EFFECT OF FOOT-ELECTROSHOCK STRESS ON CHOLINERGIC ACTIVITY, TISSUE GLYCOGEN AND BLOOD SUGAR IN ALBINO RATS

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Summary : The immediate or 24 hr delayed effects of 1-day (1-DS) or (7-DS) foot-electroshock stress in albino rats were studied on cardiac acetylcholine (ACh), blood and cardiac cholinesterase (ChE) activities, cardiac, hepatic and muscle glycogen contents and blood sugar concentrations. The effects of physostigmine (PHY), atropine, 6-hydroxydopamine (6-HD), vagotomy and adrenalectomy on 1-DS induced changes were also studied.

1-DS produced an increase in cardiac ACh content which lasted for 24 hr but repeated stress showed phenomenon of adaptation. There seems to be activation of autonomic cholinergic system in stress. 1-DS and 7-DS produced a short-lived inhibition of blood ChE activity and 7-DS also of cardiac ChE activity. Inhibition of ChE activity was probably related to release of adrenaline from adrenal medulla. 1-DS produced hepatic and muscle glycogenolysis with slight hypoglycaemia but without any effect on cardiac glycogen. Following repeated stress there was a phenomenon of adaptation. The hepatic and muscle glycogenolysis produced by stress is due to the release of adrenaline from adrenal medulla. Normally functioning cardiac cholinergic system seems to have a protective effect on heart against stress, in the absence of which cardiac glycogenolysis is induced by stress.

Key words: stress glycogen acetylcholine cholinesterase blood sugar

INTRODUCTION

Stress is known to give rise to a variety of non-specific biochemical, physiological and behavioral responses. There are enough evidences to support the aetiological role of stress in various diseases in general and cardiovascular diseases in particular. Psychological stresses are associated with the causation as well as influencing the clinical course of ischaemic heart disease (3, 25, 31). Stress has been shown to produce myocardial electrolyte changes and cardiomyopathy (14, 23). Various workers have studied the changes in adrenergic system, glycogen, cholesterol, etc. of the heart in experimental stress to correlate with stress-induced cardiac derangements. A study of literature shows that various workers have used different stressors in different species of animals. The results are, therefore, not always unanimous partly because of species variations and partly because of some specific effects of the stressors.

In the present study foot-electroshock model in albino rats has been used on the premise that this stressor is likely to produce minimum of specific changes peculiar to the stressor (4). The studies were planned with the following aims in view — (a) immediate and 24 hr delayed effects of single exposure to stress on cardiac acetylcholine (ACh), cholinesterase activity (ChE) of blood and heart, tissue glycogen and blood sugar concentrations, (b) phenomenon of adaptation, and (c) role of adrenergic and cholinergic systems on the changes produced by acute stress.

MATERIALS AND METHODS

Adult albino rats of either sex and weighing between 100 and 150 g were used. The animals

were kept in separate cages, not more than five in one cage for one week before the commencement of specific experimental procedure, to acclimatise the animals with the environmental conditions. All the animals were kept on food and water *ad lib*, except the adrenalectomised rats which were subsequently kept on normal food and water containing 0.9% sodium chloride.

Stress was induced by a method essentially the same as used by Chattopadhyay *et al.* (4). Two rats were kept in a plastic chamber (22 cm x 20 cm x 25 cm) having electric grids in the floor connected through an Autovariac to A.C. main supply. An alternating make and break system was interposed so as to provide to the electric grid 72 shocks per min with 10 msec pulse width. In initial experiments, a current of 70 volts was found to be optimum to produce a stressful state signified by jumping, fighting, defaecation, piloerection and irritability. In all the experiments, stress was induced by giving foot-electroshock of 70 volts, 72 pulses per min, pulse width 10 msec for a total period of 15 min. Animals were subjected to either single electroshock exposure (1-DS) or one exposure every 24 hr for 7 days (7-DS).

The rats were sacrificed by cervical spinal fracture for biochemical estimations, 15 min after 1-DS or last shock of 7-SD (1-DS and 7-DS respectively), or 24 hr after 1-DS or last shock of 7-DS (1-DS-24 and 7-DS-24 respectively).

The following estimations were made :

(a) ACh of auricles and ventricles was extracted by the method of Nachmanson as described by Anand (1) and assayed on eserenised frog rectus abdominis muscle by the method of Richter and Crossland (24). (b) Blood and ventricular ChE activity was estimated by the method described by Quastel (22). (c) Glycogen contents of the ventricular apex, middle lobe of liver and upper part of gastrocnemius muscle were determined by the method of Montgomery (19). (d) Samples of blood were collected before and after each experiment from the tail vein and sugar was estimated by the method of Folin and Wu (9).

The effects of the following drugs or procedures affecting autonomic nervous system were studied on 1-DS.

(a) Physostigmine salicylate (PHY 0.1 *mg/kg* ip) was administered 30 min before stress. b) Atropine sulphate (AT 2.0 *mg/kg* ip) was administered every 24 hr for 3 days, and stress was given 30 min after the last dose. c) 6-Hydroxydopamine (6-HD 20 *mg/kg* ip) was administered 24 hr before stress. d) Hydrocortisone (HC 2.0 *mg/kg* ip) was administered to normal rats 1 hr before collection of blood. e) Initially bilateral vagotomy was performed but post-operatively the rats died within 24 hr. For this study, therefore, left unilateral vagotomy (VT) was chosen as the right vagus primarily supplies the sino-auricular node. The rats were exposed to stress 5 days after VT (32). Sham vagotomy (SVT) was also performed in control rats 5 days before stress. f) Bilaterally adrenalectomised (BA) (13) rats were subjected to stress 48 hr later. Sham bilateral adrenalectomy (SBA) was also performed in control rats 48 hr before stress.

The effects of the above agents and procedures were also studied on the biochemical

parameters in control rats. Tissues and blood were collected 1 hr after PHY and the last dose of atropine.

The results are expressed as mean±S.E. and significance was estimated by using Student's 't' test except in the case of blood sugar where paired 't' test was also employed.

RESULTS

Effects on body weight and adrenal gland weight :

Exposure of rats to stress for 7 days reduced their body weight by 9.38±1.26%. The adrenal gland weight in the control rats was 0.286±0.014 *mg/g* of body weight. Fifteen min. after 1-DS there was a significant reduction (0.189±0.012 *mg/g*) while 24 hr after 1-DS and 15 min after 7-DS there was a significant increase (0.409±0.036 and 0.367±0.028 *mg/g*, respectively) in the adrenal gland weight.

Effects of 1-day and 7-day stress :

The effects of 1-DS and 7-DS on the cardiac ACh, blood and cardiac ChE activity, tissue glycogen contents and blood sugar concentrations are summarised in Table I and II.

TABLE I: Effect of stress on cardiac acetylcholine and cholinesterase activity.

Groups	*Acetylcholine µg/g*			*ChE activity µM ACh hydrolysed*		
	n	*Auricle*	*Ventricle*	*n*	*Blood per ml/hr*	*Ventricle per g/hr*
1. Control	(30)	3.37±0.15	0.66±0.05	(15)	78.1±3.7	73.4 ±4.7
2. 1 DS	(13)	6.01±0.31*	1.0 ±0.07*	(12)	50.1±3.9*	80.2 ±3.3
3. 1 DS-24	(12)	5.89±0.42*	0.99±0.08*	(10)	72.2±4.6	80.2 ±3.9
4. 7 DS	(15)	3.45±0.11	0.68±0.08	(10)	51.7±3.2*	58.2 ±4.2@
5. 7 DS-24	(14)	3.02±0.13	0.56±0.07	(11)	76.6±4.8	70.2 ±5.3

P — *<0.001, @<0.025 in relation to control group.

TABLE II : Effect of stress on tissue glycogen and blood sugar concentration in albino rats

Groups (with n)	*Glycogen mg/g*			*Change in blood sugar mg%*
	Ventricle	*Liver*	*Muscle*	
1. Control (30)	4.9±0.3	11.7±2.0	5.2±0.3	+ 1.4±2.9
2. 1 DS (13)	5.0±0.4	6.2±1.0@	3.2±0.5*	—16.3±5.1**
3. 1 DS-24 (12)	5.0±0.4	13.5±2.0	3.6±0.3*	+ 8.1±3.0@
4. 7 DS (15)	4.7±0.3	12.9±2.0	5.7±0.5	— 2.8±4.7
5. 7 DS-24 (14)	4.7±0.8	14.2±2.0	5.4±0.4	+ 2.1±5.0
	P in relation to control group			P by paired "t" test.

P values : *<0.001, @<0.025, **<0.01

There was a significant increase in auricular and ventricular ACh concentrations 15 min (78% and 55%) and 24 hr (75% and 50%) after 1-DS, but after 7-DS there was no change in cardiac ACh content. Fifteen min after 1-DS as well as 7-DS there was a significant reduction (36% and 28%) in blood ChE activity, but in both the cases 24 hr later, the ChE activity had returned to normal. 1-DS did not produce any change in cardiac ChE activity but 15 min after 7-DS there was a significant reduction (20%) in cardiac ChE activity.

1-DS produced a significant reduction in hepatic and skeletal muscle glycogen contents by 47% and 39%, respectively without any significant change in ventricular glycogen content. Twenty four hr later the hepatic glycogen content returned back to normal but muscle glycogen continued to be depleted (30%). There was no effect on tissue glycogen contents either 15 min or 24 hr after 7-DS. Fifteen min after 1-DS there was a significant hypoglycaemia but 24 hr later there was slight but significant hyperglycaemia. 7-DS had no significant effect on blood sugar concentrations.

Effects of drugs and procedures affecting autonomic nervous system on the changes induced by 1-day stress :

The results are summarised in Tables III and IV.

TABLE III : Interactions with stress-induced changes in cardiac acetylcholine and cholinesterase activity levels

Groups	*Acetylcholine µg/g*			*ChE activity µM ACh hydrolysed*		
	n	*Auricle*	*Ventricle*	*n*	*Blood per ml/hr*	*Ventricle per g/hr*
1. Control	(30)	3.37±0.15	0.66±0.05	(15)	78.1±3.7	73.4±4.7
2. 1 DS	(13)	6.01±0.31 (P_1*)	1.02±0.07 (P_1*)	(12)	50.1±3.9 (P_1*)	80.2±3.3
3. PHY	(10)	6.63±1.1 (P_1**)	1.14±0.19 (P_1@)	(10)	72.4±5.6	58.5±2.6 (P_1@)
4. PHY-1 DS	(12)	—	2.01±0.15 (P_2*, P_3**)			
5. SVT	(10)	3.35±0.21	0.73±0.03	—	—	—
6. SVT-1 DS	(13)	5.83±0.32 (P_5*)	0.98±0.04 (P_5*)			
7. VT	(10)	2.72±0.15 (P_5@)	0.56±0.03 (P_5@)	—	—	—
8. VT-1 DS	(14)	3.73±0.12 (P_6*, P_7*)	0.78±0.03 (P_6*, P_7*)	—	—	—
9. BA	(9)	3.47±0.19	0.67±0.03	(10)	45.9±4.3 (P_1*)	
10. BA-1 DS	(12)	5.69±0.21 (P_9*)	1.21±0.05 (P_9*)	(10)	50.1±3.5	—
11. HC	—	—	—	(9)	102.1±3.8 (P_1*)	

P values : *<0.001, @<0.025, **<0.01

P_1, P_2, P_3, P_5, P_6, P_7 and P_9 in relation to group numbers 1, 2, 3, 5, 6, 7 and 9, respectively.

TABLE IV : Interactions with stress-induced changes in tissue glycogen and blood sugar levels.

Group and n		*Glycogen mg/g*			*Change in blood sugar mg%*
		Ventricle	*Liver*	*Muscle*	
1. Control	(30)	4.9±0.3	11.7±2.0	5.2±0.3	+ 1.4±2.9
2. 1 DS	(13)	5.0±0.4	6.2±1.0 (P_1@)	3.2±0.5 (P_1*)	−16.3±5.1 (P**)
3. PHY	(10)	4.6±0.3	15.3±3.1	4.5±0.4	—
4. PHY-1 DS	(12)	4.2±0.3	3.3±0.3 (P_3*) (P_2**)	3.0±0.3 (P_3@)	− 3.2±5.8
5. AT	(10)	4.1±0.4	9.5±1.0	4.4±0.3	− 2.9±1.9
6. AT-1 DS	(13)	2.8±0.2 (P_5**, P_2*)	5.3±0.2 (P_5**)	3.2±0.3 (P_5**)	− 6.9±2.0 (P**)
7. SVT	(10)	4.9±0.3	10.8±0.2	5.0±0.2	—
8. SVT-1 DS	(13)	5.0±0.4	6.8±0.6 (P_7*)	3.4±0.3 (P_7*)	—
9. VT	(10)	3.6±0.3 (P_7**)	9.2±0.5 (P_7**)	4.4±0.3 (P_7**)	—
10. VT-1 DS	(14)	3.4±0.3 (P_9**)	6.2±0.4 (P_9*)	3.2±0.3 (P_9**)	—
11. SBA	(10)	4.8±0.3	12.1±1.1	5.3±0.3	+ 0.9±1.4
12. BA	(9)	2.9±0.5 (P_{11}**)	3.1±0.4 (P_{11}*)	4.5±0.4	+ 7.6±4.3
13. BA-1 DS	(12)	2.8±0.4 (P_2*)	3.8±0.5 (P_2@@)	3.9±0.5	− 2.1±3.2
14. 6-HD	(10)	3.2±0.3 (P_1*)	11.8±1.4	3.1±0.3 (P_1*)	+30.7±5.7 (P*)
15. 6-HD-1 DS	(13)	3.2±0.3 (P_2**)	12.8±1.2 (P_2*)	3.2±0.2	+32.6±4.6 (P*)

P values : *<0.001, @<0.025, **<0.01, @@<0.05
P by paired t test. P_1, P_2, P_3, P_5, P_7, P_9 and P_{11} in relation to group numbers 1, 2, 3, 5, 7, 9 and 11, respectively.

(*a*) *Physostigmine :*

PHY, one hr after administration, had no effect on blood ChE activity but reduced cardiac ChE activity by 20%. It markedly increased cardiac ACh contents. Stress in PHY pretreated rats increased ventricular ACh content by 71% as compared to PHY control and by 205% as compared to untreated control.

PHY treatment had no effect on muscle glycogenolysis produced by stress. However, stress produced slightly more hepatic glycogenolysis but no hypoglycaemia in PHY pretreated rats.

(*b*) *Atropine :*

In atropine pretreated rats stress not only produced hepatic and muscle glycogenolysis but

also cardiac glycogenolysis by 32%. In atropine pretreated rats stress also produced slight hypoglycaemia.

c) Vagotomy (VT) :

The effects of 1-DS on cardiac ACh, tissue glycogen and blood sugar concentrations in rats subjected to SVT were the same as in the control rats. VT significantly reduced auricular (19%) as well as ventricular (23%) ACh contents. In animals subjected to VT stress produced a significant increase in cardiac ACh concentration but the increase was much smaller than that seen in control stressed rats. The glycogen contents of the liver and muscle in 1-DS vagotomised rats were the same as in 1-DS control rats but the ventricular glycogen content was significantly lower (36%).

d) 6-Hydroxy-dopamine (6-HD) :

Treatment with 6-HD produced cardiac and muscle glycogenolysis with hyperglycaemia without any effect on hepatic glycogen. In 6-HD pretreated rats stress had no effect on tissue glycogen contents and blood sugar concentrations.

e) Bilateral adrenalectomy (BA) :

BA reduced the blood ChE activity by 41% without any effect on cardiac ACh concentration. Treatment of the normal rats with HC (2 *mg/kg*) raised the blood ChE activity by 37%. In adrenalectomised rats stress produced marked increase in auricular (64%) and ventricular (80%) ACh concentrations without having any effect on blood ChE activity.

BA reduced cardiac (40%) and hepatic (74%) glycogen contents without any significant effect on muscle glycogen and blood sugar. In adrenalectomised rats stress did not produce any change in tissue glycogen and blood sugar concentrations.

DISCUSSION

The adrenal gland weight changes give some indication that foot-electroshock acted as stress in rats. The decrease in the adrenal gland weight soon after 1-day stress might be due to sudden discharge of the contents of the gland into the blood stream. The inrease in the adrenal gland weight 24 hr after 1-day stress and following 7-day stress indicates adrenal gland hypertrophy due to stress. The adverse effect of 7-day stress on the body as a whole was evident from the significant reduction in the body weight.

Inhibition of myocardial ChE activity has been reported following cold stress in rabbit (28) and in rat (27) as well as following heat stress in rat (11). But chronic electroshock stress in rat has been reported to increase myocardial ChE activity (16). In the present study, however, myocardial ChE activity was unaffected by 1-day foot electroshock stress and inhibited by 7-day stress. In addition, following 1-day as well as 7-day stress there was a short-lived inhibition of blood ChE activity. The decrease in ChE activity does not seem to be due to the release of corticosteroids from adrenal glands during stress because hydrocortisone treatment increased and adrenalectomy decreased blood ChE activity. A decrease in ChE activity of kidneys (12) and brain (20) has also been shown following adrenalectomy. The short-lived decrease in blood ChE activity following

stress could be due to the release of adrenaline from adrenal glands because in adrenalectomised rats stress did not reduce blood ChE activity. In addition, adrenaline has been shown to inhibit ChE activity (2).

Acute stress increased cardiac ACh concentration supporting the findings of several workers (11, 15, 16, 26, 27, 28). In addition, the present study shows that, following acute stress, the increased cardiac ACh continues at least upto 24 hr, but after repeated stress there was no effect on cardiac ACh contents indicating a phenomenon of adaptation. The increased cardiac ACh is not secondary to inhibition of ChE activity was the changes in these two parameters have not been found to be related. In addition, in PHY-pretreated rats when ChE activity was already inhibited, stress produced further increase in cardiac ACh contents. In unilaterally vagotomised animals, the increase in cardiac ACh following stress was much lower than in animals with intact vagus. These findings indicate that increased cardiac ACh following acute stress is neurogenic in nature. Thus in stress there is not only well-known adrenergic discharge but also activation of cholinergic system.

In rat cold stress (27), heat stress (33), electroshock stress (16) and restraint stress (21) produce myocardial glycogenolysis. In addition, heat stress in rat has been shown to produce generalised tissue glycogenolysis (33). In the present study, however, acute electroshock stress produced glycogenolysis of liver and muscle with significant hypoglycaemia, without any effect on the heart. The effects on liver and blood sugar disappeared within 24 hr but not on muscle. Following repeated stress there was neither any glycogenolysis nor hyperglycaemia indicating phenomenon of adaptation. In rats, cold stress (29) and restraint stress (21) have been reported to produce hypoglycaemia. It is possible that during the initial stage of stress the suddenly liberated growth hormone exerts "insulin-like action" enhancing glucose utilisation and producing hypoglycaemia (10).

Stress-induced hepatic and muscle glycogenolysis was absent in adrenalectomised rats or in those treated with 20 *mg/kg* 6-hydroxydopamine, which at this dose is reported to cause nearly complete disappearance of adrenergic fluorescence (17, 18). These findings strongly suggest that adrenergic system is involved in stress-induced tissue glycogenolysis.

ACh completely antagonises the cardiac glycogenolysis induced by adrenaline and theophylline and partially that of anoxia in guinea pigs (34). Physostigmine antagonises cardiac glycogenolysis induced by hypothermia in dogs (7) and frogs (30), following light petroleum plus adrenaline or coronary ligation in dogs (5) and following hypoxia (8) and isoprenaline (6) in rats. In the present study, however, inhibition of ChE activity by PHY-treatment did not affect stress induced muscle glycogenolysis. However, the slight increase in hepatic glycogenolysis and absence of hypoglycaemic effects of stress could be due to potentiated adrenergic responses of stress mediated through nicotinic effects of PHY at the sympathetic ganglia. However, the cardiac glycogen content following stress in atropinised or vagotomised animals was significantly lower than in normal rats. This might suggest that the presence of normally functioning cholinergic system might be protecting the heart from glycogenolysis during stress.

The results suggest that acute stress produces activation of autonomic cholinergic system, and hepatic and muscle glycogenolysis due to release of adrenaline from adrenal medulla. Repeated stress produces a phenomenon of adaptation for both the effects. Acute stress can also induce cardiac glycogenolysis if cardiac cholinergic activity is inhibited. The short-lived inhibition of ChE activity produced by stress is most probably due to release of adrenaline from adrenal medulla. There is no adaptation to this effect.

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