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## Age and Heat Stress Related Changes in Monoamine Contents and Cholinesterase Activity in Some Central Nervous System Regions of Albino Rat Newborns

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**Abstract:** The normal monoamine [norepinephrine (NE), epinephrine (E), dopamine (DA) and serotonin (5-HT)] contents and cholinesterase (chE) activity were significantly and gradually increased with age progress between postnatal days 7 and 21 in cerebrum, cerebellum, medulla oblongata and spinal cord of rat newborns. The daily exposure of the newborns to  $40\pm 1^\circ\text{C}$  for 2 h induced deteriorated effects and the withdrawal period of 7 days failed to return these altered variables to normal levels. On the other hand, the high temperature exerted its most potent decreased effect on monoamine contents at 21 days old. This decrease may be attributed to the elevated activity of monoamine oxidase and/or the decreased activity of the key enzymes responsible for monoamine synthesis. The chE activity exhibited different effects in the tested CNS regions as a result of high temperature exposure; the enzyme activity was decreased markedly at days 7, 14 and 21 in cerebellum and medulla oblongata and lowered only at days 7 and 14 in cerebrum and at day 14 in spinal cord. The subsequent withdrawal for 7 days beyond day 21 produced marked weakening of effect of high temperature exposure on monoamine contents in all examined CNS regions except NE and 5-HT contents in cerebellum and DA level in medulla oblongata. In spite of this attenuation, the values recorded in the withdrawal group were still significantly lower than the normal levels. On the other hand, the chE activity became more deleteriously affected at day 28 in the treated CNS regions except in the medulla oblongata where it was profoundly ameliorated after the withdrawal period.

**Key words:** Heat stress, rat newborns, monoamines, cholinesterase

### INTRODUCTION

Temperature is one of the most encountered stressful factors in the environment. Hyperthermia is thought to be a teratogenic in many animal species and also in humans (Milunsky *et al.*, 1992; Sasaki *et al.*, 1995; Lee *et al.*, 2000; Hirobumi *et al.*, 2002; Edwards *et al.*, 2003; Zhu *et al.*, 2004). Exposures of embryos experimentally to elevated temperature during organogenesis has long been to be embryotoxic (Hutchinson and Bowler, 1984; Edwards, 1986; Upfold *et al.*, 1989; Sharma *et al.*, 2003; Zhu *et al.*, 2004).

Brain catecholamines and acetylcholine have an important role in the regulation of several functions (Deutsh, 1971; Drachman and Levitt, 1974; Siegel *et al.*, 1989; Herlenius and Lagercrantz, 2001). It was reported that the Central Nervous System (CNS) serotonin (5-hydroxytryptamine, 5HT) and catecholamines play crucial roles in the heat loss and heat production mechanisms in mammals (Bligh *et al.*, 1971; White *et al.*, 1985). On the other hand, cholinesterase (chE) inhibition elicits cholinergic stimulation in the central nervous system and in peripheral tissues and organs, which lead to marked dysfunction of homeostatic system, including temperature regulation (Gordon, 1996).

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Increases of catecholamines and serotonin contents in different brain regions and spinal cord with development in various animals were revealed by many authors (Karki *et al.*, 1962; Lauder *et al.*, 1982; Rajaofetra *et al.*, 1989; Abdel-Raheem *et al.*, 1995; Abdelmelek *et al.*, 2000). Also, according to Muller *et al.* (1985), the brain acetylcholinesterase (AChE) activity increased mostly postnatal in rat, but in human brain, it reached a maximum at birth.

Changes of catecholamines and serotonin concentrations in various CNS regions as a result of high temperature exposure or hyperthermia in newborns and adults are still controversial. Some authors reported a decrease (Merritt *et al.*, 1977; Kregel *et al.*, 1988; Yuan *et al.*, 1989; Kregel *et al.*, 1993), an increase (Tor-Agbidye *et al.*, 2001; Zhao *et al.*, 2001) and no change in these concentrations (Merritt *et al.*, 1977; Yuan *et al.*, 1989; Dib *et al.*, 1991). Also, the effect on chE activity is equivocal (Menon and Dandiya, 1969; Aly *et al.*, 1986).

Thus, the aim of the present study was to assess the effect of 2 h daily exposure to  $40\pm 1^{\circ}\text{C}$  for 3 post-natal weeks and subsequent withdrawal for 1 week on monoamines content and chE activity in cerebral hemispheres, cerebellum, medulla oblongata and spinal cord of albino rat newborns.

## MATERIALS AND METHODS

### Experimental Animals

White albino rats (*Rattus norvegicus*) were supplied from the National Research Institute of Ophthalmology, Giza (Egypt). The adult rats were kept under observation in the department animal house for 2 weeks to exclude any intercurrent infection and to acclimatize the new conditions. They were also kept under good hygienic conditions and were given standard balanced diets and water *ad libitum*. Males and females were allowed to meet for 2 consecutive days, then the pregnant females were transferred into separate cages till delivery.

### High Temperature Exposure and Animals Grouping

Within 24 h after delivery, the newborns were randomly selected and divided into two main groups:

#### Normal Individuals Group

They were maintained at room temperature ( $25^{\circ}\text{C}$ ) with their mothers. These selected numbers were sacrificed by decapitation at days 7, 14, 21 and 28 after birth.

#### Treated Individuals Group

They were subjected to heat stress by housing the newborns from each mother in a separate woody perforated cage and placed in a good aerated incubator set at  $40\pm 1^{\circ}\text{C}$  for 2 h daily. The newborns were returned to their nursing mothers after each exposure. The decapitation was done at ages 7, 14 and 21 days. Subsequent withdrawal for 1 week was performed beyond 21 days on sex newborns to assess the recovery process.

### Excision of the Brain and the Spinal Cord

After sacrifice, the brain was removed carefully from the skull base after cutting all cranial nerves, then dissected into its differential regions and each was kept in a deep freezer at  $20^{\circ}\text{C}$  until being used in the biochemical investigations.

Also after scarification, the vertebral column was dissected, then the spinal cord was removed carefully and kept in a deep freezer at  $20^{\circ}\text{C}$  pending biochemical examinations.

### **Biochemical Examination**

The brain regions (the cerebral hemispheres, cerebellum and medulla oblongata) as well as the whole spinal cord of the normal, treated and withdrawal newborns were quickly divided into two longitudinal equal halves. One half was homogenized by using teflon homogenizer (Glas-Col, Terre Haute in USA) in 75% methyle alcohol for monoamine determinations and the other was homogenized in an isotonic solution (0.9% NaCl) to be used for chE activity estimation.

### **Estimation of Monoamine Concentrations**

The dissected tissue samples were weighed and homogenized in 1/10 weight/volume of 75% aqueous HPLC grade methanol. The homogenate was spun at 3000 rpm for 10 min and the supernatant was immediately extracted from the trace elements and lipids by the use of solid phase extraction CHROMABOND column NH<sub>2</sub> phase Cat. No. 730031. The sample was then injected directly to the AQUA HPLC column, 150×4.6 mm, C18, purchased from phenomenex USA under the following conditions: mobile phase 97/3 20 mM potassium phosphate, pH 3.0/Methanol, flow rate 1.5 mL min, UV 270 nm. NE, E, DA and 5-HT were well separated after 12 min. The resulting chromatogram for each sample identified each monoamine position and area under curve was compared to that of the standard curve by Eurochrom HPLC Software, Version 1.6. The content of each monoamine in µg g<sup>-1</sup> tissue was then calculated for each CNS region (Pagel *et al.*, 2000).

### **Estimation of Cholinesterase (chE) Activity**

Isotonic solution homogenate was centrifuged at 3000 rpm. for 15 min The activity of cholinesterase (butyrylcholinestrace, which hydrolyses acetylcholine as well as butyrylcholine) in the supernatant was determined by a colourimetric method of Den Blaauwen *et al.* (1983) using a reagent kit purchased from Quimica Clinica Aplicada, Spain. The initial absorbance and the absorbance after 30, 60 and 90 sec of addition were read at 405 nm using an UV spectrophotometer (Humalyzer, 2000, USA) at 37°C. The activity in U 100 mg tissue was calculated for each sample.

### **Statistical Analysis**

The obtained data were analyzed using one - and two - way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985). Variables having a significant F-value were compared using the Least Significant Difference (LSD) test.

## **RESULTS**

### **Changes in Monoamines Concentration**

The normal concentration of the tested monoamines exhibited similar behavioral patterns in all examined CNS regions. They were gradually and significantly increased as the age progressed to reach their maximum values at the 28 days old (Table 1-4).

As a result of daily exposure to high temperature for 7 or 14 post-natal days, the NE content was increased in cerebellum and medulla oblongata and decreased in cerebrum and spinal cord as compared with the corresponding controls. As the period of exposure extended to 21 days, the NE level was significantly ( $p < 0.01$ ) reduced in all tested regions; the most potent effect was achieved in cerebrum (-46.811%). The stopping of exposure for 1 week beyond day 21 induced marked attenuation of the effect of heat stress on NE concentration in the cerebrum, medulla oblongata and spinal cord where the percentage changes were -26.243, -36.657 and -22.677 at day 28 instead of -46.811, -41.483 and -37.281 at day 21, respectively. However, in the cerebellum, the effect was more or less obvious as a result of withdrawal for 1 week. In spite of these changes, the values of the NE level remained significantly lower in the withdrawal group when compared to their normal newborns (Table 1).

Table 1: Age and temperature exposure effect on norepinephrine content ( $\mu\text{g g}^{-1}$ ) in different CNS regions of rat newborns

Periods	Regions	Cerebrum	Cerebellum	Medullaoblongata	Spinal cord
7 days	NC	0.929±0.0705 <sup>f</sup>	0.555±0.0468 <sup>g</sup>	0.449±0.093 <sup>e</sup>	0.692±0.058 <sup>d</sup>
	T	0.853±0.0235 <sup>f</sup>	0.846±0.01887 <sup>f</sup>	0.528±0.0132 <sup>e</sup>	0.544±0.073 <sup>d</sup>
	%	- 8.180	+52.432	+7.105	-21.387
14 days	NC	1.546±0.084 <sup>d</sup>	1.052±0.059 <sup>ef</sup>	1.608±0.091 <sup>c</sup>	1.158±0.056 <sup>c</sup>
	T	1.254±0.126 <sup>e</sup>	1.138±0.048 <sup>ab</sup>	1.840±0.121 <sup>bc</sup>	1.064±0.172 <sup>c</sup>
	%	-18.887	+8.174	+14.427	-8.117
21 days	NC	2.211±0.043 <sup>b</sup>	2.037±0.0376 <sup>b</sup>	2.090±0.135 <sup>b</sup>	1.714±0.067 <sup>b</sup>
	T	1.176±0.107 <sup>c</sup>	1.365±0.0037 <sup>e</sup>	1.223±0.042 <sup>d</sup>	1.075±0.052 <sup>c</sup>
	%	-46.811	-32.989	- 41.483	-37.281
28 days	NC	2.553±0.065 <sup>a</sup>	2301±0.0358 <sup>a</sup>	2.788±0.068 <sup>a</sup>	2.368±0.061 <sup>a</sup>
	Wd	1.883±0.086 <sup>c</sup>	1.557±0.199 <sup>f</sup>	1.766±0.056 <sup>c</sup>	1.831±0.055 <sup>b</sup>
	%	- 26.243	-32.333	-36.657	-22.677
LSD at 5% level		0.226	0.229	0.250	0.242
LSD at 1% level		0.305	0.309	0.337	0.326

Data are expressed as mean±standard error. Number of animals in each group is six. For each region, means with a common superscript letter(s) are not significantly different. N: Normal group; T: Treated group; withdrawal period is one week beyond day 21

Table 2: Age and temperature exposure effect on epinephrine content ( $\mu\text{g g}^{-1} 10^{-2}$ ) in different CNS regions of rat newborns

Periods	Regions	Cerebrum	Cerebellum	Medullaoblongata	Spinal cord
7 days	NC	0.0084±0.0003 <sup>g</sup>	0.0096±0.00022 <sup>e</sup>	0.0175±0.0001 <sup>d</sup>	0.0056±0.0003 <sup>e</sup>
	T	0.0117±0.0002 <sup>f</sup>	0.0224±0.0009 <sup>b</sup>	0.0099±0.0002 <sup>f</sup>	0.0128±0.0031 <sup>d</sup>
	%	+39.285	+133333	-43.428	+128.571
14 days	NC	0.0180±0.0003 <sup>d</sup>	0.0180±0.0011 <sup>ed</sup>	0.0262±0.0012 <sup>c</sup>	0.0170±0.0024 <sup>e</sup>
	T	0.0170±0.0010 <sup>db</sup>	0.0120±0.0010 <sup>e</sup>	0.0136±2.875 <sup>e</sup>	0.0156±0.0012 <sup>cd</sup>
	%	- 5.555	-33.333	-43.091	-8.235
21 days	NC	0.0309±0.0010 <sup>b</sup>	0.0361±0.0003 <sup>a</sup>	0.0336±0.0006 <sup>b</sup>	0.0282±0.0012 <sup>b</sup>
	T	0.0151±0.0008 <sup>g</sup>	0.0160±0.0021 <sup>d</sup>	0.0199±0.0003 <sup>d</sup>	0.0018±0.0002 <sup>c</sup>
	%	-51.132	-55.678	-40.773	-93.617
28 days	NC	0.0366±0.0009 <sup>a</sup>	0.0396±0.0007 <sup>a</sup>	0.040±0.0002 <sup>a</sup>	0.0348±0.0004 <sup>a</sup>
	Wd	0.0260±0.0016 <sup>c</sup>	0.0216±0.0021 <sup>bc</sup>	0.0315±0.0008 <sup>b</sup>	0.0275±0.0013 <sup>b</sup>
	%	-28.961	-45.454	-21.250	-20.977
LSD at 5% level		0.00268	0.00377	0.00339	0.00336
LSD at 1% level		0.00362	0.00508	0.00457	0.00454

The epinephrine (E), on the other hand, was significantly elevated in cerebrum ( $p<0.05$ ), cerebellum ( $p<0.01$ ) and spinal cord ( $p<0.01$ ), but it was significantly depleted in medulla oblongata ( $p<0.01$ ) at the 7th day of exposure as compared with the corresponding controls. As the time of exposure extended to 14 and 21 days, the epinephrine content was profoundly decreased in all the examined regions. The withdrawal of exposure for one week led to a substantial attenuation of the high temperature effect on epinephrine concentration; the calculated percentage changes were -28.961, -45.454, -21.250 and -20.977 at the 28th day versus -51.132, -55.678, -40.773 and -93.617 at day 21 in the cerebrum, cerebellum, medulla oblongata and spinal cord, respectively (Table 2).

The dopamine concentration of the treated newborns exhibited significant decrease at all tested periods except at day 7 concerning medulla oblongata in comparison with their corresponding controls. The stopping of exposure for one week beyond day 21 resulted in a marked attenuation of the decreased effect in all CNS regions except in the medulla oblongata where this deleterious effect was strengthened to become -67.213% at day 28 instead of -51.061% at day 21. In spite of the aforementioned attenuation in the most studied regions, the values as a result of withdrawal remained significantly lower than those of their corresponding controls (Table 3).

The normal behavioral pattern of changes in 5-HT concentration was disrupted as a result of high temperature exposure. At the 7th day of exposure, while the 5-HT level was significantly increased in the cerebrum, it was non-significantly ( $p>0.05$ ) decreased in other CNS regions in comparison with

Table 3: Age and temperature exposure effect on dopamine content ( $\mu\text{g g}^{-1}$ ) in different CNS regions of rat newborns

Periods	Regions	Cerebrum	Cerebellum	Medullaoblongata	Spinal cord
7 days	NC	0.396±0.0202 <sup>f</sup>	0.457±0.0145 <sup>f</sup>	0.527±0.018 <sup>f</sup>	0.339±0.0390 <sup>h</sup>
	T	0.276±0.0105 <sup>e</sup>	0.311±0.0102 <sup>e</sup>	0.683±0.104 <sup>d</sup>	0.303±0.058 <sup>g</sup>
	%	-30303	-31.947	+46.802	-10.619
14 days	NC	0.634±0.0264 <sup>d</sup>	0.625±0.041 <sup>d</sup>	0.824±0.0211 <sup>c</sup>	0.648±0.0197 <sup>e</sup>
	T	0.454±0.0124 <sup>e</sup>	0.485±0.0078 <sup>f</sup>	0.543±0.048 <sup>g</sup>	0.393±0.0218 <sup>h</sup>
	%	-28391	-22.400	-34.101	-39351
21 days	NC	0.853±0.018 <sup>b</sup>	0.933±0.0206 <sup>b</sup>	0.036±0.043 <sup>b</sup>	0.867±0.0354 <sup>b</sup>
	T	0.479±0.0114 <sup>e</sup>	0.537±0.0082 <sup>e</sup>	0.507±0.0168 <sup>ef</sup>	0.412±0.0147 <sup>d</sup>
	%	-43.845	-42.443	-51.061	-52.479
28 days	NC	1.007±0.021 <sup>a</sup>	1.040±0.01674 <sup>a</sup>	1.220±0.0160 <sup>a</sup>	1.122±0.826 <sup>a</sup>
	Wd	0.692±0.0188 <sup>g</sup>	0.798±0.0236 <sup>f</sup>	0.400±0.029 <sup>f</sup>	0.682±0.0279 <sup>f</sup>
	%	-31.281	-23.269	-67.213	-39.215
LSD at 5% level		0.05284	0.05967	0.13436	0.09180
LSD at 1% level		0.07116	0.08036	0.18096	0.12364

Table 4: Age and temperature exposure effect on serotonin content ( $\mu\text{g g}^{-1}$ ) in different CNS regions of rat newborns

Periods	Regions	Cerebrum	Cerebellum	Medullaoblongata	Spinal cord
7 days	NC	0.193±0.0074 <sup>e</sup>	0.159±0.0160 <sup>ef</sup>	0.182±0.0104 <sup>f</sup>	0.179±0.0111 <sup>ef</sup>
	T	0.230±0.011911 <sup>d</sup>	0.145±0.0064 <sup>ef</sup>	0.169±0.0076 <sup>f</sup>	0.160±0.0199 <sup>f</sup>
	%	+19.170	-8.805	-7.142	-10.614
14 days	NC	0.285±0.0129 <sup>e</sup>	0.269±0.0111 <sup>e</sup>	0.224±0.0055 <sup>e</sup>	0.294±0.0132 <sup>c</sup>
	T	0.168±0.0062 <sup>e</sup>	0.173±0.0073 <sup>e</sup>	0.221±0.0062 <sup>e</sup>	0.204±0.0090 <sup>h</sup>
	%	-41.052	-35.687	-10.339	-30.612
21 days	NC	0.356±0.0067 <sup>b</sup>	0.395±0.0101 <sup>b</sup>	0.341±0.0112 <sup>c</sup>	0.403±0.011 <sup>b</sup>
	T	0.191±0.0029 <sup>g</sup>	0.140±0.0025 <sup>f</sup>	0.259±0.0060 <sup>d</sup>	0.161±0.0029 <sup>f</sup>
	%	-46.348	-64.556	-24.046	-60.049
28 days	NC	0.490±0.025 <sup>a</sup>	0.531±0.0109 <sup>a</sup>	0.479±0.0142 <sup>a</sup>	0.551±0.0195 <sup>a</sup>
	Wd	0.233±0.0036 <sup>d</sup>	0.229±0.0164 <sup>d</sup>	0.387±0.0156 <sup>b</sup>	0.232±0.0164 <sup>d</sup>
	%	-52.448	-56.873	-19.206	-57.894
LSD at 5% level		0.03416	0.03185	0.02969	0.04059
LSD at 1% level		0.04602	0.04289	0.03998	0.05467

their corresponding control. At days 14 and 21, the 5-HT concentration was decreased markedly when compared to the corresponding controls in all the studied CNS regions. On the other hand, the 5-HT concentration was more deteriorated in the cerebrum after stopping of exposure for one week beyond day 21 (Table 4).

With regard to ANOVA, it was found that the general effect on monoamine concentrations between groups was highly significant ( $p < 0.01$ ) in all tested regions throughout the experiment. On the other hand, two-way ANOVA, on the other hand, indicated that the effect of temperature, time and their interaction on NE, DA and 5-HT content was, at least, highly significant ( $p < 0.01$ ). With the exception of the effect temperature on spinal cord where the effect is only significant ( $p < 0.05$ ), the changes of epinephrine as a result of temperature, time and their interaction were highly significant ( $p < 0.01$ ) in all the examined CNS regions (Table 6).

#### Changes in Cholinestrase (chE) Activity

The data of the normal newborn rats indicate a gradual increases in chE activity in the investigated CNS regions with the age progress. The treated newborns exhibited an obvious decrease of the enzyme activity in both cerebellum and medulla oblongata at all ages when compared to the normal controls. In cerebrum, the enzyme activity was significantly decreased due to high temperature exposure for 14 days, but it was non-significantly affected at days 7 and 21. The spinal cord chE activity showed an increase as a result of exposure for 7 and 21 days but it was detectably decreased at day 14 (-35.741%). The withdrawal led to a marked increase of enzyme activity in cerebrum, but there was significant decrease in other examined regions of CNS as compared with their corresponding control (Table 5).

Table 5: Age and temperature exposure effect on cholinestrase activity (U/100 mg) in different CNS regions of rat newborns

Periods	Regions	Cerebrum	Cerebellum	Medullaoblongata	Spinal cord
7 days	NC	1.443±0.088 <sup>d</sup>	1.681±0.083 <sup>d</sup>	1.615±0.162 <sup>f</sup>	1.574±0.147 <sup>od</sup>
	T	0.95±0.022 <sup>d</sup>	1.162±0.172 <sup>e</sup>	1.534±0.069 <sup>f</sup>	2.366±0.045 <sup>b</sup>
	%	-34.164	-30.874	-5.015	+50.317
14 days	NC	2.029±0.073 <sup>c</sup>	1.951±0.094 <sup>od</sup>	2.658±0.146 <sup>c</sup>	2.325±0.069 <sup>bc</sup>
	T	1.369±0.120 <sup>d</sup>	1.868±0.12 <sup>d</sup>	1.858±10.70 <sup>e</sup>	1.494±0.045 <sup>b</sup>
	%	-32.528	-4.254	-30.097	-35.741
21 days	NC	2.432±0.193 <sup>c</sup>	2.32±0.024 <sup>b</sup>	2.933±0.091 <sup>b</sup>	2.74±0.355 <sup>b</sup>
	T	2.491±0.278 <sup>c</sup>	2.02±0.228 <sup>bcod</sup>	2.306±0.059 <sup>d</sup>	2.865±0.198 <sup>b</sup>
	%	+2.425	-12.931	-21.777	+4.562
28 days	NC	3.652±0.249 <sup>b</sup>	2.823±0.094 <sup>a</sup>	3.366±0.055 <sup>a</sup>	3.695±0.576 <sup>a</sup>
	Wd	4.425±0.339 <sup>a</sup>	2.283±0.069 <sup>bc</sup>	2.910±0.053 <sup>b</sup>	2.323±0.210 <sup>bc</sup>
	%	+21.166	-19.128	-13.547	-37.131
LSD at 5% level		0.578	0.364	0.2480	0.773
LSD at 1% level		0.779	0.489	0.335	1.040

Table 6: One- and Two-way analysis of variance (ANOVA) for monamine contents and cholinestrase activity after exposure to high temperature in different brain regions and spinal cord of rat newborns

Regions	Source of variation	F-Probability					chE
		NE	E	DA	5-HT		
Cerebrum	General effect						p<0.01
	Temp.	p<0.01	p<0.01	p<0.01	p<0.01		p<0.01
	Time						p>0.01
	Temp. - Time						p<0.05
Cerebellum	General effect						p<0.01
	Temp.	p<0.01	p<0.01	p<0.01	p<0.01		p<0.05
	Time						p<0.01
	Temp. - Time						p>0.05
Medulla oblongata	General effect						p<0.01
	Temp.	p<0.01	p<0.01	p<0.01	p<0.01		p<0.01
	Time						p<0.01
	Temp. - Time						p<0.01
Spinal cord	General effect						p<0.01
	Temp.	p<0.01	p<0.01	p<0.01	p<0.01		p<0.01
	Time		p<0.01				p<0.01
	Temp.-Time		p>0.01				p<0.01

NE: Norepinephrine; E: Epinephrine; DA: Dopamine; 5-HT: 5-Hydroxytryptamine; chE: Cholinestrase. p<0.05: Significant; p<0.01: Highly significant

One-way ANOVA revealed that the effect on chE activity between groups was highly significant (p<0.01) in all examined regions throughout the experiment. The two-way ANOVA also indicated the highly significant effect of temperature-time interaction in medulla oblongata and spinal cord, but it was non-significantly affected in cerebrum and cerebellum. Moreover, with the exception of the effect temperature in spinal cord, which was non-significant, the other changes were at least significant as a result of temperature or period effect in all the tested CNS regions (Table 6).

## DISCUSSION

The present study revealed that the normal monoamines (NE, E, DA and 5-HT) concentrations were increased tremendously in the investigated CNS regions between days 7 and 28. This coincides with the results of several authors. According to Lauder *et al.* (1982), the monoamines are one of the earliest developing neurotransmitter systems in the mammalian brain. Moreover, it was revealed that the serotonin content increased by the development of the brain in chick, rat (Karki *et al.*, 1962; Bennett and Giarman, 1965) and guinea pig (Karki *et al.*, 1962). Also, as recorded by Abdel-Raheem *et al.* (1995), the dopamine (DA) level showed its highest profile in cerebellum and pons of young rats and it declined with aging. In addition, the neurotransmitters, like serotonin and

norepinephrine, increased gradually with age progress as postulated by Rajaofetra *et al.* (1989). In contrast, Abdelmelek *et al.* (2000) found that the DA levels and serotonin (5-HT) were lowered by the age in the cervical region and whole spinal cord of Muscovy duckling, respectively. The gradual increase in normal monoamines in the present investigation may be due to the increase in differentiation and maturation of neurons with age progress (Ahmed, 2004). The progressive increase in monoamine concentrations in the investigated rat CNS regions may also reflect the increase in sympathetic activity with age progress.

In the present study, the exposure to high temperature produced marked decrease of NE level at all the studied ages in cerebrum and spinal cord. Otherwise, in the cerebellum and medulla oblongata, the NE level exhibited mild increase at days 7 and 14, but showed a profound depletion at later ages if compared with normal values. These results go parallel with other investigators as a following; 1) Kregel *et al.* (1988 and 1993) recorded that the severe hyperthermia caused exhaustion of noradrenergic activity, which is increased during physiological heat stress; 2) Yuan *et al.* (1989) found that the exposure of mice to 45°C for 15 min reduced the brain content of NE; 3) the NE content in the basal hypothalamus was found by Merritt *et al.* (1977) to be reduced in concomitant with microwave-induced brain hyperthermia and 4). Bliss *et al.* (1968) and Abdel Hamid *et al.* (1994) reported that a variety of acute stresses (food, shock, cold, anoxia, aggregation and radiation) caused a decrease in the level of NE in the brains of mice, rats, guinea pigs, dogs, rabbits and monkeys.

In the present investigation, the epinephrine level was increased in the studied regions at day 7 except in medulla oblongata due to heat stress. Myers and Chinn (1973) and Yaksh and Myers (1972) recorded that when rats, cats and monkeys exposed to heat, the secretion and turnover of catecholamines are increased. The present epinephrine level decreased deleteriously in all the studied regions of the central nervous system of 14 and 28 days old rats due to high temperature exposure. Similar observation was also reported by Abdel Hamid *et al.* (1994) who noticed a reduction in the level of epinephrine in different brain parts of rats exposed to physical stress factor (radiation).

The dopamine (DA) level, which is an intermediate in the synthesis of the NE and E, was also decreased vigorously in the CNS regions at all ages as a result of hyperthermia. The only exception being recorded in the medulla oblongata at day 7 where the value recorded was increased as compared with the normal one. The decrease in DA level may be attributed to a fall in the release of DA from brain synaptosomes (Shouman, 1989). Moreover, as recorded by Merritt *et al.* (1977), DA content of the basal hypothalamus is reduced as a result of microwave-induced brain hyperthermia. In contrast, Tor-Agbidye *et al.* (2001) found that hyperthermia alone markedly potentiated DA release and hyperthermia together with seizure activity potentiated the increases in extracellular DA levels in the amygdala during exposure to d-amphetamine. The present results are also in disagreement with Yuan *et al.* (1989) who reported that brain DA content was unchanged in mice exposed to 45°C for 15 min. Also, Dib *et al.* (1991) showed that the catecholamine content in the plexuses remained unchanged during hyperthermia and emotional stress. These latter observations were supported by the results of Rauschenbach *et al.* (1997) who found out that neither DDC (DOPA decarboxylase, the enzyme responsible for dopamine synthesis) nor NAT (N-acetyltransferase, the enzyme responsible for dopamine degradation) activity changed under short-term heat stress. Also, Sukhanova *et al.* (1997) revealed that tyrosine decarboxylase and DOPA decarboxylase activities were unchangeable under short-term heat stress. On the other hand, Zhoa *et al.* (2001) noticed that striatum dopamine content increased significantly in male Wistar rats placed in small hot chambers till rectal temperature reached 41 to 43°C. Such discrepancies in the previous reports may be attributed to the difference of the animal species, the degree of hyperthermia, the exposure duration and the studied brain regions.

In the present study, the treated rats also showed marked decrease of the serotonin (5-HT) levels in all the studied CNS regions between days 7 and 28, but it was only increased in cerebrum at day 7 as compared to the corresponding normal controls. Concomitant with the present study, Yuan *et al.*



(1989) found that when mice exposed to 45°C for 15 min, the content of brain 5-HT was markedly reduced. Also, Mohamed and Rahman (1982) revealed marked decrease of 5-HT content following the second heat dose in various brain regions of *Gerbillus pyramidum*. In contrast, Merritt *et al.* (1977) noticed that serotonin content of the basal hypothalamus is not reduced as a result of microwave-induced brain hyperthermia. This latter inconsistency with our results may be attributed to the difference of the animal species, the degree of hyperthermia, the exposure duration, the method of hyperthermia induction and the exposed brain areas.

The general decrease of monoamine (MA) concentrations in the present treated rats, in various regions of the CNS may be attributed to the increase of the degradative enzyme, monoamine oxidase activity (Ma *et al.*, 2004) which was increased by heat stress as indicated by Aly *et al.* (1986) in cerebrum, cerebellum, midbrain and pons plus medulla of the *Gerbillus pyramidum*. The decrease may be also attributed to the reduced activity or the suppression of *de novo*-synthesis of enzymes involved in the formation of MA. This assumption was supported by the findings of Miller and Callaghan (1995), Che *et al.* (1995) and Fleckenstein (1997) who found that drug-induced hyperthermia decreased the activity of tyrosine hydroxylase and tryptophan hydroxylase, the key enzymes responsible for catecholamines and serotonin formation. Another possible explanation that the depletion of MA content in the CNS regions may be due to the histological lesions like gliosis, oedema, vacuolation and necrosis and delay in the neurogenesis and dendritic arborization development as a result of heat stress (Britt *et al.*, 1984; Lackovic *et al.*, 1988; Sminia *et al.*, 1989; Lundgren *et al.*, 1994; Ahmed, 2004).

The present monoamines concentration increases at day 7 in some CNS regions after exposure may indicate that the high temperature may act as stimulant at that age. This latter evidence was supported by Hardebo and Hindfelt (1981) who found a transient rise in transmitter monoamine levels of cerebrospinal fluid occurs in fever. Moreover, Sharma *et al.* (1992) found that after exposure of young rats to 4 h heat stress at 38°C in a biological oxygen demand incubator, there was a profound increase in the levels of 5-HT in brain.

An important aspect in this study on Central Nervous System (CNS) of rats is the measurement of the cholinesterase (chE) activity. In the present results, the cholinesterase activity increased enormously with the age progress in all examined CNS regions. These results coincide with several publications. Choline acetylase and acetylcholinesterase activities have been found to increase with age in the developing brain of rabbit (McCaman and Aprison, 1964), chick (Marchisia and Giacobini, 1969; Iqbal and Talwar, 1971) and rat (Maletta and Timiras, 1966; Singh and McGeer, 1977). The acetylcholinesterase and butyrylcholinesterase enzyme increases mostly postnatally in rat brain, but in human brain, acetylcholinesterase reaches a maximum activity at birth (Muller *et al.*, 1985).

It was reported that changes in acetylcholine content could be produced by change of temperature (Lagerspetz, 1974; Kornynshenko, 1976; Aly *et al.*, 1986) as well as other stress factors (Artemenko, 1967). The chE activity, which control the acetylcholine content and cholinergic activity was markedly decreased as a result of heat exposure in most cases in all the studied CNS regions. The withdrawal of high temperature exposure in this study for 7 days failed to return the altered enzyme activity to normal level. These results are in agreement with Aly *et al.* (1986) who found that heat stress provoked a decrease in the chE activity of the cerebrum region of the gerbil (*Gerbillus pyramidum*). Contrary to these observations, Menon and Dandiya (1969) reported that the activity of chE was significantly increased in the brain of rats kept at a higher ambient temperature (40°C). So, the heat stress may cause the disturbance in the cholinergic functions. These changes may, in turn, cause impairment in the development of neurons, oligodendrocytes and the tissues of the CNS (Rao *et al.*, 1990).

In conclusion, the heat stress at 40±1°C deleteriously affected the monoamine content and cholinesterase activity in the examined CNS regions and the withdrawal for 1 week beyond day 21 failed to return these perturbations to normal values.

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