CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

CHANGES IN AUTONOMIC RESPONSE OF THE CEREBRAL CIRCULATION AFTER NORMOTHERMIC EXTRACORPOREAL CIRCULATION

Frank W. Sellke, MD
Steven Y. Wang, MD, PhD
Alon Stamler, MD
Robert G. Johnson, MD
William E. Cohn, MD
Ronald M. Weintraub, MD

Patients who undergo cardiopulmonary bypass frequently have neuropsychologic dysfunction. This study was undertaken to determine whether altered cerebral perfusion and vascular responses may in part lead to these neuropsychologic changes. Pigs were placed on normothermic cardiopulmonary bypass for 2 hours. Basal cerebral blood flow and in vivo responses to administration by internal carotid artery of neuronally released vasoactive substances were evaluated before and 5 to 15 minutes after termination of cardiopulmonary bypass. Another group of pigs were placed on cardiopulmonary bypass for 2 hours and then perfused off bypass for 1 additional hour. In vitro responses of cerebral arterial microvessels (100 to 175 μm) from both groups were examined in a pressurized (40 mm Hg) no-flow state with videomicroscopy. Vessels from uninstrumented pigs served as control preparations for in vitro studies. Cerebrovascular resistance and cerebral perfusion were maintained constant during cardiopulmonary bypass and after separation from bypass. The internal carotid artery infusion of acetylcholine (cholinergic agonist) caused increased internal carotid artery blood flow before cardiopulmonary bypass but decreased blood flow after cardiopulmonary bypass. After 2 hours of cardiopulmonary bypass, the increase in internal carotid artery blood flow induced by isoproterenol (a β-adrenoceptor agonist) was reduced, whereas the response to sodium nitroprusside (a guanylate cyclase activator) was unchanged. In vitro acetylcholine-induced microvascular vasodilation was converted to a contractile response and isoproterenol elicited less relaxation after 2 hours of cardiopulmonary bypass. One hour of cerebral perfusion after cardiopulmonary bypass caused a further reduction in isoproterenol-induced relaxation but had no further effect on the cholinergically mediated response. In vitro relaxation responses to sodium nitroprusside and forskolin (an adenylate cyclase activator) were similar in all experimental groups, suggesting that second-messenger mechanisms remain intact after normothermic cardiopulmonary bypass. In conclusion, basal cerebrovascular resistance and internal carotid artery blood flow are maintained if the systemic circulation and pressure are supported with fluid administration after cardiopulmonary bypass. Agonist-induced vasodilation of cerebral microvessels to cholinergic and β-adrenoceptor stimulation are selectively impaired after normothermic cardiopulmonary bypass, whereas second-messenger mechanisms remain intact. (J Thorac Cardiovasc Surg 1996;112:450-61)

From the Division of Cardiothoracic Surgery, Beth Israel Hospital and Harvard Medical School, Boston, Mass.

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Address for reprints: Frank W. Sellke, MD, Division of Cardiothoracic Surgery, Beth Israel Hospital, Dana 905, 330 Brookline Ave., Boston, MA 02215.

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Extracorporeal circulation has been a critical component in the development of modern cardiovascular surgery. Unfortunately, patients undergoing cardiac operations in which cardiopulmonary bypass (CPB) is used frequently showed signs of overt neuropsychologic dysfunction or stroke after an otherwise uneventful operation. These changes have been reported to occur in 11% to 79% of patients undergoing cardiac operation, depending on the method of examination.[1-4] The rate of overt stroke after cardiac operations is approximately 4%.[2] The causes of neuropsychologic dysfunction after cardiac operations may be related to air or particulate embolization,[5] insufficient regional perfusion from obstructive cerebral vascular disease, or altered vascular reactivity leading to vasoconstriction after prolonged CPB. The effects of extracorporeal circulation, hypothermia, and pulsatile perfusion on baseline cerebrovascular flow and autoregulation have been examined,[4, 6, 7] and most clinical and experimental studies have revealed preserved cerebral blood flow. No studies, however, have directly examined the effects of extracorporeal circulation on modulation of vascular tone in the cerebral circulation by neurohumoral substances.

Endothelium-derived nitric oxide[8] and other endothelium-derived vasoactive substances influence the regulation of cerebral arteriolar tone. A balance exists between factors that tend to constrict the arterioles and the release of nitric oxide and other endothelium-derived vasodilating substances, which tend to maintain these vessels in a relaxed state. Akopov, Sercombe, and Seylaz[9] recently reported that leukocyte-induced acute endothelial dysfunction of the cerebral arteries of rabbits can predispose them toward acute vasospasm during platelet activation. Because platelets and leukocytes are activated during CPB,[10] this may create a condition predisposing either large or small cerebral arteries toward constriction, significantly reducing brain perfusion during and after cardiac operations. In addition, the use of exogenous catecholamines to augment cardiac function may influence the vasomotor state of cerebral vessels and of vessels in other vascular beds.

The primary goal of this study was to determine whether cerebral responses to neuronal substances were altered after prolonged (2 hours) extracorporeal circulation. To evaluate relaxations mediated by β-adrenoceptors and cholinergic receptors, in vivo and in vitro responses to isoproterenol and acetylcholine, respectively, were studied. To examine the state of second-messenger mechanisms, the responses to adenylate cyclase and guanylate cyclase activation were assessed in vitro.

Materials and methods

Animal preparation. Yorkshire pigs (20 to 25 kg) of both sexes were medicated intramuscularly with ketamine (10 mg/kg) and anesthetized intravenously with α-chloralose and urethane (60 mg/kg and 300 mg/kg initially and 15 mg/kg and 60 mg/kg every 60 minutes as needed, respectively). Pigs were tracheally intubated and mechanically ventilated. In the control group ($n = 6$), a frontal-parietal craniotomy was performed and the animal was given heparin (500 U/kg). A 20 gm portion of brain tissue (cortical gray matter) from the middle cerebral artery (MCA) distribution was rapidly excised for microvessel examination and immediately placed in cold (5° to 10° C) 3-(*N*-morpholino)-propanesulfonic acid (MOPS) buffer solution of the following composition: 145.0 mmol/L sodium chloride, 4.7 mmol/L potassium chloride, 2.0 mmol/L calcium chloride, 1.2 mmol/L magnesium sulfate, 1.2 mmol/L sodium phosphate, 3.0 mmol/L MOPS, 0.02 mmol/L ethylenediaminetetraacetic acid, and 5.0 mmol/L glucose. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Responses 15 minutes after separation from CPB. In seven pigs, after induction of anesthesia and tracheal intubation a fluid-filled catheter was introduced into the femoral artery and advanced to the aorta for pressure monitoring. A femoral vein was cannulated for vascular access. The right carotid artery and internal jugular vein were exposed through a vertical neck incision for carotid artery pressure monitoring, oxygen saturation measurements, and drug administration. The catheter in the internal jugular vein was advanced to the region of the venous sinus. In preparation for internal carotid artery (ICA) drug administration, a silicone elastomer catheter (inner diameter 0.3 mm, outer diameter 0.5 mm) was introduced into the common carotid artery and advanced into the ICA. The external carotid artery was ligated at its origin. ICA blood flow was measured with an ultrasonic flow probe (Transonic Systems, Inc., Ithaca, N.Y.), which was placed around the ICA just distal to the bifurcation (Fig. 1). After a sternotomy was performed, pigs were given heparin intravenously (500 U/kg initially and 300 U/kg each 90 minutes) and cannulated through the distal ascending aorta and the right atrium. Activated clotting time was measured at regular intervals and was maintained at greater than 400 seconds. Total CPB was instituted with a bubble oxygenator (Bentley Bio-2; Baxter Healthcare Corp., Irvine, Calif.) and a standard roller pump (Cardiovascular Instrument Corp., Wakefield Mass.). An arterial filter (Bentley Bio-1025; Baxter) was inserted into the circuit distal to the roller pump. Blood flow was maintained at from 2.0 to 3.0 L/min (2.6 to 4.2 $L \cdot min^{-1} \cdot m^{-2}$) to maintain a mean perfusion pressure of

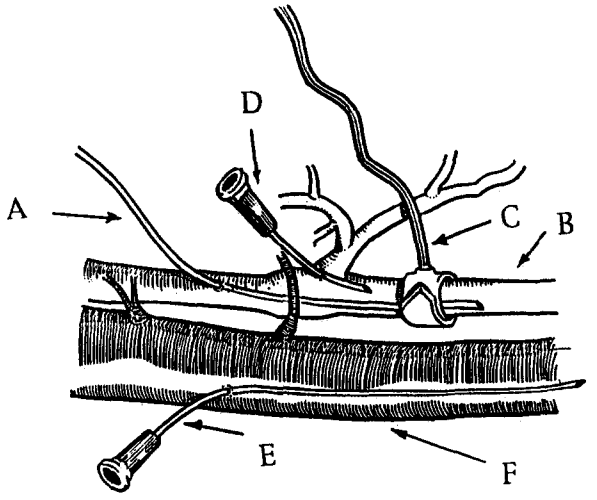

Fig. 1. Schematic of method to measure blood flow responses to internal carotid artery infusion of vasoactive substances (in vivo). A silicone elastomer catheter *(A)* was placed into the common carotid artery and advanced into the proximal internal carotid artery *(B)* for drug infusion. A perivascular Doppler flow probe *(C)* was used to measure internal carotid blood flow under baseline conditions and during the administration of vasoactive substances. Additional catheters *(D, E)* were placed in the internal carotid artery and high in the internal jugular vein *(F)* for pressure monitoring. The external carotid artery was ligated at its origin.

50 to 80 mm Hg. Systemic blood temperature was maintained at 37° C. Arterial blood gases were obtained before commencement of CPB and at approximately 20-minute intervals thereafter. Arterial blood gases were adjusted by ventilatory rate, tidal volume, and inspired oxygen fraction to maintain oxygen tension at >100 mm Hg, pH between 7.25 and 7.45, and carbon dioxide tension between 25 and 45 mm Hg. After 120 minutes of total CPB, all animals were separated from extracorporeal circulation by increasing cardiac filling. Lactated Ringer's solution was administered for 5 minutes to maintain mean systemic blood pressure at greater than 50 mm Hg.

In vivo studies. In vivo responses to the administration by means of a Harvard infusion pump (Harvard Apparatus, Inc., South Natick, Mass.) of isoproterenol (6, 60 $\mu g \cdot kg^{-1} \cdot min^{-1}$), acetylcholine (0.6, 6 $mg \cdot kg^{-1} \cdot min^{-1}$), and sodium nitroprusside (45, 450 $\mu g \cdot kg^{-1} \cdot min^{-1}$) were studied before initiation of CPB and 5 minutes after discontinuation of CPB. The recordings were first taken after the vascular responses had stabilized. Pressure and flow signals were recorded on an eight-channel recorder (Honeywell–Electronics for Medicine Research, Natick, Mass.). Cerebrovascular resistance was calculated according to the following formula: $CVR = BF \times 80/(ICAP - IJVP)$, where *CVR* is cerebrovascular resistance in dynes $\cdot sec^{-1} \cdot cm^{-5}$), *BF* is unilateral ICA blood flow in L/min, *ICAP* is ICA pressure in mm Hg, and *IJVP* is internal jugular venous pressure in mm Hg.

In two pigs, vehicle (normal saline solution) was administered at the same rate as that used during drug administration. The vehicle caused no change in unilateral ICA blood flow and had no effects on systemic hemodynamic parameters.

After completion of the in vivo vascular studies (approximately 15 minutes), a frontal-parietal craniotomy was performed on the left side (the side opposite that on which in vivo experiments were performed). Brain tissue in the distribution of the MCA was rapidly excised and immediately placed in cold MOPS buffer solution (CPB-15min group).

Responses 60 minutes after separation from CPB. To determine whether brain perfusion after separation from normothermic CPB would affect the altered vascular responses caused by extracorporeal circulation, the same procedure was followed as in the CPB-15min group, except that the administration of lactated Ringer's solution was continued for 60 minutes as necessary to support the systemic circulation after discontinuation of CPB ($n = 7$). Unilateral ICA blood flow, ICA and internal jugular vein oxygen contents, and aortic, carotid artery, and jugular venous pressures were measured and recorded at 15-minute intervals. In vivo vascular studies were not performed on animals in this experimental group. Mean systemic blood pressure was maintained at more than 50 mm Hg. Sixty minutes after separation of pigs from CPB, a frontal-parietal craniotomy was performed and brain tissue was rapidly excised and immediately placed in cold MOPS buffer solution (CPB-60min group). After harvest of brain tissue, animals were killed by exsanguination.

In vitro cerebral microvessel studies. Cerebral arterial microvessels (100 to 175 μm internal diameter) were dissected from the MCA region of the cortical gray matter of each brain under a 10× to 60× dissecting microscope (Olympus Optical Co. Ltd., Tokyo, Japan). The MCA-dependent region on the side opposite that used for in vivo experiments was selected to minimized any effects of tachyphylaxis. Microvessels were placed in an isolated acrylic plastic organ chamber, cannulated with dual glass micropipettes measuring 30 to 80 μm in diameter, and secured with 10-0 nylon monofilament suture (Ethicon, Somerville, N.J.). MOPS buffer solution aerated with room air and warmed to 37° C was continuously circulated through the organ chamber and a reservoir (total volume 100 ml) (Fig. 2). The vessels were pressurized to 40 mm Hg in a no-flow state by means of a burette manometer filled with MOPS buffer solution. With an inverted microscope (40× to 200×; Olympus) connected to a video camera, the vessel image was projected onto a black-and-white television monitor (Hitachi Ltd., Tokyo, Japan). An electronic dimension analyzer[11] (Living Systems Instrumentation, Burlington, Vt.) was used to measure internal lumen diameter. Measurements were recorded with a strip chart recorder (Western Graphtec, Irvine, Calif.). Vessels were allowed to equilibrate for 30 minutes in MOPS buffer solution before pharmacologic intervention.

Evaluation of β-adrenergic and cholinergic responses. In all experimental groups, relaxation responses of microvessels to isoproterenol, forskolin, acetylcholine, or sodium nitroprusside were examined after precontraction of vessels by 25% to 50% of the resting baseline diameter

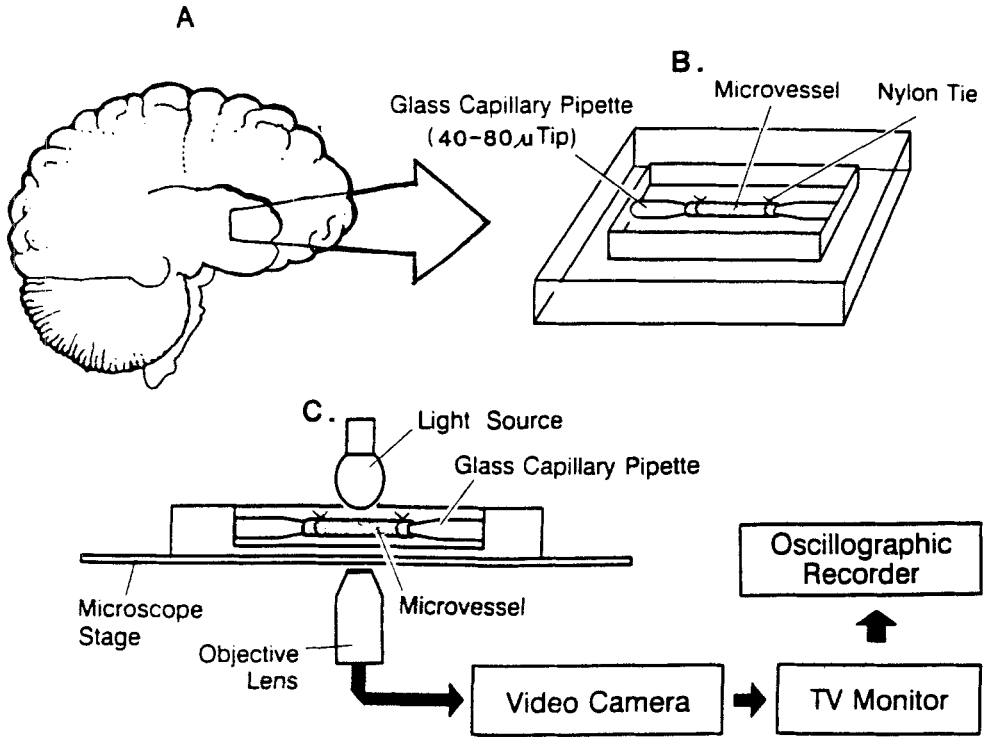

Fig. 2. Schematic of in vitro microvessel apparatus. Cerebral microvessels were cannulated and pressurized in a no-flow state. With an inverted microscope connected to a video camera, the vessel image was projected onto a television monitor. An electronic dimension analyzer was used to measure internal lumen diameter, which was recorded on an oscillographic strip chart recorder. **A,** Brain; **B,** organ chamber; **C,** imaging apparatus.

with the thromboxane A_2 analog U46619. Once the steady-state tone was reached, the dose responses to isoproterenol (10^{-12} – 10^{-4} mol/L), forskolin (10^{-9} – 10^{-5} mol/L), acetylcholine (10^{-9} – 10^{-4} mol/L), and sodium nitroprusside (10^{-9} – 10^{-4} mol/L) were examined. In addition, relaxation responses to acetylcholine were examined in control vessels pretreated with the nitric oxide synthase inhibitor N^G-nitro-L-arginine (10^{-4} mol/L), the muscarinic receptor blocker atropine (10^{-6} mol/L), or after mechanical denudation of the endothelium. The removal of the endothelial cells was accomplished by advancing a human hair (60 μm diameter) into the lumen and abrading the surface, followed by the intraluminal injection of air bubbles. The vessels denuded of endothelium showed a normal relaxation response to sodium nitroprusside (95 ± 2%, 10^{-4} mol/L) but failed to relax in response to adenosine 5′-diphosphate (4% ± 6%, 10^{-4} mol/L). Responses to isoproterenol were also examined in the presence of the β-adrenergic receptor blocker propranolol (10^{-6} mol/L). Antagonists were added at least 15 minutes before a dose-response intervention. Two or three interventions were performed on each vessel, and the order of drug administration was random. The dose response to each agonist was examined only once per vessel to avoid tachyphylaxis. All drugs were applied extraluminally. Measurements were obtained 2 to 3 minutes after the drug was administered, when the response had stabilized. The vessels were washed three times with MOPS buffer solution and allowed to equilibrate in drug-free buffer solution for 10 to 15 minutes between experimental interventions.

Drugs. Isoproterenol, acetylcholine, U46619, adenosine 5′-diphosphate, N^G-nitro-L-arginine, and sodium nitroprusside were obtained from Sigma Chemical Company (St. Louis, Mo.). Forskolin, atropine, and propranolol were obtained from Research Biomedicals International (Natick, Mass.). Forskolin was dissolved in dimethyl sulfoxide. U46619 was dissolved in ethanol to make a stock solution and was stored at −20° C. Other drugs were dissolved in ultrapure distilled water for in vitro experiments. Drugs for in vivo experiments were dissolved in normal saline solution. All solutions were prepared on the day of the study.

Data analysis. The response of microvessels to each agent was examined only once in each animal. Response data were pooled for each dose in each experimental group and an average was calculated. The relaxation responses were expressed as percentage relaxation of the U46619-induced precontraction of the vessel diameter. All values were expressed as mean ± standard error of the mean (SEM). Comparisons of in vitro and in vivo dose responses of all experimental groups were performed by

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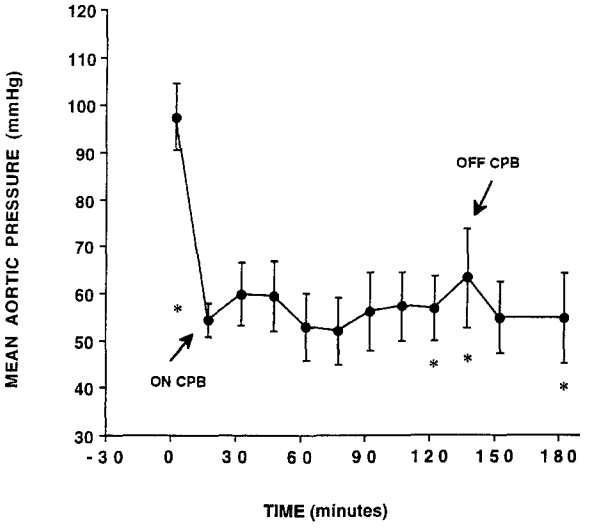

Fig. 3. Plot of mean aortic blood pressure as a function of time. Time of onset of CPB is designated as 0 minutes. Values are mean ± SEM. *Asterisks* represent $p < 0.05$ versus initial prebypass value.

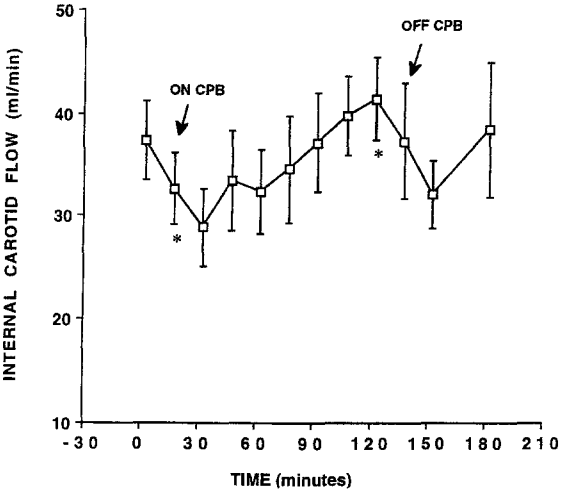

Fig. 4. Plot of internal carotid artery blood flow (unilateral) as a function of time. Time of onset of CPB is designated as 0 minutes. Values are mean ± SEM. *Asterisks* represent $p < 0.05$ versus initial prebypass value.

two-way (group, drug concentration) analysis of variance for repeated measures. In addition, comparisons were performed at each drug concentration with one-way (group) analysis of variance. Scheffe's multiple comparison test was used post hoc to determine significance between groups. Hemodynamic and blood flow data were compared with Student's *t* test (two-tailed, paired). A *p* value of less than 0.05 was considered to be significant for all comparisons.

Results

In vivo studies

Effect of CPB on ICA blood flow and vascular resistance. Initiation of CPB was associated with a marked reduction in mean aortic blood pressure (Fig. 3) and a small but significant reduction in baseline cerebral blood flow (Fig. 4). Concomitant with a reduction in systemic vascular resistance was a reduction in cerebral vascular resistance (Fig. 5), allowing brain perfusion to be relatively maintained. During the 2-hour period of CPB and subsequent 1-hour period of perfusion after separation from CPB, mean aortic pressure was depressed compared with that observed before CPB, but it remained relatively constant if the systemic hemodynamics were supported with fluid administration (Fig. 3). In this case, ICA blood flow gradually increased after an initial decline (Fig. 4).

Arterial blood gas analysis revealed an oxygen tension of 401 ± 26 mm Hg, a carbon dioxide tension of 42 ± 3 mm Hg, and a pH of 7.41 ± 0.03 before CPB.

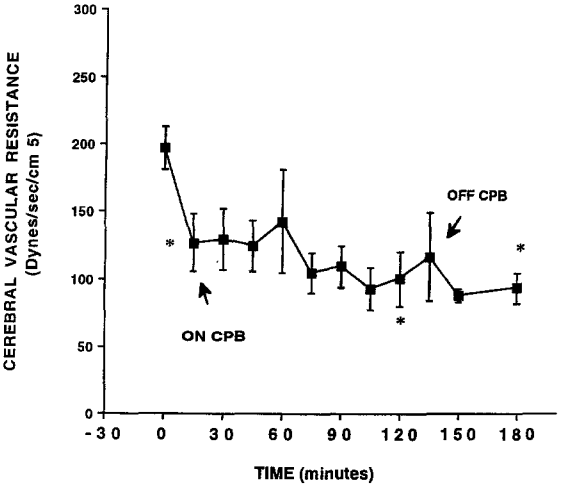

Fig. 5. Plot of cerebral vascular resistance (unilateral) as a function of time. Time of onset of CPB is designated as 0 minutes. Values are mean ± SEM. *Asterisks* represent $p < 0.05$ versus initial prebypass value.

It revealed an oxygen tension of 216 ± 32 mm Hg, a carbon dioxide tension of 38 ± 3 mm Hg, and a pH of 7.32 ± 0.02 15 minutes after termination of CPB. Hematocrit was 32% ± 2% before CPB and 23% ± 2% 15 minutes after termination of CPB.

In vivo cerebral blood flow responses. Infusion of acetylcholine into the ICA before onset of CPB increased ICA blood flow (Fig. 6). After CPB, acetylcholine caused a small but significant decrease in ICA blood flow, compared with that before CPB.

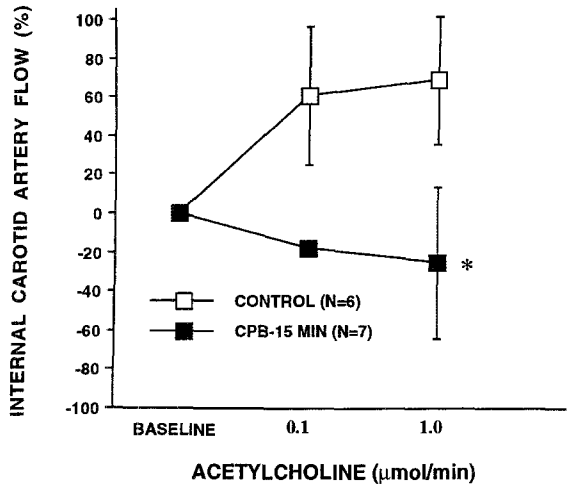

Fig. 6. Change in internal carotid artery blood flow (percentage change from baseline) in response to the intraluminal administration of acetylcholine before CPB and after 2 hours of total CPB and 15 minutes of post-CPB perfusion (CPB-15min). Data are mean ± SEM. *Asterisk* represents $p < 0.05$ versus before CPB.

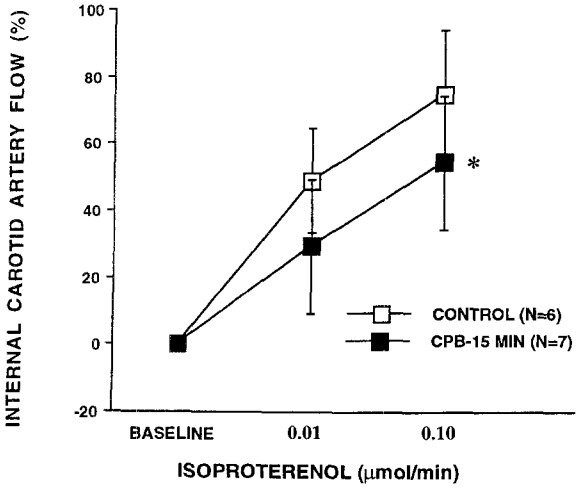

Fig. 7. Change in internal carotid artery blood flow (percentage change from baseline) in response to the intraluminal administration of isoproterenol, before and after 2 hours of total CPB and 15 minutes of post-CPB perfusion (CPB-15min). Data are mean ± SEM. *Asterisk* represents $p < 0.05$ versus before CPB.

Before CPB, infusion of isoproterenol caused an increase in ICA blood flow (Fig. 7). After CPB, the same concentration of isoproterenol increased ICA blood flow significantly less than it had before CPB, indicating a reduced sensitivity after CPB of the cerebral circulation to β-adrenergic stimulation. In-

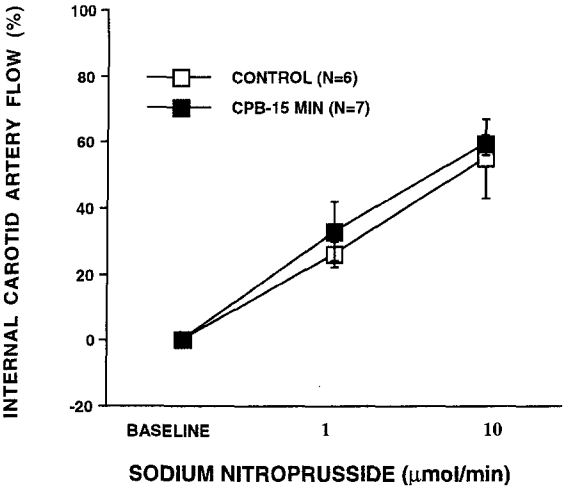

Fig. 8. Change in internal carotid artery blood flow (percentage change from baseline) in response to the intraluminal administration of sodium nitroprusside, before and after 2 hours of total CPB and 15 minutes of post-CPB perfusion (CPB-15min). Data are mean ± SEM.

fusion of sodium nitroprusside into the ICA before and after CPB increased ICA flow from baseline slightly and similarly (Fig. 8). The intraarterial infusion of acetylcholine or sodium nitroprusside had no effect on systemic pressure or hemodynamics, whereas the infusion of isoproterenol caused only a slight tachycardia at the highest concentration administered.

In vitro studies: Microvascular responses

Vessel characteristics. Cerebral microvessels averaged 139 ± 7 μm, 144 ± 6 μm, and 136 ± 6 μm in diameter in the control, CPB-15min, and CPB-60min groups, respectively. The percentage precontractions of vessels were 35% ± 2%, 36% ± 2%, and 37% ± 3% in the control, CPB-15min, and CPB-60min groups, respectively. The concentration of U46619 needed to induce this degree of contraction was similar among groups (log mol/L U46619 = −6.3 ± 0.1 [control], −6.5 ± 0.1 [CPB-15min], −6.4 ± 0.1 [CPB-60min]).

Cholinergic response to acetylcholine. Acetylcholine elicited a dose-dependent relaxation (in vitro) of control cerebral microvessels, which was inhibited in the presence of N^G-nitro-L-arginine or after endothelial denudation. Atropine, which blocks muscarinic receptors on both microvascular endothelium and smooth muscle, produced a lesser degree of inhibition of acetylcholine-induced relaxation than did either N^G-nitro-L-arginine or endothelial denudation (Fig. 9). Acetylcholine therefore causes

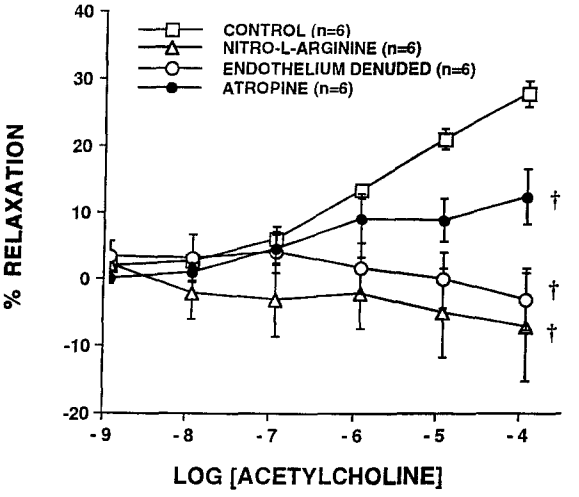

Fig. 9. Plot of in vitro responses of precontracted porcine cerebral microvessels to the cholinergic agonist acetylcholine from uninstrumented animals in the absence ($n = 6$) or presence of N^G-nitro-L-arginine (100 μmol/L, $n = 6$) or atropine (1 μmol/L, $n = 6$), or in microvessels denuded of endothelium ($n = 6$). Vessels were pressurized to 40 mm Hg in a no-flow state. Drugs were applied extraluminally. Responses are expressed as percentage relaxation of the U46619-induced vascular contraction. *Daggers* represent $p < 0.01$ versus microvessels with intact endothelium in the absence of any antagonist (control).

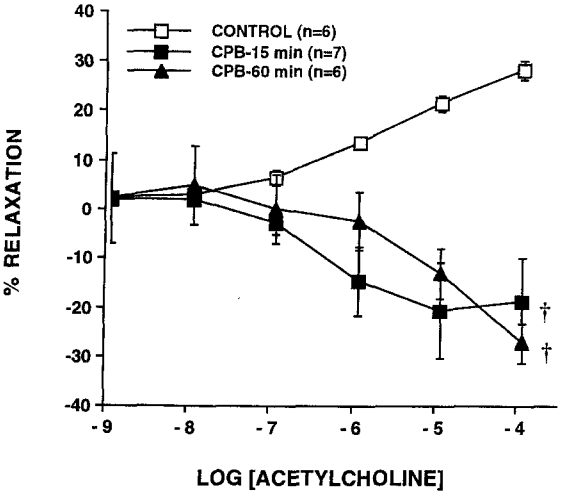

Fig. 10. Plot of in vitro responses of precontracted porcine cerebral microvessels to the cholinergic agonist acetylcholine from control animals ($n = 6$), animals after 2 hours of CPB and 15 minutes of perfusion after CPB (CPB-15min, $n = 7$), and animals after separation from CPB and 1 hour of cerebral perfusion after separation from CPB (CPB-60min, $n = 6$). Microvessels were pressurized to 40 mm Hg in a no-flow state. Drugs were applied extraluminally. Responses are expressed as percentage relaxation of the U46619-induced vascular contraction. *Daggers* represent $p < 0.01$ versus control.

relaxation of cerebral microvessels through an endothelium-dependent, muscarinic receptor–mediated release of nitric oxide. After CPB, acetylcholine elicited microvascular contraction instead of relaxation. One hour of additional perfusion after CPB did not alter this pattern of increased contraction (Fig. 10).

β-Adrenergic response to isoproterenol. Isoproterenol caused a potent relaxation of control cerebral microvessels (Fig. 11). This relaxation (76% ± 4% and 93% ± 2% of U46619-induced contraction at 0.1 μmol/L and 10 μmol/L isoproterenol, respectively) was inhibited in the presence of 1 μmol/L propranolol (7% ± 4% and 24% ± 6% at 0.1 μmol/L and 10 μmol/L isoproterenol, respectively), suggesting that the isoproterenol-induced relaxation of cerebral microvessels is mediated through a β-adrenoceptor mechanism. After CPB, this relaxation was significantly reduced. In microvessels from pigs perfused an additional hour after separation from CPB (CPB-60min group), isoproterenol induced less relaxation than seen in vessels from the CPB-15min group. This suggests that the altered β-adrenergically mediated relaxation is not resolved soon after separation from CPB but is further impaired with time.

Guanylate cyclase and adenylate cyclase activation. Sodium nitroprusside, which causes smooth muscle vasodilation through an endothelium-independent cyclic guanosine monophosphate–mediated mechanism, relaxed vessels similarly in all experimental groups (Fig. 12). This suggests that CPB does not cause a significant change in the ability of vascular smooth muscle to relax by way of activation of guanylate cyclase.

The adenylate cyclase activator forskolin caused a dose-dependent relaxation of cerebral microvessels (Fig. 13). The responses were similar in vessels from control animals and those from pigs subjected to 120 minutes of CPB.

Discussion

The primary findings of this study are that cholinergically mediated endothelium-dependent relaxation and β-adrenoceptor–mediated endothelium-independent relaxation are reduced after 2 hours of normothermic extracorporeal circulation. Furthermore, these altered responses have not returned to

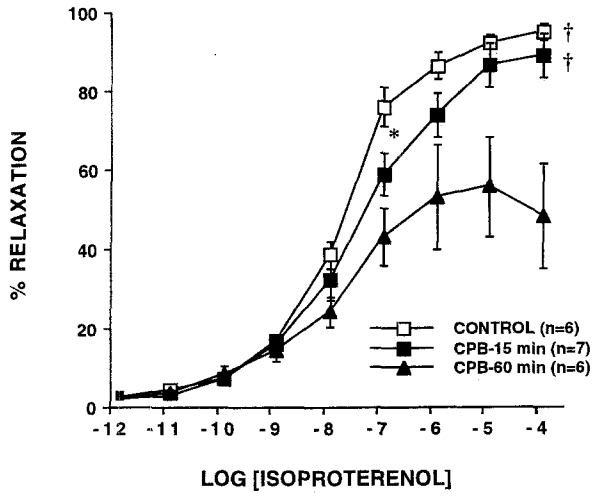

Fig. 11. Plot of in vitro responses of precontracted porcine cerebral microvessels to the β-adrenoceptor agonist isoproterenol from control animals (n = 6), animals after 2 hours of CPB and 15 minutes of perfusion after separation from CPB (CPB-15min, n = 7), and animals after CPB and 1 hour of cerebral perfusion after separation from CPB (CPB-60min, n = 6). In addition, the response of control vessels to isoproterenol in the presence of propranolol (1 μmol/L, PROP, n = 6) was examined. Microvessels were pressurized to 40 mm Hg in a no-flow state. Drugs were applied extraluminally. Responses are expressed as percentage relaxation of the U46619-induced vascular contraction. *Asterisk* represents $p < 0.05$ vs control at 0.1 μmol/L concentration; *daggers* represent $p < 0.01$ versus CPB-60min.

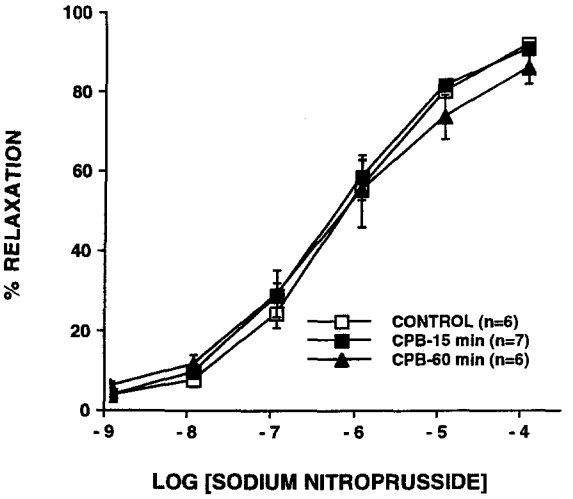

Fig. 12. Plot of in vitro responses of precontracted porcine cerebral microvessels to the guanylate cyclase activator sodium nitroprusside from control animals (n = 6), animals after 2 hours of CPB and 15 minutes of perfusion after separation from CPB (CPB-15min, n = 7), and animals after CPB and 1 hour of cerebral perfusion after separation from CPB (CPB-60min, n = 6). Microvessels were pressurized to 40 mm Hg in a no-flow state. Drugs were applied extraluminally. Responses are expressed as percentage relaxation of the U46619-induced vascular contraction.

normal after 1 hour of perfusion off CPB. Indeed, cerebral microvascular relaxation elicited by the β-adrenergic agonist isoproterenol was reduced significantly more 1 hour after CPB than it was immediately after separation from CPB. Microvascular responses to activators of guanylate cyclase or adenylate cyclase were unchanged, suggesting that CPB-related alterations in vascular relaxation are not caused by changes related to second-messenger mechanisms. This could be a result of alterations in receptor number or affinity or a consequence of uncoupling of receptors to G-protein or second-messenger mechanisms. Importantly, cerebrovascular resistance increased after separation from CPB if cardiac filling was not maintained. If cardiac filling was maintained with fluid administration, baseline ICA blood flow and cerebral vascular resistance remained relatively constant both during CPB and after separation from CPB. Alterations in autonomic regulation may, however, have implications for cerebral blood flow regulation after operation and during catecholamine infusion to augment cardiac performance.

Several hundred thousand cardiac operations involving extracorporeal circulation are performed each year in the United States, and an equal number are performed in the rest of the world. Overt stroke and neuropsychologic disturbances are a disastrous but unfortunately not uncommon complication of cardiac operations involving CPB. Previous studies examined the effects of temperature on cerebral metabolism and blood flow during CPB. Most[7, 12] reported that cerebral blood flow is maintained during CPB. Several studies, however, reported that cerebral blood flow decreases with time.[13, 14] This may be a result of progressive cerebral vasoconstriction or possibly of embolic obstruction occurring as a consequence of prolonged CPB. The effects of extracorporeal circulation on autonomic control of the cerebral circulation have not previously been examined.

Previous studies examined the neurobehavioral outcomes and cerebral pathophysiology of patients after CPB. Townes and colleagues[1] examined neu-

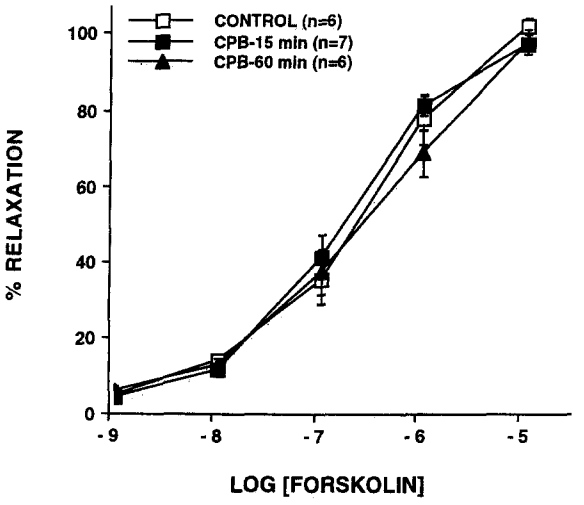

Fig. 13. Plot of in vitro responses of precontracted porcine cerebral microvessels to the adenylate cyclase activator forskolin from control animals ($n = 6$), animals after 2 hours of CPB and 15 minutes of perfusion after separation from CPB (CPB-15min, $n = 7$), and animals after CPB and 1 hour of cerebral perfusion after separation from CPB (CPB-60min, $n = 6$). Microvessels were pressurized to 40 mm Hg in a no-flow state. Drugs were applied extraluminally. Responses are expressed as percentage relaxation of the U46619-induced vascular contraction.

ropsychologic outcomes in patients undergoing operations involving CPB and determined that a significant decline in neuropsychologic variables developed between preoperative and postoperative testing in 11% of patients. Results did not correlate with the degree of hypothermia or the extent of hypotension during the operation. The only predictor of poor outcome in the study was advancing age. McLean and coworkers[2] were able to detect significant deterioration in psychomotor speed in 48% of all patients, with an overt stroke rate of 4%. These investigators were unable to demonstrate any benefit of moderate hypothermia. Newman and colleagues[4] studied the effect of aging on cerebral autoregulation during CPB. Although they determined that advancing age does predispose the patient toward impaired cognition after CPB, this predisposition could not be explained by impaired cerebral autoregulation. A documented association between increased oxygen extraction and a decline in cognition, however, suggests that an imbalance in the cerebral tissue oxygen supply may occur during CPB. The vasomotor state of the cerebral circulation, and specifically the state of the resistance vessels, could cause or contribute to an inadequate supply relative to the demand of cerebral blood flow and oxygen delivery during and after CPB. In further support of this hypothesis, Rogers and colleagues[13] found a progressive reduction in cerebral blood flow at constant temperature over a period of 1 hour in patients undergoing hypothermic CPB. Furthermore, Prough and colleagues[14] found a reduction in cerebral perfusion during stable hypothermic CPB. These authors suggested that cerebral blood flow reductions were unlikely to be caused by continuous undetected brain cooling but were more probably caused by progressive cerebral vasoconstriction or embolic obstruction.

To determine whether CPB at constant temperature caused a progressive decline in cerebral blood flow, Hindman and coworkers[6] studied a rabbit model of extracorporeal circulation. They found a significant inverse correlation between cerebral blood flow and CPB duration. Eliminating the earliest time points, however, reduced the correlation between reduced cerebral blood flow and time. These investigators concluded that the early dependence of cerebral blood flow and CPB duration was most probably a result of undetected brain cooling in the early phase of CPB. A subsequent study by the same investigators[11] found that brain blood flow and metabolism do not decrease at constant brain temperature during CPB in rabbits. In that study the rabbits were anesthetized with halothane, however, which was found by Chen and coworkers[15] to markedly blunt neurohumoral vascular responses. Finally, animals were not separated from extracorporeal circulation. In our experiments, the porcine model of extracorporeal circulation used an α-chloralose–based anesthetic. This agent is known to affect cardiovascular responses only minimally.[16] Furthermore, all animals were separated from CPB, and in vivo blood flow and vessel reactivity studies were performed after CPB in addition to in vitro reactivity experiments. The clinical applicability and relevance of the model thus may be better than those of previous models of extracorporeal circulation.

Possible mechanisms causing cerebrovascular dysfunction. Although we did not investigate the causes of CPB-related cerebral vasomotor dysfunction in this study, several possible mechanisms deserve mention. Complement and neutrophil activation have been studied extensively. Stahl, Reenstra, and Frendl[17] reported that activation of the alternate complement pathway may cause selective endothelial dysfunction while not altering relaxation responses to sodium nitroprusside. Complement

activation may attenuate endothelium-dependent relaxation responses through one of several mechanisms. Activation of the alternate complement pathway results in the production of C5a, a potent chemotactic agent and activator of neutrophils. Activation of the alternate complement cascade during and after CPB could therefore impair endothelium-dependent relaxation indirectly through the activation of neutrophils. The formation of the C5b,6,7,8,9 complex (complete membrane attack complex) could impair cholinergic endothelium-dependent relaxation directly through action on the cell membranes.[17] Furthermore, complement activation has been shown to regulate upward P-selectin adhesion molecules on endothelial cells.[18]

Activated leukocytes may cause parenchymal dysfunction after tissue ischemia[19] or after CPB.[20] Recent work has demonstrated that both inhibition of neutrophil adherence[21, 22] and neutrophil depletion[23, 24] improve myocardial performance after brief periods of ischemia. Pulmonary parenchymal injury has been found to be mediated by leukocytes during CPB.[20, 25] The mechanism whereby neutrophils cause tissue injury is multifactorial, but it probably involves the generation of oxygen-derived free radicals[19, 26] and the release of cytotoxic and proteolytic enzymes[27] after initial activation and adherence of neutrophils to endothelial cells.[28, 29] CPB has been associated with upward regulation of adhesion molecules, possibly in response to inflammatory mediators. This may increase neutrophil adherence to endothelial cells and transcapillary migration, leading to associated tissue injury. Activated neutrophils have been shown to have toxic effects on endothelial cells. In the heart and lung, inhibition of neutrophil adhesion can reduce myocardial damage seen after ischemia.[20-22, 25] In the absence of interruption of cerebral blood flow, the contribution of adhesion and activation of neutrophils to the alteration of cerebral vascular reactivity has not been determined.

Another cause of altered vascular reactivity may be the increased adrenergic stress associated with extracorporeal perfusion. Increased levels of circulating catecholamines may cause the downward regulation of adrenoceptor activity.[30, 31] Indeed, elevated catecholamine levels have been reported during CPB.[31, 32] The further decrease in the relaxation response to β-adrenergic stimulation after 1 hour of perfusion after CPB compared with that immediately after separation from CPB may be caused by continued elevation of circulating catecholamine levels as a result of persistently low mean systemic pressure.

The release of cytokines[33] and other inflammatory mediators may lead to altered vascular responsiveness during and after CPB. Interestingly, a similar pattern of altered endothelium-dependent relaxation[34] and uncoupling of β-adrenoceptor–mediated relaxation from second-messenger mechanisms[35] occurs in the coronary microcirculation after infusion of endotoxin. The release of tumor necrosis factor-α (TNF-α) and other cytokines occurs after either CPB[33] or endotoxemia.[36, 37] The altered endothelium-dependent and β-adrenergic responses as a consequence of either extracorporeal circulation or endotoxemia may thus occur through similar mechanisms. In addition, CPB may alter the response of cerebral vessels as a result of changes in the stimulated release of prostaglandin substances. The contractile responses to endothelium-dependent vasodilators to serotonin and acetylcholine after endotoxemia[38] or CPB,[39] respectively, were reduced in the presence of cyclooxygenase inhibition. The response of cerebral microvessels after CPB observed to acetylcholine may thus be related in part to the stimulated release of a contractile product of cyclooxygenases such as thromboxane.

Finally, Dauber and coworkers[33] found that peripheral bypass (not involving diversion of cardiac or pulmonary perfusion) causes pulmonary and coronary vascular dysfunction, as manifested by increased protein leakage. Increased circulating levels of TNF-α were associated with the increase in vascular permeability, again suggesting that the increased release of cytokines such as TNF-α may mediate the vascular effects of prolonged CPB. In a related study, Butler and colleagues[40] assessed the systemic inflammatory response of patients to CPB. A strong interleukin-6 elevation was observed after initiation of CPB with either membrane or bubble oxygenators.

Advantages and limitations. In contrast to previous studies, which examined the consequences of CPB on cerebrovascular function, this report describes a clinically applicable model of extracorporeal circulation in which all animals were separated from CPB. Furthermore, we examined both in vivo and in vitro responses to agonist stimulation. Whereas in vivo experiments examining internal carotid blood flow or cerebral perfusion are more clinically and perhaps physiologically relevant, in vitro studies eliminate metabolic and autoregulatory influences and other extravascular factors. In addition, in vitro studies allow more detailed examination of specific vascular mechanisms. Previous studies examined alterations in coronary and pulmonary

vascular reactivity after CPB and cardioplegic arrest. It may be inappropriate to extrapolate these findings to the cerebral circulation, however, because pulmonary vessels are deprived of blood flow during total CPB, and coronary vessels are exposed to hyperkalemic cardioplegic solutions that alter membrane potential and other physiologic properties.

This study has several limitations. First, although most cardiac operations are performed with moderate hypothermia during CPB, we examined vascular reactivity after normothermia to reduce the number of experimental variables. This study may, however, have direct applicability to normothermic heart operations. Indeed, normothermic heart operations appear to be associated with an increased incidence of stroke and neurologic dysfunction after operation.[41] Second, we did not examine specific pathologic processes responsible for altered vasomotion. These processes may include leukocyte activation and adherence, complement activation, and increased circulating levels of TNF-α and other cytokines. They will be the topic of future investigation.

Another possible source of error was the use of a perivascular Doppler flow probe around the internal carotid artery to determine cerebral blood flow. A portion of cerebral blood flow arises from the vertebral arteries and may cross through the circle of Willis and other collateral channels. Furthermore, blood flow through the internal carotid arteries may provide perfusion to the midbrain and other deeper brain structures. In addition, a small but significant amount of ICA blood flow may supply extracranial structures.[42] Although this method may not precisely measure unilateral blood perfusion to the cerebral hemisphere, it is useful to determine in vivo responses to the intraluminal infusion of vasoactive substances. Alternative methods, such as the use of microspheres, were not practical. In this study, a close correlation existed between the results of in vitro microvascular studies and those of in vivo carotid blood flow experiments. We believe that the two methods were complementary and support the conclusion of the study that normothermic extracorporeal circulation leads to the altered autonomic regulation of the cerebral microcirculation. Another potential limitation of the study is that the range of carbon dioxide tensions was quite broad. Carbon dioxide tension is an important variable in determining cerebral perfusion, and it would have been preferable to control it more tightly. Furthermore, hematocrit after CPB was significantly less than that before CPB, as is the case clinically. This may also have affected vascular responses, at least in vivo. Finally, pH in the period after CPB was slightly but significantly less than that before CPB. It is known that the pH may have a significant impact on adrenergic responsiveness. Further study is required to determine the contributions of these variables to the alteration of cerebrovascular reactivity during CPB.

Although most patients recover after cardiac operations with few if any significant neurologic sequelae, many have subtle alterations in mental status and fine cognitive function. Although baseline cerebral blood flow has been found to be preserved during CPB in most clinical and laboratory studies, this investigation has determined that responses to substances released during autonomic stimulation are markedly abnormal. The response of cerebral blood flow to the endogenous release of catecholamines or the exogenous administration of inotropic drugs such as dopamine and norepinephrine may be affected differently depending on the extend of vascular preservation. Study of the potential beneficial effects of hypothermia and inhibitors of complement and other pathologic influences will provide interesting and potentially clinically relevant information. Whether this altered vasomotor regulation has an impact on the neuropsychologic outcomes of patients after cardiac operations (especially normothermic heart operations) remains to be determined.

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