Platelet Alpha-adrenergic Receptors Are Not Down-regulated during Cardiopulmonary Bypass

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Hemodynamic instability after extracorporeal circulation for cardiac surgery frequently requires substantial pharmacologic intervention to overcome cardiovascular depression, despite marked increases in endogenous plasma epinephrine, norepinephrine, and dopamine concentrations.[1,2] There is poor correlation between the elevated plasma catecholamines and hemodynamic variables during cardiopulmonary bypass.[2] One of the possible mechanisms of the apparent reduced responsiveness to α-adrenergic stimulation by elevated catecholamine concentrations might be down-regulation of α-adrenergic receptors, i.e., a decrease in the number of receptors. This would be similar to the convincingly demonstrated down-regulation of β-adrenergic receptors on white blood cells that occurs after continued exposure to agonist.[3] Since regulation of platelet α-adrenergic receptors may be common to all α-adrenergic receptors,[4] we measured platelet α-adrenergic receptors before and after cardiopulmonary bypass in patients undergoing coronary artery bypass grafting (CABG) surgery.

METHODS

After informed consent was obtained, seven ASA class III patients undergoing multiple-vessel elective coronary artery bypass graft surgery were studied according to a protocol approved by our Human Subjects Review Committee. General anesthesia was induced with fentanyl 50–60 μg/kg, diazepam 0.2 mg/kg, and pancuronium 0.1 mg/kg iv. Ventilation was maintained with oxygen and enflurance as needed. The approximate duration of cardiopulmonary bypass was 2.5 h. No positive or negative inotropic or vasopressor drugs were used during the study period. Arterial blood was sampled a first time from the radial arterial catheter before the induction of general anesthesia and was sampled a second time from the oxygenator when the patient's nasopharyngeal temperature reached approximately 31° C during rewarming, before the administration of any blood products.

Each of the two 20-ml blood samples was withdrawn into a plastic syringe containing 2 ml of 4% sodium citrate and was immediately centrifuged at 150 × g for 10 minutes in a polypropylene test tube. All further specimen handling was with polypropylene plasticware. The platelet-rich plasma was aspirated immediately and kept at 25° C until further processing within 4 h. It was centrifuged at 8,000 × g at 4° C for 10 min and resuspended carefully in 5 ml fresh incubation buffer containing 50 mM TRIS-HCl, 100 mM NaCl, 5 mM EDTA, and 0.8 mM ascorbic acid (pH 7.5). The platelet suspension was immediately centrifuged at 6,000 × g at 4° C for 10 min and resuspended in 5 ml buffer. The platelets were thus washed four times to eliminate a potential effect on the assay of any drugs in the sampled plasma. The final volume of platelet suspension at 25° C in buffer was adjusted empirically to yield a platelet count of 200–400 thousand/μl, within the range of linearity previously established for this assay.

[³H]Yohimbine binding was performed immediately by incubating 0.2 ml of the platelet suspension with [³H]Yohimbine (0.5–20 nM) in a total volume of 0.25 ml at 25° C for 30 min. Binding reactions were terminated by adding 4 ml of incubation buffer (25° C) and immediately filtering the contents of the tubes over glass fiber filters (Whatman GF/C) that had been presoaked in 0.5% bovine serum albumin. The test tubes and filters were washed with two additional 4 ml aliquots of buffer, and the radioactivity retained on each filter was determined in a liquid scintillation counting system at an efficiency of 34% after being air-dried overnight. Filtering and washing required less than 15 s and were performed with the use of a filtration manifold with a constant vacuum. Platelet count on the final suspension was performed by the hospital clinical chemistry laboratory in a Coulter Counter® with a 70-μm aperture tube. Specific binding was defined as binding that could be competed for by 10 μM phentolamine and was calculated by subtracting mean nonspecific binding (in the presence of phentolamine) from mean total binding (in the absence of phentolamine). Total and nonspecific binding were each performed in triplicate. All counts were within ± 4% of the mean. In general, nonspecific binding was less than 40% of total [³H]Yohimbine binding. The normality of distribution of the data was confirmed and the significance of specific binding (sites/cell) before and after bypass was assessed with the paired t test.

RESULTS

Using the methods described, preliminary data obtained from Scatchard analysis of 6 point specific [³H]Yohimbine binding to platelets obtained from healthy laboratory personnel gave a B_{max} of 345 (SEM ± 48) binding sites/cell and a K_D of 2.8 (SEM ± 0.4) nM. The values obtained are in accord with quoted literature values.[5-7] Since specific binding was saturated in the presence of 10 nM ligand, binding to patients' platelets was measured at 20 nM ligand and 2 nM ligand, only to conserve the volume of blood required for the study. The results are displayed in table 1; 20 nM binding sites ranged from 145 to 324 sites/cell with a mean of 226 (SEM ± 25) before cardiopulmonary bypass and a mean of 237 (SEM ± 23) during rewarming. Paired t test revealed no statistical significance between these values.

In addition to approximating the B_{max}, the ratio of saturation binding at 20 nM ligand to binding at 2 nM ligand was calculated before bypass and during rewarming for each patient. This ratio was 2.03 (SEM ± 0.21) and 1.95 (SEM ± 0.30), respectively (see table 1), and was not statistically different.

DISCUSSION

Human platelet adrenergic receptors are exclusively of the α_2-adrenergic variety and as such have been studied extensively using [³H]Yohimbine binding.[5-7] Early reports of down-regulation of platelet α-adrenergic receptors[4] may have resulted from retained agonist competing for receptors at nonsaturation kinetics.[6] To overcome this potential interference, we washed the platelets extensively before assay. This treatment was designed to eliminate the likelihood of substances from the plasma interfering with the assay. Washing the platelets also eliminated the need to screen patients on the basis of confounding conditions in the receptor microenvironment, which might have influenced the assay before and also after cardiopulmonary bypass. This allowed each patient to act as his or her own control for changes occurring during bypass. Additionally, we approximated B_{max} by measuring ligand binding at conditions well in excess of saturation kinetics for the receptor. This eliminated any potential interference that altered affinity, or minor fluctuations in ligand concentration, would have on specific binding, since the only variable capable of altering specific binding would be the number of receptors. Our results show no evidence of down-regulation of platelet α-adrenergic receptors during cardiopulmonary bypass. Another way that the receptors might have been modified could be by reduced affinity of the receptors for the ligand. An analysis of specific binding in the presence of varying ligand concentrations would reveal a very marked reduction in binding in the presence of the lower 2 nM ligand concentration re-

sulting from such an alteration of K_D or dissociation constant. On the other hand, such an alteration of K_D would not affect the determination of B_{max}, as described before, and would have been reflected in a higher ratio of 20 nM to 2 nM binding after cardiopulmonary bypass. No such intrinsic change in receptor affinity could be demonstrated. By measuring receptor binding at two selected ligand concentrations, we have been able to perform an analysis of B_{max} and K_D changes without the need for excessive amount of patient blood sampling. It would have been interesting to know if the patients tested actually had high endogenous catecholamine levels without a hypertensive response. However, we were able to show that there is no change in platelet α-adrenergic receptors to explain a lack of hypertensive response to high levels of circulating catecholamines previously demonstrated[1,2] to occur during cardiopulmonary bypass.

Down-regulation of brain[8] and platelet[9] α_2-adrenergic receptors have been reported in response to chronic agonist stimulation. Down-regulation of platelet adrenergic receptors has also been reported in a case of orthostatic hypotension,[10] however, this finding is controversial,[7,11] especially in the light of a wide range for normal values. Acutely elevated plasma catecholamines reported to occur in the absence of a hypertensive response in patients during cardiopulmonary bypass are not associated with down-regulation of platelet α-adrenergic receptors. This finding is consistent with results from other workers who found no change in the number of platelet α-adrenergic receptors in patients with chronically or acutely elevated plasma levels of norepinephrine.[7] The platelet adrenergic receptor system has been widely studied as a model for all adrenergic receptors, however, this relationship awaits confirmation. Changes in α-adrenergic receptors occurring elsewhere may not be reflected in changes at the platelet α-adrenergic receptors. Neither do these results rule out reversible changes of receptor function such as desensitization during bypass. Conditions affecting the environment of the receptors during bypass may play a role. Several factors such as intracellular Mg^{++} and guanosine tri-

TABLE 1. Specific Binding of [³H]Yohimbine to Platelets

	Sites/Cell		Ratio of $\frac{20\ nM}{2\ nM}$ Specific Binding	
	Before Bypass	After Bypass	Before Bypass	After Bypass
Patient 1	232	268	1.78	1.75
Patient 2	324	283	1.60	1.54
Patient 3	237	245	3.19	3.68
Patient 4	150	158	2.03	1.95
Patient 5	213	296	1.66	1.79
Patient 6	268	264	1.66	1.42
Patient 7	160	145	2.26	1.52
Mean ± SEM	226 ± 25	237 ± 23	2.03 ± 0.21	1.95 ± 0.30

phosphate (GTP) and extracellular Na^+ are known to regulate receptor affinity.[12,13] In addition, hypothermia, among other factors, could conceivably alter the affinity of the receptors reversibly in a way that would not be detected by our assay. Similarly, we are unable to exclude the possibility of an agonist-induced change in affinity of the receptor that might have inhibited receptor cycling and that would have been reversed by washing off the agonist. In any event, the evidence suggests that there is no acute down-regulation or irreversible alteration of α-adrenergic receptors in platelets during cardiopulmonary bypass.

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Elective Coronary Bypass Surgery without Pulmonary Artery Catheter Monitoring

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Increasing concern over the cost of medical care has prompted reexamination of the indications for many clinical procedures, including pulmonary artery catheterization for hemodynamic monitoring. Recently Loop *et al.*[1]

reported that one of the principal techniques employed at the Cleveland Clinic to contain the cost of coronary artery bypass graft (CABG) surgery has been to limit the use of the pulmonary artery (PA) catheter to patients with severely impaired left-ventricular function. The authors presented no data to support their limited use of the PA catheter.

The indications for PA catheterization in coronary artery surgery have been debated for several years.[2,3] Mangano[4] found that the PA catheter offered little advantage over the central venous (CV) catheter in managing CABG patients with ejection fractions greater than 50% and without angiographically demonstrable ventricular dyssynergy preoperatively, because the CV and PA-occluded pressures were highly correlated. However, in similar pa-