These results are of considerable interest for the interpretation of the Neolithic elm decline. First, they show that the death of elm trees by disease results in a very similar proportional reduction in elm pollen to that found in fossil samples. Second, they indicate that the increased diversity of fossil pollen types at the time of the elm decline, particularly those types often associated with human disturbance, such as *Plantago laceolata*, may come about as a result of the alteration of those physical conditions, such as tree canopy structure, which previously restricted their pollen dispersal.

That weed pollen first comes into prominence in fossil pollen diagrams at the Neolithic elm decline could therefore be a consequence of the loss of elm trees and the resulting general effect on pollen dispersion. Evidence for forest clearance and cultivation before the elm decline is becoming increasingly strong and fits in well with this hypothesis[14,15]. This possibility also weakens the case for a man-induced elm decline, which is based on just such circumstantial evidence. The argument for a disease-induced elm decline in Neolithic times (once strongly rejected by Heybroek[16]) has been strengthened by the historical and dedrochronological work of Rackham. It has also been given greater credence by the recent finding of the beetle vector in Neolithic deposits in Hampstead, London[17], although one beetle does not prove the existence of the disease. The data presented here, however, show that an elm disease could generate pollen changes consistent with those observed in fossil pollen diagrams.

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Ligand binding to the β-adrenergic receptor involves its rhodopsin-like core

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Recently the genes for several hormone receptors that interact with guanine nucleotide binding proteins (G proteins) have been cloned, including the hamster β_2-adrenergic receptor (β_2AR)[1], a human βAR[2], the turkey erythrocyte βAR[3] and the porcine muscarinic acetylcholine receptor (MAR)[4]. All these receptors share some amino-acid homology with rhodopsin, particularly in 7 hydrophobic stretches of residues that are believed to represent transmembrane helices[5]. To determine whether differences in ligand specificity result from the divergence in the sequences of the hydrophilic regions of these receptors, we have expressed in mammalian cells genes for the wild-type hamster and human βAR proteins, and a series of deletion mutant genes of the hamster β_2AR. The pharmacology of the expressed receptors indicates that most of the hydrophilic residues are not directly involved in the binding of agonists or antagonists to the receptor. In addition, we have identified a mutant receptor that has high agonist affinity but does not couple to adenylate cyclase.

In Fig. 1 the amino-acid sequences of the hamster β_2AR, a human βAR and the turkey βAR, all of which bind catecholamines, are compared with those of the porcine MAR and bovine opsin, which interact with acetylcholine and retinal, respectively. The hamster β_2AR and the turkey βAR both couple to adenylate cyclase but differ in their specificity towards catecholamines[6]; the hamster β_2AR being of the β_2-subclass and the turkey βAR of the β_1-subclass. Previously, the pharmacology of the human receptor was unknown. Each group of receptors modulates different intracellular second messengers through interactions with specific G proteins; G_s for the βARs, G_o and G_i for MAR[7] and transducin for opsin[8]. The different receptors vary in amino-acid sequence mostly in their C-terminal regions and their putative third intracellular segments. To investigate the role of these hydrophilic regions in the binding of ligands by the hamster β_2AR, we constructed the series of deletion mutants listed in Table 1.

The genes for the hamster β_2AR, its human homologue and the deletion mutants were inserted into the SV40-derived vector pSVL as described in Fig. 2, legend. Transfection of the expression vector clones containing either the human or the hamster βAR cDNAs into COS-7 (ref. 9) cells resulted in an increase in the specific binding of the β-adrenergic antagonist iodocyanopindolol (^{125}I-CYP) to plasma membranes prepared from the cells (Fig. 2, legend). The expressed βARs displayed affinities for ^{125}I-CYP of 30-50 pM (see Table 1), approximating that of the naturally occurring β_2AR in control COS-7 cells and equivalent to that reported for purified receptor[6,10]. Agonist competition binding experiments (Fig. 2*a*) revealed that the β-adrenergic ligand binding sites resulting from expression of either the hamster or the human gene were indeed of the β_2-subtype, with the relative affinities for agonists being isoprenaline >adrenaline >noradrenaline, in agreement with published values[10].

The expression of the genes encoding the mutant βARs resulted in increases in both ^{125}I-CYP and isoprenaline binding sites (see Table 1). All the expressed mutant receptors were capable of binding ^{125}I-CYP with wild-type affinities with the exception of HAM(del 274-330) which lacked binding and HAM(del 179-187) which bound antagonist and agonist with reduced affinity. HAM(del 274-330) lacks the region encompassing the transmembrane helices VI and VII and HAM(del 179-187) has a deletion within the third external segment. A deletion at the N terminus, HAM(del 21-30), removal of 65 C-terminal residues, HAM(trun 354), and elimination of the third intracellular region, HAM(del 229-236) and HAM(del 239-272), did not dramatically alter ligand binding.

Membrane proteins were electrophoresed and immunoblotted with antisera raised against a peptide corresponding to the C-terminal sequence of the hamster β_2AR protein (Fig. 2, legend). For cells transfected with the wild-type hamster β_2AR gene (plasmid pSVHAM) a strongly reactive band with an apparent relative molecular mass of 67,000 (M_r 67K) was observed which comigrated with purified β_2AR (Fig. 2*b*). A similar immunoreactive band was barely visible for cells transfected with the plasmid pSVL alone, corresponding to the endogenous COS-7 cell β_2AR. In addition to the 67K band,

Table 1 Ligand binding to mutant β_2AR proteins

Protein	Region	COS-7 cells			L cells					
		^{125}I-CYP		Isoprenaline	^{125}I-CYP		Isoprenaline		Adenylate cyclase	
		B_{max} (fmol mg^{-1})	K_D(pM)	EC_{50}(nM)	B_{max} (fmol mg^{-1})	K_D(pM)	$R_H:R_L$	K_D(H): K_D(L)(nM)	K_{act}(nM)	Stimulation
Control		48	20	ND	0	—	—	—	—	0 (5)
HUM		2,000	50	300	ND	ND	ND	ND	ND	ND
HAM		3,300	45	300	110	70	55:45	1:50	20	3-6 (6)
HAM(del 21-30)	O1 (N)	2,200	60	300	510	ND	45:55	7:160	10	2 (1)
HAM(del 179-187)	O3	4,400	600	10,000	610	430	55:45	60:7,100	220	5 (1)
HAM(del 229-236)	i3	3,100	60	100	840	ND	60:40	8:190	5	3 (1)
HAM(del 239-272)	i3	3,100	30	10	200	10	100:0	6	—	0 (3)
HAM(del 250-259)	i3	1,600	ND	ND	180	ND	70:30	3:190	30	3 (1)
HAM(del 259-262)	i3	4,100	ND	ND	130	ND	70:30	1:190	50	3 (1)
HAM(del 274-330)	VI-VII	70	ND	ND	ND	ND	ND	ND	ND	ND
HAM(del 343-348)	i4	3,000	ND	ND	73	ND	55:45	1:70	40	4 (1)
HAM(trun 354)	i4 (C)	1.300	50	300	350	150	70:30	2:210	10	2-6 (5)

Membranes were prepared from COS-7 cells and L cells expressing wild-type or mutant βAR, and ligand-binding and adenylate cyclase assays were performed as in Figs 2 and 3. Names of mutant proteins are as in Fig. 2, and of the regions deleted as in Fig. 1. Values of K_D were determined for ^{125}I-CYP binding by Scatchard analysis[17] based on a single class of binding sites. Where no K_D is shown, the density of binding sites was determined in the presence of a single concentration of 225 pM ^{125}I-CYP. In all cases, nonspecific binding was <5%. Measurements of isoprenaline binding and of adenylate cyclase stimulation were as in Fig. 3. For COS-7 cell membranes EC_{50} values were obtained by visual inspection of binding isotherms. For L cells, competition binding data were analysed using the LIGAND nonlinear regression programs of Munson and Rodard[18]. Results are reported for two affinity states of the receptor only in cases where a two-site fit was significantly better than a one-site fit ($P<0.01$). Adenylate cyclase stimulation parameters were determined by nonlinear regression analysis. The fold-stimulation by adenylate cyclase is given as a range in cases where more than one clonal isolate expressing the same mutant βAR was studied. The number in parentheses indicates the number of clonal isolates of each mutant characterized. All data shown are the means of 1-5 experiments, each done in triplicate. Standard errors were <15%. Abbreviations: N, N-terminus; C, C-terminus; ND, not determined; EC_{50}, value at 50% maximal binding; B_{max}, saturation value for ^{125}I-CYP binding to membranes; $R_H:R_L$, ratio of receptors in the high- and low-affinity states.

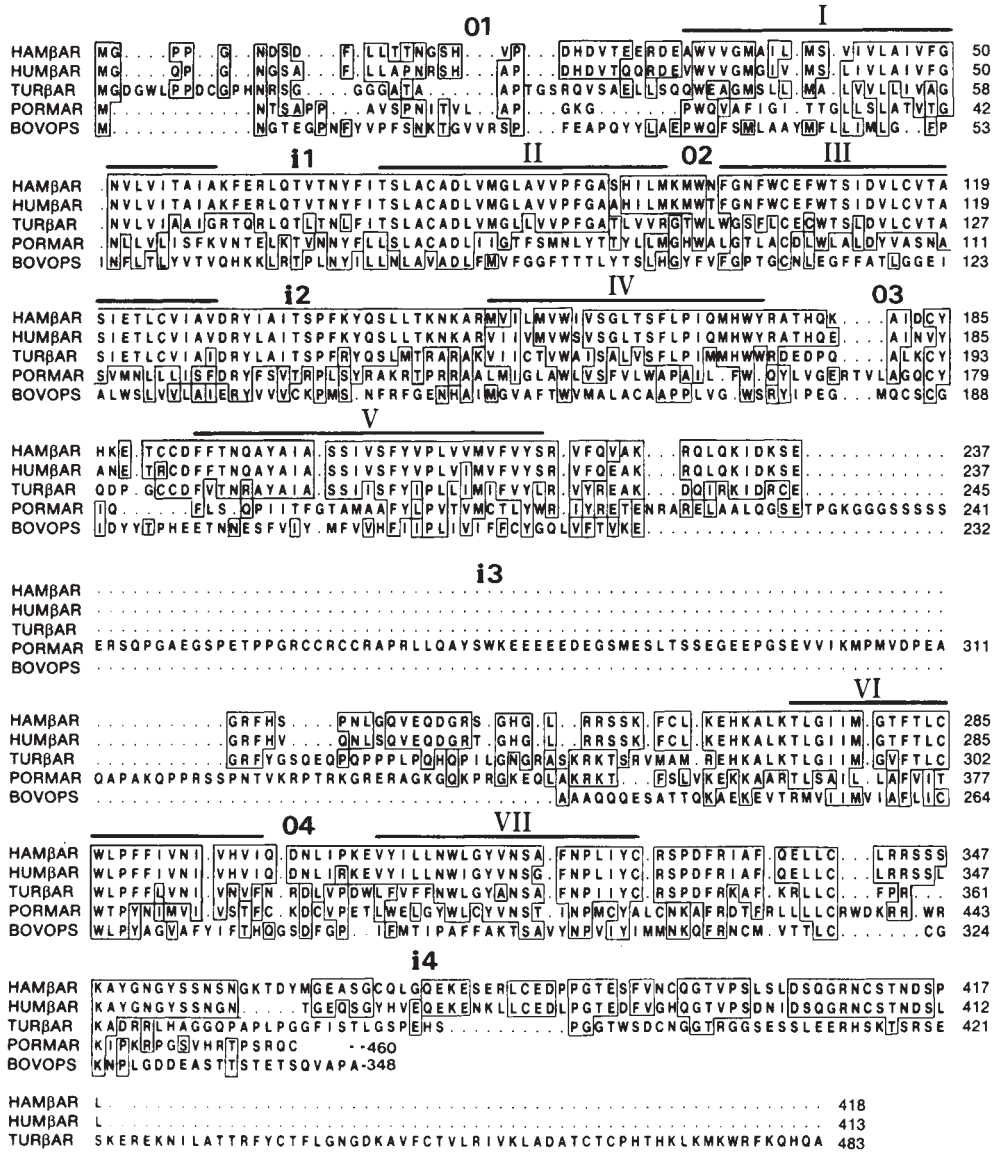

Fig. 1 Comparison of amino-acid sequences for G-protein coupled hormone receptors and rhodopsin. The amino-acid sequences for the hamster β_2-adrenergic receptor (HAMβAR)[1], human β-adrenergic receptor (HUMβAR)[2], turkey β-adrenergic receptor (TURβAR)[3], porcine cerebral muscarinic acetylcholine receptor (PORMAR)[4], and bovine opsin (BOVOPS)[5] are shown. The entire sequence for each receptor is shown with gaps (·) introduced to obtain the best alignment. Amino acids have been aligned to display regions of maximum homology among all the sequences, with identities indicated by boxes around individual amino acids. Regions I-VII correspond to hydrophobic residues postulated to be transmembrane helicies. Based on the rhodopsin model the hydrophilic residues between each transmembrane helix are designated extracellular (O) or intracellular (i) to indicate the proposed orientation of each segment of the protein with respect to the membrane[19].

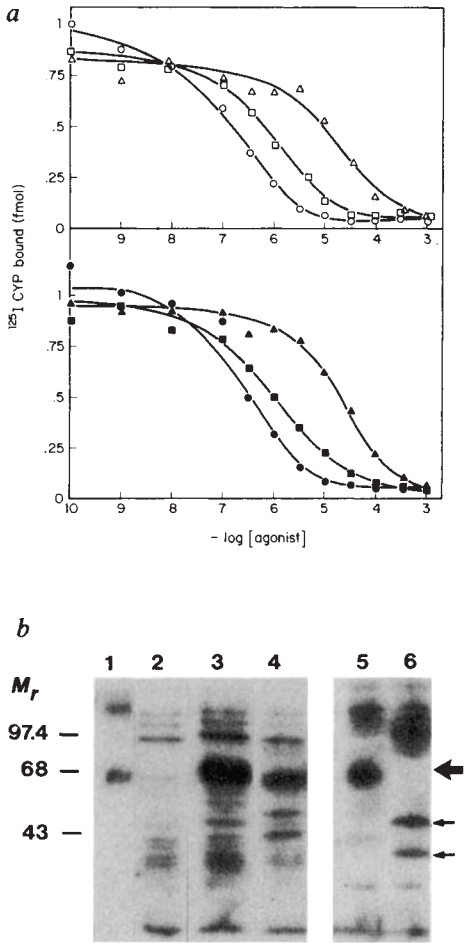

Fig. 2 Expression of wild-type and mutant βAR in COS-7 cells. *a*, Competition for the binding of ^{125}I-CYP by (−)isoprenaline (○), (−)adrenaline (□), and (−) noradrenaline (△) to membranes from COS-7 cells transfected with pSVHAM (open symbols, above) or pSVHUM (closed symbols, below). Total ^{125}I-CYP binding at saturation was 3,300 fmol per mg protein (pSVHAM) and 2,000 fmol mg^{-1} (pSVHUM). Control COS-7 cell transfected with pSVL had 48 fmol per mg protein ^{125}I-CYP binding sites. The data shown are a representative example of three separate experiments, each point performed in duplicate. Nonspecific binding of ^{125}I-CYP in the presence of 10 μM alprenolol was <5%. *b*, Protein immunoblot of βAR-related proteins expressed in cells transfected with pSVL (lanes 2), pSVHAM (lanes 3, 5), pSVHAM(del 239-272) (lane 4) and pSVHAM(del 274-330) (lane 6). Lane 1 contains purified hamster lung β_2AR. Numbers at left, size standards in thousands; large arrow, position of wild-type β_2AR; small arrows, positions of mutant proteins.

Methods. Mutations were introduced into the hamster β_2AR gene (contained in pSVHAM) by oligonucleotide-directed mutagenesis[20]. Deletions were made using oligonucleotides 30 nucleotides (nt) in length containing sequences evenly flanking the desired deletion. These mutants are designated as pSVHAM(del *A*-*B*), where *A* and *B* are the first and last amino acid of the hamster β_2AR sequence included in the deleted region. Single base substitutions were introduced using appropriate mismatched oligonucleotides 16-20 nt in length. The mutant HAM(trun 354) contains a single base substitution converting Tyr 354 to a termination codon. Authenticity was confirmed by dideoxy sequencing[21]. The plasmid pSVL contains the SV40 early promoter and the 16S intron derived from the Okayama-Berg vector pL[22] cloned[23] into the *Hin*dIII and *Pst*I sites of pUC13. The hamster β_2AR cDNA and the deletion mutants were cloned between the *Sma*I and *Eco*RI sites of pSVL as a 1.9-kb fragment originating at the *Eag*I site 86 bp 5′ to the initiator Met and extending 23 bp 3′ to the polyadenylation signal, giving pSVHAM. Similarly, cDNA for the human βAR was cloned into the same sites of pSVL as a 1.8-kb fragment beginning at the *Nco*I site corresponding to the initial Met and encompassing the polyadenylation signal to give pSVHUM. Transfection of plasmids into COS-7 cells was carried out using the $CaPO_4$ precipitation method[24]. Plasmid DNA (15 μg) was mixed with 15 μg of calf thymus DNA and precipitated with 125 mM $CaCl_2$. Precipitates were applied to 10^6 COS-7 cells and incubated at 37 °C for 4 h. Cells were glycerol shocked and incubated in fresh media for 72 h, at which time they were collected by scraping and washing once with phosphate-buffered saline. For a rapid determination of ligand-binding properties of various mutants, COS-7 cells expressing the receptor were lysed by freeze-thawing in 15 mM Tris, *p*H 7.5, 2 mM $MgCl_2$, 0.3 mM EDTA. Membranes were pelleted by centrifugation at 40,000*g* and resuspended at 1-3 mg protein per ml in 75 mM Tris, *p*H 7.5, 12.5 mM $MgCl_2$, 0.3 M EDTA. Saturation binding of ^{125}I-CYP (NEN) was measured at a final protein concentration of 20 μg ml^{-1} using 10-400 pM ^{125}I-CYP, with nonspecific binding defined in the presence of 10 μM alprenolol[25]. Isoprenaline binding was measured in competition with 35 pM ^{125}I-CYP, at a final βAR concentration of 5-7 pM, as described[25]. Protein concentrations were determined by the method of Lowry[26]. Protein immunoblot analysis of the membranes was performed as described[1]. Polyclonal antisera raised against a peptide corresponding to the C terminus of the hamster β_2AR protein was used to detect the βAR-specific polypeptides. A peptide having the sequence CLDSQGRN(norleucine)STNDSPL (amino-acid residues 404-418 of the hamster β_2AR) was synthesized using an Applied Biosystems peptide synthesizer and purified by reversed phase HPLC[1]. This peptide was coupled through its N-terminal cysteine to thyroglobulin (R. Mumford and C.D.S., in preparation) and used as an immunogen in rabbits. Total rabbit serum was used as the first antibody in the immunoblot at a 1 : 500 dilution followed by detection with ^{125}I-protein A as described[1]. Purification of β_2AR was described[10].

specifically immunoreactive proteins with apparent M_r 46K and 55-58K were also observed in the cells transfected with pSVHAM, presumably representing nonglycosylated and partially glycosylated β_2AR proteins, respectively[10]. Immunoreactive bands of higher apparent M_r were occasionally detected and probably represent aggregates of the receptor. HAM(del 239-272), which bound ligand, was expressed at levels similar to that of the wild-type receptor and had the expected M_r. For HAM(del 274-330), which did not bind ligand, immunoreactive peptides of lower M_r were observed. The M_rs of the peptides were consistent with both a deletion in the proteins and a defect in their glycosylation. Whether the lack of ligand binding for this mutant receptor is due to the loss of sequences directly involved in ligand binding or to a defect in protein folding and glycosylation is unknown.

Although the transient expression of the β_2AR genes in COS-7 cells permitted the binding properties of the expressed proteins to be measured rapidly, with minimal selection, the low frequency of expressing cells in combination with the βAR background in this cell line prevented characterization of the coupling of the receptor with the adenylate cyclase system. Consequently, clonal lines of mouse L cells[11] stably expressing the wild-type receptor and mutants which exhibited ligand binding in COS-7 cells, were established by the procedure described in Fig. 3 legend. Mouse L cells were chosen because they have undetectable levels of βAR as assessed by ligand binding (Fig. 3*a*) and they lack a β-adrenergic-stimulated adenylate cyclase, but contain measurable levels of both prostaglandin E_1- and NaF[12]-stimulated adenylate cyclase activity (data not shown). The density of receptor sites for each of the cell lines was measured by ^{125}I-CYP binding (Table 1). Because L cells lack an endogenous βAR background, the K_D values for ^{125}I-CYP and isoprenaline binding to each of the cell lines could be calculated. The affinities for ^{125}I-CYP of wild-type and mutant hamster β_2AR proteins expressed in L cells were similar to those observed in COS-7 cells (Fig. 3*a* and Table 1). In these cells, HAM(del 239-272) had a slightly greater affinity for ^{125}I-CYP than the wild-type receptor and mutant HAM(del 179-187) exhibited the same reduced affinity for ^{125}I-CYP as observed for the COS-7 cells (Fig. 3*a*).

Data for isoprenaline competition binding and stimulation of adenylate cyclase in membranes containing the wild-type and

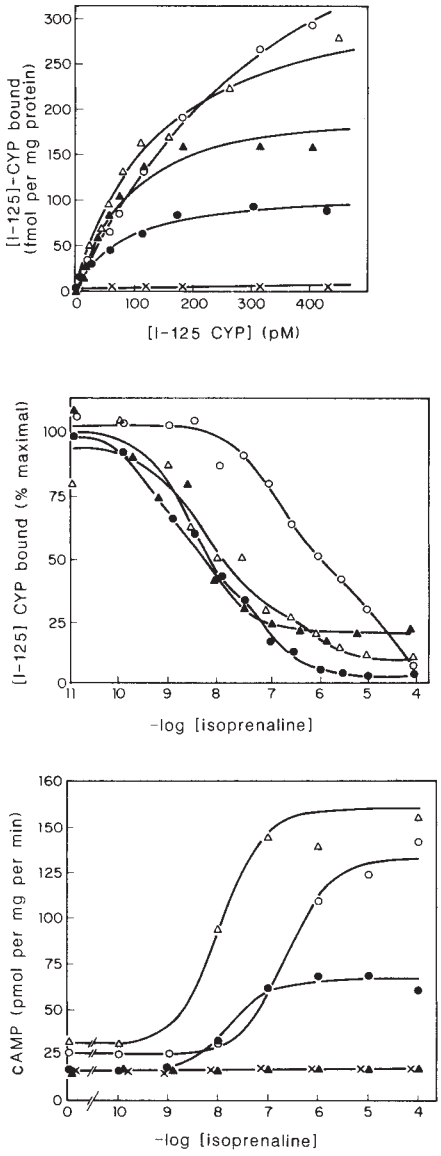

Fig. 3 Saturation binding of ^{125}I-CYP (*a*), isoprenaline competition for ^{125}I-CYP binding (*b*), and isoprenaline stimulation of adenylate cyclase (*c*) in membranes from L cell lines containing pSVL (×), pSVHAM (●), pSVHAM(del 179-187) (○), pSVHAM (del 239-272) (▲), and pSVHAM(trun 354) (△). Adenylate cyclase activity (pmol cAMP per mg per min) in the same membranes stimulated by 10 mM NaF was 46 for pSVL, 87 for pSVHAM, 124 for pSVHAM(del 179-187), 73 for pSVHAM(del 239-272), and 101 for pSVHAM(trun 354). Each experiment shown is a representative of 2-5 experiments for each clone.

Methods. To obtain L cell lines expressing the βAR, each plasmid was cotransfected with 5 μg of pRSVneo[27] as described in Fig. 2. Plasmid-bearing cells were selected by the addition of 500 μg ml^{-1} Geneticin (gentamicin, G-418, Gibco) to the media 24 h posttransfection[28]. Geneticin-resistant colonies (12-24) were picked from each of two separate transfections and assayed for ^{125}I-CYP binding as in Table 1. In all experiments 30-70% of the geneticin-resistant clones co-expressed the βAR. Positive clones (4-10) for each mutant were characterized for ligand binding. Plasma membranes were prepared from L cells by hypotonic lysis in 1 mM Tris, *p*H 7.5. After centrifugation at 40,000*g*, the membranes were resuspended at 1-3 mg protein per ml in 75 mM Tris, *p*H 7.5, 12.5 mM $MgCl_2$, 1.5 mM EDTA. Ligand-binding experiments were performed as described in Fig. 2. Adenylate cyclase stimulation in the presence of isoprenaline or 10 mM NaF was measured as described elsewhere[29].

several of the mutant receptors are shown in Fig. 3*b* and *c*. The wild-type receptor exhibited both high- and low-affinity binding sites for isoprenaline (see Table 1)[13]. The occurrence of two agonist affinity sites has been attributed to an equilibrium between free receptor and receptor complexed with G_s. Membranes prepared from cells expressing the wild-type receptor also demonstrated a dose-dependent activation of adenylate cyclase by isoprenaline and NaF, but membranes derived from the control cells showed stimulation of adenylate cyclase only by NaF. Although the maximum stimulations of adenylate cyclase by isoprenaline varied from 3- to 6-fold for different clonal isolates of each mutant, they were identical to the NaF stimulations in the same membranes (Fig. 3*c*) indicating that the expressed receptors were fully coupled to adenylate cyclase[13,14]. In sharp contrast with the wild-type receptor and HAM(trun 354), HAM(del 239-372) containing membranes exhibited a single high-affinity isoprenaline binding site and did not show any stimulation of adenylate cyclase by isoprenaline (Table 1 and Fig. 3). It is not known whether the affinity exhibited by this site reflects the K_D of the free mutant receptor or of an inactive complex between the receptor and G_s. The lack of adenylate cyclase coupling to this mutant receptor might be due to either the elimination of a site on the receptor for G-protein interaction or to the importance of the deleted region in maintaining an active conformation. HAM(del 179-187), which showed a reduced affinity for isoprenaline, also required higher levels of isoprenaline for cyclase stimulation (Table 1 and Fig. 3). The remaining mutants showed both high- and low-affinity states and stimulated adenylate cyclase by 2-8-fold in an isoprenaline-dependent manner (Table 1). The variation in the absolute level of stimulation between different clonal isolates allows for only a qualitative analysis of receptor-cyclase coupling. Future experiments measuring the stimulation of the GTPase of G_s[14] or the effect of GTP analogues on hormone binding will be necessary to compare quantitatively the abilities of the mutant receptors to couple with G proteins.

The absence of a large hydrophilic domain involved in ligand binding implies that ligands interact either with a site located close to the membrane or within the hydrophobic core of the protein. This hypothesis is supported by the observation that in the case of the turkey βAR, an affinity label bound to the receptor can covalently react with associated glycolipids[15]. Previously we had proposed, on the basis of the homology between the hamster β_2AR and bovine opsin that the β-adrenergic ligands interact with the receptor in a manner analogous to that of retinal interacting with opsin[1]. In rhodopsin retinal binds within the hydrophobic transmembrane region of the protein rather than to external hydrophilic residues[16]. The results described here lend further support to this model.

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The condensing vacuole of exocrine cells is more acidic than the mature secretory vesicle

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A number of intracellular, membrane-bound compartments in both the endocytic and exocytic pathways of eukaryotic cells have an acidic internal *p*H[1]. In endocrine cells, the mature secretory vesicle has an acidic *p*H; secretory vesicles isolated from exocrine cells, however, appear to have a neutral *p*H[2]. Recently we have used a newly developed immunocytochemical technique[3] to map low-*p*H compartments in insulin-secreting islet cells with the electron microscope and find that during the maturation of the secretory vesicle there is a progressive acidification of these vesicles that begins as soon as the *trans* Golgi condensing vacuoles form[4]. Now we have used this technique to examine two exocrine cells: the pancreatic acinar cell and the parotid serous cell. In both cell types, the *trans* Golgi condensing vacuoles are acidic and accumulate the low-*p*H probe to the same extent as condensing vacuoles of insulin-secreting islet cells. Unlike insulin-secreting cells, however, maturation of the granules is accompanied by a return of luminal *p*H to near neutrality. Therefore, although the *p*H of storage granules in exocrine and endocrine cells is different, the *p*H of the condensing vacuoles in both cells is acidic.

DAMP (3-(2,4-dinitroanilino)-3′-amino-*N*-methyldipropylamine) accumulates in acidic compartments and can be localized in plastic-embedded tissue sections with an antibody against dinitrophenol (DNP)[5]. To visualize acidic compartments in exocrine pancreatic cells by indirect immunofluorescence, isolated acini were incubated with 30 μM DAMP, fixed and embedded in Epon. Serial thick sections (0.5 μm thick) were prepared; one section was incubated with anti-amylase serum to localize secretory granules and the adjacent section was incubated with anti-DNP immunoglobulin G (IgG) to localize acidic compartments. DAMP was located in vacuoles that were scattered in the cytoplasm of each cell (Fig. 1*a*). In the companion section that was processed to localize amylase, there were numerous brightly fluorescent granules clustered together in the apex of the cells (Fig. 1*b*). Comparing the two images reveals that most of the amylase-positive granules were not positive for DAMP. Only a few amylase-positive granules just above the nucleus contained DAMP. Therefore, only a subpopulation of the amylase-positive granules appeared to be acidic.

Both amylase and DAMP can be localized in Epon thin sections by an indirect protein A-gold procedure. Figure 1*c* and *d* shows the same juxtanuclear region of an exocrine cell from an acinus examined on adjacent serial thin section (80–100 nm thick) for the presence of DAMP (Fig. 1*c*) and amylase (Fig. 1*d*). The density of anti-amylase serum binding sites in the proteinaceous core of both condensing vacuoles and mature granules was the same. In contrast, only the proteinaceous core of the condensing vacuoles had a high density of anti-DNP IgG binding sites.

These qualitative results were confirmed by a quantitative analysis of the distribution of DAMP-specific gold particles in various cellular compartments of the exocrine cell (Table 1). The density of anti-DNP IgG binding sites over the mature secretory vesicles was nearly the same as the density over the nucleus, which indicates these vesicles were not acidic. In contrast, the density of gold particles in the condensing vacuoles was, on average, ninefold greater than in the mature vesicles. The density of DAMP-specific gold particles in lysosomes was higher than in the condensing vacuole. All anti-DNP IgG binding was abolished if the DAMP-treated acini were post-incubated in the presence of monensin (Table 1). Therefore, in these

Table 1 Density of monoclonal anti-DNP binding sites on pancreatic acinar cells

Cellular compartment	Number of gold particles per μm^2 of cell compartment							
	Experiment 1		Experiment 2				Experiment 3	
			Control		Monensin			
Golgi stack	2 ± 1	(21.58)	3 ± 1	(18.41)	1 ± 0.2	(26.38)	2 ± 1	(17.87)
	$n = 10$		$n = 7$		$n = 10$		$n = 9$	
Condensing vacuoles	55 ± 9	(15.42)	53 ± 12	(10.40)	2 ± 0.6	(16.29)	60 ± 10	(9.87)
	$n = 19$		$n = 11$		$n = 10$		$n = 11$	
Granules	6 ± 1	(19.13)	6 ± 1	(15.02)	2 ± 1	(19.13)	7 ± 1	(15.57)
	$n = 14$		$n = 10$		$n = 10$		$n = 10$	
Lysosomes	111 ± 15	(6.09)	74 ± 11	(4.86)	2 ± 1	(8.93)	99 ± 14	(10.76)
	$n = 15$		$n = 4$		$n = 9$		$n = 9$	
Nucleus	2 ± 0.3	(27.12)	3 ± 1	(27.78)	2 ± 0.3	(36.21)	1 ± 0.3	(30.51)
	$n = 7$		$n = 5$		$n = 7$		$n = 5$	

Values are means ± s.e.m.; *n*, no. of pictures evaluated. The no. of μm^2 evaluated for each compartment is shown in parentheses. Dispersed pancreatic acini, prepared as described[14] were incubated in a KRB-HEPES medium containing 0.1% bovine serum albumin (BSA) in the presence of 30 μM DAMP for 30 min at 37 °C (experiment 1). In experiment 2, the cells were incubated an additional 30 min in the presence or absence of 25 μM monensin. In experiment 3, acinar cells were incubated with DAMP for 2 h. At the end of each incubation, cells were rinsed, fixed for 2 h at room temperature with 1% glutaraldehyde buffered with 0.1 M sodium phosphate *p*H 7.4, dehydrated and embedded in Epon. Thin sections were collected on nickel grids and immunostained by the protein A-gold technique[15]. To localize DAMP, sections were incubated for 2 h with monoclonal anti-DNP antibody (0.1 μg IgG ml^{-1}) followed by rabbit anti-mouse IgG (12 μg ml^{-1}) and protein A-gold (1:70 v/v) for 1 h at room temperature. Sections were stained sequentially with uranyl acetate (20 min) and lead citrate (10 min). The distribution of DAMP in cellular compartments was quantified on electron micrographs at a calibrated magnification of 44,100. The number of gold particles and the areas of respective compartments were recorded with an electronic pen on a graphic tablet (Tektronix, type 4933) connected to a microcomputer (IBM PC) programmed to calculate the number of particles per μm^2 of the compartment.