Cardiovascular Drugs and Therapy 4: 808-809, 1990

Antiischemic Effect of Trimetazidine: Enzymatic and Electric Response in a Model of In-vitro Myocardial Ischemia

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The major aim of treatment in acute or chronic ischemic heart disease is to preserve cell integrity and to limit functional repercussion of ischemia (ventricular arrythmia, conduction disturbance, ventricular function impairment). The concept of cardioprotection is based on clinical observations on the prevention of myocardial infarction, in which appropriate therapeutics are found to diminish the areas of ischemic zones [1]. An attempt has been made to evaluate the effects of some of these agents on enzymatic leakage and electric responses of ventricular myocardial to controlled cells submitted to ischemia, since these represent good monitors of the functional repercussion of ischemia [2] and are widely used in intensive care units. Moreover enzymatic leakage is a good index of ischemia-induced structural damage [3]; a quantitative assay provides information on the protective or delaying effect of the cellular damage. The aim of the present work was to study in vitro the so-called cardioprotective effect of two calcium antagonists (verapamil and diltiazem), piridoxilate, and trimetazidine, during ischemia and reperfusion.

Guinea-pig hearts were immersed in oxygenated Tyrode's solution of 20°C. The left ventricule was dissected and pinned, endocardial surface upwards, in an experimental chamber. The preparation was superfused at 4 ml/min, at 37 ± 0.5°C; carbogene (95% O_2, 5% CO_2) was bubbled into the inflow and experimental compartments. The pO_2 (510 ± 20 mmHg), pCO_2 (34 ± 2 mmHg), and pH were monitored using an automatic pH/blood gas analyzer. The preparations were paced at a cycle length of 900 ms with rectangular pulses, 2 ms in duration and twice diastolic threshold intensity. Electric activity was recorded using glass microelectrodes filled with 3 M KCl. Samples from the trapped outflow solution were kept at 4°C and the creatine-phosphokinase activity (CPK) was measured 30 minutes following the sampling using a Rotochem 2A36 centrifuge analyzer with Olivier's method.

Preparations were subjected to a) stabilization for 180 minutes at 37°C with normal oxygenation; b) superfusion for 120 minutes with ischemic modified Tyrode's solution (pO_2 80 ± 10 mmHg), with the flow rate decreased to 0.15 ml/min; c) restoration of normal conditions for 30 minutes to simulate reperfusion; the flow rate was maintained at 0.15 ml/min to allow CPK measurement; d) after drying at 70°C for 15 hours, the samples were weighed, allowing CPK activity to be expressed in UI/g of dry tissue weight.

The drugs (verapamil 10^{-5} M, diltiazem 10^{-5} M, piridoxilate 10^{-3} M, or trimetazidine 10^{-6} M) were added, respectively, to the superfusion during the last hour of stabilization and maintained throughout the rest of the procedure.

Under normal oxygen conditions, the preparation showed stable electric parameters. Trimetazidine did not significantly modify the electric activity. Ischemia produced a significant decrease in resting potential, action potential duration, upstroke velocity, and amplitude, and an increase in threshold stimulation; decremental responses were commonly seen, leading to an absence of response after several stimuli.

Both piridoxilate and trimetazidine demonstrated efficient cytoprotection during ischemia: They produced a significant improvement in action potential characteristics. The 1/1 response duration fell to 12.1 ± 2.5 minutes in control and was maintained at 42.0 ± 14.3 minutes ($p < 0.01$) and 29 ± 8 minutes ($p < 0.05$) with trimetazidine and piridoxilate, respectively. During ischemia the persistence of the total electric response was also significantly greater with trimetazidine and piridoxilate. During reperfusion trimetazidine allowed a transient persistence of normal electrophysiologic characteristics in 6 of 7 preparations as compared with only 1 of 10 control preparations.

CPK leakage was greatly increased during ischemia: 7 UI/g dry weight at 30 minutes ($p < 0.05$), 26 at 60 minutes ($p \leq 0.01$), 79.5 at 120 minutes. Trimetazidine 10^{-6} M, piridoxilate 10^{-3} M, verapamil 10^{-5} M, and diltiazem 10^{-5} M were able to significantly reduce CPK release at 120 minutes to 37 ± 10, 30 ± 5, 42 ± 6, and 47 ± 11 UI/g dry weight, respectively ($p < 0.01$). During reperfusion only verapamil and piridoxilate showed a protective effect on CPK leakage.

Several experimental works have shown that the guinea-pig papillary muscle subjected to hypoxia, acidosis, and high K^+ concentration presents electrophysiologic alterations that are almost identical to those observed in myocardial ischemia [4]. This model shows that CPK leakage is correlated to cell membrane damage, and therefore is a good marker for assessing cell protection. In this model trimetazidine (10^{-6} M) produced a significant improvement in action potential characteristics and decreased CPK leakage during ischemia. During reperfusion, electric activity was recovered, although CPK leakage was not reduced.

References

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ANTI-ISCHAEMIC EFFECT OF TRIMETAZIDINE* ENZYMATIC AND ELECTRICAL RESPONSE IN A MODEL OF IN-VITRO MYOCARDIAL ISCHAEMIA

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INTRODUCTION

Preservation of the heart from ischaemia-induced damage during myocardial infarction or coronary heart disease is a constant concern. In man, cardioprotection in myocardial infarction and cardiac surgery is mainly evaluated by enzymatic and electrical responses since these represent good indexes of the functional repercussion of ischemia.

AIM OF THE STUDY

Study of the in vitro effects of several cardioprotective drugs on enzyme leakage and electrical activity in a guinea-pig ventricular myocardium.

METHODS

Guinea-pig left ventricular tissue was pinned down, with the endocardial surface upwards, in an experimental chamber.

After stimulation with a rectangular pulse, electrical activity was recorded using glass microelectrode. A response was designated 1/1 (Figure 1: trace 1) when each stimulus achieved an action potential, and decremental when a progressively declining response leading to inexcitability was produced (Figure 1: trace 2 to 6).

Creatine phosphokinase activity (CPK) was determined enzymatically during ischaemia and reperfusion according to the study design (Figure 2).

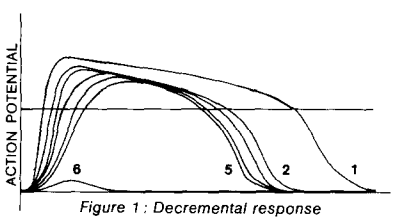

Figure 1 : Decremental response

STUDY DESIGN

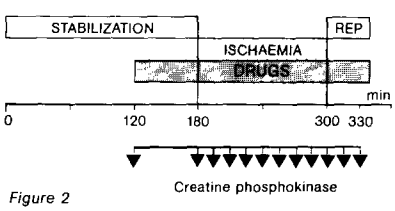

Figure 2

RESULTS

1. EFFECT ON ELECTROPHYSIOLOGICAL ACTIVITY

During stabilization, TMZ 10^{-6} M did not significantly alter the electrical activity.

Ischaemia produced a significant decrease in electrical activity. TMZ 10^{-6} M was able to significantly increase the duration of the 1/1 response (Figure 3) and the total (Figure 4) duration of electric activity.

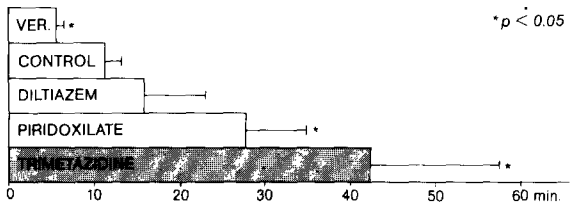

Figure 3 : Duration of 1/1 response during ischaemia after electrical stimulus

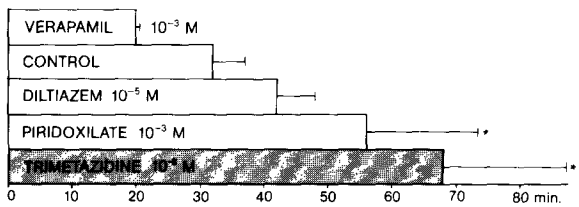

Figure 4 : Total duration of electrical activity during ischaemia

2. CPK LEAKAGE DURING ISCHAEMIA

During ischaemia, enzymatic leakage was substantial as shown by the increase in CPK activity in the outflow solution. It was still greater during reperfusion.

CPK release during ischaemia was significantly reduced by TMZ 10^{-6} M, piridoxilate 10^{-3} M, verapamil 10^{-5} M, and diltiazem 10^{-5} M (Figure 5)

Verapamil and piridoxilate significantly reduced CPK leakage during reperfusion; neither trimetazidine nor diltiazem showed any beneficial effect during that period although trimetazidine seemed to have had a transitory functional protective effect (transient persistance of normal electrophysiological characteristics).

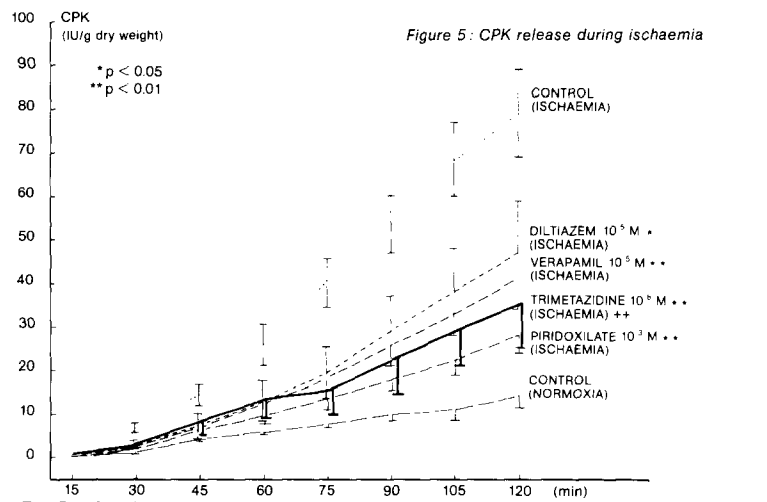

Figure 5 : CPK release during ischaemia

CONCLUSION

Trimetazidine (10^{-6} M) and piridoxilate (10^{-3} M) resulted in effective cytoprotection during ischaemia, leading to a significant improvement in action potential duration and a decrease in CPK leakage.

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