Alterations in seizure mechanisms caused by oxygen high pressure, 1,1-dimethylhydrazine, and pyridoxine

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Segerbo, B. E. 1979. Alterations in seizure mechanisms caused by oxygen high pressure, 1,1-dimethylhydrazine, and pyridoxine. Undersea Biomed. Res. 6(2): 167–174.—High pressure oxygen (HBO) and 1,1-dimethylhydrazine (UDMH) both cause grand mal seizures, brain glycogen degradation, and inhibition of glutamic acid decarboxylase (GAD). Brain glycogen degradation is a sudden process that is perhaps initiated by convulsions in the case of UDMH-poisoning, but a gradual decrease in glycogen is detectable before the onset of hyperbaric oxygen toxicity symptoms. UDMH injection causes consecutive convulsions that follow a predictable sequence. (Time to convulsions is referred to as the induction period, and time between convulsions as the interictal period.) After a single injection of UDMH, there is a gradual decrease in resistance to HBO during the induction period, measured as time to convulsions breathing 100% oxygen at 6 ATA; in the first interictal period, this time is only 4 1/2 min in comparison with a control value of 26 min for untreated rats. Administration of pyridoxine, a B6-vitamin, 2 h after UDMH injection in the first interictal period, resulted in an immediate tenfold increase in resistance to oxygen toxicity, from 4 1/2 to 48 min. Pyridoxine may reverse the inhibitory effect of UDMH on GAD, and there is perhaps an accumulation of substrate, which is made available when GAD inhibition is diminishing. Simultaneous injection of pyridoxine and UDMH causes no convulsions, no change in brain glycogen levels, and an unchanged or increased resistance to HBO, measured two and three hours after injection.

brain glycogen  hyperbaric oxygen
glutamic acid decarboxylase  oxygen toxicity
rats  convulsions
1,1-dimethylhydrazine

Glycogen is an energy reservoir for the brain during emergencies (Coxon 1970). The first reliable method for the measurement of glycogen in brain was published by Kerr in 1936. He and his co-workers found that a profound insulin hypoglycemia reduced brain glycogen levels in dogs, cats, and rabbits (Kerr and Ghantus 1936; Kerr, Hampel, and Ghantus 1937). This change was confirmed in rats by Schiller (1958) and by Carter and Stone (1960) in mice after insulin-induced as well as drug-induced convulsions. A single injection of 1,1-dimethylhydrazine (UDMH) in rats causes brain glycogen degradation and repeated convulsions. The intervals between consecutive convulsions follow a fairly predictable sequence that allows the different phases of convulsive activity to be investigated (Minard, Kang, and
Mushawar 1965). Comparative studies on hyperbaric oxygen-induced convulsions and drug-induced convulsions have used thiosemicarbazide, strychnine, picrotoxin, pentylentetrazol, and hydroxylamin (Wood, Watson, and Stacey 1965; Woodhall, Kramer, Currier, and Sanders 1971), and hydrazine and its derivatives, to change GAD activity and brain GABA levels (Wood and Peesker 1974; Wood, Gorecki, Dimmock, and Hawes 1975). Brain glycogen degradation has been demonstrated to be another effect of HBO-induced convulsions (Segerbo, Ericson, and Röckert 1976). The aim of the present investigation was to compare the convulsogenic and brain glycogen-reducing properties of HBO and UDMH.

METHODS

Young male Sprague-Dawley rats (240–320 g) were used. They were all maintained on a laboratory chow diet (Astra-Ewos R4) unless otherwise indicated. The exposures were carried out in a pressure chamber. The chamber was flushed with oxygen for 1 min and the pressure was then increased steadily over a 1-min period until it reached 6 ATA. This pressure was maintained for the required length of time or until convulsions, and decompression to ambient pressure was then carried out over a 2-min period.

A solution containing 40 mg/ml of UDMH was prepared immediately before injection: 1.27 ml of UDMH was diluted to 25 ml with a 27 mM solution of NaHCO₃ in 0.9% NaCl. The solution was injected subcutaneously at a dose of 100 mg/kg of body weight. Pyridoxine (Bendone,® Roche) was injected intramuscularly at a dose of 150 mg/kg of body weight.

For glycogen analysis the brains were rapidly removed from decapitated animals, frozen in liquid nitrogen and powdered. The method of freezing the brains for glycogen determination immediately after removal was used by Smialek, Sikeroka, Korthals, Bicz, and Mossakowski (1973). Total glycogen was determined on duplicate samples from each brain, as described by Wolfe, Klatzo, Miguel, Tibias, and Haymaker (1962) and modified by Adolfsson, Isaksson, and Hjalmarsson (1972). The weighed frozen brain powder, about 100 mg, was transferred to a tube containing 30% KOH and digested at 100% in a boiling water bath for 30 min and precipitated overnight at 4°C in 99% ethanol and 8% Na₂SO₄. The precipitate after centrifugation was washed twice in 66% ethanol, dried and dissolved in distilled water. Aliquots were added to the reaction mixture containing amyloglucosidase to hydrolyze the glycogen into glucose, and the usual reagents of the glucose oxidase method were used for spectrophotometric determination of the glucose. Glycogen values are expressed as μg glycogen/g wet brain tissue. The significance of differences was estimated using the Student’s t-test (Bailey 1964).

RESULTS

Mixed tonic and clonic convulsions occur after a single injection of UDMH at a dose of 100 mg/kg (Table 1). Because some rats were withdrawn for experimentation, there are fewer animals in the later groups. Death usually occurred after three or more convulsions, often in a terminal period of continuous convulsion. After the administration of UDMH, three phases of activity were delineated: the induction period before the first convulsion, the convulsions themselves, and the periods between convulsions. The mean induction period was determined to be 107 min, and the first interictal period was 29 min. The first convulsive period was of short duration, lasting from a few seconds to a minute, and the animals recovered well from the seizures. During the following phases, the convulsions tended to become more severe, the period between convulsions shorter, and the animals suffered from post-ictal lethargy.
ALTERATIONS IN SEIZURE MECHANISMS

TABLE 1
PERIODICITY OF CONVULSIONS AFTER A SINGLE INJECTION OF UDMH

<table>
<thead>
<tr>
<th>Time between injection and first convolution, min</th>
<th>n</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period between convulsions, min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First — second</td>
<td>51</td>
<td>15–45</td>
<td>29</td>
<td>0.9</td>
</tr>
<tr>
<td>Second—third</td>
<td>39</td>
<td>5–30</td>
<td>15</td>
<td>0.9</td>
</tr>
<tr>
<td>Third — fourth</td>
<td>10</td>
<td>2–18</td>
<td>13</td>
<td>1.6</td>
</tr>
<tr>
<td>Fourth — fifth</td>
<td>7</td>
<td>2–14</td>
<td>9</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Each rat was given a subcutaneous injection of UDMH at a dose of 100 mg/kg.

Brain glycogen was measured at predetermined times after injection of UDMH. Values of the same magnitude as from the control group were obtained until after the first convolution, when a considerable decrease occurred, a reduction to 430 μg/g from the control value of 586 μg/g (Table 2). During the consecutive phases of convulsive activity, there seemed to be a slight but insignificant further decrease, the value at the onset of the fourth convolution being 401 μg/g. No significant increase in brain glycogen was recorded in interictal periods. Table 3 shows the effect of HBO on brain glycogen content. Group A rats were maintained on ordinary diet and group B had a 24-h fast period before HBO exposure when only water was accessible ad libitum. There were no significant differences between brain glycogen content in the two groups before HBO exposure or in susceptibility to HBO toxicity expressed as time to HBO-induced convulsions. After 20 min of exposure to 6 ATA, i.e., before the onset of convulsive activity, there was a significant decrease in brain glycogen in both groups compared to control values. This brain glycogen degradation appeared to continue, and after onset of convulsions mean values of 394 μg/g and 361 μg/g were recorded, respectively. Table 4 shows the effect of UDMH on the resistance to HBO toxicity expressed as time to HBO-

TABLE 2
EFFECT OF A SINGLE SUBCUTANEOUS INJECTION OF UDMH (100MG/KG BODY WEIGHT) ON GLYCOCEN OF WHOLE BRAIN

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Mean Time After UDMH Injection, min</th>
<th>No. UDMH Convulsions</th>
<th>Brain Glycogen, μg/g</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>no injection</td>
<td>0</td>
<td>420–830</td>
<td>586</td>
<td>11.7</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>0</td>
<td>440–800</td>
<td>605</td>
<td>28.9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>80</td>
<td>0</td>
<td>500–730</td>
<td>589</td>
<td>19.5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>103</td>
<td>1</td>
<td>360–550</td>
<td>430</td>
<td>13.4</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>131</td>
<td>1*</td>
<td>360–540</td>
<td>463</td>
<td>12.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>139</td>
<td>2</td>
<td>350–550</td>
<td>436</td>
<td>14.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>146</td>
<td>3*</td>
<td>310–470</td>
<td>384</td>
<td>12.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>149</td>
<td>3</td>
<td>350–570</td>
<td>433</td>
<td>18.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>158</td>
<td>4</td>
<td>320–460</td>
<td>401</td>
<td>21.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = Animals decapitated in interictal period.
induced convulsions. The experimental rats were divided into three groups and exposed to HBO during the induction period at 30, 60, and 90 min, respectively, after injection of UDMH and the time to HBO-induced convulsions was recorded. There was a highly significant decrease in resistance to HBO 60 min after injection of UDMH, to a mean value of 13 min of exposure to HBO, compared to the control value of 26 min for untreated rats. Table 5 shows that the animals were very sensitive to hyperbaric oxygen during the first UDMH-interical period. The mean time to HBO convulsions was only 4 1/2 min. Hyperbaric oxygen was started 10 min after the cessation of the first UDMH convulsion, when the animals had recovered.

The injection of pyridoxine (PYR) 5–8 min after the first UDMH convolution resulted in a more than tenfold increase in resistance to HBO, the mean time to seizures being 48 min when HBO was started within 5 min after PYR injection (Table 5). Simultaneous UDMH and PYR injection produced no UDMH convulsions, and the brain glycogen content remained unchanged, while the resistance to HBO was unchanged or slightly increased (Table 6).

### TABLE 3

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Diet</th>
<th>Time of HBO Exposure, min, s</th>
<th>Brain Glycogen, µg/g</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 30</td>
<td>R4</td>
<td>0'</td>
<td>420–830</td>
<td>586</td>
<td>11.7</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>R4</td>
<td>20'</td>
<td>440–620</td>
<td>519</td>
<td>12.6</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>R4</td>
<td>25'57''</td>
<td>280–620</td>
<td>394</td>
<td>12.9</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>B 11</td>
<td>F***</td>
<td>0'</td>
<td>420–780</td>
<td>546</td>
<td>23.0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>20'</td>
<td>420–540</td>
<td>474</td>
<td>11.9</td>
<td>0.1***</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>29'43''</td>
<td>250–510</td>
<td>361</td>
<td>12.6</td>
<td>0.001***</td>
<td></td>
</tr>
</tbody>
</table>

* = Mean time to HBO convulsions; ** = 24-h fast before exposure; *** = compared to group B.

### TABLE 4

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Mean Time After UDMH Injection, min</th>
<th>Time to HBO Convulsions, min, s</th>
<th>Range</th>
<th>Mean</th>
<th>SEM</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>—</td>
<td>16'33''–36'57''</td>
<td>25'57''</td>
<td>01'00''</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>11'20''–39'49''</td>
<td>22'19''</td>
<td>04'56''</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>60</td>
<td>11'48''–16'42''</td>
<td>13'24''</td>
<td>00'35''</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>06'41''–10'26''</td>
<td>07'59''</td>
<td>00'38''</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>107</td>
<td>First UDMH convolution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

UDMH was injected at a dose of 100 mg/kg. Experimental rats were submitted to HBO 30, 60, and 90 min after injection.
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TABLE 5

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Rats</th>
<th>Time After UDMH Injection, min</th>
<th>No. of UDMH Convolusions</th>
<th>Time to HBO Convulsions, min, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>—</td>
<td>—</td>
<td>16'33&quot;</td>
</tr>
<tr>
<td>UDMH</td>
<td>11</td>
<td>90–185</td>
<td>1</td>
<td>01'37&quot;</td>
</tr>
<tr>
<td>UDMH + PYR</td>
<td>13</td>
<td>95–155</td>
<td>1</td>
<td>16'00&quot;</td>
</tr>
</tbody>
</table>

Injections were as follows: UDMH 100 mg/kg s.c. Pyridoxine 150 mg/kg i.m. 5–8 min after first UDMH convulsion. HBO at 6 ATA 10 min after first UDMH convulsion.

DISCUSSION

When brain tissue is exposed to the stress of hyperbaric oxygenation or convulsant drugs, there is a fall in the energy available, demonstrable as a decrease in the concentration of brain energetics (Klein and Olsen 1947; Kaplan and Stein 1957; Woodhall et al. 1971). Brain glycogen is believed to be degraded in an effort to balance an increased need for substrate under the stress of HBO, providing protection similar to that offered by exogenously administered substrates such as sucinate (Sanders, Hall, and Woodhall 1965) or starch (Segerbo et al. 1975; Segerbo et al. 1976). UDMH apparently causes no degradation of brain glycogen until the onset of convulsions but significantly decreases the resistance to HBO toxicity in rats both during the induction period before the first convolution and in the periods between convulsions. In the induction period the glycogen reserves are intact and the site of action of UDMH is probably in the enzymatic processes of the Krebs cycle. UDMH is known to be a strong inhibitor of glutamic acid decarboxylase (GAD), an enzyme of crucial importance in the GABA-shunt pathway (Medina 1963). Hyperbaric oxygen is also known to inhibit GAD activity in brain tissue (Shcherbakova 1962; Wood et al. 1965), thus possibly intensifying the inhibition of GAD when HBO is used on UDMH-poisoned rats, though the site of action is not identical.

Radioactivity appears quickly in many tissues after injection of (14C) UDMH, with a peak occurring in brain tissue about 60 min after injection (Minard et al. 1965). The preconvulsive toxic effects of UDMH reflected by decreased HBO resistance match this time schedule closely. The enzyme inhibition process is assumed to proceed throughout the period, explaining the gradual decrease in HBO resistance despite intact glycogen reserves. In the first interictal period, when HBO is used after UDMH-induced convulsions and brain glycogen is reduced by approximately 25%, seizures occur after only a few minutes. The degradation of brain glycogen by UDMH is perhaps secondary to the onset of convulsions, which cause the release of epinephrine within the brain, activating the phosphorylase enzymes (Minard et al. 1965). The resulting sudden increase in available substrate probably accounts for the periodicity of convulsions as long as appropriate doses of UDMH are used. Because of severe enzyme inhibition in the GABA-shunt, the utilization of substrate in the intermediate metabolism is likely to be hampered.

Therefore, the susceptibility to oxygen toxicity after UDMH-induced convulsions may imply increased access to substrate in the glycolysis process but lack of sufficient electron flow in the respiratory chain. Injection of PYR after the first UDMH-induced convulsion
<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Rats</th>
<th>No. of UDMH + PYR, Convulsions, min.s</th>
<th>Time to HBO Convulsions, min.s</th>
<th>Brain Glycogen, µg/g</th>
<th>Brain Glycogen, SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>30</td>
<td>120</td>
<td>30(^{±})</td>
<td>1633(^±)−3657(^±)</td>
<td>0.00(^±)</td>
<td>NS</td>
</tr>
<tr>
<td>UDMH + PYR</td>
<td>10</td>
<td>0</td>
<td>0.00(^±)</td>
<td>25(^±)−36(^±)</td>
<td>25.5(^±)</td>
<td>11.7</td>
</tr>
<tr>
<td>UDMH + PYR</td>
<td>5</td>
<td>120</td>
<td>0.00(^±)</td>
<td>30(^±)−36(^±)</td>
<td>30.0(^±)</td>
<td>3.2</td>
</tr>
<tr>
<td>UDMH + PYR</td>
<td>3</td>
<td>180</td>
<td>0.00(^±)</td>
<td>0.00(^±)−80.00(^±)</td>
<td>0.00(^±)−80.00(^±)</td>
<td>595</td>
</tr>
</tbody>
</table>

Injections were as follows: UDMH 100 mg/kg s.c. simultaneously with pyridoxine 150 mg/kg i.m. HBO was started 120 and 180 min, respectively, after injection. Rats decapitated for brain glycogen determinations not exposed to HBO.
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produced a sudden tenfold increase in resistance to oxygen toxicity. This may indicate that PYR is capable of reversing the inhibition of GAD. If so, it is reasonable to assume a rapid re-establishment of substrate turnover, which would account for the improvement in resistance to HBO. Simultaneous injection of UDMH and PYR resulted in an unchanged resistance to HBO 2 h after injection, followed by a slight increase in resistance at 3 h. This phenomenon can be explained by the prevention of UDMH inhibition on GAD activity. Evidence of this was given by Medina (1963), who found no significant change in GAD activity in rat brain 90 min after simultaneous intraperitoneal injection of 1.6 mM UDMH (98 mg/kg) and 0.5 mM PYR (85 mg/kg). Minard et al. (1965) found no change in rat brain glycogen and no convulsions 90–209 min after simultaneous i.p. injection of UDMH at a dose of 100 mg/kg and PYR at a dose of 87 mg/kg.

Segerbo, B. E. 1979. Modification des crises convulsives par l’oxygène hyperbare, la 1,1-dimethylhydrazine (UDMH) et la pyridoxine. Undersea Biomed. Res. 6(2): 167–174. — L’oxygène hyperbare et la 1,1-dimethylhydrazine (UDMH) provoquent chacun des crises convulsives du type grand mal, la dégradation du glycogène cérébral, et l’inhibition de la glutamate-décarboxylase (GAD). La dégradation du glycogène cérébral est un événement abrupte, initié peut-être par les crises, dans l’intoxication à UDMH. Une réduction lente peut être observée avant le commencement des symptômes de l’intoxication à l’oxygène hyperbare. L’injection de UDMH provoque des convulsions consécutives dont le cours est bien connu. Après une injection unique de UDMH il y a une réduction lente de la résistance à l’oxygène hyperbare pendant la période d’induction (délai entre l’administration de l’UDMH et l’apparition des crises) à 6 ATA 100% oxygen; entre crises (période interictale) ce délai ne dure que 4½ min, tandis que pour les témoins il est de 26 min. L’administration de la pyridoxine (la vitamine B6) 2 heures après l’injection de l’UDMH dans la première période interictale, a augmenté la résistance à la toxicité de l’oxygène hyperbare de 4½ à 48 min. La pyridoxine pourrait s’opposer à l’inhibition de la glutamate-décarboxylase par UDMH, et il s’accumulerarait du substrat, qui serait de nouveau libre quand l’inhibition de la glutamate-décarboxylase diminuerait. Deux et trois heures après injection simultanée de pyridoxine et de l’UDMH, aucune convulsion n’est observée, le glycogène cérébral est inchangé, et la résistance à l’oxygène hyperbare est inchangée ou augmentée.

glycogène cérébral
 glutamate-décarboxylase
 rats

REFERENCES


