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EFFECT OF THE CHRONIC ADMINISTRATION OF ETHINYL ESTRADIOL AND NORGESTREL ON BIOGENIC AMINE(S) LEVEL AND MONOAMINE OXIDASE ENZYME ACTIVITY IN RAT BRAIN

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Abstract—Adult female virgin rats were administered contraceptive steroids orally for a long period, and the effect of this on the brain biogenic amine(s) content was studied. The amine(s) content of the brain during the course of chronic treatment with the hormones was estimated at various time intervals. In addition to this, the noradrenaline (NA) and 5-hydroxytryptamine (5-HT) content of rat brain regions was assayed after three months of hormone treatment. The onset of the brain amine depletion was observed by the end of the second month of hormone administration. The amine(s) depleting action was found to be due to the norgestrel component of the pill. Studies of the regional distribution of amine(s) showed that the depletion was more prominent in the hypothalamus and pons/medulla oblongata. The assay of the monoamine oxidase enzyme did not reveal any significant change, though the estrogenic and progestogenic hormones were found to have opposing effects on the enzyme levels.

The putative neurotransmitters of the brain have been implicated with the action of gonadal hormones in regulating the secretion of pituitary hormones by hypothalamic neuroendocrine transducer cells [1–5]. Ulrich *et al.* [6] observed elevated levels of both noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in medial hypothalamus of neonatal rats after administering cortisone. Giuilian *et al.* [7] reported that testosterone propionate administration in neonatal rats increased the brain 5-HT content, whereas the administration of female steroidal hormone had an opposite effect. Thus, there are reports to indicate the possible involvement of both catecholaminergic and 5-hydroxytryptaminergic neuronal systems in the neuroendocrine transducer function of the hypothalamus [3,6,7].

Apart from having the neuroendocrine transducer function in the central nervous system (CNS), these putative neurotransmitters have been implicated in the pathogenesis of depressive states [8]. The reported cyclic changes in the brain monoamine content during the estrous cycle in the mouse [9], and also the observed variation in the monoamine oxidase (MAO) activity in the hypothalamus of the rats during the estrous cycle [10] are suggestive of the possibility of the female gonadal hormones affecting the biogenic amine(s) metabolism in the brain. As there are reports to indicate that women taking oral contraceptives for long periods do experience a state of depression [11–13], it is of interest to look into the metabolic status of biogenic amines in rat brain after the chronic administration of contraceptive steroids. The present report deals with our findings on biogenic amine(s) level, as well as the MAO activity in rat brain after chronic administration of the hormones.

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METHODS AND MATERIALS

Adult female albino rats (bred at Haffkine Institute) weighing between 150 and 160 g were used in these experiments. They were maintained in groups of 6 animals each and provided with pellet food (supplied by Hindustan Lever Ltd., Bombay, India) and tap water *ad lib*. There were four groups, the control receiving olive oil as a vehicle and the experimental groups receiving either ethinyl estradiol (1 μg/kg body wt) alone, norgestrel (10 μg/kg body wt) alone, or a combination of both ethinyl estradiol (1 μg/kg body wt) and norgestrel (10 μg/kg body wt) in olive oil. The animals were killed after the scheduled course of hormone administration and the biogenic amine(s) content as well as the monoamine oxidase enzyme activity was assayed.

Tissue sampling and extraction for amine assay. In all the experiments, the animals were sacrificed between 10.00 and 11.00 a.m. to avoid possible fluctuation in the amine content of the brain because of diurnal variation. Tissue sampling was carried out by decapitation and quick dissection involving the opening of calvarium followed by the removal and freezing of the whole brain in methanol maintained at −80° by means of dry CO_2 solidic/acetone mixture. Dissection of brain regions was carried out in a semifrozen state over a glass plate mounted on a piece of dry CO_2 as per Zeman and Maitland Innes [14]. The extraction and further processing of the extract for biogenic amine(s) assay was carried out essentially by the method described by Shellenberger and Gordon [15].

Spectrofluorometric assay of biogenic amines. The adsorption and elution of the catecholamines on alumina as well as further processing for 5-HT assay was carried out according to Shellenberger and Gordon [15]. The fluorophore development of dopamine and noradrenaline was done by the trihydroxyindole

method of Laverty and Taylor [16]. The fluorescence of dopamine was read at 375 nm after excitation at 313 nm in a Carl-Zeiss spectrophotofluorometer. The fluorescence of noradrenaline was measured at 485 nm after excitation at 365 nm.

The fluorophore of 5-HT was developed by the ninhydrin condensation method as described by Ansell and Beeson [17], and the fluorescence was measured at 485 nm after excitation at 365 nm.

Crude enzyme preparation of monoamine oxidase (MAO :EC 1.4.3.4). Rats treated with the contraceptive steroids for three months were decapitated and the whole brain was dissected out and made free of blood with cold saline. The tissues were homogenized with 0.25 % v/v Triton X-100 solution at 4° in a teflon coated glass homogenizer for 3 min at 2500 r.p.m. with 15 strokes per min. The homogenate was centrifuged for 15 min at 700 *g* in a cold centrifuge. The supernatant was used as crude enzyme.

Assay of monoamine oxidase (MAO: EC 1.4.3.4). The enzyme assay was based on measuring the rate of disappearance of substrate (kynuramine) in the reaction mixture by measuring the absorption maximum at 360 nm in a Carl-Zeiss spectrophotometer as described by Weissbach *et al.* [18].

The protein content of the enzyme solution was estimated by the method of Lowry *et al.* [19].

RESULTS

The administration of hormones for three months resulted in an overall depletion of brain biogenic amine(s) content. As shown in Table 1, the first month of hormone treatment did not result in any changes in biogenic amine content. However, the depletion in the brain amine content was evident in animals treated with steroids for two months or more.

Effect on catecholamine content. During the first month of hormone treatment, both ethinyl estradiol and norgestrel, when administered alone or in combination, did not affect the dopamine content of the brain. However, by the end of the second month of hormone treatment, the animals treated with the combination of the steroids showed decreased levels of dopamine content (5.07 ± 0.08 nmoles/g; $P<0.05$) as compared to the control value (5.39 ± 0.07 nmoles/g) of the same batch. On the other hand, the animals treated with ethinyl estradiol (5.46 ± 0.10 nmoles/g) and norgestrel (5.21 ± 0.67 nmoles/g) did not show any change in dopamine content of the brain. It is of interest to note that further continuation of norgestrel alone for the third consecutive month resulted in a significant depletion ($P<0.05$) of brain dopamine content (5.15 ± 0.05 nmoles/g) as compared to the control group (5.60 ± 0.07 nmoles/g). Though ethinyl estradiol administration by itself did not affect the brain dopamine content, it seems to act synergistically with norgestrel to deplete DA content as there was a significant decrease in DA content of the animals treated with the combination of both the steroids (4.99 ± 0.10 nmoles/g; $P<0.01$).

The profile of the brain NA content of hormone treated animals followed the same pattern as that of dopamine. As shown in Table 1, the first month of hormone administration did not produce any change in the brain NA content. By the end of the second month, the administration of norgestrel alone (1.87 ± 0.12 nmoles/g) or in combination with the ethinyl estradiol (2.07 ± 0.05 nmoles/g) caused a marked depletion in brain NA content ($P<0.05$ and $P<0.01$, respectively), whereas ethinyl estradiol when administered alone did not cause any significant change in the amine content (2.44 ± 0.12 nmoles/g) as compared to the control group 2.49 ± 0.13 nmoles/g). Further continuation of the ethinyl estradiol alone for the third month also did not affect the brain noradrenaline content, whereas the administration of norgestrel alone (1.90 ± 0.07 nmoles/g) or in combination with ethinyl estradiol (2.06 ± 0.05 nmoles/g) resulted in a significant ($P<0.01$) depletion of brain NA content.

Regionwise study of the NA content in hormone treated animals showed that the cerebellum is the least affected (Table 2), whereas the cerebral cortex showed a slight, though not significant, increase in NA content in all the groups of hormone treated animals. Norgestrel, when administered alone, was found to have a slight NA depleting effect on pons/medulla oblongata (3 per cent less), while the

Table 1. Effect of contraceptive steroids on biogenic amine(s) level of rat brain at various time intervals during the chronic administration of the hormone(s)*

Hormone administration	Biogenic amine(s)	Control	Eth-estradiol	Norgestrel	Combination
First month	Dopamine	5.33 ± 0.13	5.31 ± 0.09	5.33 ± 0.12	5.55 ± 1.04
	Noradrenaline	2.58 ± 0.10	2.54 ± 0.12	2.59 ± 0.07	2.57 ± 0.07
	5-Hydroxytryptamine	2.00 ± 0.04	2.14 ± 0.04	2.21 ± 0.06	2.02 ± 0.04
Second month	Dopamine	5.39 ± 0.07	5.46 ± 0.10	5.21 ± 0.07	5.07 ± 0.08†
	Noradrenaline	2.49 ± 0.13	2.44 ± 0.12	1.87 ± 0.12‡	2.07 ± 0.05†
	5-Hydroxytryptamine	2.22 ± 0.11	2.16 ± 0.06	2.03 ± 0.07	1.88 ± 0.04‡
Third month	Dopamine	5.60 ± 0.07	5.47 ± 0.01	5.14 ± 0.05†	4.98 ± 0.10‡
	Noradrenaline	2.44 ± 0.07	2.48 ± 0.08	1.90 ± 0.07‡	2.06 ± 0.05‡
	5-Hydroxytryptamine	2.28 ± 0.05	2.20 ± 0.04	2.11 ± 0.04†	1.97 ± 0.03‡

* Amine(s) values are expressed as nmoles/g tissue ± S.E.M.
† $P<0.05$.
‡ $P<0.01$.

Table 2. Noradrenaline content of rat brain regions after chronic administration (three months) of hormone(s)*

Region	Control	Eth-Estradiol	Norgestrel	Combination
Cerebral cortex	1.66 ± 0.11	1.72 ± 0.08	1.76 ± 0.12	1.83 ± 0.11
Cerebellum	1.28 ± 0.05	1.25 ± 0.06	12.6 ± 0.06	1.23 ± 0.07
Pons/med. oblongata	2.89 ± 0.07	2.83 ± 0.09	2.78 ± 0.08	2.66 ± 0.08
Hypothalamic region	7.21 ± 0.32	6.89 ± 0.26	6.55 ± 0.21†	6.38 ± 0.23

* Amine values expressed as nmoles/g tissue ± S.E.M.
† $P<0.05$.

presence of ethinyl estradiol seems to have a synergistic effect on the NA depleting action of norgestrel as the NA content of the combination group was found to be further decreased (7 per cent less) as compared to the control.

The hypothalamic region was the most affected as far as the NA depletion was concerned. The animals treated with the combination of steroids showed a significant decrease ($P<0.01$) in the NA content of this region. However, there was no significant change in NA content of other groups of animals.

Effect on 5-hydroxytryptamine content. Contraceptive steroids administration also had a similar effect on 5-HT content. Hormone treated animals did not show any change in the brain 5-HT level during the first month of steroid administration. By the end of the second month a significant decrease in the brain 5-HT content ($P<0.05$) was observed in animals treated with the combination of steroids. The administration of the individual hormones alone did not bring about any change in the 5-HT level of the brain. However, by the end of third month, the norgestrel alone could decrease the brain 5-HT content (2.11 ± 0.04 nmoles/g; $P<0.05$), whereas ethinyl estradiol did not affect the amine content even after its administration for the third consecutive month.

As shown in Table 3, regionwise 5-HT content of hormone treated animals did not show any significant changes in cerebellum and cerebral cortex of the brain. Norgestrel, when administered alone, was found to have resulted in a significant depletion of amine in pons/medulla oblongata (2.9 ± 0.04 nmoles/g; $P<0.05$), while in combination with ethinyl estradiol, the 5-HT content of this region was found to be further depleted (2.81 ± 0.07 nmoles/g; $P<0.01$) as compared to the control (3.15 ± 0.04 nmoles/g). Ethinyl estradiol administration as such did not have any effect on 5-HT content of pons/medulla oblongata, whereas when administered alone ethinyl estradiol was found to have depleted the 5-HT content of the hypothalamic region of the brain ($P<0.05$) as compared to the control value (4.85 ± 0.09 nmoles/g). The 5-HT depleting action of norgestrel was more marked in the hypothalamic region ($P<0.01$). The combination of steroids resulted in a further depletion of 5-HT in the hypothalamic region of the brain.

Effect on MAO (EC: 1.4.3.4) enzyme activity. A wide variation in individual values of MAO activity was observed in control as well as hormone treated animals. The control group of animals showed an average enzyme activity of 121.25 ± 5.45 units/mg protein. Ethinyl estradiol treatment was found to have resulted in an overall 11 per cent decrease in enzyme activity (108.20 ± 3.53 units/mg protein), whereas the norgestrel treated group showed a tendency to increased (7 per cent more) enzyme activity (130.08 ± 3.68 units/mg protein) as compared to the control. At the same time, the animals treated with the combination of steroids did not show any change in the enzyme activity (119.12 ± 5.64 units/mg protein).

DISCUSSION

There is a lot of evidence to indicate the involvement of brain neurotransmitters in mediating the neuroendocrine transducer function of the hypothalamus [3–5]. Apart from all this, the neurotransmitters of the brain have drawn the attention of psychopharmacologists regarding their role in the pathophysiology of psychological disorders [8, 20–22]. In the present context, the incidence of depression and other kinds of psychotic disorders among

Table 3. 5-HT content of rat brain regions after chronic administration (three months) of hormones*

Region	Control	Eth-Estradiol	Norgestrel	Combination
Cerebral cortex	2.33 ± 0.03	2.29 ± 0.13	2.25 ± 0.09	2.21 ± 0.05
Cerebellum	1.08 ± 0.05	1.04 ± 0.05	1.00 ± 0.02	1.01 ± 0.02
Pons/med. oblongata	3.15 ± 0.05	3.05 ± 0.04	2.90 ± 0.04†	2.81 ± 0.07‡
Hypothalamic region	4.85 ± 0.04	4.46 ± 0.11†	4.31 ± 0.12‡	4.29 ± 0.03‡

* Amine value expressed as nmoles/g of tissue ± S.E.M.
† $P<0.01$.
‡ $P<0.05$

users of the contraceptive pill [11–13], raises the question regarding the role of these hormones in the genesis of psychotic disorders.

Present findings indicate that the chronic administration of contraceptive steroids results in the depletion of both catecholamines (DA and NA) and the 5-HT content of the brain. These changes were seen during the second month of hormone treatment, and became more marked by the end of the third month. The amine depleting action of the steroid hormones was found to be due to the progestogenic component (norgestrel) of the pill. Ethinyl estradiol when administered alone did not have any effect on brain biogenic amine(s) content even after its administration for three consecutive months. It is also probable that the differential dose of the estrogenic (1 μg/kg body wt) and progestogenic (10 μg/kg body wt) hormones might be the reason for their difference of action on biogenic amine(s) level of the brain. (It may be mentioned here that this particular dose of hormone for animal administration was selected in keeping with the formulation used in the oral contraceptive preparations.) This is more so because, although ethinyl estradiol by itself did not affect the amine(s) content of the brain to any extent, it seems to have a synergistic action on the amine(s) depleting action of norgestrel as evidenced by the further decrease in the amine(s) content in animals receiving the combination of steroids.

Some interesting findings were observed in study of the biogenic amine(s) levels in brain regions in hormone treated animals. Hormone treatment did not affect the NA and 5-HT content of cerebellum and cerebral cortex. In fact, the hormone treatment resulted in a slight, though not significant, increase in NA content of the cerebral cortex. Pons/medulla oblongata and hypothalamic region showed a marked depletion in both NA and 5-HT levels. These regions of the brain demand a special attention because of their richness in both noradrenergic and 5-hydroxytryptaminergic neurones [23–25], as well as their being the seat of neuroendocrine transducer function because of the concentrated localization of the receptors for gonadal hormones [26, 27]. A significant decrease ($P<0.05$) in NA content of hypothalamus of the animals treated with the combination of steroids was observed, while the individual hormones did not affect the NA level of this region of the brain. Interestingly, both the hormones administered individually or in combination showed a marked 5-HT depletion of the hypothalamic region (see Table 3). While ethinyl estradiol alone did not have any effect on whole brain 5-HT content, it had a significant 5-HT depleting action on the hypothalamic part of the brain. It seems to be due to the high affinity of the hormones to the 5-hydroxytryptaminergic rich region of the hypothalamus which suggests the role of 5-HT in regulating the reproductive cycle. At least in rats, the ovulation seems to be controlled by 5HT and NA. This has been demonstrated by administering 5-HT antagonists such as cyproheptadine to rats, which inhibited the secretion of luteinising hormone (LH) and subsequently ovulation [28]. Droua and Gollo [29] demonstrated the involvement of hypothalamic NA in the episodic release of LH, and NA has been suggested to play an excitatory role in the neural events controlling the release of LH [30–32]. Norgestrel alone as well as in combination with ethinyl estradiol had a marked 5-HT depleting action on the hypothalamic region.

The studies involving the assay of monoamine oxidase enzyme did not help in arriving at any convincing answer that may help in explaining the possible mechanism of alteration in the amine(s) content of the brain. This is mainly because of the wide variation in the individual values of the control group. Although there is no significant difference in the MAO activity of the different hormone treated animals, the antagonistic action of estrogenic and progestogenic components of the pill hormones could be very well appreciated. Though there is only an 11 per cent decrease in the enzyme activity in the ethinyl estradiol treated group, the reverse was the case with the animals treated with norgestrel alone, whereas the combination group showed an average enzyme activity the same as that of the control. With these findings, it is rather premature to conclude by measuring the gross MAO activity by using a single substrate. This is because there are reports to indicate that steroidal hormones of different kinds differentially affect the isoenzymes of multiple forms of MAO enzymes [33, 34]. Moreover, Southgate [35] reported that uterine MAO activity of progesterone treated rats was found to be more enhanced when dopamine was used as a substrate than when benzylamine or tyramine were used. On the other hand, estradiol treatment seemed to be without effect when dopamine used as substrate, whereas by using benzylamine the enzyme activity was found to be decreased.

It is possible that the biogenic amine(s) depletion in brain as observed in the present study may be due to a decrease in biosynthesis or the reuptake of the amine(s).

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