VEGF modulates NMDA receptor function and synaptic localization in the hippocampus

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ABSTRACT

The vascular endothelial growth factor (VEGF) is well known to play a critical role during vascular development but recent evidence indicates that VEGF has also direct effects on neurons, regulating among other processes hippocampal synaptic transmission and plasticity. Little is known about the underlying molecular mechanisms involved in the VEGF- dependent action on synaptic transmission. Recently, we showed that a novel interaction between the glutamate receptor NMDA (NMDAR) and the VEGF receptor Flk1 facilitated neuronal migration (Meissirel et al. 2011), and hypothesized that VEGF signaling might regulate NMDAR function in hippocampal synaptic transmission. Our results revealed that VEGF and Flk1 are expressed in the Stratum Pyramidale in CA1 and CA3 regions of the hippocampus. Whole-cell patch clamp experiments in acute hippocampal slices demonstrated that VEGF potentiates NMDAR mediated synaptic transmission through a postsynaptic modulation of NMDAR function. To explore the underlying mechanisms we undertook a Cell-free system exploration which highlighted an interaction between the extracellular domain of Flk1 and the GluN2B subunit of NMDAR. In addition, high-resolution imaging revealed that NMDAR and Flk1 co-activation induces enrichment of NMDAR-2B at synaptic sites and promotes synapse formation. Altogether, our results indicated a VEGF-dependent mechanism for regulating NMDAR localization and function at hippocampal synapses, suggesting that this molecular mechanism could be relevant for long-term synaptic plasticity, learning and memory.

METHODS

Electrophysiological recordings: Whole cell patch clamp recordings were performed on acute coronal hippocampal slices from P15 C57/Bl6 mice. The perfusion solution contained picrotoxin and NBQX. NMDARs mediated responses were blocked by using D-APV or MK801, NMDAR2B by Ifenprodil and NMDAR2A by NVP-AAM077. Pyramidal cells were stimulated with a stimulating electrode placed near the proximal apical dendrite. Base line activity was recorded for 10min and VEGF was puffed locally. For statistical analysis, data were expressed as mean +/- SEM and paired student t test was used.

Cell surface immunostaining: After 15 DIV, low density hippocampal cell cultures were treated with NMDA (50 µM), VEGF (50ng/ml) or NMDA + VEGF for 15min. Cells were lightly fixed and immunolabelled in non permeabilizing buffer with αFlk1, αGluN2B or αGluR1, then permeabilized and immunostained for Synapsin or PSD95.

Image acquisition and quantitative analysis of cell surface immunostaining: Images were acquired using a Zeiss microscope equipped with apotome technology and analyzed using Image J. Subsequently images were processed for deconvolution and thresholded to assess dendritic area. The regions of interest (ROI) were centered on the apical dendrite and receptor cluster density was quantified. Co-localized clusters were identified using the co-localization analysis plug-in of Image J and their location validated for each channel. Synaptic clusters were defined as being colocalized with a synapsin or PSD95 cluster. Data were expressed as mean +/- SEM and one way ANOVA was used with post hoc t-test.

CaMKII immunoblotting and analysis: After 15 DIV, high density hippocampal cell cultures were exposed to pharmacological blockers for 1h or control medium and treated with NMDA (50µM), VEGF (50ng/ml) or NMDA + VEGF for 15min. Hippocampal cells were lysed and processed for immunoblotting with anti-pCaMKII, anti-CaMKII and anti-Actin. Semi-quantitative western blot analyses were performed with optical densitometry using Image J.

VEGF effect on NMDAR-mediated synaptic activity in the hippocampus ?

Expression of VEGF and its receptor Flk1 in the hippocampus

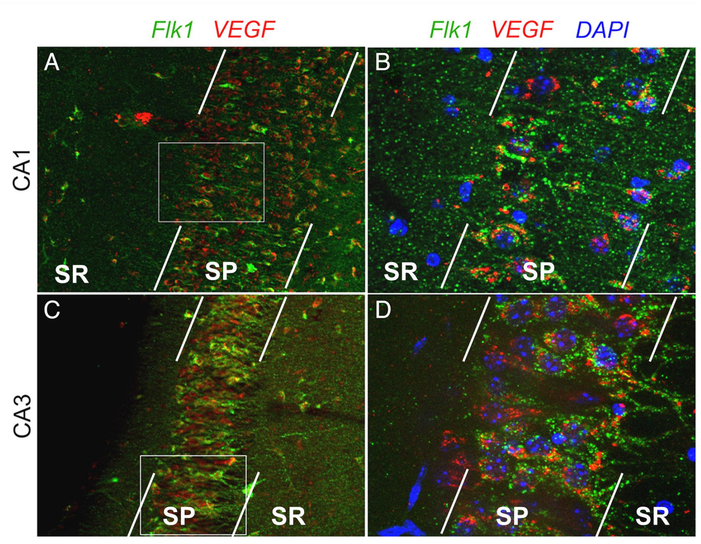


Figure 1: **VEGF and Flk1 expression in hippocampal CA1 and CA3 regions.** Immunohistochemistry on P15 hippocampal slices revealed the presence of both VEGF (in red) and Flk1 (in green) in CA1 (**A** and **B**) and CA3 (**C** and **D**). VEGF and Flk1 are expressed in the Stratum Radiatum (**SR**) and Stratum Pyramidale (**SP**) in CA1 (**A**) and CA3 (**C**). Note that the proximal part of the pyramidal apical dendrites expressed both VEGF and Flk1 in CA1 (**B**) and CA3 (**D**).

VEGF as well as its receptor Flk1 are expressed in pyramidal cell layer of CA1 and CA3

VEGF effect on NMDAR-mediated synaptic activity in hippocampal pyramidal cells?

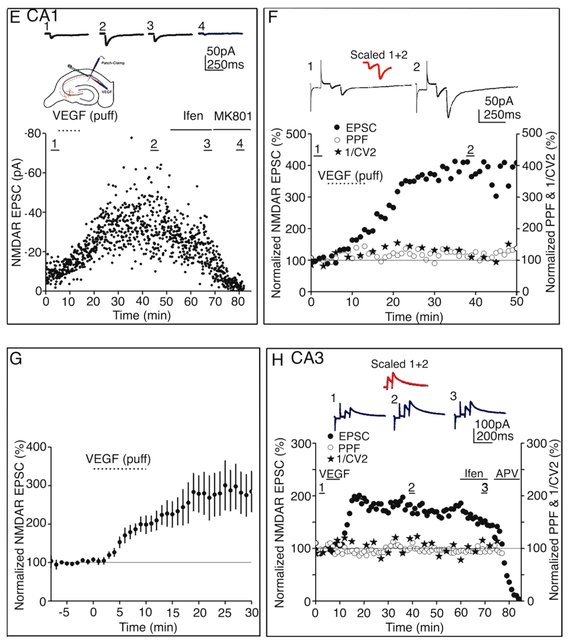


Figure 2: **VEGF-induced increase in NMDAR-mediated synaptic transmission in hippocampal CA1 and CA3 pyramidal cells. E)** Representative time course of the VEGF effect on NMDAR-mediated Excitatory Postsynaptic Currents (EPSCs) at Schaffer collateral-CA1 synapses, in baseline condition (1), after VEGF application (2), ifenprodyl (3) and MK801 (4) treatments. Note the enhancement of EPSCs in presence of VEGF and their blockade when the NMDAR antagonist ifenprodyl and the NMDAR open blocker MK801 were applied **F)** Paired Pulse facilitation protocol applied in CA1; **G)** Summary graph of the VEGF effect on 8 CA1 pyramidal cells. The amplitude of NMDAR-mediated EPSCs displayed a 3 fold increase after VEGF local application. **H)** Paired Pulse Facilitation protocol applied in CA3.

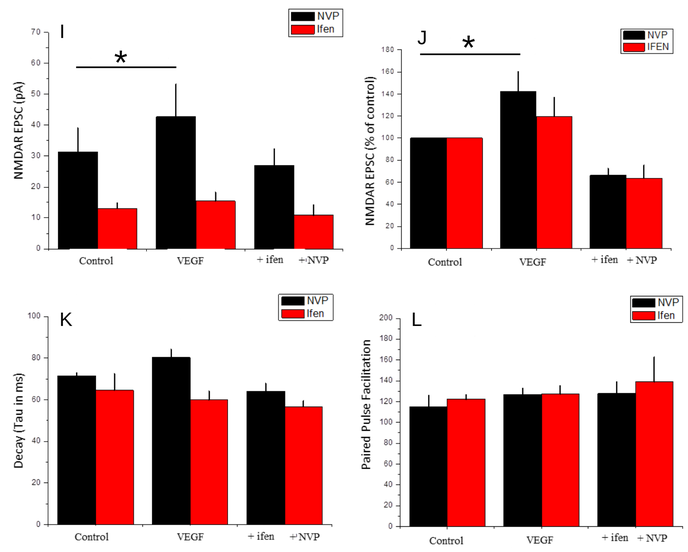


Figure 3: **VEGF-induced increase in synaptic activity is NMDAR2B dependent. I and J)** NMDAR Excitatory Post Synaptic Current recorded in presence of a specific NMDAR2A (NVP-AAM-077) or NMDAR2B antagonist (Ifenprodyl), with respectively raw (I) and normalized values (J). Note the enhancement of EPSCs in presence of VEGF when NMDAR2A are selectively blocked. **K)** NMDAR decay was quantified in presence of NMDAR2A or NMDAR2B antagonists, showing a tendency toward an increase when NMDAR2A are selectively blocked. L) Paired Pulse Facilitation tested in presence of NMDAR2A or NMDAR2B antagonists showed that the VEGF-dependent effect is mainly a postsynaptic effect.. Significant difference between conditions are indicating by (*) for p<0.05.

The VEGF-dependent increase in NMDAR mediated synaptic activity mainly involves postsynaptic NMDAR2B

Underlying mechanisms of the VEGF effect: NMDAR trafficking? Expression of synaptic plasticity markers?

Effect of Flk1 and NMDAR co-activation on NMDAR cell surface expression

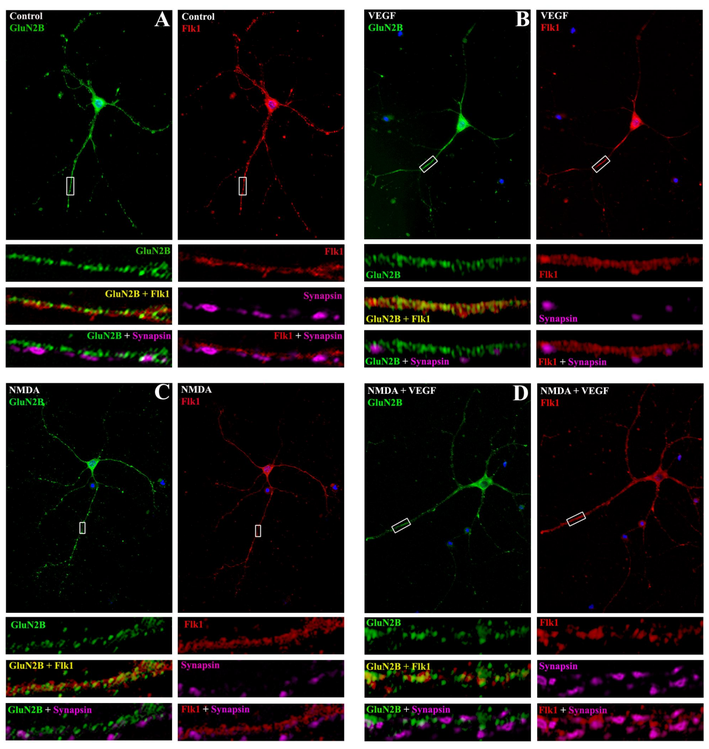


Figure 5: **Cell surface immunostaining of GluN2B and Flk1 in hippocampal pyramidal cells: A)** Pseudo-confocal image of a representative hippocampal pyramidal cell immunostained for GluN2B (green) and Flk1 (red), in control condition, with white rectangle indicating region of interest (ROI) on the apical dendrite. ROI is illustrated below for each immunostaining, including the presynaptic marker synapsin (magenta). **B, C, D,** Representative immunostainings after treatments respectively with VEGF, NMDA or combined NMDA and VEGF.

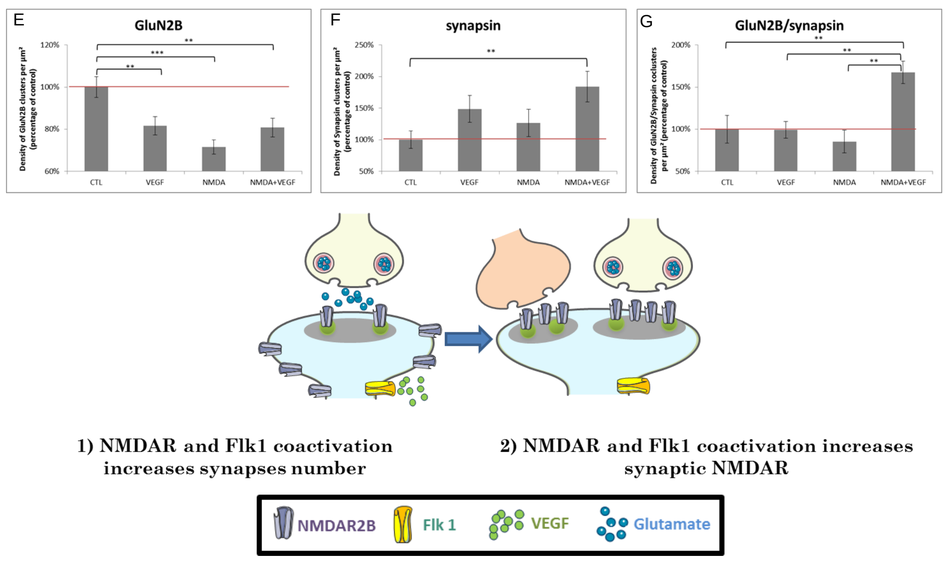
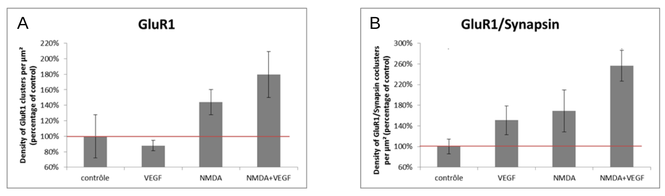
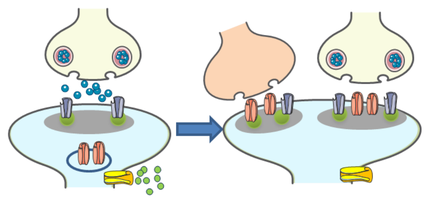


Figure 6: **Quantitative analysis of GluN2B, Flk1 and synapsin cluster distribution: E-G)** Histograms showing GluN2B and synapsin cluster densities, as well as GluN2B/synapsin co-clusters densities, in each condition. All values were normalized to control and represented as mean ± SEM, n=17, 12, 10, 8 pyramidal cells respectively in control, VEGF, NMDA and NMDA+VEGF conditions. Data shown are derived from 2 independent experiments. Significant difference between conditions are indicating by (*) for p<0.05; (**) for p<0.01; (***) for p<0.001.

Co-activation of Flk1 and NMDAR induces synapse formation and NMDAR2B synaptic targeting

Effect of Flk1 and NMDAR co-activation on AMPA receptor cell surface expression ?





Flk1 and NMDAR coactivation induces an increase in synaptic sites with AMPAR and NMDAR

Figure 7 : **Quantitative analysis of GluR1 cluster distribution. A,B)** Histograms showing respectively GluR1 cell surface expression (A) and GluR1/Synapsin colocalisation (B). All values were normalized to control and represented as mean ± SEM, n=3, 5, 4, 5 pyramidal cells respectively in control, VEGF, NMDA and NMDA+VEGF conditions. Data shown are derived from 1 experiment.

Effect of Flk1 and NMDAR co-activation on CaMKII activation

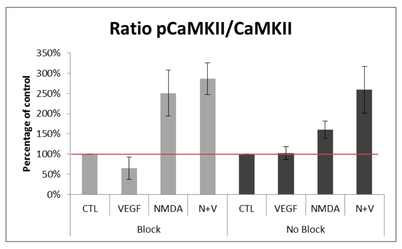


Figure 8 : **Semi quantitative analysis of CaMKII activation.** Western blot analysis of the phosphorylated form of CaMKII (phospho T286), total form of CaMKII and actin expression used as loading control ; Semi quantitative analysis of CaMKII activation (ratio of pCaMKII/CaMKII). All values were normalized to control and represented as mean ± SEM. Data shown are derived from 4 independent experiments.

Co-activation of Flk1 and NMDAR increases CaMKII activation and enriches AMPAR-GluR1 at the cell surface and synaptic sites

Conclusion

We showed that VEGF and its receptor Flk1 are expressed together in the Stratum Pyramidale of CA1 and CA3 in hippocampus. VEGF induces an increase in NMDAR-mediated postsynaptic activity in pyramidal cells, involving NMDAR2B function. This VEGF-dependent effect could involve NMDAR2B synaptic targeting and synapse formation. Indeed, both are triggered during Flk1 and NMDAR co-activation. In addition, Flk1 and NMDAR co-activation promotes synaptic plasticity marker expression. All together, these findings demonstrates for the first time that VEGF increases NMDAR mediated synaptic activity in the hippocampus through recruitment of postsynaptic NMDAR2B.

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