EFFECTS OF GLYCINE ON PROTEIN SYNTHESIS AND DEGRADATION IN C2C12 MUSCLE CELLS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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Glycine is an amino acid that is used for the synthesis of muscle proteins. In the present study, we conducted an experiment to determine the optimal amount of glycine for C2C12 cells to be synthesized and prevent degradation. The experiment required the cells to be cultured in medium of radioactive phenylalanine ^{3}H-labeled phenylalanine (*Phe) and one of 5 concentrations of glycine (10, 100, 250, 500, or 1000 μM). By counting the radioactivity of *Phe in protein, we were able to determine protein synthesis in these cells, and determined what concentration of glycine worked best. Our findings show that 100 to 1000 μM of glycine stimulated protein synthesis in C2C12 cells in a dose-dependent manner ($P<0.05$). C2C12 cells were also used to determine protein degradation in the presence of 0 to 1000 μM glycine. Specifically, the cells were cultured in *Phe and 0, 100, 250, 500 0r 1000 μM glycine. After culturing for 24 h, the release of *Phe from the prelabeled protein was determined as an indicator of protein degradation. Glycine (0-1000 μM) reduced protein degradation in a dose-dependent manner. These results indicate that glycine is an anabolic amino acid to enhance protein accretion in muscle cells. Supported by USDA-NIFA grants and Texas A&M University.

NOMENCLATURE

mL	Milliliter
μL	Microliter
mM	Millimolar
Gly	Glycine
[^{3}H]-Phe	Tritium treated Phenylalanine
TCA	Trichloroacetic acid
DMEM/F-12	Dulbeco's Modified Eagle Medium
NaOH	Sodium Hydroxide
mTORC1	mammalian target of rapamycin complex 1

CHAPTER I

INTRODUCTION

Glycine is the most abundant amino acid in the plasma of pigs and is used for the synthesis of tissue proteins. Although most mammals are able to synthesize glycine on their own, some young animals may need additional supplementation (Wang et al. 2013; Brosnan et al. 2016). Glycine has been found to increase the rate of DNA replication and the activation of mTORC1 activation (Sun et al. 2016). This information shows that glycine should be able to promote the synthesis of protein within the cell when in sufficient amounts. Glycine is also partially responsible for the synthesis of creatine; a component of cellular energy by way of shuttling high energy phosphates (Brosnan et al. 2007). The goal of this study was to determine a role for glycine in regulating protein synthesis and degradation in C2C12 muscle cells. C2C12 cells are mouse myoblast cells that readily form myotubules in culture. Results of this study are expected to enhance our knowledge of amino acid nutrition and the design of new diets to improve animal growth and feed efficiency.

CHAPTER II

METHODS

Protein Synthesis

C2C12 cells were cultured in a medium of DMEM/F-12 until a population of 5.00 X 10^6 cells were obtained. These cells were placed in 12 well culture plates and cultured in media containing 0.2 mM Gly for 4 days. This media was removed and replaced with a new solution of 0.1 mM Gly for 6h. The medium was removed and cells were analyzed for protein content using a BSA assay kit. Medium was then replaced with a new solution containing radioactive [^{3}H]-Phe and varying amounts of Gly (10, 100, 250, 500, or 1000 μM) for 3h. These cells were scraped with 1mL TCA and centrifuged. The supernatant fluid was removed leaving only a cell pellet, which was then dissolved in 1 M NaOH. 500μL of the dissolved pellet mixture was then transferred to a scintillation vial containing 15 mL of hionic fluor cocktail, and placed in a scintillation counter, to then count the degradations per minute of [^{3}H]-Phe.

Protein Degradation

C2C12 cells were cultured in 6- well plates containing DMEM/F-12 for 16 h. The medium was replaced with a solution containing variable amounts of Gly depending on group (10, 100, 250, 500, or 1000 μM) for 24 h. This medium was replaced with a similar solution containing variable amounts of glycine, but this time containing [^{3}H]-Phe to label cellular proteins. 500 μL of the medium were mixed with 15 mL of Hionic fluor and placed in a scintillation counter used to determine [^{3}H]-Phe as an indicator of intracellular protein degradation. The cells were then scraped using TCA and centrifuged. After the supernatant fluid

was removed; the pellet was dissolved in 1M NaOH and used to determine protein [^{3}H]-Phe by mixing 500 μL of pellet solution with 15mL of Hionic fluor and placed in a scintillation counter.

Statistical Analysis of Data

Values are expressed as means ±SEM. Data were analyzed by one-way analysis of variance, as described by Assaad et al. (2014). Differences among the means were determined by the Duncan multiple comparison test.

CHAPTER III

RESULTS

Protein Synthesis

In the protein synthesis portion of the experiment, 100 to 1000 μM glycine enhanced ($P < 0.05$) the rate of protein synthesis in C2C12 cells in a dose-dependent manner, compared with 10 μM glycine (Table 1). However, the rate pf protein synthesis did no differ ($P > 0.05$) between the 100 and 250 μM glycine groups. It is not known whether glycine activates the mechanistic target of rapamycin signaling pathway in muscle cells.

TABLE 1 Effects of Glycine on rates of protein turnover in C2C12 cells[1]

Protein turnover	Gly concentration in incubation medium (μmol/L)				
	10	100	250	500	1000
Protein synthesis (nmol/mg protein/3h)	35.8 ± 1.5^{d}	43.5 ± 1.7^{cd}	48.6 ± 2.0^{c}	56.4 ± 2.8^{b}	64.1 ± 3.3^{a}
Protein degradation (%/3h)	5.31 ± 0.23^{a}	4.80 ± 0.19^{b}	4.52 ± 0.16^{bc}	4.28 ± 0.15^{cd}	3.75 ± 0.13^{e}

[1]Values are means ± SEM, n = 8 independent experiments.

[a-d] Means in a row without a common letter differ ($P < 0.05$). Gly, glycine.

Protein Degradation

In the protein degradation portion of the experiment, 100 to 1000 μM glycine reduced ($P < 0.05$) the rate of protein degradation in C2C12 cells in a dose-dependent manner, compared with 10 μM glycine (Table 1). However, the rate of protein degradation in the 250 μM glycine group did not differ ($P > 0.05$) from that of 100 or 500 μM glycine group. Thus, Physiological concentrations of glycine (250-100 μM) had an anabolic effect to enhance protein accretion in C2C12 muscle cells. It is not known whether glycine suppresses autophagy or proteases in muscle cells.

CHAPTER IV

CONCLUSION

Glycine is a physiologically and nutritionally important amino acid for muscle-cell growth. This amino acid, which is deficient in corn- and soybean-based diets fed to young pigs, can stimulate protein synthesis and inhibit proteolysis in C2C12 cells. As a functional amino acid, glycine holds promise for improving muscle protein accretion in animals. We are currently doing experiments to determine the mTOR signaling pathway in C2C12 cells cultured in the presence of 10 to 1000 μM glycine.

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