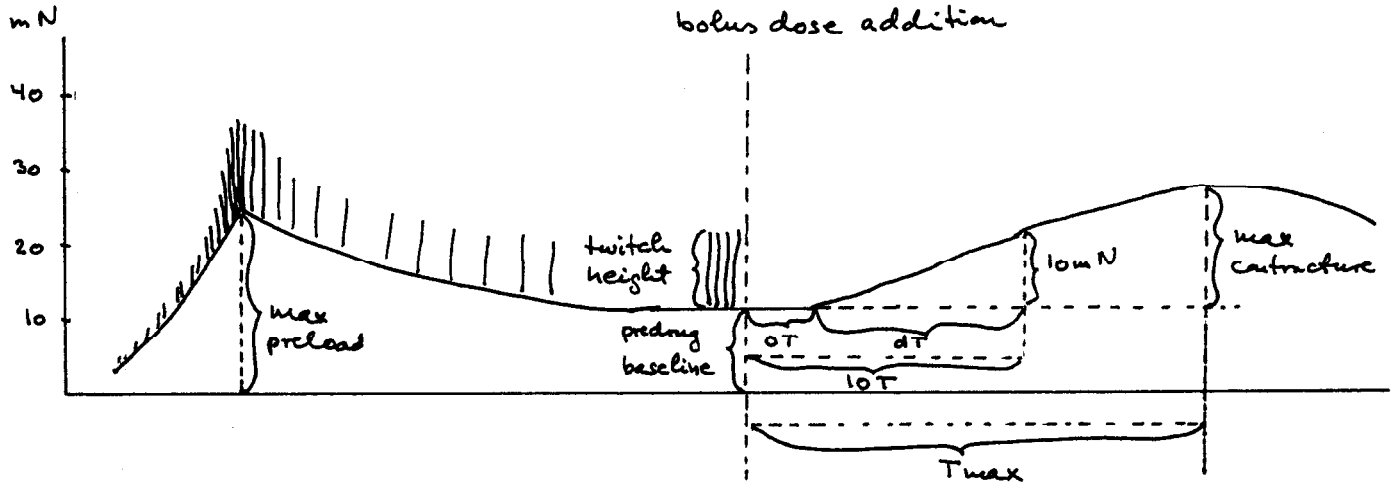
THE EUROPEAN MALIGNANT HYPERTHERMIA GROUP

Ryanodine test - optional and standardized for the EMHG

Ryanodine	High purity (Calbiochem).
Stock solution	100 micromol litre^{-1} in distilled water.
Storage	Refrigerator at 4°C.
Test concentration	Final bath concentration of 1 micromol litre^{-1}.
General guidelines	The ryanodine test is performed similarly to other contracture tests described in the EMHG protocol: 1. A fresh muscle specimen must be used. 2. The specimen is electrically stimulated with a 1 ms supramaximal stimulus at a frequency of 0.2 Hz. 3. The test is performed at optimal length (l_o). 4. Baseline must not vary more than 2 mN (0.2 g) within a 10 min period before the addition of ryanodine. 5. A single bolus dose technique is used to reach a final bath concentration of **1 micromol litre^{-1}**. If the tissue bath is continously perfused with Krebs solution, either the perfusion is stopped or a Krebs-ryanodine solution is perfused. The final bath concentration of ryanodine must be reached **within 1 min**.
Test parameters	1. Similar to other tests: weight (mg) and length (mm) of specimen, twitch height (mN), maximum preload (mN), predrug baseline (mN), lowpoint baseline (mN). 2. Specific parameters: onset time (OT) of contracture (min), time for development of a 10 mN (1 g) contracture (10T) (min), contracture velocity (dT = 10T-OT) (min), and optionally: time for development of contracture maximum (Tmax) (min), maximum contracture (mN).
Quality control	The concentration of ryanodine in the tissue bath must be periodically checked by HPLC.



In this example, predrug baseline = low point baseline.