EFFECT OF CALCIUM ON THE DURATION OF APNEA INDUCED BY SUCCINYLCHOLINE

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Succinylcholine is a neuromuscular blocking compound with a short duration of action, presumably because of its rapid hydrolysis by cholinesterase (1). The rapidity of its metabolism has made it a particularly suitable muscle relaxant for use as an adjuvant to anesthesia and electroconvulsive therapy. Although the duration of action in the anesthetized human being following administration of a sufficient amount to produce apnea is about two or three minutes (2), many cases of prolonged apnea have occurred after its administration (3). Excessive dosage, hyperventilation with oxygen, trauma, and a low plasma cholinesterase have been proposed as conditions which explain the extended periods of apnea (3–5). Mayrhofer (6) reported that the administration of calcium to a patient who was apneic after receiving succinylcholine was followed by the return of breathing. The patient was subsequently found to have a low blood calcium level. The report on this patient and the lack of an effective pharmacologic antagonist to succinylcholine suggested the present experiments.

Method

The experiments with dogs were performed on mongrel females (7.5 to 15.5 kg.) conditioned to submit to anesthesia (1 per cent thiopental [Pentothal®] sodium) without excitement. After induction the
head was placed in a chamber and the dog breathed oxygen for 30 minutes (denitrogenation period) during which a light anesthesia was maintained. Denitrogenation and a glottic atmosphere of oxygen are conditions which allow an adequate uptake of oxygen during at least 30 minutes of apnea without the use of artificial respiration (7). Respiratory arrest was produced by the intravenous administration of 0.3 mg./kg. of succinylcholine chloride (Anectine®). The duration of apnea in response to this dose of succinylcholine was determined one

TABLE 1

Effect of CaCl₂ Infusion on the Duration of Apnea Following Administration of Succinylcholine Chloride (0.3 mg./kg.) to Dogs

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Control</th>
<th></th>
<th></th>
<th>Experimental</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration of</td>
<td>Total Thiopental Sodium</td>
<td>Respiration Rate/Min.</td>
<td></td>
<td>Duration of</td>
<td></td>
<td>Total Thiopental Sodium</td>
<td>Respiration Rate/Min.</td>
</tr>
<tr>
<td></td>
<td>Apnea—1 Min.*</td>
<td>(mg./kg.)</td>
<td>Before Apnea</td>
<td></td>
<td>Apnea—1 Min.*</td>
<td></td>
<td>(mg./kg.)</td>
<td>Before Apnea</td>
</tr>
<tr>
<td>1</td>
<td>14.3</td>
<td>22.4</td>
<td>12</td>
<td>4.8</td>
<td>.129</td>
<td>21.3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>23.2</td>
<td>12</td>
<td>2.8</td>
<td>.109</td>
<td>20.0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>20.0</td>
<td>10</td>
<td>6.0</td>
<td>.106</td>
<td>20.0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13.0</td>
<td>18.9</td>
<td>18</td>
<td>4.5</td>
<td>.106</td>
<td>19.2</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.8</td>
<td>18.9</td>
<td>12</td>
<td>3.0</td>
<td>.103</td>
<td>19.2</td>
<td>20</td>
<td></td>
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<tr>
<td>4</td>
<td>15.5</td>
<td>33.8</td>
<td>32</td>
<td>4.0</td>
<td>.102</td>
<td>28.8</td>
<td>24</td>
<td></td>
</tr>
<tr>
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<td>28.1</td>
<td>18</td>
<td>5.5</td>
<td>.102</td>
<td>28.8</td>
<td>26</td>
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<tr>
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<td>11.8</td>
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<td>12</td>
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<td>.107</td>
<td>20.0</td>
<td>16</td>
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<tr>
<td>6</td>
<td>12.0</td>
<td>33.3</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>11.5</td>
<td>27.3</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>8.5</td>
<td>24.2</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>12.2±1.68</td>
<td>24.6</td>
<td>17</td>
<td>4.7±1.36</td>
<td></td>
<td>22.2</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as duration of apnea minus one, since calcium infusions were begun one minute after the onset of apnea.

± Standard deviation.

or more times for each dog. Experiments on the same animal were performed at least one week apart. The return of visible rhythmic diaphragmatic movements was considered the termination of apnea. To determine the effect of calcium on the apneic response from succinylcholine an intravenous infusion of a 10 per cent solution of calcium chloride was begun one minute after the onset of apnea and continued until spontaneous breathing resumed. Each animal was given a comparable amount of thiopental sodium during the calcium and control experiments (table 1).
The experiments with rats were performed on Osborn-Mendel males (190 to 210 Gr.). The trachea was cannulated under ether anesthesia and a polyethylene tube was inserted into a jugular vein. Anesthesia was continued using a 0.5 per cent solution of thiopental sodium administered through the jugular cannula. The tracheal cannula was joined to a plastic chamber fitted with a flutter valve through which oxygen flowed. The rats breathed oxygen from this chamber for 30 minutes and thus were largely denitrogenated when respiratory arrest was produced. The chamber was connected to a rubber membrane

### TABLE 2

**Effect of CaCl₂ or Disodium Ethylenediaminetetraacetic Acid (EDTA) Infusion on the Duration of Apnea Following Administration of Succinylcholine Chloride (0.8 mg./kg.) to Rats**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Infusion</th>
<th>No. of Rats</th>
<th>Method of Administration</th>
<th>Duration of Apnea—1 Min.* (mean ± S.D. (S.E.))</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>45</td>
<td>No calcium chloride.</td>
<td>5.7 ± 2.04 (0.308)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ca ++ 0.31 mEq./kg./min.</td>
<td>20</td>
<td>Infusion began 1 minute after onset of apnea and continued during apnea.</td>
<td>3.8 ± 1.04 (0.228)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>3</td>
<td>Ca ++ 0.33 mEq./kg./min.</td>
<td>10</td>
<td>Infusion 7 minutes preceding apnea. No calcium during apnea.</td>
<td>1.3 ± 1.53 (0.579)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>4</td>
<td>Ca ++ 1.80 mEq./kg./min.</td>
<td>5</td>
<td>Infusion for 1 minute preceding apnea. No calcium during apnea.</td>
<td>1.0 ± 0.67 (0.299)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>5</td>
<td>EDTA 0.04 mEq./kg./min.</td>
<td>12</td>
<td>Infusion 7 minutes prior to onset of apnea and continued during apnea.</td>
<td>9.8 ± 2.80 (0.810)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

* Expressed as duration of apnea minus one to make all groups directly comparable to group 2 in which the calcium infusion was begun one minute after the onset of apnea.
† Six of the ten animals did not develop apnea although respiratory volume was reduced.
± S.D. = Standard Deviation. S.E. = Standard Error.

which supported an ink writing pen that recorded the inspiration of as little as 0.02 ml. of oxygen. Intra-arterial blood pressure obtained from the carotid artery was recorded by means of an ink writing lever activated by a double membrane manometer. Respiratory arrest was induced by the administration of 0.8 mg./kg. of succinylcholine chloride through the jugular cannula. Calcium chloride or disodium ethylenediaminetetraacetic acid was administered intravenously as indicated in table 2.

The procedures used in the present experiments differ in several respects from other methods used to determine antagonism against a neuromuscular blocking compound. No artificial respiration is needed
since an adequate oxygen uptake continues during apnea under the present experimental conditions. The procedure is carried on during anesthesia and in the absence of extraneous electrical stimuli, the normal phrenic and intercostal nerve impulses supplying the stimuli for the contractions of muscles affected by the blocking compound. The measured response is therefore related to respiratory rather than limb or neck musculature. Moreover, the duration of apnea would be influenced by reflex or central actions of a compound if such were present. The procedure used in the present study thus approximates somewhat the conditions of anesthesia in which the neuromuscular compounds are used.

The respiratory principle upon which the above experiments are based (diffusion respiration) has previously been found to occur in dogs and rabbits (7, 8), human beings (9, 10), and in the present experiments in rats. A denitrogenated animal with oxygen constantly available at the glottis continues to take in oxygen after respiratory movements stop by means of a physiological mechanism, referred to as the "hemoglobin-oxygen pump" (7). The function of this mechanism is dependent upon an adequate pulmonary circulation and the chemical affinity of reduced hemoglobin for oxygen. As long as blood flows through the pulmonary capillaries a constant stream of reduced hemoglobin is presented to the capillary membranes for gas exchange. The oxygenation of the reduced hemoglobin removes oxygen from the alveoli. The gaseous contents of the respiratory passages and the atmosphere at the glottis moves inward to replace the oxygen assimilated from the alveoli. In contrast no active physiological process exists to move endogenously formed carbon dioxide outward during apnea. Therefore, carbon dioxide accumulates, and when apnea is prolonged beyond thirty minutes, its retention in alveoli and blood may interfere significantly with oxygen uptake. In the present study the duration of apnea was always less than twenty minutes and oxygenation remained good or even abnormally high throughout the experiments.

The neuromuscular observations were made on a rat sciatic-gastrocnemius preparation with the animal under thiopental anesthesia.

RESULTS

The effect of the administration of calcium on the duration of apnea induced by succinylcholine in dogs is presented in table 1. The infusion of calcium after the onset of the neuromuscular block decreased the time that each dog remained in apnea. A comparison of the duration of apnea in the control experiments with the duration when calcium was given shows that no overlap occurs between the two groups. The data indicate that no significant difference in the depth of anesthesia or the respiratory rate occurred between the control and the experimental groups. After the resumption of breathing the re-
spiratory movements were less regular in the dogs that received calcium. However, the irregularities of the respiratory pattern did not decrease appreciably the time from the beginning of rhythmic breathing to return of an adequate respiratory exchange. Cardiac arrhythmias and bradycardia occurred in the dogs during the infusion of calcium. One animal died after four minutes of the calcium chloride infusion, and another died from aspiration of vomitus following the return of spontaneous respiration. The administration of calcium in this manner is thus accompanied by considerable risk. Vomiting occurred frequently during recovery from anesthesia when calcium was given. When anesthesia was extended for a few minutes after breathing resumed no vomiting occurred.

The effects of the infusion of calcium chloride on the duration of apnea induced by succinylcholine in rats is presented in table 2. The data show that the administration of calcium after respiratory arrest occurred significantly reduced the duration of apnea (group 2). A more pronounced effect was observed when the calcium was administered before the induction of apnea (groups 3 and 4). Although the period of apnea was reduced, the animals in group 2 failed to return to an adequate respiratory exchange as rapidly as those in the control group. When the animals (groups 3 and 4) received calcium prior to the succinylcholine and no calcium during the apneic period adequate respiratory exchange returned after breathing resumed as promptly as in the control series. Figure 1 shows the respiratory response to two identical succinylcholine injections with and without the effect of calcium. An infusion of CaCl₂ before succinylcholine administration (group 3) increased respiratory volume as much as 10 per cent in some rats. Larger increases were seen when more calcium was infused (group 4). Systemic blood pressure always increased after calcium infusion in both breathing and apneic rats. The administra-
tion of glucose in a solution osmotically equal to the calcium solutions used in groups 2 and 3, and given at the same rate, prolonged apnea (average of 7 rats), but the duration was not significantly different from the controls (P = .001). No appreciable differences occurred in the amount of thiopental sodium used in each group.

Disodium ethylenediaminetetraacetic acid (EDTA) chelates calcium preferentially to other divalent ions at a physiological pH. Popovici et al. (11) have shown that a slow infusion of EDTA effectively lowers the blood level of calcium. When EDTA was infused for several minutes before and during apnea the duration of apnea was significantly increased (group 5). In contrast to calcium, the EDTA infusion lowered blood pressure in most animals. A remarkably rapid return of arterial blood pressure to preinfusion levels occurred when the infusion was stopped.

DISCUSSION

The present study shows that in dogs and rats an infusion of calcium chloride has a pronounced antagonism to the respiratory depressant effects of succinylcholine. When the calcium is infused prior to the administration of succinylcholine the antagonism is more pronounced.

The experiments reported here offer no information as to the antagonistic mechanisms, although several facts point to a peripheral rather than a central effect. Only small changes in the respiratory pattern accompanied the administration of calcium to animals that received no succinylcholine. Furthermore, an increase in calcium is known to depress rather than enhance some of the central vasomotor reflexes (12). That the antagonistic effect of calcium is more pronounced when tested in the sciatic-gastrocnemius preparation would also suggest that a peripheral action might be predominate (fig. 2).

An increase in the concentration of calcium has multiple effects on muscle and the neuromuscular junction. The threshold of stimulation of the muscle membrane is raised (13). In a curarized muscle the motor end-plate potential is enlarged, presumably by liberation of more acetylcholine from the nerve endings (14, 15). However, if increased release of acetylcholine was the mechanism at work in the experiments reported here one would expect that the block by succinylcholine would be enhanced rather than antagonized since neostigmine, a cholinesterase inhibitor which slows the destruction of acetylcholine at the neuromuscular junction, prolongs the action of succinylcholine.

The amount of calcium infused, although it produced a slight increase in respiratory volume, did not affect the tension of the supramaximal twitch before the onset of the neuromuscular block. However, muscle contractions during inspiration do not resemble muscle twitches but are gradually developing tetanic responses arising from
increased repetitive activity and recruitment of motor units during the progress of inspiration.

An increase in the concentration of the calcium ion has long been known to increase cholinesterase activity in vitro (16). This occurs mostly when the concentration of calcium is below the physiological level. When the concentration of calcium is raised above the physiological level only a small increase in cholinesterase activity occurs. It seems unlikely that this effect of calcium is important in the present experiments since the injection of cholinesterase does not effectively antagonize the action of succinylcholine.

Woods et al. (17) and others (18, 19) have reported that the administration of calcium releases epinephrine from the adrenal medulla. An increase in systemic blood pressure always occurred in the present experiments when calcium was infused. This response could have been, at least in part, due to epinephrine release. Under certain con-

![Diagram](image_url)

**Fig. 2.** The effect of calcium chloride on the neuromuscular action of succinylcholine chloride in the rat. (A) The effect of the administration (i.v.) of 0.25 mg./kg. of succinylcholine chloride on the supramaximal twitch and (B) the effect of the same dose during a 6 minute calcium chloride infusion (i.v. 18 mg./kg./min.) which was started 5 minutes prior to the second injection of succinylcholine. (Gastrocnemius muscle stimulated supramaximally through the nerve with a duration of 0.5 milliseconds.) Records A and B from the same animal.

ditions epinephrine is known to have an anticholinergic action and has been reported to antagonize succinylcholine (20). Brown et al. (21) have found the anticholinergic action of epinephrine to be referable to its effect upon the muscle fibers rather than the motor end-plates. Such an effect would be expected to increase the tension of the supramaxial twitch.

The calcium ion has been shown to antagonize the action of a number of substances, including crude curare, which produce a neuromuscular block (22). The mechanism of this antagonism has not been explained. It has been postulated that both succinylcholine and decamethonium may produce a neuromuscular block by persistent depolarization of the motor end-plate and that α-tubocurarine, which apparently does not depolarize the end-plate, blocks transmission at the neuromuscular junction by preventing the depolarizing effect of acetylcholine. That succinylcholine and decamethonium (Synecurine®)
cause a neuromuscular block by persistent depolarization has been questioned by Thesleff (23) who found that a neuromuscular block existed after repolarization of the end-plate and by Jenden (24) who showed that, except for the early phase, a block by decamethonium resembles one by \(d\)-tubocurarine. This being the case perhaps the antagonism to succinylcholine seen in the present experiments is similar to the antagonism calcium exerts against \(d\)-tubocurarine.

**SUMMARY**

A method was developed to determine the duration of activity of neuromuscular blocking compounds under various experimental conditions. The state of clinical anesthesia is more closely approximated by this than by previously used methods since no extraneous electrical stimuli or artificial respiration is necessary. This method was used to observe the effects of the administration of calcium or ethylenediaminetetraäetic acid (EDTA) on the duration of apnea induced by succinylcholine.

When calcium chloride is infused into dogs during apnea induced by succinylcholine, the duration of apnea is markedly decreased. An infusion of calcium chloride into rats given either before or after an injection of succinylcholine reduces the duration of apnea. When disodium ethylenediaminetetraäetic acid, a chelating compound that lowers the calcium level of the blood, is infused into rats, the duration of apnea is increased.

**REFERENCES**


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