EFFECT OF PRACTOLOL AND ATENOLOL ON LYSOSOMAL ENZYME ACTIVITY IN THE RAT VENTRICULAR MYOCARDIUM

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Among the factors determining the successful use of β-adrenoblockers in cardiovascular pathology, their effect on myocardial lysosomes may perhaps play a role. It is claimed that β-adrenoreceptors located on lysosomes are involved in the realization of this effect [3, 4].

The aim of this investigation was to study the character of action of two cardioselective β-adrenoblockers, practolol and atenolol on ventricular myocardial lysosomes of intact rats.

EXPERIMENTAL METHOD

Free and total activity of five lysosomal enzymes and the ratio between the levels of free and total activity after preincubation of myocardial homogenates for 15-45 min with β-adrenoblockers, added to the homogenates up to final concentrations of between 1×10^{-9} and 1×10^{-5} M, were studied. The center of the chosen range of final concentrations corresponds to values of dissociation constants of the between β-adrenoblocker atenolol, with atenolol, with specific binding sites on lysosomes of ventricular myocardium of intact rats, determined previously [4]. Ventricular myocardium of male Wistar rats weighing about 250 g was used. Myocardium from the ventricles of two or three animals, pooled and washed free from blood with ice-cold potassium chloride solution, was used for each separate test. The finely shredded myocardium was washed in homogenization medium (0.25 M solution of sucrose, containing 0.001 M EDTA, pH 7.35-7.40, 0-4°C), forced through a press, made into a 10% homogenate and centrifuged (1100*g*, 10 min, 4°C). Some of the supernatants obtained in this way formed the control series, whereas practolol (ICI, England) or atenolol (All-Union Pharmaceutical Chemical Research Institute, USSR) was added to the rest in final concentrations of 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6} and 1×10^{-5} M. From the control supernatants and a mixture of supernatants with the preparations, the necessary quantity of material was immediately selected to determine the protein content and the initial free and total enzyme activity, whereas most of the material was preincubated (without substrates) at 37°C in a water thermostat, taking samples for determination of enzyme activity after 15, 30, and 45 min of preincubation. Acid phosphatase (EC 3.1.3.2, substrate glycerol-2-phosphate, from "Merck"), acid deoxyribonuclease (EC 3.1.4.6, substrate chick erythrocyte DNA, from "Reanal"), cathepsin D (EC 3.4.23.5, substrate bovine hemoglobin, from "Reakhim"), β-glucosidase (EC 3.2.1.21, substrate 4-nitrophenyl-β-D-glucopyranoside, from "Serva"), and β-galactosidase (EC 3.2.1.23, substrate 4-nitrophenyl-β-D-galactopyranoside, from "Serva") were determined spectrophotometrically [2] in two or three parallel tests. The number of independent experiments to determine enzyme activity in the presence of each concentration of preparations was not less than 5-7. The results were subjected to statistical analysis by Student's *t* test.

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TABLE 1. Effect of β-Adrenoblockers on Initial Total Activity of Lysosomal Enzymes in Rat Ventricular Myocardium (M ± m)

Enzyme	Control	Practolol, M					Atenolol, M				
		$1 \cdot 10^{-9}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-7}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-5}$	$1 \cdot 10^{-9}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-7}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-5}$
Acid phosphatase	6,36±0,90	4,16±0,62	5,88±0,87	8,13±1,02	1,36±0,20*	0,94±0,22*	2,04±0,30*	0,48±0,07*	4,51±0,82	2,22±0,71*	1,20±0,15*
Acid deoxyribonuclease	6,43±0,92	9,11±1,36	12,15±2,22*	12,14±1,36*	41,00±6,20*	26,90±4.01*	6,78±1,02	6,94±0,72	5,04±0,90	3,87±0,98	8,62±1,00
Cathepsin D	0,058±0,006	0,015± 0,004*	0,021± 0,005*	0,028± 0,001*	0,022± 0,001*	0,012± 0,002*	0,018± 0,004*	0,024± 0,006*	0,016± 0,007*	0,012± 0,002*	0,013±0,004*
β-Glucosidase	19,90±2,05	5,13±0,27*	9,24±1,50*	5,28±0,40*	2,16±0,44*	2,96±0,21*	12,91±2,55*	38,76±1,63*	21,29±3,02	8,80±1,07*	8,98±1,02*
β-Galactosidase	29,71±4,63	10,53±1,62*	5.46±0,62*	2,79±0,24*	3,75±0,37*	6,48±0,34*	12,01±1,44*	8,19±0,44*	26,75±3,66	7,06±0,85*	2,92±0,44*

Legend. *p < 0.05: Significance of differences compared with control; acid phosphatase activity expressed in nanomoles inorganic phosphate/min/mg protein at 37°C, of acid deoxyribonuclease in nanomoles acid-soluble mononucleotides/min/mg protein at 37°C, of cathepsin D in ΔE_{280}/min/mg protein at 45°C, and of β-glucosidase and β-galactosidase, in nanomoles *p*-nitrophenol/min/mg protein at 37°C.

*p < 0.05: Significance of differences compared with control.

TABLE 2. Effect of Preincubated of Homogenates for 15 min with β-Adrenoblockers on Ratio (in %) of Free to Total Enzyme Activity (X ± m)

Enzyme	Control	Practolol, M					Atenolol, M				
		$1 \cdot 10^{-9}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-7}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-5}$	$1 \cdot 10^{-9}$	$1 \cdot 10^{-8}$	$1 \cdot 10^{-7}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-5}$
Acid phosphatase	61,8±4,7	12,6±0,8*	10,8±0,3*	13,5±1,8*	25,0±0,6*	65,9±3,5	17,7±0,04*	56,2±5,6	13,8±0,5*	'2,4±1,5*	35,6±4,8*
β-Galactosidase	79,4±8,5	95,5±1,2	45,0±12,6*	39,4±0,5*	62,6±6,2	26,1±5,0*	31,2±4,4*	23,8±2,4*	29,1±2,3*	76,2±4,2	32,9±5,6*

EXPERIMENTAL RESULTS

The average tendencies of samples of initial total activity of four of the enzymes (except cathepsin D) differed appreciably under the influence of practolol and atenolol. For example, acid deoxyribonuclease activity did not exceed the control level under the influence of atenolol over the whole concentration range, whereas in the presence of practolol it rose; under the influence of atenolol in a concentration of 1×10^{-7} M β-glucosidase activity was high, whereas under the influence of practolol, in the corresponding concentration, it fell, and so on (Table 1). However, preincubation of homogenates with preparations smoothed out the differences characteristic of the original activity. For instance, after preincubation total β-glucosidase activity was below the control level under the influence of both practolol and atenolol in all concentrations tested. After preincubation for 30 min the differences in the effect of the two β-adrenoblockers on acid deoxyribonuclease activity began to disappear: despite the fact that in the presence of atenolol activity of this enzyme was lower than in the presence of practolol, it nevertheless exceeded the control level. For example, after 30 min total acid deoxyribonuclease activity in the presence of practolol (1×10^{-8} M) amounted to 11.13 ± 2.31 nmole acid-soluble mononucleotides/min/mg protein, whereas in the presence of atenolol in the same concentration it was 5.05 ± 0.72 nmole compared with 2.83 ± 0.65 nmole in the control (in both cases $p < 0.05$).

Calculation of the ratio of free to total enzyme activity, which enables the stability of the lysosomal membranes to be judged to a certain degree, showed that addition of β-adrenoblockers to the homogenates at least did not destabilize the lysosomal membranes. Peak values of the ratio of free to total enzyme activity in the control corresponded to the time from 15 to 30 min of exposure of the homogenates to heat. After preincubation of the homogenates for a similar duration with the preparations, the ratio of free to total activity was lower than or, at least, equal to the control level. Values of the ratio calculated for activity of two enzymes are given by way of example in Table 2.

Both β-adrenoblockers, added to homogenates of ventricular myocardium, basically altered the activity of the lysosomal enzymes studied in the same direction: activity of acid phosphatase, cathepsin D, β-glucosidase, and β-galactosidase, under the influence of the preparations, developed a tendency toward inhibition, whereas acid deoxyribonuclease activity tended to increase. Differences in the effects of practolol and atenolol on enzyme activity were most frequently abolished by lengthening exposure of the homogenates to the preparations: our observations showed that practolol could cause marked changes in activity of the test enzymes immediately after addition to the homogenates, but to obtain similar

changes in enzyme activity through the action of atenolol the homogenates had to be preincubated with the preparation. Practolol and atenolol, which are cardioselective β_1-adrenoblockers, differ structurally (only slightly, but nevertheless this is reflected in their lipid solubility), in relation to their β-adrenoblocking effect and the presence of internal sympathomimetic activity (practolol possesses this property whereas atenolol does not [1]). In the intact organism, these differences may be very slight, especially if changes in integral parameters are examined, but in vitro, taken together they may be sufficient to bring to light individual differences in the effect of the preparations on biochemical characteristics. The different lipophilicity (less for atenolol) evidently was responsible for the difference in the length of contact of the preparations with homogenates required to exhibit appreciable changes of enzyme activity. We know that when atenolol is given by the bolus method, it cannot inhibit the response of the isolated perfused rat heart to noradrenalin, but the prolonged action of atenolol is effective [8]. In the present experiments the duration of preincubation of atenolol with the hydrogenates had to be increased, for because of its low lipophilicity, it evidently could not "enter" into interaction with the biological structures.

In our view, on the addition of atenolol and practolol to the homogenates their effect on lysosomal enzyme activity and on the redistribution of enzyme activity between lysosome-bound and the free state was realized primarily through complex formation with the β-adrenoreceptors of the lysosomes. These were evidently the β_1-adrenoreceptors because there are no β_2-adrenoreceptors in the ventricular cardiomyocytes of rats [6]. By carrying out the experiment in this way we ruled out the possibility of a change in enzyme activity after their accumulation in the lysosomes without the intervention of β-adrenoreceptors (lysomotropism), for accumulation of this type requires structural integrity of the cell [5, 7]. Both in homogenates and in the whole animal, "screening" of β-adrenoreceptors by β-adrenoblockers from the action of catecholamines may lead to a change in the function state of the lysosomal system of the cardiomyocytes.

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