cGMP synthesis and mRNA expression of PDE2, 5 and 9 in cultured rat cholinergic forebrain neurons

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cGMP might be an important second messenger in the cholinergic neurons of the basal forebrain (J. de Vente et al. Exp. Brain Res. 136, 480-491 (2001)). We cultured cholinergic neurons from rat and mouse embryos of E16 and E15 respectively. Cells were cultured in a defined medium for 8 day and were challenged with different PDE inhibitors and NO-donors, fixed with 4% formaldehyde and immunostained for cGMP. mRNA expression of PDE2, 5 and 9 was studied using a non-radioactive in situ hybridization method as described (Van Staveren et al. J. Comp. Neurol. 467, 566-580 (2003)). All cells obtained the cholinergic phenotype as demonstrated by immunostaining of the cells for choline acetyltransferase (ChAT) or the acetylcholine transporter molecule (VAchT). cGMP level in the cultured neurons was low. Incubation in the presence of 1 mM IBMX raised cGMP levels slightly, similarly when using a selective PDE2 or PDE5 inhibitor. NO-mediated cGMP synthesis was largest in the presence of 1 mM IBMX, followed by PDE2 inhibition and was lowest in the presence of a PDE5 inhibitor. 1uM ODQ inhibited the response. Combination of PDE2 and PDE5 inhibition enhanced the cGMP response in the presence of 100uM DEANO. Immunostaining of the cells for nNOS showed that all cells expressed nNOS. NMDA activated nNOS in a subpopulation of the cells. mRNA of PDE2, 5 or 9 was expressed by these cells to different extents. In these cells NO may function as an autocrine messenger.
Supported by grant 02506 of the Internationale Stichting voor Alzheimer Onderzoek

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