A Pharmacological in Vitro Model of Malignant Hyperpyrexia

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SUMMARY

Caffeine causes contracture in muscle. Drugs which initiate malignant hyperpyrexia in susceptible pigs, lower the threshold concentration of caffeine at which this contraction occurs. The implications of and uses for this finding are described.


Anaesthetic-induced malignant hyperpyrexia has been shown to be a disorder of muscle. Certain pigs of the Landrace strain in South Africa and Australia, the Poland China strain in America, and Landrace Wessex in England, have been shown to suffer from a similar disorder, and have served as useful models of this puzzling lethal condition. But pigs as experimental animals are expensive, and supplies of animals of the specific susceptible strain sporadic. Attempts at direct breeding of this specific strain have not proved very successful in our hands, and so we searched for a more readily obtainable and cheaper animal experimental model.

Whatever the precise nature and site of the defect of malignant hyperpyrexia myopathy, Kalow et al. showed that it produces responses in muscle rather similar to those which follow application of caffeine, a conclusion which gained pharmacological support from my demonstration that initiation of the syndrome in susceptible pigs could be effectively blocked by procaine. Strobel and Bianchi demonstrated that if frog sartorius muscle was exposed to subcontraction-producing doses of caffeine, subsequent administration of halothane to the preparation led to rigor in a manner analogous to the response of the muscle of hyperpyrexia-susceptible pigs. As the only known subjects of malignant hyperpyrexia are mammals, I chose to investigate rat rectus as an in vitro model. The rat is a readily available and inexpensive laboratory animal, and the rectus is easily cut into strips of parallel fibres.

METHOD

The apparatus is shown diagrammatically in Fig. 1. One-gram strips of rat rectus were mounted in a vertical bath of approximately 120 ml volume, containing pharmacological solution (Table I), to which 5 mg/100 ml of calcium chloride was added. This solution was oxygenated with carbogen (3% CO₂ and 97% O₂) bubbled through a dispersion grid. The pH, monitored by a pH probe (Metrohm) was buffered to 7.4 by the addition of tri-hydroxymethylaminomethane as needed. Temperature was maintained at 22°-32°C. Muscle strips were mounted vertically between a fixed lower clamp and a platinum wire attached to the cross beam of a Devices displacement transducer, counterbalanced by a 1.5 g weight, this arrangement permitting isotonic contraction of the muscle. The muscle attachments served as electrodes of a stimulator, programmed to stimulate the muscle supramaximally at 0.25 Hz. Muscle contractions were recorded on a Beckman 10" recorder. Caffeine stock solutions were made by the acid solution method of Frank et al.

Anaesthetic agents were added by passage of the carbogen stream through the appropriate vaporizer, provision being made by a venting side arm to keep gas flows within the performance tolerance of the vaporizer. Relaxant drugs used were from commercially available solutions.

<table>
<thead>
<tr>
<th>TABLE I. COMPOSITION OF SOLUTION</th>
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<tr>
<td>Salt (mEq/L)</td>
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<tr>
<td>Sodium</td>
</tr>
<tr>
<td>Magnesium</td>
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<tr>
<td>Sulphate</td>
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<td>Chloride</td>
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<td>Phosphate</td>
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RESULTS

Caffeine in increasing concentration causes enhanced muscle contraction, followed by shortening and rigor of the muscle (Fig. 2). If a muscle is exposed to a subcontraction-producing concentration of caffeine, subsequent exposure to halothane causes shortening (Fig. 3). If, on the other hand, the muscle strip is first exposed to halothane and caffeine is then added, a reciprocally synergistic effect is evident and shortening of the muscle occurs at a lower concentration of caffeine than when caffeine alone is added (Fig. 4). By noting the concentration of caffeine at which shortening occurred after exposure to halothane—or any other anaesthetic—this effect could be quantitated. I have called this concentration of caffeine at which shortening started to develop the 'caffeine threshold'. As this level differed slightly between rats, I have in each case used muscle from the same rat as its own control. The normal caffeine threshold was found to be 1-1.5 mM caffeine.

The effect of various anaesthetic agents and relaxants on the caffeine threshold was investigated. Results are given in Table II. From these it will be seen that agents which have been shown to initiate the syndrome of malignant hyperpyrexia in susceptible pigs lower the caffeine...
threshold of this muscle preparation. There is discrepancy between the agents that have been incriminated as trigger agents in humans and those that have been shown to act in susceptible pigs. Though many more agents have been incriminated in the human disease, the commonest agents in humans—halothane and succinylcholine—are those which strongly initiate the condition in pigs and lower the caffeine threshold in this preparation. (Chloroform, which does so in pigs and which lowered the caffeine threshold preparation in our in vitro experiment is no longer used in humans). A notable exception is methoxyflurane. Though this agent markedly lowered the caffeine threshold in our preparation and has been recorded as being a strong inducing agent in susceptible human subjects, I have failed to initiate malignant hyperpyrexia with it in susceptible pigs.

It is also of interest to note that though Chalstry and Edwards have described pancuronium as a trigger agent in pigs, I have failed to confirm this.

**CONCLUSIONS**

These observations appear to support Kalow's hypothesis that the functional lesion of malignant hyperpyrexia in susceptible subjects is a caffeine-like defect in the function of the sarcoplasmic reticulum. It seems that certain anaesthetics cause a similar alteration in membrane function of the sarcoplasmic reticulum. If susceptible human subjects, pigs, or muscle preconditioned by caffeine, are exposed to such agents, the defect is accentuated by a

synergistic action, so causing rigor and precipitating malignant hyperpyrexia.
A practical use for this in vitro rat muscle preparation would be as a screening test for new anaesthetic agents for malignant hyperpyrexia-inducing properties. Further uses of this model are the investigation of the efficiency of agents such as procaine to block initiation of malignant hyperpyrexia, or perhaps of even more importance in the therapeutic field, the development from this of a preparation of sustained rigor on which agents which might actively relax rigor could be sought and tested.

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REFERENCES