



SHORT COMMUNICATION

Verapamil Potentiate the Cardiodepressor Effect of Ethanol in Cat Papillary Muscle

José L. Martínez^{1*}, Hiran Cabrera² and Claudio Laurido³

¹University of Santiago of Chile, Office of the Vice Rector, Alameda 3363, Est. Central, Santiago, Chile; ²Central Laboratory of Pharmacology, Faculty of Medical Sciences Dr. Salvador Allende, University of Medical Sciences, Havana, Cuba; ³University of Santiago of Chile, Faculty of Chemistry and Biology, Santiago, Chile

*Corresponding author: editor.blacpma@usach.cl

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ABSTRACT

The possible additive effect of ethanol (EtOH) on verapamil on isolated papillary muscle of cats weighing 1200-1500g kept in oxygenated Ringer Locke solution was studied. Papillary muscles were dissected from the right ventricle, mounted vertically in an acrylic support and stimulated electrically driven at a constant rate by means of silver at 37°C (pH=7.4). We studied the effect on peak tension development (PTD) of verapamil (5.4 X 10⁻⁴ mM) and two EtOH concentrations (48.6 and 97.2 mM) in cat papillary muscle bathed in a normocalcic medium (2.2 mM) of Ringer-Locke solution. EtOH in both concentrations potentiated the decrease of PTD (inotropic effect), (-53.31±2.07% and -60.00±3.50%, respectively) compared to verapamil previously incubated in bath (-38.1±2.26%). It was concluded that verapamil induces a decrease in myocardial contraction by a posterior consumption of sufficient EtOH enough to put the patient in the risk of a lamentable induction of cardiac failure.

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INTRODUCTION

The regulation of myocardial contractility is under the control of calcium (Ca²⁺), which initiates contraction by binding to troponin C. Changes in Ca²⁺ delivery to this contractile protein, by two modulators, inorganic phosphate and hydrogen ions can also play regulatory roles by decreasing Ca²⁺ efficiency to activate contraction (Bers, 2001).

The effect of EtOH in the depression of myocardial contractility has been seen in experimental animals, both *in situ*, and in isolated heart and cardiac muscle (Bennett *et al.*, 2001). It is now widely recognized that the heart is a major locus for the adverse effects of excessive EtOH consumption. The most prevalent form of alcoholic heart disease is cardiomyopathy, characterized by depressed cardiac output, reduced myocardial contractility and dilation of all heart chambers (Wold *et al.*, 2001). These hearts are enlarged, and frequently present thickened endocardium regions. Interstitial fibrosis, hypertrophy and myocytes atrophy, are commonly observed features (Thomas *et al.*, 1994). The inotropic effect of calcium blockers and the effects of EtOH have been reported (Dul and Gajkowska, 1998). We have previously shown the cardiac depressant effect of the interaction of verapamil

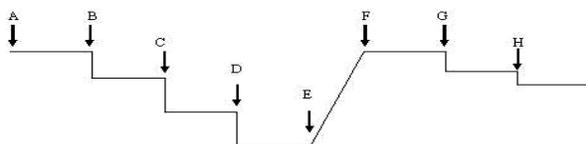
with EtOH in electrically stimulated cat papillary muscle, using three Ca²⁺ concentrations, in a different experimental method as compared to literature. These results suggested that the greater negative inotropic effect of EtOH on PTD was related with a decrease in Ca²⁺ influx. Verapamil induced a significant depression of ventricular muscle force *in vitro* increasing the negative inotropic EtOH-effect (Martínez and Penna, 1992). The main aim of this work was to study the possible additive effect of EtOH on verapamil in normocalcic medium in electrically stimulated cat papillary muscle.

MATERIALS AND METHODS

Isolated cat papillary muscle was prepared with some modifications (Martínez and Penna, 1992). Cats weighing 1200-1500g were anesthetized with diethyl ether in accordance with the Guide for the Care and Use of Laboratory Animals of National Institutes of Health (NIH) and the rules of the International Association for the Study of Pain (IASP) and based in the model of using sedative drugs in accordance to Bu *et al.* (2011). The heart was immediately removed and immersed in oxygenated (95% O₂, 5% CO₂) Ringer Locke solution. Papillary muscles (with a diameter of 1 mm or less) were dissected from the

right ventricle, mounted vertically in an acrylic support and stimulated by means of silver electrodes in contact with one end of the muscle, in a 50 ml bath tissue filled with Ringer-Locke oxygenated solution at 37°C (pH=7.4). The preparation was driven at a constant rate with a Grass Stimulator model S 88 through a Grass SIU isolation unit, with squared wave pulses of 3 msec duration at 1 Hz. The stimulation voltage was set up slightly above the threshold. Isometric tension was recorded by a Grass FT 03 force transducer coupled to a Grass model 5D Polygraph. The experiments were performed after a stabilization period of at least 1 h, measuring the PTD before and after the addition of dl-verapamil and EtOH (Merck, Darmstadt, Germany).

The effect of verapamil (5.4×10^{-4} mM), EtOH (48.6 and 97.2 mM) or both, on PTD compared to the control tension observed after 1 h of an stabilization period in Ringer-Locke solution were conducted. Firstly, experiments with 5.4×10^{-4} mM of verapamil in the presence of two ethanol-concentrations (48.6 and 97.2 mM) were carried out. Immediately, preparations were washed with physiological solution and ethanol was added. The experimental sequence is shown in the following scheme:



A: Control without drugs (Stabilization 1 h); B: Verapamil; C: Ethanol 48.6 mM; D: Ethanol 97.2 mM; E: Washed; F: Control similar stabilization; G: Ethanol 48.6 mM; H: Ethanol 97.2 mM

The results are expressed as percentage of decrease in PTD. The means \pm SEM of the response in control and experimental groups, were compared for statistical significance using ANOVA followed by Student's t-test in a GB Stat 3.0 computer program. A minimum of 6 different preparations were used for each group of experiments. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Table 1 shows the influence of verapamil on the effect of two different EtOH-concentrations in isolated cat papillary muscles. It is shown that verapamil alone causes a significant decrease in PTD ($-38.1\pm 2.26\%$), which were in agreement with previously published results (Martínez and Penna, 1992). Addition of EtOH causes a significant synergistic effect on verapamil-induced decrease in PTD, which is more evident at the highest EtOH concentration.

After, preparations were thoroughly washed and then two doses of EtOH concentration were added. As can be seen from Table 1, the effects of EtOH were lower than the original effects (19.5 ± 4.3 vs $35.05\pm 2.14\%$, respectively). Statistical analysis revealed that in both ethanol concentrations, the inhibitory effects were significantly different with $P<0.001$ and $P<0.05$ for 48.6 mM and 97.2 mM, respectively.

The cardiovascular effects of ethanol administration alone are still intriguing. The cardiac depressor effect of EtOH in experimental animals and in human has been

Table 1: Influence of verapamil (A) and verapamil plus ethanol (B) in normocalcic medium on the negative inotropic of electrical stimulated cat papillary muscle in 6 experiments. EtOH only (C)

| Ethanol | A | B | C |
|---------------|-----------------|--------------------|--------------------|
| Concentration | Verapamil | Verapamil+ EtOH | EtOH |
| 0.0 mM | -38.1 ± 2.26 | - | - |
| 48.6 mM | - | $-53.31\pm 2.07^*$ | -19.58 ± 4.32^a |
| 97.2 mM | - | $-60.00\pm 3.50^*$ | -35.05 ± 2.14^b |

Values are mean \pm SEM in %; * $P<0.05$ compared to verapamil only; ^a $P<0.001$ compared ethanol with verapamil plus ethanol; ^b $P<0.05$ compared ethanol with verapamil plus ethanol.

well documented (Vinet *et al.*, 2012). Those studies demonstrated changes in myocardial function, which were consistent with subclinical heart disease.

The contraction force depends on extracellular Ca^{2+} concentration changes across the plasma membrane. Marban and Wier (1985) blocked the reticular endoplasmic Ca^{2+} with the alkaloid ryanodine and concluded that the contraction force is mainly regulated by extracellular Ca^{2+} . On the other hand, previous results showed that the inotropic action of EtOH was increased in hypocalcic medium as compared to normocalcic solution (Martínez and Penna, 1992). This implicates that the effect of EtOH depends on a decrease of Ca^{2+} influx and/or that the cardiodepressant action of EtOH is in relation to an inhibition of the electromechanical coupling in the myofilament- Ca^{2+} interaction (Schulman *et al.*, 1991). It has been suggested that the effect of EtOH is reverted by washout and removal of EtOH by a reduction in the Ca^{2+} influx in the membrane, because EtOH produce expansion and fluidization of the excitable membrane (Yun *et al.*, 1993).

The negative inotropic effect of EtOH has been described in myocardium (Martínez and Penna, 1992), smooth muscle (Briner *et al.*, 1993) and skeletal muscle (Altura *et al.*, 1990). The present study was performed in normocalcic conditions. In this condition, EtOH induced a negative inotropic effect on electrically stimulated cat papillary muscle. The data suggest that verapamil pretreatment in this experiment produced decrease contraction force from $-38.1\pm 2.26\%$ (only verapamil) to $-53.31\pm 2.07\%$ at 48.6 mM EtOH and $-60.00\pm 3.50\%$ at 97.2 mM EtOH which alters the cardiovascular activity. In previous studies, these results were confirmed but in pretreatment with EtOH previous to verapamil (Martínez and Penna, 1992). Our experiments showed that the depressant effect of EtOH is increased in the presence of verapamil. Verapamil is a drug that represents the classical Ca^{2+} antagonists in myocardium with high potency and specificity (Morales *et al.*, 1988). The major action of verapamil is the blockade at Ca^{2+} entry through the L-type Ca^{2+} channel. During excitation, this effect could be related with the suppression of Ca^{2+} -dependent slow current responses, which may be generated in ischemic myocardium.

The present work constitutes a contribution to the comprehension of the negative inotropic effect of EtOH in heart. These results are also of clinical importance as a patient in treatment with a safe dose range of verapamil might induce a decrease in myocardial contraction by a posterior consumption of sufficient EtOH whose effects in heart and vessels will put the patient in the risk of a lamentable induction of cardiac failure.

Conclusion: This paper supports a pharmacological basis to explain the increased myocardial depression which can appear after the concomitant ingestion of ethanol with a calcium-channel antagonist, such as verapamil and may contribute with EtOH's effects on heart to the increased cardiac disease and mortality observed in alcoholics, as well as to risk factors for excessive EtOH consumption.

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