Hypertension-associated Point Mutations in the Adducin α and β Subunits Affect Actin Cytoskeleton and Ion Transport

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Abstract

The adducin heterodimer is a protein affecting the assembly of the actin-based cytoskeleton. Point mutations in rat adducin α (*F316Y*) and β (*Q529R*) subunits are involved in a form of rat primary hypertension (MHS) associated with faster kidney tubular ion transport. A role for adducin in human primary hypertension has also been suggested. By studying the interaction of actin with purified normal and mutated adducin in a cell-free system and the actin assembly in rat kidney epithelial cells (NRK-52E) transfected with mutated rat adducin cDNA, we show that the adducin isoforms differentially modulate: (*a*) actin assembly both in a cell-free system and within transfected cells; (*b*) topography of αV integrin together with focal contact proteins; and (*c*) Na-K pump activity at V_{max} (faster with the mutated isoforms, 1281±90 vs 841±30 nmol K/ h · mg pt., $P < 0.0001$). This co-modulation suggests a role for adducin in the constitutive capacity of the epithelia both to transport ions and to expose adhesion molecules. These findings may also lead to the understanding of the relation between adducin polymorphism and blood pressure and to the development of new approaches to the study of hypertension-associated organ damage. (*J. Clin. Invest.* 1996. 97:2815–2822.) Key words: rat • genetics • blood pressure • adhesion molecules • Na(+)-K(+)ATPase

Introduction

A long series of experiments, carried out in both rats and humans have led to the identification of adducin as a candidate protein for the study of the genetic molecular mechanisms of primary hypertension (1). A large deal of these experiments were carried out in a strain of genetically hypertensive rats (MHS, Milan hypertensive strain)[1] that we developed along with their normotensive controls (MNS, Milan normotensive strain) (1). These studies, based on experimental procedures like MHS/MNS kidney cross-transplantation (2, 3), renal function measurements performed both in isolated kidneys (4, 5) and in intact animals (6, 7) as well as in whole cells (8, 9) and cell membranes (10, 11), indicated that the primary defect leading to MHS hypertension was a faster ion transport rate across tubular cells (1). As the same transmembrane ion transport differences between MHS and MNS were also found in erythrocytes (1), these cells were used to demonstrate that the MHS abnormalities were genetically determined within the stem cells and genetically associated to hypertension (12). Further experimental work showed that the difference in ion transport rate could be abolished upon removal of the membrane skeleton (13, 14). In addition, cross-immunization studies between MHS and MNS membrane skeleton led to the identification of an immunochemical difference in a 105-kD protein subsequently identified as adducin (15). Adducin has been extensively characterized by Bennett and co-workers who established that the protein is a heterodimer composed of related but not identical α and β subunits (16, 17). The corresponding genes map on different chromosomes both in rats and in humans (16, 18, 19). Sequencing of the full-length α and β adducin cDNAs in MHS and MNS lead to the identification of a missense mutation in each adducin subunit (18) and further studies using genetic crosses showed that these mutations in α(*F316Y*) and β(*Q529R*) subunits were involved in causing a portion of the blood pressure difference between MHS and MNS (20).

In view of many similarities in pathophysiological changes between MHS rats and a subgroup of hypertensive patients (21) and the very high aminoacid homology between rat and human adducins, a case-control study with hypertensive and normotensive subjects was carried out to test alleles-disease association using multiallelic markers surrounding the α-adducin locus located on the short arm of chromosome 4 (4p16.3). The marker (*D4S95*) which maps closest (20 Kb) to α-adducin locus was associated with hypertension, whereas the marker (*D4S228/E24*) which maps furthest from this locus (∼ 2500 kb) was not associated (22).

Also the genotyping of French families with the above mentioned DNA markers has been carried out. The results of an alleles-sharing analysis demonstrated a significant excess of alleles ($P = 0.005$) in 127 affected pairs with marker *D4S95* and no excess of alleles for marker *D4S228/E24* (C. Barlassina, F. Soubrier, and G. Bianchi, unpublished data in preparation). Taken together these findings are very strong indications that α-adducin polymorphisms play a role also in human hypertension.

However, the cellular mechanisms by which adducin may cause hypertension, possibly through an alteration of the transmembrane Na^+ transport is unknown. Adducin favors the

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1. *Abbreviations used in this paper:* MHS, Milan hypertensive strain; MNS, Milan normotensive strain; nt, nucleotide(s).

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actin–spectrin assembly (17), and binds and bundles actin filaments (23). Its activity is modulated by Ca^{2+}/Calmodulin (24). Because of this and other characteristics, adducin has been included in the group of MARCKS proteins involved in cell signal transduction (25). Very recently, a link between adducin and transmembrane ion transport has also been suggested (26).

The objective of the present study was to establish whether adducin isoforms differentially affect actin cytoskeleton assembly and transmembrane ion transport. We studied actin polymerization and bundling in a cell-free system and actin cytoskeleton and Na-K pump activity in rat epithelial cell lines transfected with normal or mutated α-adducin cDNA. Because of the well-known indirect interaction between adhesion molecules and actin cytoskeleton assembly (27, 28), we also studied the surface expression of the integrin αv in transfected cells on the basis of the concept that adhesion mechanisms could be controlled by changes chiefly affecting the submembraneous cytoskeleton.

The results of this study show an involvement of mutated α-adducin in all these phenomena and suggest a cellular basis for its implication in blood pressure regulation.

Methods

In vitro cell-free system assays

Protein purifications. Erythrocyte adducin was purified from blood of 100 rats from each of three inbred and one recombinant inbred strains, differing for mutant alleles in genes coding for α and β adducin subunit according to the following genotypes: MNS/Gib-*αFβQ/αFβQ*, MHS/Gib-*αYβR/αYβR*, MNS/Gib-*αFβR/αFβR* and MHND/Brb-*αYβQ/αYβQ*. Blood was filtered, erythrocytes lysed in hypotonic solution, and membranes collected. The purification was carried out as described (29).

Actin was prepared from an acetone powder of rabbit skeletal muscle in buffer A (0.2 mM $CaCl_2$; 0.2 mM ATP; 0.5 mM NaN_3; 0.5 mM β-mercaptoethanol; 2 mM Tris-HCl, pH 8.0) as described (30) and further purified by gel filtration on a Sephadex G-150 column (31) Pyrenyl-actin was prepared as described (32). The pyrene/actin molar ratio varied between 0.65 and 0.9 in the various preparations. Both unlabeled and labeled actin were kept as small aliquots in liquid nitrogen. Thawed actin was used within 24 h. Actin was never exposed to sequential freeze-thaw cycles.

Fluorescence measurements of actin polymerization. Fluorescence data were obtained using an LS50 spectrofluorometer (Perkin-Elmer, England). The excitation wavelength was 365 nm and the emission wavelength was 407 nm. The excitation and emission slits were set at 2.5 and 10 nm, respectively. The sample temperature was maintained at 25°C by using a water-jacketed cuvette holder and a circulating water bath. The samples were allowed to equilibrate in the cuvette for 10 min, then the polymerization reaction was initiated by the addition of salts (KCl and $MgCl_2$) and either adducin or adducin storage buffer. The experimental conditions were: 15 mM NaCl, 30 mM KCl, 1 mM $MgCl_2$, 0.1 mM ATP, 140 μm EGTA, 0.007% Tween 20, 2.8% sucrose, 1.4 mM Na-phosphate buffer pH 7.4, 140 μM dithiothreitol, 0.25 mM β-mercaptoethanol, 0.25 mM NaN_3, 4.5 mM Tris-HCl pH 7.6. The sample size was 1.5 ml. The data are plotted as arbitrary fluorescence values relative to the fluorescence of the sample before polymerization. In the experiments in which the Ca^{2+}-dependence of the effect of adducin on actin polymerization was to be monitored, the free Ca^{2+} concentration was buffered by the addition of appropriate amounts of EGTA or EDTA which were estimated by a computer program using the stability constants (33). Freshly purified G-actin generally polimerized faster than that which had been stored for some days, however, polymerization curves run in the same conditions were highly reproducible within the same experimental session.

Other procedures. High-speed sedimentation and electron microscopy of negatively stained actin-adducin mixtures were performed as previously described (32). Protein concentrations were determined as described (34) or spectrophotometrically using an $E^{1\%}$ of 6.5 for actin (35). The concentration and labelling stoichiometry of pyrenyl-actin were determined as described (36).

Cell culture, plasmid DNA constructs, and stable transfection

NRK-52E cells (Normal Rat Kidney, epithelial-like cells) (37), were purchased from the European Collection of Animal Cell Cultures (ECACC, CRL 1571). Cells were cultured in monolayer in Dulbecco's modified Eagle's medium (GIBCO BRL, Gaithersburg, MD) supplemented with 5% Foetal Calf Serum (Myoclone, Life Technologies), 1% non-essential aminoacids (Sigma Chemical Co., St. Louis, MO), penicillin/streptomycin 100 IU/ml (GIBCO BRL) and were kept in a 37°C humidified incubator with 5% CO_2.

The rat α-adducin coding region cDNA (corresponding to nucleotides 70-2322 of the full-length cDNA) (18), was amplified by reverse transcriptase PCR (18) from kidney RNA (RNA Extraction Kit, Pharmacia) of either normotensive or hypertensive rat and subcloned into the XhoI site of the mammalian expression vector pKG5 (a gift of F.E. Baralle ICGEB, Trieste). In this vector the expression of exogenous gene is directed by the SV40 early region promoter.

DNA transfection was performed via calcium phosphate coprecipitation methods with 20 μg of normal and mutated α-adducin-expression construct as described (38). Stable transfectants were selected by addition of G418 (0.6 mg/ml). Neomycin-resistant clonal cell lines were generated from isolated individual colonies.

Northern and Western blot analyses

Northern blot. 20 μg of total RNA were purified as described before, from cell lines grown to confluency in 55 cm^2 petri dishes, size-fractionated on formaldehyde/agarose gel, transferred onto nylon membrane (Genescreen) according to the manufacturer's instructions and probed with ^{32}P-labeled cDNA (a 1.7-kb PstI and HindIII fragment corresponding to nucleotides 253–1963 of rat α-adducin cDNA sequence) (18). Exogenous and endogenous α-adducin mRNAs were visualized as two mRNA species of ~ 2500 and 3900 nt, respectively, and quantified by electronic autoradiography (InstantImager 2024, Packard).

Western blot. Cells were lysed in SDS-sample buffer and 100 μg of total protein was resolved on 7–15% gradient SDS-PAGE gels, transferred to nitrocellulose membrane, and probed with a rabbit polyclonal anti α-adducin serum (a gift of C. Hughes and V. Bennett, Duke University), followed by iodinated anti–rabbit second antibody (Amersham). Endogenous and exogenous α-adducins migrated as a single band around 105 kD. Using the autoradiography as template, the bands were excised and counted.

Immunofluorescence analysis

Transfected NRK-52E cell lines were grown to confluence on glass coverslips and treated as previously described (39). Monolayers were washed twice with PBS and fixed (5 min at room temperature) with formaldehyde (3%) solution (from paraformaldehyde, electron microscope grade), pH 7.6, containing 2% sucrose, and then permeabilized (3 min, 0°C) by Triton X-100 buffer (20 mM Hepes, 300 mM sucrose, 50 mM NaCl, 3 mM $MgCl_2$, 0.5% Triton X-100, pH 7.4). The latter step leading to extensive membrane permeabilization was omitted in cells stained for Na-K ATPase to preserve immunoreactivity; however, some permeabilization occurred also upon plain formaldehyde fixation. Staining for F-actin was performed with fluorescein-labeled phalloidin (F-PHD, Sigma) (200 nM for 20 min at 37°C in the dark). Integrin αv and the α subunit of Na-K ATPase were respectively detected with a rabbit antiserum to a synthetic peptide of the αv cytodomain (a gift of G. Tarone, University of Torino) and a mAb directed against an undefined epitope of the α subunit of Na-K ATPase (a gift of A. Mc Donough, Southern California University of Los Angeles) followed by rhodamine-tagged swine anti-rabbit and rabbit anti–mouse IgGs, respectively. The primary antibody was replaced by

nonimmune mouse IgGs for control purposes. Coverslips, mounted in Mowiol (Hoechst, Frankfurt am Main, FRG), were observed in a Zeiss Axiophot microscope equipped for epifluorescence and interference reflection (Antiflex) optics.

Na-K pump and cell volume measurements

For ion transport experiments, cells were seeded at 5×10^5 cell/cm^2 grown on Transwell filter inserts (Costar, 0.4 mm pore size). Cells were used for experiments 7 d after seeding. The filters were washed in K^+-free saline and incubated without external K^+ for different times in order to load the cells with Na^+ and reach the maximal activation of the Na-K pump. This was achieved in the different preparations at times ranging from 20 to 45 min of incubation in K^+-free medium. The Na-K pump activity was measured as ouabain-sensitive $^{86}Rb^+$ uptake (8 μCi/ml) during the initial 10 min from restoration of normal external K^+ concentration (5.4 mM) as described (40). Ouabain (10 mM) was added on the basolateral side 5 min before the assay. Ouabain-sensitive Rb^+ uptake was expressed as the equivalent K^+ transport in nanomoles per hour per milligram protein.

Cell line volumes were obtained by means of Coulter Counter connected to 256 Channelyzer and expressed in femtoliter.

Results

Interaction of adducin with actin in a cell-free system. To evaluate the possibility that normal ($\alpha F\beta Q$) and mutated hypertensive adducin ($\alpha Y\beta R$) subunits might differentially interact with the actin-based cytoskeleton, we have employed several well-established in vitro assays for the study of actin polymerization and assembly.

Solutions containing 5 M G-actin (5% labeled with *N*-(1-pyrenyl) iodoacetamide) were polymerized by the addition of 30 mM KCl and 1 mM $MgCl_2$. Polymerization was monitored by recording the enhancement of pyrenyl-actin fluorescence. Each experiment was repeated 3–6 times. The curves shown in the figures represent the results from typical experiments. The addition of adducin immediately prior to the nucleating salts caused a noticeable change in the kinetics of actin polymerization. When the assay was carried out at physiological Ca^{2+} concentration (100 nM), normal adducin strongly inhibited the rate of polymerization (Fig. 1, *left panel*). In contrast, double-mutated adducin exerted only a slight inhibitory effect on the

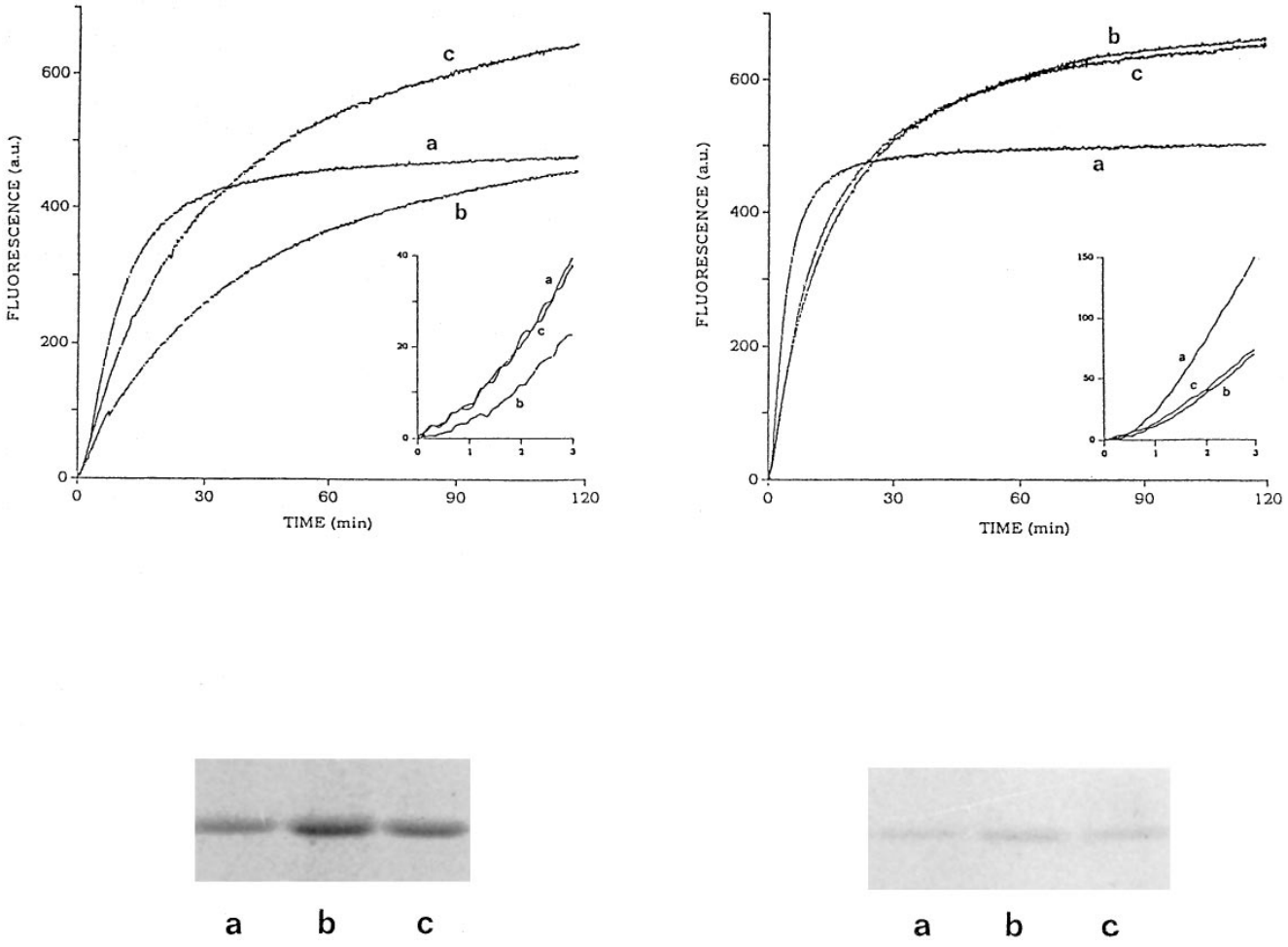

Figure 1. Effects of normal and double-mutated adducin on actin polymerization. (*Upper panels*) The polymerization of pyrenyl G-actin (5 M; 5% labeled) was followed at 25°C by monitoring the increase in fluorescence occurring after the addition of KCl and $MgCl_2$ at time 0. The inserts show the initial 3 min of the experiments on an expanded scale. Data are plotted as arbitrary fluorescence values relative to the fluorescence of the sample before polymerization. (*Lower panels*) After performing the fluorescence assays, the samples were sedimented at high speed and the supernatants were analyzed by Coomassie blue staining of SDS 10%-polyacrylamide gels in order to determine the amount of unsedimentable actin. Samples from experiments performed in the presence (*left panels*) or absence (*right panels*) of 100 nM $CaCl_2$ are shown. Lane *a*, actin alone; lane *b*, actin plus 200 nM normal adducin; lane *c*, actin plus 200 nM α- and β-mutated adducin.

rate of polymerization. In addition, in the presence of double-mutated adducin (but not of the "normal" isoform) the final extent of polymerization attained was much higher with respect to either the control or the normal adducin sample.

Since many actin-binding proteins are Ca^{2+} dependent in their actins, the effect of adducin on actin polymerization was also analyzed at a buffered Ca^{2+} concentration below 10^{-9} M. In agreement with previous data (41), the chelation of free Ca^{2+} per se shortened the lag phase (corresponding to the activation and nucleation of actin monomers) and increased the rate of actin polymerization. In the absence of Ca^{2+}, adducin was still effective in decreasing the initial rate of polymerization. However, under this condition the differential effect of normal and double-mutated adducin was lost, both isoforms showing a behavior similar to that observed with double-mutated adducin in the presence of Ca^{2+} (Fig. 1, *right panel*).

The effects of adducin isoforms bearing single mutations on either the α or the β subunit were also evaluated. Adducin heterodimer mutated only in the β subunit ($\alpha F\beta R$) essentially behaved as normal adducin, whereas adducin heterodimer mutated only in the α-subunit ($\alpha Y\beta Q$) exhibited an intermediate phenotype (Fig. 2).

In high-speed sedimentation assays, a substantial fraction of adducin was found to co-sediment with actin filaments (data not shown). This fraction did not significantly differ between the two adducin isoforms, thus suggesting that the ability of adducin to bind directly to F-actin (23) is not influenced by the point mutations. Under the conditions used for the high-speed sedimentation assays, actin filaments, but not actin monomers or oligomers, are sedimented. Thus, this assay allows to determine both the binding of a protein to actin filaments and its effect on the unpolymerized/polymerized actin ratio (32). Indeed, analysis of the supernatants indicated that the amount of unpolymerized actin was increased in the presence of adducin and more so in the case of the normal form of the protein. (Fig. 1, *lower panels*).

The ability of adducin to influence actin network formation was evaluated by electron microscopy. When the preparations of rat adducin were incubated in the presence of actin filaments and examined after negative staining, double-mutated adducin, but not normal adducin, was found to cause the formation of actin bundles (Fig. 3).

Expression of normal and mutated α-adducin in NRK-52E cells transfectants. Normal Rat Kidney epithelial cell line (NRK-52E) (37) was selected because its adducin genotype ($\alpha F\beta R$) allowed the reconstitution of the full hypertensive double-mutated adducin by simply transfecting it with mutated α-adducin cDNA. These cells were transfected with the rat normal and mutated α-adducin cDNA under the control of the early SV40 promoter and several stable lines were obtained. Exogenous and endogenous α-adducin mRNAs (∼ 2500 and 3900 nt, respectively) and the corresponding levels of total protein expression have been quantitated (Fig. 4). In all the clones tested, a correlation existed between α-adducin mRNA and protein levels. The levels of endogenous α-adducin mRNA are similar in untransfected and transfected lines (not shown). In

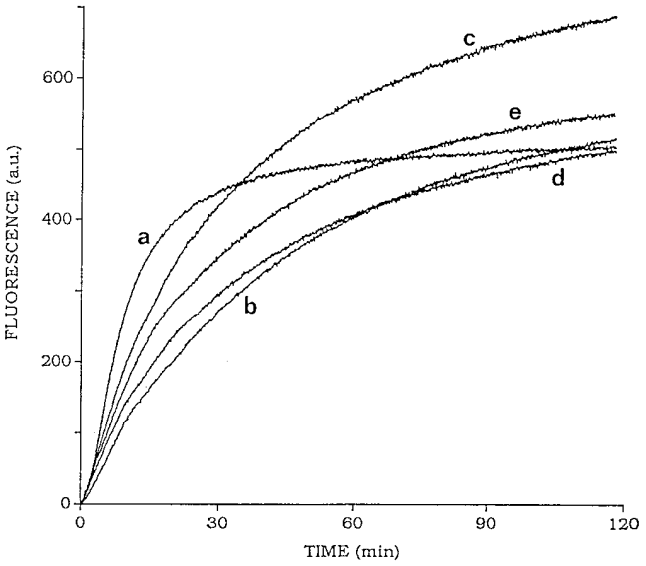

Figure 2. Effects of adducin mutations on the kinetics of actin polymerization. *a*, Actin alone; *b*, actin plus normal adducin; *c*, actin plus α- and β-mutated adducin; *d*, actin plus α-normal and β-mutated adducin; *e*, actin plus α-mutated and β-normal adducin. Experimental conditions were as described in the legend to Fig. 1 (100 nM $CaCl_2$ present).

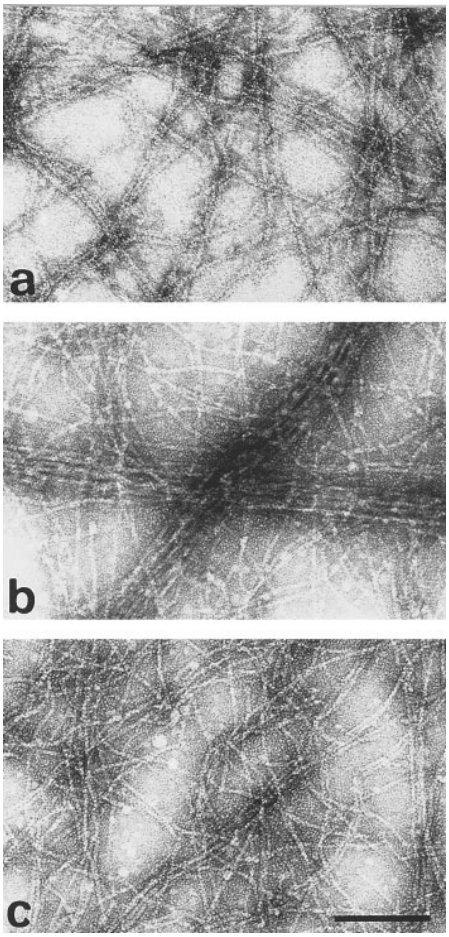

Figure 3. Effects of normal and double-mutated adducin on actin network formation. Actin (5 M) was polymerized for 30 min at 25° C by the addition of 30 mM CKl and 1 mM $MgCl_2$ in the absence (*a*) or presence of 200 nM either double mutated (*b*) or normal (*c*) adducin. Bar; 0.1 μm.

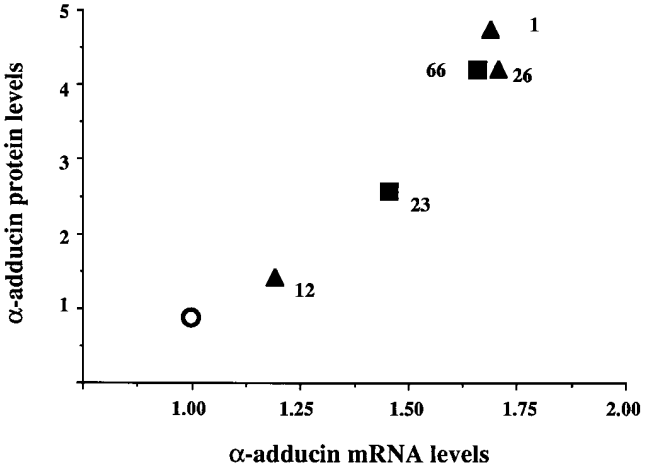

Figure 4. Relationship between α-adducin mRNA and total amount of corresponding protein in NRK-52E untransfected cells (*open circle*), normal (*closed squares*), and mutated (*closed triangles*) α-adducin stable transfected lines. Results are expressed as fractional changes with respect to untransfected cells. Northern blot assay was done to quantify abundance of exogenous and endogenous α-adducin mRNA (two mRNA species of ~2500 and 3900 nt, respectively). Western blot assay was done to quantify abundance of total protein expression, as described in Methods. Numbers represent the identification codes of the different transfected lines.

all the selected lines, the α-adducin exogenous mRNA has also been tested by allele-specific hybridization as previously described (20), to confirm the nucleotide substitution, (corresponding to *F316Y* aminoacid substitution).

Immunofluorescence analysis of normal and mutated α-adducin transfected lines. To determine whether normal and mutated adducin could display, in vivo, the same properties produced in vitro, we studied the microfilamentous cytoskeleton in transfected cell lines. As untransfected cells were similar to transfected cells overexpressing normal α-adducin, we shall refer here only to comparisons between transfected cells. When cells overexpressing normal adducin were compared to those overexpressing the mutated α-adducin chain, the major finding was that in the latter the average size of microfilament bundles was much increased (Fig. 5 *d*) whereas in the former the microfilament bundles were organized in a much looser texture (Fig. 5 *a*). Also, cells overexpressing the mutated α-adducin chain exhibited larger focal contacts identified by their vinculin or talin content (not shown). Next, we studied the expression of the αv integrin chain and found that, in analogy with vinculin and talin, αv formed patchy aggregates concentrated in the large focal contacts of cells overexpressing the mutated α-adducin chain (Fig. 5 *e*). In cells overexpressing normal α-adducin the αv was present in a diffuse punctate pattern with small focal contacts (Fig. 5 *b*). Metabolic labelling and immunoprecipitation of both cell types with αv antibodies did not show any obvious quantitative change in the expression of αv and its associated β chains (not shown) indicating that the observed αv changes were due only to redistribution of the molecule(s) on the cell surface.

In comparison to normal α-adducin transfected cells (Fig. 5 *c*), we found that the surface expression of Na-K pump α subunits in the cells overexpressing the mutated α-adducin chain was considerably increased (Fig. 5 *f*).

Na-K pump activity in normal and mutated α-adducin transfected lines. In agreement with the immunofluorescence data, in the cell lines overexpressing mutated α-adducin the Na-K pump activity at V_{max} was increased with respect to normal transfected lines with similar protein expression levels and to untransfected cells (cell lines: **26**, 1281±90: **66**, 841.1±30 $P <$ 0.0001: untransfected cells, 847.4±45 nmol K/ h · mg pt, see also Fig. 6). The Na-K pump activity was also dependent upon the expression levels of mutated protein, while it was not affected by the expression levels of normal protein (Fig. 6). All the selected lines showed cellular volume similar to that of the untransfected cells (cell lines: **1**, 1171±86 femtol, **12**, 1162±69 femtol, **26**, 1193±83 femtol, **23**, 990±89 femtol, **66**, 1095±51 femtol, untransfected cells, 932±54 femtol).

Discussion

In this report we describe the properties of adducin as a protein affecting actin polymerization and assembly and show that these properties are altered by the point mutations found in the adducin of the MHS genetically hypertensive strain of rats. The results suggest that, at physiological Ca^{2+} concentrations, normal adducin exerts an inhibitory action on actin dynamics. In contrast, double-mutated adducin interferes with actin assembly and polymerization in a more complex manner: it maintains an inhibitory effect on the rate of polymerization, although quantitatively decreased, leading however to a higher final level of filamentous actin, only part of which is sedimentable. In addition, it binds to the sides of actin filaments, aggregating them into bundles.

The ability of actin monomers to polymerize into filaments is essential for many biological functions of the cell. Nonmuscle actin filaments are highly dynamic structures and undergo self-assembly and disassembly processes which are finely tuned by the cell complement of actin-binding proteins (42). In addition, many of the actin-binding proteins are regulated in their activities by second messengers (43). In this respect, it is noteworthy that, whereas the activity of normal adducin is modulated by Ca^{2+}, mutated adducin has lost this mechanism of regulation.

The altered ability of double-mutated adducin to affect actin polymerization can be primarily ascribed to the mutation present on the α subunit. In fact, adducin mutated only on the β subunit behaves similarly to normal adducin, whereas adducin mutated only on the α subunit exhibits an intermediate behavior. Interestingly, these in vitro activities of the different adducin isoforms roughly parallel the associated level of systolic blood pressure previously described (20). In fact, the β-adducin mutation per se is not associated with significant changes in blood pressure but, when this mutation is combined with the α-adducin mutation, the associated level of blood pressure is higher than that observed in presence of the α-adducin mutation alone (20).

Several proteins, initially described as actin-binding proteins, have been subsequently found to have no actin-binding activity within the cell (42). It seemed therefore important to test the activity of normal and mutated adducin in a cellular context. The changes of actin cytoskeleton observed in transfected cells were consistent with the results obtained in the cell free system. Namely the actin fibres detected by fluorescent phalloidin were much thicker and abundant in cells overexpressing normal adducin.

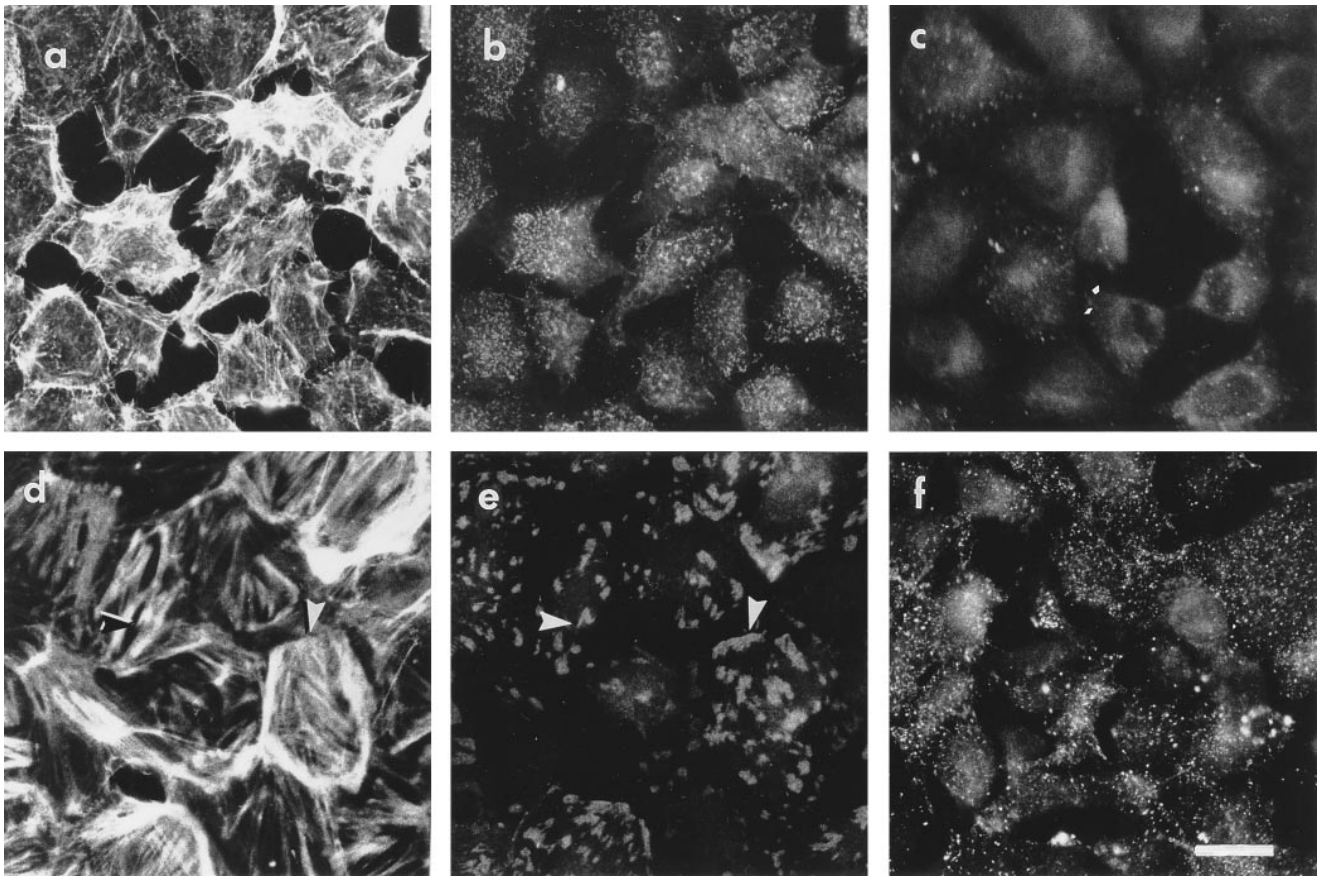

Figure 5. Immunofluorencence analysis of transfected NRK-52E cells overexpressing normal (*a–c*) or mutated α-adducin (*d–f*). Coverslip-attached cells were double stained for F-actin (*a* and *d*) and integrin chain αv (*b* and *e*) or immunostained for the α chain of the Na-K ATPase (*c* and *f*). The microfilament pattern in *a* is based on a rather loose texture of thin microfilament bundles while in *d* numerous thick stress fibers are present. In the latter sample, integrin αv is concentrated at the termini of stress fibers (*arrowheads* in *e*, compare *d* and *e*) in large speckles that correspond to focal contacts on the basis of interference-reflection microscopy and vinculin or talin localization (not shown). In overexpressing normal α-adducin cells, αv is concentrated in tiny focal contacts and in diffuse multiple tiny clusters (*b*). The surface exposure of the Na-K ATPase is markedly different in the two cell types being lower and weaker in controls (*c*) and much more marked in cells overexpresing mutated α-adducin (*f*). Bar; 20 μm.

An important role of cytoskeletal proteins is that of assisting the formation of microdomains of membrane, which differ in their molecular composition. In this regard, cytoskeletal structures are believed to function as anchoring points for membrane proteins, limiting their random diffusion in the plane of the membrane and helping their aggregation and compartmentalization. Many previous data obtained in polarized cells, such as kidney epithelial cells, support the idea that the actin cytoskeleton may either directly regulate the activity of ion channels on the luminal membrane (44) or modulate the Na-K pump activity on the baso-lateral membrane (45, 46). In renal epithelial cells transfected with mutated adducin we have found an increase in Na-K pump activity at V_{max}. Although the molecular basis of this effect remains to be established, the findings that (*a*) in a cell-free system, mutated adducin has an altered ability to interact with actin and (*b*) in intact cells overexpressing mutated adducin enhances the formation of actin bundles as well as redistribution of Na-K pump molecules in the plasma membrane, suggest that adducin is instrumental in determining the location and in modulating (either directly or indirectly, as a secondary response to changes in Na entry) the activity of the Na-K pump. Actin polymerization per se in a cell-free system stimulates the isolated Na-K pump (47). This may offer an additional link among adducin, actin filaments and Na-K pump activity. Moreover, previous data obtained both in basolateral membrane vesicles or isolated tubuli from either MHS or MNS rat kidneys showed an increased activity at V_{max} of Na-K pump in MHS (9, 11). The data described here are in agreement with the previous results and offer further arguments to support the existence of links among adducin mutations, faster transtubular ion transport and hypertension.

Arterial hypertension is a risk factor for many complications in various organs: heart, kidney, brain and retina. Lowering blood pressure with various drugs results in a decrease of these complications. However cardiac and renal complication with an incidence above the level found in the normal population may persist in patients despite good control of their hypertension (48–51). Although the interpretation of this observation may be still controversial (52), the results described here could suggest a possible explanation. Integrin and other adhesion molecules have been suggested to be involved in many cardiac (53) and renal diseases (54). It is tempting to speculate that the same genetic molecular mechanism, i.e., a mutation within the adducin genes, may produce hypertension through

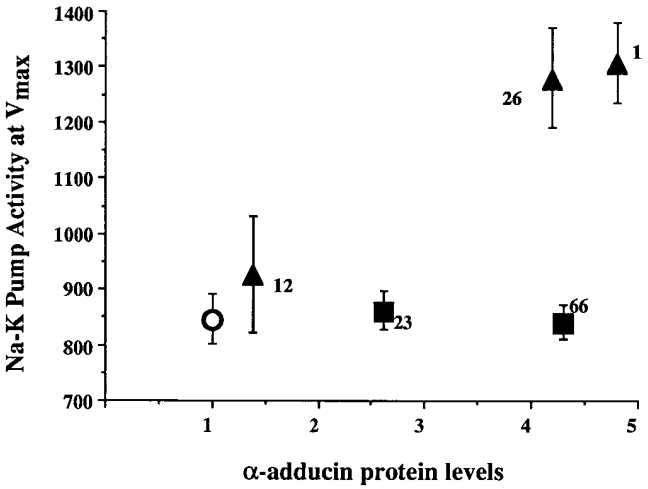

Figure 6. Relationship between the Na-K pump activity at V_{max} and the different α-adducin protein expression levels in NRK-52E untransfected cells (*open circle*), normal (*closed squares*), and mutated (*closed triangles*) α-adducin stable transfected lines. Na-K pump activity was measured as ouabain-sensitive Rb uptake in cells Na-loaded in the absence of external K. Results are expressed as mean±SEM (bars) of at least four replications. The α-adducin protein levels are expressed as fractional changes from untransfected cells (see Fig. 2). Numbers represent the identification codes of the different transfected clones (see Fig. 2).

its effect on sodium transport and may favor the development of organ complication through its effect on adhesion molecules. Therefore organ complications may arise not only from the increased hydrostatic pressure within the vessel, but also by a peculiar alteration of adhesion molecules caused by a constitutive change in their cell surface expression and, possibly, by production of altered intracellular signals.

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