EXPERIMENTAL STUDY OF MAGNETICALLY CONTROLLED TRANSPORT OF NEUROMUSCULAR BLOCKING AGENTS DIADONIUM AND DIPYRONIUM IN ANIMALS

D. A. Kharkevich, R. N. Alyautdin,
V. I. Filippov, A. Yu. Nemirovskii,
S. A. Kasparov, and A. A. Kuznetsov

UDC 615.216.5:615.451.234].033

KEY WORDS: targeted transport, liposomes, ferromagnets, muscle relaxants.

The use of magnetically controlled carriers for targeted transport of drugs in the vascular bed affords new prospects for increasing the selectivity of action of these preparations and reducing side and toxic effects. Meanwhile the range of such carriers is still small. For instance, magnetically controlled microcapsules made from polymer materials have been developed for these purposes [4, 5]. Albumin microspheres are used to transport antitumor preparations and radioactive labels [6, 7]. The selective action of curare-like agents, incorporated into magnetically controlled microspheres made from an insoluble polyelectrolyte complex has been demonstrated [1]. The problem of the use of liposomes as carriers for drugs has been intensively studied in recent years [2, 3]. The possibility of using liposomes containing ferrocolloid for the targeted transport of the muscle relaxants diadonium and dipyronium in cats was studied in the investigation described below.

EXPERIMENTAL METHOD

The possibility of creating selective muscle relaxation with the aid of curare-like agents, incorporated into magnetically controlled liposomes (MCL) was investigated in experiments on cats weighing 2.7-3.5 kg. As a first step, under ether anesthesia tracheotomy was performed, a catheter introduced into the jugular vein, and the sciatic nerves isolated in both hind limbs. The animals were then given an intravenous injection of pentobarbital (30 mg/kg). To exclude any effect of the hypoxia arising through the use of curare-like drugs, the animals were artificially ventilated until the experiment began. The state of neuromuscular transmission in the cats' gastrocnemius muscles was assessed by the magnitude of the evoked responses. The sciatic nerves were stimulated by supramaximal square electric pulses applied with a frequency of 0.5 Hz. Evoked potentials recorded in the muscles by means of bipolar needles were averaged (10 consecutive responses). One of the animal's hind limbs was placed in a magnetic field with an intensity of 2.5 kG, whereas the other limb, outside the magnetic field, served as the control. After a stable initial background had been recorded for 15-20 min, MCL containing the curare-like drugs diadonium and dipyronium were injected into the jugular vein. Evoked responses were recorded for 20-30 min until they were fully restored.

The liposome-ferromagnet complexes used in the experiment were made from ferrocolloid and liposomes consisting of egg phosphatidylcholine and cholesterol. The ferrocolloid was obtained by an electrocondensation method, followed by stabilization with gelatin. The concentration of ferroparticles in the suspension was 3-5%, and the dimensions of the ferroparticles were between 20 and 200 nm. The liposomes were prepared by the method in [8], with simultaneous incorporation of the curare-like drugs and ferroparticles into the vesicles. The dimensions of the liposomes varied from 0.01 to 2 μ. The degree of incorporation of the drugs into MCL was assessed quantitatively by spectrophotometry in the UV region. To assess the quantitative distribution of MCL in the tissues of the body, ^{14}C-oleic acid incorporated into the MCL bilayer was used; it was estimated in the tissue hydrolysate by liquid scintillation analysis. The distribution of radioactive label was studied in the gastrocnemius muscles of both limbs, the liver, spleen, and lungs.

Department of Pharmacology, Faculties of Internal Medicine and Hygiene, I. M. Sechenov First Moscow Medical Institute. Laboratory of Biophysical Engineering, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 48-49, July, 1986. Original article submitted September 17, 1985.

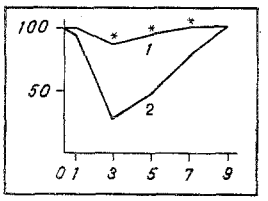

Fig. 1. Effect of MCL containing diadonium on amplitude of evoked responses in cat's gastrocnemius muscles. Abscissa, time (in min); ordinate, magnitude of response relative to initial (in %). 1) Amplitude of evoked responses in control limb; 2) amplitude of evoked responses in limb placed in magnetic field. Asterisk indicates statistical significance of difference between magnitude of responses. Experiments conducted on cats anesthetized with pentobarbital (40 mg/kg, intravenously).

EXPERIMENTAL RESULTS

After intravenous injection of MCL containing the curare-like drug diadonium in a dose of 115 μg/kg (diadonium causes complete neuromuscular block in a dose of 350 μg/kg), a reduction in amplitude of the neuromuscular potentials was observed in the gastrocnemius muscles of both hind limbs of the animal, and the magnitude of the responses in the limb placed in the magnetic field was reduced by 70%, compared with only 15% in the control limb. The maximal effect was observed 3 min after injection of the drug, and complete recovery occurred after 9 min (Fig. 1). Similar results were obtained in the experiments with dipyronium. Injection of MCL containing dipyronium (20 μg/kg) caused inhibition of responses in the limb placed in the magnetic field by 45%, compared with only 5% in the control limb. In the control experiments the magnetic field alone did not affect neuromuscular transmission. MCL without curare-like drugs and ferrocolloid likewise did not affect the state of neuromuscular conduction. Injection of the curare-like drugs without liposomes, and also of liposomes containing the curare-like drugs but without the ferromagnets, caused equal changes in neuromuscular transmission in both limbs. In the series of five control experiments on animals with spontaneous respiration the effect of the muscle relaxation produced on pCO_2 in the expired air was studied. During inhibition of neuromuscular transmission in the magnetic field with the aid of MCL by 70%, pCO_2 was raised by only 0.2 vol. % (5% of its initial level). Meanwhile inhibition of neuromuscular transmission by a similar amount by the use of diadonium, not incorporated into MCL, was accompanied by an increase in pCO_2 up to 6 vols. % (by 50%).

When the distribution of labeled MCL was studied, they were found to be five times more numerous in the limb placed in the magnetic field than in the control limb. About 33% of the injected label was found in the liver and about 6% in the spleen, but when calculated per gram of tissue, the uptake of MCL by the spleen and liver was approximately the same (1.3:1).

The experiments thus confirmed that MCL may be used for directing the supply of drugs toward a target organ. Advantages of MCL are their stability on keeping and the adequate rate of elimination of the preparations. The MCL used, moreover, consist of relatively nontoxic compounds and they can be used as carriers for drugs differing in chemical structure.

The experimental model used is sufficiently adequate and sensitive for determination of the selectivity of action of drugs for several reasons. First, curare-like drugs are well investigated substances which have only one point of application of their action, namely neuromuscular synapses. Changes in the amplitude of the potentials can therefore be interpreted unequivocally as an indicator of the degree of inhibition of neuromuscular transmission, i.e., of the effect of the curare-like agents. Second, all the experiments were accompanied by an adequate control (evoked potentials in the opposite limb), so that signs of the systemic action of the drug are easily detected.

The use of MCL is a promising method of improving the effectiveness and safety of certain types of drug treatment. At the same time, a further study of the properties of MCL as carriers of drugs, the kinetics of elimination of the preparations from MCL *in vivo* and *in vitro*, and the choice of therapeutic agents most suitable for incorporation into them, is essential. Furthermore, optimal values of the intensity of the magnetic field ensuring the necessary retention of MCL for complete release of the drug from them to occur, must be established.

LITERATURE CITED

1. R. N. Alyautdin, S. A. Kasparov, A. Yu. Nemirovskii, et al., in: Proceedings of the 3rd All-Union Seminar-School on Magnetic Fluids [in Russian], Ples (1983), pp. 6-8.
2. G. Gregoriadis and A. C. Allison, Liposomes in Biological Systems, Wiley (1980).
3. V. P. Torchilin, V. N. Smirnov, and E. I. Chazov, Vopr. Med. Khim., No. 1, 3 (1982)-
4. T. Kato, Japanese Patent No. 51411/81.
5. Y. Morimoto, M. Okumura, K. Sugibayashi, and Y. Kato, J. Pharm. Dyn., 4, 624 (1981).
6. K. Widder, G. Flouret, and A. Senyei, J. Pharm. Sci., 68, 79 (1979).
7. V. Zimmermann, FEBS Lett., 140, 254 (1982).

PRODUCTS OF ALKALOID OXIDATION BY BLOOD PLASMA HEMOPROTEIN

N. R. Elaev and A. V. Ryzhakov

UDC 616.153.1:577.152.34]-07

KEY WORDS: alkaloids, blood plasma hemoprotein, N-oxides.

A heme-containing protein with mol. wt. of about 75,000 daltons, inactivating alkaloids of the atropine group, was isolated previously from blood. The abbreviation HP_{aa} (antialkaloid hemoprotein) is suggested for this protein.

In the present investigation the mechanism of the reaction of HP_{aa} with alkaloids was studied by identifying inactivation products of atropine and also of the alkaloid arecoline, which differs from it in its pharmacologic action and structure.

EXPERIMENTAL METHOD

The method of isolating the enzyme from rat blood plasma, including fractionation of the proteins by precipitation with ammonium sulfate, ion-exchange chromatography, and gel-chromatography, was described previously [1]. The concentration of the enzyme was 1/12,000 of the total blood plasma proteins.

To study inactivation of atropine and arecoline the reaction mixture contained 0.3-7.7 μmoles of substrate and 20 μg of enzyme in 0.3 ml of 0.025 M K-phosphate buffer, pH 7.4. Samples were incubated for 60 min at 37°C, 0.3 ml of ethanol was added, and the mixture was dried in vacuo. The material was extracted with 0.2-4.8 ml of ethanol and aliquots, each of 0.025-0.05 ml, were applied to Silufol chromatographic plates (length of run 7 cm). In the case of atropine fractionation was carried out in a system of 96% ethanol—chloroform—25% ammonia (15:10:4), and in the case of arecoline, 80% ethanol—25% ammonia (97:3). One pole of the chromatogram was developed with Dragendorf's reagent [3] or with iodine vapor. Zones corresponding to the reaction products found were cut out and the material eluted with 7 ml of 0.05 M Na,K-phosphate buffer, pH 7.4. Atropine, arecoline, and their conversion products were estimated quantitatively by the reaction with bromthymol blue [2]. For preparative isolation of the reaction products they were eluted from the sorbent with 0.5 ml of ethanol. The eluate was centrifuged and dried in vacuo. The residue was dissolved in a small volume of ethanol, the solution was applied to KRS-5 glass, and dried in vacuo at 45°C. IR spectra were recorded on the UR-20 and Specord 75-UR spectrophotometers.

EXPERIMENTAL RESULTS

After incubation of atropine and arecoline with the enzyme, besides the original substances (R_f 0.70 and 0.58, respectively), single products with R_f values of 0.58 and 0.27, respectively, also were found in the reaction mixture (Fig. 1).

IR spectra of atropine and its enzymic conversion product are shown in Fig. 2. The only difference in principle is the appearance of a strong and narrow absorption band for the lat-

Department of Biological and Organic Chemistry, Petrozavodsk University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. N. Golokov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 7, pp. 50-51, July, 1986. Original article submitted April 30, 1985.

 0007-4888/86/0007-0928$12.50