PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 5 (6): 544-547, 2006
ISSN 1680-5194

Isoprenaline Reverses Glycyrrhizic Acid - Induced Inhibition of 11β-hydroxysteroid Dehydrogenase Bioactivity

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Abstract: Glycyrrhizic acid (GCA) exerts its effect by inhibiting 11beta-hydroxysteroid dehydrogenase (11β-HSD) which catalyses the oxidation of cortisol to cortisone in man and corticosterone (B) to 11-dehydrocorticosterone (A) in rats. This GCA induced inhibition of 11β-HSD activity can be overcome by repetitive stress. Since catecholamines are among the mediators of stress, this study was carried out to determine the effect of isoprenaline on GCA-induced inhibition 11β-HSD bioactivity. Intact male Sprague Dawley rats received either drinking solution containing 1 mg/ml GCA or tap water for 10 days. On the day of killing, the rats received an intraperitoneal injection of vehicle, isoprenaline or propranolol. Hypothalamus, liver and kidney homogenates were assayed for 11β-HSD1 and 11β-HSD2 bioactivity by determining the percentage conversion of B to A in the presence of NADP and NAD respectively. Isoprenaline or propranolol had no effect on basal activity of 11β-HSD in all tissues. Isoprenaline reversed the GCA induced inhibition of 11β-HSD1 activity in the hypothalamus and kidney, whereas in the liver it reversed the GCA induced inhibition of both 11β-HSD1 and 11β-HSD2. Thus, catecholamines may be one of the mediators opposing inhibition of 11β-HSD by GCA during stress.

Key words: glycyrrhizic acid, isoprenaline, 11β-HSD

Introduction

Liquorice, derived from the root of Glycyrrhiza glabra, has been used for more than 4 millennia as a flavoring agent in foods, beverages, and tobacco (Ploeger *et al.*, 2001). Also known as "sweet root," liquorice root contains a compound that is roughly 50 times sweeter than sugar. Common uses of liquorice include treatment of stomach ulcers, dry cough, arthritis, and adrenal insufficiencies. The active component of liquorice, glycyrrhizic acid (GCA), is converted in the body to glycyrrhetinic acid, which is responsible for most of the pharmacological properties of liquorice. Liquorice-derived glycyrrhizic acid (GA) is a well-known inhibitor of 11ß-HSD2 (Ploeger *et al.*, 2000).

11beta-hydroxysteroid dehydrogenase (11β-HSD) is regarded as a novel modulator of corticosteroid hormone action, by regulating the metabolism and thus the accessibility of corticosteroids to receptors in target tissues (Seckl, 1997). Two forms of 11ß-HSD have been identified as separate gene products, HSD1 and HSD2, characterized by specific cofactor requirements for NADP and NAD, respectively (Mune *et al.*, 1995). 11β-HSD1 acts predominantly as a reductase enzyme, generating active 11β-hydroxycorticosteroids by converting inactive 11-dehydrocorticosterone in rats to active corticosterone (Lakshmi and Monder, 1985). 11β-HSD2, on the other hand primarily acts as an oxidative enzyme, converting corticosterone to 11-dehydrocorticosterone (Shimojo *et al.*, 1996a; 1996b). Research on factors determining the bioactivity of 11β-HSD often poses problems, as *in vitro* enzyme activity may not reflect *in vivo* activity. In addition, the activity of these enzymes depends on cosubstrate NADPH and NAD levels.

Studies by our group have shown that various factors (Ruszymah *et al.*, 1995; Ainsah *et al.*, 1999) influence enzyme activity and that corticosteroids appear not to play a role in modulating activity during stress (Farihah *et al.*, 2000), other hormones released during stress, including catecholamines, thus need to be considered (Pignatelli *et al.*, 1998). We have previously shown that the 11β-HSD bioactivity decreases with stress, that this can be prevented by both mineralocorticoids and glucocorticoids (Ainsah *et al.*, 1999) and that GCA inhibits 11β-HSD activity in liver and kidneys (Farihah *et al.*, 2000). During repetitive stress, however, the inhibition of the 11β-HSD is overcome, an effect not due to corticosteroids. Since stress also involves the release of catecholamines, we investigated the effects of isoproterenol, beta-adrenergic receptor agonist, in normal and GCA treated rats to explore possible roles for catecholamines in modulating the activity of the enzymes 11β-HSD1 and 11β-HSD2 in various tissues in the rat.

Materials and Methods

Male Sprague Dawley rats weighing 180-230g from a pathogen-free colony bred in the Animal House, Institute

of Medical Research of Malaysia were used in this study. The rats were housed two per cage, lined with sawdust bedding and maintained on a regular day/night cycle, with the natural light period from 0700-1900 hours. Rodent chow and tap water were available ad libitum. Rats were randomly divided into seven groups. Group 1, were control rats given water to drink, and Group 2, were given glycyrrhizic acid (GCA) in the drinking solution at a dose of 1mg/ml for 10 days. The average amount consumed was 40-50 ml/day per rat. Groups 3 rats were given an intraperitoneal injection of isoprenaline on the day of sacrifice after 10 days treatment with GCA. Groups 4 and 5 rats were control rats receiving an intraperitoneal injection of isoprenaline or propranolol on the day of sacrifice. Group 6 were GCA treated rats receiving intraperitoneal injections of isoprenaline followed by propranolol on the day of sacrifice and group 7 were GCA treated rats receiving an intraperitoneal injection of propranolol followed by isoprenaline on the day of sacrifice. The doses used in these experiments have previously been shown to cause maximal effects (Khalid *et al.*, 1987; Lim *et al.*, 1982; Nabishah *et al.*, 1990).

All experiments were performed in the morning and the animals were sacrificed by decapitation between 0800 and 0900 hours.

Assay for 11β-HSD1 and 11β-HSD2 enzyme activity: The hypothalamus, liver and kidneys were removed and dissected on ice. All other procedures were done on ice unless otherwise stated. Tissues were homogenized in Krebs-Ringer bicarbonate buffer, and total protein content estimated calorimetrically (Bio-Rad, Hercules, CA, USA) on aliquots of each homogenate. Enzyme activity was measured by the method of Moison *et al.*, 1990a; 1990b with some modifications. Two hundred micromolar NADP for 11β-HSD1 activity or NAD for 11β-HSD2 activity and 12nM [1,2,6,7-^{3}H] B (specific activity:84 Ci/mmol; Amersham, Buckinghamshire, England) were added to tissue homogenates containing 0.5 mg protein, (Moison *et al.*, 1990a; 1990b) the cofactors NADP or NAD drive the enzymic reaction towards oxidative activity resulting in 11-dehydrocorticosterone. Krebs-Ringer bicarbonate buffer containing 0.2% glucose and 0.2% BSA were added to make up the total assay volume of 250 µl. The required protein concentration and incubation period were determined from the standard curve using various concentrations. After incubation in a shaking water bath at 37°C for 10 min, the reaction was terminated by the addition of 1 ml of ethyl acetate and steroids were then extracted. The organic layer was separated by centrifugation at 4°C and 3000rpm for 10 min. The top layer was then transferred into glass tubes and evaporated to dryness at 55°C with Techne Dri-Block DB.3A. Steroid residues were dissolved in ethanol containing nonradioactive carrier corticosterone and 11-dehydrocorticosterone and separated by thin layer chromatography (Merck, Darmstadt, Germany) in chloroform and 95% ethanol in the ratio of 92:8. The fractions corresponding to the steroid were located by UV lamp absorption at 240nm, scraped, transferred to scintillation vials and counted in scintillation fluid (Cocktail T) in a Kontron Betamatic fluid scintillation counter. Enzyme activity was calculated as the percentage conversion of [^{3}H]corticosterone to [^{3}H]11-dehydrocorticosterone from the radioactivity of each fraction. The lower limit of detection of 11β-HSD bioactivity was taken as 10% (Moisan *et al.*, 1990b).

The study were approved by the Medical Research and Ethics Committee of the Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM), Malaysia and all data were tested for normal distribution and are presented as mean ± standard error of mean (SEM). Differences in enzyme activity were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple group comparisons. A P-value of <0.05 was taken as significant.

Results

Effects of isoprenaline on 11β-HSD1 and 11β-HSD2 enzyme activity in the liver: Administration of isoprenaline to the normal control rats increased ($P<0.005$) hepatic 11β-HSD1 activity and GCA alone as expected decreased ($P<0.0005$) 11β-HSD1 activity. Isoprenaline given to GCA treated rats increased hepatic 11β-HSD1 to control levels, an effect that was not altered by injection of propranolol prior to or after isoprenaline administration (Table 1). There was however no significant difference in the bioactivity of the 11β-HSD1 enzyme when isoprenaline was given before or after propranolol to these GCA treated rats (Table 1).

Similarly, hepatic 11β-HSD2 activity was significantly decreased ($P<0.0005$) in GCA treated rats. Administration of isoprenaline to GCA treated rats on the day of sacrifice 11β-HSD2 enzyme was significantly increased ($P<0.0005$). However, this increase in the bioactivity of the 11β-HSD2 in the GCA treated rats with isoprenaline was still significantly lower ($P<0.0005$) than the bioactivity of 11β-HSD2 when isoprenaline only was given to the normal control rats. There was also no significant difference in the bioactivity of the 11β-HSD1 enzyme when isoprenaline was given before or after propranolol (Table 1).

Effects of isoprenaline on 11β-HSD1 and 11β-HSD2 enzyme activity in the kidney: In the kidney, GCA decreased ($P<0.0005$) 11β-HSD1 activity but not that of 11β-HSD2. Isoprenaline significantly increased ($P<0.0005$) the bioactivity of the renal 11β-HSD1 enzyme in the GCA treated rats but it did not do so when given to the normal control rats. There was no significant difference in the bioactivity of the 11β-HSD1 enzyme when isoprenaline was given before or after propranolol (Table 1).

Table 1: Effects of isoprenaline on glycyrrhizic acid induced bioactivities of 11β-HSD1 and 11β-HSD2 enzymes in hypothalamus, liver and kidneys, C : normal control rats, C-I : normal control rats injected with isoprenaline on the day of sacrifice, C-P : normal control rats injected with propranolol on the day of sacrifice, GCA : rats treated with glycyyrhizic acid, GCA-I : rats treated with glycyyrhizic acid and injected with isoprenaline on the day of sacrifice, GCA-I-P : rats treated with glycyyrhizic acid and injected with isoprenaline followed by propranolol on the day of sacrifice, GCA-P-I : rats treated with glycyyrhizic acid and injected with propranolol followed by isoprenaline on the day of sacrifice. Data are expressed as mean ± SEM. Significance values compared to control group, GCA treated rats and control rats injected with isoprenaline: **, P<0.005 versus control rats, ***, P<0.0005 versus control rats, єєє, P<0.0005 versus GCA treated rats, www P<0.0005 versus control rats injected with isoprenaline, ##, P<0.005 versus control rats injected with isoprenaline, δδ, P<0.005 versus control rats injected with isoprenaline were determined with multiple comparisons using ANOVA and *post hoc* tests.

	Liver		Kidneys		Hypothalamus	
GROUPS	11βHSD1	11βHSD2	11βHSD1	11βHSD2	11βHSD1	11βHSD2
C	65.7±1.2	66.1±2.2	76.8±1.0	80.3±0.8	23.0±1.0	5.0±0.5
C-I	76.6±0.9**	74.3±0.8	85.9±2.3	81.5±3.2	11.0±1.6***	13.4±1.7***
C-P	74.1±0.7	60.0±2.9	82.6±0.5	75.0±2.3	14.5±1.4	0.8±0.3
GCA	47.1±2.9***	32.0±2.8***	45.3±0.8***	80.0±3.7	14.8±0.4*	4.9±0.7
GCA-I	65.5±3.0єєє	49.9±2.6єєєωωω	80.1±1.5єєє	84.7±1.9	20.9±2.0##	6.0±0.8δδ
GCA-I-P	65.4±1.1	55.8±1.0	78.6±1.2	79.7±1.2	13.9±0.2	6.8±0.1
GCA-P-I	65.7±1.0	45.2±1.0	83.8±0.9	88.5±0.5	13.4±0.5	2.6±0.3

Effects of isoprenaline on the bioactivities of the 11β-HSD1 and 11β-HSD2 enzymes in the hypothalamus: In contrast to the liver and kidney, in the hypothalamus, isoprenaline significantly decreased (P<0.0005) the bioactivity of the hypothalamic 11β-HSD1 enzyme in the normal control rats. This decrease could be partially blocked by propranolol. GCA significantly decreased (P<0.05) the bioactivity of the 11β-HSD1 enzyme. Paradoxically when isoprenaline was injected on the day of sacrifice to the GCA treated rats, there was a significant (P<0.005) increase in the 11β-HSD1 bioactivity as compared to isoprenaline treatment given to normal control rats. (Table 1).

For 11β-HSD2 enzyme, the bioactivity in the hypothalamus is very low in normal control rats, at about 5% only. This is below the minimal detection level of the assay. In contrast to the bioactivity of 11β-HSD1, isoprenaline significantly (P<0.0005) increased the 11β-HSD2 bioactivity. GCA had no significant effect on 11β-HSD2 bioactivity in the hypothalamus and in contrast to 11β-HSD1, the GCA inhibited the increase in 11β-HSD2 bioactivity seen with isoprenaline. There was no significant difference in the bioactivity of the 11β-HSD1 enzyme when isoprenaline was given before or after propranolol (Table 1).

Discussion

The most significant finding in this set of experiments is that isoprenaline could block or reverse the inhibition of both 11β-HSD1 and 11β-HSD2 bioactivity in the liver and 11β-HSD1 bioactivity in the kidneys by GCA. This effect of isoprenaline was evident even when propranolol was given prior to the isoprenaline suggesting that it was not mediated via beta-adrenergic receptors. It is postulated that isoprenaline exerted its effects via other mediators which then modulated the effects on the 11β-HSD enzyme.

Secondly, GCA inhibited only the 11β-HSD1 activity in the kidneys, not the 11β-HSD2. This finding was similar to our previous study in rats subjected to repetitive stress (Pignatelli *et al.*, 1998).

Another significant finding are the different effects of isoprenaline on 11β-HSD enzymes types 1 and 2 in the hypothalamus. In contrast to the liver and kidneys, where isoprenaline had no effect on 11β-HSD1 and 11β-HSD2 activities, in the hypothalamus, isoprenaline decreased the bioactivity of 11β-HSD1. These effects of isoprenaline are therefore more specific to beta-adrenergic receptors in the hypothalamus. Thus in stress, the net effect in the hypothalamus when catecholamines are increased would be to decrease the tissue availability of inactive 11-hydroxycorticosteroids such as cortisol or cortisone hence limiting them to bind to the type I and type II receptors in the hypothalamus.

In the hypothalamus, GCA inhibited the bioactivity of 11β-HSD1 which could be overcome by isoprenaline as for liver and kidneys. We have previously found that GCA blocked the response to acute repetitive stress, similar to the effects of administering deoxycorticosterone. It is therefore surprising to find that isoprenaline reversed the inhibition of 11β-HSD1 in the hypothalamus. The significance of this finding in terms of adaptation to repetitive stress remains to be elucidated.

Isoprenaline reverses the inhibition of 11β-HSD1 bioactivity induced by the enzyme inhibitor GCA in the liver, kidney and hypothalamus. This could explain the increase in 11β-HSD1 bioactivity with repetitive stress in similar rats given GCA, which could not be explained by giving steroids to these rats. Isoprenaline, a catecholamine, results in increased active 11-hydroxycorticosteroids such as corticosterone and cortisol which are required for stress and adaptation to stress. The 11β-HSD2 in the kidneys are not affected by stress nor by GCA (Farihah *et al.*, 2000) Hence the

effects on blood pressure and salt retention in the kidneys are not modulated during stress or by exogenous inhibitors such as GCA. The mineralocorticoid-like effects on blood pressure and hypokalaemia in GCA treated animals are probably mediated by the increase in plasma and tissue hydrocorticosteroids, cortisol and corticosterone, acting in the renal tubular mineralocorticoid receptors rather than inhibition of renal 11β-HSD2 oxoreductase activity. The increase in 11β-HSD2 in the hypothalamus with isoprenaline which is increased during stress suggests that stress induced further oxoreductive activity in the hypothalamus resulting in greater increase in tissue glucoccorticoids to counter the decrease in 11β-HSD1. Clearly effects of stress on the tissues must include the effects of catecholamines mediating or modulating enzymes which affect tissue levels of corticosteroids.

Acknowledgements

This work was supported by Universiti Kebangsaan Malaysia research grant F27/96. The authors gratefully acknowledge the technical assistance of Fadzillah and Zahariah.

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