Relationship between adenosine concentration and oxygen supply in rat brain

RAFAEL RUBIO, ROBERT M. BERNE, EMMA L. BOCKMAN, AND RICHARD R. CURNISH
Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia 22901

AN abstract

Since adenosine is present in normal brain tissue and cerebrospinal fluid and since it dilates the pial vessels, it is possible that adenosine, in addition to H+ is also a mediator of the metabolic regulation of cerebral blood flow. Evidence supporting this hypothesis was obtained under various experimental conditions characterized by a change in brain oxygen supply. The brain was frozen in situ by means of a small bone rongeur precooled in liquid \( \text{N}_2\) and the tissue was processed for adenosine determination (nmol/g of tissue). Electrical stimulation of the cortex at 0, 15, 30, and 45 Hz yielded adenosine levels of 5.4 ± 0.7, 10.5 ± 1.7, 13.0 ± 1.2, and 9.0 ± 2.1 nmol/g. Arterial pressures of 87, 60, and 40 mmHg gave adenosine levels of 7.5 ± 0.76, 13 ± 2.6, and 26.6 ± 3.3, respectively. Ventilation with 29.7, 20, 10.7, and 3.5% \( \text{O}_2\) significantly increased the adenosine levels to 9.4 ± 3.0, 6.4 ± 1.2, 30.0 ± 9.3, and 63.3 ± 18.2 nmol/g, respectively. Hyperventilation significantly increased adenosine from 6.7 ± 1.0 to 11.8 ± 1.4 nmol/g. This increased adenosine level was reduced by addition of \( \text{CO}_2\) to the ventilating gas mixture. Lactate, the main \( \text{H}^+\) donor, pyruvate, and cAMP changed in a fashion parallel to adenosine. However, cAMP showed only a small increase with increases in adenosine. These findings are in accordance with the concept that adenosine and \( \text{H}^+\) may act synergistically to regulate cerebral blood flow and that endogenous adenosine may exert a small effect on cAMP formation.

Intrinsic regulation of blood flow most probably is dependent on several factors (19, 40), but it remains to be elucidated how these factors become integrated to produce the precise regulation observed in such organs as the brain and heart. Therefore, the models proposed may not be simple; they are complicated by the fact that these variables are interdependent. This is probably the reason for the controversy regarding the proposal that \( \text{H}^+\) is the sole controlling factor for brain blood flow regulation (2, 7, 12, 31, 46).

To further complicate the situation, adenosine has recently been suggested as a factor involved in the regulation of brain blood flow (6). Adenosine is found in brain tissue and cerebrospinal fluid and its concentrations increase in parallel fashion during cerebral ischemia. Therefore, it appeared appropriate to determine whether adenosine levels are related to changes in vascular resistance. In this report adenosine brain tissue levels were measured under different physiological conditions known to be associated with changes in brain vascular resistance.

METHODS

Experiments were performed in two groups of rats (250–400 g). One group of animals was anesthetized with pentobarbital intraperitoneally at an initial dose of 50 mg/kg, and supplementary doses were given when required (the maximal dose was 70 mg/kg). The other group was first anesthetized with ether and then given pentobarbital (40 mg/kg iv). With the latter method of induction, a more uniform level of anesthesia was obtained. The anesthetized rats were tracheotomized and the skin and muscles of the skull were surgically removed. Windows of about 6 x 9 mm were cut in the skull on each side of the midline and the dura was left intact. In all animals ventilation was maintained with a small-animal respirator pump adjusted to a tidal volume of 5 ml and a rate of 60/min. In some experiments the rate was increased to 100/min. In the animals initially anesthetized with ether, the experimental period was initiated 60 min after induction of ether anesthesia.

In some experiments the brain was stimulated electrically by placing a pair of electrodes on the surface of the dura approximately above both the right and left motor cortex. Stimulation consisted of square-wave pulses of 5–10 V, 3 ms in duration, and at frequencies ranging between 15 and 60 Hz. In most experiments the electrodes were made of two platinum plates (3 x 5 mm).

Variation in arterial \( \text{Po}_2\) and arterial \( \text{Pco}_2\) were produced by respiring the animals with different gas mixtures. When arterial \( \text{Po}_2\) was varied, the different ventilating gas mixtures contained 29.7, 21, 10.7, and 5.5% \( \text{O}_2\), with the difference made up with \( \text{N}_2\). When arterial \( \text{Pco}_2\) was varied, the different ventilating gas mixtures contained 0, 10, and 20.6% \( \text{CO}_2\); \( \text{O}_2\) was maintained at about 20% and the difference was made up with \( \text{N}_2\).

Variation in arterial pressure was produced by bleeding the animal. To accomplish this, heparin was administered, the lower segment of the abdominal aorta was cannulated, and the cannula was connected to a mercury manometer and to a 2-ml syringe. The blood pressure was adjusted to the desired level by bleeding the animal into the syringe.
and the pressure was maintained constant by withdrawing or reinfusing blood.

At the end of a 3-min experimental period, tissue enzyme inactivation was produced by freezing the brain in situ with small bone rongeurs precooled in liquid N₂. The rongeurs were introduced through the window opened in the right side of the skull. The frozen pellet of brain tissue trapped in the rongeur weighed between 0.35 and 0.45 g. This procedure of tissue sampling proved to be a more efficient way of enzyme inactivation than that in which the brain was scooped out with a spatula (6) and to increase significantly \( P < .04 \)–0.1) in the stimulated cerebral pressure was reduced (Fig. 1). Similar results were obtained in the stimulated and nonstimulated brains (Fig. 1).

Effect of arterial \( \text{PO}_2 \) on adenosine levels. A decrease in arterial \( \text{PO}_2 \) induced by reduction of the percent \( \text{O}_2 \) in the ventilating gas mixture caused the brain adenosine levels to increase significantly \( (P < .04–0.01) \) in the stimulated and nonstimulated brain (Fig. 2). However, an increase in the \( \text{PO}_2 \) of the ventilating gas mixture from 21 to 29.7% did not produce changes in adenosine levels.

Effects of arterial \( \text{PO}_2 \) on adenosine levels. Increase in the ventilation rate from 60 to 100/min significantly \( (P < .01) \) elevated the adenosine level, but this increase in cerebral

### Table 1. Rat brain adenosine levels at different rates of electrical stimulation of brain in two separate experiments

<table>
<thead>
<tr>
<th>Rate of Stimulation, Hz</th>
<th>Adenosine, nmol/g</th>
<th>Exp I</th>
<th>Exp II</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.3±0.2 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>10.5±1.7 (9), ( P &lt; .02 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>9.3±1.2 (5), ( P &lt; .01 )</td>
<td>13.0±1.2 (20), ( P &lt; .02 )</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>9.0±2.1 (10), NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>7.2±0.6 (5), ( P &lt; .01 )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Number in parentheses is number of animals. In all animals ventilation was kept constant at 60/min; tidal volume, 5 ml. Brains in animals of experiment I were stimulated using 2 platinum wires (0.5 mm diam) resting on the brain cortex. Parameters of stimulation: 10 V, 3 ms. In experiment II the electrodes were made of 2 platinum plates (3 x 5 mm) resting on both brain hemispheres; stimulation: 5 V, 5 ms.

![Fig. 1. Increase in brain adenosine levels produced by lowering of systemic arterial pressure. Each point represents average of 10 observations except for open circle at high pressure which is average of 5 observations. Open circles represent animals in which brains were not electrically stimulated, and filled circles represent experiments with electrical stimulation at 30/Hz, 5 V, 3 ms. Ventilation was kept constant at 60/min, tidal volume = 5 ml.](http://ajplegacy.physiology.org/)

**RESULTS**

**Effect of frequency of electrical stimulation on adenosine levels.** As the frequency of stimulation was increased, adenosine levels first increased to a maximum at 30 Hz, then declined slightly (Table 1). Although the two sets of values given in Table 3 are qualitatively similar, there is a small quantitative difference. The reason for this difference is unknown, although the different types of electrodes used may have played a role (see footnote to Table 1).
adenosine was reduced to values below control by adding CO₂ to the ventilating gas mixture (Fig. 3).

Effects of arterial P₀₂ or P_CO₂ on lactate and pyruvate. As the percent O₂ of the ventilating gas mixture was reduced, lactate and pyruvate increased, whereas the lactate-to-pyruvate ratio remained constant (Fig. 4A). Increase in the ventilation rate from 60 to 100/min with room air increased lactate and pyruvate (P < .01), whereas a reduction of these levels was produced by adding 10% CO₂ to the ventilating gas mixture (Fig. 4B). However, in contrast to the levels of adenosine (Fig. 3), further increase in P_CO₂ of the ventilating gas to 20.6% did not reduce lactate and pyruvate levels further (Fig. 4B). In the group of animals in which CO₂ was changed, the lactate-to-pyruvate ratio also remained constant, and this ratio was not significantly different from that obtained when P₀₂ was varied.

Correlation between adenosine and cAMP levels. The increase in adenosine levels associated with lowering arterial P₀₂ correlated (r = 0.63, n = 26, P < .01) with brain cAMP levels (Fig. 5). This linear representation obtained by regression analysis, although empirical, shows that a 10-fold increase in adenosine was associated with a 0.4 nmol/g increase in cAMP, which represents approximately an 80% increase above control cAMP values. Similar results were obtained when adenosine levels were changed by varying arterial P_CO₂.

Correlation between adenosine and 5'-adenosine monophosphate. The changes in adenosine levels paralleled changes in 5'-AMP when either arterial pressure, P₀₂, P_CO₂, or the rate of brain stimulation was varied in two groups of experiments (Fig. 6, r = 0.90, n = 150, P < .01). In one group, arterial pressure, rate of electrical stimulation, or P₀₂ was changed and in the other group only arterial P₀₂ or P_CO₂ was changed. Calculation of the regression lines for both groups yielded two lines that were not significantly different from each other. Therefore, the results were combined (broken line, Fig. 6). This empirical relation shows that a 7.1 nmol/g change in 5'-AMP is paralleled by a change of 1 nmol/g of adenosine.
DISCUSSION

Changes in brain tissue adenosine levels were associated with changes in either arterial pressure, $P_{O_2}$, or $P_{CO_2}$. All these procedures induce different degrees of cerebral vascular tone in different mammalian species (20, 30, 38) and are suggestive of a regulatory role of adenosine in cerebral blood flow. Although it would be helpful to have cerebral blood flow studies in conjunction with the adenosine measurements, this would have greatly complicated the experimental design. Furthermore, the few reports on cerebral blood flow in rats indicate that their cerebral vasculature responds to $CO_2$ and perfusion pressure (11, 34, 36) in a qualitatively similar manner as in other mammalian species.

Interdependence among variables affecting cerebral blood flow. Ideally, studies of blood flow regulation should be performed under conditions in which the independent variables do not trigger changes in the other variables that also influence vascular resistance. In the case of the brain it is difficult to achieve the experimental conditions for selectively changing one variable without triggering others that also affect the brain resistance vessels. Arterial pressure, $P_{O_2}$, and $P_{CO_2}$ and brain oxygen consumption are variables that affect brain vascular conductance and indirectly each other through circulatory, respiratory, and metabolic changes. Furthermore, brain blood flow correlates well with its oxygen consumption (42) and the oxygen consumption in turn is proportional to neuronal electrical activity (9, 28, 32). Thus, ideal brain blood flow regulation studies where the independent variable is perfectly defined are difficult to achieve.

Controlled neuronal electrical stimulation could be the means of approaching the ideal situation of maintaining oxygen consumption constant in the face of a change in any of the parameters ($P_{O_2}$, $P_{CO_2}$, or arterial pressure) known to affect cerebral vascular resistance and neuronal activity as well (17, 27, 29, 42). This was attempted in our experiments. The brain was electrically stimulated, and an increase in the rate of stimulation up to about 20 or 30 Hz raised adenosine levels which then decreased slightly as frequency was increased above 30 Hz. Similar changes in neuronal oxygen consumption induced by varying the frequency of stimulation have been demonstrated in brain cortical slices (32) and sympathetic ganglia (28), suggesting that a possible correlation exists between neuronal oxygen consumption and adenosine levels. Pull and McIlwain (37) have shown that electrical stimulation of brain cortical slices induces the release of adenosine into the bathing medium and that the release is blocked by tetrodotoxin. These observations suggest that the production of adenosine is associated with membrane depolarization, specifically, in this case, the generation of neuronal action potentials.

Nature of intrinsic control of cerebral blood flow. The exact mechanism(s) responsible for the regulation of cerebral blood flow is unknown and its causal nature is a matter of controversy (2, 7, 12, 25, 31, 46). It has been proposed that the H+ in the extracellular fluid is the sole agent mediating cerebral blood flow regulation (7, 46) and that lactate may constitute the main H+ donor (23). On the one hand, changes in cerebral blood flow brought about by altering arterial $P_{CO_2}$ or by brief periods of severe hypoxia (7) correlated closely with changes in cortical fluid pH. In addition, direct alterations of the pH of perivascular fluid can affect the caliber of pial vessels (46). On the other hand, sustained hyperventilation induced an increase in cerebrospinal fluid pH associated with a fall in cerebral blood flow during the first half hour (31). Thereafter, the pH gradually fell without a concomitant increase in cerebral blood flow. In fact, cerebral blood flow continued to decrease.

FIG. 5. Parallel changes in cAMP and adenosine levels produced by varying percent $O_2$ of ventilating gas mixture. A semilogarithmic scale was arbitrarily chosen for purposes of convenience. Regression line is represented by following equation: $cAMP = 0.52 + 0.40 \log_{10}$ adenosine. There was a high degree of correlation between cAMP and adenosine with a correlation coefficient $r = 0.63$ and a $P < .01$. Similar results were obtained in animals ventilated with gases containing different amounts of $CO_2$. This empirical linear approximation indicates that a 10-fold increase in adenosine is associated with only a $0.40$-nmol/g increase in cAMP. The brain tissues are same as used in Fig. 2 (g). Brains were electrically stimulated at 30/Hz, 10 V, 3 ms.
This secondary decrease in pH (0.08 U) would be expected to increase cerebral blood flow by 20%. In dogs, with prolonged or repeated reductions of brain perfusion pressure, cerebrospinal fluid hydrogen ion concentrations always increased (30). However, when perfusion pressure was returned to normal levels, the elevated lactic acid in the cerebrospinal fluid outlasted the reactive hyperemia by several hours (30). In the monkey, reduction of arterial pressure within the range of perfect autoregulation (115 to 71 mmHg) did not cause a reduction in CSF pH (2). Similarly, in the rat, reduction of systemic arterial pressure from 132 to 45 mmHg was not associated with a decrease of either the CSF or the intracellular pH (12).

Schmidt et al. (42) suggested that regulation of cerebral blood flow most probably is "achieved by summation of all of the vasodilator products of metabolism...and that no one agent should be singled out as being solely responsible." Adenosine has recently been added to the list of vasodilator products of brain metabolism and has been suggested as a regulator of cerebral blood flow (6). The present study shows that brain adenosine levels decrease when the independent variable (either arterial pressure, Po_2, or PCO_2) is increased (Figs. 1-3). However, an increase in arterial Po_2 from its normal value to that obtained by respiring animals with 30% O_2 (which does not cause a change in vascular resistance) did not reduce adenosine levels below controls (Fig. 2). Increases in arterial pressure above normal induce a proportional increase in vascular resistance, and it remains to be elucidated if adenosine levels decrease below control levels or remain constant with increased perfusion pressure. If adenosine levels were unchanged, then constancy of brain blood flow in the face of perfusion pressures greater than normal could be attributed to metabolites other than H+ or adenosine or possibly to a myogenic response.

If adenosine were involved in the control of the cerebral vasculature, a correlation would be expected between the cerebral adenosine concentration and either the blood flow, the vascular conductance, or the oxygen supply (flow X O_2/ml blood), which in turn are all a function of vascular smooth muscle tone. The three curves obtained for cerebral blood flow (or vascular conductance) when either arterial pressure, arterial Po_2, or PCO_2 was independently varied (19, 20, 30, 38) are different; however, in the case of brain adenosine levels they are similar (Figs. 1-3). Therefore, it is not possible to establish a unique correlation between either cerebral blood flow or vascular conductance and cerebral adenosine levels. However, with respect to oxygen supply the three curves (when either arterial pressure, Po_2, or PCO_2 was independently varied) follow the same trend: oxygen supply increases as the independent variable increases. Thus, an inverse correlation exists between oxygen supply and adenosine levels. However, the curve(s) relating oxygen supply and adenosine have to be defined between limits. For instance, in the cases in which arterial pressure and Po_2 are varied, the oxygen supply starts at zero and terminates at a fixed value, since further increases in aortic pressure or Po_2 above their normal values do not cause any greater increase in cerebral blood flow or blood oxygen content. With either decreases or increases in PCO_2, the limits of oxygen supply are about 0.6 times normal to 2 times normal (20), respectively, and these limits are associated with levels of adenosine higher and lower than control (Fig. 3). The representation of the relation between brain adenosine and oxygen supply most probably will be a family of curves. The configuration of a given curve would probably depend on the means of changing oxygen supply and the degree of interplay of other factors such as H+, K+, osmolality, and myogenic and neurogenic factors.

Coupling between cerebral metabolism and blood flow: a unifying hypothesis. The parallelism observed between the changes in adenosine, lactate, and pyruvate levels is in accordance with the idea that a common mechanism controls their production. This mechanism may be the bal-

![Graph](https://example.com/graph.png)
ance of adenine nucleotides, as revealed by in vitro studies with 5'-nucleotidase and several glycolytic enzymes (3, 26, 45). These enzymes are either inhibited by ATP or stimulated by AMP. Thus, the high 5'-AMP and low ATP levels that prevail during a decreased oxygen supply or increases neuronal activity (33) may increase the production of adenosine, lactate, and pyruvate. The fact that adenosine increased when the oxygen supply dropped and that 5'-AMP changed in parallel with adenosine levels gives support to this hypothesis. In our studies, the changes in lactate and pyruvate when arterial PO2 was reduced followed the same trend as those reported by Granholm and Siesjö (16). However, our lactate, pyruvate, and lactate/pyruvate ratios are higher than their corresponding values. In addition, whereas in our studies lactate/pyruvate ratios remained constant, theirs appeared to increase with hyperventilation and with acute hypoxia, as in the case of the studies of Gottesfeld and Miller (14). There are a number of differences between our experimental protocol and the ones used by these authors, such as the procedure of freezing and electrical stimulation of the brain. In our experiments the brain was electrically stimulated, and this caused increases in the metabolism of brain tissue and its lactate release (17, 37).

How the adenosine and H+ -generating systems may respond to cerebral oxygen balance is illustrated in Fig. 7. In this scheme are represented a series of interdependent variables, H+ (arising mostly from lactate) adenosine, cerebral blood flow, O2 supply, and O2 consumption. The H+ and adenosine generating-systems are dependent upon the O2 supply and the O2 consumption which are integrated by the cell metabolic processes. The O2 supply represents an inhibitory factor for the production of lactate and adenosine as indicated by the minus sign at the input of the integrator, whereas the O2 consumption represents a stimulating factor (plus sign). In addition to its metabolic source, H+ also arises from exogenous CO2. A decrease in either cerebral blood flow or arterial O2 content would induce a reduction in O2 supply, which in turn would exert less inhibition on the H+ and adenosine-generating systems, thereby increasing their concentrations. The elevated levels of these two substances would act synergistically to increase blood flow and tend to restore the cerebral oxygen balance. Similar results would be obtained if cerebral oxygen consumption were increased by metrazol-induced convulsions or electrical stimulation of the brain. However, an increase in arterial PCO2 through an elevation of H+ would increase cerebral blood flow and hence the brain O2 supply. This would increase the inhibition of the H+ and adenosine-generating systems, thereby reducing their production. The decrease in endogenous H+ and adenosine would tend to induce vasoconstriction, which would act to buffer the vasodilator effects of exogenous H+.

This model is compatible with the fact that responsiveness of the cerebral vasculature to PCO2 is gradually lost when oxygen supply is decreased by lowering perfusion pressure and the loss of response is complete when perfusion pressure reaches about 50 mmHg (20). Similarly, elevation of arterial PO2 to about 60 mmHg abolishes autoregulation and the brain blood flow then changes linearly with perfusion pressure (19, 20). Finally, it is possible that the decline in adenosine content at high stimulation rates (Table 1) is caused by a disproportionate increase in blood flow. Hence, brain adenosine levels appear to function as an error signal to indicate the amount of oxygen required by the organ as has been proposed for the heart (4, 39).

Relationship between adenosine and cAMP in situ. Incubation of cerebral cortical slices in a medium containing adenosine at a concentration of about 100 nmol/ml caused a 30-fold increase in cAMP levels (41, 44). Furthermore, electrical stimulation of rat cortical slices and of mouse brain in situ also elevated the cAMP levels, and this increase was attributed to production of adenosine (40). However, in this study (40) adenosine levels were not measured. Although we observed a positive correlation between brain cAMP and adenosine levels, a 10-fold increase in adenosine was associated with only a 0.4 nmol/g increase of cAMP (80% increase above control values), and 100 nmol/g of adenosine were associated with a two- to threefold increase in cAMP. There is an obvious numerical discrepancy between our observation and those in brain slices, which could be attributed to differences in experimental protocol. However, it is possible that in the case of the slices, addition of adenosine to the bath caused the adenosine concentration within the slice to rise above that in the medium because the tissue also produces adenosine. If this were the case, these elevated adenosine concentrations are not reached under physiological conditions, and it remains to be elucidated whether the small changes in cAMP associated with the physiological changes of adenosine are of functional significance.

This investigation was supported by National Heart and Lung Institute Grant 10584.

Preliminary reports of this work have appeared in abstract form (Circulation 48: IV-311, 1974, and Federation Proc. 33: 316, 1974).

Received for publication 29 August 1974.
REFERENCES


