ESTROGEN EFFECTS ON THE LIPID PROFILES OF CEREBRAL CORTEX, CEREBELLUM, BRAIN STEM AND SPINAL CORD

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ABSTRACT

Brain lipids were estimated after the intramuscular administration of 100 μg estrogen daily for 30 days to each female rabbit. Significantly depleted levels of free fatty acids, esterified fatty acids and triglyceride were observed in the cerebral cortex and cerebellum. Also, a marked alteration in triglyceride level was found in brain stem and spinal cord. Cholesterol level was lowered in brain stem and spinal cord, but it was elevated in cerebellum. On the otherhand, gangliosides were decreased in cerebellum and spinal cord, but an increment in their level was discernible in the cerebral cortex and brain stem. Cholesterol/phospholipid ratio was increased in cerebellum, but decreased in brain stem and spinal cord. The results suggest that the estrogen affects the lipid content differentially in the various parts of the brain.

INTRODUCTION

NIELSEN[1] has reported that it was mainly the estrogen component of the contraceptive that caused changes in lipid metabolism, particularly increased triglyceride concentration. Also, Islam *et al*[2] have observed an increased concentration of triglyceride in liver, heart, ovary, uterus and adrenals after intramuscular administration of 100 μg ethinylestradiol to each female rabbit daily for 30 days. In the same study they reported significant alteration in levels of cholesterol, free fatty acids and esterified fatty acids in these organs. There is no previous report specifying the effects of estrogen on the lipid levels of the discrete areas of the central nervous tissue. The main aim of the presnt study is to provide data pertaining to the effect of estrogen on regional brain total lipids, phospholipids, cholesterol, triglyceride, free fatty acids, esterified fatty acids, gangliosides and cholesterol/phospholipid (C/P) ratio.

MATERIALS AND METHODS

Twelve adult female albino rabbits of average weight 1.5 kg were used for this study. All the animals were allowed free access to pellet diet (Hindustan Lever, India) and water. They were divided into two groups, each having 6 animals. 17α-Ethinylestradiol (100 μg/0.1 ml peanut oil/day) was injected intramuscularly (i.m.) daily for 30 days, to each rabbit of the experimental group. The control animals were injected with identical volume of peanut oil to obviate any effects of the vehicle. Overnight fasted animals were anesthetized with ether and the brains were rapidly removed and cleaned of adhering blood in an ice-cooled petridish. Cerebral cortex, cerebellum, brain stem and spinal cord were dissected out. Different parts of the brain and spinal cord were weighed and homogenized in a glass homogenizer with chloroform-methanol (2:1, v/v) according to the method of Folch *et al*[3]. Each homogenate was shaken periodically for an hour at room temperature. Further isolation of lipids was carried out as described by Islam *et al*[4]. Total lipids (TL) were estimated colorimetrically according to the method of Woodman and Price[5]. Colorimetric procedures of Fletcher[6] and Marinetti[7] were used for the determination of triglyceride (TG) and phospholipids (PL) respectively. Cholesterol (CH) was estimated according to the method of Bloor *et al*[8]. Esterified fatty acids (EFA) and gangliosides (GS) were estimated according to the methods of Stern and Shapiro[9] and Pollet *et al*[10] respectively. The method of Mosinger[11] was used for the estimation of free fatty acids (FFA). All the reagents used were of analytical grade. Estrogen (17α-Ethinylestradiol) was purchased from Sigma Chemical Co. (USA). The data were analysed using student's *t* test. Significant differences between means of treated and control groups were calculated and *p* values were obtained.

RESULTS

The data presented in table 1 show significantly depleted levels of triglyceride, esterified fatty acids and free fatty acids in cerebral cortex and cerebellum in the

Table 1 *Changes induced by estrogen in the lipid levels of cerebral cortex and cerebellum (Values expressed as mg/g fresh weight, Mean ± S.E. of 6 animals)*

Lipids	Cerebral Cortex		% Change	Cerebellum		% Change
	Control	Experimental		Control	Experimental	
Total lipids	131.5 ± 5.1	112.7 ± 2.3	−14.3	106.3 ± 6.1	117.5 ± 8.1	+10.5
Phospholipids	54.6 ± 1.1	52.6 ± 2.5	− 3.6	51.1 ± 1.6	55.0 ± 2.9	+ 7.6
Cholesterol	13.2 ± 0.5	12.7 ± 0.8	− 3.8	12.1 ± 0.3	17.8 ± 1.9[c]	+47.1
Triglyceride	15.6 ± 0.9	12.2 ± 0.3[a]	−21.8	15.2 ± 0.9	11.9 ± 0.4[b]	−21.7
Free Fatty Acids	5.8 ± 0.5	3.6 ± 0.1[b]	−37.9	5.2 ± 0.2	4.0 ± 0.2[b]	−23.1
Esterified*						
Fatty Acids	0.22 ± 0.01	0.15 ± 0.01[a]	−31.8	0.20 ± 0.01	0.17 ± 0.01[a]	−15.0
Gangliosides	0.71 ± 0.03	0.87 ± 0.05[d]	+22.5	0.62 ± 0.02	0.51 ± 0.03[d]	−17.7
C/P Ratio	0.24 ± 0.02	0.24 ± 0.02	Nil	0.24 ± 0.01	0.32 ± 0.02[d]	+33.3

Values: $^{a}P < 0.05$, $^{b}P < 0.02$, $^{c}P < 0.01$, $^{d}P < 0.001$.
* Expressed as meq/g fresh wt.

experimental group. Gangliosides decreased in the cerebellum but increased in the cerebral cortex. Interestingly, cholesterol concentration was found to be raised in the cerebellum. No marked alterations in the concentration of triglycerides and phospholipids were observed. However, C/P ratio was significantly increased in the cerebellum.

Table 2 shows marked decrement of cholesterol in the brain stem and spinal cord. The content of triglyceride was decreased in brain stem but increased in spinal cord. On the other hand, gangliosides were elevated in the brain stem but depleted in spinal cord.

DISCUSSION

The results presented in tables 1, 2 revealed an increment of total lipids and phospholipids in cerebellum, brain stem and spinal cord, after the estrogen administration. But their concentrations were decreased in the cerebral cortex. There is no general agreement among different investigators regarding the effects of steroid contraceptives on the brain total lipid and phospholipid levels. Islam *et al*[2] from this laboratory, have reported a decreased concentration of total lipids in uterus and phospholipids in liver, heart, ovary and adrenals following 100 μg ethinylestradiol administration to female rabbits. In the same study, Islam *et al*[2] have also reported an increment of the total lipids in the ovary and adrenals. After the administration of estradiol benzoate to rats, Fewster *et al*[12] found a reduction in the level of phospholipids in the plasma and heart, but its level was increased in the liver. An indirect support to this finding is available in the reported increase of serum lipids after estrogen administration[13].

There are variable reports regarding the effects of

Table 2 *Estrogen induced changes in the lipid levels of brain stem and spinal cord (Values expressed as mg/g fresh weight, Mean ± S.E. of 6 animals)*

Lipids	Brain stem		% Change	Spinal cord		% Change
	Control	Experimental		Control	Experimental	
Total lipids	180.3 ± 11.7	232.4 ± 15.5	+28.9	270.2 ± 12.4	307.9 ± 21.7	+13.9
Phospholipids	73.2 ± 3.6	79.4 ± 5.3	+ 8.4	103.4 ± 4.7	117.9 ± 3.3	+14.0
Cholesterol	27.4 ± 1.6	21.5 ± 0.8[a]	−21.5	42.0 ± 2.1	30.8 ± 1.8[c]	−26.6
Triglyceride	26.4 ± 0.5	19.9 ± 0.2[c]	−24.6	11.3 ± 0.4	15.6 ± 1.8[b]	+38.0
Free Fatty Acids	7.6 ± 0.3	7.1 ± 0.3	− 6.6	12.1 ± 0.7	11.0 ± 0.4	− 9.1
Esterified*						
Fatty Acids	0.28 ± 0.04	0.26 ± 0.03	− 7.1	0.30 ± 0.02	0.29 ± 0.04	− 3.3
Gangliosides	0.40 ± 0.03	0.51 ± 0.03[c]	+27.5	0.24 ± 0.05	0.20 ± 0.01[a]	−16.6
C/P Ratio	0.37 ± 0.02	0.27 ± 0.02[c]	−27.0	0.41 ± 0.03	0.26 ± 0.02[c]	−36.6

Values: $^{a}P < 0.05$, $^{b}P < 0.02$, $^{c}P < 0.001$.
* Expressed as meq/g fresh wt.

estrogens on serum cholesterol[14]. Most investigators believe that estrogen lowers plasma cholesterol or that its effect is highly variable because of its divergent action on cholesterol content in the alpha and beta lipoprotein fractions. An earlier report from this laboratory[4] has shown a significantly increased level of the cholesterol in the hypothalamus and also its decrement in gyrus cinguli after the i.m. administration of Primovlar to female rabbits. In the present study, the level of cholesterol showed regional heterogeneity in exhibiting a depletion in the brain stem and spinal cord but elevation in the cerebellum and cerebral cortex.

Ramsey and Nicholas[15] have suggested that gangliosides are involved in nerve impulse conduction. Also, the binding of serotonin to synaptic vesicles may be mediated by gangliosides. According to Irwin and Samson[16] certain types of behavioral stimulation seem to be accompanied by alteration of ganglioside metabolism, compared to corresponding control animals. In the present study gangliosides were increased in cerebral cortex and brain stem, but reduced in the cerebellum and spinal cord. This shows a differential effect of estrogen on the regional brain gangliosides.

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NEWS

RESEARCH QUALITY *VS.* AGE OF RESEARCHER?

. . . "Scientific productivity is constant as a scientist ages according to recent studies relying mainly on quantity measures of productivity. An economic model of the life-cycle productivity of scientists is presented which implies that the number of citations made to a scientist's previous work will decline with age. The implication could be consistent with the finding of constant quantity of output with age if the decline in quality (as measured by number of citations per article) is large enough." (Reproduced with permission from *Press Digest, Current Contents*®, No. 37, September 10, 1984, p. 11, Copyright by the Institute for Scientific Information®, Philadelphia, PA, USA.)