Neonatal Seizures in Monkeys and Rabbits: Brain Glucose Depletion in the Face of Normoglycemia, Prevention by Glucose Loads

BARNEY E. DWYER AND CLAUDE G. WASTERLAIN

Epilepsy Research Laboratory, V.A. Medical Center, Sepulveda 91343 and the Department of Neurology and the Brain Research Institute, U.C.L.A. School of Medicine, Los Angeles, California 90024

ABSTRACT. Sustained convulsive seizures were induced with bicuculline in newborn rabbits and marmoset monkeys. In both species, seizures were predominantly tonic, with generalized polyspikes on the EEG. Brain glucose concentration fell dramatically during seizures in both species, and in many normoglycemic animals reached levels usually associated with severe hypoglycemia, suggesting that glucose transport from blood to brain could not keep pace with glucose utilization. Glucose loads given to rabbits induced hyperglycemia and during seizures maintained brain glucose well above concentrations needed to saturate hexokinase so that glycolytic rates were probably never limited by substrate availability. Blood and brain lactate concentrations rose during seizures but never reached levels considered cytotoxic. These data suggest that epileptic seizures can deplete brain glucose in normoglycemic neonates of several species, including subhuman primates. (*Pediatr Res* 19: 992–995, 1985)

Neonatal status epilepticus in rats can result in death or in stunted brain growth (1). During seizures brain glucose fell in these animals despite normal blood glucose levels (2). If rats were made hyperglycemic by injecting them with an isotonic glucose solution before seizure onset the mortality rate from status epilepticus was reduced by 90% during the 1st wk of life, brain glucose was not depleted and deficits in brain growth were reduced (2). The purpose of this investigation was to determine whether these phenomena are peculiar to the rat or occur in other species including subhuman primates. We have induced generalized seizures with bicuculline, a plant alkaloid, which blocks the postsynaptic inhibitory action of GABA on neuronal firing rates (3). We have shown that continuous seizures can deplete brain glucose in newborn rabbits and marmoset monkeys, in the presence of normal blood glucose concentrations, and that brain glucose depletion in seizing rabbits can be prevented if animals are made hyperglycemic prior to seizure onset.

MATERIALS AND METHODS

Animals. Rabbits of either sex (12–24 h old) were purchased from Curd's Caviary and Animal Supply (LaPuente, CA). Marmoset monkeys (*Callithrix jacchus*) aged 4–7 days were obtained from our breeding colony at the Sepulveda V.A. Medical Center.

Epileptic Seizures. Animals were maintained in a 37° C water bath during the experiment. Seizures were induced by the intramuscular injection of bicuculline HCl (5 mg/kg). Bicuculline obtained from Calbiochem (LaJolla, CA) was dissolved in 0.1 N HCl and the pH was adjusted to about 5 with 0.1 N NaHCO₃ shortly before use. The time of onset of seizures was taken as the time of the first tonic seizure, usually 2–3 min after bicuculline injection.

Tissue Sampling. Rabbits were subjected to microwave fixation (1–1.3 s) in the Metabostat model 4094 focused beam microwave oven (Gerling Moore; Palo Alto, CA) at various times after seizure onset. Blood was obtained from the decapitated trunk and fixed brain tissue was dissected from the skull. Marmosets (30–50 g) were immersed in liquid nitrogen with rapid agitation at times indicated in the text. Shortly before freezing, blood was obtained from the tail. Instruments were chilled in liquid nitrogen and the brain was chipped from the skull on dry ice. Tissues were repeatedly immersed in liquid nitrogen during the dissection and stored at −70° C until processed.

Chemical Assays. Whole blood and brain tissue were homogenized in 1.2 N perchloric acid chilled in a dry ice-acetone bath. The sample was centrifuged at $100,000 \times g$ for 30 min. The clear supernatant was neutralized with KOH and the insoluble KClO₄ was pelleted and discarded. Glucose was measured using an oxidase/peroxidase colorimetric assay purchased in kit form from the Sigma Chemical Co. (St. Louis, MO). Lactate was measured by a spectrophotometric assay also purchased in kit form from Sigma. Brain glucose and lactate concentrations were corrected for the contribution from blood which was estimated to represent 3% of brain wet weight.

Experimental Design. Rabbits. Three groups of rabbits were studied: group 1 consisted of untreated animals and are referred to in the text as the control group. Animals in groups 2 and 3 received seizures induced with bicuculline. Group 2 rabbits were injected with 5% of body weight of an isotonic glucose solution [5.4% D-glucose (w/v) pH 7.2] 30 min prior to bicuculline administration and are referred to in the text as "glucose-treated." Group 3 rabbits received an equivalent volume of 0.9% NaCl 30 min prior to bicuculline and served as a volume control. They are referred to in the text as "saline-treated."

Marmosets. Two groups of marmosets were studied: the control group (n = 3) was untreated. The seizure group (n = 9) received bicuculline alone to induce convulsions and were otherwise untreated.

The statistical significance of experimental data was determined by standard *t* test analysis.

RESULTS

Epileptic seizures. Immediately after intramuscular injection of bicuculline, rabbits were quiet with a normal EEG; then the
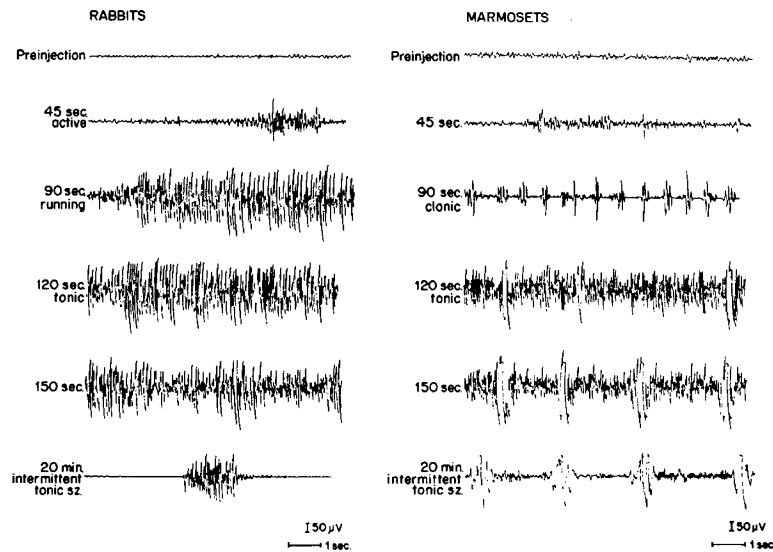
Fig. 1. Electroencephalograms of newborn rabbits and marmoset monkeys recorded from scalp electrodes during seizures induced by bicuculline.

animals became restless and agitated, as bursts of higher-voltage paroxysmal activity appeared in the tracing (Fig. 1). This hyperactivity evolved into constant running movements of all limbs, associated with loss of posture, so that the animal was lying on its side, paddling with all four limbs, with an EEG made of nearly continuous polyspikes (Fig. 1). As those increased in intensity, a tonic seizure developed and lasted approximately 15 s. These seizures were followed by a few multifocal clonic seizures interrupting postictal depression of behavioral and EEG activity. Tonic clinical and EEG seizures recurred periodically throughout the experiment with a progressive increase in the duration and intensity of postictal depression. Little difference in seizure intensity was noted between glucose and saline-injected animals. Marmosets had a similar response to bicuculline, characterized by increased activity with bursts of high voltage EEG activity, followed by clonic movements of the head and all four limbs with synchronous bursts of high voltage polyspikes, then by a tonic seizure with continuous high-voltage spikes, itself followed by clonic limb activity and EEG bursts (Fig. 1). Subsequent seizures were predominantly tonic but retained a more prominent clonic component than in rabbits.

Glucose. The effect of bicuculline seizures on blood and brain glucose concentration in newborn rabbits is shown in Figure 2. In untreated control rabbits, both blood and brain glucose concentrations were higher than expected; this may have reflected the stress of transportation and handling. Blood glucose concentration in the saline-treated rabbits remained close to control levels for the first 10–15 min of seizures, falling to 33% of control ($p < 0.01$) by 30 min (Fig. 2). Glucose-treated rabbits were hyperglycemic throughout the duration of the experiment.

Brain glucose in saline-treated rabbits fell rapidly after seizure onset (20% of control, $p < 0.01$—2 min) despite the absence of hypoglycemia. After 10 min of seizure activity, when blood glucose was still in the normal range, mean brain glucose concentration was 0.08 ± 0.077 mmol/kg, less than 3% of control levels and it remained at this level after 15 or 30 min of seizure activity. Glucose injection prior to seizures prevented most of the severe brain glucose depletion found in saline-treated rabbits except at 2 min when it was 26% of control levels ($p < 0.001$) (Fig. 2). Unlike the results in the saline group, brain glucose steadily increased in glucose-treated rabbits as the duration of seizures lengthened. Even though the glucose concentration in glucose-treated rabbits was still relatively low after 5 and 10 min of seizures (41 and 43% of control, respectively, $p < 0.01$), it was still greater than expected for unstressed rabbits.

Figure 3 depicts blood and brain glucose concentrations in newborn marmoset monkeys during bicuculline seizures. After
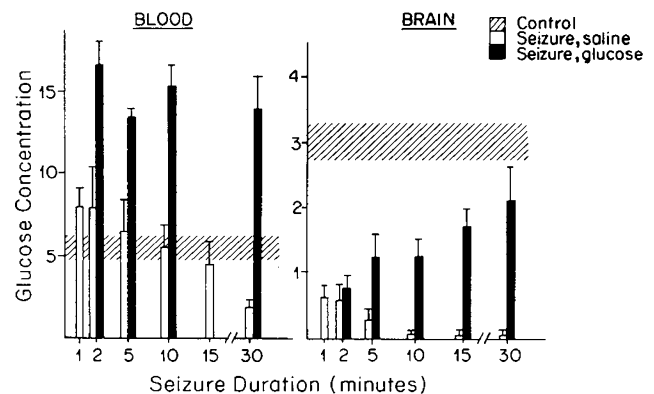

Fig. 2. Blood and brain glucose concentration during status epilepticus in newborn rabbits. Rabbits in which seizures were induced were injected with solutions of isotonic glucose or NaCl (5% of body weight subcutaneously) 30 min prior to bicuculline injection (5 mg/kg, intramuscular). Experimental animals were sacrificed by focused microwave irradiation at the times after seizure onset indicated in the figure. Blood glucose is expressed as mmol/l and brain glucose as mmol/kg. The *hatched area* represents the mean value ± SEM for untreated control rabbits ($n = 18$). Other points represent mean ± SEM for following: Glucose treated—blood and brain: $n = 3$ for each time point. Saline-treated: blood: 1 min ($n = 4$), 2 min ($n = 7$), 5 min ($n = 15$), 10 and 30 min ($n = 5$), 15 min ($n = 10$). Saline treated—brain: 1 and 30 min ($n = 4$), 2 min ($n = 7$), 5 min ($n = 12$), 10 min ($n = 5$), 15 min ($n = 9$). For statistical analysis, data obtained from rats in the control group were pooled.

30 min of seizures, severe reductions in brain glucose concentrations were observed. In fact, two animals had brain glucose concentrations of 0.06 and 0.08 mmol/kg, respectively. Blood glucose in seizing marmosets while generally lower than in controls (seizure: 5.87 ± 3.38 *versus* control: 8.53 ± 3.09 mmol/ l ± SD) never fell below 2 mM (36 mg/dl) in any animal. Thus in newborn marmosets, as in newborn rabbits, brain glucose can be severely reduced by continuous seizures while blood glucose concentration is maintained close to physiological levels.

Lactate. Bicuculline seizures produced a sharp increase in blood lactate in both saline- and glucose-treated rabbits (Fig. 4). Most of the increase in both groups occurred within 2 min after seizure onset when lactate was elevated close to six times above control levels, compared to about an 8-fold increase after 30 min of seizures. Remarkably, blood lactate increased to about the same concentration in both glucose- and saline-treated rabbits
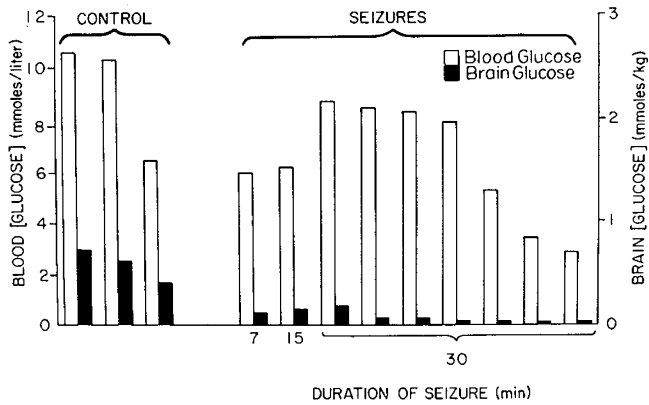
Fig. 3. The effect of status epilepticus on blood and brain glucose in newborn marmoset monkeys. Marmoset monkeys (4–7 days old) were injected with bicuculline (5 mg/kg, intramuscular) to induce seizures or the equivalent volume of isotonic NaCl (controls) and were sacrificed by immersion in liquid nitrogen at various times after seizure onset as indicated on the abscissa. Each pair of *bars* represents values of blood and brain glucose for a single marmoset.
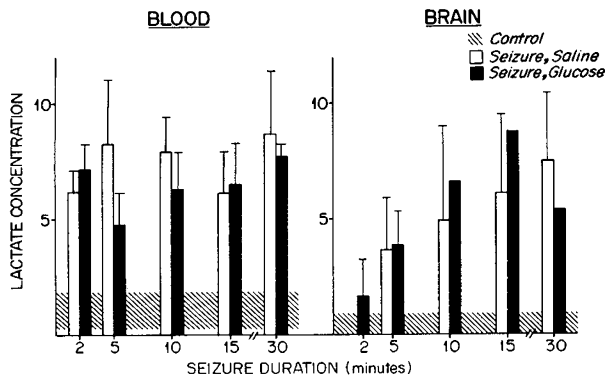

Fig. 4. Blood and brain lactate concentration during status epilepticus in newborn rabbits. Experimental conditions were the same as those described in the legend to Figure 2. Blood lactate is expressed as mmol/l and brain lactate as mmol/kg. The *hatched area* represents the mean ± SEM for untreated control rabbits—blood ($n = 15$), brain ($n = 12$). The n for each group is given below. Where standard error bars are not included in the graph $n < 3$ and both n and individual measurements are given. Glucose treated—blood: $n = 3$ for each time point. Brain: 2 and 5 min ($n = 3$), 10 min (6.62), 15 min (10.08, 7.47), 30 min (7.51, 11.27). Saline-treated—blood: 2 and 30 min ($n = 3$), 5 and 10 min ($n = 5$), 15 min ($n = 4$). Brain: 5 and 10 min ($n = 4$), 15 and 30 min ($n = 3$).

despite the greatly increased blood glucose concentrations in the former group. Brain lactate accumulation during seizures was similar in both glucose- and saline-treated rabbits. It did not bear a simple relationship to brain glucose since by 10 min after seizure onset brain glucose in saline-treated rabbits was depleted while brain lactate was slowly increasing. More likely, brain lactate in both groups of seizing rabbits reflected blood lactate concentrations.

DISCUSSION

The occurrence of brain glucose depletion during seizures in newborn marmosets (a species more mature at bith than the human), rats, and rabbits (species less mature at birth than humans) suggests that this phenomenon is seen in neonates of many species including primates. Our data suggest that mild hyperglycemia may be beneficial for the newborn during repetitive seizures as long as circulation is maintained, and underscore the dangers of hypoglycemia during seizures. Hypoglycemia can

have devastating consequences for the neonate (4–6) and it is conceivable that the combination of brain glucose depletion with seizure activity, may be even more damaging to the brain than glucose depletion alone.

The therapeutic value of elevated blood glucose has been seriously questioned. Hyperglycemia has been associated with an adverse outcome from cerebral ischemia in adult rodents (7–11) and in humans following cardiac arrest (12). It was associated with more severe CNS damage following total asphyxia in the term monkey fetus (13, 14), and did not alter the outcome of neonatal hypoxia-ischemia (15). In contrast, hyperglycemia reduced the mortality rate of immature rodents during prolonged convulsions (2) and anoxic anoxia (16, 17). Presumably the harmful effects of hyperglycemia in ischemia or asphyxia arise from the local lactic acidosis. However, brain lactate concentrations in excess of 20 mmol/kg which are associated with cell damage (18, 19) are far above those found in our animals. Seizures in adult rats (20), newborn dogs (21), and newborn monkeys (22) result in increased cerebral blood flow. The immature blood-brain barrier contains a high content of monocarboxylic acid carrier (23) by which lactate can enter or leave the brain. This combined with sustained blood flow may insure that lactate cannot accumulate to levels which exceed the buffering capacity of brain tissue (24) and are incompatible with cell survival. Under physiological conditions brain glucose utilization is limited by glucose flux through phosphofructokinase and not by transport into brain. Hexokinase may play a secondary role in the regulation of glucose flux in the glycolytic pathway, but its low K_m for glucose estimated at from 8 to 45×10^{-6} M (25–27) assures almost total saturation of the enzyme by this substrate at normal intracellular brain glucose concentrations. During seizures, glucose in rabbit brain fell to a mean value of 8×10^{-5} M within 10 min despite normoglycemia. In seizing marmosets, brain glucose fell to less than 5×10^{-4} M, and in two animals to 6×10^{-5} and 8×10^{-5} M, respectively. These values likely overestimate true intracellular glucose concentrations. The 3% correction used for contamination of brain tissue by blood is probably a lower limit during seizures when cerebral blood flow is increased and the brain vasculature is dilated. Furthermore, no correction was applied for glucose in the brain extracellular fluid. This compartment was estimated to comprise 15% of brain mass in the adult and to have a glucose concentration about half of that in blood (28). Even if the glucose concentration in the extracellular fluid compartment were substantially less than one-half that in blood calculated brain intracellular glucose concentrations would approach zero. This calculation underscores the likelihood that true brain intracellular glucose concentration fell during seizures to levels which could not saturate hexokinase so that glucose transport into brain became rate-limiting for cerebral glucose utilization. In contrast, when animals were made hyperglycemic, brain glucose concentration remained well above 10^{-3} M during seizures.

The basis for these results likely reflects the physical nature of the immature blood-brain barrier. In rats during the 1st wk of life glucose flux into brain from blood is only about 20% of that in adults (29, 30). These data suggest that the concentration of the glucose carrier per unit of brain weight is lower in the immature brain, probably as a result of lower capillary density. This is presumably why, when glycolysis is increased by seizures, more glucose is utilized than can be transported in. The protective effect of hyperglycemia can be understood by considering the kinetic properties of the transport system in the blood-brain

barrier. From the Michaelis-Menten equation $\left(V = \dfrac{V_{max}(S)}{K_m + (S)} \right)$

it can be calculated that at physiological blood glucose concentration (5 mM) the glucose carrier whose K_m is about 7 mM is never saturated (3) and the blood-brain barrier is able to utilize only 42% of its maximal transport capacity for glucose. If blood glucose concentration were to fall to 1.5 mM glucose transport

across the blood-brain barrier would operate at an efficiency of 18%. However, if blood glucose was elevated to 11 mM then 61% of the maximal transport capacity for glucose would be available. Hyperglycemia did not modify the intensity of the convulsive seizures in the newborn rabits, and presumably the rate of glucose utilization in brain. Thus the presence of significant amounts of glucose in the brains of glucose-treated rabbits during prolonged seizures likely resulted from increased glucose transport associated with hyperglycemia.

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