

# Metoprolol Reduces Cerebral Tissue Oxygen Tension after Acute Hemodilution in Rats

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**Background:** Perioperative  $\beta$ -blockade and anemia are independent predictors of increased stroke and mortality by undefined mechanisms. This study investigated the effect of  $\beta$ -blockade on cerebral tissue oxygen delivery in an experimental model of blood loss and fluid resuscitation (hemodilution).

**Methods:** Anesthetized rats were treated with metoprolol ( $3 \text{ mg} \cdot \text{kg}^{-1}$ ) or saline before undergoing hemodilution with pentastarch (1:1 blood volume exchange,  $30 \text{ ml} \cdot \text{kg}^{-1}$ ). Outcomes included cardiac output, cerebral blood flow, and brain ( $P_{\text{BrO}_2}$ ) and kidney ( $P_{\text{KO}_2}$ ) tissue oxygen tension. Hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) protein levels were assessed by Western blot. Systemic catecholamines, erythropoietin, and angiotensin II levels were measured.

**Results:** Hemodilution increased heart rate, stroke volume, cardiac output (60%), and cerebral blood flow (50%), thereby maintaining  $P_{\text{BrO}_2}$  despite an approximately 50% reduction in blood oxygen content ( $P < 0.05$  for all). By contrast,  $P_{\text{KO}_2}$  decreased (50%) under the same conditions ( $P < 0.05$ ).  $\beta$ -blockade reduced baseline heart rate (20%) and abolished the compensatory increase in cardiac output after hemodilution ( $P < 0.05$ ). This attenuated the cerebral blood flow response and reduced  $P_{\text{BrO}_2}$  (50%), without further decreasing  $P_{\text{KO}_2}$ . Cerebral HIF-1 $\alpha$  protein levels were increased in  $\beta$ -blocked hemodiluted rats relative to hemodiluted controls ( $P < 0.05$ ). Systemic catecholamine and erythropoietin levels increased comparably after hemodilution in both groups, whereas angiotensin II levels increased only after  $\beta$ -blockade and hemodilution.

**Conclusions:** Cerebral tissue oxygen tension is preferentially maintained during hemodilution, relative to the kidney, despite elevated systemic catecholamines. Acute  $\beta$ -blockade impaired

the compensatory cardiac output response to hemodilution, resulting in a reduction in cerebral tissue oxygen tension and increased expression of HIF-1 $\alpha$ .

CARDIOVASCULAR morbidity remains a leading cause of postoperative mortality.<sup>1,2</sup> Published recommendations<sup>3</sup> regarding the use of perioperative  $\beta$ -blockade to reduce cardiac morbidity and mortality are largely based on two landmark trials.<sup>4,5</sup> However, collective analysis of subsequent randomized trials suggest that the benefit of perioperative  $\beta$ -blockade (*myocardial protection*) may be offset by an increase in cardiovascular side effects (*bradycardia and hypotension*).<sup>6,7</sup> The associated hemodynamic instability may limit cardiac output (CO), reduce vital organ perfusion, and cause the increase in stroke incidence recently reported in the PeriOperative ISchemic Evaluation (POISE) trial and other studies.<sup>8-10</sup> Collectively, these studies<sup>4-7,11,12</sup> have demonstrated that the balance between the therapeutic benefit and the detrimental side effects associated with perioperative  $\beta$ -blockade requires further investigation. Indeed, the appropriate application of perioperative  $\beta$ -blockade must be reassessed.<sup>13-15</sup> A better understanding of the mechanisms involved is required to achieve this goal.

Anemia, blood loss, and hemodilution occur frequently in surgical patients<sup>16,17</sup> and are also associated with increased stroke incidence and mortality.<sup>17-24</sup> The clinical trend to tolerate lower hemoglobin thresholds in perioperative patients is supported by the association between allogeneic blood transfusions and increased mortality.<sup>25,26</sup> These factors have led to an increased incidence of acute hemodilution in surgical patients. Such hemodilution activates compensatory adrenergic responses to maintain organ perfusion<sup>27</sup> (increased heart rate, stroke volume, and cardiac output).<sup>28-31</sup> In humans, both heart rate and stroke volume increase in proportion to the reduction in hemoglobin concentration until very low hemoglobin levels, at which point the heart rate response becomes predominant.<sup>31</sup> A large percentage of this CO response is directed to the brain.<sup>30</sup> Thus, cerebral blood flow (CBF) increases in proportion to the degree of anemia, as demonstrated in clinical<sup>32</sup> and laboratory<sup>33</sup> studies. These and other compensatory mechanisms ensure that adequate brain tissue oxygen tension is maintained during acute hemodilution.<sup>29,31,32</sup> However, it is not understood whether or how  $\beta$ -blockade may alter these compensatory responses.

The mechanism by which  $\beta$ -blockers confer myocardial protection in perioperative patients is thought to be

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predominantly by reducing heart rate and oxygen demand.<sup>34,35</sup> Such therapies that limit cardiac responsiveness during episodes of acute blood loss (or other stress) may lead to inadequate perfusion of vital organs. In clinical terms,  $\beta$ -blockade could restrict the cardiac response to hemodynamic stress during surgery (blood loss, fluid resuscitation, and hemodilution) and increase the risk of tissue hypoxia/ischemia. The purpose of the current study was to assess the effect of  $\beta$ -blockade on cardiac output and tissue oxygen delivery in an experimental model of acute hemodilution.<sup>36</sup> We hypothesize that acute  $\beta$ -blockade will limit the cardiovascular response to acute hemodilution, resulting in a reduction in cerebral tissue oxygen tension.

## Materials and Methods

### *Animal Model of Acute Hemodilution*

All animal protocols were approved by the Animal Care Committee at St. Michael's Hospital (Toronto, Ontario, Canada). Male Sprague-Dawley rats (450–500 g) were anesthetized with 2% isoflurane (Abbott Laboratories, St. Laurent, Province of Quebec [PQ], Canada) in 50% oxygen, tracheotomized (terminal experiments) or intubated (14-gauge angiocath, recovery experiments) and ventilated to achieve normocapnia and normoxia. All incision sites were infiltrated with 2% xylocaine. The tail artery and vein were cannulated to measure mean arterial blood pressure, arterial blood gases, and hemoglobin concentration by cooximetry (Radiometer ALB 500 and OSM 3; London Scientific, London, Ontario, ON, Canada) and to perform acute hemodilution. Brain and rectal temperature were maintained near 35°C and 36°C, respectively, to approximate mild interoperative hypothermia. All physiologic data were acquired digitally (Power Lab 16/30; ADInstruments, Colorado Springs, CO). A total of 108 rats were used ( $n = 6$  to 8 per group) in five different experimental protocols.

After establishing a stable baseline, the  $\beta_1$ -antagonist (metoprolol 3 mg  $\cdot$  kg<sup>-1</sup>) or saline-vehicle were administered intravenously, based on previous experimental studies,<sup>37</sup> targeting a 20% reduction in heart rate. Rats were then either monitored (no hemodilution) or hemodiluted by simultaneously exchanging 30 ml  $\cdot$  kg<sup>-1</sup> of arterial blood (50% blood volume) with an equivalent volume of 10% pentastarch (Pentaspan, osmotic, and oncotic pressure 326 mOsm and 40 mmHg, respectively<sup>38</sup>; Bristol-Myers Squibb, Montreal, PQ, Canada) at a fixed rate over 10 min by using a push-pull infusion pump (PHD 2000; Harvard Apparatus, Saint-Laurent, PQ, Canada). Physiologic measurements were recorded for an additional 100 min. Arterial blood gas and cooximetry samples were taken before and after hemodilution at 30-min intervals.

### *Effect of Metoprolol on Cerebral and Renal Perfusion (Experimental Protocols 1 and 2)*

Protocol 1 assessed the effect of hemodilution on cerebral perfusion. Anesthetized rats ( $n = 6$ –8 per group) were placed in a stereotaxic frame, and bilateral burr holes were trephined as previously described.<sup>39</sup> A combined oxygen-sensitive microelectrode and laser Doppler flow probe (100  $\mu$ m; Oxford Optronix, Oxford, United Kingdom) was inserted 2–3 mm past the dura into the hippocampus by using stereotaxic coordinates to measure normalized CBF and brain tissue oxygen tension ( $P_{BrO_2}$ ). Rats were then randomized to receive  $\beta$ -blocker or saline before undergoing hemodilution. A control group of rats received saline or metoprolol (3 mg  $\cdot$  kg<sup>-1</sup>) without hemodilution to assess the stability of cerebral oxygen tension and flow measurements over time. These measurements were performed *post hoc* after completion of the hemodilution experiments. Protocol 2 assessed the effect of hemodilution on cerebral and renal cortical microvascular oxygen tension. Under isoflurane anesthesia, two different groups of rats were used to measure cerebral cortical or renal cortical microvascular oxygen tension ( $PO_2$ ,  $n = 6$  rats per group). For brain measurements, the dura was exposed through two burr holes 2–3 mm apart. For kidney measurements, the kidney was exposed by a dorsal flank incision and decapsulated. In both cases, two 4-mm-diameter fiberoptic light guides were used to measure phosphorescence lifetime at a depth of 700  $\mu$ m, which corresponded to the cerebral or renal cortex. A 0.2-ml solution containing 1 mg of Oxyphor G2 [Pd-meso-tetra-(4-carboxyphenyl)-tetrabenzoporphyrin; Oxygen Enterprises, Philadelphia, PA] was used to measure cerebral and renal cortical microvascular oxygen tension ( $PO_2$ ) as previously described.<sup>40,41</sup> Rats were randomized to receive  $\beta$ -blocker or saline before undergoing hemodilution.

### *Effect of Metoprolol on Cardiac Output and Carotid Blood Flow (Experimental Protocol 3)*

Control and  $\beta_1$ -blocked anesthetized rats underwent hemodilution ( $n = 8$  per group). Cardiac output was assessed by echocardiography with a Sonos 5500 echocardiographic system (Philips Medical Systems Canada, Markham, ON, Canada) equipped with a high-frequency (5–12 MHz broad-bandwidth) phased-array transducer (S12, Phillips Ultrasound). Two-dimensional and M-mode echocardiographic imaging along with spectral Doppler acquisition were performed by using standard parasternal and apical windows as previously described.<sup>42</sup> Left ventricular end-diastolic and end-systolic dimensions were obtained while fractional shortening [(left ventricular end diastolic diameter – left ventricular end systolic diameter)/left ventricular end diastolic diameter] and stroke volume (left ventricular outflow tract area  $\times$  left ventricular outflow tract velocity time integral) were calculated. Cardiac output was determined by multiplying stroke volume and heart rate. Carotid

**Table 1. Arterial Blood Gas and Cooximetry Data after Hemodilution and  $\beta_1$  Blockade**

Time (min)	Description	pH	Paco <sub>2</sub> (mmHg)	Pao <sub>2</sub> (mmHg)	Hb (g/ l)	% Saturation	MetHb (%)	Oxygen Content (mm)	Lactate (mm)
Saline (n = 30)									
10	Baseline	7.42 ± 0.04	36.1 ± 4.9	168.2 ± 31.9	135 ± 13	99.8 ± 0.5	1.4 ± 0.5	7.9 ± 1.0	2.1 ± 0.8
30	Saline	7.40 ± 0.06	37.6 ± 5.7	171.0 ± 28.3	124 ± 22	99.8 ± 0.6	1.6 ± 0.8	7.4 ± 1.4	2.3 ± 0.7
50	Post	7.37 ± 0.07	40.5 ± 6.2	170.9 ± 34.7	56 ± 14*	99.9 ± 0.4	2.0 ± 0.7*	3.5 ± 1.1*	2.1 ± 0.7
hemodilution 1									
100	Post	7.38 ± 0.07	39.4 ± 7.5	163.2 ± 35.0	57 ± 15*	99.9 ± 0.7	2.1 ± 0.6*	3.3 ± 0.9*	2.3 ± 0.9
hemodilution 2									
$\beta_1$ blocker (n = 30)									
10	Baseline	7.42 ± 0.05	36.1 ± 4.2	171.1 ± 34.5	133 ± 13	99.9 ± 0.4	1.5 ± 0.2	7.8 ± 0.9	1.7 ± 0.5
30	Metoprolol	7.41 ± 0.08	37.9 ± 5.1	159.8 ± 32.3	122 ± 23	99.7 ± 0.6	1.7 ± 0.1	7.2 ± 1.4	1.9 ± 0.5
50	Post	7.40 ± 0.06	36.8 ± 6.2	166.2 ± 35.1	56 ± 10*	100.0 ± 0.1	2.1 ± 0.7*	3.3 ± 0.6*	2.0 ± 0.9
hemodilution 1									
100	Post	7.39 ± 0.06	38.7 ± 6.3	157.9 ± 30.0	55 ± 13*	100.0 ± 0.2	2.0 ± 0.6*	3.2 ± 0.8*	2.0 ± 0.6
hemodilution 2									

Data from experimental protocols 1, 2, 3, and 5 (n = 8, 6, 8, 8, respectively).

\*  $P < 0.05$ , relative to Baseline (10 min).

Hb = hemoglobin; MetHb (%) = methemoglobin percent; Paco<sub>2</sub> = partial pressure of carbon dioxide; Pao<sub>2</sub> = partial pressure of oxygen.

blood flow was simultaneously recorded using a flow probe (TS420; Transonic Systems Inc, Ithaca, NY).

#### *Effect of Metoprolol and Hemodilution on Hypoxic Cerebral Cellular Responses (Experimental Protocol 4)*

For Western blot and immunohistological analysis (n = 6 per group), rats underwent hemodilution alone,  $\beta$ -blockade alone, or hemodilution and  $\beta$ -blockade (n = 6 per group). Western analysis and immunostaining were performed as previously reported 3 and 18 h after recovery from hemodilution, respectively.<sup>36</sup> For Western blot analysis, protein extracted from cerebral cortical tissue was quantified (Lowery Assay; Bio-Rad, Mississauga, ON, Canada) before running samples on SDS-PAGE followed by protein transfer to nitrocellulose membranes as previously described.<sup>36</sup> Specific hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) protein bands were identified by using an appropriate primary antibody (AF 1935; R&D Systems, Toronto, ON, Canada). Band densities were quantified by densitometry and normalized to  $\alpha$ -tubulin (T6199;  $\sigma$ -Aldrich Canada, Oakville, ON, Canada). Immunohistochemical and fluorescent staining was performed on 10- $\mu$ m fixed tissue sections (4% paraformaldehyde) by incubating slides overnight at 4°C with diluents of specific primary monoclonal antibodies for neuronal nitric oxide synthase (nNOS) (610309; BD Biosciences, Mississauga, ON, Canada),  $\beta_1$ -receptor (SC-568; Santa Cruz Biotechnology, Santa Cruz, CA), and HIF-1 $\alpha$  (NB 100-131; Novus Biologicals, Cedar Lane Labs, Burlington, ON, Canada) and specific binding detected with an appropriately labeled secondary antibody. Microscopy was performed by using fluorescent and confocal microscopes (Nikon ECLIPSE 90i and Bio-Rad Radiance 2100; SMH Medical Imaging Facility, Toronto, ON Can-

ada). Cerebral cortical sections immunostained for nNOS with a specific primary antibody (610309; BD Biosciences) and then incubated with a secondary biotinylated antibody (1:200 vectastain kit; Vector Labs, Burlington, ON, Canada). An avidin/biotinylated enzyme complex and enzyme substrate diaminobenzadine (DAB) were added to visualize the immunoreactive cells. nNOS-positive cell counts were then counted by blinded observers (TR, AT). The number of nNOS-positive cerebral cortical cells per coronal section were compared in nonhemodiluted and hemodiluted rats treated with saline or  $\beta$ -blocker.

#### *Effect of Metoprolol on Systemic Catecholamines and Vascular Hormone Levels (Experimental Protocol 5)*

After plasma collection, the simultaneous determination of norepinephrine, epinephrine, and dopamine was measured by high-performance liquid chromatography (Waters

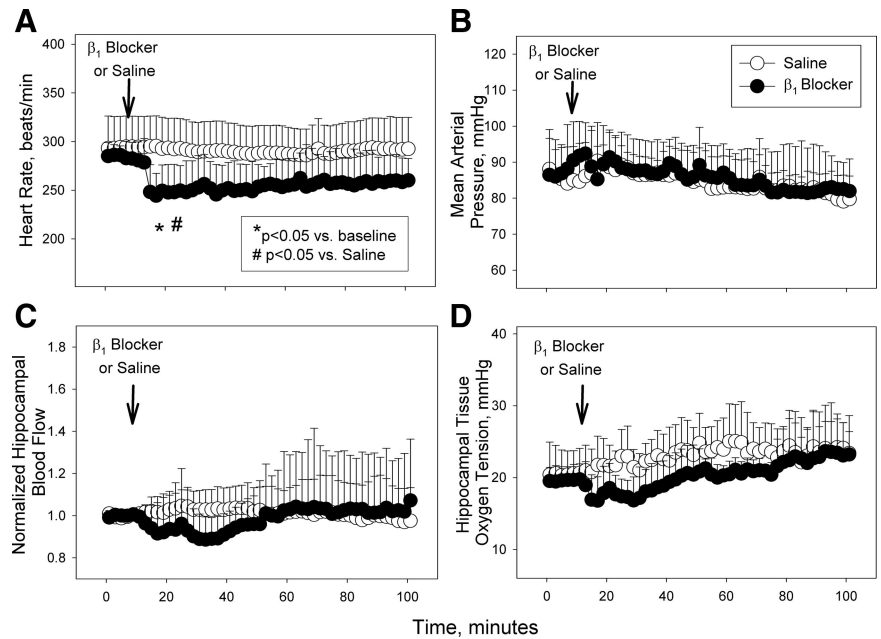
**Table 2. Hippocampal and Rectal Temperature after Hemodilution and  $\beta_1$  Blockade**

Time (min)	Description	Rectal Temperature, °C	Hippocampal Temperature, °C
Saline (n = 8)			
10	Baseline	36.0 ± 0.6	34.8 ± 1.0
30	Saline	36.2 ± 0.7	35.1 ± 1.2
50	Post hemodilution 1	35.4 ± 1.6	35.6 ± 1.1
100	Post hemodilution 2	35.9 ± 0.6	35.6 ± 1.3
$\beta_1$ blocker (n = 8)			
10	Baseline	36.0 ± 0.6	34.6 ± 0.9
30	Metoprolol	35.8 ± 0.4	34.3 ± 0.9
50	Post hemodilution 1	35.1 ± 0.7	34.9 ± 0.5
100	Post hemodilution 2	36.0 ± 0.6	35.1 ± 1.3

Data from Protocol 1.



**Fig. 1.** (A) Heart rate decreased after β-blockade (closed circles) but was stable in the saline-treated controls (open circles). (B–D) Mean arterial pressure, normalized hippocampal blood flow, and tissue oxygen tension were stable over the duration of the experimental protocol (n = 6 rats per group).



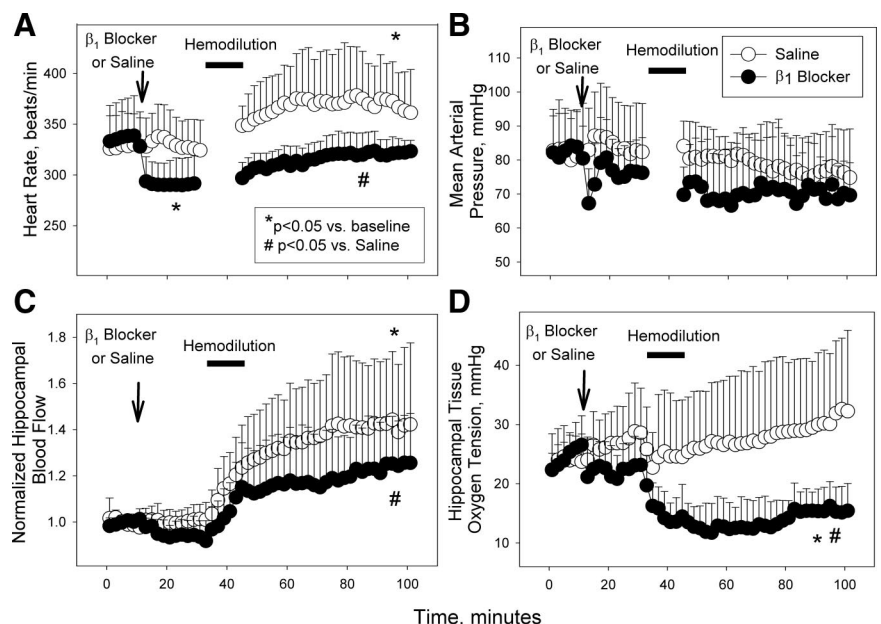
Spherisorb®, 3.0 μm ODS2, 4.6-mm × 100-mm column; Waters Associates Inc., Milford, MA) coupled with electrochemical detection (Coulochem II; ESA, Inc., Bedford, MA) (n = 8 per group) as previously described (Toronto Medical Laboratories, Toronto General Hospital).<sup>43</sup> Plasma erythropoietin and angiotensin II levels were measured in duplicate by enzyme-linked immunosorbant assay (ELISA) (Quantikine MEP00; R&D Systems, Minneapolis, MN).

#### Statistical Analyses

Data were analyzed by using SAS version 9.1 (SAS Institute, Inc., Cary, NC). Data from each of the five protocols were assessed independently by analysis of variance (ANOVA) for time, group, and interaction effects. No cor-

rection for multiple testing was performed. For arterial blood gas and physiologic data, a two-way repeated measure ANOVA was used to assess changes in values after β-blockade and hemodilution. Physiologic data, included heart rate, mean arterial pressure, tissue blood flow and oxygen tension, stroke volume, cardiac output, catecholamine, angiotensin II, and erythropoietin levels. A one-way ANOVA was used to analyze Western blot density and histologic cell count data. In all cases, a *post hoc* Tukey test was used to compare means when an adequate F ratio was achieved. The Spearman rank correlation coefficient was used to demonstrate significant correlations. All data are presented as the mean ± SD. Significance was assigned at a *P* < 0.05.

**Fig. 2.** (A) Heart rate (HR) increased after hemodilution in controls (open circles). β-blockade (closed circles) reduced baseline HR and attenuated the increase in posthemodilution HR. (B) The mean arterial blood pressure was maintained in both groups after hemodilution. (C) The characteristic increase in cerebral blood flow after hemodilution was attenuated by β-blockade. (D) Hippocampal tissue oxygen tension was maintained after hemodilution in control rats (open circles). Metoprolol pretreatment did not affect tissue oxygen tension at baseline. After hemodilution, there was a profound drop in cerebral tissue oxygen tension in β-blocked rats (closed circles, n = 8 rats per group).



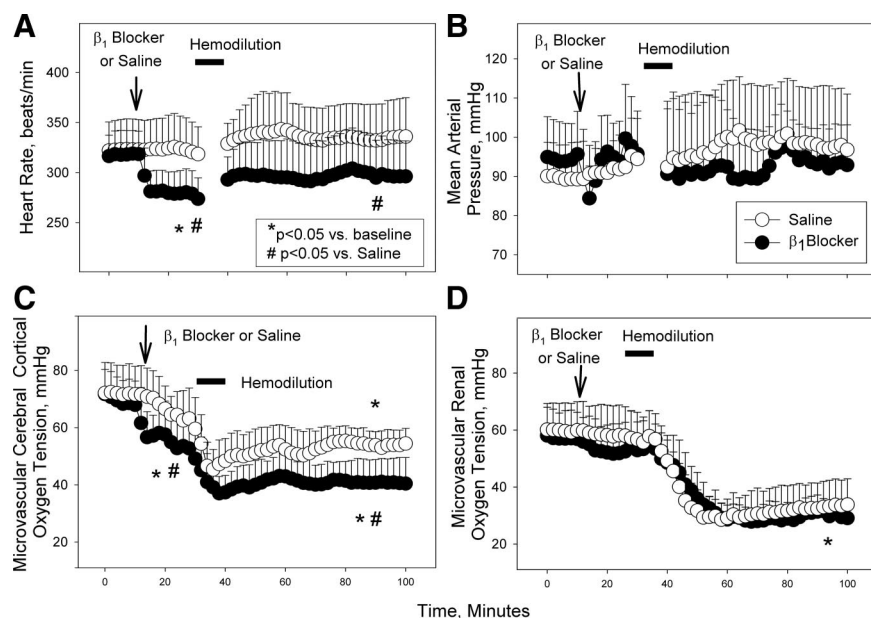


Fig. 3. (A and B) After hemodilution in control rats, heart rate and mean arterial pressure (MAP) were maintained (open circles). After  $\beta$ -blockade, heart rate decreased and MAP remained stable. (C) Cerebral cortical microvascular oxygen tension decreased by about 25% in control rats after hemodilution (open circles). Metoprolol reduced the baseline tissue oxygen tension relative to controls. After hemodilution, there was a further decrease in cerebral cortical oxygen tension in the  $\beta$ -blocked rats relative to non- $\beta$ -blocked controls ( $n = 6$  rats per group). (D) There was a comparable reduction in renal microvascular oxygen tension in both groups to values lower than that observed in the cerebral cortex.

## Results

### Effect of Acute Hemodilution on Arterial Blood Gas and Cooximetry Values

For each experimental protocol, pH,  $P_{aCO_2}$ , and  $P_{aO_2}$  remained stable without any significant differences

among control and  $\beta$ -blocker groups (table 1). After hemodilution, the hemoglobin concentration and blood oxygen content decreased comparably in both control and  $\beta$ -blocked rats (baseline hemoglobin concentration:  $135 \pm 13$  vs.  $133 \pm 13$  g  $\cdot$  l<sup>-1</sup>; posthemodilution hemo-

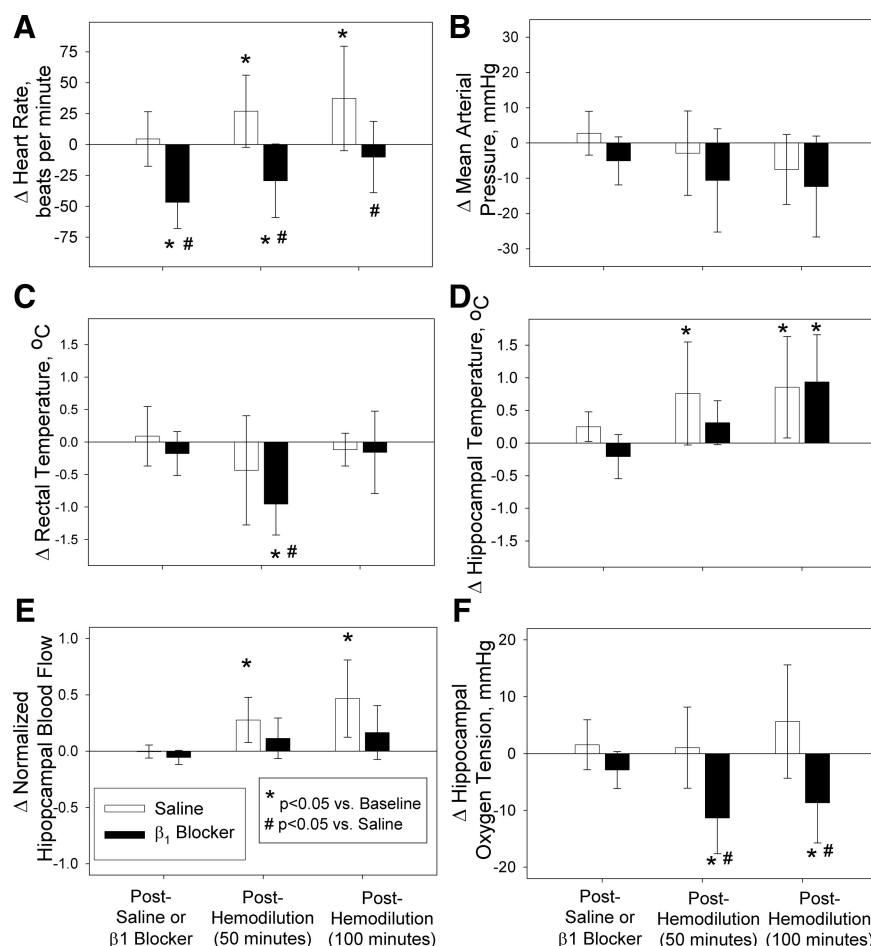


Fig. 4. (A) Heart rate decreased after  $\beta$ -blockade and hemodilution (black bars) but increased in controls after hemodilution (open bars). (B) Mean arterial pressure did not change significantly in either group. (C and D) In control rats, rectal temperature remained stable, and cerebral temperature increased after hemodilution (open bars).  $\beta$ -blockade resulted in a decrease in rectal temperature and a delayed increase in hippocampal temperature after hemodilution (closed bars). (E) Hippocampal blood flow increased in control but not  $\beta$ -blocked rats after hemodilution. (F) Hippocampal tissue oxygen tension was maintained in control rats but decreased in  $\beta$ -blocked rats after hemodilution ( $n = 8$  rats per group).

globin concentration:  $57 \pm 15$  vs.  $55 \pm 13$  g · l<sup>-1</sup>;  $P < 0.05$ , relative to baseline) (table 1). A small increase in methemoglobin was observed in all hemodiluted rats (table 1).

# *Metoprolol Reduces Cerebral Tissue Oxygen Tension in Hemodiluted Rats (Experimental Protocols 1 and 2)*

All baseline values were comparable among groups. Brain and rectal temperatures were maintained near 35°C and 36°C, respectively, without any difference between groups at any time (table 2). Metoprolol treatment reduced the heart rate by approximately 20% relative to baseline ( $P < 0.05$ ), but it did not affect hippocampal CBF and tissue oxygen tension over time in nonhemodiluted rats (fig. 1). After hemodilution, there was a significant increase in the heart rate observed in control but not in β-blocked rats (fig. 2,  $P < 0.05$ ). No significant differences in mean arterial pressure were observed between control and β-blocked rats before or after hemodilution (fig. 2B). Hemodilution caused an increase in CBF in control rats ( $P < 0.05$ ) that was attenuated in

β-blocked rats (fig. 2C,  $P < 0.05$ ). After hemodilution in control rats, hippocampal tissue oxygen tension was maintained (tissue probe, fig. 2D). After hemodilution in β-blocked rats, there was a significant reduction in hippocampal tissue  $P_{Br}O_2$  (50%, fig. 2D,  $P < 0.05$ ). The baseline cerebral and renal cortical microvascular  $PO_2$  values (intravascular oxyphor G2) were near 70 and 60 mmHg, respectively (fig. 3). In non-β-blocked rats, hemodilution resulted in a marginal reduction in cerebral cortical microvascular  $PO_2$  (25%, fig. 3C,  $P < 0.05$ ) and a more severe reduction in renal microvascular  $PO_2$  (50%, fig. 3D,  $P < 0.05$ ). Before hemodilution, β-blockade caused a small reduction in cerebral, but not renal cortical microvascular  $PO_2$ . Subsequent hemodilution caused a further and more pronounced reduction in the cerebral cortical microvascular oxygen tension in the β-blocked group (45% relative to the baseline, fig. 3C,  $P < 0.05$ ). The reduction in cerebral cortical microvascular  $PO_2$  was greater in the β-blocked group relative to non-β-blocked controls (fig. 3C,  $P < 0.05$ ). The microvascular kidney  $PO_2$  decreased comparably to values below the cerebral

**Fig. 5.** (A) β-blockade reduced heart rate before and after hemodilution, relative to saline-treated rats. (B) Mean arterial pressure was maintained after hemodilution in control (open bars) and β-blocked rats (black bars). (C) The increase in carotid blood flow after hemodilution was attenuated by β-blockade. (D) The increase in cardiac output (CO) after hemodilution was attenuated by β-blockade. (E and F) The positive linear relationship between changes in CO and carotid blood flow observed after hemodilution in control rats (open circles) was not observed after β-blockade (closed circles) (n = 8 rats per group).

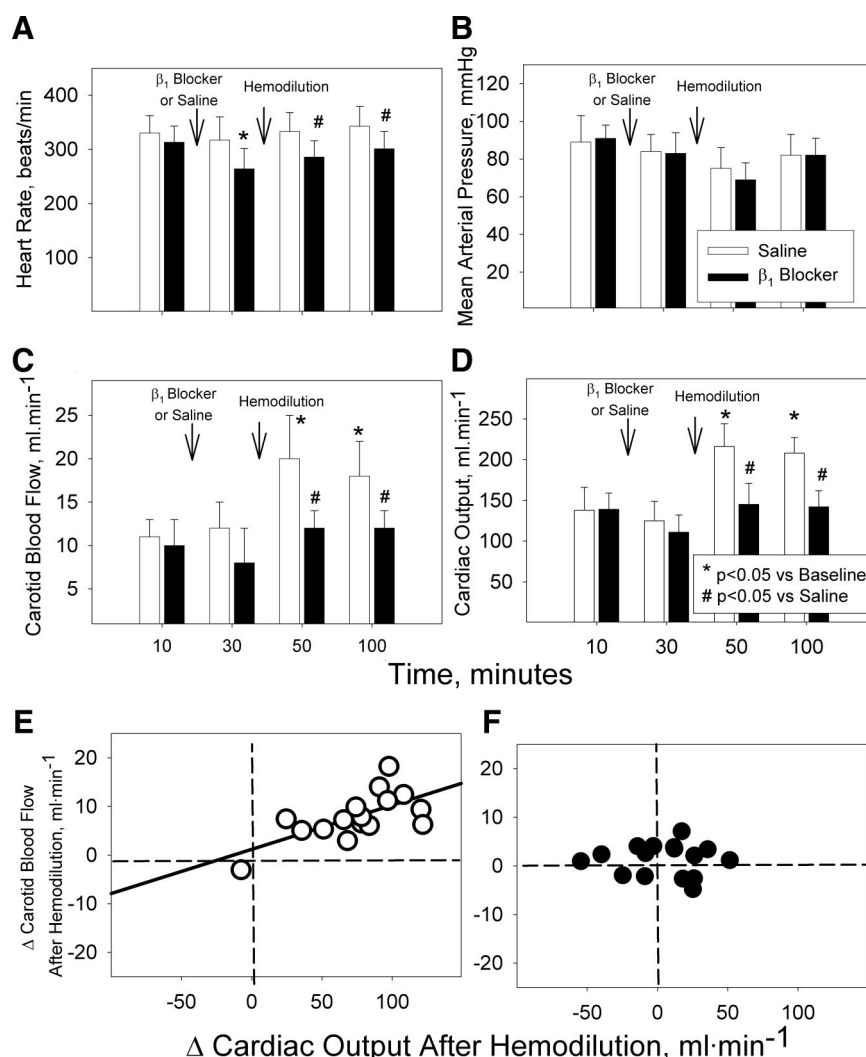




Table 3. Echocardiography Data after Hemodilution and  $\beta$ -blockade

Time (minutes)	Group	LVEDD (cm)	LVESD (cm)	FS (%)	LVEF (%)	LVEDA (cm <sup>2</sup> )	LVESA (cm <sup>2</sup> )	FAC (%)	LVOT (cm)	LVOT VTI (cm)	SV (ml)
10	Saline	0.75 ± 0.08	0.40 ± 0.07	0.46 ± 0.06	0.84 ± 0.06	0.47 ± 0.08	0.16 ± 0.04	0.66 ± 0.07	0.30 ± 0.01	6.07 ± 1.45	0.42 ± 0.01
	$\beta_1$ -blocker	0.73 ± 0.06	0.36 ± 0.09	0.51 ± 0.09	0.87 ± 0.06	0.47 ± 0.10	0.12 ± 0.04	0.75 ± 0.05	0.29 ± 0.01	6.65 ± 0.97	0.44 ± 0.07
30	Saline	0.76 ± 0.05	0.39 ± 0.04	0.49 ± 0.06	0.86 ± 0.05	0.48 ± 0.08	0.15 ± 0.02	0.68 ± 0.04	0.29 ± 0.01	6.09 ± 1.56	0.41 ± 0.10
	$\beta_1$ -blocker	0.75 ± 0.04	0.43 ± 0.07	0.43 ± 0.07*	0.80 ± 0.07*	0.49 ± 0.09	0.16 ± 0.04	0.67 ± 0.06*	0.29 ± 0.01	6.63 ± 0.72	0.43 ± 0.05
50	Saline	0.84 ± 0.05*	0.33 ± 0.05*	0.60 ± 0.08*	0.93 ± 0.04*	0.59 ± 0.10*	0.12 ± 0.02*	0.79 ± 0.04*	0.30 ± 0.01	9.29 ± 1.06*	0.64 ± 0.06*
	$\beta_1$ -blocker	0.81 ± 0.03*	0.39 ± 0.07	0.52 ± 0.08	0.88 ± 0.06	0.56 ± 0.06*	0.15 ± 0.03	0.74 ± 0.04	0.29 ± 0.02	7.65 ± 0.59†	0.51 ± 0.08†
100	Saline	0.79 ± 0.06*	0.31 ± 0.06*	0.61 ± 0.07*	0.93 ± 0.04*	0.53 ± 0.07	0.11 ± 0.02*	0.79 ± 0.04*	0.30 ± 0.01	8.66 ± 0.04*	0.60 ± 0.08*
	$\beta_1$ -blocker	0.78 ± 0.04*	0.38 ± 0.06	0.52 ± 0.05†	0.88 ± 0.05	0.52 ± 0.05	0.14 ± 0.03†	0.73 ± 0.06	0.29 ± 0.01	7.26 ± 0.06†	0.47 ± 0.05†

\*  $P < 0.05$  relative to baseline (10 min); †  $P < 0.05$  relative to saline group.  
FAC = fractional area change; FS = fractional shortening; LVEDA = left ventricular end diastolic area; LVEDD = left ventricular end diastolic diameter; LVEF = left ventricular ejection fraction; LVESA = left ventricular end systolic area; LVESD = left ventricular end systolic diameter; LVOT = left ventricular outflow tract; LVOT VTI = left ventricular outflow tract velocity time integral; SV = stroke volume.

cortical microvascular  $Po_2$  in both groups after hemodilution (50%, fig. 3D,  $P < 0.05$ ).

Relative Changes from Baseline

After  $\beta$ -blockade and hemodilution, heart rate decreased while mean arterial blood pressure was maintained relative to hemodiluted controls (fig. 4). Immediately after  $\beta$ -blockade and hemodilution, rectal temperature decreased (50 min) before returning to baseline at 100 min (fig. 4C). By contrast, hippocampal temperature increased after hemodilution in  $\beta$ -blocked rats by 100 min (fig. 4D). However, this increase in brain temperature occurred more rapidly in the non- $\beta$ -blocked rats after hemodilution (50 minutes, fig. 4D). Relative hippocampal blood flow increased in control rats after hemodilution (fig. 4E,  $P < 0.05$ ). This was not observed in  $\beta$ -blocked rats. Concurrently, hippocampal tissue oxygen tension was maintained in control rats but decreased significantly in  $\beta$ -blocked rats after hemodilution (fig. 4F,  $P < 0.05$ ).

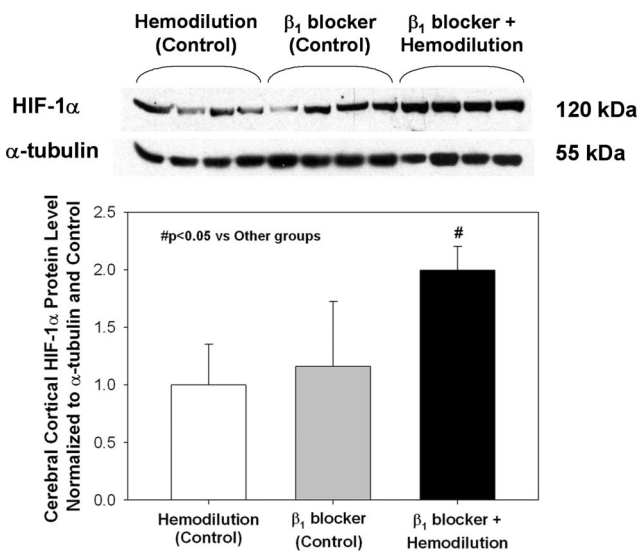


Fig. 6. Cerebral cortical HIF-1 $\alpha$  protein levels were increased twofold in hemodiluted and  $\beta$ -blocked rats (black bar) relative to hemodilution only (open bar) or  $\beta$ -blocker only (gray bar) controls (n = 6 per group).

Metoprolol Attenuates the Cardiac Output and Carotid Blood Flow Response to Acute Hemodilution (Experimental Protocol 3)

The heart rate and mean arterial blood pressure followed the same patterns observed after hemodilution in the previous protocols (fig. 5). Carotid blood flow and cardiac output were significantly increased in control rats after hemodilution (fig. 5C,  $P < 0.05$ ). Both responses were prevented in  $\beta$ -blocked rats. Left ventricular end-diastolic diameter and area both increased after

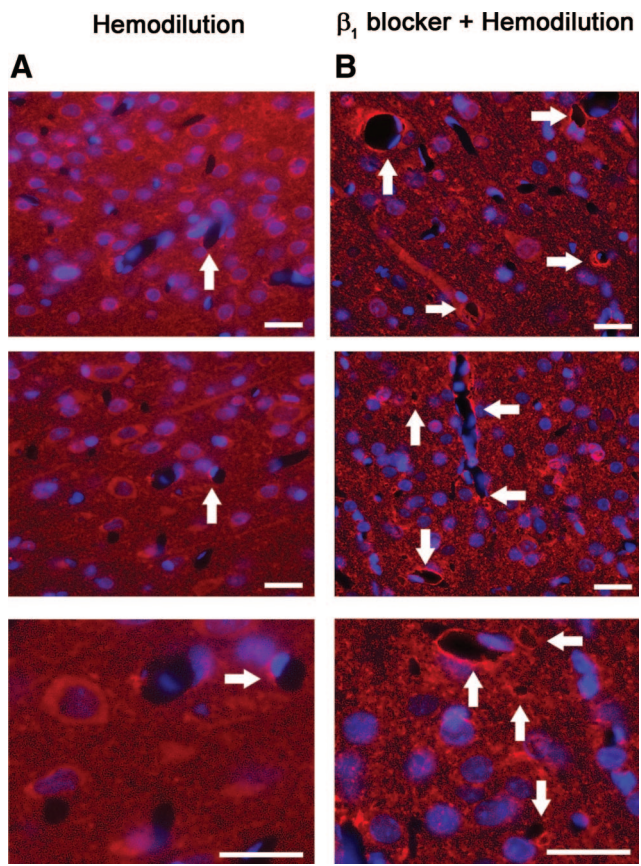


Fig. 7. Immunostaining for hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) protein demonstrates increased perivascular HIF-1 $\alpha$  after hemodilution in  $\beta$ -blocked rats (B, white arrows) relative to controls (A). (White bars = 50  $\mu$ m.)

hemodilution and saline treatment, but they did not increase after hemodilution and β-blockade (table 3,  $P < 0.05$ ). The decrease in left ventricular end-systolic diameter and area observed after hemodilution ( $P < 0.05$ ) were not observed after β-blockade and hemodilution (table 3). Therefore, hemodilution alone increased fractional shortening, left ventricular ejection fraction, fractional area change, and stroke volume (table 3,  $P < 0.05$ ). These increases were not observed after β-blockade and hemodilution.

A clear linear relationship was demonstrated between the change in CO and change in carotid blood flow after hemodilution in control rats (fig. 5E, correlation coefficient = 0.606,  $P < 0.05$ ). This relationship was not observed after β-blockade and hemodilution (fig. 5F).

#### Metoprolol and Hemodilution Increase Cerebral Cortical HIF-1α Levels (Experimental Protocol 4)

Cerebral cortical tissue extracted from rats 3 h after recovery from hemodilution and β-blockade demonstrated a twofold increase in HIF-1α levels, relative to hemodiluted rats treated with saline or nonhemodiluted rats treated with metoprolol (fig. 6,  $P < 0.05$ ). Different groups of rats that had been recovered for 18 h demonstrated an increase in HIF-1α immunostaining in the perivascular regions after hemodilution and β-blockade relative to tissue from rats that underwent hemodilution

without β-blockade (fig. 7). After 18 h, the number of nNOS-positive cerebral cortical cells increased comparably in both groups of hemodiluted rats, independent of β-blockade (fig. 8A). Double-labeling studies demonstrated the presence of β<sub>1</sub>-adrenergic receptors on nNOS- and NeuN-positive cells (neurons) within the cerebral cortex of hemodiluted rats after saline treatment or β-blockade (fig. 8B).

#### Effect of Systemic Catecholamine, Erythropoietin, and Angiotensin II Levels after Hemodilution and β-blockade (Experimental Protocol 5)

A rapid and sustained increase in plasma norepinephrine levels were observed in both control and β-blocked rats immediately after the completion of hemodilution at 70 min (fig. 9A,  $P < 0.05$  for both). This increase in norepinephrine was sustained for the duration of the experiment. By contrast, plasma epinephrine (fig. 9B) and dopamine levels (fig. 9C) increased more slowly, reaching significance by the end of the experimental protocol in both hemodiluted groups at 160 min ( $P < 0.05$  for both). Systemic erythropoietin levels increased comparably from baseline values ( $133 \pm 12$  and  $142 \pm 7$  pg · ml<sup>-1</sup>) in both groups after hemodilution ( $158 \pm 16$  and  $159 \pm 6$  pg · ml<sup>-1</sup>,  $P < 0.05$  relative to baseline). Relative to control values ( $593 \pm 153$  and  $486 \pm 80$  ng · ml<sup>-1</sup>), plasma angiotensin II increased after β-blockade

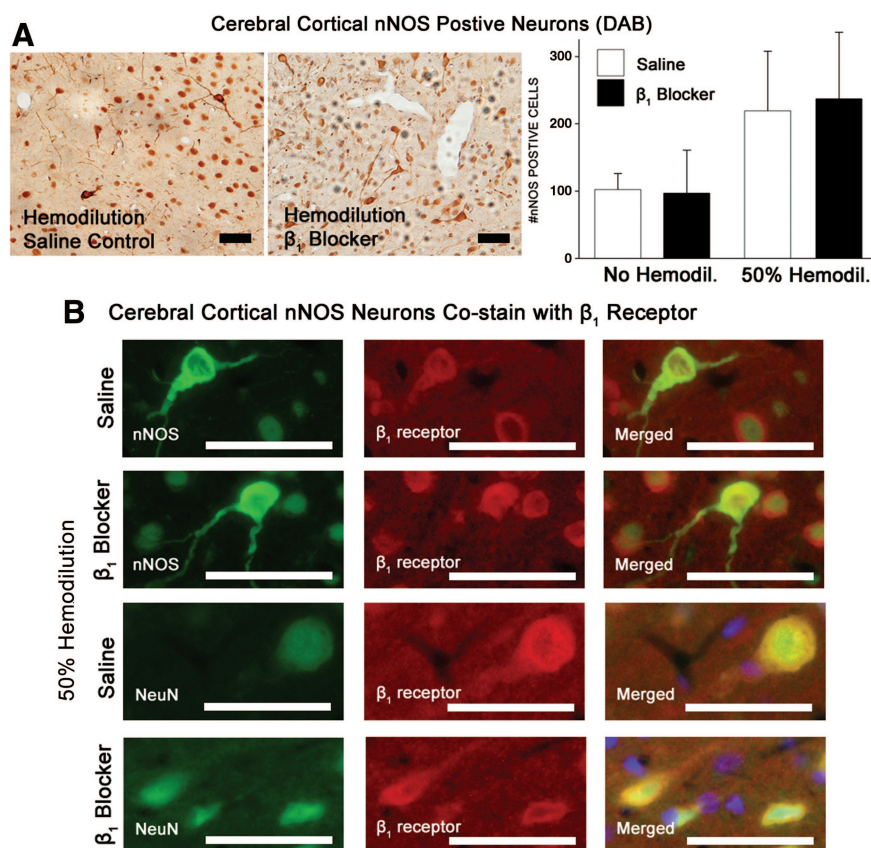
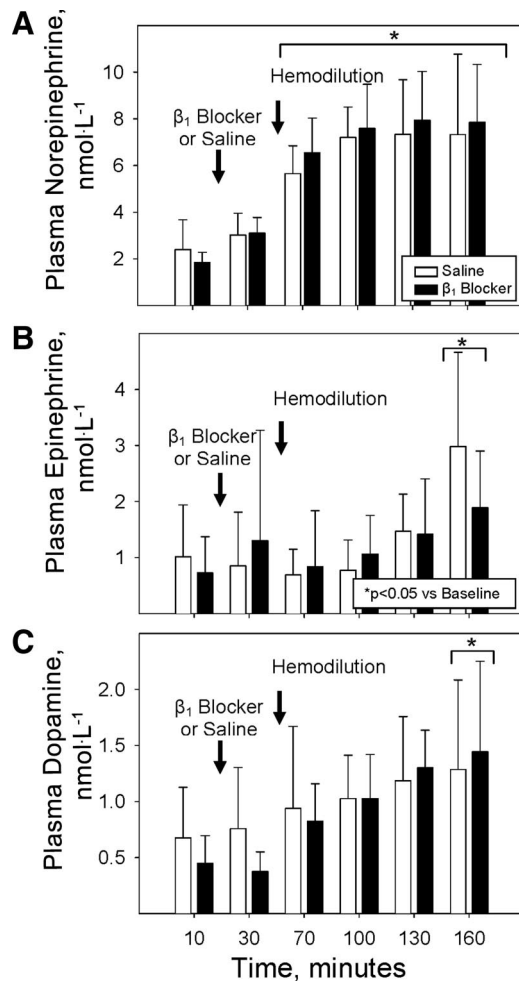


Fig. 8. (A) Diaminobenzidine (DAB)-positive neuronal nitric oxide synthase (nNOS) staining in the cerebral cortex of hemodiluted rats demonstrates a comparable increase in the number of nNOS-positive cells after hemodilution in both control (open bars) and β-blocked rats (black bars) ( $n = 6$  per group; black bars = 50 μm). (B) Double-labeling studies demonstrate that both nNOS-positive cells and neurons (green) colabel for β<sub>1</sub> adrenoceptors (red) (white bars = 50 μm).





**Fig. 9.** (A) Systemic norepinephrine levels increase rapidly after hemodilution in both control (open bars) and  $\beta$ -blocked rats (black bars). (B and C) Epinephrine and dopamine increase more slowly and to a lesser degree than norepinephrine in both groups of hemodiluted rats ( $n = 8$  per group).

and hemodilution ( $810 \pm 103 \text{ ng} \cdot \text{ml}^{-1}$ ,  $P < 0.05$ ) but not after hemodilution alone ( $574 \pm 95 \text{ ng} \cdot \text{ml}^{-1}$ ).

## Discussion

In this model of acute hemodilution (50% reduction in blood oxygen content), cerebral tissue oxygen tension was relatively maintained by a characteristic increase in CO (60%) and CBF (50%). Under these experimental conditions, hippocampal tissue oxygen tension, as measured by an invasive tissue probe, was maintained as previously demonstrated (figs. 1 and 2).<sup>44</sup> However, under the same conditions, cerebral cortical microvascular oxygen tension, as measured by a noninvasive intravascular oxyphor, decreased marginally (25%) (fig. 3). By using this intravascular method, the renal microvascular oxygen tension decreased to a greater degree (50%), suggesting that the compensatory increases in CO and CBF preserved cerebral  $\text{Po}_2$  more effectively than renal  $\text{Po}_2$ . The direct relationship between the increase in

cardiac output and carotid blood flow observed after hemodilution also supports the concept that central mechanisms act to preferentially maintain cerebral perfusion (fig. 4).<sup>30</sup> This pattern of preferential brain perfusion occurred in the face of a fourfold increase in systemic norepinephrine levels, suggesting that the cerebral and renal vascular beds react differentially to high levels of systemic vasoconstrictors (fig. 9). It also suggests that important vasodilatory mechanisms may help to perfuse the brain during acute hemodilution. Our data relate to other experimental<sup>45,46</sup> and clinical studies<sup>23,47</sup> that suggest the kidney may be vulnerable to hypoxic injury during acute hemodilution and anemia.

After acute  $\beta$ -blockade, the compensatory increase in CO and CBF were severely attenuated, leading to inadequate cerebral perfusion, as indicated by a reduction in cerebral tissue ( $\text{P}_{\text{BrO}_2}$ ) and microvascular  $\text{Po}_2$  relative to hemodiluted controls. Reduced brain tissue oxygen tension was documented by three independent measurements in  $\beta$ -blocked and hemodiluted rats: (1) An invasive tissue oxygen probe demonstrated a reduction in hippocampal  $\text{P}_{\text{BrO}_2}$ ; (2) an intravascular oxyphor demonstrated a reduction in cerebral cortical microvascular  $\text{Po}_2$ ; and (3) cerebral cortical HIF-1 $\alpha$  protein levels were increased (fig. 6, 7). In addition, relative rectal and cerebral temperature changes during hemodilution also support the conclusion that  $\beta$ -blocked rats experience impaired cerebral perfusion during hemodilution (fig. 4). These data strongly support our hypothesis that  $\beta$ -blockade limits the cardiovascular response to acute hemodilution, resulting in a reduction in cerebral tissue oxygen tension.

Our baseline values of cerebral cortical microvascular  $\text{Po}_2$ , measured with the intravascular oxyphor G2 (70 mmHg), were similar to previously reported values in studies that used microelectrodes placed in the vicinity of cerebral microvessels.<sup>48,49</sup> These baseline values were slightly higher than we observed for renal microvascular  $\text{Po}_2$  (60 mmHg), but they are within the expected range of variability for tissue oxygen tension measurements. Our microvascular kidney  $\text{Po}_2$  values also correspond to previously reported measurements in the kidney using this methodology.<sup>45,50</sup> Previous studies have differentiated renal cortical  $\text{Po}_2$  (70 mmHg) from renal medullary  $\text{Po}_2$  (50 mmHg) by using two distinct excitation wavelengths. Our measurements are primarily from the renal cortex, but they may include some signal from the renal medulla, possibly explaining the intermediate baseline microvascular kidney  $\text{Po}_2$  measurement (60 mmHg). More interestingly, the relative changes in both the microvascular cerebral cortical and renal  $\text{Po}_2$  values after  $\beta$ -blockade and hemodilution demonstrate a differential and organ-specific response to hemodilution in which brain  $\text{Po}_2$  is more tightly defended than renal  $\text{Po}_2$  (fig. 3). In addition, brain tissue oxygen tension values ( $\text{P}_{\text{BrO}_2}$ ) that were obtained with invasive tissue probes were

lower than those obtained by the intravascular oxyphor, partially resulting from tissue injury associated with probe placement (fig. 2). However, these values also correspond to previously reported cerebral tissue  $\text{Po}_2$  values in brain tissue that was remote from cerebral microvessels.<sup>48,51</sup> In addition, the relative reduction in hippocampal tissue and microvascular cerebral cortical tissue  $\text{Po}_2$  observed after  $\beta$ -blockade and hemodilution provided evidence of reduced cerebral oxygen delivery to two distinct brain regions.

$\beta$ -blockade attenuated the heart rate, stroke volume, and cardiac output response after hemodilution. The concurrent reduction in heart rate and stroke volume responses suggested that both chronotropic and inotropic mechanisms were impaired (fig. 5, table 3). As a result, there was a significant attenuation of the CBF response and a reduction in brain tissue  $\text{Po}_2$ . The reduction in hippocampal tissue oxygen tension observed in this study was greater in magnitude than that observed in a previous study in which selective inhibition of vascular  $\beta_2$ -adrenergic receptors was achieved during hemodilution.<sup>44</sup> This comparison supports the interpretation that the  $\beta_1$ -adrenergic impairment of cardiac responsiveness is of primary importance in reducing cerebral perfusion during acute hemodilution.

The observed cerebral cortical HIF-1 $\alpha$  response was sustained from 3 to 18 h after recovery from  $\beta$ -blockade and hemodilution. In a previous study, HIF-1 $\alpha$  protein levels increased 18 h after acute hemodilution, but not at earlier time points.<sup>36</sup> However, after  $\beta$ -blockade and hemodilution a significant increase in HIF-1 $\alpha$  protein was observed at an earlier time point (3 h), relative to controls (fig. 6). This suggests that a significant relative reduction in cerebral cortical tissue  $\text{Po}_2$  had activated hypoxic cellular responses after  $\beta$ -blockade and hemodilution. Immunostaining characterized the cellular location of HIF-1 $\alpha$  to the perivascular regions near cerebral blood vessels (fig. 7). The reduction in cerebral tissue oxygen tension likely contributed to the observed increase in HIF-1 $\alpha$  by a protein-stabilizing mechanism.<sup>36</sup> Alternately, increased systemic angiotensin II may have also contributed to the elevation in HIF-1 $\alpha$  protein, by promotion of HIF-1 $\alpha$  transcription.<sup>52</sup> An increase in nNOS-positive cell counts was also observed in the cerebral cortex of both groups of hemodiluted rats.<sup>36</sup> However, no specific effect of  $\beta$ -blockade was observed on the relative number of nNOS-positive cells (fig. 8). Collectively, these data suggest that  $\beta$ -blockade and hemodilution lead to a significant reduction in cerebral tissue oxygen tension during the early postoperative period after anesthesia and surgery. These data may help to explain the increased incidence of stroke in  $\beta$ -blocked patients that was observed in the POISE trial.

The reduction in renal microvascular oxygen tension observed after hemodilution was similar in both  $\beta$ -blocked and non- $\beta$ -blocked groups, as confirmed by

comparable increases in systemic erythropoietin levels. Systemic erythropoietin levels increased within 2 h of recovery from hemodilution, demonstrating a rapid systemic endocrine response. Interestingly, angiotensin II was only increased in the hemodiluted rats that had received metoprolol. Thus, the combined effect of hemodilution and  $\beta$ -blockade may have accentuated the systemic response of the renin angiotensin system. Comparable increases in systemic catecholamine levels were observed in both hemodiluted groups. An early and dramatic increase in norepinephrine, but not epinephrine, suggested that spillover from sympathetic nerve terminals was the likely source (fig. 9). Thus, hemodilution with or without  $\beta$ -blockade provides a powerful stimulus to activate neurohormonal stress responses, possibly by initiating afferent neuronal signals to the brain. Subsequent activation of the sympathetic nervous system would contribute to the increase in CO observed in hemodiluted animals. Systemic  $\beta$ -blockade limited this CO response and reduced cerebral and renal tissue oxygen tension. These combined experimental data may help to explain the observed increase in the incidence of stroke and renal failure during acute hemodilution in surgical patients.<sup>21,47,53</sup>

Alternate mechanisms may also explain our results. The finding that the CBF response was not completely attenuated by  $\beta$ -blockade would suggest that additional mechanisms (hypoxia) had maintained some degree of cerebral vasodilation during hemodilution (fig. 2). Although metoprolol is relatively  $\beta_1$ -specific, some degree of  $\beta_2$  cross-reactivity can occur.<sup>54</sup> Thus, metoprolol could have directly affected the normal vasodilatory responses of cerebral resistance arteries. In addition, previous studies have demonstrated that  $\beta$  receptor blockade with propranolol reduced the cerebral metabolic rate for oxygen in isoflurane-anesthetized rats.<sup>55</sup> This may have occurred by the binding of propranolol to abundant  $\beta_1$  receptors located on cerebral cortical neurons (fig. 8). As a result of its ability to cross the blood brain barrier, metoprolol could also have a direct effect on the central nervous system. This might impair autonomic responses to hemodilution. Although we did not directly measure cerebral metabolic rate of oxygen, brain tissue oxygen tension remained stable after metoprolol administration in control experiments. This suggests that metoprolol may not have had a profound effect on cerebral metabolism, as previously reported in humans.<sup>56</sup> In addition, isoflurane anesthesia can impair the compensatory increase in CO during hemodilution<sup>29</sup> and dynamic CBF autoregulation in humans.<sup>57</sup> This may place anesthetized patients at even greater risk of cerebral tissue hypoxia during acute hemodilution.

Our results have direct relevance to the POISE trial because bleeding increased the relative risk of stroke in these patients (adjusted odd ratio 2.18 [1.06–4.49]).<sup>9</sup> Our experimental data suggest that impairment of the

cardiovascular responses to acute blood loss may jeopardize cerebral perfusion, resulting in an increased risk of cerebral injury. The risk of tissue hypoxia associated with anemia may be greatest at the time of acute blood loss. This risk extended into the postoperative period in our study (increased HIF-1 $\alpha$ , 3 h after recovery) and may help to explain the increased incidence of stroke in  $\beta$ -blocked subjects in the early postoperative period (POISE). The high correlation between perioperative stroke and mortality and the severity of the neurologic impairment in surviving stroke victims suggest that the degree of neurologic injury associated with  $\beta$ -blockade was profound.<sup>9</sup> This degree of neurologic injury could be the result of global hypoperfusion, as indicated by our experimental data. The increase in stroke risk associated with anemia<sup>21-23,53</sup> and  $\beta$ -blockade<sup>8,9</sup> suggests that higher hemoglobin concentrations may be required to maintain cerebral oxygen delivery in specific patient populations who are exposed to both conditions.

Given the established cardioprotective benefit of  $\beta$ -blocker therapy after acute myocardial infarction,<sup>58</sup> a clearer understanding of situations in which  $\beta$ -blockers may not be indicated must be defined. For example, metoprolol has been associated with an increase in stroke and mortality relative to other  $\beta$ -blockers,<sup>59,60</sup> possibly as a result of its relatively high penetration into the central nervous system.<sup>61</sup> The use of  $\beta$ -blockers, compared to other antihypertensive agents, for primary hypertension treatment may predispose patients to an increase in stroke incidence.<sup>62</sup> Early initiation of  $\beta$ -blockade in patients with an acute myocardial infarction may increase the incidence of cardiogenic shock.<sup>63</sup> Finally, our data and that of the POISE trial suggest that  $\beta$ -blockade in perioperative patients who experience acute blood loss and fluid resuscitation may have an increased risk for vital organ hypoxia and injury.<sup>9</sup> These studies support an ongoing reevaluation of the appropriate clinical indications and timing for  $\beta$ -blocker therapy and the use of blood products.

There are some limitations to the current study. The rat has a much higher baseline heart rate and may be more profoundly affected by  $\beta$ -blockade. However, the observed cardiovascular response to hemodilution in our model closely reflects that observed in humans.<sup>29,31</sup> In addition, the reduction in heart rate (20%) was clinically relevant and comparable to patients who have undergone acute  $\beta$ -blockade. These patients have evidence of reduced cerebral oxygen saturation relative to non- $\beta$ -blocked controls.<sup>64</sup> Only one dose of metoprolol was used, based on previous pharmacokinetic studies in rats.<sup>37</sup> The effect of metoprolol on vascular  $\beta_2$ -adrenergic receptors was not directly assessed.<sup>65</sup> Cerebral blood flow was assessed by using a nonquantitative methodology. The two methods of measuring oxygen tension provide different baseline  $P_{Br}O_2$  values. The tissue  $PO_2$  is higher when measured by the oxyphor G2, possibly as a

result of its intravascular location and the lack of tissue damage associated with the invasive probes. In addition, all studies were undertaken in young healthy rats without associated comorbidities.

In summary, our data demonstrate that acute blood loss and fluid resuscitation represent significant hemodynamic stress in which adequate cerebral perfusion is maintained by an increase in CO and preferential redistribution of blood flow to the brain. Acute metoprolol therapy severely attenuated the cardiac output response, resulting in cerebral tissue hypoxia. This mechanism may explain the increased incidence of ischemic events observed in  $\beta$ -blocked perioperative patients. The use of perioperative  $\beta$ -blockade to reduce the risk of myocardial ischemia must be balanced with the potential negative outcomes associated with an impaired cardiovascular response to acute blood loss and fluid resuscitation. In clinical operative settings associated with acute blood loss, close monitoring of hemodynamic responses and the maintenance of intravascular volume and tissue oxygen delivery by aggressive hemodynamic therapies may allow clinicians to take advantage of the potential beneficial effects of  $\beta$ -blockers (*myocardial protection*) while minimizing their detrimental effects (*tissue hypoxia*). Such a hypothesis may be amenable to testing in a clinical trial.

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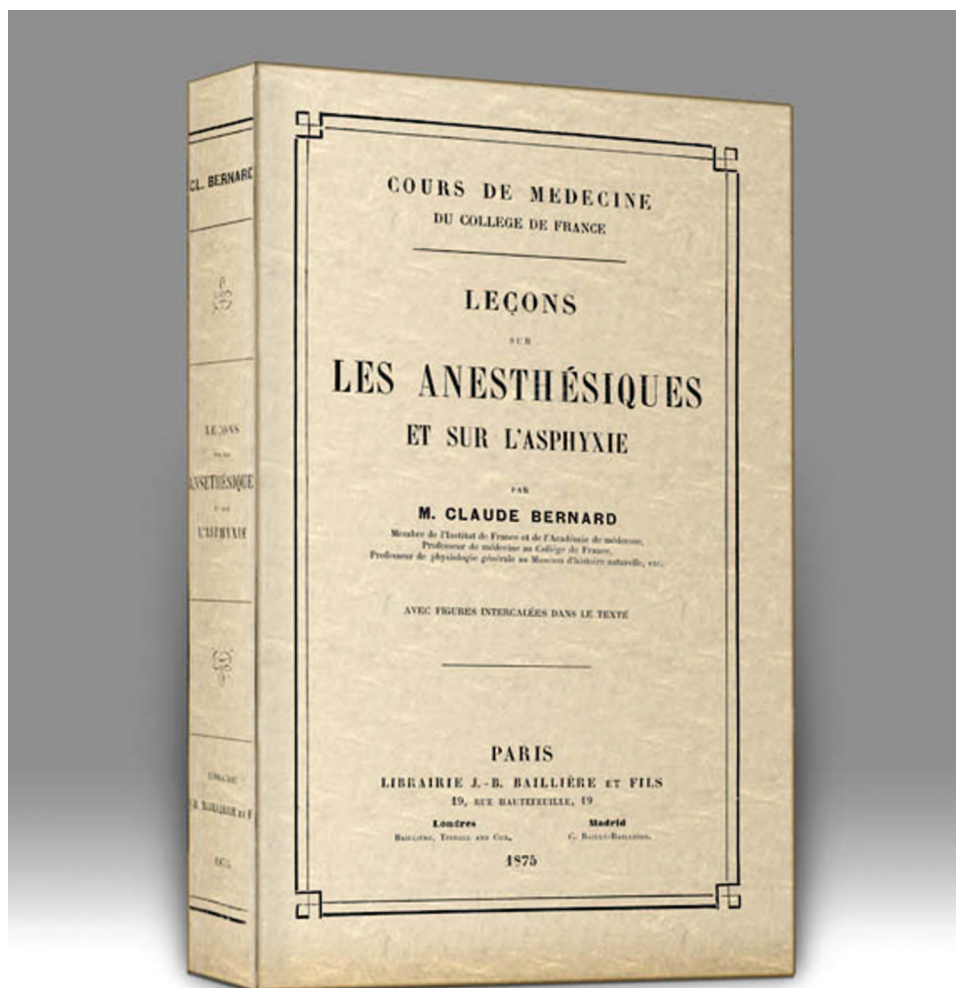
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## ■ ANESTHESIOLOGY REFLECTIONS

### Bernard on Anesthetics and Asphyxia



A French dramatist-turned-physiologist, Claude Bernard (1813–1878) promoted use of the scientific method and of observers blinded to treatments rendered. He conducted pioneering research in pancreatic, hepatic, and vasomotor physiology. As the first chair of physiology at the Sorbonne, Bernard received laboratory funding from Louis Napoleon himself. In 1989 the Wood Library-Museum published Bernard's classic 1875 work (*see above*) not in French, but in English as *Lectures on Anesthetics and on Asphyxia*, courtesy of his translator and namesake, Bernard Raymond Fink, M.D. (Copyright © the American Society of Anesthesiologists, Inc. This image appears in color in the *Anesthesiology Reflections* online collection available at [www.anesthesiology.org](http://www.anesthesiology.org).)

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