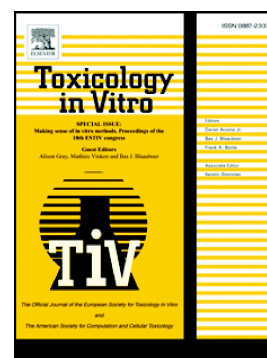


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Effect of phenol on the GABA_AR-coupled Cl⁻/HCO₃⁻-ATPase from fish brain: an *in vitro* approach on the enzyme function

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ABSTRACT

Phenol (C₆H₅OH) has a toxic effect on the central nervous system of animals and humans. The Cl⁻/HCO₃⁻-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⁻/HCO₃⁻-ATPase isolated from the fish brain. The enzyme is insensitive to phenol in the presence of Cl⁻ or HCO₃⁻ in the incubation medium. By contrast, in the presence of Cl⁻/HCO₃⁻, phenol inhibits (I₅₀=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 β3 subunits. SDS-PAGE showed that one subunit with a molecular weight of 56 kDa was directly phosphorylated by γ-³²P-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⁻/HCO₃⁻-ATPase can be a protein marker for assessment of the toxicity of phenolics on the central nervous system.

Keywords: phenol; Cl⁻/HCO₃⁻-ATPase; Cl⁻-transport; phosphorylation; fish brain

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