Biochemical Pharmacology, Vol. 30, pp. 565–569.

0006-2952/81/060565-05 $02.00/0

CHARACTERIZATION AND QUANTITATION OF ALPHA-ADRENERGIC RECEPTOR SUBTYPES IN RAT HYPOTHALAMUS

ANDRIES C. NEETHLING, BRIAN W. MCCARTHY and JOSHUA J. F. TALJAARD

MRC Neurochemistry Research Group, Department of Chemical Pathology, University of Stellenbosch, P.O. Box 63, Tygerberg 7505, South Africa

(*Received* 21 *August* 1980; *accepted* 7 *October* 1980)

Abstract—The binding of [^{3}H]dihydroergocryptine ([^{3}H]-DHE) to rat hypothalmic membranes was examined. Displacement of [^{3}H]-DHE by 100 nM phentolamine, prazosin and clonidine can be used to assay for total α-, α_1- and α_2-adrenergic receptor sites respectively. The α_1-adrenergic receptor content of the hypothalmus is 4.1 pmoles/g tissue whereas the α_2-level is 6.5 pmoles/g tissue. [^{3}H]-WB 4101 (α_1 selective) and [^{3}H]clonidine (α_2 selective) binding yielded similar levels of 3.2 and 5.8 pmoles/g tissue. It is concluded that [^{3}H]-DHE is a suitable ligand for the assay of α-adrenergic receptor subtypes under the conditions determined in this study.

The hypothalamus acts as a control centre for many behavioural and endocrine processes. In many cases the catecholamines noradrenaline and dopamine play an important role in these control mechanisms. A study of the hypothalamic receptors for the catecholamines and the way in which the number or affinity of these receptors can be modulated by other factors (e.g. hormones) could add to our knowledge of the way in which hormones and neurotransmitters act in concert to regulate hypothalamic control mechanisms.

The ergot alkaloid [^{3}H]dihydroergocryptine [^{3}H]-DHE), has been available for receptor assays for some years but conflicting reports on the specificity of the ligand for different neurotransmitter receptors have lead to some confusion. The ligand has been reported to bind to dopaminergic [2], α-adrenergic as well as to serotonergic receptors [2] in the peripheral and central nervous system [3]. Pharmacological evidence tends to support the biochemical finding that DHE binds to different receptors [4]. Recent reports have claimed that the binding of [^{3}H]-DHE can be used to estimate the α-adrenergic receptors in the brain [5]. However, subject to adequately controlled conditions with the aid of specific pharmacological agents it has been shown that [^{3}H]-DHE can also be used to estimate the dopamine receptor in the striatum [1] and pituitary [6]. Furthermore, it is also possible to distinguish between α_1 and α_2-adrenergic receptor subtypes in brain [7–9] and other tissues [10] if the different α-adrenergic receptor subtypes are blocked with specific agonists and antagonists.

In the face of these seemingly conflicting reports, this study was undertaken to validate the use of [^{3}H]-DHE for the quantitation of different α-adrenergic receptor subtypes in the hypothalamus of the rat as a prerequisite for an investigation of the effects of hormones on the hypothalamic α-adrenergic receptor.

MATERIALS AND METHODS

Membrane preparation. Female Wistar rats aged 70–100 days were used throughout the study. Animals were killed by decapitation between 0800 and 0900 a.m., the brain quickly removed from the cranium, washed in buffer, blotted dry and the hypothalamus dissected out on a chilled surface. Hypothalamic cubes were obtained by cutting the brain rostrally through the optic chiasm and caudally anterior to the mammary bodies. The hypothalmic sulci formed the lateral boundaries and the dorsal plane of the hypothalamic cube was formed by a section immediately above the anterior commisures as viewed from the rostral surface (±2 mm). Tissue was kept in ice-cold buffer until all the animals had been processed. The procedure usually took less than 60 seconds per animal.

Hypothalamic membrane preparations were prepared by homogenizing in 10 vol. 0.05 M tris–HCl buffer, pH 7.7 (25°) by 4 up and down strokes of a motor-driven Teflon pestle in a glass tube (clearance 0.15 mm; 2000 r.p.m.). Centrifugation at 40,000 *g* and rehomogenisation by the above procedure was repeated twice. The final membrane pellet was suspended in 50 volumes Tris–HCl buffer.

Ligands. [9,10(n)-^{3}H]Dihydroergocryptine (20 Ci/mmole) was purchased from the Radiochemical Centre, Amersham, whereas [phenoxy-3-^{3}H(N)]-WB-4101 (24.4 Ci/mmole) and [4-^{3}H]clonidine (22.2 Ci/mole) were obtained from New England Nuclear Corp., Boston, Mass. (−) Norepinephrine, dopamine and yohimbine were products of Sigma Chemical Co. The following drugs were donations from the companies mentioned in parentheses: dihydroergocryptine, apomorphine (Sandoz); haloperidol (Janssens Pharmaceuticals); pimozide (Ethnor Laboratories); clonidine (Boehringer, Ingelheim); prazosin (Pfizer) and phentolamine (Ciba-Geigy). Radio-active ligands were diluted

with ethanol and stored at −20°. Final dilutions were made with ethanol/tris (1:1) prior to use. Drugs were dissolved in ethanol at a concentration of 1 mM, stored at 4° and diluted with ethanol/buffer (1:1) prior to use. Catecholamine solutions were prepared freshly in 0.1% ascorbic acid on the day of use.

Binding assay. All binding experiments were carried out in triplicate. The concentration [^{3}H]-DHE was varied between 0 and 10 nM in saturation analyses experiments and a [^{3}H]-DHE concentration of 0.5 nM was employed for single point estimations and ligand displacement experiments.

Radioactive ligand (20 μl) were added to glass tubes containing 460 μl Tris–HCl buffer and 500 μl membrane preparation added to start the binding assay. It was found that the presence of ethanol (4%) in the incubation medium prevented adsorption of [^{3}H]-DHE to glassware without quantitavely influencing the results obtained. After incubation for 1 hr at 25° the contents of the tubes were filtered through Whatman GF/C glass fibre filters under vacuum and washed 3 times with 4 ml ice-cold Tris–HCl buffer. The separation step took less then 25 seconds. The filters were placed into counting vials and the residual radioactivity determined in 10 ml Instagel (Packard Instruments Co.) with a Beckman LS 9000 Liquid Scintillation spectrometer employing the H-number principle for automatic conversion of c.p.m. to d.p.m. The displacement of [^{3}H]-DHE by 100 nM phentolamine, 100 nM prazosin and 100 nM clonidine is defined as total α-, α_1- α_2-adrenergic binding sites respectively. The binding of [^{3}H]clonidine and [^{3}H]-WB-4101 was estimated by a procedure described in detail by U'Prichard *et al.* [12].

RESULTS

Assay of total α-adrenergic receptor sites. In the initial studies specific binding of [^{3}H]-DHE to the α-receptor was defined as the fraction of the ligand displaceable by 0.1 mM (−) norepinephrine [5]. Scatchard analysis of the data obtained from saturation equilibrium binding of [^{3}]-DHE to hypothalamic membranes yielded a dissociation constant (K_D) of 1.05 nM with no indication of more than one binding site being present. This is in agreement with previous reports [5]. The validity of this approach came under suspicion when it was found that dopaminergic drugs could not appreciably increase the displacement of [^{3}H]-DHE from rat striatal membranes in the presence of 0.1 mM (−) norepinephrine (Table 1). The striatum contains both α-adrenergic and dopaminergic sites. It is well-known that [^{3}H]-DHE binds to both sites with equal affinity [1, 14]. At the concentrations employed (100 nM) the dopaminergic drugs should specifically block dopaminergic receptors [1] although not necessarily quantitatively. If displacement by 0.1 mM (−) norepinephrine can be regarded as selective for the α-adrenergic sites [5] displacement of [^{3}H]-DHE by 0.1 nM (−) norepinephrine and dopaminergic drugs should be additive. The fact that this prediction is not borne out in practice (Table 1) can only be interpreted that 0.1 mM (−) norepinephrine is also displacing [^{3}H]-DHE from dopaminergic sites, leading to an overestimation of the α-adrenergic receptor.

Phentolamine, a potent α-adrenergic antagonist, has also been used extensively to block α-adrenergic receptor sites in *in vitro* binding studies. Recent reports [7, 8, 12] that phentolamine binds to different subclasses of the α-receptor with the same affinity prompted us to investigate the use of this drug for the estimation of total α-adrenergic receptors. The concentrations of phentolamine employed for the specific displacement of [^{3}H]-DHE from α-adrenergic receptor sites by different laboratories range from 10 μM to 100 nM [8, 11, 13, 21]. Displacement by these different phentolamine concentrations lead to widely differing results (Fig. 1). As phentolamine also has a relatively high affinity for the dopamine receptor (K_i for [^{3}H]-DHE = 1.5 μM) [14] it is important to estimate the phentolamine concentration that can selectively displace [^{3}H]-DHE from α-receptors. Phentolamine displacement of [^{3}H]-DHE from hypothalamic membranes is biphasic, suggesting the displacement from more than one binding site (Fig. 1(a)). The biphasic nature of the displacement curve is however not as clear as in the striatum [14]. Careful resolution of the displacement by lower phentolamine concentrations (1–500 nM) does nevertheless yield unequivocal proof for the presence of more than one binding site (Fig. 1(b)). The IC_{50} calculated for the site with high affinity for phentolamine was found to be 4.6 ± 0.4 nM ($n = 3$) whereas the IC_{50} for the low affinity site was found to be about 600 nM.

Table 1. Effects of dopamine agonists and antagonists on the displacement of 0.5 nM [^{3}H]-DHE from striatal membranes in the presence or absence of 0.1 mM (−) norepinephrine*

Displacing drug	% Displacement of [^{3}H]-DHE
(−)Norepinephrine	100
Haloperidol	77
Apormophine	16
Pimozide	75
Norepinephrine + haloperidol	108
Norepinephrine + apomorphine	103
Norepinephrine + pimozide	107
Haloperidol + pimozide + apomorphine	94

* Displacement by 0.1 mM (−) norepinephrine is defined as 100%. Dopaminergic drugs were added at concentrations of 100 nM. Results represent the mean of 2 experiments done in triplicate.

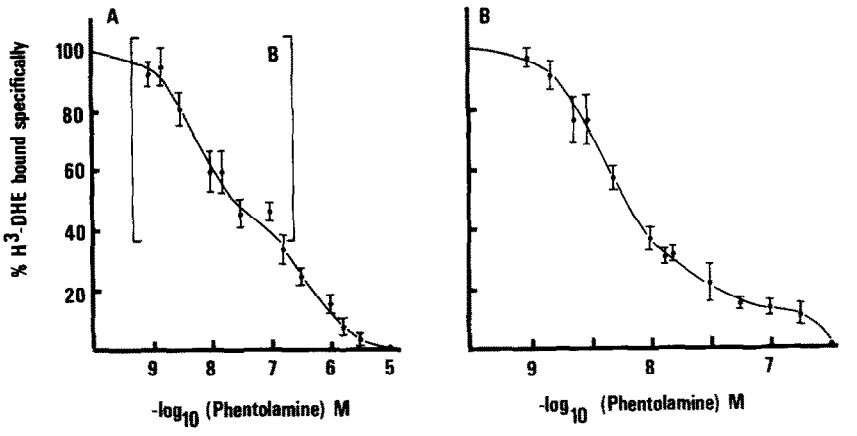

Fig. 1. Displacement of [^{3}H]-DHE (0.5 nM) from hypothalamic membranes by phentolamine (A). Displacement by phentolamine concentrations ranging from 1 nM to 10 μM. Displacement by 10 μM phentolamine defined as 100%. (B) Displacement by phentolamine concentrations ranging from 1–500 nM. Displacement by 500 nM defined as 100% displacement. Each point represents the mean + S.D. of three experiments (A) or four experiments (B) done in triplicate.

From the displacement curve (Fig. 1(b)) it was decided to investigate whether a concentration of 100 nM phentolamine could be used to displace [^{3}H]-DHE from the α-adrenergic binding sites selectively. Pharmacological proof for this assumption is presented in Fig. 2. If 100 nM phentolamine does displace [^{3}H]-DHE selectively from total α-adrenergic receptor sites, addition of other α_1, and α_2-specific ligands should not increase the displacement of [^{3}H]-DHE by phentolamine. Clonidine and prazosin (both at 100 nM) quantitatively blocks α_1- and α_2-adrenergic receptor sites respectively (Fig. 3; Table 2). Prazosin (100 nM) is about 5 times less efficient than phentolamine in displacing [^{3}H]-DHE from hypothalamic binding sites (Fig. 2 (2)) whereas clonidine is about 60 per cent as efficient as phentolamine (Fig. 2 (3)). Although the displacement of these two drugs are additive (Fig. 2 (4)) neither can increase the displacement of [^{3}H]-DHE by phentolamine Figs. 2 (4), (5), (6)). Furthermore, [^{3}H]-DHE displacement by haloperidol (Fig. 2 (10)) is additive to the phentolamine displacement (Fig. 2 (9), (11)) proving that 100 nM phentolamine does not displace [^{3}H]-DHE from dopamine receptors. Scatchard analysis of [^{3}H]-DHE binding in the presence of 100 nM yields a $K_D = 1.18 \pm 0.23$ nM and $B_{max} = 11.3 \pm 0.2$ pmoles/g hypothalamic tissue ($n = 3$).

Assay of α_1-adrenergic receptors. It has been reported that α-adrenergic receptors can be specifically blocked by relatively low concentrations of prazosin, making this drug a very suitable candidate for the selective displacement of [^{3}H]-DHE from hypothalamic α_1-adrenergic sites [7–9]. Displacement of [^{3}H]-DHE from hypothalamic membranes by prazosin is depicted in Fig. 3. Two sites can clearly

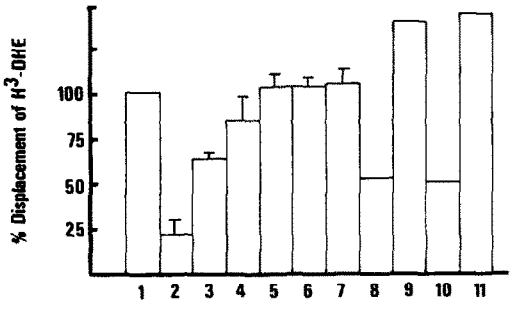

Fig. 2. Displacement of [^{3}H]-DHE (0.5 nM) from hypothalamic membranes by various α-adrenergic and dopaminergic drugs. All drugs were tested at concentrations of 100 nM. Experiments involving α-adrenergic drugs clonidine and prazosin represent the mean ± S.D. of 5 experiments in triplicate. Displacement by the dopamine blockers haloperidol and pimozide was the mean of 2 experiments done in triplicate. Displacement by phentolamine (1), prazosin (2), clonidine (3), prazosin plus clonidine (4), phentolamine plus prazosin (5), phentolamine plus clonidine (6), phentolamine plus clonidine plus prazosin (7), pimozide (8), phentolamine plus pimozide (9), haloperidol (10), haloperidol plus phentolamine (11). Displacement by 100 nM phentolamine represents 100%.

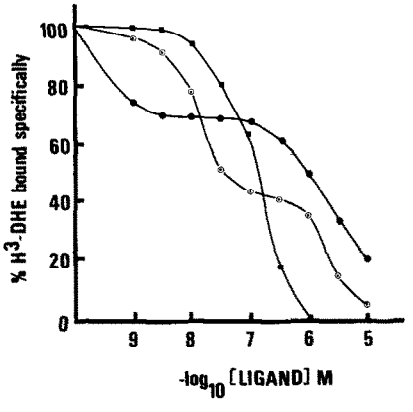

Fig. 3. Displacement of [^{3}H]-DHE (0.5 nM) from hypothalamic membranes by prazosin (—●—), clonidine (—○—) and yohimbine (—■—). Displacement by 100 nM phentolamine is defined as 100% displacement from α-adrenergic receptors in each case. Each point represents the mean of two experiments done in triplicate.

Table 2. α-Adrenergic receptor content of rat hypothalami and affinity of ligands for receptor subclasses*

Receptor	Ligand	Displacing drug	K_D (nM)	B_{max} pmoles/g hypothalamus
α Total	[^{3}H]-DHE	Phentolamine 100 nM	1.17	11.33
α_1	[^{3}H]-DHE	Prazosin 100 nM	1.07	4.1
α_1	[^{3}H]-WB 4101	Norepinephrine 0.1 mM	0.28	3.2
α_2	[^{3}H]-DHE	Clonidine 100 nM	0.97	6.5
α_2	[^{3}H]Clonidine	Norepinephrine 10 μM	2.54	5.8

* All parameters were obtained from Scatchard analyses of data obtained from 10–12 concentration points done in triplicate from at least 2 different experiments. Hypothalami from 8–10 animals were pooled form each binding isotherm.

be distinguished—a high affinity site with an IC_{50} for [^{3}H]-DHE (0.5 nM) of 0.8 nM and a second low affinity site with an IC_{50} of about 8 μM. This represents a 10,000-fold difference in affinity for these two sites making prazosin a powerful discriminator between these two binding sites. Scatchard analyses of saturable [^{3}H]-DHE binding to hypothalamic membranes in the presence of 100 nM prazosin (Table 2) which would be selective for the high-affinity site yields a $K_D = 1.1$ nM and a $B_{max} = 4.1$ pmoles/g hypothalamus ($n = 2$). The high-affinity binding for prazosin has been characterized pharmacologically in previous studies and has been classified as so-called α_1-adrenergic sites corresponding to pre-synaptic receptors in the peripheral nervous system [7]. It has been claimed that the α-blocker WB-4101 binds to the same site as the high-affinity prazosin site. Scatchard analyses of [^{3}H]-WB-4101 binding to hypothalamic membranes (Table 2) yielded a K_D of 0.28 nM, virtually the same as a previously published value [15]. The number of binding sites determined with this ligand (3.2 pmoles/g hypothalamus) compares favourably with the number estimated by [^{3}H]-DHE binding in the presence of 100 nM prazosin (4.1 pmoles/g hypothalamus).

Assay for α_2-adrenergic receptors. Previous claims that yohimbine exhibits some selectivity towards the α_2-adrenergic receptor were reinvestigated [7, 8]. The displacement of [^{3}H]-DHE from hypothalamic membranes by yohimbine did not give any indication of the presence of more than one site (Fig. 3). The reason for the difference in the results between the present study and previous reports [7, 8] is not clear. [^{3}H]Clonidine, a potent α-adrenergic agonist, is currently used to assay for α_2-adrenergic sites [5, 8, 12]. The displacement of [^{3}H]-DHE by clonidine was thus studied to ascertain whether this drug could be used to distinguish between α_1- and α_2-adrenergic sites. A marked biphasic displacement of [^{3}H]-DHE by clonidine from hypothalamic membranes was found (Fig. 3). IC_{50} for the high affinity site is 20 nM whereas the IC_{50} for the lower affinity is approx. 40 μM. The K_D of [^{3}H]-DHE for the high affinity site assayed by displacement with 100 nM clonidine was 0.93 nM and the B_{max} is obtained by Scatchard analyses was 6.5 pmoles/g hypothalamus. [^{3}H]Clonidine binding to hypothalamic membranes was found to be 5.8 pmoles/g hypothalamus (Table 2), comparing favourably to the abovementioned value obtained by [^{3}H]-DHE binding. The [^{3}H]-DHE binding displaceable by 100 nM clonidine thus represents a population of binding sites clearly dissociated from a lower affinity site. These high affinity sites are defined as α_2-adrenergic sites in the present study.

α-Adrenergic receptors in the rat hypothalamus. The quantitation of α-adrenergic receptors in rat hypothalami has been attempted before but previous values are either regional comparisons in different brain areas using single point estimations [5, 2] or can be criticized on the grounds of overestimation of α-adrenergic sites [13, 21]. No data is available on the relative α_1 and α_2-adrenergic receptor content of the rat hypothalamus. The levels as determined in the present study are depicted in Table 2.

DISCUSSION

Although the approach to the determination of receptor subtypes followed in the present investigation has been advocated before [7–9], a number of points in the practical application of the technique have come to light in the present study. Because of the relative lack of specificity of [^{3}H]-DHE for different receptor types the choice and concentration of drug for the displacement of [^{3}H]-DHE from specific receptor types need careful consideration. We have shown that the use of 0.1 mM (−) norepinephrine to displace [^{3}H]-DHE from α-adrenergic receptors [5, 16] leads to erroneous results. At this concentration of norepinephrine [^{3}H]-DHE, which binds to α-adrenergic and dopaminergic sites with equal affinity, is also displaced from the dopaminergic sites. None of the dopamine agonists and antagonists tested, could increase the displacement of [^{3}H]-DHE from striatal membranes to a significant extent in the presence of 0.1 mM (−) norpinephrine (Table 1). The same criticism can be levelled at the use of phentolamine at excessively high concentrations (1–10 μM) [13] as this drug can also displace [^{3}H]-DHE from dopaminergic receptors at high concentrations [1]. Evidence is presented in this investigation for a biphasic displacement of [^{3}H]-DHE from hypothalamic membranes by phentolamine which at 10^{-7}M selectively blocks the high-affinity site. Addition of the α_1-blocker prazosin and/or the α_2-agonist clonidine at concentrations that quantitatively displace [^{3}H]-DHE from α_1 and α_2 sites respectively (Fig. 3) does not increase phentolamine displacement (Fig. 2). Total displacement by these two drugs corresponds quantitatively to phentolam-

ine displacement. We feel confident that 100 nM phentolamine is able to displace [^{3}H]-DHE selectively from α-adrenergic sites. The finding that the displacement by the dopamine blockers pimozide and haloperidol is additive to phentolamine displacement (Fig. 2) lends additional proof to the α-adrenergic specificity of the displacement of [^{3}H]-DHE by 100 nM phentolamine.

Displacement of [^{3}H]-DHE by prazosin distiguishes very clearly between two sites. The high-affinity site is definitely not the same as the sites occupied by clonidine (Fig. 2) and is quantitatively similar to the number of sites estimated by [^{3}H]-WB 4101 binding. Unequivocal pharmacological characterization of the site as the so-called α_1-adrenergic receptor has been done in similar investigations [7–10] and no further proof of the identity of this site is necessary.

The displacement of [^{3}H]-DHE by yohimbine in the present study does not agree with previous findings that yohimbine displaces [^{3}H]-DHE from brain membranes in a biphasic pattern [7, 8] and that the high affinity site can be equated to the α_2-adrenergic receptor. A reasonable explanation for this conflicting finding is that the [^{3}H]-DHE concentration employed in the previous study [7] was much higher than in the present study. The serotonin receptor is also labelled by [^{3}H]-DHE at higher concentrations and it is well known that yohimbine can also interact with serontoninergic systems. Secondly, it is quite conceivable that the hypothalamus does not contain all the yohimbine displaceable receptor sub-types present in rat brain [8]. Thus, although yohimbine is undoubtly of use of distinguish between α-adrenergic receptor sub-types in other organs [10] it is of no use for this purpose in the hypothalamus.

[^{3}H]Clonidine is currently used to assay for α_2-adrenergic receptors. An investigation of the displacement of the [^{3}H]-DHE by clonidine yielded a biphasic displacement curve with a high-affinity site that is similar to the [^{3}H]-clonidine (α_2) binding site for the reasons outlined below. The displacement of [^{3}H]-DHE by 100 nM clonidine is additive to the displacement by 100 nM prazosin (Fig. 2) and quantitatively the same as the binding of [^{3}H]-clonidine to membranes (Table 2). Furthermore, it is quantitatively the same as the difference between phentolamine (total α sites) and prazosin (α_1 sites) displacement and not additive to displacement of [^{3}H]-DHE from hypothalamic membranes by 100 nM phentolamine (Fig. 2). We thus feel confident that displacement of [^{3}H]-DHE by clonidine is a suitable approach to the quantitation of the α_2-receptor.

Previous estimations of the α-receptor in the hypothalamus have either been over-estimations through use of inappropriately high concentrations of specific competitor [13, 21] or were comparative studies of the receptor content in different brain areas using a single point estimation [1, 12–14]. In the present study the relative amount of the two receptor subtypes in the hypothalamus was found to be 6.5 pmoles/g and 4.1 pmoles/g for the α_2 and α_1 species respectively. This seems to differ from the situation in whole rat brain preparations where the α_1 receptor content was found to be about 33 per cent higher than the α_2 receptors [17]. The *in vitro* estimation of α-receptor subtypes in the hypothalamus in the present study corresponds qualitatively to the findings of a recent autoradiographic study [18] where high densities of α_2 receptors were found in the limbic system and arcuate nucleus whereas high densities of the α_1 receptor were found in different areas of the brain (hippocampus, dentate gyrus). α-Adrenergic receptors seem to be localized both pre- and post-synaptically [19] and lesioning experiments with 6-hydroxy-dopamine have not been able to clarify the localization of α_1 and α_2 sites to pre- or post-synaptic membranes [20]. It is thus difficult to assign specific roles to the two receptor types in the hypothalamus.

The possible effects of gonadal hormones on hypothalamic α-adrenergic receptors during neonatal hypothalamic differentiation and during control of the reproductive cycle is currently under investigation.

Acknowledgements—We thank Ms M. Swanepoel for secretarial assistance. This research was supported by the South African Medical Council and the Provincial Administration of the Cape of Good Hope.

REFERENCES

1. M. Titeler, P. Weinreich and P. Seeman, *Proc. natn. Acad. Sci. U.S.A.* **74**, 3750 (1977).
2. A. Closse and D. Hauser, *Life Sci.* **19**, 1851 (1976).
3. L. T. Williams and R. J. Leftowitz, *Science* **192**, 791 (1976).
4. K. Fuxe, B. B. Fredholm, S.-O. Ögren, L. F. Agnati, T. Hokfelt and J.-Å. Gustafsson, *Fedn Proc.* **37**, 2181 (1978).
5. D. A. Greenberg and S. H. Snyder, *Molec. Pharmac.* **14**, 38 (1978).
6. M. G. Caron, J. Drouin, V. Raymond, P. A. Kelly and F. Labrie, *Clin. Res.* **24**, 256A (1976).
7. P. J. Miach, J.-P. Dausse and P. Meyer, *Nature* **274**, 492 (1978).
8. T. Haga and K. Haga, *Life Sci.* **26**, 211 (1980).
9. B. B. Hoffman and R. J. Leftkowitz, *Biochem. Pharmac.* **29**, 452 (1980).
10. B. B. Hoffman, A. De Lean, C. L. Wood, D. D. Schonken and R. J. Lefkowitz, *Life Sci.* **24**, 1739 (1979).
11. P. Seeman, J. L. Tedesco, T. Lee, M. Chau-wong, P. Muller, J. Bowles, P. M. Whitaker, C. McManus, M. Titeler, P. Weinreich, W. C. Friend and G. M. Brown, *Fedn Proc.* **37**, 130 (1978).
12. D. C. U'Prichard, D. A. Greenberg and S. H. Snyder, *Molec. Pharmac.* **13**, 454 (1977).
13. M. Wilkinson, H. Herdon, M. Pearce and C. Wilson, *Brain Res.* **168**, 652 (1979).
14. M. Titeler and P. Seeman, *Proc. natn. Acad. Sci. U.S.A.* **75**, 2249 (1978).
15. D. C. U'Prichard and S. Snyder, *Eur. J. Pharmac.* **51**, 145 (1978).
16. D. C. U'Prichard, D. A. Greenberg, P. Sheehan and S. H. Snyder, *Brain Res.* **138**, 151 (1977).
17. M. J. Morris, J.-P. Dausse, M. A. Devynek and P. Meyer, *Brain Res.* **190**, (1980).
18. W. Scott Young III and M. J. Kuhar, *Eur. J. Pharmac.* **59**, 317 (1979).
19. J. S. Aguilar, M. Criado and E. De Robertis, *Eur. J. Pharmac.* **61**, 47 (1980).
20. D. C. U'Prichard, W. Dietrich Bechtel, B. M. Rauot and S. H. Snyder, *Molec. Pharmac.* **16**, 47 (1979).