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EFFECT OF THYROID STATE ON HISTAMINE H_1 RECEPTORS IN ADULT AND DEVELOPING RAT BRAIN*

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Abstract—The effect of thyroid status on histamine H_1 receptors in adult and developing rat brain was investigated using the (^{3}H) mepyramine binding assay. Hypothyroidism induced by treatment with 6-*n*-propyl-2-thiouracil resulted in a 31% decrease in the density and total content of adult rat brain (^{3}H) mepyramine binding sites and a significant retardation of the developmental increase in H_1 receptor binding in neonates. At 30 days of age, when euthyroid rats reached binding levels of the adult, hypothyroid animals presented reductions of 22 and 39% in (^{3}H) mepyramine bound per unit weight and per brain respectively. In contrast, hyperthyroidism induced by treatment with L-thyroxine did not alter H_1 receptor numbers in the adult rat brain but accelerated the developmental increase in (^{3}H) mepyramine bound per unit weight that reached normal adult levels by 21 days of age. The results suggest that thyroid dysfunction during early life and adulthood may cause derangements of the histaminergic system in the brain.

Several lines of evidence, including morphological, biochemical and behavioral, have established that thyroid hormones have a marked influence on the functional development of the CNS [1]. In general, maturation of the brain is retarded in neonatal thyroid deficiency and advanced in hyperthyroidism [2]. The critical effect that thyroid hormones have on neuronal differentiation is probably a manifestation of their regulation of protein biosynthesis for the elaboration of structures characteristic of the differentiated state, as are those involved in neurotransmission [3–5]. Neonatal and adult thyroid activity influence the levels and turnover rates of brain catecholamines and serotonin [6–9], as well as the density and the sensitivity of the monoamine receptors [10–18]. Thus, imbalances in neurotransmitter function may be of relevance in the mental disturbances resulting from thyroid dysfunction during the critical period of postnatal development.

Histamine (HA) is now believed to play a neurotransmitter role in the CNS and it has been implicated in processes such as arousal, locomotor activity, cardiovascular function and thermoregulation, which are altered in dysthyroid states. Both types of HA receptors H_1 and H_2 mediate HA responses in the brain (for reviews see [19–21]).

Thyroidectomy has been reported to greatly increase the activity of the neuronal histamine-synthesising enzyme, histidine decarboxylase, in rabbit hypothalamus [22]. Furthermore, studies in our laboratory have shown that hyperthyroidism accelerates, while hypothyroidism retards, the ontogenic development of HA levels in the rat brain [23, 24]. Thus, thyroid activity may also affect the histaminergic system in brain.

The direct labeling of HA H_1 receptors with the antagonist (^{3}H)mepyramine has permitted to establish the presence of H_1 receptors in mammalian brain, and has provided information on their numbers, characteristics, distribution and ontogenic development [25–28].

In the present study we have used the (^{3}H) mepyramine binding assay to investigate the effect of experimentally induced hyper- and hypothyroidism on rat brain HA H_1 receptors in the adult state and during ontogenic development.

A preliminary account of this work has been published in abstract form [29].

MATERIALS AND METHODS

Chemicals. (^{3}H) Mepyramine ((^{3}H) MEP), 24 Ci/mmole, was obtained from the Radiochemical Center (Amersham, U.K.). L-thyroxine (T_4), sodium salt, 6-*n*-propyl-2-thiouracil (PTU) and mepyramine maleate were purchased from Sigma Chemical Co. (St. Louis, MO). Tripolidine hydrocloride was a generous gift from Gayoso–Wellcome, S.A.

Treatments. Studies were carried out using Sprague–Dawley rats. Adult animals were age and weight (200–250 g) matched male rats. Control animals were maintained on a normal diet. Hypothyroidism was induced by supplying PTU (0.1% w/v) in the animals' drinking water for 21 days as described by Krawietz *et al.* [30]. Hyperthyroidism

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Abbreviations: HA, histamine; (^{3}H) MEP, (^{3}H) mepyramine; T_4, L-thyroxine; PTU, 6-*n*-propyl-2-thiouracil.

was induced by daily subcutaneous injections of T_4 at a dose of 0.75 μg/g body wt for 3 weeks [31]. A group of PTU-treated animals received daily subcutaneous injections of T_4 (0.75 μg/g body wt) simultaneously, as replacement therapy.

Newborn animals (male and female), 8–10 per litter, were kept with their mothers throughout the study period at 24° and a day–night cycle of 12 hr. Hypothyroidism was induced in infant animals by substituting PTU solution (0.05% w/v) for their mother's drinking water 10 days before parturition and maintaining them on this throughout the weaning period [32]. Litters of untreated rats born on the same day and reared by normal mothers under similar ambient conditions constituted the control groups. To induce hyperthyroidism animals were given daily subcutaneous injections of T_4 at a dose of 0.3 μg/g body wt [11]. Half the animals of each litter were injected with the same vol. of the vehicle (50 μl NaCl 0.9% w/v) and used as controls. Replacement therapy was given to half litters of PTU treated animals, by daily subcutaneous injections of T_4 (0.3 μg/g body wt). Since no significant differences were observed in preliminary experiments between control animals of each experimental group for any of the parameters measured, for practical purposes controls were pooled.

Treatments were continued until 24 hr before sacrifice.

Receptor assay. Animals were killed by decapitation at specific ages up to 30 days (newborns) and 2 months (adult). Brains were rapidly removed and chilled. Tissue was homogenized with a polytron (Rafer $T_{10/20'}$ setting 5) for 30 sec in 30 vol. of ice-cold 50 mM Na/K phosphate buffer pH 7.5 and membranes were sedimented at 50,000 *g* for 15 min. The pellet was resuspended and resedimented twice with the same vol. of fresh buffer.

Binding of (^{3}H) MEP to brain membranes was assayed essentially as described by Tran *et al.* [25]. Membrane suspensions (0.7 mg of protein) were incubated with 7–9 concentrations of (^{3}H) MEP, ranging from 1 to 20 nM, in a total vol. of 0.5 ml of Na/K phosphate pH 7.5. Incubations were carried out at 25° for 45 min and terminated by adding 4 ml of ice-cold phosphate buffer. Samples were rapidly filtered under vacuum through Whatman GF/B glass fiber filters which had been previously soaked with 2 μM mepyramine. Filters were washed three times with 4 ml of ice-cold buffer and the radioactivity retained was counted in 10 ml of a toluene–triton X-100 (2:1, v/v) scintillation fluid at 36% efficiency. Samples were always run in triplicate.

Specific binding was defined as the difference between binding of the radioligand in the absence and in the presence of 2 μM triprolidine. At 4 nM (^{3}H) MEP, specific binding amounted to 65 and 55% of total binding in the adult and 5-day-old rat brain, respectively. Absolute values of non-specific binding were not affected by the treatments. Protein was estimated by the method of Lowry *et al.* [33].

Statistics. The significance of the differences between means was evaluated using a one-way analysis of variance and a Newman–Keuls multiple range test [34] or the Student's *t*-test. Differences were considered statistically significant when $P \leq 0.05$. Least squares linear regression analysis was used to derive parameters from Scatchard plots [35].

RESULTS

General effects of PTU and T_4 treatments

The treatments utilized to induce experimental hypo- and hyperthyroidism, which have been shown to be effective in modifying the levels of circulating thyroid hormones [30, 32, 36], caused typical behavioral and somatic alterations in adult and newborn rats. Whereas PTU-treated animals appeared hypoactive, T_4-treated rats were clearly hyperactive. Maturational changes such as opening of the eyes, snout elongation and appearance of incisors were delayed by PTU treatment and accelerated by T_4 treatment. In addition, both treatments caused reductions in body and brain wts in developing animals, which became significant after the second postnatal week. Retardation in body wt gain was more pronounced in hypothyroid animals, which showed 30% of the control body wt at 30 days of age compared to 75% in the hyperthyroid rats. The reduction in brain wt was of similar magnitude in both experimental groups (around 20% at 30 days of age). No

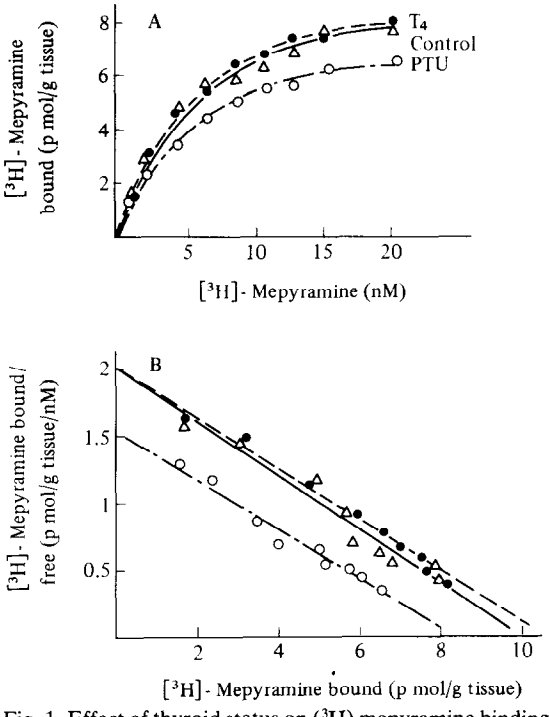

Fig. 1. Effect of thyroid status on (^{3}H) mepyramine binding to membranes from adult rats. A. Specific binding of (^{3}H) mepyramine ((^{3}H)MEP) to brain membranes from euthyroid (△——△), 6-*n*-propyl-2-thiouracil (PTU) treated (○—·—·—○) and L-thyroxine (T_4) treated (●———●) rats as function of increasing (^{3}H)MEP concentrations. Membrane suspensions (corresponding to 10 mg wet tissue) were incubated in triplicates, at 25° for 45 min, with (^{3}H)MEP concentrations ranging from 1 to 20 nM. Non-specific binding was determined in the presence of 2 μM triprolidine. Data are from a representative experiment run on six pooled brains for each experimental condition. B. Scatchard analysis of the same data.

Table 1. Effect of thyroid status on the maximal number of binding sites (B_{max}) and apparent dissociation constant (K_D) for (^{3}H) mepyramine in membranes from rat brain

(^{3}H) Mepyramine binding	Treatments			
	Control (N = 6)	PTU (N = 6)	T_4 (N = 6)	PTU + T_4 (N = 3)
B_{max}: fmoles/mg protein	156.80 ± 8.20	109.40 ± 7.10*	143.20 ± 6.40†	140.40 ± 3.60†
pmoles/g tissue	11.02 ± 0.57	7.59 ± 0.49*	9.93 ± 0.44†	9.87 ± 0.25†
K_D: nM	6.4 ± 0.5	3.9 ± 0.6*	4.7 ± 0.3	5.2 ± 0.5

Animals were treated with thyroxine (T_4), 6-*n*-propyl-2-thiouracil (PTU) or both simultaneously (PTU + T_4) as described in Materials and Methods. B_{max} and K_D values were determined by Scatchard analysis. Values represent means ± S.E.M. of *N* separate experiments like the one represented in Fig. 3. Statistically significant differences, $P < 0.05$ (Newman–Keuls test).

* vs controls, † vs PTU-treated.

changes were observed in the brain wts of treated adult animals.

In agreement with other studies [10, 12, 14], thyroid status did not affect the protein content in the membrane fraction (50,000 *g* particulate) used for receptor assays, which increased from 27.9 ± 0.7 mg/g wet tissue (N = 6) in the 5-day-old animal to 68.2 ± 4.0 mg/g wet tissue (N = 5) in the 21-day-old, a value not significantly different from the adult 69.7 ± 4.0 mg/g (N = 6).

Effect of dysthyroidism on (^{3}H) *MEP binding in the adult rat brain*

Specific binding of (^{3}H) MEP to brain membranes was saturable in controls as well as PTU-treated and T_4-treated rats (Fig. 1A). Hypothyroid animals presented lower (^{3}H) MEP binding values than euthyroid rats, while the saturation curve for hyperthyroid rats overlapped that of controls. Scatchard analysis (Fig. 1B) of the saturation data resulted in linear plots indicating a single class of (^{3}H) MEP binding sites in brain membranes from eu- and dysthyroid animals. The maximal number of binding sites (B_{max}) and the equilibrium dissociation constants (K_D) were calculated from individual Scatchard analysis of a number of experiments and average values are shown in Table 1. Results demonstrate that experimental hypothyroidism induced in adult rats by treatment with PTU caused a significant decrease of 31% in the maximal number of binding sites for (^{3}H) MEP expressed as fmoles/mg of protein or pmoles/g wet tissue, whereas T_4 treatment had no effect. Thus, hypothyroidism causes a diminution of both the density and total content of H_1 receptors in rat brain. PTU-treated animals receiving replacement therapy with T_4 presented B_{max} values no different than control euthyroids (Table 1), indicating that the effect of PTU treatment on the density of (^{3}H) MEP binding sites is mainly due to a deficit of thyroid hormone.

The equilibrium dissociation constant (K_D) of the PTU-treated group was also significantly lower than the K_D of controls. However, this change in affinity may not be real since the K_D of the PTU-treated animals did not differ significantly from the K_D of the T_4- and PTU + T_4-treated groups which are, moreover, similar to that of controls.

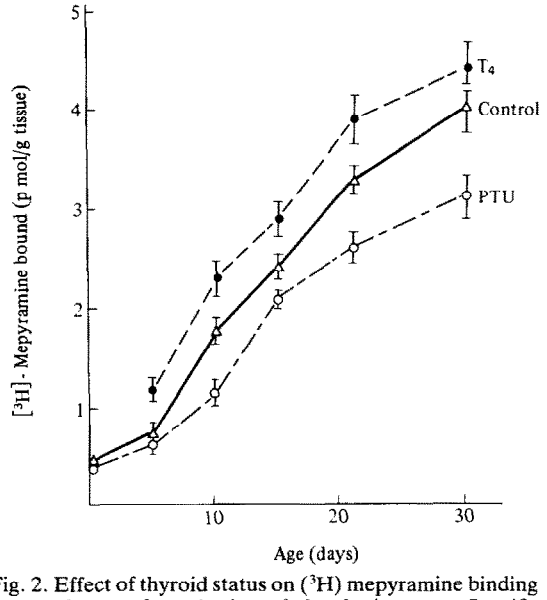

Fig. 2. Effect of thyroid status on (^{3}H) mepyramine binding to membranes from brains of developing rats. Specific binding was measured at 4 nM (^{3}H)MEP. Results are average values ± S.E.M. of 5–20 individual determinations performed in triplicate. △——△ control; ○—·—·—○ hypothyroid (PTU); ●———● hyperthyroid (T_4). Significant effects of the treatments $P < 0.05$ (Newman–Keuls test): PTU vs control at 10, 21 and 30 days; T_4 vs control at 5, 10, 15 and 21 days; PTU vs T_4 at all ages.

Effect of dysthyroidism on (^{3}H) *MEP binding in the developing rat brain*

Specific binding of (^{3}H) MEP (4 nM) to membranes from newborn rat brain was detectable at birth and increased gradually with age (about 8-fold), reaching adult values around 30 days of age (Fig. 2). Neonatal hypothyroidism resulted in a significant retardation of the developmental increase in (^{3}H) MEP binding per unit wet wt (Fig. 2). At 30 days of age hypothyroid animals presented a reduction of 22% in the (^{3}H) MEP bound/g tissue. The effect was more pronounced when (^{3}H) MEP binding was calculated per whole brain; thus, at 30 days only 61% of the control value was attained [3.92 ± 0.2 pmoles/brain (N = 6) for PTU-treated and 6.45 ± 0.19 pmoles/brain (N = 7) for controls]. In contrast, hyperthyroidism resulted in an acceleration of the developmental increase in (^{3}H) MEP bound/g tissue, with

Table 2. Effect of thyroid status on the maximal number of binding sites (B_{max}) and apparent dissociation constant (K_D) for (^{3}H) mempyramine in brain membranes from newborn rats

Age	Treatments		
	Control	PTU	T_4
5 days			
B_{max} (fmoles/mg protein)	130.9 ± 16 (5)	131.9 ± 19 (3)	114.9 ± 27 (3)
(pmoles/g tissue)	3.70 ± 0.55	3.71 ± 0.50	3.20 ± 0.75
(pmoles/brain)	2.18 ± 0.32	1.97 ± 0.27	1.79 ± 0.42
K_D (nM)	6.0 ± 1.1	8.0 ± 2.1	3.7 ± 1.6
21 days			
B_{max} (fmoles/mg protein)	137.8 ± 5 (5)	96.1 ± 4 (4)*	168.9 ± 9 (3)*†
(pmoles/g tissue)	8.98 ± 0.24	6.5 ± 0.18*	10.96 ± 0.24*†
(pmoles/brain)	12.77 ± 0.34	7.80 ± 0.22*	13.70 ± 0.30†
K_D (nM)	6.0 ± 0.6	4.6 ± 0.6	6.8 ± 0.2

Results are average values ± S.E.M. of the number of separate experiments indicated in parenthesis. Each experiment was run on 5–8 brains of the same litter and treatment. B_{max} and K_D values were determined by Scatchard analysis of saturation curves obtained using seven (^{3}H) mepyramine concentrations ranging from 1 to 14 nM. Statistically significant differences, $P < 0.05$ (Newman–Keuls test).
* vs controls, † vs PTU-treated.

levels significantly higher than controls from 5 to 15 days of age and reaching (^{3}H) MEP binding values of the adult euthyroids at 21 days [3.9 ± 0.2 pmoles/g tissue (N = 7)]. However, T_4 treatment did not alter the developmental pattern of (^{3}H) MEP bound per whole brain.

In order to investigate if the differences observed in the developmental increase in (^{3}H) MEP binding between euthyroid and dysthyroid animals involved an alteration in the affinity of the receptor for the ligand or in the number of binding sites, we examined binding at increasing (^{3}H) MEP concentrations at two selected ages, 5 and 21 days. B_{max} and K_D values calculated by Scatchard analysis of saturation isotherms are shown in Table 2. Thyroid status had no significant effect on the density or total content of histamine H_1 receptors or the affinity for ^{3}H MEP in the 5-day-old rat brain. However, in the 21-day-old rats, hypothyroidism resulted in a significant decrease of the density (−30%) and total content (−39%) of brain H_1 receptors, whereas hyperthyroidism significantly increased the density (+24%) but not the total content of the receptors.

Table 3. Effect of thyroxine (T_4) treatment on B_{max} and K_D for (^{3}H) mepyramine binding to membranes from brains of 6-*n*-propyl-2-thiouracil (PTU)-treated 21-day-old-rats

Treatment	(^{3}H) Mepyramine binding	
	B_{max} (fmoles/mg protein)	K_D (nM)
PTU	89.9 ± 3	5.1 ± 0.6
PTU + T_4	111.2 ± 2*	6.1 ± 0.1

Half litters of PTU-treated animals received daily subcutaneous injections of T_4 (0.3 μg/g body wt) from birth. Results are average values ± S.E.M. of three separate experiments run on four brains of the same litter and treatment.
* Statistically significant difference from PTU-treated animals; $P < 0.05$ (Student's *t*-test).

No alteration in the affinity for the ligand was observed in either case.

In a separate set of experiments, the density of H_1 receptors in the brain of 21-day-old PTU-treated rats was significantly increased by 24% when the animals received replacement therapy with T_4 (Table 3), indicating that the reduction in HA H_1 receptors observed in the PTU-treated animals is most probably due to a deficit of thyroid hormones.

DISCUSSION

In the present work the triprolidine-sensitive (^{3}H) MEP binding has been used to determine alterations in brain H_1 receptor levels in dysthyroid rats. The concentration of triprolidine employed to define non-specific binding (2 μM) has been reported to overestimate (^{3}H) MEP specific binding in membranes from guinea pig lung parenchyma [37] and rat cerebral cortex [38]. However, under our experimental conditions when the inhibition of 2 nM (^{3}H) MEP by increasing concentrations of triprolidine was examined in whole rat brain, we observed a well-defined plateau from 4×10^{-7} M to 10^{-6} M triprolidine, before further inhibition was detected (not shown). Thus, the overestimation of specific binding by the use of 2 μM triprolidine was of little significance (3–4%) and does not affect our findings since the absolute values of non-specific binding were not altered by the treatments utilized to induce experimental hypo- and hyperthyroidism.

The results of this study indicate that thyroid hormones regulate HA H_1 receptor levels in the rat brain. A deficit of thyroid hormones causes a significant reduction in H_1 receptors labeled with (^{3}H) MEP in the adult animal. Furthermore, the developmental increase in brain H_1 receptors is significantly accelerated in neonatal hyperthyroid rats and depressed in hypothyroidism. While hyperthyroid animals reach brain H_1 receptor densities of the normal adult rat at 21 days of age, thyroid deficient rats show significantly decreased (^{3}H) MEP

binding even at 30 days, when control animals reach adult levels. Whether the reduced numbers of H_1 receptors observed in the brains of the 30-day-old hypothyroid rats are simply due to retarded maturation or will remain low at later ages, was difficult to ascertain due to the high mortality rate of the animals when the treatment was continued further. However, the second possibility is supported by the observation that in the adult rat, experimental hypothyroidism induces similar decreases in brain H_1 receptor numbers, while hyperthyroidism has no effect.

Although specific lesions in the rat brain have given controversial results about the cellular location of HA H_1 receptors [39], autoradiographic [40], and subcellular fractionation studies [27] indicate the association of a major part of these receptors with neuronal elements. Also, the developmental pattern of (^{3}H) MEP binding in whole brain and several brain regions [27, 28] closely parallels the rise in the activity of histidine decarboxylase which is considered a marker for histaminergic neurons [20, 41]. Additionally, the development of H_1 receptors parallels the development of the stimulation of phospholipid turnover elicited by intracisternal administration of HA, a process mediated by H_1 receptors presumably at the neuronal comparment [28]. Thus, the significant reduction in H_1 receptors in the developing hypothyroid rat brain may be related to the impaired growth and arborization of neuronal processes and reduced synaptic density [42, 43]. On the other hand, the stimulation of neuronal protein synthesis and structural and biochemical differentiation induced by excess thyroid hormone [1] may explain the acceleration in the acquisition of the adult levels of HA H_1 receptors in the developing hyperthyroid rat.

Retardation and acceleration of the normal development of neurotransmitter receptors as a result of neonatal hypo- and hyperthyroid states, respectively, have been also reported for muscarinic-cholinergic and GABA receptors in the rat cerebelum [11]. However, only hypothyroidism had a delaying effect, in the development of β-adrenergic receptors [10], whereas both conditions reduced dopamine receptors in rat striatum [12] and increased brain $5HT_1$ and $5HT_2$ receptors [13]. Also, similar reductions to those observed by us, have been reported for α and β-adrenergic receptors in the hypothyroid rat brain cortex [14, 18].

In the case of the catecholamines and serotonin, alterations in the neurotransmitter systems at the presynaptic level resulting from adult and neonatal thyroid dysfunction have been described [6–9] and in some instances, attempts have been made to explain the changes in receptor density or sensitivity as adaptations to increased or decreased neurotransmitter synthesis or turnover [12, 13, 18].

During ontogenic development, the HA concentration in the rat brain is influenced by the thyroid status. In the newborn rat, HA levels which are 5–6 times higher than in the adult, are reduced in hyperthyroid animals and elevated in hypothyroidism, indicating an accelerated or retarded development of HA levels, respectively [23, 24]. However, it is not possible to speculate about the influence this alteration in HA levels could have on the development of HA receptors since most of the HA present in the newborn rat brain is of mast cell origin [44, 45]. On the other hand, thyroidectomy causes a 100% increase in the activity of the histamine-synthesising enzyme, histidine decarboxylase, in the hypothalamus of the rabbit [22]. If a similar alteration occurred in the hypothyroid rat brain, the reduced H_1 receptor density could represent an adaptive mechanism to an increased transmitter concentration at the synaptic site. However, it is not known at present if the thyroid state affects the synthesis or turnover of neuronal histamine in the adult rat. Another possibility is that thyroid hormone deficiency directly affects the synthesis of the receptor protein.

Although the functional state of the brain HA H_1 receptors would have to be examined, the distortion in the developmental pattern of H_1 receptors and the reduced H_1 receptor numbers in the brain of adult hypothyroid rats, observed in the present study, suggest that histaminergic neurotransmission may be impaired by thyroid dysfunction. This could be one more factor that contributes to the brain functional disorders associated to thyroid dysfunction during the neonatal period as well as in the adult state.

REFERENCES

1. D. H. Ford and E. B. Cramer, in *Thyroid Hormones and Brain Development* (Ed. Gilman D. Grave), p. 1. Raven Press, New York (1977).
2. R. Balazs, P. D. Lewis and A. J. Patel, in *Growth and Development of the Brain* (Ed. M. A. B. Brazier), p. 83. Raven Press, New York (1975).
3. S. E. Geel and P. S. Tamiras, in *Protein Metabolism of the Nervous System* (Ed. A. Lajtha), p. 335. Plenum Press, New York (1970).
4. R. Balazs, A. J. Patel and F. Hajós, *Psychoendocrinology* **1**, 25 (1975).
5. T. Valcana and N. L. Eberhardt, in *Thyroid Hormones and Brain Development* (Ed. Gilman D. Grave), p. 271. Raven Press, New York (1977).
6. R. L. Singhal, R. B. Rastogi and P. D. Hrdina, *Life Sci.* **17**, 1617 (1975).
7. A. Vaccari, T. Valcana and P. S. Timiras, *Pharmac. Res. Commun.* **9**, 763 (1977).
8. G. Engström, T. H. Svensson and B. Waldeck, *Brain Res.* **77**, 471 (1974).
9. J. H. Jacoby, G. Mueller and R. J. Wurtman, *Endocrinology* **97**, 1332 (1975).
10. R. M. Smith, A. J. Patel, A. E. Kingsbury, A. Hunt and R. Balazs, *Brain Res.* **198**, 375 (1980).
11. A. J. Patel, R. M. Smith, A. E. Hunt and R. Balazs, *Brain Res.* **198**, 389 (1980).
12. A. Vaccari and P. S. Timiras, *Neurochem. Int.* **3**, 149 (1981).
13. A. Vaccari, R. Biassoni and P. S. Timiras, *Eur. J. Pharmac.* **95**, 53 (1983).
14. G. Gross, O. E. Brodde and H. I. Schümann, *Eur. J. Pharmac.* **61**, 191 (1980).
15. G. Gross, O. E. Brodde and H. I. Schümann, *Archs Int. Pharmacodyn.* **244**, 219 (1980).
16. G. Gross, O. E. Brodde and H. J. Schümann, *Archs Pharmac.* **316**, 47 (1981).
17. G. Gross and H. J. Schümann, *J. Pharm. Pharmac.* **33**, 552 (1981).
18. C. K. Atterwill, S. J. Bunn, D. J. Atkinson, S. L. Smith and D. J. Heal, *J. Neural. Transm.* **59**, 43 (1984).

19. J. E. Taylor and E. Richelson, in *Neurotransmitter Receptors,* Part 2 (Ed. H. I. Yamamura and S. J. Enna), p. 71. Chapman and Hall, London (1981).
20. J. C. Schwartz, G. Barbin, A. M. Duchemin, M. Garbarg, C. Llorens, H. Pollard, T. T. Quach and Ch. Rose, in *Pharmacology of Histamine Receptors* (Ed. C. R. Ganellin and M. E. Pearsons), p. 351. Wright PSG, Bristol (1982).
21. J. P. Green, *Handbook of Psychopharmacology* 18 (Ed. L. Iversen, S. D. Iversen and S. H. Snyder), p. 385. Plenum Press, New York (1983).
22. A. Björklund, R. Hakanson, A. Nobin and N. O. Sjöberg, *Experientia* **28**, 1232 (1982).
23. I. Blanco, E. Rodergas, F. Picatoste, J. Sabriá and A. García, *Agents and Actions* **8**, 384 (1978).
24. J. Sabriá and I. Blanco, *X Congreso Sociedad Española de Bioquímica* (1982).
25. V. T. Tran, R. S. L. Chang and S. H. Snyder, *Proc. natn. Acad. Sci. U.S.A.* **75**, 6290 (1978).
26. J. J. Hill and J. M. Young, *Br. J. Pharmac.* **68**, 687 (1980).
27. V. T. Tran, A. D. Freeman, R. S. L. Chang and S. H. Snyder, *J. Neurochem.* **34**, 1609 (1980).
28. N. Subramanian, W. L. Whitmore, F. J. Seidler and T. A. Slotkin, *J. Neurochem.* **36**, 1137 (1981).
29. R. Codolà and A. García, *Neurosci. Lett.* **110**, S119 (1982).
30. W. Krawietz, K. Werdan and E. Erdmann, *Biochem. Pharmac.* **31**, 2463 (1982).
31. T. Ciaraldi and G. V. Marinetti, *Biochem. biophys. Res. Commun.* **74**, 984 (1977).
32. P. J. Burstein, B. Drazmin, C. J. Johnson and D. S. Schalch, *Endrocrinol.* **104**, 1107 (1979).
33. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
34. G. W. Snedecor and W. G. Cochran, *Statistical Methods,* 6th edn, p. 271. Iowa State University Press, Ames (1967).
35. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
36. A. J. Patel, P. D. Lewis, R. Balazs and M. Lai, *Brain Res.* **172**, 57 (1979).
37. H. Carswell and S. R. Nahorski, *Eur. J. Pharmac.* **81**, 301 (1982).
38. M. Garbarg, H. Pollard, T. T. Quach and J. C. Schwartz, in *Methods in Biogenic Amine Research* (Ed. S. Parvez, T. Nagatsu, I. Nagatsu and H. Parvez), p. 623. Elsevier (1983).
39. R. S. L. Chang, V. T. Tran and S. H. Snyder, *Brain Res.* **190**, 95 (1980).
40. J. M. Palacios, J. K. Wamsley and M. J. Kuhar, *Neurosci.* **6**, 15 (1981).
41. T. Watanabe, Y. Taguchi, S. Siosaka, I. Tanaka, H. Kubota, Y. Terana, M. Tohyana and H. Wada, *Brain Res.* **295**, 13 (1984).
42. J. L. Nicholson and J. Altman, *Brain Res.* **44**, 13 (1972).
43. C. A. Battie and M. A. Verity, *Devl Brain Res.* **3**, 219 (1982).
44. M. P. Martres, M. Baudry and J. C. Schwartz, *Brain Res.* **83**, 261 (1975).
45. I. Ferrer, F. Picatoste, E. Rodergas, A. García, J. Sabriá and I. Blanco, *J. Neurochem.* **32**, 587 (1979).