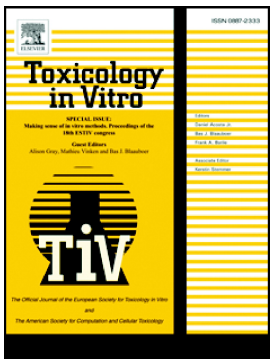

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Effect of phenol on the $GABA_AR$-coupled Cl^-/HCO_3^--ATPase from fish brain: an *in vitro* approach on the enzyme function

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ABSTRACT

Phenol (C_6H_5OH) has a toxic effect on the central nervous system of animals and humans. The Cl^-/HCO_3^--ATPase from the plasma membranes of animal brains is the primary active P-type Cl^--transporting system that is coupled to $GABA_A$ receptor ($GABA_AR$). In this paper, we used an *in vitro* approach to assess the effects of phenol (1–500 μM) on the functional parameters of the Cl^-/HCO_3^--ATPase isolated from the fish brain. The enzyme is insensitive to phenol in the presence of Cl^- or HCO_3^- in the incubation medium. By contrast, in the presence of Cl^-/HCO_3^-, phenol inhibits (I_{50}=27 μM) both the enzyme activity and its participation in ATP-dependent Cl^- transport through the membranes of artificial liposomes. Enriched plasma membranes and purified enzyme preparations were separated using hrCNE-PAGE. The ATPase activity in native gels was detected in the presence of phenol (100 μM). Detection of ATPase activity in a purified preparation, showed a native protein of 300 kDa, in agreement with western blot analysis with antibodies against $GABA_AR$ $\beta 3$ subunits. SDS-PAGE showed that one subunit with a molecular weight of 56 kDa was directly phosphorylated by γ-^{32}P-ATP and dephosphorylated in the presence of phenol. The *in vitro* approach described in this work allowed the first demonstration that $GABA_AR$-coupled Cl^-/HCO_3^--ATPase can be a protein marker for assessment of the toxicity of phenolics on the central nervous system.

Keywords: phenol; Cl^-/HCO_3^--ATPase; Cl^--transport; phosphorylation; fish brain