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Blood Flow in Seven Regions of the Brain during Endotoxin Shock in the Dog (39907)

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Introduction. Recent work from this laboratory shows that cerebral blood flow (CBF) is severely decreased (50–60% of control) during endotoxin shock in the artificially (1) or spontaneously (2) ventilated dog; cerebral metabolic disorders, such as increased cerebral O_2 uptake and decreased glucose uptake, have also been indicated in a preliminary study (3).

Since these studies provide no information relevant to regional cerebral blood blow (rCBF), particularly to blood flow in the cardiovascular regulating centers of the hindbrain, it was considered important to investigate this problem. The current study was therefore undertaken to determine blood flow in the pons, medulla, hypothalamus, thalamus, cortex, cerebellum, and pituitary gland during irreversible endotoxin shock.

Methods. Twenty-six mongrel dogs of both sexes, weighing 8.2 ± 3 kg, were anesthetized with sodium pentobarbital (30 mg/kg). An endotracheal tube was inserted and animals were respirated with a positive-pressure respirator. Pressure recordings were made using Statham pressure transducers connected to a Sanborn direct-writing recorder.

rCBF was calculated by the radioactive-labeled particle distribution technique (4). Polyethylene cannulas were placed into the right femoral artery (for the measurement of systemic arterial pressure), into the aorta through the left femoral artery (for withdrawal of reference blood after each microsphere injection), into the left femoral vein (for the infusion of endotoxin and drug administration), and into the left ventricle via the left carotid artery (for microsphere injection).

Purified *E. coli* endotoxin (Difco), 2 mg/kg (LD_{100}), was diluted in normal saline to yield a 20-ml suspension. Following the cannulations and first microsphere injection, the endotoxin was administered over an approximate 5-min period.

Four- to five-tenths milliliter of ^{141}Ce and ^{85}Sr microspheres (15 ± 5μm; 3M Company) was thoroughly mixed in a glass tube with 3 ml of 20% dextran using an ultrasonic mixer. A small amount of Tween 80 was added to each microsphere vial to limit aggregation of microspheres. Approximately 2×10^5 microspheres were injected at 0.1 mCi/ml. The suspension was then injected into the left ventricle as a bolus, and flushed with 3.5 ml of saline. At the same time, a 3-min reference blood sample was withdrawn (3.88 ml/min) from the aorta using a Harvard withdrawal pump. Duplicate tissue samples (approximately 0.5 g each) were taken from the pons, medulla, hypothalamus, thalamus, cortex, cerebellum, and pituitary, placed into plastic γ counting tubes, and weighed. Counts per minute of the blood and tissue samples were determined using a Searle Model 1185 γ counter.

rCBF was calculated by dividing counts per minute (cpm) per gram of brain tissue by counts per minute of the 3-min reference blood sample (ref. bld.) and multiplying by the withdrawal rate of the reference blood sample (RBWR) (5).

$$\text{rCBF} = (\text{cpm/g of brain/cpm ref. bld.}) \times \text{RBWR}.$$

The two blood flows calculated from the two samples of each area were averaged into one regional blood flow; regional cerebral vascular resistance (rCVR) was calculated by dividing the arterial pressure by the appropriate rCBF.

Three separate groups of dogs were utilzied: (i) 4-hr control (N = 6); (ii) 2-hr shock (N = 10); (iii) 4-hr shock (N = 10).

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All groups were prepared and treated identically, except that the control group received 20 ml of normal saline intravenously, whereas the experimental groups received 2 mg/kg of *E. coli* endotoxin suspended in 20 ml of normal saline. The 5-min intravenous infusions were started within a few minutes of the first microsphere injection. The second microsphere was injected either at 4 h after saline administration (group 1), at 2 hr (group 2), or at 4 hr after endotoxin administration (group 3). Each animal was sacrificed by Nembutal overdose within a few minutes after the last microsphere injection.

Data were analyzed using Student's t test modified for paired replicates. A P value less than 0.05 was considered significant.

Results. The following are average values ± standard errors of rCBFs (ml/min/100 g of brain) obtained at the first microsphere injection of all three series of dogs (N = 26): pons = 15 ± 3; medulla = 21 ± 3; hypothalamus = 18 ± 3; thalamus = 24 ± 3; cortex = 25 ± 3; cerebellum = 30 ± 3; pituitary = 120 ± 10.

Control group 1. Average values for arterial blood pressure, rCBF, and rCVR before and 4 hr after saline infusion are shown in Fig. 1. The open bars denote values 4 hr after saline infusion. In all regions of the brain sampled, the measured parameters did not change significantly ($P > 0.05$) over the 4-hr observation period.

Group 2 (2-hr shock). Average values for arterial blood pressure, rCBF, and vascular resistances before and 2 hr after endotoxin infusion are shown in Fig. 2. The solid bars denote values before endotoxin and the open bars denote values 2 hr after. Average blood flow after shock induction was significantly lower than preshock values ($P < 0.05$) in all regions. Specific decreases were: pons, 32%; medulla, 29%; hypothalamus, 23%, thalamus, 36%; cortex, 32%, cerebellum, 31%; and pituitary, 39%. rCVR was decreased significantly after 2 hr of endotoxin shock in the pons, medulla, and hypothalamus ($P < 0.05$) by 37, 38, and 39%, respectively, but was not significantly different from control values in the thalamus, cortex, cerebellum, and pituitary ($P > 0.05$). Arterial pressure was decreased 53% ($P < 0.05$).

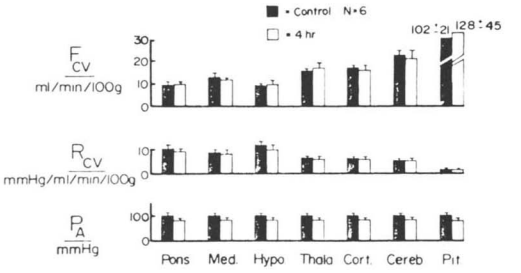

FIG. 1. Average hemodynamic responses of the cerebral vascular bed before and 4 hr after intravenous administration of saline.

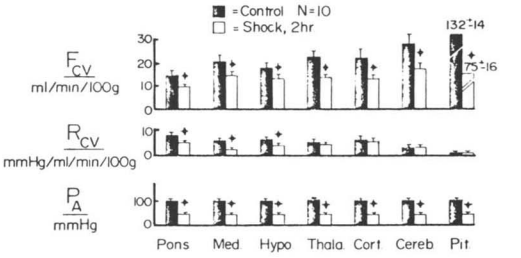

FIG. 2. Average hemodynamic responses of the cerebral vascular bed before and 2 hr after intravenous administration of 2 mg/kg of *E. coli* endotoxin.

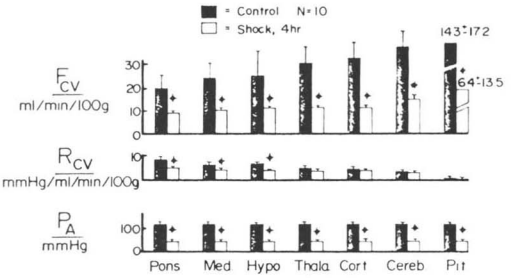

FIG. 3. Average hemodynamic responses of the cerebral vascular bed before and 4 hr after intravenous administration of 2 mg/kg of *E. coli* endotoxin.

Group 3 (4-hr shock). Average values for arterial blood pressure, rCBF, and rCVR before and 4 hr after endotoxin infusion are shown in Fig. 3. The solid bars denote values before endotoxin and the open bars denote values 4 hr later. Blood flow was decreased significantly ($P < 0.05$) in all regions. Specific decreases were: pons, 39%; medulla, 43%; hypothalamus, 46%; thalamus, 51%; cortex, 51%; cerebellum, 50%; and pituitary, 52%. rCVR was decreased significantly after 4 hr of endotoxin shock in the pons, medulla, and hypothalamus ($P < 0.05$) by 68, 18, and 32%, respectively, but was not significantly differ-

ent from control values in the thalamus, cortex, cerebellum, and pituitary ($P > 0.05$). Average systemic blood pressure was decreased 63% ($P < 0.05$).

Discussion. The current study was completed to characterize changes in rCBF during 2 and 4 hr of endotoxin shock in the dog. A review of the literature indicated the appropriateness of the radioactive-labeled microsphere technique for our purposes (6–9). Reports of CBF vary widely between and within a variety of direct and indirect techniques (10, 11). While many of the techniques in use are admittedly subjected to criticism, the wide range of "normal" values emphasizes a key problem in research involving the cerebral circulation, which was recognized in 1890 by Roy and Sherrington (12). While our rCBFs are lower than those reported by Tschetter *et al.*, who used microspheres of 25-μm diameter (6), and by Heistad and co-workers (7, 8), who used microspheres ranging in average diameter from 10 to 50 μ, they are in close agreement with flows reported by Roth *et al.* (9), who used microspheres with an average diameter of 50 μm.

The most important finding in the current study is that blood flow was severely decreased in the pons, medulla, thalamus, hypothalamus, cortex, cerebellum, and pituitary gland at 2 and 4 hr of endotoxin shock. The average decrease in blood flow at 4 hr of shock was 40%, the greatest being 52% in the pituitary. This average value is approximately the level of ischemia at which cerebral metabolic dysfunctions are clearly evident (3). It is also of interest that blood flow decrease in the hindbrain was less than in the midbrain and cortex. This lesser decrease in blood flow in the systemic cardiovascular and respiratory control centers of the brain was due to a decrease in systemic arterial blood pressure which was only partially offset by a decrease in rCVR. However, rCVR in the midbrain regions and cortex did not decrease at either 2 or 4 hr of shock, resulting in a much greater decrease in blood flow in these areas. It is indeed difficult to interpret or understand this observation, which was also noted previously by our group when cerebral venous outflow from the cortex and midbrain was measured (1, 2). In these studies (1, 2), CVR remained at near-control level until about the fourth hr of shock, at which point it increased significantly. Results from neither the current nor the past studies (1–3) provide evidence to explain this paradox. Changes in arterial CO_2 tension do not appear to be involved, since the resistance change occurs in both constantly ventilated dogs in which P_aCO_2 is maintained constant (1) and in spontaneously breathing dogs in which P_aCO_2 decreases during shock (2). Also, it occurs concomitantly with an increase in brain O_2 uptake and a decrease in brain glucose uptake (3), and the autoregulatory response is maintained for at least 4 hr of shock (2). While several explanations are possible, their presentation would be speculative.

Earlier work from our laboratory demonstrated in the dog that cerebral venous outflow from the dorsal and straight sinuses was decreased by 63, 52, and 55% 4 hr after injection of 1, 2, and 5 mg/kg of *E. coli* endotoxin, respectively (2). Wyler *et al.* (13) reported that CBF decreased 30% in the endotoxin-stressed monkey, but that blood flow in the cerebellum and brain stem was maintained after 1 hr of endotoxin shock. Weiner (14) reported a decrease in CBF from 35 to 7 ml/min/100 g 3 hr after administration of 10 mg/kg of *E. coli* endotoxin.

Comparison of CBF changes during endotoxin and hemorrhagic shock is important, but the data are conflicting. Green and Rapela (15) found no significant decrease in CBF during either hemorrhagic hypotension or the subsequent phase of shock. On the other hand, in a recent and extensive review of CBF and brain function during shock, Kovách and Sándor (16) summarize several studies which report changes in rCBF similar to those reported herein during endotoxin shock. They conclude that most available evidence suggests that a functional impairment of cortical and hypothalamic regulatory mechanisms can contribute to the irreversibility of shock (16).

We support Kovách and Sándor's interpretation, and previously proposed a similar hypothesis for gram-negative endotoxin shock (1); subsequent studies from our laboratory have consistently supported the view that malfunction of brain cardiovascu-

lar regulatory systems, subsequent to a decreased cerebral blood flow and possible other factors, may contribute to the irreversibility of endotoxin shock (1, 2).

Summary. This study was performed to determine regional cerebral blood flow during endotoxin shock using the labeled-microsphere particle distribution technique. The labeled microspheres were 15 μm in diameter. Twenty-six anesthetized and ventilated dogs were given 2 mg/kg of *E. coli* endotoxin. Regional flows were determined before endotoxin and at 2 or 4 hr of shock. Perfusion pressure and blood flow in all areas of the brain sampled were significantly decreased at 2 and 4 hr of shock. The percentage decrease in blood flows at 4 hr of shock of the regions sampled were: pons, 39%; medulla, 43%; hypothalamus, 46%; thalamus, 51%; cortex, 51%; cerebellum, 50%; and pituitary, 52%. Regional resistances of the pons, medulla, and hypothalamus were significantly decreased at 2 and 4 hr of shock. On the other hand, resistances of the cerebellum, cortex, thalamus, and pituitary were not significantly different from control values at either time. We conclude that blood flows to all brain regions measured are severely depressed during endotoxin shock.

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