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RAPID AND TRANSIENT INDUCTION OF c-fos, c-myc AND c-Ha-ras IN RAT LIVER FOLLOWING GLYCINE ADMINISTRATION

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Administration of glycine (2.5 mmoles/100 g., i.p.) results in an increased expression of several cell cycle dependent genes such as c-fos, c-myc and c-Ha-ras in the rat liver. The increased expression could be noticed as early as 20-40 minutes and declined by 2 hours following glycine administration. The rapid rise and decline in the mRNA levels of c-fos, c-myc and c-Ha-ras in response to glycine is of significance because in response to a wide variety of growth stimuli, these proto-oncogenes exhibit a temporal sequence in their expression; for example, the expression of c-fos precedes that of c-myc, which in turn precedes the increased expression of c-Ha-ras. The experimental model using a simple amino acid such as glycine will be useful in exploring some of the mechanisms of regulation of expression of these proto-oncogenes.

Recent studies have revealed that in response to a growth stimulatory signal certain genes including some proto-oncogenes exhibit increased expression in a temporal sequence during the cell cycle (1-3). Examples are expressions of (a) c-fos and c-myc which are expressed in early G1 (4-6), (b) ODC (7,8), ß actin (9), thymidine kinase (10) etc., which are involved in DNA synthesis and expressed in late G1 and (c) c-Ha-ras which is expressed during S phase (11,12). During the course of our studies on the mechanism by which amino acids such as glycine induce the synthesis of orotic acid, a liver tumor promoter (13,14), we observed in rat liver that administration of glycine resulted in an increase within 15 minutes not only the synthesis of orotic acid but also ornithine decarboxylase (ODC) (15,16), an enzyme associated with cell cycle. Since the induction of ODC was so rapid it became of interest to determine whether glycine would induce in a rapid fashion the expression of other cell cycle dependent genes. Interestingly, glycine induced a rapid expression of c-fos, c-myc and c-Ha-ras without any temporal sequence.

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Materials and Methods

Animals: Male Fischer 344 rats weighing 140-150g (Charles River Breeding Laboratories, St. Constant, Quebec, Canada) were acclamatized to basal diet (No. 101, DYETS Inc., Bethlehem, Pa.) and daily cycles of light and darknes for one week before the start of the experiment. Animals were injected intraperitoneally with a neutral solution of glycine (2.5 mmoles/100 g) in 0.9% NaCl. Control rats recieved an equal volume of saline. Animals were sacrificed under ether anasthesia and the livers were frozen in liquid nitrogen.

Preparation of messenger RNA and Northern blotting: Total RNA was extracted (17) from pooled livers of 3 animals sacrificed per time point. Polyadenylated RNA was isolated by 2 cycles of oligo (dT)- cellulose coloumn chromatography (18). The amount of poly A+ RNA in each sample was quantitated by O.D. measurements and by dot blot hybridization with oligo U probe. Samples of 10 µg poly A+ RNA were denatured and subjected to electrophoresis on 1% agarose gels (19). RNA was then transferred to gene screen plus (New England Nuclear) and immobilised with UV (20). c-DNA probes were labelled with ^{32}p dCTP by random priming (21). The blots were prehybridised and then hybridised at 65° C in the absence of formamide and washed in 2X SSC at 65° C. The bands were revealed by autoradiography (22).

Probes: Probes for c-fos, c-myc (3rd exon), c-Ha-ras and ß actin were purchased from Oncor Inc., Maryland. Ribonucleotide reductase and ornithine decarboxylase were generous gifts from Enrico Wensing, Dept. of Microbiology, Univ. of Toronto.

Results and Discussion

The results presented in Fig. 1 Panel A clearly indicate that administration of glycine resulted in increased levels of poly A RNA transcripts of c-fos, c-myc, c-Ha-ras and ODC. However, glycine did not induce the expression of ß actin and ribonucleotide reductase.

The kinetics of expression of c-myc, c-Ha-ras and ODC were similar. The increased expression was detectable as early as 20 minutes after the administration of

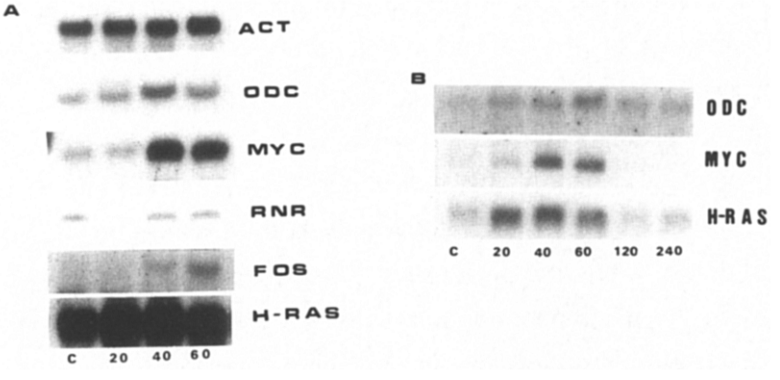

Fig. 1: Expression of cell-cycle dependent genes in glycine treated livers. Experiment was repeated at least 3-4 times with similar pattern of results. RNR- ribonucleotide reductase, ACT- ß actin.

glycine. Their expression peaked by 40-60 minutes and declined by 2 hours indicating the transient nature of expression (Fig. 1 Panel B). The expression of c-fos was detectable at 40 minutes, peaked at 60 minutes (Fig. 1 Panel A) and was undetectable at 2 hours (data not shown).

Actinomycin D (75 μg/100 g b.w., i.p.) given 30 minutes prior to the administration of glycine inhibited glycine-induced expression of c-myc and c-Ha-ras (data not presented). Further, glycine did not inhibit protein synthesis as monitered by the incorporation of tritiated leucine into hot trichloroacetic acid precipitable material. These results suggest that the increased expression of c-myc and c-Ha-ras seen in this study is a reflection of increased synthesis rather than a stabilisation of the transcripts due to an inhibition of specific nucleases as postulated for the increased c-myc transcripts following the administration of cycloheximide (17,18).

Surprisingly, even though glycine induced c-fos, c-myc, c-Ha-ras and ODC there was no DNA synthesis as measured by both specific activity 24 hours after glycine treatment and cumulative labelling index for one week. In the light of this fact, a point that merits consideration is the temporal sequence of expression of c-fos, c-myc, c-Ha-ras and ODC during cell cycle. Both in vivo and in vitro cells respond to growth stimuli by expressing these cell-cycle dependent proto-oncogenes in a temporal sequence, that is, the expression of c-fos precedes that of c-myc, which in turn precedes the increased expression of c-Ha-ras. In addition, even though the rise in expression of c-fos is transient, that of c-myc and c-Ha-ras are more sustained (19-22). These changes in the expression of genes are perhaps related to the regulation of a restriction point in the cell cycle or a response to a cell cycle specific regulatory signal. A lack of temporal sequence together with the transient nature of increased expression, especially of c-Ha-ras may in part account for the failure of DNA synthesis following glycine administration.

Our observation that the expression of c-fos, c-myc, c-Ha-ras and ODC can be induced in a rapid and non-coordinated fashion by a simple amino acid such as glycine is not only new but also provocative. The mechanism by which glycine induces the expression of proto-oncogenes associated with cell cycle is not clear. Nevertheless, it poses the question whether amino acids singly or in a mixture can serve as transducer molecules for signals affecting cell proliferation and thus be involved in the events of the cell cycle. Induction of ODC by growth factors has already

shown to be mediated by amino acids (23). Long known yet not well recogonised effect of proteins on cell proliferation (24) requires to be reevaluated in terms of induction of expression of cell cycle dependent genes by individual amino acids.

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