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Purification and subunit structure of the [^{3}H]phenamil receptor associated with the renal apical Na^+ channel

(amiloride/kidney)

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ABSTRACT **Sodium crosses the apical membrane of tight epithelia through a sodium channel, which is inhibited by the diuretic amiloride and by analogs such as phenamil. Target size analysis indicated that the functional size of the [^{3}H]phenamil binding sites associated with the epithelial Na^+ channel from pig kidney is 90 ± 10 kDa. The [^{3}H]phenamil receptor was solubilized by using 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. The solubilized material displayed the same properties of interaction with amiloride and its derivatives as the membrane-bound receptor. A two-step purification of the epithelial Na^+ channel was achieved by using QAE Sephadex chromatography and affinity chromatography on a *Bandeiraea simplicifolia* lectin column. It results in an 1100-fold purification of the Na^+ channel as compared to pig kidney microsomes with a yield of 15% ± 5%. The maximal specific activity was 3.7 nmol/mg of protein. $NaDodSO_4$/polyacrylamide gel electrophoresis of the purified Na^+ channel under nonreducing conditions showed the presence of a single major polypeptide chain of apparent molecular mass 185 kDa. Under disulfide-reducing conditions, the purified epithelial Na^+ channel migrated as a single band of apparent molecular mass 105 kDa. It is suggested that the epithelial Na^+ channel from pig kidney has a total molecular mass of 185 kDa and consists of two nearly identical 90- to 105-kDa polypeptide chains crosslinked by disulfide bridges.**

The vectorial transport of Na^+ across the distal tubular epithelium of the kidney involves two distinct transport systems. Na^+ first enters kidney cells by way of conductive Na^+ channels that are localized at the apical membrane. It is then extruded at the basolateral membrane by the Na^+,K^+-ATPase.

The epithelial Na^+ channel is inhibited by amiloride, a well-known diuretic (1). The biophysical properties of this Na^+ channel have been extensively studied using electrophysiological techniques (2–4). However, its biochemical properties and the molecular mechanisms of its regulation by hormones such as aldosterone and vasopressin are still largely unknown (5, 6).

Amiloride-sensitive Na^+ channels can be titrated in epithelial membranes by using radiolabeled amiloride derivatives such as benzamil (7–9), methylbromoamiloride (10, 11), and phenamil (12, 13). Phenamil is more potent than benzamil or methylbromoamiloride for inhibiting epithelial Na^+ channels in frog skin (14) and in pig kidney microsomes (12). In a previous study (12) we have characterized a [^{3}H]phenamil receptor in pig kidney membranes and shown its close correlation with Na^+ channel blockade measured from $^{22}Na^+$ uptake experiments. This paper reports the target size analysis and the solubilization, purification, and identification of the subunit structure of the [^{3}H]phenamil binding protein from pig kidney.

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MATERIALS AND METHODS

Materials. Amiloride [3,5-diamino-6-chloro-*N*-(diaminomethylene)pyrazine carboxamide], benzamil {3,5-diamino-6-chloro-*N*-[amino(benzylamino)methylene]pyrazine carboxamide}, phenamil {3,5-diamino-6-chloro-*N*-[amino(phenylamino)-methylene]pyrazine carboxamide}, *N*-5-ethylisopropylamiloride [3-amino-5-ethylisopropylamino-6-chloro-*N*-(diaminomethylene)pyrazine carboxamide], and [4-^{3}H-*phenyl*]-phenamil (2.8 Ci/mmol; 1 Ci = 37 GBq) were synthesized as described (15, 16). QAE Sephadex A-25 and Sephadex G-50 were purchased from Pharmacia. 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) was from Boehringer Mannheim. *Bandeiraea simplicifolia* lectin (BS1) (as a mixture of the A_3B and AB_3 forms) coupled to Sephadex A-25 was generously provided by F. Delmotte (Centre National de la Recherche Scientifique, Orléans, France).

Membrane Preparation. Pig kidneys were obtained from the local slaughterhouse and kept frozen at −70°C until use. Membranes were prepared from the cortex in the presence of protease inhibitors (1 mM iodoacetamide, 1 μM pepstatin A, 0.1 mM phenylmethylsulfonyl fluoride, and 10 μM leupeptin) as described (12).

Molecular Mass Determination with the Radiation-Inactivation Technique. Kidney membranes were lyophilized and irradiated *in vacuo* with high-energy electrons using a clinical MEL SL 75/20 20-MeV linear accelerator at a dose of 2 Mrad/min (1 rad = 0.01 gray), as described by Ellory *et al.* (17). Calibration of the radiation dosage was performed by using the Perspex optical density method (18). Following irradiation, each sample was resuspended in 1 ml of deionized water by dispersal in a Vortex before measuring the specific [^{3}H]phenamil binding. Maximal binding capacities and K_d values were determined from Scatchard plots. The molecular mass of alkaline phosphatase was determined as an internal control. It was found to be 84 ± 5 kDa. This value is in agreement with the known molecular mass of the enzyme subunit [80 kDa (19)].

Solubilization of the [^{3}H]Phenamil Binding Sites. Kidney membranes (10 mg of protein per ml) were mixed slowly at 4°C with an equal volume of 1% CHAPS/20% glycerol/15 mM triethanolamine, pH 7.0, and gently agitated for 1 hr. The solution was then centrifuged at 100,000 × *g* for 45 min. The supernatant was collected and diluted 1:10 in an ice-cold medium of 15 mM triethanolamine/1 mM ethylenediaminetetraacetic acid, pH 7.0.

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; BS1, *Bandeiraea simplicifolia* lectin 1; D_{37}, radiation dose at which 37% of the initial specific [^{3}H]phenamil binding activity remained.

For equilibrium binding studies, solubilized membranes (0.3 mg of protein per ml) were incubated in the presence of increasing concentrations of [^{3}H]phenamil for 1 hr at 4°C. The assay medium was 15 mM triethanolamine/1 mM ethylenediaminetetraacetic acid, pH 7.0. Triplicate aliquots of 200 μl were then loaded onto 2-ml Sephadex G-50 columns that were centrifuged for 45 sec at 1000 × *g* on an MSE clinical centrifuge. The bound radioactivity was measured by counting the eluted buffer. Specific [^{3}H]phenamil binding was assessed by subtraction of the nonspecific binding component from the total binding. In competition experiments between [^{3}H]phenamil and unlabeled amiloride derivatives, solubilized membranes were incubated for 1 hr at 4°C in the presence of 20 nM [^{3}H]phenamil and of various concentrations of unlabeled compounds.

QAE Sephadex and BS1 Lectin Chromatography. QAE Sephadex A-25 columns (3.6 × 20 cm) were equilibrated in 0.05% CHAPS/1% glycerol/15 mM mannitol/15 mM triethanolamine, pH 7.0 (buffer A). The solubilized membranes (60 ml, 180 mg of protein) diluted 1:10 into 15 mM triethanolamine at pH 7.0 were loaded on the column. After washing with 200 ml of equilibration buffer, the elution was performed by using a linear NaCl gradient from 0 to 200 mM. Ten-milliliter fractions were collected and assayed for [^{3}H]-phenamil binding and for protein concentration.

The active fractions were pooled, supplemented with 1 mM $CaCl_2$, and applied to a BS1 lectin-Sephadex A-25 column. The column was equilibrated overnight in buffer A supplemented with 50 mM NaCl/1 mM $CaCl_2$ (buffer B). After washing with 20 column volumes, the column was eluted with 10 ml of buffer B supplemented with 150 mM NaCl/50 mM melibiose. One-milliliter fractions were collected.

Protein concentrations were measured according to Peterson (20) using bovine serum albumin as a standard. Iodination of the purified protein was carried out using Iodo-Gen as supplied by Pierce (21).

Gel Electrophoresis. Gel electrophoresis was performed using 4–14% continuous gradient polyacrylamide gels (22). Two-dimensional gel electrophoresis was carried out according to O'Farrell (23). Protein samples were denatured for 10 min at 95°C in a 75 mM Tris·HCl buffer at pH 6.8 containing 2% $NaDodSO_4$, 7.5% glycerol, and 4% 2-mercaptoethanol. In nonreducing conditions, 2-mercaptoethanol was omitted and replaced by 8 mM iodoacetamide. Gels were stained with Coomassie blue or silver (24).

RESULTS

Estimation of the Target Size of the [^{3}H]Phenamil Receptor Site by Radiation Inactivation. Equilibrium binding studies of [^{3}H]phenamil to freshly prepared kidney membranes revealed the presence of a single class of binding sites with a K_d value of 15 ± 5 nM and a maximal binding capacity of 6 ± 2 pmol/mg of protein (12). The same binding parameters were observed after freezing the membranes into liquid nitrogen, lyophilization, and rehydration.

Upon irradiation, the specific [^{3}H]phenamil binding activity decayed exponentially as a function of the irradiation dose (Fig. 1*B*). No significant variation in the dissociation constant of the [^{3}H]phenamil receptor complex was observed as a function of irradiation dose (Fig. 1*A*). This indicated that the loss of binding activity shown in Fig. 1*B* was due to a loss of binding sites and not to a change in the affinity of these binding sites for phenamil. The radiation dose for which 37% of the initial specific [^{3}H]phenamil binding activity remained (D_{37}) was 7.4 Mrad in two independent experiments. Using the empirical equation (25) $M_r = 6.4 \times 10^5/D_{37}$, the target size of the functional [^{3}H]phenamil receptor was calculated to be 90 ± 10 kDa.

Solubilization of the [^{3}H]Phenamil Binding Sites. A variety of detergents has been assayed for solubilization, including

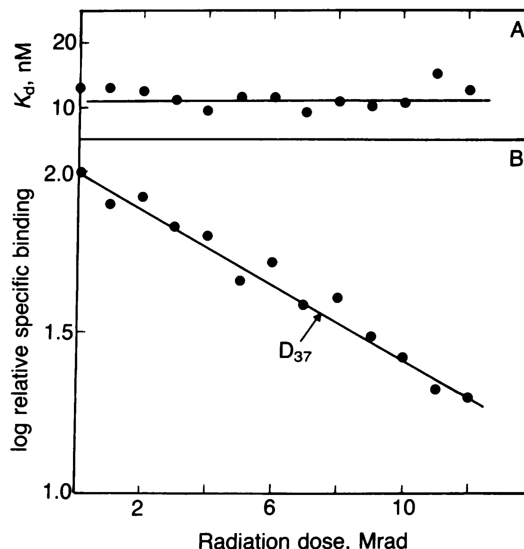

FIG. 1. Target size analysis of the epithelial Na^+ channel. (*A*) Effect of increasing radiation doses on the value of the dissociation constant of the [^{3}H]phenamil receptor complex. (*B*) Effect of increasing radiation doses on the maximal specific binding of [^{3}H]phenamil to pig kidney membranes. The arrow indicates the radiation dose at which 37% of the activity remained (D_{37}).

digitonin, $NaDodSO_4$, CHAPS, cholate, desoxycholate, Triton X-100, Triton X-114, Tween 20, and Lubrol. CHAPS was selected as the most useful detergent to solubilize the [^{3}H]phenamil binding sites (*i*) because of its low nonspecific absorption for [^{3}H]phenamil (<1%), (*ii*) because of its rather high critical micelle concentration (26), and (*iii*) because it provided the most reproducible results. It was found that the most successful solubilization of the [^{3}H]phenamil receptor sites was obtained at a final CHAPS concentration of 0.5% (8 mM). Addition of glycerol (10%) during solubilization was found to increase the solubilized [^{3}H]phenamil specific binding activity by about 50%, possibly by stabilizing the [^{3}H]phenamil binding sites. A similar observation had been made for the 1,4-dihydropyridine receptor associated with the voltage-sensitive Ca^{2+} channel (27). Under these conditions, the solubilized [^{3}H]phenamil receptor was stable at 4°C for several days. In contrast with the situation found for the Na^+ channel of excitable cells (28), the addition of phospholipids did not increase the stability of the receptor. A solubilization of 53% ± 3% of the membrane proteins was achieved by the CHAPS/glycerol procedure described above. All subsequent purification steps were performed within 2 days after the solubilization.

Fig. 2*A* shows the results of typical [^{3}H]phenamil equilibrium binding studies using CHAPS-solubilized membranes. The Scatchard plot for the specific [^{3}H]phenamil binding component shows that [^{3}H]phenamil binds specifically to a single class of noninteracting sites in solubilized as in intact membranes (Fig. 2*A Inset*). The apparent dissociation constant (K_d) of the solubilized [^{3}H]phenamil receptor complex was 20 nM. It was the same as for the membrane-bound receptor (24 nM). Maximal binding capacities (B_{max}) in the solubilized membrane preparation were 1.9 pmol/mg of protein. This value was 38% of the maximal binding capacities measured for the membrane-bound receptor (5 pmol/mg of protein).

Fig. 2*B* shows the results of competition experiments between [^{3}H]phenamil and unlabeled amiloride and amiloride analogs for the occupancy of the solubilized [^{3}H]phenamil receptor site. Half-maximal inhibition ($K_{0.5}$) of specific [^{3}H]phenamil binding was observed at 80 nM, 400 nM, 8 μM, and 4 μM for unlabeled phenamil, benzamil, amiloride, and

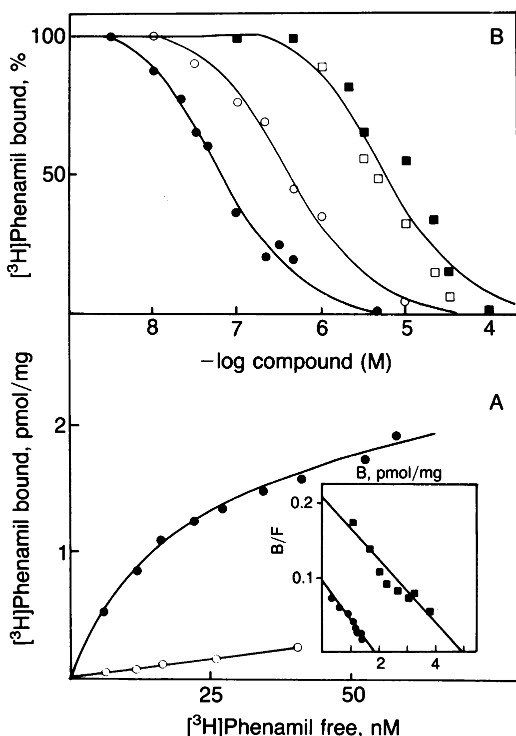

FIG. 2. Properties of the solubilized [^{3}H]phenamil receptor sites. (*A*) Equilibrium [^{3}H]phenamil binding to solubilized kidney membranes. ●, Total binding; ○, nonspecific binding. (*Inset*) Scatchard plots for the specific [^{3}H]phenamil binding to pig kidney membranes (■) and to solubilized pig kidney membranes (●). B/F, bound/free. (*B*) Competition between [^{3}H]phenamil and compounds in the amiloride series for binding to the solubilized receptor. Compounds used were unlabeled phenamil (●), benzamil (○), amiloride (■), and *N*-5-ethylisopropylamiloride (□).

N-5-ethylisopropylamiloride, respectively. The true K_d values for these compounds is given by:

$$K_{0.5} = K_d\{1 + [[^3\text{H}]\text{phenamil}]/K_{d([^3\text{H}]\text{phenamil})}\},$$

where [[^{3}H]phenamil] is the concentration of [^{3}H]phenamil used in the experiments (20 nM) and $K_{d([^3H]phenamil)}$ is the dissociation constant of the [^{3}H]phenamil receptor complex. K_d values were 40 nM, 200 nM, 4 μM, and 2 μM for unlabeled phenamil, benzamil, amiloride, and *N*-5-ethylisopropylamiloride, respectively. These values are in close agreement with the corresponding values found for the membrane-bound receptor (12).

Purification of the [^{3}H]Phenamil Binding Activity. The [^{3}H]phenamil binding activity was purified by conventional protein separation techniques. The main panel of Fig. 3 presents a typical elution profile of a QAE Sephadex column. Under conditions of low ionic strength the [^{3}H]phenamil binding activity was completely retained on the column. Elution was achieved by using a linear NaCl gradient. The [^{3}H]phenamil binding activity was eluted as a single peak (Fig. 3). The conductivity of the effluent at the time of elution of [^{3}H]phenamil binding site was usually between 3.5 and 6.0 millisiemens. *Insets A* and *B* of Fig. 3 show the results of typical equilibrium binding studies using this purified material. The Scatchard plot for specific [^{3}H]phenamil binding indicates a K_d value of 12 nM for the [^{3}H]phenamil receptor complex and a maximal binding capacity of 70 pmol/mg of protein (Fig. 3 *Inset B*). A purification factor of 30–50 was obtained routinely at this step with a yield of 40% ± 5%.

A second purification step was performed by using a column of BS1 lectin coupled to Sephadex A-25. The main

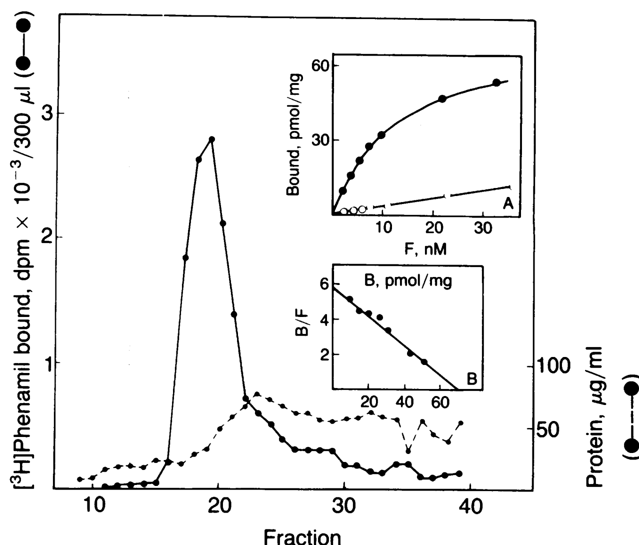

FIG. 3. Purification of the [^{3}H]phenamil receptor on QAE Sephadex column. Elution profile of the [^{3}H]phenamil specific binding (●—●) and protein concentration (●- - -●). Nonspecific binding was determined for each fraction and subtracted from the total binding component. [^{3}H]Phenamil was used at a concentration of 20 nM. (*Inset A*) Equilibrium binding of [^{3}H]phenamil to the QAE Sephadex-purified material. ●, Specific binding component; ○, nonspecific binding component. (*Inset B*) Scatchard plot for the specific [^{3}H]-phenamil binding to the QAE Sephadex-purified material. B/F, bound/free.

panel of Fig. 4 presents a typical elution profile from the BS1 lectin column. *Inset A* of Fig. 4 shows the Scatchard plot for specific [^{3}H]phenamil binding to the purified material from the BS1 lectin column. It indicated a K_d value of 10 nM for the [^{3}H]phenamil receptor complex and a maximal binding capacity of 3.7 nmol/mg of protein. This corresponded to a 52-fold purification. The purification yield was 40% ± 10%. *Inset B* of Fig. 4 shows the dose–response curve for amiloride inhibition of the specific [^{3}H]phenamil binding to the material purified from the BS1 lectin column. Half-maximal inhibition was observed at 9 μM, which corresponded to a K_d value of 3 μM. The migration of the [^{3}H]phenamil receptor protein in a sucrose gradient resulted in a single peak centered on fractions 8 and 9 (Fig. 5), corresponding to an apparent sedimentation coefficient of 9 S.

NaDodSO$_4$/polyacrylamide gel electrophoresis under disulfide-reducing conditions was run at the different steps of the purification. Fig. 6 compares typical gel electrophoresis patterns of the peak QAE Sephadex fraction (lane 1), of the peak BS1 lectin column fraction (lane 2), and of the peak fraction of the sucrose gradient (lane 3). It shows that the purified [^{3}H]phenamil receptor site consists of a single major component of 105 kDa when electrophoresis was carried out under reducing conditions. Under nonreducing conditions, the material from the peak fraction of the sucrose gradient migrated as a single band of 185 kDa (lane 4). When the 185-kDa band was electroeluted, reduced with 2-mercaptoethanol, and rerun, it migrated as a single band of 105 kDa (lane 5). Lane 6 shows that a rerun of the electroeluted 185-kDa band under nonreducing conditions resulted in a single 185-kDa band.

DISCUSSION

Phenamil is a derivative of amiloride that is the most potent inhibitor known so far for the epithelium Na$^+$ channel in frog skin (14) and in pig kidney microsomes (12).

An initial way to estimate the molecular size of membrane proteins is the radiation-inactivation technique. It has already served to determine the size of a number of other ionic

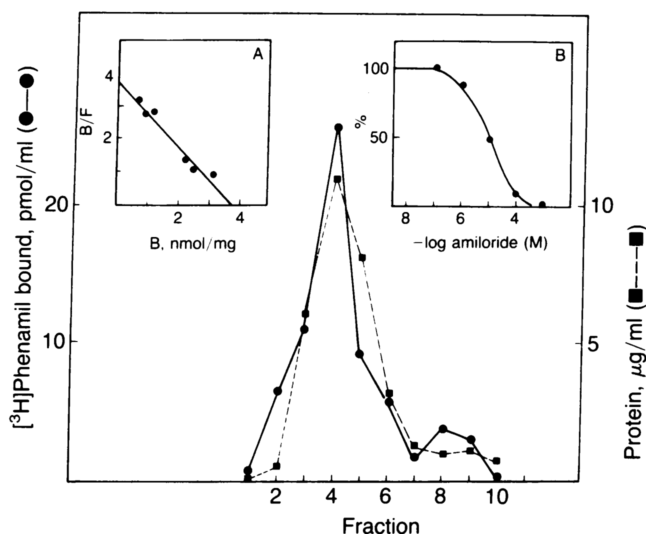

FIG. 4. Affinity chromatography on BS1 lectin column of the QAE Sephadex-purified [^{3}H]phenamil receptor. During washing of the column >98% of the proteins were eluted. Fractions were collected after the application of the 150 mM NaCl/50 mM melibiose buffer. [^{3}H]Phenamil was used at a concentration of 20 nM. (*Inset A*) Scatchard plot for the specific [^{3}H]phenamil binding to the BS1-purified material. (*Inset B*) Competition between amiloride and [^{3}H]phenamil for the binding of [^{3}H]phenamil to the BS1-purified Na^+ channel. B/F, bound/free.

channel proteins, such as the voltage-sensitive Na^+ and Ca^{2+} channels (29–31). Data presented in Fig. 1 indicate that the membrane-bound [^{3}H]phenamil receptor has a functional molecular mass of 90 ± 10 kDa.

The main purpose of this work was to purify and determine the subunit structure of the apical Na^+ channel. [^{3}H]Phenamil receptor sites were solubilized by using CHAPS as detergent and glycerol as a stabilizing agent. The binding characteristics of the solubilized [^{3}H]phenamil receptor are very similar to those of the membrane-bound receptor. The equilibrium dissociation constant, K_d, of the interaction between [^{3}H]phenamil and its receptor is 20 nM. Amiloride derivatives recognize the solubilized [^{3}H]phenamil receptor sites with the same order of efficacy and in the same range of concentrations as the membrane-bound receptor (12).

A two-step purification of the [^{3}H]phenamil receptor was

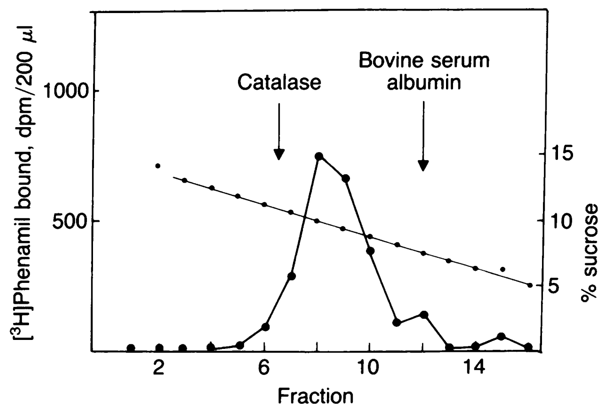

FIG. 5. Sedimentation profile of the [^{3}H]phenamil binding sites on a sucrose gradient. The BS1 lectin-purified material was layered onto a continuous 5–15% sucrose gradient in buffer A supplemented with 50 mM NaCl and centrifuged for 3 hr at 50,000 rpm in a Beckman VTI 50 rotor. Catalase (232 kDa) and bovine serum albumin (66 kDa) were used as standard proteins and sedimented in parallel gradients on the same rotor.

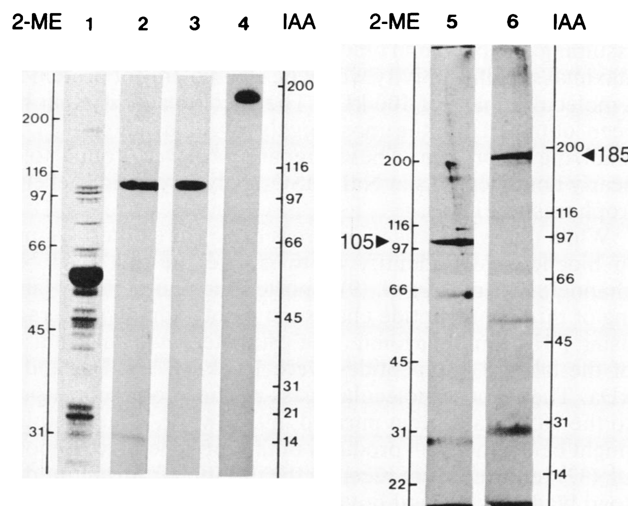

FIG. 6. $NaDodSO_4$/polyacrylamide gel electrophoresis of the purified Na^+ channel. Electrophoresis was carried out in 4–14% polyacrylamide gels under nonreducing and reducing conditions. Size standards were myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase B (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21 kDa), and lysozyme (14 kDa). Lane 1, peak QAE Sephadex fraction; lane 2, peak BS1 lectin fraction; lanes 3 and 4, peak sucrose gradient fraction. Lane 1 was Coomassie blue stained, lane 2 was silver stained, and lanes 3 and 4 are autoradiograms of iodinated samples. Lanes 1–3 were treated with 2-mercaptoethanol (2-ME), whereas lane 4 was treated with iodoacetamide (IAA). Lanes 5 and 6, after a first run of the peak BS1 lectin fraction under nonreducing conditions, the region corresponding to the 185-kDa band was cut off and the protein was electroeluted. Half was reduced with 2-mercaptoethanol (lane 5) and half was treated with iodoacetamide (lane 6). After the second run, proteins were silver stained. The bands at about 30 and 50 kDa were artifacts and seen over the entire gel.

set up that resulted into an 1100-fold purification. The first purification step was ion-exchange chromatography using QAE Sephadex. It was followed by affinity chromatography on a column of BS1 lectin coupled to Sephadex A-25. The specific adsorption of the [^{3}H]phenamil receptor site on the lectin column indicated that it is likely to be a glycoprotein.

$NaDodSO_4$/polyacrylamide gel analysis of the purified material under nonreducing conditions revealed the presence of a major polypeptide component at 185 kDa. When fresh preparations were analyzed, no evidence for material of larger molecular mass could be detected at the top of the gels or in the stacking gel. However, upon storage, the purified Na^+ channel tended to aggregate into larger molecular mass forms (not shown). Under reducing conditions, the 185-kDa polypeptide chain was no longer observed. The purified [^{3}H]phenamil receptor migrated as a 105-kDa protein. No evidence for heterogeneity of the 105-kDa component could be detected in two-dimensional gel electrophoresis (not shown). The slight discrepancy between the apparent molecular mass obtained by radiation inactivation (90 ± 10 kDa) and that of the purified material after reduction with 2-mercaptoethanol (105 kDa) might be due to the well-known tendency of reduced glycoproteins to migrate anomalously in $NaDodSO_4$/polyacrylamide gel electrophoresis. When the electroeluted 185-kDa protein was reduced with 2-mercaptoethanol and reelectrophoresed, it transformed into a single 105-kDa band (Fig. 6). These observations suggest that the epithelial Na^+ channel has a molecular mass of 185 kDa and is composed of two presumably identical 90- to 105-kDa subunits linked by disulfide bridges. Proteolytic cleavage of the 185-kDa protein to generate two distinct polypeptides during purification and/or membrane assembly cannot be excluded at the present time. The maximal specific activity of

the [^{3}H]phenamil receptor would be 5.6 nmol/mg of protein, assuming one phenamil binding site per 180-kDa protein. The maximal specific activity would be 10 nmol/mg of protein for a molecular mass of 100 kDa. The specific activity that has been obtained for the most highly purified material is 3.7 nmol/mg of protein. These preparations were found to be nearly homogeneous in NaDodSO_4/polyacrylamide gel electrophoresis (Fig. 6).

While this work was in progress there were other attempts to biochemically identify or to isolate the epithelial Na^+ channel. Kleyman *et al.* (9) reported the photoaffinity labeling of three polypeptide chains in bovine kidney membranes using [^{3}H]bromobenzamil. The estimated molecular masses of the labeled polypeptides were 176 kDa, 77 kDa, and 47 kDa. The highest molecular mass peptide could correspond to the 185-kDa protein purified in this work. Other peptides might be degradation products of this large peptide. Benos *et al.* (32) reported more recently the isolation from cultured A6 toad bladder cells and bovine kidneys of a very high molecular mass protein (700 kDa) that binds [^{3}H]methylbromoamiloride. This high molecular mass is not in agreement with the data reported in this paper and no other membrane protein with such a high molecular mass has been isolated up until now. When our purified material was analyzed by sucrose density gradient centrifugation (Fig. 5) and by size-exclusion HPLC, no evidence for [^{3}H]phenamil binding sites of >300 kDa could be obtained. We observed, however, that upon prolonged storage, purified fractions containing the epithelial Na^+ channels tended to aggregate into larger components.

Aldosterone is known to increase the synthesis of the epithelial Na^+ channel (5). Rossier *et al.* (33) have found that in the A6 cell line, aldosterone stimulated the rapid synthesis of a 98-kDa polypeptide chain. This polypeptide was localized in the apical membrane and was expressed in parallel with a decrease in transepithelial resistance. This observation is particulary interesting, knowing now that the purified Na^+ channel contains a 105-kDa polypeptide (this work) that may be identical to the 98-kDa protein identified in the work of Rossier *et al.*

The molecular mass (185 kDa) of the putative epithelial Na^+ channel is in the range of sizes found for other membrane ionic channels. It is smaller than that of the voltage-sensitive Na^+ channel from excitable tissues (270 kDa) (34, 35) and that of the complete acetylcholine receptor (250–270 kDa) (36). It is similar to the molecular mass of the voltage-dependent Ca^{2+} channel from skeletal muscle cells (170 kDa) (37, 38).

The subunit structure of the [^{3}H]phenamil receptor site presumably associated with epithelium Na^+ channel is different from that of the voltage-sensitive Na^+ channel responsible for the generation of action potentials. The fundamental unit of the voltage-sensitive channel is a single polypeptide chain of 270 kDa (34, 35). However, since the 270-kDa subunit of the voltage-sensitive Na^+ channel contains four sequence repeats (39), it may still be that there are structural homologies between the apical and voltage-sensitive Na^+ channels.

The purification of the [^{3}H]phenamil receptor associated with the apical Na^+ channel opens the way for the preparation of polyclonal and monoclonal antibodies that will be useful for the localization and for the analysis of the regulation of the biosynthesis of the channel. It is also an important step toward the cloning of the corresponding cDNA and the elucidation of the primary structure of the protein.

P.B. dedicates this paper to the memory of Dr. A. Barbry. We thank the staff of the linear accelerator at Addenbrooke's Hospital, Cambridge, for their assistance in providing irradiation facilities. We particularly wish to thank Prof. F. Delmotte (Centre National de la Recherche Scientifique, Orléans) for having kindly provided us with generous quantities of BS1 lectin. The skillful technical assistance of N. Belhacene, S. Megis, N. Boyer, and C. Roulinat-Bettelheim is gratefully acknowledged. This work was supported by the Centre National de la Recherche Scientifique, the Fondation pour la Recherche Médicale, and the Institut National de la Santé et de la Recherche Médicale. P.B. is a recipient of a grant from *Rhône-Poulenc Santé*.

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