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CHARACTERIZATION OF SOLUBILIZED ISOQUINOLINE BINDING SITES FROM RAT INTESTINE USING 6,7-DIMETHOXY 4-(4′-AMINO, 3′(^{125}I)IODOBENZYL)ISOQUINOLINE

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Abstract—In rat tissues, the specific binding of 6,7-dimethoxy 4-(4′-amino, 3′(^{125}I)iodobenzyl)-isoquinoline is distributed as follows: aorta > pancreas > liver > intestine > stomach > lung > skeletal muscle > heart > brain. In aorta and intestine ^{125}I-DMABI is specifically covalently incorporated after direct u.v. photolabeling, in a major polypeptide of Mr 36,000 daltons, and a minor polypeptide of Mr 52,000 daltons. In intestine another smaller minor polypeptide of Mr 26,000 is observed. In intestine a variety of isoquinolines are tested for their ability to inhibit the covalent photo-incorporation of ^{125}I-DMABI. Inhibitory potency is influenced by 6,7-substitutions, e.g. 6,7-dimethoxy, and by the presence of benzyl ring in C-1 and C-4 positions. Isoquinoline is much more potent than tetrahydroisoquinoline. ^{125}I-DMABI intestinal binding site is solubilized using Triton X-100. Layered on Sephadex G-25 column, a high specific peak of radioactivity is eluted in the void volume of the column. The ^{125}I-DMABI binding protein loaded onto a Sephacryl S-300 column is eluted as a single peak corresponding to a species with a Stokes radius of 43.5 Å. The sedimentation coefficient of the ^{125}I-DMABI binding protein is measured by ultracentifugation of 5.5 S, using 5–20% sucrose gradient. The calculated molecular weight of the intestinal ^{125}I-DMABI binding protein is estimated at 110,000 daltons.

The opium alkaloïde papaverine [1–5] and other 2 and 4-benzyl isoquinolines [6–8] relax smooth muscle in several mammalian tissues, e.g. aorta, duodenum and ileum. Using one of these isoquinoline derivatives: 6,7-dimethoxy 4-(4′-chloro, ^{14}C benzyl)-isoquinoline, we have shown *in vivo* that the binding sites of this probe are specifically distributed in rat cerebral vessels, aorta and intestine [9]. This distribution specifics of tissues which contains smooth muscle agree well with the observation of Kimura *et al.* [10], which shows an uptake of ^{3}H-papaverine in protein plasma-membrane of hog biliary smooth muscle. More recently, for further insight into the intestinal membranous isoquinoline site of action, we have developed a high specific affinity probe: 6,7-dimethoxy 4-(4′-amino, 3′(^{125}I)iodobenzyl)isoquinoline (^{125}I-DMABI) [11]. Using this probe we have identified in rat intestinal membranes the isoquinoline binding sites. Elucidation of the structure and molecular properties of drug receptors has become an important tool in unraveling the mechanism of drug action at molecular and biochemical levels. During the last decade, two approaches to this problem have allowed major advances in the elucidation of intrinsic membrane protein structures. One is the affinity or photoaffinity labeling of the receptor and the other is the biochemical purification of the protein binding site. The two techniques are complementary and together provide the potential of gaining information about the molecular properties of the receptor macromolecules. Purification of the isoquinoline binding protein from solubilized rat intestinal membranes is an important step toward understanding of the molecular aspects of the structure and mechanism of this action site.

We report in the present study the tissular distribution of ^{125}I-DMABI binding in the rat tissues, the identification of the molecular size of ^{125}I-DMABI binding proteins in smooth muscle tissues using direct u.v. photolabeling method, and the hydrodynamic properties of the rat intestinal membranous isoquinoline binding protein, solubilized using the non-denaturing detergent Triton X-100.

MATERIALS AND METHODS

Sprague–Dawley rats were obtained from Charles River. Carrier free Na^{125}I (IMS 300, 14.7 mCi/μg) was purchased from Amersham. DMABI was iodinated by the Chloramine T method and purified by LH 20 chromatography as described [11]. The specific activity of ^{125}I-DMABI was 2175 Ci/mmol. Sephadex G-25 fine and Sephacryl S-300 were from Pharmacia. Triton X-100 and protease inhibitors were from Sigma. Standard proteins from gel filtration and sucrose density sedimentation were from Sigma and Pharmacia. Chemicals and unstained protein markers for SDS–gel electrophoresis were from Bethesda Research Laboratories. Papaverine, isoquinoline and 6,7-dimethoxy 1,2,3,4 tetrahydroisoquinoline were from Aldrich. PK 11195 was a generous gift from Dr. G. Le Fur (Pharmuka Laboratories, France).

Preparation of tissular membranes

Male Charles River rats (body wt 200–300 g) were killed by decapitation and the liver, heart, pancreas,

lung, stomach, brain, skeletal muscle and intestine of each animal were removed rapidly and washed with ice-cold buffer (Tris–HCl 50 mM, $MgCl_2$ 5 mM, NaCl 100 mM, EDTA 1 mM and protease inhibitors, pH 7.5). The tissues were minced and homogenized with Polytron in the same buffer. The homogenate was filtered through four layers of cheese cloth. The tissue homogenate was washed five times by centrifugation at 35,000 *g* for 10 min at 4° (Sorvall RC5-B, rotor SS-34) followed by resuspension of the pellet in fresh buffer. The final pellet was resuspended to a concentration of 100 mf original wet tissue weight/ml buffer and frozen −80° until use. The intestinal epithelial cells are isolated with EGTA washing of the reversed intestine [12] and sedimentation of the extracted cells. The remaining intestine is considered as crude muscular preparation.

Protease inhibitors

In order to avoid proteolytic degradation of the isoquinoline binding proteins a mixture of protease inhibitors was added to each buffer used both for membrane preparation and in purification steps of the solubilized binding proteins. These protease inhibitors were bacitracine (100 μg/ml), tosyl-L-lysine chloromethyl ketone (10 μg/ml), phenylmethylsulfonyl fluoride (100 μM), leupeptine (10 μg/ml) and EGTA (1 mM). These concentrations of protease inhibitors did not interfere with the binding of ^{125}I-DMABI.

Radioligand binding assays.

Prior to assay, the crude tissular membranes were thawed and diluted 10-fold in buffer identical to membrane preparation without EGTA, and washed twice. The assays were performed as described: 60 min, 30°, pH 7.5, 0.75 nM ^{125}I-DMABI [11]. Specific binding was defined as that displaced by 100 μM of unlabeled DMABI. Each measurement was performed in triplicate in three or four experiments. Measurement of proteins was determined by the method of Bradford [13] using serum albumin as standard.

Photoaffinity labeling

Crude aorta and intestinal membranes (600 μg of proteins) are incubated in the presence of 5–10 nM of ^{125}I-DMABI with or without competitor agents. At equilibrium (60 min) the preparations were washed with a large excess of Tris-buffer. After centrifugation at 35,000 *g* the pellets resuspended in Tris-buffer were irradiated for 15 min in an ice-bath with a u.v. lamp (R-52G, UVP Inc.) at a distance of about 5 cm. Prior to application to acrylamide gel electrophoresis, the cross-linked samples were solubilized in Tris–HCl buffer (60 mM) pH 6.8 containing 10% (wt/vol.) glycerol, 0.001% (wt/vol.) bromophenol blue, and 3% (wt/vol.) sodium dodecyl sulfate; and boiled for 30 min. Each solubilized sample was electrophorized with 5–20% SDS polyacrylamide gel by the method of Laemmli [14]. After electrophoresis (16 hr), the gels were dried and exposed for 8–15 days at −80° to a Trimax type film (3M Co.) with a Trimax intensifying screen. Molecular weight standards are alpha-chymotrypsinogen (25.7 KD), ovalbumin (43 KD), bovine serum albumin (68 KD), phosphorylase B (92.2 KD) and myosin (200 KD).

Solubilization

Intestinal membranes (1 mg of protein in 2 ml) were incubated at 30° for 60 min in Tris–HCl buffer previously described, in the presence of 1.40 nM of ^{125}I-DMABI. Specific ^{125}I-DMABI binding was defined as that displaced by 100 μM unlabeled DMABI. At the end of incubation, the labeled membranes were washed with 20 ml ice-cold Tris–HCl buffer containing 0.5% bovine serum albumin. The labeled membranes were removed by centrifugation for 10 min at 40,000 *g*, and resuspended in Tris–HCl buffer containing 1% (v/v) Triton X-100. The suspension is placed for 15 min in ice and the solubilized material is separated from the unsolubilized by centrifugation for 30 min at 40,000 *g*. The usual centrifugation for solubilization procedure, e.g. 100,000 *g*, gives the same results. Whatever the conditions of solubilization, 75% of proteins were solubilized and 90% of the radioactivity bound to the membranes was recovered in the supernatant.

Gel filtration

Sephadex G-25. Free and bound ^{125}I-DMABI in the soluble extract were further separated by rapid gel filtration at 4° on Sephadex G-25 fine. A Sephadex G-25 fine column (30 × 0.9 cm) was equilibrated and eluted (60 ml/hr) with Tris–HCl buffer containing 0.1% of Triton X-100. An aliquot of each fraction (0.7 ml) was counted in a Packard Autogamma counter with 61% efficiency. Blue dextran 2000 and $K_3Fe(SCN)_6$ are used to determine the void volume (V_o) and the total volume (V_t) of the column, respectively.

Sephacryl S-300. A Sephacryl S-300 column (50 × 1 cm) was equilibrated with Tris–HCl buffer containing 0.1% Triton X-100 and eluted with the same buffer at 4° (30 ml/hr). An aliquot of the peak eluted in the void volume of the G-25 column (2 ml) was layered onto the Sephacryl S-300 column. Fractions (1 ml) were counted in an autogamma counter. The column was calibrated using the following market proteins: catalase (Stokes radius, rs = 52 Å), gamma globulin (rs = 51 Å), bovine serum albumin (rs = 35.5 Å), ovalbumin (rs = 30.5 Å) and cytochrome *c* (rs = 16 Å). The elution of ^{125}I-DMABI–protein complexes and marker proteins was expressed according to Laurent and Killander [15] in terms of $K_{av} = (V_e - V_t/V_t - V_o)$ where V_e is the elution volume of the protein considered.

Sucrose density centrifugation. Linear gradients (4 ml) were prepared from 5% and 20% sucrose made up in H_2O containing 0.1% Triton X-100 and buffered at pH 7.5 with 50 mM Tris–HCl. Samples of peak eluted by gel filtration on Sephacryl S-300 (0.2 ml) were applied to the tops of gradients and were centrifuged at 4° in a Beckman SW 60 rotor for 16 hr at 43,000 rpm. After centrifugation, gradients were eluted from the top with an Auto-densiflow II C gradient fractionator (Searle). Twenty-three fractions (180 μl) were collected. The sucrose gradient was calibrated using the following marker proteins: catalase (sedimentation coefficient $S_{20,w}$ =

Table 1. Tissue distribution of ^{125}I-DMABI binding in rat tissular membranes

Tissue	Specific binding (fmol/mg of protein)
Aorta	1112 ± 104
Pancreas	277 ± 20
Liver	209 ± 6
Intestine	134 ± 6
Stomach	88 ± 2
Lung	65 ± 2
Skeletal muscle	34 ± 2
Heart	32 ± 3
Brain	6 ± 1

Membranes are essayed for ^{125}I-DMABI binding at 40–100 μg of protein in total vol.) of 0.2 ml of Tris–HCl buffer (pH 7.5, 60 min at 30°) with 0.4 μCi/ml ^{125}I-DMABI in the absence and presence of 100 μM unlabeled DMABI to determine total and non-specific binding, respectively. Specific binding is defined as the difference between total anl non-specific binding. Results are means of at least three or four independent determinations.

11.4 S), bovine gamma globulin ($S_{20,w}$ = 7.2 S), bovine serum albumin ($S_{20,w}$ = 4.6 S), and cytochrome *c* ($S_{20,w}$ = 1.71 S). The molecular weight was calculated according to the following equation:

$$M_r = \frac{6\pi N \eta_{20,w}}{(1 - v\rho_{20,w})} \cdot a \cdot S_{20,w}$$

where N is Avogadro's number, $\eta_{20,w}$ the viscosity of water at 20° (0.01002 g/cm × sec) and $\rho_{20,w}$ the density of water at 20° (0.99823 g/ml).

RESULTS

Tissues distribution

Table 1 shows the rat tissular distribution of ^{125}I-DMABI binding. Specific binding defined as that displaced by 100 μM of unlabeled DMABI is high in aorta (96% of total binding), lung (95%), pancreas (80%), stomach (81%), intestine (80%) and liver (79%). Intestinal distribution of ^{125}I-DMABI binding is higher in intestinal muscle than intestinal epithelium (4/1) (not shown). Other tissues, e.g. skeletal muscle, heart and brain, have low levels of specific binding. Skeletal muscle and heart contain 50% of specific binding and the brain is the only one tissue that presents higher non-specific binding than specific.

Molecular size and pharmacological specificity

For further characterizing the isoquinoline binding site in smooth muscle tissues, we have utilized the cross-linking method to identify receptor–ligand complexes by direct u.v. irradiation method. Figure 1 shows ultraviolet absorption spectra for DMABI following increasing times of exposure to u.v. light. The control, which represents the absorption spectrum of DMABI prior to photolysis, shows a strong absorption maximum at 235 nm. Following increasing time of exposure to short wavelength u.v. light, there is a progressive decrease in the absorption at 235 nm. This decrease reflects molecular changes of DMABI. By contrast, the most well investigated isoquinoline, papaverine, shows no changes of this spectrum after 60 min of u.v. exposure.

When rat aorta and intestinal membranes are incubated with ^{125}I-DMABI, photolysed and subjected to SDS–PAGE, one major protein band of Mr 36,000 daltons (aorta and intestine) and two minor protein bands of Mrs 26,000 (intestine) and 52,000 (aorta and intestine) are labeled (Fig. 2). The evidence of specific labeling is demonstrated by the ability of unlabeled DMABI (100 μM) to inhibit ^{125}I-DMABI covalent photoincorporation. In order to examine the pharmacological specificity of the peptide labeling, intestinal membrane are incubated with ^{125}I-DMABI in the presence of isoquinolines structurally related for DMABI (Fig. 3). Isoquinoline (lane 1), 6.7-dimethoxy 1,2,3,4-tetrahydroisoquinoline (lane 3), 1-(2-chlorophenyl-*N*-methyl-*N*-(1-methyl,2-propenyl)-3isoquinoline carboxamide (lane 4) do not affect the labeling. 6,7-Dimethoxyisoquinoline (lane 2), papaverine (lane 5) and 6,7-dimethoxy-4-(3′,4′-dimethoxybenzyl) isoquinoline (lane 7) partially prevent the labeling. 6,7-Dimethoxy-4-(4′-chlorobenzyl) isoquinoline (PV 2) (lane 6) prevent all the labeling of the three protein bands.

Hydrodynamic properties of the solubilized intestinal isoquinoline binding site

In the present study we have demonstrated that the best source of isoquinoline binding site is the aorta. However, the very small size of the rat aorta renders this tissue an unpractical source for studying the isoquinoline binding site. Therefore, we have used intestine for characterizing the structure of the DMABI binding site. Indeed this tissue is available in large amounts, possessess the same sensitivity as aorta to the relaxant effect of isoquinolines [7, 8], and contains a high concentration of ^{125}I-DMABI binding sites. Application of the Triton solubilized intestinal membranes prelabeled by ^{125}I-DMABI to a Sephadex G-25 column gave a peak of ^{125}I-DMABI protein complex which appeared in the void volume (Fig. 4). Another minor peak which is observed in the salt peak represents the dissociated ^{125}I-DMABI during the solubilization by Triton X-100. When binding of the tracer to membranes is performed in the presence of 100 μM of unlabeled DMABI, the elution profile of the detergent extract shows a dramatic decrease of the high peak of radioactivity. This probably indicates that the ^{125}I-DMABI–protein complex is present in the void volume of the Sephadex G-25 column.

The ^{125}I-DMABI–protein Triton complex eluted in the void volume of the Sephadex G-25 column is loaded onto a Sephacryl S-300 column. The elution profile of a typical run is shown in Fig. 5. The ^{125}I-DMABI binding protein is eluted as a major peak between the void volume and the salt peak, at equal distance from gammaglobulin and bovine serum albumin with a distribution coefficient (K_{av}) of 0.4. A small peak also appeared to be present in the salt peak. It is characterized as ^{125}I-DMABI dissociated during the chromatographic run. Excellent recovery of applied ^{125}I-DMABI–protein is obtained. A plot of Stokes radius versus the distribution coefficient for a series of marker proteins gave a straight line (Fig. 5, inset). In four experiments, the binding

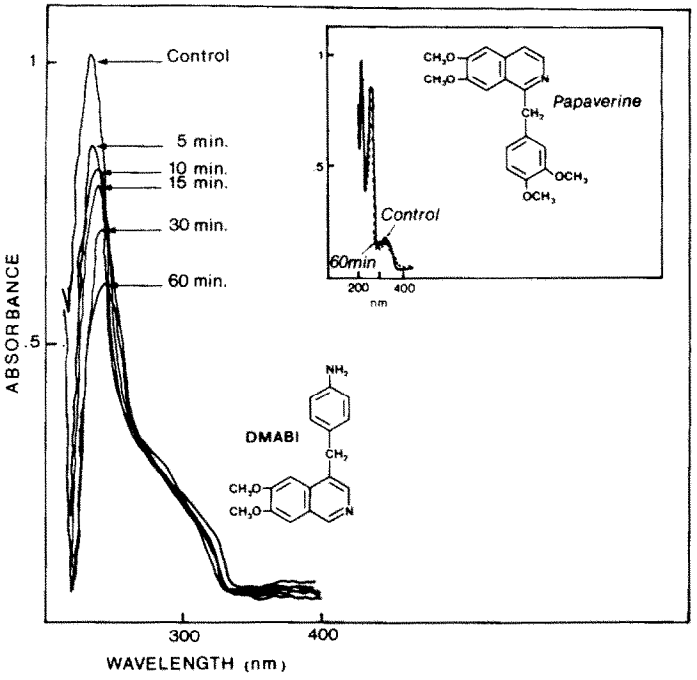

Fig. 1. Effect of photolysis on the u.v. absorption spectrum of DMABI. A 5 μM solution of DMABI was irradiated for various period of times with a R-52G UVP lamp, and the absorption spectra was recorded with a Cary 210 spectrophotometer. Inset: effect of 60 min of photolysis on the u.v. absorption spectrum of 5 μM papaverine

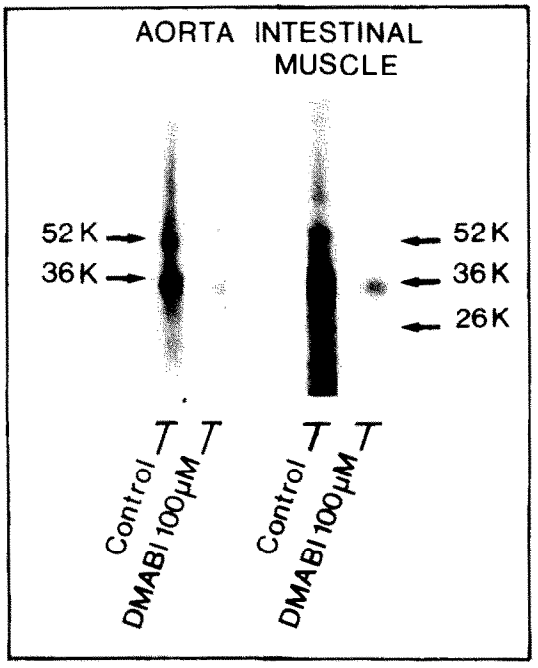

Fig. 2. Photoaffinity labeling of ^{125}I-DMABI into rat aorta and intestinal membranes. Membranes were incubated (60 min, 30°, pH 7.5) with ^{125}I- DMABI (5–10 nM) in the absence or presence (100 μM) of unlabeled DMABI, washed, photolysed and electrophorized on 5–20% slab gel as described under Methods 3.

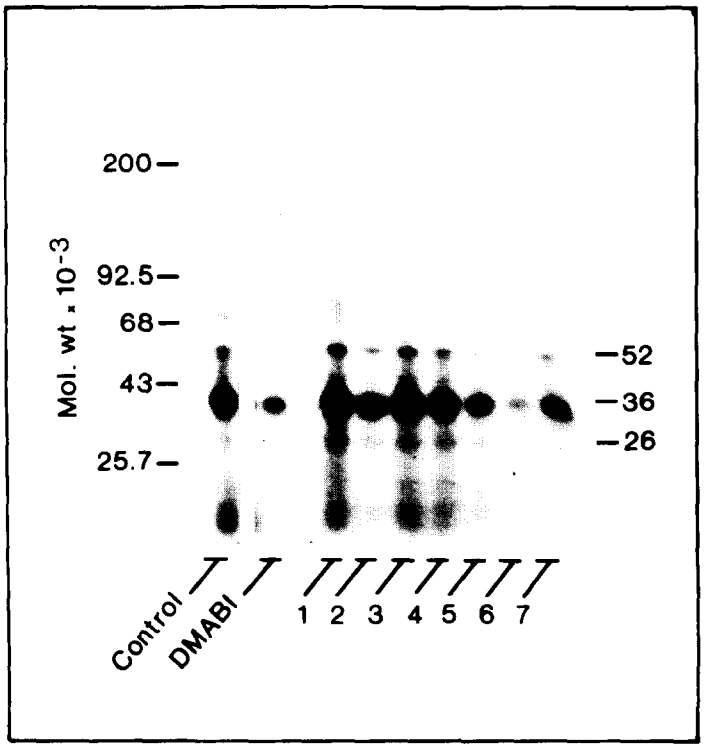

Fig. 3. Pharmacological specificity of the ^{125}I-DMABI rat intestinal binding sites. Membranes were incubated (60 min, 30°, pH 7.5) with ^{125}I-DMABI (5–10 nM) in the absence or presence of various isoquinoline derivatives. DMABI (10 μM); Isoquinoline (100 μM) lane 1; 6,7-dimethoxy isoquinoline (100 μM) lane 2; 6,7-dimethoxy 1,2,3,4 tetrahydrisoquinoline (100 μM) lane 3; PK 11195 (100 μM) lane 4; papaverine (10 μM) lane 5; 6,7-dimethoxy 4-(4′-chlorobenzyl) isoquinoline (10 μM) lane 6; 6,7-dimethoxy 4-(3′,4′-dimethoxybenzyl) isoquinoline (100 μM) lane 7.

protein peak eluted with the similar K_{av} corresponding to a Stokes radius of 43.5 Å.

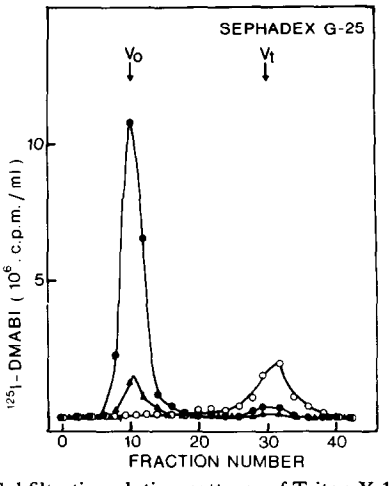

Fig. 4. Gel filtration elution patterns of Triton X-100 solubilized membranes prelabeled by ^{5}I-DMABI in presence (▲) or absence (●) of unlabeled DMABI (100 μM). Radioactive profile (○) represents the elution of free ^{125}I-DMABI from the column. Arrows indicate the elution peak of the calibration marker used: the void volume (V_0) and the total volume (V_t) of the column are determined by the elution of Blue dextran 2000 and $K_3Fe(SCN)_6$, respectively.

Estimation of the molecular weight of the ^{125}I-DMABI-binding protein is performed using a gradient of 5–20% sucrose. A sample peak obtained by gel filtration on Sephacryl S-300 loaded on the sucrose density gradient revealed a small peak of radioactivity after ultracentrifugation (Fig. 6). This peak appeared between bovine gammaglobulin (7.2 S) and bovine serum albumin (4.6 S). This peak is specific since when membranes are incubated in the presence of unlabeled DMABI (100 μM), the radioactivity of the ^{125}I-DMABI protein complex is dramatically decreased.

A plot of the sedimentation coefficients versus the gradient fractions for a series of marker proteins gave a straight line (Fig. 6, inset), and by comparison, the position of the radioactivity peak corresponds to a sedimentation coefficient ($S_{20,w}$) of 5.5 S. The fact that, using this technique, the peak of ^{125}I-DAMBI binding protein is relatively small is presumably due to the dissociation of the ligand from the protein binding site during the sedimentation procedure. Using Stokes radius, the sedimentation coefficient and the relevant equation, the calculated molecular weight of the entire DMABI–protein–Triton complex is 110,000 daltons.

DISCUSSION

The present paper describes the successful analysis of ^{125}I-DMABI–protein complex in denaturing and

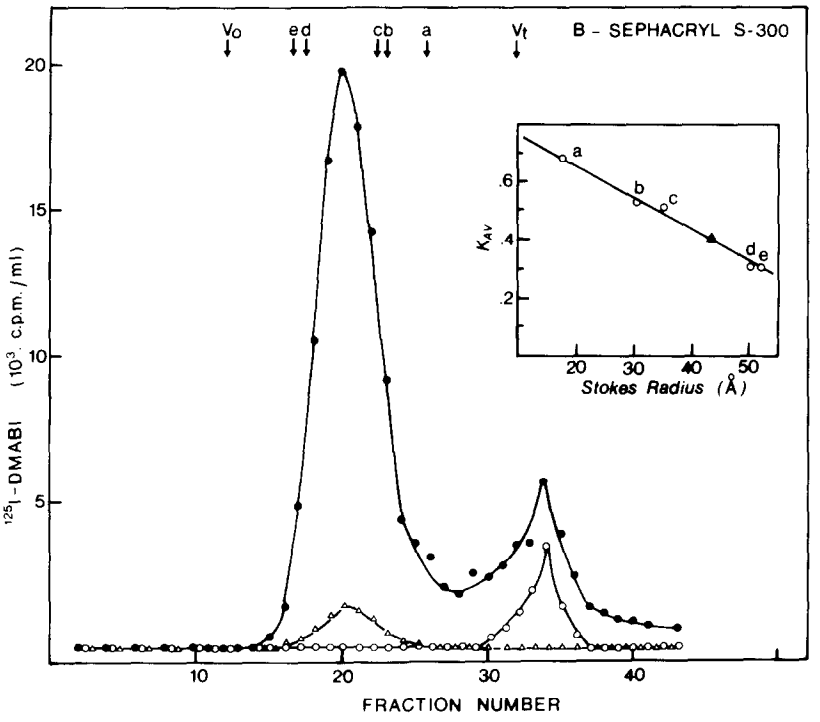

Fig. 5. Gel filtration chromatography of the ^{125}I-DMABI–protein complex. Two ml of the radioactive peak of G-25 chromatography are loaded in the Sephacryl S-300 column. Radioactive profiles represent the elution of purified ^{125}I-DMABI–protein complex from Triton X-100 solubilized intestinal membranes incubated in presence (△) or in absence (●) of unlabeled DMABI (100 μM), an (○) free ^{125}I-DMABI. Arrows indicate the elution position peaks of the marker proteins. Inset: estimation of the Stokes radius of the ^{125}I-DMABI binding protein (▲) determined by comparison with the known Stokes radii (○) of marker proteins plotted against their distribution coefficient K_{av} (a: cytochrome *c* 16 A; b: ovalbumin 30.5 A; c: bovine serumalbumin 35.5 A; d: gammaglobulin 51 A; e: catalase 52 A).

non-denaturing conditions. It is particularly interesting that the tissues in which isoquinolines exert their relaxant effect are those which contain high levels of specific ^{125}I-DMABI binding sites, i.e. aorta and intestine. Moreover, the fact that skeletal muscle contains a low level of specific binding sites is in agreement with the observation that isoquinolines derived from papaverine relax more efficiently smooth than skeletal muscle. Our data yield essential information on the structure of the ^{125}I-DMABI binding sites in smooth muscle tissues. The usefulness of direct u.v.irradiation to covalently cross-link ^{125}I-DMABI to its protein binding sites is consistent with previous results obtained with various ligands such as benzodiazepin, trimethisoquin, chlorpromazine, nitrendipine, bepridil and diltiazem (for review see ref. 16). Our results show that a major protein band of 36,000 daltons and a minor band of 52,000 daltons are specifically labeled in aorta and intestine. A smaller minor peptide of Mr 26,000 daltons is also observed in intestine. Our data also indicate that, in intestine, ^{125}I-DMABI binds to sites specific for papaverine derivatives. Indeed the absence of competition with isoquinoline and PK 11195 demonstrates the importance of 6- and 7-dimethoxy groups for the recognition of the binding sites. The unsaturation of the C-1 and N bond and the benzyl ring in C-1 or C-4 in the isoquinoline nucleus also play an important role in the interaction of isoquinoline derivatives with the binding sites. Similar results have been obtained in structure-activity studies in isoquinoline series by Hanna *et al.* [17] and Van Inwegen *et al.* [18] concerning their ability to inhibit cAMP phosphodiesterase. However, we have previously demonstrated that ^{125}I-DMABI does not bind to either the catalytic site of the membrane cAMP phosphodiesterase or receptors known to be involved in the relaxation process of smooth muscle, e.g. adrenergic, muscarinic and adenosine receptors, and calmodulin [19].

The solubilization of the ^{125}I-DMABI binding sites constitutes a useful point for their purification. Our strategy of solubilization turned toward the solubilization of pre-formed ^{125}I-DMABI receptor complex. Indeed, previous studies of various drugs and hormone receptors pointed out the ability of pre-formed ligand–receptor complex to survive solubilization, e.g. beta-adrenergic [20], dopamine [21], dihydropyridine [22, 23], prostaglandin [24], beta-endorphin [25] and CCK [26]. The present study shows the feasibility of solubilizing the ^{125}I-DMABI binding protein from rat intestinal membranes with

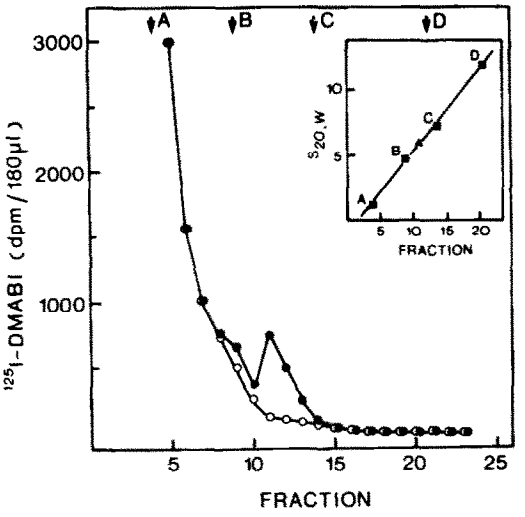

Fig. 6. Sucrose density gradient centrifugation of the ^{125}I-DMABI–protein complex. Sephacryl S-300 purified ^{125}I-DMABI binding protein was loaded onto 5–20% sucrose gradient and centrifuged 16 hr at 43,000 rpm in Beckman SW 60 rotor at 4°. Membranes were incubated in the absence (●) and presence (○) of unlabeled DMABI (100 µM). Inset: the elution profile of the ^{125}I-DMABI labeled protein (■) and marker proteins (Λ) are plotted against the gradient fractions. (a: cytochrome *c* 1.71 S; b: bovine serum albumin 4.6 S; c: bovine gamma globulin 7.2 S; d: catalase 11.4 S).

the non-denaturing detergent Triton X-100. Many lines of evidence favour this interpretation:

(i) soluble extract of membranes preincubated with ^{125}I-DMABI leads to the elution of a high peak radioactivity in the void volume of the Sephadex G-25 column, which is dramatically decreased when membranes are incubated in the presence of an excess of unlabeled DMABI;

(ii) the ^{125}I-DMABI protein complex analysed by gel permeation chromatography on Sephacryl S- 300 leads to elution of one single peak of radioactivity corresponding to a complex of Stokes radius of 43.5 Å.

(iii) sucrose density gradient centrifugation revealed a specific peak corresponding to a protein with a sedimentation coefficient of 5.5 S.

Using these data the molecular weight of the ^{125}I-DMABI binding protein is estimated at 110,000 daltons. Since ^{125}I-DMABI labeled, in denaturating conditions, one major polypeptide of Mr 36,000 daltons and two minor polypeptides of Mrs 52,000 and 26,000 daltons, after direct u.v.photolabeling, these results probably suggest that the intestinal membranous isoquinoline binding protein consist mainly in a protein of Mr 110,000 daltons composed of three subunits of 52,000, 36,000 and 26,000 daltons.

In regard to the high significant correlation which has been demonstrated for a series of 4-benzyl isoquinolines between inhibition constants of ^{125}I-DMABI binding and inhibition of contraction in rat intestine [19]; it is most likely that the isoquinoline membranous binding site which has been solubilized and characterized in this study is that involved in the relaxing effect of isoquinolines derived from papaverine.

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