Changes in Brain Adenosine during Bicuculline-Induced Seizures in Rats

Effects of Hypoxia and Altered Systemic Blood Pressure

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SUMMARY We analyzed brain tissue in 139 rats for adenosine and its metabolites, inosine and hypoxanthine, during the initial 120 seconds of seizures induced by bicuculline. We also measured ATP, ADP, AMP, phosphocreatine (PCr), and lactate. We divided the rats into four groups by adjustment of their preictal arterial oxygen tension: group I, PaO₂ > 200 mm Hg; group II, PaO₂ = 50 mm Hg; and group III: PaO₂ = 100 mm Hg. We treated a fourth group whose PaO₂ = 100 mm Hg with phentolamine to block the 44% rise in blood pressure which occurred with the onset of seizures. PaCO₂ was maintained between 30 and 40 mm Hg in all groups. Brain tissue was sampled rapidly after 0, 10, 20, 30, 60, and 120 seconds of seizures by the freeze-blow technique. With normoxia (PaO₂ = 100 mm Hg) or hyperoxia (PaO₂ > 200 mm Hg), adenosine increased within ten seconds of the onset of seizures and remained elevated even after 120 seconds. Elevations in inosine and hypoxanthine were delayed compared to the increases in adenosine. A reduction in PaO₂ (50 mm Hg) or systemic blood pressure during seizures caused a further augmentation in the increase in brain adenosine levels. During the seizure period, transient changes in adenine nucleotides and energy charge were observed, but PCr remained depressed and lactate continued to rise. The rapid and sustained increase in cerebral adenosine levels, temporally paralleling the changes in cerebral blood flow, supports the role for adenosine in the regulation of cerebral blood flow. Circ Res 47: 568-577, 1980

IN RATS, cerebral blood flow (CBF) increases several-fold within 30 seconds of the onset of epileptic seizures induced by bicuculline (Meldrum and Nilsson, 1976). However, the mechanism whereby this rapid and substantial increase in CBF occurs is unknown, but presumably the change in flow matches the increase in metabolism caused by the convulsion (Plum and Duffy, 1975). Local metabolic factors, such as H⁺, CO₂, lactate, potassium, and calcium, have been suggested as playing a role in the regulation of CBF during seizures (Meldrum and Nilsson, 1976). By dilating cerebral vessels, a metabolic agent would decrease cerebral vascular resistance, and hence, increase CBF.

Adenosine has recently been added to the list of chemical factors that may link blood flow to metabolism (Berne et al., 1974). In brain, adenosine is a dilator of pial vessels (Berne et al., 1974; Wahl and Kuschinsky, 1976) and is rapidly (ca 5 seconds) elevated following ischemia (Winn et al., 1975). Constant low voltage stimulation of the rat brain in vivo doubles cerebral adenosine levels (Rubio et al., 1975), whereas a 10-second electric shock (100 V) results in a 30-fold increase in adenosine in awake rats (Schultz and Lowenstein, 1978). Moreover, electrical stimulation of cortical brain slices produces a release of adenosine into the surrounding medium (Pull and McIlwain, 1972). Thus, it would be logical to propose that metabolic control of CBF during seizures could occur by means of the vasodilator action of adenosine.

However, Rehncrona et al. (1978), who sampled rat brain by the in situ technique, failed to find any increase in adenosine following the onset of seizures induced by bicuculline. The stable levels of adenosine found by Rehncrona et al. (1978) is unexpected and would exclude a role for adenosine in the regulation of CBF during seizures. Consequently, the present study was designed to resolve the previous disparate observations and to define the role of adenosine in the regulation of CBF during the first 60 seconds of epileptic seizures induced by bicuculline, a plant alkaloid and a competitive antagonist of γ-aminobutyric acid (GABA) at postsynaptic sites. In addition, since cerebral adenosine levels are inversely related to PaO₂ (Rubio et al., 1975; Winn et al., 1979a) and systemic blood pressure (Rubio et al., 1975; Winn et al., 1979c), we studied the effect of altered arterial oxygen and blood pressure on adenosine production during seizures.

Methods

Studies were performed on 139 rats weighing between 300 and 400 g that had free access to tap water and commercial rat pellets prior to surgery. Rats were initially anesthetized with 2–3% halothane. The femoral artery and vein were cannulated and used for continuous recording of blood pressure.
and the intravenous administration of drugs, respectively. Tracheotomies were performed, and the rats were paralyzed with pancuronium bromide (1 mg/kg, iv) and mechanically ventilated to prevent systemic acidosis, which occurs during seizures in spontaneously breathing animals (Plum and Duffy, 1975). After the operative sites had been infiltrated with lidocaine HCl (80 mg/ml) and the animals comfortably positioned, the halothane was withdrawn because of its possible effects on cerebral metabolism and CBF (Nilsson and Seisjo, 1971). Preictal MABP was standardized (110–130 mm Hg) by removal of small aliquots of blood from the arterial line.

Arterial blood (0.2 ml) was withdrawn anaerobically and PaO₂, PaCO₂, and pH measured. Rats were divided into groups by adjusting the inspired oxygen to obtain PaO₂ > 200 mm Hg (group I), PaO₂ = 50 (group II), PaO₂ = 100 (groups III and IV). Animals in group IV were given phentolamine (1 mg/kg) to block the rise in mean arterial blood pressure (MABP) associated with the onset of convulsions. PaCO₂ was maintained between 30 and 40 mm in all groups. Rectal temperature was measured and kept near 38 ± 0.5°C by means of a heat lamp. The EEG was recorded continuously via bitemporal percutaneous electrodes.

Seizures were induced by intravenous bicuculline (1.2 mg/kg), and brain samples were obtained after 10, 20, 30, and 60 seconds of seizure activity. In a few animals, brains were also sampled after 5 (group II) and 120 seconds of convulsions (groups II and III). The onset of seizures was delayed 6–8 seconds after an intravenous injection of bicuculline; thereafter, seizure activity remained continuous. Control rats were treated identically, except that their brains were sampled before the onset of seizures but after the injection of bicuculline (groups I, II, and III) or bicuculline and phentolamine (group IV). In some rats (groups I and II), arterial blood was obtained at the end of 60 seconds of seizures, and arterial O₂, CO₂, and pH were compared to initial values.

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 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 immediately frozen between two aluminum plates, pre-cooled in liquid N₂. A frozen wafer of brain about 1 g in weight and 1 mm thick is obtained. Because of the rapid removal and homogenization of brain tissue by the air, freezing of the brain sample occurs in less than 1 second.

The frozen wafer of brain was pulverized under liquid N₂ in a mortar. The brain powder was weighed in tubes pre-cooled in liquid N₂ and mixed vigorously with 2 ml of cold (−20°C) 0.1 N HCl in absolute methanol. Four milliliters of 1.23 N HClO₄, containing 5 μM EGTA, then were added, and the tissue was homogenized with a glass homogenizer. During homogenization, the tubes were kept in a −20°C bath. The perchloric acid homogenate was centrifuged for 15 minutes at 10,000 g, and the supernatant fraction was neutralized with KOH and allowed to stand for 12 hours at 10°C. The resultant precipitate was removed by centrifugation, and the supernatant fraction kept frozen in liquid N₂ until assayed for different compounds.

Adenosine, inosine, and hypoxanthine were measured sequentially and in duplicate by selective enzymatic methods on a dual-beam spectrophotometer (Perkin-Elmer model 356) as previously described (Dobson et al., 1971). ATP, phosphocreatine (PCr), ADP, AMP, and lactate were measured spectrophotometrically (Gutmann and Wahlefeld, 1965; Javoreh et al., 1965; Lamprecht et al., 1965). Energy charge was calculated (E.C. = ATP + 0.5 ADP/ATP + ADP + AMP) as described by Atkinson (1968).

Within each group, concentrations of brain metabolites before the onset of convulsions were compared by analysis of variance to values obtained during seizures. Statistical analysis of differences between values in different groups (I vs. II, and III vs. IV) was made, using Student's t-test for non-paired data.

Results

Physiological Parameters

Table 1 illustrates the MABP and PaO₂, PaCO₂, and pH prior to the onset of seizures in all groups. There was no difference within each group or between different groups, except for adjusted PaO₂ in groups I, II, and III. Arterial blood gases and pH obtained after 60 seconds of seizures revealed a decrease in PaCO₂ in groups I and II, but no change in PaO₂ or pH (Table 2). A similar change in PaO₂ was observed by Meldrum and Nilsson (1976).

MABP measured at the time of brain sampling is illustrated in Table 3. By 10 seconds of seizure activity, MABP rose an average of 44% in groups I, II, and III, and remained elevated for the entire 60 seconds. In animals given phentolamine in addition to bicuculline (group IV), the MABP dropped after 10 seconds of seizure activity to 81 ± 10 mm Hg (P < 0.001), as compared to the increase in blood pressure to 181 ± 8 mm Hg observed after 10 seconds of seizure in the rats in group III which had a similar PaO₂ but which were not given phentolamine. MABP in group IV remained depressed, compared to group III (P < 0.005), for the entire 60 seconds of convulsion. At all time periods, MABP in group IV never decreased below the autoregulatory blood pressure range.

Adenosine, Inosine, and Hypoxanthine

Prior to the onset of seizures, adenosine levels were similar in the four groups of animals. Within 10 seconds of the onset of seizures, adenosine levels
were elevated significantly in all groups from the control values (Table 4). In two rats (group III) killed after 5 seconds of seizure activity, adenosine concentrations were 1.20 ± 0.15 nmol/g, more than double preictal levels of 0.52 ± 0.11 (SEM). In animals with a PaO₂ > 200 mm Hg, concentrations of adenosine remained elevated and stable between 20 and 60 seconds (Table 4). A similar pattern was observed in animals whose preictal PaO₂ approximated 100 mm Hg (group III). However, in group II, adenosine concentrations increased after 10 seconds, reached a peak at 30 seconds, and then decreased at 60 seconds (Table 4). As compared to group I, the adenosine concentrations in group II were significantly greater at 20 (P < 0.025) and 30 (P < 0.001) seconds (Table 4). During seizures, adenosine levels in group IV were always higher than those in group III, with significant differences observed at 10 seconds (P < 0.025) and 20 seconds (P < 0.025). Elevations in inosine and hypoxanthine occurred in all groups, but the increases were delayed compared to the changes in adenosine (Table 4). In the hypoxic rats (group II), the increases in inosine and hypoxanthine at 20 seconds were greater than those with a PaO₂ > 200 mm Hg. Although inosine and hypoxanthine were elevated in group IV with seizures, the concentrations were not significantly different from animals whose MABP was not depressed (Table 4).

Because of the downward trend of adenosine concentrations after 60 seconds of seizure activity in all groups, additional animals were studied after 120 seconds of convulsions in groups II and III. In both hypoxic and normoxic rats, adenosine remained elevated [2.88 ± 0.31 nmol/g (SEM) and 2.36 ± 0.30 nmol/g, respectively], compared to preictal concentrations. The inosine and hypoxanthine values were also increased (Table 4).

### Adenine Nucleotides, PCr, and Lactate
Among all groups, there were no differences in preictal adenine nucleotides, PCr, and lactate or energy charge (E.C.), except for a higher ADP (P < 0.001) and lower E.C. (P < 0.025) in the hypoxic rats (group II), compared to animals whose PaO₂ > 200 mm Hg (group I) (Fig. 1).

In all groups, there was a fall in ATP and PCr after the onset of seizures. ATP was depressed at 10 seconds in groups I and III and at 20 seconds in
groups II and IV. However, by 30 seconds, ATP values in all groups had increased and were not significantly different from control values. PCR, like ATP, was also rapidly reduced, but unlike ATP, PCR remained depressed in all groups for the entire 60 seconds (Fig. 1). A reciprocal relationship existed for adenosine and PCR for all groups, and linear regression analysis revealed adenosine = 0.87 [PCR] + 4.83 (r = 0.57, P < 0.001). Paralleling the changes in ATP at 20 seconds, PCR concentrations in groups II and IV were lower, compared to those in groups I (P < 0.01) and II (P < 0.05) (respectively).

AMP and ADP concentrations rose initially but, by 60 seconds, did not differ from control values. In the hypoxic animals, AMP, like adenosine, continued to rise and reached a peak at 30 seconds. The difference at 30 seconds between group I and II was significant (P < 0.01). The AMP values in groups III and IV were similar at all time periods, unlike those of Rubio et al. (1975). Rehncrona et al. (1978) failed to observe any increase of adenosine in hypoxic rats, whereas Rubio et al. (1975) froze the brain with isobaric hyperoxia existed or when MABP rose spontaneously as a result of the seizure activity.

The changes in adenine nucleotides were reflected by the changes in calculated energy charge, which in all groups decreased within 10 seconds of the onset of seizures (Fig. 1). After 10 seconds, energy charge returned toward control values in all groups except the hypoxic rats. In these animals (group II), energy charge continued to fall until 20 seconds, reflecting the greater alteration in adenine nucleotide metabolism.

Brain lactate concentrations rapidly (10 seconds in groups III and IV; 20 seconds in Groups I and II), became elevated, and continued to rise for the duration of the seizures (Fig. 1). There were no differences in lactate values between the hyperoxic and hypoxic animals or between the hypertensive animals (group III) and those treated with phentolamine (group IV).

Discussion

The present study demonstrates that during the first 10 seconds of bicuculline-induced seizures, brain adenosine concentrations are significantly elevated. In addition, adenosine, a potent dilator of pial vessels, continues to remain elevated even after 120 seconds of convulsions. Under similar conditions, Meldrum and Nilsson (1976) reported a 900% increase in CBF within 15 seconds of the onset of seizures. Decrease in preictal PaO₂ and depression of ictal MABP in the present study resulted in greater increases in brain adenosine than when hyperoxia existed or when MABP rose spontaneously as a result of the seizure activity.

The preictal concentrations of adenosine are similar to those reported in rats by Rehncrona et al. (1978) and Nordstrom et al. (1977), but lower than those of Rubio et al. (1975). Rehncrona et al. (1978) and Nordstrom et al. (1977) froze the brain in situ, whereas Rubio et al. (1975) froze the brain with bone rongeurs pre-cooled in liquid N₂. The latter technique is slower than the freeze-blowing method and may cause tissue injury prior to freezing, which may account for the higher values of adenosine.

However, using the in situ technique, Rehncrona et al. (1978) failed to observe any increase of adenosine during bicuculline-induced seizures whereas, in the present study—under similar conditions, but using the more rapid freeze-blowing technique of tissue sampling—adenosine levels were significantly elevated. Schultz and Lowenstein (1978), also using the freeze-blowing technique, found profound and

### Table 4

<table>
<thead>
<tr>
<th>Time of seizure activity (sec)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.37 ± 0.30</td>
<td>2.54 ± 0.28</td>
<td>1.78 ± 0.45</td>
<td>1.57 ± 0.30</td>
</tr>
<tr>
<td>10</td>
<td>2.54 ± 0.43</td>
<td>3.03 ± 0.22</td>
<td>3.10 ± 0.21</td>
<td>1.58 ± 0.18</td>
</tr>
<tr>
<td>20</td>
<td>3.00 ± 0.43</td>
<td>2.54 ± 0.28</td>
<td>1.78 ± 0.45</td>
<td>1.57 ± 0.30</td>
</tr>
<tr>
<td>30</td>
<td>3.00 ± 0.43</td>
<td>3.03 ± 0.22</td>
<td>3.10 ± 0.21</td>
<td>1.58 ± 0.18</td>
</tr>
<tr>
<td>60</td>
<td>3.00 ± 0.43</td>
<td>3.03 ± 0.22</td>
<td>3.10 ± 0.21</td>
<td>1.58 ± 0.18</td>
</tr>
<tr>
<td>120</td>
<td>3.00 ± 0.43</td>
<td>3.03 ± 0.22</td>
<td>3.10 ± 0.21</td>
<td>1.58 ± 0.18</td>
</tr>
</tbody>
</table>

All values = mean ± se of compounds in nmol/g wet tissue. As compared to zero time:

* P < 0.05; † P < 0.01; ‡ P < 0.005. Group I vs. II; III vs. IV; ○ = P < 0.025; ● = P < 0.005; Δ = P < 0.001.
rapid increases in brain adenosine concentration after electrically induced convulsions in non-ventilated rats. In contrast to the study of Rehncrona et al. (1978) in rats, Wahl et al. (1978), also employing an in situ method, but in cats, noted an increase in adenosine following bicuculline seizures, which was in agreement with our initial observations (Winn et al., 1978). However, the control values in this preliminary study of Wahl et al. (1978) were at least 3-fold greater than those obtained by Nordstrom et al. (1977) and Rehncrona et al. (1978).

In rats, the differences in sampling technique may, therefore, account for the disparate results of the present study, in which we used the freeze-blowing technique, and that of Rehncrona et al. (1978), who employed the slower in situ method. Under normal conditions (i.e., when CBF is not increased), freezing time at the surface of the cortex in rat with the in situ technique is almost 20 seconds (Ponten et al., 1973). Perfusion of the brain with blood of body temperature opposes the movement of the freeze front through cerebral tissue. Increased blood flow, such as occurs with seizures, should prolong the time required to freeze the brain tissue. Moreover, seizure discharges and metabolic hyperactivity will decrease greatly as the temperature falls (ten Cate et al., 1949). However, due to the increased CBF, an interval of some length may exist between cessation of seizure activity and cessation of metabolic activity and tissue freezing. Conceivably, uncoupling of CBF and metabolism could therefore occur. If adenosine is acting as a chemical link between blood flow and metabolism, once seizures stop and metabolic demand decreases, adenosine concentrations should fall rapidly if the signal for production of adenosine ceases. Rapid decreases have been observed in other organs, such as the heart, in which adenosine concentrations increase and decrease during each phase of the cardiac cycle (Thompson et al., 1980). With the in situ method of freezing, the disparity with time between cessation of seizure activity, continuance of CBF, and reduction in metabolic demand—but persistence of metabolic capability—may allow adenosine values to decrease and thus give falsely lower values. Even if uncoupling of CBF and metabolism does not occur with the in situ technique, the effects of temperature on the enzymes which are directly involved in
adenosine metabolism are not similar. For example, with a decrease in temperature from 40°C to 20°C, 5'-nucleotidase activity is decreased 82% and adenosine kinase activity 95%, whereas adenosine deaminase activity decreases only 15% (Arch and Newsholme, 1978). Thus, with the relatively slower method of tissue freezing, such as the in situ degradation of adenosine, would continue, whereas production and rephosphorylation would be affected more greatly.

In non-convulsing rats, adenosine concentrations increase only when PaO2 is less than 50 mm Hg (Winn et al., 1979b), a level at which CBF is also increased (Borgstrom et al., 1975). During seizures, however, brain adenosine concentration can be augmented by decreasing PaO2 to 50 mm Hg. Similar changes in adenosine are suggested by work in guinea pig brain slices (Pull and McLwain, 1972) and in mice (Sattin, 1971). After labeling brain slices with 14C-labeled adenosine, Pull and McLwain (1972) observed that 14C in the bathing medium was increased by electrical stimulation of the slices. Decreasing the PO2 of the bathing medium further augmented the output of 14C with electrical stimulation. Although the 14C compounds were not further characterized during hypoxic stimulation, adenosine or its metabolites constituted the majority of labeled compounds after stimulation of nonhypoxic brain slices. Adenosine production during seizures and its augmentation by hypoxia are also suggested by the observation in mice that cyclic AMP levels are increased after electrically induced seizures, and that the increment in cyclic AMP can be increased by decreasing inspired oxygen (Sattin, 1971). Moreover, Sattin (1971) significantly reduced the rise in cyclic AMP with pretreatment of the mice with theophylline. In brain, adenosine produces a 4-fold increase in cyclic AMP, and this effect of adenosine is competitively blocked by theophylline (Sattin and Rall, 1970). The increase in adenosine concentrations during seizures and their augmentation by hypoxia observed in the present study supports Sattin's suggestion (1971) that "increases in postictal cyclic AMP are produced, in part, by the increased formation and release of adenosine."

The increase in adenosine in the hypoxic as compared to the hyperoxic rats may not be due to a direct effect of limited oxygen on adenosine production. Perhaps the decrease in preictal PaO2 results in a lower seizure threshold and more profound convulsions in group II, despite similar dosages of bicuculline in all groups. Therefore, augmentation in cerebral adenosine levels with hypoxia may reflect either the lack of substrate or an increase in activity.

Decreases in arterial blood pressure during seizures also resulted in augmented adenosine production. In the non-convulsing brain, adenosine concentrations increased with a decrease in blood pressure (Rubio et al., 1975) even within the autoregulatory blood pressure range (Winn et al., 1979c). In the present study, brain perfusion pressure was affected by blocking the systemic vasoconstriction with phentolamine, an α-adrenergic blocker. Since the cerebral vascular bed appears to be dilated maximally during seizures (Meldrum and Nilsson, 1976), blocking the increase in MABP normally observed with seizures would result in a relative brain ischemia in group IV compared to the hypertensive animals (group III). The continued depression of PCr in group IV as compared to group III is compatible with a relative brain ischemia. However, a direct effect of phentolamine on metabolism and CBF cannot be excluded, although there were no differences in metabolite levels in the control animals that received phentolamine (group IV) as compared to the concentrations in the rats in group III which were not treated with phentolamine. Moreover, a direct effect on pial vessels and CBF has not been observed with either intravascular or peripial application of phentolamine in non-convulsing animals (D'Alecy, 1973; Kuschinsky and Wahl, 1975). Another possible cause for the differences in metabolites between groups III and IV is that, with relative ischemia as with hypoxia, alteration in seizure threshold may occur.

Although adenosine production during seizures is effected by PaO2 and MABP, the mechanisms which control the release of adenosine are unclear. Adenosine levels could be related to changes in enzyme activity or to an alteration in substrate (AMP) availability. Multiple enzymes (5'-nucleotidase, adenosine kinase, adenosine deaminase) are capable of affecting adenosine concentrations. Kreutzberg et al. (1978) have demonstrated that the glial foot plates that surround the blood vessels contain 5'-nucleotidase in their cell wall. Thus, the enzyme that produces adenosine is in close proximity to the vessel wall. Moreover, this enzyme in vivo appears to be greatly suppressed, and is inhibited by many factors, including ADP > ATP (Ipata, 1968; Burger and Lowenstein, 1975), whereas Mg2+ reverses the inhibition by ADP and ATP (Sullivan and Alpers, 1971; Kluge et al., 1972). The falling ATP values during the initial seconds of seizure activity conceivably could disinhibit 5'-nucleotidase activity, but this disinhibition would be opposed by the rising ADP concentrations. The complex changes in the adenine nucleotide concentrations, therefore, do not allow ready assessment of their effects on 5'-nucleotidase activity in vivo. In addition to the effects of adenine nucleotide and magnesium levels, 5'-nucleotidase activity is related inversely to PCr concentrations in the ranges found in the present study (Rubio et al., 1979). The rapid and sustained decrease in PCr combined with release of free Mg2+ from ATP could cause an increase in 5'-nucleotidase activity. An inverse relationship exists in the present study for adenosine and PCr [adenosine = -0.87 (PCr) + 4.83], which is similar to that observed [adenosine = 0.89 (PCr)
Adenosine levels also may be affected by adenosine deaminase and adenosine kinase. The former enzyme, which degrades adenosine to inosine, has a low activity in brain (Brady and O'Donovan, 1965; Arch and Newsholme, 1978) and is restricted to an intracellular location for both neuronal (Pull and McIlwain, 1974) and glial (Trams and Lauter, 1975) cells. In contrast, a proportion (20%) of adenosine kinase, which phosphorylates adenosine to AMP, is associated with the cell wall in heart (DeJong and Kalkman, 1973) and erythrocyte ghosts (Schrader et al., 1972), and a similar location has been suggested, in part, in brain (Schimizu et al., 1972). Moreover, an increase in potassium, from 0 to 10 mM, has been shown to inhibit the activity of adenosine kinase by 40% (Arch and Newsholme, 1978). Thus, an increase in extracellular potassium from 3.5 to 7 mM such as occurs with seizures (Astrup et al., 1978) conceivably could inhibit adenosine kinase significantly and thereby decrease phosphorylation of adenosine to AMP, and hence, increase the amount of adenosine in the extracellular fluid.

Although alteration in enzyme activity may affect adenosine levels, changes in substrate (AMP) concentration may also influence adenosine values. In the present experiment, adenosine levels in general reflect the increases in AMP concentrations: both AMP and adenosine are elevated rapidly during seizures and are augmented further by hypoxia. However, in the relatively hypotensive rats (group IV), despite a greater increase in adenosine levels after 10 and 20 seconds of convulsions, compared to the hypertensive rats, AMP was stable. A similar lack of increase in AMP, despite an increase in adenosine, was observed during the initial seconds of ischemia (Winn et al., 1979a) and with sustained alteration in perfusion pressure (Winn et al., 1979c). Moreover, in the present study, after 60 seconds of seizure activity, AMP levels were not different from control concentrations, but adenosine, like CBF, remained elevated. Therefore, it is unlikely that adenosine concentrations are related solely to AMP levels, as suggested by Nordstrom et al. (1977).

Although the major source for adenosine production is assumed to be adenine nucleotides (Pull and McIlwain, 1973), hydrolysis of S-adenosylhomocysteine (SAH) by SAH-hydrolase will also produce adenosine (Fox and Kelley, 1978). SAH is produced by removal of a methyl group from S-adenosylmethionine (SAM) which serves as a major methyl group donor in brain (Stramentinoli et al., 1978). Transmethylation occurs in the metabolism of catecholamines (norepinephrine, epinephrine, dopamine) and other purported neurotransmitters, such as histamine and serotonin, as well as in the metabolism of fatty acids phospholipids, nucleic acids, and proteins (Saavedra, 1978). In catecholamine catabolism, SAM acts as a cofactor (Axelrod, 1971) for catechol-O-methyltransferase (COMT). COMT is responsible for extraneuronal inactivation of catecholamines (Axelrod and Tomchick, 1958), and recently, a similar location has also been suggested for SAM (Andreoli et al., 1978). In seizures, with increased neuronal activity and synaptic transmission, greater demethylation of SAM to SAH would occur due to the increased inactivation of catecholamines by COMT. Increased amounts of SAH would be available, therefore, for subsequent degradation to adenosine and homocysteine. In vivo, further catabolism of SAH occurs because SAH is a strong inhibitor of methyl transfer reactions (Hurtwitz et al., 1964) and because homocysteine can be removed easily by further degradation to methionine (Finkelstein, 1979). Methionine, in turn, is phosphorylated to SAM, completing a cycle and replenishing the source for methylation (Zeisel and Wurtman, 1979). Adenosine produced by hydrolysis of SAH could become available to regulate CBF or to modulate synaptic transmission if it reaches the extracellular space intact. Thus, during neuronal activity, adenosine may be produced from either an adenine nucleotide source (AMP) involving 5'-nucleotidase or from a non-nucleotide source (SAH) involving SAH hydrolyase. Although the relative concentration of AMP (40 nmol/g) and SAM (30 nmol/g) (Baldessarini and Kopin, 1966) in brain are known, the contribution of each substrate to the production of adenosine remains to be determined.

Like the increment in adenosine, CBF increased dramatically within the first 15 seconds of bicuculline-induced seizures in rats, and thereafter remained elevated. The changes in CBF and adenosine levels are, therefore, temporally similar. Moreover, after topical micropipette application of adenosine, Wahl and Kuschinsky (1976) found a sigmoid-shaped dose-response curve for feline pial vessels with maximal dilation occurring between 10^-7 and 10^-5 M. If adenosine exists mainly in the extracellular space, as studies from red cell ghosts (Schrader et al., 1972) and dispersed embryonic chick hearts (Mustafa et al., 1975) suggest, and if the extracellular space in brain is assumed to be 20%, then brain adenosine concentrations observed in the present study would range from at least 2 X 10^-6 M prior to seizures to almost 3 X 10^-5 M during seizures. These concentrations are thus within the vasoactive range found by Wahl and Kuschinsky (1976) for vessels >50 |im. Smaller pial vessels (<50 |im) were found by Berne et al. (1974) to be more responsive than the larger vessels studied by Wahl and Kuschinsky (1976). These smaller pial vessels, as well as intracerebral vessels, are thought to play a large role in CBF regulation (Stromberg and Fox, 1972; Kontos et al., 1978; Rosenblum, 1977; MacKenzie et al., 1979). The increases in adenosine concentrations, therefore, could account for a significant degree of the vasodilation and decrease in the cerebrovascular resistance which occurs with seizures (Meldrum and Nilsson, 1976).
However, Wahl and Kuschinsky (1977) subsequently noted that the vasodilating action of adenosine was significantly reduced by 6 μM potassium and completely attenuated with 10 μM potassium. In rats, Astrup et al. (1978), using extracellular interstitial electrodes, recorded an increase in potassium from 3.4 to 7 μM during the first 15 seconds of bicuculline-induced seizures. Therefore, the effect of adenosine on the cerebral vessels during the initial period of seizure may be attenuated.

However, eliminating the possible role of adenosine in the regulation of CBF during seizures would be valid only if the response of the intraparenchymal vessels (<50 μm) is similar to that of the larger (>50 μm) pial vessels measured by Wahl and Kuschinsky (1977), and only if the changes in extracellular potassium concentrations observed by Astrup et al. (1978) reflect the changes in perivascular concentrations. Unlike the pial vessels, the smaller intraparenchymal vessels (<50 μm), which appear to play a significant role in the regulation of cerebrovascular resistance (Stromberg and Fox, 1972; Kontos et al., 1978; Rosenblum, 1977; MacKenzie et al., 1979), are invested by glial foot plates (Maynard et al., 1978). Henn et al. (1972) have suggested that glial cells exert active control of extracellular potassium by serving as "spatial buffers" for brain extracellular space (Orkland et al., 1966). Buffering by the glial cell would occur by conduction of potassium ions away from local areas in which extracellular potassium increases due to neuronal activity (Henn et al., 1972). Any areas either wholly or partially surrounded by glia, such as the perivascular space, would be expected to have stable concentrations of potassium, despite large changes in extracellular potassium. In addition, as noted previously, the cell wall of the glial foot plate contains 5'-nucleotidase, the enzyme responsible for production of adenosine from AMP. Therefore, because of the unique glial-vascular cytoarchitecture of intraparenchymal vessels, adenosine may be higher and potassium lower in perivascular fluid than in the interstitial fluid.

The changes in inosine and hypoxanthine, metabolites of adenosine, reflect the alterations in adenosine concentration. The rise of inosine and hypoxanthine after 60 seconds of convulsions is in contrast to the upward trend of inosine and stability of hypoxanthine concentrations after 60 seconds of ischemia (Winn et al., 1979a). Only after prolonged ischemia (20 minutes) were elevations in hypoxanthine observed (Berne et al., 1974). The rapid rise in hypoxanthine during seizures may be related to the alteration in the blood-brain barrier known to occur during bicuculline-induced convulsions (Johansson and Nilsson, 1977). Alteration in the permeability of the vascular endothelium may allow inosine access to nucleoside phosphorylase. This enzyme, which degrades inosine to hypoxanthine, is confined, in brain, to an intracellular site within the vascular endothelium (Rubio et al., 1978). Therefore, in the non-convulsing state, limited degradation of inosine occurs, limiting the salvage pathway of incorporation of inosine into brain nucleotides (Winn et al., 1980).

Recently, Skolnick et al. (1978) found that both inosine and hypoxanthine are inhibitors of diazepam binding sites in brain and that inosine is capable of antagonizing pentylenetetrazole-evoked seizures. In an earlier report, Phillips et al. (1975) noted that iontophoretically applied adenosine depressed the excitability of cortical neurons. Thus, during seizures, adenosine may be increasing CBF and, together with its metabolites, may also be decreasing neuronal activity and, therefore, metabolic demand.

The changes observed in the present study in adenine nucleotides, PCr, and lactate during seizures are comparable to the findings of previous investigators (Minard and David, 1962; King et al., 1967; Ferrendelli and McDougal, 1971; Duffy et al., 1975; Plum and Duffy, 1975; Chapman et al., 1977). In general, these earlier studies revealed a transient change in ATP (decrease), ADP and AMP (increase), and a persistent alteration in PCr (decrease), and lactate (increase) during the first 60 seconds of seizures induced by either electroshock or a variety of chemical substances. As in the present study, Chapman et al. (1977) induced seizures by bicuculline, but used the in situ technique to sample rat cortex. The changes during 60 seconds of bicuculline seizures in adenine nucleotides, PCr, and lactate observed by Chapman et al. (1977) are similar to the results of the present study. In contrast to adenosine, these metabolites do not appear to be affected by the method of tissue sampling, supporting earlier observations of Ferrendelli et al. (1979).

Previous attempts to understand the control of CBF during seizures have concentrated on a metabolic explanation, but the factors coupling CBF and metabolism are unclear. For example, recently, both an increase (Kuschinsky and Wahl, 1979) and a decrease (Astrup et al., 1978) in H⁺ ion have been demonstrated during the first several seconds of seizures. Potassium, also suggested as playing a role in CBF regulation, is increased initially during seizures but may not account for the persistent increases in flow (Astrup et al., 1978). Lactate, another candidate for linking CBF and metabolism (Plum and Duffy, 1975) may play a role in CBF regulation despite its weak vasodilator action. Some of the lactate measured in brain reflects blood concentrations, since lactate is freely diffusible. Furthermore, lactate concentrations continued to rise for several minutes after the onset of convulsions, unlike CBF, which stabilized at increased levels within seconds of the onset of seizures. If adenosine regulates CBF during seizures, it may act in concert with other factors, as suggested by Rubio et al. (1975). In addition, since adenosine is elevated within 5 seconds of the onset of seizures, adenosine
may have a more significant role during the initial phases of seizures than during subsequent periods, although it remained elevated even after 2 minutes of convulsions. However, the rapid, sustained increase in adenosine concentrations, which are within the vasoactive range and which temporally parallel the changes in CBF, support the thesis that this potent pial dilator is involved in the regulation of CBF during seizures.

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Changes in brain adenosine during bicuculline-induced seizures in rats. Effects of hypoxia and altered systemic blood pressure.

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