Quantitative autoradiography of β_1 - and β_2 -adrenergic receptors in rat brain

(norepinephrine/epinephrine/¹²⁵I-labeled pindolol/Ultrofilm)

THOMAS C. RAINBOW, BRUCE PARSONS, AND BARRY B. WOLFE

Department of Pharmacology/G3, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Communicated by Louis B. Flexner, November 21, 1983

ABSTRACT We have used quantitative autoradiography to localize in rat brain β_1 - and β_2 -adrenergic receptors. These receptors were labeled in vitro with ¹²⁵I-labeled pindolol, an antagonist of *B*-adrenergic receptors that binds nonselectively to both β_1 and β_2 subtypes. The selective inhibition of ¹²⁵Ilabeled pindolol binding with specific antagonists of β_1 and β_2 receptors allowed the visualization of β -adrenergic receptor subtypes. High levels of β_1 receptors were observed in the cingulate cortex, layers I and II of the cerebral cortex, the hippocampus, the Islands of Calleja, and the gelatinosus, mediodorsal, and ventral nuclei of the thalamus. High levels of β_2 receptors were found in the molecular layer of the cerebellum, over pia mater, and in the central, paraventricular, and caudal lateral posterior thalamic nuclei. Approximately equal levels of β_1 and β_2 receptors occurred in the substantia nigra, the olfactory tubercle, layer IV of the cerebral cortex, the medial preoptic nucleus, and all nuclei of the medulla. The pronounced differences in the ratio of β_1 to β_2 receptors among brain regions suggests that the subtypes of β -adrenergic receptors may play different roles in neuronal function.

 β -Adrenergic receptors were originally subclassified by Lands et al. (1) into β_1 and β_2 receptors on the basis of the rank order of potency of a series of catecholamines in stimulating responses in several tissue preparations. Thus, catecholamine-stimulated responses of cardiac and adipose tissues were shown to be mediated by β_1 receptors; these responses in bronchiolar and vascular smooth muscle were found to be mediated by β_2 receptors. More recently, Minneman et al. (2) and Hancock et al. (3) characterized and quantified the subtypes of β -adrenergic receptors using the binding of radioligands to tissue homogenates. Minneman et al. (4) have also shown that it is likely that only two subtypes of β -adrenergic receptors exist in mammalian species. In addition, Stiles et al. (5) have shown that the primary protein structures of mammalian β_1 and β_2 receptors are distinct, but the proteins of, for example, cardiac β_1 -adrenergic receptors were conserved over a wide range of species including humans, dogs, pigs, rabbits, and rats (6).

In the central nervous system, β -adrenergic receptors are asymmetrically distributed with β_1 receptors being found in highest concentrations in forebrain structures, such as the cerebral cortex, caudate, and hippocampus (2). In the cerebellum, the β_2 subtype predominates (2). Up to the present, the measurement of these receptor subtypes has been done using homogenates of crude membrane preparations from gross brain structures, such as the cerebral cortex or the cerebellar cortex. Recently, however, a quantitative autoradiographic method has been devised to measure neurotransmitter receptors in brain (7–10). This method involves manual or computerized densitometric measurement of autoradiograms made with LKB Ultrofilm. Using a series of density stan-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

dards, the optical density readings can be quantitatively converted to amount of radioligand bound per mg of protein (11). Thus, in the present study we have combined the techniques of quantitative autoradiography and quantitative analysis of β -adrenergic receptor subtypes to allow determination of subtype densities with a resolution of $\approx 100 \ \mu$ m. These studies, in addition to confirming the previous reports on the distribution of subtypes in gross areas, such as the entire cerebral cortex or cerebellum, have also disclosed that β_1 and β_2 receptors are distributed heterogeneously among anatomically discrete nuclei and subregions of rat brain. This new technology will enable investigators to examine β -adrenergic receptor subtypes in the brain at a much higher anatomical resolution than was previously possible.

METHODS

Male Sprague-Dawley rats (200-250 g) were decapitated, the brains mounted on a cryostat chuck, and $32-\mu m$ sections were cut, thawed, and mounted onto microscope slides as described (10). The sections were stored overnight at -20° C and then stored at -70° C for up to a week before use. The slides were allowed to warm to room temperature 20 min before use. They were then immersed in a Coplin slide jar containing 35 ml of a Tris-saline buffer (20 mM Tris-HCl/135 mM NaCl, pH 7.4), ¹²⁵I-labeled (¹²⁵I-pindolol) pindolol (prepared as described in ref. 12), and various competing drugs. After incubation for 70 min at 23°C, the slides were washed $(3 \times 20 \text{ min})$ in 4°C Tris-saline buffer, rinsed quickly in cold distilled water to remove buffer salts, and rapidly dried on a slide warmer at 60°C. We either removed labeled brain sections for scintillation counting or exposed them for either 4.5 or 24 hr against LKB Ultrofilm, as described (10), to generate autoradiograms. Autoradiograms were quantified using a microcomputer-assisted densitometer at an anatomical resolution of 100 μ m. The optical density readings were converted into the amount of radioligand bound per mg of protein, using 32-µm-thick ¹²⁵I-labeled brain-mash standards (11, 14). Detailed inhibition curves constructed from optical density readings were analyzed using a nonlinear least-squares method via the computer program FITSITES on the NIHsupported PROPHET network. The untransformed data were tested for homology with one, two, and three separate binding-site models, and F-test analysis was used to determine the most appropriate fit (15). This method yielded estimates of the densities of the separate binding sites and of the affinities of each site for competing drugs. The linear leastsquares method was used for regression analysis of Scatchard (16) plots made from optical density measurements. For inhibition and saturation analysis, sections corresponding to Paxinos and Watson (13) stereotaxic levels 10-14 and 35-40 were used for receptor measurements of the caudate putamen, and the molecular layer of cerebellum, respectively, as initial studies indicated little or no rostral-caudal vari-

Abbreviation: ¹²⁵I-pindolol, ¹²⁵I-labeled pindolol.

ation in ¹²⁵I-pindolol binding. Optical density measurements were averaged across the entire dorsoventral and mediolateral extent of these structures.

RESULTS

Specificity of ¹²⁵I-Pindolol Binding to Brain Regions. Using a scintillation counter to determine the amount of ¹²⁵I-pindolol bound to tissue sections, we determined that the binding of the ligand was at equilibrium after a 70-min incubation at room temperature and that optimum ratios of total to nonspecific binding occurred after three separate washes of 20 min each in 4°C Tris-saline buffer. This washing procedure did not result in any loss of specific binding. All subsequent characterization of ¹²⁵I-pindolol binding was made by analysis of optical density readings from autoradiograms of the caudate putamen and the molecular layer of the cerebellum. Optical density measurements were averaged from the entire rostral-caudal extent of these structures. Analysis of inhibi-tion curves indicated that ¹²⁵I-pindolol binding to frozen brain sections was inhibited by isoproterenol with an affinity comparable to that observed in membrane studies (17, 18) $(K_{\rm d} = 0.3 \times 10^{-6} \text{ M})$ and was stereospecifically inhibited by propranolol with *l*-propranolol being 100 times more potent than d-propranolol (data not shown). Based on these results, we defined the nonspecific binding of ¹²⁵I-pindolol as the binding that occurs in the presence of 100 μ M isoprotere-nol. Scatchard analysis indicated that ¹²⁵I-pindolol binding was saturable $[B_{\text{max}} \text{ (caudate)} = 153 \pm 14 \text{ fmol per mg of}$ protein] and of an affinity similar to that found in membrane studies (17) ($K_d = 51 \pm 5 \times 10^{-12}$ M; n = 3). It was also observed that 1 μ M *l*-propranolol reproducibly inhibited more ¹²⁵I-pindolol binding than 100 μ M *l*-isoproterenol (data not shown). Since there always appeared to be a plateau in the inhibition curves of *l*-propanolol and *l*-isoproterenol around 5–20% of total binding, and since 1 μ M d-propranolol inhibited ¹²⁵I-pindolol binding to a great extent, we felt that the extra inhibition by 1 μ M *l*-propranolol represented inhibition of nonspecific (nonreceptor) binding. This observation was not completely unexpected, because Leichtling et al. (19) reported similar phenomena in other tissues.

Selective Labeling of β_1 and β_2 Receptors. In contrast to inhibition curves produced by such nonselective β -adrener-gic antagonists as propranolol, the inhibition of ¹²⁵I-pindolol binding by selective β_1 - and β_2 -receptor antagonists produced markedly biphasic curves, with Hill coefficients of <1(Fig. 1). ICI-89,406, a selective β_1 antagonist (20), was more potent in competing for ¹²⁵I-pindolol binding in caudate putamen than in the cerebellum, while ICI-118,551, a selective β_2 antagonist (21), showed the reverse regional specificity, reflecting the relative distribution of β_1 - and β_2 -receptors in these brain regions. Computer-iterative analysis of inhibition curves, such as those shown in Fig. 1, indicated that each drug was 1000-fold more specific for one β -adrenergic receptor subtype than the other, allowing us to define conditions in which the binding of 125 I-pindolol could be blocked from one subtype with minimal interference to binding to the other subtype. Thus, the IC₅₀ values for ICI 89,406 for inhibiting ¹²⁵I-pindolol (150 pM) binding to β_1 and β_2 receptors, respectively, were $(n = 12) 1.8 \pm 0.6$ nM and $2.9 \pm 0.7 \mu$ M, and the IC₅₀ values for ICI 118,551 for the same receptor subtypes were $(n = 11) 1.5 \pm 0.8 \mu M$ and $1.5 \pm 0.4 nM$. The binding of ¹²⁵I-pindolol (150 pM) to β_1 -adrenergic receptors was there-fore defined as the amount of ¹²⁵I-pindolol bound in the presence of 50 nM ICI-118,551 minus the nonspecific binding. We defined the binding of ¹²⁵I-pindolol to β_2 receptors as the binding of ¹²⁵I-pindolol in the presence of 70 nM ICI-89,406, minus the labeling in the presence of 100 μ M isoproterenol. These conditions of labeling resulted in $\approx 97\%$ occupancy of one subtype by the ICI drug with <3% occupancy of the other.



FIG. 1. Inhibition of ¹²⁵I-pindolol binding by ICI 89,406 and ICI 118, 551. ¹²⁵I-Pindolol (150 pM) was incubated with slide-mounted cerebellar sections in the presence and absence of various concentrations of the β_1 -selective antagonist ICI 89,406 (\odot) or the β_2 -selective antagonist ICI 118,551 (\bullet). Autoradiograms of the molecular layer of the cerebellum were analyzed, and the data are plotted as a percent of the binding occurring in the absence of any competing ligand. The lines are the computer-generated best fit to a two-site model. This is a representative experiment of 11 or 12 similar experiments.

Anatomical Distribution of β_1 and β_2 Receptors. The distribution of total ¹²⁵I-pindolol binding to β -adrenergic receptors was similar to previous descriptions of the binding of $[^{3}H]$ dihydroalprenolol, a nonselective β -receptor antagonist (22, 23). We observed high levels of total binding in the caudate putamen, the olfactory tubercle, the superficial layers of the cerebral cortex, and the superior colliculus, the cingulate cortex, the substantia nigra, the dorsal caudal subiculum, and the caudal lateral posterior thalamic nucleus (Table 1 and Fig. 2). The levels of nonspecific binding were uniform over most brain regions, ranging from 5% to 20% of the total binding (data not shown). Relatively high levels of nonspecific binding (25% to 40% of the total binding) were observed in the ventral pallidum, the globus pallidus, the dorsal caudal subiculum, the entopeduncular nucleus, and the substantia nigra. Because isoproterenol, like other catecholamines, has a limited ability to penetrate cell membranes (27), it is possible that some of the β receptors in these regions are internal to cell membranes or are located within axons. Alternatively, it is possible that these structures contain molecules that bind ¹²⁵Î-pindolol with high affinity but are not β -adrenergic receptors, and thus isoproterenol does not inhibit the binding.

We observed pronounced regional differences when β_1 and β_2 receptors were separately visualized (Table 1; Figs. 2) and 3). All regions of the rat brain contained both receptor subtypes, with the relative percentages of each receptor ranging from 50:50 to 90:10. High (80% to 90% of total) levels of β_1 receptors and low levels of β_2 receptors were found in the cingulate cortex, layers I and II of all regions of the cerebral cortex, all regions of the hippocampus, and the ventral, gelatinosus, posterior, and mediodorsal nuclei of the thalamus. By contrast, high levels of β_2 receptors and low levels of β_1 receptors were observed in all parts of the cerebellum, and over pia mater. Slightly lower levels of β_2 receptors (70% to 80% of total) occurred in the central, reticular, paraventricular, and caudal lateral posterior nuclei of the thalamus. Moderate to high levels of β_2 receptors (60% to 70% of total), and thus low to moderate levels of β_1 receptors, were observed in the olfactory tubercle (Fig. 3) and the superficial layer of the superior colliculus. A large number of brain re-

Table 1. Quantitative autoradiography of β_1 and β_2 receptors in rat brain

	ol.			
	fmol per m	2		
Region	of protein	~ % β1	% Вл	
Erontonoristal cortax	•		12	
Laver I	59.8 + 4	87 + 3	10 + 1	
Layer IV	55.0 ± 4	32 ± 3	17 - 1	
Layer VI	50.8 ± 2	49 ± 1 72 ± 1	31 ± 2 37 ± 1	
Cingulate cortex	30.3 ± 3	$\frac{72 \pm 1}{99 \pm 2}$	$\frac{2}{12} + 1$	
Thelemus	00.3 ± 2	00 <u>-</u> 2	15 ± 2	
Anterior medial n	28 5 + 2	47 + 5	52 + 5	
Anterior ventral n	26.3 ± 3	4/± 3	د <u>۲</u> در ۱۹ ۲ ۵	
Centrol n	34.1 ± 2	32 ± 3	40 ± 3	
Dercel lateral conjoulate n	47.5 ± 5	20 ± 3	$\frac{13 \pm 3}{23 \pm 3}$	
Colotinoque n	50.0 ± 7	// ± /	23 ± 2	
Leteral dereal n	01.0 ± 4	00 ± 3	20 ± 1	
Lateral mostarian n (acudal)	29.0 ± 2	73 ± 3	23 ± 2	
Madiadamal n	90.8 ± 4	31 ± 2	09 ± 2	
Mediodorsai n.	44.3 ± 8	74 ± 3	20 ± 1	
Paraventricular n.	37.5 ± 4	39 ± 9	69 ± 1	
Reticular n.	30.5 ± 2	31 ± 2	69 ± 2	
ventroposterior n.	49.5 ± 7	82 ± 2	19 ± 3	
Cerebellum	7 0 0 1 0			
Molecular layer	70.3 ± 3	9 ± 1	91 ± 1	
Purkinje-granular layer	29.2 ± 2	7 ± 1	93 ± 2	
Pons-medulla				
Medial vestibular n.	24.2 ± 1	58 ± 4	42 ± 4	
N. spinal V	21.7 ± 3	59 ± 5	41 ± 5	
N. tractus solitarius	24.0 ± 1	58 ± 2	43 ± 1	
Reticular n.	16.2 ± 2	62 ± 7	38 ± 7	
Midbrain				
Central grey	22.8 ± 5	55 ± 1	44 ± 1	
Interpenduncular n.	26.3 ± 4	83 ± 4	14 ± 1	
Medial geniculate	46.2 ± 6	76 ± 2	24 ± 2	
Substantia nigra				
pars reticularis	43.3 ± 1	52 ± 2	47 ± 2	
Superior colliculus,				
superficial layer	60.7 ± 7	36 ± 1	62 ± 1	
Hippocampus				
CA1 (rostral)	40 ± 5	81 ± 3	19 ± 3	
CA3 (rostral)	22 ± 1	60 ± 7 3	35 ± 9	
Dentate gyrus (rostral)	41.6 ± 4	75 ± 4 2	25 ± 4	
Subiculum (dorsal-caudal)	51.7 ± 5	86 ± 5	l4 ± 5	
CA1 (caudal)	76.5 ± 2	90 ± 4 1	10 ± 3	
CA3 (caudal)	33.7 ± 1	70 ± 10 3	30 ± 10	
Dentate gyrus (caudal)	53.4 ± 5	85 ± 5 1	5 ± 4	
Hypothalamus-preoptic area				
Medial preoptic n.	26.7 ± 7	$45 \pm 6 5$	i4 ± 6	
Lateral preoptic n.	25.7 ± 5	$42 \pm 6 5$	8 ± 6	
Anterior hypothalamic n.	20.7 ± 3	51 ± 7 4	9±7	
Ventromedial hypothalamic n.	13.3 ± 2	60 ± 8 4	0 ± 5	
Arcuate n.	13.6 ± 1	55 ± 8 4	5 ± 7	
Amvgdala				
Medial n.	25.8 ± 2	$53 \pm 9 4$	7 ± 9	
Cortical n.	20.7 ± 3	60 ± 64	0 ± 6	
Basolateral n.	47.1 ± 5	65 ± 73	4 ± 7	
Zona incerta	18.2 ± 1	77 ± 12	4 ± 1	
Basal forebrain	10.2 - 1			
Diagonal band	303 + 4	52 + 4 4	8 + 4	
Islands of Calleia	58.9 ± 4	79 ± 7 2	1 ± 7	
Olfactory tubercle	42.6 ± 1.0	43 ± 3 5	, 7 ± 3	
Lateral septal n	38.4 ± 2	67 ± 73	3 ± 7	
Globus pallidus	49.0 ± 9	65 ± 630	0 ± 3	
Caudate putamen	79.4 ± 6	$72 \pm 6 2$	8 ± 6	
	-		-	

Results are means \pm SEM for measurements made on three rats. Quadruplicate 32- μ m-thick sections were taken at 500 μ m intervals throughout a rat brain, in accord with the figures in the stereotaxic atlas of Paxinos and Watson (13). Total ¹²⁵I-pindolol binding, β_1 and β_2 displaceable binding, and nonspecific binding were determined

gions possessed moderate to high levels of β_1 receptors. These regions included the caudate putamen, the nucleus accumbens, the Islands of Calleja (Fig. 3), layer VI of the cerebral cortex, the lateral and medial geniculate nuclei, and all nuclei of the hypothalamus, septum, and amygdala. The remaining brain regions possess roughly comparable levels of β_1 and β_2 receptors. Among these regions were layers IV and Vb of the cerebral cortex, the nuclei of the preoptic area, and the bed nucleus of the stria terminalis, the anterior dorsal, ventral, and medial nuclei of the thalamus, the central grey, the substantia nigra, the ventral tegmental area, and all nuclei of the pons medulla (Table 1; Figs. 2 and 3).

DISCUSSION

Our study revealed that β_1 and β_2 receptors were distributed heterogeneously among brain regions, with large anatomical differences in the relative amount of each subtype (Figs. 2 and 3; Table 1). Previous studies on homogenates of grossly dissected brain regions determined that the β_1 receptor subtype predominated in forebrain areas, accounting for 80% of the total β receptor population; in the cerebellum, by contrast, β_2 -adrenergic receptors were the dominant subtype, representing 80–90% of the total number of β receptors (2). The increased anatomical resolution of quantitative autoradiography has revealed marked anatomical variations in the distribution of the low level of β_2 receptors in rat forebrain. In some forebrain nuclei, such as the central nucleus of the thalamus, β_2 receptors account for 80% of the total content of β receptors. By contrast, the low level of β_1 receptors in the cerebellum appears to be uniformly distributed across all portions of the cerebellar cortex, with equivalent levels observed in the molecular and Purkinje granular layers (Table 1). As there is evidence that β_1 receptors in the cerebellum are confined to Purkinje cells (28), our findings suggest that β_1 receptors are present on the dendritic fields and axons of Purkinje cells, which extend into the molecular and granular layers.

As in previous studies (22, 23) on the localization of total β -adrenergic receptor binding, we observed no pronounced correlation between the known locations of norepinephrine or epinephrine terminals and regions high in β_1 or β_2 receptors. High levels of norepinephrine terminals are found within the ventral portion of the bed nucleus of the stria terminalis, the dorsomedial hypothalamic nucleus, and the anterior ventral nucleus of the thalamus (29, 30), regions with low to moderate levels of either β -receptor subtype. High levels of β_1 receptors occur in the caudate putamen, and high to moderate levels of β_2 receptors are found in the olfactory tubercle, both regions with low contents of norepinephrine. High levels of epinephrine-containing neurons occur in various regions of the hypothalamus including the dorsomedial, paraventricular, and arcuate nuclei, and in the median eminance (30, 31). These regions also have low to moderate levels of β_1 and β_2 receptors (Table 1). It is possible that the location of α -adrenergic-receptor subtypes might correlate better with regions of high norepinephrine or epinephrine content.

The heterogeneous distribution of β_1 and β_2 receptors in rat brain suggests that the subtypes of β -adrenergic receptors may mediate different neuronal responses to norepi-

by quantitative autoradiography for brain structures on each group of four sections. Five to eight densitometric readings were taken for each structure on a single brain section. The values for nonspecific binding for brain structures at this anatomical level were subtracted from all measurements of total β -adrenergic receptors and β -adrenergic receptor subtypes. Unless noted, values represent the average concentration of receptor across all stereotaxic levels in which the brain structure appears. Rostral hippocampus refers to figures 19-23 and caudal hippocampus refers to Fig. 24-27 in the stereotaxic atlas of Paxinos and Watson (13).



FIG. 2. Autoradiographic visualization of ¹²⁵I-pindolol binding to β -adrenergic receptors. (A) Total binding of ¹²⁵I-pindolol. A 32- μ m-thick section from rat brain was incubated with 150 pM ¹²⁵I-pindolol and exposed for 24 hr against LKB Ultrofilm. Both β_1 - and β_2 -adrenergic receptors are visualized in this autoradiogram. This section corresponds to Konig and Klippel (25) level A4380 or Paxinos and Watson (13) level 20. There are high levels of total ¹²⁵I-pindolol. A serially cut 32- μ m-thick section was incubated with 150 pM ¹²⁵I-pindolol in the presence of 100 μ M *l*-isoproterenol. The entopeduncular nucleus shows higher levels of nonspecific binding of ¹²⁵I-pindolol to β_1 -adrenergic receptors or binding not associated with β -adrenergic sites. (C) Binding of ¹²⁵I-pindolol to β_1 -adrenergic receptors was visualized by incubating a 32- μ m-thick section with 150 pM ¹²⁵I-pindolol to β_1 -adrenergic receptors. There are high levels of β_1 -receptors in layers I and II of the cerebral cortex, the caudate nucleus, and in particular nuclei of the thalamus. (D) Binding of ¹²⁵I-pindolol to β_2 -adrenergic receptors was visualized using the same labeling procedure as described above, except the section was coincubated with 70 nM ICI 89,406, a selective β_1 -receptor antagonist. β_2 receptors are high in layer IV of the cerebral cortex, displaying an irregular distribution that may correspond to "whisker barrels" previously identified in rat somatosensory cortex (26). High levels of β_2 receptors are found in thalamic nucleus; DG, dentate gyrus; G, gelatinosus nucleus of β_2 receptors are visible in layer 1V of the cerebral cortex, γ_2 receptors are visible in layer 1V of the cerebral cortex, γ_2 receptors are visible in layer 1V of the cerebral cortex, γ_2 receptors are visible in layer Vb of the cerebral cortex, γ_2 receptors are visible in layer 1V of the cerebral cortex, γ_2 receptors are found in thalamic nucleus; DG, dentate gyrus; G, gelatinosus nucleus o



FIG. 3. Autoradiographic visualization of β_1 - (A and C) and β_2 - (B and D) adrenergic receptors in rat brain. β_1 - and β_2 -adrenergic receptors were separately visualized by inhibiting ¹²⁵I-pindolol binding with selective antagonists. (A and B) Konig and Klippel (25) level A 8920 or Paxinos and Watson (figure 12 in ref. 13). High levels of β_1 -adrenergic receptors are found in the cingulate cortex, layers I and II of the cerebral cortex, the caudate putamen, and the Islands of Calleja. β_2 receptors are most concentrated in layer IV of the cerebral cortex and the olfactory tubercle. The lateral septum has higher levels of β_1 receptors than β_2 receptors. The nucleus accumbens shows high levels of both β -adrenergic receptor subtypes. (C and D) Paxinos and Watson level 39. Very high levels of β_2 receptors are found in the molecular layer of the cerebellum. Low levels of β_1 receptors occur in all layers of the cerebellar cortex. Moderate levels of β_1 receptors occur throughout the medulla. The pia mater around the brainstem shows high levels of β_2 receptors. I, cortical layer 1; ICj, islands of Calleja; IV, cortical layer 4; ML, molecular layer of the cerebellar cortex; Ve, vestibular nucleus.

Neurobiology: Rainbow et al.

nephrine or epinephrine. While there is a great deal of evidence that norepinephrine influences brain function via activation of β -adrenergic receptors (32, 33), few studies have identified the subtype of the β receptor responsible for these effects. Our results suggest that the relative contributions of β_1 and β_2 receptors to β -adrenergic-receptor-mediated effects might vary among different brain regions. Thus, the actions of norepinephrine in the hippocampus, a region low in β_2 receptors, may be mediated by β_1 receptors, while in regions such as the central nucleus of the thalamus the influence of norepinephrine might occur via β_2 receptors. Alternatively, it is possible that low levels of one receptor subtype could mediate some β -adrenergic effects. For example, the facilitation by norepinephrine of y-aminobutyric acid-induced inhibition of Purkinje cell firing in the cerebellum appears to occur via the small number of β_1 receptors present in this tissue (34). Similarly, it has been suggested (24, 35, 36) that β_1 receptors in the central nervous system may receive endogenous noradrenergic input, while β_2 receptors do not and that, consistent with the suggestion of Yeh and Woodward (34), cerebellar Purkinje cell activity may be modulated by β_1 receptors (24, 28). The question of specific subtypes mediating specific responses could be clarified by studies in which specific β_1 - and β_2 -receptor drugs are used to characterize the receptor subtype involved in electrophysiological or behavioral responses mediated by β -adrenergic receptors.

In a previous study designed to localize β_1 and β_2 receptors by autoradiography (22), Palacios and Kuhar reported that rat forebrain possessed a diffuse distribution of β_2 receptors, while there was a marked regional heterogeneity of β_1 receptors. β_1 -adrenergic receptors were visualized indirectly in this study by the inhibition of [³H]dihydroalprenolol labeling by the β_2 selective drug zinterol. We attribute our ability to observe regional differences in β_2 receptors in forebrain to the greater selectivity of ICI drugs for the respective subtypes (2). The use of 125 I-pindolol to produce autoradiograms of β -adrenergic receptors decreased exposure times from 60 days with the ligand [³H]dihydroalprenolol (unpublished observations) to 4.5 hr. This marked decrease in exposure time results from both the high specific activity of 1^{2} pindolol and the greater sensitivity of LKB Ultrofilm for ¹²⁵I (37, 38). The availability of additional ¹²⁵I radioligands to shorten exposure times would eliminate the main obstacle for the routine use of quantitative autoradiography for receptor measurements. The ability to rapidly obtain by computerized densitometry quantitative maps of β -adrenergic and other types of neurotransmitter receptors among thousands of micron-sized brain regions is likely to provide new insights into the molecular functions of the brain.

This work was supported by Grants GM 31155, NS19597, and NS20006 from the National Institutes of Health (B.B.W. and T.C.R.). B.B.W. is an Established Investigator of the American Heart Association. T.C.R. holds fellowships from the Esther A. and Joseph Klingenstein Fund and the Alfred P. Sloan Foundation

- 1. Lands, A. M., Arnold, A., McAuliff, J. P., Luduena, F. P. & Brown, T. G. (1967) Science 214, 597-598.
- Minneman, K. P., Hegstrand, L. R. & Molinoff, P. B. (1979) Mol. Pharmacol. 16, 34-46.
- Hancock, A. A., DeLean, A. L. & Lefkowitz, R. J. (1979) Mol. Pharmacol. 16, 1-9.
- Minneman, K. P., Hedberg, A. & Molinoff, P. B. (1979) J. Pharmacol. Exp. Ther. 211, 502-508.

- Stiles, G. L., Strasser, R. H., Caron, M. G. & Lefkowitz, R. J. (1983) J. Biol. Chem. 258, 10689-10694.
 Stiles, G. L., Strasser, R. H., Lavin, T. N., Jones, L. R.,
- Stiles, G. L., Strasser, R. H., Lavin, T. N., Jones, L. R., Caron, M. G. & Lefkowitz, R. J. (1983) J. Biol. Chem. 258, 8443-8449.
- Palacios, J. M., Niehoff, D. L. & Kuhar, M. J. (1981) Neurosci. Lett. 25, 101–105.
- Penney, J. B., Jr., Pan, H. S., Young, A. B., Frey, K. A. & Dauth, G. W. (1981) Science 214, 1036–1038.
- Quirion, R., Hammer, R. P., Jr., Herkenham, M. & Pert, C. B. (1981) Proc. Natl. Acad. Sci. USA 78, 5881-5885.
- Rainbow, T. C., Bleisch, W. V., Biegon, A. & McEwen, B. S. (1982) J. Neurosci. Methods 5, 127–138.
- Unnerstall, J. R., Niehoff, D. L., Kuhar, M. J. & Palacios, J. M. (1982) J. Neurosci. Methods 6, 59-73.
- 12. Barovsky, K. & Brooker, G. (1980) J. Cyclic Nucleotide Res. 6, 297-307.
- 13. Paxinos, G. & Watson, C. (1982) The Rat Brain in Stereotaxic Coordinates. (Academic, NY).
- 14. Reivich, M., Jehle, J. W., Sokoloff, L. & Kety, S. (1969) J. Appl. Physiol. 27, 296-300.
- 15. Zar, J. H. (1974) *Biostatistical Analysis* (Prentice Hall, Englewood Cliffs, NJ).
- 16. Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660-672.
- 17. Wolfe, B. B. & Harden, T. K. (1981) J. Cyclic Nucleotide Res. 7, 303-312.
- Hegstrand, L. R., Minneman, K. P. & Molinoff, P. B. (1979) J. Pharmacol. Exp. Ther. 210, 215-221.
- Leichtling, B. H., Su, Y. F., Wimalasena, J., Harden, T. K., Wolfe, B. B. & Wicks, W. D. (1978) *J. Cell. Physiol.* 96, 215– 224.
- Engle, G., Hoyer, D., Berthold, R. & Wagner, H. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 317, 277-285.
- Mattsson, H., Andersson, T., Carlsson, E., Hedberg, A., Lundgren, B. & Olsson, T. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 321, 302-308.
- 22. Palacios, J. M. & Kuhar, M. J. (1982) Neurochem. Int. 4, 473-490.
- 23. Palacios, J. M. & Kuhar, M. J. (1980) Science 208, 1378-1380.
- Wolfe, B. B., Minneman, K. P. & Molinoff, P. B. (1982) Brain Res. 234, 474–479.
- 25. Konig, J. F. R. & Klippel, R. A. (1963) The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. (Williams & Wilkins, Baltimore).
- 26. Durham, D. & Woolsey, T. A. (1977) Brain Res. 137, 169-174.
- Weiner, N. (1980) in *The Pharmacological Basis of Therapeutics*, eds. Gilman, A. G., Goodman, L. S. & Gilman, A. (Mac-Millan, New York), pp. 138-175.
- Minneman, K. P., Pittman, R. N., Yeh, H. H., Woodward, D. J., Wolfe, B. B. & Molinoff, P. B. (1981) Brain Res. 209, 25-34.
- Swanson, L. W. & Hartman, B. (1975) J. Comp. Neurol. 163, 467-506.
- Moore, R. Y. & Bloom, F. E. (1979) Annu. Rev. Neurosci. 2, 113-168.
- Saavedra, J. M., Palkovitz, M., Brownstein, M. J. & Axelrod, J. (1974) Nature (London) 248, 695–696.
- Redmond, D. E. (1977) in Animal Models in Psychiatry and Neurology, eds. I. Hanin, I. & Usdin, E. (Pergamon, New York), pp. 293-302.
- Zornetzer, S. F. (1978) in Psychopharmacology: A Generation of Progress, eds. Lipton, M. A., DiMascio, A. & Killam, K. F. (Raven, New York), pp. 637-649.
- 34. Yeh, H. H. & Woodward, D. J. (1983) Neuropharmacology 22, 629-639.
- Minneman, K. P., Dibner, M. D., Wolfe, B. B. & Molinoff, P B. (1979) *Science* 204, 866–868.
 Minneman, K. P., Wolfe, B. B. & Molinoff, P. B. (1982) *Brain*
- Minneman, K. P., Wolfe, B. B. & Molinoff, P. B. (1982) Brain Res. 252, 309–314.
- 37. Rogers, A. W. (1979) *Techniques of Autoradiography* (Elsevier/North Holland, Amsterdam).
- 38. Enh, E. & Larsson, B. (1979) Science Tools 26, 24-29.