ORIGINAL ARTICLE

EFFECTS OF HIGH DOSES OF CONTRACEPTIVE DRUGS AND THEIR HORMONAL COMPONENTS ON BRAIN MONOAMINES AND PHYSIOLOGICAL PARAMETERS IN RATS

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SUMMARY

Brain monoamine (5-hydroxytryptamine, 5-HT; dopamine, DA; norepinephrine, NE) concentrations and several physiological parameters were determined in normal diestrous control female rats, ovariectomized rats and female rats treated with high doses of contraceptive drugs or sex steroids for 14 days. Progesterone alone (8 mg/kg/d) or estrogen (0.4 mg/kg/d) plus progesterone (8 mg/kg/d) caused a significant increase in the brain NE concentration. High dose of Neogynon (ethinyl estradiol 20 µg/kg/d, d-norgestrel 100 µg/kg/d) caused a significant decrease in the brain 5-HT but not in the brain DA and NE. There was no significant alteration in the brain 5-HT, DA and NE concentration by Microgynon 30 (12 µg/kg/d ethinyl estradiol, 60 µg/kg/d d-norgestrel) The uterine weights were reduced in ovariectomized rats. The ovarian and uterine weights in the ovary-intact rats were increased by high doses of estrogen or estrogen plus progesterone treatment but not with progesterone alone. Neogynon in high dose increased the uterine weight but not the ovarian weight. On the other hand, Depo-provera (60 mg/kg, I.M. single dose) decreased the ovarian weight but not the uterine weight. Combined treatment of estrogen plus progesterone caused significant decrease in rat hematocrit. Ovariectomy for 2 weeks caused significant decrease in the brain protein concentration. Body weight gain was reduced by high doses of estrogen plus progesterone administration but not by other contraceptive drugs used. High dose of estrogen induced mostly estrous appearance of the vaginal smear. Depo-provera, progesterone and Neogynon induced mostly diestrous whereas Microgynon-30 induced mostly metestrous appearance. Effects of treatments used may be both direct and indirect by modifying the hypothalamo-hypophysial-gonadal axis of the intact animals and differences in effects may be caused by differences in dosages and mechanisms of actions of various components in the drugs used.

The actions of contraceptive drugs on the hypothalamus-pituitary-gonadal axis are well documented (1-4). Many of their side effects have also been reported (4-7). In spite of the increasing use of the contraceptive drugs (8), a limited number of studies on effects of high dose contraceptive drugs as well as their steroid hormone components on brain neurotransmitters such as 5-hydroxytryptamine (5-HT) (9, 10), dopamine (DA) and norepinephrine (NE) (11) had been reported. The major goal of the present work was to compare the effects of high doses of Neogynon, Microgynon-30 and Depo-provera as well as estrogen and progesterone on the levels of brain neurotransmitters and on several physiological parameters in female rats.

MATERIALS AND METHODS

A. Animals and chemicals:

Female Fischer rats about 45-55 days of age (100-130 g) were used. O-Phthaldehyde, Folin reagent and serotonin creatinine sulfate were purchased from Sigma Chemical Co., U.S.A. Dopamine hydrochloride and l-norepinephrine bitartrate were from Pfaltz & Bauer, N.Y. Neogynon and Microgynon were purchased from Schering Co., Thailand and Depo-provera was from Korea Upjohn Ltd. Estradiol benzoate was from Nutrition Biochemicals Corp.,Ohio, and progesterone was from Sigma Chemical Co.

B. Experimental Designs

At the onset of the drugs treatments only diestrous rats were used. They were divided into ten groups, 6-8 animals per group, namely:

Group 1: Ovariectomized rats. Rats were ovariectomized using procedure described by Zarrow et al (12) and they were used in measurements 14 days later.

Group 2: Sham operated control for group 1. They were used in measurements 14 days later.

Group 3: Original diestrous rats were fed daily via stomach tube for 14 consecutive days with Neogynon at the dose equivalent to 20 times of that used in human (see the dosage in the result).

Group 4: Original diestrous rats were fed daily via stomach tube for 14 consecutive days with Microgynon 30 at the dose equivalent to 20 times of that used in human (see dosage in the result).

Group 5: Original diestrous rats were injected once with Depo-provera (I.M.) which is equivqlent to 20 times of that used in human (see dosage in the result)

Group 6: Diestrous control rats for group 3-5. They were fed daily via stomach tube for 14 consecutive days with distilled water equal in volume to that in group 3-4.

Group 7: Diestrous rats were injected (s.c.) daily for 14 days with 0.4 mg estradiol benzoate/kg/d which was equal in effectiveness to ethinyl estradiol in group 3 (see reference 13 for equipotent doses).

Group 8: Diestrous rats were injected (s.c.) daily for 14 days with 8 mg progesterone/kg/d which was equal in effectiveness to d-norgestrel in group 3 (see reference 13 for equipotent doses).

Group 9: Diestrous rats were injected (s.c.) daily with 0.4 mg estradiol/kg/d and 8 mg progesterone/kg/d

Group 10: Diestrous control (for group 7-9) were injected (s.c.) daily with equal volume of alcoholic corn oil used as solvent in group 7-9.

On the 14^{th} day of treatments of group 1-10, vaginal smears were determined for the appearance stages of estrous cycle of all rats. Rats were then decapitated (9-noon), trunk blood was collected, brains, ovaries and uteri were dissected and kept on ice. Hematocrit, serum protein, body weight, ovarian and uterine weights were then determined. Brain neurotransmitters were measured using combined methods of several investigators (14-18.) Protein concentration was determined using Lowry et al's technique (19).

C. Statistical Analysis

The mean ($\bar{X}$) and standard error of the mean (S.E.M.) for each set of data were calculated. Student's t-test was employed to test the difference between the control and the treated groups. Finally, all the significantly changed data were calculated as percentage of appropriate control and shown in Table I.

RESULTS

A. Effects of Ovariectomy

No significant changes in brain 5-HT, DA and NE concentrations were observed 14 days following ovariectomy (Table I). Fourteen days following the operation, the brain weight, and body weight gain in the ovariectomized rats were the same as that in the sham controls (Table I). The brain protein concentration, but not the serum protein level, in the ovariectomized group was significantly less than in the sham controls. Furthermore, the uterine weight of the ovariectomized rats was significantly less than that of the sham controls.

B. Effects of High Doses of Contraceptive Drugs

1. Microgynon 30

As show in Table I, the levels of brain 5-HT, DA and NE concentrations in rats treated with Microgynon at doses equivalent to 20 times of that used in man (containing 12 µg ethinyl estradiol/kg/d and 60 µg d-norgestrel/kg/d) were not statistically different from the diestrous control levels.

The body weight gain, brain weight, hematocrit, brain and serum proteins of the Microgynon-treated rats were not significantly different from those of the diestrous control rats treated with distilled water (Table I). Similarly, no differences in ovarian and uterine weights of the two groups were observed.

Table I. Summary of results on effects of contraceptive drugs and their derivative components on phyiological parameters of rats. The value of appropriate control was set as 100%. Changes with numbers included are the results which have significant differences and are means ± S.E.M.

Group of rats	Body weight gain	Brain weight	Brain protein concentration	Serum protein concentration	Hematocrit	Uterine weight	Ovarian weight	Brain 5-HT concentration	Brain DA concentration	Brain NE concentration
1. Ovariectomy	-	-	↓ 69.9±3.0%	-	ND	↓ 45.5±4.6%	ND	-	-	-
2. Microgynon 30 (20x)	-	-	-	-	-	-	-	-	-	-
3. Neogynon (20x)	-	-	-	-	-	↑ 133.3±10.4%	-	↓ 84.4±4.5%	-	-
4. Depo-provera (60 mg/kg)	-	-	-	-	-	-	↓ 78.6±6.3%	-	-	-
5. Estrogen (0.4 mg/kg/d)	↓ 37.5±3.0%	-	-	-	-	↑ 202.2±12.2%	↑ 146.5±17.6%	-	-	-
6. Progesterone (8 mg/kg/d)	-	-	-	-	-	-	-	-	-	↑ 117.2±6.9%
7. Estrogen Progesterone	↓ 68.4±4.9%	-	-	-	↓ 90.0±2.0%	↑ 193.3±11.1%	↑ 152.1±7.1%	-	-	↑ 117.2±6.9%

ND = not detected., - = not significantly changed, ↓ = significant decrease
↑ = significant increase when compared to the respective controls. Microgynon 20x = ethinyl estradiol 12 µg/kg/d, d-norgestrel 60 µg/kg/d. Neogynon 20x = ethinyl estradiol 20 µg/kg/d, d-norgestrel 100 µg/kg/d.

At the end of experiment, 50%, 38%, and 12% of the Microgynon-treated rats were, respectively, in the metestrus, diestrus and estrus.

2. Neogynon

Brain DA and NE concentrations in the Neogynon-treated rats were not statistically different from the diestrous control; but Neogynon at this dose (20 µg/kg/d ethinyl estradiol and 100 µg/kg/d d-norgestrel) caused a significant decrease in brain 5-HT concentration after 14 days of treatment (Table I).

The ovarian weight (Table I), body weight gain, hematocrit, brain weight, and brain and serum proteins of the Neogynon-treated rats were not significantly different from the control group. However, the uterine weight of the drug-treated group was significantly heavier than the control, the Microgynon-treated, and the Depo-provera-treated groups.

At the end of the experiment, 12%, 63% and 25% of the Neogynon-treated rats were, respectively, in the stages of metestrus, diestrus, and estrus.

3. Depo-provera

It was found that at 14 days after a single injection of 60 mg/kg of Depo-provera, brain DA, 5-HT and NE concentrations in the drug-treated rats were not significantly different from the control levels (Table I). There was a significant decrease in ovarian weight 14 days after a single I.M. Depo-provera injection. However, no significant changes in the uterine weight, body weight gain, brain weight, hematocrit, and brain and serum protein concentrations were observed.

At the termination of this experiment, all Depo-provera treated rats were in the diestrus stage.

C. Effects of High Doses of Pure Sex Hormones

1. Estrogen

As shown in Table I, daily injections of 0.4 mg of estrogen/

kg for 14 days produced no significant changes in brain 5-HT, DA or NE concentrations. Similarly, brain weight, and brain and serum protein concentrations of the estrogen-treated rats were similar to their diestrous controls. Estrogen significantly decreased the body weight gain of the animals. The estrogen-treated rats had a significantly greater uterine and ovarian weights than did the controls.

At the termination of the experiment, 82% of the estrogen-treated rats were in the estrous stage and the rest 18% were in proestrus.

2. Progesterone

As show in Table I, injections of progesterone at the dose of 8 mg/kg/d for 14 days significantly increased the brain NE concentration above the control level but it caused no significant change on either brain 5-HT or DA concentrations. Injections of progesterone alone had no significant effect on either the uterine or ovarian weights. Furthermore, progesterone injections produced no significant alterations in body weight gain, brain weight, hematocrit, and brain and brain and serum protein concentration.

At autopsy, 50%, 33%, and 17% of the progesterone-treated rats were respectively, in the diestrus, metestrus and estrus.

3. Estrogen and Progesterone in Combination

Combined injections of estrogen with progesterone significantly increased brain NE concentration but had no marked effects on brain 5-HT and DA levels which were similar to those produced by progesterone injections alone (Table I). Contrastly, the effects on uterine and ovarian weights of the rats receiving combined injections of estrogen and progesterone were similar to those receiving estrogen alone (i.e. they significantly increased both the uterine and ovarian weights above the control levels). Moreover, combined injections of estrogen and progesterone caused significant reduction in hematocrit and body weight gain as compared to the normal control values. However,

brain weight, and brain and serum protein levels were not affected by combined injections of these two hormones.

At the end of this experiment, 50%, 17%, and 33% of rats treated with both estrogen and progesterone were, respectively, in the estrus, proestrus and metestrus.

DISCUSSION

Various effects produced by the contraceptive drugs and sex steroids observed in the present study could be both the direct effects on the body functions detected and the indirect ones, since there have been ample evidence supporting that the hypothalamo-hypophysial-gonadal axis can be modified by some of these drugs. For example, it has been shown in human that ethinyl estradiol (20 µg/d, orally) can reduce the folicular stimulating hormone (FSH) and luteinizing hormone (LH) in the serum (20); consequently, estrogen and progesterone synthesis by the ovary is reduced in both human and rats (21). Franchimont (20)also shows that unlike ethinyl estradiol, progesterone does not reduce serum FSH and LH. Enovid (98.5% norethylnodrel and 1.5% mestranol) treatment for 10-20 days causes a reduction in the ovarian weight and also inhibits ovulation in rats. It is thought that enovid works through the hypothalamus to reduce gonadotropin releasing hormone and then to reduce the FSH and LH production by the anterior pituitary gland, since this effect of Enovid on the ovaries was not seen in the hypophysectomized rats (21). It was also shown that Depo-provera (medroxyprogesterone acetate) decreased the ovarian weight by reducing estrogen receptor of the ovary and therefore reduce its responses to estrogen (22).

It has been shown that brain contains receptors for several steroids distribute unevenly in the brain (23-25). The presence of sex steroid receptors in the brain could partly explain why steroid treatment or ovariectomy can alter the brain chemistry. For instance, ovariectomy for 12 weeks caused an increase in the DA and NE concentrations in the median eminence (26-28), and a reduction in the midbrain

5-HT concentration (6 weeks post-operatively) in rats (10). Progesterone (0.8 mg) with ethinyl estradiol (5 µg) administered daily for 4-32 days decreased the midbrain and forebrain NE and 5-HT and forebrain DA concentrations (8 days) in rats (11). The action of hormones on steroid receptors in the brain could also partly responsible for the findings in this study that whole brain NE concentration was increased by progesterone and progesterone plus estrogen; brain 5-HT was decreased by Neogynon and brain protein concentration was decreased by ovariectomy. However, not all sex steroids and contraceptive drugs used in this report alter concentrations of brain substances measured. Therefore, such differences in effects may be due to differences in actions of these drug component other than simple interactions of these drugs to steroid receptors in the brain.

The reduction in brain 5-HT concentration by high dose of Neogynon could be partly due to two major mechanisms which were suggested by several investigators (5, 9). Firstly, by the vitamin B_6 (pyridoxal phosphate) deficiency of the brain, since many contraceptive drugs containing estrogen-progesterone derivatives were shown to stimulate vitamin B_6 uses by many organs and may cause a physiological deficit of vitamin B_6 which is a coenzyme essential for the brain 5-HT synthesis (5). Secondly, by the reduction of tryptophan (which is a precursor of 5-HT) uptake by the brain as shown by Nistico et al (9), therefore reductions in brain 5-HT synthesis may be resulted.

The decrease in the brain 5-HT concentration and increase in the uterine weight by Neogynon but not by Microgynon-30 could be due to the 1.5 times higher dosage of the ethinyl estradiol and/or d-norgestrel in Neogynon than in Microgynon-30. It is also difficult to explain why Depo-provera (60 mg/kg medroxyprogesterone, I.M., single injection) decreased the ovarian weight while progesterone (8 mg/kg/day, s.c. 14 days), which is a similar compound, did not show such effect. These differences could be partly due to differences in dosage, schedule and route of the treatments.

The decrease in the body weight gain, increase in the uterine and ovarian weight and induced estrous-like vaginal smear by estrogen plus progesterone should be due to the estrogen component since such effects were similar to those of the estrogen but not to the progesterone. It has been shown that estradiol (2.5 µg, s.c. for 3 days) may have direct effect on stimulation of ovarian growth (increase in weight) since the effect is still seen in hypophysectomized rats (22), whereas the progesterone (1 mg) does not have such effect. It has also been shown that the decrease in body growth rate in rats by estrogen treatment may be partly due to the reduction in food consumption (29, 30). This supports the above statements that the estrogen component was the major cause of the reduction of body weight gain.

The increase in the uterine weight by Neogynon, estrogen and estrogen plus progesterone could be due to the estrogenic action since the effect was similar to the estrogen but not the progesterone alone. In addition, it has been shown that uterus contains estrogen receptors (31) and that estrogen increases the uterine protein synthesis and myometrial cell division (32-34). The decrease in the ovarian weight by Depo-provera (60 mg/kg medroxy-progesterone, I.M., single dose) indicates that the drug may have ovarian suppressive effect possibly by reducing the number of estrogen receptor in the in the ovary and consequently the response of the ovary to estrogen was suppressed (22).

The unseeing change in the uterine weight by Depo-provera and progesterone treatments in this study does not mean that there was no histological or biochemical changes in the uterus since it has been shown that progesterone administration at appropriate time after estrogen treatment can increase the number and size of the uterine stromal cells (32). In addition, the 100% and 50% diestrous vaginal smear appearance by Depo-provera and progesterone treatments, respectively, indicate histological changes of the uterus.

The finding that Neogynon and Depo-provera induced majority of diestrous appearance of vaginal smear could be due to dominant progesterone-like effects since it was similar to the effect of progesterone alone. The majority of estrous-like vaginal smear by estrogen plus progesterone treatment should be due to the dominant of the estrogen component since it behaved like the estrogen not the progesterone. The majority of metestrous-like vaginal smear induced by Microgynon 30 but diestrous-like by Neogynon eventhough the two drugs have identical components (ethinyl estradiol and d-norgestrel), may be due to the differences in dosage of their components since the components of the Neogynon were 1.5 times more than those of the Microgynon 30.

The increase in the brain NE concentration by estrogen plus progesterone treatment should be caused by the progesterone component since such effect was similar to that of progesterone treatment alone but not to the estrogen treatment. The significant decrease in hematocrit by estrogen plus progesterone treatment may be due to synergistic effects of the two steroids since estrogen or progesterone alone did not show such effect. The decrease in hematocrit may be due to either decrease in red blood cell production by bone marrow or increase in plasma volume or both. The above change in hematocrit may or may not be comparable to the decrease in hematocrit in human using contraceptive drugs since in the latter case the period of drug administration was given for 5 years (7). It has been shown by another group of investigators that there was no significant change in the hematocrit and the blood viscosity in 70% of the women using contraceptive drugs for 2-10 months (34). Only 17% of the women in the above study (34) showed increase in the two parameters.

The decrease in brain protein concentration by ovariectomy may be due to reduction in endogenous circulation of sex steroid levels. Many steroid receptors have been found in brain areas and sex hormones have been shown to modify brain protein synthesis mediating through these receptors (23, 25). However, it is difficult to explain why sex

steroids and contraceptive drugs used failed to alter brain protein concentration. It is possible that some brain proteins may be increased in accompany with decreases in some other brain proteins therefore no net change in whole brain protein concentration could be observed. No net change in total serum protein concentration by the above treatments could be similarly explained. It has been previously shown that Microgynon and d-norgestrel treatment (for 6 months) in human caused increase in hormone binding proteins, plasminogen, and prealbumin, however, Depo-provera and progesterone did not have significant effects (6).

In conclusion, effects of treatments with high dose of two oral and one injectible contraceptive drugs, along with pure sex hormones were studied. Several significant changs in physiological and biochemical parameters in female rats were observed. Effects of contraceptive drugs were discussed basing on effects of pure sex hormones used and on lights of existing experimental results.

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