Phosphorylated $\beta$-guanidinopropionate as a substitute for phosphocreatine in rat muscle

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FITCH, COY D., MAX JELLINEK, R. H. FITTS, K. M. BALDWIN, AND J. O. HOLLOSZY. Phosphorylated $\beta$-guanidinopropionate as a substitute for phosphocreatine in rat muscle. Am. J. Physiol. 228(4): 1123–1125. 1975.—To evaluate phosphorylated $\beta$- guanidinopropionate ($\beta$-GPAP) as a substitute for phosphocreatine (PC), hypoxic tibialis anterior muscles were stimulated to contract isometrically in situ until twitch tension fell to 25% of the peak value. Muscles from rats fed $\beta$-guanidinopropionic acid ($\beta$-GPA) failed to exhibit the staircase phenomenon, and they developed 28% less tension than control muscles. In control muscles lactate increased from 0.75 to 20.99, ADP increased from 0.89 to 1.20, ATP decreased from 5.09 to 2.73, and PC decreased from 15.78 to 1.52 $\mu$mol/g. In muscles from rats fed $\beta$-GPA, lactate increased from 0.85 to 14.31, ADP increased from 0.86 to 1.05, ATP decreased from 2.69 to 1.71, PC decreased from 0.73 to 0.30, and $\beta$-GPAP decreased from 30.34 to 19.45 $\mu$mol/g. From these measurements, the use of high-energy phosphate was calculated to be reduced 32% in muscles from rats fed $\beta$-GPA. The relationships between the use of high-energy phosphate and tension development conformed experimentally the ability of $\beta$-GPAP to substitute for PC as a source of energy to sustain muscle contraction.

RATS FED $\beta$-GUANIDINOPROPIONIC ACID ($\beta$-GPA) as 1% of their diet appear healthy (2, 8) despite a lowering of phosphocreatine (PC) in skeletal muscle to less than 10% of the normal concentration and accumulation of phosphorylated $\beta$-GPA ($\beta$-GPAP) to a concentration of approximately 30 $\mu$mol/g (2). $\beta$-GPA is a structural analogue of creatine which competes with creatine for transport into muscle via a specific membrane transport system (3, 4). It is also phosphorylated by creatine kinase to yield $\beta$-GPAP, although at a relatively slow rate in vitro (2). This phosphorylated guanidino derivative, like PC, is hydrolyzed during anoxic muscle contraction (2).

On theoretical grounds, $\beta$-GPAP would be expected to have phosphate-bond energy similar in magnitude to that of other phosphorylated guanidino derivatives, such as PC and phosphorylguanidine (1), but the ability of $\beta$-GPAP to substitute for PC as a source of energy for muscle has not been evaluated experimentally heretofore. Therefore, the present study was undertaken to compare the relationships between the use of high-energy phosphate and tension development in control muscles and in muscles of rats fed $\beta$-GPA.

METHODS

Male rats of a Wistar strain (specific pathogen-free CFN rats) weighing approximately 210 g were obtained from Carworth and provided with food and water ad libitum. One group of rats was fed powdered Purina rat chow plus 1% by weight of $\beta$-GPA (Cyclo Chemical Corp., Los Angeles) for 6 wk prior to use in the present experiments. A second group was fed powdered Purina chow alone.

At the end of the 6-wk feeding period, the rats were anesthetized with Na pentobarbital, 5 mg/100 g of body weight given intraperitoneally. The tibialis anterior muscles were surgically exposed, and the left one was dissected free and immediately frozen with Wollenberger tongs (9) cooled in liquid nitrogen; this is the resting muscle. The right tibialis anterior muscle was used for studies of the effects of contractile activity in situ. Its distal tendon was severed and tied to a jeweler's chain, which in turn was connected to a Sanborn model FTA 100 isometric force transducer. The leg was immobilized by pinning the proximal and distal portions of the tibia to a brace, and approximately 35 g of tension were placed on the muscle to stretch it to its normal resting length. Platinum electrodes then were placed on the common peroneal nerve, which had been isolated earlier; and single square-wave shocks of 0.2 ms duration were applied, using a Grass model S4K stimulator, to establish supramaximal voltage. Next, the right femoral artery was ligated. The muscle then was stimulated to contract at a frequency of 3/s until twitch tension fell to 25% of its peak value, at which time the muscle was rapidly excised and frozen with Wollenberger tongs cooled in liquid nitrogen.

Perchloric acid extracts of the muscles were prepared and neutralized with KOH (7) for measurement of lactate (6) and inorganic phosphate (5) enzymatically and for measurement of ATP, ADP, PC, $\beta$-GPA, and $\beta$-GPAP concentrations by an automated chromatographic procedure (2, 7).

RESULTS

The average twitch tensions developed during the first 130 s of stimulation of hypoxic tibialis anterior muscles from rats fed $\beta$-GPA and of comparably treated muscles from control rats are shown in Fig. 1. By 140 s, the twitch tensions of several muscles had already dropped to 25% of their peak tensions, at which point they were frozen.
The initial twitch tensions were the same in control muscles and in muscles from rats fed \( \beta \)-GPAP. However, during the first 50 s of stimulation, control muscles exhibited the staircase phenomenon, with a 30-g increase in twitch tension per gram of muscle, before tension started to decrease. In contrast, tension began to decrease immediately in muscles from rats fed \( \beta \)-GPAP. Consequently, the peak tension and final tension (25% of peak tension) were significantly higher in control muscles \((P < 0.02)\), and the area under the curve of twitch tension, plotted as a function of time, was 28% lower for muscles from rats fed \( \beta \)-GPAP than for control muscles.

The stimulation of these hypoxic tibialis anterior muscles caused large changes in the concentrations of lactate, ATP, PC, Pi, \( \beta \)-GPA, and \( \beta \)-GPAP (Table 1). Of particular interest is the finding that, despite roughly similar decreases in the concentrations of \( \beta \)-GPAP and PC as a result of contractile activity, the concentration of \( \beta \)-GPAP in muscles from rats fed \( \beta \)-GPAP was still higher after stimulation than the PC concentration in resting control muscles. It is also noteworthy that feeding \( \beta \)-GPA caused decreases in the concentrations of ATP and PC and accumulation of \( \beta \)-GPA and \( \beta \)-GPAP in resting tibialis anterior muscles (Table 1) to the same extent as has been reported previously for gastrocnemius muscles (2).

**DISCUSSION**

The relationships between the use of high-energy phosphate and tension development confirm the ability of \( \beta \)-GPAP to substitute for PC as a source of energy to sustain muscle contraction. The total use of high-energy phosphate per gram of stimulated muscle can be estimated by taking the sum of the decreases in the concentrations of ATP and PC and the difference between the expected increase in ATP concentration and that found, plus 1.5 times the increase in lactate concentration (assuming that 1.5 \( \mu \)mol of ATP was formed for each \( \mu \)mol of lactate produced). This amounts to 49.0 \( \mu \)mol for the control muscles and to 22.4 \( \mu \)mol for muscles from rats fed \( \beta \)-GPAP. In addition, the muscles from rats fed \( \beta \)-GPAP had a decrease of 10.9 \( \mu \)mol/g in the concentration of \( \beta \)-GPAP. Including this decrease in \( \beta \)-GPAP, the use of high-energy phosphate may be calculated to be 32% lower in muscles from rats fed \( \beta \)-GPAP than in control muscles. This apparent reduction in energy use corresponds closely to the 28% reduction in ATP concentrations in the presence of high \( \beta \)-GPAP. Whether or not ATP concentrations in the vicinity of the actin-myosin crossbridges are low enough to cause such a rapid decline in twitch tension and absence of the staircase phenomenon remains to be determined. Also to be determined is the reason for low ATP concentrations in the presence of high \( \beta \)-GPAP concentrations. For example, the ATP concentration was only 2.7 \( \mu \)mol/g in resting muscles from rats fed \( \beta \)-GPAP when the \( \beta \)-GPAP concentration was 30.3 \( \mu \)mol/g. With stimulation of the muscles, the ATP concentration decreased to 1.7 \( \mu \)mol/g although the \( \beta \)-GPAP concentration (19.4 \( \mu \)mol/g) remained higher than that of PC in resting control muscles. In contrast, the ATP concentration was maintained at 2.7 \( \mu \)mol/g in the stimulated control muscles although the PC concentration decreased from 15.8 to 1.5 \( \mu \)mol/g. Possibly, the \( \beta \)-GPAP is either inaccessible to creatine kinase or is a poor substrate for the enzyme.

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