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The Pattern of 5-Hydroxytryptamine Receptor Subtypes Mediated Epidermal Growth Factor Receptor Transactivation In Rat Aorta

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$G_q$protein-coupled 5-HT_{2A} and G_i protein-coupled $5HT_{1B}$ receptor subtypes are mainly found in vascular tissue and they mediate contractile responses in vascular smooth muscle. Epidermal growth factor receptors (EGFR) are transactivated by many G protein-coupled receptors. We have previously shown transactivation of EGFR by α_1-adrenergic receptor and 5-HT receptors in vascular smooth muscle(1,2). In this study, we aimed to investigate $5HT_{2A}$ and $5HT_{1B}$ receptor mediated EGFR transactivation profile in rat aorta. For this purpose, we examined the effect of the selective $5HT_{2A}$ agonist α-Methyl-5HT (10μM) and the selective $5HT_{1B}$ agonist Sumatriptan (Suma, 10μM) on EGFR phosphorylation with and without the EGFR inhibitor AG1478 (10μM) in endothelium-denuded rat aorta. 14-16 week Male-Wistar rats were anesthetized with ketamine/xylasine (100mg/kg,10mg/kg IP), and thoracic aorta were obtained. All animal experiment were performed following approval by Ethics Commitee of Ankara University.Statistical comparison was performed in at least 3 independent experiments using unpaired Student's t test.Furthermore, we evaluated α-Methyl-5HT and Suma stimulated auto-phosphorylation ($pEGFR_{1173}$) and Src kinase-specific phosphorylation ($pEGFR_{845}$) of EGFR in the presence of Src-kinase or PI-3 kinase inhibitors (PP2, 10μM, LY 294002, 10μM, respectively). Both α-Methyl-5HT and Suma increased $pEGFR_{1173}$ (Control (C), 100±4,8% vs α-Methyl-5HT; 233±13%; C, 100±32% vs Suma 326±28%) and $pEGFR_{845}$ (α-Methyl 5HT 166±10%; Suma, 385±15%) compared to unstimulated tissue (n=3). Moreover AG1478 incubation inhibited α-Methyl 5HT and Suma mediated phosphorylation of EGFR ($pEGFR_{1173;}$α-Methyl-5HT+AG, 90±15%, Suma+AG, 124±15.5% and $pEGFR_{845;}$α-Methyl-5HT+AG; 70±11% Suma+AG, 153±2.9%). PP2 and LY294002 partially and completely inhibited α-Methyl 5HT-induced phosphorylation of $EGFR_{1173}$ and $EGFR_{845,}$ respectively.On the other hand, while Suma-induced phosphorylation of $EGFR_{845}$and$EGFR_{1173}$ was inhibited by the PI-3 kinase inhibitor, LY 294002, PP2 partly inhibited and did not inhibit phosphorylation of $EGFR_{845}$ and $EGFR_{1173}$, respectively. Our results show that Src kinase and PI-3 kinase have similar roles in $5HT_{2A}$ mediated EGFR transactivation whereas PI-3 kinase has more prominent effect in $5HT_{1B}$ dependent EGFR transactivation.

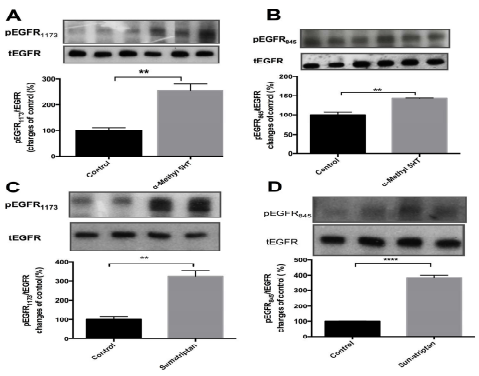

Figure 1. Alpha-Methyl-5HT (10μM, 5 minutes) stimulated A. $EGFR_{1173}$ and B. $EGFR_{845}$phosphorylation and Sumatriptan (10μM, 5 minute) stimulated C. $EGFR_{1173}$ and D. $EGFR_{845}$ phoshorylation in endotelium-denuded rat throcic aorta.Net intensities of pEGFR and tEGFR blots were calculated three separated experiments. Bar graphs were represented ratio of pEGFR-to tEGFR compared to control (unstimulated tissue). Data were shown as a (Mean ± S.E.M). Statistical differences ***P*< 0,01 *****P*< 0,0001 vs**C** group.

1. Ulu N et al (2013). The Journal of Pharmacology and Experimental Therapeutics 347(1)47-56
2. Guner S et al (2014). The FASEB Journal (28 no. 1 Supplement 1065.7)