REFERENCES AND NOTES

1. World Health Organization, *WHO Tech. Rep. Ser.* **752**, 1 (1987).
2. A. Prost, *Trans. R. Soc. Trop. Med. Hyg.* **80**, 525 (1986).
3. W. C. Campbell, M. H. Fisher, E. O. Stapley, G. Albers-Schönberg, T. A. Jacob, *Science* **221**, 823 (1983).
4. M. A. Aziz, S. Diallo, I. M. Diop, M. Lariviere, M. Porta, *Lancet* **ii**, 171 (1982).
5. B. M. Greene *et al.*, *N. Engl. J. Med.* **313**, 133 (1985).
6. M. Lariviere *et al.*, *Lancet* **ii**, 174 (1985).
7. A. T. White *et al.*, *J. Infect. Dis.* **156**, 463 (1987).
8. M. C. Pacqué *et al.*, *Bull. WHO* **67**, 721 (1989).
9. H. R. Taylor and B. M. Greene, *Am. J. Trop. Med. Hyg.* **41**, 460 (1989).
10. E. W. Cupp *et al.*, *Science* **231**, 740 (1986).
11. J. Remme *et al.*, *Trop. Med. Parasitol.* **40**, 367 (1989).
12. M. Trpis *et al.*, *Am. J. Trop. Med. Hyg.* **42**, 148 (1990).
13. We are grateful to P. N. Williams, K. Gerhart, J. Belcher, J. Barchue, and the staff of LAC, and A. P. Hanson of the Liberia Institute for Biomedical Research for their support and assistance. Supported by funds from the United Nations Development Programme/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and from the NIH.

14 May 1990; accepted 30 July 1990

Extrageniculate Vision in Hemianopic Humans: Saccade Inhibition by Signals in the Blind Field

R. RAFAL,* J. SMITH, J. KRANTZ, A. COHEN, C. BRENNAN

The functional competence of extrageniculate visual pathways in hemianopic humans was demonstrated by showing that distractor signals in the blind half of the visual field could inhibit saccades toward targets in the intact visual field. This inhibitory effect of unseen distractors in patients occurred only when distractors were presented in the temporal half of the visual field, was specific to oculomotor responses, and did not occur in normal subjects. These results show that a peripheral visual signal activates retinotectal pathways to prime the oculomotor system and that these pathways can mediate orienting behavior in hemianopic humans.

THE ENCEPHALIZATION OF VISUAL function in the cerebral cortex is a relatively new development in phylogeny. The geniculostriate pathway is fully developed only in mammals. The dominance of this pathway in human vision over the older retinotectal pathway to the midbrain is striking in neurologic patients who have suffered complete unilateral destruction of the striate cortex or its geniculostriate afferents. They are blind in the half of the visual field contralateral to the lesion and cannot see even salient signals within the scotoma (blind area).

However, some visual processing may be preserved in the hemianopic field. Researchers have demonstrated this "blindsight" by requiring hemianopic subjects to move their eyes or reach toward signals that they cannot "see" and by using forced-choice discrimination tasks (*1*). Although light-scatter artifact (*2*) has been excluded as the cause for at least some of these effects (*3*), the physiologic mechanisms of blindsight remain uncertain. In some patients, residual vision may be mediated by spared geniculostriate fibers and could reflect degraded cortical vision near the perceptual threshold (*4*). On the other hand, some blindsight phenomena may reflect processing of visual input from retinotectal afferents to the superior colliculus (*5*). The current investigation shows that signals in the hemianopic field activate the oculomotor system and that retinotectal pathways can mediate orienting behavior in hemianopic humans.

As a test of extrageniculate mediation, we exploited a lateralized neuroanatomic arrangement of retinotectal pathways that distinguishes them from those of the geniculostriate system. When compared to the geniculostriate system, the retinotectal pathway has more crossed fibers from the contralateral eye, and the temporal hemiretina (nasal hemifield) has a smaller direct input to the superior colliculus. In cats, this pathway is almost entirely monocular (*6*), and cats with bilateral occipital ablations in which extrageniculate vision is restored by intercollicular section orient only toward signals in the temporal hemifield (*7*). In monkeys, this anatomic asymmetry is much less complete (*8*). Nevertheless, the functional relevance of this anatomic asymmetry in humans was shown by demonstrating that newborns (in whom the geniculostriate pathways are not developed) have a strong bias to saccade to signals in the temporal hemifield (*9*). Even in adults, the bias to saccade toward the temporal hemifield persists under conditions of bilateral, simultaneous stimulation

Table 1. Median saccade latency in milliseconds for each patient. Data are expressed as median ± SEM.

Patient	Distractor-target interval (ms)				
	−500	0	50	150	250
			Temporal distractor		
1	326 ± 25	372 ± 18	360 ± 17	265 ± 18	273 ± 14
2	368 ± 28	415 ± 23	395 ± 19	239 ± 19	328 ± 35
3	268 ± 11	291 ± 12	269 ± 8	264 ± 11	265 ± 12
			Nasal distractor		
1	299 ± 14	282 ± 11	291 ± 14	243 ± 16	258 ± 18
2	358 ± 20	324 ± 14	357 ± 15	316 ± 25	321 ± 37
3	278 ± 8	276 ± 11	256 ± 10	284 ± 12	278 ± 9

Table 2. Mean reaction time in milliseconds for the hemianopic patients in the key press task. Data are expressed as mean ± SEM.

Distractor-target interval (ms)				
−500	0	50	150	250
		Temporal distractor		
458 ± 64	413 ± 60	403 ± 62	381 ± 28	418 ± 46
		Nasal distractor		
448 ± 66	446 ± 66	377 ± 46	372 ± 69	376 ± 68

R. Rafal, J. Smith, J. Krantz, Department of Clinical Neurosciences, Brown University, Providence, RI 02908.
A. Cohen, Department of Psychology, Indiana University, Bloomington, IN 47405.
C. Brennan, Cornell University School of Medicine, New York, NY 10021.

*Current address: Department of Neurology, University of California, Davis, and Martinez Veterans Administration Medical Center, 150 Muir Road, Martinez, CA 94553.

(*10*). This hemifield asymmetry is specific to oculomotor responses; no temporal hemifield advantage was found in a perceptual (temporal-order judgment) task. Saccades produce a subsequent inhibition of responses to signals at a recently attended location (*11*). This "inhibition of return" is abolished by midbrain lesions in patients with progressive supranuclear palsy (*12*), and in normal subjects it is enhanced by visual stimulation of the temporal hemifield (*11*). In the current study, we measured the effect of unseen distractor signals presented in the temporal and nasal blind hemifield of hemianopic patients (*13*).

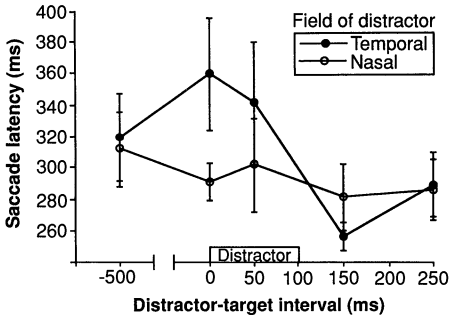


Fig. 2. Mean latency of the saccades to targets in the intact visual field, for three hemianopic patients, as a function of the interval between onset of the unseen distractor and onset of the target. Analysis of variance (ANOVA) reveals an interaction between the field of the distractor and the distractor-target interval [$F(4,8) = 5.04$, $P = 0.025$], which is due to an effect of interval in the temporal distractor condition [$F(4,8) = 4.65$, $P < 0.05$]; the effect of interval in the nasal distractor condition is not significant [$F(4,8) = 1.32$, P = not significant]. An ANOVA of the data from the temporal distractor condition comparing the no-distractor (−500 ms) and the distractor present (0 ms and 50 ms) intervals confirmed that saccade latency with the distractor present was longer than with no distractor [$F(2,4) = 12.37$, $P < 0.025$]. Data are mean ± SEM; $n = 3$.

We studied three neurologic patients and ten neurologically intact subjects who volunteered to participate after giving informed consent. All three patients had dense, homonymous hemianopia from a stroke in the distribution of the calcerine branch of the posterior cerebral artery that involved striate cortex (Fig. 1). These patients were totally unaware of a hand being waved in their hemianopic field until it crossed the midline (*14*). To ensure that the patients were blind to the distractors to be presented in the hemianopic visual field, they were first tested, at the beginning of each experimental session, on a present-absent screening task of 80 trials. They maintained fixation on a "+" at the center of the screen. After a warning signal, a bright "*" target, 1.8° in diameter, was presented in the blind hemifield at an eccentricity of 10°. The subjects were told that the target would appear in half of the trials. All denied seeing the target at any time. When the experimenter required that a "yes" or "no" response be given in every trial, the subjects were reluctant to give anything but a "no" response; and when the experimenter further required that they guess "yes" half the time, none guessed at better than chance level. In the subsequent experimental sessions, the signals were of the same size and eccentricity as those used in this screening procedure, and the luminance used was less than that in the screening test (*15*).

The patients were then tested under monocular conditions in ten blocks of 50 trials each (*16*). The eye occluded by a patch was alternated across blocks so that the effects of distractors in the blind temporal hemifield could be compared with the effects of distractors presented to the nasal hemifield. The display consisted of a + that remained constantly present in the center of the display, flanked by two dim, unfilled, white squares. The squares subtended 1.8° of visual angle across and were positioned 10° to the right and left of the + (although, of course, the subjects only saw one of these boxes). After an intertrial interval that varied randomly from 1000 to 1800 ms, both boxes brightened for 100 ms, either simultaneously or with a variable onset time interval. The subjects' task was to maintain fixation on the + in the center of the screen and to make a saccade quickly to the box as soon as they saw it brighten in the intact visual field (*17*).

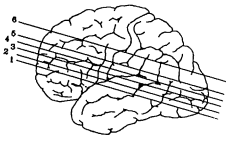
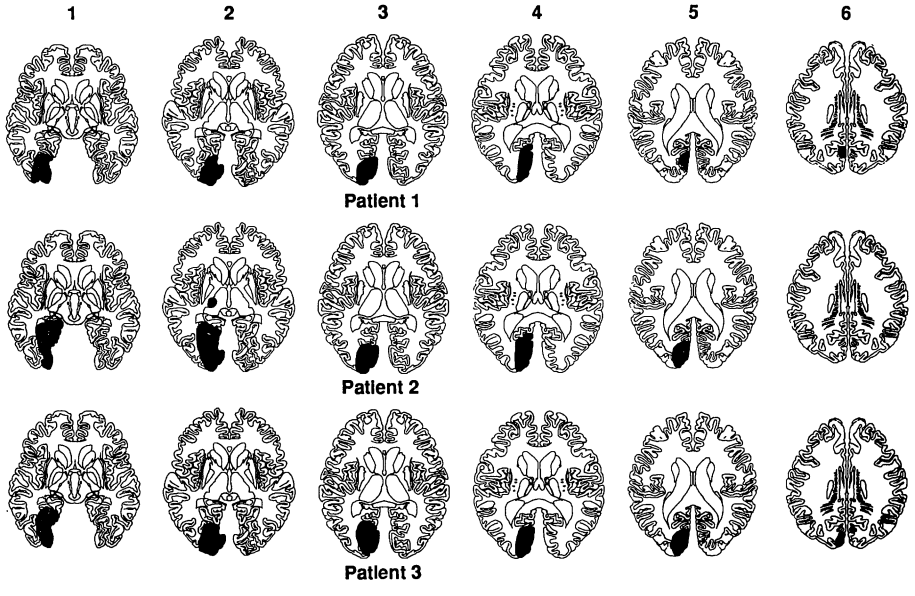


Fig. 1. Reconstructions of the brains of three patients with dense homonymous hemianopia. Extent of lesion is shown in black for each patient. The lines on the lateral view indicate the corresponding axial cuts. The reconstructions (*22*) are based on computerized tomography scans in patients 1 and 3, and from magnetic resonance imaging scans in patient 2.



Although both boxes brightened on every trial, in 20% of trials, the onset of the distractor in the blind field occurred 500 ms after that of the target in the normal field. Because nearly all saccades occurred with a latency of less than this, these trials in effect constituted a no-distractor condition; that is, the signal in the blind hemifield occurred too late to affect the latency of a saccade to the target in the intact field. In 20% of trials, the two boxes brightened simultaneously, and, in the remaining 60% of trials, the distractor onset occurred 50, 150, or 250 ms before the target. In half of the blocks, the hemianopic field in which the distractor was presented was temporal, and in the other half it was nasal.

None of the three patients ever saw the box brighten in the hemianopic field. In the no-distractor conditions, there was no statistically significant difference between the temporal and nasal presentation blocks (Fig. 2). There was no statistically significant effect of distractors presented in the nasal hemifield. However, when the unseen distractor was presented in the temporal hemifield, either simultaneously with or 50 ms preceding the target, there was an increase in saccade latency relative to the no-distractor condition for all three subjects (Table 1).

These findings in hemianopic humans are consistent with other observations (*10*) that

indicate that an eccentric luminance change activates a retinotectal pathway that biases or primes the oculomotor system to make a saccade. This activation of the oculomotor system by the distractor inhibits the saccade to the contralateral target and increases its latency. To confirm that this effect in our hemianopic patients was specific to oculomotor activation, we also tested them in a simple detection task with no eye movements. The display was identical, but the subjects' task was to maintain steady fixation on the center of the display and to make only a simple reaction time key press response with the index finger of their right hands when they detected brightening of the target. If the inhibitory effect found in the saccadic task were not related specifically to oculomotor activation but instead to a covert shift of attention to the distractor that delayed perception of the target, the same inhibitory effect would be expected in the simple detection task. There were no significant effects or interactions, and no inhibitory effect of the distractor (Table 2). Reaction times were not slower for any of the distractor trial conditions than for the no-distractor conditions. Rather, in contrast to the saccadic task [and consistent with previous findings in some hemianopic patients (*18*)], there was a tendency for distractors to produce faster responses in this detection task.

The contrast between the results of the saccadic and key press tasks indicates that the inhibitory effect found in the saccade task was not due to covert orienting of attention to the distractor but was specific for the condition where eye movements were made (*19*). The specificity of the inhibitory effect for the oculomotor task is consistent with collicular mediation. Moreover, the response dependence of the effect excludes the possibility that the effect was due to imperfections in the presentation of the stimuli (for example, light scatter to the normal field), since the stimuli used in the saccadic task and in the key press task were identical and the same subjects participated in both studies.

To obtain further evidence that the inhibitory effect on saccade latency found in the hemianopic patients was mediated by extrageniculate pathways, we tested ten normal subjects (in whom the geniculostriate pathways were intact) in the saccadic task experiment. There were no significant effects of distractors on latency of the saccade, nor was there any asymmetry between performance with distractor presentation in nasal and temporal hemifields (Fig. 3). We can be confident, therefore, that the asymmetric effects of temporal and nasal distractor presentation in the hemianopic patients was not due to a cortically mediated subliminal perception. In fact, the effect found in hemianopic patients may be critically dependent on the absence of perceptual awareness of the distractor and may reflect the activity of an isolated extrageniculate visual system (*20*).

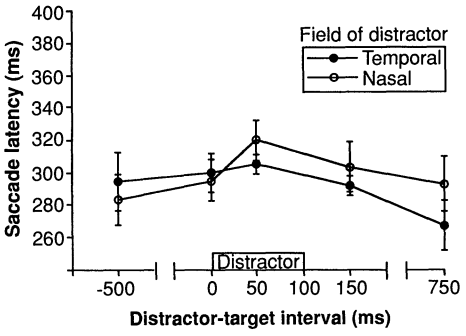


Fig. 3. Mean latency of the saccades for ten normal subjects as a function of the interval between onset of the distractor and onset of the target. Data are mean ± SEM; $n = 3$.

Our observations indicate that blindsight in hemianopic humans may be mediated by visual information through retinotectal pathways. Collicular mediation of the effect is supported by the fact that it occurred in patients with no awareness of visual signals in their hemianopic fields (as confirmed in the screening task), was specific to oculomotor responses, and was not manifest in normal subjects in whom geniculostriate pathways were intact. These results show that an eccentric visual signal reflexively activates retinotectal pathways to prime the oculomotor system and provide direct evidence that retinotectal pathways can influence orienting behavior in hemianopic humans. They also suggest that temporal-nasal hemifield asymmetries may provide a useful marker for extrageniculate visual functions in humans (*21*).

REFERENCES AND NOTES

1. E. Pöppel, R. Held, D. Frost, *Nature* **243**, 295 (1973); L. Weiskrantz, E. K. Warrington, M. D. Sanders, J. Marshall, *Brain* **97**, 709 (1974); M. T. Perenin and M. Jeannerod, *Neuropsychologia* **13**, 1 (1975); J. Zihl, *ibid.* **18**, 71 (1980); ——— and D. von Carmon, *Behav. Brain Res.* **1**, 287 (1980); A. J. Marcel, *Cognit. Psychol.* **15**, 197 (1983).
2. J. Campion, R. Latto, Y. M. Smith, *Behav. Brain Sci.* **6**, 423 (1983).
3. J. Zihl and R. Werth, *Neuropsychologia* **22**, 1 (1984).
4. J. L. Barbur, P. M. Forsyth, J. M. Findlay, *Brain* **111**, 63 (1988); J. L. Barbur, K. H. Ruddock, V. A. Waterfield, *ibid.* **103**, 905 (1980); P. Stoerig and A. Cowey, *ibid.* **112**, 1123 (1989).
5. L. Weiskrantz, *Blindsight: A Case Study and Implications* (Oxford Univ. Press, Oxford, 1986).
6. P. Sterling, *Brain Res.* **54**, 347 (1973).
7. S. M. Sherman, *Science* **185**, 355 (1974).
8. M. E. Wilson and M. G. Toyne, *Brain Res.* **24**, 395 (1970); D. H. Hubel, S. LeVay, T. N. Wiesel, *ibid.* **96**, 25 (1975); J. G. Pollack and T. L. Hickey, *J. Comp. Neurol.* **185**, 587 (1979).
9. T. L. Lewis, D. Maurer, K. Blackburn, *Vision Res.* **25**, 943 (1985); M. K. Rothbart, M. I. Posner, A. Boylan, in *The Development of Attention: Research and Theory*, J. Enns, Ed. (North-Holland, Amsterdam, in press).
10. M. I. Posner and Y. Cohen, in *Tutorials in Motor Behavior*, G. E. Stelmach and J. Requin, Eds. (North-Holland, Amsterdam, 1980), pp. 243–258; G. Shulman, *Acta Psychol.* **55**, 53 (1984).
11. R. Rafal, P. Calabresi, C. Brennan, T. Sciolto, *J. Exp. Psychol. Hum. Percept. Perform.* **15**, 673 (1989).
12. M. I. Posner, R. Rafal, L. Choate, J. Vaughan, *Cognit. Neuropsychol.* **2**, 211 (1985).
13. In a pilot experiment, ten normal subjects made saccades to unilateral targets presented in either the temporal or the nasal hemifield. The stimuli used were the same as those used in the experiments reported here. There was no difference in saccade latency to targets in temporal or nasal hemifields.
14. In two patients, the stroke had occurred more than 10 years before; in the third patient, the stroke had occurred 3 months before testing. Except for hemianopia, all three had otherwise normal vision and mentation and intact oculomotor and pupillary function.
15. With the stimuli used, these subjects did not demonstrate blindsight on the basis of conventional criteria usually used in forced-choice discrimination tasks. It is possible that our patients might have done so with appropriate stimuli and training. It was our purpose, however, to ensure the most stringent criteria for blindsight and to minimize the possibility that any blindsight effects found could be ascribed to near-threshold perception.
16. Each subject sat 40 cm from a black-and-white video display with his or her head supported on a chin rest. Eye position was recorded with an Eye-Trac 210 infrared recording device mounted on spectacle frames. The experimenter monitored an eye position cursor on a separate slave scope that simultaneously showed the same display viewed by the subject. The Eye-Trac was interfaced with a microcomputer that controlled the display and recorded saccade latency triggered by a velocity transformation of the Eye-Trac signal. Testing was conducted in a quiet room with normal ambient lighting to minimize any effect of light scatter. The unfilled squares in each field had luminances of approximately 4 cd/m^2. Both the target and the distractor were signaled by brightening these squares to a luminance of approximately 16 cd/m^2.
17. If a saccade was made before or within 100 ms of the target signal, the trial was excluded and an error signal was displayed. Each session began with a practice block of 25 trials, after which anticipatory errors were very rare. Two of the patients did not make any errors. The third patient made mistakes in 9% of the trials; most of these mistakes were due to eye blinks.
18. C. A. Marzi, G. Tassinari, S. Agliotti, L. Lutzemberger, *Neuropsychologia* **24**, 749 (1986).
19. This result should not be interpreted to mean that extrageniculate pathways do not play a role in the covert orienting of visual attention. Peritectal lesions do slow the movement of visual attention [R. Rafal, M. Posner, J. H. Friedman, A. W. Inhoff, E. Bernstein, *Brain* **111**, 267 (1988)]. Also, under some circumstances, normal subjects show a temporal hemifield advantage for orienting visual attention (R. Rafal, A. Henik, J. Smith, *J. Cognat. Neurosci.*, in press). The lack of inhibition in the key press task in the hemianopic patients only indicates that the inhibition they exhibited in the saccadic task was not due to covert attention shifts under the conditions used here.
20. In patient 2, who showed the same pattern of results as the other two subjects, part of the lateral pulvinar nucleus of the thalamus was damaged by the stroke (Fig. 1). Definition of the neural substrate of the inhibitory effect reported here will require more investigation.
21. The patient studied most intensively by Weiskrantz and associates (*5*), who has a left hemianopia, tended to show most consistent blindsight effects when the stimulus was viewed with the left eye, that is, when the hemianopic field was temporal. Also, in two studies in which no evidence of blindsight was found in hemianopic patients, the test stimuli were prepared in the blind nasal hemifield [J. D. Holtzman, *Vision Res.* **24**, 801 (1984); R. F. Hess and J.

S. Pointer, *Brain* **112**, 871 (1989)].
22. R. Frey, D. Woods, R. Knight, D. Scabini, *Soc. Neurosci. Abstr.* **13**, 1266 (1987); R. Knight, D. Scabini, D. Woods, C. Clayworth, *Encephalogr. Clin. Neurophysiol.* **70**, 499 (1988).

23. This study was conducted at Roger Williams General Hospital in Providence, RI, and was supported by Public Health Services grant MH 41544.

16 January 1990; accepted 22 June 1990

Control of Yeast Mating Signal Transduction by a Mammalian β_2-Adrenergic Receptor and G_s α Subunit

KLIM KING, HENRIK G. DOHLMAN, JEREMY THORNER, MARC G. CARON, ROBERT J. LEFKOWITZ*

To facilitate functional and mechanistic studies of receptor–G protein interactions by expression of the human β_2-adrenergic receptor (hβ-AR) has been expressed in *Saccharomyces cerevisiae*. This was achieved by placing a modified hβ-AR gene under control of the galactose-inducible *GAL1* promoter. After induction by galactose, functional hβ-AR was expressed at a concentration several hundred times as great as that found in any human tissue. As determined from competitive ligand binding experiments, hβ-AR expressed in yeast displayed characteristic affinities, specificity, and stereoselectivity. Partial activation of the yeast pheromone response pathway by β-adrenergic receptor agonists was achieved in cells coexpressing hβ-AR and a mammalian G protein (G_s) α subunit—demonstrating that these components can couple to each other and to downstream effectors when expressed in yeast. This in vivo reconstitution system provides a new approach for examining ligand binding and G protein coupling to cell surface receptors.

THE ACTIONS OF MANY EXTRACELLULAR signals (for example, neurotransmitters, hormones, odorants, and light) are mediated by receptors with seven transmembrane domains and by heterotrimeric G proteins (*1, 2*). Such G protein–mediated signaling systems have been identified in organisms as divergent as yeast and man (*1–3*). The mammalian β-adrenergic receptor (β-AR) is a member of the class of ligand-binding receptors with seven transmembrane segments. In response to epinephrine or norepinephrine, the β-AR activates the G protein G_s, which in turn stimulates adenylyl cyclase and adenosine 3′5′-monophosphate production (*1, 2*). G protein–coupled pheromone receptors in yeast control a developmental program that culminates in mating (fusion) of **a** and α haploid cell types to form the **a**/α diploid (*3, 4*).

To attain high level expression of the human β_2-adrenergic receptor (hβ-AR) in yeast, we placed a modified hβ-AR gene under control of the *GAL1* promoter in the multicopy vector YEp24, to give pYβAR2 (Fig. 1). Maximal expression required several manipulations including (i) expression of a transcriptional transactivator protein (the *LAC9* gene product); (ii) replacement of the 5′ untranslated and extreme NH_2-terminal coding sequence of the hβ-AR gene with the corresponding region of the yeast *STE2* (α factor receptor) gene; (iii) induction with galactose when cell growth reached late exponential phase; and (iv) inclusion of a β-AR ligand in the growth medium during induction.

A primary function of cell surface receptors is to recognize only appropriate ligands among other extracellular stimuli. Accordingly, we determined ligand binding affinities to establish the functional integrity of hβ-AR expressed in yeast (Fig. 2). An antagonist, ^{125}I-labeled cyanopindolol ([^{125}I]CYP), bound in a saturable manner and with high affinity to membranes prepared from pYβAR2-transformed yeast cells (Fig. 2A). By displacement of [^{125}I]CYP with a series of agonists, the order of potency and stereospecificity expected for hβ-AR were observed (Fig. 2B). Binding affinities in yeast were nearly identical to those observed previously for hβ-AR expressed in mammalian cells (Table 1).

A second important function of a receptor is agonist-dependent regulation of downstream components in the signal transduction pathway. Because the pheromone-responsive effector in yeast is not known, indirect biological assays are the most useful indicators of receptor function (*3, 4*). In yeast cells expressing high concentrations of hβ-AR, no agonist-dependent activation of the mating signal transduction pathway could be detected by any of the typical in vivo assays; for example, imposition of G_1 arrest, induction of gene expression, alteration of morphology (so-called "shmoo" formation), or stimulation of mating (*5*). A likely explanation for the absence of responsiveness is that the hβ-AR was unable to couple with the endogenous yeast G protein.

Expression of a mammalian G_s α subunit can correct the growth defect in yeast cells lacking the corresponding endogenous protein encoded by the *GPA1* gene (*6*). Moreover, because specificity of receptor coupling in mammalian cells is conferred by the α subunit of G proteins (*2*), we reasoned that coexpression of hβ-AR and a mammali-

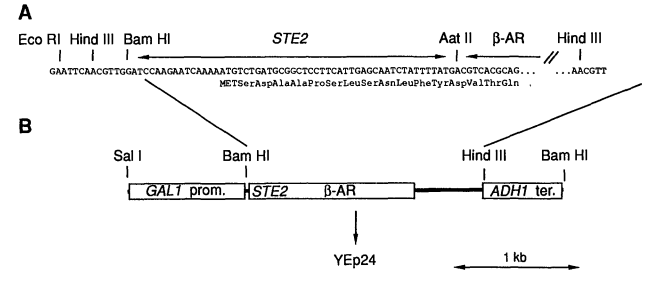


Fig. 1. Construction of yeast expression plasmid pYβAR2, in which expression of the hβ-AR gene is under control of the *GAL1* promoter (prom.). (**A**) The 5′ untranslated region and the first 63 bp of coding sequence of the hβ-AR gene in the plasmid pTZNAR (*12*) was removed by Aat II cleavage and replaced with a synthetic oligonucleotide corresponding to 11 bp of noncoding and 42 bp of coding sequence from the *STE2* gene (*13, 14*). The resulting plasmid, pTZYNAR, contains the modified hβ-AR gene flanked by Hind III sites in noncoding sequences. The Hind III–Hind III fragment was isolated from pTZYNAR and inserted into plasmid pAAH5 (*15*) such that the 3′ untranslated sequence of the modified hβ-AR gene was followed by 450 bp containing termination sequences (ter.) from the yeast *ADH1* gene (*15*). (**B**) The plasmid pYβAR2 was constructed by inserting the Bam HI–Bam HI fragment containing hβ-AR and *ADH1* gene sequences into YEp24 (*16*). Where maximum expression was sought, cells were cotransformed with plasmid pMTL9 containing *LAC9*—a homolog of the *S. cerevisiae GAL4* gene, which encodes a transactivator protein required for *GAL1*-regulated transcription (*17*). Cells grown to late exponential phase were induced in medium containing 3% galactose and alprenolol, and grown for an additional 36 hours. Standard methods for the maintenance of cells were used (*18*).

K. King, M. G. Caron, R. J. Lefkowitz, Departments of Medicine (Cardiology), Biochemistry, and Cell Biology, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710.
H. G. Dohlman and J. Thorner, Division of Biochemistry and Molecular Biology and Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

*To whom correspondence should be addressed.