Br. J. Pharmac. (1979), **65**, 663–670

THE CHARACTERISTICS OF [^{3}H]-CLONIDINE BINDING TO AN α-ADRENOCEPTOR IN MEMBRANES FROM GUINEA-PIG KIDNEY

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1 [^{3}H]-clonidine binds to membranes prepared from guinea-pig kidney.

2 At 25°C the binding is rapid and saturable.

3 Scatchard analysis of the binding data showed that the K_d for [^{3}H]-clonidine binding in kidney membranes is 8.54 nM and the density of binding sites 12.5 pmol/g wet wt. tissue.

4 Hill plots of the binding data showed that there were no cooperative site interactions associated with binding.

5 [^{3}H]-clonidine binding could be displaced by drugs, the most potent being drugs with a high affinity for the α-adrenoceptor. The neuroleptic drugs (+)-butaclamol, *cis*-clopenthixol and *cis*-flupenthixol at high concentration also displaced [^{3}H]-clonidine binding.

6 Drugs acting as agonists or antagonists of β-adrenoceptors, histamine receptors, acetylcholine receptors as well as prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$, angiotensin II, arginine vasopressin, naloxone, nalorphine and pargyline had little effect on binding.

7 It is likely that the binding site labelled by [^{3}H]-clonidine in guinea-pig kidney membranes is an α-adrenoceptor similar in some pharmacological aspects to an α_2-adrenoceptor.

Introduction

It has been known for some time that clonidine inhibits renin release from the kidney both in man (Hökfelt, Hedeland & Dymling, 1970; Onesti, Schwartz, Kim, Paz-Martinez & Swartz, 1971) and in animals (Reid, MacDonald, Pachnis & Ganong, 1975; Pettinger, Keeton, Campbell & Harper, 1976). Not only is basal release of renin affected but also that induced by diuretics or stimulation of the sympathetic nervous system (Pettinger *et al.*, 1976). The effect of clonidine seems to be at least partly peripheral rather than central in origin, since it is not affected by either sympathetic neurone or ganglion blockade and in addition inhibition of renin secretion is seen in the isolated perfused kidney (Vandongen & Greenwood, 1975). Since α-adrenoceptor antagonists prevent the inhibition of renin release by clonidine, it has been suggested that the mechanism involved is stimulation of an intrarenal α-adrenoceptor (Berthelsen & Pettinger, 1977). The location of the intrarenal α-adrenoceptor has not yet been determined but clonidine could inhibit renin release by an action directly on receptors located at the surface of the granular cells of the juxtaglomerular apparatus or indirectly by a baroreceptor mechanism involving constriction of renal blood vessels (Vander, 1967).

There is now much pharmacological evidence to support the hypothesis that there are at least two groups of α-adrenoceptors: α_1-receptors which are usually present postsynaptically on membranes of noradrenergically innervated smooth muscle cells and α_2-receptors which are present on noradrenergic nerve terminals (Langer, 1974) in the rat superior cervical ganglion (Caulfield, 1978) and on melanocytes in frog skin (Pettinger, 1976).

These conclusions are largely based on the relative effectiveness of a series of α-adrenoceptor agonists and antagonists on pre- and postsynaptic receptors in a wide variety of tissues (for review see Starke, 1977). This method of classification would classify the intrarenal adrenoceptors as α_2 since the preferential α_2-agonist, clonidine, is a powerful inhibitor of renin secretion whereas the preferential α_1-agonist, methoxamine, has less than one sixtieth the potency in the same system (Pettinger *et al.*, 1976).

The development of binding assays utilising radioligands such as [^{3}H]-noradrenaline, [^{3}H]-adrenaline, [^{3}H]-clonidine, [^{3}H]-WB 4101 and [^{3}H]-dihydroergokryptine has considerably facilitated the characterization of α-adrenoceptors in brain (U'Prichard &

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0007-1188/79/040663-08 $01.00

Snyder, 1977; U'Prichard, Greenberg & Snyder, 1977; Greenberg & Snyder, 1978) and in the periphery (Williams, Mullikin & Lefkowitz, 1976, U'Prichard & Snyder, 1978; Summers, Jarrott & Louis, 1978a, b). In the experiments described below high specific activity [^{3}H]-clonidine was used to characterize its receptor binding sites in guinea-pig kidney membranes. Preliminary reports of some of these findings have been published (Summers *et al.*, 1978*a*, *b*).

Methods

Preparation of membranes

Male guinea-pigs (500 to 800 g) were rapidly anaesthetized with halothane and killed by exsanguination. The kidneys were excised, placed on ice, and the fat and connective tissue removed. The tissue was homogenized in 20 vol of 1 mM $MgCl_2$ with a Polytron PT10 homogenizer (full speed for 30 s). The pH of the homogenate was 7.4 and membranes prepared in this way exhibited the greatest specific binding of ligand. The homogenate was centrifuged at 45,000 *g* for 15 min at 4°C and the pellet resuspended in 20 vol 1 mM $MgCl_2$ before repetition of the centrifugation step. Finally the pellet was resuspended in 50 vol of 1 mM $MgCl_2$. Membrane suspensions were always prepared on the day of use since, unlike brain homogenates, binding was reduced by freezing and subsequent thawing.

Radioligand binding assay

Membrane suspension (1 ml) was added to an equal volume of 50 mM Tris/HCl pH 7.6 in disposable 4 ml polystyrene tubes. [^{3}H]-clonidine (5.29 Ci/mmol) was added to give a final concentration of 2.5 to 3 nM (Scatchard and Hill analysis) or 15 to 20 nM (drug displacement studies). The total [^{3}H]-clonidine binding to membranes expressed as a percentage of the total label added was always $<2\%$ for Scatchard & Hill analysis or $<1.2\%$ in drug displacement studies. Preliminary experiments showed that [^{3}H]-clonidine did not bind to the polystyrene tubes. The mixture was then incubated at 25°C for 30 min. Non-specific binding was estimated in assay tubes containing an identical mixture to which was added 1 or 10 μM unlabelled clonidine. In control experiments in which 10 μM phentolamine was used to estimate non-specific binding, similar values were obtained to those using clonidine. In addition none of the drugs which were effective displacers of specific binding had any significant effect on non-specific binding when tested at a concentration of 10 μM.

At the end of incubation the membranes were rapidly harvested by filtration on to Whatman GF/B glass fibre filters under vacuum at 4°C. The filters were rapidly washed with 3 × 5 ml aliquots of 50 mM Tris/HCl pH 7.6 containing 1 μM clonidine at 4°C. The inclusion of 1 μM clonidine in the washing solution was found to be essential in that it significantly lowered the filter blanks to non-specific binding levels without affecting specific binding.

[^{3}H]-clonidine was eluted from the filters with a toluene based scintillant (PPO 0.4%, POPOP 0.01%) in which clonidine is soluble and counted at approximately 55% efficiency with a Nuclear Chicago Isocap 300 scintillation counter. Conversion to d/min was made by the channels ratio method.

Calculation of results

The method of calculation of the results of Scatchard analysis was based on that described by Rodbard (1974). Hill analysis was performed on data involving addition of increasing amounts of unlabelled clonidine to membrane suspensions containing 2.5 nM [^{3}H]-clonidine. Drug inhibition studies were performed by subtracting non-specific binding from total binding and expressing binding as a percentage of that in the absence of inhibitors. Probit analysis was used to linearise plots and obtain an estimate of the IC_{50} for inhibition. This value was then used to calculate the inhibition constant (Ki) for each inhibitor using the expression (Cheng & Prusoff, 1973):

$$IC_{50} = K_i \left(1 + \frac{[D]}{K_d}\right)$$

where

IC_{50} = concentration producing 50% inhibition of binding
K_i = inhibition constant
[D] = [^{3}H]-clonidine concentration
K_d = dissociation constant for [^{3}H]-clonidine binding to kidney membranes.

Drugs

Drugs were dissolved in 0.01 M HCl on the day of experiment to give a 1 mM solution. Dilutions to the appropriate concentration were made in 50 mM Tris/HCl pH 7.6 immediately before use. Prostaglandins and prazosin were insoluble in aqueous solutions and were dissolved in 1 part alcohol and made up to 5 parts with water to give 1 mM solutions. Alcohol, in the highest concentration used (0.02%), had no effect on [^{3}H]-clonidine binding. In the experiments in which noradrenaline and adrenaline were tested for their ability to displace [^{3}H]-clonidine, the guinea-pigs were pretreated with pargyline (100

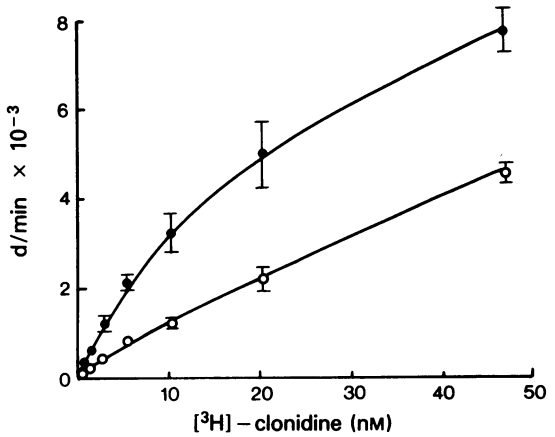

Figure 1 The effect of [^{3}H]-clonidine concentration on total (●) and non-specific binding (○) of [^{3}H]-clonidine to membranes prepared from guinea-pig kidney. Non-specific binding was defined as binding in the presence of excess (1 or 10 μM) clonidine. Error bars indicate the s.e. mean (n = 6, except for the highest concentration where n = 4).

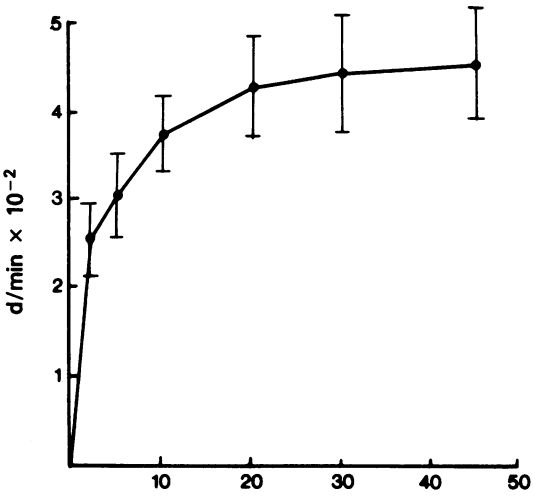

Figure 2 The time course of specific [^{3}H]-clonidine binding to membranes prepared from guinea-pig kidney. [^{3}H]-clonidine concentration ~2.5 nM, incubation temperature 25°C. Specific binding is defined as the difference between total [^{3}H]-clonidine binding and non-specific binding. Error bars indicate s.e. mean (n = 4).

mg/kg i.p.) 1 h before they were killed, to inhibit monoamine oxidase. In these experiments the 50 mM Tris/HCl pH 7.6 contained disodium edetate (EDTA) 1.5 mg/l and ascorbic acid 20 mg/l to prevent autoxidation of catecholamines. The following drugs were used: clonidine hydrochloride (Boehringer Ingelheim); naphazoline hydrochloride, xylometazoline hydrochloride, phentolamine hydrochloride, angiotensin II (Ciba); (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, (−)-isoprenaline bitartrate, dopamine hydrochloride, histamine dihydrochloride, pargyline hydrochloride (Sigma); oxymetazoline hydrochloride, atropine sulphate (Merck); phenylephrine hydrochloride (Koch-Light); piperoxane hydrochloride (Rhone-Poulenc); yohimbine hydrochloride (Baird Pharmaceuticals Ltd.); prazosin (Pfizer); labetalol hydrochloride, salbutamol (Allen and Hanbury); tazolol (Syntex); propranolol hydrochloride, pempidine tartrate (ICI); metoprolol bitartrate, H35/25 (1-(*p*-tolyl)-2-isopropylamino-1-propanol) (A.B. Hassle); apomorphine hydrochloride, (+)-butaclamol hydrochloride, (−)-butaclamol hydrochloride (Ayerst); *cis*-flupenthixol hydrochloride, *cis*-clopenthixol hydrochloride (H. Lundbeck); 4-methylhistamine dihydrochloride, metiamide (Smith, Kline and French); diphenhydramine hydrochloride (Parke-Davis); (+)-tubocurarine chloride (Burroughs-Wellcome); carbachol, nicotine bitartrate (British Drug Houses); prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ (Dr John E. Pike, Upjohn); arginine vasopressin (Ferring A.G.); naloxone hydrochloride, nalorphine hydrochloride (Endo Laboratories (Australia) Pty Ltd.); [^{3}H]-clonidine (5.29 Ci/mmol) (Roche).

Results

Specific and non-specific binding

Non-specific binding was estimated by addition of an excess (1 or 10 μM) of unlabelled clonidine to tubes containing a buffered suspension of kidney membranes and [^{3}H]-clonidine. Specific binding was taken to be the total amount of [^{3}H]-clonidine bound minus the non-specific binding. The changes in specific and non-specific binding for [^{3}H]-clonidine concentrations up to 47 nM is shown in Figure 1.

In the experiments designed to evaluate the dissociation constant (K_d) and the number of binding sites (B_{max}), specific binding represented 71.6 ± 1.3% (n = 7) of total binding whereas in the drug displacement studies in which higher concentrations of [^{3}H]-clonidine were used (at least 2 × K_d) specific binding represented 55.3 ± 1.3% of total binding (n = 45).

Time course of specific [^{3}H]-clonidine binding

The association of [^{3}H]-clonidine to its binding sites in guinea-pig kidney membranes was rapid as shown in Figure 2. Owing to the limitations of the filtration techniques it was not possible to examine binding accurately at times less than 2 min but even at this time, binding had reached a level more than half the eventual maximum. Equilibrium was reached 20 min

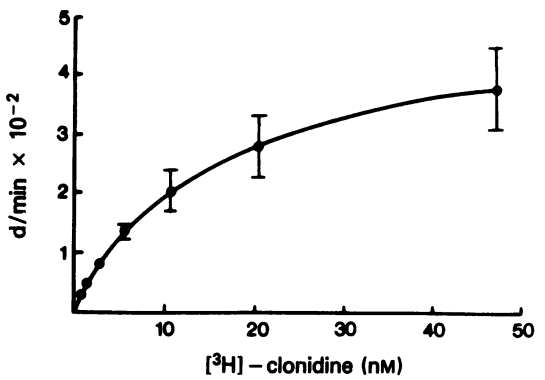

Figure 3 Saturation of specific [^{3}H]-clonidine binding to membranes prepared from guinea-pig kidney. Incubation temperature 25°C, incubation time 30 min. Error bars indicate s.e. mean (n = 6 except for the highest concentration where n = 4).

after the start of incubation so a time of 30 min was therefore routinely chosen as the time of incubation in subsequent experiments.

Saturation of [^{3}H]-clonidine binding

[^{3}H]-clonidine was added to buffer containing suspended kidney membranes to give concentrations varying from 0.65 to 47 nM and incubated for 30 min at 25°C. Non-specific binding was estimated in identical samples containing in addition 1 or 10 μM unlabelled clonidine. As seen in Figure 3 the binding isotherm is a hyperbolic curve which is approaching saturation at [^{3}H]-clonidine concentrations above 50 nM. From this graph an estimate can be made of the dissociation constant (K_d) for [^{3}H]-clonidine binding in guinea-pig kidney membranes which was approximately 10 nM.

Analysis of the characteristics of [^{3}H]-clonidine binding

[^{3}H]-clonidine (2.5 to 3 pmol) was added to buffer containing kidney membranes and also to identical tubes containing from 1 to 30 pmol of unlabelled clonidine. After incubation under equilibrium conditions, Scatchard analysis of the data indicated that in the concentration range used, the binding of [^{3}H]-clonidine is of high affinity to a single population of sites as shown for a typical experiment in Figure 4.

Scatchard analysis was carried out in each individual experiment and the values for the pooled data in all experiments gave a K_d of 8.54 ± 0.6 nM (n = 7) and B_{max} of 12.5 ± 0.7 pmol/g wet wt. tissue (n = 7).

Hill plots of the data in Figure 4 are shown in Figure 5. The line of best fit (estimated by the method

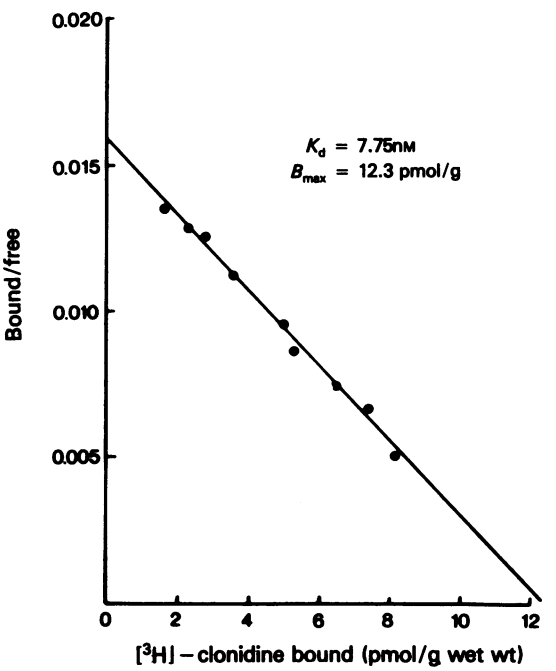

Figure 4 Scatchard plot of data obtained from a typical experiment by addition of increasing amount of clonidine to kidney membrane suspensions containing ~2.5 nM [^{3}H]-clonidine.

of least squares) was a straight line (r = 0.999) of gradient 0.993. The pooled data from seven experiments gave a mean gradient of 0.993 ± 0.004. These results strongly suggest that there are no co-operative site interactions involved in the binding of [^{3}H]-clonidine to sites in kidney membranes.

Displacement of [^{3}H]-clonidine binding by drugs

[^{3}H]-clonidine binding to guinea-pig kidney membranes could be displaced by drugs. Estimation of the potency of drugs as displacers is described in Methods. The most effective displacers were those drugs known to be agonists or antagonists of α-adrenoceptors. Table 1 shows that the order of potency with regard to α-adrenoceptor agonists was clonidine > naphazoline > adrenaline > noradrenaline > xylometazoline > oxymetazoline > phenylephrine. Antagonists of α-adrenoceptors were also effective displacers of binding the order of potency being phentolamine > yohimbine > piperoxane > labetalol > prazosin.

Another group of drugs which did displace binding, albeit at high concentrations, were drugs which are thought to act primarily on dopamine receptors. The dopamine receptor agonists, dopamine and apomorphine, displaced binding with inhibition constants

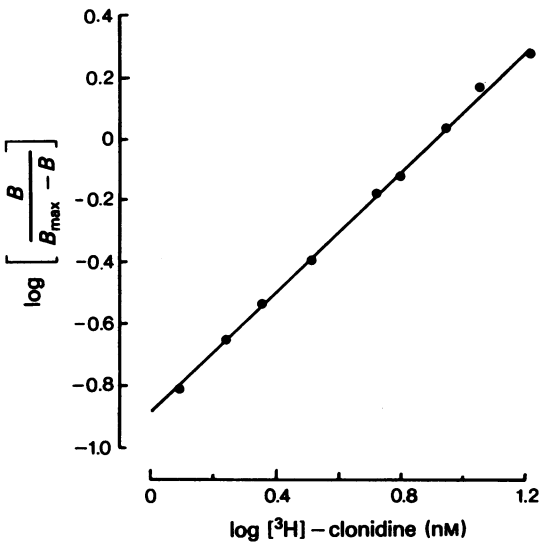

Figure 5 Hill plots of the data illustrated in Figure 4. The line of best fit was estimated by the method of least squares (r = 0.999); mean gradient = 0.993.

(K_i) of 2300 and 960 nM respctively (Table 2). Among dopamine receptor antagonists (+)-butaclamol and *cis*-clopenthixol were as effective as some of the α-adrenoceptor antagonists. The (−)-isomer of butaclamol which has little dopamine receptor antagonist potency was without effect on [^{3}H]-clonidine binding in concentrations up to 1000 nM.

It was of particular interest to examine the effects of drugs acting on histamine receptors since it has been suggested that clonidine stimulates histamine H_2-receptors in a number of tissues. However, the results provided little evidence to support the presence of H_2-receptors in the kidney since histamine, 4-methylhistamine and diphenhydramine were found to have K_i values in excess of 1 μM and metiamide in excess of 10 μM as shown in Table 2.

Drugs acting on other receptor systems were largely without effect. The following drugs, which act on β-adrenoceptors did not displace binding at 1000 nM: the β_1 and β_2-agonist isoprenaline, the β_1-agonist tazolol, the β_2-agonist salbutamol, the β_1- and β_2-antagonist propranolol, the β_1-antagonist metoprolol and the β_2-antagonist H35/25.

Drugs acting on acetylcholine receptors such as carbachol, nicotine, atropine, pempidine and (+)-tubocurarine had little effect on binding as did the monoamine oxidase inhibitor, pargyline, or prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$, or angiotensin II and arginine vasopressin. Naloxone and nalorphine which interact with opiate receptors were also ineffective at 1 μM concentrations.

Discussion

The binding of [^{3}H]-clonidine to membranes prepared from guinea-pig kidney is higher than that to any other tissue tested, except brain. The specific binding, using the filtration techniques represented a high proportion of total binding with the high specific activity [^{3}H]-clonidine used in these experiments. There can be problems associated with the use of an excess of unlabelled ligand to estimate non-specific binding, in particular that one could be looking at a 'clonidine receptor' rather than an α-adrenoceptor. Although such an approach could under certain conditions lead to erroneous results, as for instance in the case of [^{3}H]-prazosin and [^{3}H]-WB 4101 binding

Table 1 Inhibition of [^{3}H]-clonidine binding to membranes from guinea-pig kidney by α-adrenoceptor agonists and antagonists

Drug	IC_{50} (nM)	K_i (nM)
α-Adrenoceptor agonists		
Clonidine	86	27
Naphazoline	95	30
Adrenaline	450	140
Noradrenaline	560	170
Xylometazoline	790	260
Oxymetazoline	2400	780
Phenylephrine	13,000	4200
α-Adrenoceptor antagonists		
Phentolamine	71	24
Yohimbine	620	220
Piperoxan	1300	430
Labetalol	2500	880
Prazosin	3900	1400

in guinea-pig kidney membranes (Summers, unpublished observations), it is not a serious problem when [^{3}H]-clonidine is used as the ligand for the following reasons. The use of other drugs such as phentolamine with known actions on α-adrenoceptors gave similar non-specific binding values to clonidine, and the displacement of [^{3}H]-clonidine binding was almost exclusively by drugs which interact with α-adrenoceptors.

The binding of [^{3}H]-clonidine to membranes prepared from guinea-pig kidney has many characteristics which suggest binding to a single type of site. Association is rapid, saturable, and the process displays a high affinity and is reversible. It is interesting to compare the affinity of the [^{3}H]-clonidine binding in different tissues. The K_d value for binding in kidney membranes is about twice that observed in rat brain (U'Prichard *et al.*, 1977). The density of binding sites (12.5 pmol/g wet wt.) is similar to that observed in rat cortex using the same radioligand (U'Prichard *et al.*, 1977), but about four times greater than that in rat kidney membranes (U'Prichard, personal communication). The linearity of the Scatchard plot would support the argument that in the concentration range used, binding is to a single class of independent sites (De Meyts & Roth, 1975) as would the Hill analysis which gave straight lines of gradient ~1. The results of the Hill analysis would also indicate that there are no co-operative interactions involved in the combination of [^{3}H]-clonidine with its binding site i.e. the combination of each molecule of [^{3}H]-clonidine with its binding site does not affect the affinity of the remaining unoccupied sites for further interactions (De Meyts & Roth, 1975). Studies using a wide range of drugs indicate that the [^{3}H]-clonidine binding is to a site which resembles an α-adrenoceptor since it was most easily displaced by α-adrenoceptor agonists and antagonists. The binding was specific for an α-adrenoceptor since drugs acting on other receptor systems, including β-adrenoceptors, histamine receptors, acetylcholine receptors, opiate receptors and prostaglandins had little effect. It is worth commenting on the lack of effect of drugs acting on histamine H_2-receptors such as histamine, 4-methylhistamine and metiamide since it has been suggested that clonidine stimulates histamine H_2-receptors in brain (Karppannen, Paakkari, Paakkari, Huotari & Orma, 1976) and those controlling gastric acid secretion (Karppannen & Westermann, 1973). Apparently histamine H_2-receptors are not present in the kidney or the affinity of clonidine for this receptor is low compared with that for the α-adrenoceptor.

The absence of effect of nalorphine and naloxone is also noteworthy since it has been shown that clonidine possesses analgesic effects (Paalzow, 1974) and that [^{3}H]-naloxone binds to membranes from guinea-pig kidney (Simantov, Childers & Snyder, 1978). Clearly, any interaction between clonidine and naloxone is absent in the kidney and they are likely to bind at different sites.

It is also interesting to compare the relative potencies of the drugs acting on α-adrenoceptors in two systems, firstly displacement of [^{3}H]-clonidine from kidney membranes and secondly on the presynaptic α-adrenoceptor system in the rabbit pulmonary artery (Starke, 1977). The most effective displacers of binding in the kidney such as naphazoline, phentolamine, yohimbine and piperoxane also tend to be those drugs which interact preferentially with presynaptic α-adrenoceptors (Drew, 1976; 1977; Starke, 1977; Blakeley & Summers, 1978) whereas drugs such as phenylephrine, prazosin and labetalol which are poor

Table 2 Effects of agonists and antagonists of dopamine and histamine receptors on [^{3}H]-clonidine binding to membranes from guinea-pig kidney

Drug	IC_{50} (nM)	K_i (nM)
Dopamine receptor agonists		
Dopamine	6600	2300
Apomorphine	2900	960
Dopamine receptor antagonists		
(+)-Butaclamol	990	350
(−)-Butaclamol	⩾1000	—
cis-Clopenthixol	1200	410
cis-Flupenthixol	3100	1100
Histamine receptor agonists		
Histamine	13,000	4600
4-Methylhistamine	3700	1300
Histamine receptor antagonists		
Diphenhydramine	7100	2500
Metiamide	⩾10,000	⩾10,000

displacers of [^{3}H]-clonidine binding, all interact preferentially with postsynaptic α-adrenoceptors (Starke, 1977; Cambridge, Davey & Massingham, 1977; Blakeley & Summers, 1977). However, there are a few anomalies, in particular the imidazoline α-adrenoceptor agonist, oxymetazoline, which is a powerful inhibitor of transmitter release (Starke, 1977) but which is a relatively poor displacer of [^{3}H]-clonidine binding in the kidney. This is of particular interest in view of the structural similarity between oxymetazoline and clonidine and may suggest that the receptor, although similar to the presynaptic α_2-receptor in some respects, is not identical.

In view of the pharmacological similarity between the binding site in the kidney and the presynaptic α-adrenoceptor, it is worth considering whether the binding is to a presynaptic site in the kidney. We consider this unlikely since removal of the sympathetic nerve terminals by 6-hydroxydopamine pretreatment had no significant effect on [^{3}H]-clonidine binding (Jarrott & Summers, unpublished observations). However, the fraction of presynaptically located receptors compared to the total receptor population is likely to be small so that it is possible that any change induced by denervation may be difficult to observe against changes which occur in the receptor population as a whole. The α-adrenoceptor in kidney membranes may be analogous to the α-adrenoceptor which inhibits renin release in the kidney (Pettinger *et al.*, 1976). In both systems clonidine has high potency whereas the preferential α_1-adrenoceptor agonist, methoxamine, is relatively ineffective at reducing renin release and another α_1-adrenoceptor agonist with similar properties, phenylephrine, is similarly ineffective as regards displacement of [^{3}H]-clonidine from kidney membranes. In addition the α- adrenoceptor inhibiting renin release, like the binding site, appears to be located postsynaptically since the inhibition is not affected by adrenergic neurone or ganglion blockade (Pettinger *et al.*, 1976).

The neuroleptic drugs, (+)-butaclamol, *cis*-flupenthixol and *cis*-clopenthixol displaced [^{3}H]-clonidine binding at high concentrations. These observations in the kidney are similar to those in rat brain membranes with thioridazine, chloropromazine, haloperidol and trifluoperazine (U'Prichard *et al.*, 1977) and also those in similar preparations but using another adrenoceptor radioligand [^{3}H]-WB 4101 with *cis*-flupenthixol and (+)-butaclamol (Peroutka, U'Prichard, Greenberg & Snyder, 1977). There are a number of reasons for concluding that the mechanism whereby the neuroleptic drugs displace [^{3}H]-clonidine binding in brain are not the same as those utilised by drugs acting on α-adrenoceptors. Firstly, the potency of these drugs as displacers of [^{3}H]-clonidine is considerably less than that as displacers of dopamine-receptor ligands such as [^{3}H]-haloperidol (Peroutka *et al.*, 1977). Secondly, Hill plots of the data obtained in these experiments using brain membranes gave gradients which were always >1 suggesting the presence of positive co-operativity for this type of interaction.

In conclusion, [^{3}H]-clonidine binds rapidly to a single class of sites in membranes from guinea-pig kidney by a high affinity process which has many of the characteristics of an α_2-adrenoceptor.

The authors would like to thank the National Health and Medical Research Council of Australia for support and Ms Jeanette Brewer for technical assistance.

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(Received August 29, 1978.
Revised October 19, 1978.)