schizophrenia (*17*). With knowledge of the localization of MAO A and B in specific neuronal tracts, retrospective examination of past basic and clinical studies may clarify the patterns of altered neurotransmitter function and metabolism that are thought to occur in some of these disorders.

Our localization studies may also contribute to explaining the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which produces parkinsonian symptoms in humans and monkeys (*18, 19*). This substance is thought to be oxidized by MAO B (*20*) to the active metabolites 1-methyl-4-phenyl-2,3-dihydropyridine ($MPDP^+$) and 1-methyl-4-phenyl-pyridine (MPP^+) (*21*), yet we find that MAO B is localized primarily in serotonin-containing neurons, which are not affected by MPTP (*19, 22*). Thus MAO B is not observed in the dopamine-containing neurons in the substantia nigra, which are selectively destroyed by the drug (*19, 22*). However, $MPDP^+$ or MPP^+ might be selectively taken up by these neurons (*23*) after release from serotonin-containing neurons that project onto the substantia nigra or the striatum (for example, cells of the nucleus raphe dorsalis) or from MAO B–containing astrocytes. Localization of MAO A and MAO B and clarification of their physiological roles in the brain may help resolve the molecular mechanisms underlying a variety of monoamine-related neurological and psychiatric disorders.

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Externalization of β-Adrenergic Receptors Promoted by Myocardial Ischemia

Abstract. *β-Adrenergic receptors were identified in two fractions of guinea pig myocardium: a purified sarcolemmal fraction and a light vesicle (presumably intracellular) fraction. In the light vesicle fraction, which contained approximately 25 percent of the myocardial receptors under control conditions, the receptors appeared to be segregated from the stimulatory guanine nucleotide binding and catalytic components of adenylate cyclase. During myocardial ischemia, β-adrenergic receptors were redistributed from the intracellular vesicles to the sarcolemmal fraction, where isoproterenol-stimulated adenylate cyclase activity was increased. These findings should facilitate further studies on cellular and molecular mechanisms that regulate adrenergic receptor traffic in the myocardium and may explain the rapid enhancement in adrenergic receptor expression that occurs with myocardial ischemia.*

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Cell surface receptors for hormones and neurotransmitters are actively regulated by processes such as synthesis, internalization, recycling, and degradation. Because the population of receptors on target cell surfaces is under dynamic control, steady-state changes in receptor number reflect an alteration in one or more aspects of the receptor life cycle (*1, 2*). Ischemia is one example of an altered cellular environment with important physiological and pathological ramifications. Earlier studies have shown that myocardial ischemia is associated with a rapid (<1 hour) increase in the number of β-adrenergic receptors in crude cardiac membranes, and this increase in receptors may account for the enhanced effects of catecholamines in the ischemic heart (*3*). However, the mechanism mediating this up-regulation of receptors is unknown. Not all β-ad-

Table 1. Plasma membrane marker enzymes in plasma membranes and vesicles from control and ischemic left ventricles prepared from guinea pig. The results are means ± standard error of the mean for three experiments performed in triplicate for all values except those for adenylate cyclase in the presence of Gpp(NH)p (n = 2). 5′-Nucleotidase, expressed as nanomoles of inorganic phosphate per minute per milligram of protein, was assayed by the method of Purdum and Dixon (*12*). Adenylate cyclase was assayed by the method of Salomon (*13*) in a buffer containing 50 m*M* tris-HCl, 10 m*M* $MgCl_2$, 0.3 m*M* cyclic adenosine monophosphate, 1 m*M* adenosine triphosphate (ATP), 20 m*M* creatine phosphate, creatine phosphokinase (50 unit/ml), and [^{32}P]ATP at 800,000 count/min. The other compounds listed were used at the following concentrations: guanosine triphosphate (GTP) (10 m*M*), isoproterenol (100 μ*M*), Mn^{2+} (10 m*M*), forskolin (100 μ*M*), and Gpp(NH)p (10 μ*M*).

Condition	5′-Nucleotidase (nmol min^{-1} mg^{-1})	Adenylate cyclase (pmol min^{-1} mg^{-1}) in the presence of			
		GTP	Isoproterenol plus GTP	Mn^{2+} plus forskolin plus GTP	Gpp(NH)p
		Plasma membranes			
Control	10 ± 3	123 ± 6	150 ± 6	430 ± 9	172, 177
Ischemia	14 ± 3	113 ± 5	190 ± 5	367 ± 8	171, 182
		Light vesicles			
Control	0.016 ± 0.01	12 ± 3	13 ± 4	18 ± 3	8, 14
Ischemia	0.009 ± 0.02	12 ± 4	10 ± 4	22 ± 4	8, 12

renergic receptors sediment with plasma membrane fractions, but instead can be found in membrane fractions isolated at high speeds (100,000*g* to 200,000*g*); occupancy of receptors by agonists promotes movement (internalization) of receptors from the plasma membrane to those presumably intracellular sites (*4*, *5*). We propose that myocardial ischemia is associated with the reverse process: a translocation of intracellular β-adrenergic receptors to the sarcolemma, thereby increasing binding and functional activity at the myocardial cell surface.

We first isolated and defined two distinct fractions from guinea pig heart: a purified sarcolemmal fraction, in which β-adrenergic receptors are responsive to agonist, and a lighter, presumably intracellular, pool of receptors that are distinct from the sarcolemmal fraction. Using modifications of reported techniques (*4*–*6*), we extracted contractile proteins from homogenized hearts and prepared a 40,000*g* sarcolemmal fraction. From the supernatant we then prepared a light membrane fraction that sedimented in sucrose buffer at 137,000*g*. On electron microscopy these light membrane fractions appeared as small vesicles. The specific activities of two plasma membrane markers, 5′ nucleotidase and adenylate cyclase were very low in the vesicle preparation as compared to sarcolemmal membranes (Table 1). The specific activity of both basal and isoproterenol-stimulated adenylate cyclase in vesicles was only 8 to 10 percent of that found in surface membranes. In the light vesicle fraction, adenylate cyclase activity was unresponsive to the guanine nucleotide guanylyimidodiphosphate [Gpp(NH)p] and to forskolin, suggesting that this fraction is devoid of guanine nucleotide binding stimulatory (G_s) proteins and deficient in catalytic components of the adenylate cyclase system.

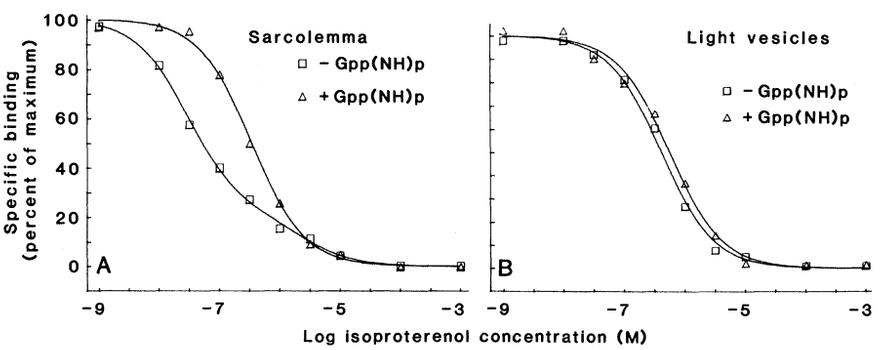

Fig. 1. Competition of (−)-isoproterenol for [^{125}I]ICYP in (A) plasma membranes from control guinea pig left ventricles and (B) light vesicles from control guinea pig left ventricles. The two membrane fractions were prepared by mincing left ventricular tissue, extracting contractile proteins with 750 m*M* NaCl and 10 m*M* histidine (*p*H 7.5), and centrifuging samples at 14,000*g*. The pellet was then suspended and washed once in 10 m*M* $NaHCO_3$ and 10 m*M* histidine. The material was vigorously homogenized and centrifuged at 45,000*g* for 30 minutes. The supernatant was centrifuged at 137,000*g* for 90 minutes, and the white rim above the pellet was aspirated and suspended in 50 m*M* tris-HCl, 8 m*M* $MgCl_2$, and 0.5 m*M* EGTA at *p*H 7.5, and used as the light vesicle fraction. The pellet from the 45,000*g* spin was resuspended, washed, and centrifuged at 17,000*g*. The resulting supernatant was centrifuged at 137,000*g*, and the rim above the pellet was aspirated and used as purified sarcolemmal membranes. The binding of 50 p*M* [^{125}I]ICYP to membrane and vesicle fractions were performed in the absence or presence of increasing concentrations of (−)-isoproterenol and in the absence and presence of 100 μ*M* Gpp(NH)p, in a total volume of 0.25 ml. Binding reactions were maintained at 25°C for 1 hour. Binding was terminated by diluting samples to 10 ml with ice-cold buffer, filtering over Whatman GF/B filters that had been soaked in 2 percent polyethyleneimine (*14*) and washing of filters with 10 ml of buffer. Values for total and isoproterenol-competed [^{125}I]ICYP binding were corrected for nonspecific binding [in the presence of 1 μ*M* (−)-propranolol (Ayerst)]. The data points represent the mean of triplicate determinations and are representative of values obtained in three separate experiments.

Data from cultured tumor cells and amphibian erythrocytes indicate that the interaction of β-adrenergic receptors in light vesicles is different from that in plasma membrane fractions (*4*). Our findings in the light vesicle fraction from guinea pig heart were similar (Fig. 1). In competitive radioligand binding studies conducted with plasma membrane (sarcolemmal) fractions, agonists recognized two classes of β-adrenergic receptors, a high-affinity class of receptors coupled to G_s and a low-affinity class of receptors apparently uncoupled from G_s (Fig. 1A). Addition of Gpp(NH)p reduced the apparent affinity of β-adrenergic receptors [recognized by the iodinated β-antagonist [^{125}I]iodocyanopindolol ([^{125}I]ICYP)] for the agonist isoproterenol, a result compatible with conversion of a high-affinity state of the receptor to a low-affinity state (Fig. 1A). By contrast, the competitive binding curve for isoproterenol in the light vesicle preparation (Fig. 1B) showed only low-affinity binding of isoproterenol, and this binding was not modulated by Gpp(NH)p. Thus, receptors in these light vesicles appeared to be functionally (and presumably physically) uncoupled from G_s (*4*).

To test whether ischemia would alter the distribution of receptors between sarcolemmal and light vesicle fractions, we conducted the following studies. We

performed a thoracotomy on anesthetized guinea pigs and then ligated the proximal left anterior descending coronary artery, thus creating intense cyanosis in the anterior left ventricular region. Recordings from an electrocardiographic lead demonstrated ST-segment elevations over the affected region, a pattern typical of ischemia. Ischemia was maintained for periods of 15, 30, 60, or 90 minutes. The animals were then killed and membrane fractions were prepared as described above. Sham-operated guinea pigs were used as controls. Specific binding of β-adrenergic receptors to [^{125}I]ICYP was measured in sarcolemmal and light vesicle fractions. In control hearts, 85 and 92 fmol of [^{125}I]ICYP were bound per milligram of protein in sarcolemmal membrane and light vesicle fractions, respectively. After 15 minutes of ischemia there was no significant change in the number of β-adrenergic receptors in either fraction (Fig. 2). However, after 30 to 90 minutes, the number of β-adrenergic receptors in the surface sarcolemma membranes increased 45 percent with a concomitant 60 percent decrease in the light vesicle fraction. This is evidence for an ischemia-induced redistribution or externalization of β-adrenergic receptors to the cardiac cell surface. The number of β-adrenergic receptors detected in control membranes (sarcolemma plus light vesicle) was 96 percent of that in ischemic hearts (Fig. 3); thus only the distribution, not the total number, of β-adrenergic receptors was changed by ischemia. Isoproterenol-stimulated adenylate cyclase activity was also significantly higher in the ischemic as compared to control sarcolemmal membranes ($P < 0.003$ by one-tailed unpaired t test, Table 1), but basal, Gpp(NH)p- and forskolin-stimulated adenylate cyclase activities were unaltered in ischemia. Thus, the "new" sarcolemmal β-adrenergic receptors were functionally active.

These data provide evidence that β-adrenergic receptors of the heart, as well as other classes of cell surface receptors (*1, 7*) in other tissues, may exist in several cellular environments, including the plasma membrane and an intracellular membrane (light vesicle) location. Under control conditions, in guinea pig heart, we find about 25 percent of β-adrenergic receptors in these light vesicle fractions. Thus the routine practice of preparing only crude membranes at 30,000*g* discards a substantial portion of myocardial cell β-adrenergic receptors. Our observations in ischemic tissue and previous work involving agonist treatment (*3–5*) suggest a reciprocal relation between surface and light vesicle receptors. The specific subcellular location and detailed biochemical properties of the light vesicle receptors will require further study, but the recognition of the existence of such a fraction should facilitate further studies on mechanisms that regulate adrenergic receptor traffic in myocardium.

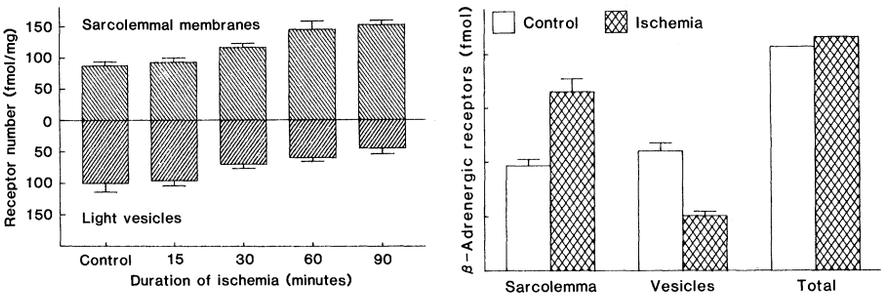

Fig. 2 (left). Ischemia leads to an externalization of β-adrenergic receptors in guinea pig heart. Male Wistar guinea pigs (400 to 500 g) were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally), intubated, and ventilated with supplemental oxygen on a Harvard respirator. A left thoracotomy was performed, the pericardium was opened, and the proximal left anterior descending coronary was ligated with 6-0 prolene suture, so as to create an anterior left ventricular region with intense cyanosis. An epicardial ECG lead was sutured over the area, confirming ST-segment elevations. Ischemia was maintained for 15, 30, 60, or 90 minutes, and then the animals were killed. Sham-operated animals were used as controls. The two membrane fractions were prepared as described in Fig. 1. Binding of β-adrenergic receptors to [^{125}I]ICYP was measured by incubating membranes with eight to ten concentrations of radioligand (50–600 p*M*) at 25°C for 1 hour. Binding reactions were terminated as described in the legend to Fig. 1. The maximal binding determined from Scatchard analysis of the binding isotherms (dissociation constants were unaltered) is plotted. Each point is the mean ± standard error of the mean of at least three experiments, with two left ventricles used in each experiment and triplicate determinations at each point. To determine statistical significance, we calculated unpaired two-tailed t tests at each time point. Because the data obtained at each of the four time points were compared against the same control hearts, we multiplied the resulting P values by 4 in accordance with the Bonferroni method. The resulting P values for sarcolemmal membranes were: 15 minutes, >0.10; 30 minutes, 0.09; 60 minutes, 0.03; and 90 minutes, 0.01. For light vesicles, the corresponding P values were >0.10, 0.07, 0.02, and 0.01. Fig. 3 (right). β-Adrenergic receptors during control conditions and ischemia in guinea pig heart. One hour of ischemia was created as described in the legend of Fig. 2. The two membrane fractions were prepared and the binding of β-adrenergic receptors to [^{125}I]ICYP was measured as previously described. The total number of receptors recovered from two control (left) and two ischemic (middle) hearts are plotted. The results are shown as the mean ± standard error of the mean of four experiments.

The evidence that myocardial β-adrenergic receptors can "move" between two subcellular compartments provides a means by which the heart may rapidly modulate its ability to respond to neuronally released or circulating catecholamines. In the setting of ischemia the molecular basis of the cellular redistribution is not yet defined, but possibilities that should be considered include enhanced breakdown of membrane phospholipids to membrane-active agents (*8*), altered cytoskeletal function (*9*), and formation of heat shock proteins (*10*).

During acute ischemia, the effects of catecholamines can be extremely deleterious. Alterations in the cardiac action potential can lead to life-threatening arrhythmias, and sinus tachycardia and increased contractility lead to increased myocardial oxygen consumption, which may extend the zone of ischemia or infarction (*11*). Our results provide a means to explain the enhanced sensitivity of ischemic myocardium to catecholamines. It will be of interest to explore mechanisms that regulate, and therapeutic interventions that may prevent, externalization in ischemia.

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Acquisition by Innervated Cardiac Myocytes of a Pertussis Toxin–Specific Regulatory Protein Linked to the α_1-Receptor

Abstract. *During development, the chronotropic response of rat ventricular myocardium to α_1-adrenergic stimulation changes from positive to negative. The α_1-agonist phenylephrine increases the rate of contraction of neonatal rat myocytes cultured alone but decreases the rate of contraction when the myocytes are cultured with functional sympathetic neurons. The developmental induction of the inhibitory myocardial response to α_1-adrenergic stimulation in intact ventricle and in cultured myocytes was shown to coincide with the functional acquisition of a substrate for pertussis toxin. A 41-kilodalton protein from myocytes cultured with sympathetic neurons and from adult rat myocardium showed, respectively, 2.2- and 16-fold increases in pertussis toxin–associated ADP-ribosylation (ADP, adenosine diphosphate) as compared to controls. In nerve-muscle cultures, inhibition of the actions of this protein by pertussis toxin–specific ADP-ribosylation reversed the mature inhibitory α_1-adrenergic response to an immature stimulatory pattern. The results suggest that innervation is associated with the appearance of a functional pertussis toxin substrate by which the α_1-adrenergic response becomes linked to a decrease in automaticity.*

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The effects of α_1-adrenergic catecholamines on cardiac automaticity change as a function of development. Purkinje fibers from dogs less than 1 day old often exhibit an increase in automaticity after exposure to the α-agonist phenylephrine. In contrast, adult canine Purkinje fibers show predominantly a decrease in rate in response to α-adrenergic input (*1*). Similarly, the neonatal rat heart responds to α-adrenergic stimulation by an increase in rate, whereas the adult rat ventricle responds by a decrease in rate (*2*). In both species, the developmental change in the α-adrenergic response from an increase to a decrease in rate parallels the maturation of functional sympathetic innervation in the heart. This observation prompted the suggestion that the cardiac sympathetic nerves are responsible for the change in cardiac responsiveness to the α-adrenergic catecholamines (*3*).

This hypothesis was tested in primary cultures of neonatal rat ventricular cells grown alone or cocultured with nerves from sympathetic ganglion cells (*2*). The neonatal rat ventricular cells showed an exclusive, positive chronotropic response to phenylephrine when cultured alone. However, when cultured with functional (*4*) sympathetic neurons, the myocytes showed a predominantly negative chronotropic response to the α-agonist (*2*).

The developmental induction of an inhibitory chronotropic response to α-adrenergic catecholamines provides an opportunity to examine potential changes in the biochemical regulators of the response. One specific candidate is the inhibitory guanine nucleotide binding protein, designated N_i, which mediates effects linked to the inhibition of adenylate cyclase (*5*). This inhibitory protein is also important in the function of effector systems not mediated by adenosine 3′,5′-monophosphate (cyclic AMP) (*6*). The latter observation is pertinent to a consideration of α_1-adrenergic catecholamines that are not believed to be regulated by cyclic AMP under most circumstances (*7*). N_i can be monitored directly with pertussis toxin, which catalyzes the transfer of ADP-ribose from NAD (ADP, adenosine diphosphate; NAD, nicotinamide-adenine dinucleotide) to the α subunit of N_i (*8*). The ADP-ribosylation of N_i inactivates it and permits unopposed utilization of stimulatory pathways (*9*). Thus, pertussis toxin can be used in pharmacological experiments to assess the functional relevance of N_i.

We present data supporting the hypothesis that the developmental conversion of the α-adrenergic chronotropic response in the heart from positive to negative is due to an increase in the functional inhibitory guanine nucleotide binding protein, presumably N_i. We used two experimental systems: (i) neonatal and adult rat ventricles and (ii) neonatal rat myocardial cells cultured alone and in the presence of sympathetic neurons.

The effect of pertussis toxin on the α_1-adrenergic response in muscle cultures and in nerve-muscle cocultures is shown in Fig. 1. Pertussis toxin did not alter significantly the basal autonomic rate of noninnervated neonatal rat myocytes. Similarly the concentration-response relation to phenylephrine was not significantly changed by pertussis toxin (Fig. 1A). The positive chronotropic response to phenylephrine, in the presence or absence of pertussis toxin, was inhibited by the α_1-adrenergic antagonist prazosin (data not shown). Thus, in neonatal muscle cells cultured alone, pertussis toxin had no significant action. In muscle cells cultured with sympathetic nerves (Fig. 1B), pertussis toxin, which did not change basal automaticity, altered the response to phenylephrine so that all concentrations were associated with a positive chronotropic response. The responses of both the control and the pertussis toxin–treated nerve-muscle cultures to phenylephrine were inhibited by prazosin.

It is possible that pertussis toxin enhances either basal cyclic AMP levels or cyclic AMP accumulation in response to stimulatory agents. Such elevations of myocyte intracellular cyclic AMP concentration are associated with an increase in automaticity (*10*). Thus, the effect of pertussis toxin could simply have been due to an indirect action to raise cyclic AMP levels in the nerve-muscle cultures. Several results, however, make this suggestion unlikely. First, the experiments were performed in the presence of both the β-adrenergic antagonist propranolol (0.1 μ*M*) and the muscarinic inhibitor atropine (0.1 μ*M*), negating a cyclic AMP response through a β-adrenergic mechanism or through reversal of a muscarinic receptor–dependent effect. Second, cyclic AMP levels in muscle and nerve-muscle cultures were measured by a sensitive radioimmunoassay and found not to be changed under any of our experimental conditions. Muscle and nerve-muscle cultures had similar basal cyclic AMP levels that did not change after addition of phenylephrine. Furthermore, there was no significant increase in cellular cyclic AMP concentration in innervated or noninnervated myocytes after pertussis toxin ex-