The Anticonvulsant Activity of Acetone, the Major Ketone Body in the Ketogenic Diet, is Not dependent on its Metabolites Acetol, 1,2-Propanediol, Methylglyoxal, or Pyruvic Acid

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The Anticonvulsant Activity of Acetone, the Major Ketone Body in the Ketogenic Diet, Is Not Dependent on Its Metabolites Acetol, 1,2-Propanediol, Methylglyoxal, or Pyruvic Acid


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Summary: Background: Acetone, one of the principal ketone bodies elevated during treatment with the ketogenic diet, exhibits anticonvulsant properties that may contribute to the seizure protection conferred by the diet. The anticonvulsant mechanism of acetone is unknown, but it is metabolized to several bioactive substances that could play a role.

Methods: Acetone and its major metabolites—acetol, 1,2-propanediol, methylglyoxal, and pyruvic acid—were assessed for anticonvulsant activity in two mouse seizure models. Various doses of the substances administered intraperitoneally were characterized for their ability to elevate the threshold for clonic seizures induced by intravenous infusion of pentylenetetrazol (PTZ) and for protection against tonic seizures induced by subcutaneous bolus administration of 4-aminopyridine (4-AP). The inverted-screen test was used to assess acute neurological toxicity.

Results: Acetone (1–32 mmol/kg, i.p.), in a dose-dependent fashion, elevated the PTZ threshold and conferred protection against 4-AP seizures (ED50, 26.3 mmol/kg). Effective doses of acetone (10–32 mmol/kg) did not cause motor impairment in the inverted-screen test (TD50, 45.7 mmol/kg). In doses 10-fold greater than the minimally effective dose of acetone (3.2 mmol/kg), the metabolites acetol, 1,2-propanediol, and pyruvic acid were inactive in the PTZ model. At higher doses that produced motor impairment, acetol and 1,2-propanediol (but not pyruvic acid) did elevate the PTZ threshold. Methylglyoxal had both proconvulsant and anticonvulsant actions, and had substantial toxicity, producing respiratory distress, motor impairment, and death. None of the acetone metabolites protected against 4-AP seizures.

Conclusions: This study confirms the broad-spectrum anticonvulsant properties of acetone and indicates that the seizure protection conferred is unlikely to result from its major metabolic products. Key Words: Ketogenic diet—Acetone—Acetone metabolites—Pentylenetetrazol—4-aminopyridine—Seizure.

It has been known for centuries that starvation can temporarily suppress seizures. Biochemical changes during periods of the limited food availability can be mimicked by the ketogenic diet (KD), composed of 80–90% fat and 10–20% carbohydrates and protein (Kossoff, 2004). Today, the KD is an important nonpharmacological treatment for epilepsy that is particularly effective in children with drug-resistant seizures (Vining et al., 1998; Stafstrom, 2004; Henderson et al., 2006). However, the mechanisms that underlie the anticonvulsant efficacy of the KD remain largely speculative (Stafstrom, 1999; Freeman et al., 2006).

Ketosis—an elevation in serum levels of the ketone bodies acetone, acetoacetate, and β-hydroxybutyrate—is the most apparent biochemical consequence of starvation and the KD (Withrow, 1980). Rising blood levels of ketone bodies coincide with a reduction in seizure activity. Further, seizure protection often ends rapidly when the diet is broken and levels of ketone bodies decrease (Huttenlocher, 1976). Thus, ketone bodies may contribute to the seizure protection conferred by the diet. Experimental findings in animals support this conclusion. Animals maintained on the KD exhibit ketosis and decreased seizure susceptibility to various convulsant stimuli (Stafstrom, 1999; Thavendiranathan et al., 2003; Bough et al., 2005). As in epileptic patients, the protection against seizures and the presence of ketone bodies in the plasma rapidly dissipate when the KD is replaced by a regular diet in animals (Uhlemann and Neims, 1972).
Studies with exogenously administered ketone bodies have raised the possibility that acetone itself may contribute to seizure protection conferred by the KD (Likhodii and Burnham, 2002; Likhodii et al., 2003). Thus, acetone has been demonstrated to protect against seizures in a broad range of epilepsy models, including seizures induced by the subcutaneous pentylentetrazol (PTZ) and AY-9944, the Frings audiogenic seizure model, the maximal electroshock test, amygdala kindling, and the 6 Hz model (Rho et al., 2002; Likhodii et al., 2003; A.L.H., M.G. and M.A.R., unpublished).

Acetone is not the final product of free fatty acid metabolism (Fig. 1). Up to two-thirds of circulating blood acetone undergoes further metabolism (Reichard et al., 1979; Owen et al., 1982; Kalapos, 2003; Morris, 2005). Not surprisingly, levels of intermediate metabolites of acetone, including acetol, 1,2-propanediol, and methylglyoxal, have been observed to increase when the supply of acetone increases during limited availability of carbohydrates and in diabetic patients (Reichard et al., 1979; Kalapos, 2003; Beisswenger et al., 2005) or after exogenous administration of acetone in experimental animals (Casazza et al., 1984; Peinado et al., 1986). During exposure to acetone, several key enzymes in degradative pathway are induced, which facilitate the production of metabolites (Kalapos, 2003).

Metabolites of acetone participate in various physiological functions, including the maintenance of pH balance, as intermediates in glucogenesis, and as insulin-independent sources of energy to peripheral tissues (Kalapos, 2003). However, it is not known whether these metabolites contribute to the anticonvulsant activity of acetone. Therefore, in the present study we sought to determine whether the major metabolites of acetone are protective in two animal seizure models where acetone shows robust anticonvulsant efficacy. Our study confirms that acetone has anticonvulsant properties, but does not support a major role of its metabolites in the seizure protection it confers.

MATERIALS AND METHODS

Animals

Male NIH Swiss mice (19–30 g) were kept in a vivarium under controlled laboratory conditions (temperature: 22–26°C; humidity: 40–50%) with an artificial 12-h light/dark cycle and free access to food and water. Animals were allowed to acclimate to the vivarium for at least 3 days before they were used for experiments. The experiments were performed during the light phase of the light/dark cycle after a minimum of 30-min period of acclimation to the experimental room. Animals were maintained in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and studies were performed under protocols approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke (NINDS) in strict compliance with the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Academy Press, Washington, DC; http://www.nap.edu/readingroom/books/labrats/).

Test substances and chemoconvulsants

Acetone, acetol, L-1,2-propanediol, methylglyoxal, and pyruvic acid were dissolved in sterile 0.9% saline and were administered intraperitoneally (i.p.) in a volume of 0.1 ml/10 g body weight. PTZ and 4-aminopyridine (4-AP) were also dissolved in saline and were administered intravenously (i.v.) and subcutaneously (s.c.), respectively. All chemicals were from Sigma-Aldrich (St. Louis, MO, U.S.A.).

PTZ seizure test

Mice were gently restrained in a closed plastic cylinder (4.5-inch long, 1.2-inch inner diameter) with a port for the tail at one end and a plunger at the other (Rotating Tail Injector, Braintree Scientific, Braintree, MA, U.S.A.). The lateral tail vein was catheterized with a 0.5-inch long, 30-gauge needle attached to a 12-inch length of polyethylene tubing (PE-10). Correct needle placement was verified by the appearance of blood in the tubing. The needle was gently secured to the tail using a plastic tape. The tubing was attached to a 12-ml plastic syringe containing the PTZ solution (10 mg/ml), which was mounted on an infusion pump (KD Scientific, Holliston, MA, U.S.A.). Following catheterization, unrestrained mice were placed in Plexiglas cages (11 in × 8 in × 6 in) for behavioral observation during the infusion.

Three signs of seizure activity typically occur in sequence during i.v. PTZ infusions: (1) tail twitch (rapid upward flick of rigid tail), (2) clonus (repeated jerking movements of all four limbs) with loss of the righting reflex (clonic seizures), and (3) tonic hindlimb extension (tonic seizures). The times between the start of the infusion and the onset of these signs were recorded for each seizure sign separately. The infusion rate was 0.05 ml/min, which permitted a reliable assessment of the times of onset of all three seizure signs (Steppuhn and Turski, 1993). Threshold dose values [mg/kg] were calculated using the formula (PTZ concentration [mg/ml] × infusion rate [ml/s] × infusion duration [s] × 1000) / body weight [g]. Infusions were stopped at the beginning of tonic seizures, which were usually lethal, or at 10 min if no tonic seizures occurred. All surviving animals were euthanized immediately after the end of the infusion.

4-AP seizure test

The 4-AP seizure test was carried out as previously described (Yamaguchi and Rogawski, 1992) using a 13 mg/kg dose, which as confirmed in the present study produced tonic seizures in 93.9% of control mice (see...
FIG. 1. Metabolic pathways for production and degradation of the three ketone bodies (acetone, acetoacetate, and \(\beta\)-hydroxybutyrate) that are elevated in the KD. Fatty acids undergo a sequential removal of 2-carbon moieties in the process of \(\beta\)-oxidation (a). Under normal conditions, the end product of this reaction, acetyl-CoA, would be further oxidized in the tricarboxylic acid (TCA) cycle to CO2 (not shown). Generation of large amounts of acetyl-CoA during high rates of fatty acid oxidation in the KD exceeds the metabolic capacity of the TCA cycle (schematically indicated by a block of condensation of oxaloacetic acid with acetyl-CoA to form citric acid that is catalyzed by citrate synthetase). As a result, acetyl-CoA is converted to acetoacetyl-CoA by thiolase (b), which is further metabolized to acetoacetate by hydroxymethylglutaryl CoA synthetase and lyase (c). Acetoacetate can be converted to \(\beta\)-hydroxybutyrate by \(\beta\)-hydroxybutyrate dehydrogenase (d) or can undergo spontaneous decarboxylation to acetone (e). Acetoacetate can also be converted to acetyl-CoA via acetoacetyl-CoA and enter the TCA cycle (not shown). Acetone is further metabolized in a series of interconnected enzymatic reactions. The following enzymes are involved in acetone degradation: acetone monooxygenase (largely CYP1E1) (A); acetol monooxygenase (B); glyoxalase I and glyoxalase II (C); D-2-hydroxyacid dehydrogenase (D); \(\alpha\)-oxoaldehyde dehydrogenase (E); methylglyoxal reductase (F); acetol kinase, L-1,2-propanediol-1-P dehydrogenase, glycerol-1-P-phosphatase (G); alcohol dehydrogenase and lactaldehyde reductase (H); aldehyde dehydrogenase, L-lactaldehyde dehydrogenase, L-lactate dehydrogenase (I); and pyruvate dehydrogenase complex (J). Compounds marked in bold were tested in the present study. Modified from Kalapos (2003).

Table 2). Mice were pretreated subcutaneously with either saline (control group) or test compounds 30 min before receiving 4-AP. Mice that failed to exhibit tonic seizures (tonic extension of hind limbs) within 45 min after 4-AP administration were considered to be protected.

Inverted-screen test
The inverted-screen test, a rapid and easily administered measure of gross motor impairment adapted from Coughenour et al. (1977), was performed immediately before the PTZ infusion test. Mice were individually placed on a wire screen held approximately 25 cm above the lab bench and the screen was slowly rotated until inverted. Mice were scored as passing the test if they were able to maintain their position on the inverted screen for 10 s. Untreated control mice never fell off the screen.

Statistical analysis
Group means ± S.E.M. of threshold values in the PTZ infusion test were plotted as a function of the test compound’s dose or pretreatment time. Statistically significant differences among category group means were assessed by one-way analysis of variance followed by specific post hoc comparisons with control values (mean threshold values for vehicle-treated mice) using Dunnett’s test. Doses (in mmol/kg) with 95% confidence limits (95% C.L.) for producing seizure protection (ED50) and motor impairment (TD50) in 50% of mice tested in the 4-AP and inverted-screen tests, respectively, were estimated by a log-probit analysis (Litchfield and Wilcoxon, 1949). Statistical significance of difference in the 4-AP seizure test was estimated by Fisher’s exact test. In all cases, differences were considered statistically significant when the probability of error was less than 0.05 (\(p < 0.05\)). Experimental groups consisted of at least eight mice.

RESULTS
Effects of acetone in seizure models and inverted-screen test
In the PTZ threshold test, acetone at a dose of 32 mmol/kg (previously reported to reliably attenuate seizures in various seizure models; Likhodii et al., 2003) caused an elevation in the mean threshold values for all three seizure signs, including tail twitch \(F(4,41) = 6.914,\)
FIG. 2. Effects of acetone on seizure sign thresholds in the PTZ infusion model. A: Acetone was administered at a dose of 32 mmol/kg at different times (15–240 min) before intravenous infusion of PTZ. The threshold for tail twitch, clonic seizures, and tonic seizures was determined as described in Materials and Methods. Each point represents the mean ± S.E.M. of the threshold values for 8–12 mice. Vertical dotted lines indicate time point used in the dose–response study. Open symbols and horizontal dotted lines indicate threshold values following administration of sterile saline (‘V’ symbols) in a group of animals tested in conjunction with acetone. ∗Significantly different (p < 0.05) from control value by Dunnett’s test after demonstration of a statistically significant main effect by one-way analysis of variance. B: As in A, except that acetone was administered at doses of 1 to 32 mmol/kg 30 min before infusion of PTZ.

p < 0.001], clonic seizures $F(4,41) = 14.008$, p < 0.001], and tonic seizures $F(4,41) = 11.762$, p < 0.001 (Fig. 2A). Although increases in mean threshold were obtained at all time points (15–240 min) for the three seizure signs, the time points at which statistically significant changes were detected by post hoc analysis were different for each of the three seizure signs. Significant effects on clonic and tonic seizure threshold were observed at the first time point (15 min) after administration of acetone and at time points up to 60 and 240 min, respectively. In contrast, a significant elevation of tail twitch threshold was obtained only at the 30 min time point. Therefore, in the subsequent dose–response study (Fig. 2B), a pretreatment time of 30 min was used to maximize detection of significant effects on all the three seizure signs.

In the dose–response study, administration of increasing doses of acetone resulted in a dose-dependent increase in the thresholds for all the three seizure signs: tail twitch $F(4,45) = 9.565$, p < 0.001, clonic seizures $F(4,45) = 14.621$, p < 0.001, and tonic seizures $F(4,45) = 23.027$, p < 0.001 (Fig. 2B). A post hoc analysis indicated significant effects on all signs at doses of 10 and 32 mmol/kg; the effect of the 3.2 mmol/kg dose on tonic seizures was also significant. The 10 mmol/kg dose of acetone caused increases from 40% (tail twitch) to 83% (clonic seizures) in the threshold value with respect to control; the 32 mmol/kg dose of acetone caused increases from 89% (tail twitch) to 133% (tonic seizures). Higher doses of acetone induced significant motor impairment, which was evident within a few minutes after acetone administration. Consequently, doses greater than 32 mg/kg were not examined in the PTZ seizure test. In the horizontal-screen test, acetone treatment (3.2 to 100 mmol/kg) caused a dose-dependent impairment of motor performance (Table 1), but only at doses >32 mg/kg; smaller doses did not cause motor impairment in any animals.

Acetone conferred a dose-dependent protection against tonic seizures induced by 4-AP (Table 2). The protective ED$_{50}$ value was 26.3 mmol/kg (95% C.L., 21.3–32.5).

| Table 1. Comparison of doses of acetone and its metabolites that induce PTZ threshold elevation and produce motor impairment |
|----------------------|--------------------|----------------------|
| Metabolite            | Significant elevation of PTZ threshold (mmol/kg)* | TD$_{50}$ (95% C.L.) (mmol/kg)$^\dagger$ |
| Acetone               | 3.2, 10, 32         | 45.7 (38.9–53.7)     |
| Acetol                | 56, 100             | 73.7 (55.4–97.9)     |
| 1,2-Propanediol       | 56, 100             | 80.6 (58.3–115.5)    |
| Methylglyoxal         | 10                  | 9.64 (7.27–12.8)     |
| Pyruvic acid          | Reduced threshold   | 22.6 (12.6–40.3)     |

*Significantly different (p < 0.05 vs. control) elevation in seizure threshold in the PTZ infusion test (Figs. 2 and 3). $^\dagger$Doses with 95% confidence limits (95% C.L.) in parentheses predicted to produce motor impairment in 50% of mice tested as determined by means of the inverted-screen test. For acetone, acetol and 1,2-propanediol, the ED$_{50}$ values are based on doses of 32, 56, 100 mmol/kg. For methylglyoxal and pyruvic acid, the TD$_{50}$ values are based on doses of 3.2, 5.6, 10, 14 mmol/kg, and 10, 32, 56 mmol/kg respectively. At a dose of 17.8 mmol/kg, methylglyoxal was lethal within a few minutes after injection in all animals tested. For all test compounds, at least eight animals were evaluated at each dose.
TABLE 2. Effect of acetone and its metabolites on tonic seizures induced by 4-AP

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Dose (mmol/kg)</th>
<th>Percent of animals exhibiting tonic seizures</th>
<th>Number of animals tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
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<td>33</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>100</td>
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<td>40‡</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>0‡</td>
<td>10</td>
</tr>
<tr>
<td>Acetol</td>
<td>32</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>32</td>
<td>91.7</td>
<td>12</td>
</tr>
<tr>
<td>Methylglyoxal</td>
<td>5.6</td>
<td>91.7</td>
<td>12</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>10</td>
<td>83.3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Lethal</td>
<td>12</td>
</tr>
</tbody>
</table>

Mice were treated with saline or metabolite 30 min before receiving 4-AP (13 mg/kg, s.c.) and were observed for the occurrence of tonic seizures for 45 min following 4-AP administration.

†p < 0.05 vs. saline-treated control groups (Fisher’s exact test).
‡Of 12 mice tested, four died shortly after 4-AP administration; all survivors exhibited tonic seizures.

**Effects of acetol in seizure models and inverted-screen test**

Doses of acetol equimolar to those of acetone that significantly elevated all three PTZ threshold measures (10 and 32 mmol/kg) were inactive in the same pretreatment time of 30 min. Acetone also increased the threshold and at 100 mmol/kg it significantly elevated all threshold values. However, these higher doses of acetol also produced motor impairment in the inverted-screen test (Table 1). Thus, following a 100 mmol/kg dose, most mice (87%) failed the test. Additional studies confirmed the lack of effect of 32 mmol/kg of acetol on PTZ thresholds with pretreatment times of 15 and 60 min (nine animal per time point; one-way analysis of variance, p > 0.05). Acetol at a dose of 32 mmol/kg was inactive in the 4-AP model (Table 2).

**Effects of other metabolites in seizure models and inverted-screen test**

1,2-Propanediol, methylglyoxal, and pyruvic acid were evaluated for effects on PTZ threshold using a pretreatment time of 30 min. At a dose of 32 mmol/kg, 1,2-propanediol was inactive on all threshold measures (Fig. 3B). At higher doses (≥ 56 mmol/kg), 1,2-propanediol significantly increased the thresholds for clonic and tonic seizures, but not tail twitch. These higher doses of 1,2-propanediol produced a high incidence of motor impairment in the inverted-screen test (Table 1).

Methylglyoxal had mixed effects on PTZ threshold. At 3.2 mmol/kg there was a slight reduction in the threshold for tail twitch, indicating a proconvulsant effect. No effect on threshold was observed at 5.6 mmol/kg either at the 30 min pretreatment time or with a longer (60 min) pretreatment time (p > 0.05 vs. control; data not shown). At 10 mmol/kg there was a significant increase in clonic and tonic seizure threshold. However, this dose of methylglyoxal was associated with motor impairment in 50% of mice tested (Table 1). It also produced severe breathing difficulty in all mice tested and was lethal in 20% of the animals. Higher doses of methylglyoxal (17 and 32 mmol/kg) produced even more severe breathing impairment and were lethal within 4 min.

Pyruvic acid did not increase PTZ seizure threshold at any dose. At doses of 32 and 56 mmol/kg, which were associated with motor impairment in a high proportion of animals (Table 1), there was a small but significant reduction in threshold for tail twitch and clonic seizures (Fig. 3D).

1,2-Propanediol, methylglyoxal, and pyruvic acid were inactive in the 4-AP model (Table 2).

**DISCUSSION**

This study provides additional evidence for the broad-spectrum anticonvulsant properties of acetone. We have confirmed the protective activity of acetone against clonic seizures induced by a fixed dose of PTZ (Likhodii et al., 2003) in the more sensitive threshold test at doses as low as 3.2 mmol/kg and now for the first time show that acetone is also active against tonic seizures induced by 4-AP. Acetone has previously been shown to confer protection in the rat maximal electroshock model, which uses tonic hindlimb extension as the endpoint (Likhodii et al., 2003). We found that acetone was also protective against tonic seizures in the PTZ threshold model. Thus, acetone is protective against both clonic and tonic seizures in diverse models, and has a broader spectrum of activity than many clinically important antiepileptic drugs, expect perhaps valproate and barbiturates (White et al., 2002). Moreover, our study demonstrates that acetone has a remarkably long duration of anticonvulsant action that persists up to 240 min after a single injection of doses that do not cause overt toxicity. This result is in agreement with a prior study showing seizure protection lasting up to 4 to 5 h in the AY-9944 model (Likhodii et al., 2003). The prolonged duration is consistent with acetone’s metabolic half-life, which is in the range of 2–5 h in mice (Wigaeus et al., 1982). The effective doses of acetone in the present study are similar to those reported previously to protect against seizures in mice (Rho et al., 2002) and rats (Likhodii et al., 2003). In rats, acute doses of acetone in the range of 2–4 mmol/kg were associated with plasma levels of 2–3 mM (Likhodii et al., 2003). A recent study in children receiving the KD for the treatment of epilepsy found mean serum acetone levels of 4 mM, with values in some children reaching 8 mM (Musa-Veloso et al., 2006). Therefore, plasma
FIG. 3. Effects of acetone metabolites on seizure sign thresholds in the PTZ infusion model. Metabolites were administered at the doses indicated 30 min before the onset of the PTZ infusion. The threshold for tail twitch, clonic seizures, and tonic seizures was determined as described in Materials and Methods. Each point represents the mean ± S.E.M. of the threshold values for 8 to 12 mice. Open symbols and horizontal dotted lines indicate threshold values following administration of sterile saline (V) symbols) in a group of animals tested in conjunction with the corresponding metabolite. *Significantly different (p < 0.05) from control value by Dunnett's test after demonstration of a statistically significant main effect by one-way analysis of variance. Acetone data from Fig. 1 are shown in gray in panel A for reference; vertical dotted lines at 32 mmol/kg indicate the maximum molar dose of acetone that increased PTZ threshold without toxicity.

Concentrations of acetone achieved by patients receiving the KD may be sufficient to exert a clinically significant effect on seizures and contribute to the efficacy of the diet. However, an association between acetone levels and seizure control remains to be demonstrated.

Acetone is a relatively nontoxic substance that, in animals and humans, is well tolerated except at very high doses (WHO, 1970; Freeman and Hayes, 1985). Indeed, doses of acetone that exhibited protection in the PTZthreshold model as high as 32 mmol/kg were completely devoid of toxicity in the inverted-screen test. Such a separation between efficacy and toxicity has been noted previously (Kohli et al., 1967; Likhodii et al., 2003). Modestly higher doses of acetone did, however, cause motor toxicity. Although the TD$_{50}$ value in the motor toxicity test was 14-fold greater than the minimum dose active in the PTZ threshold model, the TD$_{50}$ value was only 1.7-fold greater than the ED$_{50}$ in the 4-AP model. Overall, acetone does not appear to have a greater safety margin than many clinically used antiepileptic drugs (Rogawski and Porter, 1990; Löscher and Nolting, 1991; White et al., 2002).

In contrast to acetone itself, none of acetone's metabolites were devoid of toxicity at doses that were effective in the PTZ model. Although acetol and 1,2-propanediol produced threshold elevations in the PTZ model, they did so only at relatively high doses (≥ 56 mmol/kg). Not only were these doses several-fold higher on a molar basis than the minimally effective doses of acetone, but they also...
resulted in a marked behavioral impairment not seen with effective doses of acetone. Although plasma levels of acetone’s metabolites increase several-fold while on the KD or during starvation, their absolute concentrations in the plasma remain 1–2 orders of magnitude lower than that of acetone (Kalapos, 1999b; Beisswenger et al., 2005). Since attenuation of PTZ-induced seizures was observed only after administration of acetal and 1,2-propanediol in doses likely to produce plasma levels greater than protective plasma levels of acetone, these metabolites are unlikely to contribute to the seizure protection conferred by the KD.

Our results indicate that the other two metabolites of acetone tested, pyruvic acid and methylglyoxal, are also unlikely to contribute to the anticonvulsant effects of acetone in vivo. Pyruvic acid had no effect in the seizure models in doses that were comparable to the effective protective doses of acetone; higher doses had a small but statistically significant proconvulsant effect. Methylglyoxal did increase the PTZ seizure threshold at 10 mmol/kg, but it produced substantial toxicity and was lethal at higher doses. The higher relative toxicity of methylglyoxal seen in the present study is consistent with the existing literature (Kalapos, 1999a). Taken together, the present results fail to support the hypothesis that the anticonvulsant activity of acetone is mediated by a metabolite. This conclusion is tempered by the recognition that we did not test all acetone metabolites (Kalapos, 2003). However, the four metabolites tested represent those that have been reported to accumulate during the KD (Kalapos, 1999b; Beisswenger et al., 2005). Other metabolites are transient and are not known to attain measurable levels.

In summary, our results indicate that the anticonvulsant activity of acetone is unlikely to result from its major metabolic products. However, these metabolites may have other biological activities including effects on pH and glucose regulation, and may serve as sources of energy (Kalapos, 1999b). Moreover, accumulation of acetone metabolites may contribute to adverse health effects of the KD (Beisswenger et al., 2005).

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