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AT-1015, a newly synthesized 5-HT_2 receptor antagonist, dissociates slowly from the 5-HT_2 receptor sites in rabbit cerebral cortex membrane

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Abstract

The purpose of this study was to investigate the association and dissociation kinetics of [^{3}H]AT-1015 from 5-HT_2 receptors in rabbit cerebral cortex membranes using a radioligand binding assay method and to make a comparison with those of [^{3}H]ketanserin binding. Scatchard analysis of [^{3}H]AT-1015 binding in rabbit cerebral cortex membranes indicated the existence of a single class of binding sites (dissociation constant, K_d = 2.18 nM). The specific binding of [^{3}H]AT-1015 increased slowly with time and the association rate constant of [^{3}H]AT-1015 binding (k_1 = 0.1229 min^{-1} nM^{-1}) was two times slower than that of [^{3}H]ketanserin binding (k_1 = 0.2451 min^{-1} nM^{-1}). The dissociation rate constant of [^{3}H]AT-1015 binding ($t_{\frac{1}{2}}$ = 37.03 min) was six times slower than that of [^{3}H]ketanserin binding ($t_{\frac{1}{2}}$ = 6.29 min), when the addition of excess unlabelled ligands were AT-1015 and ketanserin, respectively. The dissociation rate constant of [^{3}H]AT-1015 was slowed to a greater degree ($t_{\frac{1}{2}}$ = 163.40 min and $t_{\frac{1}{2}}$ = 198.12 min) by the addition of ketanserin and sarpogrelate as excess unlabelled ligands than was that of [^{3}H]ketanserin ($t_{\frac{1}{2}}$ = 17.76 min and $t_{\frac{1}{2}}$ = 18.45 min) by the addition of AT-1015 and sarpogrelate as an excess unlabelled ligand, respectively. These findings on the dissociation kinetics of [^{3}H]AT-1015 have confirmed and supported previously reported evidence of the slower dissociation of AT-1015 from 5-HT_2 receptors.

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Introduction

It has been reported (Kihara et al 2000) that AT-1015 (*N*-[2-[4-(5*H*-dibenzo [a,d] cyclohepten - 5 - ylidene) - piperidino]ethyl] - 1 - formyl - 4 - piperidinecarboxamide monohydrochloride monohydrate) is a potent 5-HT_{2A} receptor antagonist. It has also been suggested that it might have a slow dissociation from the 5-HT_2 receptor binding sites in rat aorta and its insurmountable antagonism might be relevant to its therapeutic potential in peripheral vascular disease. AT-1015 selectively inhibited 5-HT_{2A}-receptor-mediated platelet aggregation in-vitro and this inhibitory activity of AT-1015 was almost equivalent to that of ketanserin and 100 times more potent than that of sarpogrelate. We also reported (Gong et al 2000) that AT-1015 was a strong noncompetitive 5-HT_2 antagonist, which inhibited the maximum contraction responses in porcine coronary arteries without endothelium induced by 5-HT and α-methylserotonin. On the other hand, ketanserin induced rightward shifts of contraction response without significant inhibition of maximum response.

In our previous studies (Rashid et al 2001a), it was revealed that AT-1015 had high binding affinity to 5-HT_2 receptors in rabbit cerebral cortex membranes and the blockade of specific [^{3}H]ketanserin binding sites in the rabbit cerebral cortex membranes induced by AT-1015 was not readily reversed by washing. However, the inhibition of [^{3}H]ketanserin binding induced by ketanserin and sarpogrelate was readily reversed by washing. It was also suggested in our report that AT-1015 might strongly bind to 5-HT_2 receptor sites with the pretreatment of the membrane fractions and was not readily

dissociated from bound 5-HT_2 receptors after washout, unlike ketanserin and sarpogrelate. Recently, Kihara et al (2001) reported that AT-1015 was a potent and long acting oral antithrombic agent with a low risk of bleeding-time prolongation in a photochemically induced arterial thrombosis model in the rat femoral artery. The duration of the antithrombic effect of AT-1015 was much longer than that of sarpogrelate. The author also suggested that this effect of AT-1015 might be elicited by its potent and long-acting inhibition of vasoconstriction through the 5-HT_{2A} receptor.

The purpose of this study is to investigate the association and dissociation kinetics of the binding of [^{3}H]AT-1015, a radioligand of AT-1015, to 5-HT_2 receptors in rabbit cerebral cortex membranes and make a comparison with those of [^{3}H]ketanserin binding. The investigation may provide further evidence of its slower dissociation that has been reported previously in washout experiments using the method of centrifugation, resuspension and radioligand binding assay. The study was also performed to compare the dissociation of [^{3}H]AT-1015 caused by addition of excess unlabelled AT-1015, with that induced by the addition of excess unlabelled other 5-HT_{2A} selective antagonists such as ketanserin and sarpogrelate.

Materials and Methods

Materials

[^{3}H]Ketanserin (63.3 Ci mmol^{-1}) was purchased from NEN Life Science Products, Inc. (Boston, MA). [^{3}H]AT-1015 (1.81 TBq mmol^{-1}) was obtained from ADME/TOX Research Institute, Daiichi Pure Chemicals Co. Ltd, Ibaraki, Japan. AT-1015, (*N*-[2-[4-(5*H*-dibenzo[a,d]cyclohepten-5-ylidene)-piperidino]ethyl]-1-formyl-4-piperidinecarboxamide monohydrochloride monohydrate) used in the study was synthesized and donated by Ajinomoto Co. Ltd, Tokyo, Japan. Ketanserin was obtained from RBI (Research Biochemical Incorporated, Natick, MA) and sarpogrelate was obtained from Mitsubishi Chemical Corporation, Tokyo, Japan.

Preparation of membrane-enriched fraction

Membrane-enriched fraction of rabbit cerebral cortex was prepared by the method described previously (Hosohata et al 1995). In brief, the brain was removed from a male rabbit (Japanese white, 3–4 kg), after the rabbit had been anaesthetized with sodium pentobarbital (25 mg kg^{-1}, i.v.) and exsanguinated. The cerebral cortex was then separated from the whole brain and minced in 10 volumes of sucrose buffer (10 mM Tris-HCl, 0.25 M sucrose, pH 7.4) and was homogenized by using a glass homogenizer. The homogenate was then centrifuged at 40 000 *g* for 30 min at 4°C. The supernatant was discarded and the resultant pellet was resuspended in the incubation medium containing 120 mM Tris-HCl and 40 mM $MgCl_2$ (pH 7.4). The membrane-enriched fraction was then immediately frozen in liquid nitrogen and stored at −80°C until used. Membrane protein concentrations were determined by the method of Bradford (1976) with bovine serum albumin used as standard.

Radioligand binding studies

5-HT_2 receptor binding assays

The radioligand binding assay for 5-HT_2 receptor subtypes was performed according to the method described previously (Nagatomo et al 1988). Experiments were performed at a final volume of 200 μL of assay buffer (60 mM Tris-HCl, 20 mM $MgCl_2$, 0.1% ascorbic acid and 1.0 μM pargylline, pH 7.4) with 50 μL of [^{3}H]AT-1015, 100 μL of membrane preparation (0.1 mg of protein) and 50 μL of vehicle or test solution containing various concentrations of the test compounds. The concentration of [^{3}H]AT-1015 ranged from 0.03 nM to 6.0 nM for the saturation binding experiments. After 60 min incubation at 23°C, the reaction mixture was terminated by the addition of 2.0 mL of ice-cold buffer (50 mM Tris-HCl, pH 7.4) and rapidly filtered under vacuum through a Whatman GF/C glass fiber filter, pre-soaked in 0.1% polyethyleneimine (PEI). The filters were washed five times with 2.0 mL of ice-cold buffer (50 mM Tris-HCl, pH 7.4). The retained radioactivity in the vial that contained the filter with 2 mL of toluene-trinitron based scintillation fluid was determined using a liquid scintillation counter (Packard 2200 Tri-Carb Scintillation Analyzer, Packard Instrument Co. Inc., Mariden, CT). The specific binding was determined by subtracting the remaining nonspecific binding in the presence of 1.0 μM unlabelled AT-1015 from the total binding.

Kinetic studies of [^{3}H]AT-1015 and [^{3}H]ketanserin binding to 5-HT_2 receptors

Kinetic studies of [^{3}H]AT-1015 and [^{3}H]ketanserin binding to 5-HT_2 receptors were carried out according to the method of Ojima et al (1997) with slight modification. In association experiments, the amount of specific binding of [^{3}H]AT-1015 (1.0 nM) and [^{3}H]ketanserin (1.25 nM) to the rabbit cerebral cortex membranes were determined at a final volume of 0.2 mL of assay buffer (60 mM Tris-HCl buffer, 20 mM $MgCl_2$, 0.1% ascorbic acid and 1.0 μM pargylline, pH 7.4) with 50 μL of [^{3}H]AT-1015 and [^{3}H]ketanserin, 100 μL of membrane preparation (0.1 mg of protein) and 50 μL of vehicle or nonspecific agent after various time intervals between 1 and 90 min and 1 and 60 min, respectively, at 23°C. After incubation at the various time intervals at 23°C, the reaction mixture was terminated by the addition of 2.0 mL ice-cold buffer (50 mM Tris-HCl, pH 7.4) and rapidly filtered under vacuum through a Whatman GF/C glass fiber filter, pre-soaked in 0.1% PEI. The filters were washed five times with 2.0 mL of ice-cold

buffer (50 mM Tris-HCl, pH 7.4). The retained radioactivity in the vial that contained the filter with 2 ml of toluene-trinitron based scintillation fluid was determined using a liquid scintillation counter (Packard 2200 Tri-Carb Scintillation Analyzer, Packard Instrument Co. Inc., Mariden, CT).

In dissociation experiments, [^{3}H]AT-1015 (1.0 nM) and [^{3}H]ketanserin (1.25 nM) were incubated to steady state with rabbit cerebral cortex membranes (0.1 mg protein) at a final volume of 0.2 mL of 60 mM Tris-HCl buffer (pH 7.4) containing 20 mM $MgCl_2$, 0.1% ascorbic acid and 1.0 μM pargylline for 60 and 30 min, respectively, at 23°C. Then dissociation was induced by the addition of excess unlabelled ligands (10 μM). After incubation with excess unlabelled ligands at various time intervals, the reaction mixture was terminated by the addition of 2.0 mL of ice-cold buffer (50 mM Tris-HCl, pH 7.4) and rapidly filtered under vacuum through a Whatman GF/C glass fiber filter, pre-soaked in 0.1% PEI. The filters were washed five times with 2.0 mL of ice-cold buffer (50 mM Tris-HCl, pH 7.4). The retained radioactivity in the vial that contained the filter with 2 mL of toluene-trinitron based scintillation fluid was determined using a liquid scintillation counter (Packard 2200 Tri-Carb Scintillation Analyzer, Packard Instrument Co. Inc., Mariden, CT).

Data analysis

Data were given as the means ± s.d. The dissociation constant (K_d) and the density of 5-HT$_2$-receptor binding sites (B_{max}) in the membrane preparation of the rabbit cerebral cortex were determined by linear regression analysis of the saturation binding curves transformed to Scatchard plots. The dissociation rate constant (k_{-1}) was determined from the first-order plot of ln (B_t/B_{eq}) versus time, where B_{eq} and B_t are the amount of specific binding at equilibrium and time t (the time after the addition of unlabelled ligand) using Sigma Plot Program (Jandel Scientific, San Rafael, CA). The half-life of the ligand–receptor complex ($t_{\frac{1}{2}}$) was calculated using $t_{\frac{1}{2}} = 0.693/k_{-1}$. The observed rate constant (k_{obs}) was determined from the pseudo-first-order plot of the specific binding data versus time. k_{obs} was calculated using nonlinear regression to fit the specific binding data to the one-phase exponential association equation: $Y = Y_{max}.(1-e^{-kobs.t})$, where Y_{max} is the maximum specific binding and Y is the specific binding that increases to a maximum plateau (at equilibrium) equal to Y_{max}. The association rate constant (k_1) was calculated from $k_1 = (k_{obs} - k_{-1})/[L]$, where [L] is the concentration of the radioligand.

Statistical analysis

Data were given as mean ± s.d. Statistical significance of the data was evaluated using Mann–Whitney U-test for the comparisons of two groups and one-way analysis of variance followed by Tukey's test for comparison of more than three groups. k_{obs} was calculated by nonlinear regression curve fit and one-phase exponential association equation using GraphPad Prism (GraphPad Software Inc., San Diego, CA).

Results

Saturation analysis of [^{3}H]AT-1015

The specific binding of [^{3}H]AT-1015 to rabbit cerebral cortex membranes was saturable in the range 0.03–6.0 nM. Specific binding represented 60–80% of the total radioactivity bound to the cerebral cortex membranes. A Scatchard analysis of saturation binding data yielded a linear plot, suggesting the presence of a single population of binding sites. The K_d value for specific [^{3}H]AT-1015 binding was 2.18 ± 0.84 nM and the B_{max} was 1269.61 ± 228.47 fmol (mg protein)$^{-1}$ (n = 6).

Kinetic studies of [^{3}H]AT-1015 and [^{3}H]ketanserin binding

The time-course of association and dissociation of [^{3}H]AT-1015 (1.0 nM) and [^{3}H]ketanserin (1.25 nM) binding to rabbit cerebral cortex membranes is depicted in Figures 1 and 2, respectively. The specific binding of [^{3}H]AT-1015 increased slowly with incubation time and reached equilibrium after approximately 60 min. This equilibrium was maintained at a steady level for up to 180 min of incubation. The specific binding of [^{3}H]ketanserin increased very rapidly and reached equilibrium after approximately 30 min of incubation. The association rate constants (k_1) of both radioligands were

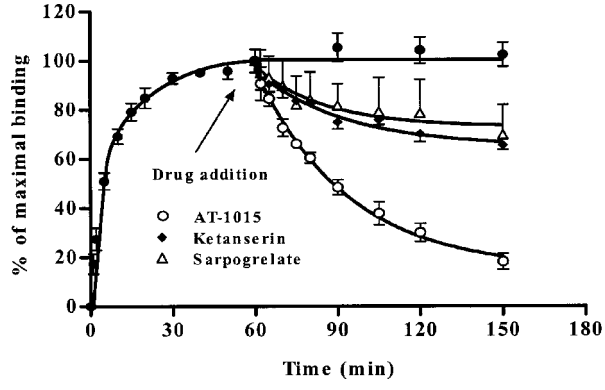

Figure 1 Time-course of the association and dissociation of [^{3}H]AT-1015 binding to rabbit cerebral cortex membranes. After [^{3}H]AT-1015 (1.0 nM) binding to membranes had reached equilibrium (at 60 min), dissociation was induced by the addition of unlabelled AT-1015 (10 μM; ○), ketanserin (10 μM; ◆) or sarpogrelate (10 μM; △). The specific binding is expressed as a percentage of the specific binding at equilibrium (maximal binding). Data are the means (± s.d.) of 4 or 5 experiments, each performed in duplicate.

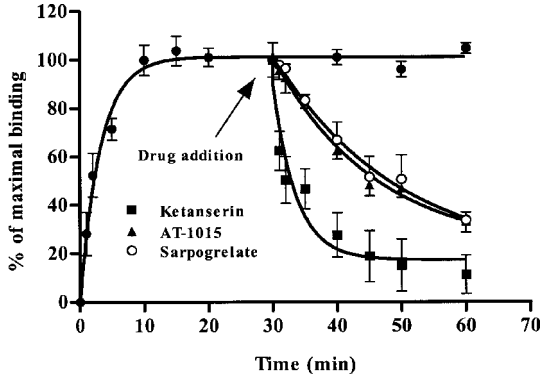

Figure 2 Time-course of the association and dissociation of [^{3}H]ketanserin binding to rabbit cerebral cortex membranes. After [^{3}H]ketanserin (1.25 nM) binding to membranes had reached equilibrium (at 30 min), dissociation was induced by the addition of unlabelled ketanserin (10 μM; ■), AT-1015 (10 μM; ▲) or sarpogrelate (10 μM; ○). The specific binding is expressed as a percentage of the specific binding at equilibrium (maximal binding). Data are the means (±s.d.) of 4 or 5 experiments, each performed in duplicate.

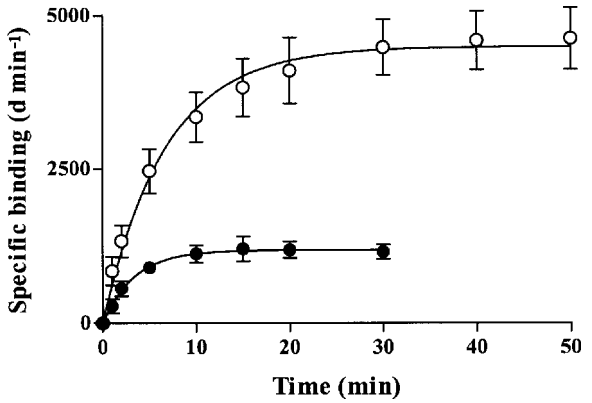

Figure 3 Association kinetics of the specific binding of [^{3}H]AT-1015 and [^{3}H]ketanserin to rabbit cerebral cortex membranes. Pseudo-first-order kinetic plots of initial binding of [^{3}H]AT-1015 (1.0 nM; ○) and [^{3}H]ketanserin (1.25 nM; ●) to membranes. The observed rate constant (k_{obs}) was calculated using nonlinear regression to fit the specific binding data to the one-phase exponential association equation. The second-order association rate constant (k_1) was calculated from $k_1 = (k_{obs} - k_{-1})/[L]$, where k_{-1} is the dissociation rate constant and [L] is the concentration of the radioligand.

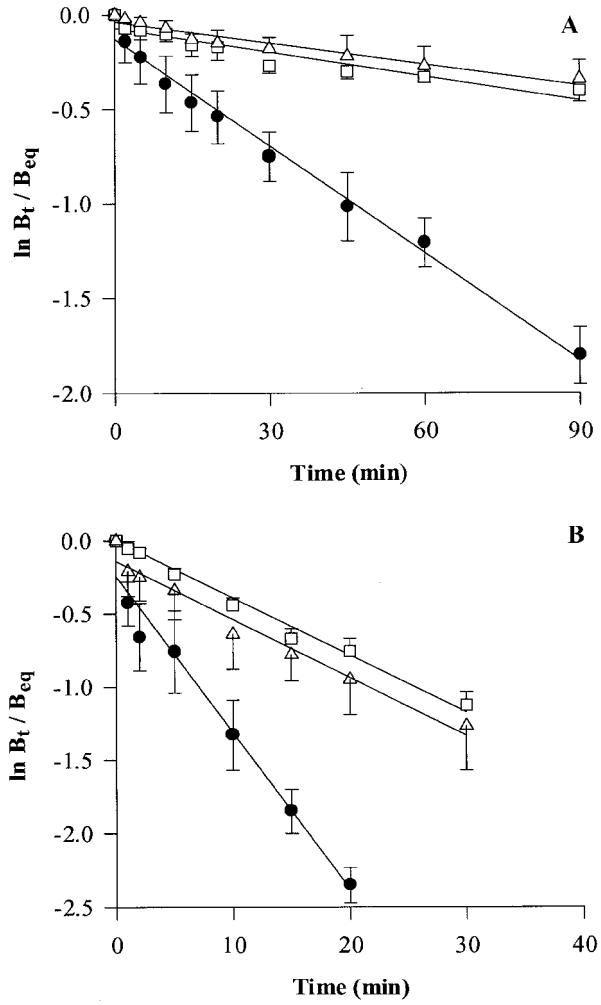

Figure 4 Dissociation kinetics of specific binding of [^{3}H]AT-1015 (1.0 nM; A) and [^{3}H]ketanserin (1.25 nM; B) to rabbit cerebral cortex membranes. A. After [^{3}H]AT-1015 (1.0 nM) binding to membranes reached equilibrium (at 60 min), dissociation was induced by the addition of unlabelled AT-1015 (10 μM; ●), and also induced by the addition of unlabelled ketanserin (10 μM; □) or sarpogrelate (10 μM; △). B. After [^{3}H]ketanserin (1.25 nM) binding to membranes reached equilibrium (at 30 min), dissociation was induced by the addition of unlabelled ketanserin (10 μM; ●) and also induced by the addition of unlabelled AT-1015 (10 μM; △) or sarpogrelate (10 μM; □). On the ordinate, B_{eq} and B_t are binding at equilibrium and time t (time after addition of the unlabelled ligand). The slope of the first-order plot is the dissociation rate constant (k_{-1}). Data are the means (±s.d.) of 4 or 5 separate experiments, each performed in duplicate.

Table 1 Association kinetics of specific [^{3}H]AT-1015 and [^{3}H]ketanserin binding to 5-HT_2 receptors in rabbit cerebral cortex membrane.

Radioligand	Observed rate constant (k_{obs}) (min^{-1})	Association rate constant (k_1) (min^{-1} nM^{-1})
[^{3}H]AT-1015	0.1418±0.0128	0.1229±0.0128
[^{3}H]Ketanserin	0.4199±0.0209*	0.2451±0.0166*

Data are the means (±s.d.) of 4 or 5 experiments, each performed in duplicate. *$P < 0.05$ vs [^{3}H]AT-1015, determined by Mann–Whitney U-test.

calculated from these studies (Figure 3). The association rate constants (k_1) of [^{3}H]AT-1015 and [^{3}H]ketanserin were found to be 0.1229 and 0.2451 min^{-1} nM^{-1}, respectively (Table 1).

When the specific binding of [^{3}H]AT-1015 (1.0 nM) and [^{3}H]ketanserin (1.25 nM) reached a steady level or equilibrium, then dissociation was induced by the addition of 10 μM unlabelled AT-1015 and ketanserin, respectively (Figure 4a, b). Table 2 summarizes the dissociation rate constant (k_{-1}) and half-life ($t_{\frac{1}{2}}$) of both

Table 2 Dissociation kinetics of specific [^{3}H]AT-1015 and [^{3}H]ketanserin binding to 5-HT$_2$ receptor in rabbit cerebral cortex membrane.

Addition of unlabelled ligand	Dissociation rate constant (k_{-1}) (min^{-1})		Half-life ($t_{\frac{1}{2}}$) (min)	
	[^{3}H]AT-1015	[^{3}H]Ketanserin	[^{3}H]AT-1015	[^{3}H]Ketanserin
AT-1015	0.0189±0.0019	0.0399±0.0063##	37.03±3.82	17.76±3.03##
Ketanserin	0.0043±0.0003**	0.1141±0.0252	163.40±9.85**	6.29±1.26
Sarpogrelate	0.0037±0.0012**	0.0377±0.0021##	198.12±48.02**	18.45±1.10##

Values are mean (±s.d.) of 4 or 5 experiments, each performed in duplicate. **$P < 0.001$ vs AT-1015 when the radioligand was [^{3}H]AT-1015 and ##$P < 0.001$ vs ketanserin when the radioligand was [^{3}H]ketanserin (one-way analysis of variance followed by Tukey's test).

radioligands. AT-1015 produced slow release of [^{3}H]AT-1015 from the [^{3}H]AT-1015–receptor complex with a dissociation rate constant (k_{-1}) and half-life ($t_{\frac{1}{2}}$) of 0.0189 min^{-1} nM^{-1} and 37.03 min, respectively. The dissociation of [^{3}H]AT-1015 was further slowed down (5–6 times) with a $t_{\frac{1}{2}}$ of 163.40 and 198.12 min by the addition of ketanserin and sarpogrelate as an excess unlabelled ligand, respectively. In contrast, the dissociation of [^{3}H]ketanserin caused by the addition of excess unlabelled ketanserin was very rapid, with a dissociation rate constant (k_{-1}) of 0.1141 min^{-1} nM^{-1} and a $t_{\frac{1}{2}}$ of 6.29 min. The dissociation of [^{3}H]ketanserin was slightly slowed ($t_{\frac{1}{2}}$ = 17.76 and 18.45 min) by the addition of AT-1015 and sarpogrelate as an excess unlabelled ligand, respectively. Table 2 shows that the dissociation rate of [^{3}H]AT-1015 from rabbit cerebral cortex membrane was six times slower than that of [^{3}H]ketanserin.

Discussion

We investigated the association and dissociation kinetics of [^{3}H]AT-1015 binding, a radioligand of AT-1015, using rabbit cerebral cortex membranes and made a comparison with those of [^{3}H]ketanserin binding. This preparation was used because 5-HT$_2$ receptor subtypes were found in high concentration in the cerebral cortex, blood vessels and gastrointestinal and uterine smooth muscles (Conn & Sanders-Bush 1987; Zifa & Fillon 1992), the cerebral cortex being the most highly enriched in 5-HT$_{2A}$ receptor (Pazos et al 1985; Roth et al 1987). In our previous findings, it was observed that AT-1015 had demonstrated high binding affinity in both the rabbit cerebral cortex membranes and platelet membranes using [^{3}H]ketanserin as a radioligand (Rashid et al 2001a, b). AT-1015 was a potent 5-HT$_{2A}$ receptor antagonist and it selectively inhibited 5-HT$_{2A}$-receptor-mediated platelet aggregation (Kihara et al 2000). It was also reported that AT-1015 was a strong non-competitive 5-HT$_2$ antagonist and it reduced the maximal contraction in a 5-HT-induced vasoconstriction in both rat thoracic artery (Kihara et al 2000) and porcine coronary artery (Gong et al 2000), whereas ketanserin inhibited the contraction without significant effect on maximum response. Several hypothetical mechanisms have been proposed to explain insurmountable antagonism, one of which is that it is thought to be produced by slow dissociation of the antagonist–receptor complex (Robertson et al 1992; Wienen et al 1992; Panek et al 1995). To examine this hypothesis, we had previously investigated the dissociation ability of AT-1015 and compared it with other 5-HT$_2$ antagonists. AT-1015 slowly dissociated from 5-HT$_2$ receptors in rabbit cerebral cortex membranes using washout experiments. In this study, we have investigated the association and dissociation rate constants and half-life of [^{3}H]AT-1015 binding to the rabbit cerebral cortex membranes and emphasized further evidence of its slower dissociation from 5-HT$_2$ receptor sites.

In saturation binding studies, Scatchard- and Hill-plot analyses of [^{3}H]AT-1015 binding indicated the presence of a single population of binding sites (K_d = 2.18 nM; B_{max} = 1269.61 fmol (mg protein)$^{-1}$) and the absence of negative or positive cooperation. In preliminary experiments, we also used a very large concentration range (0.025–17 nM) of [^{3}H]AT-1015 for examining its probable two sites of binding to rabbit cerebral cortex membranes and the saturation curve did not match with the two-site binding. We also observed that [^{3}H]AT-1015 binding in rabbit cerebral cortex membranes reached equilibrium within 60 min incubation at 23°C using the reaction mixture mentioned in the Materials and Methods section. We also used a concentration of 1.0 nM of [^{3}H]AT-1015 for the association and dissociation kinetics studies, because receptor occupancy was high and equilibrium binding was reached at the same incubation time as in saturation binding.

In our kinetic studies, [^{3}H]AT-1015 associated slowly and time dependently with an association rate constant of 0.1229 min^{-1} nM^{-1} in rabbit cerebral cortex membranes and its association rate constant was two times slower than that of [^{3}H]ketanserin (k_1 = 0.2451 min^{-1} nM^{-1}), a known 5-HT$_2$ radioligand of rapid association with the rat prefrontal cortex (Leysen et al 1981). In dissociation kinetics, [^{3}H]ketanserin dissociated rapidly with an initial half-life of dissociation ($t_{\frac{1}{2}}$) of 6.29 min in rabbit cerebral

cortex membranes, which is almost similar to the results of other authors' reports ($t_{\frac{1}{2}}$ of [^{3}H]ketanserin = 3.5 and 5.8 min) (Leysen et al 1981; de Chaffoy de Courcelles et al 1986). On the other hand, the dissociation rate of [^{3}H]AT-1015 was six times slower than that of [^{3}H]ketanserin. The kinetic data (i.e., dissociation rate constant and half-life) of [^{3}H]AT-1015 have given a quantitative value for the slower dissociation of AT-1015, which was previously reported qualitatively. Therefore, this investigation correlates with our previous report and provides further evidence of its slower dissociation from 5-HT$_2$ receptors in rabbit cerebral cortex membranes. Kihara et al (2001) also reported the long-lasting antithrombic effect of AT-1015 and this effect was much longer than that of sarpogrelate.

Another possible explanation for this insurmountable antagonism is the allosteric modification of the receptors (Wienen et al 1992; Panek et al 1995). To evaluate this hypothesis, we examined the dissociation of [^{3}H]AT-1015 from 5-HT$_2$ binding sites of the rabbit cerebral cortex induced by the addition of excess unlabelled ketanserin and sarpogrelate. The rate of dissociation of [^{3}H]AT-1015 from 5-HT$_2$ receptors after the addition of excess unlabelled AT-1015 was significantly different from that following the addition of excess unlabelled other 5-HT$_{2A}$ selective antagonists such as ketanserin and sarpogrelate. Figures 1 and 2, respectively, clearly show that, at equilibrium, ketanserin did not completely displace [^{3}H]AT-1015 and that AT-1015 did not completely displace [^{3}H]ketanserin. Thus, AT-1015 is a non-competitive antagonist that allosterically modulates the binding of ketanserin. However, further studies are required to clarify the exact mechanism of this phenomenon.

In conclusion, this study has demonstrated that AT-1015, a newly synthesized 5-HT$_2$ receptor antagonist, dissociates slowly from 5-HT$_2$ receptor sites and this result provides further evidence of its previously reported slower dissociation from rabbit cerebral cortex membranes. These findings have also revealed possible explanations for the insurmountable antagonism of AT-1015 on vasoconstriction in the rat artery (Kihara et al 2000) and porcine coronary artery (Gong et al 2000).

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