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# Acidosis antagonizes intracellular calcium response to $\kappa$ -opioid receptor stimulation in the rat heart

JIAN-MING PEI, XIAO-CHUN YU, JIN-SONG BIAN, AND TAK-MING WONG  
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**Pei, Jian-Ming, Xiao-Chun Yu, Jin-Song Bian, and Tak-Ming Wong.** Acidosis antagonizes intracellular calcium response to  $\kappa$ -opioid receptor stimulation in the rat heart. *Am. J. Physiol.* 277 (*Cell Physiol.* 46): C492–C500, 1999.—To study the effects of  $\kappa$ -opioid receptor stimulation on intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) homeostasis during extracellular acidosis, we determined the effects of  $\kappa$ -opioid receptor stimulation on  $[\text{Ca}^{2+}]_i$  responses during extracellular acidosis in isolated single rat ventricular myocytes, by a spectrofluorometric method. U-50488H (10–30  $\mu\text{M}$ ), a selective  $\kappa$ -opioid receptor agonist, dose dependently decreased the electrically induced  $[\text{Ca}^{2+}]_i$  transient, which results from the influx of  $\text{Ca}^{2+}$  and the subsequent mobilization of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum (SR). U-50488H (30  $\mu\text{M}$ ) also increased the resting  $[\text{Ca}^{2+}]_i$  and inhibited the  $[\text{Ca}^{2+}]_i$  transient induced by caffeine, which mobilizes  $\text{Ca}^{2+}$  from the SR, indicating that the effects of the  $\kappa$ -opioid receptor agonist involved mobilization of  $\text{Ca}^{2+}$  from its intracellular pool into the cytoplasm. The  $\text{Ca}^{2+}$  responses to 30  $\mu\text{M}$  U-50488H were abolished by 5  $\mu\text{M}$  nor-binaltorphimine, a selective  $\kappa$ -opioid receptor antagonist, indicating that the event was mediated by the  $\kappa$ -opioid receptor. The effects of the agonist on  $[\text{Ca}^{2+}]_i$  and the electrically induced  $[\text{Ca}^{2+}]_i$  transient were significantly attenuated when the extracellular pH ( $\text{pH}_e$ ) was lowered to 6.8, which itself reduced intracellular pH ( $\text{pH}_i$ ) and increased  $[\text{Ca}^{2+}]_i$ . The inhibitory effects of U-50488H were restored during extracellular acidosis in the presence of 10  $\mu\text{M}$  ethylisopropyl amiloride, a potent  $\text{Na}^+/\text{H}^+$  exchange blocker, or 0.2 mM  $\text{Ni}^{2+}$ , a putative  $\text{Na}^+/\text{Ca}^{2+}$  exchange blocker. The observations indicate that acidosis may antagonize the effects of  $\kappa$ -opioid receptor stimulation via  $\text{Na}^+/\text{H}^+$  and  $\text{Na}^+/\text{Ca}^{2+}$  exchanges. When glucose at 50 mM, known to activate the  $\text{Na}^+/\text{H}^+$  exchange, was added, both the resting  $[\text{Ca}^{2+}]_i$  and  $\text{pH}_i$  increased. Interestingly, the effects of U-50488H on  $[\text{Ca}^{2+}]_i$  and the electrically induced  $[\text{Ca}^{2+}]_i$  transient during superfusion with glucose were significantly attenuated; this mimicked the responses during extracellular acidosis. When a high- $\text{Ca}^{2+}$  (3 mM) solution was superfused, the resting  $[\text{Ca}^{2+}]_i$  increased; the increase was abolished by 0.2 mM  $\text{Ni}^{2+}$ , but the  $\text{pH}_i$  remained unchanged. Like the responses to superfusion with high-concentration glucose and extracellular acidosis, the responses of the  $[\text{Ca}^{2+}]_i$  and electrically induced  $[\text{Ca}^{2+}]_i$  transients to 30  $\mu\text{M}$  U-50488H were also significantly attenuated. Results from the present study demonstrated for the first time that extracellular acidosis antagonizes the effects of  $\kappa$ -opioid receptor stimulation on the mobilization of  $\text{Ca}^{2+}$  from SR. Activation of both  $\text{Na}^+/\text{H}^+$  and  $\text{Na}^+/\text{Ca}^{2+}$  exchanges, leading to an elevation of  $[\text{Ca}^{2+}]_i$ , may be responsible for the antagonistic action of extracellular acidosis against  $\kappa$ -opioid receptor stimulation.

sodium/hydrogen exchange; sodium/calcium exchange; sarcoplasmic reticulum

ACIDOSIS IS WELL KNOWN TO affect the cardiac functions (9, 12, 24). During myocardial ischemia, extracellular acidosis occurs with a lowered intracellular pH ( $\text{pH}_i$ ), which decreases contractility (3, 33). It has been shown that intracellular acidosis activates  $\text{Na}^+/\text{H}^+$  exchange, leading to an increased extrusion of  $\text{H}^+$  in exchange for an influx of  $\text{Na}^+$  (9, 25, 29). The increased activity of  $\text{Na}^+/\text{H}^+$  exchange in turn activates the reverse mode of  $\text{Na}^+/\text{Ca}^{2+}$  exchange, leading to an increased influx of  $\text{Ca}^{2+}$  and an elevation of intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) (5, 30). The altered  $[\text{Ca}^{2+}]_i$  homeostasis may account, at least in part, for the altered contractility during extracellular acidosis (31, 42).

Both  $\kappa$ -opioid receptors (27, 44, 45, 53, 54) and  $\kappa$ -opioid peptides (48) are present in the heart. Previous studies have shown that during myocardial ischemia the  $\kappa$ -opioid receptor is activated presumably because of an increased release of  $\kappa$ -opioid peptides from the heart (50, 51). It has been shown previously that  $\kappa$ -opioid receptor stimulation with a selective  $\kappa$ -opioid receptor agonist increases  $[\text{Ca}^{2+}]_i$  by mobilizing  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum (SR) via the phospholipase C/ $\text{Ca}^{2+}$  pathway (7, 36–38, 43, 46, 47). It has been shown that  $\kappa$ -opioid receptor stimulation also negatively modulates the stimulatory effects of  $\beta$ -adrenoreceptor stimulation on cardiac contractility (52). Depletion of  $\text{Ca}^{2+}$  from SR and negative modulation of the  $\beta$ -adrenoreceptor are responsible, at least in part, for the inhibitory action of  $\kappa$ -opioid receptor stimulation on cardiac contractility.

Because both acidosis and  $\kappa$ -opioid receptor activation occur during myocardial ischemia, it would be important to study the interaction between acidosis and  $\kappa$ -opioid receptor stimulation and the underlying mechanisms. The effect of acidosis on  $\kappa$ -opioid receptor stimulation is, however, not known. In the present study we determined the  $\text{Ca}^{2+}$  responses to  $\kappa$ -opioid receptor stimulation in the heart during extracellular acidosis. We measured the  $[\text{Ca}^{2+}]_i$  transients induced electrically and with caffeine, in addition to the resting  $[\text{Ca}^{2+}]_i$ , in a single isolated ventricular myocyte preparation. The electrically induced  $[\text{Ca}^{2+}]_i$  transient results from the influx of  $\text{Ca}^{2+}$  on membrane depolarization, which triggers  $\text{Ca}^{2+}$  release from the SR via a  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release mechanism, and has been shown to correlate directly to the contraction of the myocyte (52). The caffeine-induced  $[\text{Ca}^{2+}]_i$  transient is an index of the  $\text{Ca}^{2+}$  content in the SR because caffeine depletes SR of  $\text{Ca}^{2+}$  (4, 40). We also determined the  $\text{Ca}^{2+}$  responses to  $\kappa$ -opioid receptor stimulation after manipulations that stimulate or mimic  $\text{Na}^+/\text{H}^+$  exchange and  $\text{Na}^+/\text{Ca}^{2+}$  exchange in normal extracellular

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lar pH ( $pH_e$ ). In addition, we determined the  $Ca^{2+}$  responses during extracellular acidosis when these two exchanges were inhibited. Results from the present study showed that extracellular acidosis antagonizes the effects of  $\kappa$ -opioid receptor stimulation on  $Ca^{2+}$  responses in the heart. This action may result from activation of  $Na^+/H^+$  and  $Na^+/Ca^{2+}$  exchanges, which elevates the  $[Ca^{2+}]_i$ .

## MATERIALS AND METHODS

**Isolation of ventricular myocytes.** Ventricular myocytes were isolated from the hearts of male Sprague-Dawley rats (190–210 g), by a collagenase perfusion method described previously (14). Immediately after decapitation, the hearts were rapidly removed from the rats and perfused in a retrograde manner at a constant flow rate (10 ml/min) with oxygenated Joklik MEM supplemented with 1.25 mM  $CaCl_2$  and 10 mM HEPES, pH 7.2, at 37°C for 5 min; this was followed by 5 min with the same medium free of  $Ca^{2+}$ . Collagenase was then added to the medium to a concentration of 125 U/ml with 0.1% (wt/vol) BSA. After 35–45 min of perfusion with a medium containing collagenase, the atria were discarded. The ventricular tissue was transferred to an oxygenated solution that was the same as that described above but without collagenase; the tissue was cut into small pieces with a pair of scissors, and then the solution was stirred with a glass rod for 5 min to separate the ventricular myocytes from each other. The residue was filtered through 250- $\mu$ m mesh screens, sedimented by centrifugation at 100 *g* for 1 min, and resuspended in fresh Joklik solution with 2% BSA. More than 70% of the cells were rod shaped and impermeable to trypan blue. The  $Ca^{2+}$  concentration of the Joklik solution was increased gradually to 1.25 mM in 40 min.

**Measurement of  $[Ca^{2+}]_i$ .** Ventricular myocytes were incubated with fura 2-AM (5  $\mu$ M) in Joklik solution supplemented with 1.25 mM  $CaCl_2$  for 30 min. The unincorporated dye was removed by washing the cells twice in fresh incubation solution. The loaded cells were kept at room temperature (24–26°C) for 30 min before measurements of  $[Ca^{2+}]_i$  to allow the fura 2-AM in the cytosol to deesterify. Loading with a low concentration of fura 2-AM and at a relatively low temperature of 24–26°C was done to minimize the effects of the compartmentalization of the esters (39).

The ventricular myocytes loaded with fura 2-AM were transferred to the stage of an inverted microscope (Nikon) in a superfusion chamber at room temperature. The inverted microscope was coupled with a dual-wavelength excitation spectrofluorometer (Photo Technical International). The myocytes were perfused with a Krebs bicarbonate buffer containing (in mM) 118 NaCl, 5 KCl, 1.2  $MgSO_4$ , 1.2  $KH_2PO_4$ , 1.25  $CaCl_2$ , 25  $NaHCO_3$ , and 11 glucose, with 1% dialyzed BSA and a gas phase of 95%  $O_2$ -5%  $CO_2$ , pH 7.4. To produce extracellular acidosis, HCl was used to adjust to pH 6.8, which was chosen on the basis of previous studies (1, 11, 28). The myocytes selected for the study were rod shaped with clear striations. They were quiescent but exhibited a synchronous contraction (twitch) in response to suprathreshold 4-ms stimuli at 0.2 Hz delivered by a stimulator (Grass S88) through two platinum field stimulation electrodes in the bathing fluid. Fluorescence signals obtained at 340-nm ( $F_{340}$ ) and at 380-nm ( $F_{380}$ ) excitation wavelengths were stored in a computer for data processing and analysis. The  $F_{340}/F_{380}$  ratio was used to represent  $[Ca^{2+}]_i$  changes in the myocytes. In some experiments, the resting  $[Ca^{2+}]_i$  was observed while the electrical stimulation was off.

**Measurement of  $pH_i$ .** The  $pH_i$  was measured in a single myocyte as described previously (10). The apparatus and optical arrangement used for the measurement of fluorescent light emission and the preparation procedure were similar to those described in the previous section except that the cells were loaded with the membrane-permeable 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM as the fluorescence indicator at 5  $\mu$ M for 30 min. The loaded cells were transferred to the stage of an inverted microscope in the superfusion chamber at room temperature. Myocytes were continuously superfused with a Krebs solution (normal solution;  $pH_e = 7.4$ ) or an acidic solution ( $pH_e = 6.8$ ), as described above, according to the requirement of the experiment. The pH-dependent signal of BCECF was obtained by illuminating at 490 and 435 nm, and the fluorescence emission wavelength was measured at 520 nm. The ratio of fluorescence at 490 nm ( $F_{490}$ ) to  $F_{435}$  was used to represent  $pH_i$ .

At the end of each experiment, the calibration of BCECF signals was performed.  $pH_i$  was set to the  $pH_e$  with 10  $\mu$ M nigericin in the calibration solution (in mM: 12 HEPES, 140 KCl, 1  $MgCl_2$ , 11 glucose). The  $pH_e$  values were adjusted to 8, 7, 6, and 5 with KOH or HCl.

**Drugs and chemicals.** U-50488H [trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide], fura 2-AM, type I collagenase, and nigericin were purchased from Sigma. The nor-binaltorphimine (nor-BNI) was purchased from Tocris Cookson. BCECF-AM and ethylisopropyl amiloride (EIPA) were purchased from Research Biochemicals Incorporated.

Fura 2-AM, BCECF-AM, and EIPA were dissolved in DMSO, nigericin was dissolved in ethanol, and the rest were dissolved in distilled water.

U-50488H at the dose range of 10–30  $\mu$ M was administered for 10 min because preliminary studies showed that the effects of the opioid were obvious at 2–3 min and reached maximum before 10 min. In experiments conducted during extracellular acidosis the  $\kappa$ -opioid agonist was administered for 10 min after the perfusion of a solution at  $pH_e$  6.8 for 10 min because we also found that the effect of acidosis reached the maximum at  $\sim$ 10 min. The dose range used in the present study has been shown to increase the  $[Ca^{2+}]_i$  and inositol 1,4,5-trisphosphate level, effects antagonized by 1–5 mM nor-BNI (51, 54, 55), which itself had no effect on any of the preparations studied. In a preliminary experiment, 10  $\mu$ M EIPA did not alter the autofluorescence of the cell at the BCECF excitation wavelength as previously reported (29). The final concentration of DMSO was 0.1%, and at this concentration DMSO had no effect on either  $[Ca^{2+}]_i$  or  $pH_i$  (29).

**Statistical analysis.** Values are expressed as means  $\pm$  SE. The paired Student's *t*-test was used to determine the difference between control and drug treatment groups. The unpaired Student's *t*-test was employed to determine the difference among groups. The significance level was set at  $P < 0.05$ .

## RESULTS

**Effects of extracellular acidosis on  $pH_i$  and  $[Ca^{2+}]_i$ .** When the ventricular myocyte was superfused with a Krebs solution at  $pH_e$  6.8, the  $pH_i$  dropped slowly to a steady state (from  $7.03 \pm 0.03$  to  $6.65 \pm 0.04$ ;  $P < 0.01$ ) with a half time of  $2.2 \pm 0.42$  min ( $n = 5$ ). After this short period, during which the  $pH_i$  remained at the low level, the  $pH_i$  rose gradually by  $0.14 \pm 0.04$  ( $n = 8$ ;  $P < 0.01$ ) within 10 min during acidosis (Fig. 1). The recovery in  $pH_i$  was abolished by 10  $\mu$ M EIPA, a blocker of  $Na^+/H^+$  exchange (29) (Fig. 1).

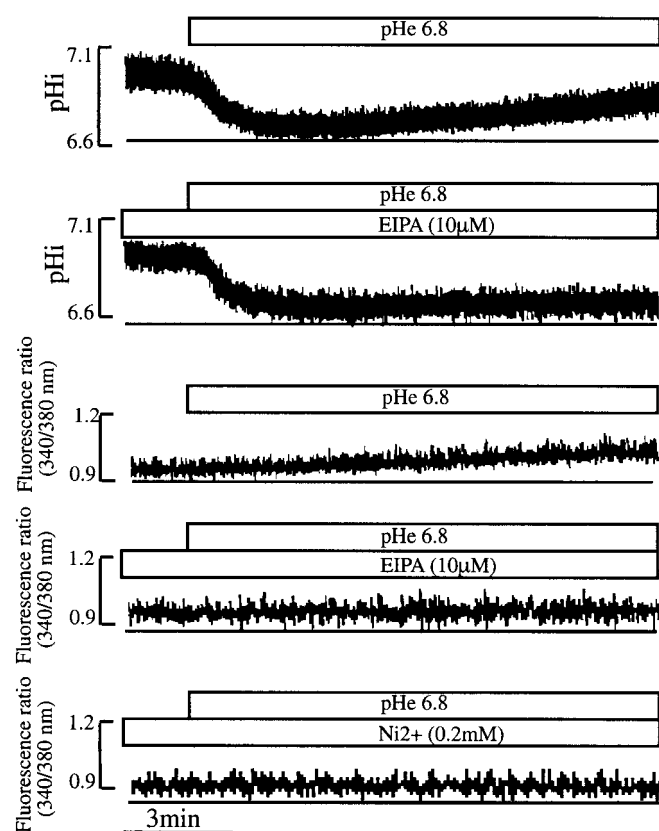


Fig. 1. Typical tracings from 4–8 experiments showing time course changes in intracellular pH ( $pH_i$ ) and resting fura 2 fluorescence ratio during extracellular acidosis in presence and absence of ethylisopropyl amiloride (EIPA) and  $Ni^{2+}$  in single ventricular myocyte.  $pH_e$ , extracellular pH.

As shown in Fig. 1, extracellular acidosis also gradually increased the fura 2 fluorescence ratio from  $0.95 \pm 0.07$  to  $1.1 \pm 0.08$  ( $n = 4$ ;  $P < 0.01$ ) within 10 min, indicating a gradual increase in the resting  $[Ca^{2+}]_i$ . The elevation was blocked by 10  $\mu M$  EIPA and 0.2 mM  $Ni^{2+}$ , a putative blocker of  $Na^+/Ca^{2+}$  exchange (6).

**$[Ca^{2+}]_i$  responses to U-50488H during extracellular acidosis.** In this series of experiments we determined the resting  $[Ca^{2+}]_i$  and the  $[Ca^{2+}]_i$  transients induced electrically and with caffeine in a single ventricular myocyte. In view of the fact that the changes in the electrically induced  $[Ca^{2+}]_i$  transient were large and easily quantified, the dose-related response was determined by observing the responses of the electrically induced  $[Ca^{2+}]_i$  transient. In agreement with previous observations (23, 38), 10–30  $\mu M$  U-50488H, a selective  $\kappa$ -opioid receptor agonist, suppressed the electrically induced  $[Ca^{2+}]_i$  transient in a concentration-dependent manner (Fig. 2). The effect of the  $\kappa$ -agonist at 30  $\mu M$  was abolished by 5  $\mu M$  nor-BNI, a selective  $\kappa$ -opioid receptor antagonist (Fig. 2).

U-50488H at 30  $\mu M$  also increased the resting  $[Ca^{2+}]_i$  (Fig. 3) and inhibited the caffeine-induced  $[Ca^{2+}]_i$  transient (Fig. 4). The effect of 30  $\mu M$  U-50488H on the resting  $[Ca^{2+}]_i$  was also abolished by 5  $\mu M$  nor-BNI (Fig. 3). The observations are in agreement with previous findings (37, 47).

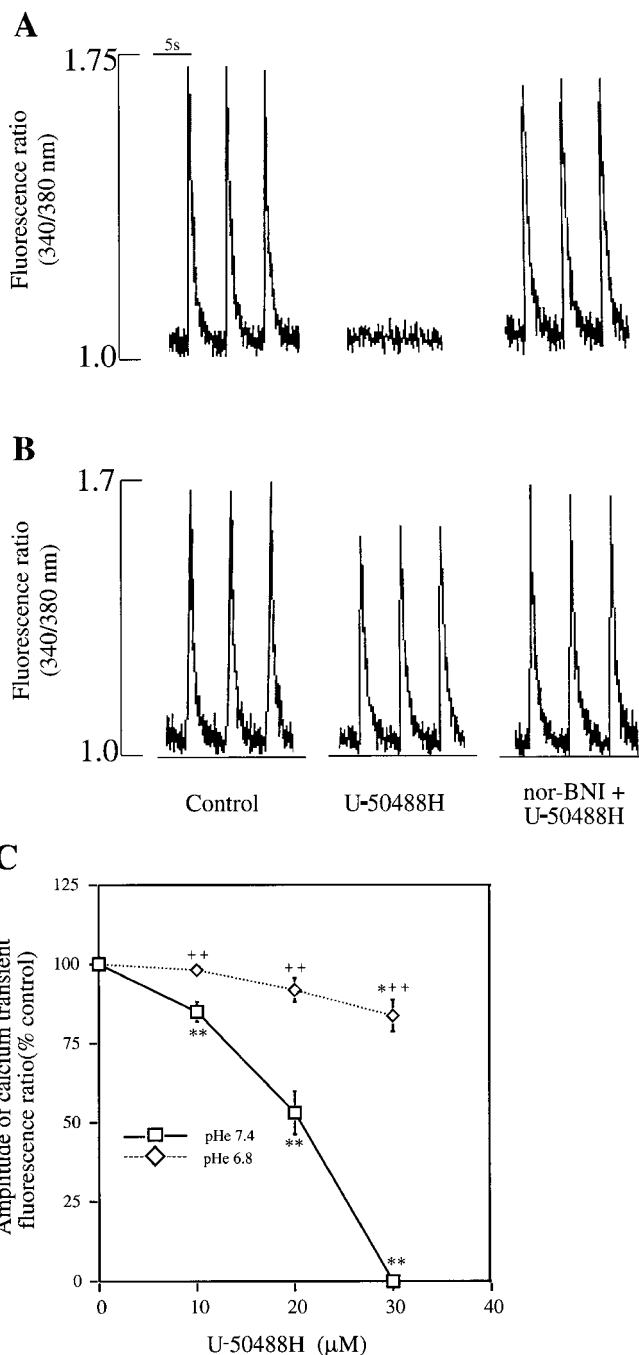


Fig. 2. Effects of  $\kappa$ -opioid receptor stimulation with U-50488H on electrically induced intracellular  $Ca^{2+}$  concentration ( $[Ca^{2+}]_i$ ) transient during extracellular acidosis in presence and absence of nor-BNI (5  $\mu M$ ) in a single ventricular myocyte. The ventricular myocyte was superfused with a solution at  $pH_e$  6.8 for 10 min when effects of low pH reached a plateau as shown in Fig. 1. U-50488H was then administered. For measurement of electrically induced  $[Ca^{2+}]_i$  transient, cell was electrically stimulated before administration of U-50488H. In experiments involving nor-BNI, drug was administered 5 min before and together with U-50488H. Both resting  $[Ca^{2+}]_i$  and electrically induced  $[Ca^{2+}]_i$  transients were recorded at  $\sim 10$  min after administration of U-50488H. Same procedure was employed for measurement of caffeine-induced  $[Ca^{2+}]_i$  transient except that myocyte was given a bolus dose of 10 mM caffeine after electrical stimulation. Shown are representative tracings of effects of 30  $\mu M$  U-50488H at  $pH_e$  7.4 (A) and 6.8 (B). C: dose-related effects of U-50488H. Values are means  $\pm$  SE;  $n = 4$ . \* $P < 0.05$ , \*\* $P < 0.01$  vs. corresponding control without U-50488H; +++ $P < 0.01$  vs. corresponding control group in normal pH.

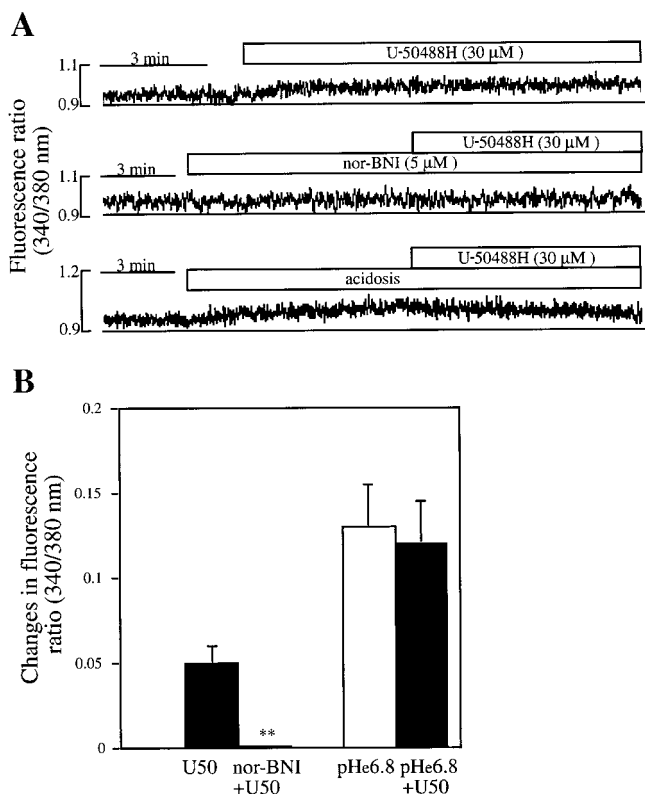


Fig. 3. Effects of κ-opioid receptor stimulation with U-50488H (U50) on resting  $[Ca^{2+}]_i$  in presence of 5 μM nor-BNI and in absence of nor-BNI. *A*: representative tracings showing effect of 30 μM U-50488H. *B*: group results. Values are means ± SE;  $n = 6$ . \*\* $P < 0.01$  vs. corresponding control.

After the ventricular myocytes had been superfused with a solution at  $pH_e$  6.8 for 10 min, during which time the cells stabilized, the effects of 30 μM U-50488H on the resting  $[Ca^{2+}]_i$  (Fig. 3) were abolished, and those on the  $[Ca^{2+}]_i$  transients induced electrically (Fig. 2) and with caffeine (Fig. 4) were significantly attenuated.

The attenuating effect of the κ-opioid receptor agonist on the electrically induced  $[Ca^{2+}]_i$  transient during extracellular acidosis was restored by 10 μM EIPA and 0.2 mM  $Ni^{2+}$  (Fig. 5).

*Effects of U-50488H on  $[Ca^{2+}]_i$  and electrically induced  $[Ca^{2+}]_i$  transients during superfusion with a high-glucose solution.* To further delineate the role of  $Na^+/H^+$  exchange in mediating the action of extracellular acidosis on the  $Ca^{2+}$  responses to κ-opioid receptor stimulation, a solution containing a high glucose concentration, known to activate  $Na^+/H^+$  exchange as a result of acute osmotic stimulation (49), was superfused. Both the  $[Ca^{2+}]_i$  and electrically induced  $[Ca^{2+}]_i$  transient in response to U-50488H were determined. In agreement with previous observations (34, 41), both the  $pH_i$  (Fig. 6A) and the fura 2 fluorescence ratio (Fig. 6B) increased gradually on superfusion with a 50 mM glucose solution. The  $pH_i$  and the resting fura 2 fluorescence ratio increased by  $0.14 \pm 0.04$  ( $n = 5$ ;  $P < 0.01$ ) and  $0.12 \pm 0.05$  ( $n = 5$ ;  $P < 0.01$ ), respectively, within 10 min. More importantly, the high glucose concentration abolished the effects of 30 μM U-50488H on  $[Ca^{2+}]_i$  (Fig.

6B), and significantly attenuated its effects on the electrically induced  $[Ca^{2+}]_i$  transient (Fig. 6, C and D), in the ventricular myocyte. The effects were similar to those of extracellular acidosis.

*Effects of U-50488H on  $[Ca^{2+}]_i$  and electrically induced  $[Ca^{2+}]_i$  transients in extracellular high  $Ca^{2+}$ .* This series of experiments was performed to further determine the roles of  $[Ca^{2+}]_i$  and  $Na^+/Ca^{2+}$  exchange

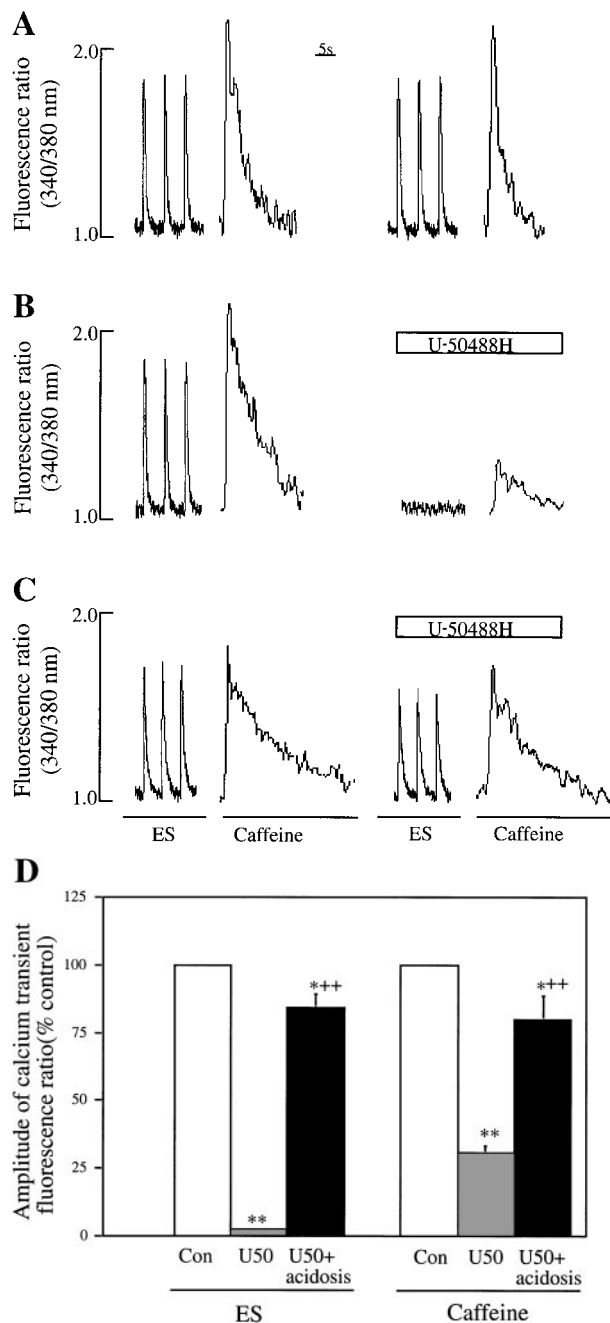


Fig. 4. Effects of κ-opioid receptor stimulation with U-50488H on caffeine-induced  $[Ca^{2+}]_i$  transient. *A-C*: representative tracings showing effect of 30 μM U-50488H at pH 7.4 (*A* and *B*) and at pH 6.8 (*C*). *D*: group results showing effects of 30 μM U-50488H on  $[Ca^{2+}]_i$  transients induced both electrically and with caffeine. ES, electrical stimulation; con, control. Values are means ± SE;  $n = 4$ . \* $P < 0.05$ , \*\* $P < 0.01$  vs. corresponding control without U-50488H; +++ $P < 0.01$  vs. corresponding control group in normal pH.

in mediating the action of acidosis. Superfusion with a high (3 mM) extracellular  $\text{Ca}^{2+}$  solution gradually increased the resting fura 2 fluorescence ratio by  $0.21 \pm 0.06$  within 10 min ( $n = 5$ ;  $P < 0.01$ ; Fig. 7B), whereas the  $\text{pH}_i$  remained the same (Fig. 7A). The rise in  $[\text{Ca}^{2+}]_i$  was abolished by 0.2 mM  $\text{Ni}^{2+}$  (Fig. 7B).

In the 3 mM  $\text{Ca}^{2+}$  solution, the effects of 30  $\mu\text{M}$  U-50488H on  $[\text{Ca}^{2+}]_i$  in the ventricular myocyte were abolished (Fig. 7B) and those on the electrically induced  $[\text{Ca}^{2+}]_i$  transient significantly attenuated (Fig. 7, C and D).

## DISCUSSION

The most important finding of the present study was that the effects of  $\kappa$ -opioid receptor stimulation with the selective  $\kappa$ -opioid receptor agonist U-50488H on resting  $[\text{Ca}^{2+}]_i$  and on  $[\text{Ca}^{2+}]_i$  transients induced electrically and with caffeine were significantly attenuated during extracellular acidosis, which decreased  $\text{pH}_i$  (13, 20) but increased the resting  $[\text{Ca}^{2+}]_i$  (10, 18, 21) via activation of the  $\text{Na}^+/\text{H}^+$  and  $\text{Na}^+/\text{Ca}^{2+}$  exchanges (2, 15, 22, 32, 35). The observations demonstrate for the first time that extracellular acidosis antagonizes the  $\text{Ca}^{2+}$  responses to  $\kappa$ -opioid receptor stimulation in the heart. This is also the first demonstration that extracellular acidosis antagonizes the  $\text{Ca}^{2+}$  response to stimulation of a receptor.

Previous studies have shown that  $\kappa$ -opioid receptor stimulation increases the resting  $[\text{Ca}^{2+}]_i$  and decreases the electrically induced  $[\text{Ca}^{2+}]_i$  transient, which is due to an initial influx of  $\text{Ca}^{2+}$  upon membrane depolarization and a subsequent release of  $\text{Ca}^{2+}$  from the SR, and the caffeine-induced  $[\text{Ca}^{2+}]_i$  transient, which is an index of  $\text{Ca}^{2+}$  content in the SR (37, 38, 47). The observations indicate that  $\kappa$ -opioid receptor stimulation mobilizes  $\text{Ca}^{2+}$  from its intracellular store, leading to an increase in the cytosolic  $\text{Ca}^{2+}$ . In the present study we found that extracellular acidosis antagonizes all these responses to  $\kappa$ -opioid receptor stimulation, suggesting that extracellular acidosis may inhibit mobilization of  $\text{Ca}^{2+}$  from the intracellular store upon  $\kappa$ -opioid receptor stimulation.

In the present study we made two interesting observations. First, blockade of these two exchanges by their respective blockers, EIPA and  $\text{Ni}^{2+}$ , restored the effects of U-50488H during extracellular acidosis. Second, activation of  $\text{Na}^+/\text{H}^+$  exchange with an osmotic stimulation by a high extracellular glucose concentration, which increased  $\text{pH}_i$  and resting  $[\text{Ca}^{2+}]_i$  (34, 41, 49), or an increase in extracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_o$ ), known to activate the reverse mode of  $\text{Na}^+/\text{Ca}^{2+}$  exchange and to increase the resting  $[\text{Ca}^{2+}]_i$  (26), mimicked the influence of extracellular acidosis on the  $\text{Ca}^{2+}$  responses to U-50488H. The observations suggest that extracellular acidosis may activate  $\text{Na}^+/\text{H}^+$  exchange,

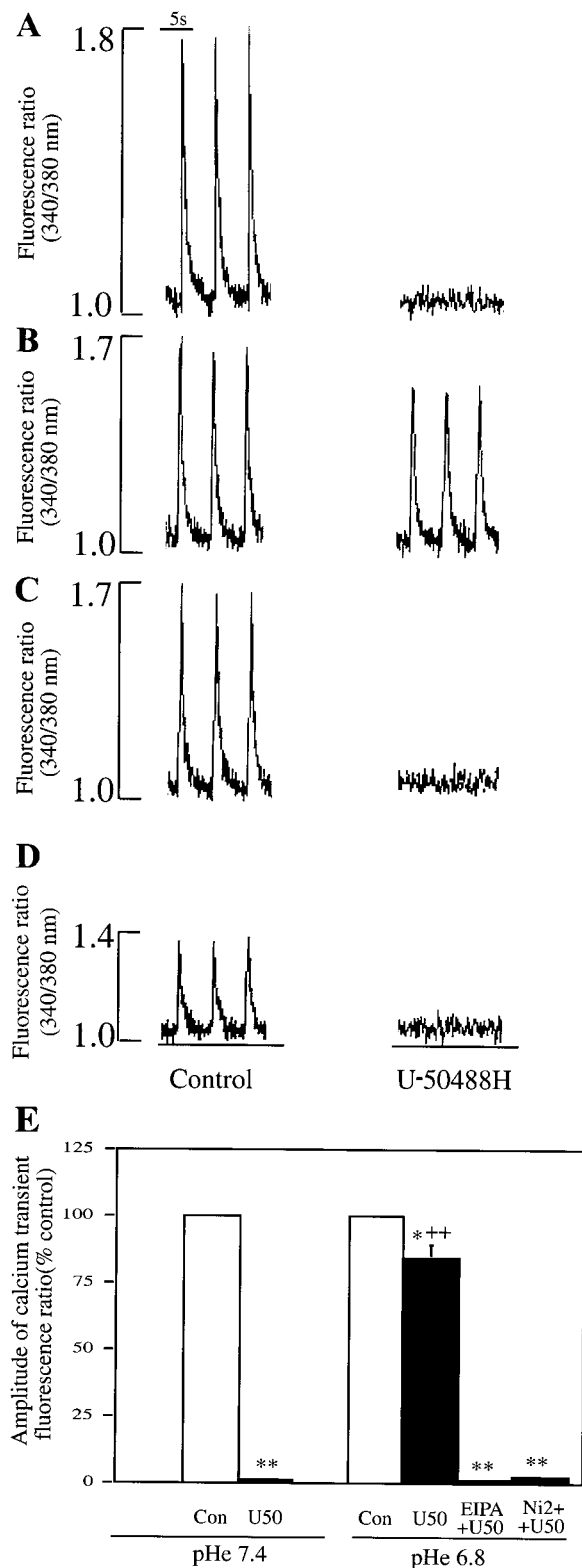


Fig. 5. Effects of  $\kappa$ -opioid receptor stimulation with U-50488H on electrically induced  $[\text{Ca}^{2+}]_i$  transient upon blockade of  $\text{Na}^+/\text{H}^+$  or  $\text{Na}^+/\text{Ca}^{2+}$  exchange during extracellular acidosis in a single ventricular myocyte. A-D: representative tracings at pH 7.4 (A) and at pH 6.8 either without EIPA or  $\text{Ni}^{2+}$  (B), with EIPA (C), or with  $\text{Ni}^{2+}$  (D). E: group results showing effects of 30  $\mu\text{M}$  U-50488H at pH 7.4 and at pH 6.8 in presence of either 10  $\mu\text{M}$  EIPA, a  $\text{Na}^+/\text{H}^+$  exchange blocker, or 0.2 mM  $\text{Ni}^{2+}$ , a  $\text{Na}^+/\text{Ca}^{2+}$  exchange blocker. Experimental procedure was exactly the same as that described in legend for Fig. 2. EIPA and  $\text{Ni}^{2+}$  were administered together with the acidic solution. Values are means  $\pm$  SE;  $n = 8$ . \*  $P < 0.05$ , \*\*  $P < 0.01$  vs. corresponding control without U-50488H. +++  $P < 0.01$  vs. group at  $\text{pH}_e$  7.4.

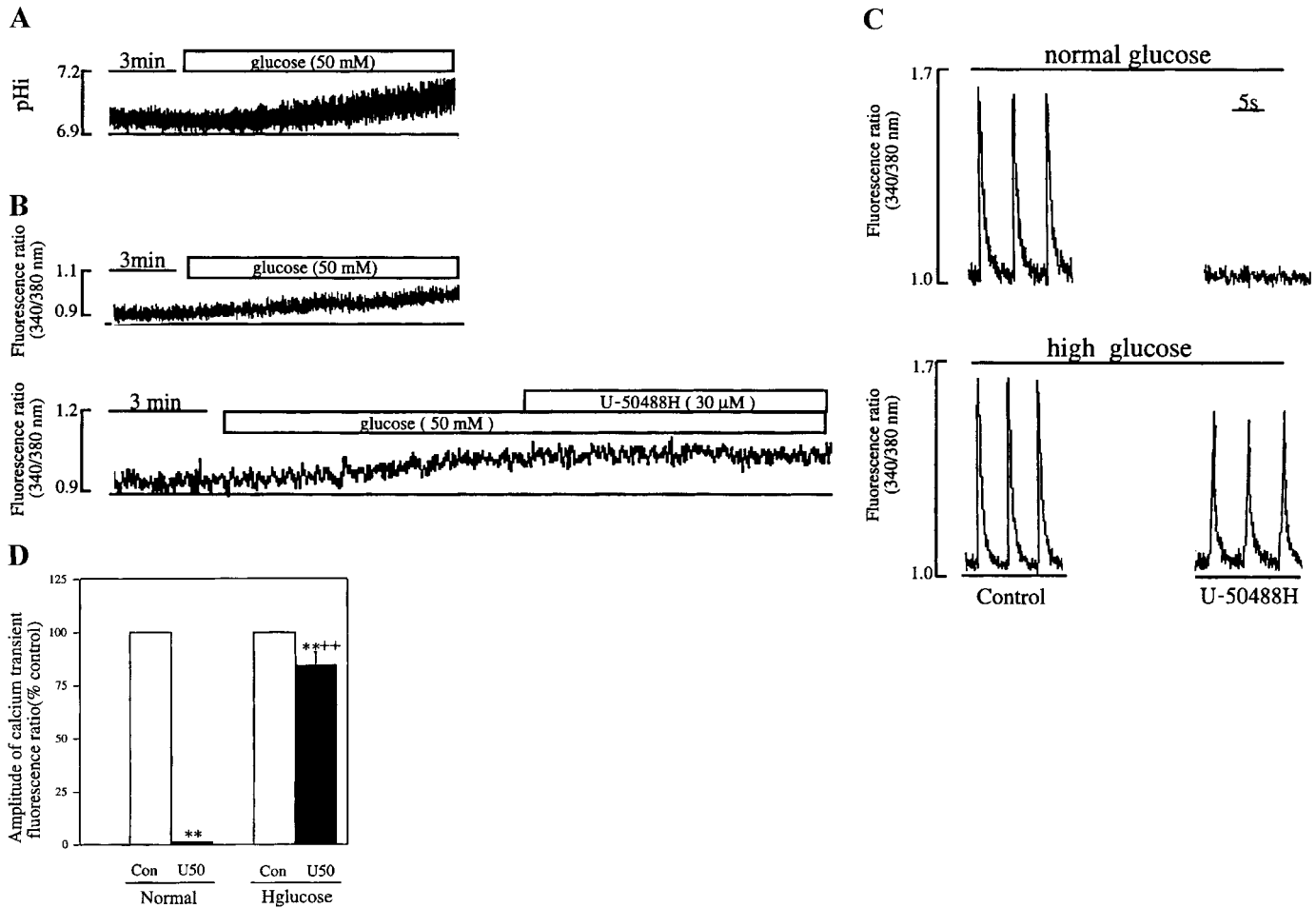


Fig. 6. Effects of  $\kappa$ -opioid receptor stimulation with U-50488H on resting  $[Ca^{2+}]_i$  and electrically induced  $[Ca^{2+}]_i$  transient during extracellular high-glucose (50 mM) perfusion in single ventricular myocyte. Experimental procedure was same as that in legend for Fig. 2 except that high-glucose solution was superfused for 10 min before electrical stimulation and/or administration of U-50488H. *A*: typical tracing of 5 experiments on changes in pH<sub>i</sub>. *B*: typical tracing of 6 experiments on changes in resting fura 2 fluorescence ratio. *C*: typical tracings showing effects of 30  $\mu$ M U-50488H on electrically induced  $[Ca^{2+}]_i$  transient. *D*: group results showing effects of 30  $\mu$ M U-50488H on electrically induced  $[Ca^{2+}]_i$  transient. Values are means  $\pm$  SE;  $n = 6$  in all groups. Hglucose, high glucose. \*\*  $P < 0.01$  vs. corresponding control without U-50488H. +++  $P < 0.01$  vs. corresponding group without high glucose.

which in turn may activate  $Na^+/Ca^{2+}$  exchange. In summary, extracellular acidosis may antagonize the effects of  $\kappa$ -opioid receptor stimulation on  $Ca^{2+}$  mobilization from its intracellular store via  $Na^+/H^+$  and  $Na^+/Ca^{2+}$  exchanges, thus reducing the inhibitory effects of  $\kappa$ -opioid receptor stimulation on muscle contraction. On the other hand, acidosis is well established to inhibit the binding of  $Ca^{2+}$  to troponin, thus decreasing contractility (8, 19, 31). However, the functional implication of the antagonism of extracellular acidosis against  $\kappa$ -opioid receptor stimulation and its effect on the contractility of the heart, especially during myocardial ischemia, need further study.

Another interesting observation in the present study is that extracellular acidosis, which reduced pH<sub>i</sub> and elevated  $[Ca^{2+}]_i$ , high extracellular glucose, which increased both pH<sub>i</sub> and  $[Ca^{2+}]_i$ , and high  $[Ca^{2+}]_o$ , which only elevated resting  $[Ca^{2+}]_i$  without affecting pH<sub>i</sub>, all attenuated the  $Ca^{2+}$  response to U-50488H. The observations suggest that the elevation of resting  $[Ca^{2+}]_i$ , not

pH<sub>i</sub>, may mediate the inhibitory action of extracellular acidosis on the  $Ca^{2+}$  response to  $\kappa$ -opioid receptor stimulation. Because it was found that cytosolic  $Ca^{2+}$  inhibits the release of  $Ca^{2+}$  from the SR (16, 17), further studies are needed to determine whether or not the elevation in the resting  $[Ca^{2+}]_i$  during extracellular acidosis inhibits directly the mobilization of  $Ca^{2+}$  from the SR on  $\kappa$ -opioid receptor stimulation.

One possible shortcoming of the present study was the use of a high glucose concentration to activate  $Na^+/H^+$  exchange because a high glucose concentration has metabolic effects. In the present study we found that a high glucose concentration, which increased  $[Ca^{2+}]_i$ , attenuated the action of  $\kappa$ -opioid receptor stimulation, an effect similar to that of extracellular acidosis and a high  $[Ca^{2+}]_o$ . In view of the fact that there is no specific activator of  $Na^+/H^+$  exchange, a high glucose concentration is the only choice available.

In conclusion, the present study has provided evidence for the first time that acidosis antagonizes the

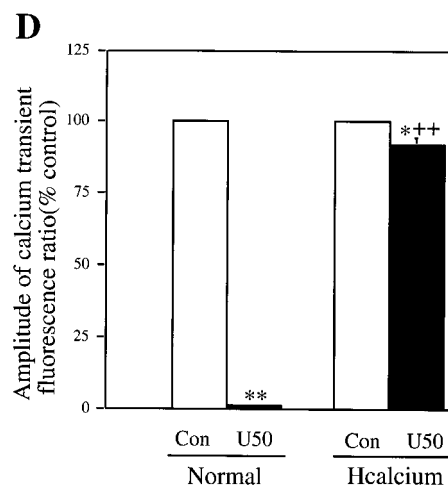
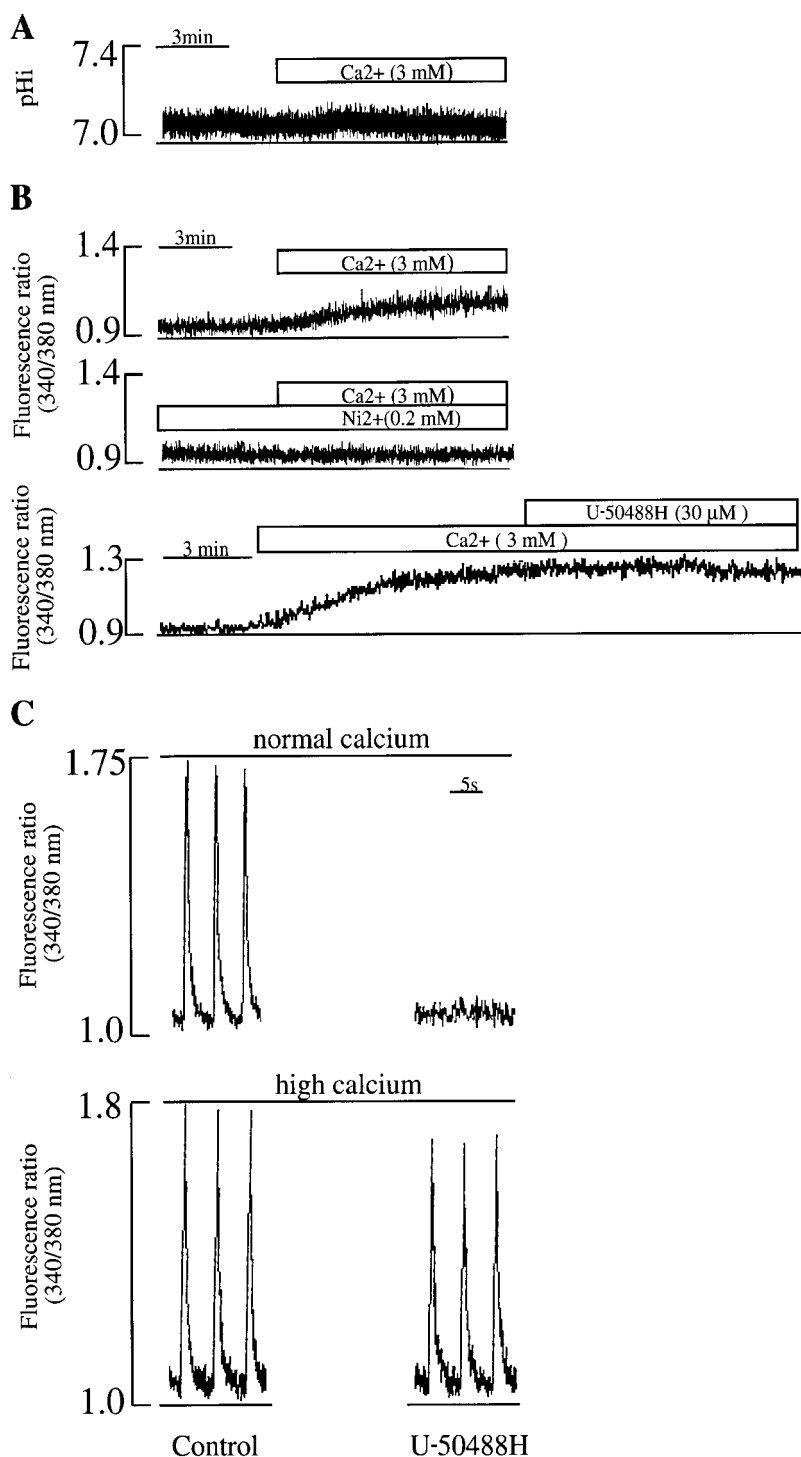


Fig. 7. Effects of  $\kappa$ -opioid receptor stimulation with U-50488H on resting  $[Ca^{2+}]_i$  and electrically induced  $[Ca^{2+}]_i$  transient during high extracellular  $Ca^{2+}$  concentration ( $[Ca^{2+}]_o$ ; 3 mM) superfusion in single ventricular myocyte. Experimental procedure was same as that described in legend for Fig. 2 except that high- $Ca^{2+}$  solution was superfused for 10 min before electrical stimulation and/or administration of U-50488H. *A*: typical tracing of 4 experiments on changes in pH<sub>i</sub>. *B*: typical tracing of 6 experiments on changes in resting fura 2 fluorescence ratio in presence of 0.2 mM Ni<sup>2+</sup> or in absence of Ni<sup>2+</sup>. *C*: typical tracings showing effects of 30  $\mu$ M U-50488H on electrically induced  $[Ca^{2+}]_i$  transient. *D*: group results showing effects of 30  $\mu$ M U-50488H on electrically induced  $[Ca^{2+}]_i$  transient. Values are means  $\pm$  SE;  $n = 6$  in all groups. Hcalcium, high calcium. \* $P < 0.05$ , \*\* $P < 0.01$  vs. corresponding control without U-50488H. ++ $P < 0.01$  vs. corresponding group without high  $[Ca^{2+}]_o$ .

effects of  $\kappa$ -opioid receptor stimulation on mobilization of  $Ca^{2+}$  from SR in the heart. Both  $Na^+/H^+$  exchange and  $Na^+/Ca^{2+}$  exchange are involved in the process, leading to an elevation of  $[Ca^{2+}]_i$ , which may inhibit  $Ca^{2+}$  release from the SR induced by  $\kappa$ -opioid receptor stimulation. Further study is needed to determine the physiological implication of the antagonism of extracellular acidosis on the effects of  $\kappa$ -opioid receptor stimulation and to verify the role of  $[Ca^{2+}]_i$  in the mobilization

of  $Ca^{2+}$  from its intracellular store and delineate the underlying mechanisms.

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