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Effects of Methylpalmoxirate on Hypoxic Rat Atria

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Isolated rat atria in hypoxia released lactate into the bathing medium and underwent a decline of the contraction frequency which, in some cases led to a complete cessation of the pacemaker activity. A pronounced fall in the peak developed tension and a rise in the resting tension also appeared. The atria from 24 h fasted rats, which oxidize faster their reserve lipids than those from fed rats, exhibited greater functional disturbances during hypoxia, a lower lactate output and a smaller recovery of peak tension upon reoxygenation. Methylpalmoxirate, which is a selective inhibitor of carnitine palmitoyltransferase I, attenuated the decline of the beating rate and the rise of the resting tension in both groups of rats and the incidence of atrial arrest in the fasted rat group. The fall in the peak tension, lactate output and recovery upon reoxygenation were not altered by the inhibitor. These data indicate that methylpalmoxirate alleviates some of the hypoxic functional derangements. Hence, it may be inferred that inhibiting the oxidation of the fatty acid derived from the endogenous triacylglycerol is beneficial during oxygen-limited conditions and that these effects could not be ascribed to changes in the glycolytic flux.

Key words: Methylpalmoxirate, Hypoxia, Fasting, Triacylglycerol.

Under hypoxic conditions, the isolated atria of fasted rats undergo a faster impairment of their contractile and pacemaker activities than those from fed rats (9, 11, 20). These functional disorders were also exacerbated by incubating the atria in the presence of short chain fatty acids (20). Since the atria from fasted rats exhibit greater triacylglycerol stores and a faster lipolysis during well-oxygenated conditions (10, 19), these findings strongly suggest that fatty acids, either exogenous or derived from endogenous triacyl-

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glycerol, are deleterious for the hypoxic atria. On the other hand, it is well-documented that methylpalmoxirate (MP) inhibits fatty acid oxidation through specific inactivation of carnitine palmitoyltransferase I (4, 16, 17). Therefore, it seemed plausible to infer that MP might be able to ameliorate the functional derangements evoked by hypoxia. Coinciding with this hypothesis, MP reduced the size of anoxic zones in the perfused rat heart exposed to oleic acid and low oxygen pressure (13). Furthermore, even though the effects of MP on the functional properties were not accurately assessed, MP tended to improve the contractile performance during hypoxia. On the contrary, MP had no favourable effects on the ischemic swine heart (8). Similar controversial data were reported using other inhibitors of carnitine palmitoyltransferase I (5, 12, 15). In view of this uncertainty, it was considered interesting to further examine the effects of MP on the hypoxic atria from fed and fasted rats. We had already found in this preparation that MP completely suppressed the triacylglycerol lipolysis under aerobic conditions (21). Therefore, the main purpose of this investigation was to ascertain whether inhibiting the oxidation of fatty acid derived from triacylglycerol would attenuate the disturbances occurring during the hypoxic incubation.

Materials and Methods

Female albino rats weighing 180-220 g maintained on a 12 h dark/light cycle, either fed *ad libitum* or fasted 24 h, were killed by decapitation. The atria were quickly excised and mounted isometrically under a resting tension of 750 mg. The bathing medium was a Krebs-Ringer bicarbonate solution containing 1.6 mM Ca and 11 mM dextrose, kept at 31 °C and continuously bubbled with 95 % O_2-5 % CO_2. The contraction frequency of spontaneously beating atria was measured from 30-s samples of the recorded contractions. Peak developed tension and changes of the resting tension were measured in the whole atria paced at 3.3 Hz or left atria paced at 1 Hz, with 10 V, 0.6 ms square pulses. After 45 or 60 min recovery periods, the atria were exposed to MP 16 μM (methyl 2-tetradecyloxirane carboxylic acid, generously provided by McNeil Pharmaceutical, Spring House, Pennsylvania) added dissolved in 25 μl of dimethylsulfoxide. Since the control atria were exposed to the vehicle alone, the medium in all groups contained 17.6 mM dimethylsulfoxide. In the atria allowed to recover for 45 min, hypoxia started 30 min after the addition of MP whereas when the recovery period continued during 60 min, hypoxia began 10 min after the addition of the drug. Hypoxia was attained by bubbling the organ bath with N_2 instead of O_2 and the PO_2 in the bathing medium declined to 65 ± 5 mmHg.

The triacylglycerol content was measured in the paced atria by means of a previously described enzymatic method (19). The lactate released into the medium and contained in the tissue was measured according to Hohorst's method (3). Results are expressed as mean values ± SEM. The triacylglycerol content was statistically compared by ANOVA followed by the procedure of Scheffé and lactate by a two factors ANOVA followed by the Tukey's test. Changes of the contractions frequency and developed and resting tensions were compared by a three factors ANOVA for repeated measures in one factor followed by the Tukey's test, and the ratio of the arrested/beating atria using the chi-square test (22). Significance was set at a P-value less than 0.05.

Results

As shown in table I, changes of the triacylglycerol content were almost negligible

during the hypoxic incubation. Lipolysis was patent only in the left atria of fasted rats after a 60 min hypoxic period and was abolished by MP.

Contractions frequency declined progressively throughout the hypoxic incubation; this decline being faster in the fasted rats atria (table II). In addition, in some of these experiments, hypoxia led to a complete cessation of the spontaneous contractions which involved a larger fraction of atria in the fasted rat group (table II). The addition of MP 10 min before the onset of hypoxia, did not exhibit any pro-

Table I. *Triacylglycerol content in the hypoxic rat atria.*
Medium contained 11 mM dextrose. Control medium contained 17.6 mM dimethylsulfoxide. MP refers to 16 µM methylpalmoxirate plus dimethylsulfoxide. Values shown are means ± SEM, expressed as µmoles/g wet weight. Drugs were added 30 min before the hypoxic incubation. Animals per group, 12.

Incubation condition	Fed rats	24-h fasted rats
Whole atria paced at 3.3 Hz:		
30 min hypoxic incubation in the control medium	4.2 ± 0.4	5.0 ± 0.2
30 min hypoxic incubation in the MP medium	4.7 ± 0.2	5.0 ± 0.4
Left atria paced at 1 Hz:		
60 min hypoxic incubation in the control medium	7.7 ± 0.5	5.3 ± 0.9[a]
60 min hypoxic incubation in the MP medium	9.8 ± 0.9	9.2 ± 1.1

a: $p < 0.05$ vs the atria incubated in the MP medium.

Table II. *Changes in the atrial frequency during the hypoxic incubation.*
Media composition as in table I. In the first column between brackets the control rate after the 30 min prehypoxic exposure to methylpalmoxirate, and the ratio between this rate and the rate at the end of the recovery period. In the other columns between brackets the number of atria which remained beating. N: 10, each group. Values shown are the mean ± SEM, expressed as beats/min.

Metabolic condition	Hypoxic incubation (min)					
	10	20	30	40	50	60
Fed rats						
Control medium (173 ± 3; 0.97)	[a][g]91±14	[a][g]47±15 (7)	[a][g]25±15 (5)	7±4 (4)	[c][h]14±6	[d]12±6 (5)
MP medium (180 ± 5; 1.02)	100±16	40±16 (7)	16±5 (5)	15±6 (6)	25±8 (8)	25±7
Fasted rats						
Control medium (163 ± 3; 0.99)	[b]65±19 (7)	[b][f]18±5 (3)	[b][c]0 (0)	[f]14±14 (1)	[b][c]0 (0)	2.8±2.8 (1)
MP medium (176 ± 4; 1.01)	90±18	51±21 (7)	34±14 (7)	26±12 (6)	25±12 (6)	15±6 (4)

a: $p < 0.01$ vs control fasted atria; b: $p < 0.01$ vs fasted atria in MP medium; c: $p < 0.05$ vs control fasted rats; d: $p < 0.05$ vs fed rats in MP medium. Ratio of arrested / beating atria, e: $p < 0.01$ vs fasted rats in MP medium; f: $p < 0.05$ vs fasted rats in MP medium; g: $p < 0.01$ vs the fasted rats in the same medium; h: $p < 0.05$ vs the fasted rats in the same medium.

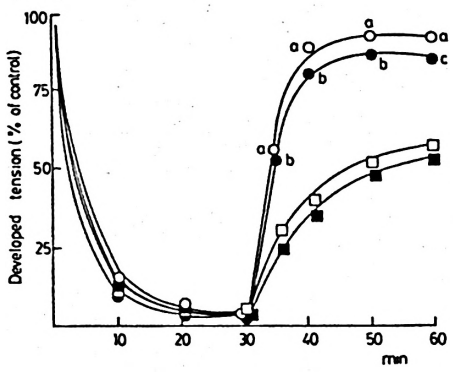

Fig. 1 *Rise in resting tension of the whole atria paced at 3.3 Hz.*
Squares: 24-h fasted rats. Circles: fed rats. Open symbols: atria incubated in the control medium containing 17.6 mM dimethylsulfoxide. Closed symbols: atria incubated in the medium containing 16 μM methylpalmoxirate (MP) plus dimethylsulfoxide. Drugs were added 30 min before the onset of hypoxia. Zero time refers to the end of the prehypoxic incubation. Values are the average of 16 atria±SEM. a: $p < 0.01$ vs the fed rats atria in the control medium. b: $p < 0.01$ vs the fed rats atria in the MP medium. c: $p < 0.05$ vs the fasted rats atria in the MP medium. d: $p < 0.01$ vs the fasted rats atria in the MP medium. e: $p < 0.01$ vs the fed rats atria in the MP medium.

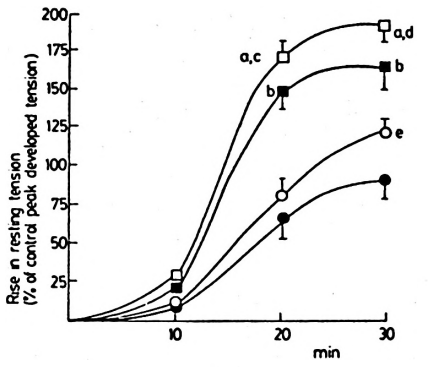

Fig. 2. *Effects of hypoxia and reoxygenation on the peak developed tension of left atria paced at 1 Hz.*
Squares: 24-h fasted rats. Circles: Fed rats. Closed symbols: atria incubated in the control medium containing 17.6 mM dimethylsulfoxide. Open symbols: atria incubated in the medium containing 16 μM methylpalmoxirate (MP) plus dimethylsulfoxide. Drugs were added 30 min before the onset of hypoxia. Reoxygenation started after a 30 min hypoxic interval. Zero time refers to the end of the prehypoxic incubation. Ratio of the peak developed tension at the end of the prehypoxic exposure and at the end of the recovery period after isolating the atria: ● 1.07 ± 0.08; ○ 1.06 ± 0.06; ■ 1.05 ± 0.07; □ 1.13 ± 0.10. Values shown are the average of 9 atria ± SEM. a: $p < 0.01$ vs the fasted rats atria in the control medium. b: < 0.01 vs the fasted rats atria in the MP medium. c: $p < 0.05$ vs the fasted rats atria in the MP medium.

tective effect on the pacemaker activity (data not shown, n = 9 each group). However, when the exposure to MP before the onset of hypoxia lasted 30 min, it ameliorated the decline of the pacemaker rate in both groups of atria and reduced the occurrence of the atrial arrest in the fasted rat group (table II).

The paced whole atria underwent a pronounced depression (> 95 %) of their peak developed tension 5 min after the onset of hypoxia. This depression was similar in the atria from fed and fasted rats, either in the presence or the absence of MP. However, towards the end of the experiments, MP attenuated the rise in the resting tension (fig. 1). In order to decrease the energy demand, experiments were also performed using left atria paced at 1 Hz. In this condition, throughout a 60 min hypoxic period, the decline of the peak tension did not exhibit differences between the atria from fed and fasted rats and MP showed unable to alleviate this decline (data not shown, n = 8, each group). In another group of atria after a 30 min hypoxic interval, a 30 min recovery period was allowed by reoxygenating the organ bath. Recovery of the contractile strength developed faster in the fed rats atria and MP did not exert a beneficial effect (fig. 2).

Data recorded in table III show that in the spontaneously beating atria from fast-

Table III. *Atrial lactate content and output during hypoxia.* Media (control and MP) and expression of results as in table I.

Incubation condition	Fed rats	24-h fasted rats
	Lactate output	
Control spontaneously beating atria exposed to hypoxia 60 min (n = 6)	53.2 ± 4.0	41.5 ± 3.3[a]
Spontaneously beating atria in the presence of MP since 30 min before hypoxia (n = 6)	49.6 ± 4.5	41.4 ± 3.7[a]
Control atria paced at 3.3 Hz exposed to hypoxia 30 min (n = 10)	38.1 ± 1.8	33.3 ± 2.9
Atria paced at 3.3 Hz in the presence of MP since 30 min before hypoxia (n = 10)	41.2 ± 3.0	34.8 ± 3.2
	Lactate content	
Control spontaneously beating atria exposed to hypoxia 60 min (n = 6)	3.7 ± 0.4	1.9 ± 0.2[a]
Spontaneously beating atria in the presence of MP since 30 min before hypoxia (n = 6)	3.9 ± 0.6	2.0 ± 0.1[a]

a: $p < 0.05$ vs fed rats atria.

ed rats, the atrial lactate content and the lactate released into the medium were lower than in those from fed rats. In the paced atria, even though lactate output tended to be lower in the fasted rats group, differences were not significant. In all of these groups, MP did not alter either lactate accumulation or output.

Discussion

Coinciding with previous findings, throughout the hypoxic incubation, when compared to those from fed rats, the atria from fasted rats displayed a smaller lactate output, a faster decline of their pacemaker frequency, a higher incidence of atrial arrest and a larger rise in resting tension (9, 11, 20). In addition, this investigation showed that upon reoxygenation, the fasted rat atria recovered their contractile strength more slowly. Since the fasted rats atria oxidize at a faster rate their reserve lipids (10, 19), it may be inferred that the atrial performance was impaired by the accumulation of fatty acid metabolites. Consequently, it was to be expected that inhibiting the fatty acid oxidation by means of MP would be beneficial for the hypoxic atria. Confirming this issue, the inhibitor decreased the fall in the pacemaker frequency and the occurrence of atrial arrest. In accordance with previous data concerning normoxic atria, the effects needed a 30 min prehypoxic exposure to the drug (14). Probably, this is the time required for the conversion of MP to its CoA ester which is the active inhibitor of carnitine palmitoyltransferase 1 (4).

MP attenuated the rise in the resting tension displayed by the atria paced at 3.3 Hz. In contrast, the fall in the peak tension as well as its recovery upon reoxygenation were not improved by the inhibitor even in the atria paced at 1 Hz. These findings may be explained by the large energy expenditure of the contractile function. Hence, the hypoxic energy shortage probably overwhelmed some of the putative favourable effects of MP. On the contrary, the inhibitor was more ef-

fective on the pacemaker cells which exhibit a lower energy demand with respect to the contractile fibres.

Since the effects of MP did not correlate with the production of lactate, most likely the functional improvements did not result from an enhanced energy supply from the anaerobic glycolysis. This conclusion is also supported by previous findings which established that several fatty acid metabolites that accumulate during oxygen-limited conditions did not alter the glycolytic flux of soluble heart extracts (18). Therefore, the mechanisms underlying the beneficial effects of MP cannot be satisfactorily explained on the basis of present data. However, they might be the consequence of a lowered level of fatty acid metabolites which are known to inhibit several enzyme systems and membrane functions (1, 2, 7, 14, 23). Despite these considerations, present data demonstrate that suppression of the triacylglycerol lipolysis alleviates some of the deleterious effects of hypoxia.

As shown in table II and fig. 2, and agreeing with previous observations, during the prehypoxic incubation the atrial functions remained unaltered. In addition, the responses of the control atria to hypoxia were similar to those attained in the absence of drugs (10, 20). Hence, it can be concluded that dimethylsulfoxide lacks effects by itself. On the contrary, other investigators demonstrated protective effects of dimethylsulfoxide on the hypoxic rabbit atria (6). However, these effects required, at least, a 23-fold higher concentration of the solvent which elicited a pronounced depression of the contractile strength that probably biased the responses to hypoxia.

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Resumen

Se determina mediante metilpalmoxirato, un inhibidor selectivo de la carnitina palmitoiltransferasa I, si la inhibición de la oxidación de los ácidos grasos derivados de los triglicéridos endógenos reduce las perturbaciones producidas por la hipoxia en las aurículas de ratas alimentadas o con ayuno de 24 h. Durante la hipoxia las aurículas liberan lactato y sufren una disminución de la frecuencia que en muchos casos culmina en un paro total. También se produce una gran caída de la fuerza máxima y un aumento de la tensión de reposo. En las aurículas de ratas en ayunas, que consumen más rápidamente los lípidos endógenos, estas perturbaciones son más acentuadas y se asocian con una menor producción de lactato y una menor recuperación de la fuerza al reoxigenar. El metilpalmoxirato atenúa la disminución de la frecuencia y la amplitud de la contractura en ambos grupos y reduce la incidencia del paro en las aurículas de ratas en ayunas. La caída de la fuerza y la recuperación al reoxigenar no se modifican por el inhibidor. Estos resultados muestran que el metilpalmoxirato reduce algunos de los trastornos provocados por la hipoxia, sugiriendo que la inhibición de la oxidación de los ácidos grasos resulta beneficiosa durante la escasez de oxígeno, efectos que no pueden atribuirse a cambios de la glucolisis.

Palabras clave: Metilpalmoxirato, Hipoxia, Ayuno, Triacilglicerol.

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