Pharmacology Biochemistry & Behavior, Vol. 21, pp. 561–565, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Alterations in the Dopaminergic System and Behaviour in Rats Reared on Iodine-Deficient Diets

D. H. OVERSTREET,* A. D. CROCKER,† C. A. LAWSON‡
G. H. McINTOSH‡ AND J. M. CROCKER§

**School of Biological Sciences and †Department of Clinical Pharmacology*
The Flinders University of South Australia, Bedford Park, South Australia 5042
‡C.S.I.R.O. Division of Human Nutrition, Glenthorne Laboratory, Majors Road
O'Halloran Hill, South Australia 5158
and §Department of Clinical Chemistry, Queen Elizabeth Hospital
Woodville, South Australia 5011

Received 23 August 1984

OVERSTREET, D. H., A. D. CROCKER, C. A. LAWSON, G. H. McINTOSH AND J. M. CROCKER. *Alterations in the dopaminergic system and behaviour in rats reared on iodine-deficient diets.* PHARMACOL BIOCHEM BEHAV **21**(4) 561–565, 1984.—Male rats raised on an iodine-deficient diet were found to be retarded in growth rate, to have lowered body temperature, and to have poorer memory retention of a passive avoidance task than rats raised on a similar diet but with adequate iodine concentration. In addition, the iodine deficient rats showed increased locomotor activity and sniffing frequency after the dopamine agonist, apomorphine; while their operant performance was inhibited to a greater degree after apomorphine. However, the hypothermic effects of apomorphine were comparable in both iodine-deficient and normal rats. At sacrifice the iodine-deficient rats were found to have significantly depressed thyroxine levels (<10% of normal), significantly elevated TSH levels (>700% of normal), and a significantly greater concentration of dopamine receptors in the striatum (28% increase). Thus, rats raised on idoine-deficient diets have considerable behavioural and physiological alterations, including an increased concentration of dopamine receptors.

Iodine-deficient diets Dopamine Avoidance Locomotor activity Body temperature

IT is well established that thyroid hormones play an important role in the normal development of the human brain and that neurological abnormalities are associated with untreated congenital hypothyroidism such as that described in groups of people living on diets deficient in iodine [14]. These observations have stimulated interest in the effects of hypothyroidism on the brain and studies have been carried out in experimental animals fed iodine-deficient diets. Recent studies in rats suggested that there may be a critical period between 0 and 21 days after birth during which iodine deficiency produced changes in the central nervous system [2,11]. Thus, rats reared on iodine-deficient diets during this period had reduced plasma concentrations of thyroxine (T_4) associated with elevated plasma concentrations of thyroid stimulating hormone (TSH) and reduced core body temperatures, and a delayed development of the central nervous system [9,10].

There appear to be relatively few studies on the mechanisms underlying the physiological and behavioural consequences of iodine deficiency. It is postulated that abnormalities in one or more neurotransmitter systems may be associated with the behavioural deficits. In recent years there have been a number of studies which have indicated that the dopaminergic neurotransmitter system may be modified by altered thyroid status [1, 8, 17]. In particular, it has been shown that adult rats made hypothyroid by the administration of propylthiouracil (PTU) are less sensitive to the effects of chronic haloperidol treatment and have an increased dopamine receptor concentration in the striatum when compared with euthyroid controls [4].

The present series of experiments was designed to assess the degree of behavioural and physiological change that occurs in male rats raised on iodine-deficient diets from conception into adulthood, and to determine whether these changes could be accounted for, in part, by alterations in the dopaminergic neurotransmitter.

METHOD

Animals and Diet

The rats were from the Hooded-Wistar strain. At weaning four breeding pairs of rats were placed on a diet low in iodine (15–20 μg/kg) and another four pairs were placed on an iodine-replete diet (1 mg/kg). At 90 days of age the animals were mated and the male progeny arising from these matings were used in the experiments described below. The animals were maintained on their respective diets from birth until sacrifice at approximately 130 days of age. There were 8

iodine-deficient and 12 iodine-replete rats in the experiments.

All of the rats were provided with a double distilled water and their respective diets, in powdered form, in ceramic feeding dishes within the confines of their chrome plated cages. The cages had raised steel mesh floors to eliminate any access to faeces or urine. The animals were housed in the airconditioned animal colony at C.S.I.R.O. Division of Human Nutrition on a 12/12 hour light/dark cycle 23°C and constant humidity until 4 weeks of age and then were housed in a separate room at Flinders University under conditions of constant temperature (21°C) and humidity. All behavioural and physiological measures were taken between 9 a.m. and 11.30 a.m.

Body Weight

Body weight was recorded to the nearest gram on a Sartorius 1203 balance attached to a 7080 printer. Weights were taken at weekly intervals beginning at five weeks of age and continuing until the animals reached 12 weeks of age.

Core Body Temperature

To record core body temperature, a thermistor probe was inserted 6–8 cm into the rectum and attached to a CRL digital temperature recorder. The temperatures were recorded on three occasions in untreated animals and also after the administration of the dopamine agonist apomorphine, to determine its hypothermic effects.

Spontaneous Motor Activity

Animals were placed in a perspex open field, 60×30 cm, with a grid of 10×10 cm squares on the base. The number of squares entered in three minutes was used as the index of locomotor activity. Activity was recorded in untreated animals and in the animals after various drug treatments, as described below.

Passive Avoidance Task

To determine the rat's ability to remember an aversive stimulus a Y-maze apparatus [6] was used. Initially the rat was placed in the lighted alley facing the light and the time taken to enter one of the dark alleys was recorded. Upon entry the rat received a footshock of 1 mA and the time taken for the animal to escape to the lighted alley was recorded. The animal was then required to remain in the maze for a further three minutes and the number of shocks received during this period was recorded. A retention test was carried out 24 hours later; the rat was placed in the lighted alley and the time taken to enter the dark alley was again recorded. If the rat failed to enter one of the dark alleys within three minutes it was considered to have a good memory and was removed from the maze.

Operant Performance

To test the animal's learning of an appetite motivated task, the rats were tested in operant chambers fitted with levers and water drippers after 24 hours deprivation of water. The operant chambers were controlled by a TRS 80 microcomputer attached to Lehigh Valley interface; a line printer gave an ouput of reinforcements received at 5-minute intervals. Two indices were used to estimate learning ability: the time required to obtain 10 reinforcements and the number of total reinforcements in 45 min sessions.

After all animals had learned the task, they were maintained on daily 15 min operant sessions followed by 15 min of water in the home cage. When the majority of animals reached a stable level of responding on an FR5 (Fixed Ratio 5) schedule of responding, the influence of dopamine agonists and antagonists on this task was assessed, as described later.

Drugs

Apomorphine hydrochloride (0.2, 0.3 and 0.4 mg/ml) was dissolved in ascorbic acid solution (0.1% w/v). Amphetamine sulfate (2 mg/ml) was dissolved in isotonic saline. Haloperidol (0.05 mg/ml) was diluted in isotonic saline from a stock solution of 5 mg/ml. All drugs were administered subcutaneously in volumes of 1 ml/kg.

Procedure

The rats were transferred from the Glenthorne Laboratories of C.S.I.R.O. to Flinders University at weaning. They were weighed at weekly intervals, as described above. Baseline body temperatures were taken in untreated animals when they were 8 and 11 weeks of age. Baseline activity was recorded during a three min exposure to the open field when the animals were 11 weeks of age and in addition to the number of squares entered, the number of rears was also recorded. The influence of d-amphetamine sulphate (2 mg/kg) on these measures of activity was also examined by observing the rats in the open field 30 minutes after injection.

Passive avoidance training and retention were also measured when the rats were 11 weeks of age. In the next week the rats were trained to bar press in the operant chambers while they were deprived of water for 24 hours. After all rats had learned to obtain water on a continuous reinforcement schedule, they were shifted to a FR5 schedule of reinforcement and maintained on this schedule for the remainder of the experiment. Operant sessions were 15 min in duration, and were followed by 15 min water supplements in the home cage.

After the majority of the rats established a stable baseline (less than 10% variability in responding over three consecutive days), injections of apomorphine (0.2, 0.3 and 0.4 mg/kg) and haloperidol (0.05 mg/kg) were given subcutaneously. At least three days separated each drug treatment. Apomorphine was given 15 min before the operant session; immediately after the session, temperatures were recorded and a one-minute open field activity score was taken. Haloperidol was given 1 hr before the operant session, a one-minute open field activity score was taken immediately after the session.

At the conclusion of these behavioural and psychopharmacological studies the rats were sacrificed, by cervical dislocation, between 9 a.m. and 11 a.m. Blood samples were taken by cardiac puncture, allowed to clot, centrifuged and serum collected and frozen at −20°C. T_4 and TSH levels were measured by radioimmunoassay as described previously [4].

Brains were removed and the striatum was dissected out, weighed and homogenized in 10 volumes of ice cold 50 mm Tris buffer (pH 7.4). Striata from four rats in each group were pooled and the homogenates stored at −20°C. Total and nonspecific ^{3}H-spiroperidol (Radiochemical Centre, Amersham) binding was measured, in the presence of 1 μM (−)- and (+)-butaclamol, respectively. Aliquots containing 10 mg of striatal tissue were incubated with concentrations of

TABLE 1

BODY TEMPERATURES, AND THYROXINE AND TSH LEVELS IN IODINE-DEFICIENT AND IODINE-REPLETE RATS

Measure		Iodine-Deficient	Iodine-Replete
Body Temperature (Mean°C ± s.e.m.)	(week 8)	38.15 ± 0.13 (8)	38.46 ± 0.09 (10)
	(week 11)	38.30 ± 0.04 (8)	38.80 ± 0.04 (12)
Tyroxine levels (Mean nmol/l ± s.e.m.)		<5 (8)	50 ± 2.6 (12)
TSH levels (Mean μg/ml ± s.e.m.)		1751 ± 467 (8)	235 ± 97 (12)

TABLE 2

BEHAVIOURAL CONSEQUENCES OF IODINE-DEFICIENCY IN RATS

Measure	Iodine-Deficient	Iodine-Replete
Squares entered (Means ± s.e.m.)	36.6 ± 2.3 (8)	31.9 ± 1.1 (12)
Rears (Means ± s.e.m.)	8.6 ± 1.1 (8)	6.9 ± 1.0 (12)
Training latency (Median sec ± s.i.r.)	15 ± 6.5 (8)	17 ± 7.5 (12)
Escape latency (Median sec ± s.i.r.)	1.5 ± 1.1 (8)	2 ± 1.1 (12)
Retention latency (Median sec ± s.i.r.)	37.5 ± 80 (8)	180 ± 0 (12)

^{3}H-spiroperidol ranging from 0.1–2.0 nM in a final volume of 5 ml for 15 min at 37°C. Incubations were terminated by the addition of 5 ml of ice cold 50 mM Tris buffer (pH 7.7), followed by rapid vacuum filtration through GF/B filters (Whatman). Filters were washed with 1.5 ml aliquots of the same buffer and counted by liquid scintillation spectrometry. Binding data were analysed by Scatchard analysis to obtain K_D in nM and B max, the maximum number of binding sites, expressed as fmol/mg protein.

RESULTS

Indices of Hypothyroidism

The rats raised on the iodine-deficient diet weighed less than rats raised on an iodine-replete diet at the start of the experiment (week 5) and this difference was still evident at week 11. This reduction in body weight in the iodine-deficient rats is consistent with them being hypothyroid.

Table 1 summarises the data for body temperatures and hormonal levels, which support the conclusion that the rats raised on the iodine-deficient diet were hypothyroid. The body temperature and T_4 levels in these rats were lower than those in the rats raised on iodine-replete diets, while the TSH levels were substantially higher.

Behavioural Measures

There was a tendency for the iodine-deficient rats to enter more squares and to rear more frequently in the open field than the control rats, as indicated in Table 2. In addition, the iodine-deficient rats had a much poorer memory on the passive avoidance task (Table 2). In fact, only 3 of the 8 iodine-deficient rats had perfect memories compared with all 12 of the iodine-replete rats. It should be stressed that the groups performed very similarly on the training trial (Table 2) so the deficit on the retention trial probably reflects a true deficit in memory in the iodine-deficient rats.

Psychopharmacological Effects

The effects of the various dopamine agonists and antagonists on open field behaviour in the iodine-deficient and iodine-replete rats are summarised in Table 3. It can be seen that the iodine-deficient rats tend to enter more squares under all conditions.

The iodine-deficient rats also exhibited a higher incidence of intense sniffing after the injection of apomorphine than did their iodine-replete counterparts.

Despite the greater locomotor activity in the iodine-deficient rats following apomorphine, there were no differ-

TABLE 3

EFFECTS OF DOPAMINE AGONISTS AND ANTAGONIST ON OPEN FIELD BEHAVIOUR OF IODINE-DEFICIENT AND IODINE-REPLETE RATS

	Iodine-Deficient Squares entered (Mean ± s.e.m.)	Rears (Mean ± s.e.m.)	Iodine-Replete Squares entered (Mean ± s.e.m.)	Rears (Mean ± s.e.m.)
Apomorphine				
0.2 mg/kg	29.3 ± 2.9 (8)	4.9 ± 0.7 (8)	18.5 ± 2.6 (8)	3.8 ± 1.1 (8)
0.3 mg/kg	34.6 ± 2.2 (8)	8.4 ± 1.6 (8)	25.8 ± 1.4 (8)	5.9 ± 0.8 (8)
0.4 mg/kg	36.3 ± 4.2 (8)	5.0 ± 1.4 (8)	24.1 ± 4.3 (8)	3.8 ± 0.9 (8)
Amphetamine				
2.0 mg/kg	92.0 ± 5.3 (8)	20.3 ± 2.6 (8)	82.1 ± 4.9 (12)	17.9 ± 1.6 (12)
Haloperidol				
0.05 mg/kg	34.5 ± 3.2 (8)	10.1 ± 1.2 (8)	26.6 ± 4.8 (8)	7.5 ± 1.8 (8)

TABLE 4

EFFECTS OF APOMORPHINE AND HALOPERIDOL ON OPERANT RESPONDING FOR WATER REWARD IN IODINE-DEFICIENT AND IODINE-REPLETE RATS

	Mean % Baseline Responding ± s.e.m. Iodine-Deficient	Iodine-Replete
Apomorphine		
0.2 mg/kg	77.4 ± 6.4 (8)	70.5 ± 5.7 (8)
0.3 mg/kg	60.6 ± 5.7 (8)	52.1 ± 6.2 (8)
0.4 mg/kg	22.9 ± 5.2 (8)	38.2 ± 5.1 (8)
Haloperidol		
0.05 mg/kg	52.9 ± 4.6 (8)	65.2 ± 4.8 (8)

ences in the hypothermic responses. The rats from both groups exhibited decreases in temperatures of approximately 1.2° after 0.2 and 0.3 mg/kg and of 2.0° after 0.4 mg/kg. Thus, even though the baseline temperature of the iodine-deficient rats was about 0.4° lower than that of the iodine replete rats, there were no differences in their hypothermic responses to apomorphine.

The effects of apomorphine and haloperidol on bar pressing for a water reward are summarised in Table 4. In general, there are few differences between the two groups. The largest difference was observed after 0.4 mg/kg apomorphine, when the iodine-deficient rats exhibited a lower rate of bar pressing than did the iodine-replete group. This suggests that the iodine-deficient rats may be more sensitive to apomorphine than are the iodine-replete rats.

Receptor Binding

The results of Scatchard analysis of ^{3}H-spiroperidol binding to striatal homogenates revealed similar K_D's in the two groups (varying between 0.2–0.7 nM). However, the iodine-deficient rats had a significantly greater concentration ($p<0.05$) of dopamine receptors (82±0.4 fmol/mg protein) than the iodine-replete rats (64±0.5 fmol/mg protein).

DISCUSSION

Previous work by McIntosh *et al.* [9,10] established the effectiveness of an iodine-deficient diet in producing hypothyroidism in rats up to 35 days of age. The present study confirms that this hypothyroid state is continued as long as rats are maintained on a low iodine diet, as reflected by lowered T_4 levels, body weight and body temperatures.

The general locomotor activity of the rats raised on the iodine-deficient diet was not reduced; in fact, they tended to be more active than the rats raised on an iodine-replete diet. This finding is not consistent with findings of previous investigators who have reported lowered metabolic rates and lethargy [5]. Nevertheless, the iodine-deficient rats did exhibit a deficit in memory for the passive avoidance task (Table 2); this finding is consistent with earlier reports of learning deficiencies in rats made hypothyroid in the neonatal period [15].

Although there have been several studies of neurochemical changes in hypothyroidism [2, 3, 7, 12, 13, 16], these have not been related to behavioural effects. Because of the recent evidence that thyroid hormones may modulate the sensitivity of dopamine receptors [1,17], we explored the possibility that iodine-deficient rats may exhibit an altered response to dopamine agonists and antagonists. Recently we

reported that hypothyroid rats were less sensitive to the effects of haloperidol, a dopamine antagonist, whereas hyperthyroid rats were more sensitive [4]. We predicted that hypothyroid, iodine-deficient rats would be more sensitive to apomorphine and the present findings support this prediction.

This increased sensitivity may be related to the increase in dopamine receptors in the iodine-deficient group. It is possible that the increase in dopamine receptors is secondary to a decrease in turnover of dopamine in hypothyroid, iodine-deficient rats. Direct studies of dopamine turnover in hypothyroid rats are inconclusive at present [8], but there is evidence from other groups that hyperthyroidism stimulates the turnover of dopamine [1,17].

The increase in dopamine receptors seen in the hypothyroid iodine-deficient rats contrasts with the observation that there were no changes in dopamine receptor concentration in adult rats made hypothyroid by chornic treatment with PTU [4]. The degree of hypothyroidism was similar in the two treatments, but the iodine-deficient rats were hypothyroid for a much longer period, including the proposed critical period of 0–21 days of age. Preliminary experiments revealed few effects when the iodine deficiency was begun after 21 days of age. It is possible, therefore, that the hypothyroidism during the early development of the rats may have produced these changes. However, it should also be pointed out that increases in dopamine receptors could be brought about in the PTU treated hypothyroid rats by chronic injection of a low dose of haloperidol that did not lead to an increase in receptors in euthyroid rats [4].

In conclusion, idoine-deficient diets can lead to hypothyrodism and a consequence of this hypothyroidism is an increase in dopamine receptor sensitivity. Further studies must be conducted before conclusions concerning the changes in behaviour observed in iodine-deficient rats can be related to these increases in dopamine receptor sensitivity.

ACKNOWLEDGEMENTS

We wish to thank Wayne Looker, Felicia Buliman, Bob Frost and Robert Westphalen for technical assistance. This work was supported in part by grants from the Australian Research Grants Scheme and the Flinders University Research Budget.

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