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EFFECT OF TAURINE ON PROPERTIES OF GUANYLATE CYCLASE OF THE SARCOPLASMIC RETICULUM OF THE HEART

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The role of taurine in the regulation of cardiac contractile activity is interesting to the cardiologist. Contractile processes in muscle tissue are known to be regulated by the intracellular distribution of calcium ions. There is evidence to suggest that the role of taurine is to control the movement of Ca^{++} in myocardial cells [3, 6]. Outflow of Ca^{++} and its accumulation in the sarcoplasmic reticulum (SPR) of the heart are processes which determine contraction and relaxation of the myocardium.

It has been shown on skeletal and cardiac muscles that with an increase in the taurine concentration the inflow of Ca^{++} into SPR and its binding are increased [4, 7]. In addition to these observations it has been found that in the presence of taurine activity of transport Ca-ATPase in SPR isolated from the rat and dog heart is unchanged [2, 5]; taurine does not affect ATP-dependent Ca^{++} transport [14].

High activity of guanylate cyclase (GC) in the SPR of the heart has recently been reported [11, 15]. The connection between GC activity and Ca^{++} transport in the microsomes of the heart is not yet clear, but it seems that the cyclic guanosine monophosphate (GMP) formed as a result of the guanylate cyclase reaction must influence movement of Ca^{++} in the SPR of the myocardial cells, if it is recalled that cyclic GMP increases the outflow of Ca^{++} from smooth muscle microsomes [1]. It can be tentatively suggested that the effect of taurine mentioned above on the inflow and binding of Ca^{++} in the SPR is effected through GC.

In the present investigation the effect of taurine was studied on GC activity in SPR isolated from the guinea pig heart.

EXPERIMENTAL METHOD

Fresh hearts, washed with physiological saline to remove blood, were homogenized in a Virtis-45 homogenizer (15 sec at 4500 rpm and 15 sec at 71,000 rpm) in isolation medium containing 10% sucrose, 25 mM

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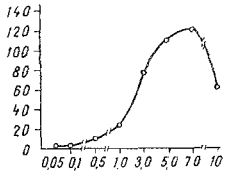

Fig. 1

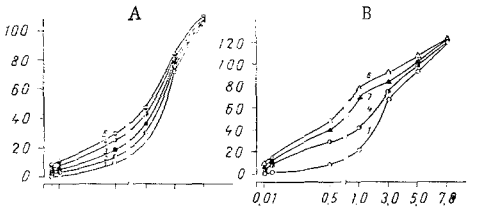

Fig. 2

Fig. 1. Effect of different concentrations of $MnCl_2$ on GC activity. SPR 6 μg per sample, GTP concentration 1 mM. Here and in Fig. 2: abscissa, Mn^{++} concentration (in mM); ordinate, GC activity (in picomoles cyclic GMP/mg protein/min).

Fig. 2. Effect of $MgCl_2$ and taurine on GC activity. A: 1) Without $MgCl_2$, 2) 0.25 mM $MgCl_2$, 3) 0.5 mM $MgCl_2$, 4) 1 mM $MgCl_2$, 5) 5 mM $MgCl_2$. SPR 7 μg per sample, final GTP concentration 1 mM. B: 1) Without $MgCl_2$, 4) 1 mM $MgCl_2$, 6) 1 mM $MgCl_2$ + 0.4 mM taurine, 7) 1 mM $MgCl_2$ + 10 mM taurine. SPR 6 μg per sample, final GTP concentration 1 mM.

Tris-HCl buffer, 4 mM sodium azide, and 0.5 mM dithiothreitol (pH 7.6). The homogenate was centrifuged for 20 min at 10,000 rpm on the RC-5 centrifuge. The supernatant was centrifuged for 20 min at 12,000 rpm and for 30 min at 38,000 rpm on the L5-50 centrifuge. The residue was suspended manually in a glass homogenizer with Teflon pestle in purification medium containing 0.6 M KCl, 10 mM Tris-HCl buffer, and 10% sucrose (pH 7.2). The suspension was centrifuged for 30 min at 38,000 rpm. The residue was suspended in keeping medium containing 40% sucrose and 10 mM Tris-HCl buffer (pH 7.2), poured in volumes of 200 μl into test tubes, frozen, and kept at -70°C. The protein concentration in the final preparation was determined by Lowry's method.

GC activity was determined from the rate of formation of cyclic GMP from guanosine triphosphate (GTP) during incubation of the SPR preparation for 10 min at 37°C in 300 μl of medium of the following composition: 50 mM Tris-HCl buffer, 1 mM theophylline, 10 mM creatine phosphate, and 19 μg creatine phosphokinase (activity 140 units/mg), pH 7.45). The protein concentration in the sample was 6-8 μg. The SPR was preincubated for 10 min at 37°C, after which GTP was added to the medium and, 10 min later, the reaction was stopped by boiling the sample for 2 min on a waterbath. The samples were centrifuged for 10 min at 4000 rpm, and a 100-μl sample of the supernatant was withdrawn to determine cyclic GMP. Cyclic GMP in the sample was assayed radioimmunologically using kits from the Radiochemical Centre, Amersham (England), in accordance with the instructions supplied. When the effect of Mn^{++}, Mg^{++}, Ca^{++}, β-alanine, and taurine on GC activity was studied the substances were added in the necessary concentrations to the incubation medium.

The following reagents were used: Tris-HCl, GTP, EGTA, theophylline, β-alanine, sucrose, dithiothreitol, and sodium azide from Sigma, USA; Mn^{++}, Mg^{++}, Ca^{++}, and taurine (from Merck, West Germany); creatine phosphate and creatine phosphokinase from Calbiochem, USA.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that GC activity increased with an increase in the Mn^{++} concentration in the incubation medium from 0.05 to 7 mM to reach a maximum at 7 mM (121.7 ± 8.7 picomoles cyclic GMP/mg protein/min). Taurine in concentrations of 0.2-20 mM (from its subcellular to its intracellular concentration in guinea pig heart cells [9]) did not affect GC activity in the presence of 0.5-3 mM Mn^{++}. In the presence of 10 mM Mn^{++} GC activity increased with an increase in the taurine concentration in the incubation medium (Table 1).

Mg^{++} ions (0.25-1.0 mM) did not affect GC activity in medium without Mn^{++} but stimulated it in the presence of Mn^{++} in concentrations from 0.1 to 1.0 mM (Fig. 2A). The maximal effect of Mg^{++} was observed when the Mg^{++}: Mn^{++} ratio was 2:1. GC activity increased by 2-2.5 times under the influence of 0.4-10 mM taurine if Mg^{++} and Mn^{++} were present at the same time in the medium compared with the effect of Mg^{++} alone (Fig. 2B).

To assess the specificity of action of taurine on GC activity in medium with Mg^{++} and Mn^{++}, the effect of β-alanine, a structural analog of taurine, on GC was investigated under the same conditions (Table 2). It was

TABLE 1. Effect of Taurine on GC Activity (in picomoles cyclic GMP/mg protein/min) in SPR of Guinea Pig Heart (M ± m; n = 10)

Experimental conditions	Taurine concentration, mM			
	0	0.2	2	20
10	62,92±2,49	75,69±5,89	89,87±7,12	108,57±3,12
3	74,15±3,4	78,72±2,86	85,53±9,1	76,80±5,4
1	21,90±2,45	19,28±1,88	21,45±1,37	22,92±1,22
0,5	9,13±1,68	7,83±3,9	9,35±2,47	9,25±1,13

TABLE 2. Changes in GC Activity (in %) under the Influence of Taurine and β-Alanine

Experimental conditions	Without β-amino acids	0.4 mM β-alanine	0.4 mM taurine
0,5 mM $MnCl_2$	100	84,1	100
0,5 mM $MnCl_2$ + 1 mM $MgCl_2$	228	130	519

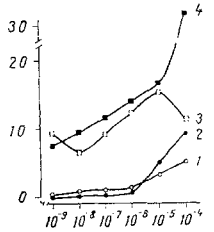

Fig. 3. Effect of different $CaCl_2$ concentration on GC activity. 1) 0.5 mM $MnCl_2$, 2) 0.5 mM $MnCl_2$ + 0.4 mM taurine, 3) 0.5 mM $MnCl_2$ + 1 mM $MgCl_2$, 4) 0.5 mM $MnCl_2$ + 1 mM $MgCl_2$ + 0.5 mM taurine. SPR 7 μg per sample, final GTP concentration 1 mM. Abscissa, Ca^{++} concentration (in mM); ordinate, GC activity (in picomoles cyclic GMP/mg protein/min).

found that 0.4 mM β-alanine inhibits GC activity by 15 and 49% respectively in the absence and presence of Mg^{++}. The effect of taurine is stimulating in character and is manifested only in conjunction with Mg^{++} and Mn^{++}.

Considering that SPR regulates the Ca^{++} concentration in the myoplasm and exhibits high guanylate cyclase activity, it was decided to investigate how GC activity depends on the Ca^{++} concentration in the medium. A Ca^{++} concentration of between 10^{-9} and 10^{-6} M was created with the aid of Ca-EGTA buffer.

As Fig. 3 shows, GC activity increased if the Ca^{++} concentration exceeded 10^{-7} M. Addition of 1 mM Mg^{++} to the incubation medium was followed by a marked increase in GC activity. Under these conditions GC activity increased proportionally to the increase in Ca^{++} concentration from 10^{-8} to 10^{-5} M and decreased if its concentration was 10^{-4} M. Taurine in medium both without Mg^{++} and containing Mg^{++} and Mn^{++} at the same time, had little effect on GC activity in the presence of Ca^{++} in concentrations of 10^{-8} to 10^{-6} M, but caused a marked increase in GC activity in the presence of 10^{-4} M $CaCl_2$.

The study of the properties of guanylate cyclase of the SPR fraction isolated from the guinea pig heart showed that GC actitivy depends on many factors: Mn^{++}, Mg^{++}, Ca^{++}, and β-amino acids. GC activity in SPR of heart tissue, just as in SPR of skeletal muscles [10], is directly dependent on Mn^{++} and increases with an increase in the concentration of these ions in the incubation medium. Without Mn^{++}, $MgCl_2$ does not affect GC activity. The results confirmed previous studies where an increase in the sensitivity of GC to taurine

was observed. In the absence of Mg^{++}, but in the presence of 10 mM $MnCl_2$, GC activity increased proportionally to the increase in the taurine concentration in the incubation medium.

These results are evidence that GC is an enzyme which reacts to many factors in the medium, and the combination of these factors, as reflected in qualitative and quantitative indices, determines the degree of activity of GC. These facts point to the possibility that the enzyme activity of GC may be regulated in vivo through a change in the intracellular concentrations of Mg^{++}, Ca^{++}, and taurine, which occurs both in normal physiological states (the change in the Ca^{++} concentration in the cytoplasm in systole and diastole [10]), and under pathological conditions (an increase in the taurine concentration in the myocardium in heart failure [8]).

Investigation of the role of Ca^{++} in activation of GC in the SPR showed that the activity of the enzyme was unchanged in the presence of 10^{-8}-10^{-7}M $CaCl_2$ and increased if the Ca^{++} concentration in the medium was 10^{-6}-10^{-4}M. A similar relationship has also been found in skeletal muscles [12].

Myofibrils are known to contract if the free Ca^{++} concentration is the sarcoplasm reaches 10^{-6}-10^{-5}M [10]. The increase in GC activity under the influence of high Ca^{++} concentrations suggests that Ca^{++}, which stimulates GC under these conditions, causes the formation of considerable quantities of cyclic GMP which, in turn, can facilitate the "pumping" of Ca^{++} into the SPR, converting the act of contraction into relaxation. Taurine evidently plays an important role in this process for, although itself it does not affect GC activity, it causes a sharp increase in such activity in the presence of 10^{-4}M Ca^{++}, irrespective of whether the medium contains Mg^{++} or not. The effect of taurine on GC is mediated by Ca^{++} and Mg^{++} ions in physiological concentrations.

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