EVALUATION OF THE ANTIANGINAL ACTION OF DRUGS BY CHANGE IN THRESHOLD OF MYOCARDIAL ISCHEMIA ARISING IN CONSCIOUS RABBITS

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KEY WORDS: method of evaluation; antianginal action; conscious animals.

The most adequate method of experimental evaluation of the antianginal activity of drugs is to study them on models of myocardial ischemia due to coronary occlusion in conscious animals. Investigations of this kind are mainly conducted on dogs or monkeys [1-4]. However, it will be evident that the great laboriousness of work on these animals makes the search for new antianginal remedies much more difficult. Accordingly the aim of the present investigation was to devise a readily reproducible and comparatively labor-saving method of evaluating the antianginal activity of drugs which, at the same time, would permit experiments to to be performed without general anesthesia.

EXPERIMENTAL METHOD

The most suitable animals for this purpose, in the writers' view, are cats and rabbits. The great laboriousness of work on larger animals limits their usefulness in screening investigations. Smaller animals, because of the smaller size of the heart and coronary vessels, also are unsuitable as models of myocardial ischemia due to coronary occlusion. Moreover it is very difficult on such animals (especially without general anesthesia) to study the cardiovascular properties of drugs.

The writers have constructed a device for occluding the coronary artery (an occluder), of suitable size for use in experiments on cats and rabbits (Fig. 1). The design of the occluder is such that it can be used not only for occlusion, but also for reperfusion. The principle of operation of the occluder is that, if the head of the screw 1 is turned anticlockwise, it is screwed out of the tube 2. Under these circumstances a loop of thread implanted around the artery is drawn tight, which narrows the lumen of the artery and reduces the blood supply to the myocardium. If the screw is turned clockwise the loop of thread is relaxed and the blood flow in the vessel is restored. The occluder is implanted under pentobarbital anesthesia. Thoracotomy is performed by retracting the ribs to widen the 4th and 5th intercostal spaces on the left side. After opening of the pericardial cavity the thread is passed around the coronary artery. The part of the thread with the needle is cut off, and the rest passed through the hole b and securely fixed. The thoracic wound is sutured. This fixes the tube 2 securely between the ribs. Part of the tube 2 and the head of the screw remain outside (Fig. 2). The postoperative wound is treated with antibiotics and iodine solution. The times of beginning of the experiments are determined by the course of the postoperative period, the severity of which can be judged from restoration of movements, reappearance of appetite, and the state of the animal's postoperative wound. In most cases experiments on rabbits can be started as early as 24 h after the operation.

The antianginal activity of drugs is evaluated on this model by studying their effect on the threshold of onset of myocardial ischemia. For this purpose, by means of special devices, notably a pointer securely fixed to the head of the screw of the occluder, and a graduated circular scale fixed to the animal's skin during the experiment, the number of turns of the screw before the appearance of ischemic changes on the ECG is recorded (Figs. 2 and 3). In this way the degree of narrowing of the coronary artery at which ischemic changes arise in the myocardium is determined. Then, by turning the head of the screw clockwise, reperfusion is allowed. The performance of these experiments calls for great accuracy in the repeated

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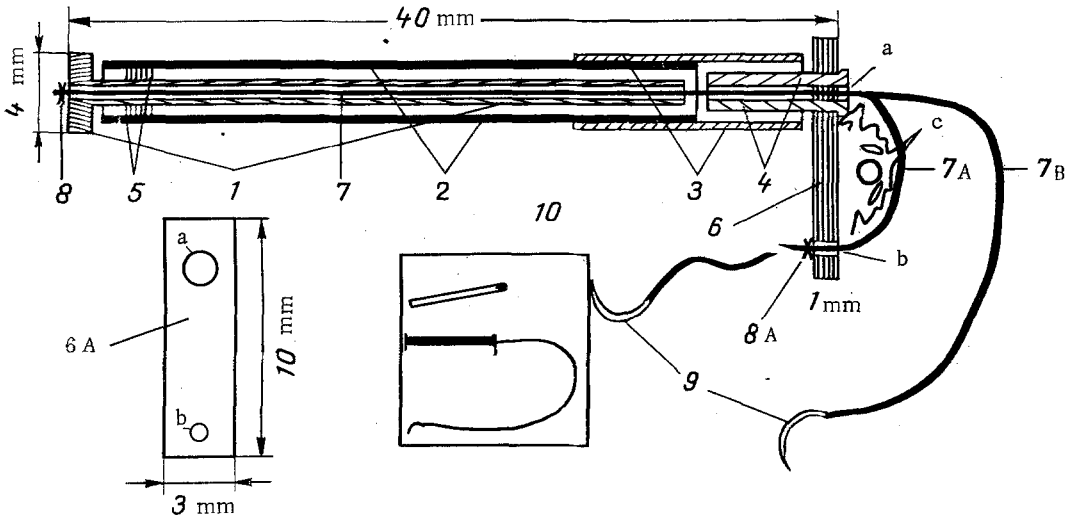

Fig. 1. Diagram of occluder. 1) Hollow metal rod with external thread and head for turning (diameter of hole in rod 0.5 mm, external diameter of rod 1.5 mm); 2) poleythylene tube (external diameter 3 mm, internal 2 mm); 3) rubber tube (nipple) designed to prevent occluder from fixing heart; 4) polyethylene tube for preventing displacement of heart during occlusion; 5) metal plug with internal thread; 6) rigid base plate for occlusion; 6A) front view of plate: *a* and b) holes in plate through which thread forming loop around artery and surrounding muscle tissue is drawn (diameter of hole *a* corresponds to diameter of tube 4, diameter of hole b is a little larger than diameter of thread, namely 0.35-0.4 mm; 7) thread (black line) 0.3 mm thick; 7A) loop of thread after implantation (artery and area of myocardial muscle tissue (a) shown inside loop; 7B) thread with needle before implantation; 8, 8A) marker of fixation of thread: thread at point 8 is fixed before operation, at point 8A during operation, after suturing of tissue around vessels, cutting off needle, and passing end of thread through hole b; 9) atraumatic needle; 10) diagram of occluder and match to show their comparative size.

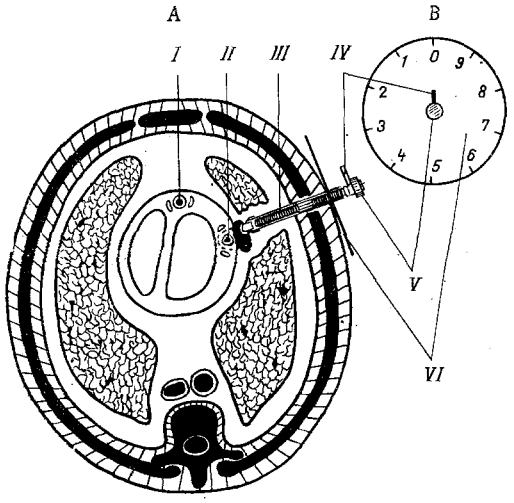

Fig. 2. Diagram of transverse section through rabbit's body at level of implanted occluder. A: I) Anterior branch of left descending coronary artery; II) lateral branch of left coronary artery, III) occluder, IV) pointer, V) head of occluder screw, VI) graduated scale, B: IV, V, IV) side view of head of screw, pointer, and graduated scale.

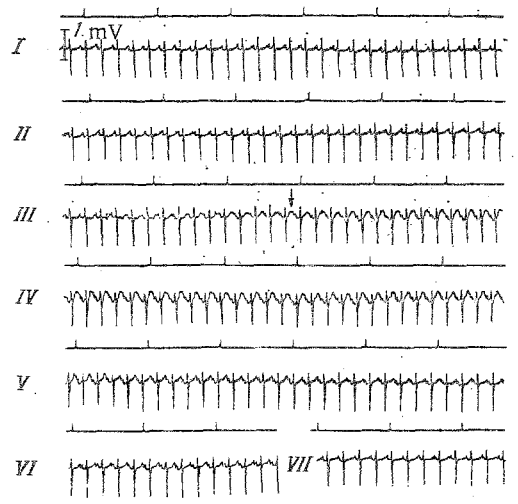

Fig. 3. ECG changes (precardial derivation) during slow occlusion and rapid reperfusion of coronary artery in conscious rabbit. I-VI) Continuous ECG trace during slow occlusion and immediately after rapid reperfusion; VII) 3 min after reperfusion. Explanations from top to bottom: time marker (1 sec), precardial ECG. Arrow indicates beginning of ischemic changes on ECG and time of reperfusion.

occlusions. In experiments on conscious animals this can be achieved only if they are fixed in a position convenient for the experimenter (on their back or right side). Because of this, the most suitable animals for use with this method are rabbits, which tolerate the enforced position comparatively easily and quickly become accustomed to it. Because of the impossibility of fixing cats, they can be used only under general anesthesia.

The following conditions must be observed when this model is reproduced.

1. The larger lateral branch of the descending coronary artery must be occluded in rabbits. Occlusion of the anterior branch, which is much smaller than the lateral branch, does not produce sufficiently rapid or distinct appearance of ischemic changes on the precardial ECG, so that exact determination of the threshold of onset of myocardial ischemia is impossible.

2. Repeated occlusion of the coronary artery must be carried out at the same speed. The results of control experiments showed that the threshold of onset of ischemia is directly dependent on the rate of occlusion of the artery. We carry out occlusion by turning the head of the screw through one division of the circular scale, divided into 10 parts (Fig. 2b) at a time, being careful to observe equal 5-sec intervals between turns. The pitch of the screw used in this occluder is 0.2 mm. Each turn thus shortens the loop of thread by 0.02 mm.

3. Equal time intervals between repeated occlusion must be observed. In our experiments the interval between successive occlusions measured 5 min.

4. The duration of the occlusions must be the same and as short as possible. In our experiments it did not exceed 1-2 sec, which is sufficient to allow visual observation of signs of ischemia on the ECG and to perform reperfusion.

EXPERIMENTAL RESULTS

Myocardial ischemia, reproduced in animals, undoubtedly differs from the ischemia observed in human patients with ischemic heart disease. Because of this situation, each new experimental model must be tested for its adequacy. For this purpose, standard antianginal preparations, nitroglycerin and propranolol, as well as dipyridamole, which has an unfavorable effect on the ischemic myocardium [5, 6], are tested on the new model. The model is considered to be adequate only if nitroglycerin and propranolol have a positive, and dipyridamole a negative effect on its working. We studied the action of these drugs on the present model. Nitroglycerin was injected intravenously by drip infusion at the rate of 30-40 μg/min. Injection of the drug began after a stable threshold of onset of ischemia had been established and it continued throughout the experiment. Propranolol was injected intravenously

in a single dose of 0.2 μg/kg, and dipyridamole intravenously in single doses of 0.2 and 0.8 mg/kg. The action of each drug was studied in six experiments on different animals. The experimental results showed that nitroglycerin and propranolol raised the threshold of onset of ischemia. Dipyridamole, in a dose of 0.2 mg/kg does not change the threshold of onset of myocardial ischemia, but in a dose of 0.8 mg/kg it lowers it. It can therefore be concluded from these observations that the present model is adequate for the study of antianginal activity of drugs. The distinguishing feature of this model compared with those using conscious dogs or mokeys is that it is much less laborious. The duration of the operation to implant the occluder does not exceed 40 min. The postoperative mortality is comparatively low (20-30%). Several experiments can be carried out on the same animal, if the necessary time intervals between administration of the drugs are observed.

The present model can therefore be used to study most drugs required to be tested within a comparatively short time, and it thus satisfies one of the essential conditions for effective screening of antianginal preparations.

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ADRENALIN-INDUCED PACEMAKER ACTIVITY IN THE ISOLATED ATRIAL MYOCARDIUM OF MITRAL STENOSIS PATIENTS AND ITS INHIBITION BY ETHMOZINE AND ETHACIZINE

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Investigations on isolated preparations of atrial and ventricular myocardium obtained from patients during surgical operations have shown that pacemaker activity is present in many cells from heart biopsy specimens, and this activity can be regarded as the prototype of an ectopic focus in the whole heart [7]. The problem of how a particular drug is able to inhibit ectopic activity can therefore be studied on fragments of the human heart.

In this investigation the action of new antiarrhythmic drugs of the phenothiazine series, namely ethmozine and ethacizine (the diethylamino analog of ethmozine), on pacemaker activity was studied in atrial preparations obtained from patients with mitral stenosis.

EXPERIMENTAL METHOD

Thin (less than 1 mm^2 in cross-section) strips of myocardium from the trabeculae of the auricles, obtained during mitral commissurotomy operations were used as the object. Altogether 80 preparations from patients with mitral stenosis were studied. The patients had stages II-IV of the disease according to A. N. Bakulev's classification. All patients had a history of circulatory disturbances, and 30% had a rhythm disturbance in the form of atrial fibrillation. After the operation the auricle was placed in cold Ringer's solution and arrived in the labora-

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