Susceptibility of the developing brain to acute hypoglycemia involving A1 adenosine receptor activation

Mina Kim, Zhao-Xue Yu, Bertil B. Fredholm, and Scott A. Rivkees. Susceptibility of the developing brain to acute hypoglycemia involving A1 adenosine receptor activation. *Am J Physiol Endocrinol Metab* 289: E562–E569, 2005; doi:10.1152/ajpendo.00112.2005.—It has been suggested that the developing brain is less vulnerable to the adverse effects of hypoglycemia than the mature brain; however, this issue remains controversial. We also do not know the magnitude or duration of hypoglycemia needed to trigger hypoglycemic brain injury during development. To address this issue a series of in vivo and in vitro studies were performed. First, we established an acute model of insulin-induced hypoglycemia in mice by administering 3 U/kg of neutral-protamine Hagedorn insulin subcutaneously. When we examined degenerating neurons in hippocampus and striatum by TUNEL labeling, injury was observed after 4 h of hypoglycemia in postnatal day (P7) mice, and we observed more cell injury in animals rendered hypoglycemic at P7 than at P21. Studies of hippocampal slice cultures revealed that reduction in glucose concentration induced more neuronal injury in slices prepared from P3 and P7 than from P14 and P21 mice. Treatment of slices with an adenosine A1 receptor (A1AR) antagonist reduced the hypoglycemic damage, whereas agonists increased damage, particularly in slices prepared from very young pups. This suggests a critically important role for A1ARs, which was further demonstrated by the reduction of hypoglycemic damage in hippocampal slices prepared from A1AR−/− mice. Furthermore, insulin-induced hypoglycemia in P7 A1AR−/− mice did not increase TUNEL-positive cells, but a major increase was seen in A1AR−/− mice. These observations show that the developing nervous system is indeed sensitive to acute hypoglycemic injury and that A1AR activation contributes to damage induced by hypoglycemia, particularly in immature mouse brain.

*These authors contributed equally to this work.

Address for reprint requests and other correspondence: S. A. Rivkees, Yale Pediatrics YCHRC, 464 Congress Ave., New Haven, CT 06520 (e-mail: Scott.Rivkees@Yale.edu).

AN EXTENSIVE BODY OF EVIDENCE suggests that acute or chronic hypoglycemia leads to neurological dysfunction and injury (8, 16, 26, 46, 54). Children and adults exposed to hypoglycemia can develop long-term impairment of cognitive function (8, 16, 24, 26, 46, 54) and are at increased risk for epilepsy (27). Vulnerable areas of the brain include the hypothalamus, striatum, cerebral cortex, and hippocampus (4, 39, 48, 49). Although it has been suggested that the developing brain is less vulnerable to hypoglycemic injury than the mature brain (16, 54), a considerable amount of clinical evidence suggests that the developing nervous system is sensitive to the adverse effects of hypoglycemia. Preterm infants with moderate hypoglycemia can have impaired neurodevelopmental outcome (35). Children with hypoglycemia due to persistent hyperinsulinism have an increased incidence of brain injury (1, 38). Infants of diabetic mothers can sustain long-term brain injury if there is a period of severe hypoglycemia in the first 12 h after birth (32, 54). In young children with diabetes mellitus, short-term episodes of hypoglycemia are associated with long-term learning problems (28, 44–46). As in brain injury associated with ischemia and neurodegenerative conditions, altered neurotransmitter action appears to play a role in hypoglycemic brain injury (2–4). Attention has been focused on glutamate as a potential mediator of hypoglycemic brain injury (2, 9, 36). Yet, other neuromodulators, such as adenosine, may play a role in this process, as hypoglycemia induces robust increases in local adenosine levels (10, 53). In studies of cultured neurons, the hypoglycemia-induced adenosine release has been observed to trigger cell death via activation of adenosine A1 receptors (A1ARs) and alteration in intracellular calcium levels (53).

A1AR activation is generally neuroprotective in adulthood (12), although deletion of the A1AR gene does not alter neuronal damage following ischemia in vivo or in vitro (41). In contrast, A1AR activation contributes to brain injury in the neonatal period (43, 52, 53), showing that A1AR action varies with developmental stage. When A1AR expression is examined, expression in the brain is present early in gestation in rodent fetuses (42), and receptor G protein coupling is present in the immediate postnatal period. There is a slight increase in A1AR expression from after birth until mature stages (42).

At present, our understanding of the effects of hypoglycemia on the developing brain is incomplete. We also do not know the magnitude or duration of hypoglycemia needed to trigger brain injury during development. To address these issues, we have examined the developmental sensitivity to acute hypoglycemia in vivo and in vitro. We have also examined the role of A1AR activation in this process.

**METHODS**

*Animals.* All studies were performed in accordance with protocols approved by the Yale Institutional Animal Care and Use Committee. C57Bl/6 mice were obtained from Charles River Laboratories. Animals were given food and water ad libitum. In some studies, previously characterized C57-Bl6/OlaHsd A1AR-deficient mice (A1AR−/−) were used (23). Animals were genotyped as reported (52).

*Glucose determinations.* Blood was obtained by pricking a paw with a sterile lancet. Glucose values were monitored using a Freestyle Navigator.
Fig. 1. Insulin-induced hypoglycemia. Changes in blood glucose levels following sc injection of 3 U/kg neutral-protamine Hagedorn (NPH) insulin (gray bars) or vehicle (filled bars) in postnatal day (P)7 mice. *P < 0.01 vs. vehicle at all time points and vs. insulin at 0 and 6 h, ANOVA.

We observed that ~10% of the pups injected with insulin died. Of the surviving pups, all were responsive to touch and spontaneously active. Fewer than 5% of pups of the surviving pups showed overt seizure activity. Pups demonstrating seizure activity were euthanized and excluded from the study to eliminate possible confounding effects of seizure activity on outcome. Mice of different ages were given different doses of insulin. After the injections, glucose levels were assessed by glucometer until the animals became euglycemic (>3.0 mM). Because the Freestyle glucometer required less blood (0.3 μl) than the glucose oxidase method (5 μl), glucose determinations were assessed by glucometer. We found that it was very difficult to repeatedly obtain more than 1–2 μl of blood from small pups with lancet sticks, necessitating glucometer use. Comparing blood glucose readings obtained by glucometer with concentrations determined by glucose oxidase assay, we validated the use of the glucometer. The lower limit of detectable glucose levels was 0.75 mM by glucometer. From 0.75 to 10 mM, there was excellent correlation between glucometer and glucose oxidase assays (r2 = 0.991, P < 0.001).

To define doses of insulin that induce hypoglycemia, postnatal day (P)7 and P21 mice were injected with 1–10 U/kg NPH insulin. We found that doses in excess of 6 U/kg resulted in profound, sustained hypoglycemia (<0.75 mM) and animal death. Doses lower than 3 U/kg failed to result in hypoglycemia (<3.0 mM). Thus 3 U/kg of insulin were used in all of the studies presented below. Control animals were injected with the insulin diluent.

At 24 h after injections, animals were killed with inhaled halothane. Brains were dissected, frozen, and stored at −80°C. Brains were cut in a cryostat at 20 μm thickness. Sections were thaw-mounted onto Super Frost Plus slides (Fisher, Pittsburgh, PA) that were pretreated with Vectabond reagent (Vector Laboratories, Burlingame, CA). Brain sections spanning the midstriatum and hippocampus were collected.

TUNEL assays. Apoptotic cell injury in tissue sections was assessed by TUNEL (terminal transferase dUTP nick end labeling; In-Situ Apoptosis Kit-POD, Roche) assay. Slides were air dried and fixed in 4% phosphate-buffered paraformaldehyde (PFA) for 15 min. Slides were washed with 1% phosphate-buffered saline (PBS) three times and treated with 0.3% Triton X-100 in PBS for 15 min. After

Table 1. TUNEL staining after 4 h of hypoglycemia as related to age

<table>
<thead>
<tr>
<th></th>
<th>P7</th>
<th>P21</th>
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<tbody>
<tr>
<td>Hippocampus</td>
<td>37 ± 3</td>
<td>124 ± 11*</td>
</tr>
<tr>
<td>Striatum</td>
<td>51 ± 5</td>
<td>180 ± 20*</td>
</tr>
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</table>

Values are means ± SE of TUNEL-positive cells/mm²; n = 5–7 animals/group. *P < 0.05 vs. control, ANOVA.

DEVELOPING BRAIN SUSCEPTIBLE TO ACUTE HYPOGLYCEMIA
containing 1 ml of culture medium consisting of neurobasal medium (NBM) with 5 mM l-glutamine (GIBCO-BRL), 6 mM d-glucose (GIBCO-BRL), 2% B27 supplements (GIBCO-BRL), and antibiotic solution (Sigma), pH 7.35. Slices were incubated for an additional 24 h. At the end of the incubation period, slices were fixed with cold 4% PFA in PBS, pH 7.4, dehydrated in 30% sucrose, and sectioned in a cryostat (14 μm thickness).

Quantification of TUNEL-positive cells in slice cultures. Cryostat-cut tissue sections were fixed with 4% PFA in PBS and treated with 0.3% Triton X-100 in PBS for 30 min. After washes with PBS, sections were incubated with fluorescein-12-dUTP and terminal transferase in the buffer provided in the TUNEL kit. Counterstaining with Hoechst or 4',6-diamidino-2-phenylindole (DAPI, Sigma) was performed to label all cells. Sections were examined using a light microscope connected to a computer. Digital images were captured and saved using Image Pro software. All images were captured using the same exposure parameters. To assess numbers of TUNEL-positive cells, each image was examined at a magnification of ×400. The percentage of TUNEL-positive cells in the hippocampal CA1 region was determined using Image Pro software. Each experimental group consisted of four or five slices prepared from at least four different animals per age.

Double-labeling studies. Sections were fixed with 4% PFA in PBS and preblocked with 3% bovine serum albumin (BSA) in PBS and 0.1% Triton X-100 after washes with PBS. Sections were incubated with a mouse monoclonal antibody against neuronal nuclei (NeuN, 1:200 dilution; Chemicon, Temecula, CA), a mouse monoclonal antibody against glial fibrillary acidic protein (GFAP, 1:200 dilution; antibody against glial fibrillary acidic protein (GFAP, 1:200 dilution; Chemicon, Temecula, CA), a mouse monoclonal antibody against glial fibrillary acidic protein (GFAP, 1:200 dilution; Chemicon, Temecula, CA), and a mouse monoclonal antibody against neuronal nuclei (NeuN, 1:200 dilution; Chemicon, Temecula, CA).

Table 2. TUNEL staining as related to duration of hypoglycemia in P7 injected mice

<table>
<thead>
<tr>
<th></th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Insulin</td>
<td>Control</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>31 ± 8</td>
<td>30 ± 4</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>Striatum</td>
<td>31 ± 7</td>
<td>31 ± 5</td>
<td>51 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE of TUNEL-positive cells/mm²; n = 5–7 animals/group. P, postnatal day. *P < 0.05 vs. control, ANOVA.
Chemicon), and a rabbit polyclonal antibody against the activated form of caspase-3 (1:200 dilution; Cell Signaling Technology, Beverly, MA) at 4°C for 24 h. Immunofluorescent samples for NeuN and GFAP were stained with secondary antibodies conjugated with Alexa fluor (Molecular Probes, Eugene, OR) and then double stained for TUNEL. Sections were covered using mounting medium containing DAPI for fluorescence (Vector Laboratories). Caspase-3 immunoreactivity products were visualized with the avidin-biotin complex kit (Vector ABC Elite) and counterstained with cresyl violet (Sigma).

**Statistical analysis.** Multiple comparisons were performed by ANOVA with Bonferroni posttest comparisons among individual groups. Paired comparisons were by Student’s *t*-test. Statistical analyses were performed using GraphPad Prism (San Diego, CA). Data presented are means ± SE.

## RESULTS

We observed that hypoglycemia (glucose <3 mM) was present at 2 and 4 h, and not at 6 h, in 80% of P7 mice injected with 3 U/kg insulin. In the remaining P7 animals, hypoglycemia was either not at all present, present at only 2 h, or present at 6 h or longer. Of the insulin-injected animals that were hypoglycemic at 2 and 4 but not at 6 h (Fig. 1), the mean blood glucose concentration 2 h after injection was 1.3 ± 0.12 mM (*n* = 21). At 2 h, control animals had mean blood glucose levels of 5.2 ± 0.21 mM (*n* = 21). Four hours after insulin injection, the pups remained hypoglycemic, with blood glucose values of 1.9 ± 0.2 mM, and control glucose levels were 5.1 ± 0.3 mM. At 6 h after injection, the insulin-injected group had mean glucose values of 4.8 ± 0.5 mM, and controls values were 5.7 ± 0.18 mM (Fig. 1).

With the background provided by the aforementioned studies, we examined effects of hypoglycemia on brain injury in animals of different ages. In other studies of the effects of A1AR activation and N-methyl-D-aspartate (NMDA) receptor blockade on brain injury at different developmental stages, apoptotic injury is observed after insults delivered during the first 2 wk of postnatal life, and injury susceptibility wanes after P14 (21, 40, 51). Thus we examined mice at P7 and P21. To assess injury, we examined TUNEL labeling in the hippocampus and striatum, which labels degenerating cells injured by apoptosis or necrosis (11). Insulin-injected animals with glucose levels lower than 3.0 mM at 2 and 4 h after insulin injection and vehicle-injected littermates were initially examined.

At P7, brain sections from hypoglycemic mice showed significantly more TUNEL labeling than sections from vehicle-injected mice (Fig. 2 and Table 1). Yet, unlike that seen in P7 animals, scant apoptosis was observed in the hypoglycemic P21 pups (Table 1).

Because we observed variability in the duration of hypoglycemia in the P7 pups, we examined neuronal injury as related to hypoglycemia duration. Animals were stratified in groups according to whether their hypoglycemia (<3.0 mM) lasted 2, 4, 6, or 8 h. In the pups hypoglycemic for 2 h or less, we did not observe differences between insulin- and vehicle-treated groups in TUNEL labeling in either striatum or hippocampus (Table 2). When hypoglycemia was present for 4 h or longer, neuronal injury was observed (Table 2).

Next, to complement in vivo studies, hippocampal brain slices were studied in a series of in vitro experiments. First, we examined slices from P7 mice treated with 0.75 or 6 mM glucose for 24 h. A glucose concentration of 0.75 mM was chosen to approximate brain glucose levels seen in hypoglycemia, as extracellular brain glucose levels are ~20% of mean glucose values in hypoglycemia (20).

### Table 3. Hypoglycemia-induced injury in hippocampal slices as related to age

<table>
<thead>
<tr>
<th></th>
<th>P3</th>
<th>P7</th>
<th>P14</th>
<th>P21</th>
</tr>
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<tbody>
<tr>
<td>6 mM</td>
<td>7±1</td>
<td>6±1</td>
<td>8±1</td>
<td>7±1</td>
</tr>
<tr>
<td>0.75 mM</td>
<td>58±6*</td>
<td>69±4*</td>
<td>18±5</td>
<td>5±2</td>
</tr>
</tbody>
</table>

Values are %total cells ± SE that are TUNEL-positive; *n* = 6–8 slices/treatment in each group. *P* < 0.05 vs. 6 mM, ANOVA.

Fig. 4. Hypoglycemia-induced injury involves neuronal nuclei (NeuN)-positive cells in cultured hippocampal slices from P7 mice. Slices were cultured in medium containing 0.75 mM glucose for 24 h and stained for TUNEL (green), NeuN (red), and DAPI to label all cells. We observe that 69 ± 10% of TUNEL-positive cells are labeled for NeuN. Note: when neuronal death occurs, NeuN reactivity is lost. Scale bar, 0.1 mm.
circulating levels (13). We observed the greatest proportion of TUNEL-positive cells in slices exposed to low glucose concentrations (Fig. 3 and Table 3). Activated caspase-3, which labels cells undergoing apoptosis (37), was also observed in the slices exposed to low glucose levels (data not shown).

To determine the identity of injured cells, double-labeling studies were performed using NeuN to label neurons and GFAP to label glial cells. We observed that TUNEL-positivity colocalized with NeuN-positive cells (69 ± 10%; Fig. 4), indicating that many of the apoptotic cells are neurons. We also found astrocytes detected by a monoclonal GFAP antibody that were TUNEL positive.

To examine developmental sensitivity to hypoglycemia, we next examined slices prepared from P7 mice. We observed that low glucose levels induced considerable TUNEL labeling at P3 and P7, and that TUNEL labeling was less at P14 and P21 (Fig. 5 and Table 3).

We next examined whether A1AR activation plays a role in hypoglycemic injury. First, we assessed effects of A1AR activation on hippocampal cultures. We examined the relative percentage of TUNEL-positive cells in hippocampal slices prepared from P7, P14, and P21 mice treated with vehicle, CPA, or DPCPX in 6 mM glucose. We observed that CPA-induced injury was more severe at P3 and P7 than at P14 and P21 (Fig. 6 and Table 4). In contrast, we did not observe effects of DPCPX on cell injury (Fig. 6 and Table 4). We also observed that DPCPX prevented CPA-induced injury (data not shown; n = 6 slices).

To evaluate the effects of A1AR activation on hypoglycemia-induced injury, slices from P3, P7, P14, and P21 mice were incubated in 0.75 mM glucose with and without DPCPX or CPA. We observed that CPA potentiated the effects of hypoglycemia on cell injury. In contrast, DPCPX reduced cell injury (Fig. 7 and Table 5).

As an additional approach for assessing whether hypoglycemic injury was A1AR mediated, brain slices were prepared from P7 A1AR+/−/− and A1AR−/− littermates, which were offspring of A1AR+/− dams paired with A1AR−/− males. When cultured in 0.75 mM glucose, the numbers of TUNEL-positive cells in A1AR+/− hippocampal slices were significantly greater than those observed in slices obtained from A1AR−/− mice (Fig. 8 and Table 6).

After the slice culture studies, we next examined insulin-treated A1AR+/− and A1AR−/− mice at P7. No differences were observed in the magnitude or duration of glucose nadirs among the different genotypes (A1AR+/− 1.8 ± 0.2 mM, A1AR−/− 1.9 ± 0.2 mM; n = 5 per group). Yet, cell counts showed more TUNEL-positive cells in the A1AR+/− mice than in the A1AR−/− mice (Table 7).

**DISCUSSION**

Our observations suggest that the developing murine brain is sensitive to the adverse effects of short-term hypoglycemia. Support for this notion is derived from in vivo studies showing that acute hypoglycemia induces more injury in younger than
Table 4. A1AR-induced injury as related to age in hippocampal slices

<table>
<thead>
<tr>
<th></th>
<th>P3</th>
<th>P7</th>
<th>P14</th>
<th>P21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6±1</td>
<td>6±1</td>
<td>8±1</td>
<td>7±1</td>
</tr>
<tr>
<td>CPA</td>
<td>55±5*</td>
<td>47±6*</td>
<td>27±4*</td>
<td>19±4*</td>
</tr>
<tr>
<td>DPCPX</td>
<td>2±1</td>
<td>6±1</td>
<td>4±1</td>
<td>8±1</td>
</tr>
</tbody>
</table>

Values are %total cells ± SE that are TUNEL positive; n = 4–6 slices/treatment in each group. A1AR, A1 adenosine receptor; CPA, N6-cyclopentyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine. All slices in 6 mM glucose. *P < 0.05 vs. vehicle or DPCPX, ANOVA.

Table 5. A1AR influences on hypoglycemia-induced injury in P7 hippocampal slices

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Vehicle</th>
<th>CPA</th>
<th>DPCPX</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0 mM</td>
<td>11±5</td>
<td>48±5*</td>
<td>87±3*</td>
</tr>
<tr>
<td>0.75 mM</td>
<td>22±4</td>
<td>47±6*</td>
<td>97±5*</td>
</tr>
</tbody>
</table>

Values are %total cells ± SE that are TUNEL positive; n = 6 slices/group. *P < 0.05 vs. 0.75 mM DPCPX and 6.0 mM, ANOVA.

in older animals, observations that are paralleled by slice culture studies.

To date, studies of acute hypoglycemia-induced injury have relied on models in which hypoglycemia is coupled with other insults, such as hypoxia (4, 16, 31, 54). In vivo and in vitro studies of isolated hypoglycemia are few (4, 16, 31, 54). We are also unaware of reports directly comparing hypoglycemic effects on brain injury among animals of different ages. By testing effects of insulin at different ages, we were able to establish a model for examining the effects of acute hypoglycemia. Like others (54), we found variability in the duration of insulin-induced hypoglycemia. Taking advantage of this variability, we found that glucose levels less than 3 mM for 4 h or more were needed to induce neuronal degeneration in the young mice.

The finding that acute hypoglycemia can induce acute neuronal injury is especially interesting considering studies showing that neonatal rats are dependent on lactate and ketones as fuels and only later convert to being glucose dependent for brain energy supply (55, 56). Glucose transporter expression in the young brain is not dependent on glucose for energy, it is possible that the brain injury that we observed is not due to the hypoglycemia itself; rather, neuronal injury may be secondary to altered neurochemical release. Our experimental findings support this notion and suggest a role for adenosine in this process. When we examined slices treated with the A1AR antagonist DPCPX, we observed protection against hypoglycemic injury, as we did in slices from A1AR−/− mice exposed to low glucose levels. We also observed reduced cell injury in A1AR−/− mice rendered hypoglycemic compared with those expressing A1ARs.

Other investigators have observed an isoelectric electroencephalogram in neonatal animals subjected to short-term hypoglycemia that results in neuronal injury, which presumably reflects brain energy depletion (19, 20). In our studies, we did not evaluate this brain electrical activity. However, although the pups were hypoglycemic they were responsive to touch and showed movement. Thus it is unlikely that they had isoelectric brain wave activity. Other factors, such as hypoglycemia-induced changes in blood pressure, oxygen availability, and brain blood flow at different glucose concentrations, also may have influenced the observed neuronal injury. We do not have data to address these issues, as the small sizes of the neonatal mice make such analysis very difficult.

Over the past several years, an evolving body of work has shown that the developing brain is sensitive to events that inhibit neuronal activity and synaptic transmission (40). For instance, blockade of NMDA receptor activity or inhibition of Na-K channel activity in rodents before P14 induces widespread neuronal injury, whereas adverse effects are not as apparent after P14 (7, 21, 22). Activation of A1ARs, which inhibit neurotransmitter release and antagonize glutamate receptor action at the postreceptor level, also causes injury in the rodent brain before P14, but not afterward (51). Because increased adenosine production is part of the metabolic response to hypoglycemia (50), our findings suggest that hypoglycemia-induced A1AR activation is also a factor in brain injury causation at young ages.

Clinical evidence supports the notion that the developing human brain is sensitive to adverse effects of hypoglycemia. Infants with hypoglycemia have an increased incidence of learning disability later in life (16, 35). If seizure activity or coma occurs in the setting of short-term neonatal hypoglycemia, the risk of mental retardation increases even more substantially (16, 35). Children with hypoglycemia due to persistent hyperinsulinism also have an increased incidence of brain injury (1, 38).

Numerous developmental studies focusing on milestones in hippocampal development show that events occurring in the P7 mouse brain occur in human and primate infants over the first...
few years of postnatal life (6). Thus our findings in rodents may have potential clinical relevance. These important developmental events include the growth of the hippocampus, synaptogenesis, and differentiation of granule and pyramidal cells (15, 17, 18, 30, 33, 34, 47).

Overall, our observations suggest that the developing nervous system is sensitive to active hypoglycemia and that A1AR action may contribute to hypoglycemic injury at early developmental stages. We also believe that reevaluation of the notion that the developing brain is not sensitive to acute hypoglycemic injury is warranted.

REFERENCES

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Table 6. Hypoglycemia-induced injury in P7 hippocampal slices as related to genotype

<table>
<thead>
<tr>
<th></th>
<th>6.0 mM</th>
<th>0.75 mM</th>
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<tr>
<td>A1AR&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>12 ± 4</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>A1AR&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>11 ± 5</td>
<td>62 ± 7*</td>
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</table>

Values are % total cells ± SE that are TUNEL positive; n = 5 or 6 slices/group. *P < 0.05 vs. all other treatments, ANOVA.

Table 7. TUNEL staining after 4 h of hypoglycemia in A1AR<sup>+/+</sup> and A1AR<sup>-/-</sup> P7 mice

<table>
<thead>
<tr>
<th></th>
<th>A1AR&lt;sup&gt;+/+&lt;/sup&gt;</th>
<th>A1AR&lt;sup&gt;-/-&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Hippocampus</td>
<td>Control</td>
<td>Insulin</td>
</tr>
<tr>
<td></td>
<td>43 ± 32</td>
<td>199 ± 64*</td>
</tr>
<tr>
<td>Striatum</td>
<td>Control</td>
<td>Insulin</td>
</tr>
<tr>
<td></td>
<td>52 ± 5</td>
<td>121 ± 10*</td>
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</table>

Values are means ± SE of TUNEL-positive cells/mm<sup>2</sup>; n = 4–6 mice/group. *P < 0.05 vs. control, ANOVA.


20. Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova T, Stofsva V, Turski L, and Olney JW. Blockade of...