the nACh-theory is also valid in case of antidepressants which function is not compatible, or partially compatible with the monoamine theory.

That is why we proposed to investigate if the previously mentioned nicotinic antagonistic property is also characteristic to atypical antidepressants, like maprotiline and the serotonin uptake enhancer tianeptine ?

To clarify this question, we studied the effect of maprotiline and tianeptine, respectively on the nicotine-evoked [³H]NA release in rat hippocampal slices.

Nicotine, at a concentration of 50 μM applied during the 6th and 7th fractions significantly increased the resting release of [³H]NA in rat hippocampal slices. This increase in the release was transient, and after 4 fractions of nicotine administration,

returned to baseline. The nicotine-evoked [³H]NA release was dose-dependently inhibited by the nicotinic receptor antagonist mecamylamine with an IC₅₀ value of 0.32 μM, in turn, at the 10 μM concentration mecamylamine produced a complete blockade of this release, which indicates, that the process was mediated through nicotinic receptors.

The noradrenaline reuptake-blocker maprotiline and the serotonin reuptake-enhancer tianeptine also blocked the nicotine-evoked NA release in a dose-dependent manner. The IC₅₀ values were 5.7 μM for maprotiline, and 11.32 μM for tianeptine.

With regard to inhibition of nicotine evoked NA-release, the IC₅₀ value of maprotiline differ in a small compass?, only with one order of magnitude from that of mecamylamine, but at the same time, the IC₅₀ value of tianeptine already shows almost two order of magnitude difference.

Since the monoamine uptake blocker substances are able to inhibit (indeed with different efficacy) the function of the noradrenaline transporter and the process of nicotine-evoked noradrenaline release, arise the possibility, that the uptake blockers (through inhibition of the noradrenaline re-uptake) influence some intracellular process and by this reduces the nicotine-evoked NA release.

To clarify this presumption, we examined the existence of an eventual correlation between the ability of NA transporter inhibition (K_i values) and the nicotine-evoked NA release inhibiting properties (IC₅₀ values) of the different transporter blocker substances. The K_i-values were taken from the literature, and the IC₅₀-values were determined in this study (in the case of maprotiline), and in our team's previous experiments. The Pearson's correlation analysis didn't established any connection between this two variables (logK_i and logIC₅₀): the value of correlation coefficient (r) was 0.17, which didn't differed significantly from zero (p=0.69). Thus, the inhibitory effect of monoamine uptake blockers (including maprotiline) on the nicotine-evoked NA release is not a consequence of the inhibition of noradrenaline transporter.

At the same time, literature data shows, that monoamine transporter blocking substances are able to inhibit (in some degree) the function of the Na⁺-channels. Theoretically, this mechanism could be an explication for the inhibition of nicotine-evoked NA release, therefore, we compared the effect of the Na⁺-channel blocker tetrodotoxine (TTX) with the effect of atypical antidepressants on the nicotine- and electrical stimulation-evoked

[³H]NA release, to investigate the possible involvement of Na⁺-channels in the inhibitory effect of these substances. In our experiments, we found, that TTX inhibited both the nicotine-evoked and the electrical stimulation-evoked NA release with same efficacy (the IC₅₀ values were 0.033 μM and 0.039 μM, respectively). These results reveals, that Na⁺-channels plays identical role in the transmitter release induced by both stimulation methods. Nevertheless, maprotiline and tianeptine also inhibited the nicotine- and electrical stimulation-evoked NA release (the IC₅₀-value for maprotiline was 177.4 μM, and 177.2 μM for tianeptine). Thus, they showed different efficacy, in a high degree, because when in the case of the nicotine-evoked release, the IC₅₀ values of these substances were higher only with one or two order of magnitude, than that of the nAChR antagonist mecamylamine. In the same time the IC₅₀ values obtained in the case of electrical stimulation showed already a three order of magnitude difference in the case of both substances in comparison to mecamylamine.

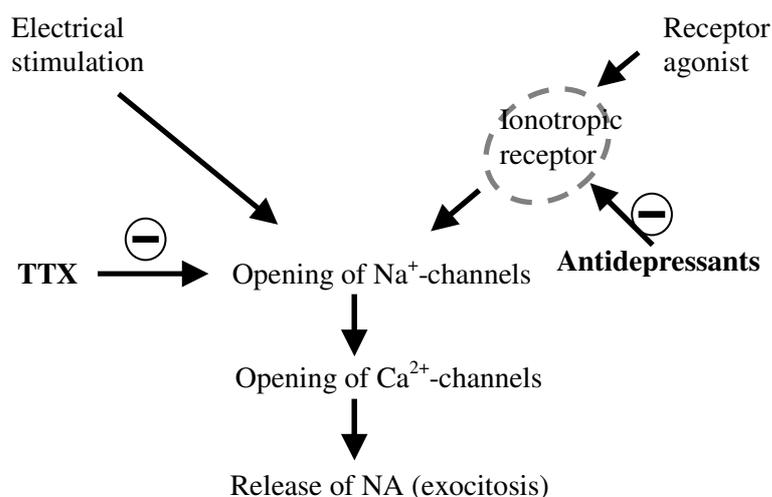


Figure 1. Comparison of the mechanism of action of tetrodotoxine (TTX) and antidepressant substances acting at monoamine uptake systems. In case of both the electrical stimulation or receptor agonist-induced NA release, the activation of Na⁺-channels is essential, because both processes were inhibited with the same efficacy by TTX.

This fact indicates, that their primary target is probably related to one event, prior to the opening of Na⁺-channels, namely, to the activation of the nAChRs, and their effect on Na⁺-channels plays only a secondary role in their NA-release inhibiting effect.

In short, besides the tricyclic and SSRI-type antidepressants, two representatives of the atypical antidepressants, the NA transporter inhibitor maprotiline and the 5-HT transporter stimulating tianeptine are also able to block the nAChRs in the central nervous system. According to this, the nAChR antagonistic property could be a general feature of the substances acting on monoamine transporter systems, independently from their selectivity.

Our results indicate the existence of pharmacological similarities between monoamine transporters and nACh receptors.

2. NMDA-R antagonistic properties of monoamine uptake blocker type antidepressants

Since it has been demonstrated, that the channel-blocker type nAChR antagonist mecamylamine is able to inhibit also the function of NMDA receptors, and that the channel-blocker type NMDAR antagonist MK-801 inhibits also the nAChRs, in addition, the antidepressants act as nAChR channel-blocker type antagonists, we proposed to investigate, whether the monoamine uptake blockers could influence the function of these ionotropic glutamate receptors. Therefore we studied the effect of the two most potent nAChR antagonist antidepressants, the tricyclic desipramine and the SSRI type fluoxetine, on the NMDA-evoked [³H]noradrenaline release from rat hippocampal slices.

NMDA, at a concentration of 100 μM applied during the 6th and 7th fractions produced a significant increase in the resting [³H]NA release, this response was transient and returned to the baseline in 21 minutes after the administration of NMDA.

The NMDA-evoked [³H]NA release was dose-dependently inhibited by the non-competitive NMDA receptor antagonist dizocilpine (MK-801) with an IC₅₀ value of 0.54 μM, in turn, at the 10 μM concentration MK-801 produced a complete blockade of this release, which indicates, that the process was mediated through NMDA receptors.

The tricyclic desipramine and the SSRI type fluoxetine also blocked the NMDA-evoked NA release in a dose-dependent manner, but with lower efficacy, than MK-801, since their IC₅₀ values were 14.57 μM for desipramine, and 41.06 μM for fluoxetine.

To investigate the possible involvement of Na⁺-channels in the inhibitory effect of these substances, we compared the effect of the Na⁺-channel blocker tetrodotoxine (TTX) with the effect of antidepressants on the NMDA- and electrical stimulation-

evoked [^3H]NA release. In our experiments, we found, that TTX inhibited both the NMDA-evoked and the electrical stimulation-evoked NA release with same efficacy (the IC_{50} values were 66nM and 55nM, respectively). These results reveals, that Na^+ -channels plays identical role in the transmitter release induced by both stimulation methods.

In contrast with this, we found that antidepressants inhibited only the NMDA-induced NA-release, but neither the tricyclic desipramine, nor the SSRI fluoxetine had any effect on the electrical stimulation-evoked response up to the concentration of 100 μM , which indicates, that under our experimental conditions the Na^+ -channels are not involved in this NA-release inhibiting effect of antidepressants.

Previously, we found that the nicotine-evoked NA release from rat hippocampal slices was inhibited by mecamylamine with an IC_{50} value of 0.19 μM , whereas in the present study the NMDA-evoked release was inhibited by MK-801 with a very similar efficacy ($\text{IC}_{50} = 0.54 \mu\text{M}$). If we compare the inhibitory effect of antidepressants to these reference values, we can conclude that the IC_{50} value of desipramine (0.36 μM) and fluoxetine (0.57 μM) for the nAChRs is in the same range as that of mecamylamine, but their efficacy is 1.5-2 orders of magnitude lower than that of MK-801 in the case of NMDA receptors. Calculation of the efficacy ratios ($\text{IC}_{50\text{NMDAR}} / \text{IC}_{50\text{nAChR}} = 56$ and 72 for desipramine and fluoxetine, respectively) also clearly indicates that these antidepressants are much weaker antagonists at the NMDA receptors than at the nAChRs.

The steady-state plasma concentration of antidepressants usually does not exceed the 1-2 μM concentration range, however, the brain concentration of monoamine uptake blockers is much higher because of the special distribution characteristics of these compounds. These data and our current results suggest that during antidepressant treatment the brain concentration of desipramine and fluoxetine might reach a concentration at which the function of NMDA receptors is substantially influenced, therefore it is reasonable to assume that the inhibitory effect of antidepressants might contribute to their therapeutic effect.

3. P2X-R antagonistic properties of monoamine uptake blocker type antidepressants

With regard to our results presented in the previous chapters, namely that antidepressants are able to inhibit both the nAChRs and the NMDA receptors, raised the question, if the uptake-blocker type antidepressants could block not only these two receptors but also the P2X receptors, which are the third representatives of ionotropic receptors family.

To clarify this presumption, we studied the effect of the two most potent nAChR antagonist antidepressants, the tricyclic desipramine and the SSRI type fluoxetine, on the ATP-evoked [³H]noradrenaline release from guinea-pig heart right atrium, using *in vitro* slice perfusion technique.

ATP, at a concentration of 1 mM applied during the 6th and 7th fractions produced a significant increase in the resting [³H]NA release. This response was transient, and after 4 fractions of ATP administration, returned to the baseline.

The ATP-evoked [³H]NA release was dose-dependently inhibited by the purinergic P2X receptor antagonist PPADS, with an IC₅₀ value of 1.70 μM, in turn, at the 30 μM concentration PPADS produced a complete blockade of this release, which indicates, that the process was mediated through P2X receptors.

The tricyclic desipramine and the SSRI type fluoxetine also blocked the ATP-evoked NA release in a dose-dependent manner, with similar efficacy to PPADS, since their IC₅₀ values were 4.45 μM for desipramine, and 2.48 μM for fluoxetine.

However, the inhibition of the ATP-evoked response didn't mean that a given substance directly inhibit the P2X receptor, because the effect could be indirect. The ATP-evoked release (similarly to nicotine- and NMDA-evoked NA-release) is also a consequence of a cascade of events, when the depolarization caused by P2X receptor activation is followed by the activation of the voltage dependent Na⁺ and Ca²⁺-channels.

Based on this, we examined the possible involvement of Na⁺-channels in the inhibitory effect of desipramine and fluoxetine. Therefore we compared the effect of these antidepressants on the ATP- and electrical stimulation-evoked [³H]NA release.

In our experiments, we found, that both desipramine and fluoxetine inhibited the electrical stimulation-evoked NA release in a dose-dependent manner (the IC₅₀-values were 16.68 μM and 141.0 μM, respectively). Thus, they showed different efficacy, in a high degree, because when in the case of the ATP-evoked release, the IC₅₀ values of these substances were in the same order of magnitude, than that of the P2X-R antagonist

PPADS, till then the IC₅₀ values obtained in the case of electrical stimulation showed already a fourfold difference in the case of desipramine, and a two order of magnitude difference in the case of fluoxetine in comparison to PPADS.

According to the fact, that activation of the Na⁺-channels play identical role in the NA release induced by both stimulation methods, our results indicate that under our experimental conditions, the inhibition of Na⁺-channels play only a secondary role in comparison to the blockade of P2X receptors in the inhibitory effect of these uptake-blocker type antidepressants on the atrial NA release.

In summary, we can conclude, that our present results demonstrate, that these two widely used monoamine uptake-blocker type antidepressants are able to inhibit also the responses mediated by purinergic P2X receptors. These data, together with our previous results with the nAChRs and the NMDA receptors suggest the possibility, that antidepressant substances have other targets in the central nervous system beyond monoamine transporters and the interaction with these other targets may contribute to development of their therapeutic and side effects.

4. DA-uptake inhibitory effect of channel-blocker type ionotropic receptor antagonist substances

In our team's previous study, we found, that the non-competitive NMDA receptor antagonist dizocilpine (MK-801) increased the striatal dopamine (DA) level, but about the mechanism of action of this effect we succeeded to elicit only one thing, that this process is independent from the action of MK-801 on the NMDA receptors. During the study of non-conventional effects of antidepressants, we found results, which can explain this prior observation.

As we previously mentioned, we observed, that monoamine uptake blocker type antidepressants, independently from their chemical structure and selectivity, could inhibit the function of neuronal nACh receptors, with a channel-blocker type mechanism of action, similar to that of the mecamylamine. Electrophysiological data also refers, that several membrane transporter proteins possess ion-channel like properties. Based on these, arises the possibility that ion-channel like structures could exist in the transporters, therefore we supposed, that the nAChR inhibitor property of the uptake blockers may be explained by the similarities existing between the transporter's and the

receptor's channel-structures. If this is true, than we can also suppose, that the channel-blockers of nAChRs are able to inhibit also the function of the monoamine transporters. In this present work, we intended to examine this hypothesis by investigating the effect of two channel-blocker type receptor antagonist substances, the nAChR antagonist mecamlamine and the NMDAR antagonist MK-801 (which is also active on the nAChR), on the striatal dopamine release using *in vivo* microdialysis technique.

In addition, we agreed to clarify, if the inhibition of the DA uptake plays any role in the previously observed increasing effect of MK-801 at the striatal dopamine level.

During our experiments, we examined the effect of intrastrially administered dizocilpine (300 μ M), the effect of mecamlamine (1mM), and also the effect of these substances in the presence of the nomifensine, on the release of DA and its metabolites in the corpus striatum of the anesthetized rats, using *in vivo* microdialysis technique. The DA uptake inhibitor nomifensine (10 μ M) administered continuously in the striatum produced an approximately eightfold increase of the resting DA concentration, and after 60 minutes stabilized it at this high level. The levels of the DA-metabolites (DOPAC and HVA) were not affected by nomifensine.

Effect of the NMDA receptor antagonist dizocilpine (MK-801) on the striatal dopamine release

Under normal conditions, the resting release of DA in the rat striatum was 12.3 ± 2.1 pg/sample (n = 18). We expressed the values of DA and its metabolites (DOPAC, HVA) release relative to the basal release (the average concentration of three samples before any treatment was taken as 100 % and all values were expressed relative to this control).

The channel-blocker type NMDA receptor antagonist dizocilpine (MK-801) administered locally through microdialysis probe at a concentration of 300 μ M produced a significant increase of striatal DA concentration. The peak effect was observed in the second fraction (85 % increase), and the DA efflux stabilized at this level. Neither DOPAC, nor HVA efflux were significantly affected by MK-801.

In the presence of the dopamine uptake blocker nomifensine (10 μ M, administered locally) dizocilpine (300 μ M, administered also intrastrially) didn't affected the striatal DA concentration and the efflux of DA metabolites.

Effect of the nAChR antagonist mecamlamine on the striatal dopamine release

The nAChR antagonist mecamylamine administered locally through microdialysis probe at a concentration of 1mM produced a significant increase in the extracellular concentration of DA in the striatum. The peak effect was observed also in the second fraction (75 % increase), and the DA efflux stabilized at this level. Neither DOPAC, nor HVA efflux were significantly affected by mecamylamine.

In the presence of the dopamine uptake blocker nomifensine (10 μ M, administered locally) mecamylamine (1 mM, administered also intrastrially) didn't affected the striatal DA concentration and the efflux of DA metabolites.

Our data shows, that the striatal DA-level increasing effect of the MK-801 and the mecamylamine is in close relationship with the function of the dopamine uptake system, because the inhibition of the transporter by nomifensine prevented their dopamine increasing effect.

The dopamine uptake inhibitory effect of MK-801 and mecamylamine is in accordance with those electrophysiological data, which reveals, that channel-like structures exists in the monoamine uptake systems, and supports the idea, that these „pores” plays an important role in the process of neurotransmitter reuptake.

CONCLUSIONS

1. We proved, that two atypical antidepressants, the NA uptake blocker type maprotiline, and the serotonin reuptake enhancer tianeptine (which don't act in accordance with the monoamine theory) are able to inhibit the noradrenergic response mediated by nAChRs in the hippocampus, in the clinically relevant concentration range. Since these substances inhibited the electrical stimulation evoked transmitter release less potently, than that of the nAChRs mediated response, we can conclude, that their primary target cannot be the Na^+ - or Ca^{2+} -channels, therefore they exert this inhibitory effect through a direct blockade of the nACh receptors. This observation confirms the nACh-theory of depression, and indicates, that inhibition of the nAChRs in the central nervous system may contribute to the development of antidepressant effect.

2. We succeeded to demonstrate, that the two most potent nAChR antagonists, the tricyclic desipramine and the SSRI type fluoxetine are able to inhibit also the NMDA

receptor mediated [³H]NA-release in rat hippocampal slices, in the clinically relevant concentration range, which means that they possess NMDAR antagonistic properties.

Because of the fact, that neither of them influenced the electrical stimulation evoked transmitter release, it is unambiguous that their primary target is the NMDA receptor itself. Our results reveals, that the inhibition of NMDARs could be also a part of the mechanism of action of antidepressant substances.

3. During our experiments on the guinea pig heart right atrium, we proved evidence, that both the tricyclic desipramine and the SSRI type fluoxetine, which possess nAChR and NMDA receptor antagonistic properties, are able to inhibit also the ionotropic ATP receptor (P2X-R) mediated [³H]NA-release, in a clinically relevant concentration range, which means that they possess also P2X receptor antagonistic properties. Based on these results, we can conclude, that the blockade of P2X receptors localized in the heart may contribute to the development of cardiovascular side effects of the antidepressant substances, and their effect on the P2X receptors in the brain may play a role in the development of the therapeutic effect.

5. Our data reveals, that antidepressants could have several targets in the central nervous system, since apart from their monoamine increasing effect, they possess receptor antagonistic properties in the case of one representative from both three receptor families (the nAChR, the NMDAR and the P2X-R), and this fact suggests the possibility, that antidepressants may influence the function of many ionotropic receptors, and their clinical effect is a sum of these interactions.

5. Using *in vivo* microdialysis technique, we demonstrated, that two ionotropic receptor antagonist substances, the nAChR antagonist mecamylamine, and the NMDAR antagonist dizocilpine (MK-801) increase the extracellular level of DA in the striatum (without any effect on the level of this neurotransmitter's metabolites), which effect is probably the consequence of their direct inhibitory effect on monoamine uptake systems. Our results confirms the idea, that the channel-like structures in the monoamine transporters could have functional importance in the process of monoamine reuptake.

LIST OF CANDIDATE'S PUBLICATIONS

Publications related to the thesis:

Mayer A, Szasz BK, Kiss JP: Inhibitory effect of antidepressants on the NMDA-evoked [³H]noradrenaline release from rat hippocampal slices. *Neurochemistry International* Ref: NCI-D-09-00053R1 (in press)

Szász BK, Mayer A, Zsilla G, Lendvai B, Vizi ES, Kiss JP. Carrier-mediated release of monoamines induced by the nicotinic acetylcholine receptor agonist DMPP. *Neuropharmacology*. 2005 Sep;49(3):400-9.

Publications not related to the thesis:

Sylvester Vizi E, Rózsa B, Mayer A, Kiss JP, Zelles T, Lendvai B. Further evidence for the functional role of nonsynaptic nicotinic acetylcholine receptors. *Eur J Pharmacol*. 2004 Oct 1;500(1-3):499-508.

Petkova-Kirova P, Rakovska A, Della Corte L, Zaekova G, Radomirov R, Mayer A. Neurotensin modulation of acetylcholine, GABA, and aspartate release from rat prefrontal cortex studied in vivo with microdialysis. *Brain Res Bull*. 2008 Sep 30;77(2-3):129-35. Epub 2008 May 7.