NON-CONVENTIONAL EFFECTS OF NICOTINIC AGONISTS AND MONOAMINE UPTAKE BLOCKERS IN THE CENTRAL NERVOUS SYSTEM

Short thesis

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effects of these drugs. Findings may have great importance in the understanding of therapeutic and adverse uptake blockers are widely used in the therapy of depressed patients, therefore our monoamine transporters and have important clinical implications. The monoamine finding may reveal some structural similarity between the ion channels of transporters action, similarly to that of mecamylamine, is a channel blocker-type antagonism. This nervous system. Based on our data and literature we propose that the mechanism of structure and selectivity are able to inhibit the function of nAChRs in the central. We provided evidence that monoamine uptake blockers with different chemical agonists (nicotine, cytisine, epibatidine, anatoxin-A) increases the vesicular release of the majority of nicotinic agonists on the hippocampal NE release from rat brain. The majority of nicotinic5.2. Conclusions

Our data indicate that different mechanisms are involved in the effect of nicotinic antidepressants (tricyclic antidepressants, SSRIs, MAOIs) as well as monoamine uptake blockers. These results may help to understand the functional properties of putative similarities between the nAChRs and monoamine transporters and the functional pharmacological consequence of this similarity. It has been shown that the monoamine uptake blockers are able to block different classes of receptors In this thesis we provided evidence that there is an interesting connection between nAChRs and monoamine transporters. We found that certain nicotine receptors have functionally expressed in most hippocampal interneurons (CA1, CA2/CA3). Multiple nicotine receptors have been found in the hippocampus. In situ 2.1. Background

It has been shown that the monoamine uptake blockers are able to block different concentration of monoamines in the extracellular fluid and the synapse). We will refer the blockage of monoamine uptake transporter (the molecule that decreases the level of monoamine in the extracellular space) to these medications as monoamine uptake blockers.

Depression is one of the most common psychiatric disorders. At any given moment, about 5-6% of the population is depressed, determined by point prevalence. An estimated of 30 % may become depressed during their lifetime. Depression is by no doubt one of the main causes of suicide or attempted suicide. Several drugs have been developed successfully for the treatment of depression including tricyclic antidepressants (like imipramine and DMI) and selective serotonin reuptake inhibitors (SSRIs as fluoxetine and citalopram) being now the drugs most widely used for treatment of depression. Several studies have shown that the monoamine uptake blockers have a pronounced effect on nAChRs. The thesis will focus on the differential effect of nicotinic agonists on the hippocampal NE release. The majority of nicotinic agonists influence the function of monoamine transporters and monoamine release. In addition lobeline has a further stimulatory effect on the hippocampal NE through stimulation of nAChRs located on noradrenergic varicosities. DMPP has a dual action because, in addition to the nAChR-mediated mechanism, it is able to induce also a carrier-mediated release through the reversal of NA transporter. Finally, nicotine, DMPP, cytisine and Anatoxin-A increased the noradrenalin release, these effect was mecamylamine sensitive showing that the release was mediated by the nicotinic receptor (87). Further experiments in superfused rat hippocampal slices 35). Also significant expression of almost all the nAChR subunits was found CA1 hippocampal neurons (36).

Experiments using hippocampal synaptosomes showed that nicotinic agonist nicotine, DMPP, cytisine and Anatoxin-A increased the noradrenalin release, these effect was mecamylamine sensitive showing that the release was mediated by the nicotinic receptor (87). Further experiments in superfused rat hippocampal slices the noradrenergic hippocampal innervation originates exclusively from the locus.
coeruleus and the majority of the varicosities do not make synaptic contact therefore this release must be non-synaptic (39).

2.2. DMPP-evoked release of NA from hippocampus
In our previous experiments we found that the DMPP increase the release of noradrenaline in a two-phase release of NA from rat hippocampal slices. The first phase was a steep increase followed by a sudden decline to a lower level that was constant in time. The release of noradrenaline in response to DMPP consists of two components. While the nicotinic receptor antagonists mecamylamine, pancuronium, piperucuronium, the nonspecific antagonist Cd2+ and tetrodotoxin completely abolished the peak response (phase I), they had no effect on the tail response (phase II). Whereas the noradrenaline uptake blocker desipramine (DMI 1-10 µM), nisoxetine (1-10 µM), and nomifensine (10 µM) inhibited both phases, nomifensine at a concentration of 1 µM selectively blocked only phase II. Our results indicate that DMPP has a dual effect on the hippocampal noradrenaline release: phase I is a transient, nicotinic receptor-mediated exocytotic release, and phase II is a maintained, transporter-mediated process (26).

3. – Aims

3.1. Comparison of the effect of nicotinic agonists on the hippocampal NA release.
The results with DMPP proved that this nicotinic agonist has a dual action (on the nAChR and the transporter). The question was whether other nicotinic agonists share the same properties. The aim was to compare the effect of a number of nicotinic agonists on the hippocampal NA release and also analyze the mechanisms of action of these compounds (nicotine, anatoxin-A, epiptabidine, cytisine, DMPP, lobeline).

3.2. Investigation of the antinicotinic effect of monoamine uptake transporters in the CNS
3.2.1. Question of selectivity
Does the nAChR antagonist effect of monoamine uptake blockers depend on the uptake transporter selectivity?
3.2.2. Quantitative analysis
In our previous work (26) we have observed the inhibitory action of monoamine uptake blockers but the effect was not characterized quantitatively therefore an additional aim of the present study was to obtain the IC_{50} values of monoamine uptake blockers

3.2.3. Search for the possible mechanism of nAChR antagonist effect.

between nAChR ion channels and transporter channels then the nAChR antagonist property of monoamine uptake inhibitors would be easily understandable. The model we propose predicts that the channel blocker nicotinic antagonists should block the monoamine uptake. Recent data that the NMDA antagonist MK-801 blocks the monoamine transporters expressed in HEK cells (80) seem to support our hypothesis since the PCP-like NMDA antagonists have been shown to bind also to the ion channels of nAChRs (74).

Figure 2. Binding sites of the nicotinic acetylcholine receptor and the suggested mechanism of action of monoamine uptake blockers.

It is a justified question why the nAChR antagonist property of different monoamine uptake blockers remained hidden in spite of the meticulous pharmaceutical research and development. Previously different uptake blockers did not show high affinity for the nAChR, apparently ruling out that these compounds could act on the receptor (54). However, it must be noted that in the binding studies investigating nAChRs almost exclusively ligands of the nicotine binding site (e.g. [3H]nicotine) were used. The uptake blockers have low affinity toward this binding site. For example, cocaine was without effect on the [3H]nicotine binding (K_i > 1mM) while competed for the mecamylamine site with a K_i of 1 µM (66). Since currently good nicotinic channel radioligands are not available, the affinity of monoamine uptake blockers to the nAChR channel was recognized only occasionally (66,74). Our data suggest that the nAChR antagonism is not a sporadic phenomenon among monoamine uptake blockers but a general characteristic of these compounds.
sustaining evidence suggests that Na⁺-channels are equally affected in both cases. The differential effect of DMI on the two types of NA release excludes that the nAChR antagonist effect of DMI would be mediated via inhibition of Na⁺-channels. Taken together, the patch clamp data and TTX-experiments suggest that Na⁺-channels are not involved in the inhibitory action of monoamine uptake blockers on the nicotine-evoked NA release. In our experiments we investigated the possible mechanism by which monoamine uptake blockers can inhibit the nicotine-evoked NA release from rat hippocampal slices. Although we excluded some reasonable assumptions (involvement of NA uptake and Na⁺-channels), our results did not give a final and definite answer. Based on the literature, however, we can propose an explanation. It has been shown that tricyclic antidepressants including DMI bind with high affinity to the ion channel of nAChRs prepared from the electric organ of Torpedo ocellata (74). The Kᵣ value of DMI in the presence of ACh was 0.2 µM, which is very close to the IC₅₀ value (0.36 µM) obtained in our experiments. This binding site within the ion channel of nAChRs is the target of the non-competitive nicotinic antagonist mecamylamine (75). The interaction of monoamine uptake blockers with the mecamylamine binding site was also supported by Lerner-Marmarosh et al. (66) who reported that behavioral effects of nicotine were antagonized by cocaine analogs in mice and demonstrated in receptor binding studies that cocaine analogs compete for the mecamylamine binding site with high affinity. These receptor binding studies suggest that DMI and cocaine may behave like channel blocker-type nAChR antagonists. Another group published similar results to us showing that fluoxetine rapidly reduced the amplitude of membrane currents elicited by stimulation of neuronal α2β4, α3β4) and muscular (α1β1γ) nAChRs expressed in Xenopus oocytes (76). In this patch clamp study single channel recordings showed that fluoxetine directly blocks the receptor channel. Recently it has been published that fluoxetine also blocks the neuronal (α7) nAChRs by a similar mechanism (77). These results indicate that monoamine uptake blockers with different chemical structure and selectivity interact directly with the ion channel of nAChRs. It is then conceivable that other monoamine uptake blockers may share the same action mechanism, that is, they bind into the ion channel of nAChRs and act as non-competitive channel-blocker type nicotinic antagonists. Posterior experiments using single patch-clamp finally confirmed all the experimental data and suggested that the inhibition of nAChR by cocaine is unit subtype-dependent (77,78).

A very interesting consequence of our findings is that it may reveal some structural similarity between nicotinic receptors and monoamine uptake carriers. Accumulating data indicate that the membrane transporter proteins contain functional channels (for a review see 79). If we assume that i.) these channels play a crucial role in the transport of molecules through the membrane, ii.) the general mechanism of uptake blockers is the blockade of these channels, and iii.) there is some structural similarity, indicating that the blockade of these channels, and iii.) there is some structural similarity,

4. – Methods

4.1. – Nicotinic agonists-evoked [¹H]noradrenaline release from rat hippocampal slices

4.2. - Electrical stimulation-evoked [¹H]noradrenaline release from rat hippocampal slices

4.3. –Electrophysiological recording of Na⁺-currents

5. - Results

5.1. Differential effect of nicotinic agonists on the [¹H]noradrenaline release from rat hippocampal slices

5.1.1. Effect of nicotinic agonists on the electrical stimulation-evoked release of [¹H]noradrenaline from rat hippocampal slices

The resting and electrical stimulation-evoked release of [¹H]NA was affected by all nicotinic agonists, however their action showed differences. Majority of the nicotinic agonists (nicotine, 100 µM; cytisine, 30 µM; epibatidine, 1 µM; anatoxin-A, 2 µM) increased only the resting release of [¹H]NA before electrical stimulation. This action was transient and it declined rapidly. Neither the electrical stimulation, nor the post-stimulation resting release was affected by these drugs. In contrast, DMPP (20 µM) increased the release of [¹H]NA in all phases, that is, it stimulated both the pre- and post-stimulation resting release and also increased the response to electrical stimulation. The effect of lobeline (30 µM) was very similar to that of DMPP, except for the time-course of action on the resting release before stimulation (fraction pairs 1 and 2). While the effect of DMPP on the pre-stimulation release showed decreasing tendency, the response to lobeline increased with time.

5.1.2 Effect of the nicotinic antagonist mecamylamine on the action of nicotinic agonists

The enhancing effect of nicotine (100 µM) on the release of [¹H]NA was completely blocked by the non-competitive nicotinic antagonists mecamylamine at a concentration of 10 µM. The effect of antagonists (cytisine, epibatidine, anatoxin-A), which similarly to nicotine, increased only the pre-stimulation resting release of [¹H]NA ('nicotine-like' antagonists), was also completely inhibited by mecamylamine (not shown). The effect of DMPP (20 µM) was only partially inhibited by mecamylamine, because a significant reduction of release could be observed only in the pre-stimulation resting phase, but the response to DMPP during the electrical stimulation and the post-stimulation resting phase was not influenced by mecamylamine. Finally, the effect of lobeline on the release of [¹H]NA was not affected at all by mecamylamine.
5.1.3 Effect of the noradrenaline uptake inhibitor desipramine on the action of nicotinic agonists

To investigate the possible role of NE transporters in the action of nicotinic agonists, we repeated the experiments in the presence of the NE uptake inhibitor DMI (10 µM). This drug was present in the Krebs solution from the beginning of superfusion, which caused a slight and non-significant upward shift in the control curve. The control ratios moved from about 90 % to about 100 %. The stimulating effect of nicotine was completely blocked by DMI. Similarly, the response to DMPP was inhibited by the NE uptake inhibitor in all phases (pre-stimulation resting, electrical stimulation, post-stimulation resting) of the experiment. In contrast, the effect of lobeline was only partially inhibited by DMI. It was completely blocked during the electrical stimulation and in the first post-stimulation sample but the action was only partially reduced in other fractions. This residual response was not influenced significantly by pargyline (10 µM), when the MAO-B inhibitor was also present in the Krebs solution from the preperfusion (not shown).

5.2. Effect of nicotine on the release of [3H]NA from rat hippocampal slices

Perfusion of nicotine at a concentration of 100 µM produced an excess release over the basal efflux of [3H]NA (AUC4-10 = 323.18 ± 44.24 %, n=28). The response was transient and the release returned to the baseline within four fractions in spite of the presence of nicotine in the solution. Although the time course of the response was not affected, the excess release in response to nicotine (100 µM) was significantly lower (AUC4-10 = 87.19 ± 6.02 %, n=8, p < 0.01 in two-tailed Welch’s t test) when the Krebs solution contained ethanol (3 mg/L) in the control experiments for the citalopram-group. The inhibitory effect of ethanol was not further investigated since the difference did not disturb the evaluation of the effect of monoamine uptake blockers.

5.3. Effect of monoamine uptake blockers on the nicotine-evoked release of [3H]NA from rat hippocampal slices

The nicotine-evoked release of [3H]NA was dose-dependently inhibited by all of the tested drugs (DMI, nisoxetine, cocaine, citalopram, nomifensine) in the concentration range of 0.03-30 µM. The IC50 values of the monoamine uptake blockers ranged between 0.36 µM (DMI) and 1.84 µM (nomifensine).

5.4. Correlation between the inhibition of nicotine-evoked [3H]NA release and the inhibition of NA uptake

To explore a possible relationship between the observed nAChR antagonist effect and the ability to block NA uptake, the logarithm of the calculated IC50 values were plotted against the logarithm of the K values for the NA uptake transporter and the coefficient of correlation (r) was determined. K values were taken from the literature.

But what is the mechanism of this antagonism? Some authors suggested that the action of nicotine may require an intact NA uptake mechanism (62), that is, the antinicotinic action of uptake blockers would be mediated through the transporter. Comparing the chemical structures and the pharmacological properties of these drugs, their only common feature is that all of them are able to block certain monoamine uptake transporters. Therefore it is justified to assume that these compounds interact with the NA uptake system and this interaction initiates some intra- and/or extracellular events which, in fact, may lead to a functional blockade of nAChRs. To investigate this hypothesis we compared the IC50 values obtained in our experiments with the inhibitory effect of these compounds on NA uptake (K, values were taken from literature) (54, 59, 68), to investigate a possible connection between these properties. The statistical analysis showed no correlation between K and IC50 values suggesting that the NA uptake system is not involved in the inhibitory effect of monoamine uptake blockers on the nicotine-evoked NA release. This conclusion is supported by our previous observation that nomifensine at a concentration of 1 µM effectively blocked the carrier-mediated component of DMPP-evoked NA release but did not affect the nAChR-mediated component (26) indicating that the actions on the transporter and on the nAChR are separated.

It has been shown that antidepressant drugs like DMI and imipramine are able to suppress fast inward sodium (Na+) current in a variety of neuronal preparations (69, 70, 71). The TTX-dependency of the nicotine-evoked release of [3H]NA (72, 73) indicates that the response to nicotine require the activation of Na+-channels therefore our next assumption was that the monoamine uptake blockers inhibited the nicotine-evoked release via inhibition of Na+ channels. Thus, in the next series of experiments we tested the inhibitory effect of the uptake blockers on the fast TTX-sensitive inward Na+ current in rat sympathetic neurons from the superior cervical ganglia. The effects of uptake blockers were studied in the concentration range (1-10 µM) which proved to be effective in the hippocampal preparation. Our data showed that only DMI had pronounced inhibitory effect on the Na+ current in the rat sympathetic neuron preparation while the rest of the monoamine uptake blockers were ineffective. Since DMI at a concentration of 10 µM inhibited about 50 % of the Na+-currents, our patch clamp data could not rule out the possibility that DMI blocks the nicotine-evoked NA release via inhibition of Na+-channels. However, if this is the case, DMI should have inhibited also the electrical stimulation-evoked release of NA, since this process is Na+-channel dependent. In contrast, our data showed that DMI blocked only the nicotine-evoked NA release but had no inhibitory effect on the electrical stimulation-evoked release. A possible explanation would be for this discrepancy that the two stimulation protocols activate the Na+-channels with different efficacy. Nevertheless, our results obtained with TTX indicated that the TTX-sensitivity of the nicotine- and electrical stimulation-evoked release was identical (the IC50 was 0.033 and 0.036 µM, respectively).
(122). This change may promote the reversal of membrane transporters and the concomitant carrier-mediated release of transmitters (123). Our data obtained with DMI indicate that a substantial part of the effect of lobeline is results indeed from the reversal of uptake; however, further factors are also involved. It has been observed that, due to the increased cytoplasmic concentration of transmitters (see above) the efflux of metabolites is also increased (122). We tested, therefore, the hypothesis that the residual tritium efflux could be metabolic. It was not reduced, however, significantly in the presence of the MAO-B inhibitor pargyline, suggesting that the DMI-insensitive release cannot be explained with the increased metabolite outflow, i.e. it is [3H]NA. It is possible that lobeline increases the efflux of [3H]NA through the cell membrane with a similar mechanism as it displaces transmitters from the vesicles into the cytoplasm. This idea is supported by the fact the lobeline-induced [3H]NA release is completely blocked at 12 °C (121). At this temperature the rigidity of biological membranes is increased, which could prevent the ‘leakage’ of transmitters through the membrane. Additional effects of lobeline (e.g. blockade of Ca2+-channels (124)) may also contribute to its action on NE release. The effects of different nicotinic agonists on hippocampal NA release are summarized on Figure 1.

6.2. Effect of monoamine uptake blockers in nicotine induced noradrenaline release
In our results we have found that monoamine uptake blockers with different chemical structure and selectivity for NA, DA or 5-HT transporters (59, 60, 61) are able to inhibit the nicotine-evoked increase of hippocampal NA release in a dose-dependent manner. The calculated IC50 values of DMI, nisoxetine, nomifensine, citalopram, fluoxetine and cocaine ranged between 0.36 and 1.84 µM.

It has already been demonstrated that some of the monoamine uptake blockers (DMI, nisoxetine and cocaine) may influence the function of nAChRs. Almost 30 years ago, Su and Bevan (62) reported that DMI and cocaine inhibited the nicotine-evoked release of [3H]NA from spiral strips of the rabbit pulmonary artery. The nicotine-induced exocytotic release of NA from isolated guinea-pig heart was blocked by DMI and nisoxetine (63). In addition, DMI inhibited the DMPP-evoked depolarization and NA release in SH-SY5Y neuroblastoma cells (64). In the same cell line whole cell patch clamp recordings proved that DMI and imipramine inhibited the nAChR-mediated currents in a non-competitive manner (65). The behavioral effects of nicotine (seizures, tremors, fasciculations, etc.) were antagonized by cocaine and cocaine analogs in mice (66). The nicotine-induced inward current (both peak current amplitude and total charge influx) was inhibited by DMI and imipramine in chromaffin cells (67). Our results confirm these findings and extend the range of monoamine uptake blockers possessing nAChR antagonistic activity with nomifensine and the SSRI citalopram.

(88,89,90). The analysis showed no correlation between the two variables (r=0.17, slope 0.02, not significantly different from zero) excluding the possibility of a connection between the uptake transporter blocker effect and nAChR-antagonist effect of these medications.

5.5. Effect of monoamine uptake blockers on Na+-currents of sympathetic neurons from rat superior cervical ganglia
In patch clamp experiments the uptake blockers (DMI, nisoxetine, nomifensine, citalopram and cocaine) were used at the concentration range (1 and 10 µM) where the inhibition of nicotine-evoked release was already about 90 %. The amplitudes of control current preceding the drug application and the amplitude of the current by the end of drug application were compared. Steady state maximal inhibition usually developed before the end of the 2 min perfusion period. The inhibitory effects of the applied drugs were reversible. Neither drug inhibited Na+-currents at 1 µM and only DMI displayed a pronounced inhibition (52 %) at 10 µM therefore the possible involvement of Na+-channel blockade in the effect of monoamine uptake blockers was further studied only in the case of DMI.

6.6. Comparison of the inhibitory effect of TTX and DMI on the nicotine- and electrical stimulation-evoked release of [3H]NA from rat hippocampal slices
The effect of DMI and the Na+-channel blocker TTX on the nicotine- and electrical stimulation-evoked release of NA were compared to test the possible involvement of Na+-channels in the action of DMI. TTX blocked both the nicotine- and the electrical stimulation-evoked release with the same efficacy (IC50 = 0.033 and 0.039 µM, respectively). In contrast, DMI blocked only the nicotine-evoked release (IC50 = 0.36 µM), whereas the electrical stimulation-evoked release was not inhibited at all (zero inhibition), moreover it was potentiated as expected from its uptake blocking effect.

6. – Discussion

6.1. Differential effect of nicotinic agonists on the [3H]noradrenaline release from rat hippocampal slices
In previous works (108, 72) we studied the effect of nicotinic agonists on the release of NE from rat hippocampal slices. We found that the major effect of these compounds is the stimulation of nAChRs located on noradrenergic varicosities (37), which induces a Ca2+-dependent vesicular exocytosis. During these studies, however, we have observed that some of the nicotinic agonists (DMPP and lobeline) are able to stimulate the release of NE also in Ca2+-free medium, indicating that the action of these drugs is more complex than that of the conventional nicotinic agonists. The aim of the present study was to investigate the mechanisms involved in the action of
different nicotinic agonists on the \[^{3}H\]NA release from rat hippocampal slices. We used a newly developed calculation method for the evaluation of experimental data, which proved to be very effective tool for the detection of fine differences between the actions of drugs.

**6.1.1. Effect of conventional nicotinic agonists**

Our data, in line with previous results, demonstrated that nicotine, cytisine, epibatidine and anatoxin–A behaved like conventional nicotinic agonists, that is, they increased the release of \[^{3}H\]NA from rat hippocampal slices exclusively through stimulation of nAChRs, because their action was completely inhibited by the nicotinic antagonist mecamylamine. These drugs had only a transient and rapidly declining effect on the resting release before the electrical stimulation but had no effect on the electrical stimulation-evoked or on the post-stimulation resting release, which was the consequence of the rapid desensitization nAChRs.

It may seem to be more surprising that the action of nicotine was also completely blocked by the NE uptake inhibitor DMI. The nicotinic antagonistic property of DMI, however, has already been described earlier (64,65,109). Results described for this thesis confirmed the findings and quantified the effect.

**6.1.2. Effect of DMPP**

Our results show that DMPP, in contrast to other nicotinic agonists, increases the hippocampal \[^{3}H\]NA release in all phases (pre-stimulation resting, electrical stimulation, post-stimulation resting) of the experiment. The observation that mecamylamine inhibits this action only partially in the pre-stimulation resting phase suggests that the effect of DMPP has more than one component. It is obvious that DMPP, similarly to other nicotinic agonists, is able to stimulate nAChRs. What can be the mechanism of the mecamylamine-insensitive effect? It has been observed that substrates of the NE uptake system are able to induce a reversed transport of NE (110). Accumulating data indicate that DMPP can be such a substrate. It has been shown that DMPP is able to release NA from rat vas deferens through a carrier-mediated mechanism but only if the vesicular uptake and monoamine oxidase (MAO) are inhibited and Ca\(^{2+}\) is omitted from the perfusion medium (111). Under normal conditions this effect is very weak in the periphery. Our data show that in the CNS, however, these manipulations are not necessary for a carrier-mediated efflux of NE. The mecamylamine-insensitive part of the DMPP-response completely disappeared in the presence of DMI, which provided convincing evidence for the involvement of uptake carriers. In addition, DMI blocked also the mecamylamine-sensitive response because of the nAChR antagonistic property of the uptake blocker (see above).

**6.1.3. Effect of lobeline**

Lobeline, similarly to DMPP, increased the resting release of \[^{3}H\]NA before and after stimulation and potentiated the response to electrical stimulation as well. The new evaluation method, however, shed light on a very important difference. While the effect of DMPP on pre-stimulation resting release showed a decreasing tendency, the response to lobeline gradually increased in this phase of the experiment. Further analysis revealed that the effect of lobeline is qualitatively different from that of DMPP because the response to lobeline was not affected at all by mecamylamine. This is surprising because lobeline has been widely used as a nicotinic agonist (112). Indeed, it has been reported that lobeline competes for the nicotine binding site with high affinity, the \(K_i\) ranges between 4-30 nM (113,114,115). Functional-level analogy and evidence for high affinity binding to nAChRs, however does not prove an agonist effect on nAChRs. Accumulating data indicate that the nature of lobeline is ambiguous, and is dependent on the type of nAChR studied: it is either a partial agonist, typically the weakest of the agonists studied (116,117), or an antagonist (115,118). Our data, in line with these findings, suggest that under our experimental conditions the nAChRs involved in the release of \[^{3}H\]NA are not stimulated by lobeline. The mecamylamine-insensitive effect of lobeline has already been observed previously. Lobeline increased the release of DA from rat (37) and mouse (119) striatal synaptosomes and slices (120) and the release of NE from rat vas deferens (121) in a mecamylamine-insensitive and Ca\(^{2+}\)-independent manner. All of these data indicate that the action of lobeline is independent of nAChRs, and the lobeline-induced release is not a vesicular exocytosis.

![Figure 1. Differential effect of nicotinic agonists on the hippocampal NA release.](image)

What kind of mechanism can be involved then? It has been shown that lobeline blocks the vesicular monoamine transporter (VMAT2) with high affinity (120,121). In addition, lobeline displaces the monoamines from synaptic vesicles, which leads to the increase of cytoplasmic transmitter concentration.