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Neuropathology of normothermic circulatory arrest in newborn dogs

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Abstract Neuropathologic findings are described, for the first time, in a neonatal dog model of circulatory arrest in normothermic conditions, and the findings are compared to those reported in neonatal dogs with hypothermic circulatory arrest. Total circulatory arrest was produced in 3- to 6-day-old anesthetized, paralyzed and ventilated, normothermic dogs either by asphyxiation or cardioplegia. Duration of circulatory arrest was 8–20 min and 10–40 min in asphyxiated and cardioplegic animals, respectively. The animals were resuscitated and maintained under controlled systemic physiologic conditions until neuropathologic examination after 8 or 24 h of recovery. The results suggest that the minimal durations of circulatory arrest for brain damage to occur following asphyxia or cardioplegia are 10 and 15 min, respectively. Ischemic lesions in both groups consisted of neuronal necrosis and involved mainly the brain stem structures, particularly the reticular nuclei and the spinal cord gray matter. The medulla was more severely involved than midbrain and pons. There was a direct correlation between the length of circulatory arrest and the severity of damage in the medulla ($P = 0.001$) and overall brain stem damage ($P = 0.004$) in animals with cardioplegia, but not in animals with asphyxia. These findings are compared to the neuropathologic changes previously described in newborn dogs subjected to hypothermic circulatory arrest, in which ischemic lesions are focused on the cerebral cortex and basal ganglia. It is concluded that hypothermia in this model not only prolongs the period of circulatory arrest that is required to produce brain damage, but also shifts the pattern of regional ischemic vulnerability from caudal to more rostral structures.

Key words Circulatory arrest · Brain stem · Spinal cord · Neuronal necrosis

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Introduction

Currently available newborn models of normothermic hypoxic-ischemic brain damage involve the ligation of one or both common carotid arteries combined with systemic hypoxia or hypotension [13, 28, 34], an insult which is not typical of the human situation [9, 14, 31]. Moreover, most non-rodent circulatory arrest models of brain damage in adult animals involve the dog as a species of choice [8, 15, 25, 38]. Therefore, we believe it appropriate to investigate the effect of circulatory arrest at normothermia in the newborn dog to ascertain age-related differences of ischemic brain damage in this species and its relationship to the human newborn infant. Accordingly, the present investigation concentrated on the presence, distribution, and severity of ischemic brain damage in newborn dogs arising from total circulatory arrest produced either by asphyxiation or cardioplegia at normothermia.

Materials and methods

Animal preparation

Pregnant, conditioned Beagle dogs were purchased from a local breeder and housed in individual kennels. Following spontaneous vaginal delivery, the newborn puppies were maintained with their bitches until time of experimental manipulation at 3–6 days of postnatal age. Body weights ranged from 275 to 365 g. Each puppy was initially anesthetized with halothane (4% induction; 1.0–1.5% maintenance), following which they underwent endotracheal intubation and muscular paralysis with succinylcholine (15 mg/kg body weight). Thereafter, the animals were artificially ventilated with a gas mixture of 0.5% halothane/70% nitrous oxide/balance oxygen. Under local anesthesia (1% procaine-HCl), a femoral artery was cannulated with polyethylene tubing (PE-50),

which was connected via a statham transducer (Gould, Oxnard, Calif.) to a dynographic recorder (Model R711; Beckman Instruments, Fullerton, Calif.) to monitor systemic heart rate and blood pressure. The diameter of catheter was 1.0 mm, and it extended to the lower portion of the descending thoracic aorta. A side arm of the arterial catheter allowed for intermittent collection of blood (0.2 ml) for analysis of PaO_2, $PaCO_2$, and pHa on a micro-blood gas analyzer (Model ABL 30; Radiometer America, Westlake, Ohio). Blood was also collected for glucose analysis on a micro-glucose analyzer (Beckman Glucostat). Oxygen and acid-base balance was maintained within a narrow range ($PaCO_2$ = 35–42 mm Hg; pHa = 7.35–7.42; PaO_2 > 60 mm Hg) by intermittent adjustments of tidal volume (± 1 ml/100 g body weight) and ventilatory rate (± 40/min). A femoral vein was also cannulated as an access route for infusion of fluids and injection of drugs. Body temperature was maintained at 37 ± 0.2°C by means of a rectal probe attached to a servo-controlled heating lamp.

Circulatory arrest and resuscitation

Once steady-state arterial normoxia and acid-base balance were achieved, the heart of each newborn dog was arrested either by asphyxiation or cardioplegia. In those randomly selected animals to be asphyxiated, artificial ventilation was discontinued, following which there was a progressive decrease in heart rate and blood pressure until such time that cardiac pulsations and blood pressure were no longer recorded on the dynograph. The animals then were subjected to total circulatory arrest for 8–20 min, following which they were resuscitated (see below). Other randomly assigned newborn dogs were arrested by the intravenous injection of KCl (25 mmol/l) in a small volume (2–3 ml). Artificial ventilation was discontinued simultaneously with the cardiac arrest. As was the case with the asphyxiated animals, complete circulatory arrest was verified by the absence of spontaneous heart rate and systemic blood pressure monitored on the dynograph. These animals remained asystolic for 10–40 min. Thereafter, all animals were resuscitated by: (1) resumption of artificial ventilation with 70% nitrous oxide/30% oxygen; (2) i.v. injection of $NaHCO_3$ (2 mmol/kg); (3) i.v. injection of 1:10 000 epinephrine (0.2 ml/kg); (4) i.v. injection of 10% calcium gluconate (1 ml/kg); and (5) closed chest cardiac massage. These maneuvers resulted in spontaneous heart action in all animals within 9 min, with progressively increasing heart rate and systemic blood pressure thereafter. Plasma glucose, hematocrit, arterial oxygen and acid-base status were determined every 15 min, up to 1 h of recovery, hourly thereafter until 4 h of recovery, and then every 2 h until animal sacrifice. The animals were weaned from the ventilator as the effect of the succinylcholine subsided. Once a stable, spontaneous respiratory pattern was achieved, the endotracheal tube was removed. Thereafter, the puppies were placed in an infant warmer and received an intravenous infusion of 10% glucose and water to maintain optimal glucose (80–140 mg/100 ml) and fluid homeostasis. All animals except for one were maintained for 8 h of recovery, at which time they underwent perfusion-fixation of their brains. One animal with KCl-induced cardiac arrest for 30 min, was allowed to survive for 24 h. This was done to see if longer survival resulted in change of the type and the distribution of cerebral lesions.

Neuropathologic methods

After 8 h of recovery from circulatory arrest, all but one of the newborn dogs were deeply anesthetized with pentobarbital (50 mg/kg i.v.), and their brains were perfusion-fixed with formaldehyde/glacial acetic acid/absolute methanol (FAM 1:1:8) for 30 min. Cerebral perfusion was performed by opening the abdomen and chest and inserting a metal cannula through the apex of the left ventricle into the proximal ascending aorta. After clamping the cannula in place, the right atrium was opened and heparinized saline infused through the ascending aorta at room temperature for approximately 30 s or until the return to the right atrium was clear. Meanwhile, the abdominal aorta was clamped. The saline infusion was followed by a solution of FAM for an additional 30 min. The pressure for perfusion was adjusted to that of the expected mean arterial blood pressure (MABP) for each animal. In one dog with KCl-induced cardiac arrest and 24 h recovery, the entire body was perfused with the fixative.

At the completion of the perfusion-fixation, the scalp was removed and the brain, together with the entire head, was fixed an additional 24 h in FAM. Then the brain, together with the first few cervical spinal cord segments, was removed from the skull and fixed in FAM for 1 week. In the animal with 24 h recovery the entire spinal cord, brain, and samples of heart, liver and kidney were removed.

FAM-fixed brains were cut coronally and spinal cords transversely, in 2- to 3-mm-thick slices and embedded in paraffin, as were the blocks from the other tissues. Sections (6 mm thick) were stained with hematoxylin and eosin and examined at the light microscopic level.

Following microscopic examination, it was noted that the ischemic lesions were located mainly in the brain stem structures (see Results). To ascertain quantitatively the extent of damage, the number of necrotic neurons in selected sections through the midbrain, pons, and medulla was determined. To ensure consistency, only damaged neurons showing acidophilic perikarya and pyknotic or karyorrhectic nuclei were counted. The brain stem was cut serially into 6-μm sections and 1 in every 20 sections was stained with hematoxylin and eosin. Selected sections were stained with cresyl violet or with acid fuchsin-cresyl violet. Following microscopic examination, three successive serial sections from each of three brain stem regions were selected for study: midbrain, the cross-sections at the level of the mid-portion of the red nucleus; pons, at the level of the middle of the trigeminal nerve exit; and medulla, at the mid-olivary level. The total number of necrotic neurons was determined in these three serial sections from each region under a × 40 objective of a microscope equipped with an ocular grid. To determine the number of the damaged neurons per mm^2, the total area of each section was determined using a planimeter (Nikon Microplan II; Laboratory Computer Systems; Cambridge, Mass.). Then, the mean of the neuronal population per mm^2 area for the three serial sections at each specified level of the brain stem was determined. An atlas of dog brain sections was used for identification of brain structures [35].

Statistical analysis

Statistical analysis of the data was accomplished using analysis of variance for sequential data (ANOVA) with Donnett's correction.

Institutional approval

The experiments described here were reviewed by the Animal Care and Use Committee of The Pennsylvania State University College of Medicine (The Milton S. Hershey Medical Center) and approved on October 22, 1993.

Results

General findings

Newborn dogs (n = 11) were subjected to asphyxia to the point of total circulatory arrest and beyond. The duration of asphyxia required to produce cardiac arrest ranged from 4 to 18 min, with the majority (n = 9) ranging from 10 to 18 min. Cardiac arrest intervals included one animal at 8 min, four at 10 min, two at 12.5 min, two at 15 min, and two at 20 min. All animals were successfully resuscitated, with the appearance of spontaneous heart rates and recordable blood pressures occurring within 1.5–7.0 min.

Table 1 Systemic physiologic variables during and following asphyxic circulatory arrest in newborn dogs. Values represent means ± SEM for 11 animals for up to 3 h of recovery, 9 animals at 4 h of recovery, and 8 animals at 6 and 8 h of recovery. Values for PaO_2 always exceeded 60 mm Hg (*MABP* mean arterial blood pressure)

* $P < 0.05$ compared to pre-arrest; ** $P < 0.05$ compared to immediately preceding value

Variable	MABP (mmHg)	Heart rate (beats/min)	pHa	$PaCO_2$ (mmHg)	Calc. HCO_3 (meq/l)	Glucose (mg/100 ml)
Pre-arrest	55 ± 2	192 ± 6	7.36 ± 0.01	37.3 ± 0.9	21.1 ± 0.8	183 ± 7
Recovery						
15 min	69 ± 3*	190 ± 6	7.00 ± 0.03*	58.1 ± 3.4*	13.4 ± 1.1*	299 ± 23*
1 h	57 ± 3	190 ± 8	7.29 ± 0.04**	40.0 ± 3.2**	18.9 ± 1.5*,**	265 ± 23*
2 h	45 ± 4	189 ± 9	7.36 ± 0.03	35.6 ± 1.9	20.2 ± 1.5	234 ± 15*
3 h	49 ± 6	185 ± 13	7.31 ± 0.03	42.0 ± 3.2	20.9 ± 1.7	173 ± 16**
4 h	46 ± 4	198 ± 12	7.27 ± 0.02	47.8 ± 3.2	21.6 ± 1.7	134 ± 12
6 h	44 ± 3	221 ± 14	7.32 ± 0.02	51.2 ± 1.8	25.6 ± 1.0*,**	149 ± 17
8 h	45 ± 5	234 ± 8*	7.31 ± 0.02	51.0 ± 4.0	24.7 ± 1.0*	153 ± 17

Table 2 Systemic physiologic variables during and following cardioplegic circulatory arrest in newborn dogs Values represent means ± SEM for 15 animals for up to 3 h of recovery, 13 animals at 4 h of recovery, and 10 animals at 6 and 8 h of recovery. Values for PaO_2 always exceeded 60 mm Hg (*MABP* mean arterial blood pressure)

**P* < 0.05 compared to pre-arrest; ***P* < 0.05 compared to immediately preceding value

Variable	MABP (mmHg)	Heart rate (beats/min)	pHa	$PaCO_2$ (mmHg)	Calc. HCO_3 (meq/l)	Glucose (mg/dl)
Pre-arrest	60 ± 2	182 ± 6	7.40 ± 0.01	35.7 ± 0.8	21.7 ± 0.7	185 ± 9
Recovery						
15 min	83 ± 2*	200 ± 5	7.04 ± 0.03*	41.3 ± 1.9	10.8 ± 0.9*	321 ± 31*
1 h	62 ± 2**	203 ± 4	7.28 ± 0.04*,**	31.6 ± 1.4	15.2 ± 1.4*,**	242 ± 26*,**
2 h	48 ± 3**	216 ± 9	7.35 ± 0.02	36.1 ± 2.4	19.5 ± 1.1	204 ± 23
3 h	55 ± 4	229 ± 12*	7.39 ± 0.03	34.7 ± 3.5	20.5 ± 2.0	156 ± 8
4 h	48 ± 6	239 ± 17*	7.34 ± 0.02	40.1 ± 3.8	21.0 ± 1.9	135 ± 20
6 h	48 ± 6	236 ± 7*	7.40 ± 0.05	36.1 ± 4.3	21.8 ± 2.6	143 ± 11
8 h	53 ± 4	223 ± 10*	7.40 ± 0.04	38.6 ± 2.3	23.7 ± 1.5	132 ± 10*

Table 3 Distribution of brain lesions following asphyxic circulatory arrest in newborn dogs (+ neuronal necrosis present, – neuronal necrosis absent, *Asphyx*. asphyxia, *Card. Arr*. cardiac arrest, *Caud*. caudate nucleus, *Puta*. putamen, *Amygd*. amygdaloid nucleus, *Thal*. thalamus, *Retic N*. reticular nuclei, *Inf. Coll*. inferior colliculus, *Trig. N*. trigeminal nucleus, *Vestib. N.* vestibular nucleus)

Animal no.	Duration (min)			Cortex		Caud	Puta	Amygd	Thal
	Asphyx	Card Arr	Total	Pyriform	Other				
1	5	10	15	–	–	–	–	–	–
2	10	10	20	–	–	–	–	–	–
3	12	12.5	24.5	+	–	+	+	–	+
4	4	20	24	+	–	–	+	–	–
5	13	12.5	25.5	+	–	–	–	–	–
6	14	15	29	+	–	–	+	+	–
7	15	15	30	+	–	–	+	–	+

Animal no.	Midbrain			Pons		Medulla			Spinal cord[a]
	Retic N	Red N	Inf Coll	Retic N	Trig N	Retic N	Trig N	Vestib N	
1	–	–	–	–	–	–	–	–	–
2	+	–	–	+	–	+	+	–	–
3	+	–	+	+	+	+	–	–	–
4	+	–	–	+	+	+	+	–	+
5	+	–	+	+	+	+	+	+	+
6	+	–	+	+	+	+	+	–	+
7	+	–	–	+	+	+	+	–	+

[a] High cervical spinal cord examined only

Eight puppies survived 8 h of recovery to undergo perfusion-fixation of their brains. Three animals expired between 3 and 5 h of recovery, owing to intractable secondary hypotension and subsequent cardiac arrest. The non-surviving animals included two which underwent prior cardiac arrest for 10 min and one for 20 min.

Of the animals subjected to total circulatory arrest by the i.v. injection of KCl (n = 15), one animal was subjected to cardiac arrest for 10 min, two for 15 min, two for 20 min, four for 25 min, two for 30 min, three for 35 min, and one for 40 min. All animals were successfully resuscitated, with resuscitation times ranging from 2 to 9 min. Ten animals survived 8 h of recovery to undergo perfusion-fixation of their brains. One animal was allowed to survive for 24 h of recovery. Five animals did not survive, owing to secondary systemic hypotension and cardiac arrest; including one previously arrested for 10 min, two for 25 min, and two for 35 min.

Table 4 Distribution of brain lesions following cardioplegic circulatory arrest in newborn dogs (+ neuronal necrosis present, – neuronal necrosis absent, *Caud*. caudate nucleus, *Puta*. putamen, *Amygd*. amygdaloid nucleus, *Thal*. thalamus, *Retic N*. reticular nuclei, *Inf. Coll*. inferior colliculus, *Trig. N*. trigeminal nucleus, *Vestib. N*. vestibular nucleus)

Animal no.	Duration of cardiac arrest (min)	Cortex		Caud	Puta	Amygd	Thal
		Pyriform	Other				
1	15	–	–	–	–	–	–
2	15	–	–	+	+	+	–
3	20	+	–	–	–	–	–
4	20	–	–	–	–	+	–
5	25	–	–	–	+	–	–
6	30	+	–	+	+	–	–
7	30	+	–	+	+	–	–
8	35	+	–	+	+	+	+
9[b]	30	+	–	–	+	–	–

Animal no.	Midbrain			Pons		Medulla			Spinal cord[a]
	Retic N	Red N	Inf Coll	Retic N	Trig N	Retic N	Trig N	Vestib N	
1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	+	–	+	+	+	+	+	–	+
4	+	–	–	+	+	+	+	+	+
5	+	–	–	+	+	+	+	–	+
6	+	–	–	+	+	+	+	–	+
7	+	–	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+
9[b]	+	–	–	+	+	+	+	–	+

[a] In all animals but in animal 9 high cervical spinal cord examined only

[b] Histologic examination in this animal unlike others was done at 24 h of recovery from cardiac arrest instead of 8 h

Table 5 Severity of brain stem lesions following asphyxic circulatory arrest in newborn dogs

Animal no.	Duration (min)			Number of damaged neurons per mm^2			
	Asphyxia	Cardiac arrest	Total	Midbrain	Pons	Medulla	Total (average)
1	5	10	15	0	0	0	0
2	10	10	20	9.30	6.22	17.89	11.14
3	12	12.5	24.5	10.34	4.84	11.22	8.80
4	4	20	24	16.90	13.62	80.95	37.16
5	13	12.5	25.5	11.97	44.09	72.91	42.98
6	14	15	29	38.42	37.95	103.37	59.91
7	15	15	30	11.00	7.98	30.69	16.56

Systemic physiologic variables

Systemic physiologic variables during and following total circulatory arrest caused by asphyxia are shown in Table 1. At 15 min of recovery from cardiac arrest, MABP was increased (+12.5%) above the pre-arrest, baseline value; subsequently MABP was not different from the baseline value. Heart rate during early recovery differed little from the baseline value. The pHa value was depressed at 15 min of recovery, owing to a combined respiratory and metabolic acidosis, both of which were corrected by 1 h. A slight respiratory acidosis was apparent between 4 and 8 h of recovery, when the animals were breathing spontaneously. Plasma glucose increased substantially above the baseline value during the early recovery interval, decreasing to the physiologic range by 3 h.

Systemic physiologic variables during and following cardioplegic circulatory arrest are shown in Table 2. The findings were comparable to those seen in the asphyxiated animals with the notable exception that the puppies subjected to circulatory arrest without prior asphyxia exhibited no respiratory acidosis but more severe metabolic acidosis at 15 min of recovery. The metabolic acidosis required up to 3 h of recovery for complete normalization. The early recovery surge in MABP and plasma glucose was seen in both groups.

Systemic physiologic variables in the single newborn dog which underwent cardioplegic circulatory arrest and recovery for 24 h were similar to those seen in the animals recovered for only 8 h (Table 2). Between 8 and 24 h of recovery, MABP, heart rate, oxygen and acid-base balance, and plasma glucose remained within the normal range for newborn dogs.

Neuropathologic findings

Gross examination of brains from both KCl-induced cardiac arrested animals (n = 10) and asphyxiated animals with cardiac arrest (n = 8) showed a mild to moderate degree of choroid plexus hemorrhage with extension into the ventricles and the subarachnoid space of the posterior fossa. Microscopic examination of brains and high cervical spinal cord showed suboptimal fixation in two animals with KCl-induced cardiac arrest for 25 and 40 min, and one animal with asphyxia and cardiac arrest for 8 min, leaving eight and seven animals in the two groups, respectively, for neuropathologic studies.

Table 6 Severity of brain stem lesions following cardioplegic circulatory arrest in newborn dogs

Animal no.	Duration cardiac arrest (min)	Number of damaged neurons per mm^2			
		Midbrain	Pons	Medulla	Total (average)
1	15	0	0	0	0
2	15	0	0	0	0
3	20	7.20	2.26	21.21	10.23
4	20	8.05	10.82	12.36	10.41
5	25	5.52	3.56	95.29	34.79
6	30	23.01	73.12	73.16	56.43
7	30	35.23	60.21	149.88	81.77
8	35	142.43	256.07	178.98	192.49

The distribution of lesions in both cardiac arrest groups was similar and mainly involved the brain stem structures, while the cerebral cortex and other regions within the cerebral hemispheres were relatively spared (Tables 3, 4). In the cerebral cortex, only the pyriform cortex was involved, and the damage consisted of a few isolated or small aggregates of acidophilic neurons. Cerebral white matter and hippocampus were not damaged. Striatal lesions consisted of a few acidophilic neurons in the lateral caudate and in the medial and inferior putamen. Amygdaloid nucleus and thalamus were infrequently involved, and the damage consisted of only a few acidophilic neurons in the lateral and ventral regions, respectively. In brain stem, the reticular nuclei were frequently involved. Among these, the nucleus reticularis profundus of the mesencephalon, nucleus reticularis pontis caudalis, and nucleus reticularis parvicellularis and ventralis in the medulla were most severely involved. The other reticular

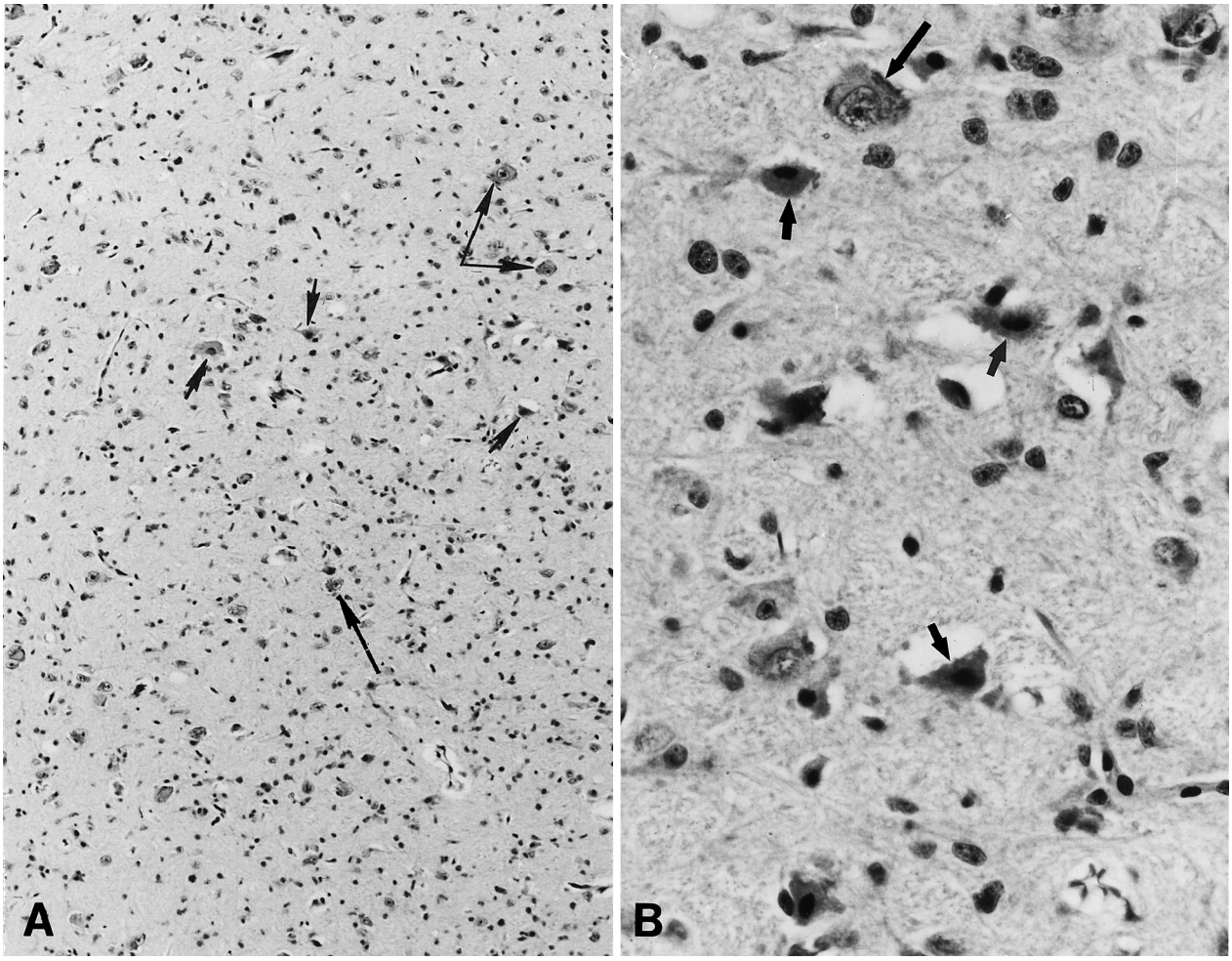

Fig. 1 Pons (region of the nucleus reticularis pontis caudalis) from an animal with normothermic circulatory arrest and 24 h of recovery showing extensive neuronal necrosis (*short arrows*). *Long arrows* indicate normal neurons. **A**, **B**. H&E; **A** × 70, **B** × 280

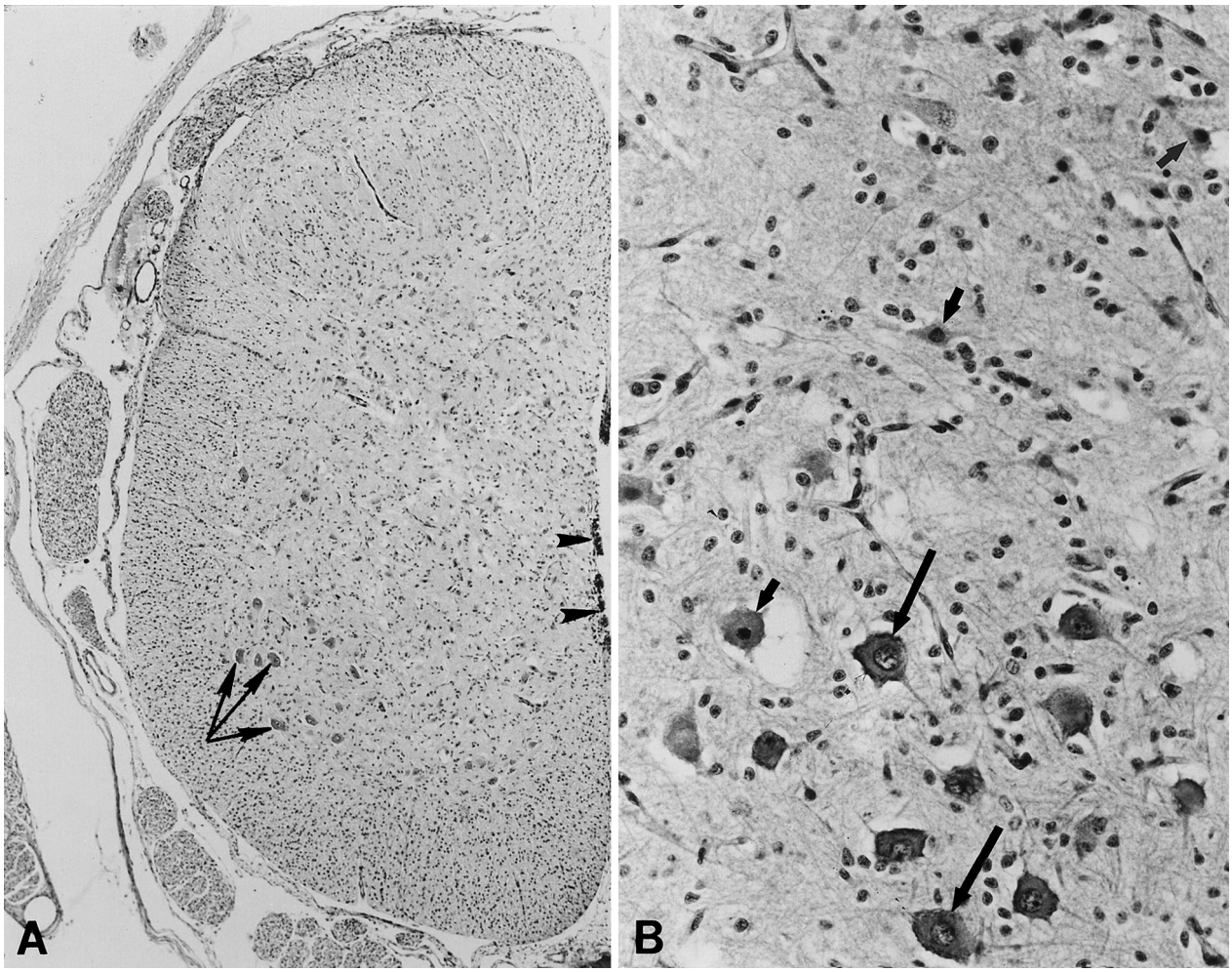

Fig. 2 Lumbosacral spinal cord from animal with normothermic circulatory arrest and 24 h of recovery. **A** Low-power view of the hemicord in cross-section showing extensive neuronal necrosis. **B** Higher-power view of same section. *Short arrows* necrotic neurons, *long arrows* normal anterior horn cells, *arrowheads* ependyma. **A**, **B** H & E; **A** × 40, **B** × 140

nuclei, including the nucleus reticularis gigantocellularis and lateral reticular nuclei in the medulla were less severely involved. Other brain stem nuclei that were damaged, according to their decreasing order of vulnerability, included the spinal trigeminal nucleus, inferior colliculi, and vestibular nucleus. The red nucleus, superior olive and posterior accessory olive were involved only in one animal that showed the most severe brain stem damage. The high cervical cord examined showed neuronal necrosis in most of the animals that had brain lesions. The cerebellum did not show lesions. The liver, kidney, and heart were examined from the animal with 30 min of cardiac arrest and 24 h recovery. The liver and kidney were normal, but the heart showed few small foci of myocardial necrosis in sub-endocardial region.

Neuronal counting showed that in most animals of both groups, the medulla was more severely involved than the midbrain and pons (Tables 5, 6). There was also direct correlation between the length of cardiac arrest and the severity of the damage in the medulla ($P = 0.001$) and the overall brain stem damage ($P = 0.004$) in animals with KCl-induced cardiac arrest. This correlation became less significant in the pons ($P = 0.017$) and midbrain ($P = 0.026$). There was no direct correlation present between the length of asphyxia combined with the period of cardiac arrest, the length of asphyxia alone, or the length of cardiac arrest alone and the severity of damage in the individual brain stem regions or the overall brain stem damage.

For the animal that underwent 30 min of KCl-induced normothermic cardiac arrest and survival for 24 h instead of 8 h post cardiac arrest, gross examination showed a mild to moderate ventricular hemorrhage and hemorrhage in the subarachnoid region, mainly in the posterior fossa. Microscopically, the type and the distribution of neuronal alterations were essentially similar to the animals with 30 min of circulatory arrest and 8 h of recovery, except that the extent of neuronal damage was less severe and the neurons were in more advanced stage of nuclear pyknosis and had bright red acidophilic perikarya. The lesions had the appearance of selective neuronal necrosis rather than infarct (Fig. 1). The other cells appeared intact as were the blood vessels, and the neuropil did not become spongy.

Cerebellar folia showed a few small foci of fresh hemorrhage. The spinal cord was examined in its entirety. Serial sections of the spinal cord showed neuronal necrosis that mostly involved the intermediate gray matter. In the severely involved segments of the cord, the lesion extended into the anterior and posterior horn cells, but never to the substantia gelatinosa (Fig. 2). The most severely involved regions were the distal half of the cervical segments and the lower lumbar and sacral regions. The upper four cervical segments were mildly involved and the entire thoracic cord together with the first two lumbar segments showed no lesions. No evidence of frank infarction is noted at this recovery interval. A small focus of fresh hemorrhage was seen in one of the spinal cord sections in the white matter. Otherwise the entire white matter, spinal roots, and sensory ganglia were normal.

Discussion

The results of the present study suggest that normothermic circulatory arrest following either asphyxia or cardioplegia with KCl in newborn dogs for intervals of 10 and 15 min, respectively, or longer, produces brain and spinal cord damage. The distribution of the cerebral lesions is similar in both groups and involves mainly the brain stem, particularly the reticular nuclei. The thalamus, striatum, amygdaloid nuclei and the entorhinal cortex are only mildly damaged. The hippocampus, neocortex, cerebral white matter and cerebellum remain intact even after 35 min of cardiac arrest.

Vulnerability of the brain stem structures and spinal cord to a hypoxic-ischemic insult has previously been demonstrated in perinatal animals of other species, including primates and guinea pigs [2, 20, 22, 27, 40]. In these models, asphyxia during fetal life was produced by interruption of the maternofetal blood supply for various durations, while preventing fetal air breathing. Asphyxia for 11–19 min in the near-term fetal monkey was associated with symmetrical lesions in the brain stem and deep cerebral and cerebellar nuclei, and the lesions frequently extended into the spinal cord [20, 27]. The cerebral cortex, cerebellar cortex, and hippocampus were relatively spared. In the spinal cord, the intermediate zone of the gray matter was more vulnerable, but in the severely involved regions, the lesion extended into the anterior horn.

Monkey fetuses of earlier gestational ages required longer periods of asphyxia to develop brain lesions, but the lesions were essentially similar to those produced by total asphyxia in near-term fetal animals [20]. Nevertheless, minor differences were noted in the distribution of the lesions in fetuses at different gestational ages. In earlier fetuses, the lower brain stem tegmentum and the spinal cord were more frequently damaged than in the near-term fetuses. In contrast, the forebrain structures became more resistant to the damaging effect of total asphyxia.

Windle et al. [40] produced a diversity of brain and spinal cord lesions in term and near-term guinea pig fetuses that had been asphyxiated for 4.5–21 min followed by resuscitation periods of a few seconds to 66 min. In some animals, the forebrain bore the brunt of the damage, while in others the thalamus, brain stem and spinal cord were primarily damaged. The topographic variation of the lesions among the animals in this study might have been related to the marked variation that existed in the duration of both asphyxia and resuscitation among the animals. In general, the lesions tended to occur in the thalamus, geniculate bodies, and the brain stem reticular formation.

The brain and spinal cord damage seen in the newborn dogs in the present investigation is essentially similar to that described in asphyxiated fetal monkeys and guinea pigs. Generally, in cases of complete cardiac arrest with or without preceding asphyxia in the perinatal period, the damage is predominantly located in the brain stem and spinal cord. In the brain stem, the tegmental nuclei and the caudal regions are particularly vulnerable. In the spinal cord, lumbosacral gray matter bears the brunt of damage. This pattern of regional vulnerability to circulatory arrest differs from that seen in adult animals,where the cerebral cortex, hippocampus, and cerebellar cortex are the most vulnerable [8, 15, 24, 37–39].

The underlying mechanism for the shift in the vulnerability of the central nervous system regions in circulatory arrest from predominantly caudal structures in fetal and newborn animals to more rostral forebrain regions in adults is not known. The shift in topography might relate to a variety of factors including the relative regional maturity of neurons, developmental changes in regional cerebral blood flow and metabolism, or other local and systemic factors that occur during development. It is well known that maturation of the structures within the central nervous system generally proceeds in a caudal to rostral direction [5, 10], and most neurons in the spinal cord and brain stem mature earlier than those in the forebrain region, particularly the cerebral and cerebellar cortical neurons and the hippocampal neurons. Relative maturity of neurons and their increased metabolic activity [4], in turn, would render the brain stem structures and the spinal cord of the immature animal vulnerable to the damaging effect of circulatory arrest, while leaving the forebrain structures, such as the cerebral cortex, relatively intact. Cardiac and other systemic complications that tend to occur in the post-circulatory arrest recovery period, particularly in adult animals, might also play a role in the determination of the distribution of lesions within the central nervous system. It has been demonstrated in adult animals that cardiac arrest followed by resuscitation is often complicated by hypotension due to ischemic myocardial damage or other factors [23, 32]. The secondary hypotension might in part be responsible for the usual adult pattern of cerebral lesions. Indeed, an uncomplicated, complete cardiac arrest in adult animals mostly damages the brain stem structures with relative preservation of the cerebral and cerebellar cortex and hippocampus [16]. Similarly, in the immature animal, the distribution of cardiac arrest damage in the central nervous system will shift from caudal to rostral forebrain regions if the cardiac arrest is not complete [21].

The pattern of distribution of central nervous system lesions is altered in the newborn dog when hypothermia is induced prior to the circulatory arrest [17–19]. We have previously demonstrated in a newborn dog model of hypothermic circulatory arrest that hypothermia (20° C) shifts the lesions from spinal cord and brain stem structures to the forebrain regions, especially the cerebral cortex. The reason for this dramatic redistribution of ischemic lesions in the hypothermic newborn dog is not known. This may be related to a redistribution of metabolic activity during hypothermia. As mentioned previously, cerebral metabolism among gray matter structures is high in brain stem nuclei in normothermic newborn dogs and declines in a caudal-to-rostral progression through the neuraxis [4]. However, during hypothermia, metabolism is substantially reduced to uniform rates in both brain stem and forebrain structures [26], rendering the cerebral cortex equally if not more vulnerable to ischemic brain damage during hypothermia.

The present findings are relevant to human subjects in that an episode of cardiac arrest uncomplicated by significant post-arrest hypotension, particularly in newborn infants [9, 14, 29], and less frequently in children and adults, [6, 11] might result in preferential involvement of the brain stem structures. In the brain stem, the reticular formation is among the most vulnerable structures to damage from cardiac arrest [14, 33]. Vulnerability of the spinal cord to asphyxia and circulatory arrest, as demonstrated in the present study, has been well documented in human newborn and older infants [3, 7, 33, 36]. At this age, the spinal cord lesions are most prominent in the lumbosacral segments [7, 36] and are often associated with ischemic lesions of the deep cerebral hemispheric structures and brain stem nuclei, with relative sparing of the cerebral and cerebellar cortex [7, 33, 36]. Similar hypoxic-ischemic spinal cord lesions may occur in adults [1]. The lesions frequently involve the spinal cord gray matter, with relative sparing of the white matter. In premature newborn infants, spinal cord lesions appear as infarcts, while in term infants, similar to the findings of the present study, the lesions have the appearance of neuronal necrosis [36]. The reason for the preferential vulnerability of the spinal cord gray matter to an ischemic insult and its predilection to occur in the lumbar region is not known.

Another interesting observation in our newborn dog model of circulatory arrest is the presence of ventricular hemorrhage. Hemorrhages mainly originated from the choroid plexus and were present in those animals with cardioplegic-induced and asphyxia-associated cardiac arrest under both the normothermic and hypothermic conditions [18]. Likewise, ventricular hemorrhage is not infrequent in the human newborn term infant following perinatal asphyxia [30, 31]. It has been suggested that most of these ventricular hemorrhages originate from the choroid plexus [12, 30].

In conclusion, we have demonstrated that in the normothermic neonatal dog model of circulatory arrest the brain stem nuclei, particularly the reticular nuclei, and the spinal cord gray matter are the most vulnerable central nervous system structures to the ischemic insult, while the cerebral cortex is relatively resistant. A reverse of this pattern of regional vulnerability is observed when the circulatory arrest is produced in the hypothermic neonatal dog. It is suggested that this dramatic shift in the regional vulnerability rendered by hypothermia during circulatory arrest is at least partly related to the changes in the regional blood flow and metabolism of the central nervous system. Further studies in this model and other models of circulatory arrest in neonatal animals are required to explain the mechanisms by which hypothermia alters the regional distribution of lesions in circulatory arrest.

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