Short communication

The Membrane Effect of Benfluron: Modulation of the Heart Sarcolemmal (Na^+, K^+)-ATPase and Mg^{2+}-ATPase Activities

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Abstract. The effect of Benfluron® on the heart sarcolemmal (Na^+, K^+)-ATPase and Mg^{2+}-ATPase activities was studied in crude membrane fraction as well as in purified sarcolemmal membranes isolated from rat heart. Benfluron in concentration range $10^{-7} - 5 \cdot 10^{-5}$ mol.l^{-1} did not exert any effect on ATPase activities studied. 10^{-4} mol.l^{-1} Benfluron was stimulatory towards (Na^+, K^+)-ATPase, while Mg^{2+}-ATPase activity was depressed. Kinetic analysis of interaction of Benfluron with (Na^+, K^+)-ATPase revealed an increase in the V_{max} and decrease in the K_m values for ATP. The possible mechanism of interaction of the drug with (Na^+, K^+)-ATPase is discussed.

Key words: Benfluron® — (Na^+, K^+)-ATPase — Heart sarcolemma

Benfluron® is a cytostatic agent recently tested in the clinical practice; however, its mechanism of action is still far from being elucidated definitely. Besides its well established cytostatic and cytolytic effects (Horáková et al. 1978) Benfluron has been also found to interfere with the amino acid metabolism, the energy generation, particularly in glycolysis, and consequently with the protein and nucleic acid synthesis in the cells (Horáková et al. 1988). Since glycolysis was shown to represent the main source of energy utilized by the sarcolemma-bound ion pumping ATPase systems (Bricknel et al. 1981), the depression of glycolysis induced by Benfluron may have considerable if not deleterious consequences for the energy supply and

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Benfluron® – 5-(2-N,N-dimenthylaminoethoxy)-7-oxo-7H-benzo (C) fluorene was synthesized at the Research Institute of Pharmacy and Biochemistry, Prague, Czechoslovakia

functional integrity of cation transport across the cell membrane.

The present study is aimed at elucidation of the effect of Benfluron on sarcolemmal membranes of cardiomyocytes with respect to:

i) the possible effect on the maintenance of transsarcolemmal gradients of cations via concentration-dependent modulation of the heart cell membrane (Na^+, K^+)-ATPase and Mg^{2+}-ATPase activities (*in vitro*);

ii) the modulation of specific properties of the sarcolemmal (Na^+, K^+)- and Mg^{2+}-ATPases by changing their kinetic parameters.

Isolated membrane fraction enriched in sarcolemma was prepared from quickly excised rat hearts using a method combining hypotonic shock with NaI treatment as described earlier (Kostka et al. 1981). The sarcolemmal fraction was contaminated to less than 3% by other subcellular membrane particles such as sarcoplasmic reticulum, mitochondria and myofibrils. The sarcolemmal membrane fraction was preincubated for 10 min at 37°C in a medium containing in mmol.l^{-1}: 2 $MgCl_2$; 100 NaCl; and 10 KCl in the absence and presence of various concentrations of Benfluron. Specific activity of (Na^+, K^+)-ATPase was established as the difference between the amount of orthophosphate liberated from ATP splitting in the

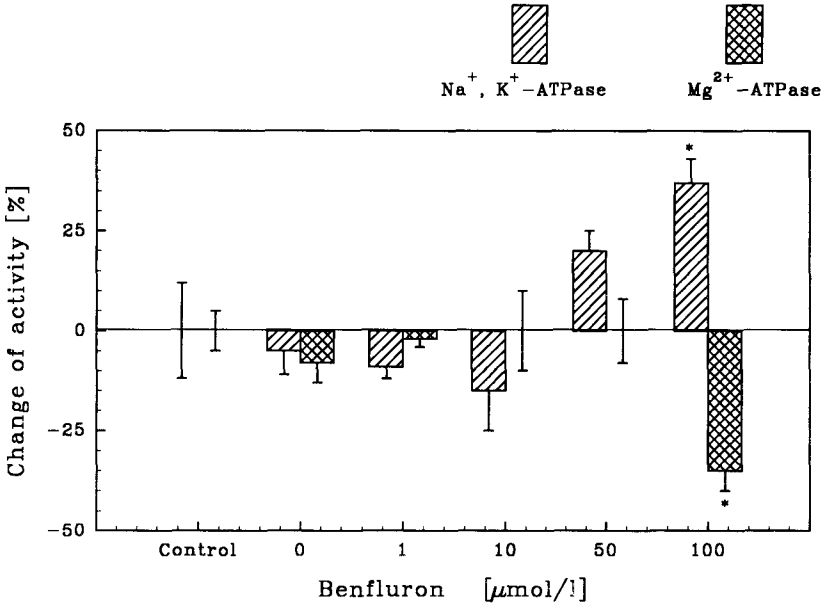

Figure 1. Influence of increasing concentrations of Benfluron on the activity of the rat heart sarcolemmal (Na^+, K^+)-ATPase and Mg^{2+}-ATPase. The specific activities of (Na^+, K^+)-ATPase amountig 11.40 ± 1.04 μmol P_i.mg^{-1}.h^{-1} and of Mg^{2+}-ATPase amounting 42.74 ± 2.5 μmol P_i.mg^{-1}.h^{-1} in untreated control preparations were referred to as 100%. Results are means ± standard deviation (n = 15); * $p < 0.001$ compared to the controls.

presence of Mg^{2+}, Na^+, K^+ (total ATPase) and in the absence of activating monovalent ions (Mg^{2+}-ATPase). Orthophosphate concentration was determined by the method of Taussky and Shorr (1953). Protein content was determined according to Lowry et al. (1951).

Lower concentrations of Benfluron within the range of 10^{-7} to $5 \cdot 10^{-5}$ mol.l^{-1} did not affect considerably the activities of the Mg^{2+}-ATPase and the (Na^+, K^+)-ATPase of the heart sarcolemma. However, upon increasing Benfluron concentration to 10^{-4} mol.l^{-1} dual effect on activities of the above enzymes could be observed (Fig. 1): an increase in the (Na^+, K^+)-ATPase activity from 11.36 ± 1.03 to 15.58 ± 1.28 μmol P_i.mg^{-1}.h^{-1} ($p < 0.001$) and on the other hand, a decrease in the activity of Mg^{2+}-ATPase from 42.74 ± 2.50 to 27.70 ± 1.30 μmol P_i mg^{-1}.h^{-1} ($p < 0.001$) as well as in the total ATPase activity from $54,10 \pm 2.67$ to 43.28 ± 2.58 μmol P_i.mg^{-1}.h^{-1}. The latter decrease amounting to 19.8% in average indicated that the Benfluron-induced net depression in specific activity of the Mg^{2+}-ATPase exceeded the corresponding elevation in the specific activity of the (Na^+, K^+)-ATPase in spite of an increase of its contribution to the total ATPase activity from originally 21% to 36%. Hence, the Benfluron-induced changes in the Mg^{2+}- and (Na^+, K^+)-ATPase activity differ in their nature. Therefore this finding cannot be considered as a simple shift in the Mg^{2+}-ATPase to (Na^+, K^+)-ATPase ratio. Similar results were obtained using higly purified sarcolemmal membranes isolated according to Džurba et al. (1993, data not shown).

The interaction of Benfluron (in effective concentration of 10^{-4} mol.l^{-1}) with the Mg^{2+}- and (Na^+, K^+)-ATPase of cardiac sarcolemma was investigated kinetically in the presence of increasing concentrations of ATP. The resulting data summarized in Fig. 2 revealed the following. In the case of (Na^+, K^+)-ATPase, a Benfluron-induced increase in the V_{max} and decrease in the K_m values for ATP ($p < 0.01$); in the case of Mg^{2+}-ATPase decreased V_{max} ($p < 0.01$) but unchanged K_m values for ATP were measured.

In spite of evidence about the molecular mechanism of action of Benfluron, which has accumulated in recent years, many aspects, especially those concerning the membrane effects of the drug, still have remained to be more elucidated (Horáková et al. 1988). Because of the well-known importance and particular vulnerability of heart sarcolemma with respect to electrogenesis, excitation-contraction coupling as well as the maintenance of cationic homeosthasis in the myocytes, the cardiac sarcolemmal cation transporting ATPases were chosen as target structures for investigation of the membrane effect of Benfluron.

Our results illustrated in Fig. 1 indicate that Benfluron exerts dual effect on the ATPases of the heart sarcolemma: i) it decreases the total- and Mg^{2+}-ATPase activity and increases the (Na^+, K^+)-ATPase activity in a manner resembling that observed after treatment of sarcolemmal preparations with ionic and/or nonionic detergents (Fedelešová et al. 1974; Kostka et al. 1981; Džurba et al. 1993); and ii)

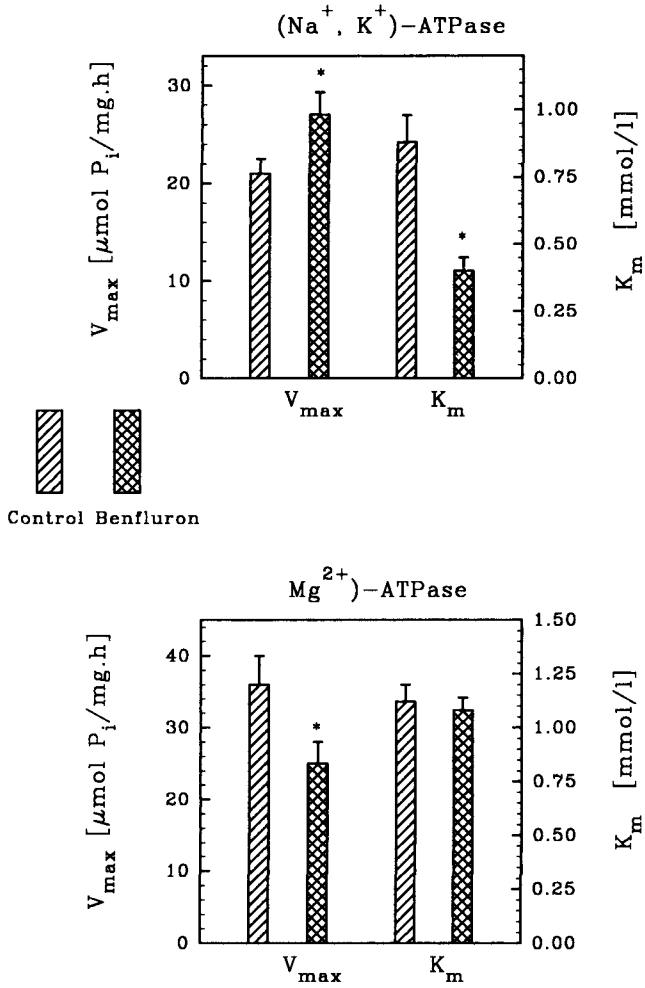

Figure 2. Influence of Benfluron on kinetic parameters of the rat heart sarcolemmal (Na^+, K^+)-ATPase and Mg^{2+}-ATPase. Stimulation of the ATPases was performed with ATP in a concentration range from 0.1 to 2.0 mmol.l^{-1}. The values of V_{max} and K_m were estimated by means of non-linear curve fitting using the Michaelis-Menten equation. Results are means ± standard deviation ($n = 6$); * $p < 0.01$ compared to the controls.

it reduces the K_m value of the (Na^+, K^+)-ATPase thus increasing the affinity of its active site to ATP and, via the latter, also the turnover of the enzyme (Fig. 2).

The results herein may contribute to a better understanding of the complexity of the mechanism of Benfluron action.

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References

Bricknel O. L., Daries P. S., Opie L. H. (1981): A relationship between adenosine triphosphate, glycolysis and ischaemic contracture in the isolated rat heart. J. Mol. Cell. Cardiol. **13,** 941—945

Džurba A., Vrbjar N., Ziegelhöffer A.(1993): Nitrendipine modulates the sodium binding site of cardiac (Na, K)-ATPase. Life. Sci. **52,** 1175—1179

Fedelešová M., Džurba A., Ziegelhöffer A. (1974): Partial purification of dog heart (Na/K)-ATPase by Lubrol WX. Acta Biol. Med. Germ. **33,** 169—180

Horáková K., Cziková S., Kernacová B. (1978): Suitability of different quantitative methods for determination of the cytotoxic activity of agents in cell cultures. Neoplasma **25,** 309—315

Horáková K., Lukáčová D., Kozanková J., Džurba A., Ziegelhoffer A., Jantová S. (1988): The mechanism of cytolytic and cytostatic activity of Benfluron. Neoplasma **35,** 177—184

Kostka P., Ziegelhöffer A., Džurba A., Vrbjar N. (1981): A comparative study of the enzyme characteristics of different sarcolemmal preparation from the heart. Physiol. bohemoslov. **30,** 173

Lowry O. H., Rosenbrough N. J., Farr A. L., Randall R. J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193,** 265—275

Taussky H. H., Shorr E. (1953): A microcolorimetric method for the determination of inorganic phosphorus. J. Biol. Chem. **202,** 195—207

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