and prophase. DNA synthesis, preparation to mitosis, and initial stages of the first and second meiotic division take place in spermatogonia and in the first and second order spermatocytes. These cells are apparently the main target of the drug. On the other hand, the low total count of mature sex cells during all periods of the experiment is indicative of damaging effect of the drug on nondividing cells of spermatogenic epithelium, spermatides, and spermatozoa.

REFERENCES

1. M. M. Belen'kii, in: *Elements of Quantitative Evaluation of Pharmacological Effect* [in Russian], Leningrad (1963), pp. 81-106.
2. A. I. Vorob'ev, *Klin. Farmakol. Ter.*, No. 4, 16-17 (1995).
3. I. F. Sanotskii and V. N. Fomenko, *Remote Effects of Chemical Compounds on the Organism* [in Russian], Moscow (1979).
4. Yu. I. Ukhov and A. F. Astrakhantsev, *Arkh. Anat.*, No. 3, 66-72 (1983).
5. S. Gundy and M. Babosa, *Mutat. Res.*, **271**, No. 2, 142 (1992).
6. B. D. Hoerni, *Ann. Endocrinol. (Paris)*, **4**, No. 4, 268-272 (1991).
7. Imarura Fumid, *Hajgan = Lung Cancer*, **33**, 95-101 (1993).
8. C. Schelonng, *Ann. Hematol.*, **64**, No. 2, 71-119 (1992).

Estimation of Na-Blocking Efficiency of Rihlocaine and Its Combinations with Low-Molecular-Weight Polymers on Isolated Rat Cardiomyocytes

A. I. Khankoeva, A. S. Dukhanin, P. A. Galenko-Yaroshevskii, and A. Yu. Reznikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 12, pp. 649-651, December, 1997
Original article submitted November 22, 1996

The effect of glucose-base polymers (dextrans with relative molecular weight 10, 40, and 70 kD) and vinylpyrrolidone (polyvinylpyrrolidone: 10, 24, and 40 kD) on changes in the sarcoplasmic Na^+ concentration in stimulated cardiomyocytes (1.0 Hz, 10 msec, 60 mV) were examined. In the concentration range of 1-100 μM, the polymer preparations produced cardioprotective effect on cells incubated under hypoxic conditions; the effect depended on the nature and molecular weight of the polymer. Rihlocaine (25 μM) inhibits by 42% elevation of intracellular Na^+ induced by plasma membrane depolarization. Dextran 40 is shown to significantly increase Na-blocking effect of rihlocaine.

Key Words: *dextran; polyvinylpyrrolidone; antiarrhythmics; cardiomyocytes*

To produce preparations with prolongated effects, both natural (carboxymethylcellulose, dextran) and synthetic (polyvinylpyrrolidone — PVP, polyvinyl alcohol) polymers with high biological compatibility and low toxicity have been used [3,6].

New preparations on the basis of traditional drugs immobilized on a carrier polymer or noncovalently bound to a polymer have the following advantages: enhanced pharmacological effectiveness, longer period of action, and lower side effects. Using the aconitine model of cardiac arrhythmia, we demonstrated antiarrhythmic activity of the sodium channel blocker rihlocaine (RHC) diluted in rheopolyglucin (10% solution of dextran 30/40) [2]. Under acute regional ischemia and reperfusion, the combination of RHC with dextran stabilized basic hemodynamic and cardiac indexes, thus demonstrating antiarrhythmic and antifibrillatory activities. At the same time, a certain decrease in the effectiveness of RHC was detected when it was combined with PVP.

Department of Molecular Pharmacology and Radiobiology, Russian State Medical University, Moscow; Department of Pharmacology, Kuban Medical Academy, Krasnodar

0007-4888/97/0012-1197$18.00

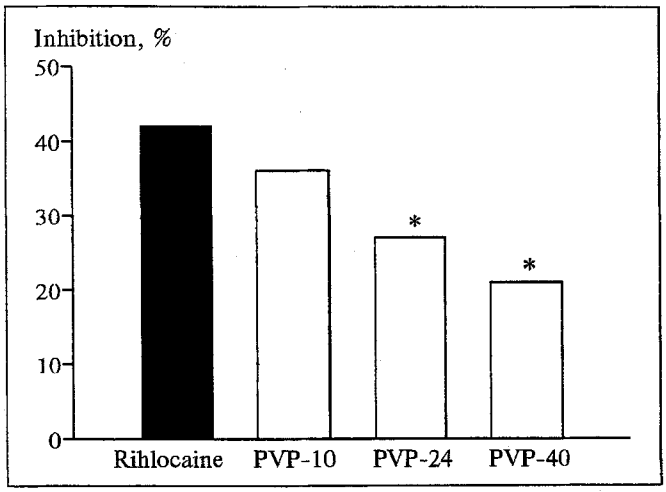

Fig. 1. The effect of polyvinylpyrrolidone (PVP, 100 μM) of various molecular weight (10, 24, and 40 kD) on sodium blocking efficiency of rihlocaine (25 μM). The mean values are given (n=5). Here and in Fig. 2: *p<0.05 in comparison with rihlocaine.

Recent studies revealed that low-molecular-weight polymer preparations (10-40 kD), dextran in particular, can be uptaken by cells by endocytosis [5,8]. In order to exclude hemodynamic effects of polymer solutions and to estimate their possible direct effect on myocardial cells, we compared the effects of different combinations of RHC with dextran and PVP on the intracellular Na^+ concentration in intact and nonoxygenated cardiomyocytes *in vitro*.

MATERIALS AND METHODS

Isolation of cardiomyocytes and determination of intracellular Na^+ in the suspension of isolated cells using the fluorescent probe SBFI was performed as described earlier [1]. To estimate the intensity of the fluorescence probe and cytoplasmic sodium concentration $[Na^+]_{cyt}$, calibration was performed according to [4]. The sarcoplasm sodium concentration was calculated from the following formula:

$$[Na^+]_{cyt}=K_d\times k\times(R-R_{min})/(R_{max}-R),$$

where R is the ratio of fluorescence intensities at excitation wavelengths of 340 nm (F_{340}) and 380 nm (F_{380}); R_{min} and R_{max} are similar ratios at zero and saturating concentrations of $[Na^+]_{cyt}$ (150 mM); k is the ratio of fluorescence intensities at 380 nm for free and complexed probe (2.1±0.1); K_d is the equilibrium dissociation constant for the probe-Na^+ complex (20.8±1.4 mM).

To simulate hypoxic conditions, the cells were incubated for 30 min at 37°C in the medium containing 0.5 mM KCN and 10 mM 2-deoxyglucose. Electrical stimulation of cardiomyocytes was performed in a MacLab System.

Working concentration of RHC (1-allyl-2,5-dimethylpiperidole-4 benzoic ether hydrochloride) was chosen to correspond the mean ED_{50} determined in the *in vivo* experiments, i.e., to the dose that produces 50% maximum pharmacological effect. The range of final concentrations of polymeric preparations in the incubation medium (1-100 μM) covered the changes in its extracellular level during the long period after intravenous administration of the preparations [7].

Na-blocking efficiency of the drugs was calculated from the formula: $[1-(\Delta Na_t/\Delta Na_c)]\times 100\%$, where ΔNa_t and ΔNa_c are changes in $[Na^+]_{cyt}$ during 30 min incubation of test and control samples in the absence of the drugs.

The results were statistically analyzed using Pharmacological Basic Statistics package. The confidence intervals of experimental values and the significance of the difference between them was calculated according to the Student's t test, taking 5% as the minimal level of significance.

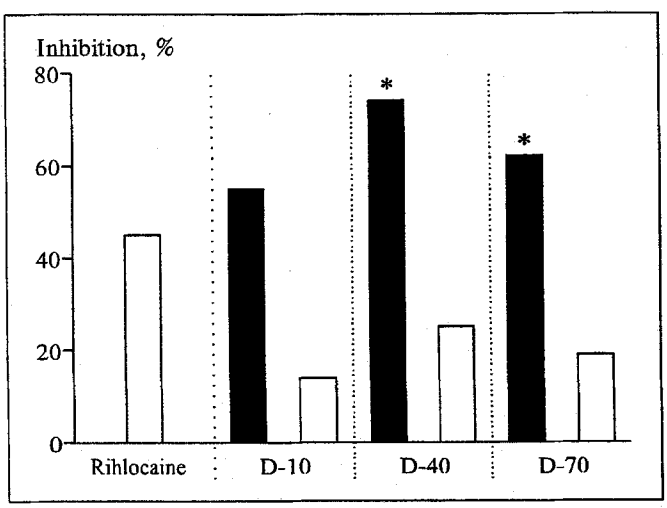

Fig. 2. The effect of dextran (D, 100 μM) of various molecular weight (10, 40, and 70 kD) on the sodium blocking efficiency of rihlocaine (25 μM) under experimental hypoxia. Open bars: relate to dextran alone; closed bars: combination of dextran with rihlocaine.

RESULTS

The diastolic $[Na^+]_{cyt}$ in the sarcoplasm of resting cells was 8.3±0.6 mM (n=5). Stimulation of cardiomyocytes at a frequency of 1.0 Hz (10 msec, 60 mV) increased intracellular $[Na^+]_{cyt}$ to 16.5±1.4 mM (n=6). Therefore, the mean difference (ΔNa) between the basal Na^+ level in the intact and stimulated myocytes was 8 mM.

The effect of polymeric preparations and RHC on induced rise of $[Na^+]_{cyt}$ was studied in two series of experiments, using intact cardiomyocytes and

nonoxygenated cells under conditions of "chemical" hypoxia.

First, we investigated the influence of natural (dextran) and synthesized (PVP) polymers on the potential-induced enhancement of $[Na^+]_{cyt}$. Dextran with molecular weights of 10, 40, and 70 kD and PVP with molecular weights of 10, 24, and 40 kD were used. In the concentration range of 1-100 μM none polymer changed significantly basal or stimulation-dependent Na^+ in intact cardiomyocytes. RHC (25 μM) inhibited elevation of $[Na^+]_{cyt}$ by 42±5% ($p<0.01$), while the basal sodium level in the presence of RHC did not significantly differ from the control.

Estimation of Na-blocking efficiency of combinations of RHC with dextran of different types (1-100 μM) did not reveal marked changes in RHC activity. Combination of PVP (100 μM) with RHC decreased the inhibitory effect of the latter (Fig. 1). There was a relationship between the ability of PVP to decrease Na-blocking activity of RHC and the length of PVP chain. In the presence of PVP with a molecular weight of about 10 kD (PVP-10) the inhibitory effect of RHC was 36±4%. However, under PVP-40 taken in the same concentration, blockade of sodium inflow into cardiomyocytes was no more than 21%. The effect of PVP results probably from formation of complexes of RHC with the polymer, which decreases the concentration of free (active) form of RHC in the test solutions.

Thus, the effects of dextran and PVP on pharmacological activity of RHC differ significantly: in contrast to PVP, which demonstrated the antagonistic effect, addition of dextran into the incubation medium did not produce significant changes in Na-blocking properties of RHC. Interpretation of the data obtained should take into account the behavior of the polymers in aqueous solutions: the molecules of PVP maintain predominantly linear form, while dextran molecules (in particular, of greater molecular weight) demonstrate a tendency to form globule-like structures. At physiological pH, RHC is a weak base, and its hydrophilic properties impede incorporation of RHC molecules into the hydrophobic dextran domains.

Under conditions of experimental hypoxia, the mean difference between the basal and induced sodium levels (ΔNa) was 17 mM. Preliminary application of polymer preparations in final concentrations of 1, 10, and 100 μM produced a dose-dependent decrease in ΔNa. The degree of Na-blocking effect of dextran decreases in the sequence dextran 40>dextran 70> dextran 10 (Fig. 2). The effect of PVP is less expressed, and dependence of PVP stabilizing effect on the size of a polymer molecule is not significant (data are not shown).

Among possible mechanisms of the membrane-stabilizing effect of polymers one can consider their ability to increase the oncotic pressure of the extracellular medium and detoxification properties resulting from the ability of polymers to bind the low-molecular-weight membrane-active substances released from cells.

Study of the combined effect of RHC and polymer preparations on $[Na^+]_{cyt}$ under hypoxia revealed the most active combination, i.e., RHC+dextran-40. After administration of this combination, sodium elevation in the cardiomyocytes was not larger than 25% of the control (drug-free) level. In the presence of either preparation alone, ΔNa was 76% (dextran) and 55% (RHC) of the control level.

Comparison of these findings with those obtained *in vivo* shows that further study of combinations of RHC with dextran is prospective, and this combination can be considered as a potential antiarrhythmic preparation.

REFERENCES

1. P. A. Galenko-Yaroshevskii, A. I. Khankoeva, and A. S. Dukhanin, *Byull. Eksp. Biol. Med.*, **122**, No. 7, 45-47 (1996).
2. P. A. Galenko-Yaroshevskii, A. I. Khankoeva, V. V. Skibitskii, *et al.*, in: *Fundamental Study as Basis in Designing of Medicinal Drugs* [in Russian], Moscow (1995), p. 99.
3. T. Oon, E. Itsuka, S. Onari, *et al.*, Biopolymers [in Russian], Moscow (1988).
4. H. Hayashi, H. Satoh, N. Noda, *et al.*, *Am. J. Physiol.*, **266**, No. 2, Pt. 1, C416-C422 (1994).
5. M. M. Lerch, A. K. Saluja, M. Runzi, *et al.*, *J. Clin. Invest.*, **95**, No. 5, 2222-2231 (1995).
6. L. G. Donaruma (Ed.), *Polymers in Biology and Medicine*, New York (1980).
7. H. P. Rang, M. M. Dale, and J. M. Ritter, in: *Pharmacology*, 3rd ed., Edinburgh (1995), pp. 286-291.
8. W. Rubas, N. Jezyk, and G. M. Grass, *J. Drug Target*, **3**, No. 1, 15-21 (1995).