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BINDING CHARACTERISTICS OF [^{3}H]GUANFACINE TO RAT BRAIN α-ADRENOCEPTORS

COMPARISON WITH [^{3}H]CLONIDINE

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Abstract—The tritium-labeled α-adrenoceptor agonist and antihypertensive drug guanfacine, *N*-amidino-2-(2,6-dichlorophenyl)-acetamide (sp. act. 24.2 Ci/mmole) was employed for a direct identification and characterization of α-adrenoceptors in rat brain membranes. Its usefulness as a radioligand was studied in comparison with [^{3}H]clonidine (sp. act. 26.7 Ci/mmole). The nonspecific binding of [^{3}H]guanfacine to rat cerebral membranes was considerably more pronounced than that observed for [^{3}H]clonidine. The specific binding of [^{3}H]guanfacine (0.1 – 20 nM) and [^{3}H]clonidine (0.1 – 20 nM) as defined as the excess over blanks containing (−)-norepinephrine (10 μM) was saturable. Scatchard analyses of these binding data indicated single populations of binding sites for both ligands. K_D values of 3.9 ([^{3}H]guanfacine) and 3.7 nM ([^{3}H]clonidine) were calculated. Maximal number of specific binding sites amounted to 220 and 195 fmole/mg protein for [^{3}H]guanfacine and [^{3}H]clonidine, respectively. In case unlabeled guanfacine (1 μM) was used to characterize the specific binding of [^{3}H] guanfacine, K_D value and maximal number of binding sites were about twice as high as determined in the presence of excess (−)-norepinephrine. The rate of association of both radioligands was rapid. Binding reached equilibrium by about 10–15 min of incubation. Half-maximal binding was attained at approximately 1–2 min. The rates of dissociation were biphasic. A rapid and a slow component were identified. The specific binding sites of [^{3}H] guanfacine in rat brain possess the general characteristics of α_2-adrenoceptors. Selective antagonists of α_2-adrenoceptors, like yohimbine and rauwolscine strongly interfered with this binding. However, preferential blocking agents of α_1-adrenoceptors, such as prazosin and corynanthine, were weak competitors. The relative potency of agonists and antagonists in displacing [^{3}H]guanfacine was identical to their effectiveness in competing for [^{3}H]clonidine specific binding sites. It is concluded that [^{3}H]guanfacine labels the same α_2-adrenoceptor population in rat brain as [^{3}H]clonidine. However, [^{3}H]guanfacine seems not as suitable as [^{3}H]clonidine for routine use in the direct identification of α_2-adrenoceptors in view of its relatively high nonspecific binding.

α-Adrenoceptor sites have been characterized in mammalian brain and peripheral tissues on the basis of binding and displacement studies employing a variety of radioligands of high specific radioactivity [1, 2]. The α-adrenoceptor agonists [^{3}H]norepinephrine [3] and [^{3}H]epinephrine [4], the antagonists [^{3}H]WB-4101 [5–8] and [^{3}H]prazosin [9–12] as well as the partial agonist [^{3}H]dihydroergocryptine [1, 13–16], have been frequently used for this purpose. [^{3}H]Clonidine and its analogue [^{3}H]para-aminoclonidine have also been successfully applied for a direct labeling of brain α-adrenoceptors [5, 6, 12, 17–20].

Guanfacine, *N*-amidino-2-(2,6-dichlorophenyl)-acetamide, is structurally related to clonidine, 2-(2,6-dichlorophenylimino) imidazolidine (Fig. 1). Like clonidine, guanfacine is an effective, centrally acting, antihypertensive drug [21]. There is considerable experimental evidence to support the view that guanfacine, like clonidine, produces its antihypersensitive effect via a central mechanism involving the stimulation of central α-adrenoceptors [22].

Recently, tritium-labeled guanfacine with a high specific radioactivity has become available to us. The present study was undertaken to characterize the binding of [^{3}H]guanfacine to rat brain membranes and to determine the usefulness of this radioligand in comparison with [^{3}H]clonidine for a direct identification of central α-adrenoceptors.

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MATERIALS AND METHODS

Drugs. [^{3}H]Guanfacine hydrochloride (24.2 Ci/mmole) and [^{3}H]clonidine hydrochloride (26.7 Ci/mmole) as well as the unlabeled reference compounds were gifts from Sandoz Ltd. and Boehringer Ingelheim, respectively. Radiochemical purity was greater than 95% as determined by thin layer chromatography. Other drugs used in the present study included coryanthine hydrochloride and rauwolscine hydrochloride (Roth), haloperidol (Janssen Pharmaceutica), (−)-norepinephrine bitartrate, (−)-phenylephrine hydrochloride and yohimbine hydrochloride (Sigma, London, U.K.), phentolamine hydrochloride (Ciba-Geigy), prazosin hydrochloride and tetrahydrozoline hydrochloride (Pfizer, Sandwich, U.K.). All other chemicals were reagent grade.

Preparation of membranes from rat brain. The method used to obtain a crude membrane fraction

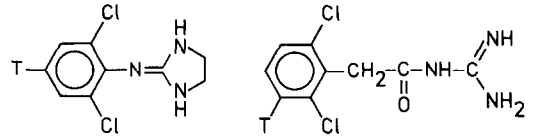

Fig. 1. Structures of clonidine, 2-(2,6-dichlorophenylimino)-imidazolidine and guanfacine, *N*-amidino-2-(2,6-dichlorophenyl)-acetamide and the position of the tritium label.

from rat brain was the same as previously described [5, 6]. Accordingly, male normotensive Wistar rats (weight 200–250 g) were killed by decapitation. Their brains (minus cerebella) were isolated and homogenized in 20 vol. (w/v) of ice-cold 50 mM Tris–HCl buffer (pH = 7.7 at 25°) in motor-driven Teflon–glass homogenizers. The homogenate was centrifuged at 50,000 *g* for 10 min at 4°. The pellet was rehomogenized in fresh cold buffer and the suspension was centrifuged again (see above). The final material was resuspended in Tris–HCl buffer at 4 mg of protein per ml for routine use in the binding assays. Protein concentration was determined by the method of Lowry *et al.* [23] using bovine serum albumin as standard and 50 mM Tris–HCl buffer as blank.

Binding assay of [^{3}H]*guanfacine and* [^{3}H]*clonidine.* Standard [^{3}H]guanfacine and [^{3}H]clonidine binding assays were run by incubating 500 μl of rat brain membrane suspension (see above) at 25° for about 60 min with [^{3}H]guanfacine (sp. act. 24.2 Ci/mmole; 0.1–20 nM) or [^{3}H]clonidine (sp. act. 26.7 Ci/mmole; 0.1–20 nM) with shaking in a total volume of 1 ml of incubation buffer. In displacement experiments, the inhibition of the specific binding of [^{3}H]guanfacine or [^{3}H]clonidine (0.4 nM of each) was determined in the presence of various concentrations of unlabeled competing drugs. Incubations were terminated by rapid vacuum filtration through Whatman GF/B filters. Filters were rapidly washed with three 5-ml portions of ice-cold Tris–HCl buffer, left to solubilize in 10 ml of Instagel® (Packard-Becker) for 24 hr and counted at an efficiency of about 40%. Nonspecific binding of [^{3}H]guanfacine and [^{3}H]clonidine is defined as binding which is not displayed by 10 μM of (−)-norepinephrine. In separate experiments unlabeled guanfacine and clonidine (1 μM of each) have also been employed to characterize the nonspecific binding of both radioligands. Nonspecific binding was subtracted from the total binding to obtain the specific binding of [^{3}H]guanfacine and [^{3}H]clonidine.

As a measure of the affinity of drugs for the specific binding sites identified by [^{3}H]guanfacine and [^{3}H]clonidine, the concentration (M) inhibiting the specific binding by 50% (IC_{50}) was calculated from the displacement curves by log probit analysis. $K_i = IC_{50}/(1 + [\text{ligand}]/K_D)$ where K_D is the radioligand equilibrium dissociation constant derived from saturation experiments.

RESULTS

Binding of [^{3}H]*guanfacine to rat brain membranes.* The total binding of [^{3}H]guanfacine to rat isolated cerebral membranes was partly inhibited by excess (−)-norepinephrine (10 μM) and excess unlabeled guanfacine (1 μM) (nonspecific binding). Figure 2 shows that total binding depended on the concentration of [^{3}H]guanfacine. Nonspecific binding increased linearly with the [^{3}H]guanfacine concentration up to 20 nM, the highest concentration studied. In the presence of excess unlabeled guanfacine, the nonspecific binding of [^{3}H]guanfacine was significantly less than measured after addition of excess (−)-norepinephrine. Excess unlabeled clonidine (1 μM) resulted in a comparable nonspecific binding of [^{3}H]guanfacine as observed in the presence of (−)-norepinephrine (results not shown). At 0.4 and 3 nM of [^{3}H]guanfacine used in standard binding assays, the nonspecific binding represented about 40 and 50%, respectively, of the total binding (unlabeled guanfacine present) and amounted to 50 and 60%, respectively, after the addition of (−)-norepinephrine (or clonidine).

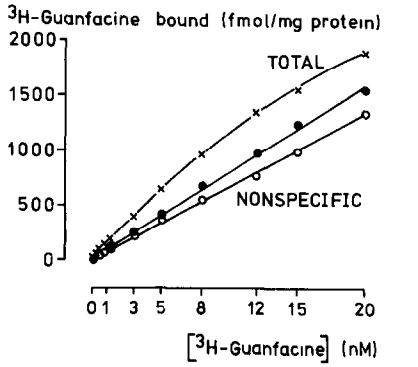

Fig. 2. Binding of [^{3}H]guanfacine to rat brain membranes as a function of the radioligand concentration. Membrane fractions (2 mg of protein per ml; total volume 1 ml) were incubated for 60 min at 25° with various concentrations of [^{3}H]guanfacine. Nonspecific binding was measured by addition of 10 μM (−)-norepinephrine (●) or 1 μM of unlabeled guanfacine (○). Each value is the mean of 6 separate saturation experiments performed in duplicate.

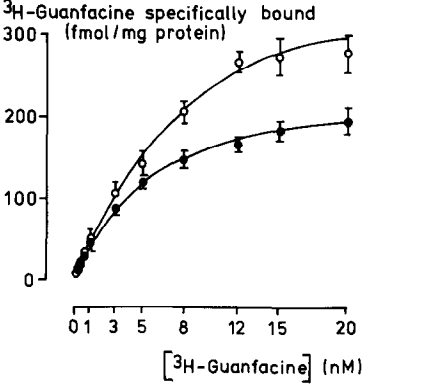

Fig. 3. Specific binding of [^{3}H]guanfacine to rat brain membranes as a function of radioligand concentration. Specific binding was obtained as the difference between total and nonspecific binding. Nonspecific binding was that occurring in the presence of 10 μM (−)-norepinephrine (●) or 1 μM or unlabeled guanfacine (○). Also see the legend to Fig. 2. Symbols represent mean values ± S.E.M.

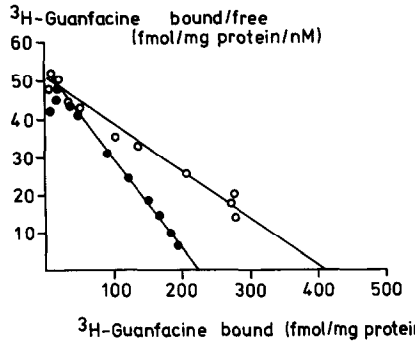

Fig. 4. Scatchard plots of specific [³H]guanfacine binding to rat brain membranes. Specific [³H]guanfacine binding was obtained by subtracting the nonspecific binding remaining in the presence of 10 μM (−)-norepinephrine (●) or 1 μM of unlabeled guanfacine (○) from the total binding (also see Fig. 3). The lines indicated were determined by linear regression analysis. The number of binding sites per mg of protein, B_{max}, was calculated from the intercept with the abscissa. The equilibrium dissociation constant at 25°, K_D, resulted from the slope of the lines. Values are means of 6 determinations.

The specific binding of [³H]guanfacine defined as the difference between total and nonspecific binding measured in the presence of excess (−)-norepinephrine as well as non-radioactive guanfacine showed saturation with increasing radioligand concentration (Fig. 3). The amount of [³H]guanfacine specifically bound to rat brain membranes was considerably higher in the presence of unlabeled guanfacine than that obtained with (−)-norepinephrine. Scatchard analysis of the saturation data (Fig. 4) gave straight lines, indicative for single populations of binding sites. The apparent dissociation constant, K_D, for [³H]guanfacine was 3.9 nM and the estimated maximum number of specific binding sites, B_{max}, amounted to 220 fmole/mg protein (nonspecific binding defined with (−)-norepinephrine).

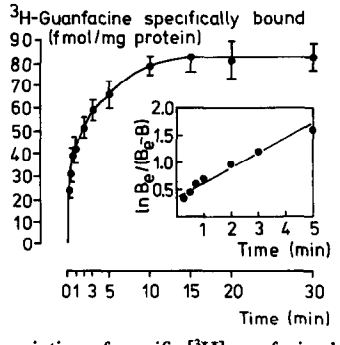

Fig. 5. Association of specific [³H]guanfacine binding to rat brain membranes. Tissue suspensions were incubated with 3 nM [³H]guanfacine at 25° for various time intervals as described under Materials and Methods. Binding in the absence and presence of (−)-norepinephrine (10 μM) was measured simultaneously. The difference between these values represented the specific binding. Association was started by addition of the brain membrane suspension and terminated by rapid vacuum filtration. Each value is the mean ± S.E.M. of 6 experiments. Inset: semilogarithmic plot of initial [³H]guanfacine binding. The slope, determined by linear regression analysis, is equal to K_{obs}, the pseudo-first order rate constant.

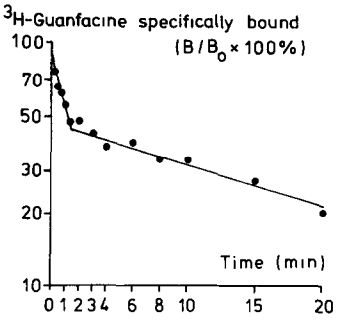

Fig. 6. Dissociation of specific [³H]guanfacine binding from rat brain membranes. Tissue suspensions were incubated at 25° with 0.4 nM [³H]guanfacine to equilibrium (about 60 min). At time zero, 10 μM (−)-norepinephrine was added to the incubation mixtures and the experiment was terminated by rapid vacuum filtration at various times. Non-specific binding was determined in parallel samples already containing 10 μM (−)-norepinephrine. Points shown are mean values of 6 individual determinations.

These values were $K_D = 8.0$ nM and $B_{max} = 410$ fmole/mg protein for the specific binding observed in the presence of nonlabeled guanfacine. Hill plots of these saturation binding experiments gave straight lines with slopes not significantly different from unity, indicating that no cooperativity is involved in the [³H]guanfacine binding.

Investigations into the kinetics of specific [³H]guanfacine binding (3 nM) showed a rapid rate of association at 25° (Fig. 5). The specific binding (nonspecific binding defined by 10 μM (−)-norepinephrine) appeared to reach equilibrium by about 10 min and was stable for at least 60 min. Half-maximal binding occurred at approximately 1 min. The observed first-order association constant, k_{obs}, was 0.32 min^{-1}.

The reversibility of the specific [³H]guanfacine binding was readily demonstrated in dissociation experiments. The rate of dissociation of [³H]guanfacine from its specific binding sites in rat brain membranes was examined by incubating membranes with the radioligand (0.4 nM) at 25° (60 min)

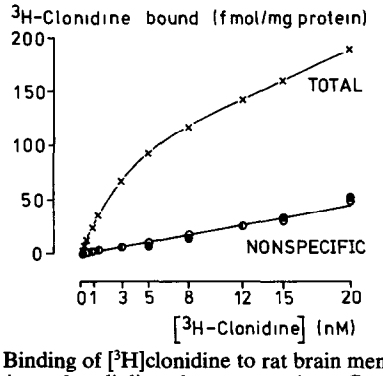

Fig. 7. Binding of [³H]clonidine to rat brain membranes as a function of radioligand concentration. Conditions of incubation were the same as described for [³H]guanfacine under Fig. 2. Nonspecific binding was that part of the total binding (×) remaining in the presence of either 10 μM (−)-norepinephrine (●) or 1 μM unlabeled clonidine (○). Symbols are given as mean values of 6 separate saturation experiments each performed in duplicate.

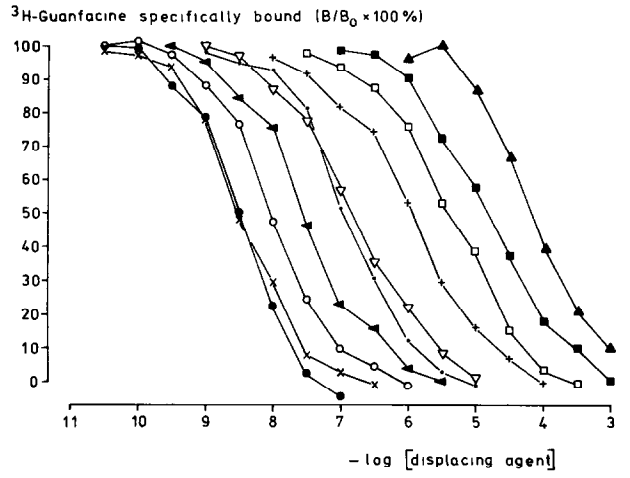

Fig. 8. Inhibition of specific [^{3}H]guanfacine binding to rat brain membranes by various drugs. Membrane suspensions were incubated with 0.4 nM [^{3}H]guanfacine in the presence or absence of 9 increasing concentrations (M) of competing nonradiolabeled drug. Nonspecific binding was that part of the total binding which was not displaced by excess (10 μM) of (−)-norepinephrine. Points are given as means of 4 individual displacement experiments each conducted in duplicate. B = fraction of [^{3}H]guanfacine specifically bound in the presence of competing displacer; B_0 in the absence of competing displacer. Key: clonidine (×), guanfacine (●), phentolamine (○), tetrahydrozoline (◀), yohimbine (•), rauwolscine (▽), (−)-phenylephrine (+), prazosin (□), corynanthine (■), and haloperidol (▲).

followed by the addition of 10 μM (−)-norepinephrine to prevent rebinding of dissociated [^{3}H]guanfacine. When plotted on a semilogarithmic scale, the dissociation of specifically bound [^{3}H]guanfacine was biphasic (Fig. 6). There was a rapid initial displacement in the first 1–2 min with a half-life of about 1 min (k_{-1} = 0.69 min^{-1}). The slower phase of disocciation had a half-life of approximately 15 min (k_{-1} = 0.05 min^{-1}).

Binding of [^{3}H]*clonidine to rat brain membranes.* The nonspecific binding of [^{3}H]clonidine determined in the presence of excess (−)-norepinephrine (10 μM) and unlabeled clonidine (1 μM) increased linearly with the [^{3}H]clonidine concentration (Fig. 7). In contrast to [^{3}H]guanfacine, the nonspecific binding of [^{3}H]clonidine was comparable in the presence of excess (−)-norepinephrine and nonradioactive clonidine. Also excess (1 μM) unlabeled guanfacine resulted in a comparable nonspecific binding of [^{3}H]clonidine (results not shown). The amount of [^{3}H]clonidine occupying specific binding sites in rat brain membranes was approximately 88 and 91% of the total binding at 0.4 and 3 nM [^{3}H]clonidine, respectively. Scatchard analysis of the specific [^{3}H]clonidine binding data (incubation concentration 0.1–20 nM) afforded a straight line. The calculated

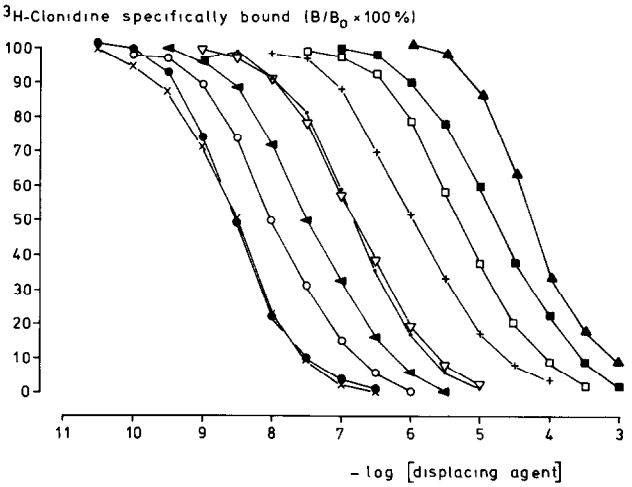

Fig. 9. Inhibition of specific [^{3}H]clonidine binding to rat brain membranes by various drugs. For details of performance and explanation of symbols see the legend to Fig. 8. The [^{3}H]clonidine concentration was 0.4 nM in all displacement experiments. Mean values of 4 separate determinations each conducted in duplicate are given. B = fraction of [^{3}H]clonidine specifically bound in the presence of competing displacer; B_0 in the absence of competing displacer.

Table 1. Inhibition of specific [^{3}H]guanfacine and [^{3}H]clonidine binding to membranes of rat brain by a number of α-adrenoceptor agonists and antagonists*

Drug	K_i (nM) [^{3}H]Guanfacine	[^{3}H]Clonidine
Clonidine	2.5	2.8
Guanfacine	2.7	2.6
Phentolamine	8.0	8.9
Tetrahydrozoline	25	27
Yohimbine	100	150
Rauwolscine	120	150
(−)-Phenylephrine	1,100	1,000
Prazosin	3,600	4,500
Corynanthine	14,000	16,000
Haloperidol	50,000	45,000

* Drugs were tested for the ability to inhibit the specific binding of [^{3}H]guanfacine and [^{3}H]clonidine to rat isolated cerebral membranes. Radioligand concentration was 0.4 nM. Each drug was studied at 9 increasing concentrations (also see Figs. 8 and 9). IC_{50} Values were calculated graphically by log probit analysis. K_i values resulted from the equation $K_i = IC_{50}/(1 + 0.4/K_D)$, where K_D is the dissociation constant for each ligand derived from Scatchard analysis (see Results). The mean value of four separate determinations is reported.

K_D was 3.7 nM and B_{max} amounted to 195 fmole/mg protein. The Hill plot was linear with a slope closely approximating unity.

Kinetic analysis of [^{3}H]clonidine binding (3 nM) showed a similar pattern of saturable, time-dependent association of specific binding as observed for [^{3}H]guanfacine. Half-maximal binding was attained at about 2 min with complete saturation evident at approximately 15 min. The first-order association rate constant, k_{obs}, was 0.21 min^{-1}.

After adding excess (−)-norepinephrine (10 μM) to an equilibrated mixture of [^{3}H]clonidine (0.4 nM) and brain membranes, specifically bound [^{3}H]clonidine dissociated biphasically with time. The rapid phase had a half-life of approximately 1.5 min ($k_{-1} = 0.46$ min^{-1}), whereas the half-life of the slower dissociating [^{3}H]clonidine was about 13 min ($k_{-1} = 0.05$ min^{-1}).

Affinities of drugs for [^{3}H]guanfacine and [^{3}H]clonidine specific binding sites in rat brain membranes. A variety of drugs were tested for their effects upon specific [^{3}H]guanfacine and [^{3}H]clonidine binding to rat cerebral membranes. The specific binding of the radioligands (0.4 nM of each) was determined in the presence of increasing concentrations of the unlabeled displacers. Figure 8 shows the results of the competition experiments for the [^{3}H] guanfacine sites. The displacement curves obtained for the drugs with respect to their inhibition of the specific [^{3}H]clonidine binding are visualized in Fig. 9. As apparent from Figs. 8 and 9, all drugs reached a 100% displacement of the specific binding of both radioligands. The curves had sigmoid shapes and were found parallel with Hill coefficients not significantly different from unity. The concentration of the competitors required to reduce [^{3}H]guanfacine and [^{3}H]clonidine specific binding by 50% (IC_{50}) were obtained from these curves by log probit analysis and K_i values were calculated (Table 1). Unlabeled clonidine as well as guanfacine were most potent and inhibited the specific binding of their radiolabeled analogues at low concentrations. The α-sympatholytic drug phentolamine and the α-adrenoceptor agonist tetrahydrozoline were also found potent inhibitors. The diastereoisomeric α-adrenoceptor blocking drugs yohimbine, rauwolscine and corynanthine had different efficacies to compete for the [^{3}H]guanfacine and [^{3}H]clonidine specific binding sites. Yohimbine and rauwolscine had approximately equal potencies and were considerably more effective than coryanthine which behaved as a very weak displacer. The α_1-adrenoceptor antagonist prazosin had moderate affinities to the sites labeled by [^{3}H]guanfacine and [^{3}H]clonidine. Haloperidol had extremely low potency and affected the [^{3}H]guanfacine and [^{3}H]clonidine binding at high concentrations only.

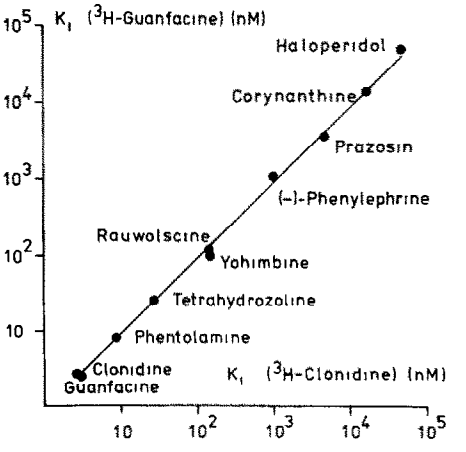

Fig. 10. Correlation between the potencies to inhibit specific [^{3}H]guanfacine and [^{3}H]clonidine binding in rat brain membranes for various drugs. K_i values were derived from displacement studies (see Figs. 8 and 9, and Table 1).

Upon comparing the K_i values of the drugs for the inhibition of [^{3}H]guanfacine binding with those for the [^{3}H]clonidine binding, a close linear relationship between both variables was found (Fig. 10). As demonstrated by Fig. 10, the relative potency of the various agonists and antagonists in displacing [^{3}H]guanfacine is identical to their efficacy in competing for the [^{3}H]clonidine specific binding sites.

DISCUSSION

A variety of radioligands have been employed to identify sites in mammalian brain having similarities to α-adrenoceptors (see Introduction). The present results establish the feasibility of a direct labeling of α-adrenoceptors in rat isolated brain membranes with the α-adrenoceptor agonists [^{3}H]guanfacine and [^{3}H]clonidine. The binding of both radioligands to cerebral membranes was found to be rapid, reversible, saturable and specific. The properties and characteristics of the [^{3}H]clonidine binding observed in this study are close to those reported by various authors [5, 6, 17, 24]. The saturation pattern and the kinetics of [^{3}H]guanfacine binding to rat brain membranes were very similar to those of [^{3}H]clonidine. In addition, the maximal number of binding sites occupied by the two radioligands were comparable and comprised a single population. The major difference between the binding of [^{3}H]clonidine and [^{3}H]guanfacine was found in the percentage nonspecific binding contributing to the total binding. The nonspecific binding of [^{3}H]guanfacine to crude membrane fractions prepared from rat brain was considerably more than that determined for [^{3}H]clonidine. This may limit the routine use of [^{3}H]guanfacine in comparison to [^{3}H]clonidine for a direct labeling of α-adrenoceptors. It may be attributed to dissimilar physicochemical properties of clonidine and guanfacine. The relatively high nonspecific binding of [^{3}H]guanfacine may also explain the finding that in the presence of excess unlabeled guanfacine a greater population of specific binding sites for [^{3}H]guanfacine was identified than defined in the presence of excess (−)-norepinephrine or clonidine. Part of this pronounced nonspecific binding of [^{3}H]guanfacine is susceptible to inhibition by nonradioactive guanfacine.

Nonlinear Scatchard plots and biphasic kinetic have been described for the binding of [^{3}H]clonidine to brain tissue [24–26]. Biphasic dissociation plots have also been published for (±) [^{3}H]epinephrine binding to rat and calf cerebral cortex membranes [4]. On the basis of these data, distinct high- and low-affinity α-adrenoceptor binding sites for [^{3}H]clonidine and [^{3}H]epinephrine in the brain have been suggested. These particular sites have been reported to differ in drug specificities, regional distribution and in relative augmentation following 6-hydroxydopamine destruction of the nerve endings [24]. Moreover, the effect of guanine nucleotides and divalent cations are different on both sides [27]. The present study, however, indicates a homogenous population of binding sites, since a linear Scatchard plot resulted for [^{3}H]clonidine as well as for [^{3}H]guanfacine. Linear Scatchard plots for [^{3}H]clonidine binding have also been obtained by other workers [19, 20, 27, 28].

Additionally, [^{3}H]para-aminoclonidine saturation is monophasic [18]. It has been discussed [4] that the radiolabeled agonist ([^{3}H]clonidine, [^{3}H]epinephrine) may convert some receptors to a higher-agonist-affinity (desensitized) state during the course of the receptor labeling. In case higher- and lower-affinity forms are interconvertible, a Scatchard plot of the saturation binding data will be linear. On the other hand, dissociation would be biphasic. This is also observed in the present study for the [^{3}H]clonidine binding. The same may apply to the binding of [^{3}H]guanfacine.

The rank order of potencies of α-adrenoceptor agonists and antagonists in competing for [^{3}H]clonidine and [^{3}H]guanfacine specific binding characterizes these specific binding sites as α_2-adrenoceptors. Unlabeled clonidine and guanfacine which are potent agonists of α_2-adrenoceptors [21, 22, 29, 30] were found much more potent displacers than (−)-phenylephrine, a selective agonist of α_1-adrenoceptors [29, 31]. Furthermore, the potent and selective antagonists of α_2-adrenoceptors rauwolscine and yohimbine [32], were effective competitors, whereas preferential α_1-adrenoceptor blocking agents, like corynanthine [32] and prazosin [33, 34] behaved as weak inhibitors. The almost perfectly linear relationship between the K_i values of the drugs tested for the inhibition of [^{3}H]clonidine and [^{3}H]guanfacine specific binding provides strong evidence for the conclusion that both radioligands bind to identical populations of α_2-adrenoceptors which make comparable demands upon agonists and antagonists. This result is in agreement with the pharmacological properties of clonidine and guanfacine which are very similar [21, 22, 35, 36]. In addition, central α_2-adrenoceptors have been identified as targets for this kind of centrally acting hypotensive drugs [12].

In conclusion, the new radioligand [^{3}H]guanfacine identifies binding sites in rat brain which possess the drug specificity of α_2-adrenoceptors. The properties as well as the kinetic aspects of the [^{3}H]guanfacine binding are comparable to those of [^{3}H]clonidine. The two radioligands label the same population of central α_2-adrenoceptors.

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