One of the obstacles in the development of transient receptor potential vanilloid-1 (TRPV1) antagonists as pain therapeutics is that these compounds have a serious side effect: they cause hyperthermia. In an earlier study in rats (Steiner AA et al. J Neurosci 27: 7459-7468, 2007), we have shown that tonic activation of TRPV1 channels in the abdominal viscera by yet unidentified non-thermal factors inhibits skin vasoconstriction and thermogenesis, thus having a suppressive effect on body temperature. When this activation by non-thermal factors is blocked by a TRPV1 antagonist, hyperthermia occurs. The present study was designed to reveal the nature of the non-thermal factors involved. We studied a large number of TRPV1 antagonists with different pharmacological profiles and quantitatively compared their potency to cause hyperthermia in rats with their reported potencies to block three different modes of activation of the rat TRPV1 channel in vitro: by capsaicin, high temperature, or low pH. Colonic temperature responses of chronically prepared male Wistar rats to a wide range of intravenous doses of A425619, AMG0347, AMG 517, AMG8163, AMG9810, JYL1421, SB366791, capsazepine, or corresponding vehicles were studied. For each dose, a temperature response index (time integral of the deviation of colonic temperature from its level at the time of injection) was calculated, and a dose-response relationship was established. We assumed that the hyperthermic response results from the blockade of the three activation modes, each contributing to the effect with a different weight, and then used a mathematical model to determine the relative contribution of each mode. A preliminary analysis has shown that the temperature mode does not contribute to the development of the hyperthermic response, thus confirming our earlier conclusion that the nature of the factors involved is non-thermal. Further analysis is currently conducted to determine the relative contributions of two other activation modes, i.e., by vanilloids and by protons.