Photoaffinity Labeling of the Monoamine Transporter of Bovine Chromaffin Granules and Other Monoamine Storage Vesicles Using 7-Azido-8-[^{125}I]iodoketanserin†

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Received July 14, 1988; Revised Manuscript Received October 28, 1988

ABSTRACT: An iodinated azido derivative of ketanserin, 7-azido-8-[^{125}I]iodoketanserin ([^{125}I]AZIK), has been used to label the monoamine transporter of bovine chromaffin granule membranes by the technique of photoaffinity labeling. In the dark, this derivative was found to bind reversibly to the membranes, with an equilibrium dissociation constant estimated to be 6 nM at 0 °C. As for ketanserin, binding occurred at the tetrabenazine site: (i) [^{125}I]AZIK was displaced efficiently from its binding site by tetrabenazine, ketanserin, and 7-azidoketanserin, whereas serotonin, which is a substrate for the transporter but has a low affinity for tetrabenazine binding site, was a poor displacer; pipamperone and pyrilamine, two antagonists of respectively serotonin S_2 and histamine H_1 receptors, were inactive. (ii) 7-Azidoketanserin was a competitive inhibitor of [^{3}H]dihydrotetrabenazine binding, and it inhibited the ATP-dependent uptake of serotonin by chromaffin granule ghosts. Irradiation of [^{125}I]AZIK with long-wavelength UV light, followed by electrophoresis on sodium dodecyl sulfate/polyacrylamide gels and autoradiography, revealed irreversible labeling of a membrane component with an apparent molecular weight of 73 000. Tetrabenazine inhibited the labeling of this 73-kDa band in a manner parallel to the binding of [^{125}I]AZIK in the dark. Such a labeling is totally compatible with previous results obtained through photolabeling with a tetrabenazine derivative or by target size analysis. Moreover, preliminary experiments showed that [^{125}I]AZIK can label the tetrabenazine binding sites of various sources including rat striatum, rabbit platelets, human pheochromocytoma, and human adrenal medulla. Therefore, this molecule appears to be an excellent probe to label the monoamine transporter of different amine storage vesicles even without purification.

In the adrenal medulla, catecholamines are stored in specialized organelles, the chromaffin granules. The mechanism by which these organelles take up not only catecholamines but also other monoamines such as serotonin or tyramine is now well understood [for a review, see Johnson (1987)]. A specific monoamine transporter of the chromaffin granule membrane catalyzes a monoamine–proton antiport, and this exchange is driven by the H^+ electrochemical gradient generated by an inwardly directed ATP-dependent H^+ pump using cytosolic ATP. A similar mechanism is found in the various monoamine storage vesicles, such as the synaptic vesicles of dopaminergic, noradrenergic, and serotoninergic neurons (Toll & Howard, 1978; Maron et al., 1979; Scherman, 1986), the serotonin-containing dense granules of blood platelets (Carty et al., 1981; Fishkes & Rudnick, 1982), and the histamine-containing granules of basophils (Schuldiner et al., 1987).

Progress in our knowledge on the monoamine transporter came largely through the use of the inhibitors tetrabenazine (TBZ)[1] and reserpine (RES), mainly in binding studies [for a review, see Henry et al. (1987)]. The two drugs have different binding sites. [^{3}H]Reserpine binds rapidly only in the presence of the H^+-electrochemical gradient generated by the proton pump, and it is efficiently displaced from its binding site by monoamine substrates (Deupree & Weaver, 1984; Scherman & Henry, 1984). On the contrary, the binding of [^{3}H]TBZOH, a TBZ derivative, is not affected by the presence of ATP, and it is poorly inhibited by the substrates (Scherman et al., 1983).

Recently, ketanserin, a drug that was reported to bind on serotonin S_2 receptors (Leysen et al., 1982), was also shown to be a good ligand of the chromaffin granule monoamine transporter (Darchen et al., 1988). [^{3}H]Ketanserin binds to TBZ binding sites with an equilibrium dissociation constant of 45 nM at 30 °C and of 6 nM at 0 °C. More recently, 7-azido-8-[^{125}I]iodoketanserin ([^{125}H]AZIK), an azido derivative of ketanserin, was found to photolabel serotonin S_2 receptors in rat frontal cortex (Wouters et al., 1987).

In the present paper, we have tested [^{125}I]AZIK as a photoaffinity marker of the TBZ binding site of the monoamine transporter, and we have compared the results to those obtained with an arylazido derivative of TBZ (Isambert & Henry, 1985).

Experimental Procedures

Chemicals. TBZ and RES were from Fluka (Buchs, Switzerland). Ketanserin and pipamperone were from Janssen Pharmaceutica (Beerse, Belgium); pyrilamine was from Rhône-Poulenc (Vitry, France). [^{3}H]TBZOH (15 Ci/mmol) was purchased from CEA (Saclay, France). [^{3}H]Serotonin (20 Ci/mmol) and [^{125}I]AZIK (1800 Ci/mmol) were obtained

† This work was supported by the Centre National de la Recherche Scientifique (UA 1112) and the Fondation pour la Recherche Médicale (grant to J.P.H. and fellowship to B.G.).
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[1] Abbreviations: AZIK, 7-azido-8-iodo-ketanserin [7-azido-8-iodo-3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,4(1*H*,3*H*)-quinazolinedione]; [^{125}I]AZIK, 7-azido-8-[^{125}I]iodo-ketanserin; TBZ, tetrabenazine (2-oxo-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b*H*-benzo[*a*]quinolizine); 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; RES, reserpine; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate.

0006-2960/89/0428-2265$01.50/0

from Amersham (Aylesbury, Buckinghamshire, U.K.).

Chromaffin Granule Membrane Preparation. Bovine chromaffin granules were purified by centrifugation on a 1.6 M sucrose layer (Smith & Winkler, 1967). Membranes were prepared by osmotic lysis of the granules, essentially as described by Giraudat et al. (1980). EDTA (1 mM), leupeptin (3 μg/mL), and aprotinin (5 μg/mL) were included in the lysis buffer to prevent proteolysis. Membranes were frozen in liquid nitrogen and stored at −80 °C.

Other Tissues. Purified granule membranes were isolated from a fragment of human pheochromocytoma (35 g) by centrifugation on a 1.6 M sucrose layer and then submitted to osmotic lysis, as previously described (Roisin et al., 1984). A crude granule fraction was prepared from a fragment of human adrenal medulla (0.2 g) by differential centrifugation (Roisin et al., 1984).

Rat brain striata (0.24 g) were dissected and homogenized in 2 mL of ice-cold 0.3 M sucrose/10 mM Hepes (Na^+), pH 7.5, with a glass–Teflon homogenizer (12 strokes at 500 rpm).

Rabbit platelets were isolated according to the method of Pletscher et al. (1966).

[^{3}H]Serotonin Uptake. Bovine chromaffin granule vesicles (0.1 mg of protein/mL) were preincubated at 22 °C in the dark in 0.3 M sucrose/10 mM Hepes (Na^+) buffer, pH 7.3, containing 5 mM ATP/2.5 mM $MgSO_4$ and increasing amounts of 7-azidoketanserin. The uptake was initiated by the addition of [^{3}H]serotonin (10 nM final concentration). Aliquots (0.5 mL) were withdrawn at intervals, diluted with 1.5 mL of ice-cold isotonic buffer, and filtered through GF/B glass fiber filters (Whatman, Clifton, NJ) preincubated in 0.3% poly(ethylenimine) (Bruns et al., 1983). The filters were washed twice with the same buffer and counted by liquid scintillation in Aqualuma (Lumac, Landgraaf, The Netherlands). Nonspecific uptake rate, determined by addition of 2 μM TBZ to the incubation medium, was subtracted from the data. The concentration of 7-azidoketanserin solutions was checked by measuring their absorbance at 240 nm (Wouters et al., 1985a).

[^{3}H]TBZOH and [^{125}I]AZIK Binding. To measure [^{3}H]-TBZOH binding, membranes (0.13 mg of protein/mL) were preincubated in the dark at 22 °C in 0.3 M sucrose/10 mM Hepes buffer, pH 7.5, and, where indicated, with 7-azidoketanserin. [^{3}H]TBZOH was then added, and the reaction mixtures (0.45 mL) were further incubated for 1 h. The amount of [^{3}H]TBZOH bound to the membranes was determined by filtration, as described above. Nonspecific binding, determined by addition of 2 μM TBZ, was subtracted.

For [^{125}I]AZIK binding measurements, chromaffin granule membranes (0.05–0.1 mg of protein/mL) were preincubated at room temperature in the dark in 0.3 M sucrose/10 mM Hepes buffer, pH 7.5, and, where indicated, with inhibitors. [^{125}I]AZIK was then added, and the membranes were incubated further in an ice-cold bath. The temperature of 0 °C was chosen because ketanserin has more affinity for bovine chromaffin granule membranes at 0 °C than at 20 °C (Darchen et al., 1988). The amount of [^{125}I]AZIK bound to the membranes was determined by filtration as above, but filters were rinsed only once. Because ketanserin dissociates rapidly from chromaffin granule membranes with a half-time of 40 s (Darchen et al., 1988), the filtration procedure was completed in less than 3 s. Radioactivity was measured either directly with a γ scintillation counter or by liquid scintillation in Aqualuma.

Photolabeling with [^{125}I]AZIK. Samples (0.03–0.3 mg of protein/mL) were incubated in the dark with [^{125}I]AZIK [(1–5 × 10^6 dpm), as described for the [^{125}I]AZIK binding assay. The incubation media were then irradiated for 11 min at an average distance of 11 cm with a universal UV lamp at position 350 nm. After irradiation, membranes were washed twice by 4-fold dilution in sucrose buffer followed by centrifugation for 20 min at 140000*g*. Samples were analyzed by SDS/polyacrylamide gel electrophoresis (Laemmli, 1970). The gels were stained with Coomassie blue and dried, and autoradiograms were obtained by exposure of Curix MR4 films (Agfa Gevaert) with Lumix MR 800 screens for 3–10 days at −80 °C. The conditions of irradiation selected were those required to flatten the 240-nm absorbance peak of 7-azidoketanserin. Irradiation by long-range UV light was preferred to irradiation at 254 nm because the former minimized the labeling of phospholipids.

Analytical Procedures. Proteins were estimated by the Bradford procedure (1976), using bovine serum albumin as a standard. The purity of [^{125}I]AZIK was checked by thin-layer chromatography on silica gel plates using chloroform/methanol/acetonitrile/acetic acid (9/9/9/2) as the developing solvent (Wouters et al., 1985a).

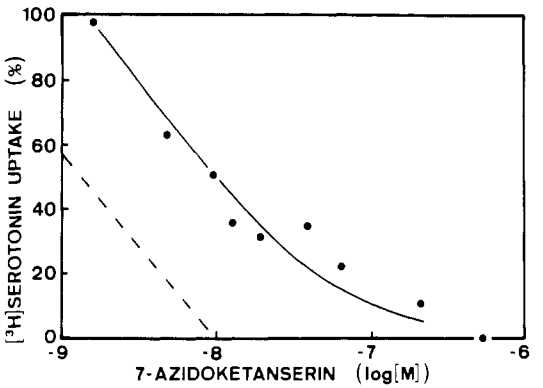

FIGURE 1: Inhibition of [^{3}H]serotonin uptake by 7-azidoketanserin. The effect of increasing concentrations of 7-azidoketanserin on ATP-dependent [^{3}H]serotonin uptake by chromaffin granule membranes was assayed in the dark as described under Experimental Procedures. Results are expressed as percentages of the uptake in the absence of inhibitor. The dotted line indicates the theoretical slope at midpoint, assuming a Hill number of 1.

Table I: Pharmacological Profile of [^{125}I]AZIK Binding

inhibitor	serotonin uptake,[a] EC_{50} (nM)	[^{3}H]TBZOH binding,[a] EC_{50} or K_I (nM)	[^{125}I]AZIK binding,[a] EC_{50} (nM)
serotonin	400[b]	300 × 10^3[d]	500 × 10^3
TBZ	12[b]	3[d]	10
ketanserin	70[c]	55[c]	10
7-azidoketanserin	5	23	10

[a] The inhibitions of serotonin uptake and of [^{3}H]TBZOH binding experiments were performed at 22 or 30 °C, whereas [^{125}I]AZIK binding was measured at 0 °C. [b] B. Gasnier, unpublished data. [c] Darchen et al. (1988). [d] Scherman et al. (1988).

RESULTS

Binding of 7-Azidoketanserin and [^{125}I]AZIK to Bovine Chromaffin Granule Membranes. 7-Azidoketanserin inhibited ATP-induced serotonin uptake by bovine chromaffin granule membranes, with an EC_{50} of 5 nM at 22 °C (Figure 1 and Table I). This compound inhibited also [^{3}H]TBZOH binding to the same membranes. A Scatchard plot of the saturation isotherms representing [^{3}H]TBZOH binding in the presence of 7-azidoketanserin revealed that this compound is a competitive inhibitor of TBZOH binding (Figure 2). The in-

Table II: Apparent Molecular Weight of the TBZ-Sensitive [^{125}I]AZIK-Labeled Polypeptide from Various Origins

organism	tissue	preparation	M_r	n^a
bovine	adrenal medulla	purified granule membrane	73 200 ± 3600	24
bovine	adrenal medulla	crude granules	68 300	1
human	adrenal medulla	crude granules	64 800	1
human	pheochromocytoma	purified granule membranes	64 800–66 700	2
rat	striatum	homogenate	76 200–72 800[b]	1
rabbit	platelets	intact cells	84 100–84 500	2

[a] Number of experiments. [b] M_r of the two components of a doublet.

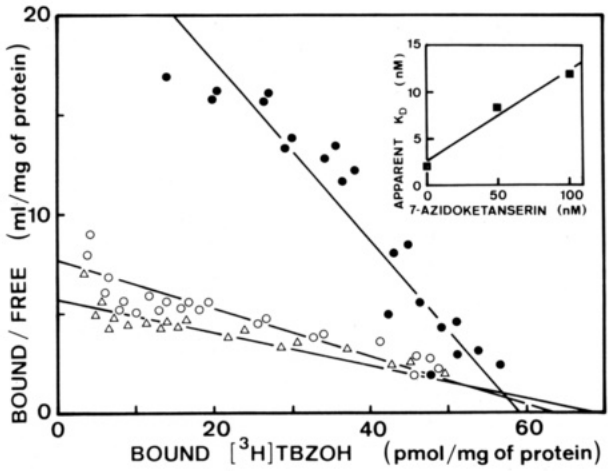

FIGURE 2: Inhibition of [^{3}H]TBZOH binding by 7-azidoketanserin. Membranes were incubated in the dark with [^{3}H]TBZOH and various concentrations of 7-azidoketanserin: (●) 0; (○) 50 nM; (△) 100 nM. The data, presented as a Scatchard plot, were analyzed by linear regression. In the absence of 7-azidoketanserin, values of 2.2 nM and 59 pmol/mg of protein were obtained for K_D and B_{max}, respectively (r = 0.95; n = 21). The apparent K_D were plotted as a function of 7-azidoketanserin concentration. (Inset) From the straight line thus obtained (r = 0.99), a K_i value of 23 nM was derived for the inhibition of [^{3}H]TBZOH binding by 7-azidoketanserin.

hibition constant, as determined from this experiment, was 23 nM at 22 °C (Figure 2, inset). These results suggested that 7-azidoketanserin, as ketanserin (Darchen et al., 1988), binds to the TBZ binding site of the vesicular monoamine transporter.

The binding of [^{125}I]AZIK was then investigated. For these experiments, membranes were incubated in the dark with subnanomolar concentrations of [^{125}I]AZIK. The incubation temperature was set at 0 °C to limit the rapid dissociation of ketanserin from the TBZ binding site (Darchen et al., 1988). Specific [^{125}I]AZIK binding, defined as the TBZ-sensitive fraction of the binding, increased linearly with the free ligand concentration in the 2–20 pM concentration range (Figure 3). This binding was not affected by addition of ATP; it was inhibited by TBZ, ketanserin, and 7-azidoketanserin, with EC_{50} values of about 10 nM at 0 °C (Figure 4 and Table I). The transporter substrate serotonin inhibited poorly [^{125}I]AZIK binding (EC_{50} = 500 μM, Table I), as previously reported for the effect of the monoamine substrates on [^{3}H]TBZOH binding (Scherman et al., 1983). Pipamperone and pyrilamine, which bind specifically to S_2 serotonin and H_1 histamine receptors, respectively, did not affect [^{125}I]AZIK binding at concentrations up to 3.5 μM, a concentration which greatly exceeds the affinity of these drugs for their own receptor (Leysen et al., 1982; Laduron et al., 1982). Therefore, it was concluded that [^{125}I]AZIK binds to the TBZ binding site of bovine chromaffin granule membranes.

Photoaffinity Labeling of Bovine Chromaffin Granule Membranes with [^{125}I]AZIK. Chromaffin granule membranes that were incubated at 0 °C with [^{125}I]AZIK and then photolabeled by UV irradiation were submitted to SDS/Polyacrylamide gel electrophoresis. Slicing the gel and counting the radioactivity revealed a major peak of iodinated material, which was not observed in the presence of 200 nM TBZ (data not shown). This material was also visualized by autoradi-

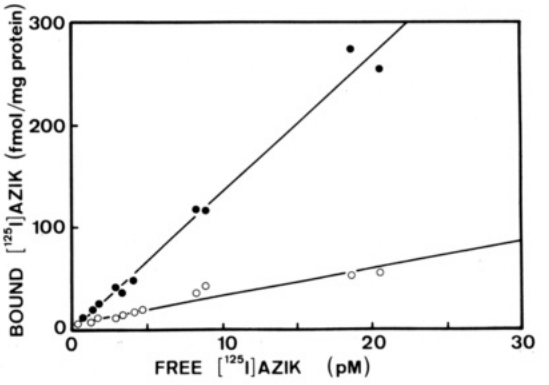

FIGURE 3: Binding of [^{125}I]AZIK to chromaffin granule membranes. Membranes were incubated in the dark with increasing concentrations of [^{125}I]AZIK in the absence (●) or presence (○) of 2 μM TBZ. The lines were obtained by linear regression of the data.

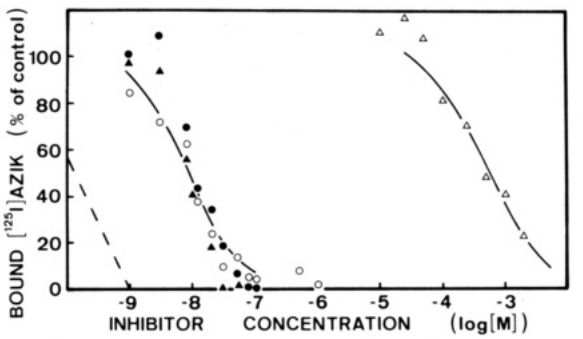

FIGURE 4: Displacement of bound [^{125}I]AZIK in the dark. Membranes were incubated with 11 pM [^{125}I]AZIK and TBZ (○), ketanserin (●), 7-azidoketanserin (▲), and serotonin (△). Results are expressed as the fraction of [^{125}I]AZIK bound in the absence of the inhibitor (100 fmol/mg of protein).

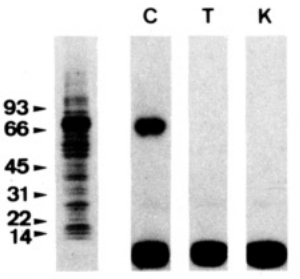

FIGURE 5: Autoradiogram of membranes photolabeled with [^{125}I]-AZIK and analyzed by SDS/polyacrylamide gel electrophoresis. Membranes (50 μg of protein/mL) were photolabeled with 40 pM [^{125}I]AZIK in the absence (lane C) or in the presence of 2 μM TBZ (lane T) or 2 μM ketanserin (lane K). The protein profile revealed by Coomassie blue staining is shown on the left. In this experiment, irradiation was performed at 254 nm.

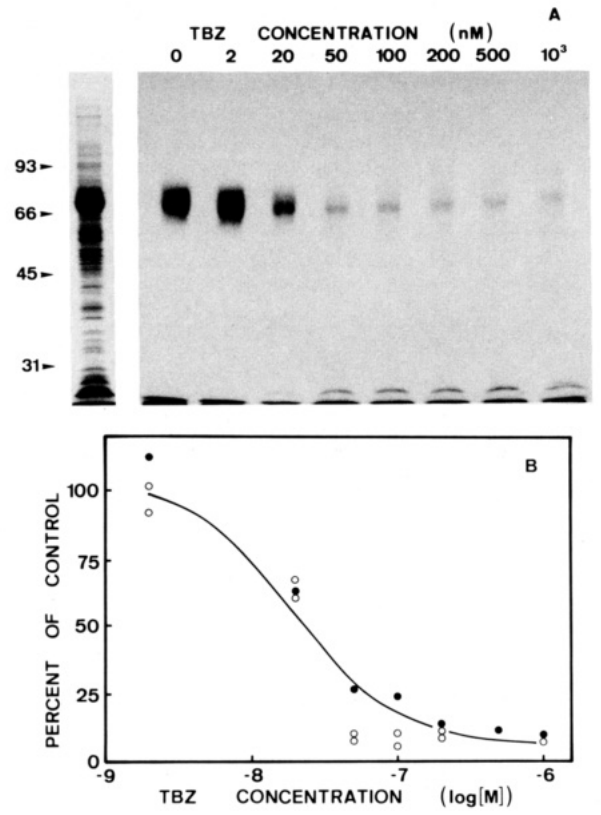

FIGURE 6: Inhibition of [^{125}I]AZIK photolabeling by TBZ. (A) Autoradiogram. The protein profile is shown in the left. (B) Dose–inhibition curve. The amount of [^{125}I]AZIK in the 69-kDa band (●) was estimated by quantitative densitometry of the autoradiogram. Reversibly bound [^{125}I]AZIK (○) was determined by filtration of aliquots before irradiation. Both curves gave an EC_{50} value of 25 nM.

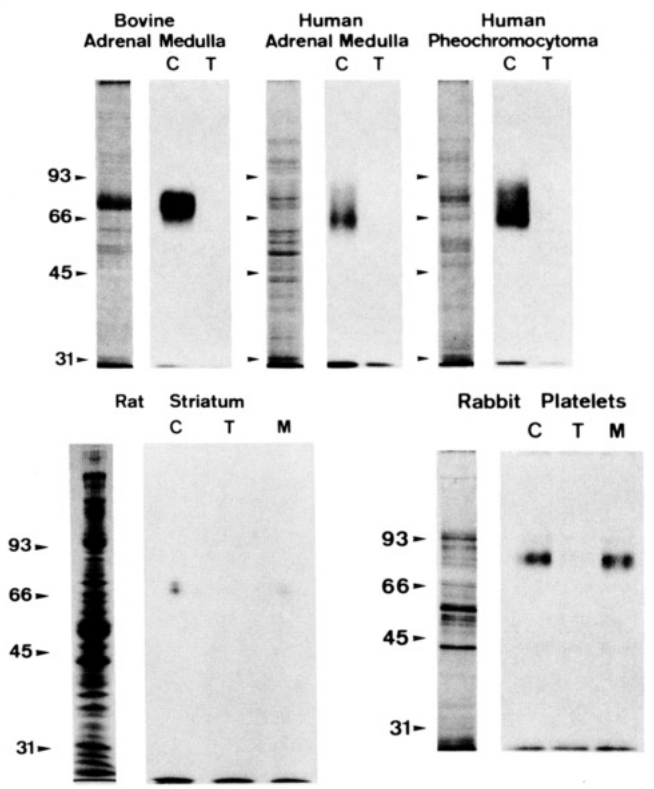

FIGURE 7: Autoradiograms of various monoamine-secreting tissues photolabeled by [^{125}I]AZIK. Crude granules from human adrenal medulla and purified granule membranes from human pheochromocytoma, rat striatum, and rabbit platelets were photolabeled with [^{125}I]AZIK in the absence of inhibitors (lane C) or in the presence of 2 μM TBZ (lane T) or 2 μM methysergide (lane M). Protein profiles, as revealed by silver staining (platelets) or by Coomassie blue staining, are shown on the left.

ography of the dried gel (Figure 5). From the data of 24 independent experiments, an apparent molecular weight of 73 200 ± 3600 was attributed to the labeled component. A component with a similar molecular weight was observed when either crude or purified intact granules were labeled under the same conditions (Table II). Extraction of the irradiated membranes with organic solvents (ethanol:acetone, 1:1) decreased the radioactivity found in the front of the gel, but did not affect that of the 73-kDa component, thus indicating a covalent labeling.

The labeling of the 73-kDa polypeptide chain by [^{125}I]AZIK was totally prevented by 200 nM TBZ, 2 μM ketanserin (Figure 5), and 2 mM serotonin (data not shown). On the other hand, this labeling was not affected by preincubation with 5 mM ATP–2.5 mM $MgSO_4$ or by preincubation with 20 nM RES, in the presence of ATP and $MgSO_4$. Preincubation of the membranes with increasing concentrations of TBZ resulted in a progressive inhibition of the incorporation of radioactivity in the 73-kDa polypeptidic chain (Figure 6A). An inhibition curve was obtained by scanning the autoradiography, from which an EC_{50} of 25 nM was derived (Figure 6B). The fact that this curve is superimposable on that describing the displacement of the reversible binding of [^{125}I]-AZIK by TBZ can be taken as evidence that the 73-kDa photolabeled component is associated with the [^{125}I]AZIK reversible binding site, i.e., the TBZ binding site of the monoamine transporter.

It may be noted that autoradiography revealed two minor components labeled in a TBZ-dependent manner: (i) The radioactivity at the front of the gel was decreased by incubation in the presence of TBZ; this labeled material might be the result of a proteolytic cleavage of the 73-kDa band. (ii) TBZ, in the nanomolar concentration range (Figure 6A), stimulated the incorporation of radioactivity in a minor band with an apparent molecular weight of 29 000 ± 1100 (n = 13); this observation has not been investigated further.

Photoaffinity Labeling of the Tetrabenazine Binding Sites in Different Tissues of Various Species. Tissues such as monoaminergic neurons, blood platelets, or pheochromocytoma tumors accumulate monoamines in their secretory vesicles through a TBZ-sensitive transporter. The ability of [^{125}I]-AZIK to label various vesicular monoamine transporters was therefore investigated. Photoaffinity labeling was performed in rat striatum, in rabbit platelets, and in human pheochromocytoma and nontumoral adrenal medullae. Different preparations were used as intact cells, homogenates, or purified vesicles. In the case of striatum and platelets, which contain serotonin S_2 receptors with a high affinity for ketanserin derivatives (Schotte et al., 1983; Leysen et al., 1983), methysergide was used as a S_2 receptor antagonist to assess the specificity of the labeling. Representative autoradiograms of the labeled material after SDS/polyacrylamide gel electrophoresis are shown in Figure 7. In all preparations, at least one polypeptidic chain was labeled by [^{125}I]AZIK in a TBZ-sensitive manner, and, where tested, in a methysergide-resistant one; a doublet was observed in the rat striatum. The apparent molecular weight of the different TBZ-sensitive [^{125}I]AZIK labeled polypeptide chains varied significantly in different tissues as compared to that of bovine chromaffin granule membranes (Table II). The apparent molecular weight of the labeled component was smaller in human adrenal medulla and pheochromocytoma and larger in rabbit platelets than in bovine

chromaffin granules.

Discussion

The main result of the present work is that an azido derivative of ketanserin can be used to label the TBZ binding site of the monoamine transporter by the technique of photoaffinity labeling. Such a result is possible because of the high affinity of this site for the probe in the dark. We have previously shown that ketanserin binds to this site with an affinity in the nanomolar concentration range (Darchen et al., 1988). The derivatization of ketanserin to 7-azidoketanserin did not change the specificity of the ligand, as shown by the fact that 7-azidoketanserin inhibited serotonin uptake and inhibited [^{3}H]TBZOH binding. Moreover, derivatization did not alter significantly the affinity (Table I).

The iodinated derivative 7-azido-8-iodoketanserin appears to have the same specificity for TBZ binding sites because its pharmacological profile, defined by displacement of [^{125}I]-AZIK bound in the dark, is similar to that of [^{3}H]TBZOH (Table I). However, although direct estimate of its affinity was difficult in the absence of unlabeled 7-azido-8-iodoketanserin, this affinity was indirectly estimated from the data of Figure 3. Assuming the presence of a unique class of binding sites, the slope of the line that represents the specifically bound ligand as a function of free ligand can be interpreted as the ratio B_{max}/K_D, where B_{max} is the density of binding sites and K_D the equilibrium dissociation constant. Assuming for B_{max} a value of 60 pmol/mg of protein, as measured with [^{3}H]TBZOH or [^{3}H]ketanserin binding (Scherman et al., 1983; Darchen et al., 1988) and using a slope value of 11 mL/mg of protein, a K_D of 5.5 nM at 0 °C was calculated. This value is similar to that obtained for ketanserin at the same temperature (Darchen et al., 1988).

When the photoaffinity technique was applied, with irradiation of the probe–membrane mixture at 0 °C with a long-wavelength UV light, a major component with an apparent molecular weight of 73 000 ± 4000 was identified by SDS/polyacrylamide gel electrophoresis. Several lines of evidence indicate that this peptide chain is associated with the TBZ binding site: (i) The labeling of the 73-kDa band was inhibited by preincubation with TBZ, with an EC_{50} of 25 nM. (ii) Ketanserin (2 μM) and serotonin (2 mM) inhibited the labeling of the polypeptide chain, as they do for [^{3}H]TBZOH binding (Scherman et al., 1983). Reserpine, which binds to another site of the transporter, did not inhibit the labeling. (iii) ATP, which is required for [^{3}H]RES binding, did not affect the labeling (Deupree & Weaver, 1984; Scherman & Henry, 1984).

The apparent molecular weight value of 73 000 is in agreement with previous work from this laboratory: (i) Using a tritiated arylazido derivative of TBZ, a polypeptide chain with an apparent molecular weight of 70 000 was labeled in chromaffin granule membranes (Isambert & Henry, 1985). (ii) A radiation inactivation study showed that these binding sites are carried by a component with a functional mass of 68 kDa (Gasnier et al., 1987). It may be noted that the probe did not label any component with a molecular weight of 40 000, as previously observed by Gabizon et al. (1982), using an arylazido derivative of serotonin.

From a methodological point of view, [^{125}I]AZIK is a better probe of TBZ binding sites than the arylazido derivative of TBZ previously used (Isambert & Henry, 1985). The TBZ derivative had an affinity of 50 nM, and the membrane-ligand complex had to be isolated before irradiation to decrease nonspecific labeling. Moreover, the probe was tritiated, and fluorography of the gels gave poor results, maybe because of the low specific activity of the probe. Nevertheless, it may be noted that the pattern of labeling was similar in the two techniques, the radioactive component migrating as a rather broad band. Such a pattern might indicate that the 73-kDa polypeptide is a glycoprotein.

If 7-azidoiodoketanserin is a good probe of the monoamine transporter, it must be emphasized that it was first developed to label serotoninergic S_2 and histamine H_1 receptors (Wouters et al., 1985b). Such sites are not present in purified bovine chromaffin granule membranes, as shown by the fact that pipamperone and pyrilamine did not affect [^{125}I]AZIK binding. However, it was of interest to determine the usefulness of this molecule in less purified extracts where ketanserin binding receptors coexist with vesicular transporter TBZ binding sites. Figure 7 shows that the transporter may be visualized in homogenates of rat striatum and in intact rabbit blood platelets. The labeled monoamine transporter was identified by its TBZ sensitivity and its resistance to methysergide.

The fact that the TBZ-sensitive band of these preparations is the major one is consistent with the choice of the starting material: the monoamine transporter is only associated with the synaptic vesicles of monoaminergic neurons (Scherman et al., 1986), and, in the striatum, TBZ-sensitive ketanserin binding sites predominate over the methysergide-sensitive ones (Darchen et al., 1988). For blood platelets, rabbit cells are known to be very rich in serotonin dense granules. It may also be noted that in previous experiments where [^{125}I]AZIK was used to label S_2 receptors (Wouters et al., 1987) that (i) cortex membranes were used, where the S_2 receptor is more abundant than the monoamine transporter (Darchen et al., 1988), and that (ii) the membrane–ligand complex was washed by centrifugation before irradiation, conditions under which ketanserin derivatives are likely to be completely dissociated from the TBZ binding site (Darchen et al., 1988).

The results of Table II indicate some heterogeneity in the molecular mass of the labeled polypeptide chain depending on the tissue and/or species. A similar study, also suggesting some structural heterogeneity in the monoamine transporter, has been initiated by Schuldiner et al. (1987), but it is difficult to compare with ours since the components labeled by the two techniques are obviously different. The labeled components of human adrenal medulla and pheochromocytoma seem to have a molecular weight smaller than their bovine counterpart, suggesting a possible species variability. On the other hand, the labeled component of rabbit platelets is significantly larger, introducing the possibility of an organ variability. Such a concept would be somehow surprising in view of the functional similarities existing between the various monoamine transporters tested: the equilibrium dissociation constants for [^{3}H]TBZOH and [^{3}H]RES binding and the turnover number for serotonin uptake have recently been shown to be the same in various rat organs and to be identical with the corresponding figures of bovine chromaffin granule membranes (Scherman & Boschi, 1988). This point will deserve a more detailed analysis.

Acknowledgments

We are indebted to Prof. J.-M. Idatte (Hôpital St. Louis, Paris) for providing human pheochromocytoma and adrenal medulla, to Dr. J.-M. Launay (Hôpital St. Louis, Paris) for a gift of rabbit blood platelets, and to Dr. H. Gozlan (Hôpital Pitié-Salpetrière, Paris) for help in dissecting rat striatum. We thank the Service Vêtérinaire des Abattoirs de Mantes for collecting bovine adrenals. We are grateful to B. Girard for the preparation of the manuscript.

Registry No. AZIK, 118798-29-1; [^{125}I]AZIK, 118798-30-4; TBZ, 58-46-8; serotonin, 50-67-9; ketanserin, 74050-98-9; 7-azidoketanserin, 95653-71-7.

REFERENCES

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Bruns, R. F., Lawson-Wendling, K., & Pugsley, T. A. (1983) *Anal. Biochem. 132*, 74–81.

Carty, S. E., Johnson, R. G., & Scarpa, A. (1981) *J. Biol. Chem. 256*, 11244–11250.

Darchen, F., Scherman, D., Laduron, P. M., & Henry, J. P. (1988) *Mol. Pharmacol. 33*, 672–677.

Deupree, J. D., & Weaver, J. A. (1984) *J. Biol. Chem. 259*, 10907–10912.

Fishkes, H., & Rudnick, G. (1982) *J. Biol. Chem. 257*, 5671–5677.

Gabizon, R., Yetinson, T., & Schuldiner, S. (1982) *J. Biol. Chem. 257*, 15145–15150.

Gasnier, B., Ellory, J. C., & Henry, J. P. (1987) *Eur. J. Biochem. 165*, 73–78.

Giraudat, J., Roisin, M. P., & Henry, J. P. (1980) *Biochemistry 19*, 4499–4505.

Henry, J. P., Gasnier, B., Roisin, M. P., Isambert, M. F., & Scherman, D. (1987) *Ann. N.Y. Acad Aci. 493*, 194–206.

Isambert, M. F., & Henry, J. P. (1985) *Biochemistry 24*, 3660–3667.

Johnson, R. G. (1987) *Ann. N.Y. Acad. Sci. 493*, 162–177.

Laduron, P. M., Janssen, P. F. M., Gommeren, W., & Leysen, J. E. (1982) *Mol. Pharmacol. 21*, 294–300.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Leysen, J. E., Niemegeers, J. E., Van Nueten, J. M., & Laduron, P. M. (1982) *Mol. Pharmacol. 21*, 301–314.

Leysen, J. E., Gommeren, W., & De Clerck, F. (1983) *Eur. J. Pharmacol. 88*, 125–130.

Maron, R., Kanner, B. I., & Schuldiner, S. (1979) *FEBS Lett. 98*, 237–240.

Pletscher, A., Bartholini, G., & Da Prada, M. (1966) in *Mechanisms of Release of Biogenic Amines* (Von Euler, U. S., Rossel, S., & Uvnas, B., Eds.) pp 165–175, Pergamon Press, Oxford.

Roisin, M. P., Isambert, M. F., Henry, J. P., Guillot, M., & Lenoir, G. (1984) *Biochem. Pharmacol. 33*, 2245–2252.

Scherman, D. (1986) *J. Neurochem. 47*, 331–339.

Scherman, D., & Henry, J. P. (1984) *Mol. Pharmacol. 25*, 113–122

Scherman, D., & Boschi, G. (1988) *Neuroscience* (in press).

Scherman, D., Jaudon, P., & Henry, J. P. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 584–588.

Scherman, D., Boschi, G., Rips, R., & Henry, J. P. (1986) *Brain Res. 370*, 176–181.

Scherman, D., Gasnier, B., Jaudon, P., & Henry, J. P. (1988) *Mol. Pharmacol. 33*, 72–77.

Schotte, A., Maloteaux, J. M., & Laduron, P. M. (1983) *Brain Res. 276*, 231–235.

Schuldiner, S., Gabizon, R., Stern, Y., & Suchi, R. (1987) *Ann. N.Y. Acad. Sci. 493*, 189–193.

Smith, A. D., & Winkler, H. (1967) *Biochem. J. 103*, 481–482.

Toll, L., & Howard, B. C. (1978) *Biochemistry 17*, 2517–2523.

Wouters, W., Van Dun, J., Leysen, J. E., & Laduron, P. M. (1985a) *J. Biol. Chem. 260*, 8423–8429.

Wouters, W., Van Dun, J., Leysen, J. E., & Laduron, P. M. (1985b) *FEBS Lett. 182*, 291–296.

Wouters, W., Van Dun, J., & Laduron, P. M. (1987) *FEBS Lett. 213*, 359–364.