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# THE ROLE OF ADENOSINE IN AUTOREGULATION OF CEREBRAL BLOOD FLOW

H. Richard Winn, Seiji Morii

Department of Neurosurgery
University of Washington
Seattle, Washington

Robert M. Berne

Department of Physiology
University of Virginia
Charlottesville, Virginia

*We have investigated the role of adenosine, a purine nucleoside and potent vasodilator of cerebral pial vessels, during both acute (0–60 sec) and sustained (2–5 min) changes in cerebral perfusion pressure. Brain adenosine concentrations are rapidly increased within 5 sec of the onset of systemic hypotension and parallel, in a temporal fashion, the changes in pial vessel diameter and alterations in cerebral vascular resistance. During sustained hypotension, brain levels of adenosine are increased even within the autoregulatory range. These data are constant with the hypothesis that adenosine is an important metabolic factor in cerebral autoregulation.*

***Keywords*** – *Adenosine, Cerebral blood flow.*

The mechanism whereby the brain regulates its own blood flow during changes in perfusion pressure is unclear. Neurogenic myogenic and metabolic theories have been suggested as playing a role in the autoregulation of cerebral blood flow (CBF). The metabolic theory (3) proposes that a chemical factor couples blood flow to metabolism, and candidates previously suggested for this chemical linkage include hydrogen ion (5,8,11), carbon dioxide (8,14), oxygen (6), potassium (10), and lactate (15).

A recent addition to this list of possible chemical factors linking blood flow and metabolism is the purine nucleoside, adenosine, which has been proposed as a metabolic regulator of coronary blood flow (1). In the heart, adenosine is a coronary vasodilator and is increased in myocardial work (14). Moreover, Thompson et al. (17) recently demonstrated changes in myocardial adenosine concentrations within a single cardiac cycle (high in systole, low in diastole), indicating a rapid metabolism of adenosine.

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Address correspondence and reprint request to Dr. Winn, Harborview Medical Center, Seattle, WA 98104.

In the brain adenosine has been proposed as a regulator of CBF (24) as well as a modulator of synaptic transmission (12). In the latter role adenosine has many functions; it has been suggested as acting as both the presynaptic (16) and postsynaptic (7) inhibitor and has been shown to depress excitability of cortical neurons (13). Its role as a regulator of CBF was suggested because, topically applied, adenosine is a potent dilator of cerebral pial vessels (2,19). Topical application of adenosine also increases cerebral blood flow (23). When adenosine is administered intraarterially no dilatation of pial vessels or increase in cerebral blood flow is noted (2). Thus, there appears to be a relative blood brain barrier for adenosine. Moreover, CSF does not contain enzymes that metabolize adenosine or its degradative products, inosine and hypoxanthine (22). Consequently, changes in brain adenosine reflect cerebral, not systemic events. Therefore adenosine appears to be, in all respects, a local metabolite and hence an ideal candidate for local regulation of cerebral blood flow. The present report summarizes the evidence recently accumulated that adenosine is increased in cerebral tissue during ischemia (20) and hypotension (21).

During a short lasting ischemia, cerebrovascular resistence decreases within 3 to 5 sec following the onset of profound hypotension (9). Consequently any factor proposed to be playing a role in alteration in cerebrovascular resistance must change within this limited time span. The first series of experiments outlined below were designed to evaluate the changes in brain adenosine concentration during the initial seconds after the onset of ischemia.

The second series of experiments were designed to evaluate the changes in brain adenosine concentrations during prolonged hypotension and to determine whether adenosine could be playing a role in cerebral autoregulation. If a metabolic regulator is playing a role in cerebral autoregulation, an increase in concentration in the proposed chemical link should occur as blood pressure is decreased. The second group of experiments summarized below were performed to test the hypothesis that adenosine is involved in autoregulation during hypotension and to determine if adenosine is increased under this condition.

## ACUTE (0–60 sec) HYPOTENSION

We lightly anesthetized 48 adult rats, weighing between 300–400 g, with sodium pentobarbital (50 mg/kg); the animals were allowed to breathe spontaneously. The axillary artery was cannulated and systemic blood pressure continuously recorded. Arterial blood gases and systemic blood pressure were measured in all animals just prior to brain sampling (mean arterial pressure = 89 ± 2[SEM]; $PaO_2$ = 39 ± 0.2; pH = 7.37 ± 0.02).

Through a left flank incision, the retroperitoneal aorta was exposed just below the diaphragm and above the junction of the renal arteries. A #3 wire was passed around the aorta and brought out through the flank incision. Both ends of the wire were then passed through a sharp #18 needle. Ischemia was

produced by pulling the wire with the enclosed blood vessel through the needle in order to transect the aorta. Preliminary studies with this method revealed that the diastolic blood pressure fell to zero within 400 msec, and that effective cerebral circulation presumably ceased within one sec, since mean blood pressure at one sec was 8 mm Hg.

Following transection of the aorta, brain samples were obtained by the freeze-blowing technique of Veech et al. (18). By this method, 2 hollow probes are driven by powerful solenoids through opposite sides of the skull. Compressed air is then blown into one hollow probe and the entire supratentorial compartment of the brain is blown out of the other probe and frozen on aluminum plates precooled in liquid nitrogen.

Using the method outlined above, brain samples were obtained at 0, 5, 10, 15, 30 and 60 sec after aortic transection. Control samples were obtained in 3 other groups of animals: (1) awake animals, (2) anesthetized animals and (3) sham-treated animals.

Brain tissue was then analyzed for adenosine and its metabolites, inosine and hypoxanthine, as well as ATP, ADP, AMP, phosphocreatine (PCr), lactate, and pyruvate. Changes in ischemic levels of metabolites were compared to zero-second values, using Student's t test for nonpaired data.

During the first 5 sec, there was a 2 1/2-fold increase in adenosine, from 0.96 nmoles/g to 2.30 nmoles/g ($p < 0.001$) (Fig. 1). At 10 sec, a peak of

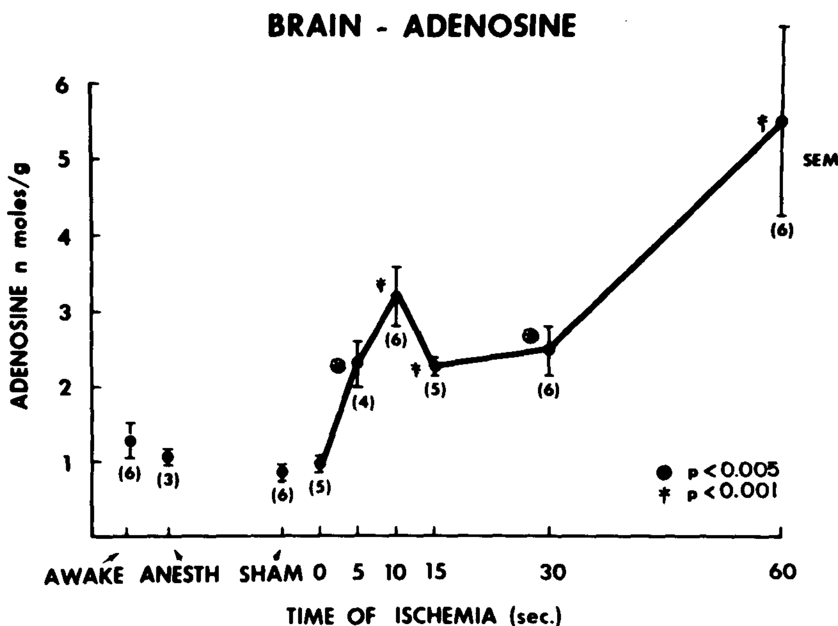


FIGURE 1. Adenosine concentrations in brain after acute ischemia. Within 5 sec, adenosine levels increased $2\frac{1}{2}$ fold with subsequent increases noted by 10 sec after aortic transection (0 sec). Control animals were sampled in the awake state, under anesthesia and after sham aortic transection.

3.20 nmoles/g was followed by a significant dip to 2.25 nmoles/g at 15 sec ($p < 0.05$). Thereafter, adenosine levels were elevated to 2.47 nmoles/g at 30 sec and 5.5 nmoles/g at 60 sec. The adenosine levels in the control animals did not differ from the levels obtained in the animals sacrificed at zero time. Inosine levels showed a progressive upward trend from 2.93 nmoles/g at zero time to 5.36 nmoles/g at 60 sec. Hypoxanthine remained constant throughout the 60 sec of ischemia at 31 nmoles/g.

AMP and ADP concentrations were significantly elevated by 30 sec, whereas ATP did not significantly decrease until 60 sec. Phosphocreatine declined 1.24 moles/g. Adenosine correlated with phosphocreatine levels (adenosine = $-0.89$ PCr + 5.0, $r = 0.9089$, $p < 0.005$). Energy charge remained stable for the first 30 sec. Lactate was significantly elevated at 10 sec ($p < 0.02$), whereas pyruvate rose slowly but not significantly until 60 sec.

## SUSTAINED (2–5 min) HYPOTENSION

Having documented that brain adenosine concentrations are rapidly increased within seconds of a change in cerebral perfusion pressure, we then evaluated the changes in brain adenosine levels during sustained alteration in perfusion pressure. Studies were performed on rats weighing between 300–400 g and initially anesthetized with 2–3% halothane. The femoral arteries and veins were cannulated and used for continuous recording of mean arterial blood pressure (MABP) and intravenous administration of drugs, respectively. Tracheostomies were performed and the rats were paralyzed, then mechanically ventilated. Because of the possible effects of Halothane on cerebral metabolism, the halothane was withdrawn, but the operative sites were infiltrated with lidocaine and the animals comfortably positioned before cessation of halothane anesthesia. Arterial blood gases and systemic blood pressures were measured continuously. Rectal temperature was maintained at $38° + 0.5°C$ by means of a heat lamp. Blood pressure was adjusted to desired level by slow withdrawal of blood in small aliquots from the arterial catheter and maintained stable for at least 5 min before brain sampling. Arterial blood gases and pH were remeasured and ventilation adjusted if any variations from specified levels occurred. Brain samples were again obtained by the freeze-blowing technique of Veech et al. (18).

Utilizing the methods outlined above, brain samples were obtained in four groups of animals: group 1 ($n = 10$) had a mean blood pressure of $135 \pm 3$ mm Hg; group 2 ($n = 11$) had a mean blood pressure of $107 \pm 4$ mm Hg; group 3 ($n = 12$) had a mean blood pressure of $72 \pm 2$ mm Hg; (4) ($n = 10$) had a mean blood pressure of $45 \pm 3$ mm Hg. Brain tissue was analyzed for adenosine and its metabolites, inosine and hypoxanthine, as well as ATP, ADP, AMP, PCr, and lactate. We used Student's t test for nonpaired data with Bonferroni correction for statistical analysis of differences between group 1 and other groups.

Adenosine levels in brain doubled ($p < 0.015$) from $0.55 \pm 0.12$ to

1.16 + 0.13 [SEM] nmole/g when blood pressure was decreased within the auto-regulatory range from 135 ± 3 to 72 ± 2 mm Hg (Fig. 2). However, there was only an upward trend in adenosine concentration between group 1 and group 2. There occurred a significant ($p < 0.05$) increase in adenosine levels between group 2 (MAVP ± 107) and group 3 (MABP ± 72 mm Hg). A greater than five fold increase ($p < 0.003$) occurred in group 4 as compared to group 1 when blood pressure reached 45 ± 3 mm Hg.

Changes in inosine and hypoxanthine, metabolites of adenosine, parallel the increases in adenosine. However, a statistically significant elevation in hypoxanthine was not observed until blood pressure was lowered to 45 ± 3 mm Hg.

Adenine nucleotides and phosphocreatine remained stable in all groups. Lactate concentrations were elevated significantly only when blood pressure was reduced to 45 mm Hg (group 4).

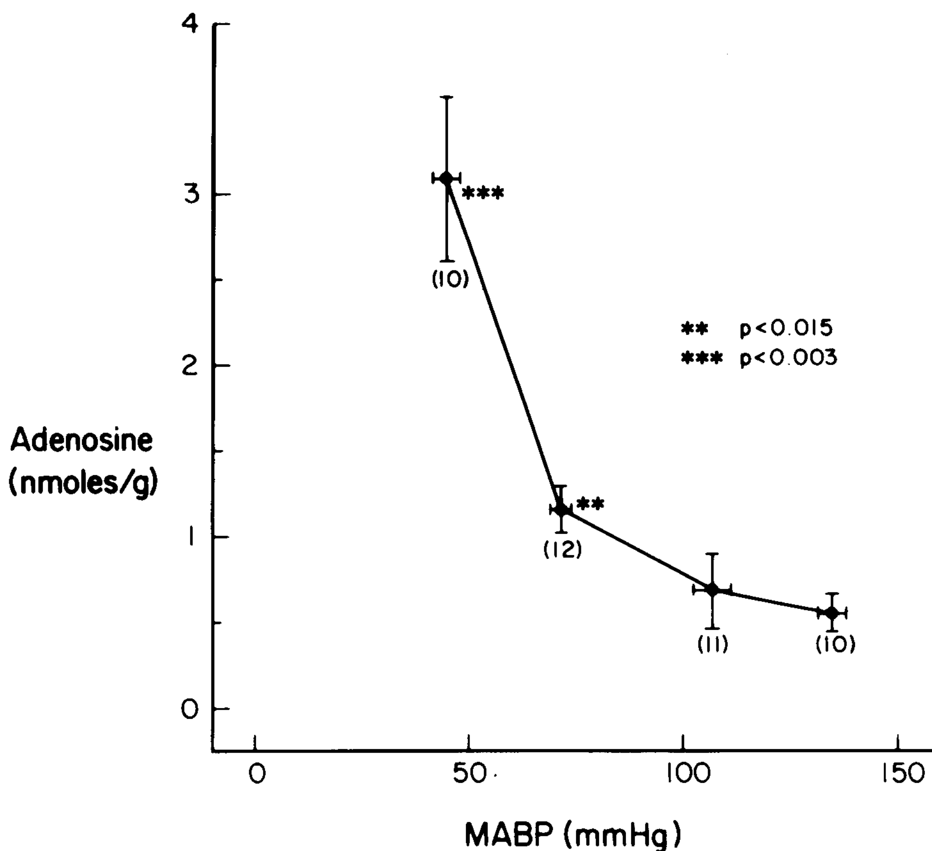


FIGURE 2. Adenosine concentrations in brain after sustained (5 min), graded, hemorrhagic hypotension. An increase in adenosine occurred when mean arterial blood pressure (MABP) was lowered to 72 mm Hg and 45 mm Hg.

## DISCUSSION

The study summarized here confirmed previous observations that ischemic brain can produce adenosine and moreover does so in a strikingly rapid fashion. Furthermore, with sustained hypotension, adenosine concentrations in the brain are elevated even within the autoregulatory range.

Having demonstrated that adenosine concentrations are increased following ischemia and during hypotension, it still remains problematical whether adenosine is involved in the vasoregulation following these stimuli. Are the concentrations in the appropriate range that would affect cerebral vessels? We estimated that the concentrations measured in our samples would have approximately $10^{-6}$ M level in resting brain (20,21). Similar levels have been measured in the extracellular space in brain (25) utilizing a dialysis technique. After topical micropipette application of adenosine, Wahl and Kuschinsky (19) found a sigmoid-shaped dose response curve for feline pial vessels. In the presence of physiologic concentrations of bicarbonate, maximum dilata-

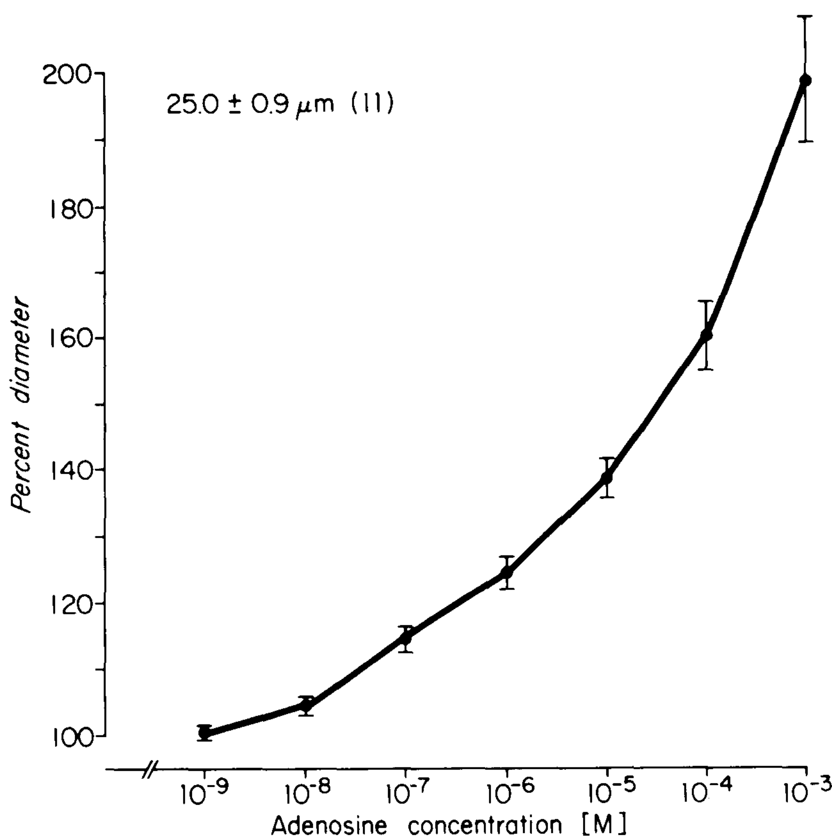


FIGURE 3. Percent dilation of pial vessels to adenosine. Studies were performed utilizing closed window technique in the rat. Mean resting vessel ($n = 11$) diameter was 25.0 ± 0.9 μm.

tion occurred between $10^{-5}$ M and $10^{-7}$ M of adenosine with the inflection point of $10^{-6}$ M. The dose response curve with topical application of adenosine in rat pial vessels is illustrated in Fig. 3. Consequently, the concentrations found in our rat brain sample utilizing the freeze-blow technique are in the appropriate range to affect significant alteration in cerebral vessels and consequently affect cerebral vascular resistance. However, the exact location (intracellular vs. extracellular), source (adenine nucleotide vs. S-adenosylhomocysteine) and the regulatory mechanisms controlling adenosine production need further clarification.

In summary, brain adenosine levels are increased with both acute and sustained reduction of arterial blood pressure. The duality of findings that adenosine is rapidly (~5 sec) increased in the brain after acute ischemia as well as within the autoregulatory range with sustained hypotension is consonant with the concept that adenosine serves as a mediator of cerebral vasodilatation during hypotension.

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