Diurnal Variation of Cerebral Blood Flow in Rat Hippocampus

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We measured local cerebral blood flow over 24 hours in 10 unanesthetized, freely moving rats to determine whether blood flow in the hippocampus fluctuated as a function of time of day. We measured hydrogen clearance at 1-hour intervals using a polyurethane-coated platinum electrode with a 1-mm bare tip implanted in the dorsal hippocampus. Individual rats displayed a wide range of local cerebral blood flow values (from 30 to 100 ml/min/100 g tissue) in a day. In seven of the 10 rats, the overall mean hippocampal blood flow for the dark cycle (7 PM–5 AM) was significantly (p<0.001, 0.01, or 0.05) greater than that for the light cycle (6 AM–6 PM), showing an average increase of 20%. Further, the maximum mean hippocampal blood flow at 11 PM in all 10 rats was 42% greater than the minimum at noon. Our study demonstrates for the first time that local cerebral blood flow in the hippocampus shows diurnal variation. (Stroke 1990;21:1464-1469)

Although total cerebral blood flow (CBF) is remarkably stable over a wide spectrum of physiologic activities ranging from intense mental work, exercise, and apprehension to sleep,1 blood flow actually changes in areas of the brain that are specifically related to each activity. Measurements have provided convincing evidence that changes in neuronal function in one part of the brain are accompanied by appropriate changes in local CBF.2 In humans, local CBF has been used effectively to detect changes in local brain activity in response to voluntary movement, speech, mental work, and somatosensory, visceral, and auditory stimulation.3-7 CBF changes accompanying the sleep-wake cycle have also been reported.2 In animals, however, few studies have specifically dealt with local CBF in relation to local brain activity. It has been shown only in rats that CBF in the cerebellum and hypothalamus changes with the sleep-wake cycle8 and that presentation of a tone increases CBF in the auditory but not the visual pathway.9

We investigated whether local CBF in the hippocampus of rats manifests diurnal variation. The hippocampus has attracted much interest because of its role in learning and memory.10 In rats, the hippocampal neurons responsive to either voluntary movement or position in the environment have been shown to fire when the hippocampal electroencephalogram (EEG) exhibits a theta wave and large-amplitude irregular activity.11-14 Such EEG activity appears predominantly during the dark cycle. Further, we have shown that multunit activity in the hippocampus, although not identified, is higher during the dark cycle than during the light cycle.15

To measure local CBF in the hippocampus repeatedly for ≥24 hours in unanesthetized, freely moving rats, we used the hydrogen clearance method based on reports by Aukland et al16 and Haining et al,17 with minor modification. Although autoradiography provides accurate and absolute CBF values,18 the measurements can be made only once, just before killing the animal. It has been reported that the hydrogen clearance method yields lower absolute values but permits an unlimited number of determinations over short intervals and is well suited to detecting time course changes in CBF.19,20

Materials and Methods

We maintained 13 adult male Wistar rats weighing 300–400 g under conditions of controlled lighting (lights on 5 AM–7 PM) and temperature (24° C) and allowed the animals free access to food and water. A 200-μm-diameter polyurethane-coated platinum electrode (Unique Medical Co., Ltd., Tokyo) with a 1-mm bare tip was implanted stereotactically under anesthesia with 31.5 mg/kg body wt pentobarbital sodium in the dorsal hippocampus of the right hemisphere, according to the coordinates of Albe-Fessard et al.21 A stainless-steel screw was used as the
CC ,//

FIGURE 1. Schematic illustration showing locations of 1-mm tip of electrode in rat brain. Each electrode is represented by bar with rat's number. CA, cornu ammonis; CC, corpus callosum; GD, gyrus dentatus.

reference electrode. The electrodes were fixed to the skull with dental cement by means of three other screws mounted in the skull to serve as anchor posts for the dental cement. CBF was first determined approximately 24 hours after surgery, when the effects of electrode implantation on CBF are reported to have disappeared.\textsuperscript{22,23} We performed surgery and the first determinations of CBF at various times of day to avoid the confounding effects of time of electrode insertion or initiation of repetitive measurement.

We constructed the chamber for measuring CBF from a metabolic cage. An upper compartment held the rat, and a lower compartment contained a duct that served either to collect feces and urine or to introduce the gas mixture. A 15\% H\textsubscript{2}-85\% air mixture was metered into the chamber at 0.6 l/min for 3 minutes while a fan on the ceiling of the upper compartment was operated. The expired H\textsubscript{2} was washed out at the same time that the H\textsubscript{2} in the atmosphere of the chamber was quickly purged through a duct in the lower compartment and another duct connected to the upper compartment. H\textsubscript{2} wash-out curves were recorded on an X-Y recorder, and CBF during the 2 minutes following the first 30 seconds of the wash-out curve was calculated with a small computer (MHG-D1, Unique Medical Co., Ltd.) based on the blood-tissue exchange theory of Kety and Schmidt.\textsuperscript{24}

Before electrode implantation and again after surgery, each rat was placed in the chamber for 2–3 days with the leads connected to the reference and measuring electrodes to adapt to the environment. During the 24-hour measurement period, the experimental room was maintained under the same conditions of controlled lighting as before. The rats were allowed free access to food and water.

A preliminary examination was performed to see whether local CBF in the hippocampus was correlated with the animal's behavior. For each CBF measurement, the rat’s behavior was scored according to our arbitrary scale as 0, resting (whether asleep or not); 1, grooming, scratching the body, or changing posture; 2, accelerating the head and neck, eating, drinking, or moving slowly; 3, moving around; or 4, moving intensively.

At the end of the experiment, the rats were anesthetized with sodium pentobarbital (31.5 mg/kg body wt) and perfused with 10\% formalin. The brains were removed for histologic identification of the electrode sites.

We calculated mean±SEM CBF for the light (6 AM–6 PM) and dark (7 PM–5 AM) cycles separately for each rat. The difference between cycles for each animal was tested by using the Student's t test. Further, analysis of variance (ANOVA) and Duncan's multiple range test were used to test the significance of fluctuations in mean CBF over the 24 hours. Cross-correlation analysis was performed to investigate whether CBF was correlated with behavior score.

Results

We completed measurements for 13 rats. No evidence of stress was seen. Figure 1 shows the locations of the tip of the measuring electrode. The entire 1-mm electrode tip was inside the hippocampus (i.e., the hippocampus proper and the dentate gyrus) in 10 rats, whereas approximately 30\% of the length of the electrode tip in one rat and 60–70\% of the electrode tip in the remaining two rats was in other structures (i.e., the corpus callosum or neocortex).
Figure 2 shows representative 24-hour profiles of CBF, with indications of the rat's behavior during measurement. The 10 rats with the entire electrode tip in the hippocampus displayed a wide range of local CBF values (from approximately 30 to 100 ml/min/100 g tissue) in a day. Further, we observed lower values of CBF (30–50 ml/min/100 g tissue) during the light cycle and higher values (70–100 ml/min/100 g tissue) from late during the light cycle into the dark cycle. Hence, the difference between the maximum and minimum CBF values was as much as approximately 50 ml/min/100 g tissue in most rats. We observed a similar tendency in the three rats with part of the electrode tip outside the hippocampus. CBF fluctuated with time of day regardless of the time measurement was started, suggesting that the time of electrode implantation and initiation of repetitive measurement was not significantly correlated with the fluctuation. We surmise that in the 10 rats with the entire electrode tip in the hippocampus CBF fluctuated with behavior. As Figure 2 shows, high and low CBF values were usually detected when the rat's behavior scores were high and low, respectively. In addition, during the dark cycle CBF was measured when the rat was moving intensively more often than during the light cycle.

TABLE 1. Local CBF of Individual Rats and Correlation With Behavior

<table>
<thead>
<tr>
<th>Rat</th>
<th>Light cycle</th>
<th>Dark cycle</th>
<th>p</th>
<th>Correlation with behavior</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>31*</td>
<td>46.8±1.4</td>
<td>46.9±2.5</td>
<td>NS</td>
<td></td>
<td>0.341</td>
<td>NS</td>
</tr>
<tr>
<td>32*</td>
<td>60.0±3.5</td>
<td>70.0±3.6</td>
<td>NS</td>
<td></td>
<td>0.342</td>
<td>NS</td>
</tr>
<tr>
<td>33</td>
<td>55.0±1.2</td>
<td>67.4±2.9</td>
<td>&lt;0.01</td>
<td></td>
<td>0.688</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>34*</td>
<td>35.4±1.0</td>
<td>43.8±1.7</td>
<td>&lt;0.001</td>
<td></td>
<td>0.501</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>36</td>
<td>56.1±2.0</td>
<td>58.5±1.6</td>
<td>NS</td>
<td></td>
<td>0.428</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>39</td>
<td>52.3±1.8</td>
<td>75.0±3.4</td>
<td>&lt;0.001</td>
<td></td>
<td>0.634</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>40</td>
<td>58.0±1.4</td>
<td>69.0±1.7</td>
<td>&lt;0.001</td>
<td></td>
<td>0.126</td>
<td>NS</td>
</tr>
<tr>
<td>42</td>
<td>48.0±1.7</td>
<td>56.8±2.6</td>
<td>&lt;0.05</td>
<td></td>
<td>0.706</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>58</td>
<td>60.9±1.7</td>
<td>74.0±2.3</td>
<td>&lt;0.001</td>
<td></td>
<td>0.512</td>
<td>&lt;0.01</td>
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<tr>
<td>62</td>
<td>55.9±1.7</td>
<td>74.0±5.0</td>
<td>&lt;0.01</td>
<td></td>
<td>0.374</td>
<td>NS</td>
</tr>
<tr>
<td>74</td>
<td>47.8±1.7</td>
<td>60.5±2.7</td>
<td>&lt;0.001</td>
<td></td>
<td>0.482</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>75</td>
<td>44.0±2.7</td>
<td>50.1±2.4</td>
<td>NS</td>
<td></td>
<td>0.021</td>
<td>NS</td>
</tr>
<tr>
<td>76</td>
<td>56.6±3.3</td>
<td>54.9±3.5</td>
<td>NS</td>
<td></td>
<td>0.417</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Student's t test was used to determine significance of difference between cycles. CBF, cerebral blood flow; NS, not significant.

*Part of the electrode tip within corpus callosum or neocortex.
Diurnal Rhythm in Hippocampal CBF

FIGURE 3. Twenty-four-hour profile of mean ± SEM hippocampal cerebral blood flow (CBF) and behavior (movement scale: 0, resting; 1, changing posture; 2, moving slowly; 3, moving; 4, moving intensively) for 10 rats with entire length of 1-mm electrode tip within hippocampus. Shaded horizontal bar shows dark cycle. *p<0.01 difference by analysis of variance and Duncan's multiple range test.

Table 1 presents mean CBF during the light and dark cycles for each rat. Seven of the 10 rats with the entire electrode tip in the hippocampus exhibited a significant difference between cycles, and the mean CBF during the dark cycle (50-75 ml/min/100 g tissue) was an average of 20% greater than that during the light cycle (44-61 ml/min/100 g tissue). Rat 34 had the electrode tip outside the hippocampus but still showed a significant difference between cycles. Cross-correlation analysis revealed a significant positive correlation between CBF and behavior score in seven of the 10 rats with the entire electrode tip in the hippocampus.

Mean CBF for the 10 rats with the entire electrode tip in the hippocampus calculated with respect to time of day is shown in Figure 3. Mean CBF during the light cycle was consistently stable and low. However, mean CBF began to rise a few hours before onset of the dark cycle and attained its maximum value at 11 PM. ANOVA revealed that this fluctuation was significant (p<0.05), and Duncan’s multiple range test indicated that the maximum value at 11 PM (mean ± SEM 68.9±3.6 ml/min/100 g tissue) differed significantly (p<0.01) from the minimum value at noon (mean ± SEM 48.6±2.5 ml/min/100 g tissue) by 42%. There was also significant fluctuation in mean behavior score (p<0.05 by ANOVA), as shown in Figure 3. Cross-correlation analysis revealed a positive correlation between CBF and behavior score (r=0.882, p<0.001).

Discussion

It is highly probable that CBF determined by our method was not influenced by its repetitive measurement since the 24-hour profiles in individual rats in which measurements were started at various times of day varied as a function of the time of day but not as a function of the number of measurements already made. Therefore, our system was confirmed to be of value for characterizing the local CBF profile over a long period in a small experimental animal.

Our study demonstrates for the first time that local CBF in the hippocampus is not stable but fluctuates considerably over a day. Each rat displayed a wide range of local CBF values, but lower values tended to occur during the light cycle and higher values during the dark cycle. In addition, the reported local CBF value for the hippocampus (50–60 ml/min/100 g tissue) as determined by the gas clearance method in conscious rats is within our range, suggesting again that our method provides relevant data. Although higher local CBF values in the hippocampus (85–148 ml/min/100 g tissue) have been reported, they were obtained in rats with other measuring electrodes in the frontal cortex and cerebellum. We also have observed that local CBF in the hippocampus is as high as 75–150 ml/min/100 g tissue when rats have another electrode implanted in the frontal cortex (unpublished observations).

The precise mechanisms for diurnal variation and an increase in local CBF in the hippocampus during the dark cycle are not clear at present. However, we propose that this increase is related to increases in neuronal function. As mentioned earlier, hippocampal electrical activity increases during the dark cycle,11–14 and it is assumed that energy-dependent changes in neuronal function are accompanied by appropriately scaled changes in local CBF.2 Isaacson26 reported that the hippocampus is implicated in the control of locomotion, exploring, rearing, and emotional behavior. Most of these activities apparently occur during the dark cycle because under normal environmental illumination rats take the greater portion of each day’s sleep during the light cycle.27

We found a significant correlation between CBF and the rat’s behavior score, showing that CBF increases with intensive voluntary movement. However, it is not certain that the movement accounts for the increase in CBF since no one has shown a relation between CBF and physical activity. Foreman et al28 found no significant increase in total and local CBF (including hippocampal CBF) during moderate and severe exercise in swine although heart rate, cardiac output, and aortic pressure rose progressively. Other studies29,30 have shown no significant...
decrease in CBF despite a sharp decrease in PaCO₂ and a severe lactic acidosis, both of which are known to alter CBF.

Alternatively, it is possible that neural factors possessing features of diurnal rhythm and regulating the function of cerebral blood vessels account for the diurnal variation of CBF. It is generally accepted that local CBF is coupled to local energy metabolism and that the CBF response to a local alteration in neuronal activity depends on local changes in the metabolic rate. However, recent reports have revealed a regulation of CBF by neural factors that is independent of the metabolic rate. Further, there has been an increase in the number of reports implicating catecholaminergic and peptidergic neurons in the regulation of the function of cerebral blood vessels. Although it is not known whether these neurons manifest diurnal rhythm in their activity, it is interesting to hypothesize that they provide hippocampal CBF with features of diurnal rhythm. Hippocampal CBF would then increase during the dark cycle, depending on either local changes in the metabolic rate related to hippocampal neuronal activity or on neural factors regulating blood vessels in the hippocampus.

A recent report based on continuous 24-hour recordings in unanesthetized rats has shown a clear diurnal variation in hemodynamic variables such as cardiac index, mean arterial blood pressure, and heart rate, with the highest values at 2 AM and the lowest at 2 PM. It is interesting that hippocampal CBF has an almost exact temporal relation to this reported hemodynamic pattern. However, it is not likely that CBF fluctuates with variations in arterial blood pressure. Because of autoregulation, CBF in rats remains constant over the mean arterial blood pressure range of 80–160 mm Hg and mean arterial blood pressure usually remains between 87 and 97 mm Hg. Smith et al have related the diurnal variation in these hemodynamic variables to diurnal changes in either the metabolic rate or autonomic nervous activity, and it is probable that diurnal variation in CBF is also related to these two factors.

References

34. Hendry SHC, Jones EG, Beinfeld MC: Cholecystokinin-immunoreactive neurons in rat and monkey cerebral cortex make symmetric synapses and have intimate association with blood vessels. Proc Natl Acad Sci USA 1983;80:2400-2404

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