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EFFECT OF ORAL CONTRACEPTIVES (LYNDIOL®) ON RAT BRAIN GAMMA AMINOBUTYRIC ACID SYSTEM

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Abstract—(1) Virgin female albino rats were treated with the estrogenic substance mestranol, the progestogenic substance lynestrenol or with a combination of these two compounds. These two drugs are the components of the oral contraceptive pill Lyndiol®. (2) Mestranol pretreatment caused a significant increase in brain GABA level, lynestrenol pretreatment caused a significant decrease in brain glutamic acid level and pretreatment with a combination of the two drugs caused a significant rise in brain GABA content. Other changes were statistically insignificant. All three types of treatment, however, caused an identical and significant decrease in the glutamic acid:GABA ratio in rat brains. (3) The changes produced by oral contraceptive steroids on two brain enzymes, namely glutamate decarboxylase and aminobutyrate aminotransferase could not satisfactorily justify the assumption that the brain GABA concentration is controlled solely by the activities of such enzymes.

In view of the worldwide use of the various oral contraceptive compounds, it was thought of interest to tesr the effect of such compounds on the brain metabolism, especially as related to the metabolism of gamma aminobutyric acid (GABA) and glutamic acid (Glu). These two amino acids were chosen since they were found to act as inhibitory and excitatory neurotransmitters respectively [1, 2]. Two enzymes known to be involved in brain GABA metabolism were also measured in this study; namely glutamate decarboxylase (L-glutamate 1-carboxylase, EC 4.1.1.15) and aminobutyrate aminotransferase (4-aminobutyrate: 2-oxoglutarate aminotransferase, EC 2.6.1.19).

The oral contraceptive compounds employed in this study were mestranol and lynestrenol, the components of the well-known oral contraceptive pill Lyndiol®, manufactured under license of N.V. Organon-Oss-Holland and containing lynestrenol 2·5 mg and mestranol 0·075 mg per tablet.

MATERIALS AND METHODS

Materials. The following drugs were used in this study: Mestranol (17α-ethinyl-17-β-hydroxy-3-methoxy-1,3,5,(10)-estratrien, Organon); Lynestrenol (17α-ethinyl-17-β-hydroxy-estr-4-en, Organon.

Animals and dosage schedule. Virgin female albino rats weighing 100–150 g were obtained from local suppliers and were allowed food and fresh water *ad lib.* The rats were divided into four groups. Treatment was started when the rats were in the estrous stage of their cycle. The drugs were suspended in 0·5% carboxymethyl cellulose and given orally for 12 consecutive days. The rats were sacrificed 24 hr after the last dose. Group I received a daily dose of 0·08 mg mestranol per rat; Group II received a daily dose of 2·5 mg lynestrenol per rat; Group III received a daily dose of 0·08 mg mestranol and 2·5 mg lynestrenol per rat; Group IV served as a control and received an equivalent volume of 0·5% carboxymethyl cellulose daily for 12 consecutive days. The dosage and duration of treatment with the two sterols ensured a maximal contraceptive effect [3–6].

Estimation of GABA and Glu of rat cerebral hemispheres. The method used was a modification of the procedures described by Maynert *et al.* [7] and Saad [8]. The rats were decapitated, the brain quickly removed and the cerebral hemispheres were rapidly separated, weighed and homogenized in 7 vol of a solution of ethanol in water (75% v/v). These operations were carried out in the cold room maintained at 0–2°. The precipitated protein was removed by centrifugation. The clear supernatant was evaporated to dryness on a water bath. After cooling the residue, it was dissolved in distilled water. The resulting turbid solution was centrifuged and an amount of the clear supernatant fluid equivalent to 60 mg of the original wet brain tissue was applied by a micropipette to a Whatman No. 1 filter paper. The chromatogram was run by the ascending method for 18–20 hr using phenol–water (4:1 v/v) as a solvent. After removal of the solvent by air drying, the strips were dipped in 0·1% ninhydrin in butanol and placed in an oven maintained at 90° for 10 min. Each GABA or Glu spot was separately cut out and eluted in a test tube containing 5 ml ethanol solution (50% v/v in water). The test tube was then centrifuged for 10 min. The optical density of the eluate was measured at a wavelength of 570 nm in a Unicam SP 500 spectrophotometer. The amount of each amino acid present in each spot was then calculated using a predetermined standard curve.

Assay of enzymatic activities. The preparation of the homogenates of cerebral hemispheres and the conditions for the incubation procedures were as those described by Sytinskii and Priyatkina [9]. Estimation

Table 1. Effect of pretreatment with oral contraceptive steroids on GABA and Glu content of rat cerebral hemispheres

Drug	Daily dose (mg/rat	GABA (μmoles/g wet brain tissue) Mean ± S.E.M.	% change from control	Glu (μmoles/g wet brain tissue) Mean ± S.E.M.	% change from control	Glu:GABA ratio Mean ± S.E.M.	% change from control
None	—	2·22 ± 0·13		8·51 ± 0·23	—	3·83 ± 0·18	—
Mestranol	0·08	2·95 ± 0·18†	+33	8·72 ± 0·06^{NS}	+2·7	2·96 ± 0·12†	−22·7
Lynestrenol	2·50	2·56 ± 0·18^{NS}	+15	7·52 ± 0·21†	−11·6	2·94 ± 0·15†	−23·2
Mestranol + lynestrenol	0·08 2·50	2·73 ± 0·13*	+23	8·21 ± 0·24^{NS}	−3·4	3·01 ± 0·11†	−21·4

Pretreatment of animals with the drugs and estimation of the amino acids were carried out as described in Methods. Figures represent the mean of at least 7 determinations ± S.E.M. Each determination was obtained from the pooled cerebral hemispheres of 2 rats. NS signifies that the difference from the corresponding control value is statistically significant ($P > 0·05$); * and † indicate that the difference is statistically significant ($P < 0·05$ and $< 0·01$ respectively).

Table 2. Effect of pretreatment with oral contraceptive steroids on the glutamate decarboxylase and aminobutyrate aminotransferase activities of rat cerebral hemispheres

Drug	Daily dose (mg/rat)	Glutamate decarboxylase activity (μmoles GABA formed/g wet brain tissue/hr) Mean ± S.E.M.	% change from control value	Aminobutyrate aminotransferase (μmoles Glu formed/g wet brain tissue/hr) Mean ± S.E.M.	% change from control value
None		16·5 ± 0·9	—	8·5 ± 0·4	—
Mestranol	0·08	19·9 ± 1·5*	−20·8	7·2 ± 0·5*	−15·4
Lynestrenol	2·50	14·1 ± 1·1^{NS}	−14·3	5·7 ± 0·7†	−32·7
Mestranol + lynestrenol	0·08 2·50	16·8 ± 1·6^{NS}	+1·8	8·1 ± 0·8^{NS}	−5·1

Pretreatment of animals with the drugs and estimation of the enzyme activities were carried out as described in Methods. Figures represent the mean of at least 8 determinations ±S.E.M. Each determination was obtained from the pooled cerebral hemispheres of 2 rats. NS signifies that the difference from the corresponding control value is statistically insignificant ($P < 0·05$); * and † indicate that the difference is statistically significant ($P < 0·05$ and $< 0·01$ respectively).

of the formed GABA (in case of glutamate decarboxylase activity) or Glu (in case of aminobutyrate aminotransferase activity) was carried out as described above.

RESULTS AND DISCUSSION

It has long been known that certain amino acids, including gamma aminobutyric acid (GABA) and glutamic acid (Glu), could greatly affect the excitability of the central nervous system [1, 2]. The concentration of these amino acids, in particular GABA, has been found to change under the influence of many factors. One of these factors is the alteration of the hormonal balance in the animal. Thus thyroidectomy was found to lower brain GABA levels [10], whereas administration of thyroid hormone resulted in an increased brain GABA and Glu levels [11]. Adrenalectomy was shown to decrease brain GABA [12, 13]. Ovariectomy was reported to increase brain GABA levels [8] while the administration of progesterone lowered these levels towards control values [14].

The steady state concentration of brain GABA has been considered to be a function of the relative rates of formation and utilization by glutamate decarboxylase and aminobutyrate aminotransferase enzymes respectively [15]. Some authors hold the view that brain GABA is entirely controlled by the glutamate decarboxylase enzyme activity [16], whereas others claim that aminobutyrate aminotransferase plays a more important role in this respect [17].

The data obtained in this study (Table 1) showed that the estrogenic steroid compound, mestranol, given alone or in combination with the progestogenic compound lynestrenol, resulted in a significant rise in brain GABA levels. Administration of lynestrenol alone caused a slight, statistically insignificant increase in this parameter. Regarding brain Glu levels, only lynestrenol caused a significant decrease amounting to 11·6% of control value. In general, the changes in the Glu levels were of a much lower magnitude than those observed with GABA. This is in fair agreement with the view held by some authors that the concentration of brain Glu, unlike that of GABA, remains more or less constant [15].

A very interesting observation was noted when the results were expressed in terms of Glu:GABA ratio rather than in terms of absolute values of individual amino acids (Table 1). That is, all types of treatment employed in this study resulted in almost an identical amount of decrease in this ratio. In other words, the steady state concentration of the two amino acids was shifted in favor of GABA so that the new Glu:GABA ratio was decreased about 20% from control value in all the three types of treatment. This finding might be of significance since one would expect that the excitability of the brain might be a function of a certain balance between the excitatory and inhibitory amino acids rather than of the absolute concentrations of any of these amino acids alone. Our results showed that all three types of treatment included in this study produced the same degree of 'imbalance' in this equilibrium.

Data concerned with the effect of oral contraceptives on brain enzyme activities are presented in Table 2. A significant change in glutamate decarboxylase activity was observed following mestranol treatment only where the activity was found to increase by about 21% over the control value. The changes following other types of treatment were statistically insignificant. The aminobutyrate aminotransferase enzyme activity was decreased in the three types of treatment, the decrease was statistically significant following mestranol or lynestrenol treatment.

From these data, it could be seen that no direct correlation between the changes in amino acid concentrations and those in enzyme activities could be established. Thus if we consider that the brain GABA level is dependent upon the glutamate decarboxylase activity alone [16], one would expect that lynestrenol should have produced a decrease in brain GABA, and that pretreatment with the mestranol lynestrenol combination should have produced no significant change, neither of which is the case in our study. The only treatment which was found to agree with this assumption was that observed with mestranol which produced an increase in brain GABA concentration. On the other hand, if we assume that aminobutyrate aminotransferase enzyme plays the dominant role in controlling the level of brain GABA [17], one would expect that all three treatments should cause an increase in brain GABA with lynestrenol causing the highest increase. Our results showed that all three types of treatment, in fact, produced an increase in this parameter. However, lynestrenol caused the least and not the highest increase in brain GABA concentration. Therefore it could be concluded that other factors may play a major role in regulating the brain GABA level in addition to the two enzymes generally held responsible for the regulation of the steady state concentration of this amino acid in the brain.

The observed changes in the level of brain GABA and Glu following oral contraceptive steroid treatment may help to explain some of the commonly seen side effects of the pills such as the mental depression that is observed more frequently in women taking these pills [18].

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