Prenatal stress alters noradrenergic modulation of LTP in hippocampal slices

Gayane Grigoryan and Menahem Segal
Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel

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EVIDENCE FOR LONG-TERM EFFECTS OF PREGNANT ENVIRONMENT on postnatal brain functions has been accumulated in recent years (Laloux et al. 2012; Mychasiuk et al. 2012; Yaka et al. 2007; Yeh et al. 2012). Exposure of pregnant dams to stressful stimulation can cause changes in neurotransmitter metabolism, brain morphology, and ability to cope with cognitive and emotional tasks in the juvenile and even in adult life (Maccari et al. 2003). It was even suggested that prenatal stress (PS) can facilitate emergence of neuropyschiatric conditions such as depression and schizophrenia (Koenig et al. 2005; Markham and Koenig 2011; Seckl and Meaney 2006; Wilson et al. 2012). The molecular mechanisms underlying these long-term changes in neuronal functions are beginning to be unraveled, and studies using gene arrays report on gene families that are altered by PS (Afadal et al. 2010; Biala et al. 2011; Bogoch et al. 2007; Weinstock 2008). Two major chemical families associated with stress are steroid hormones and the neuropeptides, including norepinephrine (NE), serotonin, and acetylcholine. There is a growing understanding of the role of steroid hormones in regulation of synaptic plasticity in the adult brain (Maggio and Segal 2007), and there is evidence that PS causes a rise of corticosterone in the mother (Abdul Aziz et al. 2012; Yaka et al. 2007), which affects steroid receptors in the pups (Tamura et al. 2011). However, the steroid receptors are developing late in embryonic age and may not play a major role in PS (Diaz et al. 1998). On the other hand, NE is known to affect plasticity (Bramham et al. 1998; Gray and Johnston 1987; Hu et al. 2007; Lim et al. 2010; Schimanski et al. 2007; Stanton and Survey 1985) and is assumed to serve an important role in cognitive processes (Sara 2009). However, there is a paucity of information on the effects of stress on NE modulation of synaptic plasticity. We hypothesized that since NE-containing neurons are formed early in embryonic age and their development can be derailed by prenatal exposure to drugs (Dey et al. 2007), they may also be affected by PS. In the present study, we compared the effects of NE, acting at β1-adrenergic receptors, on long-term potentiation (LTP) in acute slices of the dorsal and ventral hippocampus (DH and VH, respectively), shown to be differentially affected by stress (Maggio and Segal 2007) in juvenile rats that underwent PS. Our results indicate that PS caused a shift of the locus of NE involvement in plasticity from the DH to the VH.

METHODS

Animals. Animal handling and experimentation were approved by the Institutional Animal Care and Use Committee of the Weizmann Institute of Science. PS was induced by exposing pregnant Wistar rat dams of a local breeding colony (at gestation ages of 14–21 days) to 3 stressful experiences including forced swim in a water bucket (for 20 min at room temperature), placement on an elevated platform (for 30 min), and restraining tube (for 40 min) once a day, repeated twice during 6 days of pregnancy. This protocol was adapted from Yaka et al. (2007), who found a 10-fold increase of maternal plasma corticosterone level after forced swim or restraint. Control pregnant dams were left undisturbed.

To find out whether PS affects the offspring via stressed mother during pregnancy or its effects appear after birth due to changed maternal care, newborn pups were switched in one experiment between mothers, i.e., prenatally stressed pups were raised by control foster mother and vice versa. The results of this experiment are presented separately.

Electrophysiology. Transverse hippocampal slices (400 μm) were prepared from the hippocampi of 2- to 3-wk-old male rats using a McIlwain Tissue Chopper. Slices were incubated for 1.5 h in carbo- genated (5% CO2–95% O2) artificial cerebrospinal fluid (aCSF) at room temperature. The medium contained, in mM, 124 NaCl, 2 KCl, 26 NaHCO3, 1.24 KH2PO4, 2.5 CaCl2, 2 MgSO4, and 10 glucose at pH 7.4. Recording was made from slices taken from the DH and VH as described elsewhere (Grigoryan et al. 2012). Slices were slightly submerged in a standard chamber at 33.8–34.0°C with a flow rate of aCSF of 2.5 ml/min.

Extracellular recordings of population excitatory post synaptic potentials (EPSPs) in the stratum radiatum of CA1 region were made through a glass pipette containing 0.75 M NaCl (4 MΩ). Responses were evoked by stimulation of the Schaffer collaterals using bipolar electrodes positioned equidistant on both sides of the recording electrode in the s. radiatum of CA1 region such that two independent stimulation pathways were used for each slice (Fig. 1A). In previous studies, we confirm that sequential tetanic stimulation produces similar LTP in the two pathways. Tetanic stimulation was delivered at a frequency of 100 Hz using a designated number of stimuli. Before applying the tetanic stimulation, evoked EPSPs (50% of maximum amplitude) were recorded for a stable baseline period of at least 10 min.

To investigate further the effects of the PS in DH and VH slices, the paired-pulse stimulation protocol was used. Two consecutive stimul
of equal intensity were delivered at varying interpulse intervals (ranging from 10 to 100 ms) to s. radiatum.

The following drugs were used: isoproterenol, a nonselective β-adrenergic agonist (Iso; 1 μM; Sigma-Aldrich); atenolol, a selective β1-adrenergic receptor antagonist (2 μM; Sigma-Aldrich); ICI-118,551, a selective β2-adrenergic receptor antagonist (0.1 μM; Sigma-Aldrich); forskolin, used to raise levels of cAMP (0.1 μM; Calbiochem); cyclopiazonic acid, sarcoplasmic/endoplasmic reticulum Ca2+-ATPase reversible antagonist (CPA; 5 μM; Alomone Labs); and (S)-3,5-dihydroxyphenylglycine hydrate, a group 1 metabotropic glutamate (mGlu) receptor agonist (DHPG; 25 μM; Sigma-Aldrich). Stock solutions were diluted in recording medium immediately before use.

Data acquisition and offline analysis were performed using pClAMP 9.2 (Axon Instruments). All numerical data are expressed as means ± SE. Statistical comparisons were performed by one-way ANOVA followed by paired comparisons. P values of <0.05 were considered a significant difference between means.

RESULTS

Iso effects on short-term potentiation in DH and VH. Dependence of EPSP slope on stimulation intensity in the DH and VH was assessed from input/output curves. The DH (n = 6) and VH (n = 6) slices obtained from control and PS rats exhibited similar input/output relations in s. radiatum of the CA1 region (Fig. 1B). This result suggests that basal synaptic transmission in both DH and VH is not affected by PS. PS did not alter paired-pulse facilitation of population EPSPs in either DH or VH. These results are congruent with previous observations (Yaka et al. 2007; Yeh et al. 2012; but see Grigoryan and Segal 2013, where an effect on population spike recorded in s. pyramidale is found). Interestingly, Iso (1 μM) had no effect on either paired-pulse facilitation or LTP evoked by a full-length tetanic stimulation (1 s, 100 Hz), which by itself generated LTP [1.42 ± 0.004 in DH (n = 5) compared with 1.41 ± 0.003 in VH (n = 5); Fig. 1, C–F].

Short tetanic stimulation, subthreshold for LTP induction (35 pulses, 100 Hz), evoked only a transient enhancement of reactivity to the afferent stimulation (short-term potentiation, STP), which was similar in both regions and returned to baseline within 10 min after the tetanic stimulation. Iso did not have any effect on baseline reactivity to the stimulation, but in its presence, the same short tetanic stimulation now converted the STP into a full-length LTP in DH slices (1.57 ± 0.01, n = 4). Under the same conditions, Iso had no effect in VH slices (n = 4; Fig. 2, A and B).

PS switches the locus of effect of Iso from DH to VH. In a similar cohort of slices taken from PS rats, there was no difference in response between DH (n = 6) and VH (n = 6) in the input/output relations in response to the afferent stimulation (Fig. 1B). Surprisingly, in DH slices of PS rats, exposure to Iso did not convert STP to LTP. In sharp contrast, Iso was now able to convert STP to LTP in VH slices (1.41 ± 0.01; Fig. 2, C and D).

To test whether PS affects the offsprings because of their being handled by a stressed mother after delivery, we repeated some of these experiments with PS rats that were raised by an unstressed foster mother and, vice versa, normal pups raised by stressed mothers. As seen before, Iso did not have any effect on baseline reactivity to the stimulation either in DH or VH slices of both control and PS groups. However, short tetanic stimulation in presence of Iso led to an induction of LTP in DH slices of control (1.58 ± 0.01, n = 4) and VH slices of PS rats (1.36 ± 0.01, n = 5) but had no effect in either DH of PS (1.04 ± 0.01, n = 4) or VH of control rats (1.02 ± 0.02, n = 4) slices. These results indicate that PS effects on plasticity are not due to the postnatal handling by a stressed mother.

Pharmacological analysis of Iso action in the hippocampus. To examine the β-adrenergic nature of the action of Iso in the hippocampus in relation to LTP, we used the selective β2-adrenergic receptor antagonist atenolol and the β2-antagonist ICI-118,551. In either the DH slices of control rats (n = 5) or the VH slices of the PS rats (n = 6), atenolol (2 μM; Fig. 3, A and B) blocked the effects of Iso without affecting baseline response properties, indicating that the effects of low concentration of Iso are mediated by activation of a β2-adrenergic receptor, as suggested before (Schimanski et al. 2007).

To examine the possibility that a β2-adrenergic receptor is involved in the action of Iso, the selective antagonist ICI-118,551 (at 0.1 μM) was used. ICI-118,551 did not affect
Iso-induced conversion of STP into LTP in control DH (1.49 ± 0.01, n = 6) and VH slices of PS rats (1.47 ± 0.004, n = 5; Fig. 3, C and D).

β-Adrenergic responses are proposed to be mediated by activation of cAMP cascade, and so we tested the effects of forskolin (at 0.1 μM, a dose that does not affect baseline EPSPs), an activator of the cAMP system, on the ability to convert STP to LTP. Indeed, forskolin at low dose mimicked the effects of Iso, in both the control and PS rats, in the same regions where Iso was effective (1.48 ± 0.01, n = 5, in DH slices of control and 1.43 ± 0.004, n = 6, in VH slices of PS rats; Fig. 4, A and B).

Fig. 2. Effect of 1 μM Iso on EPSPs recorded in stratum radiatum of DH and VH slices from control (A and B) and PS (C and D) rats. The arrows denote the points at which short tetanic stimulation (35 stimuli at 100 Hz) was delivered, twice to 1 pathway. Short tetanic stimulation, given in the presence of Iso (1 μM), produced a full-blown LTP in DH slices of control group (A) and in VH slices of PS rats (D).

Fig. 3. Effect of atenolol, a selective β1-adrenergic receptor antagonist, and ICI-118,551, a selective β2-adrenergic receptor antagonist, on Iso-mediated conversion of short-term potentiation (STP) to LTP in DH and VH slices of control and PS rats. The effect of Iso was blocked either in the DH slices of control group (A) or in the VH slices of the PS pups (B) by the β1-adrenergic antagonist, atenolol (2 μM), but was not affected by ICI-118,551 (0.1 μM; C and D), indicating that the effects of low concentration of Iso are mediated by activation of β1-adrenergic receptors.
Finally, we have previously demonstrated that there is a marked difference between DH and VH in the involvement of calcium stores in the ability to generate LTP (Grigoryan et al. 2012). To test whether the effects of Iso are mediated by release of calcium from stores, we exposed the slices to CPA, a blocker of release of calcium from stores. Indeed, CPA (5 μM) blocked the facilitating effects of Iso in normal DH (1.05 ± 0.004, n = 7) and in VH slices of PS rats (1.04 ± 0.01, n = 6; Fig. 4, C and D).

PS enhances LTD in VH. In a further set of experiments, we examined whether PS affects long-term depression (LTD) in DH and VH slices. To this end, we evoked chemical LTD using DHPG, an agonist of group I mGlu receptors, which is likely to act by releasing calcium from stores. At concentration of 25 μM, exposure to DHPG led to depression of recorded EPSPs that persisted after 40 min of washout of the drug in DH but not in VH slices of control rats [0.82 ± 0.004 (n = 5) and 0.96 ± 0.01 (n = 4), respectively; Fig. 5]. Strikingly, VH slices from PS rats expressed significantly enhanced LTD [0.74 ± 0.01 (n = 5); P < 0.001, F = 56.74, compared with control], whereas DHPG-dependent LTD in DH slices was smaller than that of controls [0.96 ± 0.01 (n = 5); P < 0.05, F = 16.5; Fig. 5]. These results indicate that PS switches the locus of action of DHPG from DH to VH, in line with the proposal that PS makes the VH more metaplastic than the DH, and this is likely to be mediated by release of calcium from stores.

DISCUSSION

The present experiments demonstrate that selective activation of β1-adrenergic receptors, known to play an important role in regulating synaptic plasticity in the hippocampus, facilitated conversion of STP to LTP in CA1 region of the DH but not the VH of normal young rats. On the other hand, the effects of Iso were no longer seen in CA1 region of DH slices in PS rats but were found in VH. These effects were mediated by activation of a β1-adrenergic receptor- and cAMP-generating system and involved release of intracellular calcium from stores.

The effects of PS on synaptic properties in the hippocampus have been studied by several groups. It has been shown that PS reduces synaptic currents (Saboory et al. 2011) in the infant rats. On the other hand, Yaka et al. (2007) did not report on a decrease in basal EPSP size but on a reduction in ability to express LTP in the offspring of the stressed mothers. That there might be alterations in synaptic properties is indicated by the study of Afadlal et al. (2010). In our own study, we did not detect changes in basal synaptic activity but in a specific region-selective ability of NE to facilitate formation of LTP.

As noted before, different magnitudes of PS may contribute to different magnitudes of defects in synaptic properties of the affected individuals.

The present experiments confirm and extend previous studies on the facilitating role of NE in hippocampal synaptic plasticity (Izumi and Zorumski 1999; Katsuki et al. 1997) with several noted additions; the effect shown before (Izumi and Zorumski 1999) was found only in DH slices, and we found that it was absent in VH ones. On the other hand, following PS, while the facilitating action of NE in DH was suppressed, as could have been predicted from other studies showing suppression of LTP following PS, NE became effective in the VH and appeared to use the same molecular pathways as in the normal DH LTP.

The involvement of the different subtypes of the β-adrenergic receptors in synaptic plasticity is still not clear. Although β1-receptors have been found in the hippocampus, Guo and Li (2007) have shown that β1-subtype is predominantly distrib-

Fig. 4. A and B: effect of forskolin (0.1 μM) on STP in DH and VH slices of PS rats. STP was converted into LTP by application of forskolin in VH slices only. Thus, the effect of Iso was mimicked by forskolin indicating the involvement of cAMP-generating system. C and D: cyclopiazonic acid (CPA; 5 μM) blocked the Iso-mediated conversion of STP into LTP in DH of control and VH of PS slices, demonstrating the involvement of calcium stores in the Iso-mediated modulation of LTP generation. The arrows denote the points at which tetanic stimulation (35 pulses at 100 Hz) was delivered (twice to 1 pathway).
Activation of MRs is proposed to lead to enhancement of LTP dendrites and spines in the dentate gyrus. In our earlier work, are primarily postnatal (Diaz et al. 1998) and thus not likely to evolve persistent chemical LTD in DH but not in VH slices of control rats. PS switches the locus of action of DHPG from DH to VH. VH slices from PS rats expressed significantly enhanced LTD compared with control, whereas DHPG-dependent LTD in DH slices of PS rats was lower than controls. Insets are sample EPSPs recorded in either control (a and b) or PS slices (c and d) at the time indicated in the grouped data before and after exposure to DHPG.

Another promising molecular species that may be affected by PS involves the release of calcium from intracellular stores. We (Grigoryan et al. 2012) have shown a striking difference in release of calcium between DH and VH in ability of calcium released from stores to enhance LTP. Both the effects of activation of mGlu receptors and Iso share a common molecular path involving release of calcium from stores.

The functional implications of PS have been studied behaviorally by several groups. The DH is associated with cognitive functions of the hippocampus, and the connections between the DH and the cortex underlie these functions. In normal alerted animal, NE release may facilitate transfer of information between DH and VH, and its connections with the amygdala and the hypothalamus, and NE can then facilitate the activation of these pathways (Lim et al. 2010). The behavioral consequences of this switch may be exhibited by hyperactivity and hypersensitivity of the animal to otherwise neutral stimulation, which may not have adaptive value in the normal organism. In general, PS is reported to cause impairment in cognitive functions (Son et al. 2006; Wilson et al. 2012; Yaka et al. 2007). On the other hand, Fujioka et al. (2001) suggested that mild PS can in fact facilitate learning performance, which is accompanied by reduced emotionality. A similar observation was made by us recently (Grigoryan and Segal 2013), demonstrating that PS rats are more motile in an open field and acquire faster the water maze task than controls. This indicates that the magnitude of the PS as well as the type of stimulation used and the behavioral tests employed are important factors in determining the possible long-term effects of PS on brain physiology and behavior. Another important factor is the sex of the animals studied as there are major sex-dependent effects of PS on behavior. Another important factor is the sex of the animals studied as there are major sex-dependent effects of PS on behavior.
behavior (Abdul Aziz et al. 2012; Bock et al. 2011; Bowman et al. 2004; Paris and Frye 2011; Yaka et al. 2007). There is a need to study the possible DH/VH switch also in females, which we did not do in the current study for lack of resources. Further experiments are needed to explore specific behavioral functions of the DH and VH and the consequences of the apparent switch between DH- and VH-associated functions and its sensitivity to pharmaceutical intervention.

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DISCLOSURES

The authors disclose no conflict of interest whatsoever.

AUTHOR CONTRIBUTIONS

G.G. and M.S. designed the experiments; G.G. conducted the slice experiments and analyzed the data; M.S. and G.G. wrote the manuscript.

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