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Effect of pretreatment with vitamin E or diazepam on brain metabolism of stressed rats

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Abstract—The effect of vitamin E (VE) or diazepam (DZ) pretreatment on some carbohydrate metabolic aspects in the brains of stressed rats was studied. DZ and VE were given i.p. at doses of 5 mg/kg body wt for 6 days prior to subjecting the animals to single swimming stress (SSS). Pretreatment of the rats with DZ or VE diminished the stress-induced increases in plasma corticosterone and glucose levels and reversed the decrease due to stress on brain ATP, glucose, glycogen and pyruvate contents. The increase in brain ADP and lactate was brought back to levels which approached the pre-stressed values. Moreover, DZ and VE pretreatments helped in attenuating the stress-induced alteration in brain mitochondrial and cytosolic hexokinase as well as sodium, potassium adenosine triphosphatase (Na^+,K^+-ATPase) activities. The change in these metabolic parameters produced by VE pre-treatment was less than that exhibited by DZ. The effects of VE were explained in light of its antioxidant property in preventing the free radical production and lipid peroxide formation which are important factors in the pathogenesis of stress.

Acute stress produces a wide range of biochemical and behavioural changes in an organism [1]. Increased lipid peroxide formation with an accompanied stimulation of Na^+,K^+-ATPase activity has been demonstrated in brain tissues of emotionally stressed rats and such biochemical alterations initiated in the brain have been suggested as an important mechanism in the pathogenesis of stress [2, 3]. Since administration of vitamin E (VE*) has an inhibitory effect on lipid peroxide formation [4], it seems possible that pretreatment of the animal with VE may reduce the deleterious effect of stress on brain metabolism. The effect of VE on stress-induced alterations in brain metabolism was compared with changes induced by diazepam (DZ). The biochemical changes were assessed by measuring the brain contents of glucose, glycogen, pyruvate, lactate, ATP and ADP as well as Na^+,K^+-ATPase (EC 3.6.1.3), cytosolic and mitochondrial hexokinase (CHK and MHK, EC 2.7.1.1) activities. Besides these parameters, plasma corticosterone, as a sensitive index of stress and plasma glucose, was also determined.

Materials and Methods

Drugs and chemicals. DZ (Hoffmann, La Roche, U.K.) and VE (DL-α-tocopherol acetate, 1 mg = 1 IU) (Sigma Chemical Co., St Louis, MO, U.S.A.) have been used as stress modulators. For the purpose of administration of the drug, DZ was dissolved in a minimal volume of 0.1 N HCl and further diluted with saline to give a solution of 1% (w/v) [5]. VE was diluted with pure sesame oil to facilitate its injection at concentration of 1% (v/v). The other chemicals were obtained from Boehringer Mannheim (Mannheim, F.R.G.) and Sigma.

Animals and treatments. Sprague–Dawley male rats (225–250 g) were obtained from Alab Laboratory Services Ltd (Stockholm, Sweden). The rats were housed in groups of six, in a colony room maintained at 24° ± 1, humidity 50% ± 10 and under a 12-hr light/dark cycle. All rats were maintained on pelleted food and water *ad lib*. For evaluating the effect of DZ and VE as stress modulators, the model of single swimming stress (SSS) as described by Le Fur *et al.* [6] was selected. Administration of these modulators was carried out prior to the exposure of the animals to SSS. Four groups of rats, six in each group, received i.p. doses of 5 mg DZ/kg body wt [7] and four other groups received i.p. doses of 0.5 mg VE/100 g body wt [8] daily for 6 days, the last dose of DZ or VE was injected 1 hr before SSS. Comparable eight groups of normal animals from the same batches of animals were similarly treated with the vehicle in which the drugs were dissolved or diluted and used as control groups for each of the above treated groups. The drug-treated group, as well as the corresponding vehicle-treated group, were forced to swim for 3 min. Fifteen minutes after the swim, the previous groups, as well as another group of normal animals kept away from any stressful situation, were killed by decapitation between 10:00 and 12:00 a.m.

Tissue sampling and biochemical analysis. Three to four milliliters of the collected heparinized blood were centrifuged and the separated plasma was used to measure corticosterone [9] and glucose [10] levels. Then the skulls were split rapidly on an ice and salt mixture and the whole brain was separated and either frozen rapidly in liquid nitrogen or used directly for enzyme assay. The isolated frozen brain was weighed, homogenized in ice-cold 3 M perchloric acid and was then treated with 1 mM EDTA followed by centrifugation at 1000 *g* for 1 hr at 4° [11]. The supernatant was treated with ice-cold 2 M $KHCO_3$ to bring the pH to either 7.5–8 or 3.5 followed by Tris (pH 7.5–8) or formate (pH 3.5) buffers, respectively. The step of addition of Tris buffer was omitted in the case of ATP and ADP estimations. The supernatant of pH 7.5–8 was used for estimation of glucose [12] and lactate [13] while that of pH 3.5 was used for pyruvate estimation [11]. The untreated supernatant was used for estimation of ATP [14] and ADP [11]. The isolated frozen brains of another group of animals were digested in alcoholic KOH at 80° for 20 min and their glycogen content was estimated [15]. The whole brains of another group without freezing were homogenized in ice-cold solution of 0.25 M sucrose, 20 mM triethanolamine and 0.1 mM dithiothreitol at pH 7.4. The homogenates were centrifuged at 12,000 *g* for 10 min at 4° to separate mitochondria. The supernatants were further centrifuged at 105,000 *g* for 45 min to separate the cytosolic fraction [16]. The MHK and CHK activities were estimated by the method of Bennett *et al.* [17]. Moreover, the brains of another group were homogenized in ice-cold 0.32 M sucrose buffered with 50 mM Tris–HCl at pH 7.4. The homogenates were centrifuged at 1000 *g* for 10 min and the supernatants used to estimate Na^+,K^+-ATPase activity [18, 19]. The protein content of the last two experiments was determined by the method of Lowry *et al.* [20].

* Abbreviations: VE, vitamin E; DZ, diazepam; CHK, cytosolic hexokinase; MHK, mitochondrial hexokinase; SSS, single swimming stress.

Table 1. Effect of DZ or VE pretreatment on plasma corticosterone and glucose levels of rats subjected to SSS

Items	Normal	Stressed rats pretreated with: Saline	DZ	Sesame oil	VE
Plasma corticosterone (μg/DL)	27.95 ± 0.967	45.64 ± 1.588*	30.79 ± 1.738†	42.49 ± 1.408*	34.79 ± 1.317*†
Plasma glucose (mM)	4.48 ± 0.204	7.23 ± 0.174*	5.37 ± 0.232*†	7.09 ± 0.124*	5.69 ± 0.062*†

The values represent the mean ± SE for six rats.
* Significantly different from normal ($P < 0.05$).
† Significantly different from corresponding stress control ($P < 0.05$).
Both DZ and VE were injected i.p. in a daily dose of 5 mg/kg body weight for 6 days prior to SSS.

Statistical analysis was performed by Student's *t*-test. The 0.05 level of probability was used as the criterion of significance.

Results

Pretreatment of the rats with DZ or VE for 6 days prior to their exposure to SSS diminished the stress-induced increases in their plasma corticosterone and glucose levels (Table 1). These pretreatments also effectively reversed the decreasing and the increasing effects of stress on brain ATP and ADP contents, respectively. Moreover, they prevented the increases in both brain Na^+,K^+-ATPase and MHK activities as well as the decrease in brain CHK activity of stressed rats so that their activities were nearly restored to pre-stressed values (Table 2). The results also showed that administration of DZ or VE to rats prior to SSS raised the brain contents of glucose, glycogen and pyruvate and reduced the increased brain lactate content of stressed rats so that the levels of these brain metabolites were nearly normalized (Table 3). The change in these metabolic parameters after VE pretreatment was less than that produced by DZ. These results indicate that VE ameliorates, at the biochemical level, the stress-induced changes in brain metabolism.

Discussion

Pretreatment of the rats with DZ or VE prior to their exposure to SSS diminished the stress-induced increases in their plasma corticosterone and glucose levels. The effect of DZ in blocking the rise in plasma corticosterone might be mediated centrally by decreasing the release of corticotrophin [21]. However, that caused by VE may be attributed to the attenuating effect of VE on stress-induced lipid peroxidation. This is explained by the findings of Duthie *et al.* [22] that the stressed pig appears to have a defect in its antioxidant defence mechanisms and this can be partially corrected at the biochemical level by increasing the dietary intake of VE. Since plasma glucose level is strongly affected by corticosterone [23], it is to be expected that the high level of plasma glucose in stressed rats will be decreased by DZ and VE. However, these pretreatments effectively restored the levels of brain ATP and ADP to their normal values in stressed rats. They also effectively prevented the stress-induced increases in brain Na^+,K^+-ATPase and MHK activities. The effect of DZ in reversing the stress-induced increase in the brain Na^+,K^+-ATPase activity might be due to the effect of the drug on norepinephrine turnover in stress [24] since norepinephrine has been proved to have a stimulatory action on Na^+,K^+-ATPase activity *in vivo* [25]. The effect of VE in preventing Na^+,K^+-ATPase activation under stress could be explained by its peroxide terminating property. VE has been reported to quench the production of free radicals and alleviate lipid peroxidation in different tissues [4, 26]. Many types of

Table 2. Effect of DZ or VE pretreatment on brain ATP and ADP content, MHK, CHK and Na^+,K^+-ATPase activities of rats subjected to SSS

Items	Normal	Stressed rats pretreated with: Saline	DZ	Sesame oil	VE
Brain ATP (μmol/g wet wt)	1.665 ± 0.05	1.106 ± 0.080*	1.459 ± 0.09†	1.275 ± 0.055*	1.606 ± 0.06†
Brain ADP (μmol/g wet wt)	0.878 ± 0.02	0.993 ± 0.03*	0.770 ± 0.04†	1.043 ± 0.05*	0.834 ± 0.05†
Brain hexokinase (μmol NADPH,H^+/mg protein/hr)					
—MHK	13.85 ± 0.48	16.23 ± 0.58*	12.30 ± 0.83†	15.77 ± 0.41*	12.70 ± 0.50†
—CHK	4.78 ± 0.24	3.98 ± 0.14*	4.72 ± 0.21†	3.83 ± 0.21*	4.42 ± 0.23†
Brain Na^+,K^+-ATPase (μmol P_i/mg protein/min)	1.60 ± 0.09	2.48 ± 0.18*	1.70 ± 0.10†	2.50 ± 0.16*	1.99 ± 0.09*†

The values represent the mean ± SE for six rats.
* Significantly different from normal ($P < 0.05$).
† Significantly different from corresponding stress control ($P < 0.05$).
Both DZ and VE were injected i.p. in a daily dose of 5 mg/kg body weight for 6 days prior to SSS.

Table 3. Effect of DZ or VE pretreatment on brain glucose, glycogen, pyruvate and lactate content of rats subjected to SSS

Items	Normal	Stressed rats pretreated with:			
		Saline	DZ	Sesame oil	VE
Brain glucose (μmol/g wet wt)	0.99 ± 0.02	0.68 ± 0.01*	0.96 ± 0.02†	0.73 ± 0.04*	0.90 ± 0.02*†
Brain glycogen (mg/100 g wet wt)	88.49 ± 2.46	58.19 ± 3.39*	90.40 ± 2.66†	68.85 ± 2.93*	83.20 ± 3.05†
Brain pyruvate (μmol/g wet wt)	1.83 ± 0.06	1.53 ± 0.08*	2.05 ± 0.12†	1.57 ± 0.08*	2.03 ± 0.03*†
Brain lactate (μmol/g wet wt)	2.83 ± 0.06	3.46 ± 0.20*	2.44 ± 0.17†	3.58 ± 0.09*	2.33 ± 0.14*†

The values represent the mean ± SE for six rats.
* Significantly different from normal ($P < 0.05$).
† Significantly different from corresponding stress control ($P < 0.05$).
Both DZ and VE were injected i.p. in a daily dose of 5 mg/kg body weight for 6 days prior to SSS.

stress such as immobilization stress [27], emotional stress [3] and noise stress [28] are found to be associated with an increased rate of lipid peroxide formation. Involvement of lipid peroxidation in accelerating the sodium pump function in brain tissues under stress has been described by Sazontova *et al.* [2]. Moreover, this biochemical alteration was found to be responsible for the rapid decrease of VE level in the nervous tissues following prolonged physical or mental stress [29–31]. In this study, pretreatment of the animals with VE for 5 days caused a 15-fold increase in its level in the CNS as previously reported by Willmore and Rubin [32] and Means *et al.* [33]. Such an accumulation of VE in the nervous system may account for its inhibitory effect on the rate of lipid peroxide formation in the brain. Although DZ and VE are different in their mechanism of action, both can attenuate the stimulatory influence of SSS on Na^+,K^+-ATPase activity. These findings may help to explain the role of DZ or VE pretreatment in restoring the levels of brain ATP and ADP to their pre-stressed values. Another explanation for the role of VE in restoring the level of ATP in brains of stressed rats is its protective action on membrane phospholipids [24, 25]. Phospholipids appear to play a vital role in the electron transport of mitochondria. Slater *et al.* [36] and Quintanilha and Packer [37] appreciated the role of VE in stabilizing the members of electron transport chain in mitochondria against free radicals and lipid peroxidation. The presence of VE in an optimum amount in brain of stressed rats localized in the mitochondrial and microsomal fractions [38] may help in maintaining the electron transport chain in the correct spatial configuration for electron transport and oxidative phosporylation.

Restoration by DZ and VE of the levels of cerebral ATP and ADP to pre-stressed values may explain the effect of these drugs in preventing alteration in hexokinase activity, since ADP and inorganic phosphate (P_i) act as positive modulators for several rate-limiting reactions including hexokinase [39]. Pretreatment of the rats with DZ or VE prior to SSS raised the brain content of glucose, glycogen and pyruvate and reduced the brain lactate content of stressed rats. Such an increase in the glucose and glycogen contents in the brains of rats pretreated with DZ or VE with an accompanied decrease in their MHK activity suggests a decrease in cerebral glucose utilization. DZ was found to reduce local cerebral glucose utilization and depress brain metabolic activity via specific benzodiazepine receptors [40]. However, the decrease in cerebral glucose utilization after VE pretreatment could be explained in light of the antioxidant property of this vitamin. This antioxidant property will prevent the accumulation of different oxygen species which if permitted to accumulate will initiate many free radical reactions in cellular components.

We conclude that among the naturally occurring compounds which are useful as brain modulators, VE is beneficial in ameliorating the biochemical changes during stress. It is recommended to use a diet rich in VE or to administer this vitamin together with antianxiety drugs to enhance their beneficial effects in the brain during stress.

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