Ethacrynic Acid

EFFECTS ON THE COCHLEAR POTENTIALS
IN NORMAL AND HIGH BLOOD OXYGEN

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ABSTRACT The effect of ethacrynic acid (EA) at different blood O₂ saturations on cochlear potentials of guinea pigs was investigated. All 18 young healthy guinea pigs received 50 mg/kg/h of EA intravenously and were divided into three groups: first group, normal (90.00±6.30-86.17±4.83 mm Hg); second group, lower Pₒ₂ (78.00±4.74-70.00±4.42 mm Hg); and third group, high Pₒ₂ (174.40±13.41-179.00±26.15 mm Hg). The partial pressure of oxygen (Pₒ₂), the partial pressure of carbon dioxide (Pcoln), and the pH of the blood were measured before EA administration and at the end of the experiment (3 h later) by drawing blood samples from the contralateral carotid artery. Cochlear potentials—endocochlear potential (EP), cochlear microphonics (CM), and action potentials (AP)—were recorded by standard methods from the first turn of the cochlea. Experimental data seem to indicate that elevation of the Pₒ₂ to 174-179 mm Hg during relatively high doses of EA treatment prevents the declines in cochlear potentials which were observed in the first and second groups (normal and lower Pₒ₂), and preserves active ion transport which is responsible for the generation of cochlear potentials. These data suggest a means by which to reduce the ototoxic effect of EA and possibly indicates a method of treatment for hearing loss which developed after the administration of EA.

INTRODUCTION

The ototoxicity of ethacrynic acid (EA) ¹ [2,3-dichloro-4-(2-methylenebutyryl)-phenoxy]acetic acid has been well established for several years, and its toxic effect on human and animal subjects has been documented by numerous studies (1-8). The recommended therapeutic dose is 1 mg/kg of body weight. Experimental administration of EA (one dose of 30 mg/kg or two doses of 10 mg/kg, given 2 h apart) causes a reversible decrease of cochlear potentials (9, 10). This experimental information which indicates a rapid onset of EA action manifested in reduction of cochlear potentials may be correlated with clinical observations in which sudden hearing loss results shortly after EA administration. Electron microscope photomicrographs show extensive morphological changes in the stria vascularis shortly after drug administration (vacuolization and cystic swelling) (11). The descriptive diagram of the anatomy and electrophysiology (Fig. 1) demonstrates the peculiarity of the inner ear in regard to the experiments conducted in this paper.

From the previously mentioned experiment (9), in which different doses of EA were used on the same animals, it was evident that the highest dose (50 mg/kg) caused the most significant changes in the inner ear. The active ion transport system which maintains the generation of cochlear potentials was blocked for a temporary period of time. This blockage resulted in the drop of endocochlear potential (EP), cochlear microphonics (CM), and action potential (AP). The effect of the identical high level of EA was investigated to elucidate if the degree of ototoxicity would change under different levels of blood Pₒ₂.

METHODS

18 guinea pigs, each weighing between 290-400 g, were anesthetized with nembutal (30 mg/kg) given intraperitoneally. A tracheotomy was performed and artificial ventilation was effected by a respirator in which the rate was kept constant at 15/min (with a ratio of inspiration/expiration = 1/2, and volume was adjusted according to the body weight.

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¹ Abbreviations used in this paper: AP, action potential; CM, cochlear microphonics; EA, ethacrynic acid; EP, endocochlear potential; Pcoln, partial pressure of carbon dioxide; Pₒ₂, partial pressure of oxygen.
Body temperatures were maintained at approximately 37°C. On two occasions, 0.5 ml of blood from each animal was drawn from the contralateral carotid artery for blood gas analysis: at the beginning of the experiment before the electrodes were in place and at the end of the experiment, 2 h after the completion of EA infusion. Cochlear potentials CM and AP were recorded by using differential electrodes placed into the first turn of the scala vestibuli and scala tympani. EP were recorded by a glass microelectrode filled with 3 M KCl introduced through the stria vascularis into the scala media. 8 kHz 80 db sound pressure level tone pips of 40-ms duration, delivered in sequences of 3, 1 s apart, at 5-min intervals were selected for sound stimulation and transmitted to the ear through a plastic cannula from a head phones (TDH-39, Telephone Dynamics Corp.) earphone. Sound signals were generated and attenuated by a GE oscillator and attenuator (General Electric, Medical Systems, Milwaukee, Wisc.) and were gated by a Grason-Stadler switch (Grason-Stadler Co., Inc., Concord, Mass.) and interval timer combination. Cochlear potentials were monitored and preamplified inside a soundproof room by field effect transistor followers and preamplifiers that were constructed ourselves. Outside the soundproof room the CM and the AP were further amplified by Tektronix 26A2 differential amplifiers (Tektronix, Inc., Beaverton, Ore.) and, along with an amplified EP, were fed into the A/D convertor of a Nova 1220 computer (Data General Corp., Southboro, Mass.) where all data were read, analyzed, averaged, and printed out every 5 min. A schematic diagram of the cochlear potentials recordings and apparatus arrangement is illustrated in Fig. 2. All data were statistically analyzed by averaging and computing SEM, and graphs were constructed according to the results. Any significant differences between groups were determined by the t test.

The animals all received 50 mg/kg of EA (Edecrin, Merck Sharp & Dohme, West Point, Pa.) in 5 ml of 0.9% sodium chloride solution over a 1-h infusion period. The healthy young guinea pigs were divided into three groups according to their blood level Po2. Values of Po2, Pco2, and pH at the start and at the end of the experiments of all three groups are presented in Table 1.

**RESULTS**

Treatment with high concentrations of EA (50 mg/kg/h) in the first and second groups caused a substantial drop of all potentials, with the maximum decrease occurring 10 min after the completion of infusion.
—the lowering effect of EA was reversible, and potentials had a tendency to recover toward normal values. However, very different results were observed when the same concentration of EA (50 mg/kg/h) was administered in the presence of highly elevated blood oxygen levels. High Po2 levels possibly decreased the action of EA on active ion transport, which was manifested by prevention of the decline in cochlear potentials found in the normal and the lower arterial blood Po2 groups.

The results of the experiments on the three groups have been presented graphically, showing changes of potentials with time (Fig. 3). SEM have been placed at the points on the graph where differences between the groups seemed to be important; P value calculated by t test with comparison of group one and group three. The graphical data clearly indicate statistical differences of AP, CM, and AP among group three, the highly oxygenated group, and the other two groups at 70, 90, and 120 min after infusion of EA. There was not a substantial significant difference between the potentials of the first (normal Po2) and second (lower Po2) groups.

**DISCUSSION**

Several modes of EA action have been described in the post, including: increased diuresis related to the capacity of EA for binding to sulfhydryl groups (12), inhibition of Na+, K+, and Mg2+-activated ATPase (13), blockade of phosphorylation in mitochondria (14), and interruption of glycolysis (15). More recent data suggest that EA behaves like a nonspecific sulfhydryl-reacting substrate (16) and interferes with the supply of energy for the transport system (17). After the administration of EA, extensive changes were observed in the stria vascularis (vacuolization and swelling), however, no detectable changes were noted in the Corti organ. Therefore, it is possible that the primary effect of EA is in the change of EP (source in stria vascularis), and the change of CM and AP are secondary, due to the decrease of polarization within the Corti organ.

The high metabolic rate of the stria vascularis, the dependence of the stria vascularis on the oxidative mode of energy production, and the morphological

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**Table I**

<table>
<thead>
<tr>
<th></th>
<th>Group I (normal O2)</th>
<th>Group II (lower O2)</th>
<th>Group III (higher O2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Po2, mm Hg</td>
<td>Start 90.00±6.30</td>
<td>78.00±4.74</td>
<td>174.4±13.41</td>
</tr>
<tr>
<td></td>
<td>Finish 86.17±4.83</td>
<td>70.00±4.42</td>
<td>179.00±26.15</td>
</tr>
<tr>
<td>PC02, mm Hg</td>
<td>Start 27.00±3.43</td>
<td>28.83±1.19</td>
<td>30.80±0.49</td>
</tr>
<tr>
<td></td>
<td>Finish 31.50±1.78</td>
<td>29.70±4.49</td>
<td>24.75±2.78</td>
</tr>
<tr>
<td>pH</td>
<td>Start 7.48±0.04</td>
<td>7.47±0.05</td>
<td>7.56±0.04</td>
</tr>
<tr>
<td></td>
<td>Finish 7.46±0.01</td>
<td>7.37±0.06</td>
<td>7.58±0.05</td>
</tr>
</tbody>
</table>

**Figure 2** Diagramatic presentation of equipment interconnections, for recording and monitoring cochlear potentials (EP, CM, and AP). The pulse which originates from the computer triggers the oscilloscope and sound generator at certain time intervals.

**Figure 3** Data from all three groups (six animals each) are arranged according to the recorded potentials. Vertical bars (I) represent SEM and numbers 0.001, 0.01, and 0.05 represent P value as calculated by t test. All values are in millivolts.

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changes found in the stria vascularis after EA administration suggest that EA exerts an effect on membrane transport by interfering with the supply of energy source to the transport system. Therefore, an investigation of EA action under different blood O2 saturation was advisable. The normal range of arterial Po2 is considered to be in the range of 65–150 mm Hg. Our normal control group had Po2 levels of 90.00±6.30 mm Hg at the start and 86.17±4.83 mm Hg at the end of the experiment, whereas the lower Po2 group had a level of 78.00±4.42 mm Hg at the conclusion of the experiment and the high Po2 group included readings of 174.40±13.41 mm Hg at the start and 179.00±26.15 mm Hg at the end of the experiment. The lower Po2 group was selected in a range where lower Po2 levels would not affect the EP but was not low enough to show differences from the normal group. The high range of oxygenation was chosen with regard to oxygen toxicity and the effect of very high Po2 levels on vascular and enzymatic systems; the high Po2 group therefore was in the range of maximal (O2) saturation of hemoglobin. The positive effect of oxygen at increased blood concentrations is most pronounced in tissues with high aerobic metabolism which are most sensitive to anoxic conditions. Elevation of alveolar Po2 leads to an increase in oxyhemoglobin concentration until complete saturation is achieved. Hyperoxegenation of blood increased the O2 gradient between the blood and the tissue to which the O2 is delivered. A high saturation of oxyhemoglobin in the blood adversely affects the activity of several enzymes like succinic oxidase, succinic dehydrogenase, and others.

The arterialvenous oxygen-saturation difference was reduced by EA, which suggests that the oxygen utilization by skeletal muscles had been inhibited or that oxygen release from erythrocytes had been reduced (18). However, it is of interest that EA also caused simultaneous vasodilatation (19). Experiments in which oxygen tension and glucose metabolism were investigated, utilizing tissue slices of rabbit renal cortex and medulla, show that increasing high-medium oxygen tension from 32 to 530 mm Hg substantially increases stepwise glucose oxidation in the outer medulla and renal cortex (20). A direct relationship between renal O2 consumption and Na+ reabsorption has been observed (21).

The immediate time period after EA administration is probably most critical for the function of the stria vascularis and the Corti organ. Active ion transport is blocked in the inner ear by EA at the same time that the O2 supply is reduced. The primary target of EA is the stria vascularis, system of highly differentiated cells, responsible for the generation of EP+80 mV. These cells maintain a very high metabolic rate and low energy reserves (10). The electrophysiological data is supported by the morphological picture (11), where cystic changes in the stria vascularis are observed as early as the changes of the EP potentials. The decline of CM and AP is possibly secondary because the polarization of hair cells is lowered (decline of EP). According to this data, oxygen therapy may be helpful in reducing the hypoxenic conditions in the tissue. Several factors are involved when the action of high levels of EA are affected by presence of high Po2 in the blood; for example, stimulation of oxidative metabolism by high Po2; action of EA and high Po2 on sulfhydryl groups. There is also a possibility that high Po2 causes vasoconstriction in the inner ear which decreases the permeability of the tissues to EA. However, this mode of action is probably not so pronounced because of the slight elevation above normal in the Po2 level and the counteracting vasodilatory effect of EA action. More detailed investigation of the above mentioned phenomena is necessary to explain the results obtained from this study. Stimulation by high Po2 of other metabolic pathways-source of energy can be also possible. This hypothesis suggests a method of treatment for sudden hearing loss after the intravenous administration of EA.

REFERENCES


