Turk J Med Sci
35 (2005) 71-74

EXPERIMENTAL / LABORATORY STUDIES

Differential Effect of Glutathione Depletion on Glycogenolysis in Isolated Rat Hepatocytes Mediated by α-Adrenoceptor Agonists and Glucagon

Mukadder ATMACA[1], Jeffrey R. FRY[2]

[1]Department of Physiology, Faculty of Medicine, University of Dicle, Diyarbakır, Turkey

[2]School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH U.K.

Received: December 16, 2004

Abstract: Glutathione (GSH) exerts a variety of cytoprotecive effects, but is readily depleted from cells under a variety of stressful stimuli. The impact of GSH depletion on receptor-mediated activity in rat hepatocytes has been studied with regard to glycogenolysis stimulated with a-adrenoceptor agonists or glucagon, which exert their actions through different signalling pathways. Depletion of GSH content (70%) by diethyl maleate had no effect on the redox status of the cells or on basal or glucagon-stimulated glycogenolysis, but significantly reduced the response to the a-adrenoceptor agonists adrenalin and phenylephrine. These results highlight the possible modulatory effects of GSH on receptor-mediated activity in hepatocytes.

Key Words: α-adrenoceptors, glucagon, glutathione, glycogenolysis, hepatocytes receptors

Introduction

Glutathione (GSH) exerts a variety of cytoprotective effects, one such being the maintenance of protein thiol groups in a reduced state, through a role in providing the reduced milieu required by cells (1,2). This function of GSH is of particular importance in defence against oxidative stress. Such protein thiol groups are essential for the correct functioning of a variety of proteins, including those involved in receptor-mediated activity. Whilst modulation of receptor-mediated activity by GSH has been documented (3-5), there appears to be little reported in hepatocytes. These cells express a wide range of receptor-mediated activities, and GSH action is an important cytoprotective mechanism against a variety of potentially toxic insults (6).

We therefore studied the influence of GSH depletion on the glycogenolytic response of rat hepatocytes to a-adrenoceptor agonists and glucagon. Both of these agents ultimately act to activate glycogen phosphorylase but by different signalling pathways – an increase in cAMP level for glucagon and elevation of intracellular Ca^{2+} by the adrenoceptor agonists.

Materials and Methods

Adult male Wistar rats (150-180 g) were obtained from the University of Nottingham Medical School Animal Unit. They were housed at a constant room temperature of 22 ºC and had free access to standard laboratory diet and tap water. Six-well cluster dishes were purchased from Fahrenheit Laboratory Supplies (Nottingham, U.K.). Leibowitz L-15 culture medium, adrenaline, glucagon, phenylephrine, diethyl maleate (DEM), a Trinder glucose assay kit, MTT, collagen, collagenase and Percoll were purchased from Sigma Chemical Co. (Poole, Dorset, U.K.).

Hepatocytes were isolated by perfusion of liver lobes with collagenase, essentially as described by Reese and Byard (7). The hepatocyte suspension was washed with Ca^{2+}-free HEPES buffer, pH 7.4 (136.89 mM NaCl, 5.36 mM KCl, 0.44 mM KH_2PO_4, 0.38 mM Na_2HPO_4, 10 mM NaOH, 20 mM HEPES, and 50 mM phenol red). Cells were resuspended in Leibowitz L-15 medium and centrifuged (50 g for 10 min) in a 90% Percoll solution to improve the separation of viable and nonviable cells, as described by Kreamer et al. (8). The cell viability, as

determined by trypan blue exclusion, was typically greater than 95%. Hepatocytes were suspended in L-15 medium containing 10% v/v calf serum, dispensed into collagen-coated 6-well plates at a density of 10^6 cells per well, and allowed to attach to the substratum for 1-2 h. Following this attachment period, the medium was removed and hepatocytes were immediately incubated in complete HEPES buffer pH 7.4 (composition as before with the addition of 1.26 mM $CaCl_2$) containing agonists for 1 h. Alternatively, the cells were exposed to 0.6 mM DEM for 30 min, after which the cells were washed and then exposed to the agonists. After the incubation period, 100 ml of medium was assayed for glucose according to the instructions in the assay kit. Cells were treated with 15% w/v sulphosalicylic acid and the resulting solution assayed for non-protein thiols by the method suggested by Saville (9), as described previously (10). The cellular redox status was determined by MTT reduction as described by Dhanjal and Fry (11).

Data are presented as mean ± SEM of at least 4-5 experiments. Statistical analysis was undertaken using unpaired t-tests or analysis of variance with Dunnett's post-hoc test as appropriate. A value of $P < 0.05$ was considered significant. The EC_{50} value for adrenalin was defined as the concentration that produced 50% of the maximal enhancement of glycogenolysis (determined at 10^{-5} M).

Results

Treatment of hepatocytes with 0.6 mM DEM led to a 70% reduction in cellular thiol content. This had no significant effect on the basal level of glycogenolysis or the level of MTT reduction (Table). Treatment with glucagon (10^{-5} M) caused a significant 3-fold enhancement in glycogenolysis, which was not modified by prior GSH depletion (Table).

Treatment with adrenalin produced a concentration-dependent enhancement of glycogenolysis which was significantly attenuated after GSH depletion at adrenalin concentrations above 10^{-9} M (Figure). Despite this, the EC_{50} value for adrenalin remained unaltered after GSH depletion (no depletion 23.0 ± 9.9 nM; with depletion 26.3 ± 15.8 nM; n = 5 rats; $P > 0.05$).

Phenylephrine (10^{-5} M) produced a statistically significant enhancement of glycogenolysis, this again being blunted after GSH depletion (Table).

Discussion

As expected, the 3 agonists studied in this work produced significant enhancement of glycogenolysis. Adrenalin was more potent than phenylephrine in eliciting this enhancement, whilst the maximal effects observed with adrenalin and glucagon were comparable; these

Table. Influence of DEM treatment on hepatocyte thiol content, MTT reduction, and levels of basal and agonist-stimulated glycogenolysis.

Variable	Untreated Cells	DEM-Treated Cells
Thiol content (nmol/well)	36.0 ± 1.6	10.3 ± 0.5*
MTT reduction (absorbance)	0.16 ± 0.01	0.16 ± 0.01
Glycogenolysis:		
Basal (mg glucose released per well in 30 min)	0.14 ± 0.01	0.16 ± 0.01
Stimulated with glucagon (10-5 M) (fold increase above basal)	3.06 ± 0.21	2.71 ± 0.11
Stimulated with phenylephrine (10-5 M) (fold increase above basal)	1.94 ± 0.29	1.60 ± 0.20*

Values are mean ± SEM of 4-5 separate animals

* Where indicated, values for DEM-treated cells are significantly different from those incubated without DEM treatment ($P < 0.05$ or less; paired t-test).

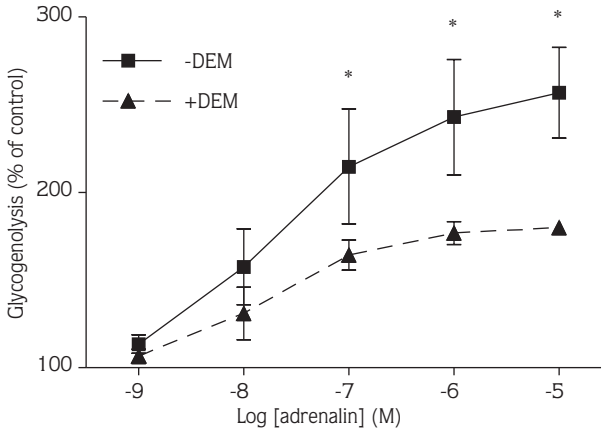

Figure. Influence of DEM treatment on adrenalin-stimulated glycogenolysis in rat isolated hepatocytes. Values are mean ± SEM of 5 separate animals.

* Where indicated, values for DEM-treated cells are significantly different from those incubated without DEM treatment ($P < 0.05$ or less).

findings are in agreement with those of other researchers (12,13).

Treatment with DEM (0.6 mM) produced the expected marked depletion of GSH, which was not associated with a gross alteration in the cellular redox status as determined by MTT reduction. GSH depletion did not impair the basal level of glycogenolysis, but did cause a reduced effect of adrenalin on glycogenolysis, as manifested by a reduction in the maximum response with no change in the EC_{50} value. Krack and et al. (14) demonstrated that DEM at a concentration of 0.9 mM by itself caused a fall in hepatocyte glycogen content. However, it seems unlikely that the reduced response to adrenalin after DEM treatment could be ascribed to a reduction in glycogen content, as the effect of glucagon, which was comparable to that of the highest concentration of adrenalin, was not modified by this treatment. This, together with the lack of effect of DEM treatment on basal glycogenolysis, suggests that the reduction in adrenalin responsiveness after DEM treatment is not mediated directly on the enzymic pathways involved in glycogen breakdown. In the adult rat, adrenalin stimulates hepatic glycogenolysis via a_1-adrenoceptors, and the finding that the stimulatory effects of another a_1-adrenoceptor agonist, phenylephrine, are also attenuated after GSH depletion points to a selective modification of adrenoceptor activity by GSH depletion in rat hepatocytes. These observations are consistent with evidence for the involvement of thiol groups in the binding site of a_1-adrenoceptors in the cerebral cortex (15).

Evidence for the presence of multiple reactive thiol groups in the glucagon-sensitive adenyl cyclase system has also been presented (16), but GSH depletion had no significant effect on glucagon-stimulated glycogenolysis (Table). As part of their studies, Lipson et al. (16) also reported that the basal adenyl cyclase activity showed greater sensitivity to thiol alkylation than did the glucagon-sensitive form. Given that basal glycogenolysis was unaffected by GSH depletion in the present study, it is thus possible that the extent of this depletion was insufficient to modify the thiol groups important for glucagon-stimulated glycogenolysis.

It is well known that adrenalin is released into the plasma in response to stress (17), and adrenalin has been reported to inhibit hepatic GSH synthesis (18,2). In the light of these observations, the present findings point to a mechanism whereby the ability of the liver to respond to stress by increasing glucose output mediated by adrenalin is limited by the concomitant GSH depletion. It is also known that hepatic GSH levels are modulated by dietary status and exposure to a variety of toxic chemicals (6,19), and these may also impinge upon the adrenalin-mediated stimulation of glycogenolysis. In a broader context, the present findings provide further evidence for the importance of GSH status in regulating receptor-mediated activity.

Corresponding author:
Mukadder ATMACA
Department of Physiology,
Faculty of Medicine,
University of Dicle,
Diyarbakır - Turkey
E-mail: heja@dicle.edu.tr

References

1. Deneke SM, Fanburg BL. Regulation of cellular glutathione. Am J Physiol 257: L163-L173, 1989.
2. Lu SC. Regulation of hepatic glutathione synthesis: current concepts and controversies. FASEB J 13: 1169-1183, 1999.
3. Piwein-Pilipuk G, Galiniana MD. Oxidative stress induced by L-buthionine-(S,R)-sulfoximine, a selective inhibitor of glutathione metabolism, abrogates mouse kidney mineralocorticoid receptor function. Biochim Biophys Acta 1495: 263-280, 2000.
4. Ogita K, Kitayama T, Okuda H et al. Effects of glutathione depletion by 2-cyclohexen-1-one on excitatory amino acids-induced enhancement of activator protein-1 DNA binding in murine hippocampus. J Neurochem 76: 1905-1915, 2001.
5. Gringhuis SI, Papendrecht-van der Voort EA, Leow A et al. Effect of redox balance alterations on cellular localization of LAT and downstream T-cell receptor signaling pathways. Mol Cell Biol 22: 400-411, 2002.
6. DeLeve LD, Kaplowitz N. Glutathione metabolism and its role in hepatotoxicity. Pharmacol Ther 52: 287-305, 1991.
7. Reese JA, Byard JL. Isolation and culture of adult hepatocytes from liver biopsies. In Vitro 17: 935-940, 1981.
8. Kreamer BL, Staecker JL, Sawada N et al. Use of a low-speed, iso-density percoll centrifugation method to increase the viability of isolated rat hepatocyte preparations. In Vitro Cell Dev Biol 22: 201-211, 1986.
9. Saville B. A scheme for the colorimetric determination of microgram amounts of thiols. Analyst 83: 670-672, 1958.
10. Atmaca M, Hammond AH, Fry JR. Evaluation of a commercial kit for glutathione determination in cellular systems. ATLA 25: 667-673, 1997.
11. Dhanjal P, Fry JR. Determinants of MTT reduction in rat hepatocytes. Biomarkers 2: 111-116, 1997.
12. Wagle SR, Ingebretsen WR. Stimulation of glycogenolysis by epinephrine and glucagon and its inhibition by insulin in isolated rat liver hepatocytes. Biochem Biophys Res Commun 52: 125-129, 1973.
13. Hutson NJ, Brumley FT, Assimacopoulos FD et al. Studies on the alpha-adrenergic activation of hepatic glucose output. I. Studies on the alpha-adrenergic activation of phosphorylase and gluconeogenesis and inactivation of glycogen synthase in isolated rat liver parenchymal cells. J Biol Chem 251: 5200-5208, 1976.
14. Krack G, Goethals F, Deboyser D et al. Interference of chemicals with glycogen metabolism in isolated hepatocytes. Toxicology 18: 213-223, 1980.
15. Reader TA, Briere R, Grondin L. Alpha-1 and alpha-2 adrenoceptor binding in cerebral cortex: role of disulfide and sulfhydryl groups. Neurochem Res 11: 9-27, 1986.
16. Lipson KE, Kolhatkar AA, Dorato A et al. N-ethylmaleimide uncouples the glucagon receptor from the regulatory component of adenylyl cyclase. Biochemistry 25: 5678-5685, 1986.
17. Wortsman J. Role of epinephrine in acute stress. Endocrinol Metab Clin North Am 31: 79-106, 2002.
18. Taylor CG, Nagy LE, Bray TM. Nutritional and hormonal regulation of glutathione homeostasis. Curr Topic Cell Reg 34: 189-208, 1996.
19. Fouin-Fortunet H, Delarue J, n'Djitoyap C et al. Nutritional status modifies glutathione levels in man. Eur J Gastroenterol Hepatol 2: 271-275, 1990.