Leonard Gregory Allen. BRAIN PROSTAGLANDINS IN EXPERIMENTAL PERINATAL ASPHYXIA. (Under the direction of Thomas M. Louis, Ph.D.) Department of Biology, July 1980.

The purpose of this study was to investigate the effects of asphyxia on brain levels of Prostaglandins E_2 and F_2-alpha in the two day old guinea pig. Animals were pretreated with either saline or indomethacin (a potent prostaglandin synthesis inhibitor) prior to asphyxiation in a nitrogen atmosphere. Following asphyxiation, animals were either killed immediately without being allowed to breathe or were resuscitated and killed fifteen minutes after resuscitation. Brains were quickly removed, frozen and stored for assay of prostaglandins by radioimmunoassay.

Results showed that brain levels of PGF_2-alpha were not altered by asphyxia alone, but that resuscitation after asphyxia caused a significant rise in brain PGF_2-alpha levels. Prostaglandin E_2 data showed that the longer period of asphyxia (3 min. 40 sec.) caused a rise in brain PGE_2 above control levels. Following resuscitation, however, brain PGE_2 levels were not different from control levels. Indomethacin pretreatment lowered brain levels of both prostaglandins below levels of non-asphyxiated control animals and prevented any asphyxia induced rise in brain prostaglandin levels. Inhibition of prostaglandin synthesis by indomethacin was found to have no effect on several survival parameters (time to primary apnea, time to last gasp, survival

following asphyxiation, and time to righting), but it was apparent from a small study that indomethacin pretreated animals initiate gasping sooner following asphyxia than do saline pretreated animals.

From these results, we speculate that a rise in brain PGF_2-alpha levels following asphyxia and resuscitation may be detrimental to the animal by limiting cerebral blood flow through its actions as a cerebral vasoconstrictor.

BRAIN PROSTAGLANDINS IN

EXPERIMENTAL PERINATAL ASPHYXIA

by

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INTRODUCTION

In humans, asphyxia during the perinatal period may lead to fetal death or neurologic abnormalities in the survivors. While better resuscitation techniques and improved methods to control blood pH have improved survival following perinatal asphyxia, the various factors which contribute to brain damage subsequent to asphyxia have not been fully investigated.

Prostaglandins are biologically active lipids which are formed in most tissues of the body. Several of the prostaglandins or prostaglandin-like molecules are potent vasoactive substances, capable of either dilating or constricting vessels. The response of the vasculature to prostaglandins varies according to the type of prostaglandin, species of test animal, and the region of the circulation into which the prostaglandin is introduced.

Several studies have demonstrated that brain levels of prostaglandins increase following trauma or insult to the brain. It has been suggested that an increase in prostaglandins in the cerebral circulation may lead to constriction of cerebral vessels, thereby lowering cerebral blood flow. Such constriction would reduce blood flow to areas already damaged by the trauma, thereby limiting the availability of oxygen and nutrients. The affected regions would then be more likely to be permanently damaged.

The present study was designed primarily to determine whether brain prostaglandin levels are changed by asphyxia, and secondly, whether inhibition of prostaglandin synthesis has an effect on brain damage following asphyxiation.

REVIEW OF LITERATURE

Perinatal Asphyxia - Human

Perinatal asphyxia is severe lowering of oxygen supply to the fetus in the antepartum or intrapartum periods. A number of factors (Dorand, 1979) may result in differing degrees as well as different forms of asphyxiation, with the common bond being the production of dangerously low oxygen tensions in the fetus. Maternal factors contributing to perinatal asphyxia may include hemorrage, placenta previa, abruptio placenta, pre-eclampsia, placental insufficiency and others. Fetal factors may include meconium aspiration at birth, intrauterine infection and erythroblastosis fetalis, while mechanical factors such as cord compression or compression of the head during delivery may also cause asphyxiation of the fetus. Extreme immaturity of the lungs or severe respirator distress syndrome may contribute to asphyxiation in the post-partum period. Brown et al. (1974), reporting on 94 cases of perinatal asphyxia, found 50% occurred antepartum, 40% intrapartum and 9% postpartum.

Rate of Occurrence

Thomson et al. (1977) reviewed 14980 birth records of the Royal Aberdeen Children's Hospital between the years, 1964 and 1968 and found 84 cases of severe birth asphyxia (as indicated by an Apgar score of 0 at birth, or less than 4 at 5 minutes). The rate of severe birth asphyxia was 5.6 per 1000 births. In another study, Brown et al. (1974) determined that 760 out of 14020 live born infants had suffered some degree of asphyxia. Only 83 out

of these 760 (or 5.9 per 1000 births) were thought to have suffered severe asphyxia, as determined by the existence of specific behavioral abnormalities in the early neonatal period. In another study, Scott (1976) found 48 severely asphyxiated infants out of 12389 live births for a rate of 3.9 cases per 1000 deliveries. These studies document the fact that severe perinatal asphyxia occurs quite frequently and is one of the major problems in the perinatal period.

Mortality and Morbidity Following Perinatal Asphyxia in the Human

Mortality in the neonatal period is high for infants that have suffered severe asphyxia. In Brown's study (1974), mortality rate was 21%. In the studies by Thomson et al. (1977) and Scott (1976), mortality rates were higher, 50% and 52% respectively. However, even though mortality was high for those infants with a history of severe asphyxia, a large percentage of the survivors were found to be normal on followup examination. Brown et al. (1974) followed the survivors to a mean age of 21 months and reported 46% of survivors to be normal. Another 20% were found to have minimal brain damage which has "little functional significance". Scott (1976) followed survivors for 3-7 years and found 74% to be normal at followup. Thomson et al. (1977) tested asphyxia survivors at ages 5-10 years and found 29 out of 31 children were normal at followup, while the remaining two were severely disabled. Another report by DeSouza and Richards (1978) showed that out of 53 infants with a history of fetal distress in labor, 78% were normal when examined at 2-5 years of age.

These studies indicate that severe birth asphyxia is associated with a high death rate in the neonatal period, but that most asphyxiated infants surviving the neonatal period recover to be essentially normal when examined later. The remaining survivors suffer some degree of neurologic handicap, from minor motor dysfunction and hyperactivity to severe crippling handicaps such as mental retardation, cerebral palsy and epilepsy.

Several studies emphasize the use of early neurologic signs following asphyxia to predict outcome of the infant. Studies by Brown et al. (1974) and Thomson et al. (1977) showed that a high positive correlation exists between the number of abnormal behaviors exhibited in the neonatal period (feeding difficulties, apneic spells, convulsions, etc.) and severity of handicap in later life. Using muscle tone in the newborn period as a predictor, Brown et al. (1974) and DeSouza and Richards (1978) found that infants with hypotonia or hypotonia followed by hyperexcitability have a more grave prognosis when compared to infants with hyperactivity or hypertonia alone. Scott (1976) suggests that the severity of the subsequent neurologic damage can be related to the duration and severity of stress suffered before or during birth. Acute stress is associated with a more favorable prognosis, while infants suffering prolonged stress or persistent fetal bradycardia were more likely to have severe neurologic handicaps later in life. (The significance of acute total asphyxia versus prolonged partial asphyxia will be discussed later in the present review.)

Neuropathologic Lesions in the Asphyxiated Human Infant

There are several neuropathologic lesions in the human infant which have been attributed to asphyxia. Gilles (1977) reviewed several sources and lists 33 different lesions which had, in the literature, been attributed to perinatal asphyxia.

In a review article, Volpe (1976) discussed four major types of brain lesions found in human infants subjected to perinatal asphyxia. These lesions were further divided into those which may result from hypoxemia and those resulting from ischemia. Volpe's premise was that hypoxia and ischemia may occur together in perinatal asphyxia.

Neuronal necrosis, a hypoxemic lesion, is marked by individual neuron death in various brain regions. In human infants, the cerebral cortex is the area most often associated with this lesion, but some reports have demonstrated neuronal necrosis in thalamus and brain stem.

Another hypoxemic lesion found in human infants is status marmoratus, in which the basal ganglia appears marbled. This marbled appearance is thought to be the result of "deranged" myelination in the area (Volpe, 1976). Such areas of increased myelination are regions which normally possess few myelinated fibers. Volpe further mentions that status marmoratus is related to specific neurologic problems of choreoathetosis and rigidity. This lesion occurs more often in asphyxiated full term infants than in asphyxiated premature infants.

The two ischemic lesions metioned by Volpe are watershed infarcts and periventricular leukomalacia. The proposed pathogenesis for these lesions involves systemic hypotension and a resulting decrease in blood flow to areas on the border between two vascular supply regions. This theory, termed the "border zone hypothesis", proposes that regions bordering arterial distributions are most vulnerable to damage during hypotensive ischemia. While no specific neurologic sequelae have been associated with watershed infarcts, periventricular leukomalacia (in which tissue necrosis is found in certain areas of periventricular white matter) is associated with the occurrence of spastic diplegia, a motor disturbance in which there is incomplete paralysis of the lower extremities.

Another type of lesion closely related to perinatal asphyxia in the premature infant is that of intraventricular (IVH) or periventricular (PVH) hemorrhage (bleeding into or around the ventricles). This hemorrhage does not develop immediately after the asphyxial episode, but may occur at one to two days of age (Ahmann, et al.1980). Usually the occurrence of an intraventricular hemorrhage is heralded by a rapid deterioration of the condition of the infant.

There are two theories concerning the pathogenesis of PVH or IVH. Both theories are based on asphyxia-induced alterations of cerebral blood flow and/or autoregulation. Friede (1975) argues that asphyxia causes circulatory collapse. This collapse

results in a shift of blood from the arterial side to the venous side. It is Friede's hypothesis that the increase in venous pressure which accompanies the circulatory collapse is the precipitating factor for venous hemorrhages. On the other hand, Volpe (1979) and Lou et al. (1979) suggest that the bleeding is from capillaries. Again, circulatory collapse due to asphyxia is given as a major factor leading to IVH. The cornerstone of their theory comes from a recent paper by Lou et al. (1979) in which he shows that autoregulation of cerebral blood flow is impaired in "distressed" infants. This means that cerebral blood flow varies passively with arterial pressure. Rapid increases in arterial pressure are then directed against the fragile capillaries without restriction by arterioles. Bleeding then occurs from the damaged capillary beds. Volpe (1979) further supports this notion by referring to recent data (McKay, 1973) which shows that capillaries are the source of periventricular hemorrhage in prematures.

Animal Studies of Perinatal Asphyxia

A number of species have been used to investigate the effects of asphyxia on the fetus and newborn (see Dawes, 1968). The literature also contains several different methods for producing asphyxia. These variations may make it difficult to compare among species and experimental techniques, but there are still several common responses to asphyxia which occur in most models. This review will be limited to the models which are most relevant

to the present project.

Respirator Changes in Total Asphyxia

Generally, all animals exhibit basically the same respiratory changes in response to acute total asphyxiation. Different methods of inducing total asphyxiation may either lengthen or shorten the duration of the respiratory alterations, but the pattern remains similar (Dawes, 1968).

With the onset of total asphyxiation (cutting off O_2 supply), the animal is hyperactive for a short period. After this hyperactive phase, the animal enters a brief phase known as primary apnea. During this brief period, the animal is still able to respond to tactile stimulation and may easily be resuscitated with few adverse effects. As asphyxiation proceeds, the animal begins gasping. At first, the gasps are shallow and slow, becoming deeper and more frequent until last gasp is reached. Following last gasp, the animal is said to be in secondary apnea. Unlike primary apnea, an animal does not respond to tactile stimulation whiel in secondary apnea. Vigorous resuscitation by positive pressure ventilation and cardiac massage are necessary to revive an animal following last gasp.

The time from removal of oxygen to last gasp (time to last gasp) is often used as an end point in research involving the ability of animals to survive or tolerate asphyxia. Treatments are sought which extend an animal's time to last gasp, thus possibly prolonging survival of the animal in asphyxia.

Circulatory Adaptations in Asphyxia

The near-term fetus is endowed with several cardiovascular adaptations to ensure survival in an asphyxial situation. Primarily, there is the physiological reaction of the circulatory system which maintains circulation to heart and brain. The importance of an intact circulation during asphyxia was demonstrated by LeGallois in 1812 (see Dawes, 1968). In rabbits, when circulation was interrupted by decapitation or removal of the heart, gasping movements persisted for 15 to 20 minutes respectively. However, if asphyxiation was induced by submersion in water or opening the thorax, gasping was prolonged to 27 to 30 minutes. Perhaps the intact circulation serves to remove CO_2 from brain tissues as well as supplying the brain with glucose.

Asphyxiation results in rapid and severe biochemical changes. Oxygen is rapidly depleted in the asphyxiated animal. Using fetal monkeys, both Dawes (1968) and Myers (1977) have demonstrated the rapid decrease in arterial pO_2, from preasphyxial levels of 25 mmHg down to 2-5 mm Hg in 3 to 5 minutes after oxygen removal. pCO_2 rises quickly during asphyxia. In fetal monkeys, pCO_2 rose from baseline levels of 40-60 mm Hg to 80-100 mm Hg at 5 minutes. Blood pH decreases steadily as pCO_2 rises (due to the interaction of CO_2 with water to form carbonic acid which dissociates, releasing H^+ ions). Generally, last gasp occurs when the blood pH has reached approximately 6.8-6.9 (Dawes, 1968).

It is probable that the accumulation of CO_2 and H^+ are important

triggers for the protective physiological response which occurs during asphyxiation. This cardiovascular response is best documented in large fetuses such as the lamb and the monkey, but a similar response is likely to occur in most mammalian fetuses. The purpose of this reflex is primarily to maintain circulation to heart and brain as well as conserve oxygen and glucose stores.

In fetal monkeys (Dawes, 1968), asphyxiation by cord clamping results in rapid heart rate slowing from a baseline level of around 170 beats per minute (bpm) to less than half of that rate within 1-2 min. Windle and Becker (1943) report a similar heart rate response in the fetal guinea pig, with heart rate falling from 164 bpm to half of that in 2 min. Prolonged asphyxia lowers the heart rate to around 52 bpm. Fetal lambs respond to a combination of hypoxemia-acidemia by heart rate slowing from 170 bpm to around 120 bpm (Cohn et al., 1974). In another report (Dawes, 1968), fetal lamb heart rate decreased from 200 to 100 rapidly, then, as asphyxia progressed, the rate fell still further to less than 50 bpm at around 50 min.

Systemic blood pressure (BP) increases with the onset of asphyxiation. Again, this has been demonstrated in both fetal lambs and monkeys. The rise in BP, however, appears to be slower than the decrease in heart rate.

Two studies (Behrman et al., 1970, and Cohn et al., 1974)

reported decreases in cardiac output with asphyxia or hypoxemia-acidemia. However, Behrman et al. (1970) reported that the heart rate did not change with asphyxia. He concluded that stroke volume is decreased with asphyxia. In fetal lambs, on the other hand, Cohn et al. (1974) measured stroke volume during hypoxemia-acidemia and showed a slight but non-significant increase. Therefore the decrease in cardiac output may be attributed to the heart rate slowing in the fetal lamb.

Several examples show that the fetus is pre-programmed to cope with an asphyxial episode. They are a) the high cardiac glycogen stores in the fetus, b) a great dependence on anaerobic metabolism in the fetus and c) differential maturation between parasympathetic and sympathetic systems.

Cardiac glycogen concentration has been shown to be higher in the fetus as compared to the newborn (Dawes, 1968). This is evident in several different species. Such high stores supply the heart with energy to continue to pump for long periods in the absence of oxygen. The bradycardia accompanying asphyxia combines with the large cardiac glycogen stores to further prolong survival.

Using selective metabolic blockers, Friedman and Kirkpatrick (1977) demonstrated that the fetal heart relies heavily on anaerobic glycolysis. When glycolysis is blocked by iodoacetate (inhibiting phosphofructokinase), the fetal heart will continue beating for a shorter time than the adult heart. However, when 2,4 dinitrophenol is used to uncouple electron transport and oxidative phosphorylation, the fetal heart will beat longer than

will the adult heart. These studies show that the fetal heart is able to survive longer than the adult heart using anaerobic glycolysis.

In fetal lambs, Friedman and Kirkpatrick (1977) also showed that there is an imbalance in the fetal myocardium with regard to autonomic innervation. The concentration of acetylcholine fibers in the fetal heart is quite similar to that of the adult heart. On the other hand, both norepinephrine and histochemical evidence of myocardial sympathetic fibers are greatly decreased in the fetus when compared to the adult. The fetal heart partially compensates for this imbalance by being more sensitive to norepinephrine than the adult heart. There remains, however, a parasympathetic dominance in the fetal myocardial tissue. This autonomic imbalance is consistent with the fetal bradycardic response and the diminished cardiac output during asphyxia. The vagal parasympathetic input to the SA node triggers the reflex bradycardia while the sympathetic system is unable to substantially increase the stroke volume, resulting in a reduction of cardiac output. In contrast to the heart, fetal peripheral vascular beds are highly innervated by sympathetic fibers which mediate the peripheral vasoconstriction response to increased sympathetic influence.

The probable trigger for the reflex response to asphyxia lies in increasing pCO_2 in the blood and tissues, and subsequent decrease in pH. Two possible sites of stimulation to evoke the reflex are the carotid chemoreceptors and/or the vasomotor center. Berne and Levy (1972) state that stimulation of the chemoreceptors

usually results in little alteration in the cardiovascular system. Direct stimulation of the vasomotor center (in the medulla and lower pons) is a more likely explanation. The response to asphyxia resembles the cerebral ischemic response (Berne and Levy, 1972) in which heart rate slows, there is peripheral vasoconstriction, and cardiac stroke volume increases. The stimulus for the cerebral ischemic response is initiated by local increases in pCO_2 and concentration of H^+ ions. The fetus exhibits the same cardiovascular changes seen in the cerebral ischemic response except for the increased stroke volume. This could be due to the lack of sympathetic innervation in the fetal myocardium.

During the fetal response to asphyxia, blood flow to the brain and heart is maintained (Behrman et al.,1970). Several adaptations, both intrinsic and extrinsic, insure adequate cerebral blood flow. Cerebral vessels are poorly innervated and therefore do not constrict in response to the sympathetic barrage which constricts peripheral vessels in asphyxia. Instead, cerebral vessels are mainly regulated by local levels of oxygen, H^+ ions, CO_2 and certain metabolites such as lactate and adenosine. Decreased oxygen, or increased H^+, CO_2, or lactate will cause dilatation of cerebral vessels in order to increase cerebral blood flow (Berne and Levy, 1972).

Behrman et al. (1970) measure the distribution of the circulation in asphyxiated fetal monkeys and found that blood flow to the brain, heart and adrenal glands was maintained at preasphyxial levels. With lowered cardiac output in asphyxia,

this represents a larger percentage of the cardiac output going to these organs. In contrast, during asphyxia, the percent of cardiac output to liver, kidneys, and GI tract were unchanged while the percent of cardiac output to spleen and lung were decreased in comparison to preasphyxial values. Using microspheres labelled with several different radioisotopes, Behrman et al. (1970) demonstrated two shunts which operate during asphyxia. First, blood from the superior vena cava is shunted through the foramen ovale of the heart for distribution to the brain and heart. Second, a large amount of umbilical blood bypasses the liver through the ductus venosus. These shunts direct blood away from less vital organs and send more blood to the brain and heart.

As asphyxia becomes prolonged, the cardiovascular responses described above begin to collapse. Blood pressure will decrease when the vasomotor center is depressed by prolonged asphyxia. Heart rate will slow still further as cardiac glycogen supplies are depleted. Unless oxygen is resupplied to the system, complete circulatory collapse will ensue, followed by death. If oxygen reaches the asphyxiated system in time, heart rate will rapidly increase to preasphyxial levels and blood pressure will gradually increase. Successful resuscitation depends largely upon reinstitution of circulation and oxygenation. Therefore, if a heart rate is still palpable, there is a chance that the animal may be successfully resuscitated.

Neuropathology- Animal Models

Relatively few investigators have studied the neuropathology resulting from experimental perinatal asphyxia in animal models. The major group contributing to the understanding of the neuropathology of asphyxia in recent years in the Division of Perinatal Physiology at NIH in Bethesda, Md. This group, headed by R.E. Myers, has been using the fetal Rhesus monkey for its studies. The purpose of Myers' studies has been first to develop a model in which the neuropathological damage is similar to that seen in the human. He has attacked this problem by using several different methods of inducing asphyxia. Myers is also attempting to determine which factors play the largest part in production of the specific neuropathological changes.

The second group examing neuropathology of asphyxia was headed by W.E. Windle. Windle's early work in the 1940's involved a total asphyxia model in the full term guinea pig fetus. His interests were broad in scope and led to investigations of neuropathology as well as studies of subsequent neurologic behaviors and psychological development of asphyxiated guinea pigs. In 1959, Windle (Bailey and Windle, 1959) repeated some of his early work in the guinea pig and also published the first studies using the acutely asphyxiated fetal Rhesus monkey for neuropathological studies (Ranck and Windle, 1959).

Neuropathology- Fetal Monkey

In 1959, Ranck and Windle compiled the results of studies

on 5 asphyxiated fetal monkeys and two control animals. The experimental animals were asphyxiated by placental separation, and resuscitation was initiated after 11-15 min of asphyxiation.

The animals were sacrificed at ages ranging from 2-9 days and brains were examined for neuropathologic lesions. While there was considerable variability in severity of damage among the five experimental animals, lesions were generally found in the same regions of all brains. The main finding was consistent damage to the inferior colliculi in all brains. Lesions were also found in several other brain stem nuclei, such as the superior olivary nucleus, superior and medial vestibular nucleus and the principal oculomotor nuceleu. A few higher nuclei (putamen, globus pallidus and ventral posterior nucleus) were also damaged. The cerebral cortex was spared from severe damage in most of the asphyxiated animals. A few minor hemorrhages were found, but the authors believed them to be fairly insignificant and suggested that the nuclear lesions were not related to vascular or hemorrhagic factors. However, the authors were not able to identify the primary cause of the lesions.

Myers (1977) has also experimented with total asphyxia in the fetal Rhesus monkey and demonstrated brain stem nuclear lesions similar to those reported by Ranck and Windle (1959). In these studies, the inferior colliculus was the first structure damaged by total asphyxiation. His studies indicated that fetal monkeys could be asphyxiated for 12-13 min with

virtually no damage, but that after a threshold 14 min of asphyxiation, the typical brain stem nuclear pathology developed. Certain structures were found to be more prone to damage during total asphyxia. As the duration of asphyxia was increased, the more resistant structures were also damaged. From these studies, Myers developed a ranking for vulnerability to asphyxia of certain structures in the brain. Those structures most vulnerable to damage from total asphyxia are typically structures with high rates of blood flow and metabolism. Myers (1977) points out that there are exceptions to this rule, since there are a few structures with high flow rates and high metabolism (he cites the cerebral cortex) which are not readily damaged by total asphyxia.

Based on the pathology produced by acute total asphyxia in the fetal Rhesus monkey, Myers concluded that the model may not be applicable as a model of human asphyxia. Reviewing the literature on human neuropathological findings following perinatal asphyxia, Myers (1977) found few references to brain stem nuclear pathology. Most of the papers describing the neuropathologic lesions found in asphyxiated human infants seem to indicate that the primary sites of damage were the cerebral hemispheres, and that there was little damage in the brainstem. The few references describing brain stem nuclear pathology in the human were written after such pathology had been found in animal models.

This conclusion led Myers to adopt a "partial asphyxia"

model (Myers, 1977). In this model, severe, prolonged fetal hypoxia, rather than acute asphyxia, is produced by experimentally limiting maternal blood supply to the placenta. The pathology resulting from prolonged (25-30 min) partial asphyxia is quite different from that of acute total asphyxia, with the cerebral hemispheres being the main sites of damage. Thalamus and brain stem are usually not damaged in prolonged partial asphyxia.

Myers showed that there is widespread cerebral edema and corresponding cerebral necrosis following partial asphyxia. Certain areas (in particular the paracentral area and posterior parietal cortex) show increased vulnerability. Myers has concluded that the damage is related to hypoxia rather than ischemia. This explanation differs from the border zone hypothesis of Brierley et al. (1973) which states that the damaged areas lay along boundaries between adjacent arterial distributions and that ischemia is the common denominator in causing damage. On the other hand, Myers found that he could produce the same type of lesion by perfusing a hemisphere with oxygen-deficient blood at slightly above the animal's recorded blood pressure. This would tend to dispute the ischemia model for cerebral damage.

Myers (1979) theorizes that partial asphyxia produces cerebral damage in the following way: decreased oxygen tension shuts down the tricarboxylic acid cycle so that cells must rely on anaerobic glycolysis for energy (ATP). This results in an increase in lactic acid within the cells. Increased intra-

cellular lactate is thought to be a factor in promoting brain edema by altering membrane permeability. Brain edema and the subsequent increase in intracranial pressure may limit cerebral blood flow by compression of cerebral vessels. By this model, the hemispheres would be subjected to more severe oxygen deprivation while subcortical structures are less vulnerable.

Neuropathology - Guinea Pigs

Two other studies on the neuropathology of experimental perinatal asphyxia employed an acute total asphyxia model in the full term fetal guinea pig (Windle et al., 1944; Bailey and Windle, 1959). These studies involved production of fetal asphyxia by completely occluding either the maternal uterine artery or the umbilical cord. This technique subjected the fetus to acute total asphyxia, unlike the partial asphyxia described by Myers.

In the first study (Windle et al., 1944), asphyxiated guinea pigs were sacrificed at intervals between one day and 13 weeks after asphyxia. Asphyxia produced a variety of neuropathological lesions in these animals. Those animals that were exposed to longer asphyxia or were difficult to resuscitate were found to have move severe pathology. The different types of pathology included small capillary hemorrhages (especially in the thalamus and brain stem), cerebral edema, various cytologic changes (most often in the thalmus, cerebral cortex, tegmentum and spinal cord), microglia reaction (beginning at 2.5 days) and cerebral atrophy (in the more severely asphyxiated animals).

Brains from all asphyxiated animals that were examined between 1.5 hours and three weeks showed neuropathological damage, with most of the damage being apparent between a few hours and about 5 days after asphyxiation. As animals aged, however, it was apparent that some repair took place since less than half of the animals which were examined three weeks after asphyxia showed no pathology.

In a second guinea pig study on neuropathology following asphyxia (Bailey and Windle, 1959), asphyxiation was produced by clamping blood vessels to the uterus for 10-19 min. Fetuses were then removed and resuscitated by a procedure that had been described in earlier work (Windle et al., 1944). Histological examination of the brains from these asphyxiated animals revealed lesions which were similar to, but less severe than the lesions found in the 1944 study. In the second study, fewer hemorrhages were found and cytologic changes were found mainly in the thalamus and brain stem.

Other Guinea Pig Studies

Fetal or newborn guinea pigs have been used to investigate several other aspects of the response to asphyxia. The most extensive work was done by W. F. Windle and his collaborators (Windle and Becker, 1943; Bailey and Windle, 1959). Windle's work was an attempt to evaluate the overall status of the nervous system after exposure to intrauterine asphyxiation. His studies and those by his colleagues involved observations of outward signs of neurologic damage damage and psychological tests of

learning and behavior in addition to the previously mentioned neuropathology.

In 1943, Windle and Becker reported their first intrauterine total asphyxia study using the fetal guinea pig. They showed that immediately following asphyxia, all animals showed weakness and tremors. Animals were also insensitive to changes in their environment. In a number of animals, these neurologic signs diminished after a brief period, but in other animals neurologic symptoms persisted. These symptoms included numerous motor deficits such as loss of control or paralysis of the hindlimbs. Other animals had decerebrate rigidity, seizure-like activity, paralysis of various muscle groups, and altered sensitivity to the environment (either hyper- or hyposensitive). These severe symptoms usually disappeared after a few days or a week.

The experimental animals were also tested for learning and behavior deficits by maze and alternation testing. Asphyxiated animals were more prone to make errors and to repeat those errors than were the controls. Their behavior was therefore more rigid and stereotyped.

Bailey and Windle reported the results of a similar study in 1959 (Bailey and Windle, 1959). In this study, the authors attempted to relate the degree of asphyxiation to the severity of subsequent neurologic deficits. They found several high correlations between measures of the degree of asphyxiation (length of asphyxiation and time until first breath) and several

indices of immediate neurologic deficits (degree of spasticity, muscle activity, tremor frequency and time of righting). However, the only statistically significant correlation was between time to first breath upon resuscitation and time until righting.

For psychological tests, Bailey and Windle repeated several tests used by Becker and Donnell (1952) and found in several instances that their results were either less definitive or not in agreement with those reported by Becker and Donnell. Nevertheless, the results showed essentially the same pattern of impaired learning in the asphyxiated animals. There is apparently some repair in learning deficit because animals show more severe deficits when tested sooner after asphyxia than when tested later on.

Another investigator to study total asphyxia in the neonatal guinea pig was J. A. Miller. The focus of his research involved asphyxiation of one day old guinea pigs in 95% N_2, 5% CO_2 atmosphere. Miller's work (Miller, 1949; Miller and Miller, 1969; Miller, 1971) centered around the use of hypothermia as a treatment to prolong survival during asphyxia. He found that many animals have optimum body temperatures at which gasping is prolonged. However, extremely higher or lower temperatures decrease the time to last gasp. This hypothermia effect is less apparent in animals which are more mature at birth (i.e. guinea pigs and piglets). Guinea pigs cooled to 5-7 degrees below normal body temperature gasp 1.5

times as long as normothermic controls. The newborn guinea pig's time to last gasp in 95% N_2, 5% CO_2 is given as 3 min 30 sec at a normal body temperature. At 5-7 degrees below normal temperature, time to last gasp is increased to 5 min 13 sec (Miller and Miller, 1962). Of the species tested, the puppy shows the most extreme prolongation of time to last gasp with cooling. At a normal body temperature of 37° C, puppies gasp for around 12 minutes, but at a body temperature of 15° C, they will gasp for 105 minutes, an increase of 775% (Miller and Miller, 1969).

Severe cold is detrimental to the animal, probably by inducing vasoconstriction. Some of the detrimental effects of cold can be overcome by either pretreating the animals with a sedative (such as sodium pentobarbital) or by cooling the animals in a hypoxic-hypercapnic (10% O_2, 5% CO_2, 85% N_2) atmosphere. Studies in which newborn guinea pigs were sedated before cooling showed that the sedation effect was more prominent at lower temperatures (Miller and Miller, 1962). For example, sedated animals cooled to 15° C gasped twice as long as their non-sedated counterparts. However, at a body temperature of 40° C, sedation caused only a 17% increase in time to last gasp. Miller et al. (1964) also showed that hypoxia-hypercapnia during cooling in the guinea pigs may increase the time of gasping from 34-48% (in those animals asphyxiated at 15-25° C body temperature).

One key to prolonged survival in asphyxia may be a large cardiac storage of glycogen which would supply the myocardium

with energy in order to maintain the circulation (Dawes, 1968). Again using one day old guinea pigs asphyxiated in 95% N_2, 5% CO_2, Miller determined that cooling prior to asphyxiation resulted in essentially no depletion of cardiac glycogen during asphyxiation (when the cooled animals were killed at the time of last gasp for normothermic animals) (Miller et al., 1964). However, in adult guinea pigs, pre-cooling in a hypoxic-hypercapnic atmosphere resulted in a substantial decrease in cardiac glycogen content, but still substantially prolonged gasping. This observation, as well as the observation that newborn guinea pigs have less cardiac glycogen but a longer survival in asphyxia than adults has led to speculation that cardiac glycogen is not as important a factor in neonatal survival in asphyxia as had been thought (Miller et al., 1964).

Prostaglandins

Prostaglandins are a family of biologically active lipids derived from arachidonic acid, a 20 carbon fatty acid with four double bonds. These substances are synthesized in all tissues of the body and have diverse actions on many different systems (see Vapaatalo and Parantainen, 1978). Briefly, various prostaglandin compounds have effects on: release of norepinephrine from adrenergic nerve terminals, stimulating or inhibiting cAMP formation in certain tissues, constricting or dilating blood vessels, maintaining the patency of the fetal ductus arteriosus, kidney function, gastric secretion, platelet aggregation, bronchial dilation, fever, threshold of epileptic seizures, luteolysis, parturition and many other

systems. While pharmacological effects have been reported for certain prostaglandins, their roles in normal physiological function are unclear in many cases. Since prostaglandin actions are quite broad in scope, this review will be limited to a brief overview of advances in prostaglandin research, prostaglandin nomenclature, synthesis and metabolism (general and in brain), and effects on cerebral vasculature.

Historical Overview

The term "prostaglandins" was coined by U. S. von Euler in the early 1930's to describe an extract from semen which had the ability to contract uterine smooth muscle. The term is actually a misnomer, for the source of the smooth muscle contracting substance is not the prostate gland but the seminal vesicles.

Few new advances in research on prostaglandins were made until the early 1960's when Bergstrom and his colleagues published several important findings. In 1960, they isolated two primary prostaglandins, PGE_1 and PGF_2-alpha (Bergstrom and Sjovall, 1960a and 1960b). The structures of these compounds were revealed in 1962 (Bergstrom et al., 1962). In 1964, Bergstrom's group first demonstrated that PGE_2 was synthesized from arachidonic acid (Bergstrom et al., 1964).

Several other major advances were made in the 1970's. In 1971, Vane's group in England showed that synthesis of prostaglandins could be inhibited with anti-inflammatory analgesics such as aspirin and indomethacin (Vane, 1971). A major step in defining the prostaglandin synthesis pathway was made

in 1973 when the intermediate endoperoxide, PGH_2, was isolated. The three most recent advances in prostaglandin biochemistry were the discoveries of several new prostaglandin-like compounds: thromboxanes (Hamberg et al., 1975), prostacyclin (Johnson et al., 1976), and leukotriene (Borgeat and Samuelsson, 1979). While much work has been done on thromboxane and prostacyclin, little is presently known about leukotriene.

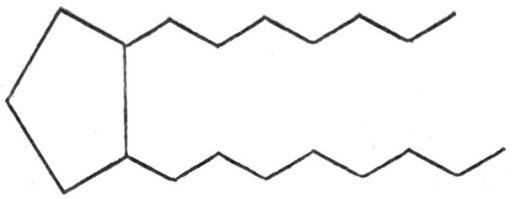
Nomenclature

The backbone for the primary prostaglandins is the hypothetical structure prostanoic acid (Fig. 1), a 20 carbon fatty acid with a cyclopentane ring between carbons 8 and 12. Primary prostaglandin nomenclature (Fig. 1) is based on 1) keto or hydroxyl substitutions on the ring structure (at carbons 9 and 11) 2) number of double bonds in the fatty acid backbone, and 3) (in the F group) the orientation of the hydroxyl group at carbon 9. E-type prostaglandins have a keto group at carbon 9 and hydroxyl group at carbon 11. F prostaglandins, on the other hand, have hydroxyl groups at both carbons 9 and 11. A, B and C prostaglandins have only a keto group at carbon 9. The difference between A, B and C prostaglandins lies in the location of the double bond in the cyclopentane ring structure. The D prostaglandins have a hydroxyl group at carbon 9 and a keto group at carbon 11.

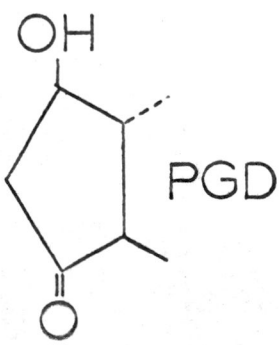
The subscript 1, 2 or 3 following the identifying letter denotes the number of double bonds in the fatty acid backbone structure. Most of the primary prostaglandins have two

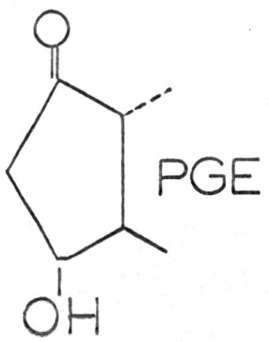
Figure 1. Prostaglandin nomenclature: Prostanoic acid and ring substitutions of the prostaglandins.

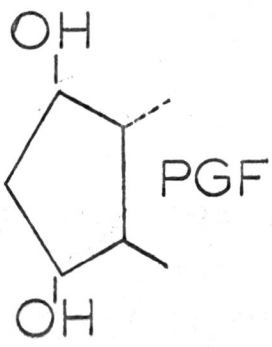
PROSTAGLANDIN NOMENCLATURE

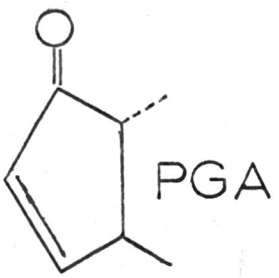


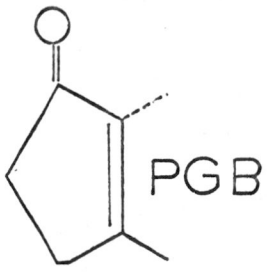
PROSTANOIC ACID

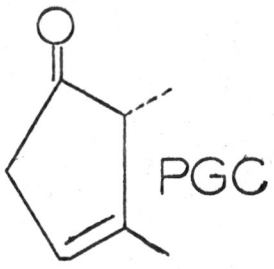


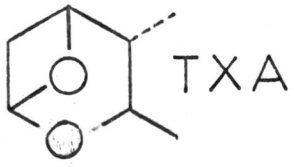


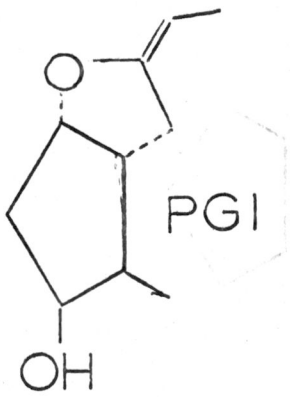












double bonds (at carbons 5 and 13). Two other characteristic features of all prostaglandins are 1) a hydroxyl group at carbon 15 and 2) a double bond between carbons 13 and 14.

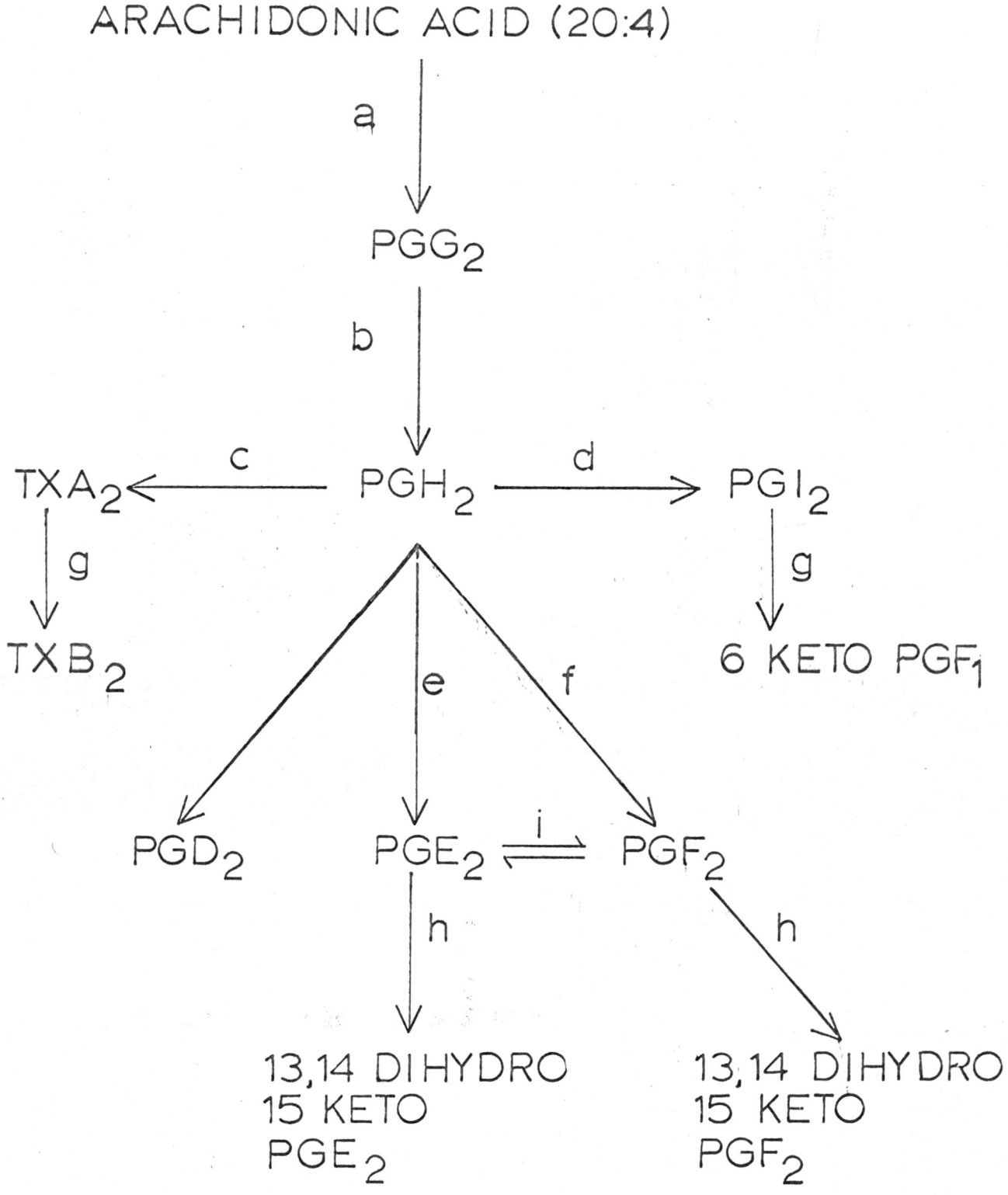
Two members of the prostaglandin family, prostacyclin (PGI_2) and thromboxane (TXA_2), have structures which are somewhat different from that of the "classic" prostaglandins but they are still included under the designation prostaglandin. The major difference between these molecules and the classic prostaglandins is that the oxygen atom at the C-9 position is involved in an oxygen bridge. Prostacyclin (or PGI_2) is similar in ways to the other prostaglandins except that the oxygen atom in the 9 position forms an oxygen bridge between carbons 9 and 6. Thromboxane A_2 has two oxygen bridges in the cyclopentane structure. One bridge is between C-9 and C-11 and the other is from C-11 and C-12.

General Prostaglandin Biosynthesis And Metabolism

The main substrate for the synthesis of prostaglandins, prostacyclin and thromboxane is arachidonic acid, a 20 carbon fatty acid with double bonds at positions 5, 8, 11 and 14. Arachidonate is typically bound at the second position in membrane phospholipids, and activation of a phospholipase is necessary to free the substrate for conversion to prostaglandins.

Figure 2 shows several of the main steps in biosynthesis and breakdown of prostaglandins. The first step in the bio-conversion of arachidonate into prostaglandins involves the enzyme complex "prostaglandin endoperoxide synthase" (other-

Figure 2. General Biosynthesis and Metabolism of Prostaglandins. Reactions: a) Prostaglandin Endoperoxide Synthetase (Cyclo-oxygenase reaction) b) Prostaglandin Endoperoxide Synthetase (Peroxidase reaction) c) Thromboxane Synthetase d) Prostacyclin Synthetase e) Isomerase f) Reductase g) Hydrolysis h) Prostaglandin 15-dehydrogenase i) 9-keto reductase


ARACHIDONIC ACID (20:4)
a
PGG2
b
c
TXA2
PGH2
d
PGI2
g
g
TXB2
6 KETO PGF1
e
f
PGD2
PGE2
i
PGF2
h
h
13,14 DIHYDRO
15 KETO
PGE2
13,14 DIHYDRO
15 KETO
PGF2

wise known as prostaglandin synthetase). This enzyme is made of 2 subunits of equal size and has a total molecular weight of about 129,000. The enzyme catalyzes two steps in the formation of prostaglandins. The first step, cyclooxygenation, involves 1) formation of the cyclopentane ring by development of a bond between C-8 and C-12 and also 2) addition of two oxygen molecules to the structure. Two oxygen molecules and a heme group are necessary for this step. One oxygen molecule adds between C-9 and C-11 while the other forms a peroxide linkage at C-15, thus forming the prostaglandin endoperoxide PGG_2. This cyclooxygenation step may be inhibited by aspirin or indomethacin. Inhibition by indomethacin is irreversible and competitive (Flower and Vane, 1974).

In the second step catalyzed by prostaglandin endoperoxide synthase, the peroxide group at C-15 is altered, leaving a hydroxyl group. This reaction requires a heme group and tryptophan as cofactors and is not inhibited by indomethacin. The product formed is PGH_2, an intermediate from which several different prostaglandins may be formed.

Five different enzyme systems compete for PGH_2. These are: 1) an isomerase which forms E-type prostaglandins, 2) a reductase which forms F prostaglandins, 3) an isomerase responsible for formation of D prostaglandins, 4) prostacyclin synthetase and 5) thromboxane synthetase. The A, B and C prostaglandins are thought to be formed from PGE_2 after removal of the hydroxyl group at C-11. There is some doubt about the exist-

ence of a reductase system which converts PGH_2 into PGF. However, the existence of a 9-keto reductase which converts PGE into PGF has been demonstrated in several tissues.

Inactivation of the primary prostaglandins (A, B, C, D, E and F) is accomplished by a specific enzyme system. This enzyme system is probably present in all tissues, but is very active in some (such as lung and kidney) and less active in other tissues (brain). The two steps involved in the enzymatic inactivation of primary prostaglandins are 1) reduction of the 13-14 double bond and 2) oxidation of the C-15 hydroxyl group to a ketone. These steps result in the formation of a 13, 14 dihydro, 15 keto prostaglandin which is much less biologically active than the primary prostaglandin. Further metabolism of the molecule can occur by beta oxidation at the carboxyl end or omega oxidation at the aliphatic end. Prostacyclin and thromboxane undergo hydrolysis rather than enzymatic inactivation. The metabolites of these molecules are 6-keto PGF_1-alpha and thromboxane B_2.

Biosynthesis And Metabolism Of Prostaglandins In Brain

Several studies have documented the synthesis and breakdown of prostaglandins in brain tissue and in cerebral vessels. Early studies by Leslie (1976), Wolfe et al. (1976 and 1978), and Pace-Asciak et al. (1976) concentrated on enzyme activity in whole brain homogenates or slices, while more recent work by Abdel-Halim et al. (1980) and Hagen et al. (1979) look also at enzymatic activity of cerebral vessels.

In 1976, Leslie incubated rat brain slices at 37° C for 60-90 min and demonstrated PGE and PGF_2-alpha formation. She also found that brain prostaglandin levels were increased by incubation with norepinephrine, epinephrine, dopamine, serotonin and tryptamine. While there was little evidence of a 15 hydroxy dehydrogenase system for catabolism of prostaglandins, she found 9-keto reductase activity which converts PGE_2 into PGF_2-alpha. Wolfe et al. (1976) also studied brain prostaglandin synthesis and breakdown in the rat brain and confirmed many of Leslie's observations. In his study, brain prostaglandin synthesis was primarily directed towards PGF_2-alpha. Synthesis was stimulated by norepinephrine, dopamine, and adrenochrome. One interesting finding was that addition of labelled arachidonate to the brain homogenate resulted in very little labelled prostaglandins being formed.

Catabolic enzymes were also studied, and the 15 hydroxy dehydrogenase activity was again found to be minimal. However, when the homogenate was incubated with labelled PGE_2, some labelled PGF_2-alpha was formed, confirming the existence of an active 9-keto reductase system in the brain.

Pace-Asciak (1976) studied prostaglandin pathways in the brain. In order to bypass problems encountered when incubating the brain with labelled arachidonate, he added labelled endoperoxides (either PGG_2 or PGH_2) to the rat brain homogenate and demonstrated the formation of PGD_2, PGE_2, PGF_2-alpha,

6-keto PGF_1-alpha and another oxidized fatty acid, 12 hydroxy heptadecatrienoic acid (Pace-Asciak and Nashat, 1976). In another study, Pace-Asciak examined the development of the PGE_2 and PGF_2-alpha synthesis and PGF_2-alpha breakdown during gestation and in early neonatal life in the sheep brain (Pace-Asciak and Rangaraj, 1976). His results showed synthesis of PGE_2 and PGF_2-alpha increasing with gestational age in the whole brain homogenates, On the other hand, PGF_2-alpha catabolic enzymes were quite active early in gestation, but decreased through gestation, until activity was negligible in neonatal life. Pace-Asciak concluded that the high activity of 15 hydroxy dehydrogenase early in gestation was to protect the developing fetal brain against possible harmful effects of circulating prostaglandins. He noted that the decline in activity of this catabolic enzyme system coincides with the development of the protective blood-brain barrier.

Abdel-Halim et al. (1980) studied prostaglandin synthesis profiles in mouse, rat and rabbit brain homogenates and also in rat cerebral blood vessels. In rats and mice, brain homogenates formed high levels of PGD_2. PGF_2-alpha and PGE_2 were also formed, but very little 6-keto PGF_1-alpha (metabolite of prostacyclin) synthesis could be demonstrated. In rabbit brain homogenates, PGE_2 and PGF_2-alpha were the dominant prostaglandins formed, followed by PGD_2 and virtually no 6-keto PGF_1-alpha production. However, in rat cerebral

blood vessels, 6-keto PGF_1-alpha was by far the major prostaglandin synthesized. Some PGF_2-alpha was produced, but there was no measurable PGD_2 or PGE_2 produced by rat cerebral vessels. The study also looked at catabolism of PGE_2 and PGF_2-alpha. No breakdown into the 13, 14 dihydro 15 keto derivatives could be demonstrated for either prostaglandin.

In 1979, Hagen et al. investigated the ability of bovine cerebral arteries to synthesize prostaglandins and thromboxanes. He observed the rate of formation of endogenous PGE_2 and PGF_2-alpha as well as the relative amounts of each prostaglandin formed following addition of labelled arachidonate. When labelled arachidonate was added to the culture, PGE_2, PGF_2-alpha, PGD_2 and 6-keto PGF_1-alpha were formed in fairly equal proportions. A small amount of TXB_2 was formed, but may represent a contribution by platelets rather than the vessel itself.

These studies tend to indicate that brain tissue has the enzyme systems necessary to synthesize several types of prostaglandins. The predominant type formed, however, may depend on animal species tested. It is interesting that several investigators found that addition of labelled exogenous arachidonate to brain slices or homogenates does not result in formation of labelled prostaglandins. The possible explanations for this are 1) that the pool of endogenous arachidonate released from membrane phospholipids post-mortem may be large, diluting out any exogenous,

labelled substrate or 2) that the cyclo-oxygenase enzyme somehow "prefers" endogenous arachidonate by being closely tied in with the phospholipase enzyme which cleaves arachidonate from membrane phospholipids. There is some evidence supporting the latter condition, for if labelled arachidonate is allowed to become incorporated into membrane phospholipids, labelled prostaglandins are formed.

The brain has very little prostaglandin catabolic activity. Such activity is present early in gestation but decreases toward term, and is virtually non-existent in the adult. Several studies demonstrated a 9-keto reductase enzyme in brain tissue. This enzyme is responsible for conversion of PGE_2 into PGF_2-alpha. Since brain tissue has no system for catabolizing prostaglandins, another method of preventing accumulation must be used. In 1976, Bito et al. demonstrated a facilitated transport mechanism for movement of prostaglandins across the blood-CSF and blood-brain barriers. This clears prostaglandins into the circulation for inactivation by organs with high catabolic activity, such as the lung.

Prostaglandin Actions On Cerebral Vasculature

Most of the studies concerning the effects of prostaglandins on cerebral vasculature and cerebral blood flow are undertaken primarily to determine the contribution of prostaglandins to specific pathological processes such as cerebral vasospasm or ischemia. Works by Denton et al.

(1972), Pennink et al. (1972), LaTorre et al. (1974) and Jarman et al. (1979) studied possible roles of various prostaglandins in cerebral vasospasm which may accompany trauma or hemorrhage. Pennink et al. (1972) experimentally produced cerebral hemorrhage in the dog by injecting blood into the chiasmatic cistern. When blood is mixed with PGF_2-alpha, the incidence of vasospasm (as determined by angiography) was increased significantly over the incidence of vasospasm associated with the injection of blood alone. Also, the injection of a mixture of cerebro-spinal fluid (CSF) and PGF_2-alpha caused vasospasm in 3 out of 3 cases, while PGE_1 did not cause vasospasm in four trials. Denton et al. (1972) studied the effects of PGE_1, PGA_1 and PGF_2-alpha on the cerebral circulation in dogs and monkeys as part of an assessment of several pharmacologic agents which may be responsible for cerebral vasospasm. When infused into the carotid artery, PGE_1 reduced cerebrovascular tone in the dog, but not in the monkey. Carotid infusion of PGA_1 had no effect on the cerebral tone of either species, but infusion of PGF_2-alpha caused potent vasoconstriction of cerebral vessels in both dogs and monkeys.

In 1974, LaTorre studied the problem of a PGF_2-alpha in cerebral vasospasm by measuring PGF_2-alpha in the CSF of 15 patients without neurologic disease and also in 8 patients with subarachnoid hemorrhage (SAH). Patients with SAH had significantly greater concentrations of PGF_2-alpha in the CSF than did patients without neurologic disease (655 pg/m)

vs 37 pg/ml). However, in those patients with SAH, there was no correlation between PGF_2-alpha level in the CSF and presence of vasospasm, as determined by angiography. While no significant relationship was demonstrated between higher PGF_2-alpha levels in the CSF and vasospasm, this study documents higher PGF_2-alpha levels in the CSF of patients with central neurologic disease, a trend which has been reported by other researchers.

More recently, Jarman et al.(1979), also investigating vasospasm, looked at the effects of prostacyclin (PGI_2) and prostaglandin endoperoxide (PGH_2) on baboon cerebral vessels both in vitro and in vivo. In vitro experiments showed that PGH_2 caused a triphasic response consisting of an initial rapid contraction, followed by relaxation, then a slower, more prolonged contraction. One in vivo experiment revealed that intra-arterial PGH_2 had no effect on the caliber of cerebral vessels.

In isolated cerebral arteries, PGI_2 had a dose dependent effect, with low doses relaxing the vessels while higher doses produced contractions. The response of cerebral vessels to infusion of PGI_2 was dependent on the site of infusion. Intra-arterial PGI_2 caused systemic hypotension and bradycardia but did not alter the caliber of cerebral vessels. When given IV, PGI_2 dilated intracranial vessels and constricted the one extracranial vessel that was measured. Jarman speculated that defective prostacyclin synthetase

systems in cerebral vessel walls may contribute to the development of vasospasm.

Several other studies focused on the role of prostaglandins in stroke or cerebral ischemia. Using a cat model for cerebral infarction, Black et al. (1979) showed that animals treated with dietary fish oil prior to experimental infarction showed less severe neurologic deficits and smaller cerebral infarctions than controls. Black speculated that this was due to competitive inhibition of the cyclooxygenase enzyme by certain metabolic products of fish oil. Cyclooxygenase inhibition prevents synthesis of prostaglandins. Though prostaglandin levels were not measured in the study, Black speculates that high levels of constrictor prostaglandins contribute to the narrowing of vascular channels which occurs in stroke and restrics cerebral blood flow. Therefore, competitive blocking of prostaglandin synthesis by fatty acid metabolites of fish oil would prevent the prostaglandin-induced constriction of cerebral vessels and allow better perfusion of brain tissue.

Spagnuolo et al. (1979) measured brain levels of constrictor prostaglandins (PGF_2-alpha and TXB_2) during cerebral ischemia (occlusion of common carotid arteries) and following decapitation. He found that during ischemia, brain prostaglandin levels did not rise above control levels, but that after 5 minutes of decapitation-induced ischemia, brain prostaglandin levels were substantially increased. He concluded that the different prostaglandin responses

between the two ischemia models were due the stimulation of central nervous system pathways with decapitation. This stimulation probably does not occur with occlusion of the carotid arteries. During the ischemic period produced by occlusion of cerebral blood flow, brain arachidonate levels increase, but there was no increase in prostaglandin levels. The authors suggested that allowing reperfusion after occlusion may be necessary for prostaglandin synthesis.

The effects of brain ischemia followed by reperfusion on brain prostaglandin levels were investigated in 1979 by Gaudet and Levine. In their study, experimental cerebral infarction was produced in the Mongolian gerbil by temporary occlusion of the common carotid artery for up to two hours. Following the period of ischemia, the occlusion was removed and brain reperfusion was allowed. Brain levels of PGE_2, PGF_2-alpha and PGFM (metabolite of PGF_2-alpha) were measured by radioimmunoassay. During the period of occlusion-induced cerebral ischemia, brain prostaglandin levels did not change from pre-ischemia control levels. However, upon reperfusion, levels of all three prostaglandins increased dramatically. This prostaglandin increase was a transient pulse, with levels returning to control values at two hours after onset of reperfusion. Pretreatment of the experimental animals with the cyclooxygenase inhibitor indomethacin successfully prevented the reperfusion-induced rise in brain prostaglandins. The animals pretreated with indomethacin were more active

during the reperfusion period than were saline pretreated animals. This evidence demonstrates that inhibition of prostaglandin synthesis may improve the neurologic status of the animal during the immediate reperfusion period.

Gaudet and Levine speculated that the reperfusion-induced rise in prostaglandins is due to the re-introduction of oxygen into a system which has a high level of the prostaglandin substrate, arachidonic acid, and an absolute requirement for oxygen. They also speculate that the rise in prostaglandins may be detrimental to the animal by restricting cerebral blood flow and by causing behavioral depression.

Further evidence that prostaglandins may be involved in the impairment of post-ischemic brain perfusion is given by Furlow and Hallenbeck (1978) and Hallenbeck and Furlow (1979). In these studies, dogs were subjected to cerebral ischemia followed by a period of reperfusion. Following 30 min of reperfusion, regional cerebral blood flow was measured by ^{14}C antipyrine autoradiography. The data indicated that the administration of indomethacin prior to ischemia, or a combination of indomethacin and prostacyclin after ischemia significantly improved cerebral blood flow during the post-ischemic period. Neither indomethacin nor prostacyclin alone had an effect on impaired cerebral blood flow when given after ischemia. Based on these results, the authors speculate that an imbalance in prostaglandin pathways at the blood-endothelial interface may be a major factor in post-ischemic impairment of brain reperfusion.

Summary

Many of the aforementioned studies demonstrated that prostaglandins are rapidly synthesized following insults to the brain such as ischemia. This increased synthesis is highly dependent on the availability of oxygen and arachidonate to the cyclooxygenase enzyme system. There is concern that the prostaglandin release which results from cerebral insult may also be instrumental in the initiation of further pathologic processes associated with prostaglandin induced restriction of cerebral blood flow. A large, sudden increase in brain levels of vasoconstrictor prostaglandins could conceivably produce widespread cerebral vasoconstriction, limiting the availability of oxygen and other energy substrates to areas which may already be damaged by tissue anoxia. This might also prevent adequate washout of CO_2 and other harmful metabolites such as lactic acid.

The study described in this thesis is the preliminary investigation into the postulated role of prostaglandins in brain damage following acute total asphyxiation of the newborn guinea pig.

MATERIALS AND METHODS

Experimental Objectives

There were two basic objectives of this preliminary study:

1) To determine if brain levels of PGE_2 and PGF_2-alpha are changed following asphyxiation induced by exposure to a nitrogen atmosphere.

2) To determine if pretreatment with the prostaglandin synthesis blocker indomethacin has any effect on prostaglandin levels following asphyxiation or survival during asphyxia.

Animals and Pretreatment

Two day old Fort Dietrick Duncan-Hartley guinea pigs (Dutchland Labs, Denver, Pa.) were used in the study. Litters of five newborns with dams were shipped from the supplier after birth on Monday afternoons. The animals arrived on Tuesdays and were kept with their mothers until a short time before testing on Wednesday afternoon.

Prior to testing, all newborns were grouped together, then randomly divided into two groups. One group (Saline) received 0.5 ml of phosphate-buffered saline (PBS) i.p. and the other group (Indomethacin) received 0.5 mg of indomethacin (Sigma Chemicals) in 0.5 ml of PBS. The indomethacin was dissolved by heating in pH7.4 PBS. Solutions were stored at 4 degrees C before use.

Experimental Procedure

The basic design for this experiment is shown in Table 1.

Thirty minutes after pretreatment with either PBS or indomethacin, animals were either used as controls or were asphyxiated by placing the animal in an incubator through which nitrogen was continually

TABLE I. Experimental Design.

		3 Min. Asphyxia		3 Min 40 Sec Asphyxia	
	Non-Asphyxiated	Non-Resuscitate	Resuscitate	Non-Resuscitate	Resuscitate
Saline Pretreated					
Indomethacin Pretreated					

flushed. Asphyxiated animals were kept in the nitrogen atmosphere for either of two times; 3 min (which is a literature value for time to last gasp of neonatal guinea pigs in nitrogen, Dawes, 1968) or 3 min 40 sec. Upon removal from the nitrogen, asphyxiated newborns were either sacrificed immediately without being allowed to breathe, or were resuscitated by positive pressure ventilation with 95% O_2, 5% CO_2 and closed chest cardiac massage. Resuscitated animals were killed 15 minutes after removal from the nitrogen.

Animals were killed by decapitation. Brains were quickly removed and frozen in liquid nitrogen in order to prevent excess post-mortem PG synthesis. While still forzen, tissue from two regions (cerebral cortex and cerebellar-colliculi areas) was taken and stored at -70 degrees C until assay of PGE_2 and PGF_2-alpha by radioimmunoassay.

In asphyxiation procedures, times to onset of primary apnea and last gasp were recorded. In a few later experiments, time to first gasp upon resuscitation and time until righting were also recorded. One small group of animals (5 indomethacin pretreated and 4 saline pretreated) was asphyxiated for 3 min 40 sec, resuscitated, then sacrificed one day later. Brains were removed, frozen, then stored for prostaglandin assay.

RIA method - extraction procedure

The technique for extraction and purification of samples for assay was similar to that of Gaudet and Levine (1979).

Brain samples (cortex and cerebellar-colliculi regions) were weighed, then freeze dried to a constant weight and reweighed.

The weight difference was used to compute water content. Following lyophilization, samples were homogenized in 2.5 ml of cold 100% ethanol using a Brinkmann polytron. The homogenate was centrifuged for 15 min at 2500 rpm and the supernatant, (containing the lipid fraction) was removed.

The ethanol was dried completely under nitrogen and one ml of PBS was added to each tube. After vortexing, approximately 1500 cpm of tritiated PGE_2 or PGF_2-alpha were added to the samples. This small amount of radioactivity was used to monitor recoveries after extraction procedures. After 30 min, 3.5 ml of petroleum ether was added to the samples and vortexed for 10 min to extract neutral lipids. After extraction, samples were centrifuged for 10 min at 2000 rpm and the supernatant was discarded. The lower phase (containing the prostaglandins) was then acidified to pH 3 or 4 using 200 ul of 2 M citric acid. 3 ml of ethyl acetate was added and vortexted for 15 min. Samples were then centrifuged and the supernatant was saved. The ethyl acetate step was repeated and the two supernatants were combined. This was dried down completely under nitrogen and warm air. The extract was then picked up in 0.5 ml of PBS. Of this 0.5 ml, 50 ul was taken for recovery estimation, 250 ul was used for PGE_2 assay and 200 ul was used for PGF_2-alpha assay.

RIA method - PG Assays

Standard curves of each prostaglandin ranged from 0 to 1000 picograms (0, 10, 25, 100, 250, 500, and 1000 pg). Prostaglandin standards (Upjohn Co., Klalmazoo, Michigan) were dissolved in 100% ethanol and stored at -20 degrees C.

Antibody for the PGE_2 radioimmunoassay (Institute Pasteur, Paris, France) was used at a dilution of 1:1250, while the PGF_2-alpha antibody (Upjohn Co.) was used at a final dilution of 1:1350. The assay procedure consisted of addition of 100 ul of appropriately diluted antibody to the samples and standard curves. This was vortexed briefly and allowed to equilibrate for 15-20 min. 8-9,000 cpm of tritiated prostaglandin in 100 ul PBS was then added to each tube. The mixture was vortexed again and incubated overnight at 4 degrees C.

Separation of antibody-bound and free radioactivity was accomplished by addition of 0.5 ml of dextran-coated charcoal. This mixture was vortexed and placed in the cold for 7 min. The mixture was then centrifuged for 10 min. at 2500 rpm. The supernatant containing the bound fraction was poured into scintillation vials, 4 ml of scintillation fluid (Scintiverse) was added and the samples were then counted in a Beckman liquid scintillation counter.

Standard curves were calculated and plotted on semilog paper as percent bound radioactivity vs. prostaglandin concentration (in picograms). Prostaglandin levels in samples were read from the standard curves and were corrected for 1) procedural losses from extraction, 2) percent of sample assayed, and 3) lyophilized weight of tissue. Final values are expressed in nanograms per gram dry weight.

Statistical Analysis of Data

For statistical analysis of the prostaglandin data, values for two brain regions were combined so that each animal had two

replicates. Therefore, if ten animals comprised a group, there would be 20 observations, unless the group had missing data. Prostaglandin data was analyzed by a one way analysis of variance for 10 groups, with each cell in the experimental design representing one group. Prior to analysis, an F_{max} test was applied to the data to determine if the variance was homogeneous. F_{max} for the PGF_2- alpha data was 330.26 and for the PGE_2 data, F_{max} was 471.03, indicating heterogeneous variance for both sets of data. A $\log_{10}$ transformation was then applied to the data. The F_{max} test was repeated on the transformed data and gave values of 1.51 for the PGF_2-alpha data and 2.577 for the PGE_2 data. The critical value for F_{max} with degrees of freedom of 10 and 20 at a 0.05 level was 4.37. This indicates that the $\log_{10}$ transformation had corrected the heterogeneity of variance problem. The transformed data was then analyzed by a one way analysis of variance for 10 groups. A Student Newman-Keuls procedure was used to test for significance of difference between individual groups.

Chi-square analysis was used to determine the effect of treatment on survival and occurrence of last gasp in the animals asphyxiated for 3 min. 40 sec. Effect of treatment on time to primary apnea (for 244 animals) was evaluated by t-test. Indomethacin effect on time to last gasp and time to first gasp upon resuscitation (for 31 and 29 animals) were also compared by t-test.

Student's t-tests were also used to compare 1) brain prostaglandin levels in animals killed at one day post-asphyxia (indomethacin

vs. saline pretreatment) and 2) brain water content between the two examined brain regions (cebral cortex vs. cerebellar-colliculi regions). One way analysis of variance and Student Newman-Keuls tests were used to compare brain water content between two day old non-asphyxiated animals and 3 day old animals which were killed at one day after asphyxia.

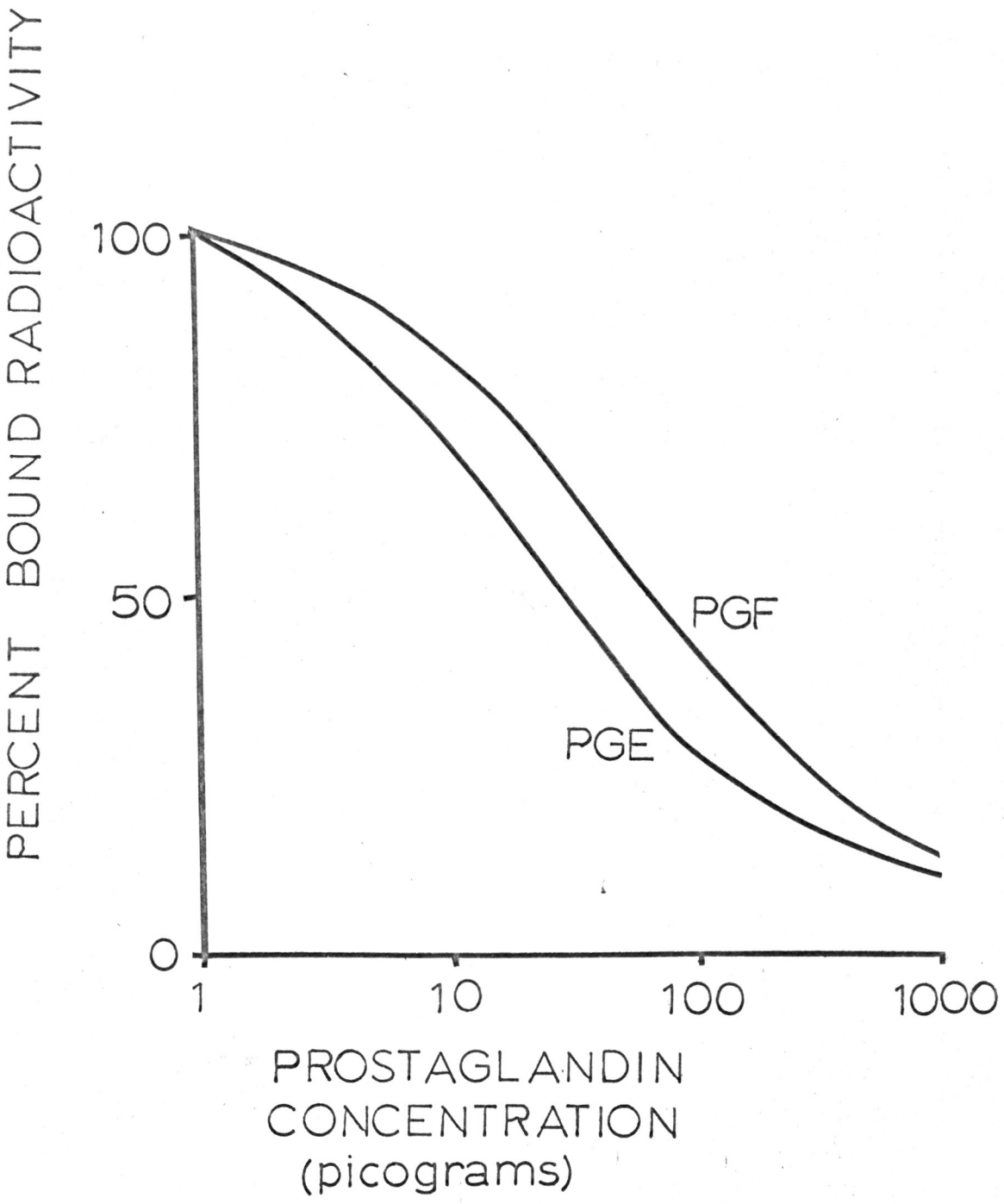
RESULTS

RIA

Brain samples were assayed in ten separate assays. Extraction and assay conditions were identical for all assays. Percent binding (cpm of bound/cpm of total radioactivity) averaged 49.0± 6.27% (mean ± standard deviation) for the PGF_2-alpha assays and 53.0± 4.59% for the PGE_2 assays. The 50% binding inhibition points (that point on the standard curve at which zero point binding is inhibited 50% by non-labelled prostaglandin) were 60.6± 7.48 picograms for the PGF_2-alpha assays and 23.0 ±2.6 picograms for the PGE_2 assays. Typical standard curves are shown in Figure 3. Recovery percentages were calculated by counting 10% of the extract, correcting for percentage of sample taken, and dividing by total cpm of added tracer. This procedure yielded recovery values of 82.9± 7.55% for PGF_2-alpha assays and 82.84± 7.51% for the PGE_2 assays.

Control tubes (containing no cold prostaglandins) were extracted and assayed along with the samples. These blanks gave values of 7.41± 4.41 pg of PGF_2-alpha and .75± 1.27 pg of PGE_2. Standard brains were also run through the extraction and assay procedures. For the first seven assays, rat brain homogenate which had been stored at -20 degrees C was used as the standard. This was not lyophilized or extracted in ethanol, but was first extracted at the petroleum ether step. Values for this standard varied widely within and between assays. For the last three assays

Figure 3. Radioimmunoassay standard curves for PGE_2 and PGF_2-alpha.


PERCENT BOUND RADIOACTIVITY
100
50
0
PGF
PGE
1
10
100
1000
PROSTAGLANDIN
CONCENTRATION
(picograms)

however, several normal guinea pig brains were frozen in liquid nitrogen, lyophilized, and extracted in ethanol. These ethanol extracts were mixed thoroughly and dispensed into one milliliter aliquots. Two identical standards were run in each of the last three assays. Values for the guinea pig brain standards were 1736± 118 pg of PGF_2-alpha and 946± 134 pg of PGE_2. This would give interassay variation coefficients of 6.8% for PGF_2-alpha and 14.2% for PGE_2. Therefore, the prostaglandin values for the guinea pig brain standard used in the last three assays were less variable than those obtained for the rat brain homogenate used in the first seven assays. This difference in variability could be due to the fact that the guinea pig brain standard was more evenly mixed than the rat brain homogenates. Therefore, since the same technique was used in the first seven assays, it is probable that variability in the rat brain homogenate prostaglandin levels was due to an inadequate brain standard.

Prostaglandin Data

PGF_2-alpha and PGE_2 results for the ten groups are shown in Figures 4 and 5.

PGF_2-alpha Results

The results of the one-way ANOVA and SNK multiple comparisons tests are shown in Table 2. The overall F value of 35.68 is highly significant ($P < .001$). Multiple comparisons results revealed the saline control, saline 3 min. non-resuscitate and 3 min. 40 sec. non-resuscitate groups to be not significantly different. Groups

Figure 4. Brain PGF_2-alpha levels for two day old guinea pigs. Values (mean $\pm$ standard error of the mean) are in nanograms per gram dry weight of tissue. Numbers in parantheses represent the number of animals used in each group. Two samples were assayed per animal. The lower case letters designate groups which are not significantly different from each other as determined by Student Newman-Keuls multiple range test.

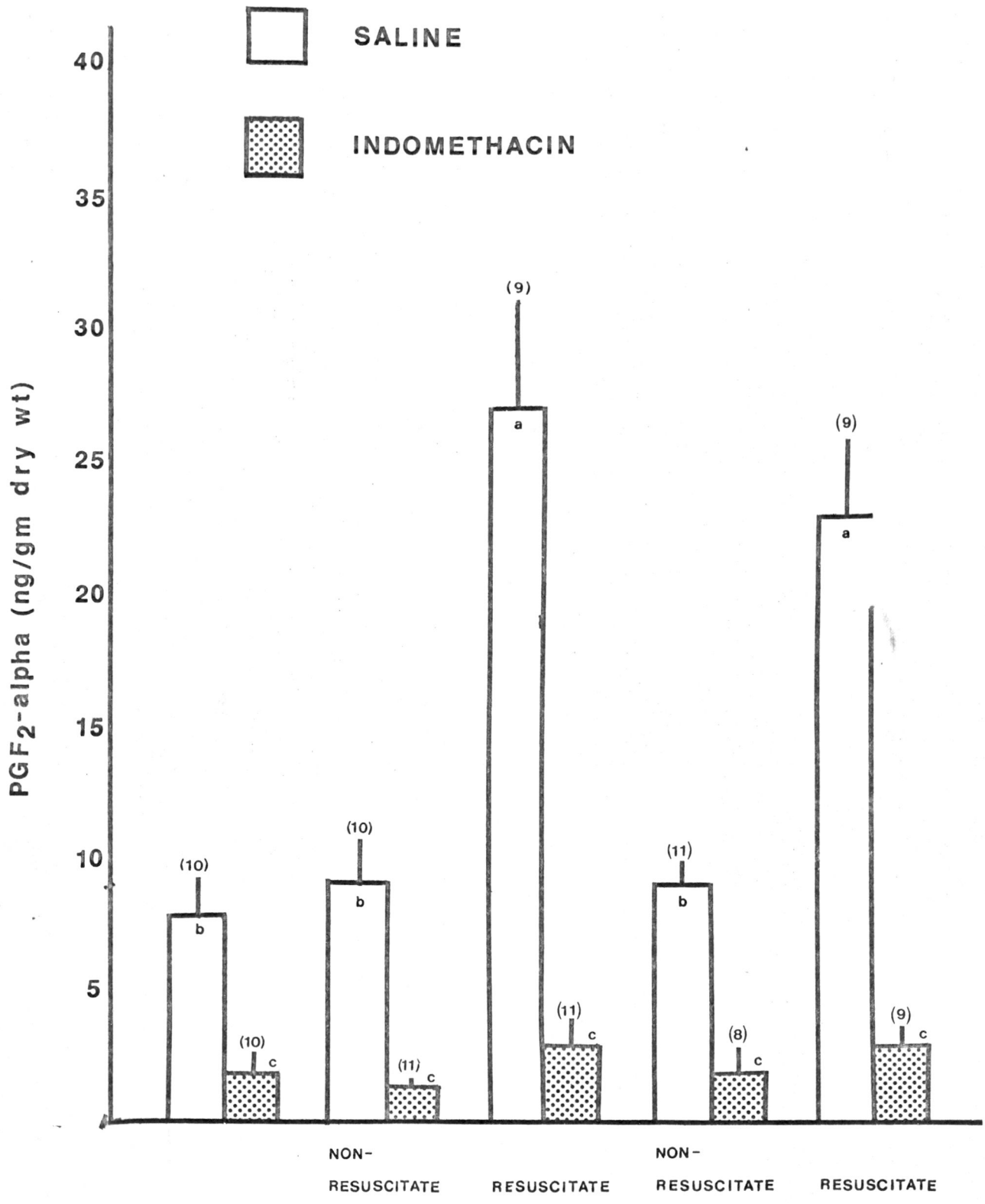

SALINE
INDOMETHACIN
PGF2-alpha (ng/gm dry wt)
40
35
30
25
20
15
10
5
(10)
b
(10)
c
(10)
b
(11)
c
(9)
a
(11)
c
(11)
b
(8)
c
(9)
a
(9)
c
NON-
RESUSCITATE
RESUSCITATE
NON-
RESUSCITATE
RESUSCITATE
NO
ASPHYXIA
3 MIN
ASPHYXIA
3 MIN 40 SEC
ASPHYXIA

TABLE II. Statistical Analysis of PGF_2-alpha Data.

Analysis of Variance

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F Ratio
Between Groups	40.17	9	4.46	35.68
Within Groups	23.63	189	.125	
Total	63.80	198		

$F_{0.05(1)9,200} = 1.93$

P 0.001

Student Newman-Keuls Multiple Comparisons Test

Subset 1: 3:00 SR and 3:40 SR

Subset 2: 0:00 S, 3:00 SNR, and 3:40 SNR

Subset 3: 0:00 I, 3:00 INR, 3:00 IR, 3:40 INR, and 3:40 IR

I= Indomethacin Pretreated

S= Saline Pretreated

NR= Asphyxiated, Not Resuscitated

R= Asphyxiated, Resuscitated

3 and 5, or the saline pretreated resuscitated groups had significantly higher prostaglandin F_2-alpha levels than the other saline groups, but they did not differ from each other. Statistical analysis also revealed the indomethacin pretreated animals to have lower PGF_2-alpha levels than the saline pretreated non -asphyxiated animals. It is also apparent that there is no trend or change with asphyxia or asphyxia and resuscitation in the indomethacin pretreated groups.

PGE_2 Data

The results of statistical analysis of the PGE_2 data are shown in Table 3. The overall F value for the PGE_2 data was 22.13. This value was also highly significant ($P < .001$). Multiple comparisons by SNK again revealed that indomethacin pretreated animals had lower PGE_2 levels than saline pretreated non-asphyxiated animals, and that there was no change in PGE_2 values with asphyxiation in the indomethacin pretreated groups. In the saline pretreated group, on the other hand, the only significant difference found was that the animals asphyxiated for 3 min. 40 sec. and not resuscitated had higher PGE_2 levels than non-asphyxiated control animals. Figure 5 shows a slight tendency for PGE_2 to rise with asphyxia and to decrease slightly upon resuscitation. There is no resuscitation-induced rise in PGE_2 as was seen in the PGF_2-alpha data.

Effect of Indomethacin Pretreatment on Survival During Asphyxia

Time to onset of primary apnea (TPA) was recorded for all

Figure 5. Brain PGE_2 levels for two day old guinea pigs. Values (mean $\pm$ standard error of the mean) are in nanograms per gram dry weight of tissue. Numbers in parentheses represent the number of animals used in each group. Two samples were assayed per animal. The lower case letters designate groups which are not significantly different as determined by Student Newman-Keuls multiple comparisons test.

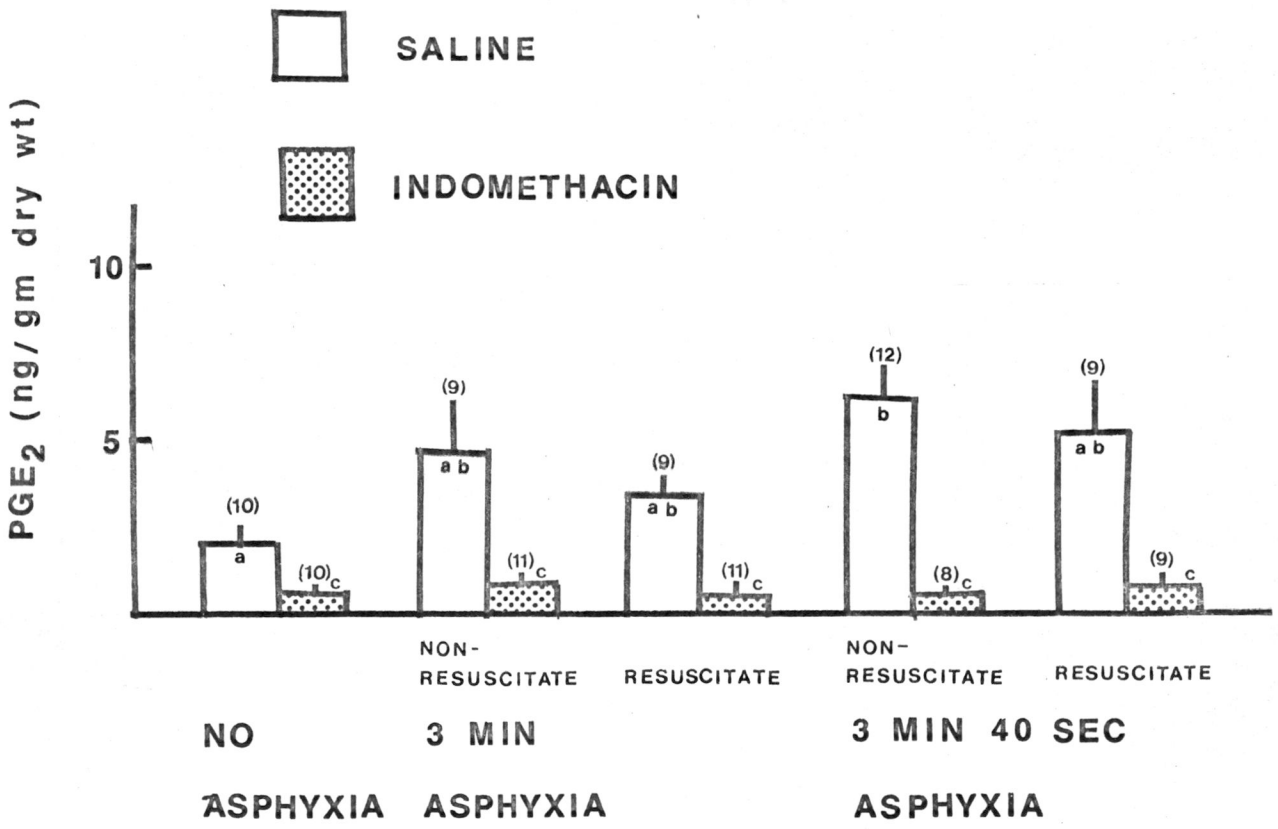

SALINE
INDOMETHACIN
PGE2 (ng/gm dry wt)
10
5
(10)
a
(10) c
(9)
a b
(11) c
(9)
a b
(11) c
(12)
b
(8) c
(9)
a b
(9) c
NON-RESUSCITATE
RESUSCITATE
NON-RESUSCITATE
RESUSCITATE
NO ASPHYXIA
3 MIN ASPHYXIA
3 MIN 40 SEC ASPHYXIA

TABLE III. Statistical Analysis of PGE_2 Data.

Analysis of Variance

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F Ratio
Between Groups	80.989	9	8.998	22.130
Within Groups	77.235	190	.4065	
Total	158.22	199		

$F_{0.05(1)9,200} = 1.93$

P 0.001

Student Newman-Keuls Multiple Comparisons Test

Subset 1: 0:00 S, 3:00 SNR, 3:00 SR, and 3:40 SR

Subset 2: 3:00 SNR, 3:00 SR, 3:40 SNR, and 3:40 SR

Subset 3: 0:00 I, 3:00 INR, 3:00 IR, 3:40 INR, and 3:40 IR

I= Indomethacin Pretreated

S= Saline Pretreated

NR= Asphyxiated, Not Resuscitated

R= Asphyxiated, Resuscitated

asphyxiated animals. For the indomethacin pretreated animals (N=118), TPA was 57.71± 6.14 sec., while TPA for the saline pretreated group (N=126) was 57.39± 6.11 sec. This difference is not significant at the .05 level.

The effect of pretreatment on occurrence of last gasp was evaluated in those animals asphyxiated for 3 min. 40 sec. Records were kept as to whether an animal had finished gasping prior to the 3 min. 40 sec. cut-off point or whether the animal was still gasping at the cut-off point. Results comparing the two treatment groups with respect to occurrence of last gasp by 3 min. 40 sec. were analyzed by Chi-square and are shown in Table 4. In the saline group, 28 of 46 animals had finished gasping by the cut-off point of 3 min. 40 sec. In the indomethacin group, 39 of 55 had finished gasping by that time. These frequencies are not significant at the .05 level.

In another experiment, animals were pretreated as usual and were asphyxiated until last gasp was observed. Results indicate no difference in time to last gasp between groups. Saline pretreated animals finished gasping at 220.50± 37.64 sec. (N=14), while indomethacin pretreated animals stopped gasping at 211.53± 33.90 sec. (N=17).

Data was also kept on whether animals survived or did not survive 3 min. 40 sec. of nitrogen asphyxiation and subsequent resuscitation. These data are shown in Table 4. 24 of 40 indomethacin pretreated animals and 27 of 50 saline pretreated

TABLE IV. Effect of Indomethacin on A) Occurrence of Last Gasp and B) Survival Following Perinatal Asphyxia. (Only animals asphyxiated for 3 min. 40 sec. are included.)

A. Occurrence of Last Gasp (Before 3 min. 40 sec.)

	Last Gasp	No Last Gasp	
Indomethacin Pretreated	28	18	46
Saline Pretreated	39	16	55
	67	34	101

Chi-Square = .3257

$X^2_{0.05,1} = 3.841$

N.S. at the .05 level

B. Survival (After Asphyxiation and Resuscitation)

	Lived	Died	
Indomethacin Pretreated	24	16	40
Saline Pretreated	27	23	50
	51	39	90

Chi-Square = 1.130

$X^2_{0.05,1} = 3.841$

N.S. at the .05 level

animals survived 3 min. 40 sec. of asphyxiation with resuscitation. Chi-square analysis shows no significant difference in survival rate between the two treatment groups.

Time to First Gasp and Recovery of Righting Reflex Upon Resuscitation From Asphyxia

The times from removal from nitrogen to first gasp and recovery of the righting reflex were recorded in animals which were 1) pretreated with indomethacin or saline, 2) asphyxiated until last gasp and 3) resuscitated. Time to first gasp in the indomethacin pretreated group was 65± 36.57 sec. (N=15), while time to first gasp for the saline pretreated group was 105± 61.92 sec. A t-test revealed the difference to be significant at the .05 level.

Time to recovery of righting reflex following asphyxiation was recorded in only one experimental session. These preliminary results show time to righting for the indomethacin group (N=8) to be 514± 185 sec. (range: 300-889 sec.) and 724± 248 sec. (range: 373-900 sec.) for the saline group (N=3).

Prostaglandin Levels and Brain Water Content in Animals One Day After Neonatal Asphyxia

In this experiment, animals were pretreated as usual, asphyxiated for 3 min. 40 sec., resuscitated and sacrificed one day later. As in the other main experiments, two brain regions were removed from each animal for prostaglandin analysis. In this study, values from these two regions were pooled so that each animal had two replicates. Results from this study are shown in Table 5. At

TABLE V. Brain Prostaglandin levels in animals killed by decapitation one day after nitrogen asphyxiation (3 min. 40 sec.). Each animal had two replicate samples. Values are mean ± standard deviation.

	Brain Prostaglandin level (ng/gm dry wt)	
	PGF_2-alpha	PGE_2
Saline Pretreated (N=4)	6.36 ± 2.54	2.08 ± .958
Indomethacin Pretreated (N=5)	4.89 ± 2.68	1.81 ± .986

Values are not different between treatment groups ($P < 0.05$)

one day post-asphyxia, PGE_2 and PGF_2-alpha levels are not different between the indomethacin pretreated and saline pretreated groups. These values were also comparable to brain prostaglandin levels in saline pretreated non-asphyxiated animals at two days of age.

Water content of the brain samples was determined by the difference between frozen and lyophilized weights. Data for the two day old non-asphyxiated animals and the three day old animals killed one day after nitrogen asphyxiation are shown in Table 6. Water content of the cerebellar-colliculi region is significantly less ($P < .05$) than water content of the cerebral cortex in all groups shown except the indomethacin pretreated and previously asphyxiated three-day old animals. Also, there is no significant difference in water content of the cerebellar-colliculi regions between the groups examined. On the other hand, water content in the cerebral cortex of the two-day old non-asphyxiated guinea pigs is higher ($P < .05$) than the cortical water content of the three-day old guinea pigs which were killed one day after asphyxiation and resuscitation.

TABLE VI. Brain water content (as percent water) in two day old non-asphyxiated guinea pigs and in guinea pigs which were asphyxiated (at 2 days of age) for 3 min 40 sec, resuscitated and killed by decapitation one day later. Water content for the two brain regions (cerebral cortex and cerebellar-colliculi regions) are shown (Mean ± standard deviation).

	Indomethacin Pretreated		Saline Pretreated	
	Cerebral Cortex	Cerebellum-Colliculi	Cerebral cortex	Cerebellum-Colliculi
2 day old Non-Asphyxiated	81.88 ± .347 (N=10)	79.345 ± .516 (N=11)	81.6 ± .572 (N=11)	78.32 ± 2.36 (N=10)
3 day old Asphyxiated and Resuscitated on day 2.	79.82 ±1.47 (N=5)	77.92 ±1.55 (N=5)	80.62 ± .680 (N=4)	76.92 ±1.61 (N=4)

Statistical Analysis: t-tests

- Cerebellar-colliculi regions have lower ($P < .05$) water content than cerebral cortex except in the 3 day old indomethacin pretreated groups.

ANOVA and Student Newman-Keuls tests

- No difference ($P > .05$) in water content of the cerebellar-colliculi regions between the groups.
- Animals asphyxiated at 2 days and killed at 3 days have lower ($P < .05$) cortical water content than non-asphyxiated 2 day old animals.

DISCUSSION

Models of Asphyxia

The present model of perinatal asphyxia is one of acute total asphyxia produced by placing the animal in an oxygen-free atmosphere. Myers (1977) has argued against the applicability of acute total asphyxia as a model of human neonatal asphyxia. This argument is based largely on comparisons of neuropathology exhibited by fetal monkeys which were subjected to total asphyxia with the neuropathology which has been seen in asphyxiated human neonates. As previously mentioned, total asphyxia results mainly in damage to the brain stem with little or no involvement of the cerebral cortex. Based on these observations, Myers has adopted a different model of asphyxiation in which fetal arterial oxygen tension is severely lowered for a long period of time ($>$30 min.). His studies demonstrated that this "partial asphyxia" may cause brain pathology which is quite similar to that observed in the asphyxiated human infant. Therefore, his conclusion was that most asphyxiated human infants have been subjected to long periods of severe hypoxia rather than acute and total restriction of oxygen supply. Several of the previously mentioned predisposing factors for neonatal asphyxia (Dorand, 1978) may be expected to cause prolonged partial rather than acute total asphyxia. However, it is possible that other factors (such as interruption of placental blood supply by umbilical cord occlusion) may result in rapid and complete disruption of oxygen supply to

the fetus. The present study investigated the effect of such sudden total asphyxia on the levels of prostaglandins E_2 and F_2-alpha in the newborn guinea pig brain.

Two different methods for producing total asphyxia in newborn guinea pigs have been described. Windle's group (Windle and Becker, 1943, Windle et al., 1944 and Bailey and Windle, 1959) produced intrauterine total asphyxia by clamping either the unbilical cord or uterine artery, while Miller's research (Miller, 1949, Miller and Miller, 1962, Miller et al., 1964, Miller and Miller, 1969, and Miller, 1971) involved placing animals in an atmosphere of 95% N_2, 5% CO_2. Dawes (1968) has mentioned that there is a slight difference between these two methods of total asphyxiation. This difference arises from the fact that animals asphyxiated in a nitrogen atmosphere hyperventilate in the early phase of asphyxiation. This hyperventilation results in respiratory alkalosis by "blowing off" CO_2. However, after the hyperventilation-alkalosis phase, the animal develops hypoxemia and hypercapnia in a manner similar to that resulting from cord occlusion asphyxiation of the fetus. The initial alkalosis may briefly protect the animal from a decrease in pH, and thus, slightly prolong gasping.

Discussion of Results

Results of the present study have shown that total asphyxiation of newborn guinea pigs (by exclusion of oxygen from the inspired air) has no effect on brain levels of PGF_2-alpha. Brain levels of

PGE_2 are also not significantly altered by 3 min. of asphyxia. However, with 3 min. 40 sec. of asphyxia, brain PGE_2 levels are significantly higher than levels of non-asphyxiated control animals. When animals are asphyxiated and subsequently resuscitated, brain PGF_2-alpha levels rise significantly while PGE_2 levels are not different from the levels of non-asphyxiated controls. This indicates that asphyxia alone has no effect on PGF_2-alpha levels, but that the longer periods of asphyxia (3 min. 40 sec.) may cause an increase in brain PGE_2 levels. Following asphyxia and resuscitation, brain PGF_2-alpha levels rise while PGE_2 levels seem to drop slightly from those levels associated with asphyxia alone.

The author is unaware of any works on the effects of asphyxia and asphyxia followed by resuscitation on brain prostaglandin levels in the fetus or neonate. A few studies are available on the effects of hypoxia on prostaglandin levels in the brain and plasma.

Steinhauer et al. (1979) measured brain levels of PGF_2-alpha and PGE_2 following decapitation-induced "hypoxia" in the adult mouse. Animals were decapitated and the brains remained in situ for up to seven minutes before being removed and frozen in liquid nitrogen. His data indicates that there is a gradual increase in both PGE_2 and PGF_2-alpha levels in brain homogenates over the time periods used. This decapitation model, however, has little relevance to our asphyxiation model and is also probably a poor representation of hypoxia since the brain is also subjected to

total ischemia. Steinhauer's results probably represent the increased prostaglandin synthesis that results from post-mortem release of arachidonic acid.

In another study of prostaglandins in acute fetal hypoxia, Challis et al. (1978) lowered the arterial oxygen tension of the fetal lamb by respiring the mother with a mixture of 9% O_2 and 3% CO_2 in nitrogen. Fetal plasma levels of prostaglandins were measured and it was found that hypoxic fetuses had plasma prostaglandin levels similar to those of control fetuses.

Lewis et al. (1978) also induced hypoxia in maternal sheep and measured fetal plasma prostaglandin levels. He reported a slight, but non-significant rise in both PGE_2 and PGF_2-alpha levels in the fetal plasma after hypoxia. Lewis also demonstrated that in the normal condition, plasma levels of PGE_2 and PGF_2-alpha were higher in the fetuses than in the mother. He speculated that this accumulation could be due to a decreased blood flow through the fetal lungs. Since the lungs are a major site for prostaglandin breakdown, the lowered fetal pulmonary blood flow would lead to higher levels of prostaglandins in the fetal plasma.

There are similarities between the total asphyxia model and models of cerebral ischemia used in investigation of stroke. Both Spagnuolo et al. (1979) and Gaudet and Levine (1979) reported that cerebral ischemia did not significantly alter brain levels of various prostaglandins even though brain levels of arachidonic acid increase during the ischemic period. Gaudet and Levine (1979) reported a

rapid rise in brain levels of PGE_2, PGF_2-alpha and PGFM (the primary metabolite of PGF_2-alpha) upon reperfusion of the brain following a period of cerebral ischemia.

The PGF_2-alpha data of Gaudet and Levine (1979) resembles our data, as both studies demonstrated increases in PGF_2-alpha upon re-oxygenation of the brain after ischemia or asphyxiation. On the other hand, both Gaudet and Levine (1979) and Spagnuolo et al. (1979) show no change in PGE_2 with ischemia, and Gaudet found a rise in PGE_2 with reperfusion after a period of ischemia. Our data show a rise in PGE_2 levels with longer periods of asphyxiation, but following resuscitation, levels resembled those of non-asphyxiated control animals. Perhaps this difference is due to the difference between ischemia and asphyxiation. In ischemia, blood flow and oxygen supply are suddenly and completely cut off. In the asphyxia model used in this study, blood flow and some oxygen supply are probably maintained for a period during the nitrogen asphyxiation. Perhaps the residual oxygen is used in preferential synthesis of PGE_2 during asphyxia, but, upon resuscitation from asphyxia, the rate of PGF_2-alpha formation is increased. It is possible that some of the PGF_2-alpha is formed from PGE_2 through the 9-keto reductase system which is found in the brain.

Gaudet's study also showed that the post-ischemic increase in brain prostaglandins was transient, with levels returning to baseline in about two hours. Though we have not followed the time

course as closely, we did find that prostaglandin levels in neonatal guinea pig brains one day after asphyxiation were similar to those prostaglandin levels in the brains of non-asphyxiated control animals.

Increased levels of prostaglandins have been demonstrated in several models of brain trauma. However, the precise role of this increase in prostaglandins is not known. The increase could be physiological, perhaps representing a compensatory mechanism aimed at stabilizing the cerebral circulation, or it may be a pathological process resulting from cell damage.

Several members of the prostaglandin family are potent vaso-active substances with the ability to alter the caliber of cerebral vessels or change the rate of cerebral blood flow when administered in pharmacological doses. It is not completely understood whether or not these substances are involved in the normal physiological control of the cerebral circulation. There are conflicting reports on the effects of the inhibition of prostaglandin synthesis on the normal cerebrovascular responses to hypercapnia or changing perfusion pressure (Wei et al., 1980, Pickard et al., 1973 and Pickard and MacKenzie, 1977). However, studies have also shown that indomethacin treatment may lower cerebral blood flow by increasing cerebrovascular resistance (see Wei et al., 1980) This suggests a role for vasodilatory prostaglandins in the normal resting tone of the cerebral vasculature.

A study by Hallenbeck and Furlow (1979) indicated that

vasoconstrictor prostaglandins may have a role in the impairment of cerebral reperfusion following a period of cerebral ischemia. In a dog model, he showed that ischemia-induced impairment of cerebral reperfusion could be prevented by either pretreating the animal with indomethacin prior to the ischemic period, or treating the animal with a combination of indomethacin and prostacyclin (a cerebral vessel vasodilator) after the ischemic episode. In the gerbil stroke model of Gaudet and Levine (1979), there was evidence that animals pretreated with indomethacin prior to cerebral ischemia were more active during recovery from ischemia than were animals pretreated with saline. Both of these studies indicate that the post-ischemic rise in brain prostaglandins may be detrimental to the animal. These works would suggest that an increase in prostaglandins is not a physiological control mechanism, but more of a pathological event with adverse effects on the animal.

This study has demonstrated that indomethacin pretreatment drastically lowers brain prostaglandin levels in neonatal guinea pigs and prevents any increase in PGF_2-alpha usually associated with asphyxia and resuscitation. If the resuscitation induced rise in PGF_2-alpha is detrimental to the animal, one would expect the indomethacin pretreated animal to be protected against the possibly harmful effects of the PGF_2-alpha surge. It has been found that time to onset of primary apnea is not affected by indomethacin pretreatment prior to asphyxiation. Some early results suggested that indomethacin pretreatment may prolong gasping in

the nitrogen atmosphere (Allen, et al., 1980). However, addition of more animals to the design did not support this tendency, and therefore, there is apparently no effect of inhibition of prostaglandin synthesis on duration of gasping in a nitrogen atmosphere. Other results obtained from a small series of animals seem to indicate that indomethacin pretreated animals have a shorter time to first gasp upon resuscitation than saline pretreated animals. This indicates that inhibition of the resuscitation-induced PGF_2-alpha surge may be beneficial by enabling the animal to recover more rapidly from asphyxia.

In 1944, Windle et al. found that intrauterine total asphyxia caused certain neuropathological changes in the guinea pig brain. In their study, brain edema occurred in the "acute period" following fetal asphyxia and was present between 8 hours and 4 days after asphyxia in all animals but one. In the present study, an attempt was made to quantitate post-asphyxial edema formation by measurement of brain water content at one day after asphyxia.

Table 6 shows the comparison of brain water content (as percent water) for the three-day old animals killed one day after asphyxia and brain water content of non-asphyxiated two-day old animals. The data show that in all groups but one (indomethacin treated three-day old animals) the cerebral cortex had a higher water content than the cerebellum-colliculi region. This difference may be due to the higher fiber content of the hind brain structures (cerebellum-

colliculi) relative to that of the cerebral cortex.

The data also shows no difference between groups with regard to the water content of the cerebellar-colliculi regions. However, it is evident that the cortical water content of the two-day old non-asphyxiated animals is significantly higher ($P < .05$) than the cortical water content of the three-day old animals killed one day after asphyxiation. There is no difference in cortical water content between treatment groups. Therefore, the observed difference in cortical water content between two-day old non-asphyxiated animals and three-day old,previously asphyxiated animals may be either due to asphyxia or age. From the experimental design, it is not possible to exactly determine which factor causes the effect. Based on Windle's observations of edema formation in the brain after asphyxia, it would be hypothesized that an asphyxia effect would cause an increase in tissue water. Therefore, if our method of determining water content were able to detect such edema formation, and if it did in fact occur in the experimental animals, the three-day old previously asphyxiated animals would have a higher cortical water content than the two-day old non-asphyxiated controls. This was not seen.

The decrease in brain water content may, therefore, be due to age. Studying the growth and development of the guinea pig brain, Dobbing and Sands (1970) show that the perinatal period is a time of rapid decrease in brain water content. The observed difference in cortical water content between two-day old and three-

day old animals may be due to normal development of the brain.

Learning and Behavior Impairment in the Asphyxiated Newborn Guinea Pig

As part of a collaborative study with the Department of Psychology, studies of learning ability and behavior were performed on control and experimental animals in both treatment groups. Preliminary results (Gray, 1980) showed that the behavior and learning ability of animals asphyxiated for three minutes was not different from that of non-asphyxiated control animals. On the other hand, animals asphyxiated for three minutes forty seconds differed from their control counterparts in spontaneous activity as well as the ability to learn a simple problem. In the spontaneous activity tests, animals aged three to twenty days were placed in a box for five minutes each day and amount of spontaneous activity was observed. Animals asphyxiated for 3 min. 40 sec. were consistently less active than both control animals and those asphyxiated for three minutes. The observed difference in activity was statistically significant only in the fifth minute. In learning tests, 90-day old animals were trained to seek reinforcement (food) in a specific goal box. After the animal had learned the task, reinforcement was switched to another goal box and the task was relearned. Animals asphyxiated for 3 min. 40 sec. took sufficiently longer to reverse and learn to seek reinforcement in the new goal box. This indicates that asphyxiated animals exhibited more stereotyped behavior once a task was learned

and were less likely to reverse that behavior when reinforcement was moved. In all of the learning and behavior tests, indomethacin pretreatment prior to asphyxia had no effect on subsequent learning and behavior impairments.

Conclusions and Speculation

The present study has shown that asphyxiation of newborn guinea pigs by nitrogen exposure, followed by resuscitation, results in a significant rise in the brain concentrations of PGF_2-alpha, a potent vasoconstrictive substance. Also, prolonged asphyxiation causes a rise in brain PGE_2 concentrations, but, following resuscitation, PGE_2 levels are not different from brain levels in non-asphyxiated control animals. These alterations in brain prostaglandin levels are transient, for levels at one day after asphyxiation are not different from those of control animals. We have also shown that administration of indomethacin, a prostaglandin synthesis inhibitor, lowers brain prostaglandin concentrations and prevents any change in brain prostaglandin levels with asphyxia or asphyxia and resuscitation. Indomethacin pretreatment had no effect on survival parameters during asphyxia (time to primary apnea and time to last gasp), but may enable a quicker recovery following resuscitation.

While the effect of asphyxia on brain prostaglandin levels has been demonstrated, it is unknown whether the increased brain PGF_2-alpha concentration following asphyxiation and resuscitation is detrimental to the animal. Two previously mentioned studies (Furlow and Hallenbeck,

1978, and Hallenbeck and Furlow, 1979) have shown that ischemia-induced rises in brain prostaglandins may cause impairment of brain reperfusion, probably through the actions of vasoconstrictor prostaglandins such as PGF_2-alpha.

Therefore, based on the literature concerning prostaglandin effects on cerebral vasculature, and the results of this preliminary investigation into prostaglandins and perinatal asphyxia, it may be speculated that an increase in vasoconstrictive prostaglandins may effect post-asphyxial cerebral blood flow in the neonate. Further works on the topic should possibly investigate other methods of asphyxiation (such as the "partial asphyxia" model of Myers) and should include determination of cerebral blood flow following asphyxiation.

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