Modulation of dopamine and noradrenaline release and of intracellular Ca\textsuperscript{2+} concentration by presynaptic glutamate receptors in hippocampus

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1 We studied the release of [\textsuperscript{3}H]-dopamine and [\textsuperscript{3}H]-noradrenaline (NA) from hippocampal synaptosomes induced by glutamate receptors and the associated Ca\textsuperscript{2+} influx through Ca\textsuperscript{2+} channels. The release of trigitated neurotransmitters was studied by use of superfusion system and the intracellular free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) was determined by a fluorimetric assay with Indo-1 as a probe for Ca\textsuperscript{2+}.

2 Presynaptic glutamate receptor activation induced Ca\textsuperscript{2+}-dependent release of [\textsuperscript{3}H]-dopamine and [\textsuperscript{3}H]-NA from rat hippocampal synaptosomes. Thus, L-glutamate induced the release of both neurotransmitters in a dose-dependent manner (EC\textsubscript{50} = 5.62 \mu M), and the effect of 100 \mu M L-glutamate was inhibited by 83.8\% in the presence of 10 \mu M 6-cyano-7-nitroquinolxaline-2,3-dioxide (CNQX), but was not affected by 1 \mu M (+)-5-methyl-10,11-diaryl-H-dibenzo[a,d,j]cyclohepten-5,10-imine (MK-801).

3 Other glutamate receptor agonists also stimulated the Ca\textsuperscript{2+}-dependent release of [\textsuperscript{3}H]-dopamine and [\textsuperscript{3}H]-NA as follows: N-methyl-d-asparate (NMDA), at 200 \mu M, released 3.65 \pm 0.23\% of the total [\textsuperscript{3}H] catecholamines, and this effect was inhibited by 81.2\% in the presence of 1 \mu M MK-801; quisqualate (50 \mu M), S-a-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) (100 \mu M) or kainate (100 \mu M) released 1.57 \pm 0.26\%, 1.93 \pm 0.17\% and 2.09 \pm 0.22\%, of the total [\textsuperscript{3}H] catecholamines, respectively.

4 The ionotropic glutamate receptor agonist, AMPA, induced an increase in the [Ca\textsuperscript{2+}+]i which was inhibited by 58.6\% in the presence of 10 \mu M CNQX. In contrast, the increase in [Ca\textsuperscript{2+}+]i due to stimulation by glutamate was not sensitive to CNQX or MK-801.

5 Nitrendipine, at 1 \mu M, did not inhibit the neurotransmitter release induced by AMPA, but, both 0.5 \mu M \omega-conotoxin GVIA (\omega-CgTX) and 100 \mu M \omega-Aga IVA reduced catecholamine release to 49.03 \pm 3.79\% and 46.06 \pm 10.51\% of the control, respectively. In the presence of both toxins the release was reduced to 12.58 \pm 4.64\% of the control.

6 The results indicate that activation of presynaptic glutamate receptors of the NMDA and non-NMDA type induces the release of [\textsuperscript{3}H]-dopamine and [\textsuperscript{3}H]-NA from rat hippocampal synaptosomes and that the release induced by AMPA involves the activation of N- and P-type Ca\textsuperscript{2+} channels which allow the influx of Ca\textsuperscript{2+} that triggers the [\textsuperscript{3}H] catecholamines release.

Keywords: Hippocampus; catecholamines release; presynaptic glutamate receptors; calcium; synaptosomes; voltage-sensitive calcium channels

Introduction

The exocytotic release of neurotransmitters depends on the Ca\textsuperscript{2+} influx into the cell (for review see: Carvalho, 1982; Augustin, 1987; Smith & Augustin, 1988; Knight et al., 1989). The influx of Ca\textsuperscript{2+} is mediated by Ca\textsuperscript{2+} channels activated upon excitation of the nerve terminal, providing a mechanism for the coupling between stimulation and neurosecretion (Tsiens et al., 1988, 1991).

Voltage-sensitive Ca\textsuperscript{2+} channels (VSCCs) are activated by membrane depolarization and two groups of VSCCs can be distinguished on the basis of the different sensitivities to membrane depolarization. Low voltage-sensitive calcium channels or T-Type VSCCS are transiently activated by small decreases in membrane potential. High voltage-sensitive calcium channels are VSCCs which are activated after large depolarizations of the membrane. Three different types of high VSCCS can be distinguished on the basis of their sensitivity to specific antagonists. The L-type Ca\textsuperscript{2+} channels are a group of channels inhibited by dihydropyridines, like nitrendipine (Tsiens et al., 1991). The N-type Ca\textsuperscript{2+} channels are blocked by \omega-conotoxin GVIA (\omega-CgTX) (Kerr & Yoshikami, 1984; Tsiens et al., 1991). The P-type VSCCS are inhibited by the polyamine FTX (Linás et al., 1989) or by the peptide \omega-Aga IVA (Mintz et al., 1992b) which is a component of the venom of the funnel web spider, Agelenopsis aspersa.

The excitatory neurotransmitter L-glutamate acts both at the postsynaptic and presynaptic membranes by interaction with its receptors. Glutamate receptors may be ionotropic, allowing the influx of ions through a receptor channel (Zorumski & Thio, 1992; Ruzicka & Jhamandas, 1993), or G protein-coupled metabotropic receptors, which are coupled to several second messenger systems (Schoepf & Conn, 1993). The metabotropic receptors are coupled to phospholipase C (Nicoletti et al., 1986; Sugiyma et al., 1987) and the activated phospholipase C catalyzes the hydrolysis of phosphatidylinositol and produces inositol phosphate which releases Ca\textsuperscript{2+} from internal stores (Zorumski & Thio, 1992). The metabotropic receptor is activated by ACPD and also by quisqualate (Zorumski & Thio, 1992). The ionotropic glutamate receptors are the N-methyl-D-aspartate (NMDA), S-a-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) and kainate receptors (Zorumski & Thio, 1992; Ruzicka & Jhamandas, 1993). The NMDA receptor is very permeable to Ca\textsuperscript{2+} (MacDermott et al., 1986; Iino et al., 1990), reversibly blocked by Mg\textsuperscript{2+} (Nowak et al., 1984) and potentiated by the co-agonist, glycin (Johnson & Ascher, 1993).
mined which high catecholamine release in dopamine and AMPA synaptosomes the localization (Malva carefully isolated crude pocampi centrifuged 2 min. The protein concentration of about 5 mg ml−1, was determined diluted at a concentration of 15800 ng ml−1. The uptake and release of 3H-catecholamines was performed as follows: synaptosomes were loaded (0.75 mg ml−1) with 100 mM dopamine, containing 5 μCi of [3H]-dopamine mmol−1, in Na+ medium (NaCl 132 mM, KCl 1 mM, MgCl2 1.4 mM, H2PO4 1.2 mM, CaCl2 100 μM, glucose 10 mM and HEPES-Tris 10 mM, pH 7.4) containing nialamide 12.5 μM, ascorbic acid 0.1% and fatty acid-free BSA 1 mg ml−1. The loading of synaptosomes proceeded for 15 min at 30°C and was stopped by pelleting the synaptosomes at 15800 g for 30 s. The supernatant was discarded and the pellet resuspended in Na+ medium (1.5 mg ml−1). Samples of the synaptosomal suspension containing 0.75 mg of protein were aspirated and collected onto Whatman GF/B filters mounted in a superfusion system as described previously (Raiteri et al., 1974). Synaptosomes were washed for 10 min, followed by pelleting 12.5 μM nialamide and 0.1% ascorbic acid, at a flow rate of 0.8 ml min−1, using a peristaltic pump. By this time a stable basal release of 3H-catecholamines was obtained and the sample collection was started. Each sample was collected for 1 min and the total experiment proceeded for 10 min. All drugs were used after 1 min before starting the sample collection and the stimulation of synaptosomes was initiated at minute 3. At the end of the experiment, all the filters containing synaptosomes were dried and transferred into scintillation vials. The radioactivity present in the samples and in the filters was determined in vials after addition of a scintillation cocktail (Univonol, ICN laboratories), using a liquid scintillation spectrometer (Packard, Tri-Carb 2000) provided with a d.p.m. correction programme. Some of the results are presented as percentage of the total 3H-catecholamines released in percentage of the total H-catecholamines accumulated. To determine the 3H-catecholamines effectively released upon stimulation, the following calculations were made: from the amount of H-catecholamines released in each sample, the average value of basal release was subtracted. All individual values above basal release were summed and the obtained value was taken as the H-catecholamines released by the stimulus. The total H-catecholamines accumulated was calculated as the sum of the H-catecholamines released by stimulation of synaptosomes and the 3H-catecholamines still present in the filters at the end of the experiment.

Catecholamine analysis by h.p.l.c.-e.d.

Dopamine, NA and dihydroxyphenylacetic acid (DOPAC) content of synaptosomes was compared in untreated synaptosomes (endogenous catecholamines) and in synaptosomes loaded with unlabelled dopamine in the conditions described in the previous section for the loading with tritiated dopamine. In both cases, 2 mg of synaptosomal protein was pelleted in order to determine the content of catecholamines. The obtained pellets were resuspended in 0.5 ml of a solution consisting of 10% acetic acid and 0.4 M perchloric acid. The samples were kept at −20°C for later high performance liquid chromatography (h.p.l.c.) analysis (Duarte et al., 1993).

Dopamine and DOPAC analysis was performed as follows: the samples were injected into a 15×0.4 cm Spherisorb ODS-1 column with 5 μm particle size and eluted at a flow rate of 1 ml min−1 with a mobile phase consisting of 50 mM NaH2PO4, 1.24 mM heptanesulfonic acid, 0.24 mM EDTA, 10% methanol, pH 2.9. The electrochemical detector used was an ESA model Couliecho II.

The same samples were used to quantify NA levels in hippocampal synaptosomes by h.p.l.c. Samples were injected
in a column Ultrosphere ODS, 4.7 x 75 mm with 3 μm particle size. The mobile phase was 50 mM NaH2PO4, 0.02% trichloroacetic acid, pH 3.5 (Levine, 1986). The flow rate of the mobile phase and the conditions for electrochemical detection were the same used for dopamine and DOPAC analysis.

**Analysis of released [1H]-NA, [1H]-dopamine and [1H]-DOPAC by h.p.l.c.-e.d.**

Hippocampal synaptosomes were loaded as described previously in this paper, but with the following modifications. The loading proceeded in the same Na⁺ medium (1 mM) with 100 nM dopamine, containing 20 μCi of [3H]-dopamine nmol⁻¹. One sample (1 min) of the basal release and another sample during the first min of stimulation, were collected. To each sample acetic acid (10% final concentration) were added, and the samples were stored at −20°C for latter analysis (Duarte et al., 1993).

The samples were injected in a column Ultrosphere ODS, 4.7 x 7.5 mm with 3 μm particle size with the same mobile phase and flow conditions as described in the previous section for NA quantification. The injected samples were prepared as following: 18% of tritiated sample were mixed with cold standards containing 100 pmol dopamine, 100 pmol NA and 50 pmol DOPAC, at a final volume of 200 μl. A volume of 10 μl of this mixture was injected into the column, using the appropriate loop. At the exit of the analytical cell, five of each 2 min samples were collected into scintillation vials and the radioactivity determined as previously described. The radioactivity in each sample was compared with the chromatogram of the cold standards (see Figure 1).

**Materials**

Nordripine was obtained from Research Biochemical International, Natick, U.S.A. The o-conotoxin GVIA (o-CgTx) was from Peninsula Laboratories Inc., Belmont, CA, U.S.A. The o-AGA IVA was from Scientific Marketing Associates, U.K. and 6-nitro-7-cyano-quinoxaline-2,3-dione (CNQX) was obtained from NOVO, Nordisk, Denmark. (+)-5-Methyl-16,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801) was purchased from Merck Sharp & Dohme Research Lab., Rahway, N.J., U.S.A. S-a-amino-3-hydroxy-4-methyl-5-isoxazolepropionic acid (AMPA) was obtained from Tocris Neuramin, Bristol, England. The acetoxyethyl ester of Indo-1 (Indo-1/AM) was obtained from Molecular Probes Inc. Eugene, OR, U.S.A. Ionomycin and fatty acid-free bovine serum albumin (BSA) were from Calbiochem-Boehringer Corp., San Diego, U.S.A. [7,8-H]-dopamine hydrochloride (40–60 Ci mmol⁻¹) was purchased from Amer sham Laborato ries, Buckinghamsh, England. All other reagents were from Sigma Chemical Co., St. Louis, MO, U.S.A. or from Merck-Schuchard, Germany. Stock solutions of Indo-1/AM, ionomycin, nitrindipine and CNQX were prepared in dimethyl sulfoxide.

**Other methods**

Results are presented as means ± s.e.mean of the number of experiments indicated. Statistical significance was determined by Student's two-tailed t test.

**Results**

**Endogenous content of NA, dopamine and DOPAC in hippocampal synaptosomes**

The hippocampal synaptosomes contain endogenous NA (2.94 ± 0.22 pmol mg⁻¹ protein), dopamine (0.69 ± 0.16 pmol mg⁻¹ protein) and small amounts of DOPAC (0.029 ± 0.027 pmol mg⁻¹ protein) (Table 1). When we loaded the synaptosomes with exogenous dopamine as described in Methods, we observed that the total content of NA was not significantly different from the content of endogenous NA (2.91 ± 0.21 pmol mg⁻¹ protein) (P > 0.05). However, after the loading with dopamine, the synaptosomal content of this catecholamine increased to 4.40 ± 0.78 pmol mg⁻¹ protein. The content of DOPAC in the synaptosomes observed after loading with exogenous dopamine was not significantly different from the content of the endogenous DOPAC (2.28 ± 0.41 pmol mg⁻¹ protein) (Table 1) than the content of the endogenous DOPAC in the synaptosomes.

**Release of [3H]-catecholamines by hippocampal synaptosomes loaded with [1H]-dopamine**

After loading the hippocampal synaptosomes with [3H]-dopamine, we observed that the main component of the tritium release in basal conditions was DOPAC (68.11 ± 2.17% of basal release), but also some dopamine (23.13 ± 1.89% of basal release) and NA (8.76 ± 0.64% of basal release) were released in the same conditions (Figure 1). However, after stimulation with L-glutamate, AMPA or KCl, the amount of DOPAC released did not increase as compared to basal dopamine release, while we observed a large increase in the tritium efflux with the retention time characteristic of dopamine (Figure 1). Thus, the increase in [1H]-catecholamines release due to stimulation with 100 μM L-glutamate, 100 μM AMPA, 5 mM KCl or 30 mM KCl was mainly due to the release of [3H]-dopamine (68.43 ± 4.03%, 74.05 ± 2.66, 69.53 ± 4.17 and 71.35 ± 3.18%, respectively) of the total tritium released, and a significant part of [3H]-NA was also released in the same conditions (31.58 ± 4.03%, 25.95 ± 2.66%, 30.48 ± 4.17% and 28.65 ± 3.19%, respectively). Thus, this methodology allow us to study in the same sample the release of both NA and dopamine induced by stimulating the glutamate receptors, although the major neurotransmitter in the efflux medium is dopamine (Figure 1c).

**Stimulation of [1H]-catecholamines release from hippocampal synaptosomes by glutamate receptors agonists**

Synaptosomes obtained from rat hippocampus released [3H]-catecholamines in response to stimulation with L-glutamate in a dose-dependent manner (Figure 2). Stimulation of synaptosomes with medium containing 1 mM Ca²⁺ released 0.658 ± 0.171% (n = 9) of the total [3H]-catecholamines accumulated. The addition of L-glutamate released 1-H-catecholamines in a Ca²⁺-dependent process, with the EC₅₀ determined as 5.6 μM and the maximum [3H]-catecholamines release of 2.17% of the total tritium accumulated (Figure 2b). The release induced by L-glutamate was dependent on extracellular Ca²⁺, since we did not observe [3H]-catecholamines release in the absence of added Ca²⁺ (Figure 2a).

**Table 1**

<table>
<thead>
<tr>
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<th>B</th>
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<tr>
<td><strong>DOPAC</strong></td>
<td>0.03 ± 0.03 (n = 6) 2.28 ± 0.41 (n = 6) *</td>
</tr>
<tr>
<td><strong>NA</strong></td>
<td>3.94 ± 0.82 (n = 6) 2.91 ± 0.21 (n = 6)</td>
</tr>
<tr>
<td><strong>Dopamine</strong></td>
<td>0.69 ± 0.16 (n = 5) 4.40 ± 0.78 (n = 5)</td>
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A, Endogenous content of dihydroxyphenylacetic acid (DOPAC), noradrenaline (NA) and dopamine. B Contents of DOPAC, NA and dopamine observed after the loading of the synaptosomes with exogenous dopamine as described in the Methods Section. The values are expressed as pmol mg⁻¹ protein.

*Significantly higher than endogenous, P < 0.05.
was correspondent (c) legends, please formed results of standards of synaptosomes by 5 catecholamines release was 1.93 (200 gM) 2.09 ± 0.23% (n = 9) (EC₅₀ = 10.9 μM and maximum ³H-catecholamines release = 2.09%), and by 100 μM kainate was 2.09 ± 0.22% (n = 6) (EC₅₀ = 4.9 μM and maximum ³H-catecholamines release = 2.22%) (Figures 2b and 3b). Quisqualate (50 μM) released 1.57 ± 0.26% (n = 5) of the total ³H accumulated. We also observed that NMDA, in the presence of 5 μM glycine and in the absence of added Mg²⁺, was effective in releasing ³H-catecholamines. Thus, NMDA (200 μM) released 3.65 ± 0.23% (n = 13) of the total ³H-catecholamines (Figure 3).

We also observed that agonists of the ionotropic glutamate receptors induced the release of ³H-catecholamines (Figures 2 and 3). Thus, the extent of tritium release by 100 μM AMPA was 1.93 ± 0.17% (n = 9) (EC₅₀ = 10.9 μM and maximum ³H-catecholamines release = 2.09%), and by 100 μM kainate was 2.09 ± 0.22% (n = 6) (EC₅₀ = 4.9 μM and maximum ³H-catecholamines release = 2.22%) (Figures 2b and 3b). Quisqualate (50 μM) released 1.57 ± 0.26% (n = 5) of the total ³H accumulated. We also observed that NMDA, in the presence of 5 μM glycine and in the absence of added Mg²⁺, was effective in releasing ³H-catecholamines. Thus, NMDA (200 μM) released 3.65 ± 0.23% (n = 13) of the total ³H-catecholamines (Figure 3).

Glutamate-induced release of ³H-catecholamines is due to receptor activation

It has been described in various cell models that L-glutamate increases the [Ca²⁺] in part due to glutamate transport, which can cause membrane depolarization, and activate VSCCs (Heinonen et al., 1989; McMahon et al., 1989). Therefore, we investigated whether it observed Ca²⁺-dependent release of ³H-catecholamines induced by L-glutamate was mediated through the L-glutamate transport or by activation of presynaptic glutamate receptors. We observed that the increase of the hippocampal synaptic basal [Ca²⁺] due to 100 μM L-glutamate was not inhibited by 10 μM CNQX or 1 μM MK-801 (Figure 4a). However, the 100 μM AMPA-induced increase in the [Ca²⁺] was strongly inhibited by 10 μM CNQX (58.6% inhibition) (P<0.05) (Figure 4b). Interestingly, when we studied the effects of L-glutamate or AMPA on the release of ³H-catecholamines, we observed that the release induced by L-glutamate or by AMPA was inhibited by CNQX (Figure 5a and b), but, in the presence of 1 μM MK-801, the observed release of ³H-catecholamines induced by 100 μM L-glutamate was not significantly different from the control (P>0.05), indicating that the release evoked under these conditions did not involve the activation of NMDA receptors. However, in the presence of 10 μM CNQX, the release induced by 100 μM L-glutamate was reduced by 83.8% (Figure 5a), indicating

Figure 2 Effect of L-glutamate (Glu) on the release of ³H-catecholamines in rat hippocampal synaptosomes. (a) Fractional release induced by Ca²⁺ (1 mM) alone (●), by 100 μM L-glutamate in medium without Ca²⁺ (▲), or by 10 μM L-glutamate (■) in the presence of 1 mM Ca²⁺. The results are the mean value ± s.e.mean of 4 to 9 experiments performed in duplicate. (b) Dose-response curves for the release of ³H-catecholamines induced by L-glutamate (■), AMPA (■) or kainate (▲). The curves were determined by the mean value ± s.e.mean of 4 to 5 independent experiments performed in duplicate.
activation of ionotropic non-NMDA glutamate receptors coupled to [3H]-dopamine and [3H]-NA release. Further evidence that presynaptic non-NMDA glutamate receptors activation release [3H]-catecholamines was obtained in a study in which we observed that a specific non-NMDA glutamate receptor agonist (AMPA) induced the release of [3H]-catecholamines and that this release was significantly inhibited ($P<0.05$) by 10 µM CNQX (61.2% inhibition) (Figure 5b).

As reported above, we did not observe any inhibition of the 100 µM L-glutamate induced [3H]-dopamine or [3H]-NA release by 1 µM MK-801 (Figure 5a). However, we observed that 200 µM NMDA in the presence of 5 µM glycine, and in the absence of added Mg²⁺, induced the release of 3.65 ± 0.23% ($n = 13$) of the total tritiated neurotransmitter accumulated (Figures 3 and 5c). The release of [3H]-catecholamines induced by NMDA was inhibited by 81.2% by 1 µM MK-801 (Figure 5c).

**AMPA induced release of [3H]-catecholamines is mediated by N- and P-type VSCCs**

The ionotropic glutamate receptors activated by AMPA allow the influx of cations, inducing membrane depolarization (Ruzicka & Jhamandas, 1993) which may activate VSCCs, allowing the influx of Ca²⁺ which may trigger the release of several neurotransmitters. In this work we observed that the L-type VSCCs blocker nitrendipine (Tsien et al., 1988; 1991) did not inhibit the release of [3H]-dopamine or [3H]-NA, but the N-type calcium channel blocker, ω-CgTx (Kerr & Yoshikami, 1984; Reynolds et al., 1986) inhibited the release of [3H]-catecholamines to 49.03 ± 3.79% of the control whereas, the ω-Aga IVA, a blocker of the P-type VSCCs (Mintz et al., 1992a,b) reduced the release of [3H]-catecholamines to 46.06 ± 10.51% of the control. When both blockers were perfused simultaneously, the release of [3H]-catecholamines was inhibited to 12.76 ± 4.64% ($n = 4$) of the control. The release observed in the presence of ω-Aga IVA or with both toxins were significantly different from the release observed in the absence of VSCC blockers ($P<0.05$) (Figure 6).

These results suggest that most of the Ca²⁺-dependent release of [3H]-dopamine and [3H]-NA, due to activation of

**Figure 3** [3H]-catecholamine release induced by L-glutamate receptor agonists. (a) Fractional release induced by Ca²⁺ (1 mM) alone (●) or by 100 µM AMPA (□), 100 µM kainate (■), 50 µM quisqualate (○), in the presence of 1 mM Ca²⁺; or 200 µM NMDA in the absence of Mg²⁺ and in the presence of 1 mM Ca²⁺ (▲). (b) [3H]-catecholamines released by L-glutamate receptor agonists (applied from min 3 to 9) as a percentage of the total tritium accumulated. The results are the mean value ± s.e.mean of 5 to 13 experiments performed in duplicate.

**Figure 4** Increases in the [Ca²⁺]i concentration in hippocampal synaptosomes induced by 100 µM L-glutamate or 100 µM AMPA. (a) Increase in the [Ca²⁺]i induced by L-glutamate (Glu) is not sensitive to either 10 µM CNQX or 1 mM MK-801. (b) AMPA-induced increase in the [Ca²⁺]i is sensitive to 10 µM CNQX. The results are the mean ± s.e.mean of 4 to 17 experiments. Significantly lower than control: *$P<0.05$.

**Figure 5** Effects of NMDA and non-NMDA glutamate receptors activation on the release of [3H]-catecholamines. (a) The release induced by 100 µM L-glutamate is inhibited by 10 µM CNQX and is not sensitive to the NMDA receptor antagonist MK-801 (1 µM). (b) The release of [3H]-catecholamines evoked by 100 µM AMPA is inhibited by 10 µM CNQX. (c) The release induced by 200 µM NMDA, in medium without Mg²⁺ and in the presence of 1 mM Ca²⁺ and 5 µM glycine, is inhibited by 1 µM MK-801. The results are the mean value ± s.e.mean of 5 to 16 experiments performed in duplicate. Significantly lower than control: *$P<0.05$. 
presynaptic receptors with AMPA, is a consequence of activation of VSCCs due to membrane depolarization induced by activation of the AMPA receptors (Figures 5 and 6).

Discussion

Dopaminergic and noradrenergic terminals are present in the hippocampus (Wyss et al., 1979; Lopes da Silva et al., 1990). It was shown previously that synaptosomes isolated from rat hippocampus are endowed with endogenous dopamine and NA, and that they release catecholamines in response to depolarization (Verhage et al., 1992). However, in the presence of dopamine in the hippocampus, the physiological role of this neurotransmitter in this area of the brain is still unclear (Lopes da Silva et al., 1990).

We have determined the endogenous content of NA and dopamine in the crude synaptosomal fraction of the hippocampus used in this work. The NA content of these synaptosomes is about four fold higher than the dopamine content (Table 1), in agreement with previous observations showing much higher levels of NA than of dopamine in purified hippocampal synaptosomes (Verhage et al., 1992). However, we find a dopamine content about 3.5 fold higher than that reported (Verhage et al., 1992). We also found that the NA content was about 18.3 fold lower than the levels described earlier (Verhage et al., 1992). These differences are probably due to different isolation methods used for preparing the synaptosomes. Apparently, we used a crude synaptosomal fraction which is much richer in dopamine and poorer in NA than the purified synaptosomes used by Verhage et al. (1992).

We used h.p.l.c.-e.d. to analyze the nature of the H-catecholamines released by hippocampal synaptosomes previously loaded with [3H]-dopamine. In the hippocampus the catecholamine nerve terminals are less than 1% of the total nerve terminals (Lopes da Silva et al., 1990), and the endogenous content of either NA or dopamine is too low to allow the study of endogenous catecholamines release stimulated by presynaptic glutamate receptors. Thus, we decided to use [3H]-dopamine loaded synaptosomes since the high sensitivity of the tritium detection allowed us to detect very small amounts of neurotransmitters released.

Monoamine oxidase can transform dopamine in DOPAC, in an enzymatic reaction inhibited by nialamide. We observed in our preparation that after loading the synaptosomes with exogenous dopamine, the synaptosomal content of DOPAC increased (Table 1). We concluded that, in spite of the presence of nialamide throughout the experiment, some dopamine was metabolized to DOPAC. In this work, we also observed that DOPAC is released in basal conditions and that the increase in [3H]-catecholamines release observed after synaptosomal stimulation was mainly due to the release of dopamine and, to a smaller extent, to release of NA (Figure 1).

After loading the synaptosomes with dopamine, we observed that the NA content of hippocampal synaptosomes did not increase (Table 1). However, we observed that after stimulation of the synaptosomes some [3H]-NA was released, indicating that in hippocampal synaptosomes some [3H]-dopamine can be transported into noradrenergic terminals and then be converted in [3H]-NA (Figure 1).

The increase in [3H]-NA release observed after [3H]-dopamine loading and the finding that NA content of the synaptosomes did not increase after loading with dopamine may be explained by the rapid turnover of NA, which allows the conversion of [3H]-dopamine in [3H]-NA without affecting total NA levels. During the loading of hippocampal synaptosomes with [3H]-dopamine the neurotransmitter can be transported into both dopaminergic and noradrenergic nerve terminals. In the dopaminergic terminals this catecholamine is stored in dopaminergic synaptic vesicles (Henry & Scherman, 1989; Moriyama et al., 1993), whereas in noradrenergic terminals the [3H]-dopamine is transported into the nerve terminal and is accumulated in the noradrenergic synaptic vesicles where it is transformed to [3H]-NA. In other brain regions with high ratios NA/dopamine, experimental evidence suggested that dopamine can also be accumulated into noradrenergic terminals and converted into NA (Kelly et al., 1985; Carboni et al., 1990).

We observed that the majority of the [3H]-catecholamines released after stimulation of the synaptosomes with 30 mM KC1 (71.4 ± 3.2%), 5 mM KC1 (69.5 ± 4.2%), 100 μM L-glutamate (68.4 ± 4.0%) or 100 μM AMPA (74.1 ± 2.7%), was in fact [3H]-dopamine, but that a significant portion of the [3H]-catecholamines was released as [3H]-NA (28.7 ± 3.2% for 30 mM KC1, 30.5 ± 3.2% for 5 mM KC1, 31.6 ± 4.0% for 100 μM L-glutamate and 26.0 ± 2.7% for 100 μM AMPA). L-Glutamate induced the release of [3H]-dopamine and [3H]-NA from rat hippocampal synaptosomes in a process which depends on extracellular Ca2+ (Figure 2). The dependence on external Ca2+ of the dopamine release induced by depolarization (Raiteri et al., 1978; Drapeau & Blaustein, 1983) is due to the requirement of Ca2+ for exocytosis, since this neurotransmitter is stored in synaptic vesicles within the synaptic terminal (Henry & Scherman, 1989; Moriyama et al., 1993).

The release of [3H]-catecholamines induced by 100 μM L-glutamate in the presence of 1 mM Mg2+ and 1 mM Ca2+ was reduced by 83.8% by 10 μM CNQX, but it was insensitive to 1 μM MK-801 (Figure 5). The sensitivity of the L-glutamate induced release of [3H]-dopamine and [3H]-NA release to the NMDA receptor inhibitor, MK-801, probably reflects the blocking of the NMDA receptor channel by Mg2+ (Nowak et al., 1984; Zorumski & Thio, 1992), and the depolarization which occurs after the non-NMDA receptors activation probably was not sufficient to remove the Mg2+ from the NMDA receptor channel. Another possibility is the localization of the NMDA and non-NMDA receptors in different synaptosomes, which could explain the finding that the activation of the non-NMDA receptors was not sufficient to release the NMDA channel from the blockade due to Mg2+.

The 100 μM L-glutamate induced change in [Ca2+]i, which reflects the influx of Ca2+ following stimulation of synaptosomes, was insensitive to 10 μM CNQX or 1 μM MK-801 (Figure 4). The insensitivity of the L-glutamate induced increase in the [Ca2+]i to the non-NMDA glutamate receptor antagonist, CNQX (Honore et al., 1988; Zorumski & Thio, 1992; Figure 4a), or to the NMDA glutamate receptor antagonist, MK-801 (Zorumski & Thio, 1992; Figure 4a) is compatible with the idea that L-glutamate transport through its carrier may lead to Ca2+ influx due to depolarization which activates VSCCs. These results are in agreement with pre-
vious observations that D-aspartate increases the [Ca^{2+}] in synaptosomes from the guinea-pig cerebral cortices (McMahon et al., 1989). However, the release of ^3H^-catecholamines induced by glutamate does not appear to be mediated by a carrier-dependent process, since it is sensitive to CNQX. This observation indicates that presynaptic non-NMDA glutamate receptors mediate the release of ^3H^-catecholamines in this preparation. We also observed that both NMDA and non-NMDA glutamate receptor agonists induced the release of ^3H^-catecholamines (Figure 3). The release induced by 100 µM AMPA was sensitive to 10 µM CNQX and the release induced by 200 µM NMDA was inhibited by 1 µM MK-801 (Figure 5). These results are consistent with both presynaptic non-NMDA and NMDA receptor activation mediating the Ca^{2+}-dependent ^3H^-catecholamine release.

Interestingly, NMDA (200 µM) is the glutamate receptor agonist which induces the highest release of ^3H^-catecholamines (Figure 3). This result apparently is contradictory to the observation that NMDA is the least effective glutamate receptor agonist in elevating the [Ca^{2+}] (not shown). Probably, the local Ca^{2+} concentrations attained in the active zones (Smith & Augustine, 1988) after the NMDA receptors activation, are sufficient to induce Ca^{2+} influx without changing the bulk Ca^{2+} concentrations to any great extent. Another possible explanation is that only a sub-population of synaptosomes respond to NMDA stimulation, inducing a large efflux of ^3H^-catecholamines, without causing large average changes in the [Ca^{2+}] of all the synaptosomes. Glutamate receptors modulate the release of several neurotransmitters and neuromodulators (Desce et al., 1993). The ionotrophic NMDA receptor stimulation was reported to induce the release of NA from rat hypothalamic slices, but had no effect on the release of dopamine in this preparation (Blaindina et al., 1992). The presence of presynaptic NMDA receptors able to induce the release of NA was also demonstrated in synaptosomes isolated from rat cerebral cortex (Fink et al., 1990). In the hippocampus, the presynaptic glutamate receptors (200 µM) are the glutamate receptor agonists which modulate the release of NA from striatal synaptosomes (Desce et al., 1993). A dihydropyridine and o-CgTx insensitive class of VSCCs, which is inhibited by FTX (Llinas et al., 1989) and by o-Aga IVA (Mintz et al., 1992a,b) the P-type calcium channels, have been shown to be the main ion channels involved in neurotransmitter release without being sensitive to the inhibition by MPA (Mintz et al., 1992a,b; Turner et al., 1992; Tareilus et al., 1993). We observed in the present study that the ^3H^-catecholamines release induced by AMPA was also strongly inhibited by o-Aga IVA (Figure 6). This observation indicates that P-type VSCCs are important in mediating the influx of Ca^{2+} following AMPA receptor activation, which induced the release of ^3H^-catecholamines (Tareilus et al., 1993). We observed that o-Aga IVA also inhibited the AMPA-induced increase in the [Ca^{2+}] as well as the KC1 induced increase in the [Ca^{2+}] (not shown). When we applied o-CgTx and o-Aga IVA simultaneously, we observed that the inhibition of ^3H^-catecholamine release was almost complete, supporting the idea that the influx of Ca^{2+} following AMPA receptor stimulation occurred mainly through N- and P-type VSCCs. Other authors have reported effects of o-Aga IVA on 4Ca^{2+} influx into synaptosomes (Mintz et al., 1992b; Tareilus et al., 1993). The release of glutamate by rat cortical or striatal synaptosomes is also sensitive to o-Aga IVA (Turner et al., 1992; 1993), also indicating the presence of P-type VSCCs in glutamatergic terminals. The release of dopamine in rat striatal synaptosomes induced by depolarization with KC1 or 4-aminopyridine and dopamine agonists was inhibited by a combination of AMPA and VSCCs (Turner et al., 1993). A synergistic inhibition of KC1 evoked [^3H^-]dopamine release was observed by combining the effects of o-Aga IVA and o-CgTx, suggesting the coexistence of P- and N-type calcium channels in dopaminergic terminals of the striatum (Turner et al., 1993).

In summary, we showed here that presynaptic glutamate receptors modulate the release of [^3H^-]dopamine and [^3H^-]NA in rat hippocampal synaptosomes, since glutamate receptor agonists were effective in releasing these catecholamines in a Ca^{2+}-dependent manner. The AMPA induced release of ^3H^-catecholamines is mediated by VSCC activation of the N- and P-type, which allows the influx of Ca^{2+} and therefore the exocytosis of synaptic vesicles. These observations are in agreement with the current idea that the function of the N-type calcium channels can be closely related to the release of catecholamines (Reynolds et al., 1986; Tsien et al., 1988), and with the coexistence of both N- and P-type VSCCs in dopaminergic terminals (Turner et al., 1993).

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References


