Sym-4

Purinergic-mediated Calcium Homeostasis and Dopamine Release in PC 12 Cells: Effect of Ethanol

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Extracelluar ATP evokes many biological processes, including neuronal excitation and neurotransmitter secretion, through activation of purinergic P2 receptors. Although excitatory and inhibitory receptor-operated channels (ROC) and voltage-dependent calcium channels (VDCC) have been reported to be altered by acute and chronic exposure to ethanol, little is known of the ethanol effects on purinergic receptor-operated channels in neuronal cells. The present study characterized the P2 receptor in PC 12 cells and investigated the effects of ethanol on the P2 receptor-mediated calcium homeostasis and dopamine release. ATP did not increase phosphoinositide hydrolysis but did increase intracellular calcium levels which were dependent on the influx of extracellular calcium via both ROC and VDCC. The pharmacological characteristics studied using the rank order of potency showed that the P2 receptor on PC 12 cells used in the present study does not fit the current classification for the P2x, P2y, P2t, P2u, and P2z receptor subtypes. Scatchard plots of the equilibrium saturation binding data are consistent with a negative cooperativity between the radioligand binding sites and/or a multi-state model where there is a rapid transition to a desensitized state that has a lower affinity for the receptor ligand. Acute addition of ethanol did not alter ATP-induced increases in $[Ca^{2+}]i$. However, a 4-day exposure to 150mM ethanol decreased the maximal response to ATP with no change in EC50 values. Voltage-sensitive calcium channel blockers reduced ATP-induced increases in $[Ca^{2+}]i$, but did not alter the response elicited by chronic ethanol treatment, suggesting chronic ethanol exposure reduces calcium influx only through P2 ROC but not through VDCC. The lack of change of VDCC after chronic ethanol treatment was confirmed by the observations that chronic ethanol exposure did not alter the depolarization-induced increase in $[Ca^{2+}]i$. The functional significance of the ethanol-induced alterations in $[Ca^{2+}]i$ was confirmed by consistent alterations of ATP- or depolarization-stimulated dopamine releases after exposure to ethanol.