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Effects of 3-hydroxybutyrate on the hypoxic and reoxygenated atria from fed and fasted rats

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When exposed to hypoxia, the isolated atria from fed rats released lactate into the medium and underwent a decline of the peak developed tension and pacemaker frequency. The atria from 24-h fasted rats showed a rise in the resting tension together with a greater decline of the pacemaker rate and a lower lactate output than those from fed rats. The exposure to 5 mM 3-hydroxybutyrate caused only a small and brief decline in the pacemaker rate in the fed rats atria indicating that ketone bodies are able to exert only a minor detrimental effect on the hypoxic atria. Since the lactate output remained unaffected, this effect cannot be ascribed to a lowering in the energy supply from anaerobic glycolysis. On the contrary, 3-hydroxybutyrate improved the post-hypoxic recovery of the peak tension in the atria from fasted rats. This finding may be reflecting an anaplerotic role of 3-hydroxybutyrate, thus suggesting that in addition to glucose a second substrate is needed to meet the energy demand in the reoxygenated atria from fasted rats.

Key words: 3-hydroxybutyrate, Hypoxia, Reoxygenation, Fasting, Rat atria.

Previous studies have shown that when exposed to hypoxia the atria from fasted rats exhibited a faster impairment of their functional properties, as well as a smaller lactate output than those from fed rats (2, 20, 22, 24). Since the fasted rats atria have greater triacyl glycerol stores and a faster *in vitro* lipolysis (19, 21, 23), their enhanced lability might have been a consequence of an increased fatty acid catabolism. However, the inhibition of fatty acid oxidation only partially reversed the effect of starvation on hypoxic atria (2, 22). This finding suggested that aside from the fatty acid oxidation another mechanism should also be involved in the effects of fasting. On the other hand, it is well-documented that ketone bodies are readily oxidized by the heart (7, 14, 15, 18, 29)

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leading in turn to a decrease in the glucose utilization (12, 18, 26, 27). In addition, the availability of ketone bodies, as energy source for the rat heart, is increased during several metabolic disturbances such as fasting and diabetes (3, 9, 16). Therefore, it seemed plausible to infer that the catabolism of ketone bodies could be detrimental during oxygen-limited conditions. On these bases it appeared interesting to assess whether 3-hydroxybutyrate affects the performance of the atria from fed and fasted rats exposed to hypoxia and reoxygenation.

Materials and Methods

Atria from decapitated 220-300 g Wistar rats of either sex, maintained on a 12-h dark/light cycle, fed *ad libitum* or fasted 24-h, were mounted isometrically at 750 mg of resting tension. The bathing medium was a Krebs-Ringer bicarbonate solution containing 1.6 mM Ca and 11 mM glucose, kept at 31 °C and continuously bubbled with 95 % O_2-5 % CO_2. The frequency of contractions of spontaneously beating whole atria was measured from 30 s samples of the recorded contraction. Peak developed tension was measured in left atria paced at 1 Hz with 5-10 V, 0.6 ms square pulses. After a 30 min recovery period the atria were exposed to 5 mM of the sodium salt of D-(-)-3-hydroxybutyric acid (Sigma). Hypoxia started 30 min after the addition of 3-hydroxybutyrate by bubbling the organ bath with N_2 instead of O_2. The concentration of 3-hydroxybutyrate measured enzymatically (28) in samples withdrawn from the organ bath, remained constant throughout the experiments (data not shown). The lactate released into the medium was assayed according to HOHORST' method (6) and was statistically compared by a two factors ANOVA followed by the Tukey's test (30). Changes of the contraction frequency and peak developed tension were statistically compared using a three factors ANOVA for repeated measures in one factor followed by the Tukey's test (30) and the rise in resting tension using the Student's *t* test.

Results

The contraction frequency decreased progressively throughout the 60 min hypoxic incubation, the decrease being greater in the fasted atria (fig. 1). The addition of 3-hydroxybutyrate did not affect the pacemaker activity in the fasted atria but 40 min after the onset of hypoxia it caused a small reduction of the atrial rate in the atria from fed rats.

Over the 60 min exposure to hypoxia the paced left atria underwent a pro-

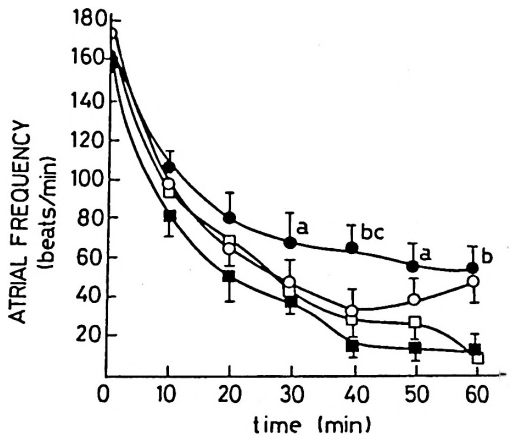

Fig. 1. *Changes in atrial rate during hypoxic incubation.*
Squares: fed rats. Circles: 24-h fasted rats. Closed symbols: normal medium. Open symbols: medium containing 5 mM 3-hydroxybutyrate added 30 min before the onset of hypoxia. Zero time refers to the end of the prehypoxic incubation. Values are the average of 9 atria ± SEM. $^{a}p < 0.05$ vs the fasted rat atria in the same medium. $^{b}p < 0.01$ vs fasted atria in the same medium. $^{c}p < 0.05$ vs fed rat atria in the medium containing 3-hydroxybutyrate.

nounced depression of their peak developed tension, which attained a similar extent in the fed and fasted groups (fig. 2). In addition, towards the end of the hypoxic incubation the fasted rats atria developed contracture, as indicated by a rise in the resting tension. Neither the peak tension nor the resting one was affected by 3-hydroxybutyrate. Figure 2 also shows that the recovery of the peak tension, occurring after reoxygenating the organ-bath, was improved by 3-hydroxybutyrate in the fasted atria.

In the spontaneously beating atria from fasted rats the lactate output (8.03 ± 0.58) was lower than in those from fed rats (9.2 ± 0.58, $p<0.05$). 3-hydroxybutyrate did not affect the release of lactate in both nutritional states (7.29 ± 0.31 and 10.0 ± 0.64 in fasted and fed rats, respectively).

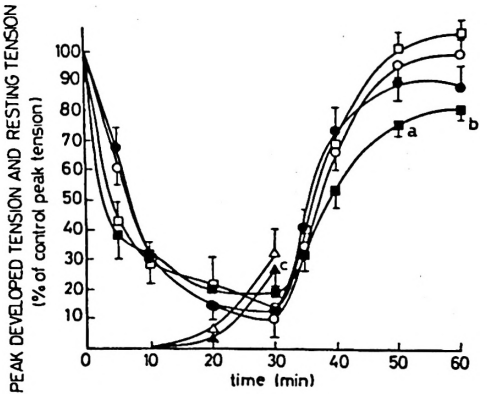

Fig. 2. *Effects of hypoxia and reoxygenation on the peak developed tension and the resting tension of left atria paced at 1 Hz.*
Closed triangles: rise in resting tension of fasted atria in normal medium. Open triangles: rise in resting tension of fasted atria in the medium containing 3-hydroxybutyrate. The other symbols as in fig. 1. Reoxygenation started after a 30 min hypoxic interval. Values are the average of 9 atria ± SEM. $^{a}p < 0.05$ vs the fasted rat atria in the medium containing 3-hydroxybutyrate. $^{b}p < 0.01$ vs fasted rat atria in the medium containing 3-hydroxybutyrate. $^{c}p < 0.02$ vs the fed rat atria in the same medium. The fed atria did not underwent any rise in the resting tension.

Discussion

In agreement with previous findings (2, 20, 22, 24) during the hypoxic incubation the atria from fasted rats exhibited a rise in resting tension together with a greater decline of the contraction frequency and a smaller lactate output than those from fed rats, whereas the fall of the peak tension attained a similar extent in both nutritional states. 3-hydroxybutyrate, even though it was tested at a concentration three-fold higher than that occurring in the 24-h fasted rat (3, 9), caused only a small and brief decline of the pacemaker rate in the fed rats atria. This effect cannot be ascribed to a reduction of the energy supply from anaerobic glycolysis, since the lactate output was not affected by 3-hydroxybutyrate. However, regardless of the mechanisms involved in this effect of 3-hydroxybutyrate and in spite of its being rather weak, ketone bodies seem able to exert at least a minor detrimental effect on the hypoxic atria.

Contrariwise, 3-hydroxybutyrate enhanced the posthypoxic recovery of the peak tension in the fasted rat atria. This finding does not agree with data from GOODWIN and TAEGTMEYER (4) in the ischemic-reperfused rat heart, but it does so with that of HEARSE *et al.* (5) in the anoxic-reoxygenated heart of diabetic rats, which are known to have a fast fatty-acid oxidation rate resembling that from fasting (10, 11, 13). Regarding the latter and the present data, it should be mentioned that depletion of the citric acid intermediates occurs during oxygen deprivation (1, 17), replenishment being one of the prerequisites needed for the recovery of the heart function after reoxygenation (25). Hence the ability of 3-

hydroxybutyrate to improve the posthypoxic recovery might be due to the previously reported anaplerotic role of this substrate (1), suggesting that, in addition to glucose, a second substrate is needed to meet the energy demand in the reoxygenated atria from fasted rats.

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A. VARELA, G. TESTONI, M. CARREGAL, V. DALAMON y E. A. SAVINO. *Efectos del 3-hidroxibutirato sobre las aurículas hipóxicas y reoxigenadas de ratas alimentadas y en ayunas.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (4), 377-382, 1997.

Las aurículas de ratas alimentadas, expuestas a la hipoxia, liberan lactato al medio de incubación y presentan una caída de la frecuencia del marcapaso y de la fuerza pico desarrollada. Las aurículas de ratas en ayuno de 24 h desarrollan contractura acompañada de una mayor caída de la frecuencia y de una menor liberación de lactato, con respecto a las de ratas alimentadas. El agregado de 3-hidroxibutirato 5 mM sólo causa una pequeña y breve disminución de la frecuencia en las aurículas de ratas alimentadas, indicando que los cuerpos cetónicos ejercen un leve efecto nocivo en las aurículas hipóxicas. Como la liberación de lactato no queda afectada por el 3-hidroxibutirato no puede atribuirse a una menor provisión de energía de la glucolisis anaeróbica. Por el contrario, el 3-hidroxibutirato mejora en las ratas en ayunas la recuperación de la fuerza pico al reoxigenarse. Este resultado sugiere el papel anaplerótico del 3-hidroxibutirato y que, además de la glucosa, se requiere un segundo sustrato para abastecer la demanda energética de las aurículas de ratas en ayunas durante la reoxigenación.

Palabras clave: 3-hidroxibutirato, Hipoxia, Reoxigenación, Ayuno, Aurícula de rata.

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