Modulation of N-methyl-D-aspartate (NMDA)-stimulated noradrenaline release in rat brain cortex by presynaptic $\alpha_2$-adrenoceptors

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Summary. Rat brain cortex slices and synaptosomes preincubated with $[^3]$H]noradrenaline were used to investigate whether the NMDA-evoked noradrenaline release is modulated by agonists or antagonists at presynaptic $\alpha_2$-adrenoceptors.

In experiments on slices, noradrenaline and the preferential $\alpha_2$-adrenoceptor agonists talipexole (former B-HT 920) and clonidine inhibited the NMDA-evoked tritium overflow whereas the selective $\alpha_1$-adrenoceptor agonists cirazoline and methoxamine were ineffective. The $\alpha_2$-adrenoceptor antagonists rauwolscine and idazoxan facilitated the NMDA-evoked tritium overflow whereas the preferential $\alpha_1$-adrenoceptor antagonist prazosin was ineffective. The concentration-response curve of talipexole for its inhibitory effect on NMDA-evoked overflow was shifted to the right by idazoxan (apparent $pA_2 = 7.5$). The EC$_{50}$ of NMDA (97 $\mu$mol/l) for its stimulating effect on tritium overflow was not substantially changed by blockade of $\alpha_2$-autoreceptors with 1 $\mu$mol/l rauwolscine (EC$_{50}$ of NMDA in the presence of the $\alpha_2$-adrenoceptor antagonist, 155 $\mu$mol/l), but the maximal overflow of tritium was increased 2.5 fold by this rauwolscine concentration.

In experiments on synaptosomes, talipexole and noradrenaline inhibited the NMDA-evoked tritium overflow. The inhibitory effect of talipexole was abolished by idazoxan which, given alone, was ineffective, as was prazosin. Talipexole did also not produce an inhibition when tritium overflow was evoked by NMDA in the presence of $\omega$-conotoxin GVIA 0.1 $\mu$mol/l; the latter, by itself, decreased the response to NMDA by about 55%. It is concluded that the NMDA-evoked release is at least partially due to a functional interaction between the NMDA receptors and $\alpha_2$-autoreceptors at the level of the same varicosities. The results obtained with $\omega$-conotoxin GVIA suggest that $Ca^{2+}$ influx via the N-type voltage-sensitive calcium channel (VSCC) occurs in response to NMDA receptor stimulation and contributes substantially to the induction of NMDA-evoked noradrenaline release. The inhibitory effect of $\alpha_2$-adrenoceptor stimulation on this release appears to be ultimately due to an inhibition of the influx of $Ca^{2+}$ via the N-type VSCC.

Key words: NMDA-receptors – $\alpha_2$-Adrenoceptors – Noradrenaline release – Presynaptic receptors – Rat brain cortex – $\omega$-Conotoxin GVIA

Introduction

The NMDA receptors represent one of the main classes of excitatory amino acid (EAA) receptors via which the endogenous neurotransmitters L-glutamate and L-aspartate exert their effects (Cotman and Monaghan 1988; Stone and Burton 1988; Collingridge and Lester 1989). Under physiological conditions, NMDA receptors are implicated in memory and learning, whereas excessive stimulation of these receptors induces neurotoxicity resulting in cerebral dysfunctions, such as ischemic damage or epilepsy (Dingledine et al. 1990; Meldrum and Garthwaite 1990).

Previous investigations in rat and human brain cortex slices revealed that NMDA induces noradrenaline release in a $Ca^{2+}$-dependent and largely tetrodotoxin-sensitive manner (Fink et al. 1989, 1992). The effect of NMDA was inhibited by competitive and noncompetitive NMDA receptor antagonists (DL-2-amino-5-phosphonovaleric acid and dizocilpine, respectively) and was abolished by physiological concentrations of $Mg^{2+}$ ions, which, like dizocilpine, block the NMDA receptor channel. These results indicate that the release-stimulating effect of...
NMDA is mediated by NMDA receptors. Analogous results were obtained in rat cortical and hippocampal synaptosomes (Fink et al. 1990; Göttert and Fink 1991; Fink and Göttert 1992; Pittaluga and Raiteri 1990, 1992). The only differences compared to slices were the resistance to tetrodotoxin, the dependence on exogenous glycine and the considerably higher potency but substantially lower maximum effect of NMDA in stimulating glycine and the considerably higher potency but substantially lower maximum effect of NMDA in stimulating noradrenaline release. According to these results, at least part of the NMDA receptors mediating stimulation of noradrenaline release are located presynaptically on the noradrenergic varicosities themselves.

Among the previously identified presynaptic receptors on noradrenergic axon terminals, inhibitory α2-autoreceptors play an important role in the regulation of noradrenaline release (Starke 1977, 1987; Langer 1981; Starke et al. 1989). Therefore, the possibility of an interaction between the presynaptic NMDA receptors and α2-adrenoceptors and its functional implications have to be taken into account. This possibility has been suggested by preliminary experiments in rat brain cortical slices (Fink et al. 1991) and by data obtained in rat hippocampal synaptosomes (Raiteri et al. 1992); in those experiments α-adrenoceptor agonists such as clonidine and/or talipexole inhibited the NMDA-evoked [3H]noradrenaline release in a manner sensitive to blockade by idazoxan.

On the basis of these findings and considerations, the aims of the present study were to examine more comprehensively, (1) whether in the rat brain cortex presynaptic α2-autoreceptors are involved in the modulation of NMDA-evoked noradrenaline release, (2) if so, whether and by which mechanism the two receptor systems can functionally interact at the level of the same noradrenergic varicosity, and (3) whether the lower potency of NMDA in stimulating noradrenaline release in slices than in synaptosomes (in which the α2-autoreceptors are not activated by endogenous noradrenaline; see Raiteri and Levi 1978) is related to the stimulation of α2-autoreceptors by endogenous noradrenaline released in response to NMDA receptor activation in the slices. Some of the results were reported at the International Symposium on Presynaptic Receptors and Neuronal Transporters, Rouen 1990 (Fink et al. 1991).

**Methods**

Brain cortical slices (0.3 mm thick, diameter 3 mm) prepared from male Wistar rats weighing 200–300 g were incubated for 30 min in Krebs’ solution (37°C) composed as follows (mM): NaCl 118, KCl 4.8, NaHCO3 25, KH2PO4 1.2, CaCl2 1.3, MgSO4 1.2, glucose 11.1, ascorbic acid 0.06, disodium EDTA 0.03 (equilibrated with 95% O2 and 5% CO2). During incubation this solution contained 50 mM [3H]noradrenaline (specific activity 43.7 Ci/mmol).

Synaptosomes were prepared essentially as described by Gray and Whittaker (1962) with slight modifications. Briefly, cortical tissue (without the frontal poles; 10% w/v) was homogenized in 0.32 M sucrose by means of a Potter-Elvehjem glass homogenizer with a rotating teflon pestle (1000 rpm, 6 strokes/2 min). The homogenate was centrifuged at 1000 •g for 10 min (4°C), and 9 ml of the supernatant plus 6 ml of Krebs-Henseleit solution were incubated for 7 min at 37°C. After addition of [3H]noradrenaline incubation was continued for another 7 min. The labelled suspension was centrifuged at 600 •g for 10 min (4°C) and the resulting pellet resuspended in 2.25 ml ice-cold Krebs’ solution (protein content 1152 ± 60 µg protein/0.1 ml, determined with the method of Lowry et al. 1951).

Single slices or aliquots of 100 µl of the synaptosomal suspension were distributed on Whatman GF/C or GF/B filters, respectively, in 24 parallel superfusion chambers. Subsequently, the slices or synaptosomes were superfused with Mg2+-free Krebs’ solution for 62 min at a flow rate of 0.6 ml/min; the superfusion technique in synaptosomes was based on that described by Raiteri et al. (1974). Tritium overflow was calculated by subtracting basal from total efflux. When unlabelled noradrenaline was studied as an α-adrenoceptor agonist, the superfusion solution contained 1 µmol/l desipramine. Substances under investigation were present in the buffer from the 20th min of superfusion onward until the end of the experiments (exception: ω-conotoxin GVIA which was present during the 2-min stimulation period only). The superfuse was continuously collected in 4-min (synaptosomes) or 5-min (slices) samples. At the end of the experiments the radioactivity in the superfuse samples and slices (solubilized with 0.5 M Soluene®) was determined by liquid scintillation counting.

Tritium efflux was calculated as the fraction of the tritium content in the slice or synaptosomes 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 12–16 (synaptosomes) or 15–20 min (slices) after onset of stimulation. Stimulation-evoked tritium overflow was calculated by subtraction of basal from total efflux during the stimulation period and the following 10 (synaptosomes) or 13 min (slices) and was expressed as percentage of the tritium content in the slices or synaptosomes at the onset of stimulation. Control experiments were run in parallel, and evoked tritium overflow was expressed as percentage of these controls.

Under the conditions applied here, i.e. superfusion of cortical slices and synaptosomes preincubated with a low [3H]noradrenaline concentration, the NMDA-evoked tritium overflow reflects the release of labelled and unlabelled noradrenaline from the noradrenergic varicosities (see Fink et al. 1989). Hence, the term release of noradrenaline (or of [3H]noradrenaline) will be used throughout the discussion.

Results are given as means ± SEM of n experiments (experiments in synaptosomes were carried out in quadruplicate). For comparison of mean values, Student’s t-test was used. In case of multiple comparisons, Dunnett’s test was applied.

**Drugs used.** (-)-[ring 2,5,6-3H]noradrenaline (NEN, Dreieich, Germany); cirazoline (Synthelabo, Paris, France); (+)-methoxamine hydrochloride (Burroughs Wellcome, London, UK); talipexole dihydrochloride (former B-HT 920; Thomas, Biberach a.d. Riss, Germany); clonidine hydrochloride (Boehringer, Ingelheim, Germany); rauwolscine hydrochloride (former B-HT 920; Thomae, Biberach a.d. Riss, Germany); desipramine hydrochloride (CIBA-Geigy, Wehr, Germany); noradrenaline (base; Farbwerke Hoechst, Frankfurt/Main, Germany); ω-conotoxin GVIA (RBI, Natick, MA, USA).

**Results**

**Basal tritium efflux**

Under control conditions, basal tritium efflux from rat brain cortex slices and synaptosomes preincubated with [3H]noradrenaline declined continuously with time. At the concentrations investigated the drugs applied in this study (see Figs. 1–7) did not affect basal efflux (results not shown).