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THE PROTECTIVE EFFECTS OF TAURINE ON HYPOXIA (PERFORMED IN THE ABSENCE OF GLUCOSE) AND ON REOXYGENATION (IN THE PRESENCE OF GLUCOSE) IN GUINEA-PIG HEART

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Abstract—In isolated guinea-pig heart submitted to hypoxia in the absence of substrate and subsequent reoxygenation 1–20 mM taurine decreases LDH release and ventricular arrhythmias, and the recovery of normal electrical and mechanical activity is increased. The taurine effect is dose-dependent, and is not mimicked by β-alanine. Moreover, taurine reduces the increase in calcium gain of reoxygenated heart.

The consequences of inducing myocardial hypoxia include a rapid decline in contractile force with a subsequent increase in resting tension if oxygen deprivation continues [1], leakage of enzyme [2] and development of ventricular arrhythmias. The reintroduction of oxygen to the heart submitted to hypoxic perfusion determines a paradoxical escalation of already existing tissue damage (oxygen paradox) [2–4]. Nakanishi *et al.* [5] found that Ca^{2+} uptake is not changed during hypoxia, but it is increased during reoxygenation. It is known that taurine is lost during hypoxia from rat heart [6], possesses antiarrhythmic activity in different models of arrhythmias [7–9] and modulates the calcium availability. In fact, it prevents the calcium paradox [10, 11] and the severity of lesions on the cardiomyopathy of the hamster [12], while in guinea-pig heart it decreases the positive inotropic effect due to an increasing concentration of calcium, but shows a positive inotropic effect at low perfusate Ca^{2+} concentrations [13]. We therefore decided to investigate the effect of taurine in a model of hypoxia performed in the absence of substrate followed by reoxygenation and re-exposure to glucose.

MATERIALS AND METHODS

Guinea-pigs (250–350 g) were used for this study. The animals had free access to standard diet and water.

Heart perfusion protocol. Animals were killed by a blow on the head and the hearts were removed and arranged for perfusion using a non-recirculating Langendorff system. The perfusion fluid had the following composition (mM): NaCl 137, KCl 2.7, $MgCl_2$ 0.11, $CaCl_2$ 1.8, $NaHCO_3$ 12, NaH_2PO_4 0.42 and glucose 5. The hearts were perfused at a constant flow of 6 ml/min with a LKB 215-multiperpex peristaltic pump. The perfusion medium was gassed with O_2–CO_2 (97–3%) except as noted below, and the entire system was thermoregulated at 37° by allowing the fluid to pass through a temperature-controlled coiled glass condenser immediately before entering the heart and by enclosing the heart in a glass water jacket. The pH of the buffer was 7.4 ± 0.02 and was unaffected by any treatment. The apex of the heart was connected to a force transducer; 1 g of resting tension was applied. In addition, surface electrical activity was obtained by attaching two platinum electrodes to the epicardium connected to a Battaglia Rangoni electrocardiograph. The hearts were initially equilibrated for 45 min, then perfused with a glucose-free medium gassed with a mixture of N_2–CO_2 (97–3%) (hypoxic phase). Following the hypoxic phase the hearts were reperfused for 30 min with the original medium gassed with O_2–CO_2 (97–3%) (reoxygenation phase). The drugs used in this study were as follows: taurine (1–20 mM) and 10 mM β-alanine; both substances were from Merck, Darmstadt, Germany. The administration of drugs started with the onset of the hypoxic phase and was maintained through the experiments. Analysis of ventricular arrhythmias was performed as follows: ventricular premature beats (VPbs), a large and aberrant QRS complex and absence of preceding P wave; ventricular tachycardia (VT) was considered if more than five VPbs consecutively occurred; ventricular fibrillation (VF), complete irregularity of the morphology of the repetitive complex for at least 10 cycles. Recovery of normal electrical activity was considered to exist when ventricular arrhythmias disappeared accompanied by a recovery of sinus rhythm.

Lactate dehydrogenase (LDH) estimation. Samples of effluent were collected at the time indicated in Fig. 1 and assayed for LDH content on the same day. LDH was measured spectrophotometrically, according to Wrobleski and La Due [14].

Calcium estimation. Dried tissues were digested in concentrated HNO_3 and the calcium concentration was measured using an atomic absorption spectrophotometer (Perkin–Elmer 303) in the presence

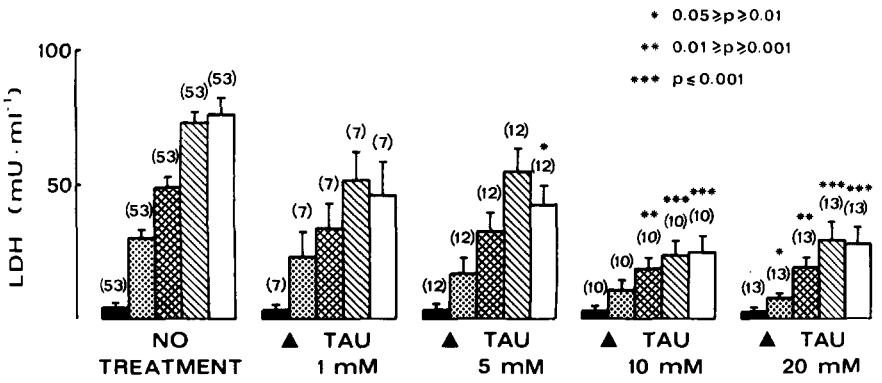

Fig. 1. LDH release during last 5 min of stabilization (■) 10th–15th (▨) and 25th–30th (▩) min of hypoxic phase and 5th–10th min (▧) and 15th–20th (□) min of reoxygenation phase. Results are mean ± S.E.; in brackets the number of experiments.

of $LaCl_3$ in dried samples as described in Dolara *et al.* [15].

Taurine estimation. Taurine was measured in the liophylized effluent after perchloric extraction. The taurine was assayed by HPLC according to Kruzs *et al.* [16].

Statistical analysis. Statistical analysis of differences in the incidence of arrhythmias and in the recovery of normal electrical activity was carried out using the Chi-square test. For changes in LDH release, contraction force, and basal tone, an unpaired Student's *t*-test was used.

RESULTS

In untreated hearts LDH release increases after the onset of the hypoxic phase, and within 10–15 min is significantly higher ($P \leq 0.001$) than that measured in the effluent obtained during the last 5 min of stabilization. The release of the enzyme is more marked during the 25–30 min of the hypoxic phase and is considerably increased on re-exposure of the hearts to a medium containing glucose and gassed with a mixture of O_2–CO_2. The administration of taurine, which starts with the onset of the hypoxic phase, attenuates the outflow of the enzyme both in hypoxic and reoxygenation phases (Fig. 1). Taurine is most active against the exacerbation of enzyme leakage caused by re-introducing glucose and oxygen. During the first minutes of the hypoxic phase (10–15 min), untreated hearts consistently increase the release of taurine, that returns to normal values during the last period of the hypoxic phase and during the reoxygenation phase. Taurine release does not run parallel to LDH release (Fig. 2). The onset of the hypoxic phase results in a rapid attenuation of the total myocardial force (Fig. 3). During

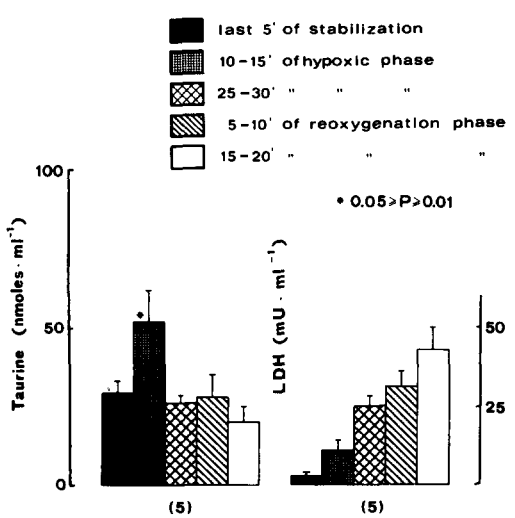

Fig. 2. Taurine and LDH release measured in the perfusate obtained from non-treated hearts submitted to hypoxia and reoxygenation. Results are mean ± S.E.; in brackets the number of experiments.

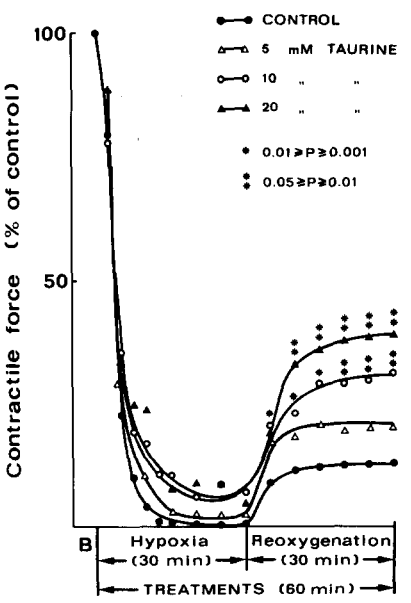

Fig. 3. % changes in contractile force of the heart perfused under the hypoxic phase for 30 min and under the reoxygenation phase for 30 min. Taurine treatment starts with the hypoxic phase and is continued throughout the experiments. Each value is the mean of at least 10 experiments.

Table 1. Percentage of hearts presenting ventricular arrhythmias during hypoxic and reoxygenation phases

	Percentage				
		Taurine (mM)			
	No drugs	1	5	10	20
(1) Hypoxic phase					
VF	31	29	8**	15*	15*
VT	35	57	8**	0**	0**
VPbs	74	86	85	54*	46**
(2) Reoxygenation phase					
VF	57	29**	13**	31**	31**
VT	69	86	38**	46**	8**
VPbs	86	100	92	92	92
N	65	7	13	13	13

The Chi-square test was used to detect significant differences between control and treated preparations. N = number of experiments; VPBs = ventricular premature beats; VF = ventricular fibrillation; VT = ventricular tachycardia.

* $0.01 \geq P \geq 0.001$; ** $P \leq 0.001$.

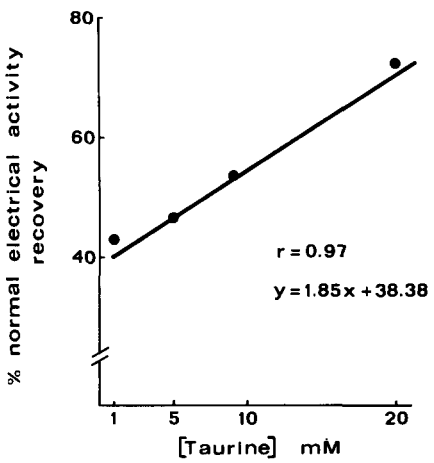

Fig. 4. Effect of taurine on the recovery of normal electrical activity after 30 min of reoxygenation. Each value is the mean of at least seven experiments.

the reoxygenation phase, the contractile force partially recovers and reaches about 11% of the initial value after 30 min of the reoxygenation phase. However, although no contractile force is evident after 15 min of the hypoxic phase in the control situation, some activity is evident in 10–20 mM taurine-treated hearts (Fig. 3). During the reoxygenation phase tension develops in the group treated with 10–20 mM taurine, reaching 31.3 and 38.3% of the pre-hypoxic value respectively. Lower taurine concentrations (1–5 mM) do not statistically increase the recovery of normal mechanical activity as shown in Fig. 3, thus showing that the taurine effect is dose-dependent. On the other hand, taurine does not produce any significant modification of the resting tension increase induced by hypoxic phase and of the resting tension decline during the reoxygenation phase. During the hypoxic phase, both in taurine-treated and control hearts the presence of atrio-ventricular blocks of different degree is constantly observed. In control hearts, ventricular arrhythmias occur during the hypoxic phase with the following incidence: VF 31%, VT 35%, VPbs 74%. The re-exposure of the heart to a solution containing glucose gassed with O_2–CO_2 produces an exacerbation in the severity of ventricular dysrhythmias (Table 1). During the hypoxic phase 5, 10 and 20 mM taurine causes a reduction in the incidence of VF and VT, while only 10 and 20 mM taurine are able to reduce the incidence of VPbs. VF is reduced by all taurine concentrations during the reoxygenation phase while only 5, 10 and 20 mM taurine reduce the incidence of VT during the reoxygenation phase (Table 1).

Table 2. Calcium content in cardiac muscle (ng/mg dry wt)

Groups		N	Calcium
(A)	Control	5	566.48 ± 36.68
(B)	30 min hypoxic phase + 30 min reoxygenation	7	1163.45 ± 73.21*
(C)	30 min hypoxic phase + 30 min reoxygenation + 20 mM taurine	4	828.08 ± 31.20**

Control: hearts perfused for 100 min with oxygenated normal solution. 20 mM taurine was added to perfusate at the beginning of hypoxia and was continued throughout reoxygenation period. Each value is the mean ± S.E.; N = number of experiments.

* Significantly different ($P \leq 0.001$) from A; ** significantly different ($0.01 \geq P \geq 0.001$) from B.

Table 3. Percentage of hearts presenting ventricular arrhythmias during hypoxic and reoxygenation phases

	Percentage		
	No drugs	10 mM Taurine	10 mMβ-alanine
(1) Hypoxic phase			
VF	31	15*	50
(2) Reoxygenation phase			
VF	51	31**	75
Recovery of normal electrical activity after 30 min reoxygenation	11	54	–
N	65	13	8

The Chi-square test was used to detect significant differences between control and treated preparations. VF = ventricular fibrillation, N = number of experiments. * $0.01 > P > 0.001$; ** $P < 0.001$.

The effect of taurine on VT in the hypoxic and reoxygenation phases is dose-dependent. At the end of the reoxygenation phase only 11% of control hearts recover normal electrical activity while the taurine treatment increases the percentage recovery of normal electrical activity in the hearts. This taurine effect is also dose-dependent (Fig. 4).

Total calcium in cardiac tissue is doubled after the exposure of the heart to 30 min of the hypoxic phase and 30 min of the reoxygenation phase. The perfusion of the heart with 20 mM taurine reduces the increase in calcium content significantly although it failed to restore basal values (Table 2). The administration of β-alanine, the carboxylic analogue of taurine increases the incidence of VF both in hypoxic and in reoxygenation phases (Table 3), while failing to increase the recovery of normal electrical (Table 3) and mechanical (Fig. 5) activities.

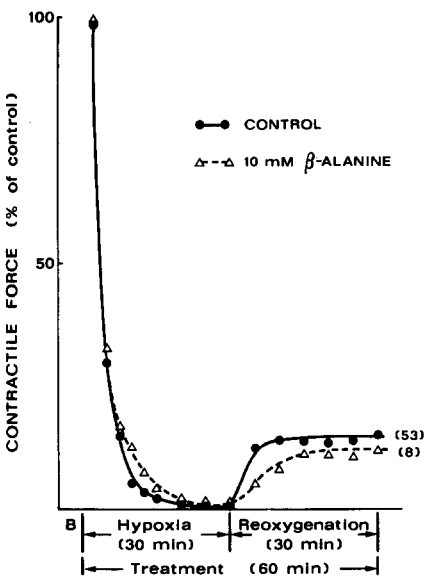

Fig. 5. Percentage changes in contractile force (developed tension) of the heart perfused under hypoxic conditions for 30 min and under reoxygenation conditions for 30 min. Each value represents the mean; in brackets the number of experiments.

DISCUSSION

The use of taurine on the hypoxic and subsequently reoxygenated myocardium has not previously been investigated. The present study describes a number of parameters associated with cardiac dysfunction during hypoxic and reoxygenation phases and the influence that taurine has on these parameters. It has also been shown that in guinea-pig heart hypoxia provokes a release of taurine. As previously described in rat heart [6], it appears that the increased outflow of taurine is a transitory event not running parallel to the LDH release measured in the same perfusate. This fact suggests that the release of taurine might not be attributable to a simple increase in membrane permeability. 10 and 20 mM taurine are able to reduce LDH release also during the hypoxic phase; this fact suggests that this compound can in some way reduce the hypoxic damage; on the other hand taurine, even at lower concentrations, reduces the LDH leakage during reoxygenation. Besides, taurine decreases the incidence of ventricular arrhythmias both in hypoxic and in reoxygenation phases, and increases the recovery of normal electrical and mechanical activities, although it has no beneficial effect on the increase in resting tension. However, 20 mM taurine reduces reoxygenation-induced calcium gain.

The mechanism by which taurine reduces tissue Ca^{2+} gain is not known but it is interesting to point out that there are many situations in which taurine prevents calcium overload, such as in the calcium paradox [10, 11] and in the cardiomyopathy in the hamster [12].

In addition, it is known that taurine is able in guinea-pig heart to prevent the positive inotropic effect caused by an increase in calcium concentrations [13]. The fact that an equimolar concentration of β-alanine is not effective indicates that taurine action is not due to an osmotic effect.

Data so far available suggest that taurine can exert its cardioprotective action by the reduction of calcium gain. But it is interesting to note that taurine decreases the positive inotropic action of phenylephrine in the presence of propanolol [17] and decreases the number of ^{3}H-prazosin binding sites [18], and in the same experimental model prazosin (α_1-antagonist) exerts an antiarrhythmic effect and

reduces the LDH release [19]. Therefore, the effect of taurine could also be attributed to its antagonistic effect on α-adrenergic stimulation, at least in our experimental model. Because taurine is lost during the hypoxic phase, replenishment could be of some importance in the protective mechanism of taurine action.

In conclusion, evidence has been provided for a certain protective effect of taurine on various parameters of guinea-pig heart subjected to hypoxic and reoxygenation phases, and these influences are associated with a reduction in calcium gain. But the protective effect of taurine may also originate from a replenishment of the taurine source in the heart.

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