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Short communication

Differential role of nitric oxide in long-term potentiation in the medial and lateral amygdala

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Abstract

The effects of nitric oxide (NO) donors on the induction of long-term potentiation in the amygdala were investigated using rat brain slice preparations in vitro. In the medial amygdala synapses, sodium nitroprusside (10 μM) and hydroxylamine (100 μM), when applied together with a weak tetanic stimulation, significantly facilitated the induction of long-term potentiation. The NO donors showed no effect in the lateral amygdala synapses. These results suggest that NO plays a role in facilitating the induction of long-term potentiation in the medial amygdala but not in the lateral amygdala.

Keywords: Nitric oxide (NO); Long-term potentiation; Amygdala, medial; Amygdala, lateral

1. Introduction

Recently nitric (NO) has been proposed to function as a neuronal messenger in the brain (Garthwaite, 1991; Bredt and Snyder, 1992). The NO-synthesizing enzyme NO synthase is constitutively expressed in neuronal cells and activated by Ca^{2+} influx following activation of NMDA receptors (Garthwaite, 1991; Bredt and Snyder, 1992). Long-term potentiation of excitatory synaptic transmission is a form of activity-dependent plasticity which may underlie learning and memory (Bliss and Collingridge, 1993). Since the induction of long-term potentiation in the CA1 region and the dentate gyrus of the hippocampus is triggered by activation of NMDA receptors and an increase of intracellular Ca^{2+} concentration in postsynaptic cells, it is possible that NO participates in long-term potentiation in these synapses. In fact, several laboratories have reported that NO synthase inhibitors block the induction of hippocampal long-term potentiation (O'Dell et al., 1991; Schuman and Madison, 1991; Mizutani et al., 1993), although a role for NO in hippocampal long-term potentiation is still controversial (Bliss and Collingridge, 1993).

The amygdala is thought to be involved in learning and memory associated with emotion (McGaugh et al., 1990). It has been demonstrated that amygdala synapses display long-term potentiation (Chapman et al., 1990; Gean et al., 1993; Watanabe et al., 1995a), but the cellular mechanism of amygdala long-term potentiation is not fully understood. Histochemical studies have demonstrated the abundant presence of NO synthase in medial amygdala neurons but very little NO synthase in lateral amygdala neurons (Bredt et al., 1991; Vincent and Kimura, 1992). Furthermore, we have recently reported that NO synthase inhibitors block the induction of long-term potentiation in the medial amygdala but not in the lateral amygdala (Watanabe et al., 1995b). These observations suggest that endogenous NO production is required for the induction of long-term potentiation in the medial amygdala but not in the lateral amygdala. However, this does not necessarily mean that the induction mechanism of lateral amygdala long-term potentiation is insensitive to NO. It is possible that lateral amygdala long-term potentiation is affected by exogenously applied NO. To further clarify the differences in cellular mechanisms underlying long-term potentiation in the medial and lateral amygdala, in the present study, we examined the effects of NO donors on the induction of long-term potentiation in these synapses by using brain slices in vitro.

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2. Materials and methods

Preparation of brain slices and recording of evoked potentials were as described in our previous paper (Watanabe et al., 1995a). Briefly, amygdala slices (400–500 μm thickness) were prepared from male Wistar rats (7–9 weeks old) and maintained in a submersion chamber (3 ml) where warmed (34°C) and oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) was continuously perfused at a rate of 1 ml/min. The composition of ACSF was as follows (in mM): 124.0 NaCl, 5.0 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.24 KH_2PO_4, 26.0 $NaHCO_3$ and 10.0 glucose. A bipolar tungsten electrode with 0.15-mm tip separation was placed on the stria terminalis or the external capsule to stimulate the afferent fibers, and the evoked potential was extracellularly recorded from the medial amygdaloid nucleus or the lateral amygdaloid nucleus, respectively. A glass microelectrode filled with 0.9% NaCl (tip resistance, 2–4 MΩ) was used for the recording. A single test stimulation (0.05 ms duration) was applied at intervals of 20 s. The stimulus intensity was adjusted to produce a population spike of about 50% of the maximum amplitude. All drugs were delivered by perfusion. Tetanic stimulation was applied at the same stimulus intensity through the same electrode as used for test stimulation. As described in our previous paper (Watanabe et al., 1995a), the negative-going field potentials recorded from the medial and lateral amygdaloid nuclei correspond to population spikes. The amplitude of the population spike was measured as shown in Fig. 1A. Long-term potentiation was considered to have occurred if the potentiation spike amplitude remained at least 20% higher than the baseline value 30 min after tetanic stimulation.

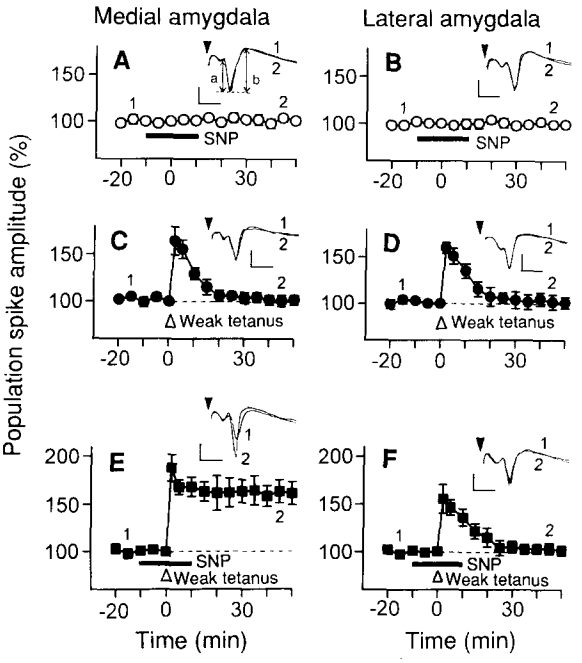

Fig. 1. The effects of an NO donor, sodium nitroprusside (SNP), on baseline synaptic responses and short-term potentiation induced by weak tetanic stimulation in the medial (A, C, E) and lateral amygdala (B, D, F). Representative records at the times denoted by the numbers are shown as insets. Calibration bars: vertical 0.5 mV, horizontal 5 ms. The amplitude of population spike was calculated as $(a+b)/2$. Ordinates of time course graphs indicate population spike amplitude expressed as a percentage of baseline value at time 0. In A and B, 10 μM SNP (bold bars) was applied without tetanic stimulation. SNP alone did not affect the baseline synaptic responses. In C and D, weak tetanic stimulation (100 Hz for 0.5 s, white arrowheads) alone was applied. It produced only short-term potentiation in both the medial and lateral amygdala. In E and F, a weak tetanic stimulation was applied in the presence of 10 μM SNP. It induced long-term potentiation in the medial amygdala but not in the lateral amygdala. The experiments illustrated in A–F were done on separate slices. All data are represented as the mean ± S.E.M. of five separate observations.

3. Results

We used two NO donors, sodium nitroprusside and hydroxylamine, which produce NO by different mechanisms. Sodium nitroprusside produces NO by simple dissociation and hydroxylamine is metabolised to NO by various cellular enzymes (Southam and Garthwaite, 1991). First we examined the effects of these NO donors on the baseline synaptic potentials evoked by test stimulation. However, neither sodium nitroprusside (1–10 μM) (Fig. 1A) nor hydroxylamine (10–100 μM) (data not shown, $n = 5$) affected the baseline synaptic responses in the medial amygdala. They also had no effect on the baseline synaptic responses in the lateral amygdala (sodium nitroprusside, Fig. 1B; hydroxylamine, data not shown, $n = 5$).

Next it was tested if NO donors facilitate the induction of long-term potentiation when applied with weak tetanic stimulation. Weak tetanic stimulation (100 Hz for 0.5 s) alone induced short-term potentiation but did not produce long-term potentiation in either the medial amygdala (Fig. 1C) or the lateral amygdala (Fig. 1D). In the medial amygdala, when weak tetanic stimulation was applied in the presence of sodium nitroprusside (1–10 μM), long-term potentiation was produced (Fig. 1E and Fig. 2A). A similar effect was observed with hydroxylamine (10–100 μM, Fig. 2A). However, in the lateral amygdala, NO donors neither enhanced nor suppressed the potentiation induced by weak tetanic stimulation (Fig. 1F and Fig. 2B). The effects of sodium nitroprusside and hydroxylamine at higher concentrations (100 μM and 1 mM, respectively) were examined in the lateral amygdala, but neither showed significant effects (Fig. 2B).

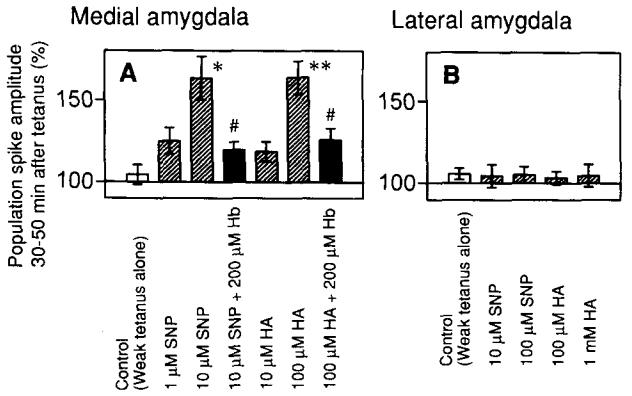

Fig. 2. Summary of the effects of sodium nitroprusside (SNP), hydroxylamine (HA) and hemoglogin (Hb) in the medial (A) and lateral amygdala (B). Experimental protocol was as shown in Fig. 1E and F. Weak tetanic stimulation (100 Hz for 0.5 s) was applied in normal ACSF (control, white columns) or in the presence of SNP or HA (hatched columns) or in the presence of NO donors and Hb (solid black columns). The average population spike amplitude 30–50 min after tetanic stimulation was calculated as a measure of long-term potentiation. All data are represented as the means ± S.E.M. of five separate observations. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control; $^{\#}P < 0.05$ vs. 10 μM SNP or 100 μM HA; Duncan's multiple range test.

To check if the effects of NO donors are mediated by NO, the effect of an NO scavenger, hemoglobin, was examined. Hemoglobin (200 μM) alone did not affect short-term potentiation induced by weak tetanic stimulation (data not shown, $n = 5$). However, the effects of NO donors in the medial amygdala were significantly attenuated by the concomitant presence of hemoglobin (200 μM) (Fig. 2A). These results confirm that the effects of NO donors observed here are mediated by NO.

4. Discussion

In our previous study (Watanabe et al., 1995b), the induction of long-term potentiation in the medial amygdala was blocked by NO synthase inhibitors, suggesting that endogenous NO production is required for the induction of long-term potentiation in the medial amygdala. The role of NO in medial amygdala long-term potentiation was further supported by the present results. NO donors, sodium nitroprusside and hydroxylamine, facilitated the induction of long-term potentiation by weak tetanic stimulation in the medial amygdala. These data suggest that the induction of long-term potentiation in the medial amygdala is regulated by NO. However, the induction of long-term potentiation in the lateral amygdala was not blocked by NO synthase inhibitors (Watanabe et al., 1995b) and was not affected by NO donors (our present observation). It can be concluded that lateral amygdala long-term potentiation does not involve endogenous NO production and is also insensitive to exogenously applied NO.

In the medial amygala, NO alone did not affect the baseline synaptic responses and facilitated the induction of long-term potentiation only when coupled with weak tetanic stimulation. This means that NO facilitates potentiation at active, but not quiescent, synapses. The activity-dependent action of NO in the medial amygdala is very similar to that reported in hippocampal synapses (Zhou et al., 1993).

The mechanism underlying NO-independent long-term potentiation in the lateral amygdala remains to be investigated. We have previously observed that lateral amygdala long-term potentiation is not blocked by an NMDA receptor antagonist but suppressed by a muscarinic receptor antagonist, scopolamine (Watanabe et al., 1995a). Lateral amygdala long-term potentiation may be induced by activation on non-NMDA glutamate receptors or stimulation of other neurotransmitter receptors including muscarinic receptors. In addition, it is possible that NO donors act on multiple cell types or pathways within the slice; thus the differential effects of NO in the medial and lateral amygdala might reflect a difference in neural circuit connections.

In summary, NO plays a role in facilitating the induction of long-term potentiation in the medial amygdala, whereas lateral amygdala long-term potentiation does not involve endogenous NO production and also is totally insensitive to exogenously applied NO. Lateral amygdala long-term potentiation may be a useful model for studying the NO-independent form of synaptic plasticity in the brain.

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