obtained by the use of free ligand in concentrations of between 0.25 and 16 nM lead to the conclusion that GABA (10 μM) reduces dissociation (K_d) and increases the density of binding sites, but with a wider range of concentrations. Although GABA reduces K_d it does not affect B_{max}, in good agreement with the model of allosteric control of the benzodiazepine receptor through GABA.

The results of this investigation thus show that when determining parameters of equilibrium binding the experiments must be conducted over the widest possible range of free ligand concentrations (as Scatchard rightly pointed out), or otherwise the experimental data will not correspond to Scatchard's suggested model and cannot be analyzed by that method. The experimental points must be first represented between Klotz' coordinates, so as to make sure that the necessary range of concentrations has been correctly chosen, after which they can be analyzed between Scatchard coordinates.

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POSSIBILITY OF PHARMACOLOGIC INTERVENTION IN POSTICHEMIC MYOCARDIAL DAMAGE

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UDC 616.12-005.4-036.8-06:616.127-085.224-036.8-092.9

KEY WORDS: postischemic damage, necrosis, pharmacologic intervention.

One of the main tasks in the treatment of ischemic heart disease (IHD) is the prevention of anginal attacks. Several antianginal drugs are used for this purpose: nitroglycerin in various therapeutic forms, Ca^{++} antagonists, β-adrenoblockers, etc. However, even with the aid of these highly effective drugs, it is by no means always possible to completely prevent the development of attacks. The termination of an attack once it has arisen is therefore no less important. Each attack of angina causes definite damage to the cardiomyocytes in that region of the myocardium affected by ischemia. For instance, occlusion of the coronary artery in dogs for 15 min leads to a marked decrease in the ATP concentration in the myocardium and to inhibition of its contractile function. These changes persist for several days after restoration of the blood supply to the ischemic zone [2]. Damaged myocardial cells, depending on the intensity and duration of ischemia, may recover their function or die.

The question arises whether this pathological process, determining the fate of the myocardium after an anginal attack, can be influenced by means of drugs. The investigation described below was undertaken to study this problem.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9, pp. 302-303, September, 1986. Original article submitted May 11, 1985.

0007-4888/86/0009-1214$12.50

TABLE 1. Development of Necrosis in Myocardium after Temporary Threshold Ischemia of Varied Duration Caused by Partial Occlusion of Coronary Artery in Conscious Rabbits

Expt. No.	Size of necrotic lesion of myocarium (in % of weight of left ventricle)		
	duration of occlusion, min		
	60	90	120
1	Absent	3,0	12,0
2	»	3,2	16,5
3	»	6,0	18,0
4	»	8,8	25,0
5	»	25,0	31,0
6	4,0	26,0	36,0
7	4,5	29,0	36,0
8	6,2	33,0	37,0
9	7,8	36,0	44,0
10	8,2	56,0	55,0
$M \pm m$	6,1±0,8	22,6±5,4	31,0±4,0

TABLE 2. Effect of Ethacizine and Nonachlazine on Volume of Myocardial Necrosis Developing after Threshold Ischemia Lasting 1.5 h and Caused by Partial Occlusion of Coronary Artery in Conscious Rabbits

Expt. No.	Size of necrotic lesion of myocardium (in % of weight of left ventricle)		
	untreated animals	ethacizine	nonachiazine
1	3,0	Absent	Absent
2	3,2	»	2,4
3	6,0	»	3,6
4	8,8	»	8,4
5	25,0	»	8,7
6	26,0	»	9,4
7	29,0	4,0	11,3
8	33,0	7,0	12,5
9	36,0	13,0	13,0
10	56,0	17.0	26,0
$M \pm m$	22,6±5,4		9,5±2,0

EXPERIMENTAL METHODS

Experiments were carried out on conscious rabbits weighing 2.5-3 kg. A model of acute transient myocardial ischemia, caused by partial occlusion of the lateral branch of the left descending coronary artery, was used. For this purpose an occluder, the design and principle of operation of which were described by the writers previously [1], was used. By means of this occluder it was possible to reduce the blood flow in the coronary artery slowly, and then to restore it again. Myocardial ischemia was produced as follows. To begin with the coronary artery was constricted until the appearance of ischemic changes in the precordial ECG. These changes, if the constriction was removed, i.e., if the dimensions of the lumen of the coronary artery achieved remained constant, disappeared in the course of a few minutes as a result of removal of the disparity developing between the oxygen supply and its demand by the heart, as a result of opening of the coronary reserve in response to ischemia. Immediately after normalization of the ECG, the lumen of the coronary artery was again slowly constricted until the appearance of signs of ischemia on the ECG. Later (after these signs had diminished) the procedure was repeated, so that a mild (threshold) degree of myocardial ischemia was maintained for long enough for irreversible injury to the cardiomyocytes to take place. At the end of this time the blood flow in the vessel was restored. Drugs were in-

jected intravenously: the first time 5 min after restoration of the blood flow, and thereafter 3 times a day for 3 days. In addition, an intramuscular injection of the drugs was given daily after the last intravenous injection, in a dose 3 times greater than the intravenous sessional dose. Thus, treatment was carried out after restoration of the blood supply to the zone of ischemia, so that it was possible to judge the effect of the drugs actually on postischemic cardiac damage. The results of treatment were judged by studying changes in the size of the area of necrosis, which was measured 72 h after production of ischemia by determination of succinate dehydrogenase activity by means of a gravimetric (planimetric) method [3]. To study the possibility of pharmacologic intervention in postischemic cardiac damage two drugs were used: the antiarrhythmic ethacizine (ethmozine diethylamine analog, E-DAA; sessional dose 0.3 mg/kg) and the antianginal druge nonachlazine (viloxazine; sessional dose 3 mg/kg). Both drugs were synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR, and they are currently being used in clinical practice.

EXPERIMENTAL RESULTS

In the experiments of series I the minimal duration of myocardial ischemia necessary for the development of irreversible cardiomyocyte damage was determined in all the rabbits in which the lesion was produced by the method described above. It was found that ischemia for 2 h leads to the development of necrotic myocardial damage in all experimental animals ($n = 10$). The area of necrosis was equivalent to 31 ± 4% of the weight of the left ventricle below the site of occlusion. After myocardial ischemia for 1.5 h necrosis of the myocardium also was found in all rabbits ($n = 10$), but the lesions were smaller than in the rabbits of the previous group (22.6 ± 5.4%; $n = 10$, $P < 0.05$). After myocardial ischemia for 1 h, areas of necrosis were discovered in only 50% of the experimental animals ($n = 10$). The results of the experiments of series I are shown in Table 1. It can be concluded from these data that ischemia lasting 1.5 h, if produced by the method described above, is at the threshold for the subsequent development of irreversible cardiomyocyte damage in 100% of animals, and is therefore the most suitable method for use when studying the possibility of pharmacologic intervention in the postischemic pathological process in heart muscle.

In the experiments of series II the possibility of pharmacologic intervention in postischemic heart damage caused by ischemia for 1.5 h was studied. For this purpose the phenothiazine derivatives ethacizine and nonachlazine were used. Treatment began 5 min after restoration of the blood study to the zone of ischemia and it continued for 3 days in accordance with the scheme described above. The experimental results showed that, during treatment with ethacizine, foci of necrosis in the myocardium were found in only four of the 10 rabbits. These results differ statistically significantly from the control ($P < 0.001$). During treatment with nonachlazine areas of necrosis were discovered in nine of the 10 animals, but they were significantly smaller in size than in the control (9.5 ± 2.0 and 22.6 ± 5.4% respectively; $P < 0.001$). Results obtained in rabbits treated with nonachlazine and ethacizine also differed statistically significantly. Ethacizine was found to be more effective than nonachlazine ($P < 0.05$). The results of these experiments are given in Table 2.

Pharmacologic intervention in IHD may thus be directed not only toward the prevention and termination of anginal attacks, but also toward treatment of postischemic heart damage, taking place under conditions of a compensated blood suppply to the myocardium. This conclusion may be taken as a starting point for the development of a new approach in the search for drugs for the treatment of IHD.

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