Influence of Intraarterial Norepinephrine on Cerebral Hemodynamics of Newborn Pigs (42905)

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Abstract. **Effects of intraarterial norepinephrine on cerebral hemodynamics were investigated in conscious newborn pigs. Intracarotid infusion of norepinephrine (100 ng/min) increased cerebral blood flow from 72 ± 5 to 82 ± 8 ml/100 g·min. The norepinephrine-induced increase in cerebral blood flow was accompanied by an increase in cerebral oxygen consumption from 2.75 ± 0.17 to 3.11 ± 0.29 ml O_2/100 g·min. Both the increase in cerebral oxygen consumption and the increase in cerebral blood flow were blocked by propranolol and unchanged by combined prazosin and yohimbine treatment. These data show that intraarterial norepinephrine increases cerebral blood flow and cerebral oxygen consumption in newborn pigs. β-Adrenergic stimulation increases cerebral oxygen consumption. This increase may produce the increase in cerebral blood flow caused by the norepinephrine.** [P.S.E.B.M. 1989, Vol 191]

Cerebral arteries have a rich supply of sympathetic nerves (1, 2). Activation of these nerves can constrict cerebral arteries and reflexly attenuate increases in cerebral flow during hypoxia, hypercapnia, and acute hypertension (3–5). We have shown that sympathetic innervation is functional in newborn pigs and that stimulation of these nerves and topical application of norepinephrine both constrict pial arterioles (6, 7). Although norepinephrine does not cross the blood-brain barrier readily, circulating catecholamines also may influence cerebral hemodynamics under certain conditions, such as stress in adults (8–10). Effects of circulating catecholamines have not been examined in the neonate. The newborn rabbit blood-brain barrier has restrictive properties similar to those of adults to substances of high molecular weight but exhibits carrier-mediated uptake of some compounds, particularly neurotransmitter precursors, that is much more active than in the adult (11). The behavior of the newborn blood-brain barrier toward norepinephrine has not been examined.

This study was undertaken to address the hypothesis that intracarotid administration of norepinephrine affects cerebral blood flow and cerebral metabolic rate in newborn pigs. The role of α- and β-adrenoceptors in norepinephrine-induced changes in cerebral hemodynamics also was investigated.

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Materials and Methods

Animal Preparation. Catheters were implanted in newborn pigs (less than 36-hr old; 1.4–2.5 kg) under aseptic surgical conditions, using a mixture of halothane, oxygen, and nitrous oxide for anesthesia. A polyurethane catheter was placed in the abdominal aorta (via an umbilical artery) for monitoring systemic arterial pressure, reference withdrawal in microsphere determination of blood flow, and for blood sampling. The right common carotid artery was ligated and cannulated superiorly for drug infusion into the internal carotid artery and Circle of Willis. Inferior to the ligature, a second catheter was placed in the left ventricle (via the carotid artery) for injection of radioactive microspheres. In piglets, ligation of one carotid artery has no detectable effect on cerebral blood flow (12, 13). All surgical and experimental procedures used were reviewed and approved by the Animal Care and Use Committee at the University of Tennessee, Memphis, Tennessee.

After surgery, the piglets received benzathene penicillin (150,000 units/kg, im), gentamicin (5 mg/kg im), and colloidal iron-dextran suspension (0.5 ml im) and were placed in cages warmed by overhead lamps. A

continual supply of pig milk substitute and water were provided. Experiments were performed on the third postoperative day. On the morning of experimentation, the scalp was anesthetized with lidocaine, and a 2-cm incision was made in the midline to expose the cranial suture. A 22-gauge Angiocath was used to cannulate the sagittal sinus for collection of cerebral venous blood. This Angiocath was then secured with Superglue. Piglets were divided into three groups that received intra-arterial injections: the vehicle group ($n = 11$) received 5 ml of saline; the propranolol group ($n = 5$) received 1 mg of propranolol/kg in 5 ml of saline to block β-adrenergic receptors; the prazosin + yohimbine group ($n = 5$) received 1 mg of prazosin/kg and 1 mg of yohimbine/kg dissolved in 5 ml of saline to provide combined α_1- and α_2-adrenergic receptor blockade. Experimentation was begun 20 min after administration of vehicle or agonist. β-adrenoceptor blockade was confirmed by observation of the depressor response to 2.5 μg of isoproterenol/kg before and after propranolol. Previously, we have shown that prazosin, in the dose used, crosses the blood-brain barrier in sufficient quantity to block responses to topically administered phenylephrine, an α_1- adrenoceptor agonist. Similarly, we have also shown that this dose of yohimbine inhibits responses to sympathetic nerve stimulation, exogenous norepinephrine, and exogenous clonidine, an α_2 agonist, but not to phenylephrine (6, 7).

Experiments were designed such that all piglets in each of the three groups received two 5-min drug infusions into the carotid artery. These infusions were vehicle and 100 ng of norepinephrine/min. This dose of norepinephrine was chosen to produce a plasma level in blood going to the brain near that measured during ventilation of newborn pigs with a low O_2/high CO_2 mixture (14). Green *et al.* (14) reported a geometric mean plasma norepinephrine concentration in unanesthetized piglets of 380 pg/ml. During asphyxia, the mean plasma norepinephrine concentration was 3802 pg/ml before indomethacin treatment and 6607 pg/ml after indomethacin treatment, although the postindomethacin value was not significantly greater than that before indomethacin. Since the average cerebral blood flow was about 28 ml/min, an infusion of 100 ng/min into that flow would produce a plasma concentration of (100 ng/min ÷ 28 ml/min) + 0.38 ng/ml = 3.95 ng/ml. Although norepinephrine is effectively removed during passage through the pulmonary circulation, some increase would be expected due to recirculation. However, the level in the blood going to the brain would still be between the two levels reported previously (14) of 3.8 and 6.6 ng/ml. Data from experiments using higher rates of infusion are not reported for two reasons. First, the present levels appear to be near the top of the physiologic range, and, therefore, higher infusion rates would not be relevant to effects of norepinephrine on cerebral circulation and metabolism during stress. Second, when a higher infusion rate was used (800 ng/min), substantial increase in arterial pressure occurred that could not be entirely eliminated with propranolol or prazosin and yohimbine, making data interpretation questionable. Microsphere infusions toward the brain through the carotid injection line confirmed that the bilateral distribution of the infusions was uniform. Microsphere injections into the left ventricle for determination of cerebral blood flow were made at the end of each 5-min infusion period, and blood samples were drawn from the aorta and sagittal sinus for determination of oxygen content. Fifteen-minute time intervals between the infusions were allowed for stabilization of the animal. All drugs were made freshly on the day of use, and the vehicle (0.9% saline) contained ascorbate (0.5 g/liter) to prevent the oxidation of norepinephrine.

Blood Chemistry and Cerebral O_2 Consumption. Blood pH, P_{O_2} and P_{CO_2} were measured using an Instrumentation Laboratories blood gas analyzer. An American Optical Reflection Oximeter (corrected to newborn pig blood) was used to determine percentage of saturation of the hemoglobin in arterial and sagittal sinus blood. Blood hemoglobin quantity was determined using a Reichert hemoglobinometer. The oxygen capacity of the hemoglobin was assumed to be 1.39 ml O_2/g of hemoglobin. Blood oxygen content was then calculated as C_{O_2} = (g hemoglobin/ml × 1.39 ml O_2/g hemoglobin × % saturation of hemoglobin with O_2) + dissolved O_2. The dissolved O_2 was calculated from the P_{O_2}. Cerebral oxygen consumption was calculated as (arterial C_{O_2} − venous C_{O_2}) × cerebral blood flow.

Radioactive Microsphere Determination of Cerebral Blood Flow. A known amount of radioactivity in 15-μm microspheres (300,000–800,000 microspheres) labeled with ^{46}Sc, ^{96}Nb, ^{103}Ru, ^{113}Sn, ^{57}Co, or ^{125}I was injected into the left ventricle, and the injection line was flushed with 1 ml of saline. Withdrawal of the reference blood sample from the abdominal aorta was begun 15 sec before microsphere injection and continued for 2 min after the injection. After the experiment, the piglet was killed with T-61, intracardiac, and the brain removed for determination of radioactivity. Blood flow to the brain at the time the microspheres were injected was calculated by using the formula:

$$Q = C \cdot R \cdot CR^{-1},$$

where Q is brain blood flow (in ml/100 g/min), C is counts per 100 g tissue, R is rate of withdrawal of reference blood sample (in ml/min), and CR is total counts in the reference arterial blood sample.

Statistical Analysis. All values are means ± SEM. Comparisons were made using analysis of variance, t test with Bonferonni correction, and t tests for planned comparisons, as appropriate. $P < 0.05$ was required for comparisons to be significant.

Results

Before drug treatment, the arterial pressure of piglets in the three groups was the same (vehicle, 68 ± 5 mm Hg; propranolol, 70 ± 5 mm Hg; prazosin + yohimbine, 75 ± 2 mm Hg). Both propranolol and prazosin + yohimbine caused decreases in arterial pressure (Table I). Intracarotid infusion of norepinephrine produced an increase in cerebral blood flow (Fig. 1). Norepinephrine increased mean arterial pressure as well (Table I). Propranolol blocked the increase in cerebral blood flow caused by norepinephrine infusion (Fig. 1). Prazosin + yohimbine did not change the cerebral blood flow response to intracarotid norepinephrine, but, similarly to propranolol, inhibited the systemic hypertensive response.

Intracarotid infusion of norepinephrine increased cerebral oxygen consumption (Fig. 2). Norepinephrine did not increase cerebral oxygen consumption following propranolol, and prazosin + yohimbine treatment did not affect the metabolic response to norepinephrine (Fig. 2).

Discussion

Results of this study show that intracarotid administration of norepinephrine increases cerebral blood flow and cerebral oxygen consumption. The norepinephrine-induced increase in cerebral oxygen consumption was blocked by propranolol, but not by α-adrenoceptor blockade, indicating that activation of β-adrenoceptors by norepinephrine produces the increased cerebral oxygen consumption. Norepinephrine-induced increases in cerebral blood flow were blocked after propranolol, suggesting that the increased cerebral metabolic rate and/or direct β-adrenergic receptor-induced vasodilation causes the increase in flow. The failure of prazosin + yohimbine to alter the cerebral vascular response to intravascular norepinephrine indicates that α-adrenergic receptors are not involved.

Cerebral arteries have a rich supply of sympathetic nerves (1, 2) that can constrict cerebral arteries and reflexly attenuate increases in cerebral blood flow during hypoxia and hypercapnia in adult cats and rabbits (3–5). Sympathetic innervation of cerebral arterioles is present in newborn pigs (6). In the newborn pig, both α_1- and postsynaptic α_2-adrenoceptors may be involved in mediating cerebrovascular responses to neurogenically released norepinephrine (6, 15). Topically applied norepinephrine constricts cerebral arterioles in newborn pigs (7). β-Adrenoceptor stimulation produces dilation of cerebral arteries of newborn (16) and older (17) pigs.

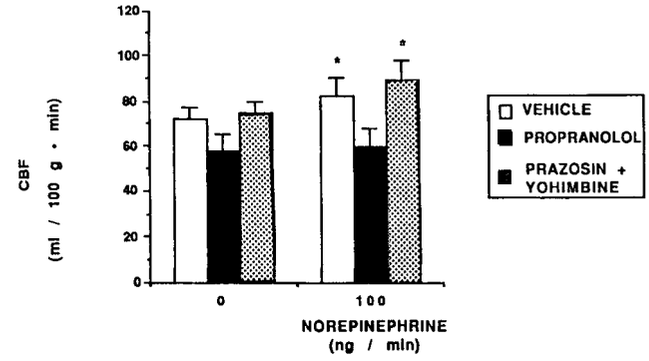

Figure 1. Influence of intracarotid infusion of norepinephrine on cerebral blood flow (CBF) in unanesthetized newborn piglets treated with vehicle (saline), propranolol (1 mg/kg), and prazosin (1 mg/kg) + yohimbine (1 mg/kg) combined. $n = 11$ for vehicle; $n = 5$ each for propranolol and prazosin + yohimbine animals. Values are means ± SEM; * $P < 0.05$ compared with 0 dose value.

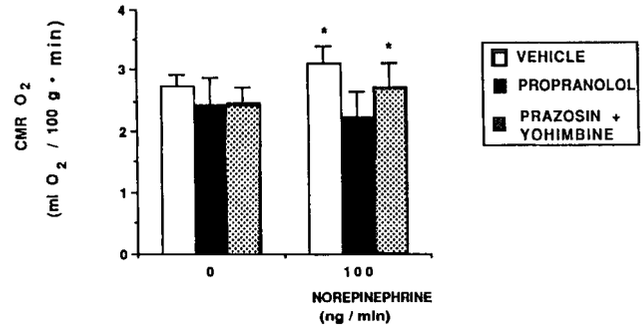

Figure 2. Influence of intracarotid infusion of norepinephrine on cerebral oxygen consumption ($CMRO_2$) in unanesthetized newborn piglets treated with vehicle (saline), propranolol (1 mg/kg), and prazosin (1 mg/kg) + yohimbine (1 mg/kg) combined. $n = 11$ for vehicle; $n = 5$ each for propranolol and prazosin + yohimbine animals. Values are means ± SEM; * $P < 0.05$ compared with 0 dose value.

Table I. Arterial Pressure and Blood Chemistry of Newborn Pigs[a]

	Norepinephrine (ng/min)	
	0	100
Mean arterial pressure (mm Hg)		
Vehicle ($n = 11$)	68 ± 2	76 ± 2*
Propranolol ($n = 5$)	53 ± 2†	54 ± 2†
Prazosin + yohimbine ($n = 5$)	54 ± 1†	56 ± 2†
Pa_{O2} (mm Hg)		
Vehicle ($n = 11$)	76 ± 2	77 ± 2
Propranolol ($n = 5$)	75 ± 3	81 ± 4
Prazosin + yohimbine ($n = 5$)	70 ± 4	73 ± 4
Pa_{CO2} (mm Hg)		
Vehicle ($n = 11$)	30.0 ± 0.6	33.0 ± 1.3
Propranolol ($n = 5$)	30.0 ± 1.0	30.0 ± 1.0
Prazosin + yohimbine ($n = 5$)	35.7 ± 1.1†	33.8 ± 0.7
pH		
Vehicle ($n = 11$)	7.41 ± 0.02	7.41 ± 0.02
Propranolol ($n = 5$)	7.42 ± 0.02	7.39 ± 0.03
Prazosin + yohimbine ($n = 5$)	7.42 ± 0.02	7.44 ± 0.02

[a] Values are means ± SEM.
* $P < 0.05$ compared with 0 norepinephrine. † $P < 0.05$ compared with corresponding value without antagonist.

Several studies have investigated the influence of circulating catecholamines in cerebral hemodynamics of adults. These studies, however, have been contradictory, both with respect to the magnitude and the direction of the response. Injections of norepinephrine or epinephrine into the carotid artery have been found to either decrease (8, 18), increase (9, 19), or have no effect on (20, 21) cerebral blood flow. Increased cerebral blood flow after norepinephrine administration was reported to occur only after disruption of the blood-brain barrier (19, 22). Newborns possess a selectively permeable blood-brain barrier that exists at birth and that differs from that of the adult (11). Since intraarterial norepinephrine produces cerebral vasodilation whereas topically applied norepinephrine constricts pial arterioles (7), a blood-brain barrier to norepinephrine appears to exist at birth in pigs. Both MacKenzie *et al.* (19) and Edvinsson *et al.* (22) suggested that increased cerebral flow caused by norepinephrine infusion was secondary to an increase in cerebral metabolism. However, in some studies, norepinephrine-induced dilation or constriction could not be fully explained by changes in metabolism or activation of vascular receptors examined (23, 24). Increased cerebral blood flow per se does not change cerebral oxygen consumption since cerebral hyperemia induced by hypercapnia is not accompanied by a change in cerebral oxygen consumption (25). Therefore, the increase in cerebral blood flow would not cause the increase in oxygen consumption during norepinephrine infusion.

The rate of norepinephrine infusion used in these experiments (100 ng/min) directly into the cerebral circulation would produce norepinephrine concentrations in the blood that are within the range measured in arterial blood of newborn pigs breathing high CO_2/low O_2 mixtures (14) (see calculation in Materials and Methods). Propranolol blocked the increase in cerebral blood flow at this infusion rate. Therefore, circulating norepinephrine may increase cerebral blood flow via β-adrenergic-mediated stimulation of cerebral oxygen consumption during severe stress in newborn pigs. Stimulation of oxygen consumption appears to occur indirectly, possibly via activation of β-adrenergic receptors on vascular endothelial cells or neurons projecting from circumventricular organs, because topical application of the catecholamine does not produce the same effect.

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