Comparison of Adrenergic Activation by Anticholinesterases and by Hypoxia

By Vladislav M. Varagić, M.D., and Dušan B. Boleslin, M.D.

Several anticholinesterases have been found to produce a blood pressure rise in the rat.\textsuperscript{1-4} This effect has been attributed to the central adrenergic activation.\textsuperscript{4,5} On the other hand, anticholinesterases are known to cause bronchoconstriction and to produce, by this action, a long-lasting hypoxia (or anoxia) which is also known to produce general sympathetic activation. Asphyxiation of normal cats leads, after 5 to 10 seconds, to a progressive rise in arterial pressure and to an enhancement of efferent sympathetic outflow.\textsuperscript{6,7} It was, therefore, of interest to compare the effects of hypoxia (and anoxia) and anticholinesterases in the same animal in order to obtain more data as to whether bronchoconstriction caused by anticholinesterases and the subsequent hypoxia (or anoxia) may be the causal factor in producing the hypertensive response to eserine and other anticholinesterases in the rat.

Methods

Rats of both sexes (180 to 320 Gm.) were used and anesthetized with 0.7 ml./100 Gm. body weight of 25 per cent urethane solution injected subcutaneously. To record the blood pressure, a cannula was inserted into the carotid artery and connected with a small mercury manometer.\textsuperscript{8} A small polyethylene catheter was inserted into the jugular vein and was used for injecting drugs. Before the experiment was started, 1 mg./100 Gm. body weight of heparin and 30 mg./Kg. gallamine were injected. All drugs were injected in 0.1 ml. and washed with the same volume of saline. The animals were kept on artificial respiration by means of a Palmer miniature respiration pump.

The resistance to lung inflation was recorded by a method previously described,\textsuperscript{9} except that the whole system was closed. Short-lasting asphyxia was produced by stopping the respiration pump for 30 to 60 seconds. Anoxia was produced by inhalation of pure nitrogen for 30 to 90 seconds. Long-lasting hypoxia was produced by inhalation of 11 per cent oxygen in nitrogen.

The following drugs were used: eserine salicylate, neostigmine methylsulfate (Prostigmine), bretylium tosylate, atropine sulfate, and gallamine (Flaxedil).

Results

EFFECTS OF VARIOUS TYPES OF HYPOXIA AND ESERINE

Different types of hypoxia (or anoxia) have been found to produce various types of circulatory responses. In seven experiments, only a rise of blood pressure was observed, but in the other three, a biphasic effect or pure hypotension was present. A typical experiment is shown in figure 1. Breathing of pure nitrogen for 60 seconds produced an initial fall of blood pressure followed by a rise lasting three to five minutes, as shown in figure 1A. A similar effect was observed after stopping the respiration pump for 30 seconds (the animal was curarized), as shown in B. Intravenous injection of 0.2 mg./Kg. eserine caused a long-lasting blood pressure rise which lasted about 30 minutes. At the same time, eserine caused an increase in lung resistance to inflation, as shown in the upper record in C. The peak of the blood pressure rise and the maximum of increase in lung resistance to inflation do not occur in a parallel manner. Bronchoconstrictor action starts by the time the vascular effect has reached its maximum, or even later. The relationship between hypertensive and bronchoconstrictor action is shown in table 1.

Breathing of 11 per cent oxygen in nitrogen for 3 to 11 minutes produced only a fall of blood pressure in all five experiments. One of these experiments is shown in figure 2. In this particular experiment, the fall of blood pressure was very small, but in the others it ranged from 5 to 25 mm. Hg. At the same time, eserine in a dose of 0.08 mg./Kg. caused a blood pressure rise without apparent...
The effects of nitrogen, asphyxia, and eserine on the blood pressure (lower record) and lung resistance to inflation (upper record) of the rat (200 Gm.).

A. Breathing of pure nitrogen for 60 seconds.
B. Respiration pump stopped for 30 seconds.
C. At the dot 40 μg. eserine injected intravenously.

Changes in lung resistance to inflation. Neostigmine methylsulfate, in a dose of 0.2 mg./Kg., caused a small rise of blood pressure, but it produced a substantial increase in lung resistance to inflation. It was possible to abolish this effect on the bronchi by atropine.

**EFFECT OF VAGOTOMY ON THE RESPONSES TO ESERINE AND NEOSTIGMINE**

If doses of eserine and neostigmine were small enough (0.057 mg./Kg. to 0.1 mg./Kg. for eserine, and 0.156 mg./Kg. to 0.25 mg./Kg. for neostigmine), the bilateral vagotomy was found to abolish the bronchoconstrictor effect of eserine and neostigmine without altering the blood pressure response to these drugs. A typical experiment is shown in figure 3. Control responses to eserine (0.07 mg./Kg.) and neostigmine (0.22 mg./Kg.) are shown in A and B. Between B and C the vagi were cut, and 10 minutes later no increase in lung resistance to inflation was observed, whereas the blood pressure response was unchanged, as shown in C and D. This type of response was obtained in five out of eight experiments.

On the other hand, large doses of eserine (0.5 mg./Kg.) increased the lung resistance to inflation even in the vagotomized animals, as shown in figure 4. At the dot, 0.2 mg./Kg. eserine was injected. This dose produced a blood pressure rise without causing an increase in lung resistance to inflation (the vagi were previously cut). At the arrow, 0.5 mg./Kg. eserine was injected, and this dose produced a large blood pressure rise accompanied by an increase in lung resistance to inflation. This type of response was obtained in four experiments.

**EFFECT OF BRETYLIUM AND ESERINE**

In previous work, bretylium was found to block the blood pressure response to eserine, while leaving intact the effect of epinephrine and norepinephrine. In the present work, bretylium was found not to affect the action of eserine on the bronchi, causing at the same time a block of the blood pressure response to eserine. One of three experiments with bretylium is shown in figure 5. At the dots, 0.3 mg./Kg. eserine was injected. At B, 10 mg./Kg. bretylium were injected, and 30 minutes later, the vascular response to eserine was blocked, whereas the effect of eserine on the bronchi was unchanged.

**Discussion**

It was found in the present experiments that the hypertensive response to eserine in the rat is accompanied by an increase in lung resistance to inflation. These two effects usually do not take place simultaneously. In the
The effects of vagotomy on the blood pressure (lower record) and lung resistance to inflation (upper record) responses to eserine and neostigmine (rat 320 Gm.). At the arrows in A and C, 20 µg. eserine intravenously. At the dots in B and D, 50 µg. neostigmine intravenously. Between B and C, vagi cut.

FIGURE 4
The effects of various doses of eserine on the blood pressure (lower record) and lung resistance to inflation (upper record) of the vagotomized rat (200 Gm.). At the dot, 40 µg. eserine intravenously. At the two dots, 100 µg. eserine intravenously.

majority of experiments, the blood pressure rise occurred before any effect on the bronchiolar muscles was observed. Nevertheless, asphyxia, which occurs as a result of a reduction of pulmonary ventilation after injection of eserine, could produce peripheral vasoconstriction through direct action of the changes in arterial blood gas tensions on the vasomotor center or on the spinal cord. Asphyxiation also leads to an enhancement of efferent sympathetic outflow. Acute hypoxemia causes an increased cardiac output, although the mechanisms responsible for this effect have not been clearly defined. The sympathoadrenal system is repeatedly taken as a factor involved in this effect of hypoxemia. In the present experiments, the effect of acute hypoxemia on the blood pressure was variable. In some experiments, a pure hypertension or hypotension occurred, whereas in other experiments, a biphasic effect was observed. Similar evidence was presented in studies on acute hypoxia in normal and atherosclerotic rabbits. On the contrary, eserine invariably caused an increase of blood pressure. It would, therefore, mean that the blood pressure responses of acute hypoxia and eserine are dissimilar. This statement is further supported by the effect of prolonged hypoxia in the rat which was found to produce only fall of blood pressure.

In previous work, bretylium, as well as 2,6-xylyl ether bromide, were found to depress or even block the hypertensive response to eserine in the rat. In the present experiments, bretylium also blocked the vascular response to eserine, at the same time leaving unchanged the effect of eserine on the bronchi. Therefore, different mechanisms are involved in the vascular and bronchiolar responses to eserine. The effect of eserine on the blood pressure depends on adrenergic activation, whereas the action of eserine on the bronchi depends on acetylcholine accumulation at the effector site and on general activation of
ANTICHOLINESTERASES AND HYPOXIA

TABLE 1

<table>
<thead>
<tr>
<th>Number of experiment</th>
<th>Dose of eserine per rat (in μg.)</th>
<th>Increase of blood pressure in mm. Hg</th>
<th>Duration of effect (minutes)</th>
<th>Maximum reached after injection of eserine (minutes)</th>
<th>Hypertension</th>
<th>Bronchoconstriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>33</td>
<td>35</td>
<td>7</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>23</td>
<td>35</td>
<td>4</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>27</td>
<td>15</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>25</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>16</td>
<td>10</td>
<td>2,5</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>12</td>
<td>30</td>
<td>7</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>43</td>
<td>40</td>
<td>7</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>30</td>
<td>35</td>
<td>6</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>40</td>
<td>15</td>
<td>35</td>
<td>9</td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>

parasympathetic outflow. On the other hand, atropine blocks both vascular and bronchiolar responses to eserine, but this might only mean that the origin of both effects is cholinergic in nature.

Neostigmine was found to produce a small rise of blood pressure and a very strong bronchoconstriction. If blood changes due to bronchoconstriction were the primary cause of the hypertensive response to eserine, then it would be difficult to understand why these changes do not produce the blood pressure rise in the case of neostigmine. It is, therefore, concluded that the bronchoconstriction and the subsequent hypoxia might contribute to the hypertensive response to eserine in the rat, but this mechanism does not seem to be of primary importance. These findings once again suggest that the hypertensive effect of eserine is central in origin. The specific implication of these results is in evaluating the symptoms of the poisoning by anticholinesterases which penetrate the blood-brain barrier.

Summary

The hypertensive response to eserine in the rat is accompanied by an increase in lung resistance to inflation. Usually, these two effects do not occur simultaneously. The effects of acute hypoxemia on the blood pressure of the rat were found to be variable, ranging from a biphasic effect to a pure hypertension or hypotension. Inhalation of 11 per cent oxygen in nitrogen produced only a fall of blood pressure.

Bretylium was found to block the hypertensive response to eserine, while leaving intact the effect of eserine on the bronchi. On the other hand, bilateral vagotomy was found to block the effect of small doses of eserine on the bronchi and not to affect the blood pressure response to eserine.

Neostigmine caused a small rise of blood pressure and a very significant bronchoconstriction. It is concluded that bronchoconstriction and subsequent hypoxia might contribute to the hypertensive response to eserine in the curarized rat, but this mechanism does not seem to be of primary importance for a hypertensive action of eserine.

Acknowledgment

We are indebted to Mr. I. Gašpar and Mr. B. Stejanović for their technical assistance.

References


Book Reviews


This is an extensive revision of the previous manual, which appeared in 1964. The discussion on the use of anticoagulants in the treatment of varicose veins is complete with opinions from both sides. The surgical treatment of venous ulcers and the stripping operations on varix are adequately illustrated.


The recording oscillometer, described in detail, makes possible the recording of the calibrated arterial volume pulse, without arterial puncture, from anywhere in the extremities, temporal artery, brain, and intrabulbar tissue. The tracings derived from patients with Raymond's syndrome, atherosclerosis obliterans, or Buerger's disease are included. The sensitivity, accuracy, and potential usefulness in experimental and clinical medicine are discussed.


The investigator interested in pulsatile flow will find this monograph contains a complete survey of arterial flow. The book starts with a historical survey, followed by a discussion of characteristics of blood flow, the disturbances in flow, and methods for measuring pulsatile flow. The appendixes include a method for calculating oscillatory flow from its pressure-gradient and a theoretical analysis of manometer behavior.

Cardiopulmonary Data for Children and Young Adults, Donald E. Cassels and M. Morse. Springfield, Illinois, Charles C Thomas, 1962, 134 pages, $7.00.

The data contained in this book are based on material and work in the author's own laboratory. The data can be readily separated into several main categories: blood, respiration at rest, and respiration during exercise. Less than a third of the 59 tables pertain to abnormal situations, such as congenital heart disease and kyphoscoliosis.
Comparison of Adrenergic Activation by Anticholinesterases and by Hypoxia
Vladislav M. Varagic and Dusan B. Beleslin

Circ Res. 1962;11:916-920
doi: 10.1161/01.RES.11.6.916
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1962 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/11/6/916

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/