

Reduction of Noise-stress-induced Physiological Damage by Radices of *Astragali* and *Rhodiolae*: Glycogen, Lactic Acid and Cholesterol Contents in Liver of the Rat

Bei-Wei ZHU,¹ Yu-Mei SUN,¹ Xia YUN,¹ Song HAN,² Mei-Lan PIAO,² Yoshiyuki MURATA,³ and Mikiro TADA^{2,†}

¹College of Bio & Food Technology, Dalian Institute of Light Industry, Dalian 116034, China

²Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

³Department of Agriculture, Okayama University, Okayama 700-8530, Japan

Received March 19, 2003; Accepted May 13, 2003

Noise is one of the factors that induces critical stress in animals. The contents of glycogen, lactic acid and cholesterol in the liver of noise-stressed rats were analyzed in order to investigate the alleviation of noise-stress-induced physiological damages by traditional medicine using *Astragali* and *Rhodiolae* radices. More than 95 dB noise ranging from 2 to 4 kHz reduced the contents of these compounds in the liver of rats not injected with the extract of *Astragali* or *Rhodiolae*, but did not change the contents in the liver of rats injected with the *Astragali* or *Rhodiolae* extract. These results show that noise induced stress in the rats via a decrease in contents of these compounds in the liver and that *Astragali* or *Rhodiolae* maintained the contents of these compounds in the liver of the noise-stressed rats. The results indicate that *Astragali* or *Rhodiolae* improved the ability for rats to resist noise stress.

Key words: noise; Chinese medicine; glycogen; lactic acid; cholesterol

Noise has auditory and non-auditory effects on the behavior of animals including humans and has therefore been extensively studied. The non-auditory and auditory effects induce a disturbance of the cardiovascular, endocrine and some movement systems, as well as sleep, neuropsychic response, mental work and so on.^{1,2} Noise, dust and nitrogen oxides have significantly increased the methemoglobin level and changed the erythrocyte metabolism of industrial workers, the activation of glucose-6-phosphate dehydrogenase and pyruvate kinase, and the *in vitro* production of lactic acid in erythrocytes.³ Noise stress has decreased the blood cholinesterase activity and the glycogen content in the liver of rats.⁴ Chronic exposure to noise has changed the activity of glucose-6-phosphatase and the contents of glycogen

and periodic acid Schiff (PAS) reaction-positive substances in the liver of guinea pigs.⁵ In respect of the hepatic noradrenergic innervation in albino rats exposed acute acoustic stress, an 8 h treatment induced a significant increase in the catecholamine content, in addition to a non-homogeneous response to the PAS reaction by hepatocytes surrounding the portal spaces or the lobular central veins.⁶ Noise stress has affected mainly the postreceptorial mechanism linked to β -adrenoceptors rather than their density or affinity.⁷

Astragali and *Rhodiolae* are two kinds of traditional Chinese medicines which have been studied more in recent years. Sodium selenite and the tincture from *Astragali* grass reduced the contents of malonic dialdehyde and dienic conjugates to inhibit free radical oxidation in whole blood and liver homogenates.⁸ Both *Acanthopanax* and *Rhodiolae* exert a strong protective action against a lethal heat shock. These adaptogens also significantly protect against the negative effect of superoxide radicals induced by menadione. The definition of phyto-adaptogens as being universal enhancers of non-specific resistance against different kinds of stress conditions has been confirmed. The phyto-adaptogens did not induce the synthesis of any heat shock proteins (hsps).⁹ Polymer proanthocyanidin from *R. semenovii*, named epihalochin, has been found to exhibit a pronounced antihypoxic effect in different models of hypoxia (hypoxic, cytotoxic and hemic) to relieve the hypoxic isolated heart contracture.¹⁰ Rhodiocyanoside A and B exhibit activity on the histamine release from rat peritoneal exudative cells sensitized against anti-2,4-dinitrophenyl IgE and rhodiocyanoside A has shown antiallergic activity in a passive cutaneous anaphylaxis test on rats.^{11,12} The antitumor and antimetastatic effects of cyclophosphan (cyclo-

[†] To whom correspondence should be addressed. Fax: +81-86-251-8309; E-mail: mtada@cc.okayama-u.ac.jp
Abbreviation: PAS, periodic acid Schiff

phosphamide) are potentiated by extracts from *Baikal skullcap*, *Rhodiolae*, common licorice, and by their principal acting components, baicalin, paratyrosol and glycyrram.¹³ The extracts from *Rhodiolae rosea* L. roots has affected learning ability and memory.¹⁴ Moreover, *Astragali* radix has suppressed the change in glycogen content in rat liver caused by non-auditory noise.¹⁵

However, there have been few studies on the change in physiological and biochemical conditions of a rat subjected to only non-auditory noise and suppression of the changes by any traditional Chinese medicine. This paper focuses on an analysis of the glycogen, lactic acid and cholesterol contents in the liver of rats exposed to and not exposed to noise, and an assessment of the improving effects of *Astragali* radix and *Rhodiolae* radix on the changes caused by noise.

Materials and Methods

Animals. Two-month-old female Wistar rats whose weights were 180 to 200 g were kindly provided by Experimental Animals Laboratory of Dalian Medical University. The rats were treated in accordance with European Union Directives (86/609/EEC) for laboratory animal care and were fasted for 60 min before noise exposure.

Noise application. The equipment to produce the noise was kindly provided by Acoustics Institute of Nanjing University. The rats were exposed to the noise (75 dB, 95 dB and 110 dB, between 2 and 4 kHz) for 30, 60, 90 or 120 min.

Preparation of the extracts from *Astragali* radix and *Rhodiolae* radix. *Astragali* radix or *Rhodiolae* radix (130 g) was added to 100 ml of distilled water. This suspension was decocted for 30 min and then the boiled solution was filtered through gauze. The residue was decocted for 30 min again and filtered through gauze. The collected filtrate was evaporated to 150 ml in a boiling water bath. The concentrated solution was added to 100 ml of 95% ethanol, shaken and kept overnight at 4°C. The supernatant was transferred to a new flask. The sediment was washed 3 times with 100 ml in total of 95% ethanol. The supernatant was mixed with the collected washing solutions. The mixed solution was put in a boiling water bath to evaporate the ethanol. The concentrated solution was diluted in physiological saline water to 65 ml to obtain the final extract solution. Two ml of the final extract solution was injected into the abdomen of each rat. Noise was applied to the rat 30 min after this injection.

Preparation of the liver homogenate. Soon after noise exposure, the rats were killed by decapitation

and the livers removed. The livers were then transferred into liquid nitrogen. The frozen liver (0.6 g) was homogenized in 5 ml of 20 mM Tris-HCl (pH 7.6) with a Teflon-glass homogenizer. The homogenate was centrifuged at 5000 × g for 60 min at 4°C, the supernatant being transferred to tubes and stored at -70°C.

Liver glycogen analysis. The hepatic glycogen was dissolved in KOH with Na₂SO₄ and precipitated with ethyl alcohol. The precipitated glycogen was then dissolved in distilled water to a final volume of 5 ml. The amount of glycogen was determined by the phenol-sulfuric acid method from the absorbance at 490 nm.^{16,17}

Liver lactic acid analysis. After 3.5 ml of water and 0.5 ml of 10% ZnSO₄ had been added to 0.5 ml of the sample, the mixture was supplemented with 0.5 ml of 0.5 M NaOH and then centrifuged at 600 × g for 10 min. One ml of the supernatant was supplemented with 3.5 ml of water, 0.5 ml of 20% CuSO₄ and 0.6 g of Ca(OH)₂ powder, the mixture being vortexed and kept at room temperature for 15 min. The mixture was then centrifuged at 600 × g for 10 min. One drop of 2% CuSO₄ and 3 ml of cooled sulfuric acid were added to the supernatant on iced water. The tube containing the mixed solution was put in boiling water for 5 min and then cooled in water. Fifty μl of 1.5% *p*-hydroxybiphenyl was added to the solution and mixed strongly. The mixture was incubated at room temperature for 45 min while being occasionally shaken. The tube containing the mixture was put in boiling water for 90 s and then cooled in water. The concentration of lactic acid was calculated from the absorbance at 570 nm.¹⁸

Liver cholesterol analysis. After 4 ml of 2-propanol and 1 g of Al₂O₃ had been added to 0.5 ml of the homogenate, the mixture was vortexed and then centrifuged at 800 × g for 5 min. Three ml of 0.05% FeCl₂ in 50% sulfuric acid was added to 2 ml of the supernatant, and the mixture was incubated at 60°C for 15 min. The concentration of cholesterol was calculated from the absorbance at 530 nm.^{18,19}

Protein measurement. The protein concentration was determined by the modified Bradford method, using bovine serum albumin (BSA) as a standard.²⁰

Statistics. All statistical analyses were performed with the TTEST program (a two-tailed, unpaired *t*-test) in EXCEL software (Microsoft, Redmond, WA, U.S.A.). Values of *P* < 0.05 are considered to show statistically significant differences.

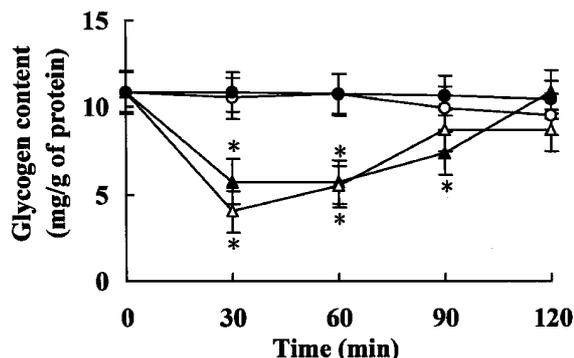


Fig. 1. Effect of Noise Stress on the Content of Glycogen in the Liver of Rats.

The rats were stimulated by 75 dB (○), 95 dB (▲) and 110 dB (△) of noise for 30, 60, 90 and 120 min. The rats not subjected to any noise were used as a control (●). Significant difference from the control is indicated by an asterisk (* indicates $P < 0.05$). Each value is presented as the mean \pm SD for 10 rats.

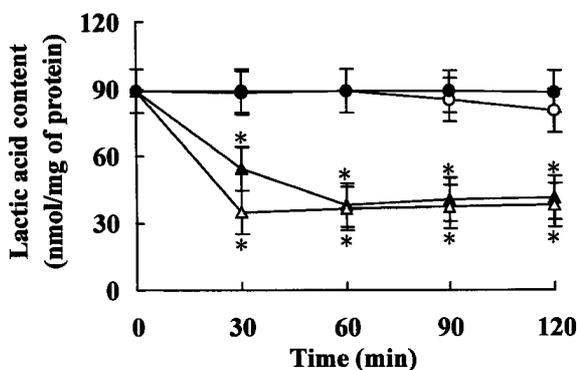


Fig. 2. Effect of Noise Stress on the Content of Lactic Acid in the Liver of Rats.

The rats were stimulated by 75 dB (○), 95 dB (▲) and 110 dB (△) of noise for 30, 60, 90 and 120 min. The rats not subjected to any noise were used as a control (●). Significant difference from the control is indicated by an asterisk (* indicates $P < 0.05$). Each value is presented as the mean \pm SD for 10 rats.

Results

Effect of noise on the contents of glycogen, lactic acid and cholesterol in the liver of rats

The rats were divided into a non-exposed group and noise-exposed group, the non-exposed group not being subjected to any noise stimulation. The noise-exposed group was divided into 12 sub-groups that were stimulated by noise of 75 dB, 95 dB or 110 dB for 30, 60, 90 or 120 min, respectively. Ten rats were employed in each group. The contents of glycogen (Fig. 1), lactic acid (Fig. 2) and cholesterol (Fig. 3) in the liver of the rats subjected to noise stress were measured.

The content of glycogen in the rat liver was not affected by the noise of 75 dB for a duration of 30, 60, 90 or 120 min. Application of the noise of 95 dB for 30 and 60 min reduced the content of glycogen in

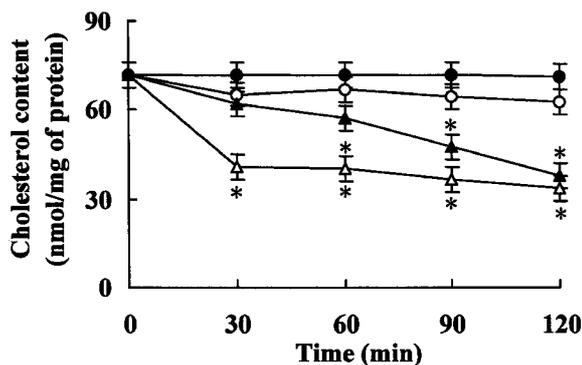


Fig. 3. Effect of Noise Stress on the Content of Cholesterol in the Liver of Rats.

The rats were stimulated by 75 dB (○), 95 dB (▲) and 110 dB (△) of noise for 30, 60, 90 and 120 min. The rats not subjected to any noise were used as a control (●). Significant difference from the control is indicated by an asterisk (* indicates $P < 0.05$). Each value is presented as the mean \pm SD for 10 rats.

the liver. However, the amount of glycogen in the liver of the rats subjected to the noise of 95 dB for 90 min was greater than that for 30 and 60 min, and the content of glycogen after application of the noise of 95 dB for 120 min was the same as that before application, showing that the noise of 95 dB transiently reduced the content of glycogen.

The content of lactic acid was also not affected by the noise of 75 dB for 30 to 120 min. However, when applying the noise of 95 dB, the content of lactic acid was reduced by 39.5% with 30 min of exposure and reduced by 57.0%, 54.4% and 53.9% with 60, 90 and 120 min of exposure, respectively. Exposure to the noise of 110 dB reduced the amount of lactic acid in the liver of the rats to a level similar to that from the noise of 95 dB for longer than 60 min.

The content of cholesterol in the liver of the rats subjected to the noise of 75 dB was slightly lower than that of the unexposed rats. However, there was no significant difference in the content of cholesterol in the liver between the unexposed rats and the rats exposed to the noise of 75 dB for 30 to 120 min. The noise of 95 dB slightly reduced the content of cholesterol by 13.4% for 30 min of exposure but not significantly. The content of cholesterol decreased with increasing duration of the application of 95 dB noise; the content of cholesterol was reduced by 20.7%, 33.9% and 47.7% by the application of 95 dB of noise for 60, 90 and 120 min, respectively. The content of cholesterol progressively decreased after exposure to the noise of 110 dB by 43.4%, 44.4%, 49.2% and 53.4% for 30, 60, 90 and 120 min of exposure.

Alleviation of noise stress by the extract of Astragali radix or Rhodiolae radix

The rats used in the experiments with these extracts were divided into 16 groups that were stimulated by

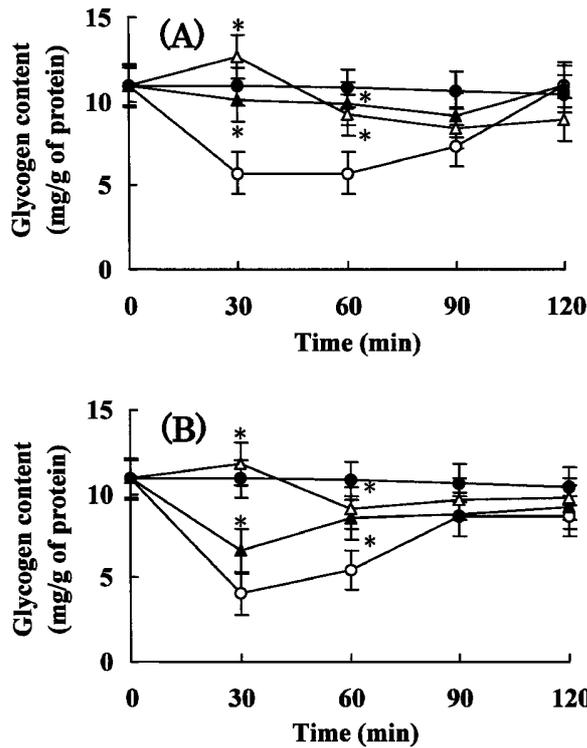


Fig. 4. Effect of the Radices, *Astragali* and *Rhodiolae*, on the Content of Glycogen in the Liver of Rats Subjected to 95 dB (A) and 110 dB (B) Noise Stress.

The glycogen content is shown in the liver of unexposed rats (●) and rats exposed to noise stress without (○) or with injection of the extract from *Astragali* (△) or *Rhodiolae* (▲). Significant difference from the control is indicated by an asterisk (* indicates $P < 0.05$). Each value is presented as the mean \pm SD for 10 rats.

the noise of 95 dB (A in Figs. 4–6) or 110 dB (B in Figs. 4–6) for 30, 60, 90 or 120 min, respectively, after being injected with the extract of *Astragali* radix or *Rhodiolae* radix. Ten rats were employed in each group. The contents of glycogen (Fig. 4), lactic acid (Fig. 5) and cholesterol (Fig. 6) in the liver of the rats treated with the extract of *Astragali* radix or *Rhodiolae* radix and subjected to noise stress were measured.

Under exposure to the noise of 95 dB and 110 dB for 30 min and 60 min, the glycogen content in the liver of the rats injected with the extract of *Astragali* radix or *Rhodiolae* radix was significantly higher than that of the non-injected rats ($P < 0.05$) and approached the level of the rats in the untreated group ($P < 0.05$). In contrast, under exposure to the noise of 95 dB and 110 dB for 90 min and 120 min, the difference in glycogen content between the rats injected and non-injected with the extract of *Astragali* radix or *Rhodiolae* radix was not significant ($P > 0.05$), because the glycogen content in the liver of the untreated rats had recovered by 90 min after exposure to the noise. It is suggested that *Astragali* radix and *Rhodiolae* radix suppressed the decrease of

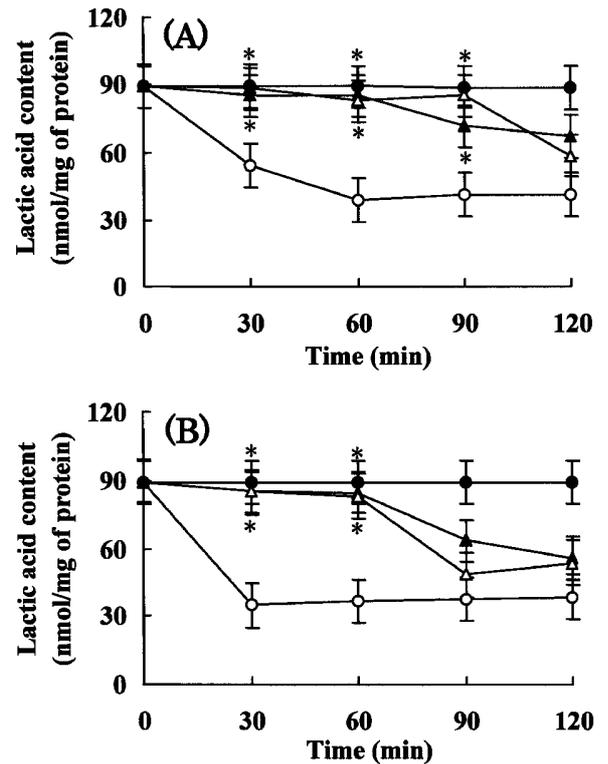


Fig. 5. Effect of the Radices, *Astragali* and *Rhodiolae*, on the Content of Lactic Acid in the Liver of Rats Subjected to 95 dB (A) and 110 dB (B) Noise Stress.

The lactic acid content is shown in the liver of unexposed rats (●) and rats exposed to noise stress without (○) or with injection of the extract from *Astragali* (△) or *Rhodiolae* (▲). Significant difference from the control is indicated by an asterisk (* indicates $P < 0.05$). Each value is presented as the mean \pm SD for 10 rats.

glycogen content in the rat liver caused by exposure to the noise of 95 dB and 110 dB for 30 min and 60 min.

Under exposure to the noise of 95 dB and 110 dB for 30, 60 and 90 min, the lactic acid content in the liver of the rats injected with the extract of *Astragali* radix or *Rhodiolae* radix was significantly higher than that of the non-injected rats ($P < 0.05$). However, the difference in lactic acid content between the rats injected and non-injected with the extract of *Astragali* radix was not significant ($P > 0.05$) under exposure to the noise of 110 dB for 90 min. The difference in lactic acid content between the rats injected and non-injected with the extract of *Astragali* radix or *Rhodiolae* radix was not significant ($P > 0.05$) under exposure to the noise of 95 dB and 110 dB for 120 min. These results show that the *Astragali* radix and *Rhodiolae* radix could markedly inhibit the decrease in lactic acid content of the rat liver caused by exposure to the noise.

Under exposure to the noise of 95 dB for 90 min and to the noise of 110 dB for 30, 60 and 90 min, the cholesterol content in the liver of the rats injected

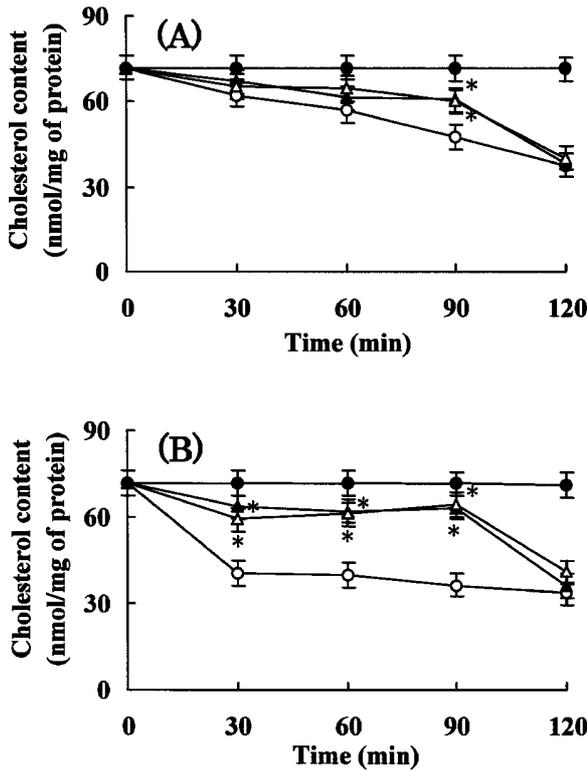


Fig. 6. Effect of the Radices, *Astragali* and *Rhodiola*, on the Content of Cholesterol in the Liver of Rats Subjected to 95 dB (A) and 110 dB (B) Noise Stress.

The cholesterol content is shown in the liver of unexposed rats (●) and rats exposed to noise stress without (○) or with injection of the extract from *Astragali* (△) or *Rhodiola* (▲). Significant difference from the control is indicated by an asterisk (* indicates $P < 0.05$). Each value is presented as the mean \pm SD for 10 rats.

with the extract of *Astragali* radix or *Rhodiola* radix was significantly higher than that of the non-injected rats ($P < 0.05$) and approached the level of the rats in the unexposed group. However, under exposure to the noise of 95 dB for 30, 60 and 120 min and to the noise of 110 dB for 120 min, the difference in cholesterol content between the injected and non-injected rats was not significant. These results reveal that the extracts of *Astragali* radix and *Rhodiola* radix could resist the decrease in cholesterol content of the rat liver caused by exposure to the noise of 95 dB for 90 min and to the noise of 110 dB for 30, 60, and 90 min.

These results demonstrate that the glycogen, lactic acid and cholesterol contents in the liver of the medicated rats exposed to the noise were higher than those in the liver of the uninjected rats exposed to the noise and similar to the contents of the control group. Moreover, the recovery effect of medication was greater as the exposure time was shorter.

Discussion

Changes in the glycogen and lactic acid contents of the rat liver caused by noise

The content of glycogen in the liver has been found to be susceptible to noise stress and electro-shock.²¹⁾ The contents of glycogen and lactic acid in the rat liver were reduced by noise stimulation of 95 dB and 110 dB. It is believed that the noise of 95 dB and 110 dB may have stimulated the excitation of the rat nerves and the secretion of glucagon that activates glycogen phosphorylase to degrade glycogen to glucose in the liver. The glycogen content in the liver after 90 min and 120 min exposure to the noise was higher than that at 60 min. It is thought that longer exposure to noise may elicit the biosynthesis of glycogen to compensate for its initial loss.²²⁾ However, the glycogen content in the liver didn't reach the normal level of the unexposed group during 120 min of noise exposure.

Noise exposure would convert lactic acid into glucose by gluconeogenesis in the liver to recover the content of glycogen. With increasing exposure time to noise, the consumption of glucose and energy would be diminished, whereas the rats would adapt and resist noise *via* gluconeogenesis. Therefore, the content of glycogen increased even during increased the exposure to the noise, but the content of lactic acid did not increase.

The noise of 75 dB did not affect the contents of glycogen and lactic acid. However, the noise of 95 dB was as effective as that of 110 dB in reducing these contents, suggesting that there could be threshold to elicit a detrimental effect.

Change in cholesterol content of the rat liver caused by noise

Exposure to the noise of 75 dB slightly decreased the cholesterol content of the rat liver, exposure to the noise of 95 dB reduced the content with increasing exposure time, and exposure to the noise of 110 dB decreased the content by approximately 50% in 30 min. The cholesterol content did not recover like that of glycogen during the application of noise stress. These results suggest that a selective recovery system could be in operation during noise stress.

Four reasons may account for the reduction in content of cholesterol by noise stress. First is that acetyl-CoA would be consumed in order to maintain homeostasis under noise stress, which would yield ATP so that the rat would be deficient in acetyl-CoA to synthesize cholesterol; that is, the rats would be unable to produce cholesterol in the liver. Second is that noise stress would inhibit the activity of HMG-CoA reductase which catalyzes HMG-CoA to produce cholesterol in the liver, resulting in a diminishing content of cholesterol. Third is that the

noise would stimulate the transport of cholesterol away from the liver cells and the conversion in the liver so that the equilibrium of cholesterol metabolism in the liver would collapse.²³⁻²⁵ Fourth is that noise-induced secretion of corticosterone could make the liver convert cholesterol into bile acids. The combined effect of these four factors could have decreased the cholesterol content of the liver in the rats subjected to noise stress.

Mitigation of the noise effect by Astragali radix and Rhodiolae radix

The experimental results demonstrate that *Astragali* radix and *Rhodiolae* radix could mitigate the reduction in contents of glycogen, lactic acid and cholesterol in the liver of the rats exposed to noise. *Astragali* radix was more effective than *Rhodiolae* radix for the metabolism of glycogen and lactic acid. These results indicate that *Astragali* radix and *Rhodiolae* radix can function as phyto-adaptogens and it is possible that both radices might non-specifically improve the damage by other types of stress. However, the physiological study of the metabolic regulation by these radices and the identification of compounds to relieve noise stress will need to be conducted further.

Conclusions

Noise stress reduced the contents of glycogen, lactic acid and cholesterol in the liver of rats to perturb their homeostasis. However, *Astragali* radix and *Rhodiolae* radix suppressed the reduction in the contents of glycogen, lactic acid and cholesterol caused by noise in the rat liver. These results suggest that both radices are effective suppressors against noise stress.

Acknowledgments

The authors thank Han Jie of Dalian Medical University for measuring the biochemical parameters. The financial support of National Natural Science Fund of China (Grant No. 39870190) is gratefully acknowledged. The authors thank Shinsuke Kuwaki for comments on the manuscript and discussions.

References

- 1) Xu, C. X., Deng, C. W., Shi, C. H., Cong, F. T., Hua, L. P., Luo, Y. D., Wang, H., and Li, W. Y., Influence of several vitamins on the mice liver glycogen content and the mice liver GPT activity caused by noise exposure. *Basic Medical Sciences and Clinics*, **11**, 29-32 (1991).
- 2) Han, G. Z., Xiang, D. Y., He, Y. J., Xu, C. X., and Zhang, P. Z., The effect of radix *Astragali* on the glycogen content induced by noise. *J. Chinese Herbal Medicine*, **14**, 46-48 (1989).
- 3) Andrzejak, R., Antonowicz, J., Lewczuk, E., Tomczyk, J., and Smolik, R., Effect of combined exposure (noise, dust, nitrogen oxides) on health status of metal workers in heavy industry. Evaluation of erythrocyte metabolism. *Med. Pr.*, **43**, 411-420 (1992).
- 4) Tsapkov, M. M., Kalistratova, V. S., and Tishchenko, G. S., Combined effect of tritium oxide, noise and heat on rats. *Radiobiologiya*, **22**, 275-277 (1982).
- 5) Jonek, J., Stanosek, J., and Waclawczyk, H., Behavior of glucose-6-phosphatase, glycogen and PAS-positive substances in the liver of guinea pigs following chronic exposure to noise. *Z. Mikrosk. Anat. Forsch.*, **72**, 256-263 (1965).
- 6) Pellegrini, A., Soldani, P., Ricciardi, M. P., and Paparelli, A., Morpho-histochemical observations on the liver parenchyma of adult albino rats subjected to acute acoustic stress. *Boll. Soc. Ital. Biol. Sper.*, **67**, 111-118 (1991).
- 7) Breschi, M. C., Scatizzi, R., Martinotti, E., Pellegrini, A., Soldani, P., and Paparelli, A., Morphofunctional changes in the noradrenergic innervation of the rat cardiovascular system after varying duration of noise stress. *Int. J. Neurosci.*, **75**, 73-81 (1994).
- 8) Oleinik, A. N., Effect of antioxidants on lipid peroxidation in combined lesions of the liver. *Farmakol. Toksikol.*, **46**, 102-105 (1983).
- 9) Boon-Niermeijer, E. K., van den Berg, A., Wikman, G., and Wiegant, F. A., Phyto-adaptogens protect against environmental stress-induced death of embryos from the freshwater snail *Lymnaea stagnalis*. *Phytomedicine*, **7**, 389-399 (2000).
- 10) Kurmukov, A. G., Azikov, M. I., and Rakhimov, S. S., Pharmacology of the plant polyphenol epigalokhin. *Farmakol. Toksikol.*, **49**, 45-48 (1986).
- 11) Yoshikawa, M., Shimada, H., Shimoda, H., Murakami, N., Yamahara, J., and Matsuda, H., Bioactive constituents of Chinese natural medicines. II. *Rhodiolae* radix. (1). Chemical structures and antiallergic activity of rhodiocyanosides A and B from the underground part of *Rhodiola quadrifida* (Pall.) Fisch. et Mey. (Crassulaceae). *Chem. Pharm. Bull.*, **44**, 2086-2091 (1996).
- 12) Yoshikawa, M., Shimada, H., Shimoda, H., Matsuda, H., Yamahara, J., and Murakami, N., Rhodiocyanosides A and B, new antiallergic cyanoglycosides from Chinese natural medicine "silie hong jing tian", the underground part of *Rhodiola quadrifida* (Pall.) Fisch. et Mey. *Chem. Pharm. Bull.*, **43**, 1245-1247 (1995).
- 13) Razina, T. G., Zueva, E. P., Amosova, E. N., and Krylova, S. G., Medicinal plant preparations used as adjuvant therapeutics in experimental oncology. *Eksp. Klin. Farmakol.*, **63**, 59-61 (2000).
- 14) Petkov, V. D., Yonkov, D., Mosharoff, A., Kambourova, T., Alova, L., Petkov, V. V., and Todorov, I., Effects of alcohol aqueous extract from *Rhodiola rosea* L. roots on learning and memory. *Acta Physiol. Pharmacol. Bulg.*, **12**, 3-16 (1986).
- 15) Han, G. Z., Xiang, D. Y., He, Y. J., Xu, C. X., and Zhang, P. Z., Influence of radix *Astragali* on noise-caused changes in hepatic glycogen content in the rat.

- China Journal of Chinese Materia Medica*, **14**, 750–752, 764 (1989).
- 16) Kato-Weinstein, J., Lingohr, M. K., Orner, G. A., Thrall, B. D., and Bull, R. J., Effects of dichloroacetate on glycogen metabolism in B6C3F1 mice. *Toxicology*, **130**, 141–154 (1998).
 - 17) Lo, S., Russell, J. C., and Taylor, A. W., Determination of glycogen in small tissue samples. *J. Appl. Physiol.*, **28**, 234–236 (1970).
 - 18) Zhong, Y., Practical medicine test, The People's Military Medicine Press, Beijing (in Chinese) (1992).
 - 19) Feng, R. F., Practical medicine test, Shanghai Press of Science and Technology, Shanghai (in Chinese) (1996).
 - 20) Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254 (1976).
 - 21) De, A. K., Dey, C., and Debnath, P. K., Studies in tissue glycogen in acute stress. *Indian J. Physiol. Pharmacol.*, **22**, 75–77 (1978).
 - 22) Furukawa, T., and Honda, S., *Gendai no seirigaku*, Kanahara Shuppan, Tokyo, pp. 343–344, 739 (1982).
 - 23) Wang, Y. L., Development of labour hygiene, vol. 2, People Press, Beijing, pp. 145–155 (in Chinese) (1985).
 - 24) Shen, T., and Wang, J. Y., *Biochemistry*, ed. 2, Advanced Educational Press, Beijing, pp. 88–420 (in Chinese) (1999).
 - 25) Hames, B. D., Hooper, N. M., and Houghton, J. D., *Instant notes in biochemistry*, Science Press, Beijing, pp. 248–286 (1999).