ACTION OF NIFEDIPINE ON CALCIUM METABOLISM OF THE FROG HEART

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The writers showed previously [2] that nifedipine (fenigidine, BAY-1040, Adalat), a known blocker of calcium channels (2×10^{-5} M), in Ringer's solution irreversibly depresses the force of contractions of the frog ventricle, which is not restored after removal of the drug from the solution. Nifedipine, in the same concentration, irreversibly blocks calcium channels [1, 2]. Admittedly, the force of contractions is partially restored by reducing the sodium ion concentration in the external solution. However, the restored force of contraction in physiological saline with a reduced sodium ion concentration and in the presence of nifedipine is considerably less than in the same solution without nifedipine.

It was concluded previously [2] that nifedipine may have a reversible effect on the sodium–calcium exchange system. The aim of this investigation was to test this conclusion with the aid of a Ca^{++}-selective electrode.

EXPERIMENTAL METHOD

In experiments on *Rana temporaria*, the heart was dissected, washed to remove traces of blood, cut in half, weighed, and placed in a cuvette containing 1 ml of Ringer's solution for 30 min, which is long enough for dynamic calcium equilibrium to be established in the heart tissue under these experimental conditions. The solution was aerated with oxygen. One half of the heart was used to study the effect of nifedipine, the other as the control. Strips were then transferred to physiological saline with nifedipine. Calcium exchange was recorded in the resting heart by Ca^{++}-selective electrodes, made by the "Burevestnik" Leningrad Scientific Instrument Combine, connected to a differential measuring circuit. The experiments were conducted in the fall and winter. The composition of the solutions was as follows. Ringer's physiological solution (in mM): NaCl 110, KCl 2.5, $CaCl_2$ 1.1, Tris-HCl 10, glucose 5.5; pH 7.4, temperature 18-20°C. Sodium-deficient solution: Ringer's solution containing 80 mM sodium chloride (replaced by sucrose). Nifedipine, in a dose of 4 mg, was dissolved in 0.3 ml of ethyl alcohol and added to physiological saline (concentration of the blocker 10^{-5} M). Other concentrations were made by the dilution method. Nifedipine was used in the form of fenigidine [4-(*o*-nitrophenyl)-3,5-dimethoxycarbonyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid] and was synthesized at the Institute of Organic Syntheses, Academy of Sciences of the Latvian SSR.

EXPERIMENTAL RESULTS

Averaged results of 6-9 experiments to measure pCA in the solution surrounding the heart tissue are given in Fig. 1. Absorption of Ca^{++} ions from the solution by the tissue is represented above the zero line, outflow of calcium from the tissue into the external solution below the line. When heart tissue was placed in physiological saline with nifedipine in a concentration of 10^{-7} M, Ca^{++} was observed to flow from it (about 0.081 ± 0.02 mmole/kg wet weight of tissue). With an increase in the nifedipine concentration by 2 orders of magnitude the Ca^{++} outflow was 2.7 times greater (0.22 ± 0.05 mmole/kg wet weight of tissue). Since calcium is pumped out of the cell by means of the sodium–calcium exchange system on account of the sodium gradient [3, 4], calcium exchange was measured in a solution deficient

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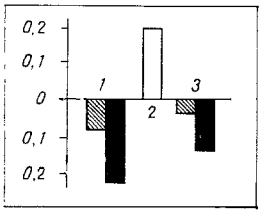

Fig. 1. Effect of nifedipine on calcium exchange in frog heart tissue. Abscissa: 1) Ringer's solution with nifedipine; 2) low-sodium solution; 3) low-sodium with nifedipine; ordinate, Ca^{++} concentration (in mmole/kg wet weight of tissue). Obliquely shaded columns 10^{-7} M nifedipine, black columns 10^{-5} M. Absorption of calcium ions from solution by tissue represented above the line, outflow of calcium from tissue into solution below the line.

in sodium. In low-sodium solution (80 mM NaCl) additional adsorption of calcium by the tissue was observed (0.21 ± 0.06 mmole Ca^{++}/kg wet weight of tissue) compared with normally. Addition of nifedipine to the low-sodium solution led to loss of calcium by the tissue: 10^{-7} M nifedipine prevented additional Ca^{++} inflow (0.062 ± 0.01 mmole/kg wet weight of tissue), whereas in a concentration of 10^{-5} M nifedipine induced the outflow of 0.14 ± 0.031 mmole Ca^{++}/kg wet weight of tissue. If, after the heart tissue had remained in low-sodium or physiological solution with nifedipine, it was transferred every 30 min (for 2 h) successively into cuvettes with the corresponding solution but without the blocker, in neither case was the deficit of intracellular calcium restored. Consequently, during the time of study the blocker was not washed out of the heart tissue. Nifedipine reduced the intracellular calcium concentration by reducing the calcium inflow into the heart cells, but had very little or no effect on calcium outflow. This decreases in calcium inflow was probably unconnected with the calcium channel, because the measurements were made with the heart resting conditions.

The outflow of calcium from the cells under the influence of nifedipine revealed by these experiments can thus be explained by blocking of calcium inflow through the sodium–calcium exchange system.

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