SUPPLEMENTARY MATERIAL

METHODS

$[Ca^{2+}]_i$ measurement in isolated dorsal root ganglion neurons. Dorsal root ganglion neuronal cultures were prepared as described previously (Depree *et al*., 1994). Briefly, ganglia were removed from spinal cords taken from embryonic day 15 (E-15) rat embryos and dissociated with 0.25% trypsin in Hank's balanced salt solution. Following trituration, cells were plated onto the Matrigel coated No. 1 cover glass slips (22 x 40 mm) in the plating medium consisting of Eagle's minimum essential medium supplemented with 10% NuSerum, 0.3% additional glucose, and crude nerve growth factor. Cultures were maintained in an incubator at 37^o with 5% CO_2 overnight and used the next day for intracellular ion activity measurements. The cover glass slips formed the bottom of the recording/perfusion chamber that was held in a chamber platform and placed on the stage of an upright Zeiss Axioskop 2 plus upright fluorescence microscope. The dorsal root ganglion neurons were perfused with the same control solution as described above. The changes in relative Ca^{2+} activity were determined by loading dorsal root ganglion neurons with Fura-2-AM. Cells were loaded with Fura-2-AM (10 µM) in the presence of 0.15% pluronic at room temperature for 4 h. Before the experiment was started, the cells were perfused on both sides with control solution for 15 min. The cells were alternately excited at 340 nm and 380 nm and imaged at 10 s intervals. The emitted light was detected with a set up containing a 430 nm dichroic beam splitter, and a 510 nm emission

filter (20 nm band pass). All measurements were performed at room temperature. The relative changes in fluorescence intensity ratio (FIR; F_{340}/F_{380}) were expressed as the mean ± SEM of N, where N = number of cells studied.

RESULTS

Effect of external pH (pH_o) on the Bz-insensitive NaCl chorda tympani response. Protons act as endogenous modulators of the VR-1 receptor. The cloned VR-1 receptor is activated by a reduction in pH_o (Caterina *et al*., 1997; Davis *et al*., 2002; Gunthorpe *et al*., 2002), and at high concentration H^+ ions can block the VR-1 receptor. However, in our studies, stimulating the tongue with rinse solution (10 mM KCl) and NaCl stimulating solutions (100 mM NaCl + 5 μM Bz) adjusted to pH values between 2 and 10 had no significant effect on chorda tympani responses (Fig. S1) (Lyall *et al*., 2002). The results suggest that in the absence of a ligand, the amiloride-insensitive NaCl chorda tympani response is not modulated by changes in pH_o.

Effect of pH_o on the temperature-induced changes in the Bz-insensitive NaCl chorda tympani response. In the absence of RTX, stimulating the tongue with 100 mM NaCl + 5 μM Bz (pH 6) at temperatures between 23^o and 55.5^o, increased chorda tympani responses with a $t_{0.5}$ of 39.9 ± 0.13^o (Fig. S2; filled circles; N = 13). Increasing pH_o from 6.0 to 9.7 gave a $t_{0.5}$ value of 40.2 ± 0.09^o (Fig. S2; open circles; N = 4; $p > 0.05$). These results indicate that in the absence of a ligand, changes in pH_o do not affect the temperature threshold of the Bz-insensitive NaCl chorda tympani response. This is consistent with the observations that in the absence of a ligand, the amiloride-insensitive NaCl chorda tympani response was insensitive to changes in pH_o (Fig. S1) (Lyall *et al.*, 2002).

RTX-induced changes in the Bz-insensitive NaCl chorda tympani response are independent of the alterations in the taste receptor cell intracellular pH (pH_i). Changes in pH_o induce changes in taste receptor cell pH_i (Lyall *et al*., 2002). In order to differentiate if changes in pH_o or pH_i modulate the effect of RTX on the Bz-insensitive NaCl chorda tympani response, we investigated if changes in taste receptor cell pH_i (while maintaining constant pH_o) can modulate the effect of RTX on the Bz-insensitive NaCl chorda tympani response. In polarized taste receptor cells switching from a HEPES-buffered Ringer's solution (pH 7.4) to a similar Ringer's solution buffered with HCO_3^-/CO_2, also at pH 7.4, reversibly decreased taste receptor cell pH_i (Lyall *et al*., 2002). Consistent with this, switching from a rinse solution buffered with HEPES (RH = 72 mM KCl + 200 mM mannitol + 10 mM HEPES; pH 7.4) to a similar rinse solution buffered with HCO_3^-/CO_2 (RCO_2 = 72 mM $KHCO_3$ + 10% CO_2 + 200 mM mannitol; pH 7.4) increased the chorda tympani response relative to RH (Fig. S3). Figure S3 further shows that stimulating the tongue with 100 mM NaCl + 5 μM Bz + 0.75 μM RTX adjusted to pH 7.4 with either HEPES (*a*-*b*-*c*) or HCO_3^-/CO_2 (*d*-*e*-*f*) produced similar increases in the magnitude of the chorda tympani response relative to NaCl alone. These results suggest that at constant pH_o, a decrease in taste receptor cell pH_i induced by the entry of dissolved CO_2 does not potentiate the effect of RTX on the amiloride-insensitive cation channel in taste receptor cells. These data are consistent with the hypothesis that protons act by binding to the extracellular surface of the VR-1 receptor (Jordt *et al*., 2000; Gunthorpe *et al*.,

2002) and modulate receptor activity by shifting the dose response curves for the vanilloid compounds to the left (Jordt *et al*., 2000).

Isolated rat dorsal root ganglion neurons respond to RTX and CPC in the same rank order as in taste receptor cells. Vanilloid receptors are expressed in rat dorsal root ganglion neurons (Caterina *et al*., 1997; Caterina & Julius, 2001). In primary cultures of rat dorsal root ganglion neurons maintained on matrigel coated glass coverslips and loaded with fura-2 (Depree *et al*., 1994), perfusing the cells with Ringer's solution containing 25 μM CPC, 2 out of 13 neurons responded with an increase in fluorescence intensity ratio (FIR; $F_{340}/F_{380;}$ Fig. S4A; inset), indicating an increase in $[Ca^{2+}]_i$. Increasing the CPC concentration to 50 μM decreased the fluorescence intensity ratio towards baseline. However, in the presence of 50 μM CPC all of the other 11 cells responded with an increase in $[Ca^{2+}]_i$ (Fig. S4A). Following a maximum increase in $[Ca^{2+}]_i$, there was a spontaneous decrease in $[Ca^{2+}]_i$ with time. In another set of dorsal root ganglion neurons, 0.25 μM RTX reversibly increased the fluorescence intensity ratio in 7 out of 8 cells studied (Fig. S4B). The results indicate that dorsal root ganglion neurons that express native vanilloid receptors are heterogeneous, responding to RTX and CPC in the same rank order of potency as observed in taste receptor cells. However, in contrast to dorsal root ganglion neurons, perfusing the apical membrane of polarized taste receptor cells produced a sustained increase in $[Na^{+}]_i$ without receptor desensitization. In contrast to Bz-insensitive NaCl chorda tympani responses, the recombinant VR-1

expressed in HEK 293 cells is not responsive to CPC (J.B. Davis, personal communication).

REFERENCES

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **389**, 816-824.

Caterina MJ & Julius D (2001). The vanilloid receptor: a molecular gateway to the pain pathway. *Annu Rev Neurosci* **24**, 487-517.

Davis JB, Smart D & Gunthorpe MJ (2002). The vanilloid receptor and vanilloid receptor-like genes: A hot topic getting hotter. *Cell Transmissions* **18**, 3-9.

Depree JL & Bigbee JW (1994). Retardation of neuritic outgrowth and cytoskeletal changes accompanying acetylcholine esterase inhibitor treatment in cultured rat dorsal root ganglion neurons. *J Neurosci Res* **39**, 567-575.

Gunthorpe MJ, Benham CD, Randall A & Davis JB (2002). The diversity in the vanilloid (TRPV) receptor family of ion channels. *Trends Pharmacol Sci* **23**,183-191.

Jordt SE, Tominaga M & Julius D (2000). Acid potentiation of the capsaicin receptor determined by a key extracellular site. *Proc Nat Acad Sci USA.* **97**, 8134-8139.

Lyall V, Alam RI, Phan THT, Russell OF, Malik SA, Heck GL *et al*. (2002). Modulation of rat chorda tympani NaCl responses and intracellular Na^+ activity in polarized taste receptor cells by pH. *J Gen Physiol* **120**, 793-815.

FIGURE LEGENDS

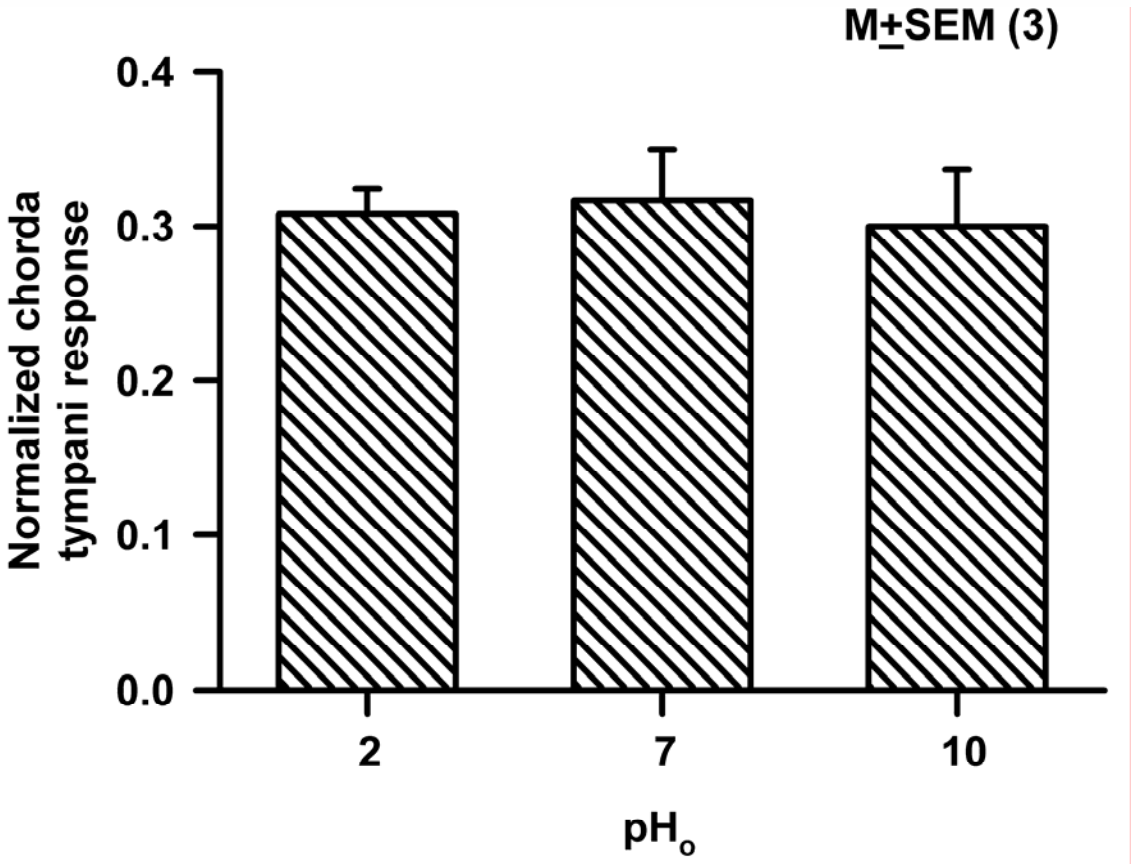
Fig. S1. Effect of external pH (pH_o) on the Bz-insensitive NaCl chorda tympani response. The lingual surface was superperfused with rinse solution (10 mM KCl + 200 mM mannitol + 10 mM HEPES) and NaCl solution (10 mM KCl + 100 mM NaCl + 5 μM Bz + 10 mM HEPES) adjusted to either pH 2, 7, or 10. In each animal the NaCl chorda tympani response was normalized to the chorda tympani response obtained with 300 mM NH_4Cl. The values are presented as the mean ± SEM of N (number of animals).

Fig. S2. Effect of pH_o on temperature-induced changes in the Bz-insensitive NaCl chorda tympani response. The chorda tympani responses were recorded during superfusion of the lingual surface with a rinse solution (R = 10 mM KCl + 200 mM mannitol + 10 mM HEPES; pH 6.0) and stimulating solutions (10 mM KCl + 100 mM NaCl + 5 μM Bz + 0.25 μM RTX + 10 mM HEPES; pH 6.0) (open circles). Similar rinse and stimulating solutions were used at pH 9.7 (open circles). While the rinse solution was superfused at 23^o, the NaCl stimulating solutions were superfused at temperatures varying from 23^o to 55.5^o. Values are presented as the mean ± SEM of N; where N = number of animals used under each condition (see text for details).

Fig. S3. **Effect of pH_i on RTX-induced changes in chorda tympani responses to NaCl.** The experiments were done at constant pH_o and osmolarity. The chorda tympani responses were recorded during superfusion of the lingual

surface with a rinse solution (RH = 72 mM KCl + 200 mM mannitol + 10 mM HEPES; pH 7.4) and with stimulating solutions containing 72 mM KCl + 100 mM NaCl + 10 mM HEPES + 5 μM Bz (pH 7.4) with and without 0.75 μM RTX. In the second part of the experiment, the chorda tympani responses were recorded during superfusion of the lingual surface with a rinse solution (RCO_2 = 72 mM $KHCO_3$ + 10% CO_2 + 200 mM mannitol; pH 7.4) and with stimulating solutions containing 72 mM $KHCO_3$ + 10% CO_2 + 100 mM NaCl + 5 μM Bz (pH 7.4) in the presence and absence of 0.75 μM RTX.

Fig. S4. Effect of CPC and RTX on $[Ca^{2+}]_i$ in isolated dorsal root ganglion neurons loaded with fura-2. (A) Isolated dorsal root ganglion neurons in primary culture loaded with fura-2 were perfused with control Ringer's solution and with Ringer's solution containing 25 μM and 50 μM CPC. The relative changes in fluorescence intensity ratio (FIR; F_{340}/F_{380}) of dorsal root ganglion neurons reflect changes in $[Ca^{2+}]_i$. Values are presented as the mean ± SEM of 11. The insert shows the effect of CPC in 2 individual dorsal root ganglion neurons. (**B**) In another set of isolated dorsal root ganglion neurons, stimulating the cells with 0.25 μM RTX increased the fluorescence intensity ratio in 7 out of 8 cells studied. Values are presented as the mean ± SEM of 7 cells.


M±SEM (3)
Normalized chorda tympani response
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0.3
0.2
0.1
0.0
2
7
10
pH$_o$

Fig. S1

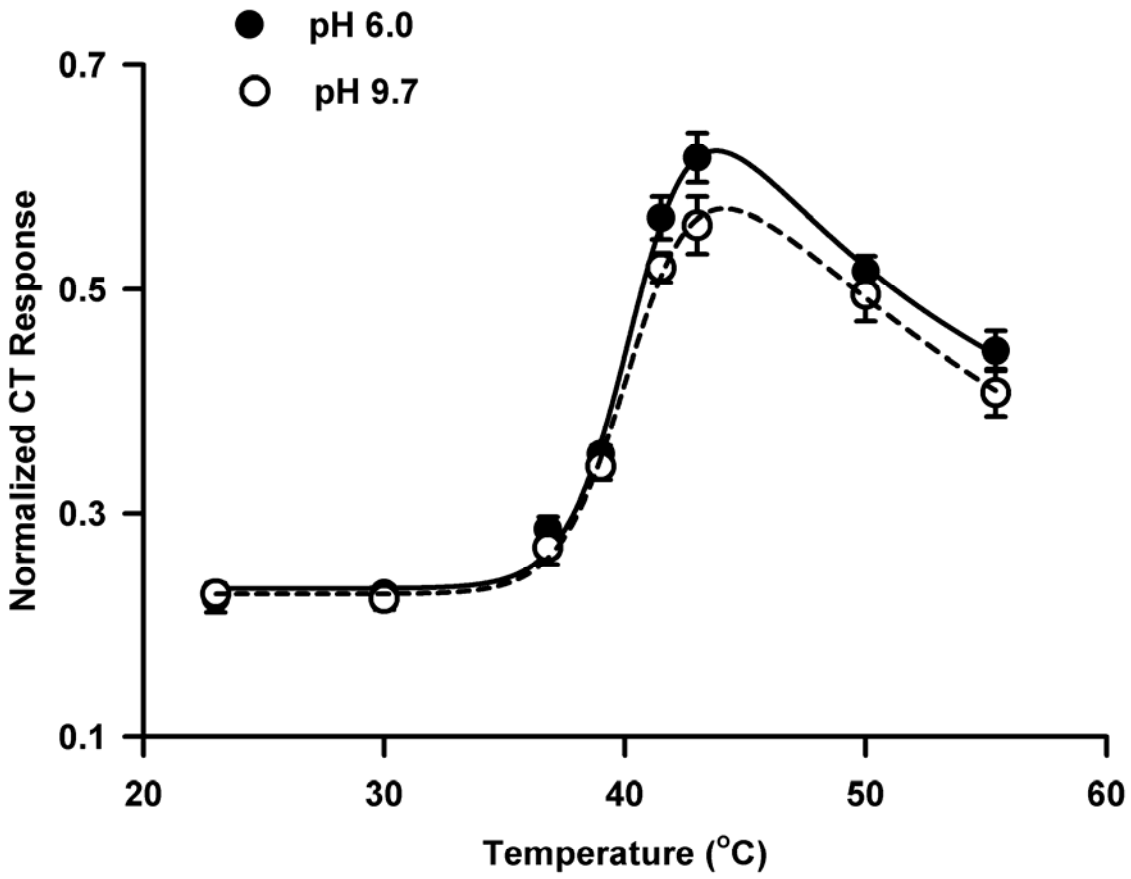
pH 6.0
pH 9.7
0.7
0.5
0.3
0.1
Normalized CT Response
20
30
40
50
60
Temperature (°C)

Fig. S2

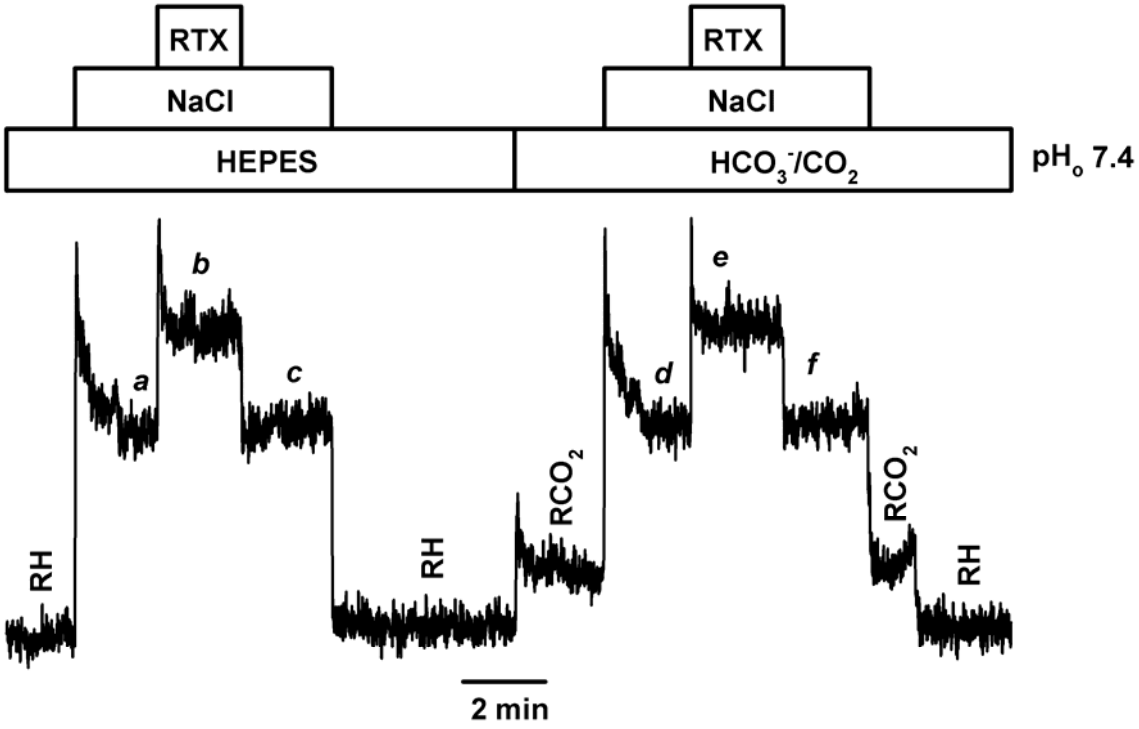
RTX
NaCl
HEPES
RTX
NaCl
HCO3-/CO2
pHo 7.4
a
b
c
d
e
f
RH
RH
RH
RCO2
RCO2
2 min

Fig. S3

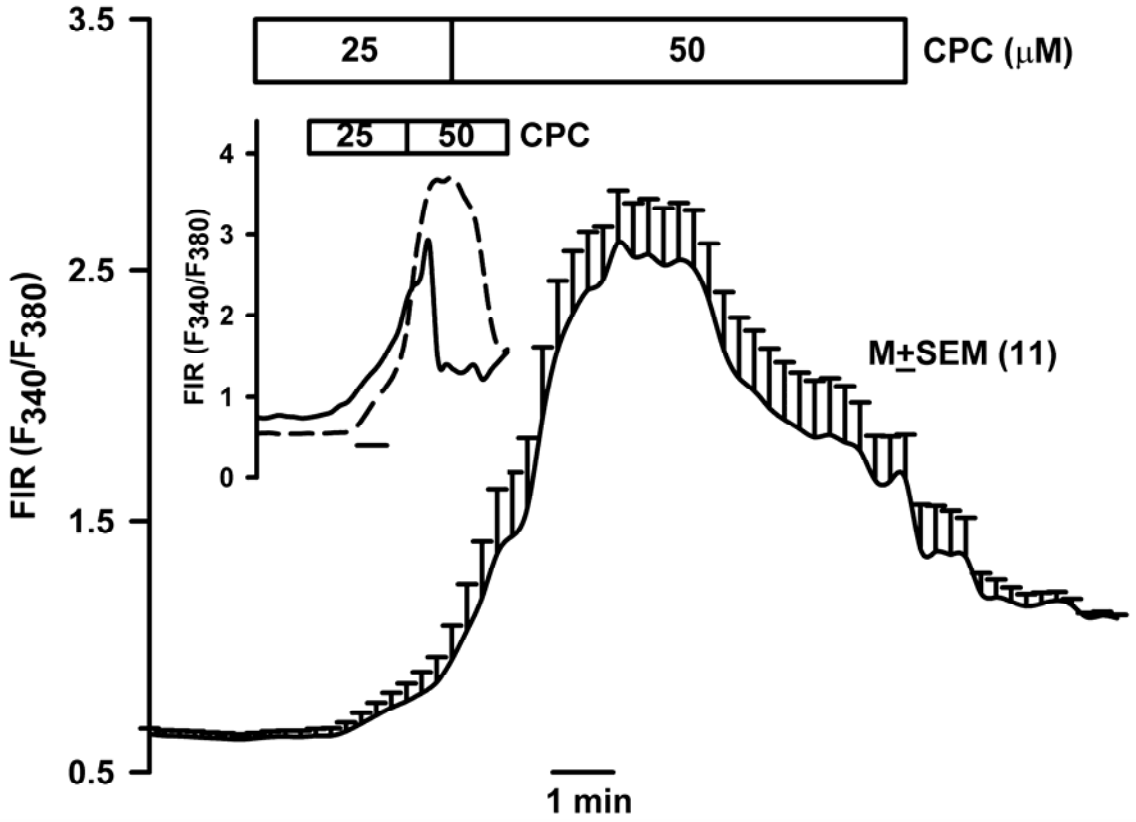
3.5
2.5
1.5
0.5
FIR (F340/F380)
25
50
CPC (μM)
25
50
CPC
4
3
2
1
0
FIR (F340/F380)
M±SEM (11)
1 min

Fig. S4A

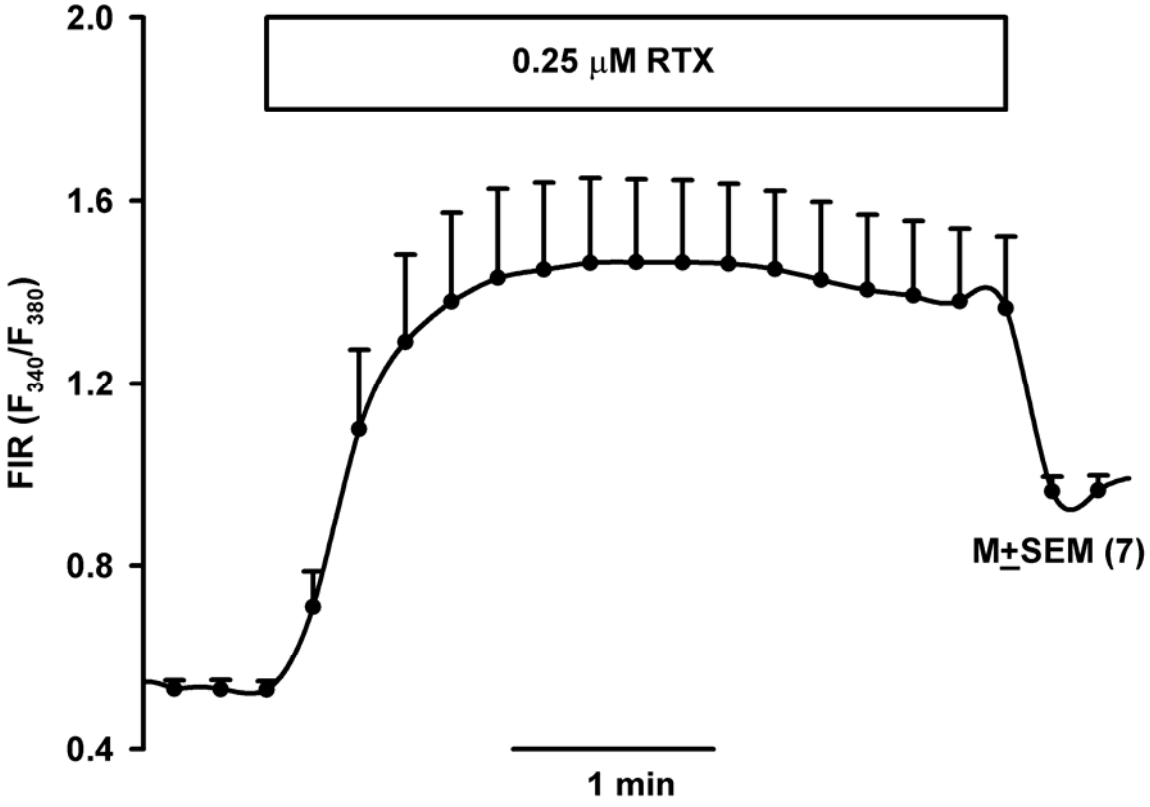

Fig. S4B