BRAIN LESIONS IN RATS AFTER INTRAUTERINE EXPOSURE TO ESTRONE AND PROGESTERONE

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Estrone (0.1 mg/kg) or progesterone (4 mg/kg) was administered to female rats on the 9th day of pregnancy. As a result of injection of these substances, 33-38% of the rat fetuses showed developmental defects of the head and brain. Administration of the hormones in doses 2-4 times smaller led to the appearance of functional defects, consisting of disturbances of behavioral responses in the absence of any morphological changes in the brain.

Preparations containing female sex hormones are known to be capable of inducing malformations in developing fetuses [1, 6-8]. However, the possibility cannot be ruled out that the progeny may suffer damage from small doses of these preparations which do not give rise to visible malformations. An expert committee of the World Health Organization has recommended that these congenital disturbances of the functions of organs and systems which can be detected only by special instrumental investigation should be called functional.

The object of this investigation was to detect functional disturbances of the brain manifested as changes in the behavior of rats exposed in utero to the action of estrone and progesterone.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 180-210 g were used. Oily solutions of estrone and progesterone were injected subcutaneously into the rats once only, on the 9th day of pregnancy, the time of appearance of the primitive central nervous system [5]. Control animals were injected with peach oil at the same period of pregnancy.

The aggressive response was recorded in the progeny as described in [2]. The number of "fights" and the presence of a squeak in response to electrical stimulation were recorded. During the formation of defensive conditioned reflexes, the unconditioned stimulus was an electric current (10 V) applied to the floor of the chamber, while the conditioned stimulus was a lamp giving white light and the differential stimulus a lamp giving green light. The number of combinations required for the first independent conditioned-reflex response (going into the other compartment of the chamber) to appear, which was interpreted as formation of the reflex, was counted, together with the number of combinations required for the appearance of 3 successive responses, which was interpreted as stabilization of the reflex. The response of choice of way of escape from electrical stimulation of the rats was assessed by Malakhovskii's method [3]. The speed at which the rats moved from the first floor to the second and the number of incorrect attempts at going into the side compartment of the chamber were studied.

EXPERIMENTAL RESULTS

In the experiments of series I the rats were injected with estrone in a dose of 0.1 mg/kg or progesterone in a dose of 4 mg/kg, both of which have been shown to give rise to malformation [4]. At autopsy on

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TABLE 1. Indices of Behavioral Responses of Month-Old Rats Born to Control Females Receiving Injection of Teratogenic Agents during Pregnancy

Experimental conditions	Aggressive response		Defensive conditioned reflexes		Response of choice of way of escape	
	number of squeak responses	number of fllghts	rate of formation of reflex	rate of stabilization of flux	rate of movements to second floor of chamber	no. of incorrect attempts to move into side compartment of chamber (in %)
	$M \pm m$					
Control	8,4±0,9	10,3±1,6	8,6±1,6	10,8±2,1	14,6±1,8	1,9
Administration of estrone, 0.05 mg/kg	4,2±0,2	3,2±1,2	2,7±1,1	4,2±1,3	4,2±1,5	3,8
P	<0,01	<0,01	<0,01	<0,01	<0,01	<0,02
Administration of progesterone, 1 mg/kg	5,1±0,7	6,3±0,7	3,5±1,3	5,5±1,5	6,3±1,1	2,6
P	<0,005	<0,01	<0,01	<0,01	<0,01	<0,025

the 18th day of pregnancy, 136 fetuses were found in the 19 females receiving estrone. Malformations of the head were present in 38% of cases (cerebral hernia 26%, anencephaly 12%). After administration of progesterone to 16 pregnant females, cerebral hernias were found in 33% of the 112 fetuses studied.

In the experiments of series II estrone was injected into 20 pregnant rats in a dose of 0.05 mg/kg and progesterone was given to another 20 animals in a dose of 1 mg/kg. These rats were not autopsied. The offspring of these rats were indistinguishable from the control both in body weight, in external appearance, and in the mean number of rats per litter. When these young rats had reached the age of 1 month (326 rats) their behavioral responses were studied. The control consisted of 116 young rats whose mothers had received a subcutaneous injection of peach oil.

The results for the behavioral responses of the rats are given in Table 1.

These results demonstrate inhibition of the aggressive response in rats exposed in utero to estrone and progesterone. The number of fights involving rats exposed to the action of the hormones was 3.2 and 1.6 times less than in the control group. The frequency of the squeak response also was reduced, although by a lesser degree (by 2 and 1.6 times). Remembering that the squeak not only characterizes the passive defensive response, but also reflects the rats' sensitivity to stimulation, presumably the decrease in the number of fights (active defensive response) in animals exposed in utero to the action of estrone is evidence of reduced sensitivity to electrical stimulation. This factor may perhaps be more important still in the case of progesterone.

The rate of formation and stabilization of the conditioned reflex was determined as the quotient obtained by dividing 100 by the number of combinations necessary to produce the conditioned-reflex response. The rate of formation of the reflex and of its stabilization in the progeny of rats receiving the hormones was 3.1 and 2.5 times less than in intact animals. In rats exposed in utero to progesterone these values were reduced by 2.4 and 1.9 times respectively. If the differential stimulus was applied immediately after stabilization of the conditioned-reflex response, the number of correct responses given by the control animals was 72 ±7.2%, the number given by rats treated with estrone 21 ±6.4%, and the number in rats exposed to the action of progesterone 33 ±7.6%.

The time t (in sec) which the rats took when choosing the correct way of escape, measured from the beginning of electrical stimulation until the rat moved from the first floor of the chamber to the second, was converted into a velocity (100/t). As a result of antenatal exposure to estrone and progesterone the velocity of the response of choosing the correct way of escape was slowed by comparison with the control by 3.4 and 2.3 times respectively. When the rats exposed to the action of the hormones moved into the side compartment of the chamber, they made 2 and 1.3 times more incorrect attempts respectively.

Macroscopic and histological (sections stained with hematoxylin and eosin and by Nissl's method) studies of the brain of rats with severely altered behavioral responses revealed no abnormality. The changes in the rats' behavior found in these experiments can thus be regarded as the result of a functional brain defect.

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