0031-3998/89/2502-0205$02.00/0
PEDIATRIC RESEARCH

Vol. 25, No. 2, 1989
Printed in U.S.A.

The Effect of Arterial P_{CO_2}-Variations on Ocular and Cerebral Blood Flow in the Newborn Piglet

TOM STIRIS, JAN-PETTER ODDEN, THOR W. R. HANSEN, CHRISTIAN HALL, AND DAG BRATLID

Neonatal Research Laboratory, Department of Pediatric Research [T.S., J.-P.O., T.W.R.H.], Institute for Surgical Research [J.S., C.H.], and Department of Pediatrics [D.B.], Rikshospitalet, University of Oslo, Oslo, Norway

ABSTRACT. The response of ocular and cerebral blood flow to different arterial P_{CO_2} levels was studied in ventilated paralyzed newborn piglets with the radionuclide-labeled microsphere method. The retina and the choroid have different blood flow responses to variations in arterial P_{CO_2} levels. Retinal blood flow (ml/g/min) was increased during hypercarbia, from 0.26 ± 0.03 at baseline to 0.51 ± 0.07 (Pa_{CO_2} 8.7 ± 0.2 kPa) and 0.62 ± 0.07 (Pa_{CO_2} 11.0 ± 0.2 kPa). However, no significant change was found in choroidal blood flow during hypercarbia. Cerebral blood flow was more responsive to Pa_{CO_2} than retinal blood flow, increasing from 0.71 ± 0.03 at baseline to 2.25 ± 0.25 (Pa_{CO_2} 8.7 ± 0.2) and 1.77 ± 0.13 (Pa_{CO_2} 11.0 ± 0.2). Hypocarbia did not influence either retinal or choroidal blood flow. (*Pediatr Res* 25:205–208, 1989)

Abbreviations

CBF, cerebral blood flow
ChBF, choroidal blood flow
CO, cardiac output
OBF, ocular blood flow
RBF, retinal blood flow
ROP, retinopathy of prematurity

ROP is still a major problem in the care of the small premature infant (1–3).

Although hyperoxia is considered a major risk factor for the development of ROP (4), experimental data and clinical studies have also pointed at other factors. Thus, hypercarbia (4), hypocarbia (5) and hypoxemia (6, 7) have been associated with the development of ROP.

At least some putative major risk factors in the development of ROP affect cerebral and ocular blood flow (8, 9). Experimental work with adult cats, as well as with newborn lambs and piglets, has shown that hypercarbia influences ocular and cerebral blood flows, although the blood flow response in the retina may differ from that of the choroid (10–12). However, studies on ocular blood flow in the neonate are few (13), and to our knowledge there are no reports on the influence of hypocarbia on ocular blood flow in the neonatal period.

Hypocarbia and hypercarbia have opposite effects on cerebral vasculature, hypercarbia increasing cerebral blood flow, hypocarbia reducing it. If the relation between both hypocarbia and hypercarbia to ROP are linked to effects on ocular blood flow, these effects may not necessarily be the same in the eye as in the brain. Thus, previous studies have shown that retinal and choroidal blood flow change differently from each other and partly differently from that of the brain (11, 12).

The present study examines the effect of arterial P_{CO_2} levels on RBF, ChBF, and total OBF as well as CBF in the newborn piglet.

MATERIALS AND METHODS

Experimental animals. Piglets have been shown to develop retinal changes similar to those seen in infants with ROP (15, 16), and were chosen as experimental animals. Ten newborn piglets (2–4 days old) weighing 1.45 ± 0.11 kg (mean ± SEM) were used. The piglets were obtained from a local farmer and removed from the sow on the morning of the day the study was performed.

Surgical procedures. Azaperon (1 mg/kg) was used as premedication and 1% xylocain as local anesthetic. A gas mixture of 70% nitrous oxide and 30% oxygen was used as general anesthesia. This type of anaesthesia does not influence cerebral or ocular blood flow significantly (17, 18). Initially, the gas mixture was delivered through a mask fitted around the nose and mouth. The animals were then tracheotomized, and an endotracheal tube (Portex 3.5) was connected to the gas flow.

Polyethylene catheters (Portex PE 50) with an internal diameter of 0.58 mm were placed in the left axillary artery, the abdominal aorta via the femoral artery and the inferior vena cava via the femoral vein. The left ventricle was catheterized via the right axillary artery with a 3.5 Charrier × 38 cm umbilical artery catheter (Argyle, Sherwood Medical Industries, Inc., St. Louis, MO, cat. no. 8888-160218). The position of the catheter was verified by blood pressure tracings and later by inspection at autopsy.

Paralysis was induced with pancuronium 0.5 mg/kg intravenously (19), and the animal was then connected to a Loosco Amsterdam Infant Ventilator Mk2. Pancuronium in doses not exceeding 0.5 mg/kg was repeated throughout the experiment as soon as the animal attempted spontaneous respiration.

Body temperature and systemic arterial blood pressure were monitored throughout the study. Before each blood flow determination, arterial blood gases (AVL-945 Blood Gas Analyzer) and hematocrit were measured. To maintain a constant hematocrit, blood losses (reference blood withdrawal and blood gas sampling) were replaced with donor pig blood immediately after each blood flow determination had been made. No continuous infusion of fluid was given during the study. However, the combined flushings of catheters with heparinized saline in connection with blood sampling and microsphere injections

Received March 25, 1988; accepted October 12, 1988.
Correspondence Tom Stiris, M.D., Neonatologisk forskningslaboratorium, Pediatrisk forskningsinstitut, Rikshospitalet, 027 Oslo 1, Norway.
Supported by a donation from Libero Diaper Fund (SABA-Mølnlycke A/S), The Norwegian Association for the Blind, and The Blix Family Medical Research Foundation.

amounted to approximately 60–80 ml in total during the whole experiment. Blood glucose levels were not routinely measured, but were found to be normal when checked. The head of the animal was covered by a black rubber bag to prevent possible effects of light on OBF (17).

Experimental protocol. The study design is shown in Figure 1. Five predetermined $Paco_2$ levels were chosen for blood flow determinations. These levels were achieved by changing the ventilator settings with or without additional carbon dioxide in the gas mixture. Because only four different labeled microspheres were available, one of the five predetermined Pco_2 levels had to be omitted in each animal studied. All animals had a baseline microsphere injection (n = 10). However, in six animals, one of the hypocarbic levels was omitted (three hypocarbia I and three hypocarbia II), and in four animals, one of the hypercarbic levels was omitted (two hypercarbia I and two hypercarbia II). Hence 40 measurements were performed in the 10 animals, 10 baseline, seven hypocarbia level I, seven hypocarbia level II, eight hypercarbia level I and eight hypercarbia level II. Hypocarbia always preceded hypercarbia due to previous work demonstrating that hypercarbia may alter the autoregulation in the brain (20, 21).

Blood flow determination. Organ blood flow was measured with the microsphere technique as described by Heymann (22). Microspheres with a diameter of 15 ± 1.5 μm (mean ± SD) labeled with one of the following radionuclides: ^{141}Ce, ^{51}Cr, ^{103}Ru and ^{95}Nb (New England Nuclear, Inc., Boston, MA) were used. The sequence of the radionuclides was randomized for each study. Approximately 1.5×10^6 microspheres were suspended in 2 ml of 10% dextran and 0.05% Tween 80. After vigorous shaking, the suspension was injected and flushed with 5.0 ml of heparinized saline into the left ventricle within a 45-s period.

A reference sample of blood was withdrawn continuously from the axillary artery catheter at a rate of 1.06 ml/min, using a constant flow rate withdrawal pump (Harvard Apparatus, Millis, MA). Blood withdrawal started 15 s before microsphere injection to ensure catheter function. Blood withdrawal lasted for 2 min.

After the final blood flow determination, the piglet was killed by a bolus injection of pentobarbital through the ventricular catheter. The eyes and the brain were carefully removed and weighed before fixation in 10% formalin. Radioactivity in the cerebral tissues, eyes and blood samples were counted in a γ-well counter (Packard Auto-Gamma Scintillation Spectrometer 5221, Hewlett-Packard Co., Palo Alto, CA). A computer program was used to calculate blood flow, correcting for spectral overlap from the different isotopes.

After counting the whole eye as such, the eye was dissected (11), and the retina and the choroid were counted separately. Blood flow to the various tissues was calculated according to the equation (22):

$$\text{tissue flow} = \frac{\text{tissue cpm} \times \text{withdrawal rate of reference blood}}{\text{cpm of reference blood}}$$

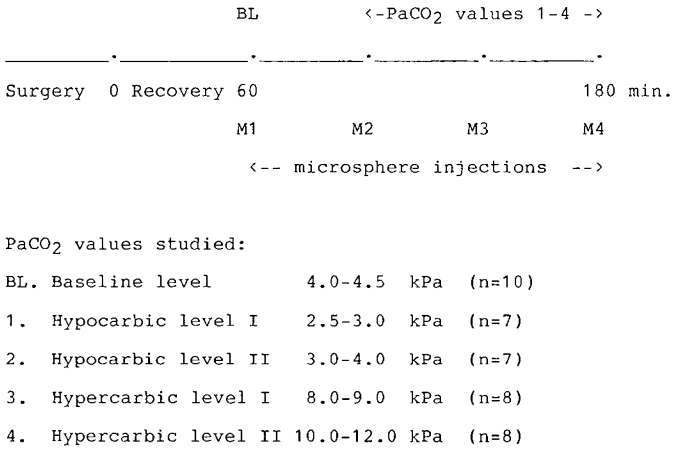

Fig. 1. Study design.

where cpm = radioactivity counts/min. All tissue samples except retina contained more than 500 microspheres and reference blood samples more than 1100 microspheres. The mean total number of microspheres in the retina was 283.

Cardiac output was calculated from the same equation, by substituting tissue cpm with the total amount of radioactivity (cpm) injected. Organ blood flow was expressed in ml/min/g, cardiac output in ml/min/kg.

Data analysis. Blood flow was calculated separately for each eye. The blood flow data were compared using Wilcoxon signed rank test (two tailed), for paired comparisons, adjusted with Bonferroni's correction for repeated measurements. All values were expressed as mean ± SEM.

RESULTS

The results are reported in Table 1. No significant effect of arterial Pco_2 variations on total OBF was found. A significant increase in RBF was found during hypercarbia, whereas no significant changes were seen during hypocarbia. ChBF, by contrast, was not significantly altered by any changes in arterial Pco_2 levels.

CBF was significantly increased by hypercarbia. However, the apparent fall in cerebral blood flow during hypocarbia did not obtain statistical significance.

CO did not change significantly either during hypocarbia or during hypercarbia. The results of blood gas and blood pressure measurements are shown in Table 2. The animals remained normoxemic, and no significant changes in systemic blood pressure (MAP) were found throughout the study.

DISCUSSION

RBF response to hypercarbia has been studied in a number of species both in adults and newborns (11, 12, 14, 23). All studies report an increase in RBF. This increase seems to be greater in adult animals (cat and monkey) than in the newborn (lamb). Milley *et al* (12) speculate that this may be due either to age or to species differences. There are, however, only few reports on this effect in the newborn period. The increase seen in the newborn piglet retina in our study agrees with those found in adult animals (11, 19). Hence the reduced neonatal RBF response described by Milley *et al* can most likely be attributed to species differences rather than age. Furthermore, in our study, hypercarbia did not change ChBF significantly, in contrast to reports from other investigators (11, 14).

Tsacopoulos (23), using a method based on dye dilution curves constructed from densitometry measurements on fluorescing vessels in fundus angiograms, detected a decrease of approximately 50% in RBF during hypocarbia in adult monkey. We failed to obtain the same results in the newborn piglet. On the contrary, we found a trend toward an increase in RBF during level II hypocarbia (Table 1). This did not obtain statistical significance. However, because the retina is a small organ with relatively low blood flow, the number of microspheres counted will be low. Hence, only relatively large changes in blood flow can be detected (25, 26).

We have not found any reports on the effect of hypocarbia on ChBF in the neonate, but in the present study ChBF was not influenced by hypocarbia.

Hypocarbia and hypercarbia are considered risk factors in the development of ROP (4, 5). The mechanism behind this is still unknown. However, excessive oxygen is regarded to play a key role in the development of ROP. With increased RBF as seen in hypocarbia, there will be an increase in the amount of oxygen offered to the retina. Alm and Bill (27) have shown that increased arterial Pco_2 causes increased vitreous body Po_2. They conclude that this increase is secondary to increased RBF, an interpretation supported by the present study. ChBF counts for approximately 60% of oxygen delivery to the pig retina and 80% to the cat retina (14). Because ChBF is not increased during hypercarbia,

Table 1. *OBF, RBF, ChBF, CBF, and CO at different $Paco_2$ (kPa) levels*

	Hypocarbia		Baseline	Hypercarbia	
	I ($n = 7$)	II ($n = 7$)	($n = 10$)	I ($n = 8$)	II ($n = 8$)
$Paco_2$	2.5–3.0	3.0–4.0	4.0–4.5	8.0–9.5	10.0–12.0
OBF†	0.65 ± 0.09	0.49 ± 0.05	0.60 ± 0.07	0.78 ± 0.10	0.60 ± 0.07
RBF†	0.34 ± 0.08	0.42 ± 0.10	0.26 ± 0.03	0.51 ± 0.07‡	0.62 ± 0.07‡
ChBF†	26.46 ± 2.15	19.20 ± 2.20	22.00 ± 2.02	30.93 ± 3.85	28.31 ± 3.52
CBF†	0.48 ± 0.03	0.62 ± 0.02	0.71 ± 0.03	2.25 ± 0.25‡	1.77 ± 0.13‡
CO§	326 ± 31	275 ± 29	374 ± 37	462 ± 50	331 ± 30

* Mean values ± SEM.
† Values in ml/min/g.
‡ $p < 0.05$ from baseline.
§ Values in ml/min/kg.

Table 2. *Blood gas values (kPa) and mean blood pressures (MAP) during hypocarbic, baseline, and hypercarbic blood flow measurements**

	Hypocarbia		Baseline	Hypercarbia	
	I ($n = 7$)	II ($n = 7$)	($n = 10$)	I ($n = 8$)	II ($n = 8$)
$Paco_2$	2.5–3.0	3.0–4.0	4.0–4.5	8.0–9.5	10.0–12.0
$Paco_2$	2.9 ± 0.1	3.7 ± 0.1	4.3 ± 0.1	8.7 ± 0.2	11.0 ± 0.2
pH	7.59 ± 0.02	7.52 ± 0.01	7.47 ± 0.01	7.16 ± 0.01	7.10 ± 0.01
PaO_2	9.6 ± 0.6	10.4 ± 0.6	11.3 ± 0.5	10.6 ± 0.7	11.0 ± 0.4
MAP	76 ± 5	89 ± 4	87 ± 3	80 ± 2	89 ± 4

* Mean values ± SEM.

the possible harmful effects of increased oxygen transport will therefore be limited to the effect of increased RBF only.

Because RBF and ChBF are not influenced by hypocarbia, our results suggest that the linkage between hypocarbia and development of ROP is not flow related. However, in clinical situations, hypocarbia is usually seen during accidental hyperventilation, which also may cause hyperoxemia. Thus, as ChBF is not reduced by hypocarbia, increased oxygen transport to the retina may be the result of hyperventilation.

Hypercarbia leads to an increase in CBF. This has also been found by others (19, 28, 29). In the present study, there apparently was a fall in CBF from level I to level II hypercarbia. This tendency to a slight decrease in CBF during prolonged exposure to hypercarbia has also been reported by others (30). This may partly be due to reduced vasodilative response of pial arterioles as suggested by Levasseur (31).

It is well known that hypocarbia causes vasoconstriction and hence reduction in CBF (28, 32). In our study we also found a trend towards reduction of CBF during hypocarbia, although this did not reach statistical significance. This might be explained by the fact that the $PaCO_2$ level studied was never below 2.5 kPa. Thus, Hansen *et al.* (19) did not find a significant reduction in CBF from baseline before the $PaCO_2$ levels dropped below 2.0 kPa.

In our study as well as in that of Hansen *et al.* (19) variations in $PaCO_2$ levels did not alter CO significantly, whereas Brubakk *et al.* (30) found an increase in CO during hypercarbia. Both in our study and in that of Hansen, the animals were paralyzed with pancuronium and mechanically ventilated. In the study by Brubakk *et al.*, the animals were not paralyzed but were breathing spontaneously. It is therefore possible that pancuronium and/or mechanical ventilation alter the response of CO to changes in arterial P_{CO_2} (33). Thus, pancuronium is known to influence organ blood flow such as to the brain (34, 35). However, its effect on OBF has not been reported.

In conclusion, our study confirms the responsiveness of CBF to variations in $Paco_2$. Also, hypercarbia was associated with an increase in RBF, whereas hypocarbia did not alter RBF significantly. Furthermore, ChBF did not react to either hypercarbia or hypocarbia.

Although the well-oxygenated mature piglet is different from the premature infant, we speculate that $Paco_2$ and its effects on ocular blood flow may be of importance in the pathophysiology of ROP.

We gratefully acknowledge the excellent technical assistance provided by Ms. Ellen Sagen.

REFERENCES

1. Cats BP, Tan KEW 1985 Retinopathy of prematurity: review of a four-year period. Br J Ophthalmol 69:500–503
2. Phelps DL 1981 Retinopathy of prematurity: an estimation of vision loss in the United States—1979. Pediatrics 67:924–925
3. Reisner SH, Amir J, Shohat M, Krikler R, Nissenkorn I, Ben Sira I 1985 Retinopathy of prematurity: incidence and treatment. Arch Dis Child 60:698–701
4. Bauer CR, Widmayer SM 1981 A relationship between $Paco_2$ and retrolental fibroplasia. Pediatr Res 15:649 (abstr)
5. Shoat M, Reisner SH, Krikler R 1983 Retinopathy of prematurity: incidence and risk factors. Pediatrics 72:159–163
6. Ashton N, Henkind P 1965 Experimental occlusion of retinal arterioles. Br J Ophthalmol 49:225–234
7. Phelps DL, Rosenbaum A 1984 Effects of marginal hypoxemia on recovery from oxygen-induced retinopathy in the kitten model. Pediatrics 73:1–6
8. Leathy FAN, Cates D, MacCallum M, Rigatto H 1980 Effect of CO_2 and 100% O_2 on cerebral blood flow in preterm infants. J Appl Physiol 48:468–472
9. Lindner W, Schaumberger M, Versmold H 1987 Ophthalmic artery blood flow velocity in neonates, relation to cerebral blood flow and cardiac output. Pediatr Res 22:241(abstr)
10. Stiris T, Hansen TWR, Mørkrid L, Bratlid D 1986 The effects of light, hyperoxia and hypercarbia on ocular blood flow in the newborn piglet. Pediatr Res 20:362 (abstr)
11. Alm A, Bill A 1972 The oxygen supply to the retina: II: effects of high intraocular pressure and of increased arterial carbon dioxide tension on uveal and retinal blood flow in cats. Acta Physiol Scand 84:306–319

12. Milley RJ, Rosenberg AA, Jones M Jr 1984 Retinal and choroidal blood flows in hypoxia and hypercarbia newborn lambs. Pediatr Res 18:410–414
13. Flower RW 1986 Perinatal retinal vascular physiology. In: Silverman WA, Flynn JT (eds) Contemporary Issues in Fetal and Neonatal Medicine, vol 2: Retinopathy of Prematurity. Blackwell Scientific Publications, Boston, pp 97–120
14. Bill A 1984 Circulation in the eye. In: Robert B. Sperelakis N (eds) Handbook of Physiology. Williams & Wilkins, Baltimore, pp 1001–1034
15. Sisson TRC, Glauser EM, Romayananda N 1984 Effect of light and various concentrations of oxygen on the retina of the newborn pig. In: Rubatelli FF, Jori G (eds) Neonatal Jaundice: New Trends in Phototherapy. Plenum Press, New York, pp 277–290
16. Sisson TRC, Glauser SC, Glauser EM, Tasman W, Kuwabara TJ 1970 Retinal changes produced by phototherapy. Pediatrics 77:221–227
17. Stiris T, Hansen TWR, Hall C, Bratlid D 1987 The effect of light and hyperoxia on ocular and cerebral blood flow in the newborn piglet. Biol Neonate (in press)
18. Hemmingsen R, Barry DI, Hertz MM 1979 Cerebrovascular effects of central depressants: a study of nitrous oxide, halothane, pentobarbital and ethanol during normocapnia and hypercapnia in the rat. Acta Pharmacol Toxicol 45:287–295
19. Hansen NB, Brubakk AM, Bratlid D, Oh W, Stonestreet BS 1984 The effects of variations in $Paco_2$ on brain blood flow and cardiac output in the newborn piglet. Pediatr Res 11:1132–1136
20. Häggendal E, Johansson B 1965 Effects of arterial carbon dioxide tension and oxygen saturation on cerebral blood autoregulation in dogs. Acta Physiol Scand [Suppl] 258:27–53
21. Iwabuchi T, Kutsuzawa T, Kyuhei K, Nakamura T 1973 Effects of blood gases on the pressure-flow relationships in canine cerebral circulation. Stroke 4:65–72
22. Heymann MA, Bruce DP, Hoffman JIE, Rudolph AM 1977 Blood flow measurements with radionuclide-labelled particles. Prog Cardiovasc Dis 20:55–78
23. Tsacopoulos M, Noble DJ 1973 The effect of arterial Pco_2 on relative retinal blood flow in monkeys. Invest Ophthalmol 5:335–347
24. Parver LM, Auker CR, Carpenter DO, Doyle T 1982 Choroidal blood flow II: reflexive control in the monkey. Arch Ophthalmol 100:1327–1330
25. Buckberg GD, Luck JC, Payne B, Hoffman JIE, Archie JP, Fixler DE 1971 Some sources of error in measuring regional blood flow with radioactive microspheres. J Appl Physiol 31:518–604
26. Dole WP, Jackson DL, Rosenblatt JI, Thompson WL 1982 Relative error and variability in blood flow measurements with radiolabeled microspheres. Am J Physiol 243:371–378
27. Alm A, Bill A 1972 The oxygen supply to the retina, I: effects of changes in intraocular and arterial blood pressures, and arterial po_2 and pco_2 on the oxygen tension in the vitreous body of the cat. Acta Physiol Scand 84:261–274
28. Busija DW, Heistad DD 1984 Factors involved in the physiological regulation of the cerebral circulation. Rev Physiol Biochem Pharmacol 101:162–211
29. Harper AM, Glass HJ 1965 Effects of alterations in the arterial carbon dioxide tension in the blood flow through the cerebral cortex at normal and low arterial blood pressures. J Neurol Neurosurg Psychiatr 28:449–457
30. Brubakk AM, Oh W, Stonestreet BS 1987 Prolonged hypercarbia in the awake newborn piglet: effect on brain blood flow and cardiac output. Pediatr Res 1:29–33
31. Levasseur JE, Wei EP, Kontos HA, Patterson JL 1976 Responses of pial arterioles after prolonged hypercapnia and hypoxia in the awake rabbit. J Appl Physiol 46:89–95
32. Wei EP, Seelig JM, Kontos HA 1984 Comparative responses of cerebellar and cerebral arterioles to changes in $Paco_2$ in cats. Am J Physiol 246:386–388
33. Stiris T, Hall C, Bratlid D 1988 Retinal (RBF) and Choroidal (ChBF) blood flow response to hypercarbia in the spontaneously breathing and mechanically ventilated newborn piglet. Pediatr Res 23:426(abstr)
34. Peabody JL 1981 Muscle relaxants-A potential danger to infants at risk for intraventricular hemorrhage. Pediatr Res 15:456(abstr)
35. Hascoet JM, Monin P, Schaefer JL, Vert P 1988 Pancuronium (P) increases cerebral blood flow (CBF) and impair autoregulation (AR) in the newborn piglets. Pediatr Res 23(suppl):410(abstr)