
THE mechanism whereby the brain regulates its own blood flow during changes in perfusion pressure is unclear. Neurogenic (James et al., 1969), myogenic (Bayliss, 1902), and metabolic (Roy and Sherrington, 1890) theories have been suggested as playing a role in the autoregulation of cerebral blood flow (CBF). The metabolic theory proposes that a chemical factor couples blood flow to metabolism, and candidates previously suggested for this chemical linkage include hydrogen ion (Lassen, 1968; Betz et al., 1973; Kontos et al., 1977a), carbon dioxide (Severinghaus and Lassen, 1967; Kontos et al., 1977b), oxygen (Courtice, 1941), potassium (Kuschinsky et al., 1972), and lactate (Siesjo and Zwetnow, 1970).

Recently, Berne et al. (1974) found that cerebral adenosine levels were elevated following 1 minute of total ischemia and that topicaly applied adenosine dilated pial vessels; the latter observation was confirmed by Wahl and Kuschinsky (1976). However, no dilation of pial vessels occurred when adenosine was administered intra-arterially (Buyniski and Rapela, 1969; Berne et al., 1974). In addition, Rubio et al. (1975) reported an increase in brain adenosine concentrations with hypotension, hypoxia, hypocarbia, and brain excitation by electrical stimulation in rats. However, production of adenosine by brain within seconds of the onset of hypotension has not been determined. If adenosine is a mediator of autoregulation of CBF, changes in adenosine levels should be observed within seconds of alteration of perfusion pressure, since CBF

SUMMARY In rats, cerebral perfusion pressure was altered abruptly by aortic transection to determine the production by ischemic brain of adenosine and its metabolites, inosine and hypoxanthine. Brain samples were obtained after 0, 5, 10, 15, 30, and 60 seconds of ischemia. Also measured were ATP, ADP, AMP, phosphocreatine (PCr), lactate, and pyruvate. Blood pressure was monitored continuously, and arterial Po2, Pco2, and pH were measured just prior to induction of ischemia. Adenosine was elevated to 2.30 ± 0.31 (st) nmol/g at 5 seconds from a control value of 0.96 ± 0.07. A significant elevation of adenosine continued to 60 seconds (5.58 ± 1.24). Furthermore, inosine showed a progressive upward trend during the entire 60 seconds of ischemia, whereas no change in hypoxanthine occurred between the moment of transection (31.81 ± 2.01 nmol/g) and 60 seconds of ischemia (34.72 ± 2.93). PCr decreased by 1.24 μmol/g within the first 5 seconds. After the onset of hypotension, significant changes did not occur in AMP and ADP until 30 seconds, and in ATP and pyruvate until 60 seconds after aortic transection; lactate was elevated by 10 seconds. The rapid rise of cerebral adenosine within 5 seconds after the onset of ischemia supports a role for adenosine in the regulation of cerebral blood flow.

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Brain Adenosine Production in the Rat during 60 Seconds of Ischemia

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changes occur rapidly in response to this stimulus (Kenskamp and Fein, 1977; Kontos et al., 1978). The present study investigated the rapidity of rise of cerebral adenosine concentrations after the onset of systemic hypotension.

**Methods**

All studies were performed on rats weighing between 300 and 400 g and with free access to tap water and commercial rat pellets prior to surgery. Since, in earlier experiments (Berne et al., 1974; Rubio et al., 1975) dealing with the effects of sustained hypotension on the production of brain adenosine, we had used barbiturate anesthesia, the same anesthetic (sodium pentobarbital, 50 mg/kg, ip) was used in the present study. Anesthesia was supplemented when necessary, and rats were allowed to breathe spontaneously. Rectal temperature was continuously monitored and kept between 37°C and 38°C by a heat lamp. The axillary artery was exposed and cannulated for continuous recording of systemic blood pressure. Any rat with mean blood pressures below 80 mm Hg was excluded. Arterial blood (0.2 ml) was withdrawn anaerobically from the axillary cannula just prior to brain sampling, and oxygen, carbon dioxide, and pH were measured. Withdrawal of this small volume of blood did not affect blood pressure. In some rats, the EEG was monitored by bitemporal, percutaneous needle electrodes.

Through a left flank incision, the retroperitoneal aorta was exposed just below the diaphragm. A no. 3 stainless steel wire was passed around the aorta and brought out through the flank incision. Both ends of the wire then were passed through a sharp 18-gauge needle. Cerebral ischemia was produced by pulling the wire with the enclosed aorta through the sharp needle to transect the vessel (Fig. 1). Preliminary studies with this method revealed that the diastolic blood pressure fell to zero within 400 msec. Since mean blood pressure at 1 second was 8 mm Hg (Fig. 2), effective cerebral circulation presumably ceased prior to 1 second.

After transection of the aorta, brain samples were obtained by the freeze-blowing technique of Veech et al. (1973). By this method, two hollow probes are driven by powerful solenoids through the opposite sides of the skull. Compressed air then is blown into one hollow probe and the entire supratentorial compartment of the brain is blown out of the other probe and immediately frozen between two aluminum plates, precooled in liquid nitrogen (Fig. 1). A frozen wafer of brain, weighing about 1 g and 1 mm thick, is obtained and represents the supratentorial compartment of the brain. Because of the rapid removal and the homogenization of the brain tissue by the air, freezing of the brain sample occurs in less than 1 second. The rapid fall in cerebral perfusion pressure following aortic laceration is similar to that following decapitation into liquid N2, but the freeze-blowing technique provides a more rapid rate of tissue freezing.

By the methods outlined above at least four brain samples were obtained at 5, 10, 15, 30, and 60 seconds after aortic transection. In addition, samples were obtained in four groups of control rats: (1) awake rats; (2) rats anesthetized with pentobarbital but without surgery (in this group, brain samples were obtained 35–50 minutes after the induction of anesthesia, a time period appropriate in relation to the surgically prepared rats); (3) sham-operated rats (this group had axillary artery catheterization and retroperitoneal dissection of the aorta, without aortic transection); (4) rats whose brains were sampled simultaneously with aortic

![Compressed air-25 PSI](http://example.com/compressed-air.png)

**Figure 1** Experimental design demonstrating rat in head holder of brain blower. Needles mounted on solenoids are shown. After needles pierce skull, compressed air is blown into the right needle, and brain is blown out of the left needle and frozen on aluminum plates, precooled with liquid nitrogen. The axillary artery cannula is used to monitor blood pressure and to obtain arterial blood for measurement of blood gases and pH. The aorta is exposed by a retroperitoneal incision. The wire with the enclosed vessel is pulled through the needle to sever the aorta with a resultant drop in blood pressure.

![Blood pressure recording from the axillary artery](http://example.com/blood-pressure.png)

**Figure 2** Blood pressure recording from the axillary artery. Arrow indicates moment of aortic transection.
TABLE 1 Physiological Data for all Groups of Rats Prior to Transection of the Aorta

<table>
<thead>
<tr>
<th>Time* (sec)</th>
<th>(n)</th>
<th>MAP (mm Hg)</th>
<th>Pco₂ (mm Hg)</th>
<th>Pco₂ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>6</td>
<td>112 ± 6</td>
<td>89 ± 2</td>
<td>42 ± 5</td>
<td>7.37 ± 0.02</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>105 ± 4</td>
<td>92 ± 4</td>
<td>43 ± 3</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>110 ± 5</td>
<td>91 ± 7</td>
<td>40 ± 4</td>
<td>7.37 ± 0.02</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>104 ± 6</td>
<td>93 ± 5</td>
<td>37 ± 5</td>
<td>7.38 ± 0.02</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>116 ± 7</td>
<td>89 ± 4</td>
<td>39 ± 2</td>
<td>7.38 ± 0.02</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>107 ± 6</td>
<td>91 ± 5</td>
<td>38 ± 3</td>
<td>7.38 ± 0.02</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>110 ± 12</td>
<td>89 ± 3</td>
<td>41 ± 3</td>
<td>7.37 ± 0.02</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>110 ± 2</td>
<td>90 ± 0.5</td>
<td>39 ± 0.8</td>
<td>7.37 ± 0.02</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se. MAP = mean arterial pressure.

* Time after aortic transection.

Physiological Parameters

No significant differences were noted among the groups of rats with respect to arterial blood oxygen, carbon dioxide, or pH or in mean blood pressure prior to aortic transection (Table 1), and no seizure discharge was noted on the EEG after the onset of ischemia. The EEG became isoelectric, on the average, by 15 seconds after aortic transection.

Adenine Nucleosides and Base

During the first 5 seconds of ischemia, there was a 2.5-fold increase \((P < 0.005)\) in adenosine from control values obtained at the moment of aortic transection (Table 2). At 10 seconds, a peak of 3.20 nmol/g of adenosine was followed by a significant depression to 2.26 nmol/g at 15 seconds. Thereafter, adenosine levels were elevated to 2.47 at 30 seconds and 5.50 nmol/g at 60 seconds.

Measurement of inosine concentrations revealed an upward trend with increasing duration of ischemia, but significant differences from control levels were not achieved by 60 seconds (Table 2). Hypoxanthine remained constant throughout the entire ischemic period (Table 2). Adenosine, inosine, and hypoxanthine were not significantly different among the awake, anesthetized, and sham-operated and zero-time rats (Table 2).

Adenine Nucleotides and PCr

ATP levels remained constant during the first 30 seconds of ischemia (Table 3). By 60 seconds, ATP

TABLE 2 Cerebral Adenosine, Inosine, and Hypoxanthine Levels before and after Aortic Transection

<table>
<thead>
<tr>
<th>Time (in sec)</th>
<th>Awake</th>
<th>Anesth</th>
<th>Sham-op</th>
<th>0*</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>(6)</td>
<td>(3)</td>
<td>(6)</td>
<td>(5)</td>
<td>(4)</td>
<td>(6)</td>
<td>(5)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>1.26</td>
<td>1.07</td>
<td>0.85</td>
<td>0.96</td>
<td>2.30*</td>
<td>3.20*</td>
<td>2.26*</td>
<td>2.47*</td>
<td>5.50*</td>
</tr>
<tr>
<td>Inosine</td>
<td>2.47</td>
<td>2.50</td>
<td>2.64</td>
<td>2.93</td>
<td>2.91</td>
<td>3.43</td>
<td>3.26</td>
<td>3.80</td>
<td>5.36</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>±0.68</td>
<td>±0.40</td>
<td>±0.61</td>
<td>±0.74</td>
<td>±0.66</td>
<td>±0.70</td>
<td>±0.27</td>
<td>±0.77</td>
<td>±0.91</td>
</tr>
<tr>
<td></td>
<td>±0.24</td>
<td>±0.09</td>
<td>±0.11</td>
<td>±0.07</td>
<td>±0.31</td>
<td>±0.38</td>
<td>±0.11</td>
<td>±0.33</td>
<td>±1.24</td>
</tr>
<tr>
<td></td>
<td>±4.21</td>
<td>±2.69</td>
<td>±3.28</td>
<td>±2.01</td>
<td>±3.00</td>
<td>±3.22</td>
<td>±4.41</td>
<td>±3.52</td>
<td>±2.93</td>
</tr>
</tbody>
</table>

Values expressed as nmol/g ± se.

* 0 second = moment of aortic transection.
† \(P = 0.005\), as compared to 0-second value.
‡ \(P = 0.001\), as compared to 0-second value.
TABLE 3  Cerebral Adenine Nucleotides, PCr, Lactate, Pyruvate Levels, and Energy Charge before and after Aortic Transection

<table>
<thead>
<tr>
<th></th>
<th>Awake</th>
<th>Anesth</th>
<th>Sham-op</th>
<th>0*</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (nmol/g)</td>
<td>2.75</td>
<td>2.65</td>
<td>2.61</td>
<td>2.87</td>
<td>2.67</td>
<td>2.65</td>
<td>2.55</td>
<td>2.50</td>
<td>1.708</td>
</tr>
<tr>
<td>±0.10</td>
<td>±0.25</td>
<td>±0.10</td>
<td>±0.10</td>
<td>±0.08</td>
<td>±0.11</td>
<td>±0.07</td>
<td>±0.11</td>
<td>±0.10</td>
<td>±0.10</td>
</tr>
<tr>
<td>ADP (nmol/g)</td>
<td>0.377</td>
<td>0.382</td>
<td>0.340</td>
<td>0.381</td>
<td>0.371</td>
<td>0.399</td>
<td>0.419</td>
<td>0.527</td>
<td>0.688</td>
</tr>
<tr>
<td>±0.222</td>
<td>±0.036</td>
<td>±0.025</td>
<td>±0.025</td>
<td>±0.044</td>
<td>±0.021</td>
<td>±0.032</td>
<td>±0.061</td>
<td>±0.027</td>
<td>±0.052</td>
</tr>
<tr>
<td>AMP (nmol/g)</td>
<td>0.038</td>
<td>0.038</td>
<td>0.040</td>
<td>0.037</td>
<td>0.040</td>
<td>0.058</td>
<td>0.062</td>
<td>0.078</td>
<td>0.489</td>
</tr>
<tr>
<td>±0.007</td>
<td>±0.004</td>
<td>±0.004</td>
<td>±0.004</td>
<td>±0.002</td>
<td>±0.004</td>
<td>±0.007</td>
<td>±0.010</td>
<td>±0.011</td>
<td>±0.076</td>
</tr>
<tr>
<td>PCr (nmol/g)</td>
<td>4.65</td>
<td>4.00</td>
<td>4.59</td>
<td>4.40</td>
<td>3.155</td>
<td>3.041</td>
<td>2.885</td>
<td>1.525</td>
<td>0.583</td>
</tr>
<tr>
<td>±0.29</td>
<td>±0.20</td>
<td>±0.16</td>
<td>±0.14</td>
<td>±0.134</td>
<td>±0.10</td>
<td>±0.18</td>
<td>±0.16</td>
<td>±0.06</td>
<td>±0.08</td>
</tr>
<tr>
<td>Lactate (μmol/g)</td>
<td>1.35</td>
<td>0.72</td>
<td>0.71</td>
<td>0.65</td>
<td>0.65</td>
<td>1.074</td>
<td>1.755</td>
<td>2.91</td>
<td>4.28</td>
</tr>
<tr>
<td>±0.28</td>
<td>±0.06</td>
<td>±0.06</td>
<td>±0.07</td>
<td>±0.04</td>
<td>±0.10</td>
<td>±0.20</td>
<td>±0.37</td>
<td>±0.64</td>
<td></td>
</tr>
<tr>
<td>Pyruvate (μmol/g)</td>
<td>0.093</td>
<td>0.062</td>
<td>0.074</td>
<td>0.078</td>
<td>0.069</td>
<td>0.090</td>
<td>0.082</td>
<td>0.089</td>
<td>0.105</td>
</tr>
<tr>
<td>±0.004</td>
<td>±0.001</td>
<td>±0.001</td>
<td>±0.006</td>
<td>±0.007</td>
<td>±0.007</td>
<td>±0.014</td>
<td>±0.014</td>
<td>±0.009</td>
<td>±0.016</td>
</tr>
<tr>
<td>Energy Charge</td>
<td>0.934</td>
<td>0.928</td>
<td>0.928</td>
<td>0.922</td>
<td>0.926</td>
<td>0.906</td>
<td>0.962</td>
<td>0.865</td>
<td>0.670</td>
</tr>
<tr>
<td>±0.003</td>
<td>±0.003</td>
<td>±0.003</td>
<td>±0.006</td>
<td>±0.007</td>
<td>±0.014</td>
<td>±0.007</td>
<td>±0.008</td>
<td>±0.028</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as nmol/g ± SE.

Energy charge = (ATP + 0.5 ADP/ATP + ADP + AMP).

* 0-second » moment of aortic transection. As compared to 0-second value: t = P < 0.05, † = P < 0.02, § = P < 0.01, ¶ = P < 0.002.

had declined to 64% of its initial value. In contrast, PCr decreased by 1.25 nmol/g after 5 seconds of ischemia. A less rapid but significant decline occurred thereafter, so that by 60 seconds, only 13% of the initial PCr remained (Table 3). A reciprocal relationship existed for adenosine and PCr throughout the entire 60 seconds of ischemia, and linear regression analysis revealed adenosine = — 0.89 PCr — 0.9089, (r = 0.934, P < 0.005).

AMP and ADP concentrations were not significantly elevated until 30 seconds after aortic transection (Table 3), but large increases in both compounds were observed between 30 and 60 seconds. The rise in AMP, the substrate for adenosine production, was particularly striking, reaching a 13-fold increase by 60 seconds.

Energy charge was constant during the first 30 seconds of ischemia (Table 3), but a 20% decrease occurred between 30 and 60 seconds.

**Lactate and Pyruvate**

With the onset of ischemia, there was a significant elevation in lactate (P < 0.002) by 10 seconds (Table 3). Pyruvate increased slowly (Table 3) but not significantly until 60 seconds (P < 0.05). Because of the stable pyruvate levels, the lactate-to-pyruvate ratio reflects the elevation in lactate.

A decrease in both lactate (49%) and pyruvate (23%) occurred in the anesthetized, as compared to the awake, rats (Table 3), presumably due to decreased glucose utilization caused by barbiturates (Hawkins et al., 1974).

**Discussion**

The present study confirms earlier observations (Deuticke and Gerlach 1966; Berne et al., 1974; Rubio et al., 1975) that the brain can produce adenosine and, moreover, does so rapidly. Within 10 seconds of the onset of ischemia, cerebral adenosine values increased by more than 3-fold, with the major increase occurring within 5 seconds. A similar rapid elevation of adenosine has been observed in ischemic heart (Berne et al., 1971). After 1 minute, brain adenosine levels were 5.5 nmol/g, and these values are lower than those reported by Rehncrona et al. (1978) following 1 minute of ischemia. However, these investigators sampled brain by the decapitation technique which, as noted above, is a slower method of tissue freezing and is associated with more autolytic changes.

The adenosine concentrations in the present study are comparable to those reported in rat brain by Nordström et al. (1977) but lower than those of Rubio et al. (1975). Nordström et al. (1977) froze the brain in situ, whereas Rubio et al. (1975) froze the brain with bone rongeurs precooled in liquid nitrogen. The latter technique is slower than the freeze-blowing method and may cause tissue injury prior to freezing, which accounts for the higher values of adenosine.

After topical micropipette application of adenosine, Wahl and Kuschinsky (1976) found a sigmoid-shaped, dose-response curve for feline pial vessels. In the presence of physiological concentrations of bicarbonate, maximal dilation occurred between 10⁻⁵ M and 10⁻⁷ M adenosine, with the inflection point at 10⁻⁶ M. Studies in red cell ghosts (Schrader et al., 1972) and dispersed embryonic chick heart cells (Mustafa et al., 1975) suggest that adenosine does not exist intracellularly and is restricted to the extracellular space. If the extracellular space in brain is assumed to be 20%, then brain adenosine concentration during ischemia observed in the present study would range between 6 X 10⁻⁶ M and 10⁻⁶ M, which is within the vasoactive range found by Wahl and Kuschinsky (1976). Moreover, Kreutzberg and Barron (1978) have demonstrated that the astrocytic footpads that surround the blood vessels contain 5'-nucleotidase in the cell wall. Thus, the enzyme that produces adenosine is in close proximity to the vessel wall, and the diffusion distance between the site of production and the vascular smooth muscle is small.
The rate of adenosine formation is a function of substrate (AMP) availability and enzyme (5'-nucleotidase) activity. During the later phases of ischemia (30–60 seconds), adenosine production reflects increases in AMP. However, during the initial seconds of ischemia, despite large increases in adenosine, the AMP content of the brain did not become elevated. Lack of a measurable rise in AMP prior to 30 seconds may be due to the barbiturate anesthesia, since during the 1st minute of complete ischemia in rats, Nordström and Siesjö (1978) observed a decrease in elevation of AMP with phenobarbital, as compared with N₂O anesthesia. A second, and perhaps more likely, explanation of the increased adenosine production without a concomitant increase in AMP is an alteration in the activity of 5'-nucleotidase. 5'-Nucleotidase has been shown in heart to be related closely to the cell membrane of 5'-nucleotidase. 5'-Nucleotidase has been shown to be related closely to the cell membrane and interaction of decreasing PCr and increasing Mg²⁺ has been proposed as coupling adenosine to AMP, rather than deamination to inosine. The stable hypoxanthine levels are in keeping with the findings of Berne et al. (1974), who noted no change in hypoxanthine levels until 20 minutes of ischemia had elapsed. This delayed rise in hypoxanthine is explained by the lack of availability of nucleoside phosphorylase (Rubio et al., 1978). Unlike heart (Rubio et al., 1972), this enzyme in brain is located solely within the vascular endothelium. Because of an endothelial barrier to inosine, its production exceeds that of adenosine (Rubio et al., 1972). In brain, the slow rise in inosine, in contrast to the rapid rise of adenosine, may account for the lack of production of inosine, perhaps the intracellular location of adenosine deaminase may account for the lack of production of inosine, or because of unequal distributions of these two enzymes. The Km of adenosine kinase in rat brain is 2 X 10⁻⁶ M, whereas that of adenosine deaminase is 3.4 X 10⁻⁵ M (Arch and Newsholme, 1978). Although the greater affinity of adenosine kinase for adenosine may account for the lack of production of inosine, perhaps the intracellular location of adenosine deaminase may account for the lack of production of inosine, which presumably is extracellular.

The increased production of adenosine, as compared to inosine in the present study, is similar to that seen with sustained cerebral hypotension and hypoxia (Rubio et al., 1978), and the reverse of that seen in experimental cerebral anoxia. The predominant role of adenosine deaminase, rather than deamination of adenosine, may account for the lack of production of inosine, perhaps the intracellular location of adenosine deaminase may account for the lack of production of inosine, or because of unequal distributions of these two enzymes. The Km of adenosine kinase in rat brain is 2 X 10⁻⁶ M, whereas that of adenosine deaminase is 3.4 X 10⁻⁵ M (Arch and Newsholme, 1978). Although the greater affinity of adenosine kinase for adenosine may account for the lack of production of inosine, perhaps the intracellular location of adenosine deaminase may account for the lack of production of inosine, or because of unequal distributions of these two enzymes. The Km of adenosine kinase in rat brain is 2 X 10⁻⁶ M, whereas that of adenosine deaminase is 3.4 X 10⁻⁵ M (Arch and Newsholme, 1978). Although the greater affinity of adenosine kinase for adenosine may account for the lack of production of inosine, perhaps the intracellular location of adenosine deaminase may account for the lack of production of inosine, because of unequal distributions of these two enzymes.
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These previous studies used different: (1) species [rats (Ljunggren et al., 1974), gerbils (Kobayashi et al., 1977), cats (Schmahl et al., 1965), mice (Lowry et al., 1964)], (2) anesthesia [N₂O or barbiturates in differing doses (Gatfield et al., 1966; Nordström and Siesjö, 1978)]; and (3) sampling methods [in situ (Ljunggren et al., 1974), immersion in liquid N₂ (Kobayashi et al., 1977), suction freezing (Schmahl et al., 1965), and decapitation (Lowry et al., 1964)]. With the exception of Nordström and Siesjö (1978), who observed only a 15% decrease in ATP and an 11-fold increase in AMP with pentobarbital anesthesia, these earlier investigators, despite the differences in techniques, found similar ranges of alterations in brain metabolites after 60 seconds of ischemia: the decline in ATP and PCr ranged from 60 to 70% and 82 to 96%, respectively, whereas the rise in ADP, AMP, and lactate was 1.6- to 4.2-, 21- to 33-, and 2.8- to 7.8-fold, respectively. The changes are comparable to alterations in PCr, ATP, and ADP at 10 seconds of ischemia. Recently, Nordström and Siesjö (1978) compared changes in cerebral metabolites after complete ischemia in rats anesthetized with phenobarbital or N₂O. In the present study, in which we used a lower dosage (50 mg/kg) of a faster acting barbiturate (pentobarbital), the changes in PCr, ATP, and ADP at 10 seconds approximate the values in their animals anesthetized with phenobarbital (150 mg/kg) or N₂O. In the present study, in which we used a lower dosage (50 mg/kg) of a faster acting barbiturate (pentobarbital), the changes in PCr, ATP, and ADP at 10 seconds approximate the values in their animals anesthetized with N₂O.

The metabolic changes emphasized by previous investigators after profound hypotension or after the initial few seconds of ischemia may not be related directly to cerebral blood flow regulation. Metabolites such as ATP, PCr, ADP, and AMP are intracellular components and, thus, unable directly to affect vascular diameter. In contrast, adenosine appears to exist extracellularly. Moreover, as the present study documents, adenosine concentration in brain is elevated rapidly following the onset of ischemia. Dilation of pial vessels is observed within 3.5-5 seconds after clamping of the carotid arteries (Kenskamp and Fein, 1977; Kontos et al., 1978). Therefore, any metabolic factor proposed to be involved in the autoregulation of CBF must change quickly to be considered as a chemical link between metabolism and CBF. Unlike extracellular pH changes which are delayed for more than 10 seconds after the onset of ischemia (Silver, 1978), increases in adenosine, as the present study illustrates, occur within 5 seconds. Adenosine, therefore, may be a primary physiological agent in the regulation of CBF.

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References

Arch JRS, Newholme EA (1978) Activities and some properties of 5'-nucleotidase, adenosine kinase and adenosine deaminase in tissues from vertebrates and invertebrates in relation to the control of the concentration and the physiological role of adenosine. Biochem J 174: 965-977


Bayliss WM (1902) On the local reactions of the arterial wall to changes of internal pressure. J Physiol (Lond) 28: 220-231


Brady TG, O'Donovan CI (1965) A study of the tissue distribution of adenosine deaminase in six mammal species. Comp Biochem Physiol 14: 101-120

Burger RM, Lowenstein JM (1975) 5'-Nucleotidase from smooth muscle of small intestine and from brain. Inhibition by nucleotides. Biochem 14: 2362-2366


Courtie FC (1941) The effect of oxygen lack on the cerebral circulation. J Physiol (Lond) 100: 186-211


Kenskamp P, Fein J (1977) Assessment of rapid changes in local...


Brain adenosine production in the rat during 60 seconds of ischemia.
H R Winn, R Rubio and R M Berne

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