RYANODINE CONTRACTURE: A POTENTIALLY SPECIFIC IN VITRO DIAGNOSTIC TEST FOR MALIGNANT HYPERTHERMIA

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SUMMARY

In vitro contracture tests used currently for malignant hyperthermia (MH) do not possess absolute specificity. This is potentially a great problem in the study of the genetic approach which offers the best prospect for the development of a non-invasive diagnostic test for the condition. The calcium release channel of the sarcoplasmic reticulum has been proposed as the site of the MH defect. Ryanodine, which binds avidly to this channel, was shown to differentiate between muscle of MH susceptible and normal patients in terms of in vitro contracture response. This ryanodine contracture response is proposed as a potentially specific in vitro diagnostic test for MH.

KEY WORDS


Current methods for diagnosis of malignant hyperthermia (MH) are based upon an abnormal in vitro contracture response both to caffeine and to halothane, by muscle taken at open biopsy. The majority of centres screening for MH susceptibility now use either the procedure of the European MH Group [1] or that of the North American equivalent [2].

The halothane contracture test, performed according to the European procedure, is a very sensitive test. The caffeine contracture test appears to produce false negative results in about 15% of MH susceptible patients, but it may provide useful information in subjects in whom the halothane result is borderline. However, both the halothane and the caffeine tests have been shown not to be specific for MH, with positive results occurring in some patients with other muscle diseases known to be genetically distinct from MH [3]. The lack of absolute specificity of the in vitro contracture tests for MH is perhaps the greatest hindrance to world-wide efforts to identify the genetic defect in this condition.

Ryanodine, a neutral plant alkaloid, has been shown to cause time- and dose-dependent contractures of normal rabbit and rat skeletal muscle [4]. This is related presumably to its affinity for ryanodine receptor protein, which is now known to incorporate the sarcoplasmic reticulum calcium release channel [5]. This protein, which spans the gap between the terminal cisternae of the sarcoplasmic reticulum and the T-tubule, has been suggested as the site of the MH defect, as [³H]-ryanodine has been shown to have greater affinity for the protein from pigs with porcine stress syndrome than that from normal pigs [6].

Any differences in the contracture response to ryanodine between muscle from MH susceptible and normal individuals may, therefore, form the basis of a test for MH more specific than either the halothane or caffeine contracture tests.

METHODS AND RESULTS

The muscle specimens used were surplus to diagnostic requirements for patients attending for investigation of MH susceptibility according to the European MH Group procedure. Experiments were performed using muscle fascicles from six MH susceptible (MHS) patients, seven MH negative (MHN) patients and two patients

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whose muscle had an abnormal contracture response to halothane but normal response to caffeine and were therefore classified as MH equivocal (MHE) according to the European procedure. For two MHS and two MHN patients, a second fascicle was available to test for reproducibility.

Immediately after excision, each specimen was placed in Kreb's solution (content (mmol litre\(^{-1}\)): NaCl 118.1, KCl 3.4, MgSO\(_4\) 0.8, KH\(_2\)PO\(_4\) 1.2, glucose 11.1, NaHCO\(_3\) 25.0, CaCl\(_2\) 2.5) at room temperature and bubbled with carbogen (95% oxygen–5% carbon dioxide). All experiments were performed within 5 h of biopsy. Muscle fascicles were placed in a muscle bath perfused with Kreb's solution at 37°C bubbled with carbogen. The muscle was stimulated directly (0.3-Hz, 2-ms square wave) at a supramaximal voltage and the response recorded on a Gould 2200 recorder using an Ormed 4157 force transducer. A baseline tension of 2 g was applied. After 5 min, ryanodine was added incrementally at doses of 0.4, 0.8, 1.6 and 10 \(\mu\)mol litre\(^{-1}\), each dose being maintained for 3 min. A contracture was recorded as the increase in baseline tension above the minimum baseline tension. Ryanodine was obtained from Agri Chemicals (Philadelphia, U.S.A.); all other chemicals were obtained from Sigma (Poole, U.K.).

No muscle fascicle from the MHN group of patients developed a contracture in response to the regimen described of exposure to ryanodine. In contrast, a contracture developed during the incremental application of ryanodine in all fascicles from MHS patients. The muscle from the two MHE patients behaved in a similar way. Details of the contractures produced are given in table I, with responses to caffeine and to halothane.

The response to ryanodine was compared with those for caffeine and halothane, using Spearman's rank correlation coefficient. Rank order was determined by the threshold concentration for contracture of each drug. Where two or more patients had the same threshold concentration, the size of contracture produced at that concentration was used for ranking. The correlation coefficient for halothane and ryanodine was 0.93 \((P < 0.01)\), while that for caffeine and ryanodine was 0.69 \((P < 0.05)\).

**COMMENT**

This study has demonstrated a method for distinguishing MH susceptible from normal individuals. By studying other members of their families, we have confirmed that the two patients classified as MHE as a result of their halothane and their caffeine contracture tests were indistinguishable from the MHS group using ryanodine as the challenge. This suggests that the ryanodine contracture test is more sensitive than the caffeine contracture test and at least as sensitive as the halothane contracture test. In conjunction with the biochemical abnormality demonstrated on the ryanodine receptor protein in MH pigs by Michelson and colleagues [6], this
implies that the ryanodine contracture test may be more specific to MH. The results of this study have important implications for our understanding of the pathophysiology and pharmacology of the ryanodine receptor protein—sarcoplasmic reticulum calcium release channel.

The number of patients involved in this study was small, however, and the results require confirmation. Because of the potential impact of a specific diagnostic test for MH on the analysis of genetic linkage data, laboratories screening for MH susceptibility should consider including a ryanodine contracture test in their procedure.

REFERENCES