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Understanding malignant hyperthermia: each move forward opens our eyes to the distance left to travel

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In this issue of the *British Journal of Anaesthesia*, Figueroa and colleagues[1] report on the relationship between muscle responses in the caffeine/halothane contracture test (CHCT) for malignant hyperthermia from a large Canadian cohort and calcium handling in myotubes derived from myoblasts obtained from muscle samples not used in the CHCT test. Based on the results of the CHCT test, the authors stratified the 121 subjects into three groups: Normal (HN—not hyper-responsive to 2 mM caffeine or 3 vol% halothane); Susceptible (HS—hyper-responsive to both 2 mM caffeine and 3 vol% halothane); and Equivocal (HH—hyper-responsive only to halothane). Surprisingly, this cohort did not contain any Equivocal patients who were only responsive to caffeine despite such individuals being identified previously in a large European cohort.[2] Figueroa and colleagues[1] also devised a new five-component clinical index to summarise the relative musculoskeletal symptoms (e.g. weakness/fatigue, myalgia, heat/exercise intolerance, elevated creatine kinase, and histopathology) of each individual in the cohort.

The first surprise from this study was the observation that HH individuals exhibited more musculoskeletal symptoms (higher clinical index) than HS and HN individuals. In addition to the clinical index, they also devised a four-component calcium index (resting calcium, frequency of spontaneous calcium release events, calcium waves, and calcium spikes after electrical stimulation) from calcium responses myotubes from a subset of 16 patients from each CHCT subgroup from a total of 95 successful patient cultures (~50% of myotube cultures). However, the rationale used to select which myoblast cultures would be chosen for each subgroup for these calcium studies was not provided.

The CHCT studies found that the force generated by muscle strips in response to halothane exposure (F_C) was greater in the HS subgroup than for either the HN or HH subgroups. In contrast, the HH subgroup exhibited the highest values for both clinical index and calcium index compared with the HN and HS subgroups, although the difference in clinical indexes between the HH and HS subgroups did not reach statistical significance.

Using Indo-1, a UV light-excited fluorescent Ca^{2+} indicator, both resting intracellular calcium concentration and the frequency of spontaneous calcium release events (calcium 'sparks' or macro sparks) were significantly increased only in myotubes derived from HH individuals. On the other hand, calcium spiking after an electrical stimulus was significantly increased both in myotubes from HH and HS individuals. As a result, the calcium index was significantly increased in both HH and HS myotubes, although the calcium index was greatest in HH myotubes. Unfortunately, the clinical and calcium indexes within a given subgroup were poorly correlated. Importantly, however, principal component analysis of the four elements that comprise the calcium index revealed that plotting component 1 against component 2 resulted in HN and HS data that fall largely on a common line, whereas data from the HH group are unequivocally separated from both the HS and HN groups. An additional intriguing characteristic of the HH subgroup is that only 12% of these individuals were found to possess an *RYR1* variant (all being variants of unknown significance), whereas previous studies indicate that *RYR1* variants are typically found in >80% of malignant hyperthermia susceptible (MHS) individuals within the Canadian population.[3]

Together, these results provide provocative new evidence to suggest that the genetic causes and calcium alterations within the HH subgroup are fundamentally distinct from that of conventionally classified MHS individuals (i.e. HS individuals). The authors further conclude that future studies will need to consider other variables not assessed in this study (e.g. calcium stores, calcium entry, and RYR1 sensitivity to calcium and voltage) in order to provide a deeper and more comprehensive understanding of what these differences actually mean.

An important strength of this study is the integration of clinical and basic science data to probe the mechanisms of malignant hyperthermia. These results also have important implications for clinical manifestations associated with different subgroups that may occur in the absence of exposure to volatile anaesthetics, such as musculoskeletal symptoms and susceptibility to acute heat stroke or exercise-induced rhabdomyolysis. The fact that resting calcium levels were significantly increased only in myotubes from HH individuals, but not HS individuals, is in contrast to the increase in resting calcium levels observed *in vivo* in sartorius muscles of MHS patients using Ca^{2+} selective microelectrodes.[4] Similar discrepancies in relative levels of resting calcium have been reported previously after heterologous[5] and homologous[6] expression of MHS-linked mutations in RyR1 and $Ca_V1.1$, and

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in muscle fibres and myotubes derived from murine models of MHS.[7–9] The reasons for these discrepancies likely result from the different conditions (room *vs* physiologic temperature; *in vivo vs in vitro* measurements), preparations (HEK293 cells *vs* myotubes *vs* adult muscle fibres), and experimental approaches (calcium dyes *vs* microelectrodes) used in these studies.

There are also several important caveats to the conclusions of this study. First, the clinical grading scale for musculoskeletal symptoms used in this study has not been validated in any previous report. Second, as mentioned above, the cohort used in this study lacked individuals with positive CHCT responses to only caffeine (an 'HC' subgroup), which have been observed previously.[2] Third, it will be important for future studies to determine if the calcium index developed here in immature cultured myotubes also applies to fully developed muscle fibres from MHS individuals. Finally, while the North American CHCT stimulates muscle electrically during exposure to 3 vol% halothane [or 2 vol% halothane for the European *in vitro* contracture testing (IVCT)], clinical MH events do not appear to require a depolarisation trigger to initiate an MH crisis. However, Zullo and colleagues[10] recently provided evidence to suggest that this may not matter as only a small depolarisation is needed to 'get the calcium-ball rolling' in MHS myotubes, at least under the conditions of their studies. Consistent with this, exposure to halothane is unable to trigger an MH episode in MHS swine under conditions of neuromuscular block using a non-depolarising neuromuscular blocker (Jones and colleagues, unpublished data).

With each move forward, it becomes increasingly clear that we still have a long way to go to completely understand the molecular mechanisms that lead to clinical MH events and to develop therapies other than dantrolene that not only prevent/reverse potentially fatal MH episodes, but are also effective in treating related muscle diseases, including exertional rhabdomyolysis and heat injury.

Authors' contributions

All authors contributed equally to the writing of this editorial.

References

1. Figueroa L, Kraeva N, Manno C, Toro S, Ríos E, Riazi S. Abnormal calcium signaling and the caffeine–halothane contracture test. *Br J Anaesth* 2018; **122**: 32–41
2. Klingler W, Heiderich S, Girard T, et al. Functional and genetic characterization of clinical malignant hyperthermia crises: a multi-centre study. *Orphanet J Rare Dis* 2014; **9**: 8
3. Kraeva N, Riazi S, Loke J, et al. Ryanodine receptor type 1 gene mutations found in the Canadian malignant hyperthermia population. *Can J Anaesth* 2011; **58**: 504–13
4. Lopez JR, Gerardi A, Lopez MJ, Allen PD. Effects of dantrolene on myoplasmic free [Ca^{2+}] measured *in vivo* in patients susceptible to malignant hyperthermia. *Anesthesiology* 1992; **76**: 711–9
5. Tong J, McCarthy TV, MacLennan DH. Measurement of resting cytosolic Ca^{2+} concentrations and Ca^{2+} store size in HEK-293 cells transfected with malignant hyperthermia or central core disease mutant Ca^{2+} release channels. *J Biol Chem* 1999; **274**: 693–702
6. Dirksen RT, Avila G. Distinct effects on Ca^{2+} handling caused by malignant hyperthermia and central core disease mutations in RyR1. *Biophys J* 2004; **87**: 3193–204
7. Durham WJ, Aracena-Parks P, Long C, et al. RyR1 S-nitrosylation underlies environmental heat stroke and sudden death in Y522S RyR1 knockin mice. *Cell* 2008; **133**: 53–65
8. Yang T, Riehl J, Esteve E, et al. Pharmacologic and functional characterization of malignant hyperthermia in the R163C RyR1 knock-in mouse. *Anesthesiology* 2006; **105**: 1164–75
9. Yuen B, Boncompagni S, Feng W, et al. Mice expressing T4826I-RYR1 are viable but exhibit sex- and genotype-dependent susceptibility to malignant hyperthermia and muscle damage. *FASEB J* 2012; **26**: 1311–22
10. Zullo A, Textor M, Elischer P, et al. Voltage modulates halothane-triggered Ca^{2+} release in malignant hyperthermia-susceptible muscle. *J Gen Physiol* 2018; **150**: 111–25

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Slow waves, cognitive disintegration, and delirium

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Delirium has recently become something of the 'diagnosis du jour' (or perhaps 'diagnosis de la décennie') in the field of anaesthesia. Delirium is underdiagnosed, and is associated with several adverse perioperative outcomes.[1] In this issue of the journal, Numan and colleagues[2] show that postoperative delirium is associated with a 50% increase in relative delta power in the EEG. They then go on to suggest that this index of the EEG could be used as an automated monitor to diagnose

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