

[³H]-GUANFACINE: A RADIOLIGAND THAT SELECTIVELY LABELS HIGH AFFINITY α_2 -ADRENOCEPTOR SITES IN HOMOGENATES OF RAT BRAIN

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- 1 [³H]-guanfacine (N-amidino-2-(2,6-dichloro 3[³H] phenyl) acetamide hydrochloride; 24.2 Ci/mmol) has been used as a radioligand in homogenates of rat cerebral cortex.
- 2 Specific binding of [³H]-guanfacine was linear with respect to tissue concentration (2.5–15 mg/ml), saturable and not markedly affected in the pH range 6.5–8.0.
- 3 Analysis of the saturation of [³H]-guanfacine binding using an iterative least squares fitting procedure gave best fits to a single site model.
- 4 [³H]-guanfacine binding was of high affinity (K_d 1.77 ± 0.24 nM; $n = 8$) to a population of non interacting sites (nH 0.99 ± 0.02 ; $n = 8$) with a density of 118.2 ± 8.4 fmol/mg protein ($n = 8$).
- 5 Highest levels of binding were achieved in cerebral cortex followed by thalamus > hypothalamus > medulla/pons > spinal cord > striatum > cerebellum.
- 6 Binding was stereoselective with regard to the (–)-isomer of noradrenaline and the order of potency for displacement of [³H]-guanfacine by agonists was naphazoline > clonidine > (–)-adrenaline > (–)- α methylnoradrenaline > (–)-noradrenaline > (\pm)- α -methylnoradrenaline > (+)-noradrenaline > methoxamine > (+)-adrenaline > phenylephrine and by antagonists was phentolamine > dihydroergocryptine > piperoxane > yohimbine > prazosin > labetalol > indoramin suggested binding to α_2 -adrenoceptors.
- 7 The monovalent cations Na⁺ and K⁺ and also guanosine 5'-triphosphate (GTP) produced concentration-dependent inhibition whereas the divalent cations Ca²⁺, Mg²⁺, and Mn²⁺ first enhanced, then inhibited [³H]-guanfacine binding.
- 8 Na⁺ (150 mM) or GTP (100 μ M) produced marked reductions and Mn²⁺ (5 mM) marked increases in the number of receptor sites labelled by [³H]-guanfacine.
- 9 It is concluded that [³H]-guanfacine preferentially labels a high affinity state of the α_2 -adrenoceptor in homogenates of rat cerebral cortex.

Introduction

The substituted phenylacetyl guanidine drug guanfacine (N-amidino-2-(2,6, dichlorophenyl)-acetamide hydrochloride) has a pharmacological profile which resembles that of clonidine in some aspects (Scholtysik, Lauener, Eichenberger, Burki, Salzmann, Muller-Schweinitzer & Waite, 1975). *In vivo* the intravenous injection of guanfacine produces an initial pressor response followed by a hypotensive phase associated with depression of sympathetic outflow (Saameli, Scholtysik & Waite, 1975) but the dose levels required to produce this effect in animals and in man are at least 10 times higher than those for clonidine (Saameli *et al.*, 1975; Waite, 1975). This lower potency is not reflected in receptor binding assays using membranes from rat cerebral cortex, in which guanfacine is equipotent with clonidine in displacing [³H]-clonidine binding (Jarrott, Louis & Summers, 1979; Summers, Jarrott & Louis, 1980a).

Rebound hypertension which occurs following cessation of treatment with clonidine in man appears to be less of a problem with guanfacine (Turner, 1974; Jaattela, 1976; Kirch & Distler, 1978). Clonidine and guanfacine have a similar powerful and selective agonist effect at prejunctional α -adrenoceptors and have been shown to reduce transmitter output from the isolated perfused heart of the rabbit (Pacha, Salzmann & Scholtysik, 1975). However, there are indications from classical pharmacological studies (Doxey, 1979) and from receptor binding studies (Summers, Jarrott & Louis, 1980b) that guanfacine may be more selective for α_2 -adrenoceptors than clonidine. Thus it was of interest to examine if [³H]-guanfacine at high specific activity could be used to characterize α_2 -adrenoceptor binding sites in membranes prepared from rat brain.

Methods

Preparation of membranes

Rats (Sprague-Dawley 175–225 g, of either sex) were stunned, decapitated and the brains removed and placed on ice. Brain areas were dissected as described by Holman, Angwin & Barchas (1976). Membranes for binding studies were prepared by a modification of the method of U'Prichard, Greenberg & Snyder (1977). The cerebral cortex was homogenized in 20 vol 50 mM Tris/HCl pH 7.6 at 4°C in an Ultra Turrax TP18/10 (TP10N Shaft) homogenizer (full speed 30 s). The homogenate was pelleted by centrifugation at 49,000 g for 15 min in an IEC B20A centrifuge at 4°C. After resuspension in 20 vol 50 mM Tris/HCl the centrifugation step was repeated. Finally the pellet was resuspended in 50 vol 50 mM Tris/HCl pH 7.6 at 25°C. In a few experiments the crude mitochondrial pellet was layered over 31% sucrose and centrifuged at 49,000 g for 15 min. High levels of binding were obtained in the plasma membrane-enriched pellet but not in the myelin-enriched fraction in the sucrose layer, indicating that binding was to plasma membranes.

Radioligand binding assay

Membrane suspension (1 ml containing 20 mg wet wt. tissue or approximately 1.5 mg protein) was incubated with an equal volume of 50 mM Tris pH 7.6 and [³H]-guanfacine (24 Ci/mmol) to give concentrations of between 0.1 and 10 nM (Scatchard & Hill Analysis) or ~1 nM (drug displacement studies) for 30 min at 25°C. Non specific binding was estimated in identical samples containing in addition 1 μM phenotolamine. After incubation, the membranes were filtered in a cold room at 4°C on to Whatman GF/B filters and washed three times with ice cold 50 mM Tris pH 7.6. [³H]-guanfacine remaining bound to the membranes retained on the filters was eluted with 2-methoxyethanol (3 ml) for at least 30 min. A toluene based scintillant (0.01% POPOP and 0.4% PPO) (10 ml) was added and the samples counted at ~40% efficiency on a Searle Analytical Delta 300 scintillation counter. Corrections for quenching were made by the channels ratio method.

In saturation experiments the binding parameters B_{\max} (maximal number of binding sites) and K_d (dissociation constant) were estimated using the iterative least squares fitting programme 'Ligand' (Munson & Rodbard, 1980). Over the concentration range used in these experiments the best fit was obtained using a single site model.

Displacement of [³H]-guanfacine binding by drugs

Potential inhibitors of [³H]-guanfacine binding were

added to buffer containing the ligand (~1 nM) followed by the suspension of rat cerebral cortex membranes. The concentration of inhibitors used was in the range of 0.1 nM to 10 μM. Incubation and filtering conditions were as previously described.

In displacement studies, curves of best fit were obtained with an iterative computer programme utilising the logistic function

$$\% \text{ radioligand displaced} = \frac{100 \cdot [D]^P}{[D]^P + [IC_{50}]^P}$$

where [D] is the concentration of displacing drug and P is the slope factor. From this relationship the value of the IC_{50} can be determined and hence the inhibition constant (K_i) using the Cheng & Prusoff (1973) equation.

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

where K_i = the inhibition constant

K_d = dissociation constant

Significance of difference between means was assessed by Student's *t* test.

Drugs

[³H]-guanfacine (N-amidino-2-(2,6-dichloro 3-phenyl) acetamide hydrochloride; 24.2 Ci/mmol) was kindly donated by Dr R. Voges, Sandoz Ltd, Ch 4002, Basel, Switzerland. The radioligand was routinely purified before use by thin layer chromatography on silica gel GF plates (Analtech, Inc.) using methylene chloride : methanol : acetic acid (8 : 1 : 1) as mobile phase.

Other drugs were obtained as follows: (–)-α-methylnoradrenaline base, (+)-noradrenaline bitartrate (Sterling Winthrop); (–)-adrenaline, (–)-noradrenaline bitartrate, (Sigma); clonidine hydrochloride (Boehringer-Ingelheim); phenotolamine hydrochloride, naphazoline hydrochloride, (Ciba); prazosin hydrochloride (Pfizer UK Ltd); methoxamine hydrochloride (Burroughs-Wellcome Australia); (±)-α-methylnoradrenaline hydrochloride (Calbiochem); piperoxane hydrochloride (Rhône Poulenc); phenylephrine hydrochloride (Koch-Light); guanosine 5'-triphosphate (Tris salt) (Sigma).

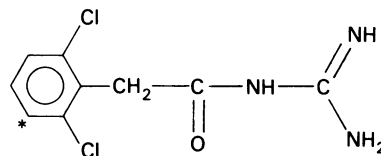


Figure 1 Structure of [³H]-guanfacine with position of tritium marked by the asterisk.

Results

Characteristics of [³H]-guanfacine binding

The structure of guanfacine is shown in Figure 1. The substance is a phenylacetylguanidine derivative which has been labelled with tritium in the 3 position to give a specific activity of 24.2 Ci/mmol. Binding of [³H]-guanfacine to membranes prepared from rat cerebral cortex was linear with respect to tissue concentration within the range of 5–30 mg (wet wt. tissue) in 2 ml of incubation solution. Results of a typical experiment are shown in Figure 2. In the experiments described below, membranes from 20 mg wet wt. of tissue were used for each determination.

Specific binding in these experiments was taken to be the total amount of [³H]-guanfacine minus non-specific binding which was defined as that in the presence of an excess (1 μ M) of unlabelled phenolamine. Total, non-specific and specific binding of [³H]-guanfacine are plotted against concentration in Figure 3. It can be seen that specific binding was saturable at ligand concentrations above 5 nM.

The effect of pH on binding was examined at a [³H]-guanfacine concentration of ~ 1 nM over a pH range of between 6.5 and 8.0. As can be seen from Figure 4 there was no clear pH optimum for binding. Both non-specific and total binding tended to increase with decreasing pH and so specific binding remained fairly constant. Since non-specific binding was at acceptable levels at physiological pH (7.6) this was chosen for the remaining experiments.

Saturation analysis of [³H]-guanfacine binding was performed by addition of a buffered suspension of membranes from rat cerebral cortex to 50 mM Tris/HCl containing radioligand. Analysis of results was by an iterative least squares fitting procedure (Munson & Rodbard, 1980). Over the range of con-

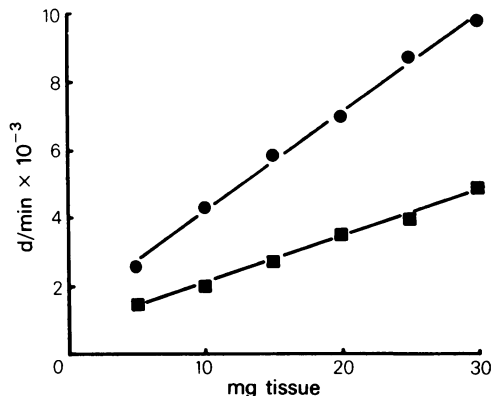


Figure 2 Effect of tissue concentration on [³H]-guanfacine binding to membranes from rat cerebral cortex: (●) total binding ($r = 0.99$); (■) non-specific binding defined in the presence of 1 μ M phentolamine ($r = 0.99$).

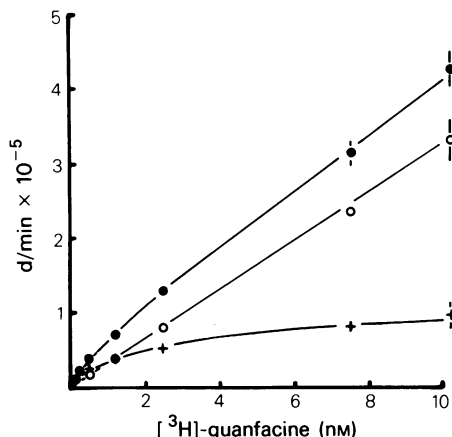


Figure 3 Relationship between total (●), non specific (○) and specific (+) (total – non specific) binding and ligand concentration. [³H]-guanfacine concentration was increased from 0.1 to 10 nM and incubation was for 30 min at 25°C in 50 mM Tris/HCl buffer pH 7.6 (at 25°C). Non-specific binding was defined in the presence of 1 μ M phentolamine. Each experiment was conducted in duplicate. Vertical bars indicate the s.e. mean ($n = 4$).

centrations used in these experiments the best fits were obtained with a single site model.

The results of a typical saturation experiment are shown as a Scatchard transform in Figure 5. It can be seen that the plot is linear indicating that there is a single population of binding sites with a density of 113.2 fmol/mg protein and a K_d of 1.81 nM. The density of binding sites in all experiments was

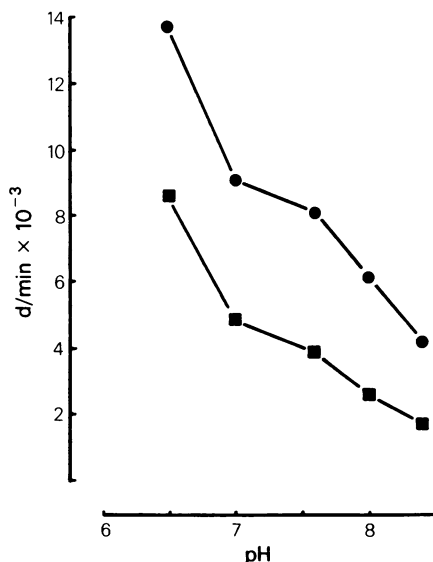


Figure 4 [³H]-guanfacine binding to membranes from rat cerebral cortex: effect of pH on total (●) and non-specific binding (■).

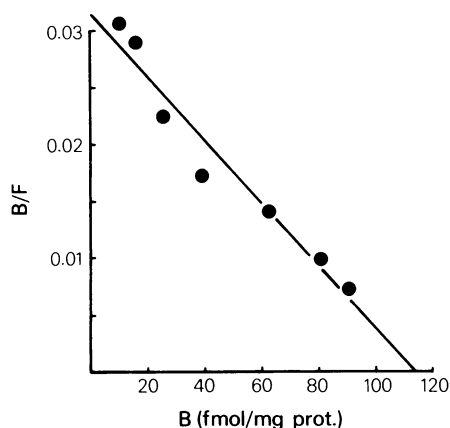


Figure 5 Scatchard plot of [^3H]-guanfacine binding to membranes from rat cerebral cortex. Each point is the mean of a duplicate determination and the line is the line of best fit determined by the iterative curve fitting programme. The maximum number of binding sites indicated by the intercept on the abscissa scale was 113.2 fmol/mg protein and the dissociation constant ($-1/\text{slope}$) 1.81 nM. A Hill plot of the data has a gradient of 1.012 indicating a lack of cooperativity in binding.

118.2 ± 8.4 fmol/mg protein ($n=8$) and the K_d was 1.77 ± 0.24 nM ($n=8$). Hill coefficients for the saturation experiments had a mean gradient of 0.99 ± 0.02 ($n=8$) not significantly different from unity.

Distribution of [^3H]-guanfacine binding in rat brain areas

There were differences in [^3H]-guanfacine binding in areas of rat brain. Highest levels of binding were obtained in membranes prepared from cerebral cortex. The rank order of binding, highest to lowest, was obtained in cerebral cortex > hippocampus > thalamus > hypothalamus > medulla/pons > spinal cord > striatum > cerebellum (Table 1).

Displacement properties of [^3H]-guanfacine binding

[^3H]-guanfacine binding to membranes prepared from rat cerebral cortex could be displaced by drugs, the most effective being those which are known to act on α -adrenoceptors. Displacement was stereoselective with respect to isomers of catecholamines. The (+)-isomers of noradrenaline and adrenaline were less effective at displacing binding than the (–)-isomers. In addition (\pm)- α -methylnoradrenaline was approx. half as effective as the (–)-isomer at displacing binding.

The order of potency for α -adrenoceptor agonists in displacing the radioligand was naphazoline > clonidine > (–)-adrenaline > (–)- α -methylnor-

Table 1 Specific [^3H]-guanfacine binding to membranes prepared from areas of rat brain

Brain region	[^3H]-guanfacine bound (pmol/g wet wt.) Control
Cerebral Cortex	3.4 ± 0.3 (5)
Hippocampus	2.2 ± 0.2 (5)
Thalamus	2.1 ± 0.1 (5)
Hypothalamus	1.9 ± 0.2 (5)
Medulla/pons	1.7 ± 0.2 (5)
Spinal Cord	1.3 ± 0.1 (5)
Striatum	1.3 ± 0.1 (5)
Cerebellum	1.0 ± 0.1 (5)

In all determinations 20 mg wet wt. of tissue was used together with a ligand concentration of 0.70 ± 0.03 nM. Values are mean \pm s.e.mean. No. of experiments in parentheses.

adrenaline > (–)-noradrenaline > (\pm)- α -methylnoradrenaline > (+)-noradrenaline > methoxamine > (+)-adrenaline > phenylephrine. Antagonists also displaced binding, those which are known to have potent actions at α_2 -adrenoceptors being particularly effective. The rank order of potency for the antagonists was phentolamine > dihydroergocryptine > piperoxane > yohimbine > prazosin > labetalol > indoramin. The K_i values for these compounds are shown in Table 2. A wide variety of drugs with actions predominantly on other receptor systems were also tested for their effects on [^3H]-guanfacine binding. Almost all were ineffective with K_i values in excess of $10 \mu\text{M}$. They included the β_1 - and β_2 -adrenoceptor antagonist, propranolol, the β_2 -adrenoceptor agonist, salbutamol and antagonists H35/25 and the β_1 -adrenoceptor antagonist, metoprolol. Drugs acting on cholinergic receptors including nicotine, carbachol, pempidine, atropine and (+)-tubocurarine had no effect as did the monoamine oxidase (MAO) inhibitor, pargyline, the catechol-*O*-methyl transferase (COMT) inhibitor, U0521, the neuronal uptake inhibitor desmethylimipramine (DMI) and the opiate antagonist, nalorphine. It was of interest to examine the effects of drugs acting on histamine receptors on [^3H]-guanfacine binding since the closely related compound, clonidine, is known to stimulate a histamine-sensitive adenylate cyclase in slices of guinea-pig and rat brain. However, histamine, cimetidine, dimaprit and triprolidine had no significant effect on binding indicating that in the concentration range used, [^3H]-guanfacine does not bind to histamine receptors in membranes from rat cerebral cortex. The adrenergic neurone blockers, guanethidine and debrisoquine, were also tested and found to be ineffective even though both drugs have a guanidine moiety similar to that of guanfacine.

Table 2 Inhibition constants (K_i) for α -adrenoceptor agonists and antagonists against [³H]-guanfacine binding in membranes from rat cerebral cortex

α -Adrenoceptor agonists	K_i (nM)
Naphazoline	1.6 \pm 0.7
Clonidine	2.0
(-)-Adrenaline	7.2 \pm 2.1
(-)- α -Methylnoradrenaline	8.1 \pm 2.4
(-)-Noradrenaline	13.3 \pm 3.4
(\pm)- α -Methylnoradrenaline	18.7 \pm 3.9
(+)-Noradrenaline	322.4 \pm 73
Methoxamine	608
(+)-Adrenaline	811 \pm 195
Phenylephrine	910 \pm 280
α -Adrenoceptor antagonists	
Phentolamine	5.5 \pm 1.3
Dihydroergocryptine	9.6 \pm 4.3
Piperoxane	169.7 \pm 28.7
Yohimbine	180 \pm 23.0
Prazosin	26,220
Labetalol	22,740 \pm 6770
Indoramin	38,540

IC₅₀ values were obtained from the iterative curve fitting function and the K_i derived using the Cheng & Prusoff equation as described in methods. Values are means obtained from 2–5 experiments conducted in duplicate \pm s.e.mean. [³H]-guanfacine concentration was 0.876 \pm 0.02 nM (n = 50).

Modulation of [³H]-guanfacine binding by ions and guanyl nucleotides

[³H]-guanfacine binding was altered by both monovalent and divalent cations. Addition of Na⁺ (0.1–150 mM) produced concentration-dependent inhibition of binding as shown in Figure 6a. The process was less affected by K⁺ with modest inhibition being produced at concentrations between 50–150 mM. In the physiological range of concentration, [³H]-guanfacine binding was significantly inhibited by Na⁺ but not by K⁺.

Divalent cations produced biphasic effects as shown in Figure 6b. Low concentrations (<10 mM) of Ca²⁺, Mg²⁺, and Mn²⁺ produced enhancement of binding which was marked in the case of the latter two cations. High concentrations (>50 mM) of all three divalent cations produced inhibition of binding.

[³H]-guanfacine binding was inhibited by guanosine 5'-triphosphate (GTP) in a concentration-dependent manner. The process was not particularly sensitive to GTP since the IC₅₀ was 30 μ M, as shown in Figure 7.

The effects of the most potent monovalent (Na⁺) and divalent cations (Mn²⁺) and GTP were studied on the saturation of [³H]-guanfacine binding. As can be seen in Figure 8, the major effect of Na⁺ (150 mM) or GTP (100 μ M) was to decrease the number of sites labelled by [³H]-guanfacine. Both these compounds produced these changes with only a small decrease in apparent affinity of binding. In contrast, the divalent cation, Mn²⁺ (5 mM), produced a marked increase in

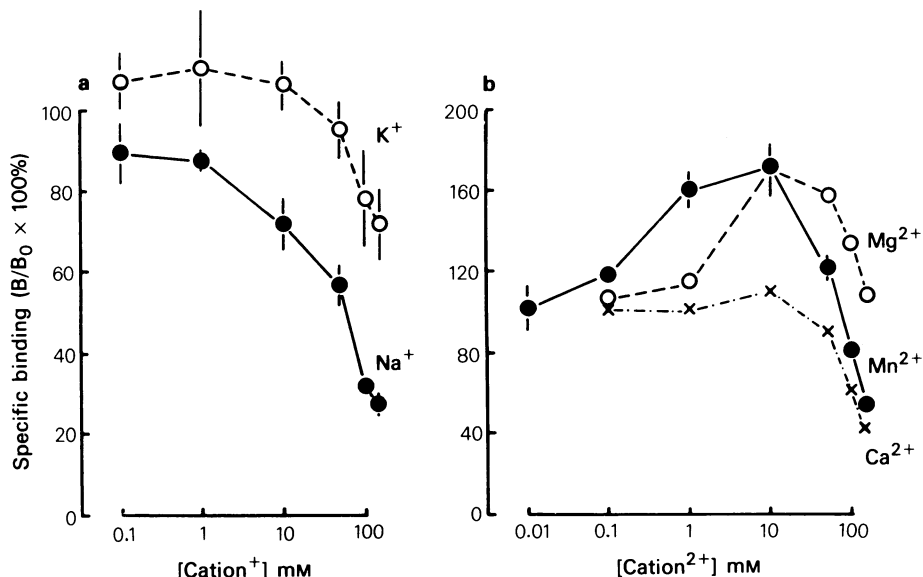


Figure 6 Effect of cations on specific [³H]-guanfacine binding to membranes from rat cerebral cortex. In (a) the effect of the monovalent cations sodium (n = 3) and potassium (n = 4) and in (b) the effect of the divalent cations Mg²⁺ (n = 3), Mn²⁺ (n = 4) and Ca²⁺ (n = 4) are shown. Vertical bars indicate the s.e.mean.

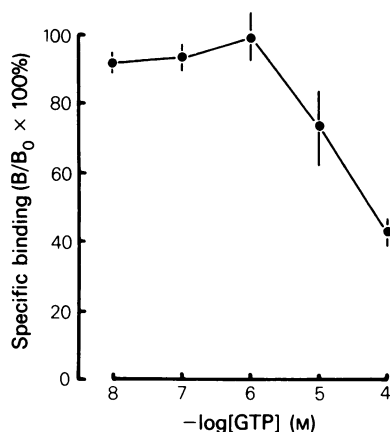


Figure 7 Effect of guanosine 5'-triphosphate (GTP) on specific [3 H]-guanfacine binding to membranes from rat cerebral cortex. Vertical bars indicate the s.e. mean ($n = 6$).

the number of receptor sites labelled with a slight increase in apparent affinity of binding. The results of all experiments are summarized in Table 3.

Discussion

Guanfacine (N-amidino-2-(2,6-dichlorophenyl)acetimide) is one of the most active of a new group of antihypertensive drugs, the phenylacylguanidines. Although the drug has a pharmacological profile broadly similar to that of clonidine, there are a number of important differences including a lack of effect on dopamine turnover, (Scholtysik, Jerie & Picard, 1980) central histamine H_2 -receptors, (Scholtysik, 1979) less central sedative effect (Scholtysik *et al.*, 1975) and recently, evidence that guanfacine is more selective for α_2 -adrenoceptors than clonidine (Summers *et al.*, 1980b). These properties would be expected to make guanfacine, in tritium labelled form, a useful tool for examining α_2 -adrenoceptors.

[3 H]-guanfacine binding met all of the criteria tested for demonstration of binding to receptors.

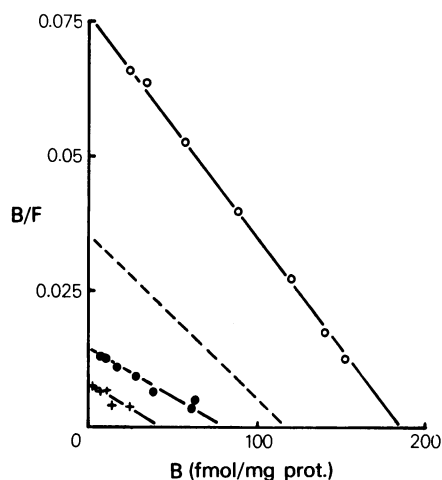


Figure 8 Scatchard plot of specific [3 H]-guanfacine binding to membranes from rat cerebral cortex showing the effect of manganese 5 mM (○), GTP 100 μ M (●), and sodium 150 mM (+). The broken line shows the mean line of best fit in the control experiments.

Binding to membranes from rat cerebral cortex was linear with respect to tissue concentration up to 15 mg/ml, saturable and present in fractions enriched with plasma membranes. Total and non-specific binding were pH-dependent although specific binding was relatively little affected. The changes in binding with pH are probably dependent on changes in non-specific binding. The rank order of displacement of [3 H]-guanfacine by drugs acting on α -adrenoceptors indicated that, as with clonidine in rat cerebral cortex (U'Prichard *et al.*, 1977; U'Prichard, Bechtel, Rouot & Snyder, 1979) guanfacine binds to a site resembling the α_2 -adrenoceptor. Drugs with actions on other receptor systems were largely without effect and even those which are structurally related to guanfacine such as guanethidine did not displace binding. None of the drugs tested which act on histamine receptors affected [3 H]-guanfacine binding which would indicate that guanfacine does not interact with this receptor population; this would support the observation that guanfacine does not

Table 3 Effect of sodium, manganese and guanosine 5'-triphosphate (GTP) on binding constants for [3 H]-guanfacine in membranes from rat cerebral cortex

Treatment	B_{max} (fmol/mg protein)	K_d (nM)	nH	n
Control	118.2 \pm 8.4	1.77 \pm 0.24	0.99 \pm 0.02	8
Manganese 5 mM	193.5 \pm 10.5*	1.35 \pm 0.11	0.97 \pm 0.02	3
GTP 100 μ M	62.2 \pm 9.8*	2.63 \pm 0.70	0.94 \pm 0.04	5
Sodium 150 mM	42.6 \pm 12.0*	2.40 \pm 0.60	0.95 \pm 0.02	4

*Values significantly different from control.

stimulate histamine-sensitive adenylate cyclase in rat brain (Scholtysik, 1980).

The regional distribution of α_2 -adrenoceptors as determined by [³H]-guanfacine binding was similar to that observed with [³H]-clonidine (U'Prichard *et al.*, 1977) with highest density of receptors in cerebral cortex and lowest in cerebellum. Rather higher concentrations of α_2 -adrenoceptors were detected in hippocampus and rather lower concentrations in hypothalamus than is the case for [³H]-clonidine.

Saturation analysis of [³H]-guanfacine binding in rat cerebral cortex homogenates revealed several interesting features. Since guanfacine is more selective than clonidine for α_2 -adrenoceptors (Summers *et al.*, 1980b) and dissociation curves reveal that binding is primarily to a single high affinity site (Summers, Jarrott & Louis, 1981) it might be expected that [³H]-guanfacine would label fewer receptors than [³H]-clonidine. However, this was not the case since the density of sites labelled by [³H]-guanfacine was greater than for [³H]-clonidine (Summers *et al.*, 1981). The explanation for this may lie in the relative efficacy of the two compounds. It is well recognized that α_2 -adrenoceptor agonists label only that fraction of receptors in the high affinity (α_H) state (Hoffman, Mullin-Kilpatrick & Lefkowitz, 1980). Compounds such as clonidine and guanfacine are partial agonists (Kobinger, 1978) which have different efficacies in different tissues. If guanfacine has a lower efficacy in rat brain it would be expected to label more sites than clonidine. There are a number of experimental observations that support this contention. Firstly guanfacine is about 1/10th as potent as clonidine in hypertensive man and in animal models of hypertension, yet it has similar physicochemical properties and partition coefficient (Summers *et al.*, 1980a) and therefore should enter the CNS relatively easily. Also in *in vitro* radioligand assays, guanfacine and clonidine have similar affinities against [³H]-clonidine binding, indicating that lower affinity at the receptor is not the explanation for the difference in potency between the compounds. In the present ex-

periments [³H]-guanfacine binding was inhibited by GTP but the concentrations of nucleotide required were higher than those reported for [³H]-clonidine (Rouot, U'Prichard & Snyder, 1980). The lower sensitivity to GTP and the labelling of more sites by [³H]-guanfacine could be interpreted as indicating that this compound has lower efficacy than [³H]-clonidine.

[³H]-guanfacine binding was reduced by Na⁺ (150 mM) or GTP (100 μ M) and increased by Mn²⁺ (5 mM). The alteration of binding was mainly brought about by alteration in the number of receptor sites labelled by [³H]-guanfacine. Previous findings with [³H]-clonidine have shown a more complex picture, ions and guanyl nucleotides produce changes in apparent affinity of binding with only minor changes in receptor density (Glossman & Presek, 1979; Rouot *et al.*, 1980). This effect is probably due to the presence of high and low affinity binding sites for [³H]-clonidine (Rouot *et al.*, 1980; Summers *et al.*, 1981); when these are differentiated it can be seen that it is only the high affinity sites that are modulated and that the number of these sites varies with Na⁺, Mn²⁺ or GTP (Rouot *et al.*, 1980). The extent of the change in receptor numbers brought about by GTP and Mn²⁺ was similar for the high affinity [³H]-clonidine site and for the [³H]-guanfacine site but the concentrations of nucleotide or ion required to produce the effect were higher for [³H]-guanfacine.

The site labelled by [³H]-guanfacine appears to resemble therefore the high affinity site labelled by [³H]-clonidine.

In conclusion, [³H]-guanfacine preferentially labels a high affinity state of the α_2 -adrenoceptor and this ligand may be a useful tool for examination of modulatory effects of cyclic nucleotides and ions.

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