MOLECULAR PHARMACOLOGY, 23:607–613

Binding of [^{3}H]Ro 11-2465

Possible Identification of a Subclass of [^{3}H]Imipramine Binding Sites

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Received August 27, 1982; Accepted December 8, 1982

SUMMARY

Ro 11-2465 is a cyanide derivative of imipramine. In cerebral cortex homogenates, [^{3}H] Ro 11-2465 displays a binding profile similar to that of [^{3}H]imipramine. Agents compete with binding of [^{3}H]Ro 11-2465 in an order of potency similar to their ability to block serotonin uptake, and raphe lesions greatly decrease the binding of [^{3}H]Ro 11-2465. These observations suggest that the sites labeled by [^{3}H]Ro 11-2465 are presynaptic. The binding of [^{3}H]Ro 11-2465 is sodium ion-dependent, as are both the binding of [^{3}H] imipramine and the serotonin uptake mechanism. In the presence of sodium ions, binding of [^{3}H]Ro 11-2465 to brain tissue or platelets at 4° is apparently irreversible. Binding is not displaced by high concentrations of the displacing agent desipramine or by repeated washing. However, by either removing sodium or increasing the assay temperature to 23°, the ligand dissociates from the tissue. In tissue where [^{3}H]Ro 11-2465 is irreversibly bound to receptor at 4°, subsequent [^{3}H]imipramine binding is decreased by about 50%. At temperatures greater than 23°, [^{3}H]Ro 11-2465 binding displays a temperature dependency similar to that of [^{3}H]imipramine; that is, when temperatures are raised from 23° to 30° or 37° there is no change in the B_{max}, but the affinity of the ligand for the receptor is decreased. These data suggest that [^{3}H]Ro 11-2465 binds to a discrete population of [^{3}H]imipramine binding sites, comprising about one-half of the total [^{3}H] imipramine binding sites.

INTRODUCTION

The receptor binding of antidepressant drugs has been of much clinical interest recently as a possible tool for predicting or assessing depression. Several laboratories have previously shown that [^{3}H]IMI[3] binds to a site with high affinity (1, 2) on the presynaptic serotonergic neuron (3–5), which is associated with the serotonin uptake mechanism. RO is a cyanide derivative of IMI (Fig. 1). Similar to IMI, RO has been shown to inhibit the uptake of serotonin in platelets and synaptosomes (6–8) and to bind to brain homogenates (9). The purpose of this study was to examine the binding characteristics of [^{3}H]RO to brain homogenates and platelets, and compare them with properties of [^{3}H]IMI binding in order to define further the [^{3}H]IMI binding site.

METHODS

Tissue preparation. Male Wistar rats (200–250 g) were decapitated and the brains removed. The cortical gray matter was scraped from the dorsal surface of the cerebral cortex. The brain tissue was homogenized in 50 volumes of 4° Tris-HCl buffer (50 mM, pH 7.4) using a glass homogenizer with a Teflon piston (0.13–0.18 mm clearance) rotated at 500 rpm with 15 up-and-down strokes. After centrifugation at 30,000 × *g* for 10 min at 4° the supernatant was discarded and the pellet was resuspended in 50 volumes of ice-cold buffer and again centrifuged at 30,000 × *g*. The pellet was then resuspended in 50 volumes of cold buffer, homogenized with a Brinkmann Polytron at setting 6 for 5 sec, and frozen at −70° until used. Directly before assay the tissue was thawed, centrifuged at 30,000 × *g* for 10 min, resuspended in the incubation buffer, and further homogenized for 20 sec.

Outdated human platelet concentrates were donated by the Toronto Red Cross. These had been rotated at room temperature for 72–96 hr. The concentrate was centrifuged at 18,000 × *g* for 10 min at 25° to obtain the platelets. The platelets were then washed three times in cold buffer [50 mM Tris-HCl (pH 7.4)/120 mM NaCl/5 mM KCl) and subsequently resuspended and homogenized by Polytron (setting 6, 20 sec). Platelet membranes were stored at −70° until used.

The incubation buffer was 50 mM Tris-HCl (pH 7.4 at 4°)/120 mM NaCl/5 mM KCl as used by Raisman *et al.* (1), unless otherwise specified.

Binding assay. Binding to cortical homogenates was determined by incubating 35 μl of [^{3}H]RO (17.1 Ci/mmole) or [^{3}H]IMI (70–80 Ci/mmole, New England Nuclear Corporation), 35 μl of buffer ± displacing drug (100 μM desipramine was used to define specific binding), and 180 μl of membrane (final concentration, 400 μg of protein per milliliter). Assay tubes containing [^{3}H]RO were incubated for 1 hr at 23° and those containing [^{3}H]IMI were incubated for 1 hr at 4°, unless otherwise

Preliminary results of this study were submitted as an abstract to the Society for Neuroscience, Minneapolis, November 1982.

[1] Supported by an Ontario Mental Health Foundation Studentship.

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[3] The abbreviations used are: IMI, imipramine; RO, Ro 11-2465.

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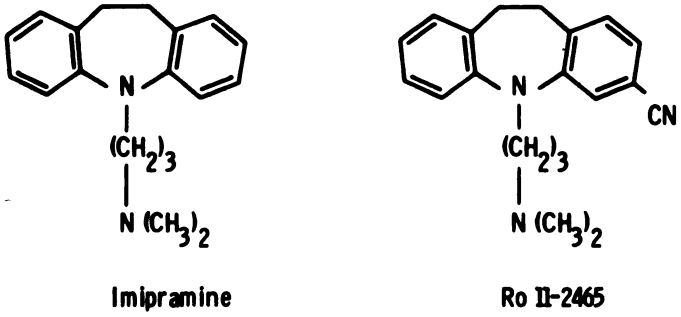

FIG. 1. *Structures of imipramine and RO*

specified. After incubation, 5 ml of ice-cold buffer were added to the incubation tube, and the total volume was rapidly filtered under vacuum through Whatman GF/B glass-fiber filters and washed with 10 ml of cold buffer. The radioactivity remaining on the filters was counted by liquid scintillation spectrometry in 9 ml of Aquasol. Binding to platelets was determined in a similar manner, except that an incubation period with [^{3}H]IMI of 2 hr at 4° was necessary for equilibration. Protein was assayed by the method of Lowry *et al.* (10).

Raphe lesions. Raphe lesions were created as described previously (3). Simultaneous dorsal and medial raphe lesions were made in brains of pentobarbital-anesthetized rats (Nembutal, 50 mg/kg) by generating 55° temperatures for 1 min through a Radionics thermal probe at the following stereotaxic coordinates: anterior-posterior, −0.35 mm; medial-lateral, 0 mm; and dorsal-ventral, −5 and −7 mm. Levels of endogenous serotonin, norepinephrine, and dopamine were measured by high-performance liquid chromatography in the hypothalamus of control and lesioned rats (11) to confirm the extent of the lesions. Hypothalamic serotonin depletion after these lesions was previously shown to parallel the reduction in both cortical serotonin uptake as well as [^{3}H]IMI binding, thus indicating destruction of presynaptic serotonin fibers.

Data analysis. Receptor density and affinity were determined by Scatchard analysis. The two components of [^{3}H]RO binding were analyzed using a nonlinear regression computer program. The kinetic constants were calculated by least-squares approximation of parameter estimates (12, 13), assuming two saturable components: $b = (B_1 \times S)/(K_1 + S) + (B_2 \times S)/(K_2 + S)$, where B_1 and B_2 are the maximal number of binding sites, K_1 and K_2 are the dissociation constants of the binding sites, and S is the ligand concentration.

RESULTS

Saturation of [^{3}H]RO binding. The saturability of [^{3}H]RO binding to cortical homogenates was examined at 23° and 4° using 16 concentrations over a range of 0.1–40 nM. The ratio of total [^{3}H]RO binding to nonspecific binding as defined by 100 μM desipramine was about 7 at a [^{3}H]RO concentration of 2 nM. This concentration was used for routine binding assays.

When cerebral cortex homogenates were incubated at 23° for 1 hr, Scatchard analyses of the data (Fig. 2) were biphasic. The dissociation constant (K_d) of the high-affinity component was 0.25 ± 0.02 nM and the maximal number of binding sites (B_{max}) was 273 ± 11 fmoles/mg of protein (3); the K_d of the low-affinity component was 195 ± 35 nM, and the B_{max} was 4471 ± 589 fmoles/mg of protein (n = 3). As the high-affinity site is more likely to be pharmacologically and physiologically relevant and almost exclusively the only site labeled in competition studies at [^{3}H]RO concentrations of 2 nM, this is the site we examined in more detail.

Binding of [^{3}H]RO in both cortical homogenates and platelets was compared with that of [^{3}H]IMI binding. In

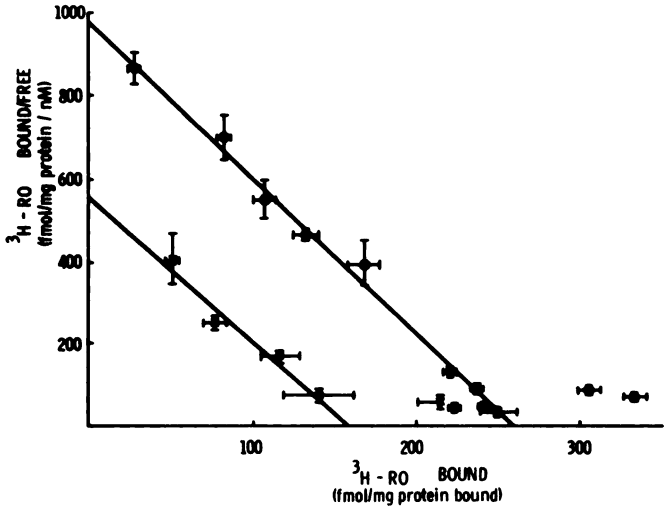

FIG. 2. *Scatchard analysis of binding of [^{3}H]RO (0.1–10 nM) to cerebral cortex homogenates from normal (●) and raphe-lesioned (×) rats*

Each point represents the mean ± standard error of four separate Scatchard analyses. Specific binding was defined by 100 μM desipramine.

studies performed in parallel, [^{3}H]RO bound with high affinity in the cortex with a B_{max} of 257 ± 11 fmoles/mg of protein (n = 4) and [^{3}H]IMI bound with a B_{max} of 416 ± 14 fmoles/mg of protein (n = 6) (Fig. 3). The B_{max}

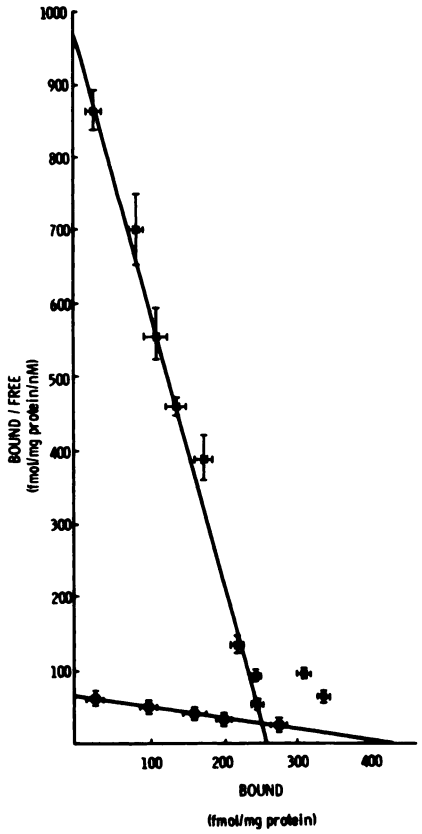

FIG. 3. *Binding of [^{3}H]RO (×) and [^{3}H]IMI (●) to cerebral cortex homogenates*

Each point represents the mean ± standard error of four and six individual experiments, respectively.

values of [^{3}H]RO and of [^{3}H]IMI in platelets were similar, being 323 ± 36 and 360 ± 25 fmoles/mg of protein, respectively (n = 4; Fig. 4). This B_{max} value for [^{3}H]IMI binding in platelets is lower than that found by other groups [Paul *et al.* (14), 460 fmoles/mg of protein; Briley *et al.* (15), 580 fmoles/mg of protein] but is within the range shown by the groups. The differences may be due to different platelet sources, ours being outdated donations from the Red Cross.

Kinetics of [^{3}H]RO binding. The rate of association of [^{3}H]RO binding in cerebral cortex homogenates at 23° is not rapid, reaching equilibrium only after 45 min (Fig. 5A). The observed rate constant for association (K_{ob}) was determined to be 0.082 nM^{-1} min^{-1}, as derived from the equation $\ln[B_e/(B_e - B)] = K_{ob} \cdot t$ (16). The slope of the line of the plot of $\ln[B_e/(B_e - B)]$ versus time in Fig. 5A represents the observed initial rate constant (K_{ob}) at 23°. At lower temperatures the rate of association was greatly reduced, so that at 4° equilibrium was reached only after 6 hr (Fig. 5B). The K_{ob} at 4° was 0.009 nM^{-1} min^{-1}.

The rate of dissociation of [^{3}H]RO was examined by incubating membranes to equilibrium and then adding 10 μl of desipramine (100 μM final concentration) at zero time, after which the reaction was terminated by filtration at various time intervals. When plotted on a semilogarithmic scale the dissociation of [^{3}H]RO in cortical tissue at 23° was monophasic, with a mean half-time of 64 min as shown in Fig. 6. The dissociation constant (K_{-1}), derived from the equation $K_{-1} = 0.693/t_{1/2}$, where

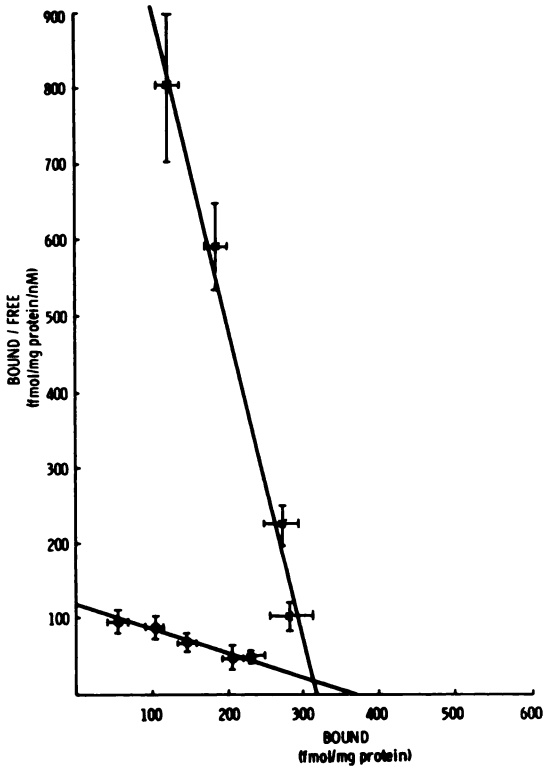

FIG. 4. *Binding of [^{3}H]RO (×) and [^{3}H]IMI (●) to platelet membrane fragments*

Each point represents the mean ± standarad error of four individual experiments.

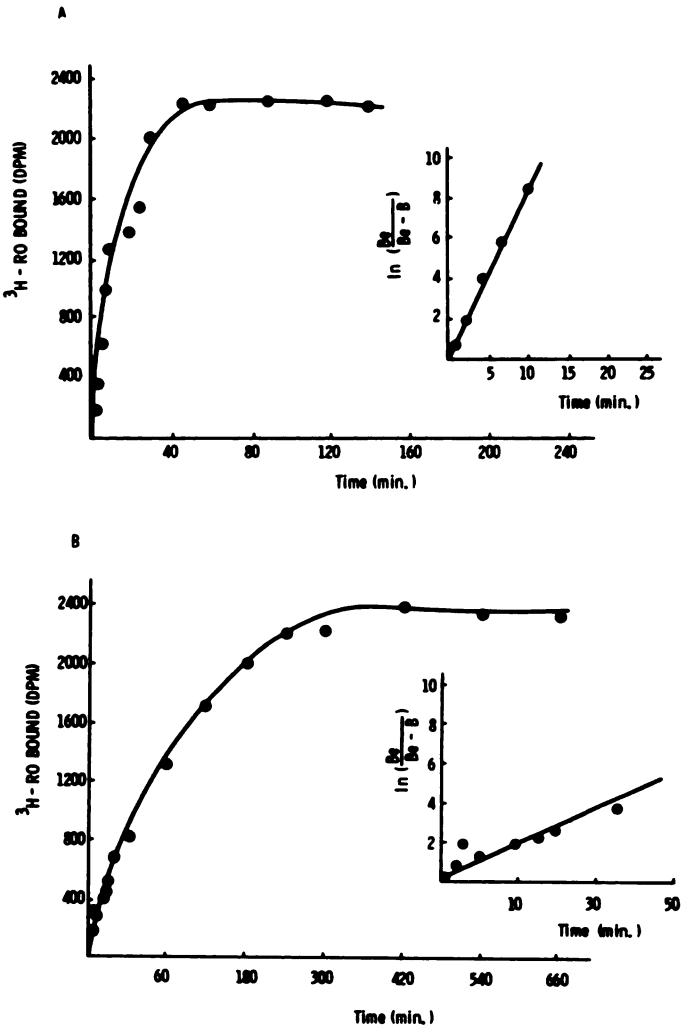

FIG. 5. *Time course of association of specific [^{3}H]RO binding to cerebral cortex homogenates at 23° (A) and 4° (B)*

[^{3}H]RO (2 nM) was incubated in the absence or presence of 100 μM desipramine. Points shown are from a single experiment performed in triplicate. *Inset*, pseudo-first order kinetic plots of specific binding. B is the amount of [^{3}H]RO specifically bound at time t, and B_e is the amount of [^{3}H]RO specifically bound at equilibrium. The slopes of these lines are K_{ob} as defined in the text.

$t_{1/2}$ is the time at which B is half that at $t = 0$, was found to be about 0.01. From the K_{-1}, the K_{+1} (the second-order rate constant), was determined from the equation $K_{+1} = (K_{ob} - K_{-1})/[RO]$ where [RO] is the concentration of [^{3}H]RO. From these values the theoretical K_d ($K_d = K_{-1}/K_{+1}$) was found to be 0.26 nM. This is very close to the experimental value of 0.25 nM as derived from analysis of saturation data.

In experiments conducted in cortical tissue at 4°, less than 10% of the [^{3}H]RO was dissociated from the tissue after 40 hr (Fig. 6). Similarly, in platelets less than 20% of the [^{3}H]RO dissociated from the tissue after allowing 105 hr of dissociation.[4]

Characteristics of the dissociation at 4° were examined. Cortical tissue was incubated at 4° with [^{3}H]RO for various time periods ranging from 1 to 16 hr, dissociated by adding 10 μl of desipramine (100 μM final concentra-

[4] A. Davis, personal communication.

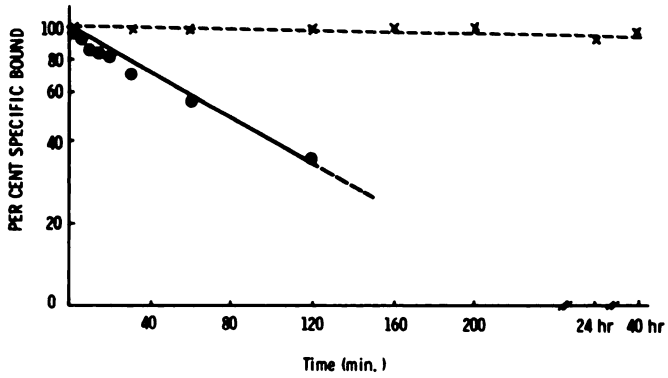

FIG. 6. *Time course of dissociation of [^{3}H]RO specific binding to cerebral cortex homogenates at 4° (×) and 23° (●)*

[^{3}H]RO was incubated to equilibrium (2 hr and 8 hr, respectively). At zero time, 10 μl of desipramine (final concentration 100 μM) were added and the reactions were terminated by filtration at various time intervals. Points shown are from a representative experiment performed in triplicate.

tion), and the proportion of nondissociated [^{3}H]RO was determined. With the longer association periods the amount of nondissociable [^{3}H]RO binding increased, reaching a maximal 90–100% of total binding after 12 hr of association (Fig. 7). However, if the temperature was raised to 23° or 37° after association, binding of [^{3}H]RO readily dissociated, 90% dissociating in 8 hr.

In order to examine whether [^{3}H]IMI binds to sites occupied by [^{3}H]RO, binding of [^{3}H]IMI was examined following preincubation with [^{3}H]RO. To accomplish this, the cortical tissue was divided into two aliquots, one of which was kept at 4°. The second aliquot was incubated with [^{3}H]RO (2 nM) for 16 hr, washed four times at 4° to remove any unbound ligand, and left for 6 hr in 400 volumes of ice-cold buffer to allow dissociation of any "loosely bound" ligand. The tissue was then centrifuged at 30,000 × *g* and resuspended in 50 volumes of cold buffer. Scatchard analysis of [^{3}H]IMI binding was then performed in parallel with the two aliquots of tissue. The percentage of nondisplaceable [^{3}H]RO binding was checked to be 90–100% of total binding in each experiment. As shown in Fig. 8, the B_{max} of [^{3}H]IMI binding was reduced by about 50% in tissue preincubated with [^{3}H]RO. The B_{max} (320 ± 22 fmoles/mg of protein in untreated tissue) was reduced to 152 ± 24 fmoles/mg of protein in [^{3}H]RO preincubated tissue. The K_d was not significantly altered.

To test whether this reduction in B_{max} was due to [^{3}H]RO's causing a desensitization of [^{3}H]IMI binding sites resulting in a reduced B_{max}, parallel assays were performed where the tissue was preincubated for 16 hr with 2 nM IMI, then washed and assayed in a manner identical with that used for tissue preincubated with [^{3}H]RO. Preincubation with IMI did not alter the B_{max} or K_d of [^{3}H]IMI binding.

Effect of temperature on [^{3}H]RO binding. In addition to the above-mentioned effects of temperature on the ability of [^{3}H]RO to dissociate from tissue, there were other reversible effects of temperature on the binding of [^{3}H]RO.

As shown in Table 1, there was a decrease in the IC_{50} values of agents competing for [^{3}H]RO binding at 4° as compared with 23°. If differences in the mean values were caused by the [^{3}H]RO not dissociating from the tissue at 4°, one would have expected the IC_{50} values at 4° to be higher, rather than lower, than those at 23°; therefore, this cannot explain the differences.

More dramatically, when the temperature of the assay incubation was increased from 23° to 37°, there was a 20-fold decrease in the affinity of [^{3}H]RO binding from 0.18 ± 0.05 nM to 1.39 ± 0.12 nM, respectively, without a

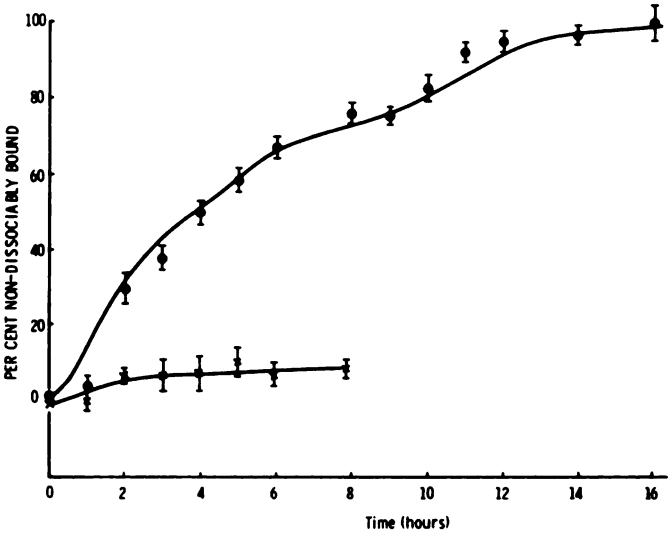

FIG. 7. *Time course of association of [^{3}H]RO bound irreversibly to cerebral cortex homogenates at 23° (×) and 4° (●)*

Tissue was incubated with [^{3}H]RO for various time periods at the specified temperature and then dissociated by adding 10 μl of desipramine (100 μM final concentration) for 6 hr (23° assay) or 12–16 hr (4° assay). Remaining [^{3}H]RO bound to the tissue was assayed as described in the text.

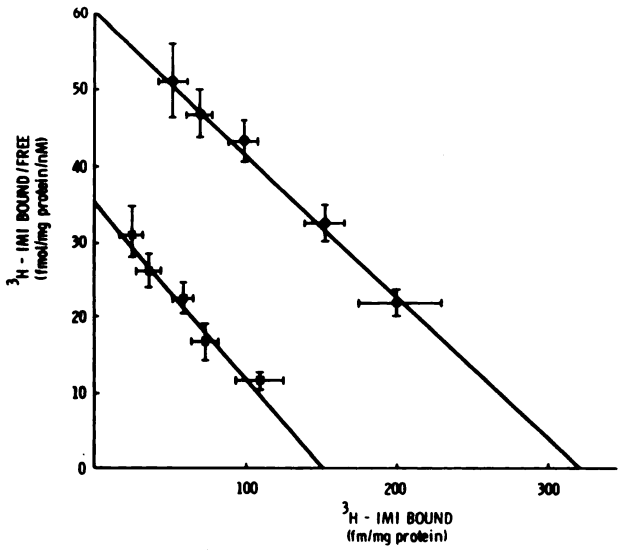

FIG. 8. *Binding of [^{3}H]IMI in the absence (●) or presence (×) of [^{3}H]RO*

An aliquot of tissue was preincubated in the presence of [^{3}H]RO (2 nM) for 16 hr, washed four times at 4°, and left 6 hr for loosely bound ligand to dissociate. The tissue was resuspended and [^{3}H]IMI binding was compared with binding in tissue not exposed to [^{3}H]RO. Each point represents the mean ± standard error of four separate experiments.

TABLE 1

Effect of temperature on [^{3}H]RO binding

Binding assays were performed in the presence of 2 nM [^{3}H]IMI or 2 nM [^{3}H]RO. Specific binding was defined by 100 μM desipramine. All values are the means of three independent determinations. Standard errors are in all cases less than 15% of the mean. Compounds are listed in decreasing order of potency as serotonin uptake inhibitors.

Compound	IC_{50}		
	[^{3}H]IMI (4°)	[^{3}H]RO (23°)	[^{3}H]RO (4°)
		nM	
RO	4	1	1
Chlorimipramine	5	18	2
Citalopram	25	6	—
Fluoxetine	15	150	12
Amitriptyline	70	400	—
IMI	6	65	8
Zimelidine	100	1400	300
Nortriptyline	100	1000	—
Protriptyline	60	2500	—
Desipramine	395	2800	—
Trimipramine	1500	9400	—

change in the B_{max} (Fig. 9). On preincubating the assay mixture for 1 hr at 37° and then lowering the temperature to 23° for 30 min, the affinity was again raised to control levels. A similar change in affinity was observed with [^{3}H]IMI binding, where the K_d increased from 9 ± 1 nM at 23° to 24 ± 4 nM at 37° with no change in B_{max}.[5] Contrary to [^{3}H]RO, however, there is a drop in B_{max} with [^{3}H]IMI binding upon raising the temperature of the assay mixture from 4° to 23° (B_{max} values of 323 ± 12 and 175 ± 16 fmoles/mg of protein, respectively) with no apparent change in affinity.

Competition of [^{3}H]RO binding. Serotonin was the only neurotransmitter tested which displaced either [^{3}H]RO or [^{3}H]IMI, and it had similar potency at the two sites (IC_{50} = 4 μM and 1.8 μM, respectively). In cortical homogenates drugs generally competed for binding of both [^{3}H]RO and [^{3}H]IMI in an order of potency similar to that of their ability to inhibit serotonin uptake (Table 1). Competition curves are shown in Fig. 10. The exception was IMI, which competed for both [^{3}H]IMI and [^{3}H]RO binding more potently than for serotonin uptake. We cannot explain this inconsistency. The data suggest that [^{3}H]IMI and [^{3}H]RO may bind to a common site.

Hill slopes of the competition curves of either ligand in the cortex were less than 1 (0.6–0.75 in all cases), suggesting that both may be binding to more than a single site. Hill slopes of [^{3}H]RO competition curves in platelets were also less than 1 (0.7), whereas those of [^{3}H]IMI in platelets were about 1 (17).

Sodium dependency of [^{3}H]RO binding. Both the uptake of serotonin by synaptosomes (18) and the binding of [^{3}H]IMI (3) are sodium-dependent. In the absence of sodium ions [50 mM Tris-HCl (pH 7.4) or 50 mM Tris-HCl (pH 7.4)/125 mM KCl], only a small amount of [^{3}H]RO binding remained, which may represent binding to a site of very low affinity or possibly nonspecific binding. Similar observations were made with [^{3}H]IMI binding in the absence of sodium.

[5] A. Dumbrille-Ross and S. W. Tang, manuscript in preparation.

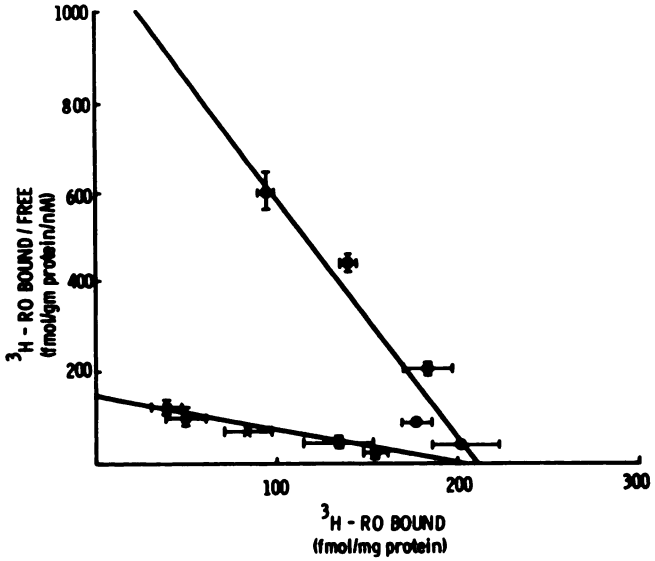

FIG. 9. *Effect of temperature on [^{3}H]RO binding*

Binding of [^{3}H]RO was examined at 23° (●) and 37° (×) as described in the text. Each point represents the mean ± standard error of four experiments.

Removal of sodium from the incubation mixture following equilibrium causes [^{3}H]RO to dissociate spontaneously at 4°, (40% within 8 hr), whereas in the presence of 120 mM NaCl, no dissociation was detectable even after four washings and an 8-hr incubation with 100 μM desipramine.

Effect of raphe lesions on [^{3}H]RO binding. Lesion of presynaptic serotonergic fibers at the level of the raphe nuclei decreases serotonin levels, serotonin uptake, and binding of [^{3}H]IMI (3, 5). Raphe lesions also reduced [^{3}H]RO binding by an amount proportional to the serotonin depletion (as measured by high-performance liquid chromatography). Figure 2 demonstrates that [^{3}H]RO binding was reduced by 50% whereas raphe lesions reduced the serotonin content by 43%.

DISCUSSION

In this study, we have shown that [^{3}H]RO binding shares many characteristics with [^{3}H]IMI binding. This suggests that the two ligands may bind to a common site. Drugs compete for binding of both ligands in an order of potency similar to that in blocking uptake of serotonin, serotonin being the only known neurotransmitter shown to compete for such binding. The majority of the binding of both ligands is sodium-dependent and, as shown by destroying presynaptic serotonin fibers with raphe lesions, is present on presynaptic serotonergic fibers.

Binding of [^{3}H]RO is also similar to that of [^{3}H]IMI in that it is sensitive to temperatures. When the incubation temperature is raised from 23° to 37°, both ligands show no change in B_{max} but there is a drop in the affinity ([^{3}H]IMI data not shown). This change is probably a thermodynamically induced change in receptor conformation, to which agonists are sensitive, similar to that of the *beta*-adrenergic system documented by Weiland *et al.* (19) and Weiland and Molinoff (20). There was also a decrease in the IC_{50} values of agents competing for [^{3}H]

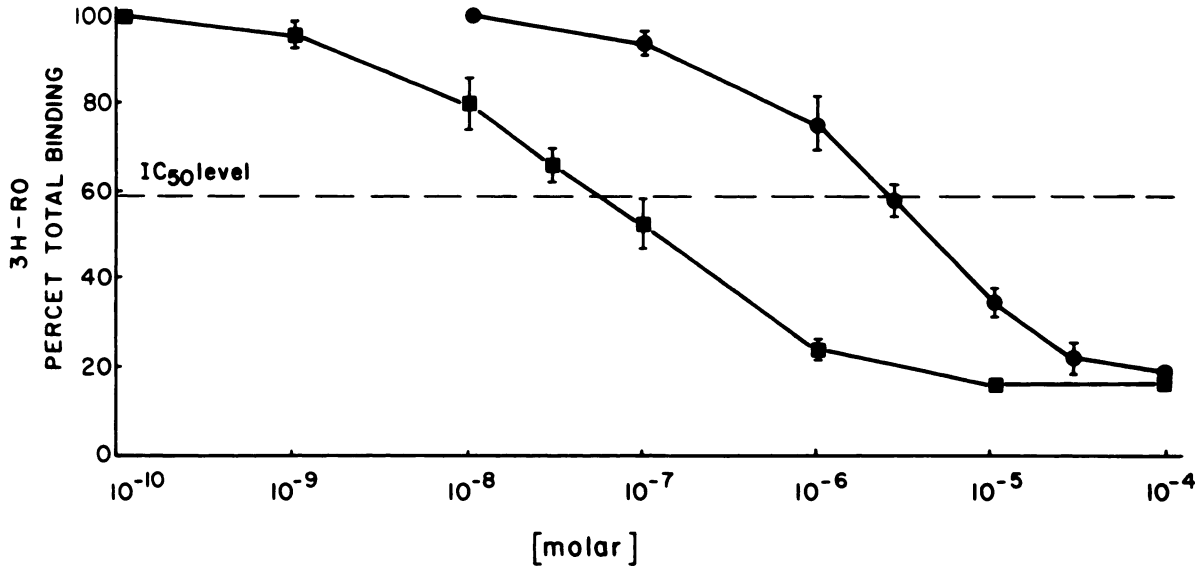

FIG. 10. *Displacement of bound [^{3}H]RO (2 nM, 23°) from rat cerebral cortex homogenate by IMI (■) and DMI (●)*
Specific binding was defined by 100 μM desipramine. All points represent means ± standard errors of three separate experiments.

RO binding at 4° as compared with 23°. These thermodynamic effects suggest that [^{3}H]RO and [^{3}H]IMI may act as agonists at the cortical binding site. We cannot explain why this temperature effect is seen for uptake inhibitors but not for RO.

Several distinct differences exist between [^{3}H]IMI and [^{3}H]RO binding. Although [^{3}H]RO and [^{3}H]IMI bind to a similar number of platelet binding sites, [^{3}H]IMI occupies nearly double the number of cortical binding sites than does [^{3}H]RO with high affinity (B_{max} of 416 ± 14 and 257 ± 11 fmoles/mg of protein, respectively). This suggests that in the cortex [^{3}H] RO may bind to a subpopulation of [^{3}H]IMI binding sites. However, although [^{3}H]RO binds to fewer sites than does [^{3}H]IMI in the cortex, high concentrations of RO displace all of the [^{3}H]IMI in competition experiments. Perhaps concentrations of RO higher than those used in the Scatchard analysis are necessary to displace [^{3}H]IMI from the second "site." A similar discrepancy was observed by Taylor and Richelson (21), who found that [^{3}H]doxepin labeled a subpopulation of [^{3}H]pyrilamine binding sites, but displaced all of the latter in competition experiments.

[^{3}H]RO displays unusual binding kinetics. At 37° or 23°, [^{3}H]RO is displaced from tissue in a normal manner by the desipramine. However, when the incubation temperature is cooled to 4°, desipramine no longer displaces the ligand. This apparent irreversibility of the drug is probably not due to alkylation of the receptor by the cyanide group on the ligand as, when the temperature is returned to 23° or when sodium is removed from the buffer by repeated washings, the [^{3}H]RO is again dissociable by desipramine. We suggest that the cyanide group may change the spatial configuration of the drug, so that it is locked in a particular conformation of the receptor, that occurring at 4°. By removing sodium or by increasing the temperature, the receptor conformation changes to allow the release of the ligand. As similar long-term incubations with [^{3}H]IMI rather than [^{3}H]RO resulted in a maximal 15% loss in binding with no irreversibility, it is unlikely that these changes in binding are due to membrane instability. It is also unlikely that [^{3}H]RO appears to bind to fewer sites than [^{3}H]IMI because of ligand degradation. Even if a proportion of [^{3}H]RO was degraded, all sites would be irreversibly bound by the ligand on incubation as saturating concentration are used. Yet [^{3}H]IMI still binds to a remaining population of sites.

In summary, these data suggest that [^{3}H]RO binds to a discrete population of cortical [^{3}H]IMI binding sites. As [^{3}H]RO binds with very high affinity and specificity (80–95%, as defined by 100 μM desipramine), it may be a more useful ligand than [^{3}H]IMI in studying subpopulations of the IMI binding site(s). The irreversibility of the binding of [^{3}H]RO under particular conditions also makes it a useful ligand in the purification of a population of [^{3}H]IMI binding sites.

ACKNOWLEDGMENTS

We thank Dr. A. Davis for his helpful discussions and advice and Dr. S. Kish for the use of his computer program for Scatchard analysis. We thank Dr. W. P. Burkard for his gifts of [^{3}H] Ro 11-2465 and Ro 11-2465. We also thank Astra Lakemedel, Ciba-Geigy, Lilly, H. Lunbeck and Company, and Merck Sharp & Dohme for their supply of drugs.

REFERENCES

1. Raisman, R., M. Briley, and S. Z. Langer. Specific tricyclic antidepressant binding sites in rat brain. *Nature (Lond.)* **281**:148–150 (1979).
2. Paul, S. M., P. Rehavi, P. Skolnick, and F. K. Goodwin. Demonstration of specific "high affinity" binding sites for [^{3}H]imipramine on human platelets. *Life Sci.* **26**:453–959 (1980).
3. Dumbrille-Ross, A., S. W. Tang, and D. V. Coscina. Differential binding of ^{3}H-imipramine and ^{3}H-mianserin in rat cerebral cortex. *Life Sci.* **29**:2049–2058 (1981).
4. Brunello, N., D. M. Chuang, and E. Costa. Different synaptic location of mianserin and imipramine binding sites. *Science (Wash. D. C.)* **215**:1112–1115 (1982).
5. Sette, M., R. Raisman, M. Briley, and S. Z. Langer. Localization of tricyclic antidepressant binding sites on serotonin nerve terminals. *J. Neurochem.* **37**:40–42 (1981).
6. Haefely, W., R. Schaffner, W. P. Burkard, M. DaPrada, H. H. Kellar, P. Polc, and J. G. Richards. Ro 11-2465, a potent and selective inhibitor of 5-hydroxytryptamine uptake. 11th C.I.N.P. Congress, Vienna, July Abstracts, 95 (1978).

7. Lenehan, T., L. M. O. Omer, and A. Darragh. Effects of Ro 11-2465, a new psychotropic agent, on the uptake of serotonin by human platelets: in vitro determination of the IC_{50}. *Arch. Int. Pharmacodyn. Ther.* **249**:147–152 (1981).
8. Lenehan, T., L. M. O. Omer, M. Kenny, R. Lambe, and A. Darragh. The effect of multiple rising doses of Ro 11-2465 (serotonin uptake inhibitor) on serotonin content of human platelets. *Psychopharmacology* **74**:1–3 (1981).
9. Burkard, W. P. Specific binding sites in rat brain for a new and potent inhibitor of 5-hydroxytryptamine uptake: Ro 11-2465. *Eur. J. Pharmacol.* **61**:409–410 (1980).
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1959).
11. Warsh, J. J., A. Chiu, and D. D. Godse. Simultaneous determination of norepinephrine, dopamine and serotonin in rat brain regions by ion-pair liquid chromatography on actyl silane columns and amperometric detection. *J. Chromatogr.* **228**:131–141 (1982).
12. Roos, H., and K. Pfleger. Kinetics of adenosine uptake by erythrocytes and the influence of dipyridamole. *Mol. Pharmacol.* **8**:417–425 (1972).
13. Kolassa, N., B. Plank, and K. Turnheim. pH and temperature dependence of adenosine uptake in human erythrocytes. *Eur. J. Pharmacol.* **52**:345–351 (1978).
14. Paul, S. M., M. Rehavi, P. Skolnick, J. C. Ballenger, and F. K. Goodwin. Depressed patients have decreased binding of tritiated imipramine to platelet serotonin transporter. *Arch. Gen. Psychiatry* **38**:1315–1317 (1981).
15. Briley, M. S., R. Raisman, D. Sechter, E. Zarifian, and S. Z. Langer. [^{3}H]-Imipramine binding in human platelets: a new biochemical parameter in depression. *Neuropharmacology* **19**:1209–1210 (1980).
16. Williams, L. T., and R. J. Lefkowitz. Alpha-adrenergic receptor identification by [^{3}H]-dihydroergocryptine binding. *Science (Wash.D.C.)* **192**:791–793 (1976).
17. Davis, A., J. Morris, and S. W. Tang. Solubilization and assay of [^{3}H] imipramine binding sites from human platelets. *Eur. J. Pharmacol.* in press (1983).
18. Bogdanski, D. F., A. Tissari, and B. B. Brodie. Role of sodium, potassium, oubain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. *Life Sci.* **7**:419–428 (1968).
19. Weiland, G. A., K. P. Minneman, and P. B. Molinoff. Fundamental difference between the molecular interactions of agonists and antagonists with the β-adrenergic receptor. *Nature (Lond.)* **281**:114–117 (1979).
20. Weiland, G. A., and P. B. Molinoff. Quantitative analysis of drug-receptor interactions. 1. Determination of kinetic and equilibrium properties. *Life Sci.* **29**:313–330 (1981).
21. Taylor, J. E., and E. Richelson. High affinity binding of [^{3}H]doxepin to histamine H_1-receptors in rat brain: possible identification of a subclass of histamine H_1-receptors. *Eur. J. Pharmacol.* **78**:279–285 (1982).

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