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Reduced number of α_2-adrenoceptors in cortical brain membranes of hypothyroid rats

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Behavioural as well as biochemical studies suggest that changes in the thyroid state influence noradrenergic neurotransmission in the central nervous system. The spontaneous and the (+)-amphetamine-induced locomotor activity of rats is increased during hyperthyroidism and decreased in hypothyroidism (Coville & Telford 1970; Emlen et al 1972). These findings agree with an enhanced noradrenaline synthesis and/or turnover in hyperthyroidism and a reduced turnover in the brains of hypothyroid animals (Engström et al 1974; Jacoby et al 1975; Strömbom et al 1977). However, there is some evidence that postsynaptic mechanisms may contribute to the alterations of central noradrenergic neurotransmission in dysthyroid states. Hyperthyroid rats displayed an increased sensitivity to the activating effect of noradrenaline administered intraventricularly (Emlen et al 1972) and repeated thyroxine treatment of mice enhanced the locomotor activity due to α-adrenoceptor stimulation by clonidine after previous depletion of endogenous catecholamines by reserpine and simultaneous inhibition of catecholamine synthesis (Strömbom et al 1977). Recently, we demonstrated that β-adrenoceptor mediated cAMP accumulation and the number of [^{3}H]dihydroalprenolol binding sites were decreased in the cerebral cortex of hypothyroid rats (Gross et al 1980a, b).

The density of α_1-adrenoceptors, determined by [^{3}-H]-WB4101 {2-(*N*-[2,6-dimethoxyphenyloxyethyl])-amino methyl-1,4-benzodioxane} binding was also reduced in hypothyroidism and increased due to triiodothyronine treatment. The affinities of α_1-adrenoceptors remained unaltered (Gross, Brodde & Schümann submitted for publication). The following experiments were performed to find out whether α_2-adrenoceptors in the cerebral cortex determined by a [^{3}H]clonidine binding assay underlie similar changes in dysthyroid states.

Methods and materials

Male Wistar rats, initially 270–320 g, were made hypothyroid by feeding pellets containing 0·15% 6-propyl-2-thiouracil (PTU) as described by Nakashima & Hagino (1972) for 8 weeks. This treatment was shown to reduce thyroxine serum levels to 28% of control values (Gross et al 1980a). Hyperthyroidism was induced by daily i.p. injections of triiodothyronine (500 μg kg^{-1}) for 10 days. [^{3}H]clonidine binding was essentially according to U'Prichard et al (1977). From the brain, immediately after decapitation, a crude membrane fraction was prepared by homogenizing 1 cerebral cortex in 5 ml 0·05 M Tris-HCl buffer pH 7·7 for 20 s using an Ultraturrax. The homogenate was centrifuged at 500 *g* for 15 min, the pellet was washed by rehomogenization in 5 ml fresh buffer solution and centrifuged as before. The final pellet was suspended by sonication. Binding was assayed by incubating 0·95 ml samples of membrane suspensions containing 20 mg of the original wet weight of the tissue with various concentrations of [^{3}H]clonidine (New England Nuclear, specific activity 22·2 Ci mmol^{-1}) ranging from 0·5 to 15 nM at 25 °C for 30 min in duplicate. The final assay volume was 1 ml. Incubations were terminated by filtration under reduced pressure through Whatman GF/C filters. Filters

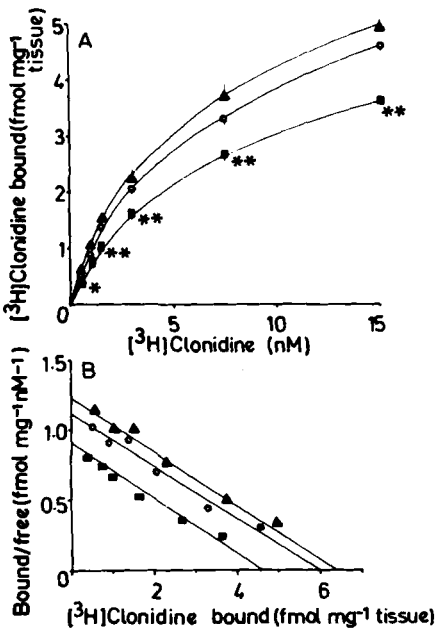

FIG. 1. Specific binding of [^{3}H]clonidine to membranes from the cerebral cortex (A) of euthyroid (—○—, n = 11), 6-propyl-2-thiouracil-fed (—■—, n = 11) and triiodothyronine-treated (—▲—, n = 6) rats. Membrane suspensions were incubated with [^{3}H]clonidine concentrations ranging from 0·5 to 15 nM. Unspecific binding was determined in the presence of 10 μM clonidine and subtracted from total binding. The means = s.e.m. of n experiments are given. $^*P < 0·05$ $^{**}P < 0·005$ compared with values from euthyroid animals. (B) Scatchard analysis of the same data. Data were subjected to linear regression analysis (r = 0·98–0·99).

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Table 1. Effect of triiodothyronine (T_3) and 6-propyl-2-thiouracil (PTU) treatment on density and affinity of [^{3}H]clonidine binding sites in membranes from the rat cerebral cortex. Values represent means ± s.e.m. of n separate experiments. The maximal number of binding sites (B_{max}) and equilibrium dissociation constants (K_D) were determined by Scatchard analysis using the data from Fig. 1.

Treatment	N	B_{max} (fmol mg^{-1} protein)	K_D (nM)
None	11	139 ± 6	5·7 ± 0·4
T_3	6	155 ± 4$^{n.s.}$	5·6 ± 0·5
PTU	11	112 ± 8* **	6·6 ± 0·8

*$P < 0·02$ compared with untreated animals, **$P < 0·005$ compared with T_3 treated animals, n.s. = not significantly different from untreated animals.

were washed three times with 5 ml ice-cold buffer and dried at 100 °C. The retained radioactivity was counted by liquid scintillation spectrometry at 49% efficiency. Specific binding was defined as that inhibited by 10 μM clonidine. It amounted to 88% at 3 nM [^{3}H]clonidine. The number of binding sites (B_{max}) and the equilibrium dissociation constant (K_D) were determined by Scatchard analysis. The protein content was measured according to Lowry et al (1951). The means ± s.e.m. of n determinations are given. Significance levels were determined by Student's *t*-test.

Results and discussion

The influence of altered thyroid states on specific [^{3}H]clonidine binding is demonstrated in Fig. 1. In saturation experiments, hypothyroidism resulted in decreased binding to cortical brain membranes at each concentration of the radioligand tested, the mean values being significantly lower than controls at 0·5 and 1·5 to 15 nM. Values obtained from triiodothyronine-treated animals were slightly higher than controls. However, these differences did not reach significance. PTU-treatment reduced the maximal number of binding sites (B_{max}) by 19%. B_{max}-values in hyperthyroid animals were 38% higher than those calculated for hypothyroid rats (Table 1). The equilibrium dissociation constants (K_D) remained unaltered in hypo- and hyperthyroidism (Table 1) suggesting unchanged properties of [^{3}H]clonidine binding sites. This assumption is further supported by the fact that K_I-values for yohimbine and prazosin in euthyroid animals were in good agreement with those from PTU treated animals (Table 2). The higher affinity of yohimbine compared with prazosin to [^{3}H]clonidine binding sites confirms that this radioligand predominantly labelled α_2-adrenoceptors. Our results demonstrate that the number of α_2-adrenoceptors in the cerebral cortex depends on the thyroid state. As already described for β- and α_1-adrenoceptors, the number of receptors is reduced in hypothyroidism. In contrast to α_1-adrenoceptors, triiodothyronine treatment failed to cause a significant increase in the number of [^{3}H]clonidine binding sites. The affinities of these binding sites were not affected by either hypo- or hyperthyroidism. The reduction in α_2-

Table 2. Inhibition of [^{3}H]clonidine binding to membranes from the cerebral cortex of untreated and 6-propyl-2-thiouracil (PTU) treated rats by yohimbine and prazosin. Inhibition of [^{3}H]clonidine binding was determined by incubating the membrane suspensions with 5 nM [^{3}H]clonidine in the presence or absence of 5–8 concentrations of the α-adrenoceptor antagonists.

	K_I (nM)	
Treatment	Yohimbine	Prazosin
None	187 ± 43	3867 ± 716
PTU	202 ± 31	4106 ± 790

K_I values were calculated as

$$\frac{IC_{50}}{1 + (S/K_D)}.$$

Values represent means ± s.e.m. of 6–8 experiments.

adrenoceptor density is somewhat smaller than those found for β- and α_1-adrenoceptors, but it may well contribute to the overall behavioural changes observed in hypothyroidism. In preliminary experiments, using additional [^{3}H]clonidine concentrations (0·1–0·4 nM), we found a second binding site with a higher affinity (K_D 0·4–0·5 nM) which has already been reported by U'Prichard et al (1979). This binding site, however, which amounted up to 20% of the total number of [^{3}H]clonidine binding sites, could not be detected in all experiments. Thus a comparison between euthyroid and dysthyroid animals concerning this very high affinity binding site was not possible. The major part of [^{3}H]clonidine binding sites in the cerebral cortex and other brain areas seems to be located postsynaptically (U'Prichard et al 1979). The function of postsynaptic α_2-adrenoceptors is not well defined. They seem to mediate the clonidine-induced locomotor changes (Nomura & Segawa 1979). A decrease in α-adrenoceptor density may thus contribute to the reduced motor response due to (+)-amphetamine (Coville & Telford 1970). The mechanism by which hypothyroidism induces a decrease in α_2-adrenoceptor number remains to be elucidated. A reduced protein and/or receptor synthesis may be responsible for this phenomenon.

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Inhibition of gastric acid secretion by sodium cromoglycate and FPL 52694

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Histamine is present in the gastrointestinal tract of nearly all vertebrates in concentrations ranging from 1 μg to over 100 μg per gram of tissue (Vugman & Rocha e Silva 1966; Lorenz et al 1973). The highest concentrations are those in the stomach, particularly in the acid secreting fundus and body of the stomach. For many years histamine has been believed to play a part in the regulation of gastric acid secretion and Code (1965) has proposed histamine as a final common mediator which stimulates the parietal cell in response to other secretagogues. More recently, belief in the importance of histamine in regulating gastric acid secretion has been reinforced by the introduction of specific histamine H_2 receptor antagonists (Black et al 1972). In the experiments reported below, the effects of two mast-cell stabilizing agents have been studied on gastric acid secretion; sodium cromoglycate and FPL 52694 (5{2-hydroxypropoxy}-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid, sodium salt). Sodium cromoglycate has been shown to inhibit the release of histamine from mast cells in response to a number of stimuli (Cox et al 1970). FPL 52694 is approximately equiactive to sodium cromoglycate in both passive cutaneous anaphylaxis induced in the rat by IgE antibody and mast cell degranulation induced in rat skin by compound 48/80 (P. A. Riley, unpublished observations).

Stomachs were perfused in anaesthetized rats using a method similar to that described by Ghosh & Schild (1958). Male rats, 200–300 g, were fasted for 18 h before being anaesthetized with urethane (7·7 g kg^{-1} intraperitoneally). The stomach was perfused via a cannula in the oesophagus with 5% dextrose solution at 37°C, at a rate of 2 ml min^{-1}, and the effluent perfusate collected from a cannula in the pylorus. The perfusate was then passed over a pH electrode to provide a continuous record of pH and, by means of an anti-log unit, H^+ concentration. In some experiments the left cervical vagus was dissected, sectioned high in the neck and placed over bipolar platinum stimulating electrodes. The nerve was stimulated at supramaximal voltage using 2 ms pulses at a frequency of 6 Hz. Experiments were also carried out in rats in which both cervical vagi were cut during the preliminary dissection. In the experiments on dogs, beagles of either sex, 12–15 kg, were anaesthetized with thiopentone sodium (25 mg kg^{-1} i.v.), a cuffed endotracheal tube inserted and anaesthesia maintained with 1–1·5% halothane in a 2:1 N_2O/O_2 mixture. An Andersen's tube was passed into the stomach via the oesophagus and the optimal position determined by means of a water recovery test in which at least 18 ml of a 20 ml bolus of water given through the tube could subsequently be recovered. Gastric juice was then continuously aspirated and collected for 15 min periods. The volume of fluid collected in each 15 min period was measured and acid concentration was determined by titrating an aliquot to pH 7·0 with 0·1 M sodium hydroxide using a Radiometer Auto-burette.

In perfused rat stomachs, both sodium cromoglycate (0·1–10 mg kg^{-1} i.v.) and FPL 52694 (1·25–20 mg $kg^{-1}h^{-1}$ i.v.) significantly inhibited gastric acid secretion in response to pentagastrin (3·8 μg $kg^{-1}h^{-1}$ i.v.). The inhibition developed gradually, reaching a peak in 20–40 min with either sodium cromoglycate or FPL 52694 and the maximum inhibition produced by both drugs was about 40% (Fig. 1). The effects of FPL 52694 (5 mg $kg^{-1}h^{-1}$ i.v.) were also studied on gastric acid secretion evoked by stimulation of the cervical vagus and by infusing histamine (3 mg $kg^{-1}h^{-1}$ i.v.); the results are shown in Table 1. The response to vagal stimulation was inhibited by 40%, but there was no significant reduction in the response to histamine. After vagotomy, the response to pentagastrin in the rat was reduced by over 30%. However, FPL 52694 still produced a significant reduction in the remaining response.

In the anaesthetized dog, gastric acid secretion in response to pentagastrin was reduced by FPL 52694 in doses of 2·5–10 mg $kg^{-1}h^{-1}$. The maximum reduction in acid output with an infusion of 10 mg $kg^{-1}h^{-1}$ of FPL 52694 was

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