

Carrier-mediated release of monoamines induced by the nicotinic acetylcholine receptor agonist DMPP

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Abstract

We have previously shown that dimethylphenylpiperazinium (DMPP) increases the release of noradrenaline (NA) from rat hippocampal slices via two distinct mechanisms: a nicotinic acetylcholine receptor (nAChR)-mediated exocytosis and a carrier-mediated release induced by the reversal of NA transporters. Our aim was to investigate whether other monoaminergic systems are also affected by the multiple actions of DMPP. In our experiments DMPP dose-dependently increased the release of dopamine (DA) and serotonin (5-HT) from rat striatal and hippocampal slices, respectively. The dual effect was observed, however, only in case of DA at a lower DMPP concentration (30 μM), where the response was partly inhibited by mecamylamine, TTX and Ca^{2+} -free medium (nAChR-mediated exocytosis) while the other part of the response was blocked only by the DA uptake inhibitor nomifensine (carrier-mediated release). In contrast, the DMPP-evoked 5-HT release and the DA release induced by high concentration DMPP was not inhibited by nicotinic antagonists, TTX and Ca^{2+} -free medium but only by selective uptake inhibitors. In addition, DMPP dose-dependently inhibited the [^3H]DA and [^3H]5-HT uptake in striatal and hippocampal synaptosome preparation with an IC_{50} of 3.18 and 0.49 μM , respectively. Our data show that DMPP interacts with monoamine transporters and induces a substantial carrier-mediated release of DA and 5-HT, therefore caution is needed for the interpretation of data, when this drug is used as a nAChR agonist.

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1. Introduction

Nicotinic acetylcholine receptors (nAChRs) play an important role in the regulation of neurotransmission in the central nervous system (Wonnacott, 1997; Vizi and Lendvai, 1999). Previously we investigated the involvement of nAChRs in the cholinergic-noradrenergic interactions in the hippocampus (Vizi et al., 1995; Sershen et al., 1997) because of its significance in

cognitive functions. We found that nicotinic agonists increase the hippocampal noradrenaline (NA) release via stimulation of presynaptic nAChRs located on noradrenergic varicosities. During these studies we observed that dimethylphenylpiperazinium (DMPP), in contrast to other nicotinic agonists has multiple effects on hippocampal NA release (Kiss et al., 1997). First, it provokes a Ca^{2+} -dependent exocytotic release through stimulation of nAChRs, similarly to conventional nicotinic agonists like nicotine, anatoxin A, cytosine and epibatidine. This part of the response is completely blocked by nicotinic antagonists and can be modulated by presynaptic α_2 -adrenoceptor ligands. Second, DMPP

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is able to induce a Ca^{2+} -independent NA efflux which is not affected by nicotinic antagonists but can be blocked by NA uptake inhibitors, indicating that this component results from the reversed transport of NA through the membrane transporter. This latter effect can be explained by the observation that DMPP is a substrate of the NA transporter (Niebler and Trendelenburg, 1990), therefore it is able to induce an exchange mechanism by which the cytoplasmic NA can be released.

The NA uptake carrier belongs to the family of Na^+/Cl^- -dependent membrane transporters containing 12 transmembrane domains (Amara and Arriza, 1993). The amino acid sequence identity of the NA and dopamine transporters (NET and DAT) is higher than 75% (Giros et al., 1992), while the 5-HT transporter (SERT) is also very similar, its homology with NET being still above 60% (Blakely et al., 1991). Because of this high degree of homology, there is a significant functional overlap between monoamine transporters. It has been shown, for example, that NET takes up DA more efficiently than DAT (Buck and Amara, 1994). Recently we have reported that SERT is able to take up NE in NET KO mice (Vizi et al., 2004). The existence of heterologous uptake between DAT, NET and SERT was supported by other studies (Carboni et al., 1990; Yamamoto and Novotney, 1998; Mundorf et al., 2001) as well.

Based upon the previously observed effect of DMPP on NET and the similarities between transporters, our aim was to investigate whether DMPP is able to influence the function of other members of the monoamine transporter family, the DA and 5-HT transporters. We, therefore, studied and analyzed the effect of DMPP on the striatal DA release and the hippocampal 5-HT release from rat brain slices.

2. Methods

2.1. [^3H]Dopamine and [^3H]serotonin release from rat brain slices

All experimental procedures were approved by the local ethical committee and were in accordance with NIH guidelines. Male Wistar rats (weight 140–160 g) were killed by decapitation and the brain was rapidly removed and immediately placed into ice-cold Krebs solution (composition in mM: NaCl 113, KCl 4.7, MgSO_4 1.2, CaCl_2 2.5, NaHCO_3 25, KH_2PO_4 1.2, glucose 11.5, Na_2EDTA 0.3, and ascorbic acid 0.03) continuously gassed with a mixture of 95% O_2 and 5% CO_2 . Then the striatum or the hippocampus was sliced to 0.4-mm thick sections with a McIlwain chopper. Slices were washed with 5 ml of Krebs solution, and loaded for 45 min with [^3H]dopamine ([^3H]DA) or

[^3H]serotonin ([^3H]5-HT) at a concentration of 10 μCi in 1 ml of Krebs solution. After the incubation they were washed three times with 5 ml of Krebs solution and transferred into a four-channel microvolume perfusion system (Vizi et al., 1985). Four slices were put into each chamber, the preparation was superfused with Krebs solution at 37 °C at a rate of 0.6 ml/min for 60 min (preperfusion period), and the effluent was discarded. Subsequently 3-min fractions were collected. DMPP was added from the 5th fraction (0 time point, see figures), mecamylamine was applied from the 3rd fraction of the sample collection period, whereas uptake blockers (nomifensine or fluoxetine) were present in the medium from the beginning of the preperfusion period. At the end of experiments slices were removed from the chamber and were homogenized in 0.5 ml of 10% trichloroacetic acid. To determine the tissue content, a 0.2-ml aliquot of this superfusate was added to 2 ml of scintillation cocktail (Ultima Gold, Packard). Tritium content was measured with a Packard 1900 TR liquid scintillation counter. Radioactivity was expressed in terms of disintegration per minute per gram of tissue (Bq/g). The fractional release was expressed in terms of the percentage of tritium present in the tissue at the beginning of a given collection period. The average FR data of different treatment groups were not significantly different, the overall value being $0.83 \pm 0.03\%$ per 3 min ($n=187$) for the DA and $0.55 \pm 0.04\%$ per 3 min ($n=102$) for the 5-HT release.

2.2. [^3H]Dopamine and [^3H]serotonin uptake in rat brain synaptosomal preparation

2.2.1. Preparation of crude synaptosomal fraction (P_2)

After decapitation of male Wistar rats (140–160 g) the brain was removed and the striatum or hippocampus was dissected over ice and homogenized in 0.32 M sucrose (1:10 g/ml) solution with up and down strokes of a Teflon pestle homogenizer. The homogenate was centrifuged at $1000 \times g$ for 10 min and the supernatant was further centrifuged at $12000 \times g$ for 20 min. The pellet containing synaptosomes was used for uptake studies.

2.2.2. Measurement of [^3H]DA or [^3H]5-HT uptake in synaptosomal (P_2) preparation

Synaptosomal (striatal or hippocampal) pellet was suspended in carbogenated Krebs assay buffer containing: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 10 mM D-glucose, 25 mM NaHCO_3 , 0.3 mM ascorbic acid and 0.01 mM pargyline (pH 7.4). Aliquots of synaptosomal suspension was preincubated with drug for 5 min at 37 °C, in a final volume of 500 μl . After preincubation [^3H]DA (50 nM) or [^3H]5-HT (50 nM) was added to the tubes and incubation was continued for 5 min. The uptake

was terminated by adding 3 ml of assay buffer. Samples were filtered through GF/B filters soaked in 0.05% polyethylenimine by using a Brandel cell harvester, and filters were washed with cold Krebs solution. Radioactivity trapped on filters was counted in a Packard liquid scintillation counter. Nonspecific uptake was determined by incubation of samples at 0 °C, the specific uptake was the difference of total and non specific uptake value.

2.3. Statistical analysis

Fractional release (FR) data were normalized in order 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. The results were analyzed using an area under curve (AUC) method. For the DMPP dose–response curves the area (A) was calculated as the surplus release over the basal efflux according to Eq. (1).

$$A = \left(\sum_{i=4-12} \text{FR}_i \right)_{\text{DMPP}(c)} - \left(\sum_{i=4-12} \text{FR}_i \right)_{\text{DMPP}(0)} \quad (1)$$

where i is the number of fractions. Expressions indexed with DMPP(c) and DMPP(0) represent the response in the presence of DMPP at a concentration of c and in the absence of DMPP (basal release), respectively. Where the response to DMPP had two components (DA release, see Section 3), the area of the first ($A_{\text{phase I}}$) and second component ($A_{\text{phase II}}$) were calculated separately according to Eqs. (2) and (3) (for further details see Kiss et al., 1997).

$$A_{\text{phase I}} = \sum_{i=4-9} \text{nFR}_i - 5 * (\text{nFR}_4 + \text{nFR}_9) / 2 \quad (2)$$

$$A - A_{\text{phase I}} = A_{\text{phase II}} \quad (3)$$

In each experiment two channels were used for control (DMPP alone) and two channels for a given treatment (nicotinic antagonists, uptake inhibitors, TTX, Ca^{2+} -free medium). Because of this design each treatment group had its corresponding control group which provided a more reliable analysis. The effect of a treatment was calculated as the percentage of the corresponding control response and each treatment group was compared to its own control by a two-tailed Student's t -test. The level of significance was set to $P < 0.05$. Values represent the mean \pm S.E.M. of 4–13 independent experiments. The EC_{50} values for dose–response curves and the IC_{50} values for uptake inhibition were calculated by nonlinear regression (GraphPad Prism 3.0).

2.4. Drugs

Dimethylphenylpiperazinium iodide (DMPP), mecamylamine hydrochloride, methyllycaconitine (MLA), fluoxetine HCl and nomifensine maleate were purchased from Sigma–Aldrich, Budapest, Hungary; tetrodotoxin citrate (TTX) was obtained from Alomone Labs, Jerusalem, Israel. [^3H] serotonin (15.7 Ci/mmol) and [^3H] dopamine (47 Ci/mmol) were purchased from Amersham, Little Chalfont, UK. All other chemicals used were of analytical grade.

3. Results

3.1. Effect of DMPP on the resting release of [^3H]dopamine from rat striatal slices

After 45-min of loading with [^3H]DA, followed by a 60-min preperfusion, the slices contained 212477 ± 12759 Bq/g ($n=63$) radioactivity. DMPP increased the striatal [^3H]DA release in a dose-dependent manner (Fig. 1); however, the response did not reach a plateau even at the highest concentration of the $1 \mu\text{M}$ – 10mM range. The time course of the response was dependent

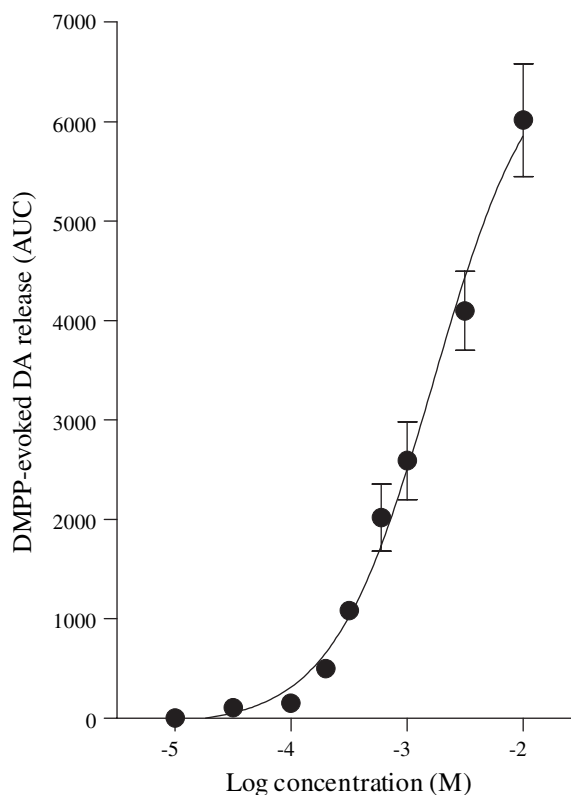


Fig. 1. Dose–response curve of the effect of DMPP on the resting release of [^3H]DA from rat striatal slices. The response (area under curve) was calculated as described in Section 2 and represents the surplus over the basal release. Each point represents the mean \pm S.E.M. of 5–8 independent experiments (in arbitrary units).

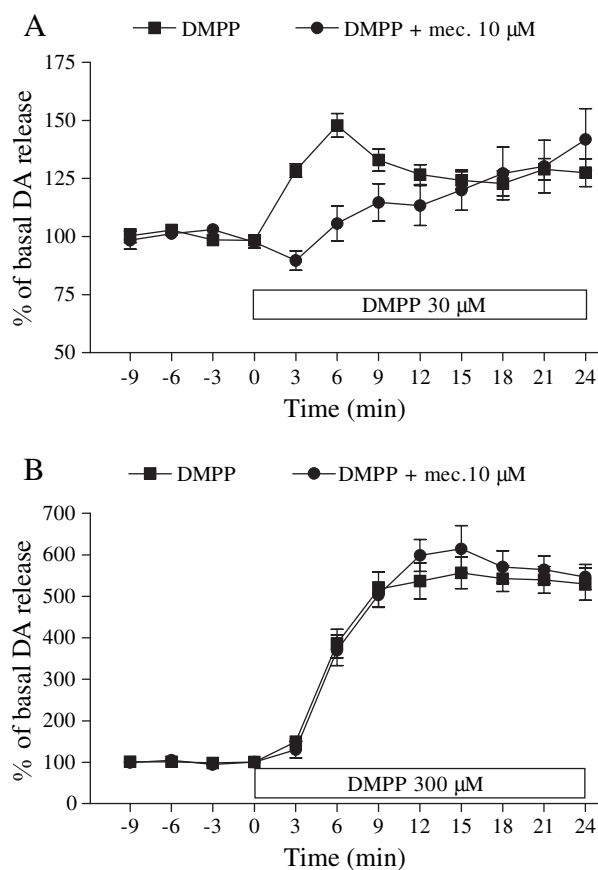


Fig. 2. Effect of mecamlamine on the DMPP-evoked release of [³H]DA from rat striatal slices. DMPP was applied from the 5th fraction (from the 0 time point) and was present in the medium as indicated by the horizontal bar. Mecamlamine (10 μM) was present from the 3rd fraction. (A) Low concentration (30 μM)-evoked release. (B) High concentration (300 μM)-evoked release. Each point represents the mean ± S.E.M. of 4–12 independent experiments.

on the concentration of DMPP. While at higher concentrations (≤ 100 μM) the release increased gradually and reached a plateau within a few minutes (3–4 fractions), at lower concentrations the response was biphasic: an immediate fast increase and a sudden decline (peak response: phase I) was followed by

a persistent release (tail response: phase II) which was still significantly higher than the spontaneous DA efflux (Fig. 2A). The biphasic response was most evident at 30 μM, therefore this concentration was used for further experiments. For the analysis of response to higher concentrations of DMPP the 300 μM concentration was selected.

3.2. Characterization of DMPP-evoked [³H]dopamine release from rat striatal slices

3.2.1. Nicotinic antagonists

We investigated the involvement of nAChRs in DMPP-evoked monoamine release using the potent nicotinic receptor antagonist mecamlamine and the $\alpha 7$ nAChR selective antagonist MLA. In the striatum the [³H]DA release evoked by low concentration DMPP (30 μM) was partly blocked by mecamlamine (10 μM). The inhibitory effect was concentrated on the peak response, since phase I was completely inhibited while the tail response (phase II) was not affected at all by the drug (Table 1, Fig. 2A). In contrast to mecamlamine, MLA had no effect on the DMPP-evoked [³H]DA release. At higher concentration (300 μM); however, the DMPP-evoked [³H]DA release was not inhibited by mecamlamine (DMPP_{control} = 100 ± 8.69, $n = 8$; DMPP_{mecam} = 104.75 ± 5.49, $n = 4$) or MLA (DMPP_{control} = 100 ± 9.99, $n = 6$; DMPP_{MLA} = 99.78 ± 15.51, $n = 6$) (Fig. 4).

3.2.2. Uptake inhibition

The possible involvement of monoamine transporters in the DMPP-evoked monoamine release was investigated by the use of specific monoamine uptake blockers. The DA uptake blocker nomifensine alone had no effect on the basal release (data not shown) but dose-dependently inhibited the [³H]DA release evoked by low concentration (30 μM) DMPP (Table 1, Fig. 3A). The inhibitory effect was stronger on phase II, which was completely inhibited by nomifensine at 10 μM, but phase I was also significantly attenuated (Table 1). The

Table 1
Characterization of the low concentration (30 μM) DMPP-evoked [³H]DA release from rat striatal slices

Drug	Whole area (%)	Phase I (%)	Phase II (%)
DMPP (30 μM)	100 ^a	100 ^a	100 ^a
DMPP (30 μM) + mecamlamine (10 μM)	74.11 ± 24.61	3.38 ± 22.11***	110.18 ± 26.85
DMPP (30 μM) + nomifensine (1 μM)	38.97 ± 4.67***	73.51 ± 9.62	21.24 ± 9.7**
DMPP (30 μM) + nomifensine (10 μM)	6.31 ± 5.87***	18.69 ± 11.17***	0.04 ± 4.51***
DMPP (30 μM) + TTX (1 μM)	76.70 ± 11.34	22.88 ± 3.53***	97.54 ± 2.48
DMPP (30 μM) + MLA (10 nM)	117.3 ± 12.72	92.5 ± 23.88	120.74 ± 12.25
DMPP (30 μM) in Ca ²⁺ -free medium	113.18 ± 36.54	16.15 ± 12.71***	133.67 ± 45.13

The area under curve was calculated as described in Section 2. Phase I and Phase II correspond to the nAChR-mediated response and the carrier-mediated release, respectively. The effect of a treatment was calculated as the percentage of the corresponding control response and each treatment group was compared to its own control group by a two-tailed Student's *t*-test. The level of significance was set to $P < 0.05$ (** $P < 0.01$, *** $P < 0.001$). Values represent the mean ± S.E.M. of 6–14 independent experiments.

^a The S.E.M. of control groups is different for every treatment group.

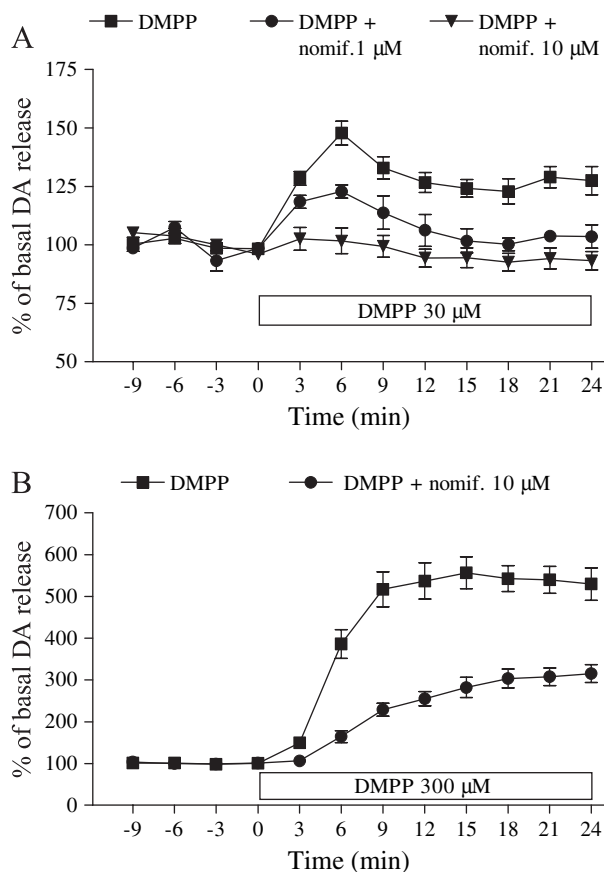


Fig. 3. Effect of nomifensine on the DMPP-evoked release of [3 H]DA from rat striatal slices. DMPP was applied from the 5th fraction (from the 0 time point) and was present in the medium as indicated by the horizontal bar. Nomifensine (1 or 10 μ M) was present from the preperfusion period. (A) Low concentration (30 μ M)-evoked release. (B) High concentration (300 μ M)-evoked release. Each point represents the mean \pm S.E.M. of 4–12 independent experiments.

[3 H]DA release evoked by high concentration DMPP (300 μ M) was also significantly inhibited by nomifensine (10 μ M), although the inhibition was not complete (Fig. 3B), the response to DMPP decreased only by about 60% (DMPP_{control} = 100 \pm 8.69, n = 8; DMPP_{nomif} = 39.34 \pm 4.56, n = 4, P < 0.001).

3.2.3. TTX and Ca²⁺-free medium

In order to differentiate between vesicular exocytosis and carrier-mediated release the effect of the Na⁺-channel inhibitor TTX and Ca²⁺-free medium was investigated on the DMPP-evoked monoamine release. In case of low concentration (30 μ M) DMPP stimulation, the phase I component of [3 H]DA release was significantly inhibited by TTX (1 μ M) or Ca²⁺-free medium (73% and 83% inhibition, respectively), whereas the phase II component was not affected by these treatments (Table 1). The high concentration (300 μ M) DMPP-evoked [3 H]DA release was not inhibited by TTX or Ca²⁺-free medium (DMPP_{control} =

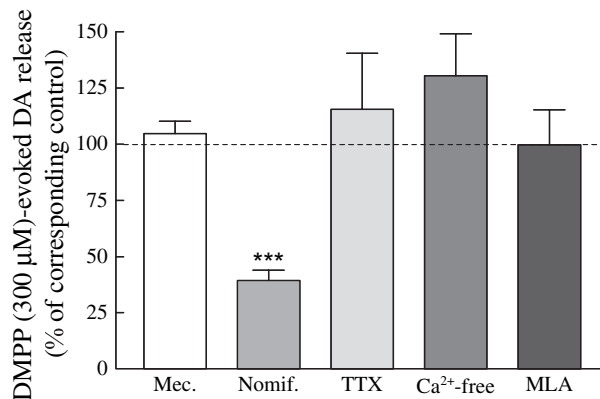


Fig. 4. Characterization of the striatal [3 H]DA release evoked by high concentration (300 μ M) DMPP. Each treatment group had its own control group (corresponding control), 100% is represented by the dotted line; for the S.E.M. of controls see Section 3. Each bar represents the mean \pm S.E.M. of 4–7 individual experiments. Two tailed Student's t -test was used for statistical analysis (***) P < 0.001. Mec, mecamlamine 10 μ M; Nomif., nomifensine 10 μ M; TTX, tetrodotoxin 1 μ M; Ca²⁺-free, Ca²⁺-free medium with 1 mM EGTA; MLA, methyllycaconitine 10 nM.

100 \pm 21.27, n = 4, DMPP_{TTX} = 115.57 \pm 24.88, n = 4; DMPP_{control} = 100 \pm 15.66, n = 8; DMPP_{Ca²⁺-free} = 130.45 \pm 18.79, n = 8) (Fig. 4). It should be noted that there was a tendency for potentiation of the DMPP-evoked [3 H]DA release in Ca²⁺-free medium both at 30 and 300 μ M (34% and 30% increase, respectively), but the difference was not significant.

3.3. Effect of DMPP on the resting release of [3 H]serotonin from rat hippocampal slices

After 45-min of loading with [3 H]5-HT, followed by a 60-min preperfusion, the slices contained 55 757 \pm 3882 Bq/g (n = 25) radioactivity. The DMPP-evoked release of [3 H]5-HT from rat hippocampal slices was also dose-dependent, but in contrast to the striatum, it reached a maximum at 50 μ M (Fig. 5) therefore the EC₅₀ value could be determined and found to be 6.58 μ M. In further experiments a near-EC₅₀ concentration (10 μ M) was used. The time course of the DMPP-evoked hippocampal [3 H]5-HT release was similar to that of the high concentration-evoked [3 H]DA release from striatal slices. It was monophasic, increased gradually and stabilized on a higher level within a few minutes (3–4 fractions) following drug application.

3.4. Characterization of DMPP-evoked [3 H]serotonin release from rat hippocampal slices

3.4.1. Nicotinic antagonists

In the hippocampus the [3 H]5-HT release evoked by DMPP (10 μ M) was not significantly affected by mecamlamine (10 μ M) (DMPP_{control} = 100 \pm 13.87,

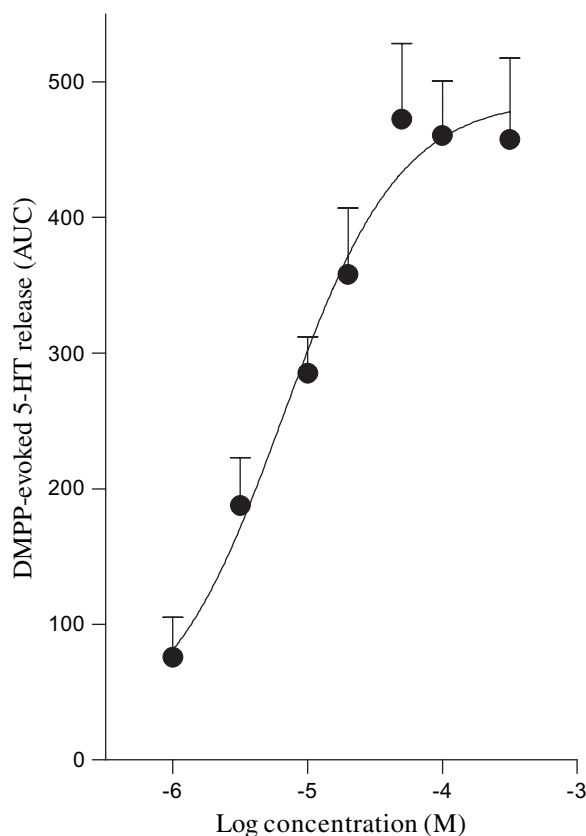


Fig. 5. Dose–response curve of the effect of DMPP on the release of [^3H]5-HT from rat hippocampal slices. The response (area under curve) was calculated as described in Section 2 and represents the surplus over the basal release. Each point represents the mean \pm S.E.M. of 4–5 independent experiments (in arbitrary units). The EC_{50} value was calculated by nonlinear regression.

$n=13$; $\text{DMPP}_{\text{mecam}}=93.93 \pm 15.82$, $n=11$) or MLA (10 nM) ($\text{DMPP}_{\text{control}}=100 \pm 25.80$, $n=4$; $\text{DMPP}_{\text{MLA}}=102.43 \pm 13.95$, $n=5$) (Figs. 6 and 8).

3.4.2. Uptake inhibition

The selective serotonin reuptake inhibitor fluoxetine alone had no effect on basal release of [^3H]5-HT from rat hippocampal slices (data not shown) but the DMPP (10 μM)-evoked [^3H]5-HT release was dose-dependently inhibited by fluoxetine. The response was significantly attenuated by the uptake blocker at 1 μM (48% inhibition) ($\text{DMPP}_{\text{control}}=100 \pm 8.96$, $n=10$; $\text{DMPP}_{\text{fluox1}}=51.63 \pm 6.54$, $n=5$, $P<0.01$) and was almost completely (96%) blocked at 10 μM ($\text{DMPP}_{\text{control}}=100 \pm 8.96$, $n=10$; $\text{DMPP}_{\text{fluox10}}=4.15 \pm 9.85$, $n=10$, $P<0.001$).

3.4.3. TTX and Ca^{2+} -free medium

The DMPP-evoked [^3H]5-HT release was not affected by TTX (1 μM) ($\text{DMPP}_{\text{control}}=100 \pm 9.68$, $n=6$; $\text{DMPP}_{\text{TTX}}=104.10 \pm 11.49$, $n=5$) or Ca^{2+} -free medium

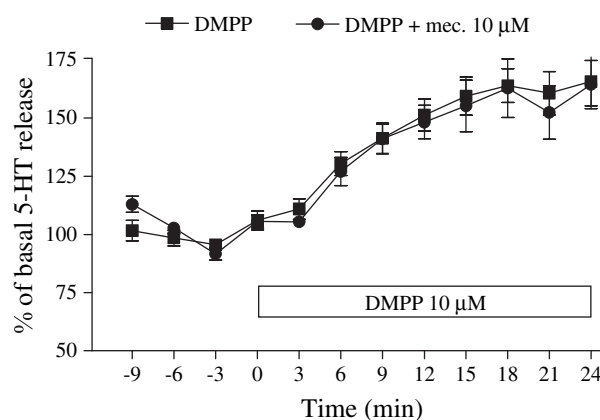


Fig. 6. Effect of mecamylamine on the DMPP-evoked release of [^3H]5-HT from rat hippocampal slices. DMPP was applied from the 5th fraction (from the 0 time point) and was present in the medium as indicated by the horizontal bar. Mecamylamine (10 μM) was present from the 3rd fraction. Each point represents the mean \pm S.E.M. of 10–12 independent experiments.

($\text{DMPP}_{\text{control}}=100 \pm 9.74$, $n=4$; $\text{DMPP}_{\text{Ca}^{2+}\text{-free}}=96.15 \pm 25.18$, $n=3$) (Fig. 8).

3.5. Effect of DMPP on the [^3H]dopamine and [^3H]serotonin uptake in rat brain synaptosomal preparation

The high-affinity uptake of [^3H]DA (50 nM) in rat striatal synaptosomes was inhibited by DMPP with an IC_{50} value of 3.18 μM . Similarly to this effect, the high-affinity uptake of [^3H]5-HT (50 nM) was also inhibited in hippocampal synaptosomes; however, the IC_{50} value was one order of magnitude lower (0.49 μM). In both cases the inhibition of monoamine uptake was dependent on DMPP concentration (Fig. 9).

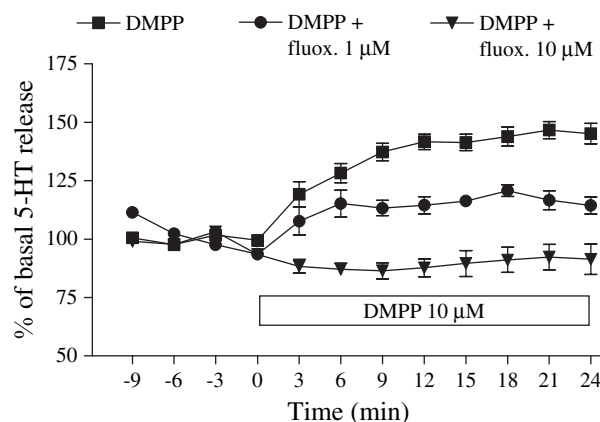


Fig. 7. Effect of fluoxetine on the DMPP-evoked release of [^3H]5-HT from rat hippocampal slices. DMPP was applied from the 5th fraction (from the 0 time point) and was present in the medium as indicated by the horizontal bar. Fluoxetine (1 or 10 μM) was present from the preperfusion period. Each point represents the mean \pm S.E.M. of 5–10 independent experiments.

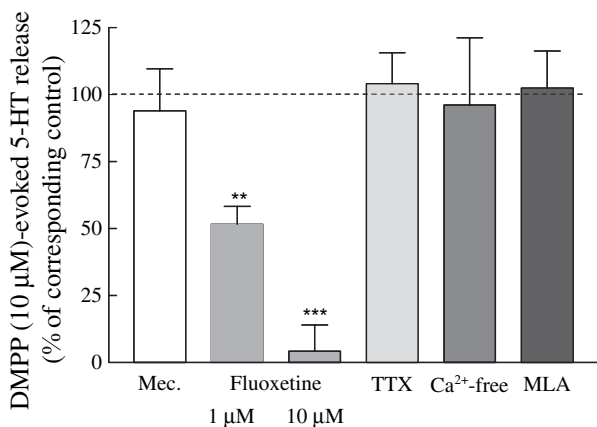


Fig. 8. Characterization of the hippocampal [³H]NA release evoked by DMPP (10 μM). Each treatment group had its own control group (corresponding control), 100% is represented by the dotted line; for the S.E.M. of controls see Section 3. Each bar represents the mean ± S.E.M. of 4–12 individual experiments. Two-tailed Student's *t*-test was used for statistical analysis (***P* < 0.01, ****P* < 0.001). Mec, mecamylamine 10 μM; TTX, tetrodotoxin 1 μM; Ca²⁺-free, Ca²⁺-free medium with 1 mM EGTA; MLA, methyllycaconitine 10 nM.

4. Discussion

The ganglionic stimulant agent DMPP is widely used in the research of cholinergic transmission as a potent nAChR agonist compound. Nevertheless, in our previous work we observed that DMPP increases the release of NA from rat hippocampal slices not only through the stimulation of nAChRs located on noradrenergic varicosities but also through the reversal of the NA transporter (Kiss et al., 1997). The major aim of the present work was to investigate whether other monoaminergic systems are also affected by the multiple actions of this drug, therefore we investigated the effect of DMPP on the striatal DA and the hippocampal 5-HT

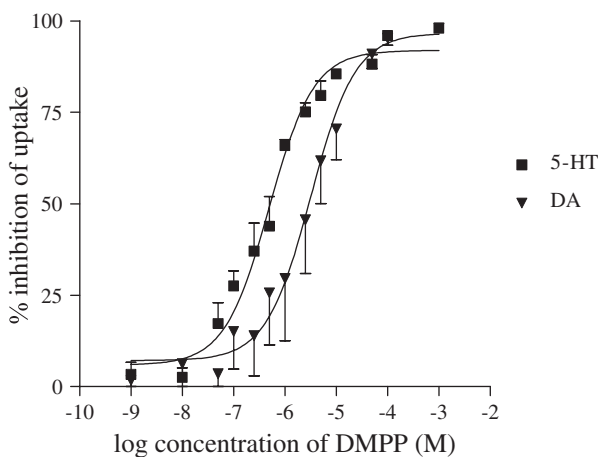


Fig. 9. Effect of DMPP on the [³H]dopamine and [³H]serotonin uptake in rat striatal and hippocampal synaptosomes. Each point represents the mean ± S.E.M. of three independent experiments. The IC₅₀ values were determined by nonlinear regression.

release. We found that DMPP is able to induce a dose-dependent increase in the release of both transmitters; however, the characteristics of these release processes showed marked differences, especially in comparison with the previously studied DMPP-evoked NA release.

4.1. Role of nAChRs in the DMPP-evoked monoamine release

In our previous work on hippocampal NA release (Kiss et al., 1997) we observed that the nAChR-mediated part of the response to DMPP dose-dependently increased and reached its maximum at a concentration of 100 μM. In contrast, the involvement of nAChRs in the DMPP-evoked striatal DA release was detectable only at a relatively low concentration (30 μM), at which the nAChR-mediated phase I response was completely inhibited by mecamylamine (but not affected by MLA), while the DA release induced by high concentration DMPP and the DMPP-evoked 5-HT release was not sensitive at all to these nAChR antagonists under our experimental conditions.

The role of nAChRs in the regulation of dopaminergic neurotransmission is well documented. It has been shown that acetylcholine increases the release of DA from rat striatal synaptosomes and this effect can be blocked by the nAChR antagonist hexamethonium (De Belleruche and Bradford, 1978). Since that observation a number of studies confirmed the presence of nAChRs on dopaminergic varicosities (Rapier et al., 1990; Grady et al., 1992; Soliakov et al., 1995; Clarke and Reuben, 1996; Kulak et al., 1997; Teng et al., 1997; Puttfarcken et al., 2000). Our observation that MLA was ineffective both at the low and high concentration DMPP-evoked DA release suggests that α7 subunit containing nAChRs are not involved in the nicotinic agonist-induced DA response. But what can be the reason for our finding that at higher concentration the DA response to DMPP was insensitive to mecamylamine although at lower concentration the phase I response was completely blocked by this drug? A possible explanation is the desensitization of neuronal nAChRs. Because of the properties of the perfusion system, the drug application is not fast enough to induce response without significant desensitization. Using an isotope tracer we determined that the final concentration of drugs develops gradually and reaches its maximum in 30–40 s in the tissue chamber. During this time the DMPP concentration might be insufficient to stimulate nAChRs but sufficient enough to desensitize them. It is interesting that under identical experimental conditions this desensitization could not be observed in the hippocampus (Kiss et al., 1997). This can be the consequence of the wide variety of nAChRs in the central nervous system. Neuronal nAChRs are pentameric structures and exist either as hetero-oligomers composed of α (α2–α6) and β (β2–β4)

or as homo-oligomers containing $\alpha 7$, $\alpha 8$, or $\alpha 9$ subunits (Lindstrom et al., 1996; Le Novère et al., 2002). Accumulating data indicate that nAChRs regulating hippocampal NA and striatal DA release have different subunit composition. The hippocampus receives noradrenergic innervation from the locus coeruleus where $\alpha 3$, $\beta 2$ and $\beta 4$ mRNA can be detected by in situ hybridization whereas in the substantia nigra (the origin of striatal dopaminergic innervation) the $\alpha 4$, $\alpha 5$, $\beta 2$ and $\beta 3$ mRNA show strong expression (Wada et al., 1989). Comparative pharmacological studies confirmed the involvement of different nAChRs (Sacaan et al., 1995) and in line with hybridization studies suggested that the hippocampal NA is modulated by $\alpha 3\beta 2$ (Sershen et al., 1997) or $\alpha 3\beta 4$ (Clarke and Reuben, 1996; Luo et al., 1998) whereas striatal DA release is regulated most probably by $\alpha 4\beta 2$ (Marks et al., 1994; Sharples et al., 2000). The subunit composition of different nAChRs determines their desensitization properties (Cohen et al., 1995; Fenster et al., 1997; Corringer et al., 1998; Quick and Lester, 2002). These variations in desensitization between hippocampus and midbrain dopamine areas have already been described (Dani et al., 2000) and might explain the differences observed in our experiments in the hippocampus and striatum.

The involvement of nAChRs in the regulation of 5-HT release is a less explored area. Only one group reported that nicotine induces 5-HT release from rat hippocampal slices (Kenny et al., 2000); however, this effect was not a classical nAChR-mediated exocytosis because it was insensitive to TTX and Ca^{2+} -free medium, and was only partially inhibited by the nicotinic antagonist mecamylamine. Other studies also failed to confirm the modulatory role of nAChRs. In our previous work (Lendvai et al., 1996) we found that the 5-HT release from hippocampal slices could not be stimulated by conventional nicotinic agonists (nicotine, cytosine, epibatidine); however, both DMPP and lobeline were able to induce a release which was Ca^{2+} -insensitive and could not be effectively antagonized by the nAChR antagonist mecamylamine. Our further studies (Kiss et al., 1997; Santha et al., 2000) revealed that these nicotinic agonists might influence the function of monoamine transporters. Later it was shown that nicotine increased 5-HT release from striatal synaptosomes but not from cerebral cortex or hippocampus synaptosomes (Reuben and Clarke, 2000). A recent report indicated that nicotinic agonists induced a vesicular exocytotic release of NA and DA from prefrontal cortex slices. In contrast, nicotine, epibatidine and cytosine were unable to induce substantial 5-HT release (Rao et al., 2003). Our results confirm these previous findings and suggest that nAChRs are most probably not involved in the regulation of hippocampal 5-HT release, since neither mecamylamine nor MLA had any effect on the response to DMPP, although the possibility

cannot be unequivocally excluded because a special subunit composition and desensitization characteristics could provide alternative hypothesis for the lack of effect of nicotinic antagonists.

4.2. Role of membrane transporters in the DMPP-evoked monoamine release

In our previous work we provided evidence that DMPP induces a carrier-mediated release of NA from rat hippocampal slices (Kiss et al., 1997). Our present data indicate that this effect of DMPP on monoamine transporters is universal because both the striatal DA and hippocampal 5-HT release proved to be insensitive to TTX and Ca^{2+} -free medium but sensitive to selective monoamine uptake inhibitors (nomifensine and fluoxetine, respectively), fulfilling all criteria of the carrier-mediated release (Levi and Raiteri, 1993). Nevertheless, there were differences in the characteristics of the effects since the DMPP-evoked carrier-mediated DA release increased in a dose-dependent manner increased and did not reach its maximum in the wide concentration range tested, while the DMPP-evoked 5-HT release reached a plateau at a relatively low concentration (50 μM). In this respect the carrier-mediated 5-HT release was similar to the previously investigated NA release since the latter was also moderate (a maximum 2-fold increase compared to resting) and reached its maximum at 100 μM of DMPP (Kiss et al., 1997). These findings might reflect the anatomical features of monoaminergic innervations, since the density of hippocampal 5-HT varicosities is similar to that of NA varicosities, about 2.7 million/ mm^3 (Oleskevich and Descarries, 1990), while the density of striatal DA varicosities is two orders of magnitude higher, about 150 million/ mm^3 (Doucet et al., 1986), which might account for the differences in the magnitude of response. Our data show that at a higher concentration (300 μM) of DMPP the DA response was only partially inhibited by nomifensine (10 μM). At this supramaximal concentration nomifensine completely blocks DA uptake since its IC_{50} is in the nanomolar range (Fielding and Szewczak, 1984). We therefore have to consider an additional component of DA efflux, which is not mediated through the transporter and also not due to the nAChR stimulation as indicated by the mecamylamine and MLA insensitivity. Moreover, the response to 300 μM DMPP is not blocked at all by TTX or Ca^{2+} -free medium, therefore the residual nomifensine-insensitive release cannot be exocytotic in nature. Most probably this release is induced by some nonspecific effect attained at high concentrations of DMPP (e.g. cytosolic pH shift, and a concomitant increased leakage through the membrane). Identification of the exact mechanism

requires further investigations and is beyond the scope of the recent work.

Another interesting finding is that nomifensine effectively antagonized not only the carrier-mediated component but also the nAChR-mediated response (phase I, Fig. 3A). This can be explained by our previous observation suggesting that monoamine uptake blockers (including nomifensine) inhibit neuronal nAChRs in the low micromolar concentration range (Hennings et al., 1999). Our findings provide an explanation for a recent report demonstrating that while conventional nicotinic agonists (nicotine, epibaridine, cytosine) fail to induce 5-HT release from prefrontal cortical slice, DMPP elicits a robust increase; however, this 5-HT efflux is insensitive to nicotinic antagonists and cannot be blocked by TTX or Ca^{2+} -free medium (Rao et al., 2003). These features are the characteristics of carrier-mediated transmitter release (Levi and Raiteri, 1993). What can be the mechanism of action of DMPP? The reversal of monoamine transporters can be induced by either an increase in intracellular Na^+ concentration (e.g. Gerevich et al., 2001), or by a substrate of the transporter, which can initiate an exchange mechanism (Langeloh et al., 1987; Trendelenburg, 1991). Theoretically the stimulation of nAChRs by DMPP could lead to depolarization and a concomitant increase of intracellular Na^+ concentration; however, in our experiments DMPP was able to induce carrier-mediated release even in the presence of nicotinic antagonists and TTX, therefore the involvement of nAChRs and depolarization-induced, Na^+ -coupled mechanisms can be excluded. Our data showing that DMPP inhibits the high-affinity uptake of both transmitters in concentrations reconcilable with the observed carrier-mediated release suggest that the mechanism of action of DMPP can be similar to that observed in case of NA transporters (Niebler and Trendelenburg, 1990), that is, DMPP might be a substrate of DAT and SERT and is able to induce an exchange mechanism resulting in a carrier-mediated efflux of monoamines. It is interesting that in the periphery (vas deferens) DMPP can induce a carrier-mediated release of NA only if the metabolism (MAO) and vesicular uptake (VMAT2) is inhibited, which suggests that DMPP, in contrast to e.g. amphetamine, is not able to mobilize vesicularly stored monoamines (Niebler and Trendelenburg, 1990), whereas in the central nervous system (CNS) the carrier-mediated release can be detected even if the function of MAO and VMAT2 is intact (Kiss et al., 1997). This might be explained by some possible differences in synthesis, metabolism and/or vesicular uptake of monoamines, which leads to a higher cytoplasmic transmitter concentration in the CNS than in the periphery, thereby facilitating the effect of DMPP.

In conclusion, the most important finding of our study is that DMPP is able to influence the function of monoamine transporters and evokes a substantial carrier-mediated release of NA (Kiss et al., 1997), DA,

and 5-HT, therefore special caution is needed for the interpretation of data obtained in experiments where DMPP is used as a nicotinic agonist.

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