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Hypothyroidism Leads to Increased Dopamine Receptor Sensitivity and Concentration

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CROCKER, A. D., D. H. OVERSTREET AND J. M. CROCKER. *Hypothyroidism leads to increased dopamine receptor sensitivity and concentration.* PHARMACOL BIOCHEM BEHAV 24(6) 1593–1597, 1986.—Rats treated with iodine-131 were confirmed to be hypothyroid by their reduced baseline core body temperatures, reduced serum thyroxine concentrations and elevated serum thyroid stimulating hormone concentrations. When hypothyroid rats were compared to euthyroid controls they were more sensitive to the effects of apomorphine (1.0 μmol/kg) on stereotypy, operant responding and body temperature and showed a smaller reduction in locomotor activity after injection of haloperidol (0.25 μmol/kg). Receptor binding studies on striatal homogenates indicated that hypothyroid rats had increased concentrations of D2 dopamine receptors but there was no change in the affinity. It is concluded that hypothyroidism increases dopamine receptor sensitivity by increasing receptor concentration.

Dopamine receptors Thyroid hormones Apomorphine Haloperidol ^{3}H-Spiroperidol

THERE have been several reports that modification of thyroid hormone status affects behavioural responses to dopamine agonists and antagonists. Thus, we have reported that rats made hypothyroid by oral treatment with propylthiouracil showed reduced behavioural responses to chronic haloperidol treatment, while hyperthyroid rats showed increased responses compared with euthyroid controls [5]. In addition, rats made hypothyroid by dietary restriction of iodine were more sensitive to the behavioural effects of apomorphine [12]. Other workers have focussed on the hyperthyroid state and have reported both increases and decreases in the behavioural responses to apomorphine [1, 9, 17], while we have consistently found decreases [13].

The object of the present study was to investigate the hypothesis that behavioural responses to the dopamine agonist, apomorphine, will be increased in rats made hypothyroid by treatment with iodine-131 and that this will be associated with an increased dopamine receptor concentration. Iodine-131 was used because it brings about a state of hypothyroidism which is not complicated by changes in body function resulting from the side effects of the procedures used. For example, surgical thyroidectomy may result in damage to the parathyroids and disturbances of calcium homeostasis. The hypothyroidism produced by chronic treatment with propylthiouracil, which blocks thyroid hormone synthesis, may be associated with changes in other metabolic events in the body [6] which could mask changes related only to the hypothyroid state. The results of this study support the hypothesis and indicate that thyroid hormones can modulate the sensitivity of dopamine receptors.

METHOD

Animals

Male Sprague-Dawley rats weighing 300–350 g at the start of experimental procedures were maintained at constant temperature and humidity and given free access to food and water. All experimental procedures and the termination of the experiments were carried out between 0900 and 1200 hours to minimise diurnal variations.

Production of Hypothyroidism

Rats were given an injection of 100 μg/kg thyrotrophin releasing hormone (TRH, Roche) into the tail vein 30–45 min prior to intraperitoneal injection of 2 mCi/kg iodine-131. The purpose of the TRH injection was to stimulate iodine uptake mechanisms in the thyroid gland and maximise the concentration of radioactive iodine in the gland. Control rats were given injections of vehicle by the same routes. Rats were then housed in pairs and their body temperature monitored weekly from 16 days after iodine-131 injection. Blood samples were taken and the concentration of thyroxine (T4) and thyroid stimulating hormone (TSH) measured by radioimmunoassay to assess the effectiveness of iodine-131 in destroying the thyroid gland.

Assessment of General Activity

General activity was assessed by placing the rats in a rectangular open field chamber (60×30 cm) made of perspex with a grid of 10×10 cm marked on its base and lit only by a

dim red light. The number of lines crossed in one minute was used as an index of activity and was measured one hour after haloperidol was injected subcutaneously.

Assessment of Stereotypy

Stereotyped behaviour was observed by placing the rats in a rectangular open field chamber 10 and 30 min after a subcutaneous injection of apomorphine (1.0 μmol/kg) and assessing the degree of stereotypy according to the 6-point scale developed by Creese and Iversen [4]. All observations were carried out in a room lit only by a dim red light.

Catalepsy

Catalepsy was assessed one hour after a subcutaneous injection of haloperidol (0.25 μmol/kg) according to the scoring method devised by Pedigo *et al.* [14].

Operant Responding

Rats were trained to press a bar to obtain a water reward in an operant chamber. Each chamber was housed in a sound-proofed box and was controlled by a TRS 80 microcomputer with Lehigh Valley interface. After being trained to bar-press the rats were maintained on an FR5 (5 bar-presses for one reward of 50 μl water) schedule of reinforcement. The operant sessions were 15 min in duration and followed by 15 min of water in the home cage. This parameter which was selected as a sensitive measure of psychomotor performance, was assessed 12 min after injection of apomorphine.

Body Temperature

Core body temperature was measured weekly using a YSI telethermometer by inserting a thermistor probe 6–8 cm into the rectum. The reading was taken when it had remained constant for 15 sec. The effects of apomorphine on core body temperature were assessed 35 min after injection.

Assay of Thyroxine and TSH

Blood samples were taken from a tail vein during the course of the experiment and by cardiac puncture at the termination of the experiment. Samples were allowed to clot, then centrifuged and the serum removed and stored at −20°C. Thyroxine (T4) was measured by a radioimmunoassay developed at The Queen Elizabeth Hospital, Woodville, South Australia. Antibody was raised in sheep using T4 conjugated to bovine serum albumin (Commonwealth Serum Laboratories, Melbourne, Australia) as the antigen. T4 standard was purchased from Henning, Berlin, FRG (9050/14209) and iodine-125-T4 from the Radiochemical Centre, Amersham, U.K. Separation of bound and free T4 was achieved by precipitation with 30% w/v polyethelene glycol (Lab Supply, Adelaide, South Australia).

Rat TSH was assayed by a double antibody radioimmunoassay developed at the Queen Elizabeth Hospital, Woodville, South Australia. Purified rat TSH for iodination (NIAMD-rat TSH-104), antiserum to rat TSH, prepared in rabbits (NIAMD-anti-rat TSH serum-5), and rat TSH reference preparation for radioimmunoassay (NIAMD-rat TSH-RP-1) were donated by the National Pituitary Agency, Baltimore, MD 21201.

Drugs

Apomorphine (Sigma) was dissolved in isotonic saline (0.15 M) prior to injection and kept at 5°C. Solutions were discarded after 30 min and replaced with a freshly made up solution. Haloperidol was prepared prior to injection by diluting a stock solution of 10 mg/ml (Serenace, Searle Aust. Pty. Ltd.) with isotonic saline. All drugs were adminstered in a volume of 1 ml/kg and control animals received isotonic saline.

Statistics

The ordinal data obtained from stereotypy and catalepsy assessment were tested using a non-parametric method, the Mann-Whitney U-test. Since non-parametric methods have been used, the stereotypy data for each group are presented as median scores in Table 2. Student's *t*-test was used to analyse differences in operant responding, body temperature, hormonal concentrations in serum and receptor concentrations.

Measurement of D2 Dopamine Receptors

Rats were killed by cervical dislocation. 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) and homogenates stored at −20°C. Total and nonspecific ^{3}H-spiroperidol binding was measured, in the presence of 1 μM (−) and (+) butaclamol (gift of Ayerst laboratories), respectively. Aliquots containing 10 mg of striatal tissue were incubated with concentrations of ^{3}H-spiroperidol (20 Ci/mmol; Amersham), ranging from 0.1–2.0 nM in a final volume of 5 ml for 15 min at 37°C. Incubation was terminated by the addition of 5 ml of ice cold 50 mM Tris buffer (pH 7.7) followed by rapid vacuum filtration using GF/B filters (Whatman). Filters were washed with four 2.5 ml aliquots of the same buffer and counted by liquid scintillation spectrometry. Binding data were analysed by the method of Scatchard using linear regression to obtain Kd in nM and B_{max}, the maximum number of binding sites, expressed as pmol/100 mg protein. Tissue protein concentration was determined using the method of Lowry *et al.* [10].

RESULTS

Hormone Concentrations and Body Temperatures

The body temperature of the iodine-131-treated rats was significantly lower than controls 16 days after injection of iodine-131 ($p<0.001$) and measurement of thyroxine (T4) showed the rats were hypothyroid (see Table 1). Subsequently body temperatures were monitored weekly to check that no recovery from the hypothyroid state occurred and this was confirmed by T4 analysis at 5 and T4 and TSH analysis at 10 weeks after initial treatment with iodine-131. These data are presented in Table 1 and show that the hypothyroid state persisted up to the time the experiment was terminated.

Effects of Apomorphine

At 5 and 10 weeks after destruction of the thyroid gland hypothyroid rats were injected with 1.0 μmol/kg apomorphine and its effects on operant responding, stereotypy and body temperature were assessed as described previously and compared with euthyroid controls. As can be seen in Table 2, the hypothyroid rats exhibited significantly greater reductions in operant responding and body temperature and significantly higher stereotypy scores than the euthyroid controls at both 5 and 10 weeks. Thus, the length of hypothyroidism

TABLE 1

THE EFFECT OF TREATMENT WITH IODINE-131 ON PLASMA THYROXINE (T4) AND TSH CONCENTRATIONS AND ON BODY TEMPERATURE

Group	T4 (nmol/l)	TSH (ng/ml)	Body temperature (°C)	N
16 days after				
Saline	53.8 ± 2.6	—	37.9 ± 0.06	10
Iodine-131	11.6 ± 2.8*	—	37.5 ± 0.08*	15
5 weeks after				
Saline	56.5 ± 2.7	—	38.1 ± 0.11	11
Iodine-131	13.2 ± 3.0*	—	37.6 ± 0.07*	15
10 weeks after				
Saline	59.3 ± 6.0	733 ± 141	38.0 ± 0.08	12
Iodine-131	13.7 ± 2.6†	3744 ± 322†	37.7 ± 0.06‡	16

*$p<0.001$, †$p<0.02$, ‡$p<0.05$ compared with control group. (Student's t-test).
All results expressed as means ± s.e.m. N=number of rats in each group.
—Not assayed.

TABLE 2

EFFECTS OF APOMORPHINE ON OPERANT RESPONDING, STEREOTYPY AND CORE BODY TEMPERATURE IN HYPOTHYROID AND CONTROL RATS

Group	% Baseline Operant Responding (mean ± s.e.m.)	Stereotypy Rating (median)	% Baseline Temperature (mean ± s.em.)	N
5 weeks after				
Saline	49.0 ± 3.8	2	97.9 ± 0.26	11
Iodine-131	34.5 ± 3.9*	3§	96.8 ± 0.18‡	15
10 weeks after				
Saline	43.0 ± 3.7	2	97.6 ± 0.22	12
Iodine-131	30.0 ± 3.5†	3§	95.8 ± 0.22‡	16

*$p<0.05$, †$p<0.02$, ‡$p<0.001$ (Student's t-test).
§$p<0.002$, Mann Whitney U test. N=number of rats in each group.

did not appear to affect the greater responses to apomorphine in the hypothyroid rats.

Effects of Haloperidol

Behavioural responses to haloperidol (0.25 μmol/kg) were assessed in hypothyroid rats eight weeks after ablation of the thyroid gland. No significant difference in catalepsy rating scores were found between hypo and euthyroid groups although the euthyroid group was significantly less active (4.2±0.7 line crossings/min) than the hypothyroid group (8.1±1.3 line crossings/min) ($t(25)=2.616$, $p<0.02$) following haloperidol injection.

^{3}H-Spiroperidol Binding

The concentration and affinity of striatal D2 dopamine receptors was determined by Scatchard analysis of binding data obtained from the incubation of striatal homogenates with ^{3}H-spiroperidol. There was no significant difference in Kd between euthyroid rats and rats made hypothyroid by iodine-131 injection ten weeks previously, range of Kd was 0.08–0.14 nM. However, there was a significant 13% increase in B_{max} in the hypothyroid group compared with the euthyroid group ($t(16)=2.779$, $p<0.02$) (Table 3).

DISCUSSION

The present findings show that stereotypic responses to apomorphine were enhanced in hypothyroidism induced by iodine-131 and associated with an increase in dopamine receptor concentration in the striatum. Since stimulation of postsynaptic dopamine receptors in the striatum has been shown to produce stereotypy [2], this increased receptor concentration in the hypothyroid rats may explain their increased behavioural responses to apomorphine. Conversely, since blockade of striatal dopamine receptors produces immobility [3], a decreased response to a dopamine antagonist might be predicted in hypothyroidism and this was observed following haloperidol injection.

Because spiroperidol was used to label receptors in this study, the changes observed in hypothyroid rats are probably an indication of alterations in the dopamine D2 recep-

TABLE 3

EFFECT OF TREATMENT WITH IODINE-131 ON THE Kd and B_{max} VALUES OBTAINED FROM SCATCHARD PLOTS OF ^{3}H-SPIROPERIDOL BINDING IN STRIATUM

Group	Kd (nM)	B_{max} (pmol/100 mg protein)	N
Saline	0.12 ± 0.01	18.1 ± 0.4	9
Iodine-131	0.11 ± 0.01	20.4 ± 0.6*	9

*$p<0.02$ compared with control group. Results expressed as means ± s.e.m. N=number of rats in each group.

tor. In our hands, the addition of ketanserin, a serotonin S2 blocker, had little influence on ^{3}H-spiroperidol binding in striatal homogenates. Consequently, we feel that the main change has been in the D2 receptors. Other recent work in our laboratory indicates that the stereotypy induced by apomorphine can be reduced by both selective D1 and D2 antagonists. This may account for the apparent discrepancy between the small increase in D2 receptors in the hypothyroid rats and the large increase in the behavioural effects of apomorphine. We are currently investigating whether there are alterations in D1 receptor sensitivity in hypothyroid rats.

In a previous study in which hypothyroidism was induced by oral administration of propythiouracil [5], we did not demonstrate a change in dopamine receptor concentration. However, an increase in behavioural responses to apomorphine associated with an increased concentration of striatal dopamine receptors was observed in another study [12] using rats maintained on an iodine-deficient diet from weaning. It is possible, therefore, that receptor changes are observed only when hypothyroidism has been maintained for a long period, as with iodine deficiency of iodine-131 treatment, and these changes could be secondary to changes in dopamine synthesis and turnover as suggested by other workers [15,17].

The present results are consistent with our earlier finding that an increase in dopamine receptor concentration was observed when hypothyroid rats were treated chronically with a dose of haloperidol which by itself had no effect on receptor concentration of affinity [5]. Thus, stimuli which have been shown to produce up-regulation of dopamine receptors, such as chronic haloperidol treatment, were more effective in the hypothyroid state.

The increased sensitivity to apomorphine in the hypothyroid rats was also manifested in increased effects on body temperature and operant responding. This result suggests that the effects of thyroid hormone deficiency were not confined only to functions attributable to stimulation of dopamine receptors in striatum.

In the present study the production of hypothyroidism by iodine-131 resulted in low levels of thyroxine and elevated levels of TSH by negative feedback control of the pituitary [7]. It would be expected that TRH release would also be increased as a result of the low T4 concentrations in plasma. Thus this experimental model differs from the clinical situation of hypothyroidism of hypothalamic or pituitary origin in which TRH and TSH may be markedly reduced, but is similar to that induced by iodine deficiency. It is possible, therefore, that the results obtained could be due to increased TRH and/or TSH release. Miyamoto and Nagawa [11] showed that TRH injected either intraperitoneally or into the nucleus accumbens stimulated locomotor activity and the frequency of stereotyped sniffing. This suggested that TRH may play a role as a modulator of dopamine activity in the nucleus accumbens. Further, TRH is known to increase prolactin release from the anterior pituitary and other workers have suggested that prolactin may increase dopamine receptor sensitivity in the striatum [8]. However, recent studies have shown that co-administration of TRH with chronic haloperidol treatment did not lead to a greater up-regulation of dopamine receptors [16].

Thus, although the results of our study show that there are increased behavioural responses to apomorphine in hypothyroidism which are associated with an increased striatal dopamine receptor concentration, it is not possible to attribute these effects to a primary lack of thyroid hormones or to the secondary changes in the release of hormones from the hypothalamus and/or anterior pituitary. These considerations make extrapolation of findings from animal studies to the clinical situation difficult and experiments are in progress to clarify which factors are involved in producing the changes in dopamine receptor responsiveness.

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