Pharmacology Biochemistry & Behavior, Vol. 28, pp. 193–196. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Post-Synaptic Changes and Increased Dopamine Receptor Sensitivity in Hypothyroid Rats

DONALD L. CAMERON AND ANN D. CROCKER[1]

Department of Clinical Pharmacology and Centre for Neuroscience, School of Medicine The Flinders University of South Australia, Bedford Park, Adelaide 5042, Australia

Received 25 February 1987

CAMERON, D. L. AND A. D. CROCKER. *Post-synaptic changes and increased dopamine receptor sensitivity in hypothyroid rats.* PHARMACOL BIOCHEM BEHAV **28**(2) 193–196, 1987.—Hypothyroid rats showed increased behavioural sensitivity to both selective and non-selective dopamine agonists. Ligand binding analysis revealed no differences in concentration or affinity of striatal dopamine receptor subtypes in hypothyroid rats. Measurement of striatal cAMP levels however, indicated that hypothyroid rats showed a greater increase in cAMP production in response to stimulation by dopamine. It is concluded that the changes in behavioural sensitivity observed may be associated with alterations in post-synaptic mechanisms.

Hypothyroidism Dopamine Selective dopamine agonists D1 and D2 receptors Adenylate cyclase

AN understanding of the alterations in brain function in response to hormonal factors may have important implications for understanding the basis of many neuropsychiatric disorders. The emergence of schizophrenia-like psychotic symptoms in myxoedematous patients that were abolished when a euthyroid state was re-established [1,14] has prompted research into the interactions between thyroid hormones and central neurotransmitter systems.

Previous work from our laboratory has established that there was an increased behavioural responsiveness to the mixed D1 and D2 dopamine receptor agonist, apomorphine, in hypothyroid rats which was associated with an increase in the concentration of striatal D2 receptors [4]. The present study was directed at investigating whether these behavioural effects were mediated through D1 and/or D2 dopamine receptor subtypes and what receptor events were associated with them. The following questions were addressed: does hypothyroidism bring about (1) changes in behavioural sensitivity to the selective D2 dopamine receptor agonist LY-171555 and the selective D1 agonist SK&F-38393?; (2) changes in the concentration and/or affinity of striatal D1 and D2 receptor subtypes?; (3) changes in dopamine receptor coupled adenylate cyclase activity?

METHOD

Animals

Male Sprague-Dawley rats were used for all experiments. These animals were housed with free access to food and water under conditions of constant illumination and temperature. All treatments and drug challenges took place between 0800 and 1200 hours. Animals were also sacrificed during this period.

Thyroid Modification

Animals were treated daily with either PTU (propylthiouracil, 200 mg/kg) or water administered via gavage for a period of 4 weeks. Thyroid status was monitored twice weekly by body temperature measurement and confirmed at the termination of the experiment by assay of serum thyroxine (T4) concentration in blood samples taken by cardiac puncture, as described previously [4]. Hypothyroidism was shown by significant decreases in both parameters (Table 1).

Drugs

LY-171555 (Lilly), SK&F-38393 (Smith, Kline and French) and apomorphine (Sigma) were dissolved in isotonic saline and injected subcutaneously into the right flank.

Behavioural Assessment

Behaviours were assessed by the fixed interval momentary sampling method of Cameron and Crocker [2], in which the presence or absence of specific target behaviours are recorded at the precise instant of an auditory cue spaced at 3 second intervals for periods of one minute. The one minute observation periods were spaced five minutes apart at varying times after injection depending on the time of peak effect for each drug. This had been ascertained from preliminary experiments and is shown on the following page.

[1]Requests for reprints should be addressed to Ann D. Crocker.

Each animal was placed in a perspex chamber measuring 600×300×300 mm situated in a room illuminated only by a dim red light where observations of the following target behaviours were made; sniffing, locomotion, grooming, rearing, vacuous oral movements, gnawing and licking. These target behaviours were chosen as they are known to be mediated through striatal areas [6, 8, 16].

For each observation period the number of times a behaviour was recorded as present (out of a maximum of 20) was expressed as a percentage of observed behaviour. This technique provides ratio level data which is amenable to parametric analysis.

Binding Assay

At the conclusion of the pharmacological studies animals were sacrificed by cervical dislocation, brains removed, and striata (caudate-putamen) dissected out and homogenized (Polytron, setting 3.5, 15 sec) in 10 volumes (w/v) of ice cold assay buffer (20 mM 4-morpholinepropanesulfonic acid (MOPS), 1 mM EDTA, 0.1% ascorbic acid, 4 mM $MgSO_4$, 19 mM Tris base, pH 7.2 at 22°C) and stored at −80°C. Binding assays were carried out within 7 days of sacrifice.

Concentrations of both dopamine receptor subtypes were measured in the striata from each animal using [^{3}H]-flupenthixol with spiroperidol as the displacing ligand. Both subtypes can be resolved using the method described by Leff *et al.* [9]. Constant amounts of tissue (1 mg/ml) and [^{3}H]-flupenthixol (1 nM, 10.2 Ci/mmol New England Nuclear) were incubated with varying concentrations of spiroperidol (0.03–1000 nM, Jansen) in glass test tubes (18×150 mm) at 22°C for 90′. Nonspecific binding was defined using 1 μM (+)-butaclamol (Ayerst). The total volume in each tube was made up to 2 ml with the assay buffer described above. This reaction was terminated by vacuum filtration and the radioactivity retained on Whatman GF/B filters, after 3×5 ml washes with ice cold buffer (Tris-HCl, pH 7.7), determined by liquid scintillation spectrometry. When [^{3}H]-flupenthixol, which has equivalent affinity for both D1 and D2 receptors, is competitively displaced by spiroperidol, a biphasic displacement curve is obtained due to the 1000-fold selectivity of spiroperidol for the D2 receptor [9]. The displacement curves resulting from the present experiment were analyzed using an extended least squares non-linear curve fitting program, using a general model for ligand-receptor interaction according to the law of mass action [7]. In agreement with Leff *et al.* [9], the data obtained from this experiment were best described by a two binding site model. Estimates of Kd (nM) and Bmax (pmol/g·protein) for spiroperidol at both high affinity (D2) and low affinity (D1) sites were subsequently obtained. Tissue protein concentrations were determined by the method of Lowry *et al.* [10].

Adenylate Cyclase Assay

Aliquots (50 μl) of striatal homogenates were incubated with and without dopamine. Extraction of baseline and dopamine stimulated cAMP was carried out using the method of Nielsen *et al.* [12]. The concentration of baseline and stimulated cAMP was determined by competitive protein binding assay using a cAMP assay kit (Amersham) and expressed, after protein estimation [10], as pmol cAMP/mg protein. Dopamine (0.1 mM) stimulated cAMP was calculated by subtracting basal cAMP concentration from the concentration of cAMP in incubations containing dopamine and expressed as percentage of basal cAMP concentration.

TABLE 1

VALUES OF SERUM THYROXINE (T4) AND CORE BODY TEMPERATURE IN RATS TREATED WITH EITHER PTU OR WATER FOR 4 WEEKS PRIOR TO SACRIFICE

Group	N	T4 (nmol/l) (mean ± S.E.M.)	Temperature (°C) (mean ± S.E.M.)
Water	7	89.3 ± 3.3	37.9 ± 0.1
PTU	6	3.3 ± 0.3*	36.5 ± 0.1*

*$p<0.001$ (U-test).

Drug	Observation Time (minutes after injection)
Apomorphine	15′ 20′ 25′
LY-171555	25′ 30′ 35′ 40′
SK&F-38393	30′ 35′ 40′ 45′

Data Analysis

Differences in the incidence of target behaviours, binding data and cAMP concentrations between PTU treated animals and controls were analyzed using the Mann-Whitney U statistic. This nonparametric method was employed due to the sample sizes of the experimental groups. As the data obtained was of ratio level in all cases, results are presented as mean±standard error of the mean (S.E.M.).

RESULTS

Effects of Apomorphine

Initially it was confirmed that PTU treated rats were more sensitive to the mixed D1/D2 agonist apomorphine (0.5 μmol/kg, assessed 20 minutes after injection) showing significantly increased sniffing ($p<0.01$) and locomotor activity ($p<0.05$) than water treated controls (Table 2). No differences were observed between groups for any of the other target behaviours.

Effects of LY-171555 and SK&F-38393

Responses to the selective D2 agonist, LY-171555 (0.5 μmol/kg), were assessed 35 minutes after injection. Increased behavioural sensitivity in the PTU treated rats was shown by significantly increased sniffing ($p<0.001$) and locomotor activity ($p<0.05$) compared with water treated controls. No differences for any of the other target behaviours were observed.

Responses to the selective D1 agonist, SK&F-38393 (2.0 μmol/kg), assessed 40 minutes after injection, were significantly increased in PTU treated rats as shown by the increased incidence of vacuous oral movements ($p<0.02$)

TABLE 2

BEHAVIORAL RESPONSES TO APOMORPHINE, LY-171555 AND SK&F-38393 IN ANIMALS TREATED WITH EITHER PTU OR WATER

Drug	Target Behaviour	Group	N	% Observed Behaviour (mean ± S.E.M.)
Apomorphine	Sniffing	Water	8	31.4 ± 5.3
		PTU	8	66.4 ± 9.5†
	Locomotion	Water	8	8.6 ± 3.2
		PTU	8	17.1 ± 2.8*
LY-171555	Sniffing	Water	8	12.9 ± 3.8
		PTU	8	40.7 ± 6.5†
	Locomotion	Water	8	5.0 ± 3.3
		PTU	8	17.4 ± 4.1*
SK&F 38393	Oral Movements	Water	8	2.9 ± 0.6
		PTU	8	12.1 ± 3.4*

*$p<0.05$, †$p<0.01$ (U-test).

TABLE 3

CONCENTRATION AND AFFINITY OF STRIATAL D1 AND D2 DOPAMINE RECEPTORS (MEAN ± S.E.M.)

Subtype	Group	N	Bmax (pmol/g protein)	Kd (nM)
D1	Water	6	473.9 ± 55.9	271.5 ± 66.4
	PTU	6	428.2 ± 41.5	261.4 ± 57.9
D2	Water	8	436.6 ± 58.3	0.07 ± 0.007
	PTU	6	320.8 ± 20.0	0.08 ± 0.007

TABLE 4

BASELINE AND DOPAMINE STIMULATED cAMP CONCENTRATIONS (MEAN ± S.E.M.) IN STRIATAL TISSUE

Group	N	Baseline (pmol cAMP/mg protein)	+0.1 mM Dopamine (% baseline increase)
Water	7	180.2 ± 8.45	57.3 ± 6.9
PTU	6	169.82 ± 13.4	93.2 ± 6.9*

*$p<0.005$ (U-test).

compared with water treated controls (Table 2). There were no differences between groups for any of the other target behaviours.

[^{3}H]-Flupenthixol Binding

No significant differences in either affinity (Kd) or concentration (Bmax) were found for either D1 or D2 receptor subtype in the striata of PTU treated rats compared with water treated controls (Table 3).

Adenylate Cyclase Assay

Although no significant differences in baseline cAMP concentrations were observed, striatal homogenates from PTU treated animals showed a significantly greater increase in cAMP concentrations in response to stimulation by 0.1 mM dopamine ($p<0.005$) compared to water treated controls (Table 4). This latter observation indicates a change in coupling between dopamine receptor and adenylate cyclase in response to altered thyroid state.

DISCUSSION

The present findings confirmed our previous observations that stereotypic responses to the mixed D1/D2 agonist, apomorphine, were enhanced in hypothyroid rats compared with euthyroid controls. Further, increased responses to both the selective D2 agonist, LY-171555, and the selective D1 agonist, SK&F-38393, were found in hypothyroid compared with euthyroid rats indicating changes in the sensitivity of both receptor subtypes. However, ligand binding analysis revealed no changes in the concentration or affinity of either D1 or D2 receptor subtypes in the hypothyroid group from control values. Previously we had shown that in rats made hypothyroid by ablation of the thyroid gland with iodine-131 ten weeks before, increased behavioural sensitivity to apomorphine was associated with an increased concentration of striatal D2 receptors [4]. In the present and a previous study [3] in which the period of hypothyroidism was shorter (3–4 weeks) although behavioural responses to apomorphine were significantly increased there was no change in dopamine receptor concentration compared with euthyroid controls. We have argued [4] that receptor changes may only occur when hypothyroidism is maintained for a long period and that they may be secondary to changes in dopamine synthesis and turnover.

The present findings of increased behavioural sensitivity to both D1 and D2 agonists in the absence of changes in receptor concentration and affinity suggest that other mechanisms may underlie the behavioural changes observed in hypothyroidism. Since such mechanisms could involve changes in second messenger systems and it has been established that D1 and D2 dopamine receptor subtypes exert opposite influences on cAMP production [13,15] (i.e., activation of D1 receptors stimulates cAMP production, and that of D2 inhibits cAMP production) our present finding that there is increased dopamine stimulated cAMP formation in hypothyroid rats is of great interest. It may be that changes in coupling may precede alterations in receptor concentration. The time course of these responses is currently under investigation.

However, the question must also be raised whether this is due to an increase in D1 activity or a decrease in D2 activity and experiments are in progress to clarify this.

The previous observation of increased D2 receptor concentration and our current finding of increased DA stimulated cAMP formation in the striata of hypothyroid rats bear striking parallels to data obtained from the post mortem brains of schizophrenics. Cross *et al.* [5] demonstrated significantly increased concentrations of D2 receptors in the caudate nuclei from the brains of drug-free schizophrenics while Memo *et al.* [11] showed increased adenylate cyclase

activity in response to D1 receptor stimulation in the same brain areas. This, together with the occurrence of myxoedema psychosis, may point to some similarities between the pathophysiology of schizophrenia and the changes taking place in the brain in response to hypothyroidism.

We conclude therefore, that hypothyroidism was associated with increased behavioural sensitivity to both D1 and D2 receptor agonists which may not always be associated with a change in receptor concentration or affinity but was associated with changes in post receptor mechanisms.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Health and Medical Research Council to A.D.C. and a Commonwealth Postgraduate Research Award to D.L.C. We gratefully acknowledge gifts of drugs from Lilly Laboratories, Ayerst and Smith, Kline and French Laboratories and the skilled technical assistance of Robert Westphalen. We also thank Chris Reilly of the Queen Elizabeth Hospital, Woodville, for the thyroxine assays.

REFERENCES

1. Asher, R. Myxoedematous madness. *Br Med J* Sep 10th: 555–562, 1949.
2. Cameron, D. L. and A. D. Crocker. A fixed interval momentary sampling method for assessing on-going behaviours induced by dopamine receptor agonists. C.I.N.P., Proceedings of 15th Congress, San Juan, 16, 1986.
3. Crocker, A. D. and D. H. Overstreet. Modification of the behavioural effects of haloperidol and of dopamine receptor regulation by altered thyroid status. *Psychopharmacology (Berlin)* **82:** 102–106, 1984.
4. Crocker, A. D., D. H. Overstreet and J. M. Crocker. Hypothyroidism leads to increased dopamine receptor sensitivity and concentration. *Pharmacol Biochem Behav* **24:** 1593–1597, 1986.
5. Cross, A. J., T. J. Crow and F. Owen. ^{3}H-Flupenthixol binding in post-mortem brains of schizophrenics: Evidence for a selective increase in dopamine D2 receptors. *Psychopharmacology (Berlin)* **74:** 122–124, 1981.
6. Fiegenbaum, J., J. Vanai, R. Blass, B. Moon and H. Klawans. The effect of drugs altering striatal dopamine levels on apomorphine induced stereotypy. *Pharmacol Biochem Behav* **16:** 235–240, 1982.
7. Holford, N. MKMODEL: A modelling tool for microcomputers. *Clin Exp Pharmacol Physiol* Suppl **12:** 179, 1984.
8. Joyce, J. Multiple dopamine receptors and behaviour. *Neurosci Biobehav Rev* **7:** 227–256, 1983.
9. Leff, S., M. Hamblin and I. Creese. Interactions of dopamine agonists with brain D1 receptors labelled by ^{3}H antagonists. *Mol Pharmacol* **27:** 171–183, 1985.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
11. Memo, M., J. E. Kleinman and I. Hanbauer. Coupling of dopamine D_1 recognition sites with adenylate cyclase in nuclei accumbens and caudatus of schizophrenics. *Science* **221:** 1304–1307, 1983.
12. Nielsen, M., V. Klimek and J. Hyttel. Distinct target size analysis of dopamine D-1 and D-2 receptors in rat striatum. *Life Sci* **35:** 325–332, 1984.
13. Onali, P., M. Olianas and G. Gessa. Characterization of dopamine receptors modulating inhibition of adenylate cyclase in rat striatum. *Mol Pharmacol* **28:** 138–145, 1985.
14. Sanders, V. Neurologic manifestations of myxoedema. *N Engl J Med* **266:** 547–603, 1962.
15. Stoof, J. and J. Kebabian. Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature* **294:** 366–368, 1981.
16. Ungerstedt, U. Central dopamine receptors and unconditioned behaviour. In: *The Neurobiology of Dopamine*, edited by A. Horn, J. Korf and B. Westerlink. London: Academic Press, 1979, pp. 132–180.