EFFECT OF CALMODULIN INHIBITORS ON CONTRACTION AND RELAXATION OF HEART MUSCLE

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Calmodulin plays an important role in the Ca^{++} transport system in many types of cells. Being highly sensitive to Ca^{++}, it can bind with a special protein kinase, which subsequently phosphorylates the kinase of myosin light chains, phospholamban, a protein of the sarcoplasmic reticulum, and the kinase of glycogen phosphorylase [7, 10, 12, 14, 15]. The role of calmodulin in myocardial contractile function has not yet been studied. This is evidently because there are as yet no methods of inhibiting the functions of this protein selectively. Known calmodulin blockers (trifluoperazine, chlorpromazine, etc.) can act also on another Ca^{++}-binding protein, namely troponin C [11], and they thus produce a complex picture of changes in myocardial contractility. In an attempt to find selective calmodulin blockers the effects of more than 30 different pharmacologic agents on calmodulin, calmodulin-dependent phosphodiesterase, troponin C, and actinomycin ATPase, controlled by troponin, have been investigated.

In the investigation described below the action of two calmodulin inhibitors — trifluoperazine and frenolon (metofenazate), differing in their affinity for troponin C, was studied.

EXPERIMENTAL METHOD

Experiments were carried out on isolated papillary muscles of the guinea pig right ventricle. The conditions of mechanical loading were such that contraction and relaxation depended maximally on binding of Ca^{++} ions with their removal from myofibrils. This condition is satisfied by isotonic contraction with a small load stretching the muscle at rest and raised by it during contraction. Under these circumstances the velocity of shortening and subsequent lengthening are maximal. The muscles had a calculated area of cross section of 0.73 ± 0.05 mm^2, and the stretching load was 0.15 ± 0.01 g/mm^2. The muscles were contracted by electrical stimulation in Krebs' solution, saturated with 95% O_2 + 5% CO_2, pH 7.3-7.4, at 29°C. The shortening signal, obtained from a photoelectric transducer, and its first derivative were recorded on a two-channel "Gould Brush 2200" recorder. Details of the technique were described previously [1]. In each experiment the frequency of contractions was increased stepwise from 0.5 Hz until the time of reaching the maximal increase in the contraction velocity, which was observed at a frequency of 2.0-2.5 Hz. Next, after the frequency had been reduced to 1 Hz, trifluoperazine (five experiments) or metofenazate (10 experiments) was added to the solution in increasing concentrations, and at each concentration (after 30 min) a stepwise increase in contraction frequency was repeated. The maximal velocity of contraction and relaxation was expressed as a ratio of muscle length in the usual way. ATPase activity of native and partially desensitized actomyocin was determined by the method of Schaub et al. [13]. A preparation of partially purified Ca^{++}-dependent cAMP phosphodiesterase from rabbit heart was obtained by Wang's method in the modification described previously [5]. cAMP phosphodiesterase activity was estimated by a modified method of Kincaid et al. [9]. Calmodulin was isolated by the method of Gopalakrishna and Anderson [8]. Binding of trifluoperazine and metofenazate was estimated by measuring fluorescence of 3',3-dipropyl-thiocarbocyanin iodide [dis-C_3-(5)] in the presence of calmodulin and troponin C [4]. A homogeneous preparation of troponin C was generously provided by N. B. Gusev (Department of Biochemistry, Faculty of Biology, Moscow University).

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TABLE 1. Parameters of Affinity of Trifluoperazine and Metofenazate for Calmodulin and Troponin

Parameter measured	Trifluoperazine, μM	Metofenazate, μM
Calmodulin-stimulated phosphodiesterase*	4	7
$K_{0.5}$ of displacement of fluorescent dis-$C_3(5)$ dye from complex:		
With calmodulin	4	6
With troponin C	24	>1000

*Inhibitory effect of trifluoperazine and metofenazate without calmodulin is exhibited only in concentrations of over 100 μM.

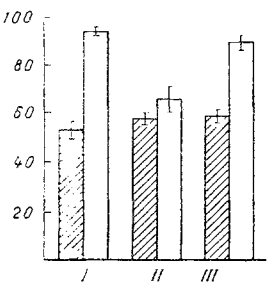

Fig. 1

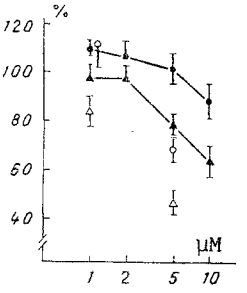

Fig. 2

Fig. 1. Action of trifluoperazine and metofenazate (100 μM) on actomyocin ATPase activity stimulated by Ca^{++} ions. I) Control, II) trifluoperazine, III) metofenazate. Shaded columns represent without Ca^{++}, unshaded columns with Ca^{++}. Activity expressed in nanomoles P_i/min/mg protein (M ± m).

Fig. 2. Dependence of velocity of contraction (circles) and relaxation (triangles) of papillary muscles on concentration of trifluoperazine (empty symbols) or metofenazate (filled symbols) in % of initial values (M ± m).

EXPERIMENTAL RESULTS

A study of the affinity of various phenothiazines (trifluoperazine, chlorpromazine, ethmozine, ethacizine, metofenazate, levomepromazine, thioproperazine) to calmodulin and troponin C led to the isolation of two inhibitors with equal affinity for calmodulin but with different affinity for troponin C (Table 1). The inhibition constants of the two calmodulin-dependent reactions for trifluoperazine and metofenazate were about equal, whereas the affinity constants for troponin C differed by more than 40 times. Their effect on Ca^{++}-stimulated ATPase activity of actomyocin also differed (Fig. 1): Trifluoperazine in a high concentration (100 μM) was able to inhibit it completely, whereas metofenazate had no effect. Thus metofenazate is a more specific blocker of calmodulin-dependent processes than trifluoperazine.

In experiments on the papillary muscles both trifluoperazine and metofenazate had a dose-dependent effect mainly on relaxation of heart muscle. At a frequency of 1 Hz the maximal velocity of relaxation (1.02 ± 0.11 of muscle length per second) was significantly higher than the maximal velocity of contraction (0.55 ± 0.03 of muscle length per second). Metofenazate, in a concentration of 1 μM, significantly increased the velocity of muscle contraction by 10%, but did not affect the velocity of relaxation (Fig. 2). In a concentration of 5-10 μM metofenazate reduced the velocity of relaxation by 21-30%, whereas the velocity of contraction was not significantly changed. The action of trifluoperazine in a concentration of 1 μM was about the same as that of metofenoazate, but in a concentration of 5 μM trifluoperazine lowered both parameters significantly more strongly than metofenazate. This effect was most probably due to the fact that trifluorperazine has much higher affinity for troponin than has metafenazate (Table 1; Fig. 1).

A common effect of both substances was a greater reduction in the velocity of relaxation than the velocity of contraction. This result suggests that the main role of calmodulin is in regulating relaxation, and not contraction, thanks to its ability to stimulate Ca^{++} uptake into the sarcoplasmic reticulum [10, 12].

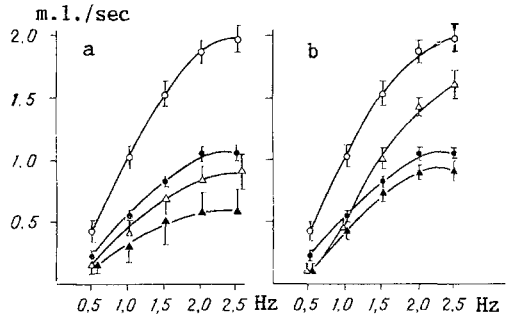

Fig. 3. Dependence of velocity of contraction (filled symbols) and relaxation (empty symbols) of papillary muscles on frequency of contractions (in Hz). Circles, control; triangles, action of trifluorperazine (*a*, 5 μM) and metofenazate (b, 10 μM). Values expressed in muscle length units/sec (M ± m).

In the initial state an increase in the frequency of contractions, accompanied by elevation of the Ca^{++} concentration in the myoplasm characteristic of the myocardium of most species of mammals [4] regularly increased the velocity of both relaxation and contraction; the difference between them, moreover, was greater at a higher frequency (Fig. 3). The action of trifluoperazine was combined with lowering of the values at any frequency, but the relative increase in the velocity of contraction remained the same as in the control, due to a decrease in the initial value at a low frequency of contraction. This result is in agreement with preservation of the relative increase in tension developed by the rat heart in response to an increase in the Ca^{++} concentration or addition of isoproterenol against the background of trifluoperazine [15].

The velocity of relaxation was reduced in the present experiments by trifluoperazine by a greater degree, and accordingly at any frequency the almost twofold difference between the velocities of relaxation and contraction disappeared (Fig. 3). Metofenazate, unlike trifluoperazine, did not change the dependence of the velocity of contraction on frequency, but it also reduced the rate of relaxation; however, at a high frequency this effect was relatively smaller, evidently because, with an increase in the Ca^{+} concentration in the myoplasm, the process of removal of Ca^{++} from the myoplasm was activated by other mechanisms also [2, 3, 15]. A self-regulating acceleration of relaxation with an increase in the intensity of contractile function is thus operated [3]. However, this basic reaction may perhaps take place not so perfectly or so quickly as that with intact calmodulin.

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