A hydrophobic dipeptide, Leu-Ile, protects against neuronal death by inducing glial cell line-derived neurotrophic synthesis

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Glial cell line-derived neurotrophic factor (GDNF) is an important neurotrophic factor that hastherapeutic implications for neurodegenerative disorders. We previously showed that Leu-Ile, ananalogue of dipeptide-like structure of FK506 (tacrolimus), induces GDNF expression both in *vivo* and in *vitro*. In this investigation we sought to clarify the cellular mechanisms underlying GDNF-inducing effect of this dipeptide. Leu-Ile transport was investigated using FITC-Leu-Ile in cultured neurons, and the results showed the transmembrane mobility of this dipeptide. By liquid chromatography-mass spectrometry and quartz crystal microbalance assay, we identified heat-shock cognate protein 70 (Hsc70) as a protein binding specifically to Leu-Ile, and molecular modeling showed that ATPase domain is the predicted binding site. Leu-Ile stimulated Akt phosphorylation, which was attenuated significantly by Hsp90 inhibitor geldanamycin (GA). Moreover, enhanced interaction between phosphorylated Akt and Hsp90 was detected by immunoprecipitation. Leu-Ile elicited an increase in cAMP response element binding protein (CREB) phosphorylation, which was inhibited by GA, indicating that CREB is a downstream target of Hsp90/Akt signaling. Leu-Ile elevated the levels of GDNF mRNA and protein expression, whereas inhibition of CREB blocked such effects. Leu-Ile promoted the binding activity of phosphorylated CREB with cAMP response element. These findings show that CREB plays a key role in transcriptional regulation of GDNF expression induced by Leu-Ile. In conclusion, Leu-Ile activates Hsp90/Akt/CREB signaling, which contributes to the upregulation of GDNF expression. It may represent a novel lead-compound for treatment of dopaminergic neuron or motoneuron diseases.