PATHOGENESIS OF SUXAMETHONIUM-INDUCED MUSCLE DAMAGE IN THE BIVENTER CERVICIS MUSCLE IN THE CHICK

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SUMMARY
Muscle damage induced by suxamethonium, and the influence of halothane on it, has been examined by measuring the efflux of creatine kinase (CK) in the biventer cervicis muscle of the chick. Whereas halothane and suxamethonium alone did not increase the enzyme efflux significantly, the combination of the two was associated with significant increase in the concentration of CK in the bathing medium by 59-157%. The increase in CK was prevented by adding chlorpromazine 100 μmol litre⁻¹ to the medium, suggesting the involvement of phospholipases in the pathogenesis of suxamethonium-induced muscle damage.

KEY WORDS

Suxamethonium has several side effects, the most common of which are muscular in origin. The association between administration of suxamethonium and delayed muscle pains has been long recognized [1]. It is also known to cause an increase in serum concentrations of creatine kinase (CK), which has been regarded as an index of muscle damage in both in vivo and in vitro studies [2-4].

Little is known about the aetiology of suxamethonium-induced muscle damage. Several theories have been proposed, but the acute nature of the condition has made it difficult to define its pathogenesis. It has been suggested that endplate depolarization by suxamethonium produces an influx of calcium ions into the myoplasm, causing activation of phospholipases and culminating in cellular damage [5]. In addition, there is evidence that similar calcium-mediated processes are responsible for muscle damage in other situations [6-8]. In the present study, we have examined the effect of suxamethonium on the efflux of CK from the biventer cervicis muscle of the young chick. This muscle contains a large proportion of multiply-innervated fibres, with cholinoreceptors distributed all over the sarcolemma, thus rendering it markedly sensitive to suxamethonium-induced membrane depolarization [9].

MATERIALS AND METHODS
The study was carried out in two phases. In the first phase, we examined the release of CK in response to suxamethonium and the effect of halothane on this. In the second phase, the influence of chlorpromazine, which inhibits phospholipase A2 [10], was assessed on limitation of muscle damage as measured by the same enzyme efflux.

Phase 1. One-day old chicks weighing 45-50 g were killed in an ether chamber and the biventer cervicis muscle dissected out as described by Ginsborg and Warriner [9]. This muscle lies in the dorsum of the neck, arises from the base of the skull and is inserted into the supraspinous ligament. It has two bellies, separated by a tendon. The lower muscle belly was removed, mounted on holders and suspended in tubes containing 6 ml of freshly prepared bicarbonate-buffered Krebs...
solution modified to produce free calcium concentration of 1 mmol litre$^{-1}$ (constituents (mmol litre$^{-1}$): NaCl 120, KCl 5.9, NaHCO$_3$ 23, Na$_2$HPO$_4$.2H$_2$O 1.2, glucose 5.5, MgCl$_2$ 1.2, and CaCl$_2$ 1.0). The medium was gassed with 5% carbon dioxide in oxygen and maintained in a thermostatically controlled water bath at 37 °C.

After isolation, each muscle was incubated for 30 min to allow re-establishment of baseline conditions, after which the medium was discarded and replaced with fresh buffer. After a further 30 min this buffer was exchanged and stored for analysis of baseline or time zero (T0) CK concentrations. The preparations were incubated for 30 min in the presence of suxamethonium 50 mmol litre$^{-1}$ (group S), 3% halothane (group H), suxamethonium 50 mmol litre$^{-1}$ and 3% halothane (group S+H) or without any additive, to serve as controls (group C). The pH of all materials added to the media was adjusted to 7.4 and checked using a Corning pH meter. The bathing medium was exchanged and replaced at the end of this time and stored for analysis as sample T30. The medium in each tube was changed at 30-min intervals for a further 60 min (samples T60 and T90), yielding a total of four samples for each preparation.

CK concentrations were measured spectro-photometrically on the same day as the experiment was performed, by measuring the catalysed conversion of NADP to NADPH at 340 nm. All chemicals were supplied by Sigma Chemical Company and were Anal-R grade.

Phase 2. After the completion of phase 1 experiments and analysis of the results, the combination of halothane-suxamethonium was adopted as the standard muscle “trauma”. The purpose of this phase was to investigate the effect of chlorpromazine on enzyme efflux caused by the combination of suxamethonium and halothane. Muscle preparations were incubated as before, without any additions to the medium to serve as controls (group C), with suxamethonium 50 mmol litre$^{-1}$ and 3% halothane added to the gassing mixture from a calibrated Fluotec mark III vaporizer (group S+H) or suxamethonium, halothane and chlorpromazine 100 μmol litre$^{-1}$ (group S+H+Ch). Replacement of the medium at 30-min intervals was performed as in phase 1, yielding four serial samples, and analyses of CK concentrations were performed promptly.

Under preset criteria, samples with a baseline CK activity of > 50 u litre$^{-1}$ were removed from analyses as they were thought to indicate occurrence of unacceptable muscle damage on dissection. Within each phase, the results were analysed statistically using two-factor repeated measures analysis of variance (ANOVA). $P < 0.05$ was considered significant.

RESULTS

A total of 70 muscle preparations yielded acceptable results in phase 1. The mean (SEM) baseline (T0) CK concentrations in the four groups were between 17.5 and 23.1 u litre$^{-1}$, with no significant difference between groups. In undamaged preparations, relatively little enzyme accumulated in the medium during this initial stabilizing period. CK concentrations remained constant or showed a slight tendency to decrease through each 30-min period (samples T30, T60 and T90) in groups C, H and S (fig. 1). In group S+H, CK values increased significantly in T30, T60 and T90 samples ($P < 0.05$–0.01). The mean CK concentration increased by a maximum of 157% (21-54 u litre$^{-1}$) at 60 min in this group.

A total of 48 muscle preparations yielded acceptable results in the second phase of the study. The baseline (T0) CK concentrations were comparable in the three groups and similar to baseline values in phase 1 of the study. The

![Fig. 1. Mean (SEM) CK concentrations with and without exposure to suxamethonium, halothane and their combination.](https://academic.oup.com/bja/article-abstract/67/6/764/358690)
FIG. 2. Baseline (□) and maximal (■) (SEM) CK concentrations in the presence of suxamethonium and halothane with or without chlorpromazine. C = Control preparations (n = 18); S + H = preparations exposed to suxamethonium and halothane (n = 17); S + H + Ch = preparations exposed to suxamethonium, halothane and chlorpromazine (n = 13). *P < 0.05 compared with respective baseline value.

maximal changes in CK in groups S + H and S + H + Ch are shown in figure 2. CK concentrations again, increased significantly in group S + H, this time by a maximum of 59% (24.6–39.1 u litre⁻¹), but showed only minimal and insignificant change in both the control (group C) and chlorpromazine-treated preparations (group S + H + Ch).

DISCUSSION

The measurement of efflux of cell-bound enzymes is an accepted method of assessing tissue damage in in vivo and in vitro preparations [4]. Isolated muscle preparations have been used with success in elucidating cellular processes involved in the pathogenesis of some muscle diseases [3, 6, 8, 11, 12]. The role of calcium and phospholipases, specifically phospholipase A2 (PLA2), in the cellular disruption associated with various muscle damaging processes has been identified from these studies. This enzyme may also have a role in the pathogenesis of malignant hyperthermia [13].

There are several problems associated with the use of an experimental model for the study of suxamethonium-induced muscle damage. The isolated muscle must be removed intact with minimal damage, the preparation must be suffi-
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It would be interesting to know if chlorpromazine prevents the contracture produced by suxamethonium in the presence of halothane, in addition to preventing the release of CK. This was not examined in the present investigation, which was designed to establish a model for the study of enzyme changes.

In conclusion, the chick biventer cervicis muscle appears suitable for investigating suxamethonium-induced muscle damage. The damage may be assessed by examining efflux of CK into a bathing medium. The increase in the enzyme was obtunded by addition of chlorpromazine to the medium before exposure to suxamethonium and halothane. It is possible that damage caused by suxamethonium is mediated by activation of the enzyme PLA2. In view of the effectiveness of chlorpromazine in the prevention of suxamethonium-induced CK efflux, and presumably muscle damage, it will be interesting to explore the effectiveness of this agent in preventing some of the side effects of suxamethonium in clinical situations.

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REFERENCES