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Ketanserin Binds to the Monoamine Transporter of Chromaffin Granules and of Synaptic Vesicles

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SUMMARY

[^{3}H]Ketanserin binding studies were performed on purified chromaffin granule membranes. Binding was found to occur on one class of sites and was temperature dependent. At 30° the equilibrium dissociation constant K_D was 45 nM. At 0°, a K_D value of 6 nM and a half-life of dissociation of 40 sec were measured. Methysergide, an antagonist of 5-hydroxytryptamine$_2$ (5-HT$_2$) receptors structurally unrelated to ketanserin, did not displace ketanserin binding. Tetrabenazine, an inhibitor of the monoamine transporter of chromaffin granules, displaced [^{3}H]ketanserin binding. Conversely, ketanserin inhibited the binding of [^{3}H] dihydrotetrabenazine, a ligand that specifically binds to the monoamine transporter. The inhibition was of the competitive type, indicating that both drugs bind to the same site. Ketanserin binding did not depend upon ATP-induced energization of chromaffin granules. ATP-dependent 5-HT uptake by chromaffin granule ghosts was inhibited by ketanserin with an IC_{50} value of 70 nM. A series of ketanserin derivatives were tested for their ability to displace [^{3}H]dihydrotetrabenazine; EC_{50} values differed by more than 2 orders of magnitude and were not correlated to affinities on 5-HT$_2$ receptors. In mouse brain, [^{3}H]ketanserin was found to bind to methysergide-sensitive and to tetrabenazine-sensitive sites. In the striatum, tetrabenazine-sensitive sites represented a larger fraction than the methysergide-sensitive ones, whereas the reverse was true in the frontal cortex. It is concluded that nonspecific displaceable binding sites of [^{3}H]ketanserin previously described in the striatum are tetrabenazine binding sites associated with the synaptic vesicle monoamine transporter.

[^{3}H]Ketanserin is considered to be a specific ligand of 5-HT$_2$ or S$_2$ receptors (1, 2). It has been currently used to identify these sites in the central nervous system (3) as well as in platelets (4). However, in these tissues [^{3}H]ketanserin also bound to another class of sites, the so-called displaceable nonspecific binding sites; [^{3}H]ketanserin was displaced from these sites by an excess of unlabeled ketanserin, but neither by methysergide, a 5-HT$_2$ receptor antagonist having another chemical structure, nor by micromolar concentrations of 5-HT. These sites were numerous in the striatum and in platelets (2).

Nonspecific ketanserin binding sites are unlikely to be neurotransmitter receptors, because all neurotransmitters tested were unable to displace [^{3}H]ketanserin from these sites. However, the membrane preparations used in these studies contained various organelle membranes in addition to the plasma membranes presumably bearing S$_2$ receptors, and the nonspecific ketanserin binding sites might be located on organelle membranes. A recent report has suggested that ketanserin binding to these sites was associated with an increase of DOPAC release (5).

We have recently described the regionalization of the TBZ binding sites associated with the monoamine transporter of synaptic vesicles, a transporter that is involved in the ATP-dependent uptake of catecholamines and indoleamines and that is inhibited by reserpine and TBZ (6–8). TBZ binding sites have a maximal density in the striatum, as have the displaceable nonspecific ketanserin binding sites.

In the present communication, we have tested the hypothesis that the two classes of sites were related. We have thus examined the binding of [^{3}H]ketanserin to purified chromaffin granule membranes, a preparation enriched in TBZ binding sites that does not contain S$_2$ receptors.

Materials and Methods

Chemicals. Ketanserin, ketanserin derivatives, and [^{3}H]ketanserin (35 Ci/mmol) were obtained from Janssen Pharmaceutica (Beerse, Belgium); ketanserin hydrochloride (10 mM) was prepared in water; TBZ was from Fluka (Buchs, Switzerland); TBZ stock solutions (2 mM) were prepared in 4 mM HCl; [^{3}H]TBZOH (12 Ci/mmol) was

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ABBREVIATIONS: The abbreviations used are: 5-HT, 5-hydroxytryptamine (serotonin); TBZ, tetrabenazine; TBZOH, dihydrotetrabenazine (2-hydroxy-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b(*H*)benzo[*a*]quinolizine; [^{3}H]TBZOH, [2-^{3}H]dihydrotetrabenazine; HEPES, *N*-(2-hydroxyethyl) piperazine-*N'*-2-ethanesulfonic acid; DOPAC, 3,4-dihydroxyphenylacetic acid.

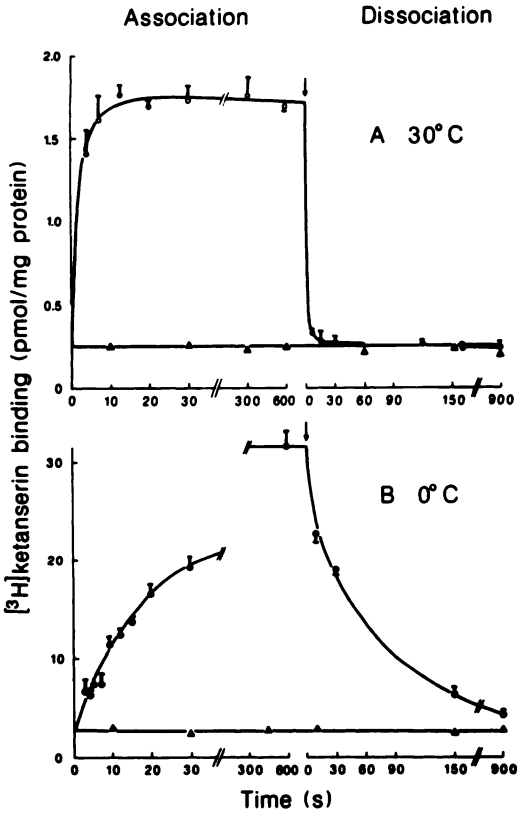

Fig. 1. Association and dissociation kinetics of [^{3}H]ketanserin binding to bovine chromaffin granule membranes at 30° (A) and 0° (B). A, Membranes (200 μg of protein/ml) were incubated with 1.6 nM [^{3}H]ketanserin in 0.3 M sucrose/20 mM HEPES KOH, pH 8.0 (○). To follow the association reaction, 60-μl aliquots were withdrawn at intervals and rapidly filtered. For the dissociation reaction, 60-μl aliquots were diluted in 1 ml of sucrose buffer containing 10 μM unlabeled ketanserin and incubated for the indicated period of time at 30° before filtration. Nonspecific binding (▲) was measured in the presence of 10 μM ketanserin. Each point is the mean of three determinations. B, Same experiment, but membranes (50 μg protein/ml) were incubated with 10.6 nM [^{3}H]ketanserin at 0° in the absence (●) or presence (▲) of 10 μM ketanserin.

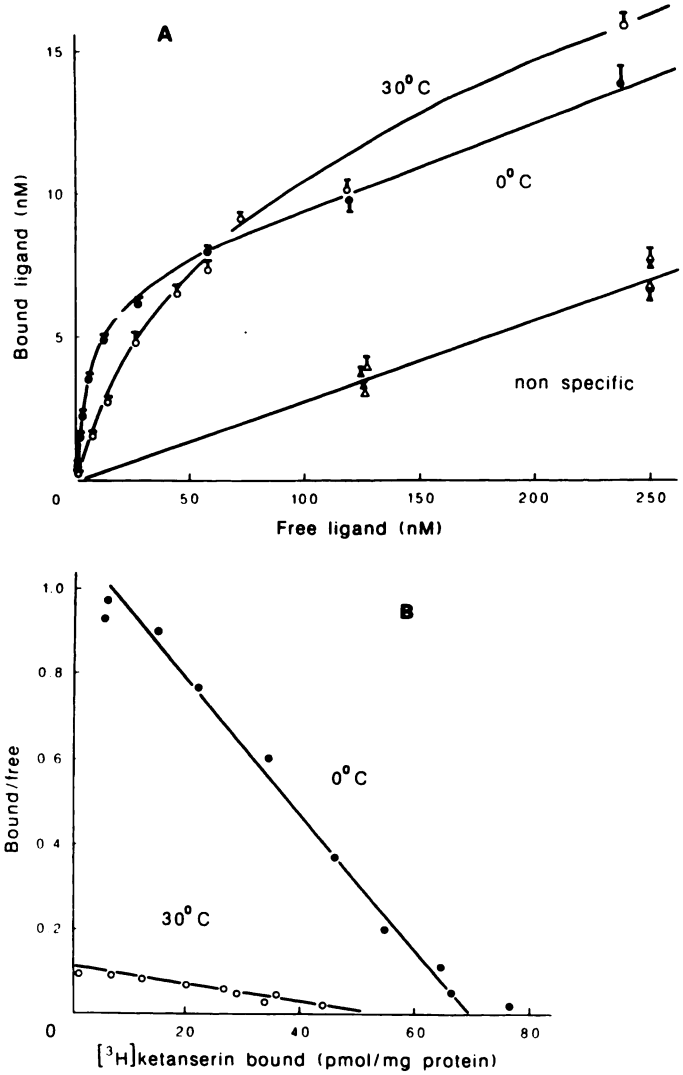

Fig. 2. Binding of [^{3}H]ketanserin to chromaffin granule membranes. A, Saturation curve. Membranes [200 (○,△) and 100 (●,▲) μg of protein/ml] were incubated with [^{3}H]ketanserin (1.2–260 nM) at 30° (○,△) or 0° (●,▲). Each point is the mean of three determinations. Nonspecific binding (△,▲) was proportional to free [^{3}H]ketanserin with a proportionality coefficient of 0.0265. B, Scatchard plot of specific binding. K_D and B_{max} values derived from the plot were as follows: at 30, K_D = 45.3 ± 2.4 nM, B_{max} = 51 ± 2 pmol/mg of protein, r = 0.93; at 0°, K_D = 6.3 ± 0.4 nM, B_{max} = 69 ± 3 pmol/mg of protein, r = 0.96.

prepared as described (7); [^{3}H]5-HT (12.3 Ci/mmol) was purchased from Amersham (Aylesbury, Buckinghamshire, England); reserpine was from Sigma (St. Louis, MO) and was dissolved in 1 M acetic acid at a concentration of 10 mM; methysergide was from Sandoz (Basel, Switzerland).

Chromaffin granule membrane preparation. Bovine chromaffin granule membranes were prepared by osmotic lysis of granules isolated by centrifugation on a 1.6 M sucrose layer (9, 10). Membranes were frozen in liquid nitrogen and were stored at −80°.

Homogenates of mouse cerebral tissues. Male OF1 mice (Iffa Credo, Lyon, France) aged 6 weeks and weighing 30–35 g were used. The striatum and the frontal cortex were dissected and homogenized as described (6, 11).

Uptake experiments. Uptake was initiated by addition of [^{3}H]5-HT (17 nM) to membranes (20 μg of protein/ml) preincubated at 30° in 0.3 M sucrose/10 mM HEPES KOH, pH 8.0, with ATP (2.5 mM), $MgSO_4$ (1.3 mM), and various concentrations of unlabeled ketanserin. Aliquots were withdrawn at 11 and 15 min, diluted 10 times in ice-cold 0.3 M sucrose buffered at the pH of the incubation medium, and filtered through GF/C filters (Whatman, Clifton, NJ). Filters were washed twice with the same volume of dilution medium. Their radioactivity was measured by liquid scintillation in Aqualuma (Lumac, Landgraaf, The Netherlands).

[^{3}H]TBZOH and [^{3}H]ketanserin binding. To study [^{3}H]ketanserin binding, membranes (40–200 μg of protein/ml) were incubated at the indicated temperature, with various concentrations of tritiated ligand in 0.3 M sucrose/20 mM HEPES KOH, pH 8.0. Bound ligand was measured by filtration on Whatman GF/C filters. The incubation mixture was diluted 10 times in ice-cold 0.3 M sucrose/10 mM Tris·HCl, pH 8, containing 10 μM ketanserin and rapidly filtered through filters previously incubated in the same buffer. The filters were then rapidly washed with 1 ml of the same ice-cold buffer, and their radioactivity was measured by liquid scintillation in Aqualuma. Specific [^{3}H]ketanserin binding was obtained by subtracting nonspecific binding determined in assays containing 5 μM unlabeled ketanserin. A similar procedure was followed for [^{3}H]TBZOH binding; filters were washed twice with 2 ml of 0.3 M sucrose/10 mM Tris·HCl, pH 8.0, containing 100 μM TBZ.

Miscellaneous. Protein was measured according to Bradford (12) with bovine serum albumin as a standard. The octanol-buffer apparent partition coefficient of ketanserin derivatives was measured as described (13).

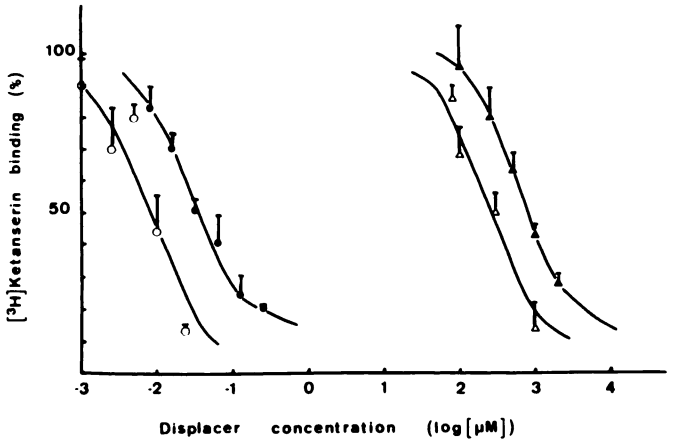

Fig. 3. Displacement of bound [³H]ketanserin. Membranes (100 μg of protein/ml) were incubated for 1 hr at 30° with 1 nM [³H]ketanserin and drugs: ○, TBZ; ●, ketanserin; △, 5-HT; ▲, noradrenaline. Nonspecific binding, measured in the presence of 5 μM ketanserin, was subtracted. Each *point* is the mean of two determinations. Hill numbers were, respectively: TBZ, 1.02 ± 0.24; ketanserin, 1.08 ± 0.10; 5-HT, 1.2 ± 0.3; and noradrenaline, 1.03 ± 0.17.

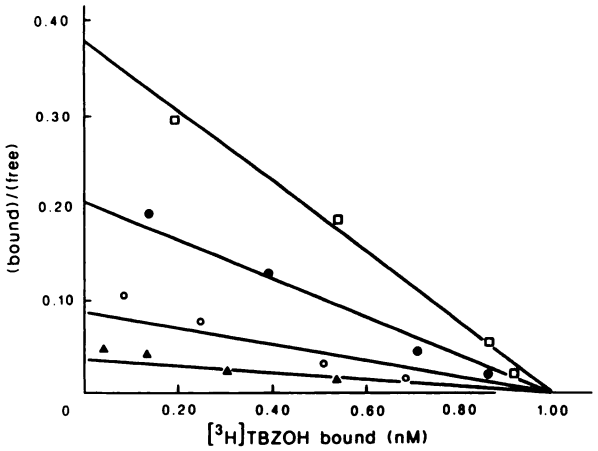

Fig. 4. Competition between [³H]TBZOH and ketanserin binding. Membranes (20 μg of protein/ml) were incubated at 30° with [³H]TBZOH (1–45 nM) and various concentrations of ketanserin: 0 (□), 40 nM (●), 120 nM (○), 360 nM (▲). Nonspecific binding was measured in the presence of 2 μM TBZ and was subtracted. Each *point* is the mean of three determinations. The data are presented as a Scatchard plot of specific [³H]TBZOH binding.

TABLE 1

Pharmacological profiles of ketanserin and TBZOH binding sites of chromaffin granule membranes and of S_2 receptors

Agent	[³H]Ketanserin binding to bovine chromaffin granules, K_i[a]	[³H]TBZOH binding to bovine chromaffin granules, K_i	[³H]Ketanserin binding to rat cortex, K_i[b]
		μM	
Ketanserin	0.045	0.055[c]	0.0004
TBZ	0.007	0.0013[d]	
5-HT	250	240[d]	0.296
Noradrenaline	800	1200[d]	150
Methysergide	>10		0.00094

[a] Calculated by the Cheng-Prusoff equation (21) from EC_{50} values derived from the displacement curves of Fig. 3.
[b] Taken from Ref. 1.
[c] Calculated from the data of Fig. 4.
[d] Taken from Ref. 7.

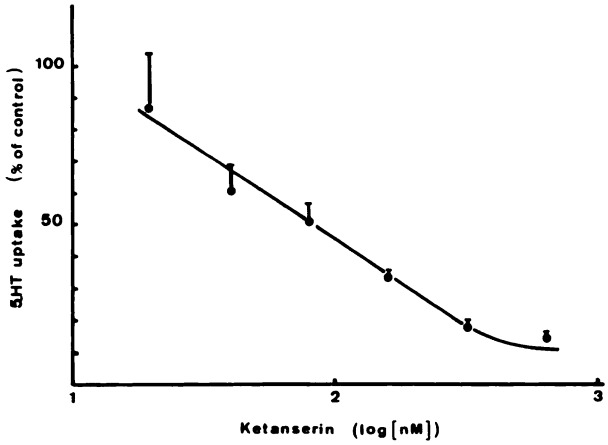

Fig. 5. Inhibition of [³H]5-HT uptake by ketanserin. The experiment was performed as described in Materials and Methods. *Points* are mean of two determinations. Hill number was 0.938 ± 0.063 (r = 0.97).

Results

Kinetics of [³H]ketanserin binding to chromaffin granule membranes. [³H]Ketanserin binds to purified chromaffin granule membranes. Binding kinetics were studied at 30° and at 0° (Fig. 1). At 30°, binding was very fast and, at 1.6 nM [³H]ketanserin, equilibrium was reached in less than 1 min. Dissociation, observed after addition of an excess of nonlabeled ketanserin, was too fast to be measured by the filtration technique. At 0°, the kinetics were slower and a dissociation rate constant k_{-1} of 0.0175 sec^{-1}, corresponding to a half lifetime of 40 sec, was derived from the dissociation curve (Fig. 1B). The association rate constant k_{+1} was derived from the binding curve, using the equation

$$\ln (B_{eq}-B)/B_{eq} = (k_{+1}\,[\text{ketanserin}] + k_{-1})t$$

where B and B_{eq} are the concentrations of bound ligand at, respectively, time t and equilibrium, [ketanserin] is the free ligand concentration, and k_{-1} the dissociation rate constant derived from the dissociation curve. A figure of 1.67×10^6 M^{-1} sec^{-1} was thus obtained. The equilibrium dissociation constant K_D, calculated from these values by the equation $K_D = k_{-1}/k_{+1}$, was 10.5 nM.

At both temperatures, the addition of 2.5 mM ATP/1.3 mM $MgSO_4$ to the incubation mixture had no effect, either on the rate of binding or on plateau value.

[³H]Ketanserin binding at equilibrium. Binding isotherms were determined at 0° and at 30° (Fig. 2A). Because of the rapid dissociation rate at 30°, the samples incubated at that temperature were diluted in ice-cold buffer before filtration and the filters were washed by ice-cold buffer. At the two temperatures tested, [³H]ketanserin binding was saturable. At 0° using 6 nM [³H]ketanserin, nonspecific binding, determined in the presence of 10 μM nonlabeled ketanserin was 8% of the bound tritiated ligand. A Scatchard plot of the data (Fig. 2B) indicated only one class of binding sites, with K_D and B_{max} values of, respectively, 45.3 ± 2.4 nM and 51 ± 2 pmol/mg of protein at 30°, and 6.1 ± 0.4 nM and 69 ± 3 pmol/mg of protein at 0°. The binding reaction is thus exothermic, and comparison of the K_D values at the two temperatures suggests ΔH and ΔS values of, respectively, −12 kcal/mol and −0.08 cal/mol, for the binding reaction.

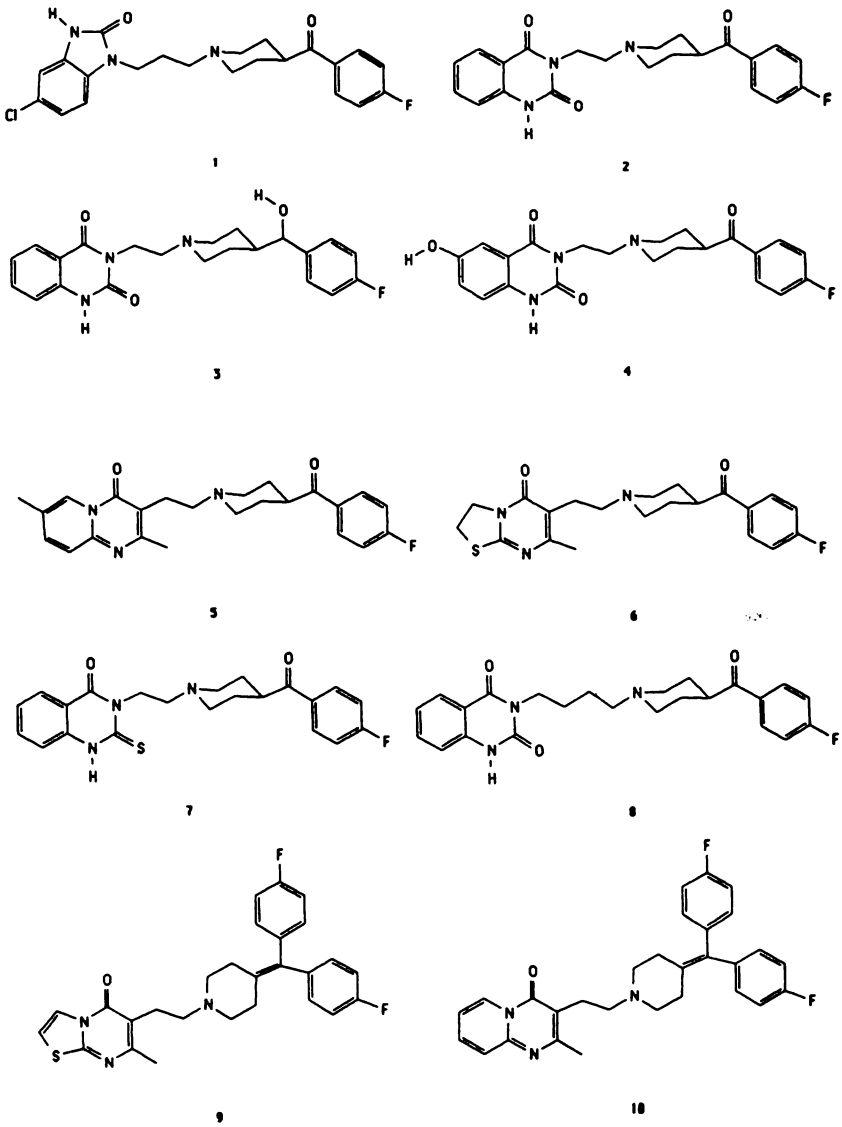

Fig. 6. Chemical structures of the tested ketanserin derivatives. Compounds 1, 2, 6, and 9 are milenperone, ketanserin, setoperone, and ritanserin, respectively.

Relationship between ketanserin and TBZ binding sites. [^{3}H]Ketanserin was efficiently displaced from its binding site on chromaffin granule membrane by TBZ (Fig. 3). The corresponding curve was characterized by an EC_{50} value of 7 nM and a Hill number of 1.02. In contrast, noradrenaline, 5-HT, and methysergide were poor displacers of [^{3}H]ketanserin (Table 1). This pharmacological profile differs from that of rat cortex S_2 sites (1), which have a high affinity for 5-HT and methysergide and a low affinity for noradrenaline (Table 1). On the other hand, this profile is similar to that of the TBZ binding site of the monoamine transporter, which has a low affinity for both 5-HT and noradrenaline.

To investigate further the relationship between TBZ and ketanserin binding sites, we examined the inhibition of [^{3}H]TBZOH binding by ketanserin (Fig. 4). The experiment was performed at various [^{3}H]TBZOH concentrations and the results are shown as a Scatchard plot. The fact that the apparent B_{max} for [^{3}H]TBZOH binding was independent of the concentration of ketanserin used indicates strongly that the two drugs compete for the same site or for two sites that cannot be simultaneously occupied. From this experiment, a K_i value for ketanserin of 55 nM at 30° was derived.

It may also be noted that ketanserin inhibited ATP-dependent 5-HT uptake with an IC_{50} value of 70 nM (Fig. 5).

Binding profiles of ketanserin derivatives. The affinity for the TBZ binding site of a series of ketanserin derivatives (see structures in Fig. 6) has been tested by comparing their potency in displacing [^{3}H]TBZOH from its binding site (Table 2). The various compounds had K_i values differing by more than 2 orders of magnitude. Two compounds (compounds 4 and 7) had a potency similar to or somewhat better than ketanserin. The addition of two methylene groups between the piperidine and the pyrimidine moieties of ketanserin (compound 8) results in a 20-fold decrease of affinity.

The TBZ binding site is hydrophobic and the apparent affinity of a ligand for this site is largely dependent upon its lipophilicity (13). The lipophilicity of a compound can be estimated from its apparent octanol/water partition coefficient (14). The apparent octanol/water partition coefficients, $p_{o/b}$, of the tested ketanserin derivatives are given in Table 2. In the same table, an estimate of the "instrincic affinity" of the various compounds, defined as described (13) and calculated as $K_i \times p_{o/b}$ is indicated. The intrinsic affinity varies by about 2 orders of magnitude.

TABLE 2
Binding profile of ketanserin derivatives

Compound[a]	Octanol/buffer apparent partition coefficient,[b] $P_{o/b}$	[^{3}H]TBZOH binding to chromaffin granule membranes,[c] K_i	Intrinsic affinity for TBZ binding site, $P_{o/b} \times K_i$	[^{3}H]Ketanserin binding to rat cortex,[d] K_i
		nM	μM	nM
1	305	950	290	
2	770	48	37	1.3
3	293	350	103	890
4	456	14	6	0.7
5	340	814	277	0.9
6	31	3600	112	1.3
7	1940	40	78	1.4
8	280	950	266	2.3
9	1470	410	603	2.8
10	150	325	49	

[a] Formulae are indicated in Fig. 6.
[b] Determined as in Ref. 13. For each sample, concentrations were determined spectrophometrically and fluorometrically.
[c] Membranes (10 μg of protein/ml) were incubated for 1 hr at 30° with [^{3}H]TBZOH (1 nM) in the presence of ketanserin derivatives at 10 different concentrations in the 3 nM–3 mM concentration range. Determinations were performed in duplicate, with a variability of less than 10%. K_i values were derived from EC_{50} using the Cheng-Prusoff equation (21).
[d] Taken from Refs. 1 and 5.

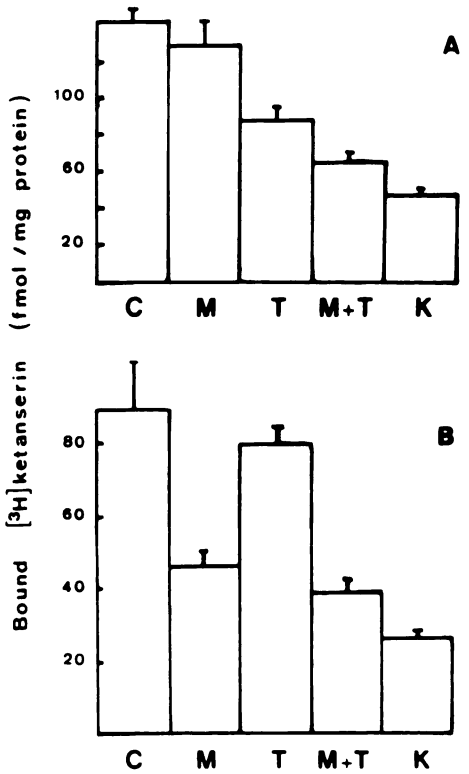

Fig. 7. Binding of [^{3}H]ketanserin to mouse cerebral homogenates. Striatum homogenates (5 mg of protein/ml) (A) and frontal cortex homogenates (4 mg of protein/ml) (B) were incubated with [^{3}H]ketanserin at 3.3 nM (A) or 2 nM (B) with or without inhibitors. *C*, total binding; *M*, 1 μM methysergide; *T*, 2 μM TBZ; and *K*, 6 μM ketanserin. Each value is the mean of three separate experiments.

The affinity of the tested ketanserin derivatives for TBZ binding sites of chromaffin granule membranes has been compared with their affinity for 5-HT S_2 sites of rat brain (Table 2). The two sets of data are not correlated.

Ketanserin binding to mouse brain homogenates. We have compared the binding of [^{3}H]ketanserin in striatum and in cortex homogenates from mouse brain (Fig. 7). As reported earlier (3), total binding was high in these two brain structures. However, specific binding to S_2 receptors, defined as the methysergide-sensitive fraction of [^{3}H]ketanserin binding was more important in the cortex than in the striatum (43 and 15 fmol/mg of protein, respectively). On the other hand, TBZ-sensitive [^{3}H]ketanserin binding was larger in the striatum than in the cortex (55 and 10 fmol/mg of protein). Moreover, the effects of the two drugs were additive, and binding to these two sites accounted for most of saturable [^{3}H]ketanserin binding.

Discussion

Purified chromaffin granule membranes bind [^{3}H]ketanserin. Binding occurs on one class of sites and is characterized by equilibrium dissociation constants of 45 and 6 nM at 30 and 0°, respectively. These sites are believed to be identical to the TBZ binding sites of the monoamine transporter for the following reasons: 1) ketanserin, as TBZ, inhibits ATP-dependent 5-HT uptake by chromaffin granule ghosts, and the inhibition constant for the uptake reaction is the same order of magnitude as the dissociation constant K_D; 2) the density of TBZ and ketanserin binding sites are identical (60 pmol/mg of protein; fig. 2) (see also Ref. 7); 3) TBZ inhibits [^{3}H]ketanserin binding in the nanomolar concentration range (consistent with a figure of 3 nM for the K_D of TBZOH) (7). Reciprocally, ketanserin competitively inhibits [^{3}H]TBZOH binding with an inhibition constant of 55 nM, which is identical to the [^{3}H]ketanserin equilibrium dissociation constant; 4) TBZ and ketanserin binding sites have a similar low affinity, in the millimolar concentration range, for the substrates of uptake 5-HT and noradrenaline; 5) photoaffinity experiments, using an arylazido derivative of TBZ (15), and radiation inactivation experiments (16) have shown that the TBZ binding site is associated with a 70-kDa polypeptide chain. Preliminary experiments performed with an arylazido derivative of ketanserin suggested a similar molecular weight for the ketanserin binding site.[1]

During monoamine uptake, the transporter is activated by the H^+ electrochemical gradient generated by an ATP-dependent H^+ pump of chromaffin granule membranes (17, 18). Two different sites have been demonstrated on this transporter, using the uptake inhibitors TBZ and reserpine (7, 19, 20). The TBZ binding site has a low affinity for the substrates and binding to this site is independent of the presence of ATP. On the contrary, reserpine binding is greatly accelerated by the H^+ electrochemical gradient, and the reserpine binding site has a high affinity for the substrates. The ketanserin binding site is similar to that of TBZ in that it is not affected by the presence of ATP and it has a low affinity for the substrates.

Estimates of the intrinsic affinity of ketanserin (13) based on the displacement of [^{3}H]TBZOH (EC_{50}) and on apparent octanol/buffer partition coefficient ($p_{o/b}$) suggest that ketanserin has a high intrinsic affinity for TBZ binding site. This recognition factor is only 10 to 20 times lower than that of the monoamine substrates or that of TBZ (13). It is at least 2 orders of magnitude higher than that of haloperidol (13) and spiperone,[2] two ligands of dopaminergic receptors. The fact

[1] M.-F. Isambert, B. Gasnier, P. M. Laduron, and J.-P. Henry, unpublished experiment.
[2] M.-F. Isambert and J.-P. Henry, unpublished experiment.

that the tested ketanserin derivatives have very different intrinsic affinities suggests that the affinity for ketanserin of TBZ binding site reflects some structural complementarity and not simply a high lipophilicity.

We propose that ketanserin sites that are not affected by S_2 ligand (the so-called ketanserin nonspecific displaceable binding sites) in brain and platelets are identical to the TBZ binding sites of the monoamine transporter. This assumption is based on the fact that TBZ displaces methysergide-resistant bound [^{3}H]ketanserin (Fig. 7). The level of [^{3}H]ketanserin binding observed in the presence of methysergide and TBZ is virtually that observed in the presence of an excess of unlabeled ketanserin. This assumption is also consistent with the reported regionalization of TBZ binding sites (6). TBZ-sensitive ketanserin binding sites have a 5-fold higher density in the striatum than in the cortex. A similar difference has been reported for TBZ binding sites, reflecting the presence of a large number of dopaminergic vesicles in the striatum.

Our hypothesis is important to consider when [^{3}H]ketanserin is used to label serotoninergic S_2 receptors. As shown in Fig. 7, addition of 2 μM TBZ to the incubation mixture saturates the nonspecific sites without interfering with S_2 receptors. Alternatively, we suggest that binding and filtration of the incubation be done at 30°, a temperature that will result in a rapid dissociation of the [^{3}H]ketanserin bound to TBZ binding sites.

Another consequence of our hypothesis is that ketanserin might have some amine-depleting effect, similar to that of reserpine or TBZ. This effect might explain the ketanserin-induced DOPAC release observed by Leysen *et al.* (5). However, such an effect can be predicted to be limited because of the rapid dissociation of ketanserin at 37°. At 30° reserpine,[3] TBZ (7), and ketanserin have K_D values of, respectively, 0.03, 3, and 40 nM. The corresponding half-time of dissociation are 16 hr, 7 min, and less than 5 sec. Because the effect of TBZ on the level of brain monoamines is less marked and of shorter duration than that of reserpine, it can be anticipated that ketanserin will have a very limited reserpine-like effect.

Acknowledgments

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References

1. Leysen, J. E., J. E. Niemegeers, J. M. Nueten, and P. M. Laduron. [^{3}H] ketanserin (R 41468), a selective ^{3}H ligand for serotonin$_2$ receptor binding sites. *Mol. Pharmacol.* **21**:301–314 (1982).
2. Leysen, J. E., D. de Chaffoy de Courcelles, F. de Clerck, J. E. Niemegeers, and J. M. Nueten. Serotonin-S_2 receptor binding sites and functional correlates. *Neuropharmacology* **23**:1493–1501 (1984).
3. Schotte, A., J. M. Maloteaux, and P. M. Laduron. Characterization and regional distribution of serotonin-S_2 receptors in human brain. *Brain Res.* **276**:231–235 (1983).
4. Leysen, J. E., W. Gommeren, and F. de Clerck. Demonstration of S_2-receptor binding sites on cat blood platelets using [^{3}H]ketanserin. *Eur. J. Pharmacol.* **88**:125–130 (1983).
5. Leysen, J. E., A. Eens, W. Gommeren, P. Van Gompel, J. Wynants, and P. A. J. Janssen. Non-serotonergic [^{3}H]ketanserin binding sites in striatal membranes are associated with a dopac release system on dopaminergic nerve endings. *Eur. J. Pharmacol.* **134**:373–375 (1987).
6. Scherman, D., G. Boschi, R. Rips, and J. P. Henry. The regionalization of [^{3}H]dihydrotetrabenazine binding sites in mouse brain and its relationship to the distribution of monoamines and their metabolites. *Brain Res.* **370**:176–181 (1986).
7. Scherman, D., P. Jaudon, and J. P. Henry. Characterization of the monoamine transporter of chromaffin granule membrane by binding of [2-^{3}H] dihydrotetrabenazine. *Proc. Natl. Acad. Sci. USA* **80**:584–588 (1983).
8. Scherman, D. Dihydrotetrabenazine binding and monoamine uptake in mouse brain regions. *J. Neurochem.* **47**:331–339 (1986).
9. Smith, A. D., and H. Winkler. A simple method for the isolation of adrenal chromaffin granules on a large scale. *Biochem. J.* **103**:480–482 (1967).
10. Giraudat, J., M. P. Roisin, and J. P. Henry. Solubilization and reconstitution of the adenosine 5′-triphosphate-dependent proton translocase of bovine chromaffin granule membrane. *Biochemistry* **19**:4499–4505 (1980).
11. Glowinski, J., and L. L. Iversen. Regional studies of catecholamines in the rat brain: the disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]dopa in various regions of the brain. *J. Neurochem.* **13**:655–669 (1966).
12. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254 (1976).
13. Scherman, D., B. Gasnier, P. Jaudon, and J. P. Henry. Hydrophobicity of the tetrabenazine binding site of the chromaffin granule monoamine transporter. *Mol. Pharmacol.* **33**:72–77 (1988).
14. Seeman, P. The membrane action of anesthetics and tranquilizers. *Pharmacol. Rev.* **24**:583–655 (1972).
15. Isambert, M. F., and J. P. Henry. Photoaffinity labelling of the tetrabenazine binding sites of bovine chromaffin granule membranes. *Biochemistry* **24**:3660–3667 (1985).
16. Gasnier, B., J. C. Ellory, and J. P. Henry. Functional molecular mass of binding sites for [^{3}H]dihydrotetrabenazine and [^{3}H]reserpine and of dopamine β-hydroxylase and cytochrome b_{561} from bovine chromaffin granule membranes as determined by radiation inactivation. *Eur. J. Biochem.* **16**:73–78 (1987).
17. Bashford, C. L., R. P. Casey, G. K. Radda, and G. A. Ritchie. The effect of uncouplers on catecholamine incorporation by vesicles of chromaffin granules. *Biochem. J.* **148**:153–155 (1975).
18. Johnson, R. G., N. J. Carlson, and A. Scarpa. ΔpH and catecholamine distribution in isolated chromaffin granules. *J. Biol. Chem.* **253**:1512–1521 (1978).
19. Scherman, D. and J. P. Henry. Reserpine binding to bovine chromaffin granule membranes: characterization and comparison with dihydrotetrabenazine binding. *Mol. Pharmacol.* **25**:113–122 (1984).
20. Deupree, J. D., and J. A. Weaver. Identification and characterization of the catecholamine transporter in bovine chromaffin granules using [^{3}H]reserpine. *J. Biol. Chem.* **259**:10907–10912 (1984).
21. Cheng, Y. C., and W. H. Prussof. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3109 (1973).

[3] F. Darchen, D. Scherman, and J.-P. Henry, unpublished experiment.

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