INFLUENCE OF APROTININ (TRASYLOL) ON THE ACTION OF SUXAMETHONIUM

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SUMMARY

Aprotinin concentrations such as occur in vivo after intravenous injection of 100,000 KIU, inhibit ChE activity by about 5–16 per cent. The degree of inhibition is greatest between 6 and 20 minutes post-injection and is negligible after 30 minutes. When the ChE baseline activity is normal, 5–16 per cent inhibition does not lead to a prolongation of the action of suxamethonium. A prolongation of the apnoeic period in connection with an administration of aprotinin is only possible if the ChE activity is close to the critical level and is lowered by aprotinin to a range in which the breakdown of suxamethonium is delayed. There is no reason to dispense with aprotinin in operations in which suxamethonium has been given or must be given. Aprotinin could lead to a lowering below the critical threshold only in those patients who have a pathologically depressed ChE activity level and therefore are already predisposed to prolonged apnoea after suxamethonium.

Chasapakis and Dimas (1966) and Marcello and Porati (1967) reported observations on three and seven patients in whom there was a return of apnoea for periods of 7–90 minutes after spontaneous breathing had recovered in the normal way at the end of the anaesthetic. These patients had been treated with aprotinin (Trasylol) near the end of the operation. Marcello and Porati (1967) had not previously encountered this complication when, apart from the use of suxamethonium for intubation, only non-depolarizing relaxants had been used. They proposed that the cause of the recurrence of apnoea was that aprotinin inhibited the activity of cholinesterase (ChE)* by which suxamethonium is hydrolyzed.

Chasapakis and associates (1968) reported an inhibition of ChE activity by aprotinin. They used Caraway's method and stated that the ChE activity in twenty-eight patients was inhibited by about 25 per cent at 30 minutes after injection of 25,000 or 50,000 KIU aprotinin. The present authors have never seen any influence on the duration of apnoea after suxamethonium following injection of aprotinin, in terms of either prolongation or recurrence, and therefore undertook studies to assess the influence of aprotinin on the action of suxamethonium. Doenicke (1968) expressed reservations about the validity of the results published by Chasapakis and associates (1968). The authors doubted whether the injection of aprotinin at the end of anaesthesia had caused the return of apnoea observed by Chasapakis and Dimas (1966) and Marcello and Porati (1967). In an extensive experience they have not observed any marked influence of aprotinin on the spontaneous respiration at whatever stage the drug was injected. Laboratory investigations were therefore undertaken to determine the influence of aprotinin on the activity of ChE which splits suxamethonium (experiments 1–4). Clinical experiments were then undertaken to investigate the interaction between aprotinin and suxamethonium (experiment 5). The methods used are outlined and the variations that were necessary are described.

*Cholinesterase = pseudocholinesterase = serum cholinesterase = non-specific cholinesterase.
EXPERIMENTAL METHODS

Determination of the ChE activity according to Kalow and Lindsay (1955).

The test is carried out with quartz cuvettes (10 mm lumen) at 240 μm in a Beckman spectrophotometer (26°C). Absorbance of the serum is eliminated with the aid of a 0 cuvette (serum 1:200 in solvent).

The enzyme activity is obtained by multiplication of the ΔU values for 3 minutes at 26°C with the factor 1,000. To prevent temperature fluctuations, heatable cuvette stands are used. Reactions in the range between 20° and 25°C can be referred to the reaction at 26°C by the formula:

\[ 0.0283 \left( \frac{t_2 - t_1}{t_2} \right) \Delta U = \Delta U \left( 26°C, t_1 \right) \]

(\(t_2=26°C, t_1=\text{reaction temperature}, v_2=\Delta U \text{ at } 26°C, v_1=\Delta U \text{ at } t_1°C\)).

Reagents.
Solvent of all solutions: 0.0667 mol phosphate buffer pH 7.4 (26°C).

Solutions:
I. Enzyme: Serum or solution of purified concentrated ChE (Behringwerke) diluted 1:100.
II. Inhibitor: Dibucaine hydrochloride 4·10⁻⁵ mol or aprotinin in different concentrations.
III. Substrate: 2·10⁻⁴ mol benzoylcholine chloride.

Test samples.
1. Test without separate inhibitor solution:
(a) completely without inhibitor;
(b) inhibitor in the enzyme solution (serum or ChE solution).
2. Test with separate inhibitor solution:
(a) with dibucaine solution for determination of the dibucaine number (DN);
(b) with aprotinin solution.

1. (a) 1.0 ml solvent;
2.0 ml solution I;
1.0 ml solution III.
(b) as I(a).
2. (a) 2.0 ml solution I;
1.0 ml solution II (dibucaine hydrochloride);
1.0 ml solution III.
(b) 2.0 ml solution I;
1.0 ml solution II (aprotinin);
1.0 ml solution III.

The dibucaine number (DN) derives from the equation:

\[ \text{DN} = 100 \left( 1 - \frac{\Delta U \text{ with inhibited reaction 2(a)}}{\Delta U \text{ with uninhibited reaction 1(a)}} \right) \]

Determination of ChE activity according to Caraway (1956).

For this photometric determination are used acetylcholine bromide in 15 per cent solution as the substrate and 0.001 per cent phenol-red solution in 0.05 molar phosphate buffer as the indicator solution.

For the test, 5 ml buffer-indicator solution, 0.5 ml serum and 0.5 ml substrate solution are mixed. The absorbance is measured immediately and after 30 min at 540 nm, 25°C, in 1 cm layer thickness.

The quotient of the second and first measured values is derived and the activity read in units from a calibration curve (normal 55-125 units). The purpose of forming the quotient is to eliminate errors resulting from the binding of dyestuff to serum proteins. A parallel test without serum gives the value of spontaneous hydrolysis which is deducted from the previous value.

For testing the action of the inhibitor this is added at the start of the test. In this case a somewhat higher concentrated buffer-indicator solution is used so that after addition of the inhibitor the dilution is the same as in the other test.

Determination of ChE activity according to Pilz (1965).

Principle.
Prepare a control (C) and two test (T) samples, both containing substrate, only the test sample containing the biological material.

After incubation, the ester that has not been split enzymatically is converted by reaction with alkaline hydroxylamine to iron-III hydroxamate and the concentration of this measured photometrically at 490 nm in 1 cm layer thickness against the blank value.

Reagents.
(a) Trichloroacetic acid 0.5 molar: 81.7 g trichloroacetic acid dissolved with water to 1,000 ml.
(b) Alkaline hydroxylamine: mixture of equal volumes of 2.5-n NaOH and 1-n NH₄OH HCl. (The solution only keeps for 1 hour and must be freshly prepared for every test.)

Hydroxylamine 1-n: 70 g hydroxylamine hydrochloride dissolved in water to 1,000 ml. (This solution remains stable in the dark for 6 weeks.)

Sodium hydroxide solution 2.5-n: 100 g NaOH dissolved with water to 1,000 ml.
(c) Buffer pH=1.4: dissolve 10.5 g citric acid (monohydrate) and 4.0 g NaOH in a little water in a 500-ml volumetric flask, add 445 ml 1-n hydrochloric acid and fill up with water to the mark.
(d) Iron solution: 337.5 g iron-III ammonium alum dissolved with 25 g potassium nitrate and mild heating on a water bath to 1,000 ml.
(e) Substrate: 150 ml standard buffer and 2 ml stock solution of the substrate are combined and the mixture is well shaken.

Standard buffer: 200 ml borate buffer and 20 ml buffer pH=4.62 diluted with water to 1,000 ml.

Borate buffer: 31.0 g boric acid B(OH)₃ dissolved in a 1,000-ml volumetric flask in approx. 700 ml water, 20 ml 1-n NaOH added, filled up to the mark.

Buffer pH=4.62: 200 ml 1-n acetic acid and 100 ml 1-n NaOH filled up in a 1,000-ml volumetric flask to the mark.

Stock solution of the substrate (according to Pilz): 10 mMol succinylcholine/50 ml H₂O = 4.02 g suxamethonium (Succinyl-Asta) succum/50 ml H₂O yields 2.66·10⁻² molar substrate.

Stock solution of the substrate as used in the reported tests: 80 mg suxamethonium chloride/50 ml H₂O yields 5.9·10⁻² molar substrate.
Incubation in a water bath at 37°C according to Pilz, 24 hours; in the test here reported 1 hour.

<table>
<thead>
<tr>
<th>Test samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
</tr>
<tr>
<td>25 ml sol. (e)</td>
</tr>
<tr>
<td>10 ml sol. (b)</td>
</tr>
<tr>
<td>20 ml sol. (d)</td>
</tr>
<tr>
<td><strong>Incubation</strong></td>
</tr>
<tr>
<td>5 ml sol. (a)</td>
</tr>
</tbody>
</table>

Filled up with water to 100 ml.

**Procedure.**

The replenished samples are well shaken and left standing for 20 minutes. Then filter through a dry, double, fluted filter (Schleicher & Schüll No. 602h) into a dry Erlenmeyer flask, discarding the first portions of the filtrate. Photometry can be carried out immediately at 490 nm with 1 cm layer thickness against the blank value.

**Calculation.**

The quantity of suxamethonium in µequiv split in 100 ml of the aqueous test solution can be calculated by multiplying the difference C extinction value minus the mean of the T extinction value by the factor 102.

**EXPERIMENTAL SERIES**

I. After determination of the initial activity the inhibition of ChE activity was measured in ten different sera and ten different solutions of purified concentrated human ChE (Behringwerke*) with the addition of different concentrations of aprotinin (method of Kalow and Lindsay, test sample 2b). Measurements were carried out immediately after combining the materials and after incubating the dilutions of the serum and ChE solution with aprotinin solution for 10 or 15 minutes.

II (a). In six experiments the initial ChE activity of healthy, fasted and rested subjects was determined. Aprotinin 100,000 KIU was then injected i.v. over 2 minutes, and thereafter the change in ChE activity in vivo was assayed, using fresh blood samples (method of Kalow and Lindsay, test sample 1b).

III. Using the modified method of Pilz the decrease of the suxamethonium concentration was measured after incubation for 1 hour at 37°C with serum or ChE solution. Measurements were made without and with aprotinin in the ratios stated in section II (c).

IV. In order to clarify whether the inhibition of ChE activity was due to aprotinin or the stabilizer, thiomersal, added to aprotinin in the proportion 1:40,000, thiomersal solutions 1:100 to 1:50,000 were used instead of aprotinin and their influence on the activity of ChE determined (test sample 2b).

V. In nineteen patients the duration of apnoea was assessed during operation after injection of suxamethonium alone and after differently timed combined injection of suxamethonium and aprotinin. These comparative measurements served to determine the influence of aprotinin on the action of suxamethonium. When spontaneous respiration had completely returned after suxamethonium, aprotinin was injected, and comparative measurements of the respiratory minute volume were made before and after the injection. The measurements of apnoea were made during intubation anaesthesia, the first at the earliest 20 minutes after intubation. In the meantime the patient was ventilated manually at his own rhythm with the

(b) In four in-vivo/in-vitro experiments on healthy fasted and rested subjects a blood sample was obtained for determination of the initial activity. Aprotinin (25,000 or 100,000 KIU) was then injected; 3 minutes later a larger quantity of blood was withdrawn and the change in ChE activity in the serum on storage in a water bath at 37°C was observed (test sample 1b).

(c) In seven in-vitro experiments, a larger quantity of blood was freshly obtained from healthy fasted and rested subjects, the serum separated, placed into a water bath (37°C), the initial activity determined, aprotinin added and the change in activity measured (test sample 1b).

In order that the addition of aprotinin in vitro corresponded as far as possible to i.v. injection in vivo, aprotinin 0.02 ml from a 10 ml (100,000 KIU) ampoule was added to 6 ml serum (equal to 100,000 KIU to 3,000 ml serum) or 0.01 ml from a 5 ml (25,000 KIU) ampoule was added to 6 ml serum (equal to 25,000 KIU to 3,000 ml serum).

* The purified concentrated human cholinesterase is produced from a pool of different sera. Only about 4 per cent of people of the investigated population have a variant of the cholinesterase. The influence of these 4 per cent can be neglected. This opinion is supported by the dibucaine number (DN) of the purified cholinesterase which is similar to normal cholinesterase.
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RMV which had been assayed as accurately as possible. The onset of apnoea was timed from cessation of the movement of the Dräger volumeter needle, and the termination as the time of return of spontaneous rhythmic movement of the chest or the needle. In the course of prolonged anaesthesia it was possible to repeat these measurements several times. Up to 250 mg suxamethonium and up to 300,000 KIU aprotinin were administered.

In eight anaesthetic experiments intercurrent determinations of ChE activity (after Kalow) were carried out.

RESULTS

As far as the ChE activity is concerned, only the results obtained by the method of Kalow and Lindsay are considered.

I. With the addition of aprotinin solution in $7 \times 10^{-7}$ mol/l. concentration (test sample 2b) no inhibition of ChE activity occurred in any of the sera and ChE solutions. With $7 \times 10^{-4}$ mol/l. aprotinin concentration no ChE activity was demonstrated in the ChE solutions and sera. The concentration which inhibited the initial activity by one-half ($I_{50}$) was for the ChE solutions between $2.8 \times 10^{-8}$ and $1.1 \times 10^{-8}$ mol/l.

The $I_{50}$ for serum fell within the aprotinin concentration range of $9.5 \times 10^{-8}$ to $1.2 \times 10^{-8}$ mol/l.

Only in the serum with the high initial activity of 244 was $1.5 \times 10^{-8}$ molar aprotinin solution needed for 50 per cent inhibition (see figs. 1 and 2 and tables I and II).

The $I_{50}$ values found for serum are in the relatively narrow range of $9.5 \times 10^{-8}$ to $1.2 \times 10^{-8}$ mol/l.

### Table I

**ChE activity in AU-1000 with added aprotinin solution after Kalow 2b without previous incubation.**

<table>
<thead>
<tr>
<th>ChE sol.</th>
<th>0</th>
<th>$7 \times 10^{-7}$</th>
<th>$7 \times 10^{-8}$</th>
<th>$3.5 \times 10^{-8}$</th>
<th>$7 \times 10^{-8}$</th>
<th>$3.5 \times 10^{-9}$</th>
<th>$7 \times 10^{-9}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>96.5</td>
<td>96</td>
<td>70.5</td>
<td>34</td>
<td>3.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>102</td>
<td>102</td>
<td>66</td>
<td>51.5</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>94</td>
<td>91.5</td>
<td>21.5</td>
<td>38</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>37.5</td>
<td>36</td>
<td>52</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>93</td>
<td>93</td>
<td>35</td>
<td>17.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>43.5</td>
<td>43.5</td>
<td>52</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>127.5</td>
<td>128</td>
<td>112</td>
<td>69</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>77.5</td>
<td>77.5</td>
<td>69</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i_L</td>
<td>237</td>
<td>237</td>
<td>123.5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_L</td>
<td>250</td>
<td>235</td>
<td>121</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$i_L$ and $k_L$ not illustrated in fig. 1.

### Table II

**ChE activity in AU-1000 with added aprotinin solution after Kalow 2b without previous incubation.**

<table>
<thead>
<tr>
<th>Serum</th>
<th>DN</th>
<th>0</th>
<th>$7 \times 10^{-7}$</th>
<th>$7 \times 10^{-8}$</th>
<th>$3.5 \times 10^{-8}$</th>
<th>$7 \times 10^{-8}$</th>
<th>$3.5 \times 10^{-9}$</th>
<th>$7 \times 10^{-9}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>85</td>
<td>63.5</td>
<td>60</td>
<td>49.5</td>
<td>39</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>81</td>
<td>105</td>
<td>104.5</td>
<td>80.5</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>89</td>
<td>114</td>
<td>110</td>
<td>91</td>
<td>71</td>
<td>30</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>88</td>
<td>59.5</td>
<td>59.5</td>
<td>56</td>
<td>38.5</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>87</td>
<td>87</td>
<td>81.5</td>
<td>60</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>88</td>
<td>103</td>
<td>96.5</td>
<td>76.5</td>
<td>67</td>
<td>15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>81</td>
<td>96</td>
<td>94.5</td>
<td>80.5</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>89</td>
<td>59.5</td>
<td>58</td>
<td>35.5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>89</td>
<td>103</td>
<td>94</td>
<td>66</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k_g</td>
<td>85</td>
<td>244</td>
<td>245</td>
<td>243</td>
<td>182.5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$k_g$ not illustrated in fig. 2. The activity of the sera b, c, e, f, g and i lie in the normal range. But also the sera a, d and h with their low activity and the serum $k_g$ with its very high activity seem to have a normal ChE, because they have a high dibucaine number (DN) which is not typical for ChE variants.
FIG. 1
Inhibition of ChE activity by aprotinin in ChE solution.

FIG. 2
Inhibition of ChE activity by aprotinin in serum.
aprotinin; the $I_{50}$ values for ChE solution are distributed within the wider range from $2.8 \times 10^{-5}$ to $1.1 \times 10^{-4}$ mol/l. aprotinin. It seems that 50 per cent inhibition of ChE activity in serum requires a somewhat higher aprotinin concentration than 50 per cent inhibition in ChE solution.

Higher ChE activities apparently require higher aprotinin concentrations for 50 per cent inhibition than do lower activities.

If in the Kalow test 2b, the enzyme and inhibitor solution were incubated for 10 or 15 minutes at 37°C before the substrate solution was added, then the $I_{50}$ values for ChE solution were in the lower concentrated part of the $I_{50}$ range for ChE solution not previously incubated. For a previously incubated serum the aprotinin concentration sufficing for 50 per cent inhibition of ChE activity is below the $I_{50}$ range found for serum not previously incubated.

Determining the $I_{50}$ with and without incubation revealed, for equal ChE solutions, that without incubation the $I_{50}$ is at about twice the aprotinin concentration of the $I_{50}$ with incubation (see fig. 3 and table III).

![Graph](https://example.com/graph.png)

**Fig. 3**
ChE activity of ChE solution immediately after the addition of aprotinin ($a+b$).
ChE activity after 15 min incubation with aprotinin at 37°C ($a'+b'$).

**Table III**

<table>
<thead>
<tr>
<th>Initial activity</th>
<th>Aprotinin (mol./l.)</th>
<th>Determination Kalow 2b without incubation</th>
<th>Determination Kalow 2b 15 min incubation at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>$7 \times 10^{-6}$</td>
<td>71.5</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>$7 \times 10^{-5}$</td>
<td>43.5</td>
<td>32.5</td>
</tr>
<tr>
<td></td>
<td>$3.5 \times 10^{-4}$</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$7 \times 10^{-4}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$I_{50} = 8.8 \times 10^{-6}$</td>
<td>$I_{50} = 4.6 \times 10^{-5}$</td>
</tr>
<tr>
<td>101</td>
<td>$7 \times 10^{-6}$</td>
<td>99.5</td>
<td>80.5</td>
</tr>
<tr>
<td></td>
<td>$7 \times 10^{-5}$</td>
<td>61</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>$3.5 \times 10^{-4}$</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>$7 \times 10^{-4}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$I_{50} = 9.7 \times 10^{-3}$</td>
<td>$I_{50} = 4.4 \times 10^{-8}$</td>
</tr>
</tbody>
</table>
Fig. 4
Tests in vivo.

Fig. 5
Administration of aprotinin in vivo; course of ChE activity in vitro.
II(a). In vivo the ChE activity temporarily decreased after an injection of aprotinin (see fig. 4). The lowest point was reached 6–20 min after injection (average 14.4). The decrease amounted to 3–12 units absolute or 4.5–16.5 per cent (average 5.9 units; 8.2 per cent) after 100,000 KIU. The initial activity values had been regained after 20–30 minutes.

II(b). Changes in ChE activity in vitro accompanying aprotinin administration in vivo resembled the results of II(a) (fig. 5). The lowest point was attained about 6–10 min (average 9) after the start of incubation in a water bath. The decrease amounted after 100,000 KIU to 9 and 9.5 units absolute or to 12.5 and 13 per cent (average 9.25 units; 12.75 per cent). After 25,000 KIU the decrease was 4 and 2 units, or 6 and 3 per cent (mean 3 units; 4.5 per cent). In the test series (b), too, the initial activities were restored after 20–30 minutes and then remained approximately constant.

II(c). Also when aprotinin was added to serum in vitro, a transient inhibition of ChE activity occurred (fig. 6). The lowest point was reached between 4 and 20 minutes, return to the initial level of activity taking 20–30 minutes. The decrease after 100,000 KIU was absolutely 4–10 units, or 5.5–13.5 per cent (average 6.8 units; 9.3 per cent). After 25,000 KIU the decrease was 4 and 2 units or 6 and 3 per cent (mean 3 units; 4.5 per cent).

The reason why in test series II(b) the point of lowest activity occurred earliest, at 9 minutes compared with 14.4 minutes for (a) and 10.6 minutes for (c), is probably that in the pre-mixing period of 3 minutes between injection and blood sampling aprotinin is already active. The weaker inhibition of ChE activity by aprotinin in vivo, compared with the in-vivo/in-vitro and the in-vitro tests, may be due to an escape from the serum into the extravascular space. Thus, the actual aprotinin concentration would be lower than under in-vitro conditions. The ChE activities in test series I and II were determined by the method of Kalow and Lindsay and by the method of Caraway.

The results of test series I and II were obtained from measuring the ChE activity only by the method of Kalow and Lindsay. When using the Caraway method in the same test series no regularity in the behaviour of ChE activity could be seen. Figures 7a and 7b show examples.

III. In the modified Pilz test, serum (ChE 80)* without aprotinin split 12 per cent suxamethonium; the same serum with aprotinin 2 per cent.

* ChE 80 means that the ChE activity is 80 units in the Kalow test.
Changes in ChE activity in vivo after injection of 100,000 KIU aprotinin.

The curve K demonstrates the course of activity obtained by the method of Kalow and Lindsay. The curve C₁ demonstrates the course of activity obtained by the method of Caraway from the same test samples as used for K. The curve C₂ demonstrates the course of activity obtained by the method of Caraway from the same test samples which had been kept at 4°C for 2 hours.

Repetition of determination of the course of ChE activity 2 hours later by the Kalow method gave almost exactly the same curve as K.

The consequences of the large standard deviation of the Caraway method (Caraway: ±15.6 per cent, Kalow: ±1.6 per cent) can be seen on inspection of curves C₁ and C₂, where the curve K has the lowest level of activity (20 and 10 min after injection respectively). The curves C₁ and C₂ differ substantially and conclusions drawn from the curve K cannot be drawn from curves C₁ and C₂.

Curve K in fig. 7A is the same as curve 10 in fig. 4. Curve K in fig. 7B is the same as curve 12 in fig. 4.

Inhibition of ChE activity by thiomersal.
On repetition the values were 16 and 3.5 per cent. The ChE solution (ChE 95) split without aprotinin 15 per cent suxamethonium; with aprotinin 4.5 per cent. On repetition the values were 20.5 and 5 per cent. In all tests, ChE without aprotinin split at least three times as much suxamethonium as ChE in the presence of aprotinin (table IV).

IV. No inhibition of ChE activity was observed when thiomersal was diluted beyond the concentration 1:1,000 (fig. 8). The 10-ml aprotinin ampoule contains per 14 mg pure aprotinin and 0.25 mg thiomersal, the ratio accordingly present in the dry substance being 56:1.

The highest concentrated aprotinin solution used (7·10⁻⁴ mol) contained 4.55 g aprotinin dissolved in 1 litre water and 0.08 g thiomersal. This corresponds to a 1:12,500 thiomersal solution in which inhibition of ChE activity does not occur. Therefore no influence of thiomersal on the experimental results is possible.

V. During nineteen anaesthetics, suxamethonium (approx. 0.7 mg/kg) was administered at different times after the injection of 100,000 KIU. The duration of the ensuing apnoea was measured and compared with the value obtained at least 20 minutes before, after the administration of suxamethonium alone in the same dose. The largest changes in the duration of apnoea were +33 per cent and -34 per cent. The arithmetic mean of all nineteen deviations was -2 per cent (table V). After the end of suxamethonium apnoea, aprotinin 100,000 KIU was given at different times and the spontaneous respiration observed. In only one case this decreased from 10 to 8 l./min. In all others it did not change, or even increased.

**TABLE IV**

<table>
<thead>
<tr>
<th>Test</th>
<th>Value (U-1000)</th>
<th>After 1 hour incubation with serum (ChE 80)</th>
<th>ChE = sol. (ChE 95)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>364</td>
<td>319.5</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>387</td>
<td>379</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>268</td>
<td>224.5</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>313</td>
<td>302</td>
<td>96.5</td>
</tr>
</tbody>
</table>

Mean of tests

| 1 and 2 (%) | 100 | Without aprotinin | 86  | 82.3 |
|             | 100 | With aprotinin    | 97.3| 95.5 |

Initial suxamethonium concentration 5.9·10⁻⁶ mol; aprotinin 100,000 KIU/3,000 ml serum or 3,000 ml ChE solution. C = control; T = test.

**TABLE V**

<table>
<thead>
<tr>
<th>Time between aprotinin and suxamethonium injection (min)</th>
<th>Change in duration of apnoea (%)</th>
<th>Mean change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to ½</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>-12</td>
<td>0**</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>-6</td>
<td>33*</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>13.5</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>-9.5</td>
</tr>
<tr>
<td></td>
<td>-23</td>
<td></td>
</tr>
<tr>
<td>6</td>
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<tr>
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<td>-16</td>
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</tr>
<tr>
<td>7</td>
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<td>0</td>
</tr>
<tr>
<td>8</td>
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<td>-5</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>-4.1</td>
</tr>
<tr>
<td></td>
<td>-17</td>
<td>0</td>
</tr>
</tbody>
</table>

* After aprotinin 25,000 KIU and suxamethonium 20 mg (4-year-old child).

** After aprotinin 200,000 KIU and suxamethonium approx. 0.7 mg/kg.

Otherwise aprotinin 100,000 KIU and suxamethonium approx. 0.7 mg/kg.

Mean of all changes: -2%.
When several comparative measurements were carried out during anaesthesia, suxamethonium and aprotinin were repeatedly administered. Cumulation of the two drugs must be considered. It appears justifiable to neglect such a possibility in the evaluation, since in the case of cumulation, any prolongation would be more marked.

The ChE determinations carried out during eight anaesthetics showed that the ChE activity was distinctly decreased during the anaesthesia compared with the initial values. This inhibition of activity did not, however, influence the duration of apnoea after suxamethonium.

**DISCUSSION**

ChE activities were measured by the method of Kalow and Lindsay and that of Caraway. The results obtained by the Caraway method could not be reproduced under standardized conditions. Likewise, the patterns that were found differed greatly from each other and failed to produce a characteristic picture. This is firstly due to the large standard deviation of the Caraway method, which according to Caraway can reach ±15.6 per cent. In our own studies it reached 10 per cent. In contrast, the results obtained by the Kalow method were almost identical in several tests; typical activity curves resulted. With the Kalow method the standard deviation reaches ±1.5-2 per cent (Kalow, personal communication). In our own studies it reached 1.6 per cent. In addition, with the Caraway method, a greater methodological error must be taken into account, since acetylcholine esterase also splits the substrate acetylcholine bromide. During the mechanical separation of the serum, AChE can escape from the erythrocytic membrane into the serum. Thus, the assessed ChE activity includes AChE activity. This source of error is eliminated in the method of Kalow and Lindsay since benzoylcholine is practically not split by AChE.

Factors of uncertainty also arise from the 30-minute period of determination with the Caraway method. It is not so easily possible to allocate a measured value to a definite point of time as it is with the method of Kalow and Lindsay.

For these reasons, only the results obtained by the Kalow tests were evaluated. Those of the Caraway tests are not reported in detail. These shortcomings of the Caraway method cast doubt on the results reported by Chasapakis and associates (1968). These authors stated that an average inhibition of ChE activity of about 25 per cent occurred 30 minutes after administration of aprotinin 25,000 or 50,000 KIU.

Although higher doses were used in the present experiments (generally 100,000 KIU) the highest degree of inhibition of activity was 16.5 per cent. Inhibition began a few minutes after the application of aprotinin and was no longer detectable 30 minutes later, i.e. at the point of time when Chasapakis measured 25 per cent inhibition.

The problems connected with the method of Pilz have been discussed by Doenicke, Schmidinger and Krumey (1968).

In test series I, the Kalow test 2b showed practically no inhibition of ChE activity with an addition of 7·10⁻⁷ aprotinin solution.

In test series II, with the addition of aprotinin 100,000 KIU to 3,000 ml serum (in vivo or in vitro) a 7·10⁻⁷ mol aprotinin solution occurs in serum as the solvent.

In the Kalow test 2b (test series I) the aprotinin solution of the concentration 7·10⁻⁷ mol/l. is diluted to 4·7·10⁻⁷ mol/l., because 1 ml of aprotinin solution is mixed with 3 ml of other solutions (see methods).

If there is an aprotinin concentration of 7·10⁻⁷ mol/l. in serum and this serum with aprotinin is treated according to the prescription of Kalow and Lindsay (1955) test 1b as in test series II, the aprotinin concentration is diluted to 1/200·7·10⁻⁷ mol/l. in the final mixture of test reagents. Because with the serum dilution the aprotinin concentration decreases to 1/100·7·10⁻⁷, the diluted serum (2 ml) is mixed with 2 ml of other solutions. The aprotinin concentration finally is then 1·1/100·7·10⁻⁷ mol/l.

In the test samples of the test series II there is only 1/50 of the aprotinin concentration of the test samples in the test series I when an aprotinin solution of 7·10⁻⁷ mol/l. is used. Therefore it is surprising that an inhibition of the ChE activity is found in test series II, whereas in test series I with an aprotinin solution of 7·10⁻⁷ mol/l. no change from full ChE activity can be seen. Nor could ChE activity be decreased by an aprotinin solution of 1/50·7·10⁻⁷=1·4·10⁻⁸ mol/l.

The inhibition of ChE activity regularly found in II tests at 7·10⁻⁷ mol aprotinin concentration
INFLUENCE OF APROTININ ON THE ACTION OF SUXAMETHONIUM

in the serum may be explained as follows. The degree of inhibition of ChE depends first on the concentration of aprotinin, secondly on the period for which aprotinin acts on the ChE until the result of the Kalow test is read. About 5–15 minutes elapse until the maximum inhibition of ChE activity by a certain aprotinin concentration appears.

As for the formation of the aprotinin-ChE complex, a certain period of time is probably needed for a certain concentration of this complex to decrease, after sudden dilution of the solution, to a level which corresponds to the molar concentrations of the reaction partners in the diluted solution.

The result of the Kalow 2b test without previous incubation is read before the maximal concentration of the complex (maximal inhibition) has been reached. The Kalow test 1b does not provide enough time between dilution and reading for the complex formed in the presence of higher concentrations to disintegrate to such a degree as would correspond according to the law of mass action to the lower concentration after dilution of the serum.

However, it is only by this behaviour of the aprotinin-ChE complex that inhibition of ChE activity by aprotinin in vivo can be demonstrated.

Despite verification of the inhibition of ChE activity by aprotinin in vivo, it was not possible to prolong suxamethonium apnoea by the additional administration of aprotinin. The results obtained by Stößel (1969, personal communication) also fail to support an enhancement of the action of relaxants by aprotinin. This author recorded the bulbar retractions after electric stimulation of the ocular motor nerves of anaesthetized rats. Aprotinin up to 40,000 KIU/kg, administered i.v. at different times did not influence the decrease in the amplitude of contractions after tubocurarine or suxamethonium injection, or the duration of the relaxant effect.

From our experience it has become known that a very low ChE activity can result in a prolonged period of apnoea after suxamethonium. Apnoea will not be influenced by a few per cent inhibition of normal ChE activity.

It is conceivable that if ChE activity is substantially lowered for other reasons (e.g., other drugs or liver damage), then additional administration of aprotinin may decrease the level below the critical threshold. In this case, the effect of aprotinin would be a contributory cause of the resulting prolongation of apnoea. However, when ChE activity is so low, the anaesthetist must, in any case, anticipate a prolonged apneic period.

It is correct that aprotinin decreases activity of ChE as Chasapakis and Dimas (1966) describe, but the inhibition is not so great or so prolonged as they found by means of a method which we consider inadequate. Our experiments do not confirm the reported influence of aprotinin injection on the action of suxamethonium. The events observed by Chasapakis and Dimas must have had another cause.

REFERENCES


INFLUENCE D'APROTININE SUR L'ACTION DE SUXAMETHONIUM

SOMMAIRE

Les concentrations d'aprotinine, existantes in vivo après l'injection intraveineuse de 100.000 KIU, inhibent l'activité ChE d'environ 5 à 16 pourcent. Le degré d'inhibition est le plus grand entre 6 et 20 minutes après l'injection et négligeable après 30 minutes. Lorsque l'activité ChE de base est normale, l'inhibition de 5–16 pourcent ne cause pas de prolongation de l'action du suxamethonium. Une prolongation de la période d'apnée en connection avec une administration d'aprotinine n'est possible que lorsque l'activité ChE
es proche du niveau critique et est réduite par aprotinine à un taux, auquel la métabolisation de suxamethonium est retardée. Il n'y a pas de raison de ne pas utiliser aprotinine au cours d'opérations, où suxamethonium a été administré ou doit l'être. Aprotinine pourrait uniquement causer une réduction en-dessous du seuil critique chez les patients, dont le taux d'activité ChE est pathologiquement bas et qui sont par conséquent déjà prédisposés à une apnée prolongée après suxamethonium.

DER EINFLUSS VON APROTININ AUF DIE WIRKUNG VON SUXAMETHONIUM

ZUSAMMENFASSUNG


CORRESPONDENCE

EAST-RADCLIFFE VENTILATOR

Sir.—Further to my recent letter (Brit. J. Anaesth. (1970), 42, 791, Sept.) I wish to add the following.

Upon examination it was found that the pushrod between the valve rod and the valve stem was not adjusted correctly. To ensure complete closure of the valve a spring-loaded override mechanism should come into operation so that the valve closure is assisted by the spring.

On those machines which were not functioning correctly it was found that there was an absence of this override adjustment and thus the valve was barely seating in its closed position. When the valve plungers were adjusted correctly the machines worked normally.

This valve setting is checked by the manufacturers on all their machines and this is a normal check when they service the machines. This is not a fault that can develop by itself and will only occur if the valve mechanism is incorrectly adjusted.

J. B. MAGNER
Liverpool

Sir.—Our attention has been drawn to a letter from Dr J. B. Magner in your September issue (Brit. J. Anaesth., 42, 791) in which he gives an account of what he considers to be "a potentially dangerous characteristic of the East-Radcliffe Ventilator". The letter describes a situation which can only occur if the setting of the inspiratory and expiratory valves has been disturbed and reset incorrectly.

From reading Dr Magnier’s letter it would be possible to draw the incorrect conclusion that this fault could develop during the use of the Ventilator.

It is impossible, when correctly set, for the reading of the respirometer to include gas from the inspiratory phase because the valve cams are arranged so that the inspiratory valve closes just prior to the opening of the expiratory valve, and vice versa. If either of the screws which are designed to come into contact with the valve plungers (only when the machine is switched to the bag position) are initially touching one of the plungers, or if the spring-assisted override mechanism on the valve pushrods is not adjusted correctly, the result would be an incomplete closure of the valve concerned, thus causing a leak-through and incorrect tidal volume reading.

All our machines are carefully tested before they leave our factory and again whenever we service them. We do not have any contract to service the machines in question.

M. F. FREEMAN,
H. G. East & Co. Ltd.
Oxford