Supplemental Materials and Methods

Drugs. Aliquots of an ethanolic stock solution of A-425619 (4.2 mg/ml), AMG0347 (3.5 mg/ml), AMG517 (3 mg/ml), AMG8163 (6.7 mg/ml), AMG9810 (33.2 mg/ml), JYL1421 (83.3 mg/ml), and SB-366791 (7.1 mg/ml) were stored at –80°C. On the day of the experiment, the stocks of AMG0347, AMG517, and AMG8163 were diluted with saline to achieve a 50% ethanol concentration. These working solutions were infused to rats intravenously at a rate of 167 μl/kg/min for 2 min to deliver the following doses: 8-512 nmol/kg for AMG0347, 16-1024 nmol/kg for AMG517, and 16-1024 nmol/kg for AMG8163. To administer A-425619 (512-8192 nmol/kg), AMG9810 (4096-131072 nmol/kg), JYL1421 (1024-262144 nmol/kg), and SB-366791 (32768 nmol/kg), either undiluted ethanolic stock solutions were used, or the stocks were diluted with saline or 1,2-propanediol to achieve an ethanol concentration of 50%. These solutions were infused at a rate of 33-167 μl/kg/min for 2-40 min. To administer capsazepine (CPZ) to rats or guinea pigs intravenously, a working solution (18.5 mg/ml) in 100% ethanol was prepared *extempore* and infused at a rate of 33 μl/kg/min for 40 min, thus resulting in a total CPZ dose of 65536 nmol/kg. To administer AMG517 intraperitoneally to mice, aliquots of an ethanolic stock solution (3 mg/ml) were diluted with saline on the day of an experiment to achieve an ethanol concentration of 10%, and AMG517 at a dose of 256 nmol/kg (or its vehicle in control experiments) was injected as a bolus. In experiments in rats, a single dose of transient receptor potential vanilloid-1 (TRPV1) antagonist was used when a further increase in the dose was impossible (due to a solubility limit) or impractical. Control animals were infused with the corresponding vehicle (10, 50, or 100% ethanol in saline or 50% ethanol in 1,2-propanediol). Due to the use of very low infusion rates and total volumes, none of these administration regiments caused any detectable hemolysis or other sign of intoxication in the present and our previous (Steiner et al., 2007; Steiner et al., 2009) studies.

Formulas. The heat loss index (*HLI*) was calculated as:

$$HLI = \frac{T_{sk} - T_a}{T_c - T_a},$$

where T_{sk}, T_a, and T_c are skin, ambient, and colonic temperatures, respectively. The *HLI* changes between 0 (maximum heat conservation due to skin vasoconstriction) and 1 (theoretical maximum heat loss due to skin vasodilation) (Romanovsky et al., 2002).

The rate of oxygen consumption (VO_2) was calculated by comparing the oxygen fraction in the air exiting the chamber occupied by a rat (F) to the oxygen fraction in the air exiting an empty chamber (F_0):

$$VO_2 = \frac{A(F_0 - F)}{M - F_0 M(1 + Q)},$$

where A is air flow, Q is the respiratory quotient (considered to be 0.71), and M is the animal mass (Steiner et al., 2007).

Supplemental Model

It was assumed that the hyperthermic effect H of a TRPV1 antagonist occurs due to a blockade of three independent modes of TRPV1 activation: by heat (M_1), protons (M_2), and vanilloids (M_3). Thus,

$$H = H(\Delta M_1, \Delta M_2, \Delta M_3),$$

where ΔM_i is the extent to which a TRPV1 antagonist blocks the i-th mode of activation (i is either 1, 2, or 3). For the j-th antagonist (j is an integer between 1 and either 7 or 8, depending on whether the data obtained with JYL1421 were excluded or included, respectively), the extent of blockade in each activation mode was considered to be a function of the antagonist dose D_j:

$$\Delta M_i = \Delta M_i(D_1, D_2, \ldots, D_8).$$

Hence, the effect H was a function of those doses:

$$H = H(D_1, D_2, \ldots, D_8) = H(\Delta M_1(D_1, D_2, \ldots, D_8), \Delta M_2(D_1, D_2, \ldots, D_8), \Delta M_3(D_1, D_2, \ldots, D_8)).$$

The $H(D_1, D_2, \ldots, D_8)$ function was tabulated for each antagonist based on the experimental data presented in supplemental Table 1. It was required to find the sensitivity k_i of the hyperthermic effect H to the extent of blockade of each mode of TRPV1 activation:

$$k_i = \frac{\partial H(\Delta M_1, \Delta M_2, \Delta M_3)}{\partial \Delta M_i}.$$

For this, we first presented the derivative of H with respect to the dose of the j-th antagonist as:

$$\frac{\partial H(D_1, D_2, ..., D_8)}{\partial D_j} = \frac{\partial H(\Delta M_1, \Delta M_2, \Delta M_3)}{\partial \Delta M_1} \frac{\partial \Delta M_1(D_1, D_2, ..., D_8)}{\partial D_j} +$$
$$\frac{\partial H(\Delta M_1, \Delta M_2, \Delta M_3)}{\partial \Delta M_2} \frac{\partial \Delta M_2(D_1, D_2, ..., D_8)}{\partial D_j} + \frac{\partial H(\Delta M_1, \Delta M_2, \Delta M_3)}{\partial \Delta M_3} \frac{\partial \Delta M_3(D_1, D_2, ..., D_8)}{\partial D_j}.$$

With j taking seven or eight different values, this gave us seven or eight equations, respectively, from which the sensitivity measure k_i for each activation mode was found based on an assumption that a partial derivative $\partial M_i(D_1, D_2, \ldots, D_8)/\partial D_j$ is inversely proportional to d_{ij}, which is the IC_{50} value of the j-th antagonist in the i-th mode of TRPV1 activation (Table 1). Then,

$$\frac{\partial H}{\partial D_j} = \frac{k_1}{d_{1j}} + \frac{k_2}{d_{2j}} + \frac{k_3}{d_{3j}}.$$

The tabular data on $H(D_1, D_2, \ldots, D_8)$ were approximated by a sigmoid function commonly used for pharmacological dose-effect relationships:

$$H(D_1, D_2, ..., D_8) = v_0 + \frac{v_1}{e^{-(w_0 + w_1 D_1 + w_2 D_2 + ... + w_8 D_8)} + 1}.$$

Because the *in vivo* antagonist dose corresponding to d_{ij} was unknown, a simplifying assumption was made that $\partial H/\partial D_j$ is proportional to w_j with a proportionality coefficient independent of j. Since only the relative sensitivity of the effect H to the blockade of each mode of TRPV1 activation was of interest, the parameters $w_1, w_2, \ldots, w_8$ were expressed using the sensitivities k_1, k_2, and k_3 in the form of the following system of seven or eight (one for each value of j) linear equations:

$$w_j = \frac{k_1}{d_{1j}} + \frac{k_2}{d_{2j}} + \frac{k_3}{d_{3j}}.$$

Hence, $H(D_1, D_2, \ldots, D_8)$ was a function that contained six unknown parameters: the sensitivities k_1, k_2, and k_3 and the constant parameters v_0, v_1, and w_0, which were identified through best-fitting the tabulated data to the mathematical model described above.

To avoid a systematic approximation error in this nonlinear model, we accounted for the statistical variance of H. Using a Monte Carlo simulation technique (Metropolis and Ulam, 1949), 1000 datasets (replicates) were generated randomly, according to the Gaussian probability distribution, with the mean and SE values taken from supplemental Table 1. The datasets generated were used to find the unknown parameters k_1, k_2, k_3, v_0, v_1, and w_0 by applying the standard least squares technique (Wolberg, 2006). Thus, 1000 sets of the six unknown parameters were obtained, from which the mean value and the variance estimates were calculated for each sensitivity.

To estimate the corresponding number of observations N (required for statistical comparisons between the mean values of the sensitivities), the following considerations were made. It was assumed that the measurements on the dose-temperature effect produced by the drug and that produced by the vehicle are mutually uncorrelated. Then, the statistical variance V of the difference between those effects should be equal to the sum of the respective variances, $V_{drug} + V_{vehicle}$. At the same time, the corresponding variance of the mean difference equals the sum of the respective variances of the mean values of the drug and vehicle dose-temperature effects:

$$\frac{V}{N} = \frac{V_{drug}}{N_{drug}} + \frac{V_{vehicle}}{N_{vehicle}},$$

where N_{drug} and $N_{vehicle}$ are the numbers of observations related to a specific dose of a drug or vehicle, respectively. Therefore,

$$N = \frac{V_{drug} + V_{vehicle}}{\frac{V_{drug}}{N_{drug}} + \frac{V_{vehicle}}{N_{vehicle}}}.$$

The calculated sum of N values across all entries of H in supplemental Table 1 equals 210 (with JYL1421) or 172 (without JYL1421). To find an equivalent for the number of observations, this sum was divided by the number of freedom degrees corresponding to the set of the six unknown parameters. To take into account any mutual cross-correlation between these six parameters, a significant dimensionality of the linear space formed by these parameters was computed as described elsewhere (Shimansky, 2006). A significant dimensionality was found to be 3.6, yielding an equivalent for the number of observations of 58.2 (with JYL1421) or of 47.7 (without JYL1421). This equivalent was used to perform statistical comparisons between the sensitivities.

Supplemental References

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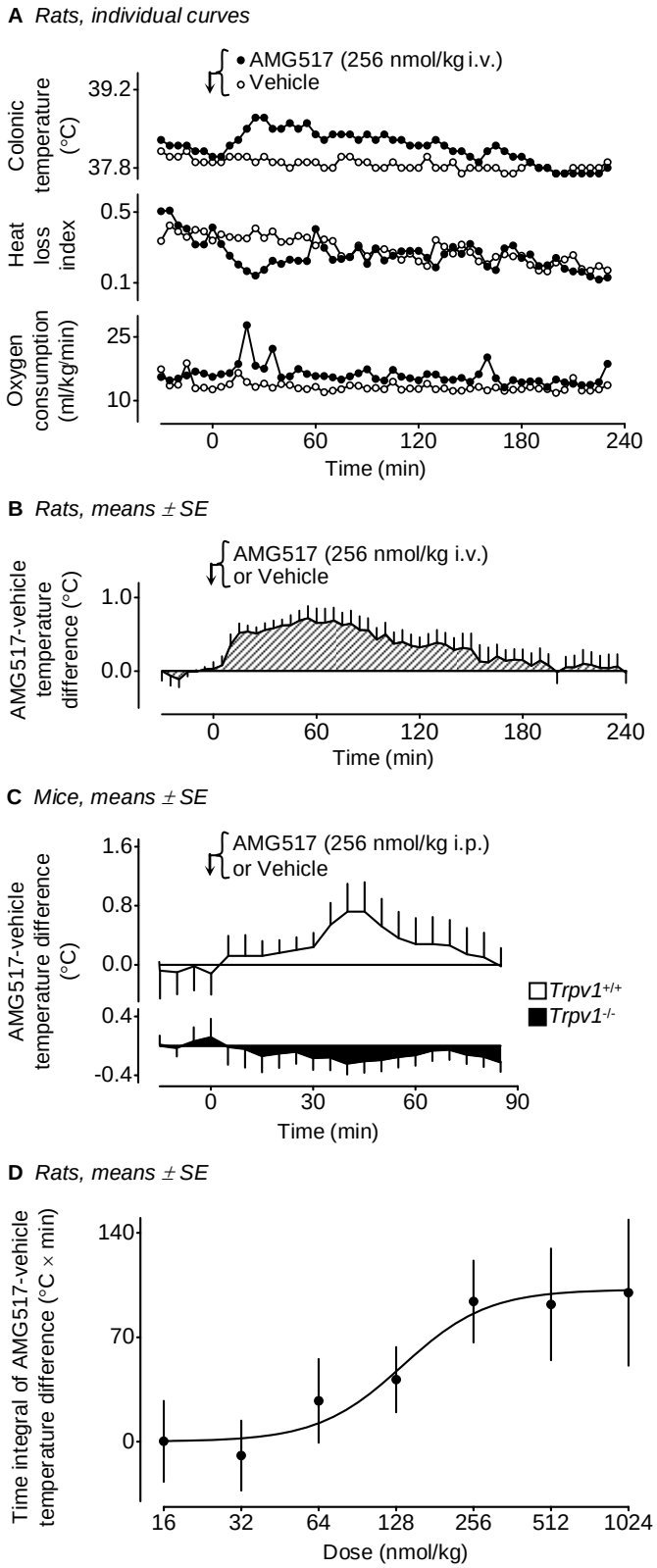
Supplemental Figure



Supplemental Figure 1. The hyperthermic response to AMG517 in rats and mice. ***A***, Recruitment of autonomic thermoeffectors in the hyperthermic response of rats to AMG517 (individual curves). The hyperthermic response (an increase in T_c) occurs in response to AMG517 (dose indicated) but not its vehicle (top panel). The response involves tail skin vasoconstriction (a decrease in the *HLI*; middle panel) and an increase in thermogenesis (VO_2; bottom panel). ***B***, The difference in body temperature between AMG517- and vehicle-treated rats. The number of animals used is 6 and 8, respectively. ***C***, The difference in body temperature between AMG517- and vehicle-treated mice with or without a homozygous targeted null mutation of the *Trpv1* gene (*Trpv1*$^{-/-}$ and $^{+/+}$, respectively). Both AMG517 and its vehicle were injected to 5 *Trpv1*$^{+/+}$ mice and 10 *Trpv1*$^{-/-}$ mice. The hyperthermic response occurred in *Trpv1*$^{+/+}$ (top panel) but not *Trpv1*$^{-/-}$ (bottom panel) mice. ***D***, The dose-dependence curve for the hyperthermic response to AMG517 in rats. The measure of the hyperthermic effect *H* was determined as a time integral of the difference between the mean T_c curve of rats treated with a given dose of a TRPV1 antagonist and the mean T_c curve of rats treated with the corresponding vehicle; calculated over 0-180 min. Each point is the result of 3-6 experiments with AMG517 and 8 experiments with the corresponding vehicle.

Supplemental Table

Supplemental Table 1. The hyperthermic effect *H* (mean ± SE) for different doses of TRPV1 antagonists (°C × min)

Dose nmol/kg	AMG0347	AMG517	AMG8163	A-425619	AMG9810	JYL1421	SB-366791	CPZ
8	-2.1 ± 20.3							
16	-9.5 ± 16.4	-12.6 ± 24.2	13.8 ± 15.9					
32	25.8 ± 28.4	-22.2 ± 20.0	-10.7 ± 13.4					
64	100.5 ± 53.4	14.5 ± 25.3	44.9 ± 38.0					
128	102.3 ± 17.8	28.7 ± 18.1	77.5 ± 38.9					
256	84.3 ± 30.5	81.1 ± 24.6	115.7 ± 23.7					
512	133.6 ± 23.9	79.2 ± 35.4	96.3 ± 14.1	-29.5 ± 21.6				
1024		87.0 ± 47.4	86.9 ± 32.6	15.8 ± 56.6		27.3 ± 42.9		
2048				44.6 ± 34.7		30.4 ± 56.8		
4096				31.9 ± 29.5	-6.2 ± 31.2	35.5 ± 29.7		
8192				108.5 ± 26.7	-0.8 ± 39.6	21.6 ± 25.2		
16384					20.1 ± 32.3	-17.5 ± 23.7		
32768					34.9 ± 29.6	-19.3 ± 27.1	3.3 ± 43.1	
65536					127.8 ± 31.3			13.8 ± 24.2
131072					122.0 ± 67.5			
262144						-84.4 ± 29.2		