Nervous Mechanisms in Ventricular Arrhythmias Induced by Calcium Chloride in Rats

By Manuel René Malinow, M.D., Fernando F. Batlle, M.D., and Bernardo Malamud, M.D.

The ventricular arrhythmias induced in rats by standardized doses of calcium chloride are largely prevented by sympatholytic and autonomic blocking drugs, by encephalic and/or upper spinal destruction and by medullospinal section. They are not prevented by bilateral vagotomy, bilateral adrenalectomy, or by lower spinal destruction.

OPERATION of a nervous mechanism in the genesis of cardiac arrhythmias secondary to the injection of calcium chloride was suggested by the observation that electrical sympathetic stimulation consistently induced ventricular arrhythmias only when calcium chloride was given simultaneously. Insight into the problem was obtained when sympatholytic and other autonomic blocking drugs were reported to prevent ventricular flutter and fibrillation induced by standardized doses of calcium chloride. Further studies on the involved mechanisms seemed to be in order, since the suspected importance of the central nervous system has not been fully determined.

MATERIAL AND METHODS

Seventy to 130 Gm. white albino rats of the Williams strain were anesthetized with Nembutal* intraperitoneally, (6 mg. per 100 Gm. of a 0.6 per cent solution). Only the main features of the method for producing ventricular arrhythmias in the rat will be mentioned here. Using a clean but not sterile technic, the femoral vein was isolated at the inguinal region in order to check the accuracy of the injection. A standard dose of 0.2 cc. per 100 Gm. of a 10 per cent CaCl₂ solution was used in all animals. During the injection and the following two minutes, the cardiac mechanism was continuously studied through a cardioscope connected to the animal with two percutaneous clamp electrodes. An electrocardiograph recorded occasional tracings when so desired.

Results were graded as follows: (a) isolated ventricular premature systoles; (b) frequent ventricular premature systoles, but not runs or bigeminal rhythm; (c) short runs of ventricular flutter or fibrillation, and (d) long runs of ventricular flutter or fibrillation. In the final interpretation, results a and b were considered as negative, c and d as positive.

Other methods used in this study are described in the text. The control group of animals was taken from a previous experimental series.

RESULTS

Effects of CaCl₂ Control Animals

When 0.2 cc. per 100 Gm. of a 10 per cent CaCl₂ solution was injected into each of 20 rats, a positive response, that is, short or prolonged runs of ventricular flutter or fibrillation, was found in 95 per cent of the cases (fig. 1). Immediately after the injection, sinus tachycardia and increased voltage of the QRS appeared. Fifteen to 30 seconds afterwards, ventricular rate increased suddenly (up to 1200 per minute or above). The nature of this arrhythmia is best shown in auricular electrograms, in which the independence of auricular and ventricular waves is demonstrated. The heart action may be regular or irregular, and it is difficult to decide at these high rates.
whether flutter or fibrillation is present. In this paper, therefore, both of these mechanisms will indiscriminately be called ventricular arrhythmias. Other effects of CaCl₂ on the electrocardiogram, such as A-V or I-V block, and S-T segment changes, will not be discussed in the present paper.

Influence of the Autonomic Nervous System

In order to demonstrate the influence of the autonomic nervous system in these ventricular arrhythmias, several procedures were employed:

(a) Ganglion Blocking Drugs. Several drugs reported to block sympathetic and parasympathetic pathways at the autonomic ganglia were used. Five mg. per 100 Gm. of tetraethylammonium chloride were injected into the femoral vein of 10 rats, three minutes before the injection of CaCl₂. In none of these animals were positive results produced by the standard injection of CaCl₂. The same was true in 10 rats that received 2.5 mg. per 100 Gm. of hexamethonium bromide three minutes before the injection of CaCl₂.

Since this protection has been attributed to the nonspecific effect of hypotension, experiments were performed to determine whether the results could be explained through blood pressure changes, per se.

In 10 rats, 2 to 4 cc. of blood were withdrawn from the previously visualized abdominal aorta through a midline incision. This massive bleeding induced a shock-like state in all animals as evidenced by pallor of the skin, collapsed femoral veins, and difficult breathing. In nine of these animals, subsequent injection of CaCl₂ induced positive results.

In order to quantitate the results, the same procedure was repeated in six additional animals, and the mean blood pressure was measured in the abdominal aorta with a mercury manometer connected to the artery through a 0.8 mm. needle and heparinized physiologic saline filled tubes. To avoid bleeding of the animal into the system, the manometer was previously set at 90 mm. Hg. The average control mean blood pressure was 73 mm. Hg (range, 60 to 90 mm. Hg). Bleeding reduced the average mean blood pressure to 22 mm. Hg (range, 20 to 30 mm. Hg). When the standard dose of CaCl₂ was given to these six hypotensive animals, a positive result was found in five. If both groups are added, 87.5 per cent of the rats showed a positive reaction, a nonsignificant difference with the control group.

(b) Vagotomy. As the ganglion blocking drugs influence sympathetic and parasympathetic pathways, a further delimitation of the involved mechanism was attempted by vagotomy. In 10 rats both vagi were sectioned in the neck. When CaCl₂ was injected two minutes after sectioning, ventricular arrhythmias appeared in all animals.

(c) Adrenalectomy. To rule out the influence of adrenal hormones, 10 rats were subjected to bilateral retroperitoneal adrenalectomy. In nine animals ventricular arrhythmias were in-
duced when CaCl₂ was injected two minutes later.

(d) Sympatholytic Drugs. Sympatholytic drugs might further delineate the pathways involved. Three cc. of Hydergine,* a sympatholytic agent, were given intravenously to 10 rats. The injection of CaCl₂, three minutes later induced ventricular arrhythmias in only one animal.

Influence of the Central Nervous System

The importance of the central nervous system in the production of these arrhythmias was evidenced with experiments destroying localized regions of the brain stem. In order to overcome apnea, the trachea was dissected and canalized with a 1.5 mm. needle and intermittent positive pressure respiration was given. A wide median posterior incision visualized the spine and the skull. Sections were made at fixed bony landmarks: for example, through the first cervical vertebra, easily identified immediately below the skull, and through the first dorsal vertebra, easily identified in the rat by the presence of a large cartilaginous process. Histologic sections showed that the first procedure severed the medulla from the spine, and that those performed below the first dorsal vertebra allowed the destruction of dorsolumbar spinal tissue.

(a) Medullospinal Section. In 10 rats, a specially designed cutting hook was introduced above the first cervical vertebra and the medulla was severed from the spinal cord. Arrhythmias followed the injection of CaCl₂ in only three rats. As it was felt that hemorrhage secondary to operative procedures might have changed the results, in five other rats the section was performed after the transfusion of 4 cc. of homologous freshly heparinized blood (obtained from other rats through the abdominal aorta). Results were positive in only one rat.

(b) Encephalic Destruction. In 15 rats, sharp scissors were introduced into the skull through the upper cervical vertebra, and the nervous tissue was destroyed by divulsion. Four rats showed ventricular arrhythmias after the injection of CaCl₂.

(c) Upper Spinal Destruction. In 15 animals the osseous dorsal aspect of the spine was lifted and the underlying nervous tissue destroyed with scissors. Positive results were obtained in only four rats after the injection of CaCl₂.

(d) Encephalic and Upper Spinal Destruction. In 19 rats the two destructive procedures were both performed. When CaCl₂ was injected, in only two instances did ventricular arrhythmias appear. It was observed that in these animals the veins were markedly collapsed. It was also felt that operative procedures might modify circulating blood volume

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* Laboratories Sandoz generously provided us with the Hydergine used in these experiments. Each cubic centimeter of Hydergine contains 0.3 mg. of dehydrogenated alkaloids of ergot.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of Animals</th>
<th>Positive Results</th>
<th>Per cent Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (standardized dose)</td>
<td>20</td>
<td>19</td>
<td>95</td>
</tr>
<tr>
<td>Tetraethylammonium chloride (5 mg./100 Gm.)</td>
<td>10</td>
<td>0</td>
<td>0*</td>
</tr>
<tr>
<td>Hexamethonium bromide (2.5 mg./100 Gm.)</td>
<td>10</td>
<td>0</td>
<td>0*</td>
</tr>
<tr>
<td>Bilateral vagotomy</td>
<td>10</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td>Bilateral adrenalectomy</td>
<td>10</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td>Hydergine (0.9 mg./rat)</td>
<td>10</td>
<td>1</td>
<td>10*</td>
</tr>
<tr>
<td>Medullo-spinal section</td>
<td>5</td>
<td>3</td>
<td>30*</td>
</tr>
<tr>
<td>Medullo-spinal section plus transfusion</td>
<td>15</td>
<td>4</td>
<td>20*</td>
</tr>
<tr>
<td>Intracranial nervous destruction</td>
<td>15</td>
<td>4</td>
<td>20*</td>
</tr>
<tr>
<td>Intracranial and upper spinal nervous destruction</td>
<td>10</td>
<td>2</td>
<td>10*</td>
</tr>
<tr>
<td>Intracranial and upper spinal nervous destruction plus transfusion</td>
<td>5</td>
<td>0</td>
<td>0*</td>
</tr>
<tr>
<td>Lower spinal destruction</td>
<td>10</td>
<td>9</td>
<td>90</td>
</tr>
</tbody>
</table>

* Statistically significant differences from control results.
and thereby increase the effective CaCl_2 levels in the blood; experiments then would not be comparable with our controls. Consequently, hypovolemia was overcorrected with homologous transfusions. In five rats, the above-mentioned procedures were repeated and 4 cc. of freshly heparinized homologous blood was administered two minutes before the CaCl_2 injection. Ventricular arrhythmias appeared in none of these animals.

(e) Lower Spinal Destruction. In order to rule out the nonspecific effects of nervous destruction, the spine was destroyed down to the tail in 10 rats. In nine of these animals, the injection of CaCl_2 induced ventricular fibrillation.

**DISCUSSION**

In the interpretation of our experiments, it is important to point out that negative results after the injection of CaCl_2 were not the effect of hypotension due to drugs or operative procedures, since acute hypotension alone, induced by massive bleeding, was not able to protect rats from the arrhythmic effects of CaCl_2. The above-mentioned experiments show that standard doses of CaCl_2 induce ventricular arrhythmias in the rat only when an intact central nervous system, nonvagal conduction pathways, and adrenergic mediators exist. These results consequently suggest the neurogenic origin of the induced arrhythmias, with probable centers within the brain stem (hypothalamus?); impulse conduction occurs through the upper spinal cord and then to the heart via sympathetic pathways.

The mechanism of CaCl_2 arrhythmias in the rat is apparently similar to those operative in the arrhythmias brought about by stimulation of the central nervous system\textsuperscript{6}--\textsuperscript{9} or by action of chloroform\textsuperscript{12}, \textsuperscript{13} and cyclopropane.\textsuperscript{14}, \textsuperscript{15} These experiments showed a sensitization to epinephrine during cyclopropane anesthesia in dogs, which depended on stimulation of an encephalic center situated above the pons, which sends impulses to the heart through the sympathetic system.\textsuperscript{14} The heart effects are initiated by the stimulation of intra-abdominal receptors by cyclopropane. Impulses are then sent to the hypothalamus through the splanchnic nerves.\textsuperscript{14}, \textsuperscript{15} This explains why the destruction of the central nervous system\textsuperscript{16} or the injection of sympatholytic agents, such as dibenamine,\textsuperscript{17}, \textsuperscript{18} prevents the appearance of arrhythmias.

Ventricular arrhythmias have also been described in rabbits under benzene inhalation, and the importance of the nervous system was demonstrated when the arrhythmias were prevented by stellate gangliectomy. The same protection was evidenced by upper thoracic root excision and by section of lateral spinal tracts down to C-7. No protection was seen when sections were performed below D-3, nor by mesencephalic sections, bilateral vagotomy or lower dorsal sympathectomy.\textsuperscript{19}, \textsuperscript{20} These experiments, performed several weeks after surgery, when no spinal shock was present, also showed no correlation between protection from the arrhythmias and blood pressure changes. They also demonstrated that trigeminal stimulation in the rabbit sent diencephalic impulses down through the spinal lateral tracts, the spinal roots, and the upper dorsal sympathetic nerves, and was thus producing bigeminal ventricular premature systoles.

Neurologic mechanisms in human ventricular arrhythmias are generally accepted;\textsuperscript{21} they have been reported during anxiety\textsuperscript{22} and emotional disturbances,\textsuperscript{23} as well as a result of reflexes.\textsuperscript{24}, \textsuperscript{25} We have found that ventricular arrhythmias may disappear in patients following the injection of autonomic blocking drugs; moreover, patients with organic lesions of the central nervous system may show ventricular arrhythmias\textsuperscript{26} that can be partially abolished with autonomic blocking drugs. It is believed that ventricular arrhythmias experimentally induced in rats are probably similar in origin to some persistent or paroxysmal cardiac disturbances found in man.

The demonstrated neurogenic mechanisms in the rat do not rule out the possibility that CaCl_2 may be able to produce arrhythmias by acting directly upon the myocardium. Such a direct effect has been demonstrated by us, using higher concentrations of CaCl_2\textsuperscript{29} in a rat heart-lung preparation.
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SUMMARY

Ventricular flutter and fibrillation, induced by standard doses of CaCl₂ in rats, are prevented by the injection of tetraethylammonium chloride, hexamethonium bromide or Hydergine, as well as by localized destructions of the central nervous system. Bilateral vagotomy, bilateral adrenalectomy, and lower spinal cord destruction did not prevent these arrhythmias. The neurogenic mechanisms and the involved pathways have been thus demonstrated, but direct arrhythmic effects of higher concentrations of CaCl₂ upon the myocardium were also noted. The pathogenic and pharmacologic similarities with other ectopic rhythms experimentally induced in different species or spontaneously arising in man are discussed. Proper caution should be used in extrapolations from experimental arrhythmias to pathologic conditions in man.

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