Physiol. Res. 44: 257–260, 1995

RAPID COMMUNICATION

Gluconeogenesis During Hypoxia in Vascular Smooth Muscle Studied by ^{13}C-NMR

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Received May 31, 1995
Accepted June 5, 1995

Summary

We investigated whether hypoxia altered the utilization of fructose-1,6-bisphosphate as a gluconeogenic or glycolytic intermediate in superfused media from hog carotid artery. Using ^{13}C-NMR, we found that although 3-^{13}C-lactate production from 1-^{13}C-glucose increased compared to that under well-oxygenated conditions, the conversion of exogenously applied 1,6-^{13}C-fructose-1,6-bisphosphate to glucose (gluconeogenesis) or to 3-^{13}C-lactate was not significantly affected by hypoxia. Since hypoxia alters the rate of glucose conversion to lactate but not the conversion of fructose-1,6-bisphosphate to glucose, we conclude that glycolysis and glycogenolysis may continue to be compartmentalized during hypoxia and that a high rate of gluconeogenesis can occur even during hypoxia.

Key words

Hog carotid artery – Glucose – Glycolysis

The highly glycolytic nature of vascular smooth muscle has made it a model system for the study of the organization of carbohydrate metabolism and for studies of cellular responses to hypoxia. Paul and his colleagues have shown that oxygen consumption and lactate production can vary, often in opposite directions (Paul 1983), and have suggested that oxidative metabolism may provide ATP specifically for contraction while a membrane-associated glycolytic pathway may provide ATP to membrane-associated ATPases such as the sodium pump (Campbell and Paul 1992) or the calcium pump (Hardin *et al.* 1992). In addition, using ^{13}C-NMR we have shown that when glucose and glycogen were ^{13}C-labeled at different positions, the intermediates of the two pathways did not completely mix despite simultaneous breakdown of glucose and glycogen (Hardin and Kushmerick 1994). Furthermore, we have recently shown that vascular smooth muscle is capable of gluconeogenic flux and that this gluconeogenic flux is compartmentalized from glycolytic flux (Hardin and Roberts 1995). Therefore carbohydrate metabolism in vascular smooth muscle appears to be structured with spatially distinct sets of glycolytic enzymes carrying out glycolysis, glycogenolysis and gluconeogenesis.

Since the rate of glycolysis (glucose conversion to lactate) typically increases during hypoxia, if glycolysis and gluconeogenesis were not compartmented, it would be predicted that gluconeogenesis would decrease or cease during hypoxia and metabolism of fructose-1,6-bisphosphate would shift to lactate. We sought to determine whether hypoxia results in a decline in gluconeogenic flux during hypoxia in vascular smooth muscle.

Hog carotid arteries were obtained from local abattoirs within ≈30 minutes of slaughter. Arteries were placed in a cold (≈5 °C) physiological saline solution (PSS), pH 7.4, pre-equilibrated with a gas mixture of 95 % O_2 and 5 % CO_2. PSS was composed of (mM): NaCl (116), KCl (4.6), KH_2PO_4 (1.16), $NaHCO_3$ (25.3), $CaCl_2$ (2.5), and $MgSO_4$ (1.16). Segments were dissected free of loose fat, connective tissue, and adventitia.

The synthesis of 1,6-^{13}C-fructose-1,6-bisphosphate was performed as previously described (Hardin and Roberts 1994). Tissues were unmounted, incubated at 37 °C and contracted and relaxed twice in the presence of glucose and twice in the absence of glucose. Contraction was elicited for 20 min by switching to PSS with 5 mM glucose with 80 mM KCl added. Tissues were relaxed for 20 min by switching the superfusate back to PSS with 5 mM glucose. Approximately 900 mg of tissue was added into capped tissue incubation chambers filled with new superfusate (with 80 mM added KCl) consisting of either 2.4 ml concentrated PSS (1.25 x concentration of PSS of all components) and 0.6 ml of 1,6-^{13}C-fructose-1,6-bisphosphate solution or PSS with 5 mM 2-^{13}C-glucose. The final concentration of 1,6-^{13}C-fructose-1,6-bisphosphate was 2.1 mM. The superfusate was equilibrated with either 95 % N_2 and 5 % CO_2 or 95 % O_2 and 5 % CO_2 throughout the incubations with label. After 180 minutes of incubation, superfusate and tissues were rapidly frozen in liquid N_2 and stored at −80 °C. Tissues were blotted dry and weighed at the end of all incubations.

Frozen superfusates (2.5 ml aliquots) containing ^{13}C-labeled compounds were lyophilized in a Speed Vac (Savant Instruments, Inc.) and resuspended in 1 ml 99 % D_2O with 25 mM 3-(trimethylsilyl)-1-propane-sulfonic acid (DSS) as a chemical shift reference (set to 0 ppm). ^{13}C-NMR spectroscopy was performed on a Bruker AMX 500 spectrometer with the following acquisition parameters: 300 scans with 16 dummy scans, 30° pulse angle at 125.77 MHz, 33,333 Hz sweep width, and a 1 second pre-delay. 32K points were acquired and processed with 1 Hz line broadening prior to Fourier transform. All spectra were broadband proton decoupled.

Statistical significance was determined using an unpaired two tailed Student's t-test with $p=0.05$ assuming unequal variances.

When hog carotid artery was incubated under hypoxic conditions, the production of lactate from glucose significantly increased compared to incubations performed under well-oxygenated conditions. Fig. 1 shows the mean 3-^{13}C-lactate production from 1-^{13}C-glucose under well-oxygenated and hypoxic conditions. Under hypoxic conditions, lactate production from glucose increased significantly ($p=0.009$), an average of 72.8 % ($n=3$) indicating that there was a substantial increase in lactate production from glucose compared to carotid segments incubated in PSS equilibrated with 95 % O_2 and 5 % CO_2.

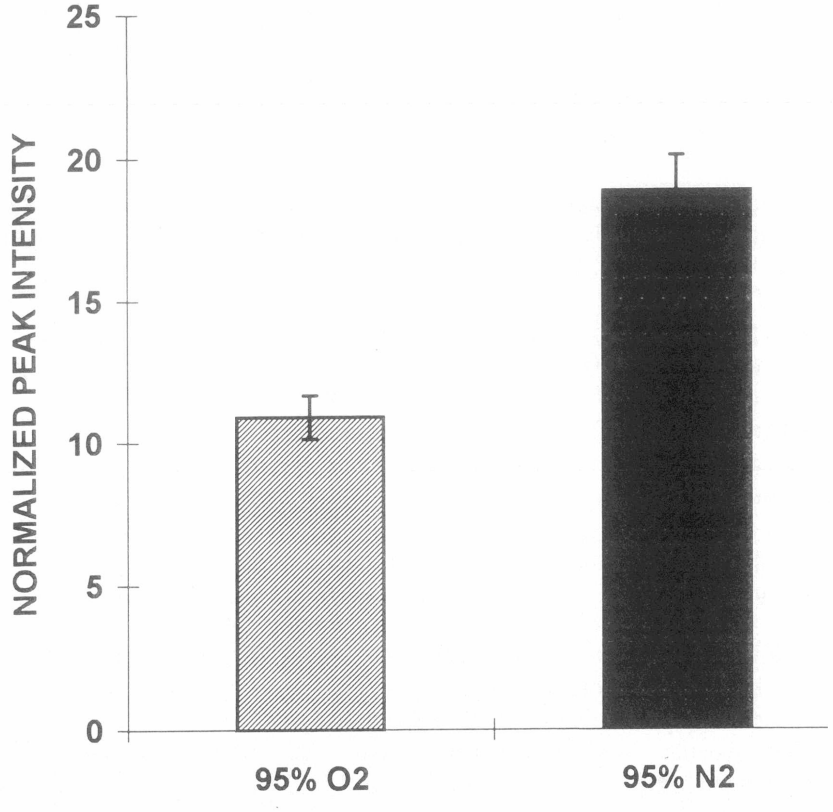

Fig. 1
^{13}C-NMR peak intensities of 2-^{13}C-lactate (from 2-^{13}C-glucose metabolism) normalized to DSS-methyl peak intensity and to tissue mass in either well-oxygenated (gray bar) or hypoxic (black bar) conditions. All peaks are expressed relative to the DSS peak at 0 ppm and no corrections were made for nuclear Overhauser effects which were assumed to be unchanged for all experiments. Bars represent the mean ± S.E.M., $n=3$.

When hog carotid artery is incubated with 1,6-^{13}C-fructose-1,6-bisphosphate under hypoxic conditions, resonances corresponding to 1,6-^{13}C-glucose and 3-^{13}C-lactate appear, consistent with fructose-1,6-bisphosphate acting as a glycolytic and gluconeogenic substrate. To determine if hypoxia results in a stimulation of glycolysis from fructose-1,6-bisphosphate and an inhibition of gluconeogenesis from fructose-1,6-bisphosphate, we incubated segments of hog carotid artery superfused with PSS containing 2.1 mM 1,6-^{13}C-fructose-1,6-bisphosphate under hypoxic and well-oxygenated conditions. Shown in Fig. 2, hypoxia resulted in no significant change in 1-^{13}C-glucose and 6-^{13}C-glucose production ($p=0.07$ and 0.33, respectively) compared to carotid segments similarly superfused but under well-oxygenated conditions. In addition, incubation of carotid segments with 1,6-^{13}C-fructose-1,6-bisphosphate under hypoxic conditions resulted in no significant change ($p=0.21$) in 3-^{13}C-lactate production compared to carotid segments similarly superfused but under well-oxygenated conditions. Therefore, in contrast to flux of glucose to lactate, the flux of fructose-1,6-bisphosphate to either glucose or lactate was not significantly altered during hypoxia.

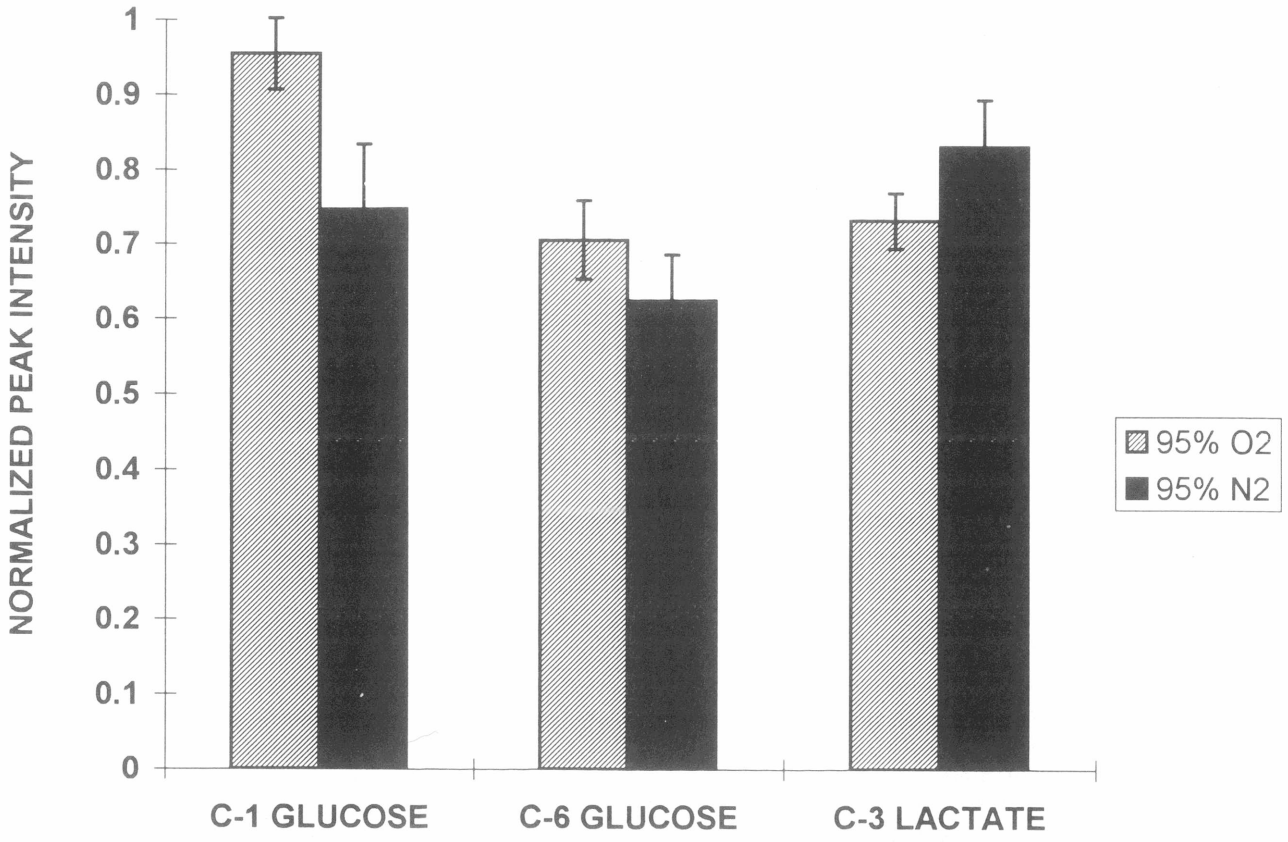

Fig. 2
^{13}C-NMR peak intensities of 1-^{13}C-glucose and 6-^{13}C-glucose (gluconeogenesis from 1,6-^{13}C-fructose-1,6-bisphosphate) and 3-^{13}C-lactate (from 1,6-^{13}C-fructose-1,6-bisphosphate) normalized to DSS-methyl peak intensity and to tissue mass in either well-oxygenated (gray bars) or hypoxic (black bars) conditions. All peaks are expressed relative to the DSS peak at 0 ppm and no corrections were made for nuclear Overhauser effects which were assumed to be unchanged for all experiments. Bars represent the mean ± S.E.M., $n=6$.

We have recently shown that hog carotid artery is capable of gluconeogenic flux from fructose-1,6-bisphosphate and that this gluconeogenic flux was compartmentalized from glycolytic flux (Hardin and Roberts 1995). It is commonly believed that glucose-6-phosphatase-phosphotransferase activity is found only in liver, kidney and intestinal mucosa. However, glucose-6-phosphatase-phosphotransferase activity is known to exist in other tissues including pancreas, brain, adrenals, and testes (for a review see Nordlie 1975). In cardiac tissue and coronary vascular tissue from dogs, glucose-6-phosphatase was also localized to the endoplasmic reticulum and the nuclear envelope

(Borgers *et al.* 1971). Therefore, gluconeogenic capability exists in vascular smooth muscle.

During hypoxia, hog carotid artery exhibits an increased conversion of glucose to lactate (Fig. 1). If the exogenously added fructose-1,6-bisphosphate accesses the same set of glycolytic enzymes as those used for glucose catabolism, then a substantial increase in lactate from fructose-1,6-bisphosphate should also be observed. However, there is no significant increase in the lactate production from fructose-1,6-bisphosphate (Fig. 2) indicating that the fructose-1,6-bisphosphate produced by glucose metabolism is a different pool of fructose-1,6-bisphosphate than that derived from exogenous fructose-1,6-bisphosphate administration. Therefore, the compartmentation of glucose utilization (glycolysis) and fructose-1,6-bisphosphate utilization (gluconeogenesis) appears to continue during hypoxia. The compartmenta-tion of glycolysis and glycogenolysis in vascular smooth muscle has been proposed to allow for localized energetic supply localized ATPases (Hardin and Paul 1995). It has been proposed that glycolytically produced ATP may support membrane ATP-requiring processes such as membrane ion pumps while glycogenolysis may provide carbon substrates to the mitochondria for support of contraction by ATP produced by oxidative phosphorylation (Hardin and Paul 1995). Presumably, the separation of the intermediates of glycolysis and gluconeogenesis, which even occurs during hypoxia, may provide for a independent regulation of glycolysis and gluconeogenesis. However, the physiological function of gluconeogenic flux in vascular smooth muscle has not been determined.

Acknowledgments

This work was supported by NIH HL48783 (CDH) and NSF Instrumentation Grant 8908304. Hog carotid arteries were graciously provided by Tyson Foods, Marshall, MO.

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