IN VITRO MUSCLE CONTRACTURES INDUCED BY HALOTHANE AND SUXAMETHONIUM

I: The Rat Diaphragm

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Malignant hyperthermia (MH) is a potentially fatal syndrome triggered by the potent volatile general anaesthetic agents and suxamethonium. Susceptibility to MH is diagnosed by the contracture response of skeletal muscle to halothane or caffeine, or both (Kalow et al., 1970; Moulds and Denborough, 1974; Rosenberg and Reed, 1983). The porcine stress syndrome resembles human MH and can also be triggered by the potent volatile general anaesthetic agents and suxamethonium (Gronert, 1980). Muscle strips from pigs susceptible to the porcine stress syndrome also exhibit a lower contracture threshold to halothane than do those from control pigs. The mechanisms underlying halothane-induced contractures of muscle are poorly understood.

A synergism exists between suxamethonium and halothane that has been observed clinically (Tammisto and Airaksinen, 1966; Innes and Strømme, 1973; Schwartz, Rockoff and Koka, 1984) and in vitro (Fletcher and Rosenberg, 1985, 1986). When examined in vitro, this interaction appears to require the liberation of fatty acids by suxamethonium (Fletcher and Rosenberg, 1986).

The access to human and pig skeletal muscle is limited. Obtaining a large number (8-12) of preparations without cut fibre ends from the same muscle is difficult with pigs and impossible in man. Also, the thickness of preparations of human and pig skeletal muscle fibres makes them less than ideal for pharmacological and physiological studies of contracture. The rat diaphragm is relatively thin, allowing a much larger percentage of the fibres to be oxygenated and exposed to the drugs introduced to the bath. The purpose of the present study was to develop a mammalian (small rodent) model system in which halothane-induced contractures and the interaction between suxamethonium and halothane could be conveniently examined in vitro. In this study agents known to antagonize phospholipase A₂ (PLA₂) activity were

SUMMARY

The rat diaphragm was used as an in vitro model for studies of contractures synergistically induced by halothane and suxamethonium. The effects of three agents reported to inhibit phospholipase A₂ activity (quinacrine, spermine and indomethacin), tubocurarine and dantrolene were examined on these contractures. Contractures induced by 1% halothane (0.26±0.02 g) (mean±SEM) were increased (0.60±0.04 g) if suxamethonium 50 mmol litre⁻¹ was also in the bathing medium. Suxamethonium-induced contractures (0.22±0.03 g) were also enhanced when halothane was present (0.51±0.03 g). Spermine, indomethacin and dantrolene antagonized both halothane- and suxamethonium-induced contractures. Quinacrine potentiated contractures induced by either halothane or suxamethonium. Contractures induced by suxamethonium were antagonized by tubocurarine; however, contractures induced by halothane were not antagonized by tubocurarine. These results suggest that free fatty acids may be involved in contractures induced synergistically by halothane and suxamethonium. Different mechanisms are involved in the induction of contractures by suxamethonium than by halothane.
used to determine whether PLA₂ activity could be involved in halothane-induced contractures of the rat diaphragm. Dantrolene, the drug of choice for epidoses of MH, was used, as dantrolene has been shown previously to block halothane-induced contractures in muscle strips from other species. Finally, the effects of tubocurarine were examined on the synergistic induction of contractures by halothane and suxamethonium (Gronert, 1980).

MATERIALS AND METHODS

Male Sprague–Dawley rats (200–400 g) were decapitated and the diaphragm was removed. Muscle strips (about 4 × 8 × 0.5 mm) were prepared by cutting the diaphragm parallel to the direction of the fibres from the rib section to the central tendon. Dissection was undertaken at 25 °C in Krebs solution composed of (mmol litre⁻¹): NaCl 118, KCl 3.4, MgSO₄ 0.8, KH₂PO₄ 1.2, glucose 11.1, NaHCO₃ 25.0 and CaCl₂ 2.5, and pH adjusted to 7.4. Usually, eight to 12 muscle strips could be prepared from a single diaphragm. Preparations were mounted in a tissue bath containing Krebs solution at 37 °C and bubbled with oxygen–carbon dioxide (95:5). The rib section of each strip was tied to a fixed position stimulating electrode and the central tendon connected to a force transducer. The muscle strips were adjusted to an initial resting tension of 1 g and directly stimulated at 0.2 Hz with supramaximal voltage pulses of 2 ms duration. Only preparations maintaining a stable resting tension for 30 min were used subsequently. Following the 30-min equilibration period, 1 % halothane was added to the gas phase. The time of exposure to halothane alone was 5 min. Suxamethonium was then added to the bath and the maximum contracture within 5 min to both agents recorded. In some experiments suxamethonium was added to the bath 5 min before halothane. When interaction studies were carried out with suxamethonium and halothane, those strips exhibiting a contracture greater than 0.5 g to the first agent added (except to 3 % halothane) were not used further in the study. This resulted in rejection of about 10 % of the total number of strips tested. These strips were eliminated because, on occasions, the resting tension of the preparation would not recover from the effect of the administration of the first drug before it was time to add the second agent. The results are expressed in terms of numbers of rats used. For each rat the average response of one to 12 strips was treated as a single value. When used, tubocurarine, spermine, indomethacin, quinacrine or dantrolene was added to the bath 2 min before suxamethonium or halothane. Krebs solution was the solvent for all drugs in the study, as solvents such as ethanol potentiate contractures induced by halothane (unpublished observations). The pH was adjusted to 7.4 for each solution of drug. Solutions of dantrolene and indomethacin were sonicated before use. Indomethacin and tubocurarine were not completely soluble at 1 and 50 mmol litre⁻¹, respectively. The halothane concentration in the gas phase was confirmed by gas chromatography, using an OV-101 (3 %) Chrom-W-HP 100–120 mesh (Perkin–Elmer) column.

Indomethacin, spermine, quinacrine, suxamethonium and tubocurarine were purchased from Sigma Chemical Co. (St Louis, MO). Caffeine was obtained from Eastman (Rochester, NY) and halothane from Ayerst (New York, NY). Dantrolene sodium was a gift of Norwich Pharmaceutical Co. (Norwich, NY).

The unpaired data in tables I and IV were each analysed using a one-way analysis of variance followed by Duncan's multiple range test. Significance was tested at the P < 0.05 level. The data in tables II and III were analysed using two-sided paired t tests.

RESULTS

The typical responses of the strips of rat diaphragm muscle to halothane and suxamethonium alone, or in combination, are demonstrated in figure 1. Preparations exhibited significantly greater contractures to either halothane or suxamethonium if the other agent was also present in the bath, than to either agent alone. The diaphragms from some rats exhibited contractures to halothane that exceeded 1.2 g. Currently, we believe the response to these agents may vary with season and age.
HALOTHANE AND SUXAMETHONIUM CONTRACTURES—RAT

1429

Suxamethonium 50 mmol litre⁻¹ on directly stimulated muscle strips isolated from the rat diaphragm. Tracings A and B are of the same muscle preparation. After the suxamethonium response (contracture) in tracing A halothane was discontinued, the preparation was washed and re-equilibrated for 30 min. Tracing B shows the drug additions following the 30 min equilibration. Notice that suxamethonium and halothane induced significant contractures only if the other agent was present.

Fig. 1. Effects of 1% halothane and suxamethonium 50 mmol litre⁻¹ on directly stimulated muscle strips isolated from the rat diaphragm. Tracings A and B are of the same muscle preparation. After the suxamethonium response (contracture) in tracing A halothane was discontinued, the preparation was washed and re-equilibrated for 30 min. Tracing B shows the drug additions following the 30 min equilibration. Notice that suxamethonium and halothane induced significant contractures only if the other agent was present.

greater in magnitude as the suxamethonium concentration was increased in the range of 1–50 mmol litre⁻¹ (table I). There was no response to suxamethonium at the concentrations of 1 or 10 mmol litre⁻¹ in the absence or presence of 1% halothane. Contractures induced by suxamethonium 50 mmol litre⁻¹ were not significantly different from those induced by 1% halothane. Suxamethonium 50 mmol litre⁻¹-induced contractures were increased significantly when preparations were preincubated with 1% halothane (table I). Contractures induced by suxamethonium 50 mmol litre⁻¹ in the presence of halothane did not increase significantly when the halothane concentration was increased to 3%. In contrast, halothane-induced contractures in the presence of suxamethonium 50 mmol litre⁻¹ were greater at a halothane concentration of 3% than at 1% (table I).

The effects of various antagonists were examined with 1% halothane and suxamethonium 50 mmol litre⁻¹. Results obtained with tubocurarine are shown in table II. Tubocurarine did not induce significant contractures in the rat diaphragm, even at a concentration of 50 mmol litre⁻¹. Contractures induced by suxamethonium in the presence of halothane were antagonized by tubocurarine (table II). However, contractures induced by halothane in the presence of suxamethonium were not antagonized by tubocurarine (table III). Dantrolene antagonized the contractures induced by either suxamethonium or halothane (tables II and III, respectively).

Two PLA₂ inhibitors, spermine and indomethacin, antagonized the interaction response to either suxamethonium (table II) or halothane (table III). A third PLA₂ inhibitor, quinacrine, did not antagonize suxamethonium-induced contractures in preparations pre-exposed to halothane (table IV). In addition, quinacrine did not antagonize halothane-induced contractures in preparations pre-exposed to halothane (table IV). In addition, quinacrine did not antagonize halothane-induced contractures in

Table I. Interaction between halothane and suxamethonium on contractures induced in the rat diaphragm. Suxamethonium and halothane were added within 5 min of each other. The values represent the maximum contracture within 5 min of addition of the second agent. Significant differences (P < 0.05) when compared with: *1% halothane alone; †Suxamethonium 50 mmol litre⁻¹ alone; ‡Suxamethonium 1 mmol litre⁻¹ followed by 1% halothane; §Suxamethonium 10 mmol litre⁻¹ followed by 1% halothane; ¶Suxamethonium 50 mmol litre⁻¹ followed by 1% halothane; **3% halothane alone

<table>
<thead>
<tr>
<th>Sequence of agent addition</th>
<th>Contracture (g) (mean ± SEM)</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>First</td>
<td>Second</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1% Halothane</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>Suxamethonium (1 mmol litre⁻¹)</td>
<td>1% Halothane</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>Suxamethonium (10 mmol litre⁻¹)</td>
<td>1% Halothane</td>
<td>0.45 ± 0.08*‡</td>
</tr>
<tr>
<td>Suxamethonium (50 mmol litre⁻¹)</td>
<td>1% Halothane</td>
<td>0.60 ± 0.04*‡</td>
</tr>
<tr>
<td>None</td>
<td>Suxamethonium 1 mmol litre⁻¹</td>
<td>0.00 ± 0.0f</td>
</tr>
<tr>
<td>None</td>
<td>Suxamethonium 10 mmol litre⁻¹</td>
<td>0.01 ± 0.0f</td>
</tr>
<tr>
<td>None</td>
<td>Suxamethonium 50 mmol litre⁻¹</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>None</td>
<td>Halothane (1%)</td>
<td>0.00 ± 0.0f</td>
</tr>
<tr>
<td>None</td>
<td>Suxamethonium 1 mmol litre⁻¹</td>
<td>0.00 ± 0.0f</td>
</tr>
<tr>
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<td>Suxamethonium 10 mmol litre⁻¹</td>
<td>0.05 ± 0.0f</td>
</tr>
<tr>
<td>None</td>
<td>Suxamethonium 50 mmol litre⁻¹</td>
<td>0.51 ± 0.0f</td>
</tr>
<tr>
<td>None</td>
<td>3% Halothane</td>
<td>0.55 ± 0.07*</td>
</tr>
<tr>
<td>Suxamethonium (50 mmol litre⁻¹)</td>
<td>3% Halothane</td>
<td>0.80 ± 0.16*‡</td>
</tr>
<tr>
<td>Halothane (3%)</td>
<td>Suxamethonium 50 mmol litre⁻¹</td>
<td>0.54 ± 0.02†</td>
</tr>
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preparations pre-exposed to suxamethonium (table IV). The response to 1% halothane following the addition of quinacrine to the bath (no suxamethonium present) was much greater than in the absence of quinacrine (table IV). Quinacrine also enhanced suxamethonium-induced contractures in the absence of halothane in the bath (table IV).

**DISCUSSION**

Suxamethonium and halothane act synergistically to induce contractures in directly stimulated muscle strips isolated from the rat diaphragm. This interaction is similar to that observed using preparations isolated from biopsies of human skeletal muscle (Fletcher and Rosenberg,
HALOTHANE AND SUXAMETHONIUM CONTRACTURES—RAT

1985, 1986). This interaction between halothane and suxamethonium does not appear to be simply additive, as the order in which the drugs were added determined the degree of response to the second agent. For example, the response to suxamethonium 50 mmol litre\(^{-1}\) was not significantly increased in preparations pre-incubated with halothane when the halothane concentration was increased from 1 to 3%. In contrast, the response to halothane in preparations pre-exposed to suxamethonium 50 mmol litre\(^{-1}\) was increased when the concentration of halothane was increased from 1 to 3%. Also, there was essentially no response to suxamethonium 10 mmol litre\(^{-1}\) in the presence of 1% halothane. When the order in which the agents were added to the bath was reversed, 1% halothane-induced contractures were increased significantly in the presence of suxamethonium 10 mmol litre\(^{-1}\). Thus, separate mechanisms may be involved in the response to each of the agents. Further evidence for separate mechanisms in halothane- and suxamethonium-induced contractures was the observation that tubocurarine antagonized suxamethonium-induced contractures, but not those induced by halothane. These results are in agreement with the clinical situation in regard to MH: that is, tubocurarine can prevent suxamethonium-induced MH, but not halothane-induced MH (Harrison, 1971; Hall, Lucke and Lister, 1976). These results may not be mediated at the endplate, as such high concentrations of suxamethonium and tubocurarine are required, respectively, to induce or antagonize contractures.

The results of the present study, using indomethacin and spermine, suggest that PLA\(_2\) activity may be involved in halothane and suxamethonium-induced contractures in the rat diaphragm. The results obtained with quinacrine on the diaphragm preparation are more complex. Studies using human muscle have demonstrated that quinacrine can block halothane and suxamethonium-induced contractures (Fletcher and Rosenberg, 1986). A recent report suggests that the increased specific activity in pigs susceptible to the porcine stress syndrome may result from a greater concentration of calmodulin (Cheah, 1984), which would stimulate calmodulin-dependent PLA\(_2\). Although we do not understand the difference in response between rat and human preparations, one possible explanation is that PLA\(_2\) activity of the rat diaphragm may not be calmodulin-dependent. Alternatively, an action of quinacrine other than the inhibition of PLA\(_2\) may account for the potentiation of the contractures induced by halothane and suxamethonium. Regardless of this discrepancy, the difference between human and rat preparations in the action of quinacrine can be used to understand better the role of lipolysis in the induction of contractures. Although PLA\(_2\) activity is presumed to be the source of fatty acids, it is premature to eliminate other lipolytic enzymes including PLA\(_1\), triglyceride lipase, diglyceride lipase, and so on.

Dantrolene is known to block Ca\(^{2+}\) release from the sarcoplasmic reticulum (Martonosi, 1984). In view of the present study, dantrolene could be a potent PLA\(_2\) inhibitor. The inhibition of PLA\(_2\) activity could be either direct or indirect. An example of an indirect mechanism would be the blocking of Ca\(^{2+}\) availability to the Ca\(^{2+}\)-dependent PLA\(_2\) enzyme. Alternatively, dantrolene may physiologically antagonize the effects of the unsaturated fatty acids on Ca\(^{2+}\) regulation.

In conclusion, muscle strips isolated from the rat diaphragm are useful in investigations of drug-induced contractures. The fibre strips are thin and are mostly uncut—highly desirable features when compared with the human and pig fibre bundles used frequently in such studies. The interaction between halothane and suxamethonium in the present studies appears similar to that observed using human skeletal muscle, except for the interactions of halothane and suxamethonium with quinacrine. In addition, the present study demonstrates that suxamethonium and halothane induce contractures through different mechanisms. These studies support a role for fatty acids in contractures synergistically induced by halothane and suxamethonium.

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