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Short communication

Presynaptic 5-HT autoreceptors modulate *N*-methyl-D-aspartate-evoked 5-hydroxytryptamine release in the guinea-pig brain cortex

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Abstract

Guinea-pig cerebral cortical slices preincubated with [^{3}H]5-hydroxytryptamine ([^{3}H]5-HT) were superfused with Mg^{2+}-free Krebs' solution. *N*-Methyl-D-aspartate (NMDA) stimulated tritium overflow in a concentration-dependent manner. The NMDA-evoked overflow was abolished by omission of Ca^{2+} or presence of 1.2 mM Mg^{2+}, but only partly inhibited by tetrodotoxin. The competitive and noncompetitive NMDA receptor antagonists, DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoic acid (CGP 37849) and dizocilpine, respectively, also blocked the stimulatory effect of NMDA. Furthermore, the NMDA-evoked tritium overflow was inhibited by 5-carboxamidotryptamine in a manner susceptible to blockade by methiothepin, which given alone facilitated overflow. This facilitatory effect was increased in the presence of 6-nitroquipazine, a selective 5-HT reuptake inhibitor. It is concluded that the release of 5-HT in the guinea-pig cerebral cortex is stimulated via NMDA receptors, which are in part located on the serotoninergic axon terminals, and that the NMDA-evoked 5-HT release is modulated via inhibitory 5-HT autoreceptors.

Keywords: NMDA receptor; 5-HT autoreceptor; Presynaptic receptor; 5-HT (5-hydroxytryptamine, serotonin) release; Cerebral cortex, guinea-pig; Serotoninergic axon terminal

1. Introduction

In slices of the rat cerebral cortex, activation of *N*-methyl-D-aspartate (NMDA) receptors induces a powerful stimulation of noradrenaline release (Fink et al., 1989), but the conditions applied in that study were not suitable to disclose a stimulatory effect of NMDA on 5-hydroxytryptamine (5-HT) release. Recently we found that NMDA receptors, when activated for a prolonged period, also elicit release of 5-HT in rat brain cortical slices, and that the NMDA-evoked 5-HT release is inhibited via presynaptic α_2-heteroreceptors (Fink et al., 1995).

It is well known that 5-HT release evoked by electrical impulses or high K^+ is also modulated via presynaptic 5-HT autoreceptors (Starke et al., 1989). However, it has not yet been investigated whether such a negative feedback control is operative when 5-HT release is stimulated by NMDA, i.e. via receptors which are, at least in part, located on the serotoninergic axon terminals (Fink et al., 1995), potentially on the same varicosities as the 5-HT autoreceptors.

Therefore, the aims of the present study were to examine whether a stimulatory effect of NMDA on 5-HT release can also be observed in the guinea-pig brain cortex and whether the NMDA-evoked 5-HT release is under the feedback control of presynaptic 5-HT autoreceptors. For the latter purpose, the guinea-pig, rather than the rat, cerebral cortical slice is considered as an appropriate model, since the 5-HT autoreceptor in the guinea-pig brain (Hoyer and Middlemiss, 1989; Limberger et al., 1991; Bühlen et al., 1995) resembles that in the human one (Maura et al., 1993) in that it belongs to the 5-HT_{1D} receptor class, whereas in the rat brain it is a 5-HT_{1B} receptor (Engel et al., 1986).

Some of the results have been reported in abstract form (Göthert et al., 1994).

2. Materials and methods

2.1. Slice preparation and superfusion

Cerebral cortical slices (0.3 mm thick, 3 mm diameter) from male Dunkin Hartley guinea-pigs weighing 250–450

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g were incubated for 30 min at 37°C in modified Krebs-Henseleit solution (composition, see below) containing 50 nM [^{3}H]5-HT and 1 μM maprotiline. Subsequently, the slices were superfused for 60 min at a rate of 0.6 ml/min with [^{3}H]5-HT-free solution. Tritium overflow was stimulated for 5 min (as in our recent study in rat brain cortical slices; Fink et al., 1995) after 40 min of superfusion by adding NMDA. As a rule, the Krebs-Henseleit solution was composed as follows (mM): NaCl 118, KCl 4.8, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 1.3, glucose 11.1, ascorbic acid 0.06, disodium EDTA 0.03 (equilibrated with 95% O_2 and 5% CO_2; pH: 7.4). In some experiments, $MgSO_4$ (1.2 mM) was added to, or $CaCl_2$ was omitted from, the solution throughout superfusion. The superfusate was collected in 5-min samples. At the end of the experiments, the slices were solubilized with Soluene, and the radioactivity of the slices and superfusate samples was determined by liquid scintillation counting.

2.2. Calculations

Tritium efflux was calculated as the fraction of the tritium content in the slices at the beginning of the respective collection period (fractional rate of tritium efflux). To quantify effects of drugs on basal efflux, the fractional rate was determined in the sample collected immediately before stimulation. Basal efflux was assumed to decline linearly from the sample before to that 15–20 min after onset of stimulation. Stimulation-evoked tritium overflow was calculated by subtraction of basal from total efflux during the stimulation period and the following 15 min and was expressed as percentage of the tritium content in the slice at the onset of stimulation.

Concentration-response curves were fitted to the data points according to a sigmoidal logistic equation by iterative nonlinear regression analysis (GraphPad InPlot 4.0); this yielded the maximal effect and either the EC_{50} or the IC_{50} (concentration producing 50% of the maximum facilitation or inhibition, respectively). Apparent pA_2 values of antagonists were calculated according to the formula for competitive reversible antagonism given by Furchgott, 1972; equation No. 4). Results are given as means ± S.E.M. of n experiments. For comparison of two series of experiments, t-test for unpaired data was applied. For multiple comparisons with one control series, one-way analysis of variance followed by Dunnett's post-tests was applied.

2.3. Drugs

5-[1,2-^{3}H(N)]Hydroxytryptamine creatinine sulfate ([^{3}H]5-HT; NEN, Dreieich, Germany); N-methyl-D-aspartic acid (NMDA), tetrodotoxin (Sigma Chemicals Co., St. Louis, MO, USA); dizocilpine (= MK-801), 6-nitroquipazine, 5-carboxamidotryptamine maleate (5-CT; Research Biochemicals Int., Natick, MA, USA); DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid (CGP 37849; Ciba Geigy, Basel, Switzerland); methiothepin maleate (Hoffmann-La Roche, Basel, Switzerland); maprotiline hydrochloride (Ciba-Geigy, Wehr, Germany).

3. Results

Unless stated otherwise, the guinea-pig cortical slices preloaded with [^{3}H]5-HT were superfused with Mg^{2+}-free solution. Basal tritium efflux decreased with time. In the collection period from the 35th to 40th minute of superfusion, i.e. immediately before stimulation with NMDA, basal tritium efflux was 0.16 ± 0.03 nCi/min, corresponding to a fractional rate of efflux of 0.0070 ± 0.0009/min. The changes in ionic conditions and addition of the drugs

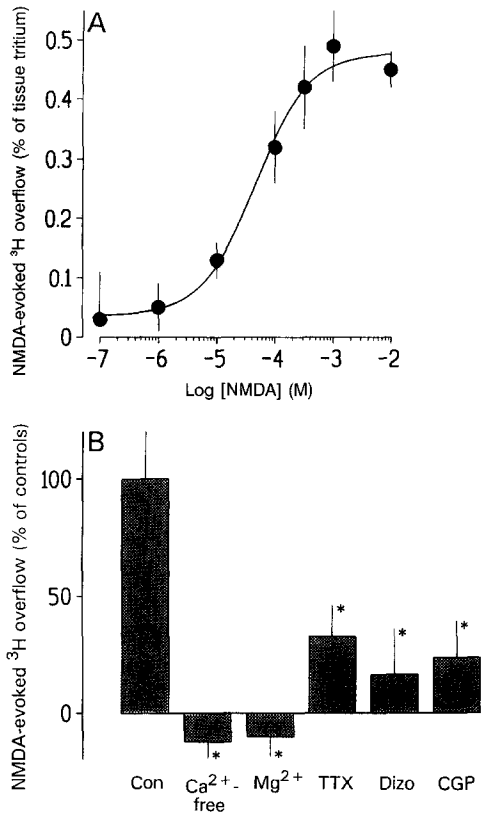

Fig. 1. N-Methyl-D-aspartate (NMDA)-evoked tritium overflow from superfused guinea-pig brain cortex slices and its changes by drugs or modification of the ionic composition of the superfusion fluid. The slices were preincubated with [^{3}H]5-HT (in the presence of 1 μM maprotiline) and, unless stated otherwise, superfused with Mg^{2+}-free medium. NMDA was added for 5 min after 40 min of superfusion. A: Concentration-response curve for NMDA. NMDA-evoked tritium overflow was expressed as percentage of tissue tritium. Means ± SEM of 5–15 experiments. B: Responses to modification of the ionic conditions or to tetrodotoxin (TTX, 0.3 μM), dizocilpine (Dizo, 0.1 μM) or CGP 37849 (CGP, 30 μM) on the NMDA (1 mM)-evoked tritium overflow (Con, controls). When relevant, Mg^{2+} was added to, or Ca^{2+} was omitted from, the medium throughout superfusion; tetrodotoxin, dizocilpine or CGP 37849 was present from the 20th minute of superfusion onward. Ordinate, overflow expressed as percentage of that in controls in which the ionic conditions were not changed or no drug was applied. Means ± S.E.M. of 5–8 experiments. * $P < 0.01$, compared to controls.

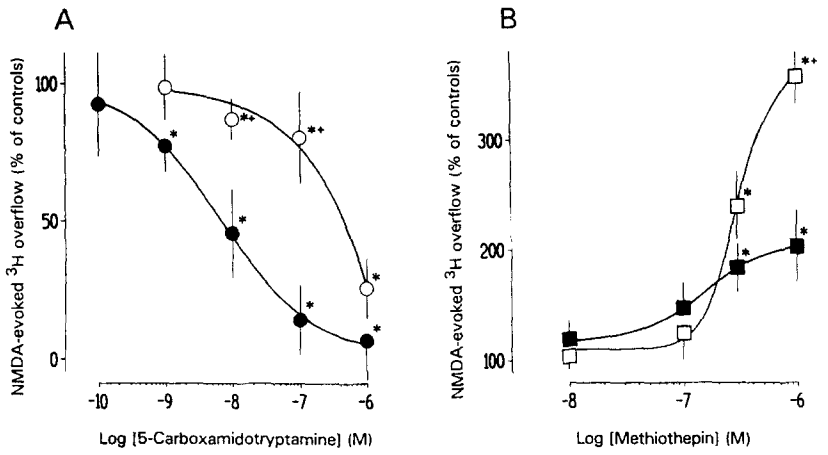

Fig. 2. (A) Effect of 5-carboxamidotryptamine (5-CT) in the absence (●) or presence of 1 μM methiothepin (○) and (B) effect of methiothepin in the absence (■) or presence of 1 μM 6-nitroquipazine (□) on the NMDA-evoked tritium overflow from superfused guinea-pig brain cortex slices. The slices were preincubated with [^{3}H]5-HT (in the presence of 1 μM maprotiline) and superfused with Mg^{2+}-free medium. NMDA was added for 5 min after 40 min of superfusion. When relevant, 5-carboxamidotryptamine or methiothepin or both was/were added to the medium from the 20th minute of superfusion onward; 6-nitroquipazine was present throughout superfusion. Ordinate, overflow expressed as percentage of that in corresponding 5-CT-free controls (in the absence or, when relevant, in the presence of methiothepin; A) or methiothepin-free controls (in the absence or, when relevant, in the presence of 6-nitroquipazine; B). Means ± S.E.M. of 5–12 experiments. * $P < 0.05$, compared to the corresponding 5-CT-free (A) or methiothepin-free controls (B). + $P < 0.05$, compared to the effect of the same 5-CT concentration in the absence of methiothepin (A) or of the same methiothepin concentration in the absence of 6-nitroquipazine (B).

at the concentrations investigated did not modify basal efflux of tritium.

NMDA stimulated the overflow of tritium in a concentration-dependent manner (Fig. 1A; maximum response: 0.49 ± 0.06% of tissue tritium, corresponding to 0.10 ± 0.01 nCi; EC_{50} 46 μM). The tritium overflow evoked by 1 mM NMDA was abolished when Ca^{2+} was omitted from, or 1.2 mM Mg^{2+} was present in, the superfusion fluid; however, 0.3 μM tetrodotoxin did not cause complete blockade of evoked overflow, but an inhibition by 67% only (Fig. 1B). Dizocilpine (0.1 μM) and CGP 37849 (30 μM), inhibited the NMDA (1 mM)-evoked tritium overflow by more than 75% (Fig. 1B).

The NMDA evoked tritium overflow was inhibited by the 5-HT_1 receptor agonist, 5-CT, in a concentration-dependent manner (Fig. 2A; IC_{50}: 6.9 nM). Methiothepin, a mixed 5-HT_1/5-HT_2 receptor antagonist, which given alone increased the NMDA-evoked tritium overflow (Fig. 2B), shifted the concentration-response curve for 5-CT to the right (apparent pA_2: 7.82; Fig. 2A). In the presence of the neuronal 5-HT reuptake inhibitor, 6-nitroquipazine, the increasing effect of methiothepin on the NMDA-evoked tritium overflow was considerably enhanced (maximum increase by 104% in the absence, versus 257% in the presence, of 6-nitroquipazine; Fig. 2B).

4. Discussion

Stimulation-evoked, Ca^{2+}-dependent and tetrodotoxin-sensitive tritium overflow from brain slices preincubated with a [^{3}H]neurotransmitter such as [^{3}H]5-HT may be assumed to reflect the exocytotic release of this transmitter from the respective neurones (for review, see Starke et al., 1989). Therefore, in the present study, the NMDA-evoked tritium overflow from the guinea-pig cortical slices preincubated with [^{3}H]5-HT was taken as a measure for the release of labelled and unlabelled 5-HT from serotoninergic neurones. Evidence has been presented that activation of NMDA receptors mediates the stimulation of 5-HT release. This conclusion can be drawn, since the receptors involved exhibited features which are characteristic for NMDA receptors. Thus, the NMDA-evoked tritium overflow was dependent on the presence of Ca^{2+} ions in the extracellular fluid, it was abolished by physiological Mg^{2+} concentrations and it was strongly inhibited by dizocilpine, an antagonist at the phencyclidine site of the NMDA-gated ion channel (Wong et al., 1986) and by CGP 37849, a competitive NMDA receptor antagonist (Fagg et al., 1990). The stimulatory effect of NMDA was rather weak. NMDA stimulated tritium overflow in the guinea-pig cerebral cortex at similar potency (EC_{50}: 46 μM) as in the rat cerebral cortex (Fink et al., 1995; EC_{50}: 17 μM).

The possibility that [^{3}H]5-HT was taken up into, and released from, the noradrenergic axon terminals contained in the cortical slices was excluded, since preincubation of the slices with [^{3}H]5-HT was carried out in the presence of the selective noradrenaline uptake inhibitor, maprotiline (Göthert et al., 1983). As previously shown in the rat brain cortex (Fink et al., 1995), tetrodotoxin did not completely block the NMDA-evoked tritium overflow in guinea-pig cerebral cortical slices. The tetrodotoxin-resistent tritium overflow may be assumed to reflect action potential-independent [^{3}H]5-HT release from 5-hydroxytryptaminergic varicosities. This assumption is compatible with the suggestion that the excitatory NMDA receptors stimulating tetrodotoxin-resistant release are also located on the sero-

toninergic varicosities themselves, i.e. that they are presynaptic heteroreceptors.

The second question addressed in this study was whether the NMDA-evoked 5-HT release resembles the release evoked by electrical impulses or high K^+ in that it is modulated via presynaptic inhibitory 5-HT autoreceptors (see Introduction). The following data clearly prove the operation of such a negative feedback control of the 5-HT release induced by NMDA:

(1) The nonselective 5-HT_1/5-HT_2 receptor antagonist, methiothepin, given alone increased the NMDA-evoked tritium overflow, probably by preventing endogenous 5-HT from activating the autoreceptors.

(2) When the selective 5-HT reuptake inhibitor, 6-nitroquipazine (Vaatstra et al., 1981; Göthert et al., 1983), was present in the superfusion fluid, the facilitatory effect of methiothepin on NMDA-evoked tritium overflow was enhanced. The most plausible explanation for this finding is that 6-nitroquipazine increased the concentration of 5-HT in the biophase of the autoreceptors, a condition under which the disinhibitory effect of methiothepin on evoked 5-HT release is more pronounced.

(3) The 5-HT_1 receptor agonist, 5-CT, inhibited the NMDA-evoked tritium overflow in a manner susceptible to antagonism by methiothepin.

Since both the inhibitory 5-HT receptors and part of the stimulatory NMDA receptors are located on the serotoninergic axon terminals, it is conceivable that the autoreceptor-mediated modulation of the NMDA-evoked tritium overflow may be due to an interaction between both receptors at the level of the same varicosities. Analogous results have been obtained for presynaptic NMDA heteroreceptors and α_2-autoreceptors on the noradrenergic neurones of the rat hippocampus and cerebral cortex (Raiteri et al., 1992; Fink and Göthert, 1993).

Taken together, the main conclusions drawn from the present data are that activation of NMDA receptors induces 5-HT release in the guinea-pig cerebral cortex and that this NMDA-evoked 5-HT release is under the control of presynaptic inhibitory 5-HT autoreceptors.

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