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Photoinactivation of Serotonin Uptake by an Arylazido Derivative of 5-Hydroxytryptamine

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SUMMARY

A potential photoaffinity probe for the substrate-binding polypeptide of the neuronal serotonin uptake system has been synthesized. Under dark conditions, 3-(β-(4-azidobenzamidino)ethyl-5-hydroxyindole (serotonin azidobenzamidine (SABA) was found to inhibit competitively [^{3}H]5-hydroxytryptamine uptake by rat cortical synaptosomes with a K_I of 130 nM. The selectivity of this action was indicated by SABA's much lower potency as an inhibitor of synaptosomal [^{3}H]norepinephrine uptake ($K_I = 7$ μM). When synaptosomes were irradiated in the presence of SABA, serotonin uptake was irreversibly inhibited in a concentration-dependent fashion with the maximum effect occurring at 1 μM SABA. At this concentration, approximately 40% of the serotonin uptake activity could not be recovered upon repeated washing of the synaptosomes. This inhibition was determined not to result from the production of a potent inhibitory photolysis product of SABA. The photoinactivation of serotonin transport by SABA was found to depend on the time of irradiation and could be prevented by the presence of agents that interact with the uptake system. Serotonin, *p*-chloroamphetamine, fenfluramine, and alaproclate protected the serotonin carrier against SABA's irreversible effects in a concentration-dependent manner. The presence of high concentrations of Tris or *p*-aminobenzoic acid, two nitrene-scavenging agents, did not reduce the level of photoinactivation of serotonin uptake by SABA, indicating that the irreversible inhibition is a result of true photoaffinity labeling of the carrier.

INTRODUCTION

While several neurotransmitter receptor proteins have been identified in recent years, there has not been much progress made in the characterization of the proteins involved in the neuronal uptake of any neurotransmitter. One reason for this discrepancy has been the lack of specific affinity reagents to label the transport sites in membranes. Selective affinity and photoaffinity probes have greatly contributed to the elucidation of the structure and molecular properties of β-adrenergic (1, 2), α-adrenergic (3, 4), muscarinic (5, 6), and benzodiazepine (7) receptors. [^{3}H]Xylamine, a 2-chloroethylamine analogue of bretylium, has been introduced as a potential marker for norepinephrine uptake sites (8), but there have been no reports of its successful application in this role to date. A nitrogen mustard analogue of hemicholinium has been reported (9), and a radiolabeled derivative of this compound may be a valuable probe for the high affinity choline transport system.

There have been attempts to photoaffinity label imipramine-binding sites in platelets and synaptosomes. Rotman and Pribluda (10) used a tritiated azido derivative of imipramine to label both preparations, but did not report experiments that could have identified a specific imipramine-binding protein. Wennogle *et al.* (11) used [^{3}H]2-nitroimipramine to label platelet membranes and reported the specific labeling of a 30-kDa band on polyacrylamide gels. However, the question arises as to whether these tricyclics, although potent inhibitors of serotonin uptake, can label the protein that is responsible for serotonin transport. It is widely believed that imipramine allosterically inhibits platelet and neuronal serotonin uptake (12, 13), and it is conceivable that [^{3}H]2-nitroimipramine labels a protein distinct from that which actively translocates serotonin across the plasma membrane. This report describes the first photodependent, irreversible inhibitor of serotonin uptake that interacts with the substrate-binding site of the transport system. The compound SABA[1] exhibits a selectivity for serotonin uptake that may make it a useful probe for identifying and characterizing the serotonin transport protein. A

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[1] The abbreviations used are: SABA, 3-(β-(4-azidobenzamidino)ethyl)-5-hydroxyindole; 5-HT, 5-hydroxytryptamine; NE, norepinephrine.

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radiolabeled derivative could also provide direct information concerning the relationship of the imipramine-binding site to the substrate-binding site of the serotonin transport system.

METHODS

Chemical ionization-mass spectroscopy was kindly performed by Dr. Kenneth Chan at the Pharmacoanalytic Laboratory of the University of Southern California Comprehensive Cancer Center using a Hewlett-Packard 5985A Quadrupole GC-MS-DS. Ammonia was used as the reagent gas. Infrared spectra were recorded with a Beckman IR4240 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Melting points were determined with a Fisher-Johns micromelting point apparatus and are uncorrected.

The commercially obtained materials used in this study were as follows: [^{3}H]5-hydroxytryptamine binoxalate (New England Nuclear), [^{3}H]norepinephrine (New England Nuclear), 5-hydroxytryptamine hydrochloride (Sigma), nialamide (Sigma), *p*-chloroamphetamine (Sigma), and methyl-4-azidobenzoimidate hydrochloride (Pierce Chemical Co.) The following drugs were generously donated by their manufacturers: fenfluramine hydrochloride (A. H. Robbins, Richmond, VA), fluoxetine hydrochloride (Eli Lilly & Co., Indianapolis, IN), indalpine (Pharmuka Laboratories, Gennevilliers, France), imipramine hydrochloride (Ciba-Geigy, Summit, NJ), desmethylimipramine hydrochloride (U.S.V. Pharmaceutical Corp., Tuckahoe, NY), and alaproclate hydrochloride (Astra Läkemedal AB, Södentälje, Sweden).

SABA synthesis. Methyl-4-azidobenzoimidate hydrochloride is commercially available but can be quite easily prepared as described by Ji (14). Methyl-4-azidobenzoimidate hydrochloride (100 mg, 0.47 mmol), 5-hydroxytryptamine hydrochloride (300 mg, 1.41 mmol), and sodium carbonate (448 mg, 4.23 mmol) were stirred under nitrogen at room temperature in 5 ml of dimethylformamide. After 6 h, the reaction mixture was filtered and the solvent was removed under reduced pressure. The residue was taken up into 2 ml of acetonitrile:water (30:70), and the product was purified by high pressure liquid chromotography. One hundred-μl aliquots were chromatographed using a reverse-phase C_{18} column (EM Hibar Prep 10, 25 cm × 10 mm (inner diameter)) with a mobile phase of 20 mM ammonium acetate in acetonitrile:water (30:70) pumped at 9 ml/min. Under these conditions, unreacted 5-HT and imidoester eluted at 2 and 20 min, respectively. The product, eluting between 7 and 12 min, was collected at each injection, and the acetonitrile was removed from the combined fractions under reduced pressure. Lyophilization of the aqueous solution yielded 72 mg (0.23 mmol, 48% yield based on the amount of starting imidoester) of product melting at 186–188° (decomposition). Chemical ionization-mass spectroscopy showed major ion fragments at *m/z* 321 (M + 1), *m/z* 293 (N_2 elimination), and *m/z* 177 (5-HT structure). Infrared spectroscopy (KBr) was also consistent with the proposed SABA structure (Fig. 1) with strong absorbances at 3590 cm^{-1} (indole N—H), 3380 cm^{-1} (broad, O—H), 2150 cm^{-1} (—N_3), and 1690 cm^{-1} (C═N).

$C_{17}H_{16}N_6O$

Calculated:	C 63.74	H 5.03	N 26.23
Found:	C 63.51	H 5.22	N 25.98

Synaptosome preparation. Male Sprague-Dawley rats (160–200 g) were sacrificed by decapitation, and the cortices were immediately dissected on ice. All subsequent steps were carried out at 0–4°. The tissue was homogenized in 10 volumes of isotonic sucrose using a glass homogenization vessel with a motor-driven Teflon pestle. The homogenate was centrifuged at 1,000 × *g* for 10 min, and the decanted supernatant was recentrifuged at 10,000 × *g* for 20 min. A washed mitochondrial-synaptosomal fraction was obtained by resuspending the pellet in 5 volumes of isotonic sucrose and centrifuging at 10,000 × *g* for 20 min. This pellet was gently resuspended in Krebs-Ringer phosphate buffer (123 mM Na^+, 115 mM Cl^-, 5.2 mM K^+, 1.3 mM Ca^{2+}, and 3.6 mM Mg^{2+}), pH 7.4, containing 0.01% ascorbic acid, 8.4 mM D-glucose, and 20 μM nialamide. The final protein concentrations were 1.6 mg of protein/ml for [^{3}H]5-HT experiments and 3.2 mg of protein/ml for [^{3}H]NE uptake experiments. Protein was determined by the method of Lowry *et al.* (15).

Uptake experiments. Incubations were performed at 37° in a total volume of 0.8 ml of the above buffer. Samples contained 100 μl of the synaptosome suspension, 100 μl of tritiated substrate, and 100 μl of inhibitor (buffer for control samples). Except for the kinetic experiments, the final [^{3}H]5-HT concentration was 5 nM. In experiments studying the kinetics of [^{3}H]5-HT uptake inhibition by SABA, the substrate concentrations used were 25, 50, 100, 265, and 500 nM. The SABA concentrations evaluated were 30 nM, 100 nM, 300 nM, and 1 μM. In the kinetic studies of [^{3}H]NE uptake inhibition, the substrate concentrations used were 100, 200, 400, and 800 nM, while the SABA concentrations tested were 3, 10, and 30 μM. All determinations were performed in triplicate. After a 2-min ([^{3}H]5-HT) or 3 min ([^{3}H]NE) incubation, uptake was terminated by the addition of 5 ml of ice-cold saline, and the samples were immediately filtered through Whatman 0.8-μm cellulose nitrate filters. The filters were washed two additional times with 5 ml of cold saline and placed in scintillation vials containing 5 ml of Liquiscint (National Diagnostics). Radioactivity retained by the filters was determined by liquid scintillation spectrometry at a counting efficiency of approximately 50%. Nonspecific binding of tritiated substrate was determined with samples incubated for 2 min on ice prior to filtration. Specific uptake of each substrate was defined as the difference between the uptake that occurred at 37° and 0° and represented about 85% of the total radioactivity. Deviations from the mean are expressed as standard errors of the mean.

Photolysis experiments. Synaptosomes were prepared as described above and resuspended at a concentration of 0.7 mg of protein/ml in Krebs-Ringer phosphate buffer. SABA or other agents were added in a small volume to the synaptosomes in Kimax tubes (10 mm inner diameter, 2-mm wall thickness), and the suspensions were incubated for 2 min at 37° to promote association with the carrier. The samples were placed on ice for 10 min and then irradiated at a distance of 20 cm from a 450-W medium pressure mercury vapor lamp (Hanovia model no. 7825-34). This lamp produces a broad spectrum of light with typical mercury lines from 222.4 to 1367.3 nm. For reference, the radiated energies at 253.7, 302.5, and 366.0 nm are 5.8, 7.2, and 25.6 Ws, respectively. The suspensions were diluted with 4 volumes of ice-cold 0.32 M sucrose and centrifuged at 10,000 × *g* for 20 min. The resulting pellets were resuspended in 15 ml of cold sucrose and centrifuged again at 10,000 × *g* for 20 min. This washing procedure was repeated once again, and the final pellets were resuspended in Krebs-Ringer phosphate buffer for determination of [^{3}H]5-HT uptake as described above. [^{3}H]5-HT uptake was normalized to the amount of protein in individual samples before comparisons between different treatments were made.

RESULTS

Reversible inhibition of [^{3}H]5-HT uptake by SABA. One advantage of photoaffinity reagents compared to electrophilic alkylating agents is that the reversible inhibitory properties of a photoaffinity probe can be assessed prior to photolysis experiments. Thus, the reversible inhibition of [^{3}H]5-HT and [^{3}H]NE uptake by SABA (for structure see Fig. 1) was studied in synaptosomes prepared from rat cortex. The apparent K_m values for

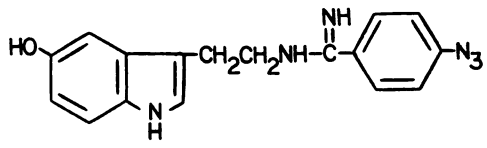

FIG. 1. *Structure of SABA*

[^{3}H]5-HT and [^{3}H]NE uptake under our assay conditions were found to be 70 and 178 nM, respectively. The kinetic studies shown in Fig. 2 demonstrate that SABA is a competitive inhibitor of neuronal serotonin transport, and the K_I value for the inhibition was determined to be 130 nM. SABA also was found to inhibit competitively the accumulation of [^{3}H]NE by cortical synaptosomes, but it was much less potent in this respect (K_I = 7 μM, Fig. 3). Thus, although SABA is not as potent as some other inhibitors of serotonin uptake such as fluoxetine or indalpine, its selectivity for the serotonin carrier versus the norepinephrine carrier is almost as high as for these other compounds (16, 17).

Irreversible inhibition of [^{3}H]5-HT uptake by SABA. Irradiation of synaptosome suspensions in the presence of SABA caused a reduction of [^{3}H]5-HT uptake capacity that could not be recovered upon washing of the synaptosomes. The concentration dependency of this irreversible effect is shown in Table 1. The maximum level of photodependent inhibition occurs at 1 μM SABA, which is consistent with reversible effects of SABA on [^{3}H]5-HT accumulation. At this concentration, SABA had no photodependent inhibitory effects on synaptosomal [^{3}H]NE uptake. In these photolysis experiments, three different control samples are always included: (1) a sample that is neither irradiated nor contains SABA, (2) a sample that is irradiated in the absence of SABA, and (3) a sample that contains SABA but is not irradiated. Typically, when the time of photolysis is 2 min or less, [^{3}H]5-HT uptake in the latter two controls is at least 95% of the control sample that does not contain SABA and is not irradiated. This indicates the absence of a direct effect of the irradiation on [^{3}H]5-HT uptake and the effectiveness of the washing procedure to remove noncovalently bound SABA. This also supports the notion of an irreversible action of SABA, when irradiated, on serotonin uptake.

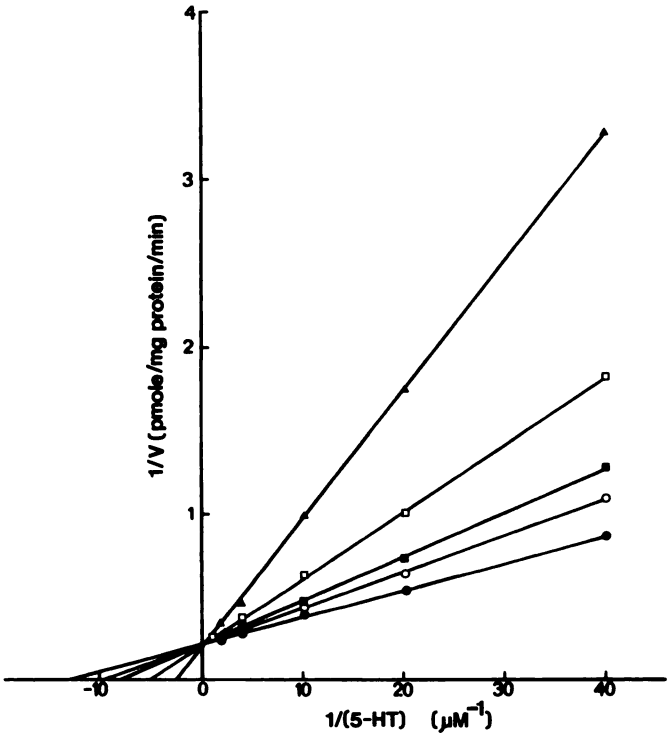

FIG. 2. *Double reciprocal plot of the reversible inhibition of [^{3}H]5-HT uptake by SABA in rat cortical synaptosomes*

The experiments were performed as described in "Methods," and the data are from one experiment that was reproduced two additional times. Each point represents the mean of triplicate samples that varied by less than 5%. The concentrations (μM) of SABA tested were 0 (●), 0.03 (○), 0.10 (■), 0.30 (□), and 1.0 (▲).

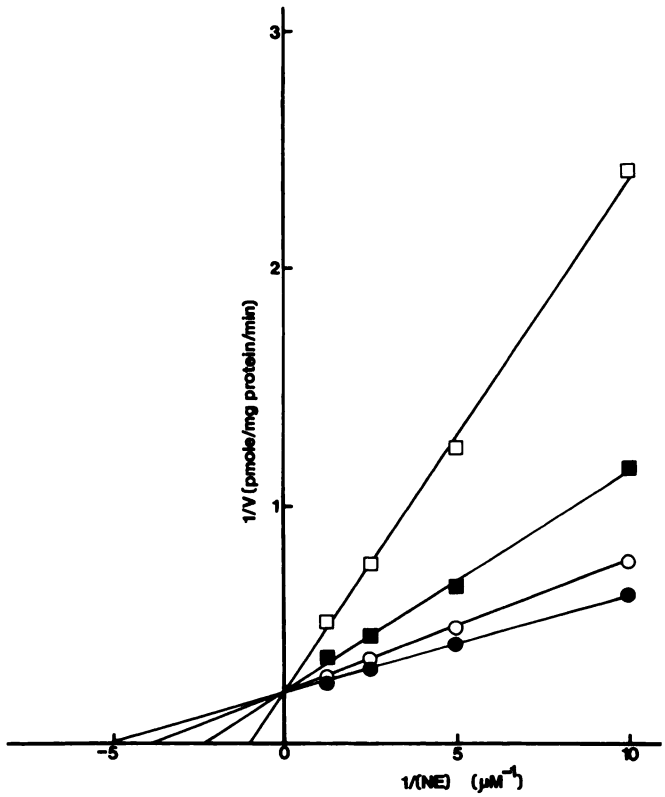

FIG. 3. *Double reciprocal plot of the reversible inhibition of [^{3}H]NE uptake by SABA in rat cortical synaptosomes*

The experiments were performed as described in "Methods." The data shown are from one experiment that was reproduced two additional times. Each point represents the mean of triplicate samples that varied by less than 5%. The concentrations (μM) of SABA tested were 0 (●), 3 (○), 10 (■), and 30 (□).

TABLE 1

Concentration dependence of the photoinactivation of [^{3}H]5-HT uptake by SABA

Synaptosome suspensions were irradiated for 1 min in the absence or presence of the indicated concentrations of SABA and washed as described in "Methods." The data show the percentage (±SE) of uptake compared to that of the controls (no SABA) from three experiments. At all concentrations tested, the uptake in samples treated with SABA and not irradiated were at least 95% of the control value. In these experiments and the experiments shown in Tables 2 and 3, the control uptake of [^{3}H]5-HT was 0.31 ± 0.02 pmol/mg of protein/min.

SABA	[^{3}H]5-HT uptake
μM	*% of control*
0	100
0.1	82 ± 4
0.3	72 ± 5
1.0	62 ± 5
3.0	60 ± 3

The observed photodependent inhibition of serotonin uptake by SABA was determined not to result from the presence of a photolysis product which has an extremely high affinity for the 5-HT carrier and is not removed upon washing of the synaptosomes. In these experiments (not shown), SABA (1 mM) was photolyzed for 2 min in the Krebs-Ringer phosphate incubation buffer, and the [^{3}H]5-HT uptake blocking properties of the photolysis product(s) were examined. The photolyzed material was about 20 times less potent than SABA as a reversible inhibitor of [^{3}H]5-HT accumulation by cortical synaptosomes.

Irreversible inactivation of the serotonin transport system was also supported by the changes in the kinetic properties of [^{3}H]5-HT uptake following irradiation in the presence of SABA. The V_{max} for [^{3}H]5-HT uptake in synaptosomes photolyzed for 1 min with 1 μM SABA and washed twice by centrifugation was 2.8 ± 0.3 pmol/mg of protein/min ($n = 3$). This value is 35% lower than the V_{max} for [^{3}H]5-HT uptake by control synaptosomes (4.3 ± 0.2 pmol/mg of protein/min). There was no difference between the K_m values (70 nM) for [^{3}H]5-HT uptake in the SABA-photolyzed samples and control tissue. The reduction in V_{max} with no change in K_m is consistent with the irreversible photoinactivation of [^{3}H]5-HT uptake sites by SABA.

The results shown in Table 1 were obtained using a 1-min irradiation period. This time of exposure was selected on the basis of the data in Table 2 which show that the maximum level of photoinactivation of [^{3}H]5-HT uptake occurred after 1 min of irradiation. This result is consistent with the rate of photolysis of the azide. Fig. 4 shows the time course of the photolytic decomposition of SABA in Krebs-Ringer phosphate buffer, pH 7.4. More than 90% of the SABA is destroyed in 1 min, and we have determined that the presence of synaptosomes at the protein concentrations normally present in the photoinactivation experiments does not appreciably alter the rate of SABA's photoinduced decomposition. Therefore, the maximum photodependent effect of SABA should occur after about 1 min of irradiation.

Protection experiments. One requirement for true affinity labels is the demonstration that irreversible inhibition is a result of an interaction of the inhibitor at the ligand (substrate)-binding site of the target macromolecule. This information can be provided by experiments in which compounds that are known to interact with the binding site of the target are shown to protect against irreversible inhibition produced by the affinity agent. Accordingly, several drugs that reversibly inhibit serotonin uptake were studied for their effects on the photoinactivation of [^{3}H]5-HT uptake by SABA. The results in Table 3 show that the photodependent inhibition produced by SABA could be blocked, in a concentration-dependent manner, by serotonin, *p*-chloroamphetamine, fenfluramine, and alaproclate. This protection was related to the potency of the irreversible inhibitors in that alaproclate blocked SABA's effects at much lower concentrations than fenfluramine or *p*-chloroamphetamine, and it is a much more potent inhibitor of serotonin uptake than these compounds. Protection experiments were also performed with fluoxetine and indalpine, but neither of these compounds could be effectively removed by washing of the synaptosomes, and the results of these experiments were not unambiguous.

The level of photoinactivation of [^{3}H]5-HT uptake could be increased if synaptosomes are irradiated twice in the presence of SABA. When synaptosomes are photolyzed for 1 min with 1 μM SABA, washed by centrifugation, and irradiated a second time in the presence of fresh 1 μM SABA, the [^{3}H]5-HT uptake activity remain-

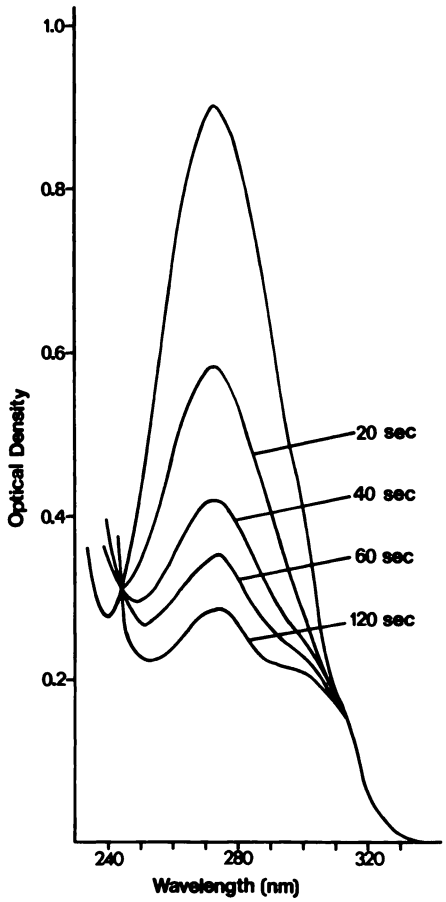

FIG. 4. *Absorption spectrum of SABA and the spectral changes that occur upon irradiation*

SABA (50 μM), in Krebs-Ringer phosphate buffer, pH 7.4, was irradiated at a distance of 20 cm from an Hanovia 450-W mercury lamp. Spectra were recorded after 0, 20, 40, 60, and 120 sec of irradiation. No change in the spectrum occurred at longer photolysis times.

TABLE 2

Irreversible inactivation of [^{3}H]5-HT uptake as a function of irradiation time

The experiments were performed as described in "Methods" at a concentration of 1 μM SABA. The data represent the percentage of uptake (±SE) compared to that of controls (no SABA) from three experiments at each time point.

Irradiation time	[^{3}H]5-HT uptake
min	*% control*
0.5	77 ± 3
1.0	64 ± 2
2.0	59 ± 3

TABLE 3

Protection against the photoinactivation of [^{3}H]5-HT uptake by SABA

Synaptosomes were incubated with 1 μM SABA alone or in the presence of the indicated agent for 2 min at 37°. The suspensions were then chilled on ice for 10 min and irradiated for 1 min. After two washes by centrifugation [^{3}H]5-HT uptake was determined as described in "Methods." The data show the percentage (±SE) of uptake compared to controls (i.e., no SABA or other agent) from three experiments for each condition listed. Uptake in samples containing SABA, the protecting agent, or both, and not irradiated, were at least 95% of the control uptake.

Agent	Concentration	[^{3}H]5-HT uptake
	μM	*% control*
	0	60 ± 2
5-HT	0.1	62 ± 1
	0.3	70 ± 3
	1.0	85 ± 3
	3.0	95 ± 4
p-Chloroamphetamine	1.0	78 ± 3
	10.0	98 ± 2
Fenfluramine	1.0	73 ± 3
	3.0	86 ± 2
Alaproclate	0.1	82 ± 3
	0.3	95 ± 2

ing was 0.11 ± 0.1 pmol/mg of protein/min ($n = 3$). This represents a 65% reduction in uptake compared to the control uptake of 0.30 ± 0.02 pmol/mg of protein/min and is consistent with the irreversible inactivation of approximately 40% of the synaptosomal uptake activity in each irradiation period. The uptake of [^{3}H]5-HT in synaptosomes photolyzed for 1 min with 1 μM SABA was 0.18 ± 0.02 pmol/mg of protein/min. If these are washed and photolyzed a second time in the presence of 1 μM SABA and 3 μM 5-HT, the [^{3}H]5-HT uptake activity was 0.17 ± 0.01 pmol/mg of protein/min. The ability of 5-HT to protect against the increased inhibition that occurs following a second irradiation period with fresh SABA demonstrates that the accrued inhibition is not due to the photoinduced destruction of the serotonin transport system.

One important observation was that at concentrations up to 3 μM, serotonin did not have a photoinhibitory effect of its own on subsequent [^{3}H]5-HT uptake. Although serotonin is photolabile itself, these results indicate that it is the arylazido portion of SABA that is responsible for the photoinactivation of serotonin transport. Also, none of the other compounds used as protecting agents were found to irreversibly inhibit serotonin uptake upon irradiation.

Effects of nitrene scavengers. The phenomenon of pseudo-photoaffinity labeling was first documented by Singer *et al.* (18). This type of inhibition can be distinguished from true photoaffinity labeling by including nitrene-scavenging agents in the irradiation mixture. Therefore, the effects of two nitrene scavengers, Tris and *p*-aminobenzoic acid (19), were examined for their effects on the photodependent inhibition of [^{3}H]5-HT uptake produced by SABA. It was found that the presence of 50 mM Tris or 1 mM *p*-aminobenzoic acid had no effect on the level of photoinactivation of uptake at 1 μM SABA. This lack of effect is evidence that SABA which is bound to the serotonin uptake site is responsible for the observed inhibition and the inhibition is not due to nitrene intermediate that is formed in solution which then diffuses to, and reacts with, the carrier's substrate-binding site.

DISCUSSION

This report has documented the irreversible photoinactivation of [^{3}H]5-HT uptake produced by SABA in rat cortical synaptosomes. The photodependent inhibition is concentration-dependent, dependent on the length of irradiation, and can be blocked by compounds that interact with the serotonin uptake carrier. Thus, serotonin, fenfluramine, *p*-chloroamphetamine, and alaproclate are shown to reduce SABA's photodependent, irreversible inhibitory effect on serotonin uptake, and this protective action is related to their potency as reversible inhibitors of serotonin uptake. The selectivity of SABA's action is indicated by its much lower potency as a reversible inhibitor of synaptosomal norepinephrine uptake compared to its reversible effects on serotonin transport. Significantly, SABA was found to inhibit serotonin uptake in a competitive manner under dark conditions. This suggests that SABA inhibits uptake by competing for substrate binding rather than by an allosteric mechanism. Imipramine and its analogues have been shown by some to inhibit serotonin uptake noncompetitively (20, 21). This, as well as other evidence, suggests that the polypeptides labeled by [^{3}H]2-nitroimipramine (11) or [^{3}H]azidoimipramine (10) may not represent that which actively translocates serotonin across the neuronal plasma membrane. Thus, the kinetic experiments with SABA indicate that it may be the first irreversible inhibitor of serotonin uptake that acts at the substrate-binding site of the carrier system. In preliminary experiments, SABA has been found to be a very poor displacer of [^{3}H]imipramine binding to cortical membranes. This also supports the notion that serotonin uptake inhibition is due to competition for substrate binding rather than a result of an interaction at the tricyclic-binding site of the transport system. This is not surprising in view of the structural similarity between SABA and serotonin.

One feature of SABA's effect on uptake that may appear to be inconsistent is that only 40% of the [^{3}H]5-HT accumulation can be inhibited when synaptosomes are irradiated in the presence of 1 μM SABA, a concentration that should occupy virtually all of the serotonin transport sites. However, this phenomenon has been observed with other photoaffinity probes. Ruoho *et al.* (19) found that only 10% of the β-adrenergic receptors in erythrocytes could be labeled with [^{125}I]iodohydroxybenzylpindolol, and Lavin *et al.* (22) found that *p*-azidobenzylcarazolol could inactivate only 30% of the β-adrenergic receptors in frog erythrocytes. Also, [^{3}H]flunitrazepam was originally observed to label only 20–25% of benzodiazepine receptors (23), although recently, using shorter wavelength irradiation, [^{3}H]flunitrazepam could be demonstrated to label at least *40%* of these receptors (24). The extent of photodependent inhibition depends on the lifetime of the nitrene intermediate, its

rate of dissociation from the target, its reactivity toward functional groups present in the combining site, and its rate of reaction with solvent that may be present in or near the combining site. All of these factors contribute to the efficiency of incorporation of the nitrene intermediate into its combining site, and it is likely that one or more of these limit the efficiency of SABA's reaction with the serotonin uptake carrier so that only 40% of the sites can be labeled in one irradiation period. We have found that a second exposure to low wavelength irradiation at 1 μM SABA results in an additional 40% inactivation of [^{3}H]5-HT uptake (65% overall inhibition). This result indicates that the limited photoinhibitory effect of SABA is probably not due to more than one population of serotonin uptake sites.

[^{3}H]SABA can be prepared from commercially available [^{3}H]serotonin for studies that may lead to identification of the serotonin carrier protein. Such experiments could be performed with either brain synaptosomes or platelets. In preliminary studies, SABA has been found to inhibit porcine platelet serotonin uptake with the same potency as it inhibits synaptosomal serotonin uptake. At this time we have not performed photolysis experiments with platelets, but there is no reason that SABA would not be a suitable photoaffinity probe for the serotonin uptake system in this cell since it appears to be identical to the neuronal serotonin uptake carrier (25). It will also be of interest to compare the protein(s) specifically labeled with[^{3}H]SABA and [^{3}H]azidoimipramine or [^{3}H] 2-nitroimipramine. Such experiments could provide direct information concerning the relationship between the tricyclic-binding site and the serotonin transport protein.

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