Mini Review

A SURVEY OF RECENT RESULTS CONCERNING GLYCYRRHIZIC ACID IN STRESS AND ADAPTATION

RUSZYMAH BHI*
KHALID BAK**

SUMMARY: Glycyrrhizic acid (GCA) the active component of liquorice acts by inhibiting the enzyme 11β-hydroxysteroid dehydrogenase (11HSD) which is a microsomal enzyme catalysing the reversible conversion of corticosterone to 11-dehydrocorticosterone. On repeated exposure to ether stress, there was a significant reduction in tail blood pressure during the second exposure followed by adaptation on the fifth to seventh exposure. This stress-induced hypotension was blocked by prior treatment with opioid antagonist Naloxone, mineralocorticoid deoxycorticosterone and GCA. It is not mediated by the β-endorphins containing neurons at the paraventricular nucleus of the hypothalamus. The 11HSD type 1 bioactivity is reduced in the anterior pituitary of rats exposed to ether stress suggesting it could be involved in blocking stress-induced hypotension. Rats exposed to restraint stress demonstrated similar pattern, that is, a significant decrease in the locomotor activity on the second exposure and adaptation on the seventh exposure. This decrease in locomotor activity was partially blocked and adaptation to the repetitive stress was enhanced in rats given GCA. It thus appears that GCA acts in reducing stress by increasing the body's corticosterone level by blocking the enzyme which breaks it down.

Key Words: Stress, glycyrrhizic acid, 11β-hydroxysteroid dehydrogenase, steroid hormones.

Everyone experiences some degree of stress most of the time. Individuals suffer from a multitude of environmental stressors: overcrowding, competition, noise, dirt, chemicals, pollution, infectious organisms and other harmful effects. Stress is a condition that is associated with feelings, but it manifests physiologically as well as in behavioral changes. Because organisms are constantly challenged or threatened by intrinsic or extrinsic disturbing stressors, the dynamic steady state required for successful adaptation is maintained by adaptational responses. Stress can thus be defined as a state that can lead to disharmony or threatened homeostasis. The endocrine system reacts to stress by secreting hormones necessary to alter metabolic processes and restore homeostasis. The hypothalamic-pituitary-adrenal (HPA) axis together with the sympathetic system connect the brain with the periphery of the body. The parvicellular corticotropin- releasing hormone (CRH) and the arginine-vasopressin (AVP) neurons of the paraventricular nuclei of the hypothalamus and the noradrenergic neurons of the locus ceruleus/

*From Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.
**From Department of Medicine, Hospital Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Cheras, 56000 Kuala Lumpur, Malaysia.

norepinephrine nuclei (LC/NE) of the brain stem innervate and stimulate each other and have both a baseline circadian and stress-related activity. The CRH/AVP and LC/NE neurons and their peripheral axes are known as the stress system (1). Activation of the stress system leads to changes that improve the organism's ability to regain homeostasis and increase survival chances. Neuroendocrine interactions in response to a stressor are the release of catecholamines; norepinephrine and epinephrine, and the hypothalamic, pituitary and adrenal hormones. These hormones include CRH, ACTH, ADH, glucocorticoids (cortisol) and mineralocorticoids (aldosterone). Growth hormone, prolactin and testosterone are also released in response to a variety of stressful stimuli. Other brain systems involved include the mesocortical and mesolimbic dopamine systems, the amygdala/hippocampus complex and arcuate pro-opiomelanocortin neurons. Stimulation of the arcuate pro-opiomelanocortin neurons in the central nervous system may increase self-analgesia due to the generation of β-endorphins. The term endorphin was coined by combining the words endogenous and morphine. Like morphine, endorphins raise the pain threshold and produce both sedation and euphoria. As endorphin levels increase, individuals experience these pleasurable sensations. The stress of exercise, the excitement of dance, the anticipation of eating delicious food, or the drama of combat or sportive contacts can all produce a concomitant increase in endorphin levels. Our research looked at the HPA axis, how it is regulated, affected by stress and modulated for eventual adaptation to stress, if the animals survive the stress period. In the final analysis, it is hoped that we can develop new drugs which can alleviate stress and promote adaptation to overcome the stress.

When Sprague-Dawley and Holtzman rats were stressed, plasma levels of immunoreactive β-endorphins were elevated four-fold above their basal levels in the first 10 minutes and remained at plateau levels thereafter (2). These stress-induced changes were abolished by glucocorticoid pretreatment. The decrease in tail blood pressure in Sprague-Dawley rats during immobilization stress was due to the release of endogenous opioids hence the opioid antagonist, naloxone, reversed the decline in blood pressure during the stress (3). The hypotensive effect of opioids had been demonstrated in septic shock (4), in which the effect on blood pressure was reversed by the administration of naloxone. The hypovolaemic shock that was reversed by naloxone was improved by glucocorticoid; dexamethasone (DM) but not by mineralocorticoid; deoxycorticosterone (DOC) (5). Studies from our laboratory demonstrated that repeated exposure to ether vapour stress caused significant decrease in tail blood pressure (6). There was a marked decrease during the second exposure followed by a subsequent increase back to normal by the 5th to 7th exposure. We termed this drop as stress-induced hypotension. This stress-induced decrease in tail blood pressure was blocked by prior treatment with naloxone, suggesting that it was due to the release of endogenous opioids such as β-endorphins and enkephalins. Repeated exposure to stress did not alter the tail blood pressure in DOC and glycyrrhizic acid (GCA) treated rats, but it was altered in DM rats. This suggests that the stress-induced hypotension due to repeated exposure to ether vapour stress could be blocked by mineralocorticoids and not glucocorticoids, and that the GCA effects were mediated via mineralocorticoid receptors (6). We went further to investigate the source of endogenous opioids and found that the stress-induced hypotension were not mediated by β-endorphins containing neurons at the paraventricular nucleus of the hypothalamus (7). This suggests that DOC and GCA blocked stress-induced hypotension via peripheral rather that central endorphins.

We showed that GCA treated rats could block the stress-induced hypotension or in other words, GCA administration increased the adaptive capacity to repeated exposure to stress. The amount of stress a person can tolerate depends on the adaptive capacity of that person. Everyone has a different adaptive capacity, and the limits of adaptability may be genetically determined. Could it be possible that GCA or

liquorice intake, increases ones' adaptive capacity when exposed repeatedly to stressful stimuli? GCA is the active component of liquorice and acts by inhibiting the enzyme 11β-hydroxysteroid dehydrogenase (11HSD). Liquorice (*Glycyrrhizia glabra*), a Mediterranean plant, has been used as traditional medication for many years (8). It is also widely used as a flavouring agent in foods, beverages and candies (9). In Malaysia, liquorice is found in many traditional medications, as sweeteners and preservative agents in 'asam boi', 'jeruk buah' and other preserved fruits and in canned drinks (10). 11HSD is a microsomal enzyme which catalyses the reversible conversion of cortisol to cortisone in man and corticosterone to 11-dehydrocorticosterone in rats. Inhibition of 11HSD results in higher tissue corticosterone or cortisol levels that will act on type 1 mineralocorticoid receptors in the kidney with resultant sodium retention and hypertension. Currently, there are two types of 11HSD namely the low affinity, NADP preferring form known as 11HSD1 (11) and the high affinity, NAD dependant form known as 11HSD2 (12). A wide distribution of 11HSD1 activity has been described in the brain, namely in the hippocampus, cortex, pituitary, hypothalamus, brain stem and spinal cord. Since GCA could block stress-induced hypotension, this enzyme 11HSD could be involved. In order to increase the mineralocorticoid action, the 11HSD bioactivity must be reduced. Reduction in the 11HSD activity will result in decreased conversion of cortisol to cortisone in man or corticosterone to 11-dehydrocorticosterone in rats. Excess cortisol or corticosterone will act on the mineralocorticoid receptor and gives rise to mineralocorticoid action. We investigated the 11HSD1 bioactivity at the anterior pituitary and hypothalamus in normal, glucocorticoid and mineralocorticoid treated rats. The 11HSD1 bioactivity is reduced in the anterior pituitary of normal rats exposed to stress suggesting it could be involved in blocking stress-induced hypotension (13).

Another study from our laboratory showed that following the first restraint stress, there was a highly significant decrease in locomotor activity which remained significantly lower till the 7th and subsequent exposure, indicating adaptation to these repeated stress had occured (14). Treatment with naloxone completely blocked the stress response and DOC and DM partially prevented the stress reaction, demonstrating that the stress response was primarily mediated by endogenous opioids. Both mineralocorticoid and glucocorticoid, which can act centrally to inhibit endorphins, partially blocked the stress response (14). This decrease in locomotor activity was partially blocked and adaptation to the repetitive stress was enhanced in the rats given GCA for 10 days (15). When GCA was given to animals, these animals were all able to withstand stress of anaesthesia or shock or immobilization, just as good as giving the steroid DOC or the morphine antagonist Naloxone. GCA thus works in reducing stress by increasing the body's cortisol by blocking the enzyme which breaks it down. The problem however, is that GCA causes systemic hypertension with hypokalemia and pulmonary hypertension (16).

Another interesting finding in this study was the increase in 11HSD1 activity in the hypothalamus of adrenalectomized rats given DOC, whereas in anterior pituitary there was a reduction in activity (13). For the first time mineralocorticoid action is shown in a non-mineralocorticoid target tissue. Since the anterior pituitary contains mineralocorticoid receptors (17), it seems possible that DOC act via mineralocorticoid receptors to decrease the bioactivity of 11HSD. GCA, by blocking the conversion of corticosterone may cause similar effects to DOC. In the hypothalamus, though glucocorticoid had no effect, the mineralocorticoid DOC caused a marked increase in 11HSD bioactivity. This demonstrated that the mineralocorticoid receptor in the hypothalamus was very sensitive to DOC, similar to the findings of others that showed that infusions of low doses of aldosterone intracerebrally in normal rats raised blood pressure with no discernible effect to that of given peripherally (18). The infusion of mineralocorticoid receptor antagonist RU28318 intracerebroventricularly in salt sensitive rats given 6% sodium chloride solution which would have suppressed

low mineralocorticoids were also found to have profound effects (19), suggesting hypersensitivity of mineralocorticoid receptor in cerebral tissues. The differential effects shown by the anterior pituitary and the hypothalamus after DOC treatment could also be due to the origin of both tissues which are entirely different. The anterior pituitary is an epithelial organ derived gland while the hypothalamus is a neural crest derived from neuronal tissue. In the anterior pituitary, 11 HSD1 bioactivity increased with adrenalectomy and returned to basal level with the administration of glucocorticoids. This shows that in the anterior pituitary, 11HSD1 is glucocorticoid sensitive whilst mineralocorticoid has no effects.

Much more needs to be investigated regarding the exact action of GCA, mineralocorticoid and glucocortiocoid in the central vs. peripheral, in respect to repetitive stress and adaptation. The precise physiologic circumstances in which these substance caused or blocked stress-induced hypotension remains to be determined.

REFERENCES

1. Chrousos GP and Gold PW : Editorial: A healthy body in a healthy mind - and vice versa - the damaging power of "uncontrollable" stress. J Clin Endocrinol Metab, 83:1842-1845,1998.

2. Lim AT and Funder JW : Stress-induced changes in plasma, pituitary and hypothalamic immunoreactive β-endorphin: effects of diurnal variation, adrenalectomy, corticosteroids and opiate agonists and antagonists. Neuroendocrinology, 35:225-234, 1983.

3. Nordin M, Morat P and Zainora M : The effects of endogenous opioids on blood pressure during stress. Clin Exp Pharm Physiol, 14:303-308, 1987.

4. Gahhos FN, Chiu RCJ, Hinchey EJ and Richards GK : Endorphins in septic shock. Arch Surgery, 117:1053-1057, 1982.

5. Khalid BAK, Paden M and Zainuddin M : The effects of naloxone, dexamethasone, deoxycorticosterone and 17-hydroxyprogesterone on blood pressure responses of normal and adrenalectomized rats during hypovolemic shock. Clin. Exp. Pharm. Physio, 14:111-117, 1987.

6. Ruszymah BHI, Nabishah BM, Aminuddin S and Khalid BAK : Mineralocorticoid and glycyrrhizic acid block stress induced hypotension in rats. Clin. Exp. Pharm. Physiol, 22:35-39, 1995.

7. Ruszymah BHI, Nabishah BM, Aminuddin S, Sarjit S and Khalid BAK : Effects of glycyrrhizic acid and steroid treatments on corticotropin releasing factor and b-endorphin containing neurons of the hypothalamus of the rat. M'sian J. Patho, 21 : 1999-in press.

8. Davis EA and Morris DJ : Medicinal uses of licorice through the millenia: the good and plenty of it. Mol. Cell Endocrinol, 78:1-6, 1991.

9. Spinks EA and Fenwick GR : The determination of glycyrrhizin in selected UK liquorice products. Food Addit. Contam, 7:769-778, 1990.

10. Ruszymah BHI, Nabishah BM and Khalid BAK : 11 β-hydroxysteroid dehydrogenase - the ubiquitous enzyme. JAMA SEA, Dec: 7-8, 1997.

11. Agarwal AK, Monder C, Eckstein B and White P : Cloning and expression of rat cDNA encoding corticosteroid 11 β-dehydrogenase. J Bioi Chem, 264:18939-18943, 1989.

12. Albiston A, Obeyesekere V, Smith R and Krozowski ZS : Cloning and tissue distribution of the human 11 β-hydroxysteroid dehydrogenase type II enzyme. Moll Cell Endocrinol, 105:R11-R17, 1994.

13. Ruszymah BHI, Nabishah BM, Paden BM, Aminuddin S and Khalid BAK : Differential effects of adrenocorticosteroids on 11 β-hydroxysteroid denydrogenase bioactivity at the anterior pituitary and hypothalamus in rats. Steroids, 61:448-452, 1996.

14. Ainshah O, Nabishah BM, Osman CB and Khalid BAK : Effects of naloxone, glycyrrhizic acid, dexamethasone and deoxycorticosterone in repetitive stress. Clin. Exp. Pharm. Physiol, 26:433-437, 1999.

15. Ainshah O, Nabishah BM, Osman CB and Khalid BAK : Short - and long - term effects of glycyrrhizic acid in repetitive stress. Clin Exp Pharm Physiol, 26:444-448, 1999.

16. Ruszymah BHI, Nabishah BM, Aminuddin S and Khalid BAK : Effects of glycyrrhizic acid on right atrial pressure and pulmonary vasculature in rats. Clin Exper Hypertension, 17:575-591, 1995.

17. Sheppard K and Funder JW : Type I receptors in parotid, colon and pituitary are aldosterone selective in vivo. Am J Physiol, 253:E467-471, 1987.

18. Gomez-Sanchez EP, Fort CM and Gomez-Sanchez CE : Intracerebroventricular infusion of RU28318 blocks aldosterone - salt hypertension. Am J Physiol, 258:E482-484, 1990.

19. Gomez-Sanchez EP, Fort CM and Thwaites D : Central mineralocorticoid receptor antagonism blocks hypertension in Dahl SIJR rats. Am J Physiol, 262:E96-99, 1992.

Correspondence:
Khalid Bin Abdul Kadir
Director and Consultant Endocrinologist
Hospital Universiti Kebangsaan Malaysia
Jalan Yaacob Latif, Bandar Tun Razak
56000 Cheras, Kuala Lumpur
MALAYSIA.
e-mail: ruszy@medic.ukm.my