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.

LITERATURE CITED

1. J.-P. Closel, P. Théroux, and M. G. Bourassa, Circ. Res., 52, No. 2, 120 (1983).
2. M. A. Gopal, W. A. Neill, and J. M. Oxendine, Cardiovasc. Res., 17, 267 (1983).
3. R. C. Hill, L. H. Kleinman, W. H. Tiller, et al., Am. J. Physiol., 244, H60 (1983).
4. M. W. Sharon and R. I. Bache, Basic Res. Cardiol., 77, 656 (1982).
5. L. Szekeres, V. Csik, and E. Udvary, J. Pharmacol. Exp. Ther., 196, 15 (1976).
6. M. M. Wimbury, in: Advances in Pharmacology, S. Garattini and P. A. Shore, eds., New York (1964), pp. 1-82.

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

T. F. Shklyar, L. V. Rozenshtraukh, V. S. Markhasin, and M. S. Savichevskii

UDC 616.126.42-06:616.125-008.318-02:615.22:547.869.2

KEY WORDS: adrenalin; arrhythmic activity; ethmozine; myocardium.

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-

Laboratory of Biophysics, Sverdlovsk Research Institute of Work Hygiene and Occupational Diseases. Sverdlovsk Inter-Regional Cardiac Surgical Center, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 462-466, October, 1984. Original article submitted March 23, 1984.

0007-4888/84/9810-1396$08.50

TABLE 1. Effect of Ethmozine and Ethacizine on Parameters of Adrenalin-Induced APs

Test substance	AAP, mV	AZ, mV	MDP, mV	(MDP – AZ)/Δt	Frequency of AP per minute	n	Total suppression of activity
Adrenalin	55,3±2,0 (30—81)	32,3±1,2 (21—55)	44,5±1,4 (31—64)	22,2±3,3 (3,3—74)	55,7±5,2 (20—130)	35	
Ethacizine:							
10^{-6}	53,9±5,4 (25—81)	35,3±2,7 (22—50)	46,2±3,7 (27—65)	10,0±5,7 (0,3—57)	26,6±6,6 (0—83)	14	3
$5\cdot10^{-6}$	53,5±9,0 (29—90)	35,2±3,3 (22—45)	45,8±4,8 (30—62)	8,6±2,8 (1,3—20)	20,3±7,2 (0—58)	11	3
10^{-5}	69,4±18 (36—84)	40,6±13,3 (26—51)	51,7±15,4 (32—60)	4,8±2,9 (1—19)	13,4±6,9 (0—46)	9	2
Ethmozine:							
10^{-6}	63±6 (44—84)	33,2±2,6 (27—40)	44,9±3,2 (37—57)	19,0±4,5 (3—35)	44,3±8,4 (23—77)	9	1
$5\cdot10^{-6}$	58,3±6,2 (38—76)	30,6±2,6 (23—34)	42,5±4,1 (34—48)	8,6±4,4 (2—9)	23,5±6,8 (20—47)	8	2
10^{-5}	60,3±7,1 (28—102)	39,2±3,9 (21—74)	49,8±4,4 (29—90)	7,9±3,0 (1,3—12)	25,2±6,0 (6—40)	6	2

Legend. Limits of variations given in parantheses.

tory after 10-15 min, after which the preparation was obtained from a suitable trabecula of the auricle. The strip was fixed in a 5-ml perfusion bath. Krebs' solution, aerated with a mixture of 95% O_2 and 5% CO_2, was passed through the bath at the rate of 20 ml/min. The temperature of the solution was 37 ± 0.5°C and its pH 7.4. The preparation was stimulated by massive platinum electrodes (field stimulation) with above-threshold pulses 5 msec in duration, with an interstimulus interval of 3.3 sec. Mechanical activity was recorded with a 6MKh1S mechanotron. The preparation in the perfusion bath was adapted for 90-120 min until the amplitude of contractions became stabilized. The muscle was then stretched by means of a micrometric device to $L = L_{max}$ (where L_{max} denotes the length at which the force of contractions is maximal) and adrenalin was added in a concentration of 10^{-4} M, as recommended in [3]. After 20-120 min stable and regular pacemaker activity appeared in about 30% of the preparations. External stimulation was switched off and a microelectrode investigation carried out, using the ordinary microelectrode technique. Electrical activity was recorded by glass microelectrodes with a resistance of 10-20 MΩ. Measured parameters of transmembrane action potentials (APs) are indicated in Fig. 1, and the results of these measurements are included in Table 1. The amplitude of AP (AAP), the amplitude of AP from the end of slow diastolic depolarization to the zero potential level (AZ), the maximal diastolic potential (MDP), and the interval from the time of reaching MDP until the end of the slow depolarization phase were measured. The rate of change of AP in phase 4 was thus (MDP – AZ)/Δt. The frequency of pacemaker APs, i.e., the number of APs per minute, also was calculated. In all cases, i.e., after the action of adrenalin or of the tested concentration of the antiarrhythmic drug, at least 20 insertions of the microelectrode into the preparation were made so that mean values of the parameters of AP could be calculated; the insertions were made at a distance of more than 1 mm from the injured ends.

EXPERIMENTAL RESULTS

Types of pacemaker activity arising in myocytes of the atrial myocardium under the influence of adrenalin in a concentration of 10^{-4} M are shown in Fig. 2. Simultaneous recordings of AP and mechanical activity of the muscle are shown in traces 1 and 2. The AP is followed by delayed postdepolarization (traces 1). Mechanical activity of the preparation correlates closely with electrical: For each depolarization there is a corresponding mechanical response. The electrical response recorded in this particular myocyte and mechanical activity of the preparation do not necessarily coincide, for the contractions are not accompanied by electrical activity (curves 2). This fact shows that in this particular preparation foci of pacemaker activity which do not spread all over the preparation are present: This is a phenomenon characteristic of the atrial myocardium of patients with mitral stenosis. Trace 3, recorded on an oscilloscope, is of a type also frequently observed in the myocardium of this group of patients. Adrenalin, added to the perfusion solution, leads to bursts of spontaneous APs, separated by a pause sometimes 10-15 sec in duration.

Trace 4 records oscillator potentials arising in partially depolarized myocytes (AP under 50 mV). In some preparations pacemaker activity may not arise under the influence of adrenalin, but it is easily induced by an external stimulus. The duration of this activity varies from a few seconds to a few minutes, then it subsides, as shown by trace 5.

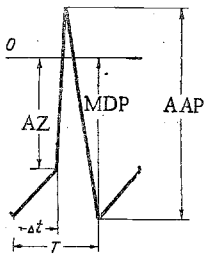

Fig. 1. Diagram showing measurement of parameters of AP (explanation in text).

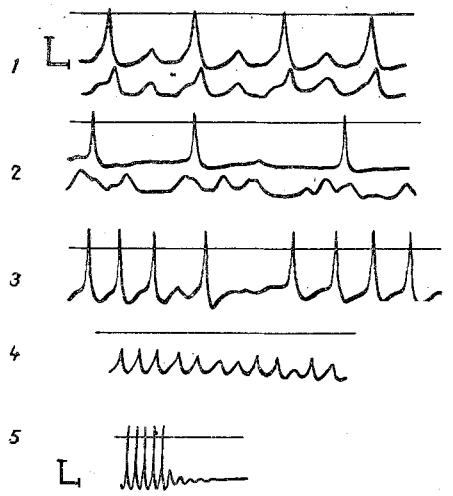

Fig. 2. Types of pacemaker activity of myocytes of atrial myocardium arising in response to adrenalin 10^{-4} M.

Table 1 summarizes data on the parameters of pacemaker electrical activity induced by adrenalin in the isolated atrial myocardium and also under the influence of ethmozine and ethacizine. Parameters of pacemaker activity induced by adrenalin serve as control values (maximal and minimal values of the parameters are shown between parentheses). It will be clear that of 80 preparations studied, pacemaker activity in response to adrenalin was obtained in 35, and in 25 of them this activity was stable. The effect of antiarrhythmic drugs was evaluated on these preparations. The action of ethmozine was studied on 11 and that of ethacizine on 14 preparations.

As Table 1 shows, under the influence of ethmozine in a concentration of 10^{-6} to 10^{-5} g/ml the amplitude of spontaneous AP and the maximal diastolic potential were unchanged, whereas the slope of phase 4 of depolarization falls steadily from 22.2 ± 3.3 mV/sec (control) to 7.9 mV/sec under the influence of ethmozine in a concentration of 10^{-5} g/ml. The decrease in the rate of spontaneous depolarization is matched by a decrease in the frequency of APs from 55.7 ± 5.2 (control) to 25.2 ± 6.0 per minute (ethmazine 10^{-5} g/ml). In precisely the same way no significant changes were found in the amplitude and MPD of spontaneous APs under the influence of ethacizine, but its effect on the rate of diastolic depolarization and the frequency of AP discharges was more marked than that of ethmozine. In a concentration of 10^{-6} g/ml ethacizine reduced the steepness of spontaneous depolarization by 2.2 times — from 22.2 ± 3 mV/sec (control to 10 ± 5.7 mV/sec (ethacizine, 10^{-6} g/ml), and the frequency of the AP discharges also fell by half correspondingly (Table 1). Incidentally, in a similar concentration ethmozine reduced the frequency of the AP discharges by only 20%. In addition, under the influence of increasing concentrations of ethmozine up to 10^{-5} g/ml pacemaker activity was completely suppressed in 33% of preparations, whereas ethacizine, when its concentration was increased from 10^{-6} to 10^{-5} g/ml, suppressed this activity in nearly 60% of of preparations.

Changes in the frequency of AP discharges with an increase in the concentration of the antiarrhythmic drugs in the perfusion solution are shown in Fig. 3. Examples of pacemaker activity induced by adrenalin (a) and changes in that activity under the influence of ethacizine (b) and ethmozine (c) are shown in Fig. 4.

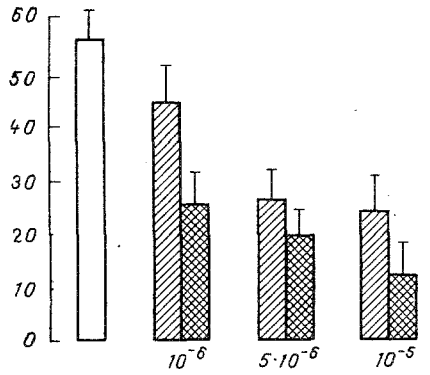

Fig. 3. Diagram of changes in frequency of AP discharge with an increase in concentration of antiarrhythmic drugs from 10^{-6} to 10^{-5} g/ml. Vertical axis: number of APs per minute. Unshaded columns — adrenalin (10^{-4} M); obiquely shaded — ethmozine; cross-hatched columns — ethacizine. Concentration of drug shown below columns (in g/ml).

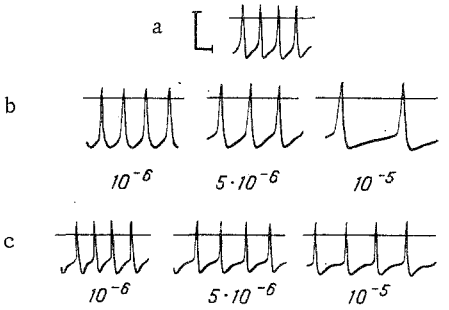

Fig. 4. Effect of ethmozine (c) and ethacizine (b) on myocardial pacemaker activity induced by adrenalin in a concentration of 10^{-4} M (a). Numbers give concentration of drug (in g/ml).

The data indicate that adrenalin-induced pacemaker activity in fibers of the working myocardium is heterogeneous. It includes pacemaker-like APs with slow depolarization in phase 4, pacemaker activity triggered by delayed postdepolarization or by the external stimulus and, finally, oscillations which, on reaching the threshold level, can also lead to a burst of electrical activity. It is important to note that these types of pacemaker activity often arise spontaneously in the pathologically changed myocardium of the human atria and ventricles and, consequently, they can be regarded as potential sources of rhythm disturbances [7].

It was concluded from this electrophysiological and pharmacological analysis of adrenalin-induced pacemaker activity in the human atrial myocardium that its mechanism differ from those observed in the specialized tissue of the heart. In particular, it is considered that pacemake activity in fibers of the pathologically changed human myocardium, treated with adrenalin, is controlled by a current of i_{x_1} type, and not of i_{x_2} type as in the case of Purkinje fibers [4].

Delayed postdepolarization can be obtained in Purkinje fibers by treatment with digitalis [5]. It has been shown that the main contribution to the development of delayed postdepolarization is made by the sodium current, which is perhaps mediated through cyclic release of Ca^{++} from intracellular sources [8]. Delayed postdepolarization arising in fibers of the human atria in response to treatment with adrenalin evidently has a different mechanism. This postdepolarization is inhibited by typical inhibitors of the slow inward calcium current (i_{s1}) — verapamil and AHR-2666 [6].

In general, the investigation of the action of antiarrhythmic drugs with different points of application on pacemaker activity of adrenalin-treated myocardial fibers, namely lidocaine, which increases potassium conductance of the membrane without changing the resting potential [3]; acetylcholine, which also hyperpolarizes the membrane [3] and reduces its conductance for the i_{s1} current; and also TTX, which blocks the inward fast sodium current, showed

that these substances cause virtually no change in the magnitude of delayed postdepolarization, but reduce the rate of development of depolarization in phase 4.

Conversely, blockers of the calcium current (verpamil and AHR-2666) reduced the steepness of spontaneous depolarization in this object and inhibited delayed postdepolarization [3]. These facts show that pacemaker activity in pathologically changed human myocardial fibers treated with adrenalin is due at least in part to the i_{s1} current. This conclusion is confirmed also by the results of the present investigation, which show that delayed postdepolarization is accompanied by a mechanical response of the muscle (Fig. 2, traces 1).

Drugs of the phenothiazine series, namely ethmozine and ethacizine, as was shown previously [1], have a TTX-like action: They reduce the fast sodium current [1]. Ethacizine also appreciably reduces i_{s1} [2]. This may be the explanation of the essentially greater effectiveness of ethacizine compared with ethmozine in reducing adrenalin-induced pacemaker activity in human atrial fibers. Ethacizine can simultaneously inhibit delayed postdepolarization and reduce the steepness of spontaneous depolarization. It will also be noted that the effects of ethacizine in partially depolarized fibers may be stronger, and this must also be taken into account, for there is a considerable population of partially depolarized fibers in the pathologically changed human myocardium [7].

LITERATURE CITED

1. L. V. Rozenshtraukh, N. V. Kaverina, E. P. Anyukhovskii, et al., Kardiologiya, No. 6, 72 (1982).
2. L. V. Rozenshtraukh and V. N. Chikharev, Byull. Éksp. Biol. Med., No. 9, 303 (1980).
3. L. Mary-Rabine, A. J. Hordof, P. Danilo, et al., Cir. Res., 47, 267 (1980).
4. D. Noble, The Initiation of the Heart Beat, Oxford (1975).
5. M. R. Rosen and P. J. Danilo, Cir. Res., 46, 112 (1980).
6. M. S. Siegal, L. Mary-Rabine, and B. F. Hoffman, Bull. N.Y. Acad. Med., 54, 333 (1978).
7. D. H. Singer, C. M. Baumgarten, and R. E. Ten Eick, Prog. Cardiovasc. Dis., 24, 97 (1981).
8. R. Tsien and D. O. Carpenter, Fed. Proc., 32, 2127 (1978).

COMPARATIVE STUDY OF THE EFFECT OF ETHACIZINE AND LIDOCAINE ON BLOOD SUPPLY AND FUNCTION OF THE INTACT AND ISCHEMIC MYOCARDIUM

G. Kh. Goldshteine and G. G. Chichkanov

UDC 616.127-005.4-085.22-036.8-07:[616.127-005+616.127-008.3

KEY WORDS: ethacizine; lidocaine; acute myocardial infarction.

Improvement of the blood supply and functional state of a focus of myocardial ischemia by the use of antiarrhythmic drugs may be an important mechanism lying at the basis of normalization of the rhythm and of the antianginal action of these drugs. However, until very recently there had been little attempt to study the effect of antiarrhythmics on the ischemic myocardium. This applies also to the new antiarrhythmic drug ethacizine, the diethylamino analog of ethmozine, synthesized in the Institute of Pharmacology, Academy of Medical Sciences of the USSR. Accordingly, in the investigation described below the effect of ethacizine on the blood supply and function of the intact and ischemic myocardium was studied and compared with that of lidocaine, a drug widely used in clinical medicine for the treatment of acute myocardial infarction [1-3, 6, 12].

Laboratory of Pharmacology of the Cardiovascular System, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 466-469, October, 1984. Original article submitted November 18, 1983.

0007-4888/84/9810-1400$08.50