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Lactate activates the somatotropic axis in rats

Rafael Barrera Salgueiro [a], Rodrigo A. Peliciari-Garcia [a], Daniella do Carmo Buonfiglio [a], Cibele Nunes Peroni [b], Maria Tereza Nunes [a,*]

[a] University of São Paulo, Institute of Biomedical Sciences-I, Department of Physiology and Biophysics, São Paulo, Brazil
[b] Nuclear and Energy Research Institute (IPEN), Biotechnology Center, São Paulo, Brazil

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ABSTRACT

Under physical activity a wide variety of cellular metabolic products and hormones are altered in the blood stream, including lactate, a metabolite of pyruvate reduction, and growth hormone (GH). Although a positive correlation between lactate and GH seems to exist during exercise, the role of lactate as a mediator of GH production has never been investigated. Thus, the aim of this study was to investigate whether lactate could activate the somatotropic axis and stimulate GH synthesis/release, contributing to the enhanced somatotropic activity described in exercise conditions.

Male adult Wistar rats were acutely treated with sodium lactate [15 or 150 μmols, i.p.] at the beginning of the active period (Zeitgeber time 13–14), and euthanized by decapitation 30, 60 and 120 min after the injections. Serum GH concentration were determined using ELISA and *Gh* and *Igf-1* mRNA expressions were quantified by qPCR.

Serum GH concentration and *Gh* mRNA expression were increased 30 min after lactate injections for both treatments. However, [15 μmols] of lactate injection kept GH serum concentration chronically high throughout the experimental period. *Igf-1* mRNA expression was increased only 60 min after challenge with [15 μmols] of lactate, time point which corresponded to 30 min after the serum GH peak.

The present results led us to conclude that lactate mediates activation of the somatotropic axis, therefore emphasizing its possible role on GH synthesis/release, and further indicating that it could play a part on the increased GH secretion observed in exercise conditions.

1. Introduction

It is well described in the literature that different types of physical activity mediate lactate release [1,2]. The lactate, produced by anaerobic glycolysis through pyruvate reduction, behaves as an intramuscular oxidizing agent, an important substrate for liver gluconeogenesis and an energy substrate for the maintenance of general cellular physiology, including neuronal activity [3,4]. Recently, several studies have shown that lactate induces prolactin release [5] and production of brain-derived neurotrophic factor [6], which is known to improve cognitive function. Under intense muscular activity, lactate production is further stimulated, resulting therefore in its release. Sports physiologists often measure blood levels of lactate as a parameter of physical exercise intensity and muscle fatigue. Like lactate, the secretion of growth hormone (GH), which is produced by the somatotropic cells of anterior pituitary, is elevated during exercise and it also seems to depend on volume and intensity of the activity [7]. The immediate action of serum GH increment is the stimulation of insulin-like growth factor 1 (IGF-1) synthesis. IGF-1 is a hepatic hormone that induces longitudinal growth, body weight gain; and together with GH contributes to improvement of body growth and development. Besides it, GH also plays an important role on the stimulation of hepatic gluconeogenesis, lipolysis in white adipose tissue, amino acid transport and gene transcription [8,9]. Several studies have shown that many biological factors related to exercise, including stress-induced growth hormone releasing hormone (GHRH) release, catecholamines, as well the rapid reduction of glycemia, are the main modulators of GH synthesis/release [10–12]. Although previous work showed a positive correlation of GH and lactate release in a heavy-resistance exercise protocol [13], the role of lactate as a mediator of GH synthesis/release has not been investigated so far.

In the present study, we investigated the effect of a single injection of lactate on the activity of the somatotropic axis. Our hypothesis is to provide novel insights of lactate as a metabolite that activates GH-IGF-1 axis, which could place it as a contributing factor to the elevation of GH in exercise, as well as a therapeutic tool to be used in disorders of the somatotropic axis.

* Corresponding author at: University of São Paulo, Institute of Biomedical Sciences, Department of Physiology and Biophysics. Av. Prof. Lineu Prestes 1524, 1st Floor, Room 129, São Paulo, SP 05508-900, Brazil. Tel.: +55 11 3091 7465.
E-mail address: mtnunes@icb.usp.br (M.T. Nunes).

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2. Methods

2.1. Animals

Male Wistar rats weighing 280–300 g were obtained from the Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil. The animals were kept on a 12 h/12 h light/dark (LD) cycle (lights on at 06:00 am, Zeitgeber time — ZT 0), in a temperature controlled room (21 ± 2 °C) and had access to food and water *ad libitum*. The animals, $n = 5$ animals/group/time point, received a single i.p. injection of sodium lactate (Sigma, St. Louis, MO, USA), [30 and 300 mM in 0.5 mL of saline 0.9%, which was used as vehicle], which is equivalent to 15 and 150 μmols of lactate, respectively, at the beginning of the active period (ZT 12–13). The animals were euthanized by decapitation after 30, 60 and 120 min of injections and the pituitary, liver and serum were collected, frozen and kept for approximately a week at −80 °C before subsequent analysis. Ethics approval was granted by the Committee of Ethics in Animal Experimentation of the Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil. # 137, p. 110, book 02.

2.2. GH determination

Serum GH concentration was quantified by the Rat/Mouse Growth Hormone ELISA kit from Millipore (St. Charles, MO, USA) as previously described [14].

2.3. Quantitative RT-PCR

Total RNA was isolated from rat pituitary and liver, using the TRIzol Reagent (Life Technologies, USA) according to the manufacturer's instructions. RNA concentration was determined by Nano Drop and integrity evaluated in a 1% (wt/vol) agarose gel containing ethidium bromide, visualized with ultraviolet transilluminator and 1 μg of total RNA was reverse transcribed (Invitrogen Corp., Carlsbad, CA, USA). *Igf-1* and *Gh* mRNA expressions were evaluated by Real Time-PCR using Rotor Gene Q (QIAGEN, Hilden, Germany) and SYBR GREEN (Applied Biosystems, Warrington, UK) as fluorescent dye. Gene expressions were evaluated by the $2^{-\Delta\Delta Ct}$ method using ribosomal protein L19 (*Rpl19*) expression as inner control. All samples were compared using the relative cycle threshold, which is the calculated cycle number where the fluorescence signal emitted is significantly above the background levels. Rat primer sequences used for *Igf-1*, *Gh* and *Rpl19* mRNA amplifications were: *Igf-1* — forward 5′-AAGCCTACAAAGTCAG CTCG-3′, reverse 5′-GGTCTTGTTTCCTGCACTTC-3′; *Gh* — forward 5′-GAGTTCGAGCGTGCCTACATTC-3′, reverse 5′-GCAGGAGAGCAGCCCATA GTTT-3′ and *Rpl19* — forward 5′-CCAATGAAACCAACGAAATCG-3′, reverse 5′-TCAGGCCATCTTTGATCAGCTT-3′.

2.4. Statistical analysis

Data were analyzed using the GraphPad Prism (GraphPad Software version 5.01, San Diego, CA, USA) statistical analysis program. Two-way ANOVA, followed by Bonferroni's post hoc test was used at a significance level of $p < 0.05$. The main effects analyzed were lactate treatments, periods and their interactions.

3. Results

According to our findings, a single injection of sodium lactate [150 μmols] evoked a significant increase in serum GH concentration in 30, 60 and 120 min when compared to its respective controls. This increase was also observed for [15 μmols] in 30 min, but not for 60 and 120 min. In these time points, serum concentrations of GH have already returned to values, similar to their controls (Fig. 1).

In order to evaluate the repercussions of lactate injection in the somatotropic axis the hepatic and pituitary mRNA expressions of *Igf-1* and *Gh* were also respectively investigated. Fig. 2A shows a significant increase of *Igf-1* mRNA expression only 60 min after lactate injection in the [15 μmols] group, when compared to its control. However, *Igf-1* mRNA expression was not different than its controls 30 min before or even 60 min after the observed effect, independently of the treatment (Fig. 2B). Interestingly, *Gh* mRNA expression presented an eight-fold higher induction 30 min after lactate injection in both concentrations (15 and 150 μmols), but no differences were observed 60 or 120 min after lactate injection in both groups (Fig. 2B).

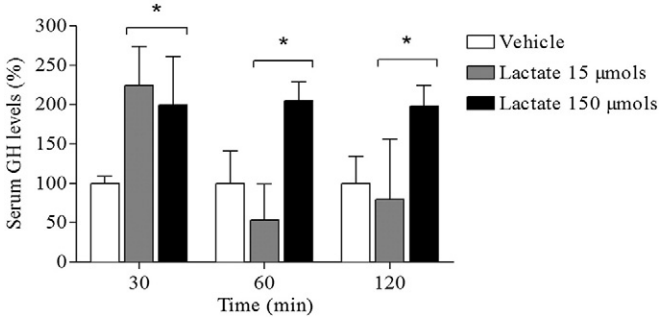

Fig. 1. Serum growth hormone (GH) concentration. GH levels are presented as % from controls (100%) ± SEM. Two-way ANOVA *p = 0.0485 for treatment, no difference for Bonferroni's post-hoc test. $n = 5$ animals/group/time point.

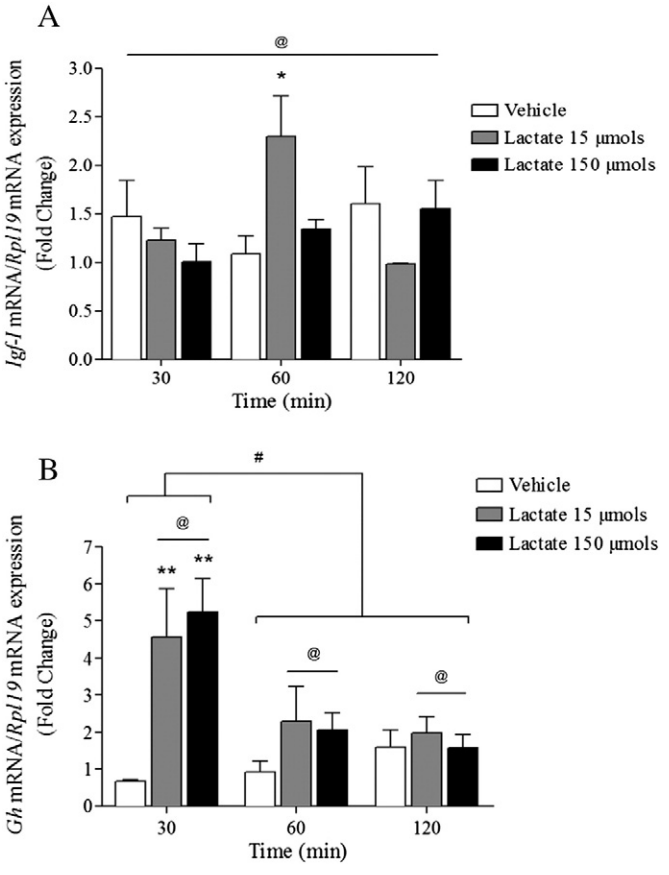

Fig. 2. Lactate induction of *Igf-1* and *Gh* mRNA expression. The values were normalized by *Rpl19* mRNA expression and are presented as fold change ± SEM. $n = 5$ animals/group/time point. (A) *Igf-1* mRNA expression in the liver. Two-way ANOVA @p = 0.0444 for interaction, *p < 0.05 *vs* its control for Bonferroni's post-hoc test. (B) *Gh* mRNA expression in the pituitary. Two-way ANOVA @p = 0.007 for treatment and #p = 0.0153 for period, **p < 0.01 *vs* its control for Bonferroni's post-hoc test.

4. Discussion

In this investigation we proposed to elucidate whether lactate injection could stimulate the somatotropic axis, based on several studies that have established a correlation of its production during exercise challenge and GH secretion. Our findings highlight lactate as a factor that activates the somatotropic axis, validating its contribution to the increase of serum GH observed in exercise, showing therefore another important physiological aspect of lactate, which is often indicted as noxious to the body after intensive exercise. These results also point to a possible role of this metabolite as a promising therapeutic tool in the management of subjects with impaired GH synthesis/secretion.

The present data show that a single lactate injection (15 μmols) leads to a quick (30 min) increment of serum GH concentration, indicating that this metabolite can act inducing GH release, which corroborates in part with previous publication [15]. This increment is transient, since at 60 min it returned to basal levels, and could be explained, in part, by the GH short half-life, which is approximately 11 min [16] and by its autocrine negative feedback regulation [17]. In fact, other studies have shown a serum GH elevation after 30 min of a stimulus [2]. This treatment also increased *Gh* mRNA expression at the same time as serum GH concentration, indicating that in parallel to the effects on GH release, lactate also increased *Gh* gene expression, which suggests that both effects were elicited independently from each other. The induction of *Igf-1* mRNA expression, observed 60 min after lactate injection, might be due to the previous elevation of serum GH levels, as already described [18].

Lactate injection in a dose 10-fold higher (150 μmols) also increased serum GH concentration at 30 min post treatment. However, this increment was maintained throughout the analyzed period (till 120 min), indicating that GH secretion was sustained, which could explain the reduction of *Gh* mRNA expression 60 and 120 min after lactate injection by its own negative feedback through GHRH and activation of somatostatin [17]. Although this dose is 10 times higher than that used before (15 μmols), the increase on *Gh* mRNA expression presented the same magnitude, which do not exclude a differential regulation of protein expression. Therefore, it is possible to infer that lactate, at this concentration, might recruit additional cellular mechanisms, maintaining a sustainable serum GH concentration [19]. Considering that liver *Igf-1* mRNA expression depends on the pulsatile secretion pattern of GH, the sustained higher serum GH levels, from 30–120 min, could explain why the expression of *Igf-1* mRNA was kept under basal conditions, as already described [20].

In fact, several reports describe a simultaneous elevation of GH and lactate during physical activity; however this is the first study showing that lactate, *per se*, activates the somatotropic axis. Therefore, it is possible to speculate that during physical exercise the released lactate could directly induce GH synthesis and secretion, contributing to the recognized increase of serum GH and IGF-1 levels detected in exercises states; furthermore, lactate is a substrate of gluconeogenesis, which is known to be activated by GH.

Although the mechanisms recruited by lactate to trigger the effects presented herein are currently under investigation, our findings show that a single injection of lactate increases *Gh* mRNA expression and serum levels, impacting on hepatic *Igf*-1 mRNA expression, in specific circumstances. The novelty of these results includes lactate in the hall of hormone/metabolites that could enhance somatotropic axis activity and strengthen evidences of its importance to a physiological context.

Disclosure

None declared.

Conflict of interest statement

The authors have no conflict of interest to declare.

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References

[1] R.F. Fregosi, J.A. Dempsey, Arterial blood acid–base regulation during exercise in rats, J. Appl. Physiol. 57 (1984) 396–402.
[2] J. Manetta, J.F. Brun, L. Maimoun, A. Callis, C. Préfaut, J. Mercier, Effect of training on the GH/IGF-I axis during exercise in middle-aged men: relationship to glucose homeostasis, Am. J. Physiol. Endocrinol. Metab. 283 (2002) E929–E936, http://dx.doi.org/10.1152/ajpendo.00539.2001.
[3] F. Tang, S. Lane, A. Korsak, J.F.R. Paton, A.V. Gourine, S. Kasparov, et al., Lactate-mediated glia-neuronal signalling in the mammalian brain, Nat. Commun. 5 (2014) 3284, http://dx.doi.org/10.1038/ncomms4284.
[4] D. Smith, A. Pernet, W.A. Hallett, E. Bingham, P.K. Marsden, S.A. Amiel, Lactate: a preferred fuel for human brain metabolism *in vivo*, J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 23 (2003) 658–664, http://dx.doi.org/10.1097/01.WCB.0000063991.19746.11.
[5] K.L. De Meirleir, L. Baeyens, M. L'Hermite-Baleriaux, M. L'Hermite, W. Hollmann, Exercise-induced prolactin release is related to anaerobiosis, J. Clin. Endocrinol. Metab. 60 (1985) 1250–1252.
[6] T. Schiffer, S. Schulte, B. Sperlich, S. Achtzehn, H. Fricke, H.K. Strüder, Lactate infusion at rest increases BDNF blood concentration in humans, Neurosci. Lett. 488 (2011) 234–237, http://dx.doi.org/10.1016/j.neulet.2010.11.035.
[7] S. Rezaee, S. Kahrizi, M. Hedayati, Hormonal responses of combining endurance–resistance exercise in healthy young men, J. Sports Med. Phys. Fitness 54 (2014) 244–251.
[8] D.R. Clemmons, J.J. Van Wyk, Factors controlling blood concentration of somatomedin C, Clin. Endocrinol. Metab. 13 (1984) 113–143.
[9] M.L. Vance, M.L. Hartman, M.O. Thorner, Growth hormone and nutrition, Horm. Res. 38 (Suppl. 1) (1992) 85–88.
[10] L. Wideman, J.Y. Weltman, N. Shah, S. Story, J.D. Veldhuis, A. Weltman, Effects of gender on exercise-induced growth hormone release, J. Appl. Physiol. 1985 (87) (1999) 1154–1162.
[11] G. Delitala, M. Palermo, R. Ross, D. Coy, M. Besser, A. Grossman, Dopaminergic and cholinergic influences on the growth hormone response to growth hormone-releasing hormone in man, Neuroendocrinology 45 (1987) 243–247.
[12] T. Shibasaki, M. Hotta, A. Masuda, T. Imaki, N. Obara, H. Demura, et al., Plasma GH responses to GHRH and insulin-induced hypoglycemia in man, J. Clin. Endocrinol. Metab. 60 (1985) 1265–1267.
[13] K. Häkkinen, A. Pakarinen, Acute hormonal responses to two different fatiguing heavy-resistance protocols in male athletes, J. Appl. Physiol. 1985 (74) (1993) 882–887.
[14] Y. Hashimoto, I. Ikeda, M. Ikeda, Y. Takahashi, M. Hosaka, H. Uchida, et al., Construction of a specific and sensitive sandwich enzyme immunoassay for 20 kDa human growth hormone, J. Immunol. Methods 221 (1998) 77–85.
[15] A. Luger, B. Watschinger, P. Deuster, T. Svoboda, M. Clodi, G.P. Chrousos, Plasma growth hormone and prolactin responses to graded levels of acute exercise and to a lactate infusion, Neuroendocrinology 56 (1992) 112–117.
[16] E. García, F. Santos, J. Rodríguez, V. Martínez, C. Rey, J. Veldhuis, et al., Impaired secretion of growth hormone in experimental uremia: relevance of caloric deficiency, Kidney Int. 52 (1997) 648–653.
[17] E. Richmond, A.D. Rogol, D. Basdemir, O.L. Veldhuis, W. Clarke, C.Y. Bowers, et al., Accelerated escape from GH autonegative feedback in midpuberty in males: evidence for time-delimited GH-induced somatostatinergic outflow in adolescent boys, J. Clin. Endocrinol. Metab. 87 (2002) 3837–3844, http://dx.doi.org/10.1210/jcem.87.8.8770.
[18] J. Isgaard, L. Carlsson, O.G. Isaksson, J.O. Jansson, Pulsatile intravenous growth hormone (GH) infusion to hypophysectomized rats increases insulin-like growth factor I messenger ribonucleic acid in skeletal tissues more effectively than continuous GH infusion, Endocrinology 123 (1988) 2605–2610, http://dx.doi.org/10.1210/endo-123-6-2605.
[19] C.K. Tuggle, A. Trenkle, Control of growth hormone synthesis, Domest. Anim. Endocrinol. 13 (1996) 1–33, http://dx.doi.org/10.1016/0739-7240(95)00059-3.
[20] D. Maiter, L.E. Underwood, M. Maes, M.L. Davenport, J.M. Ketelslegers, Different effects of intermittent and continuous growth hormone (GH) administration on serum somatomedin-C/insulin-like growth factor I and liver GH receptors in hypophysectomized rats, Endocrinology 123 (1988) 1053–1059.