Effects of Promethazine on the Energy Metabolism of Normoxic and Hypoxic Rat Brain

V. MacMillan, M.D.

SUMMARY The metabolic effects of intraperitoneal administration of promethazine on normoxic, hypoxemic and hypoxemic-oligemic rat brain were assessed by measurement of the cerebral contents of energy phosphates, and selected glycolytic-citric acid cycle intermediates. In normoxic brain promethazine (25–100 mg/kg−1) was associated with unaltered adenylates, increased glucose and aspartate and decreased pyruvate, lactate and malate; a pattern which was compatible with cerebral metabolic depression. Hypoxemic animals receiving either saline or promethazine (25 mg/kg−1) showed equivalent decreases in ATP and increases in lactate which indicated that promethazine had no significant effect on the metabolism of the acutely hypoxic brain. In animals exposed to hypoxemia plus right carotid artery occlusion (oligemia) the promethazine treated group (25 mg/kg−1) showed significantly lower ATP and higher AMP contents which suggested an adverse effect on the metabolism of the acutely hypoxic-oligemic brain. It is concluded that promethazine does not beneficially alter the energy metabolism of the acutely hypoxic or hypoxic-oligemic brain.

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A RECENT REPORT has indicated that the phenothiazine chlorpromazine has a major protective effect on ischemic liver.1 In this study although treated and untreated animals showed equivalent metabolic changes during a 3 hour ischemic exposure, animals pretreated with chlorpromazine (20 mg/kg) showed little or no evidence of liver cell necrosis during a 24 hour restitution from the ischemic exposure. In addition chlorpromazine treated animals showed improved post-ischemic regeneration of hepatic ATP stores and a reduced accumulation of tissue and mitochondrial calcium which markedly increased in the untreated post-ischemic liver. The results of these experiments suggested that the action of chlorpromazine was not related to a modifying effect on the intensity of the ischemia, but rather to a prevention of some critical cellular reaction to ischemia. Due to the prominent membrane effects of the phenothiazines,2 it was suggested that this protective action could be related to a primary prevention of ischemic membrane damage or to a blockade of calcium flux across damaged membranes. A second possibility considered was that the chlorpromazine action was related to an inhibition of phospholipase activation during or following ischemia. The sensitivity of mammalian brain to hypoxia-

References

4. Humphries AW, Young JR, Santilli PH, Beven EG, deWolfe VG: Thiazine chlorpromazine has a major protective effect on ischemic liver. The results of these experiments suggested an adverse effect on the metabolism of the acutely hypoxic-oligemic brain. It is concluded that promethazine does not beneficially alter the energy metabolism of the acutely hypoxic or hypoxic-oligemic brain.

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ischemia is well documented and an increasing number of studies have suggested that the pathogenetic mechanisms of the resultant cerebral damage may be quite similar to those advanced for the ischemic liver. Thus it has been proposed or shown that hypoxia-ischemia is associated with activation of brain tissue phospholipases, altered membrane permeability to calcium, and membrane damage due to deregulation of tissue free radicals. Due to these similarities an assessment of the effects of phenothiazine compounds on the hypoxic-ischemic brain seemed warranted and formed the rationale of this study dealing with the metabolic effects of promethazine during acute hypoxic-ischemic exposure. Since detailed studies concerning the effects of phenothiazines on cerebral carbohydrate metabolism are unavailable this report also includes a documentation of the metabolic effects of promethazine on normoxic brain. The decision to use promethazine was made on the basis of earlier experiments that showed this phenothiazine derivative was approximately 4 times more potent than chlorpromazine in blocking in vitro lipid peroxidation and associated membrane disturbances (see discussion).

Methods

Chemicals

Promethazine HCl (N, N, -trimethyl-10H - Phenothiazine - 10 - ethanamine HCl) was obtained as a 25 mg ml⁻¹ solution from Poulenac Ltd. (Montreal, Canada). Substrates and co-enzymes for fluorometric assay were obtained from Sigma Chemical Corp. (St. Louis, MO). All enzymes were obtained from Boehringer-Mannheim.

Animals and Experimental Exposures

The experiments were performed on male rats of the Wistar strain (250-300 g) that had free access to food and water. The animals were briefly anesthetized with 2% halothane, tracheotomized, paralyzed with tubocurarine chloride and artificially ventilated with 30% N₂-70% N₂O on a small animal respirator. A femoral artery was cannulated for blood pressure recording and anaerobic sampling of arterial blood. A skin incision was made over the skull for later in situ freezing of the brain and in the normoxic series the atlanto-occipital membrane was exposed for sampling of cisternal cerebrospinal fluid (CSF). In the hypoxemic-oligemic series the right common carotid artery was exposed for later occlusion with a small arterial clamp.

When animals were in a respiratory steady state, promethazine (25-100 mg/kg⁻¹) was given intraperitoneally. In the normoxic series the 30% O₂-70% N₂ gas mixture was continued, whereas in the hypoxic series the O₂ flow was reduced and replaced with N₂ to give arterial PO₂'s of about 30 mm Hg. The animals in the hypoxemia-oligemia series underwent the addition of right carotid artery occlusion at the time of reducing the O₂ flow. At the end of 30 min exposure to the various PO₂ levels and promethazine doses, CSF and arterial blood were sampled and the brain was frozen by pouring liquid nitrogen into a funnel fitted to the scalp incision. Controls were obtained by giving normoxic, hypoxicemic and hypoxemic-oligemic animals equivalent injections of 0.9% saline.

Analytical Methods

Arterial PO₂, PCO₂ and pH were measured with direct reading electrodes (Eschweiler, Kiel and Radiometer, Copenhagen) operated at 37°C and the values were corrected for variations in body temperature.

The brains were prepared for metabolite extraction in a refrigerated glove box maintained at −22°C. About 200 mg of cortex was dissected from the frozen right cerebral hemisphere, weighed, homogenized in methanol-HCl and then extracted with 0.3N perchloric acid at 0°C. The perchloric acid extracts were centrifuged and the neutralized supernatants were assayed for their metabolite contents by the enzymatic fluorometric methods of Lowry and Passionneau. Blood and CSF were processed as above and analyzed for pyruvate, lactate and glucose.

The intracellular concentrations of pyruvate, lactate and glucose were calculated from the measured tissue, blood and CSF contents on the assumption of a 3% intracellular fluid volume, a 20% extracellular fluid volume and an intracellular fluid volume of 57% of the net tissue weight. The results were statistically evaluated using Wilcoxon's rank sum test.

Results

The administration of promethazine to normoxic animals was associated with a progressive lowering of the mean arterial blood pressure (p < 0.05 for 50-100 mg/kg⁻¹ groups) in the presence of unchanged arterial PO₂ and acid-base parameters (table 1). All hypoxicemic animals showed significant reductions of arterial PO₂ (26-34 mm Hg), PCO₂ (27-32 mm Hg), pH (7.05-7.20) and mean blood pressure (75-120 mm Hg) in comparison to the normoxic groups. Values for these parameters were not significantly different in hypoxicemic and hypoxemic-oligemic animals receiving either saline or promethazine. Body temperature was artificially maintained between the limits of 36.5-37.2°C in all animals.

Hypoxicemic and hypoxemic-oligemic animals treated with 50-100 mg/kg⁻¹ promethazine experienced a 75-80% incidence of acute hypotensive collapse and death which precluded further study at these dose levels.

Energy Phosphates and Carbohydrate Metabolites

The administration of promethazine to normoxic animals resulted in a pattern of metabolic change characterized by initial decreases in pyruvate, lactate and malate (25 mg/kg⁻¹ groups) which was followed by later increases of glucose and aspartate (50-100 mg/kg⁻¹ groups) (table 2). The cerebral contents of ATP, ADP, AMP, citrate, α-ketoglutarate and glutamate were not significantly altered by promethazine.

Although promethazine was associated with increased tissue glucose, it was also associated with near
TABLE 1  Arterial PO₂, PCO₂, pH, Mean Arterial Blood Pressure (MABP) and Temperature in Rats Given i.p. Saline or Promethazine at Onset of 0.5h Exposures to Normoxia, Hypoxemia or Hypoxemia Plus Right Common Carotid Artery Clamping

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>PO₂ (mm Hg)</th>
<th>PCO₂ (mm Hg)</th>
<th>pH</th>
<th>MABP (mm Hg)</th>
<th>TEMP (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normoxia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (6)</td>
<td>115±3</td>
<td>34.8±0.7</td>
<td>7.36±0.01</td>
<td>143±2</td>
<td>36.9±0.1</td>
</tr>
<tr>
<td>Promethazine</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>25 mg kg⁻¹ (6)</td>
<td>124±6</td>
<td>35.6±1.6</td>
<td>7.33±0.02</td>
<td>133±5</td>
<td>36.8±0.1</td>
</tr>
<tr>
<td>50 mg kg⁻¹ (6)</td>
<td>105±4</td>
<td>37.1±1.2</td>
<td>7.32±0.01</td>
<td>123±4*</td>
<td>36.8±0.1</td>
</tr>
<tr>
<td>100 mg kg⁻¹ (6)</td>
<td>113±6</td>
<td>36.1±1.2</td>
<td>7.32±0.01</td>
<td>118±3*</td>
<td>36.9±0.1</td>
</tr>
<tr>
<td><strong>Hypoxemia</strong></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Saline (6)</td>
<td>30.3±0.6</td>
<td>29.2±0.4</td>
<td>7.05±0.03</td>
<td>115±6</td>
<td>36.7±0.1</td>
</tr>
<tr>
<td>Promethazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg kg⁻¹ (6)</td>
<td>32.1±1.2</td>
<td>28.8±1.1</td>
<td>7.12±0.03</td>
<td>105±12</td>
<td>36.8±0.1</td>
</tr>
<tr>
<td><strong>Hypoxemia-Oligemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (6)</td>
<td>31.4±1.2</td>
<td>28.2±0.8</td>
<td>7.10±0.02</td>
<td>95±5</td>
<td>36.8±0.1</td>
</tr>
<tr>
<td>Promethazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg kg⁻¹ (6)</td>
<td>30.5±1.8</td>
<td>30.0±1.1</td>
<td>7.11±0.04</td>
<td>87±5</td>
<td>36.9±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Saline vs promethazine groups, *p < 0.05.

equivalent increases of blood glucose (table 3) which resulted in an unchanged ratio of intracellular to blood glucose at the various dose levels (saline — 0.25 ± 0.02; 25 mg/kg⁻¹ — 0.26 ± 0.03; 50 mg/kg⁻¹ — 0.29 ± 0.02; 100 mg/kg⁻¹ — 0.26 ± 0.01). The converse was present for pyruvate and lactate in that the calculated intracellular values showed progressive decreases in the presence of increasing or unchanged blood levels. This suggests that although the increases of tissue glucose could be secondary to the increases of blood glucose, the decreases of pyruvate and lactate seem to reflect primary intracellular events.

Saline treated hypoxic animals showed a pattern of metabolic derangement that was characterized by modest decreases of ATP and increases of ADP, AMP, glucose, pyruvate and lactate (all values being significantly different at the 0.05 level from saline normoxic animals) (table 4). Hypoxic animals receiving promethazine (25 mg/kg⁻¹) showed a statistically equivalent pattern.

The combination of right carotid artery occlusion and hypoxemia resulted in metabolic alterations which signified the presence of a more advanced tissue hypoxia (table 4). Thus the saline treated group showed massive increases in lactate and AMP plus large decreases in ATP. The corresponding promethazine treated group showed further significant decreases in ATP and increases in AMP. In addition, decreases in glucose and pyruvate which suggested a more profound metabolic disturbance than that experienced by the saline treated group.

**Discussion**

The objective of this study was to assess the effects of the phenothiazine derivative promethazine on the energy and carbohydrate metabolism of the hypoxic

TABLE 2  Right Cerebral Hemisphere Contents of Energy and Carbohydrate Metabolites in Normoxic Rats 30 min After the i.p. Administration of Saline or Promethazine

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Saline</th>
<th>Promethazine mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>ATP</td>
<td>2.88±0.02</td>
<td>2.90±0.03</td>
</tr>
<tr>
<td>ADP</td>
<td>0.30±0.03</td>
<td>0.30±0.03</td>
</tr>
<tr>
<td>AMP</td>
<td>0.03±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.96±0.15</td>
<td>3.44±0.25</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.097±0.005</td>
<td>0.082±0.003*</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.49±0.07</td>
<td>0.96±0.05*</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.43±0.03</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>-ketoglutarate</td>
<td>0.101±0.014</td>
<td>0.097±0.008</td>
</tr>
<tr>
<td>Malate</td>
<td>0.32±0.01</td>
<td>0.24±0.01*</td>
</tr>
<tr>
<td>Aspartate</td>
<td>3.42±0.04</td>
<td>3.67±0.11</td>
</tr>
<tr>
<td>Glutamate</td>
<td>10.81±0.24</td>
<td>10.67±0.24</td>
</tr>
</tbody>
</table>

Values are means ± SEM in mMole kg⁻¹. Number of animals in each group = 6. Saline vs promethazine groups, *p < 0.05.
The overall results which indicated that promethazine...metabolic effects on 1) normoxic and 2) hypoxic brain.

**Metabolic Effects of Promethazine on Normoxic Brain**

As a pharmacological group the phenothiazines are associated with a bewildering diversity of physiological, biophysical and biochemical actions. In regard to cerebral oxidative metabolism the available literature indicates that although sedative doses of phenothiazines have little effect on the overall oxygen consumption of the brain, they are associated with modest depressions of regional glucose utilization (i.e. thalamus, frontal-parietal cortex, brain stem nuclei) when studied with the more sensitive C2-deoxy-glucose autoradiographic technique. Although a number of studies using isolated mitochondria and enzyme preparations have shown that phenothiazine can interact with flavin adenine dinucleotide, cytochrome oxidase and hexokinase, the extrapolation of those observations to a possible direct inhibitory effect on *in vivo* oxidative phosphorylation and glycolysis is most tenuous.

The alterations of cerebral carbohydrate metabolism associated with behavioral depressants are well documented and consist of unchanged tissue contents of ATP, increases of glucose and aspartate, and decreases of various glycolytic-citric acid cycle intermediates such as pyruvate, lactate, citrate, α-ketoglutarate and malate. The cerebral metabolic pattern observed in normoxic animals administered promethazine essentially conformed to this pattern. The results also indicated that the metabolic changes were not strictly dose related since doubling or quadrupling the dose (50-100 mg/kg) caused relatively little further quantitative change in the metabolite values observed at the 25 mg/kg level. Overall the results are supportive of a recent study showing that phenothiazines are associated with mild reductions in the rate of *in vivo* cerebral oxidative metabolism.

**Metabolic Effects of Promethazine on Hypoxic Brain**

A variety of procedures and pharmacological agents...
have been shown to have a protective action on various organ systems exposed to hypoxia-ischemia. Although the precise nature of the primary irreversible change(s) is as yet undefined, the rationale formulation and testing of therapeutic procedures has followed certain basic lines of reasoning. Thus it is well established that artificial reduction of the cerebral metabolic activity increases the brain’s resistance to hypoxia-ischemia, a fact which is believed to explain, in part at least, the beneficial effect of hypothermia and barbiturate anesthesia. More recently, experimental efforts have focused on the cellular membrane damaging actions of hypoxia-ischemia. In this scheme it is proposed that hypoxia-ischemia results in free radical induced alterations of membrane function and structure which lead to the secondary tissue destructive processes of edema, microvascular failure and activation of proteolytic enzymes (cf. Siesjö for discussion). Phenothiazine compounds have a number of actions which make them ideally suited as potential therapeutic agents against this proposed membrane damaging effect of hypoxia-ischemia. These include their ability to quench free radicals, to prevent K+ leakage from and Ca2+ entrance into cells, to reduce cellular osmotic swelling and to block release of acid hydrolyses from lysosomes.

Since phenothiazines produce metabolic evidence for a mild to moderate reduction of cerebral metabolic activity in normal animals (see above and reference 13) it seemed necessary to evaluate the influence of the effect in this hypoxic-ischemic animal prior to further detailed study of their role as membrane stabilizers. In the present study the cerebral metabolic effects of promethazine were evaluated in two hypoxic models. The first of these, unifactorial hypoxemia (PO2, 25–35 mm Hg), is a subcritical hypoxic model which although showing a moderately advanced lactacidosis maintains its ATP stores at or near control levels and upon reoxygenation shows absence of structural change. In this preparation promethazine (25 mg/kg) was without apparent beneficial effect. This again suggests that the level of cerebral metabolic depression produced by phenothiazines is rather mild and probably non-operational during stressful situations such as hypoxia. The second model, hypoxemia plus carotid artery clamping, is a critical threshold model which is associated with excessive accumulation of tissue lactate, decreased ATP and evidence of irreversible mitochondrial and cellular damage upon restitution. In this model promethazine (25 mg/kg) was associated with further depletion of tissue ATP and since the intensity of this metabolite change is frequently correlated to cellular damage it can be tentatively concluded that promethazine is potentially detrimental to the acutely hypoxemic-oligemic brain. This potentially detrimental effect was further supported by the observation that the combination of larger doses of promethazine (50–100 mg/kg) with hypoxemia or hypoxemia-oligemia resulted in a very high incidence (75–80%) of acute cardiovascular collapse and death. Although the precise reason for the enhanced sensitivity of promethazine treated animals to acute hypoxia-oligemia is not established, it may possibly be related to the documented central and peripheral cardiodepressive actions of this class of drugs.

As indicated earlier phenothiazines have a number of pharmacological actions which make them ideally suited for the evaluation of the role of free radical induced membrane dysfunction and damage in the pathogenesis of hypoxic-ischemic brain damage. Thus brain homogenates prepared from animals pretreated with promethazine (25–50 mg/kg) or chlorpromazine (100–200 mg/kg) show a significant resistance to in vitro free radical induced lipid peroxidation. In addition to this anti-free radical action these drugs seem to have the ability to block hypoxic-ischemic induced membrane translocation of Ca2+. An event which may be critical in the causation of irreversible cellular damage. The overall results of the present experiments indicate that although the administration of promethazine, in doses required for presumed suppression of in vivo free radical reactivity, cause minor or negligible effects on the metabolism of the normoxic or hypoxic brain, its administration to more advanced hypoxic models results in further significant deterioration of the cerebral energy state and in acute death of the preparation. These observations suggest that some degree of caution will be required in the interpretation of experimental trials evaluating the effectiveness or mechanism of action of phenothiazines in the amelioration of hypoxic-ischemic brain damage.

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Intravenous Digital Subtraction Angiography: An Index of Collateral Cerebral Blood Flow in Internal Carotid Artery Occlusion

ISSAM AWAD, M.D., JOHN R. LITTLE, M.D., MICHAEL T. MODIC, M.D., AND ANTHONY J. FURLAN, M.D.

SUMMARY The objective of this investigation was to correlate Xenon-133 inhalation rCBF measurements with the pattern of cortical arterial filling on intravenous DSA in 18 patients with unilateral internal carotid artery occlusion. Of 9 patients showing symmetrical filling of hemispheric cortical arteries, none showed an inter-hemispheric difference in rCBF (ΔFg) greater than 10ml/100gm/min. Of 9 patients showing delayed cortical opacification ipsilateral to the internal artery occlusion, 3 showed a ΔFg greater than 10ml/100gm/min, 3 showed a ΔFg in the 7-10ml/100gm/min range, and 3 had a ΔFg less than 7ml/100gm/min. All patients with asymmetric abnormalities in the rCBF profile had the delayed pattern of cortical filling on DSA. The presence of symmetrical hemispheric opacification of cortical arteries on DSA indicates adequate interhemispheric redistribution of rCBF and patent inter-hemispheric collateral channels, but not necessarily normal cerebral blood flow. The presence of delayed cortical arterial opacification on the side of internal carotid artery occlusion does not necessarily imply significant inter-hemispheric rCBF differences, nor does it rule out a normal rCBF. The presence of bilateral reduction of rCBF and symmetrical cortical arterial filling on DSA may represent an “interhemispheric steal.”

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AFTER UNILATERAL INTERNAL carotid artery (ICA) occlusion, regional cerebral flow (rCBF) in the ipsilateral hemisphere reflects the adequacy of collateral circulation from the contralateral ICA, the verteobasilar system and from other sources.1, 2 Quantification of collateral flow might clarify the ischemic nature of patient symptoms and could affect therapeutic decisions.1, 3, 4 Conventional angiography can only visualize patterns of collateral circulation originating from the injected artery. This may be a misleading index of overall collateral blood flow. Since intravenous digital subtraction angiography (DSA) simultaneously delivers contrast material into the territory of an occluded ICA from all possible collateral sources,5 DSA allows an examination of the distribution of arterial blood to the hemisphere on the side of the ICA occlusion. The objective of this investigation was to correlate Xenon-133 inhalation rCBF measurements with the pattern of opacification of cortical vessels on DSA in 18 patients with unilateral ICA occlusion.

Methods

Eighteen patients (mean age 57 years) with unilateral ICA occlusion underwent Xenon-133 inhalation rCBF measurement and DSA as part of their evaluation by the Cerebrovascular Section of the Cleveland Clinic. One patient had occlusion of the ICA secondary to a medial sphenoid ridge menigioma. All other patients had atherosclerotic occlusion of one ICA with varying degrees of contralateral disease. All patients presented with recent stroke, transient ischemic attacks and/or amaurosis fugax ipsilateral to the occluded ICA. The rCBF studies were carried out in a quiet, darkened room. The patients inhaled 20 mCi of Xenon-133 through a mouthpiece designed to eliminate leakage of gas. Radiation over the cranial vault was detected

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