SUMMARY  Blood flow to bilateral tissues (cranial and extracranial) was studied by means of the particle distribution method in two groups of anesthetized dogs (five using 25-μS radioactive microspheres, six using 15-μS microspheres) and five anesthetized stump-tailed Macaques monkeys (8-μS spheres) during unilateral sympathetic stimulation. The stimulatory parameters were adjusted to produce maximum pupillary dilatation.

In the five dogs hemispheric and regional cerebral blood flow decreased but not significantly. Flow to the extracranial tissues decreased 82%. Hemispheric brain blood flow averaged 0.70 ml/min/gm for Paco₂ of 40 mm Hg. In the six dogs sympathetic stimulation did not significantly decrease cerebral blood flow but decreased flow to extracranial tissues (72.3%). At an average Paco₂ of 33.5 mm Hg, hemispheric blood flow to the unstimulated side averaged 0.51 ml/min/gm. In the five monkeys findings were essentially the same as those observed in the dogs. The hemispheric blood flow averaged 0.36 ml/min/gm on the nonstimulated side for an average Paco₂ of 36.6 mm Hg. Under the conditions studied, electrical stimulation of the cervical sympathetic nerves does not appear to modify regional or total brain blood flow in dogs and Macaques monkeys. The vascular response in oral and other extracranial tissues is very dramatic, however.

METHODS  Which appear to provide a measure of blood flow have been utilized to estimate the cerebral blood flow response to sympathetic stimulation. Measuring venous outflow by flowmeter methodology, D’Alecy and Feigl* presented findings that electrical stimulation of the stellate ganglion in dogs produced an 8% decrease in cerebral blood flow. Traystman and Rapela, using similar flowmeter techniques in dogs, found little or no effect of sympathetic stimulation on cerebral blood flow. However, they were able to demonstrate a significant decrease in blood flow measured at the confluence of the sinuses when the lateral sinuses were kept patent. Their studies also demonstrated a marked decrease in blood flow in the common carotid artery during sympathetic stimulation.

In recent years the microsphere or particle distribution method has been developed for assessing organ and regional blood flow in numerous organs and tissues.* In cerebral blood flow has also been studied by various investigators using the particle distribution method. In our laboratory we have utilized 25-μS spheres to study the effect of unilateral sympathetic stimulation on hemispheric and regional cerebral blood flow as well as blood flow to noncerebral tissues of dogs. A minimal effect on both hemispheric and regional brain blood flow was noted. Blood flow to noncerebral tissues, however, was reduced on the average of 82% on the stimulated side compared with the nonstimulated side. With 25-μS spheres some preferential streaming may occur, and estimates of blood flow to small regions of the brain may be inaccurate. The number of beads trapped in any small regions being sampled also influences the accuracy of the flow estimates. We have therefore expanded these studies on dogs and monkeys using smaller microspheres during or near normocapnia but not during hypercapnia.

Methods

The validity of using radioactive labeled microspheres to assess the distribution in experimental animals of cardiac output to various organs including the brain has been established by others and ourselves. In using the particle distribution method the major assumptions are as follows: (1) The microspheres are well mixed when injected into the left heart and then distributed according to the blood flow; (2) essentially all the microspheres are trapped in the microcirculation; (3) the blood flow is not significantly altered during the time period in which the spheres are trapped; and (4) the cardiac output or a reference flow can be accurately determined. In the present study the reference flow was measured by withdrawing arterial blood at a known rate (7 to 8 ml/min) beginning prior to the injection of the microspheres and continuing for approximately 30 seconds thereafter. The withdrawal period was usually 1 minute, and the duration for injecting microspheres was usually 20 seconds beginning 10 seconds after starting arterial sampling. The number of spheres (N) withdrawn during arterial sampling divided by the reference flow (V/t) provides an estimate of the integrated value for the concentrated time curve required by the indicator-dilution techniques for calculating flow. Flow (F) per gram in any tissue i is then the ratio of the number of microspheres in the tissue to the integrated value (i.e., F = (N_i)/(V/ t)). Since the microspheres are labeled with an isotope, radioactivity (A) is proportional to the number of spheres, thus F = (A_i)/(A/ V).

Experimental details of the particle distribution method for measuring local blood flow have been published elsewhere by ourselves and others. In brief, each of one or more quantities of microspheres of a known activity is injected into the left heart by ventricular catheterization or via the left atrium by acute or chronic placement of a cannula in a pulmonary vein or atrium. Arterial pressure is usually monitored continuously throughout the experiment for assessing the hemodynamic status of the animal, especially during the reference sampling period. Arterial blood samples are taken for determining blood gases, pH, and hematocrit. In our initial studies using the 25-μS microspheres, the five animals were anesthetized with sodium pentobarbital, 30 mg/kg, and supplemented at about 30-minute intervals to maintain a surgical level of anesthesia. A short segment of the sympathetic fibers in the left cervical chain was freed just distal to the caudal ganglion. The stimulus was initiated 30 seconds prior to beginning arterial
sampling for the reference flow using 20 to 30 Hz, 5 to 10 v, and 3 to 5-msec duration pulses with a bipolar electrode. The pupil of the left eye was examined to observe the extent of pupillary dilatation, and CeM1 labeled microspheres were injected 10 seconds after initiating arterial sampling, i.e., 40 seconds after initiating the stimulus. The blood flows were therefore measured during the period 40 to 60 seconds after initiating the stimulus. About 10 seconds after completing reference flow sampling (or 100 seconds after beginning the stimulus), electrical stimulation was discontinued and the animal allowed to recover. About 25 minutes later, a second injection of microspheres labeled with a different isotope (Sr) was made to provide a means of comparing flow on the right side and left side under nonstimulatory conditions.

Subsequently, animals were injected with a lethal dose of concentrated KCL. The entire brain and selected noncerebral tissues were removed. Radioactivity per gram of tissue of the two different isotopes was determined using gamma scintillation counting techniques. Samples were taken and counted so that blood flow to the right and left hemisphere, to selected small regions of the brain, and to various noncerebral tissues could be calculated. The activity of each isotope in the respective reference flow sample was determined, and tissue blood flow was calculated by the equation cited above.

Since microspheres of 25-μ diameter may distribute differently from blood flow, a similar investigation was accomplished on another six dogs using microspheres averaging about 15 μ. Procedures in these experiments were essentially the same as in the initial study with several exceptions. The anesthesia management was the same as for the initial study with the 25-μ microspheres. In two animals the stimulus was applied to the cervical branch of the stellate ganglion after thoracotomy. These dogs were artificially resired, and microspheres were injected through a cannula placed in the pulmonary vein. The electrical current was measured in all six dogs and averaged 4.0 ma, using 4 to 5 v, 13 to 25 Hz, and 3-msec duration pulses to get maximum pupillary dilatation on the stimulated side. Since the effect of sympathetic stimulation on brain blood flow is controversial, it was our intent to minimize surgical neck dissection for isolating a portion of the cervical sympathetic system. The nerve was therefore carefully freed from surrounding tissues, using microdissection procedures to insure minimal injury to the nerve fiber.

**Results**

In the initial studies during left side sympathetic stimulation, blood flow to noncerebral tissues on the right side was not significantly different from the average of the combined right and left side control blood flow (table 1). Blood flow to these noncerebral tissues on the stimulated side decreased significantly. Brain blood flow, by combining the left and right hemispheric values during nonstimulatory conditions, averaged 0.76 ml/min/gm. During the stimulatory period the flow to the right hemisphere averaged 0.70 and 0.66 to the left hemisphere (table 1). During nonstimulatory conditions the mean PaCO2 was 39 mm Hg and 40 mm Hg during stimulation. Regional cerebral blood flow to the stimulated side, when compared with the right side (fig. 1), showed essentially a 1:1 relationship with a significant correlation (0.97). The left to right side hemispheric flow ratios during

**TABLE 1** Average Blood Flow to the Brain and Noncerebral Tissues of Five Dogs During Stimulation and Control Period Using 25-μ Microspheres

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Stimulation</th>
<th>Control (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left side</td>
<td>Right side</td>
</tr>
<tr>
<td>Brain</td>
<td>0.66 ± 0.22</td>
<td>0.70 ± 0.24</td>
</tr>
<tr>
<td>Dental pulp</td>
<td>0.24 ± 0.09</td>
<td>0.68 ± 0.06</td>
</tr>
<tr>
<td>Alveolar bone</td>
<td>0.10 ± 0.03</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td>Submandibular gland</td>
<td>0.11 ± 0.06</td>
<td>0.24 ± 0.07</td>
</tr>
<tr>
<td>Sublingual gland</td>
<td>0.04 ± 0.02</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Masseter muscle</td>
<td>0.02 ± 0.01</td>
<td>0.24 ± 0.10</td>
</tr>
<tr>
<td>Temporal muscle</td>
<td>0.02 ± 0.01</td>
<td>0.22 ± 0.10</td>
</tr>
<tr>
<td>Skull</td>
<td>0.01 ± 0.006</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>Tongue</td>
<td>0.05 ± 0.04</td>
<td>0.17 ± 0.10</td>
</tr>
</tbody>
</table>

*Average blood flow in ml/min/gm ± SE. Unilateral sympathetic stimulation (left side).
  1F = average blood flow for right and left side.
stimulatory conditions averaged 0.95 (range 0.85 to 1.05) and 1.07 (range 0.87 to 1.33) during control conditions. The average regional cerebral blood flow ratios ranged from 0.96 to 1.06 and 0.87 to 1.12, respectively, for these two conditions. With the use of standard statistical methods, these ratios are not significantly different from 1. The injection of the 25-μ spheres did not appear to produce any significant changes in the arterial blood pressure. Average cardiac output during stimulation was 1,929 ml/min and 1,817 ml/min during nonstimulatory conditions.

In the six dogs in which the 15-μ were injected, the Paco2 averaged 33.5 mm Hg. Average flow to the right (control) hemisphere was 0.51 ml/min/gm. The mean cardiac output was 1,850 ml/min. The relationship between the regional blood flow on the stimulated side and the control side again showed a 1:1 relationship (fig. 2). Left to right side flow ratios for regional areas ranged from 0.63 to 1.28 with a mean of 0.98. Blood flow to the entire left hemisphere averaged 3.4% less than flow to the right hemisphere, and the flow ratios ranged from 0.88 to 1.03. The percentage decrease in flow to noncerebral tissues on the stimulated side averaged 72.3%.

In monkeys the blood flow to the entire left side of the brain did not appear to be affected greatly, averaging about 4% less on the left or stimulated side compared with that on the right side (average 0.36 ml/min/gm). Flow to regional areas of the brain also appeared to be minimally reduced (4%), but to noncerebral tissues flow was dramatically decreased by 87.8% (fig. 3). The Paco2 averaged 36.6 mm Hg, and the cardiac output averaged 828 ml/min. In the monkeys stimulation altered blood pressure and heart rate (+13 mm Hg and +19 beats/min). During the control conditions the left and right hemispheric blood flows were the same. The Paco2 averaged 45.0 mm Hg, perhaps accounting for the higher cerebral blood flow of 0.60 ml/min/gm.

Discussion

Our studies examining cerebral and noncerebral blood flow changes during sympathetic stimulation using the particle distribution method can be contrasted with results obtained by flowmeter techniques. Our results in dogs, similar to those reported by Traystman and Rapela,8,9 indicated no change in cerebral blood flow but a marked decrease in noncerebral blood flow during sympathetic stimulation. D'Alacy and Feig1 attempted to measure only cerebral blood flow. The decrease which they found in cerebral flow was almost the same as that observed by Traystman and Rapela8,9 and ourselves for extracranial tissues. Traystman and Rapela8,9 suggested that the flow measurements observed by D'Alacy and Feig1 may represent a reflection of extracranial vascular responses rather than intracranial responses to sympathetic stimulation. They reasoned that in experiments in which extracranial contamination was allowed, cerebral blood flow decreased significantly during sympathetic stimulation. D'Alacy and Feig1 felt this is not the case because vessels were carefully tied off to exclude extracranial blood flow contamination, as presumably verified by a vessel cast technique.

The effect of sympathetic stimulation has been investigated in "monkeys" (Papio anubis), using electromagnetic flowmeters. Meyer, Yoshida, and Sakamoto10 reported an average decrease in internal carotid flow of 29.9% and a 67.7% decrease in external carotid artery blood flow during stimulation of the superior cervical ganglion. When stimulating the cervical sympathetic chain, the decreases reported were 24.8% and 68%, respectively, whereas microsphere methods in our study suggested a 4% (nonsignificant) decrease in cerebral blood flow and 87.8% for noncerebral blood flow in stump tail Macaques monkeys. Slight increases in blood pressure during stimulation were observed by Meyer et al.19 and ourselves. Harper, Deshmukh, Rowan, and Jennett20 used external monitoring techniques with Xe133 in the Papio anubis. Flows were calculated by the "height over area" method. They reported that sympathetic stimulation caused an 18% reduction in cerebral blood flow during normocapnia, although no
statistical significance could be claimed. During hypercapnia the 25% reduction observed was significant. Using Doppler ultrasonic flow probes on arterial vessels in rhesus monkeys, Hernandez, Raichle, and Stone suggested that the sympathetic system maintains the major cerebral vessels in a constant tonic state in the intact animal. Moreover, they found autoregulation in both intact and denervated preparations. They suggested that the sympathetic system modulates the process of cerebral blood flow autoregulation.

Yamaguchi and Waltz have utilized an autoradiographic method in cats to examine the effect of sympathetic stimulation on regional cerebral blood flow. They suggested that the decreases were nonuniform, occurring primarily in cortical structures. Accordingly, they felt the evidence was not convincing that the function of the sympathetic system is necessary for normal regulation of cerebral circulation.

The reasons for the conflicting evidence on changes in cephalic blood flow conditions of sympathetic stimulation are not clear. The experimental procedures in the various investigations are not the same; each method has certain assumptions and errors which influence the calculated data. The labeled microsphere technique offers a method for evaluating regional distribution of blood flow in the whole body and within a given organ or tissue. There are limitations, however, which must be assessed before claiming any significance for the data obtained. The effect of embolization on changing blood flow appears to be minimal (table 1) because during the nonstimulation period (second microsphere injection), flows to the brain and other tissues are essentially identical to the values observed on the right side during stimulation. The question of shunting of microspheres in dogs has been studied by Prosenz. He reported that microspheres larger than 7 μm are almost completely trapped in the "normal" dog brain. In one of our validity studies, microspheres having two different distributions of sizes (7 to 10 and 15 ± 5 μm) were injected simultaneously in ten dogs. For nine of the ten regions, the ratios of the fractional uptakes of the different-sized spheres in each region were essentially one. The exception was the pituitary in which the uptake of the 7 to 10-μ spheres was about 0.55 of the larger spheres.

Since the presentation of our findings at the symposium, recent studies by others and ourselves have provided further information about the use of these two sizes of microspheres. Marcus et al. found that in dogs approximately 8% of the 7 to 10-μ microspheres going to the brain appeared in the cerebral venous blood, whereas less than 2% of the 15-μ microspheres were shunted. In their investigation the animals were anesthetized with choralose and urethan, supplemented with a paralyzing agent. The dogs in Prosenz's study, as in ours, were anesthetized with pentobarbital. In the monkey experiments it is not clear whether the higher blood flow calculated during the control period using the 15-μ microspheres can be accounted for by the increased Paco2 alone. In our recent studies in monkeys, 7 to 10-μ and 15-μ spheres were injected simultaneously to examine changes following craniofacial surgery. Brain samples were also taken (unpublished data), and the total brain flow calculated from the uptake of the 15-μ size (0.45 ml/min/gm) averaged nearly 39% higher than that calculated from the uptake of the 7 to 10-μ size (0.32 ml/min/gm).

Also of interest is one recent experiment (unpublished data) involving unilateral sympathetic stimulation accomplished in a baboon (Papio anubis). Two different-sized microspheres were injected simultaneously in this baboon (using pentobarbital anesthesia) during stimulation. For the regional areas of the brain, the blood flow to the stimulated side averaged 91% of the control side from data obtained by each sized sphere. A statistical assessment suggested that this decrease was not significant. It appeared that nearly 30% of the 7 to 10-μ spheres going to the control or stimulated side were shunted if we assume that essentially 100% of the 15-μ spheres were trapped. If the assumptions are valid, then the microsphere method should provide conclusive evidence about the effect of unilateral electrical stimulation of cervical sympathetic nerves on cerebral and noncerebral blood flow, at least in the animals used and in conditions under which they were studied.

The Paco2 and the anesthetic used, as well as other conditions, may influence or bias the data obtained. The use of Ketamine in our monkey experiments may be questioned because of the evidence for transient sympathetic influences, particularly if administered intravenously. In our studies the Ketamine was given intramuscularly for induction and maintenance of anesthesia. Blood pressures and heart rates observed initially after arterial cannulation were as expected. The average initial values were 94 mm Hg and 114 beats per minute. The blood pressure is lower than that observed by Meyer et al. (about 130 to 140) and Harper et al. (100 mm Hg) in the Papio anubis. The 87.8% decrease in blood flow to noncerebral tissues following stimulation in our study would suggest minimal influence of Ketamine in producing a sympathetic effect on vessels to those regions. It is not known if cervical sympathetic fibers, which were stimulated, are the fibers leading to the brain in the animals studied. The pupil on the stimulated side was maximally dilated, suggesting that the nerve fibers were still functional after the microdissection procedure. The unstimulated side showed no change in size. The threshold for pupillary dilatation and vascular constriction of vessels in the brain need not be the same. The electrical current flow measured in 6 of 11 dogs and the 5 monkeys averaged slightly less than 4 ma, as determined from the maximum deflection on an oscilloscope tracing. There was no evidence of current spread to adjacent tissue.

In our studies using the 25, 15, and 8-μ spheres, findings demonstrate only a minimal effect of unilateral sympathetic stimulation on cerebral blood flow in dogs and monkeys. Very recent studies by others using radioactive microspheres in cats, dogs, and monkeys confirm our findings. Recently, Sercombe et al. using the thermal clearance method in two regions (caudate nucleus and lateral geniculate body) of the rabbit brain, found the blood flow to be decreased by about 25% and 15%, respectively, in these two regions. Blood flow to the caudate nucleus in our study tended to be reduced more than in other regions. The clearance recording in their study indicated that within 40 seconds the blood flow was reduced and tended to remain the same until stimulation was discontinued. Thus, in our studies, if any reduction occurred and stabilized, then within experimental errors the microsphere technique should...
provide information about the extent of reduction. The minimal response to unilateral sympathetic stimulation does not necessarily imply lack of sympathetic influence on the regulation of cerebral blood flow. The autoregulatory mechanisms for cerebral blood flow may predominate, and any effects of experimental electrical stimulation could be modified in order to maintain cerebral blood flow essentially constant. However, for extracerebral tissues in which autoregulatory mechanisms do not exist, blood flow cannot be restored during stimulation. Hernandez-Perez et al. have indicated that the peripheral sympathetic nervous system has a role in cerebral blood flow autoregulation mediated by resistance control of the extraparenchymal arteries.

Various reviewers have discussed the contradictory evidence for the neurogenic control of cerebral blood flow. Perhaps these contradictions could be resolved using combined methods on the same species of experimental animals. Such a "multimethod" approach could encompass not only studying effects of sympathetic stimulation but also effects of stimulating specific regions within the brain.

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