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Biochemistry

(^{125}I)Iodoazidococaine, a photoaffinity label for the haloperidol-sensitive sigma receptor

(cocaine/[^{3}H]cocaine binding/cocaine-binding protein)

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ABSTRACT **A carrier-free radioiodinated cocaine photoaffinity label, (−)-3-(^{125}I)iodo-4-azidococaine [(^{125}I)IACoc], has been synthesized and used as a probe for cocaine-binding proteins. Photoaffinity labeling with 0.5 nM (^{125}I)IACoc resulted in selective derivatization of a 26-kDa polypeptide with the pharmacology of a sigma receptor in membranes derived from whole rat brain, rat liver, and human placenta. Covalent labeling of the 26-kDa polypeptide was inhibited by 1 μM haloperidol, di(2-tolyl)guanidine (DTG), 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine (3-PPP), dextromethorphan, and carbetapentane. Stereoselective protection of (^{125}I)IACoc photolabeling by 3-PPP [(+)-3-PPP more potent than (−)-3-PPP] was observed. (^{125}I)IACoc labeling of the 26-kDa polypeptide was also inhibited by 10 μM imipramine, amitriptyline, fluoxetine, benztropine, and tetrabenazine. The size of the (^{125}I)IACoc-labeled proteins is consistent with the size of proteins photolabeled in guinea pig brain and liver membranes by using the sigma photolabel azido-[^{3}H]DTG. Kinetic analysis of (^{125}I)IACoc binding to rat liver microsomes revealed two sites with K_d values of 19 and 126 pM, respectively. The presence or absence of proteolytic inhibitors during membrane preparation did not alter the size of the photolabeled sigma receptor, indicating that the 26-kDa polypeptide was not derived from a larger protein. In summary, (^{125}I)IACoc is a potent and highly specific photoaffinity label for the haloperidol-sensitive sigma receptor and will be useful for its biochemical and molecular characterization.**

Cocaine is a drug of abuse that has a number of pharmacological activities. While its potential for abuse is clear, precisely how cocaine produces its various effects in humans has proved elusive. Cocaine blocks dopamine (DA), serotonin (5HT), and norepinephrine (NE) uptake into central and peripheral neurons (1–4) and at higher concentrations has also been shown to bind to muscarinic receptors (5), sigma receptors (6), and sodium channels (7). In the central nervous system (CNS), DA has been demonstrated to play an important role in the reward mechanisms of animals, and the ability of cocaine to block DA uptake has been associated with CNS stimulation and cocaine self-administration in animals (8, 9). However, mechanisms other than DA uptake blockade may contribute to cocaine euphoria and addiction, as well as to the psychiatric, cardiovascular, and perinatal complications of cocaine use (10).

One of the difficulties with studying the relative importance of various cocaine-binding sites has been an inadequate understanding of the cocaine-binding proteins themselves. By using photoaffinity probes derived from a class of DA uptake inhibitors, the 1,4-diphenyl-substituted piperazines, a glycoprotein of 58–62 kDa for the DA transporter has been identified (11, 12). Although cocaine was shown to stereospecifically block the photolabeling of the DA transporter, it was not established whether cocaine bound directly to this protein or to an associated protein. Recently, the human NE transporter has been expression-cloned as cDNA and overexpressed in HeLa cells (13). The ability of cocaine to block [^{3}H]NE uptake into these HeLa cells provides good evidence that cocaine binds directly with this transporter.

Although cocaine has been shown to block the binding of sigma ligands to rat and guinea pig brain membranes with relatively low affinity, the nature and physiological importance of this interaction is not known. Indeed, the biochemical function of the sigma receptor itself, while stimulating a great deal of interest, is also not known. Originally classified as an opioid receptor on the basis of the pharmacological effects and binding of *N*-allylnormetazocine (SKF 10,047) (14, 15), the sigma receptor has now been defined pharmacologically on the basis of the binding of a number of compounds, such as di(2-tolyl)guanidine (DTG), 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine (3-PPP), haloperidol, SKF 10,047, and cyclazocine (16). Binding sites for sigma ligands have been found in a number of areas of the brain (17), as well as in peripheral organs (18), by using (+)-3-[^{3}H]PPP, [^{3}H]pentazocine, and [^{3}H]DTG. A number of antitussives, including dextromethorphan, carbetapentane, and caramiphen, appear to bind to the sigma receptor as well (19, 20). By using the sigma-specific photolabel azido[^{3}H]DTG, a 27- to 29-kDa haloperidol-sensitive photolabeled protein was identified by SDS/PAGE in guinea pig brain and liver membranes (21, 22). The binding of compounds such as rimcazole, remoxipride, and BMY 14802 to the sigma receptor has been suggested to mediate their antipsychotic activities (23, 24).

As a step towards directly probing cocaine binding proteins in the brain and periphery, we have developed the photoaffinity label (−)-3-(^{125}I)iodo-4-azidococaine [(^{125}I)IACoc]. This compound selectively photolabels a 26-kDa haloperidol-sensitive sigma receptor in rat whole brain, rat liver, and human placental membranes.

METHODS

Synthesis of (−)-3-Iodo-4-azidococaine (IACoc). The synthesis of 3-iodo-4-azidobenzoic acid (IABA) will be detailed elsewhere (M. K. Sievert and A.E.R., unpublished results). IABA (22.5 mg) was refluxed in the dark in 200 μl of thionyl chloride at 80°C for 2 hr. The solution was cooled to room temperature, thionyl chloride was removed under reduced pressure, and the residue was washed with 1.0 ml of diethyl ether three times. (−)-Ecgonine methyl ester hydrochloride (12 mg) and 4-dimethylaminopyridine (33 mg) were then

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Abbreviations: DA, dopamine; NE, norepinephrine; 5HT, serotonin (5-hydroxytryptamine); DTG, di(2-tolyl)guanidine; 3-PPP, 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; IACoc, (−)-3-iodo-4-azidococaine; (^{125}I)IACoc, ^{125}I-substituted IACoc.

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added as a suspension in 300 μl of tetrahydrofuran (THF). After 4 hr, the THF was removed under reduced pressure. IACoc was purified by preparative silica gel thin-layer chromatography [toluene/diethylamine, 20:1 (vol/vol)]. The product (R_f = 0.45) was extracted from the silica gel with 0.5 ml of ethyl acetate three times to yield 11 mg of IACoc. The structure of IACoc was verified by NMR (deuterodimethyl sulfoxide): δ 8.21 (s, 1 H, aromatic H *ortho* to I), δ 7.92 (d, 1 H, aromatic H *para* to I), δ 7.51 (d, 1 H, aromatic H *meta* to I), and δ 5.47 ppm (m, 1 H, alkyl H at the 3 position of the tropane ring).

Synthesis of (^{125}I)IACoc. *Synthesis of (−)-4-aminococaine.* (−)-Ecgonine was prepared according to Bell and Archer (25). (−)-Ecgonine methyl ester (free base) prepared according to Singh *et al.* (26); 200 mg was dissolved in 1 ml of pyridine. To this solution was added a 5-fold excess of 4-nitrobenzoyl chloride, and the reaction was allowed to proceed at room temperature with stirring for 4 hr. Pyridine was removed under reduced pressure, and then 2 ml of water was added. The aqueous solution was rendered basic (pH 9.0) with sodium carbonate and extracted three times with 1.0 ml of ethyl acetate to yield (−)-4-nitrococaine free base, 75 mg, >90% pure on TLC, R_f = 0.8 in methanol/ethyl acetate/diethylamine (10:10:1, vol/vol). After removal of the ethyl acetate, (−)-4-nitrococaine was dissolved in 20 ml of anhydrous methanol. Pd/charcoal (100 mg) was added and H_2 gas was bubbled through the suspension at room temperature and atmospheric pressure for 5 hr. The Pd/charcoal was filtered off and the resulting solution was concentrated under reduced pressure, and components were separated by preparative TLC (methanol/ethyl acetate/diethylamine, 10:10:1) using two silica gel plates (1.0 mm thick, 20 cm × 20 cm). (−)-4-Aminococaine (R_f = 0.65) was extracted from silica with 0.6 ml of ethyl acetate three times to yield a clear oil, which crystallized at −20°C (30 mg). The chemical structure was confirmed by NMR (deuterodimethyl sulfoxide): δ 7.56 (d, 2 H, *meta* to amine), δ 6.54 (d, 2 H, *ortho* to amine), δ 5.93 (s, 2 H, aryl amino H), δ 5.03 (m, 1 H, alkyl H at the 3 position of the tropane ring) and δ 3.60 ppm (s, 3 H, methy ester).

Synthesis of (−)-3-(^{125}I)iodo-4-aminococaine. To 2.5 mCi (1 mCi = 37 MBq) of Na^{125}I (in 10 μl of 0.1 M NaOH) was added 10 μl of 0.1 M HCl and 50 μl of 0.5 M sodium acetate buffer (pH 5.6). To this was added 30 μl of a 1 mg/ml solution of (−)-4-aminococaine (0.5 mg dissolved with two drops of methanol and then diluted to 0.5 ml with sodium acetate buffer). The reaction was started by adding 30 μl of a 1.0 mg/ml solution of chloramine T (dissolved in water). After 15 min, the reaction was stopped by adding 100 μl of $Na_2S_2O_5$ (5.0 mg/ml in water). The pH was adjusted to approximately 9.0 by adding three drops of 0.1 M NaOH and the reaction mixture was extracted three times with 0.6 ml of ethyl acetate. The combined extracts were concentrated by evaporation with an argon stream to approximately 50 μl and then streaked onto a silica gel plate (0.25 mm thick, 10 cm × 20 cm). The TLC plate was developed with a toluene/diethylamine (4:1, vol/vol) solvent system, and the major band, (^{125}I)iodo-4-aminococaine, which was located by autoradiography using Kodak X-Omat AR film (R_f = 0.6; approximately 1.2 mCi), was extracted from the silica with 0.7 ml of ethyl acetate three times and stored at −20°C until further use. This compound was estimated to be 99% radiopure, as assessed visually by autoradiography of a silica gel TLC plate.

Synthesis of (^{125}I)IACoc. The (^{125}I)iodoaminococaine (1.2 mCi) in ethyl acetate was evaporated under an argon stream to dryness. To this was immediately added 50 μl of 3% (wt/wt) H_2SO_4, and the solution was cooled to 4°C. Sodium nitrite (10 μl of a 1 M solution, 4°C) was added and allowed to react in the dark at 4°C for 30 min. Sodium azide (50 μl of a 1 M solution, 4°C) was then added, and after 30 min in the dark, the reaction was stopped by adding 0.5 ml of aqueous 0.5 M $NaHCO_3$. The reaction mixture was extracted three times with 0.6 ml of ethyl acetate. The extracts were pooled and back extracted once with 0.5 ml of water to yield a single product on TLC (toluene/diethylamine, 20:1; R_f = 0.45), yielding (^{125}I)IACoc (1.0 mCi). (^{125}I)IACoc was at least 99% radiopure and cochromatographed with authentic nonradioactive IACoc on silica gel TLC.

Tissue Membrane Preparation. Crude rat whole brain membranes were prepared according to Calligaro and Eldefrawi (4), and rat liver microsomal membranes were prepared according to El-Maghrabi *et al.* (27). Both membranes were prepared in the presence of protease inhibitors (leupeptin at 20 μg/ml, soybean trypsin inhibitor at 5 μg/ml, 100 μM benzamidine, 100 μM phenylmethylsulfonyl fluoride, and 1 mM Na_2EDTA). Human placental membranes were prepared from freshly obtained human placenta according to the method of Evans *et al.* (28).

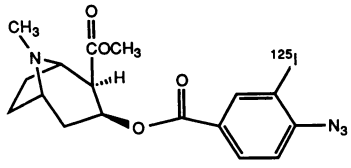
Association and Dissociation of (^{125}I)IACoc from Rat Liver Microsomes. Association and dissociation of (^{125}I)IACoc from rat liver microsomes were determined by rapid filtration using Whatman GF/B filters soaked with 0.05% polyethylenimine/10 μM cocaine/1 μM IACoc. Association of (^{125}I)IACoc was determined at 4°C by adding 50 μl of rat liver microsomes (1.0 mg/ml) to 4.95 ml of incubation buffer (10 mM sodium phosphate/120 mM NaCl/5 mM KCl/0.16 mM sucrose, pH 7.4) to a final protein concentration of 0.01 mg/ml and a final (^{125}I)IACoc concentration of 0.9 nM. Aliquots of this incubation mixture (50 μl, 0.5 μg of protein) were removed at various times (20 sec to 5 hr), diluted with 5 ml of ice-cold incubation buffer, and immediately filtered and washed twice with 5 ml of ice-cold incubation buffer. Nonspecific binding was defined by 1 μM IACoc in a parallel association experiment.

After steady-state (^{125}I)IACoc binding had been achieved (133 min), 1 ml of the association assay mixture was diluted to a final volume of 100 ml with ice-cold incubation buffer containing 1 μM IACoc. Aliquots (5 ml, 0.5 μg of protein) were filtered directly and washed twice with 5 ml of ice-cold incubation buffer. Filters were dried and radioactivity was determined by using a Packard γ counter. The observed association rate constants (k_{obs}) and the dissociation rate constants (k_{-1}) were determined for the association and dissociation experiments, respectively, by using the kinetic program of LIGAND (29). The association rate constants (k_1) were determined according to the equation $k_{obs} = k_1 \times [\text{drug}] + k_{-1}$, using an (^{125}I)IACoc concentration of 0.9 nM. The equilibrium dissociation constants were determined by the equation $K_d = k_{-1}/k_1$.

General Photolabeling Protocol. Rat whole brain and human placental membranes were suspended to a final volume of 0.1 ml in an incubation buffer (except where indicated) of 10 mM sodium phosphate/120 mM NaCl/5 mM KCl/0.16 mM sucrose, pH 7.4, at a protein concentration of 1.0 mg/ml in the presence or absence of protecting drugs. After this mixture was incubated with gentle shaking for 60 min at 20°C, (^{125}I)IACoc (final concentration 0.5–1.0 nM) was added from ethyl acetate (1% ethyl acetate final concentration) and the incubation was continued for 15 min at 20°C. Membrane suspensions were then placed on ice for 1 min and diluted with 5 ml of ice-cold incubation buffer for 10 min prior to photolysis. Rat liver microsomal membranes were incubated similarly but were kept on ice (without shaking) throughout the incubations to prevent metabolism of (^{125}I)IACoc or protecting drugs. Photolysis was performed for 5 sec with a high-pressure AH-6 mercury lamp (30), followed by the addition of 50 μl of 2-mercaptoethanol to scavenge long-lived photoproducts. Proteins were separated by SDS/PAGE (31) and exposed to X-Omat AR film at −80°C.

RESULTS

Structure of Cocaine and (^{125}I)IACoc. The chemical structure of (^{125}I)IACoc is shown below.



Carrier-free (^{125}I)IACoc was prepared as described in *Methods* in high yields at a specific activity of 2200 Ci/mmol. (^{125}I)IACoc makes use of the effectiveness of iodophenyl azides to identify and characterize receptors (32, 33) by incorporating an iodophenyl azide moiety to replace the phenyl ring of cocaine.

Association and Dissociation of (^{125}I)IACoc from Rat Liver Microsomes. The equilibrium dissociation constants (K_d) of (^{125}I)IACoc binding to liver microsomal membrane were determined from the association and dissociation rate constants. Association of (^{125}I)IACoc was rapid (Fig. 1) and computer fit to one site with a k_{obs} of 0.29 min^{-1}. Nonspecific binding, as defined by 1 μM nonradioactive IACoc, was less than 10% of total (^{125}I)IACoc binding at equilibrium and relatively constant throughout the experiment. The total (^{125}I)IACoc bound was less than 10% of the free (^{125}I)IACoc in solution. The dissociation of (^{125}I)IACoc was computer analyzed to two separate sites ($\alpha < 0.01$) with high- and low-affinity dissociation rate constants (k_{-1}) of 0.0058 and 0.034 min^{-1}. From these dissociation rate constants and the association k_{obs} were defined two association rate constants (k_1) of 3.04 $\times$ 10^8 M^{-1}·min^{-1} and 2.72 $\times$ 10^8 M^{-1}·min^{-1}, respectively. The corresponding K_d values of 19 and 126 pM were derived, representing 42% and 58% of specific (^{125}I)IACoc binding, respectively, at 0.9 nM.

Photolabeling of Rat Liver Microsomal Membranes and Whole Brain Synaptosomes with (^{125}I)IACoc. As demonstrated in Fig. 2, (^{125}I)IACoc specifically labeled a cocaine-sensitive 26-kDa protein in rat whole brain and liver microsomal membrane preparations. A minor 19-kDa cocaine-sensitive protein in liver membranes was also photolabeled (data not shown). On immunoblots neither of these liver proteins was detected by antibodies recognizing cytochrome P-450$_b$, P-450$_c$, or P-450$_{f-k}$ (data not shown).

(^{125}I)IACoc Labels the Haloperidol-Sensitive Sigma Receptor. As shown in Fig. 3*A*, a significant inhibition of (^{125}I)IACoc labeling of the rat whole brain 26-kDa protein was seen

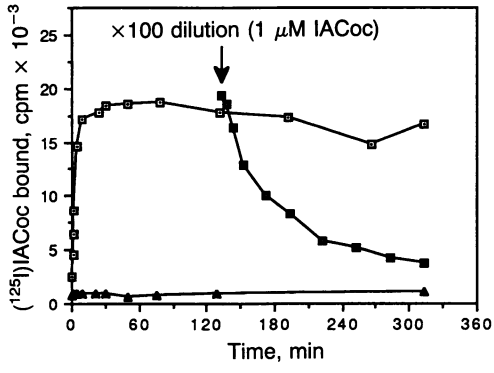


FIG. 1. Association and dissociation of (^{125}I)IACoc from rat liver microsomes. Association (□) of (^{125}I)IACoc to microsomes was determined by rapid filtration. Dissociation (■) of (^{125}I)IACoc was determined by a 100-fold dilution with 1 μM IACoc and collection of samples by rapid filtration. Association in the presence of 1 μM IACoc is shown by ▲.

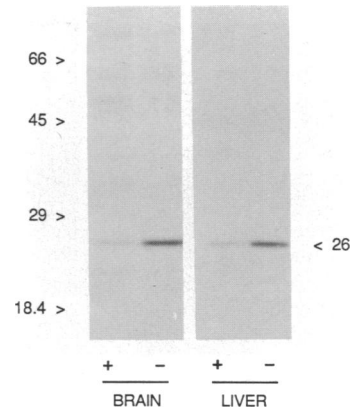


FIG. 2. Autoradiogram of cocaine-inhibitable (^{125}I)IACoc labeling of rat whole brain and rat liver microsomal proteins separated by SDS/PAGE. Incubations were performed in the presence (+) or absence (−) of 50 μM (−)-cocaine. The incubation buffer in this experiment consisted of 10 mM $NaHPO_4$ and 0.32 M sucrose with a final (^{125}I)IACoc concentration of 1.0 nM. Positions of marker proteins are shown on the left in kDa.

with the sigma ligands, (+)-3-PPP, (−)-3-PPP, and DTG, at 1.0 and 0.1 μM, while similar concentrations of imipramine were less potent. Stereospecific inhibition of (^{125}I)IACoc photolabeling was seen, with (+)-3-PPP being more effective than (−)-3-PPP. Two additional sigma-binding ligands, dextromethorphan and carbetapentane, also inhibited labeling (Fig. 3*B*). Fig. 3*C* demonstrates the labeling of this polypeptide in human placental, rat microsomal, and rat whole brain membranes with (^{125}I)IACoc. Labeling of the 26-kDa protein in these tissues was inhibited by 1 μM (+)-3-PPP, DTG, and haloperidol.

Inhibition of (^{125}I)IACoc Photolabeling of the Sigma Receptor in Membranes from Whole Rat Brain with Various Biogenic Amine Uptake Inhibitors. Crude whole rat brain membranes were photoaffinity labeled with (^{125}I)IACoc in the presence or absence of a number of amine uptake inhibitors at 10 μM under conditions that minimized nonspecific labeling. The Coomassie blue staining profile is shown in Fig. 4*A*, while the autoradiogram obtained from that gel is shown in Fig. 4*B*. The

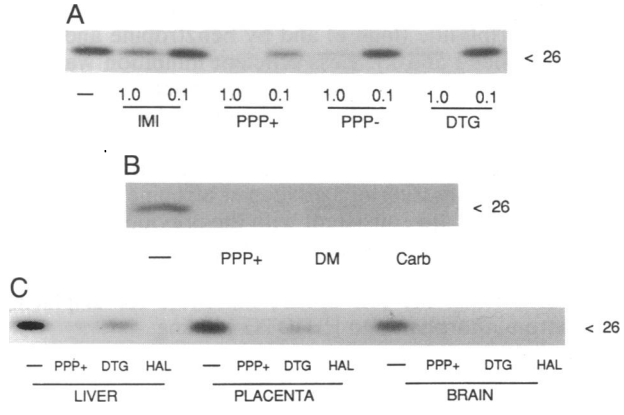


FIG. 3. Autoradiograms of the 26-kDa polypeptide photolabeled by (^{125}I)IACoc. Membrane suspensions were incubated and photolyzed, using 0.5 nM (^{125}I)IACoc in the presence or absence of inhibitors. In all cases, (−) indicates control photolabeling without inhibiting compounds. (*A*) Photolabeling of rat whole brain membrane proteins was inhibited by imipramine (IMI), (+)-3-PPP, (−)-3-PPP, and DTG at 1.0 and 0.1 μM. (*B*) Dextromethorphan (DM) and carbetapentane (Carb) also inhibited labeling at 1.0 μM. (*C*) (^{125}I)IACoc photolabeling of rat whole brain, rat liver, and human placental membrane proteins was inhibited by the sigma ligands, (+)-3-PPP, DTG, and haloperidol (HAL), at 1 μM.

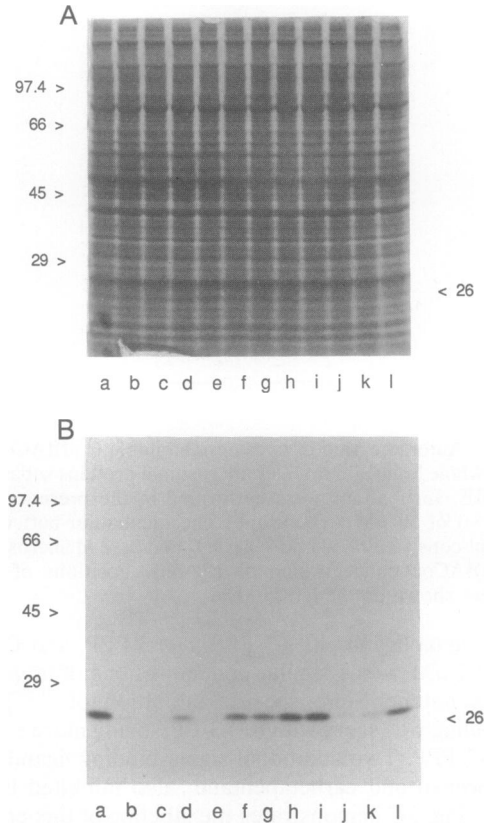


FIG. 4. Inhibition of (^{125}I)IACoc photolabeling of rat whole brain membrane proteins by various uptake inhibitors at 10 μM. (*A*) Coomassie blue staining of an SDS/9% polyacrylamide gel of rat whole brain membrane proteins photolabeled by (^{125}I)IACoc (0.5 nM) in the presence and absence of inhibitors. (*B*) The corresponding autoradiogram was obtained from the gel shown in *A*. Inhibitors were as follows: lane a, control without inhibitor; lane b, fluoxetine; lane c, imipramine; lane d, desipramine; lane 3, amitriptyline; lane f, nortriptyline; lane g, mazindol; lane h, nomifensine; lane i, atropine; lane j, benztropine; lane k, tetrabenazine; lane l, (−)-cocaine.

highly specific nature of (^{125}I)IACoc labeling is demonstrated, since only the 26-kDa protein was appreciably labeled. (^{125}I)IACoc labeling was completely inhibited by the 5HT uptake inhibitors fluoxetine (lane b), imipramine (lane c), and amitriptyline (lane e) and by benztropine and tetrabenazine (lanes j and k). Less efficient inhibition was seen with the NE uptake inhibitors desipramine (lane d) and nortriptyline (lane f), with the dopamine/norepinephrine uptake inhibitors mazindol (lane g) and nomifensine (lane h), and with cocaine (lane l). No protection was observed when the nonselective muscarinic antagonist atropine (lane i) was used. These data are consistent with the previously reported relative affinities of monoamine uptake inhibitors for the sigma receptor (19, 34), with the 5HT uptake inhibitors being more potent than the NE uptake inhibitors for displacing [^{3}H]dextromethorphan and [^{3}H]DTG binding.

DISCUSSION

To characterize cocaine-binding proteins, we have synthesized a photoaffinity probe, (^{125}I)IACoc. This compound was used to selectively label a 26-kDa protein that has the pharmacology of a sigma receptor in membranes derived from rat brain, rat liver, and human placenta. (^{125}I)IACoc labeling of the 26-kDa polypeptide from rat whole brain and rat liver microsomal membranes was inhibited by (−)-cocaine (Fig. 2). Labeling of this protein from rat whole brain membranes was also potently inhibited by a number of sigma ligands, including haloperidol, 3-PPP [(+)-3-PPP > (−)-3-PPP], DTG, dextromethorphan, and carbetapentane (Fig. 3). When the sigma photolabel azido-[^{3}H]DTG was used, a similarly sized haloperidol-sensitive protein was photolabeled in guinea pig brain and liver membranes (21, 22). The fact that (^{125}I)IACoc labeled a 26-kDa sigma receptor from a number of different tissues, whether they were prepared in the presence or absence of protease inhibitors (data not shown), suggests that the sigma receptor is not merely a proteolysis product of another protein.

Several investigators have shown that cocaine binds with low affinity to sigma receptors (6, 19, 35). Substitution of the benzoyl group of cocaine by the iodoazidobenzoyl moiety produced a ligand, (^{125}I)IACoc, with significantly increased affinity for this sigma receptor. This is consistent with the high affinity of a number of aryl-substituted 4-phenylpiperidine compounds (i.e., haloperidol) for sigma receptors. While only a 26-kDa protein was labeled at 1.0 nM (^{125}I)IACoc (Fig. 2), two sites were determined by dissociation kinetics measured at a similar (^{125}I)IACoc concentration (Fig. 1). This is similar to the biphasic dissociation obtained from both dextromethorphan-sensitive and -insensitive [^{3}H]DTG binding sites in guinea pig brain (35). The explanation for the apparent existence of multiple (^{125}I)IACoc- and [^{3}H]DTG-binding sites will require a better understanding of the biochemical function and molecular structure of the sigma receptors.

In addition to having putative antipsychotic activities, sigma receptor binding has been suggested to mediate effects on the immune system (36, 37), though the mechanisms that might mediate such responses are not clear. Recent studies have also associated the sigma receptor with monoamine oxidase type A, on the basis of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) binding (38), or the cytochrome P-450IId (debrisoquine hydroxylase), on the basis of SKF 525a and substrate binding (39, 40). The subcellular localization of sigma binding sites in rat brain [correlated with microsomal fractions and the membrane marker 5-nucleotidase (41)] is consistent with an association with either of these enzymes, as is the reported inhibition of debrisoquine hydroxylase activity by MPTP and its analogs (42). The low molecular weight of the sigma receptor in comparison with monoamine oxidase type A (43) or the cytochrome P-450IId (44) (both >45 kDa) argues against the identity of the sigma receptor with either of these enzymes, however.

A number of monoamine uptake inhibitors, including fluoxetine, imipramine, and amytriptyline (at 10 μM), also inhibited photolabeling of the 26-kDa protein by (^{125}I)IACoc (Fig. 4). Although a similarly sized (30-kDa) protein was specifically labeled by Wennogle *et al.* (45) in brain, platelet, and liver membranes by using the photolabel [^{3}H]nitroimipramine, we do not believe that the (^{125}I)IACoc-photolabeled 26-kDa polypeptide shown in these experiments is the 5HT transporter. The 5HT uptake blockers were consistently less potent than the sigma ligands at inhibiting (^{125}I)IACoc labeling of the 26-kDa protein (Fig. 2). In addition, preliminary studies of [^{3}H]5HT uptake into human platelets have shown that nonradioactive IACoc is less potent than cocaine at blocking [^{3}H]5HT uptake (data not shown). Given the unusually high density of the 30-kDa protein in liver membranes photolabeled by [^{3}H]nitroimipramine and the fact that Wennogle *et al.* showed that haloperidol also potently inhibited labeling, it is possible that [^{3}H]nitroimipramine labeled the sigma receptor rather than the 5HT transporter.

Since cocaine has been shown to bind stereospecifically and with high affinity (K_d = 2.3 nM) to a protein that is present in large amounts (88 pmol/mg of protein) in rat liver microsomal membranes (27) and since a large amount of high-affinity sigma ligand binding exists in rat liver as well (46), we assessed the sigma ligand inhibition of [^{3}H]cocaine binding to liver microsomes. While sigma ligands inhibited with moderate affinity and with stereospecificity [(+)-3-PPP

> (−)-3-PPP; (+)-cyclazocine > (−)-cyclazocine; (+)-SKF 10,047 > (−)-SKF 10,047) (data not shown)], the relative affinities of these sigma ligands for inhibiting [^{3}H]cocaine binding were low when compared with the affinities of IACoc, SKF 525a, and the 5HT uptake inhibitors. Thus, while displaying pharmacological characteristics of the rat liver sigma binding site, the high-affinity liver cocaine binding site appears to be different from the site defined by high-affinity rat liver [^{3}H]SKF 10047 binding. Still, the reported association of sigma sites with the cytochrome P-450IId/debrisoquine hydroxylase (39, 40) and the potent inhibition of debrisoquine hydroxylase activity by (−)-cocaine (47) suggest a connection between cocaine and sigma binding sites that is worthy of further investigation.

In summary, we have synthesized (^{125}I)IACoc to probe cocaine-binding proteins. This compound selectively labels a 26-kDa haloperidol-sensitive sigma receptor in membranes prepared from rat brain, rat liver, and human placenta. While the binding of cocaine to sigma receptors has been suggested to mediate the psychotomimetic properties of cocaine, this remains to be demonstrated. IACoc will be useful in the biochemical and functional characterization of the sigma receptor and other cocaine-binding proteins.

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