Department of Transportation for permission to excavate the Tomahawk site. We thank E. B. Sues and P. A. Kroehler for expert help in the field. Field crews also included M. A. Parrish, K. A. Pitt, R. W. Schlische, and B. J. Small. W. W. Amaral prepared several specimens. M. A. Parrish drew Fig. 2. We thank J. R. Bolt and J. A. Hopson for their help in the identification of certain taxa.

2 April 1990; accepted 22 June 1990

Alteration of α1 Na^+,K^+-ATPase $^{86}Rb^+$ Influx by a Single Amino Acid Substitution

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The sodium- and potassium-dependent adenosine triphosphatase (Na^+,K^+-ATPase) maintains the transmembrane Na^+ gradient to which is coupled all active cellular transport systems. The R and S alleles of the gene encoding the Na^+,K^+-ATPase α1 subunit isoform were identified in Dahl salt-resistant (DR) and Dahl salt-sensitive (DS) rats, respectively. Characterization of the S allele–specific Na^+,K^+-ATPase α1 complementary DNA identified a leucine substitution of glutamine at position 276. This mutation alters the hydropathy profile of a region in proximity to T_3(Na), the trypsin-sensitive site that is only detected in the presence of Na^+. This mutation causes a decrease in the rubidium-86 influx of S allele–specific sodium pumps, thus marking a domain in the Na^+,K^+-ATPase α subunit important for K^+ transport, and supporting the hypothesis of a putative role of these pumps in hypertension.

THE SODIUM- AND POTASSIUM-DEpendent adenosine triphosphatase (Na^+,K^+-ATPase) maintains the Na^+ electrochemical gradient across the plasma membrane by actively transporting Na^+ and K^+ ions (*1–3*). Maintenance of this Na^+ gradient is critical to other vectorial transport systems of the cell (*4*). The Na^+,K^+-ATPase therefore plays a central role in general and specialized cellular functions such as regulation of cellular volume and pH; ion and solute uptake in the kidney, intestine, liver, and bone; nerve impulse; and muscle contraction, to name a few (*5*). Three α subunit isoforms (*6, 7*) and two β subunit isoforms of Na^+,K^+-ATPase (*8–10*) with varying αβ cognate pairing in different tissues (*10–12*) have been identified, providing a versatile mechanism for the observed functional diversity. DS rats are salt-sensitive, as shown by hypertension induced by high salt (8%) diets, in contrast to the relative salt resistance of DR rats, marked by normotension despite high salt (8%) diets (*13*). We therefore investigated the possibility of a genetic difference in Na^+,K^+-ATPase activity in these rats.

Restriction fragment length polymorphism (RFLP) analysis (Fig. 1A) of genomic DNA from DS and DR rats identified homozygosity for a Na^+,K^+-ATPase α1 allele (S allele) with a 2.5-kb Pst I restriction fragment in DS rats; this is in contrast to the R allele, which is shown by a 3.0-kb Pst I restriction fragment in DR rats. Heterozygosity was marked by the presence of both the 2.5- and 3.0-kb Pst I restriction fragments in equal amounts in two non-inbred rat strains tested: Sprague-Dawley, from which the Dahl rats were derived, and Wistar rats (*14*). This Pst I RFLP is specific for Na^+,K^+-ATPase α1 gene, as the hybridization patterns of α2 and α3 Pst I restriction digest genomic fragments were unequivocally different from those of α1 (Fig. 1B).

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Analysis of the nucleotide sequence of the 2.5-kb Pst I genomic fragment from the S allele confirmed specificity for and localization within the Na^+,K^+-ATPase α1 transcription unit (*14*).

To determine whether there were functional differences between S- and R-specific α1 Na^+ pumps, equal amounts of α1 mRNA from kidney polyadenylated [poly(A)$^+$] RNA [an abundant source of α1 mRNA (*11*) and protein (*15*)] from age-matched normotensive DS and DR rats were microinjected (75 to 100 ng per 50 nl) into *Xenopus* oocytes (*16–18*). Equality of nondegraded α1 mRNAs was ascertained by Northern blot analysis and densitometric quantitation of α1-specific hybridizing signal (Fig. 2A). After 3 days of incubation, ouabain-sensitive rubidium-86 ion ($^{86}Rb^+$) influx was assayed (*19*) in the presence of 20 μM monensin, 140 mM Na^+, and 5 mM Rb^+ with and without 1 mM ouabain. The oocytes microinjected with homozygous R (RR) RNA had greater ouabain-sensitive $^{86}Rb^+$ influx as compared to the oocytes microinjected with homozygous S (SS) RNA (Fig. 2B). Results from three independent experiments were concordant, showing that the 20-min time point of incubation (20°C), SS-specific $^{86}Rb^+$ influx was 25 to 45% of RR-specific $^{86}Rb^+$ influx (Table 1). These results have been confirmed in another experimental system: ouabain-sensitive $^{86}Rb^+$ influx measurements between age-matched normotensive DS and DR rat erythrocytes (*20*).

To determine whether the decreased $^{86}Rb^+$ influx of the S-specific α1 Na^+ pump

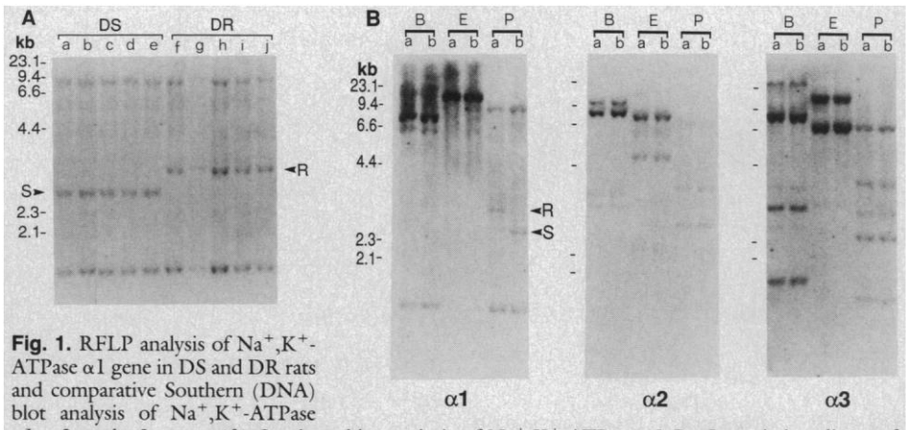

Fig. 1. RFLP analysis of Na^+,K^+-ATPase α1 gene in DS and DR rats and comparative Southern (DNA) blot analysis of Na^+,K^+-ATPase α1, α2, and α3 genes. (**A**) Southern blot analysis of Na^+,K^+-ATPase α1 Pst I restriction digest of genomic DNA from DS and DR rats detects a RFLP identifying the S allele marked by a 2.5-kb Pst I restriction fragment (S), and the R allele marked by a 3.0-kb Pst I restriction fragment (R). Five of 15 animals tested for each group, (a through e) DS and (f through j) DR, are shown. DNA molecular weight markers (in kilobases) are shown on the left. (**B**) A panel of three Southern blots showing distinct isoform-specific genomic restriction fragments for Na^+,K^+-ATPase α1, α2, and α3 genes. The Pst I RFLP (R) and (S) is detected only in the Na^+,K^+-ATPase α1 Southern blot. Restriction enzymes used: Bam HI (B), Eco RI (E), Pst I (P); genomic DNA obtained from DR (a), and DS (b) rats. The isoform-specific α subunit hybridization probes used were: α1, 1.8-kb Eco RI–Bam HI restriction fragment; α2, 3.0-kb Eco RI–Eco RI cDNA; α3, 3.0-kb partial Sac I–Eco RI restriction fragment (*12*). Southern blot analysis was performed as described (*7*) with three 15-min washes at 65°C, in 0.1× saline sodium citrate, 0.1% SDS.

was caused by a structural mutation or differences in gene expression, parallel Northern (*12*) and immunoblot (*21*) analyses were done on age-matched normotensive DS and DR rat kidney RNA and membrane preparations (Fig. 3). Equal size and amounts of α1 mRNA and protein were detected in DS and DR rats, indicating that the differences in Na^+ pump activity could not be accounted for by a difference in the levels of gene expression of S and R alleles, and that there was no observable size difference between mRNAs or polypeptides encoding the two alleles.

A DS kidney cDNA library was then made and screened (*22*) with the previously cloned α1 and β1 cDNAs as hybridization probes (*12*). Full-length cDNA clones for the S alleles of these isoforms (S-α1 and S-β1) were isolated and characterized. S-β1 revealed a nucleotide sequence within the amino acid coding region identical with the previously published (*8*) β cDNA sequence (*14*). The amino acid coding region of the S-α1 cDNA showed three nucleotide substitu-

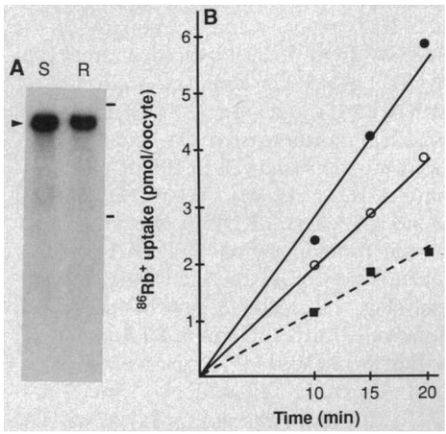

Fig. 2. $^{86}Rb^+$ influx assays of S- and R-specific α1 sodium pumps in *Xenopus* oocytes microinjected with kidney poly(A)$^+$ RNA (*21*). (**A**) Northern analysis of kidney poly(A)$^+$ RNA from DS (*S*) and DR (R) rats. The nondegraded quality of α1-specific mRNAs (arrowhead) was ascertained by Northern analysis (*12*); amounts were quantitated by densitometry and adjusted accordingly for microinjection of equal S- and R-α1 mRNA into oocytes. The 28*S* and 18*S* ribosomal markers are noted on the right. (**B**) A representative *Xenopus* oocyte expression experiment showing the respective linear plots of ouabain-sensitive $^{86}Rb^+$ uptake of oocytes microinjected with DR rat kidney poly(A)$^+$ RNA (RR). (●); oocytes microinjected with DS rat kidney poly(A)$^+$ RNA (SS) (○); and control oocytes microinjected with water (■). Age-matched 9- to 10-week-old male DS and DR rats were used for all experiments; DS blood pressure was 106 ± 8 mmHg; DR, 100 ± 8 mmHg.

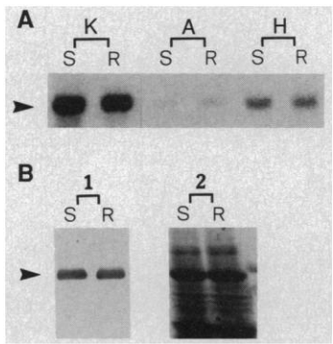

Fig. 3. (**A**) Northern analysis of equal amounts of S and R tissue RNAs determined with a 3.4-kb α1 cDNA probe (*12*). A single mRNA species (≈3.5 kb of mRNA) of Na^+,K^+-ATPase α1 is detected with equal size and amount (arrowhead) in kidney (K), aorta (A), and heart (H). (**B**) Immunoblot analysis, panel 1, of equal amounts of DS (S) and DR (R) kidney membranes (5 μg) detects a single polypeptide, of ≈100 kD, in both S and R membranes of equal size and amount (arrowhead). Panel 2, Coomassie blue–stained lanes of the S and R kidney membranes showing equal amounts of protein loaded for analysis.

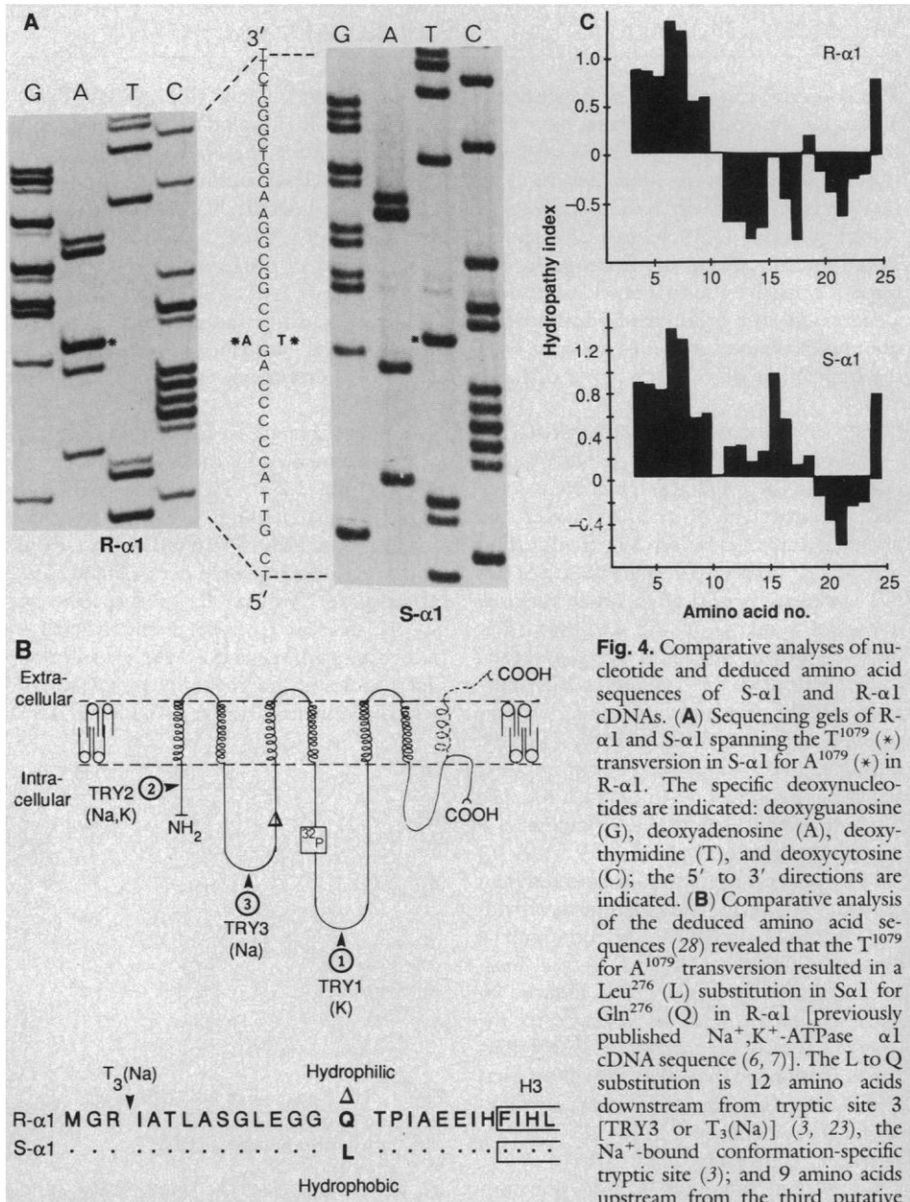

Fig. 4. Comparative analyses of nucleotide and deduced amino acid sequences of S-α1 and R-α1 cDNAs. (**A**) Sequencing gels of R-α1 and S-α1 spanning the T^{1079} (*) transversion in S-α1 for A^{1079} (*) in R-α1. The specific deoxynucleotides are indicated: deoxyguanosine (G), deoxyadenosine (A), deoxythymidine (T), and deoxycytosine (C); the 5′ to 3′ directions are indicated. (**B**) Comparative analysis of the deduced amino acid sequences (*28*) revealed that the T^{1079} for A^{1079} transversion resulted in a Leu^{276} (L) substitution in Sα1 for Gln^{276} (Q) in R-α1 [previously published Na^+,K^+-ATPase α1 cDNA sequence (*6, 7*)]. The L to Q substitution is 12 amino acids downstream from tryptic site 3 [TRY3 or T_3(Na)] (*3, 23*), the Na^+-bound conformation-specific tryptic site (*3*); and 9 amino acids upstream from the third putative membrane-spanning region as deduced from the hydropathy profile, H3 (*6*). The location of this single amino acid substitution in the putative structure [modification of putative structure diagram by Jorgensen (*25*)] of the Na^+,K^+-ATPase α1 subunit polypeptide is marked (Δ); amino terminus (NH_2), carboxyl terminus (COOH) in the intracellular location, and putative extracellular location; the location of the other tryptic sites (*3, 25*); tryptic site 2 (TRY2) detected in the presence of both Na^+ and K^+ and tryptic site 1 (TRY1), specific for the K^+- bound conformation are also indicated. (**C**) Hydropathy profile analysis of this region presented in (B) revealed a marked change in the S-α1 hydropathy profile spanning Leu^{276} to Gln^{276}. A hydrophilic segment in R-α1 spanning amino acid 276 is transformed into an hydrophobic segment in S-α1. The hydropathy index according to the Kyte-Doolittle scale (*26*) is noted on the *y*-axis and the number of the central amino acid in the seven–amino acid window averaging used on the *x*-axis.

tions [T^{1079} for A^{1079}; C^{1680} for T^{1680}; T^{2922} for C^{2922} of the published α1 cDNA sequence (*6*, *7*)] but only one (nt 1079) resulted in an amino acid substitution. The T^{1079} for A^{1079} transversion (*6*, *7*) (Fig. 4A) was observed with two different sequencing systems (*23*). The resulting amino acid substitution in S-α1 Na^+,K^+-ATPase, Leu^{276} instead of Gln^{276} (Fig. 4B), is located 12 amino acids downstream from a well-characterized tryptic site in the Na^+,K^+-ATPase α subunit polypeptide, $T_3(Na)$. As $T_3(Na)$ is exposed only in the presence of Na^+, and not in the presence of K^+, it marks a domain deduced to be involved in one or more conformational changes in the α subunit necessary for ion transport (*24*, *25*). This single amino acid substitution changes the hydropathy profile of the flanking peptide segment (Fig. 4C) from a hydrophilic to a hydrophobic segment as analyzed according to the Kyte-Doolittle scale with a window of seven amino acids (*26*).

To demonstrate unequivocally that the Leu^{276} for Gln^{276} substitution accounts for the decreased K^+ influx, equal amounts of in vitro transcribed RNAs from S- and R-α1 cDNAs [confirmed to have the same sequence as the published α1 cDNA (*6*, *14*)] were then microinjected, respectively, into oocytes in a 1:1 molar ratio with the in vitro transcribed β1 RNA and assayed for Rb^+ influx (*27*). Consistent with the data from oocytes microinjected with S and R kidney poly(A)$^+$ RNA (Fig. 2), the S-specific α1 Na^+ pumps had significantly less $^{86}Rb^+$ influx as compared to R-specific α1 Na^+ pumps (Table 1). Analysis of α1-specific immunoprecipitable products of oocyte membranes (*27*) revealed equivalent amounts of α subunit polypeptide in oocytes microinjected with S- or R-α1 RNA oocytes, both being more than that detected in control oocytes injected with water (Fig. 5).

These observations suggest that the Leu^{276} substitution of Gln^{276} affects K^+ influx of the Na^+,K^+-ATPase, thus marking a domain on Na^+,K^+-ATPase important for K^+ influx. Its location in a region that has been implicated in conformational change during Na^+,K^+-ATPase ion transport, as deduced from the presence of a sodium-bound conformation-specific tryptic site (*3*, *24*, *25*), is consistent with the hypothesis that the change in hydropathy profile of this region might alter the conformational change or changes necessary for normal Na^+/K^+ ion transport, and hence account for the observed decreased K^+ influx. Furthermore, Na^+,K^+-ATPase α3 isoform also has an amino acid substitution at this exact position as compared to α1 and α2 (*6*): a charged Lys residue instead of Gln. The ion transport properties of α3 in comparison to α1 remain to be elucidated.

Further studies will be necessary to elucidate whether this mutation, Gln → Leu^{276}, is involved in the mechanism of Na^+ transport by the Na^+ pump, as well as provide insight into ion transport mechanisms. Our hypothesis is that the S-α1 Na^+,K^+-ATPase is involved in the pathogenesis of hypertension or its sequelae as this mutation was found in a genetic salt-sensitive hypertensive strain. An alteration in Na^+,K^+-ATPase ion transport would affect the Na^+/K^+ electrochemical gradient and conceivably contribute to changes in renal function, vessel wall resistance, or cardiac rhythmogenicity and contractility as described in hypertension. Genetic linkage studies and transgenic animal experiments will be necessary to critically evaluate this hypothesis.

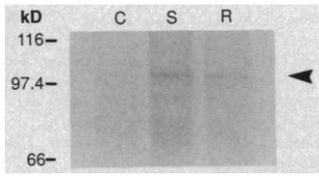

Fig. 5. Immunoprecipitation of rat α1 Na^+ pumps expressed in *Xenopus* oocytes. Autoradiogram of size-fractionated immunoprecipitated α1 subunit protein from oocyte membranes (arrowhead) isolated from [^{35}S]methionine-labeled oocytes microinjected with water as control (C), or with S-α1 in vitro–transcribed RNA (S), or R-α1 in vitro–transcribed RNA. Immunoprecipitation was done with α1-specific monoclonal antibody, MCK-1 (*27*). Cross-reactivity with endogenous *Xenopus laevis* oocyte Na^+ pumps is noted. Molecular mass markers are noted on the left.

Table 1. Ouabain-sensitive $^{86}Rb^+$ influx of S- and R-specific α1 sodium pumps in microinjected *Xenopus* oocytes. Comparison of ouabain-sensitive $^{86}Rb^+$ uptake of S- and R-specific α1 sodium pumps expressed from kidney poly(A)$^+$ RNAs (*16*). Comparison of ouabain-sensitive $^{86}Rb^+$ uptake of S- and R-specific α1 sodium pumps expressed from in vitro transcribed RNAs (*26*). Columns: *n*, number of oocytes; average ouabain-sensitive $^{86}Rb^+$ uptake by exogenous sodium pumps plus or minus standard error; CV, coefficient of variation; *d*, average difference of the means plus or minus standard error. The percent of total $^{86}Rb^+$ uptake comprised by exogenous ouabain-sensitive $^{86}Rb^+$ uptake was an average of 19.45 ± 2.4% for SS and 42.9 ± 4.7% for RR.

Alleles	*n*	$^{86}Rb^+$ uptake (fmol/oocyte)	CV (%)	*d*
		*Kidney poly(A)$^+$ RNAs**		
SS	17	900.8 ± 28.3	12.9	2300.7 ± 245.3
RR	17	3201.5 ± 265.1	34.0	
		In vitro transcribed RNA†		
SS	6	769.3 ± 87.4	27.8	
RR	7	1174.9 ± 71	16.2	

*Paired-sample *t* test: P ($t \geq 8.02$) < 0.001. †Two-sample *t* test: P ($t \geq 3.61585$) < 0.005.

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18. *Xenopus* oocyte expression experiments were as described (*16*, *17*) with the following specifications: all oocytes were mechanically stripped before microinjection and ascertained to be devoid of all vitelline membrane; integrity of oocytes assayed was ascertained visually by the presence of a nonmottled homogeneous pigmented half (*16*) and nonbrittleness; 75 to 100 ng of SS or RR kidney poly(A)$^+$ RNA was microinjected in 50 nl of doubly distilled H_2O (ddH_2O); controls were oocytes microinjected with 50 nl of ddH_2O. Incubation was in modified Barth saline with penicillin and streptomycin for 72 hours.
19. Ouabain-sensitive $^{86}Rb^+$ influx was measured as described (*17*) with the following specifications: we ascertained the integrity of each oocyte, washed them twice with excess cold 5 mM RbCl solution, and subjected them to a quick centrifugation through a dinonyl phthalate:silicon oil cushion [L. H. English, J. Epstein, L. Cantley, D. Housman, R. Levenson, *J. Biol. Chem.* **260**, 1114 (1985)]; β-emissions were measured per oocyte; ouabain-sensitive $^{86}Rb^+$ influx of exogenous Na^+ pumps was measured as total ouabain-sensitive $^{86}Rb^+$ influx minus ouabain-sensitive $^{86}Rb^+$ influx of endogenous *Xenopus* Na^+ pumps measured in control ddH_2O microinjected oocytes; incubation was at 20°C for 5, 10, 15, 20 min (empirically chosen on the basis of linearity of $^{86}Rb^+$ influx in our experimental conditions); for the determination of ouabain-sensitive $^{86}Rb^+$ uptake, we used separate preincubation and incubation media (*17*) containing 1 mM ouabain.

20. V. L. M. Herrera *et al.*, unpublished data. When various concentrations of intracellular Na^+ (5 to 25 mM) were used, $^{86}Rb^+$ influx was consistently less in DS erythrocytes as compared to age-matched DR erythrocytes. By immunoblot analysis only α1 was detected in DS and DR erythrocytes, and the amounts and size of α1 subunit polypeptide were equivalent in both types of rats. Ouabain affinities determined from erythrocyte and kidney membranes were equal in DS and DR.
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23. Nucleotide sequences of the S-α1 were obtained by the Sanger dideoxy-chain termination sequencing method of M13 single-stranded DNA [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977); J. Messing, R. Crea, P. H. Seeburg, *Nucleic Acids Res.* **9**, 309 (1981)] with the use of two sequencing systems: the Klenow fragment of DNA polymerase I (Pharmacia) (*14*) and Sequenase (U.S. Biochemical) shown here. Both sequencing gels revealed the T for A transversion unequivocally. The R-α1 nucleotide sequences were as previously published, obtained by Klenow sequencing of single-strand DNA (shown here) (*7*), and by Maxam and Gilbert sequencing (*6*). DNA and protein analyses were done with the GenePro computer software (Riverside Scientific Enterprises).
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27. In vitro transcribed RNAs were obtained by SP6 RNA polymerase transcription of respective S-α1 cDNA and R-α1 cDNA subcloned into psp73 transcription vector (Promega) according to manufacturer's specifications. Equality of S- and R-α1 transcripts were ascertained by densitometric quantitation of full-length nondegraded transcripts on Northern blot analysis with random-primed ^{32}P-labeled α1 cDNA probe (*14*). *Xenopus* oocyte expression experiments were done as described (*16–18*) with 25 ng of SS- and RR-specific in vitro transcribed α1 RNA with equimolar β1 in vitro transcribed RNA in 50 nl of ddH_2O. Microinjected oocytes were apportioned for $^{86}Rb^+$ uptake assays and immunoprecipitation with the α1-specific monoclonal antibody, MCK-1 (*21*). Immunoprecipitation was done as described (*16, 17*) with the following modifications: 12 hours after microinjection, unhealthy oocytes were removed; only intact oocytes were incubated in fresh modified Barth saline with L-[^{35}S]methionine (1 mCi/ml; specific activity, 1146 Ci/mmol) for 60 hours with one media change; before membrane isolation, oocytes were again checked for integrity; oocyte membranes were isolated by sucrose step-gradient centrifugation; membranes were incubated with MCK-1 antibody overnight at 4°C; affinity-purified goat antibody to mouse immunoglobulin G (CAPPEL) was used for precipitation of the antibody-antigen complexes.
28. Single-letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q. Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y. Tyr.
29. We thank A. V. Chobanian, J. F. Cloix, M. Canessa, and J. Navarro; K. Sweadner for the MCK-1 antibody; and R. Levenson for the β1 cDNA clone. Supported by the National Institutes of Health (HL 01967, HL 39267, and HL 18318) and the American Heart Association Grant in Aid. V.L.M.H. is a Syntex Scholar Awardee.

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Sexual Role Reversal in Mate-Finding Strategies of the Cabbage Looper Moth

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The mate-finding behavior of the cabbage looper moth *Trichoplusia ni* (Lepidoptera: Noctuidae) includes both female- and male-produced sex pheromones used in distinct mate-finding strategies. Both sexes release multicomponent pheromones attractive to the opposite sex. Male pheromone is comprised of *d*-linalool, *m*-cresol, and *p*-cresol released from abdominal hair pencils. Males exposed to host plant odor or to the female sex pheromone (*Z*)-7-dodecen-1-ol acetate are more attractive to females, suggesting stimulation of male pheromone release.

THE MATE-FINDING BEHAVIOR OF the cabbage looper moth *Trichoplusia ni* (Hübner) has been depicted as a system typical of other insects, particularly other moths. The stationary calling female produces a potent species-specific male attractant (*1–3*), and males release possible aphrodisiac pheromones during courtship interactions (*2, 4*). This pheromone communication system was one of the first studied and has served as a model for research on insect sex pheromones. However, we discovered an alternate mate-finding strategy in this species that involves attraction of females to males (*5*). Observations of cabbage loopers in field cages confirmed that male attraction to females and female attraction to males constitute separate mate-finding strategies. Female visitation at cages of males occurred principally at dusk, whereas male visitation at cages of females occurred in the fourth to ninth hours of the 10-hour night (*6*).

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We report here the identification of a male cabbage looper pheromone isolated from hair pencils that is attractive to females. We show that males release this pheromone in response to female pheromone and that males in an airstream of host odor or female pheromone are more attractive to females. Such behavior, as well as male attraction to host plants and scent-marking on host plants, suggests a resource-based mating strategy, with host plants as natural sexual rendezvous sites. We have identified a long-range sex attractant from a male moth and documented host plant kairomonal enhancement of male attractiveness in *Lepidoptera*.

Because we previously isolated material attractive to females from male cabbage looper hair pencils (*5*), hexane extracts of male cabbage looper hair pencils were fractionated with capillary gas chromatography (GC). Flight tunnel bioassays of GC fractions showed that maximum attraction of unmated females required the combination of three fractions. Attraction response rates were 0, 0, 13, and 44% for fractions 1, 2, 3, and the three fractions combined respectively ($n = 45$), with attraction to combined fractions significantly higher (Duncan's new multiple range test, $P \leq 0.05$, after arcsine transformation of the data). Spectroscopic analyses of compounds in these fractions resulted in the identification of the active components as *d*-linalool, *m*-cresol, and *p*-cresol (*7*). Bioassay results confirmed that all three compounds were required to entice the cabbage looper moths to fly upwind for contact with the pheromone dispenser. Thirty-seven percent of 35 moths were attracted to contact the dispenser baited with all three compounds, compared to none for cresols, 9% for *d*-linalool, and 34% for hair pencil extract. The configuration of *d*-linalool was established via chiral derivitization techniques (*7*).

Initial attempts to collect pheromone released by males were unsuccessful. We hypothesized that males may be stimulated to release pheromone by host plant chemicals, by female pheromone, or by both. In a previous study of cabbage looper moth attraction to cabbage plants [*Brassica oleraceae* (L.)] (*8*), males arriving at plants called and scent-marked. Also, male cabbage loopers exhibit full hair pencil displays upon approaching calling females (*9, 10*), possibly releasing the same pheromone blend we isolated from these hair pencils. Experiments were conducted to determine if male pheromone release is stimulated by host plant odor or by female pheromone and if the pheromone released is the attractant we isolated from extracts of hair pencils.

In flight tunnel experiments, noncompetitive comparisons were made of unmated female *T. ni* responses (upwind-oriented flight and contact) to males, potted cabbage