THE INFLUENCE OF CARBON DIOXIDE ON THE NEUROMUSCULAR BLOCKING ACTIVITY OF RELAXANT DRUGS IN THE CAT

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SHORTLY after the introduction of relaxants into routine anaesthetic practice Brennan (1952) observed that their action could be prolonged if the patients were over-ventilated. Clinical experience suggested that the converse might also be true, namely that when carbon dioxide was allowed to accumulate relaxation might be more difficult to obtain. To test the validity of this impression the experiments to be described were carried out.

METHOD

The sciatic nerve tibialis anterior muscle preparation in the intact cat (Brown, 1938) was used to assess the response of the muscle to known doses of relaxant in the presence of varying concentrations of carbon dioxide.

Healthy but otherwise unselected cats were used. After induction with ethyl chloride and ether, the right external jugular vein was cannulated and chloralose (80 mg/kg) given intravenously to maintain anaesthesia. Thereafter a tracheal cannula was inserted and artificial respiration established by means of a positive pressure respirator. This technique was employed to avoid the risk of hypoxia during the action of the relaxant and to facilitate the administration of carbon dioxide.

The sciatic nerve was exposed through a longitudinal incision on the posterior aspect of the thigh. It was crushed as far proximally as possible and ligated; its medial division was treated likewise. The tendon of the tibialis anterior muscle was attached to a flat steel spring myograph recording on a smoked drum. The stimulus was a square wave pulse of 0.5 m.sec duration applied to the sciatic nerve through shielded platinum electrodes at 10-sec intervals, strength 1 to 3 V.

It was intended to administer a given dose of relaxant and to compare the effect produced when the same dose was given during exposure to varying concentrations of carbon dioxide. Before this could be done it was necessary to know the effect of carbon dioxide on muscle contraction without the intervention of neuromuscular blocking agents. It was further required that the dose given and the effect produced could be measured accurately. For this purpose a divided dose technique suggested by Paton (personal communication) was adopted. In each instance a preliminary trial dose of relaxant was given to assess the approximate amount of drug required. The maximum depression of twitch without actual abolition was the objective.

The effect of three different concentrations of carbon dioxide on the action of four separate drugs was assessed. The concentrations of gas employed were 5, 10 and 20 per cent and the drugs used were suxamethonium, decamethonium, gallamine and tubocurarine.

A control series of injections of relaxants was given intravenously while the animals were ventilated with air. In a few instances oxygen was substituted for air to exclude any possible effect of the oxygen in the carbon dioxide/oxygen mixture. When the muscle twitch appeared to have recovered the appropriate mixture of carbon dioxide and oxygen was administered. The duration of exposure to carbon dioxide varied between 20 and 45 mins before the second series of injections was given, after which the carbon dioxide was discontinued. After recovery from the effects of the gas a third series of injections was given. The time interval between each series was the same and the dosage and number of injections were unaltered.

In three cats subtotal neuromuscular block was obtained by means of a continuous infusion of

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suxamethonium chloride. After establishing a satisfactory control period carbon dioxide was given; a different concentration to each cat.

In one group of seven cats the plasma potassium levels were estimated using the Eel flame photometer. In a second group of nine cats blood pH was measured continuously. For this purpose an artificial arteriovenous fistula was created by inserting a small glass chamber (capacity 3 ml) between the femoral artery and a superficial vein. This chamber contained both the measuring and reference electrodes which were connected to a Pye universal pH meter.

RESULTS

In the majority of cases the height of the muscle twitch was diminished when carbon dioxide was inhaled. In no instance was the twitch increased. Figure 1 illustrates the depression of muscle twitch obtained with different concentrations of carbon dioxide inhaled for varying periods.

Suxamethonium Chloride.

Initial injections of suxamethonium chloride increased the height of the individual twitches obtained by supramaximal shocks applied to the nerve. Further injections depressed the twitch and these injections were repeated at minute intervals until approximately 90 per cent block had been achieved. Recovery of the twitch after the last injection was rapid and frequently rose, temporarily, above the control level. Despite this apparent recovery a delay of 30 minutes at least was required between each series of injections to avoid cumulative effects.

Invariably the effect of the administration of carbon dioxide was to lessen the block. The temporary nature of the resistance to suxamethonium produced by carbon dioxide is demonstrated by the return of nearly complete block after the animal has been ventilated with air. The extent, duration and variation of the response is illustrated in figures 2, 3 and 4 and the effects of carbon dioxide on a group of nine cats given suxamethonium are outlined quantitatively in table I.

The use of a continuous infusion of suxamethonium provided a more accurate picture of the course of the resistance to the drug produced by carbon dioxide. Reference to figure 5 shows that

![Image]

FIG. 1
Sciatic nerve tibialis anterior muscle preparation in the intact cat. The illustrations show the depression in the size of the muscle twitch obtained by exposure to different concentrations of carbon dioxide.

| Percentage change in the size of the tibialis anterior muscle twitch obtained after equal doses of suxamethonium before, during and after the administration of carbon dioxide. |
|---|---|---|---|---|---|
| % Carbon dioxide | Dose in mg/kg | Control | CO₂ effect | Recovery |
| 5 | 5 x 10 | 81 | 36 | 75 |
| 10 | 4 x 10 | 89 | 70 | 98 |
| 20 | 3 x 20 | 98 | 91 | 98 |
| 4 x 10 | 97 | 67 | 97 |
| 3 x 10 | 99 | 65 | 99 |
| 3 x 20 | 97 | 88 | 99 |
| 6 x 20 | 50 | 30 | 97 |
| 5 x 10 | 95 | 12 | 94 |
antagonism began to develop as soon as the animal was exposed to 10 per cent carbon dioxide and reached its peak within 20 minutes. Immediately the carbon dioxide was discontinued the resistance to suxamethonium weakened perceptibly but the return of subtotal neuromuscular block was a gradual process and nearly 45 minutes elapsed before the control level was reached. Exposure to 5 and 20 per cent carbon dioxide produced essentially similar results.

Gallamine Triethiodide.
In nine cats given gallamine in divided doses neuromuscular block was obtained in each case without initial stimulation although some increase in the size of the twitch was noted for a brief period after the first injection in many of the experiments. This was not observed after any

**Fig. 2**
Sciatic nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression, after initial stimulation, in muscle twitch achieved by five intravenous injections of suxamethonium chloride (20 µg/kg). The middle tracing shows the resistance of muscle activity to the same dose of suxamethonium during exposure to 20 per cent carbon dioxide. The lower tracing shows the effects of the same dose of suxamethonium after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 45 minutes.

**Fig. 3**
Sciatic nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression, after initial stimulation, in muscle twitch achieved by three intravenous injections of suxamethonium chloride (20 µg/kg). The middle tracing shows the resistance of muscle activity to the same dose of suxamethonium during exposure to 10 per cent carbon dioxide. The lower tracing shows the effects of the same dose of suxamethonium after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 45 minutes.
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10 µG/KG SUXAMETHONIUM CHL.

CONTROL

AFTER 20 MINS. ON 5% CO2

RECOVERY

MINUTES

Fig. 4

Sciatic nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression in muscle twitch achieved by five intravenous injections of suxamethonium chloride (10 µG/kg). The middle tracing shows the resistance of muscle activity to the same dose of suxamethonium during exposure to 5 per cent carbon dioxide. The lower tracing shows the effects of the same dose of suxamethonium after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 45 minutes.

succeeding injection even when recovery from previous doses appeared complete. In each experiment the inhalation of carbon dioxide increased resistance to neuromuscular block with gallamine. The extent of this resistance is illustrated in figures 6, 7 and 8 and table II gives the percentage change in the size of the twitch obtained after equal doses of gallamine before, during and after the administration of carbon dioxide.

<table>
<thead>
<tr>
<th>% Carbon dioxide</th>
<th>Dose in mg/kg</th>
<th>Percentage depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2 x 0.5</td>
<td>64 19 51</td>
</tr>
<tr>
<td>10</td>
<td>2 x 0.5</td>
<td>88 37 83</td>
</tr>
<tr>
<td>20</td>
<td>1 x 0.5</td>
<td>91 20 76</td>
</tr>
</tbody>
</table>

Decamethonium.

The development of neuromuscular block with decamethonium closely followed the pattern described for block with suxamethonium. The initial stimulation, however, was more marked, as shown in figures 9, 10 and 11 and both muscle fasciculation and fibrillation were more prominent. As before, exposure to carbon dioxide antagonized the effect of the relaxant and the extent of this resistance in six cats is set out in table III.

<table>
<thead>
<tr>
<th>% Carbon dioxide</th>
<th>Dose in µG/kg</th>
<th>Percentage depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3 x 5</td>
<td>100 83 91</td>
</tr>
<tr>
<td>10</td>
<td>2 x 10</td>
<td>94 40 81</td>
</tr>
<tr>
<td>20</td>
<td>2 x 5</td>
<td>100 0 86</td>
</tr>
</tbody>
</table>
CONTINUOUS INFUSION OF SUXAMETHONIUM CHLORIDE

20 µg/kg

20 µg/minute

10% Carbon Dioxide

INFUSION ON

INFUSION OFF

MINUTES

Fig. 5

Sciatic nerve tibialis anterior muscle preparation in the intact cat. The neuromuscular block has been achieved by the continuous infusion of suxamethonium chloride. The animal was then ventilated with 10 per cent carbon dioxide and the antagonism to neuromuscular block which developed is shown, as is also the return of neuromuscular block when the carbon dioxide was discontinued.

Tubocurarine Chloride.

Tubocurarine was given in divided doses to a group of six cats. In these experiments exposure to carbon dioxide enhanced the neuromuscular blocking properties of the drug as shown in figures 12, 13 and 14. The increase in neuromuscular block obtained is shown quantitatively in table IV.

Plasma Potassium.

No consistent trend was shown in the plasma potassium levels during exposure to carbon dioxide, but during recovery from the effects of the gas it was observed that the level rose in each instance. The smallest increase was 0.4 m.equiv/l. and the largest 1.8 m.equiv/l. The average in seven cats was 0.97 m.equiv/l.

TABLE IV

Percentage change in the size of the tibialis anterior muscle twitch obtained after equal doses of tubocurarine, before during and after the administration of carbon dioxide.

<table>
<thead>
<tr>
<th>Dose in µg/kg</th>
<th>Percentage depression</th>
<th>(Control</th>
<th>CO₂ effect</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 2 x 100</td>
<td>53</td>
<td>94</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>2 x 100</td>
<td>62</td>
<td>90</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>10 2 x 200</td>
<td>43</td>
<td>81</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>2 x 200</td>
<td>39</td>
<td>42</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>20 3 x 100</td>
<td>63</td>
<td>87*</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>1 x 100</td>
<td>91</td>
<td>100</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>1 x 50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Dose: 2 x 100 µg/kg
Sciatric nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the initial stimulation followed by depression achieved by three injections of decamethonium (5 μg/kg). The middle tracing shows the resistance of muscle activity to the same dose of decamethonium during exposure to 20 per cent carbon dioxide. The lower tracing shows the effects of the same dose of decamethonium after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 60 minutes.

Sciatric nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression, after initial stimulation, in muscle twitch achieved by four injections of decamethonium (5 μg/kg). The middle tracing shows the resistance of muscle activity to the same dose of decamethonium during exposure to 10 per cent carbon dioxide. The lower tracing shows the effects of the same dose of decamethonium after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 60 minutes.

Sciatric nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression in muscle twitch obtained by three intravenous injections of decamethonium (5 μg/kg). The middle tracing shows the resistance of muscle activity to the same dose of decamethonium during exposure to 5 per cent carbon dioxide. The lower tracing shows the effects of the same dose of decamethonium after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 60 minutes.
Sciatic nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression in muscle twitch achieved by one intravenous injection of gallamine (0.5 mg/kg), without initial stimulation. The middle tracing shows the resistance of muscle activity to the same dose of gallamine during exposure to 20 per cent carbon dioxide. The lower tracing shows the effects of the same dose of gallamine after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 60 minutes.

Fig. 9

Sciatic nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression, without initial stimulation, in muscle twitch achieved by two intravenous injections of gallamine (0.5 mg/kg). The middle tracing shows the resistance of muscle activity to the same dose of gallamine during exposure to 10 per cent carbon dioxide. The lower tracing shows the effects of the same dose of gallamine after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 60 minutes.

Fig. 10

Sciatic nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression, without initial stimulation, in muscle twitch achieved by two intravenous injections of gallamine (0.5 mg/kg). The middle tracing shows the resistance of muscle activity to the same dose of gallamine during exposure to 5 per cent carbon dioxide. The lower tracing shows the effects of the same dose of gallamine after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 60 minutes.

Fig. 11
TUBOCURARINE 100 µg/Kg PER INJECTION

CONTROL

AFTER 20 MINS. ON 20% CO2

RECOVERY

MINS.

Fig. 12

Sciatic nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression, without initial stimulation, in muscle twitch achieved by three intravenous injections of tubocurarine (100 µg/kg). The middle tracing shows the marked depression achieved by only two injections during exposure to 20 per cent carbon dioxide. The lower tracing shows the effects of the original dose of tubocurarine after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 60 minutes.

TUBOCURARINE 200 µg/Kg PER INJECTION

CONTROL

AFTER 30 MINS. ON 10% CO2

RECOVERY

MINS.

Fig. 13

Sciatic nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression, without initial stimulation, in muscle twitch achieved by two injections of tubocurarine (200 µg/kg). The middle tracing shows the increased depression of muscle activity by the same dose of tubocurarine during exposure to 10 per cent carbon dioxide. The lower tracing shows the effects of the same dose of tubocurarine after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 60 minutes.

TUBOCURARINE 100 µg/Kg PER INJECTION

CONTROL

AFTER 25 MINS. ON 5% CO2

RECOVERY

MINS.

Fig. 14

Sciatic nerve tibialis anterior muscle preparation in the intact cat. The upper tracing shows the depression, without initial stimulation, in muscle twitch achieved by two injections of tubocurarine (100 µg/kg). The middle tracing shows the increased depression of muscle activity by the same dose of tubocurarine during exposure to 5 per cent carbon dioxide. The lower tracing shows the effects of the same dose of tubocurarine after the animal has been allowed to recover from exposure to carbon dioxide. The interval between each series of injections was 60 minutes.
Blood pH.

The blood pH value invariably fell on exposure to carbon dioxide. The range of this fall was 0.2 to 1.0 pH units and the average was 0.5 units. The rate and extent of the fall were directly related to the concentration of carbon dioxide employed; the higher the concentration the greater the effect.

DISCUSSION

The ability of carbon dioxide to modify the response of the tibialis muscle to single nerve stimuli is not difficult to explain. The general narcotic effect of carbon dioxide has been recognized for many years, indeed Hickman employed the gas to produce anaesthesia in animal experiments in about the year 1820, and more recently Waters (1937) used a 30 per cent concentration for anaesthetic purposes in clinical practice. More specifically Waller (1895) found that carbon dioxide interfered with neural transmission and later Lorente de No (1946) showed that when an isolated nerve was exposed to an environment of 5 per cent carbon dioxide its threshold for stimulation was raised. The depression of the muscle twitch, therefore, could reasonably have been anticipated.

The ability to alter the neuromuscular blocking action of different drugs is less easy to explain. There is a possibility that carbon dioxide modifies enzyme activity and it has even been suggested that it acts as a physiological anticholinesterase (Gesell et al., 1944). Normally the brief action of suxamethonium is due to its rapid destruction by the enzyme pseudocholinesterase and only slightly to its excretion unchanged in the urine. The reduced effectiveness of a given dose of suxamethonium in the presence of carbon dioxide could be due to its more rapid destruction by more efficient enzyme action. But the optimum pH for pseudocholinesterase activity is 8.5 (Augustinson, 1948), and in these circumstances potentiation rather than antagonism of the neuromuscular block could be expected if there were no other factors to be considered. Support for the anticholinesterase theory could be derived from the resistance to neuromuscular block with gallamine produced by carbon dioxide. For this theory to be acceptable, however, tubocurarine ought also to be antagonized. The fact that the action of tubocurarine is enhanced in the presence of carbon dioxide would seem to exclude this explanation.

Although only 5 to 15 per cent of suxamethonium is excreted through the kidneys (Norton and de Beer, 1954) the excretion of the other relaxant drugs is mainly renal. For this reason factors which modify renal blood flow have to be considered. The accumulation of carbon dioxide in the blood reduces renal blood flow and consequently relaxant drugs tend to be retained. Their action, theoretically, should be enhanced in these circumstances, but this is only true of tubocurarine. The influence of renal blood flow can therefore probably be disregarded.

The antcurare action of potassium was described by Wilson and Wright (1936), and two years later Catell and Civin (1938) showed that the inhalation of a 10 per cent concentration of carbon dioxide produced a temporary rise in blood potassium in cats. This rise, however, was not sustained and fell again even while the animal continued to breathe the gas. The observation of Catell and Civin was confirmed by Mackay in 1947, who claimed that the rise in serum potassium reached a peak about the fifth minute and gradually returned to normal within 30 minutes; when the carbon dioxide was discontinued there was a further secondary rise in serum potassium. This postinhalation rise was also observed by Brown (1955) in experiments on dogs, but he was unable to show a fall in serum potassium after the initial rise during the administration of carbon dioxide.

In the present series of experiments there was a gradual rise often preceded by a slight fall in serum potassium during the administration of carbon dioxide which continued for some considerable time after the administration of the gas ceased, whereas the resistance to neuromuscular block began to diminish immediately the carbon dioxide was discontinued (fig. 4). It is obvious, therefore, that there is no direct relationship between serum potassium levels and resistance to relaxants, a fact emphasized by the work of Davis et al. (1955). These workers concluded that, despite the known antagonism of potassium to suxamethonium, the inhalation of carbon dioxide by dogs given this drug prolonged the duration of muscle weakness. The failure of carbon dioxide
to reduce the activity of tubocurarine is further evidence that potassium is unlikely to be involved.

As well as potassium, adrenaline is readily released by carbon dioxide and its ability to support neuromuscular activity is well known. But again the enhanced activity of tubocurarine in the presence of carbon dioxide would seem to exclude any adrenaline influence.

Among the many factors that influence biological activity the degree of ionization is recognized to be particularly significant (Albert, 1952). Its importance is due to the fact that the molecules and corresponding ions of many drugs behave differently; some drugs are active in the molecular phase, others show activity only when ionized. These changes in ionization, however, are not confined to drugs; similar changes occur at the receptor sites in response to variations in the environmental pH.

In this series of experiments it has been shown that a change in blood pH follows ventilation with carbon dioxide and that this change is associated with an alteration in the response of the tibialis anterior muscle to relaxant drugs. Three of these drugs, suxamethonium, decamethonium and gallamine have pKₐ values above 13 and are therefore completely ionized within the pH range employed. Tubocurarine, although containing "onium" groups in the same pKₐ range, also contains two phenolic hydroxyl groups which have pKₐ values of 8.1 and 9.1 (Kalow, 1954). Such values are susceptible to changes in blood pH and consequently variations in blood pH will alter their degree of ionization. This may explain why the action of tubocurarine differs from that of the other relaxants. Support for this explanation comes from the observation (Payne, 1958) that the neuromuscular blocking property of dimethyl tubocurarine, which does not contain hydroxyl groups, is antagonized in the presence of carbon dioxide.

Additional support is provided by the fact that the action of mecamylamine is also enhanced by exposure to carbon dioxide (Payne and Rowe, 1957). Mecamylamine with a pKₐ value of 11.3 has a small but appreciable quantity in the unionized state in body fluids and like tubocurarine is susceptible to changes in blood pH (Milne et al., 1957). According to these authors the unionized component diffuses across cell membranes to combine with the intracellular protein and in this way a considerable quantity can be stored in tissues. A change in pH presumably upsets this balance as the inhalation of carbon dioxide leads to a rise in the plasma level of mecamylamine concomitant with a fall in tissue content (Payne and Rowe, 1957).

The combination of mecamylamine with cell proteins draws attention to another factor likely to influence the action of carbon dioxide on neuromuscular blocking agents. The effectiveness of drugs is often modified by the binding power of proteins. It is known that the intensity and duration of action of a given dose is inversely proportional to that fraction bound to protein and that alterations in plasma pH influence the ability of the plasma proteins to take up certain drugs (Goldstein, 1949). When carbon dioxide is inhaled the resulting rise in its tension in the blood raises the hydrogen-ion concentration. Such a reduction in pH may facilitate the combination of the quaternary ammonium salts with protein, thereby limiting their activity.

This would explain the resistance to suxamethonium, decamethonium and gallamine which develops in the presence of carbon dioxide without invalidating the explanation given for the enhanced activity of tubocurarine under similar conditions.

SUMMARY
Four series of experiments have been carried out to study the influence of carbon dioxide on the neuromuscular blocking properties of certain drugs. Under the conditions of the experiments carbon dioxide opposed the action of suxamethonium, decamethonium and gallamine but enhanced the activity of tubocurarine.

Concomitant studies on plasma potassium levels and blood pH values were carried out. No direct relationship could be shown between the plasma potassium levels and the modified activity of the relaxant drugs. There was, however, an invariable decrease of between 0.2 and 1 unit in the pH values which could be related to the change in response to these drugs.

No complete explanation could be given for the modified response to relaxants induced by carbon dioxide, but it is suggested that changes
in the degree of ionization and protein binding could be responsible.

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