Ca^{2+}-induced Ca^{2+} release involved in positive inotropic effect mediated by CGRP in ventricular myocytes

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Huang, Ming-He, Paul R. Knight III, and Joseph L. Izzo, Jr. Ca^{2+}-induced Ca^{2+} release involved in positive inotropic effect mediated by CGRP in ventricular myocytes. *Am. J. Physiol.* 276 (*Regulatory Integrative Comp. Physiol.* 45): R259–R264, 1999.—To investigate the effects and mechanisms of calcitonin gene-related peptide (CGRP) on ventricular contractility, ventricular myocytes isolated from adult rat and mouse hearts were exposed to CGRP. Myocyte contractility was assessed by a video edge motion detector, and the intracellular [Ca^{2+}] transients were measured by a spectrofluorophotometer in fura 2-loaded myocytes. CGRP exerted a potent concentration-dependent (10 pM–10 nM, EC_{50} = 44.1 pM) positive inotropism on rat ventricular myocytes. CGRP (1 nM) increased cell shortening during contraction by 140 ± 40% above baselines and increased maximum velocity of contraction and relaxation by 98 and 106%, respectively. CGRP failed to produce any response in the presence of the $CGRP_1$ receptor antagonist. CGRP induced similar inotropic response in mouse ventricular myocytes. CGRP increased the amplitude of [Ca^{2+}] transients of ventricular myocytes by 120 ± 25% above baseline and shortened the time of half-maximum myoplasmic Ca^{2+} clearance by 30 ± 5%. Increase in intracellular Ca^{2+} mobilization by CGRP was dependent on Ca^{2+} influx through the activation of the L-type Ca^{2+} channel, because nifedipine blocked the CGRP-induced increase in [Ca^{2+}] transients. Furthermore, CGRP failed to increase [Ca^{2+}] transients after the inhibition of protein kinase A in ventricular myocytes. These data indicate that stimulation of mammalian ventricular myocardial $CGRP_1$ receptors enhances [Ca^{2+}] transients through the activation of protein kinase A, which in turn activates voltage-dependent L-type Ca^{2+} channels. These events lead to Ca^{2+}-induced intracellular Ca^{2+} release and enhanced myocyte contraction and facilitated relaxation.

calcitonin gene-related peptide; calcium transient; myocardial contractility; protein kinase A

RECENT EVIDENCE INDICATES that neuropeptides in the autonomic nervous system may play significant roles in cardiovascular regulation (4). Calcitonin gene-related peptide (CGRP) is broadly distributed in peripheral autonomic neurons innervating cardiovascular structures (4, 6, 17, 26, 28), including human heart (6). Expression of mRNA of CGRP and $CGRP_1$ receptors has been localized in rat ventricular myocardial tissue (23) and in in situ mouse ventricular myocytes (1), respectively.

The physiological role of CGRP on the cardiovascular system has not been fully established. A relatively high amount of CGRP has been detected in rat (31) and human plasma (13). Circulating CGRP is largely derived from perivascular sensory nerve endings (28, 32). Increase in circulating CGRP occurs during diseased states such as congestive heart failure (7) and acute myocardial infarction (18). CGRP is an extremely potent coronary and peripheral vasodilator (25, 26, 28). CGRP exerts positive inotropic effects on human atrial tissue (10). It has also been reported that CGRP exerts positive inotropic effects on isolated porcine ventricular muscle strips (20, 29). Intravenous infusion of CGRP dramatically improves ventricular contractile function in heart failure patients (11). However, some studies have failed to demonstrate a CGRP-induced positive ventricular inotropism on isolated canine (26) and rat (19) hearts. Furthermore, mechanisms underlying the inotropic effects of CGRP on ventricular muscle are unclear. We have hypothesized that CGRP exerts positive inotropic effect on ventricular muscle through the activation of L-type Ca^{2+} channels, resulting in Ca^{2+}-induced intracellular Ca^{2+} mobilization.

To determine the effect and mechanisms of CGRP on mammalian ventricular muscle and species variability, we examined the effect of CGRP on contractile function of isolated ventricular myocytes from both adult rat and mouse hearts and compared its effects with those elicited by a β-adrenoreceptor agonist, isoproterenol (Iso). We used the β-adrenoreceptor antagonist atenolol and the $CGRP_1$ receptor antagonist CGRP-(7—37) to investigate CGRP receptor effects. To investigate cellular mechanisms, we studied CGRP effect on intracellular [Ca^{2+}] transients in the presence and absence of L-type Ca^{2+} channel blockade. Furthermore, CGRP was tested after inhibition of protein kinase A in ventricular myocytes to determine the role of cAMP in CGRP signal transduction.

MATERIALS AND METHODS

Preparations of ventricular myocytes. The procedure for obtaining rod-shaped and Ca^{2+}-tolerant ventricular myocytes was based on a method previously described (14). In brief, mice (n = 7, 4–6 mo old) and rats (n = 27, 3–6 mo old) were killed using pentobarbital sodium anesthesia. The heart was excised, cannulated through the aorta, and then mounted on a Langendorff perfusion apparatus. The coronary arteries were perfused with Ca^{2+}-free Tyrode containing (in mM) 132 NaCl, 4.8 KCl, 1.2 $MgCl_2$, 5 glucose, and 10 HEPES (pH 7.4) for 5 min. The heart was then perfused with collagenase II (0.4 mg/ml, Worthington Biochemical, Lakewood, NJ) in Ca^{2+}-free Tyrode for 10 min. All solutions were continuously oxygenated with a perfusion temperature of 37°C. The ventricles were cut from the atria, minced, and shaken in a waterbath. Dissociated myocytes were collected by centrifugation. The cell pellets were resuspended in Tyrode solution (0.5

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mM Ca^{2+}, 1% bovine serum albumin, and 1% taurine) before the experiment.

Measurement of cell shortening. Freshly isolated myocytes were placed in a cell chamber that was continuously perfused (37°C) on the stage of an inverted microscope. Experimental solution containing (in mM) 120 NaCl, 2.6 KCl, 1.2 $MgCl_2$, 1.2 KH_2PO_4, 5 glucose, 5 HEPES, 20 $NaHCO_3$, and 1 $CaCl_2$ was circulated through the cell chamber (Biotechs FCS2, Biotechs, Butler, PA). Platinum electrodes connected to a stimulator (Grass S48, Grass Instruments) were used to stimulate cells at a frequency of 1 Hz. A PTI system (Photon Technology International, South Brunswick, NJ) was used for the study. Cell image was continuously acquired through an objective lens and transmitted to a camera. The output from the camera was displayed on a video monitor. Myocyte contraction was measured using a video motion edge detector system (Crescent Electronics, Sandy, UT), and data were acquired, stored, and analyzed by a computer. Cells (90–120 μm in length) were chosen for study based on the criteria that rod-shaped myocytes with clear striations should be quiescent before electrical pacing. On electrical stimulation, myocytes should contract rhythmically and persistently.

Measurement of $[Ca^{2+}]$ transients. Myocytes were loaded with 4 mM of fura 2-acetoxymethyl ester (Sigma) at room temperature for 30 min in Ca^{2+}-free Tyrode solution. The loaded myocytes were washed three times and equilibrated in experimental solution for 30 min. A PTI spectrophotometer excited the cell at 340/380 nm alternately at a 240-Hz sampling rate. Measurements of 510-nm emissions were recorded as fluorescence ratios (340/380 nm), which were used to represent intracellular Ca^{2+} changes (9).

Pharmacological interventions. All chemicals were purchased from Research Biochemicals Internationals (Natick, MA). Ventricular myocytes were perfused in Tyrode solution (1 mM Ca^{2+}) for at least 10 min, and data for baseline cell shortening and $[Ca^{2+}]$ transients were collected thereafter. The effects of CGRP and Iso on contraction and $[Ca^{2+}]$ transients of myocytes were tested in a concentration-dependent manner. The effect of CGRP was tested in the presence of the β-adrenoreceptor blocker atenolol (100 nM) and the $CGRP_1$ receptor blocker CGRP-(7—37) (2×10^{-7} M) (8). Their effects on myocytes were evaluated immediately after they were added to the tissue bath perfusate. The effect of CGRP on $[Ca^{2+}]$ transients of rat ventricular myocytes was tested in the presence of the Ca^{2+} channel blocker nifedipine (5 mM) to determine whether intracellular Ca^{2+} release is dependent on Ca^{2+} influx through sarcolemmal L-type Ca^{2+} channels. Cells were exposed to nifedipine for 5 min followed by perfusion of nifedipine and CGRP (1 nM). Thereafter, CGRP (1 nM) was reapplied after nifedipine and CGRP were washed out for 20 min. To determine the role of cAMP in CGRP signal transduction, the effect of CGRP on $[Ca^{2+}]$ transients in myocytes was tested in the absence and presence of the cAMP antagonist Rp-cAMPS, which inhibited protein kinase A (30). After identification of CGRP-induced increase in $[Ca^{2+}]$ transients in myocytes, the cells were treated with Rp-cAMPS (100 mM) for 30 min before the reapplication of CGRP. CGRP was further tested in the absence and presence of caffeine (15 mM for 2 min) in beating myocytes.

Data analysis. Cell shortening was recorded during baseline and interventions. The amplitude of cell shortening and $[Ca^{2+}]$ transients during contraction and the half-maximum myoplasmic Ca^{2+}-uptake time during relaxation were calculated from individual waveforms using Matlab software. Changes in the amplitude of $[Ca^{2+}]$ transients before and after drug treatments were expressed as mean percentage changes if the mean diastolic baseline fluorescence ratio was not significantly changed. To calculate the velocity of myocyte shortening and relaxation, the derivative was calculated as a two-point slope looking back one point on individual contraction waveforms. Maximum values of negative slope ($-dL/dt_{Max}$) and positive slope ($+dL/dt_{Max}$) during contraction and relaxation were used to represent the velocity of cell shortening and relaxation, respectively. Paired Student's *t*-test and analysis of variance were used for statistical analyses. A value of $P < 0.05$ was considered significant.

RESULTS

Cell contraction. In rat ventricular myocytes, the increase in cell shortening after CGRP application was 50 ± 10 to 140 ± 40% greater than their respective baselines at concentrations of 10 pM–10 nM (Table 1 and Fig. 1*D*). Increase in CGRP concentrations beyond 100 nM led to myocyte fibrillation or contracture. Peak effect was reached 2–4 min after CGRP exposure. The EC_{50} for CGRP and Iso was 44.1 pM and 9.8 nM, respectively. CGRP (1 nM) increased maximum velocity of rat myocyte shortening ($-dL/dt_{Max}$) and relaxation ($+dL/dt_{Max}$) by 98 and 106%, respectively (12 cells, $P < 0.01$). In mice, CGRP-induced augmentation in ventricular myocyte shortening was 70 ± 24 to 200 ± 54% greater than respective baselines at concentrations of 10 pM–10 nM (Table 1 and Fig. 1*B*), with a peak effect at 2–3 min after CGRP exposure. The EC_{50} for CGRP and Iso was 55.8 pM and 8.4 nM, respectively, in mouse myocytes. CGRP (1 nM) increased $-dL/dt_{Max}$ and $+dL/dt_{Max}$ by 150 and 213%, respectively (9 cells, $P < 0.01$). CGRP did not produce any effect in the presence of the $CGRP_1$ receptor blocker CGRP-(7—37) but still elicited a 92 ± 23% increase in cell shortening in the presence

Table 1. *Dose-dependent increase in shortening of ventricular myocytes by CGRP and Iso in adult rat and mouse*

	Basal	10 pM	Basal	100 pM	Basal	1 nM	Basal	10 nM	Basal	100 nM	Number of cells
GGRP-induced positive inotropic effect											
%Cell shortening	4.6 ± 0.5	5.6 ± 0.9	4.4 ± 0.4	8.2 ± 0.8	4.9 ± 0.5	10.1 ± 0.9	4.7 ± 0.5	10.2 ± 1.0	4.1 ± 0.4	8.8 ± 0.5	20, 14 rats
%Cell shortening	4.4 ± 0.7	6.1 ± 1.0	3.9 ± 0.7	9.9 ± 2.1	4.3 ± 0.8	11.4 ± 1.8	5.0 ± 1.5	8.5 ± 2.3			14, 7 mice

	Basal	1 nM	Basal	10 nM	Basal	100 nM	Basal	1 μM	Number of cells
Iso-induced positive inotropic effect									
%Cell shortening	4.5 ± 0.6	8.2 ± 1.1	4.7 ± 0.6	9.1 ± 1.2	4.5 ± 1.0	10 ± 2.0	4.4 ± 1.0	14.0 ± 1.6	15, 8 rats
%Cell shortening	3.9 ± 0.7	9.9 ± 2.1	4.3 ± 0.8	11.4 ± 1.8	5.0 ± 1.5	8.5 ± 2.3			14, 8 mice

Values are means ± SE. Iso, isoproteranol; CGRP, calcitonin gene-related peptide.

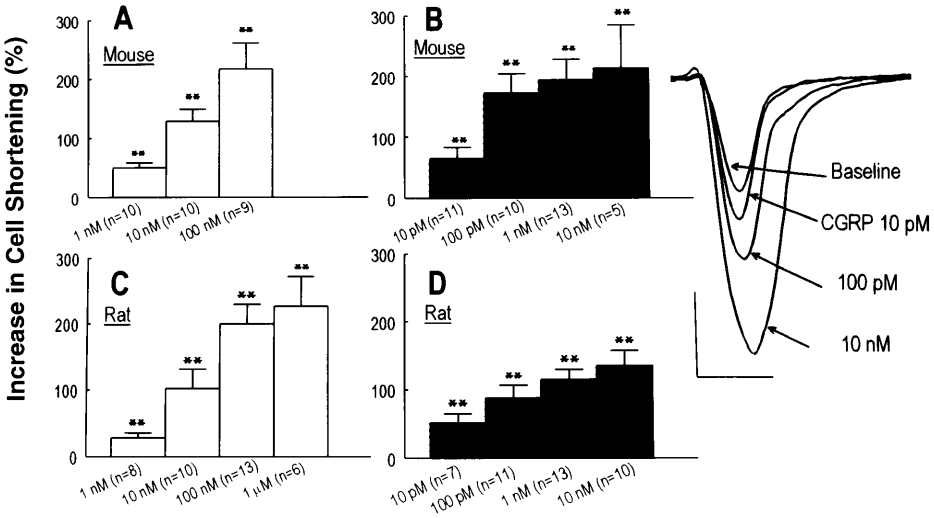

Fig. 1. Dose-dependent effect of calcitonin gene-related peptide (CGRP; *A* and *C*) and isoproterenol (Iso; *B* and *D*) on ventricular myocyte shortening in mouse (*A* and *B*) and rat (*C* and *D*). Increase in amplitude of cell shortening is expressed as %change compared with baseline contraction. CGRP increases cell shortening by 50 ± 10, 95 ± 18, 110 ± 46, and 140 ± 40% at the doses of 10 pM–10 nM, respectively, in rat. CGRP increases cell shortening by 70 ± 24, 181 ± 48, 198 ± 44, and 200 ± 54% at the doses of 10 pM–10 nM, respectively, in mice. *Inset* shows superimposed tracings of a cell responding to CGRP in a dose-dependent manner. $^{**}P < 0.0l$ (comparison is made between drug treatment and baseline, n = cell number). Vertical and horizontal bars represent 5 μm and 250 ms, respectively.

atenolol at a concentration that abolished the Iso-induced effect (Table 2).

$[Ca^{2+}]$ transients. CGRP did not significantly alter the baseline diastolic $[Ca^{2+}]$ transients at all doses tested. The systolic $[Ca^{2+}]$ transients in rat ventricular myocytes were increased by 120 ± 36% ($P < 0.01$) after 1 nM CGRP administration, an effect that was 54% greater than that produced by 1 nM of Iso (Fig. 2). Neither CGRP nor Iso changed diastolic Ca^{2+} levels. The half-maximum myoplasmic Ca^{2+} clearance time during relaxation was shortened by 30 ± 5% (141 ± 24 vs. 96 ± 17 ms; $n = 10$, $P < 0.01$) after the cells were exposed to CGRP. The exposure of myocytes to nifedipine (5 mM) for 3–5 min decreased the amplitude of basal $[Ca^{2+}]$ transients of rat ventricular myocytes. CGRP-induced increase in $[Ca^{2+}]$ transients was abolished in the myocytes in the presence nifedipine (Fig. 3).

Application of caffeine (15 mM for 2 min) to beating rat ventricular myocytes quickly reduced the amplitude of $[Ca^{2+}]$ transients from a level of 65 ± 8 to 20 ± 3% above baseline. In the presence of caffeine, CGRP induced an additional 18 ± 2% increase in $[Ca^{2+}]$ transients (Fig. 4). Incubation of rat ventricular myocytes with Rp-cAMPS (100 mM) did not affect the amplitude of basal $[Ca^{2+}]$ transients. CGRP no longer increased $[Ca^{2+}]$ transients in the myocytes after Rp-cAMPS treatment (Fig. 5).

DISCUSSION

This study demonstrates that the neurotransmitter CGRP increases the amplitude and velocity of cell contraction and increases the rate of cell relaxation in adult rodent ventricular myocytes (Table 1 and Fig. 1). This stimulatory effect is mediated through the activation of myocardial $CGRP_1$ receptors, which leads to Ca^{2+}-induced Ca^{2+} release. Contractility was assessed in terms of cell shortening profile. Evidence shows that measurements of cell length shortening in single myocytes are compatible with the systolic and diastolic contractile properties of intact cardiac muscle (5). These results are further confirmed when compared with those obtained from the β-adrenoreceptor agonist Iso.

In addition to its blood-borne hormonal property, CGRP might function as an intrinsic cardiac peptide, because abundant CGRP mRNA has been identified in rat ventricular tissue, implying a local synthesis and release mechanism independent of extracardiac innervation (23). Present data demonstrate a twofold increase in ventricular myocyte contractility compared with baseline when CGRP levels are raised to 100 pM, which is similar to serum CGRP levels in rat and human (13, 31). Thus it is possible that CGRP may exert an endocrine or a paracrine influence on ventricular myocardial performance. Peptide release from cardiac sensory nerves involves the stimulation of mechano- and chemosensitive receptors in the sensory nerve endings (15, 16). Peptides and purines such as CGRP, substance P, and adenosine are released in excessive amounts during cardiac-diseased states such as myocardial ischemia (16, 18). Inhibition of Ca^{2+}-dependent K^+ current in nerve endings may be responsible for sensory receptor activation and neuropeptide release (16, 24). Thus the sensory nervous system may exert an important regulatory function in the cardiovascular system.

Table 2. *Effects of CGRP on rat ventricular myocyte contraction in the presence of $CGRP_1$-receptor blocker CGRP-(7—37) and β-adrenoreceptor blocker atenolol*

	Basal	CGRP-(7—37) (200 nM)	CGRP-(7—37) +CGRP (1 nM)	Basal	Atenolol (100 nm)	Atenolol +CGRP (1 nM)	Basal	Atenolol (100 nM)	Atenolol +Iso (10 nM)
%Cell shortening	5.7 ± 0.3	5.0 ± 1.0	4.4 ± 0.9	4.6 ± 0.4	4.5 ± 0.4	8.0 ± 0.5*	4.4 ± 1.1	3.7 ± 0.6	4.1 ± 0.6
ΔIncrease, %			NS			92 ± 23			NS
Number of cells		5, 3 rats			10, 6 rats			6, 4 rats	

Values are means ± SE. *Significantly different from basal ($P < 0.05$). NS, not significant.

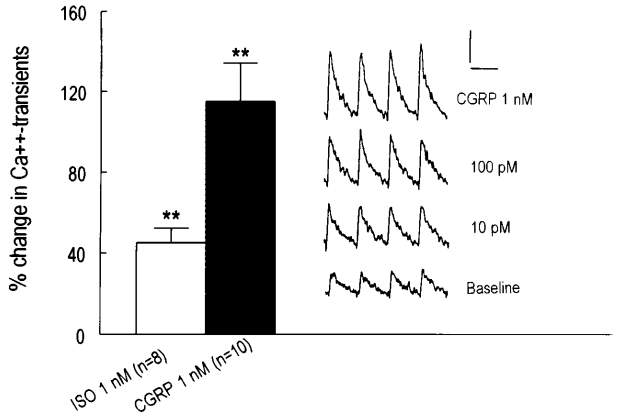

Fig. 2. Effects of CGRP and Iso on [Ca^{2+}] transients in rat ventricular myocytes. Increase in [Ca^{2+}] transients is expressed as %change in 340/380 ratio from baseline values. *Inset* is a typical tracing of a dose-dependent increase in [Ca^{2+}] transients in a rat ventricular myocyte during CGRP administration (vertical calibration bar represents [Ca^{2+}] transient ratio of 1 and time calibration bar is 1 s; $^{**}P < 0.01$).

There have been inconsistent results regarding the physiological roles of CGRP in regulating ventricular contractile function. It has been demonstrated that CGRP exerts a positive inotropic effect on isolated porcine ventricular muscle strips (20, 29) and on isolated adult rat ventricular myocytes (2). However, some studies have failed to show a CGRP-mediated positive ventricular inotropic effect on isolated blood-perfused canine (26) and isolated rat hearts (19). Many reasons may account for these different observations. A species difference in terms of CGRP receptor expression and density might exist between canine and rodent ventricular muscle. Assessment of the direct inotropic effect of CGRP in vascular-intact isolated hearts may not provide information about whether CGRP has a direct action on ventricle or an indirect action secondary to coronary vasodilation, because CGRP is a potent coronary vasodilator. The influence of coronary vasculature is minimized in strips of cardiac muscle perfused in vitro and absent in suspensions of isolated myocytes.

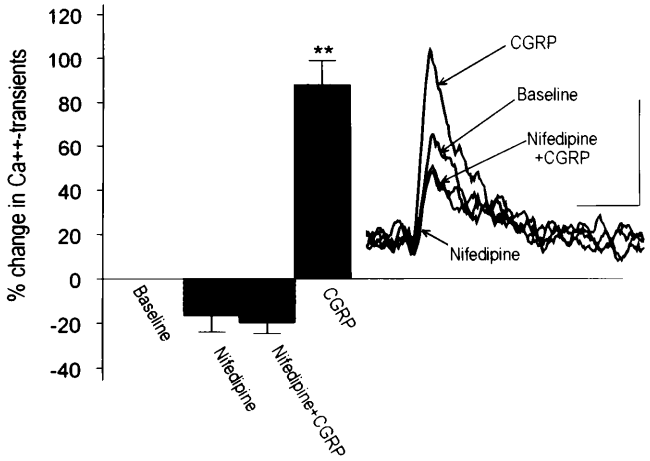

Fig. 3. Effect of CGRP (1 nM) on [Ca^{2+}] transients of ventricular myocytes (8 cells, 4 rats) with and without the Ca^{2+} channel blocker nifedipine (5 μM). Response is shown as %change in [Ca^{2+}] transients (340/380 ratio) from the baseline value of individual cells. *Inset* is a typical cell showing that the CGRP-induced increase in [Ca^{2+}] transient (CGRP) is abolished in the presence of nifedipine (nifedipine+CGRP). Experimental order is nifedipine, nifedipine+CGRP, and CGRP. Vertical and horizontal bars represent Ca^{2+} transient ratio of 1 and 200 ms, respectively. ANOVA was used; $^{**}P < 0.01$ between CGRP and baseline, Nifedipine, and Nifedipine + CGRP.

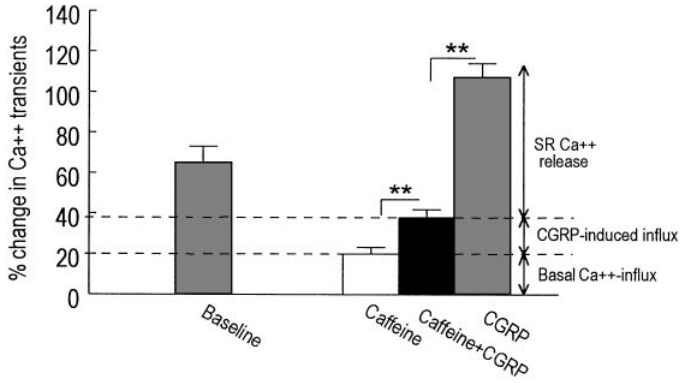

Fig. 4. Effect of CGRP on Ca^{2+} transients in the absence and presence of caffeine. Caffeine (15 mM) markedly reduces the amplitude of [Ca^{2+}] transient to a value 20 ± 3% above diastolic baseline, a level reflecting the amount of Ca^{2+} influx during depolarization, because sarcoplasmic reticulum (SR) Ca^{2+} is depleted by caffeine. In the presence of caffeine, application of CGRP induces an additional 18 ± 2% increase in [Ca^{2+}], a value reflecting CGRP-induced Ca^{2+} influx. Accordingly, SR Ca^{2+} release in the presence and absence of CGRP during each depolarization is 69 ± 6 (CGRP) and 45 ± 5% (baseline), respectively, after subtracting their Ca^{2+} influx amount, suggesting increased Ca^{2+} release from SR after CGRP stimulation. $^{**}P < 0.01$ between bracketed columns.

Mechanisms underlying CGRP-mediated positive inotropic effect on ventricular myocytes were examined in the present study. We found that CGRP-induced positive ventricular inotropic effect was not mediated through the β-adrenoreceptors, because CGRP induced a 92% increase in cell shortening in the presence of atenolol (Table 2). That CGRP no longer elicited a positive inotropic response in the presence of $CGRP_1$ receptor blockade confirmed that CGRP-induced inotropic effect was mediated specifically through the activa-

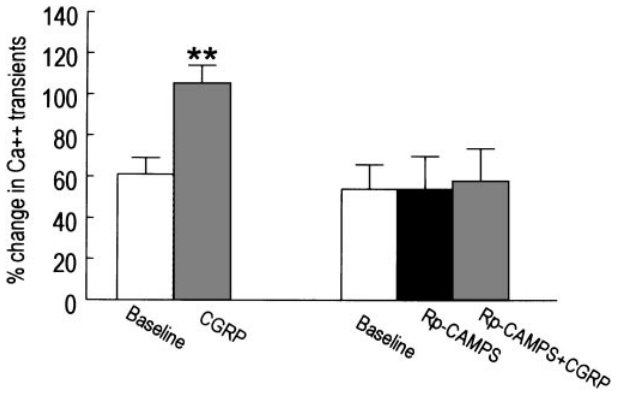

Fig. 5. Effects of CGRP on [Ca^{2+}] transients in rat ventricular myocytes in the absence and presence of a protein kinase A inhibitor Rp-cAMPS. CGRP (100 pM) increases [Ca^{2+}] transients by 86 ± 31%. Treatment of myocytes ($n = 6$) with Rp-cAMPS (100 mM for 30 min) abolishes CGRP-induced increase in [Ca^{2+}] transients. (*t*-test and ANVOA are used for baseline CGRP- and Rp-cAMPS-treated groups, respectively; $^{**}P < 0.01$).

tion of $CGRP_1$ receptors. CGRP produced a concentration-dependent increase in the magnitude of the $[Ca^{2+}]$ transients in beating rat ventricular myocytes (Fig. 2). This effect was abolished in the presence of blockade of the L-type Ca^{2+} channel by nifedipine (Fig. 3), suggesting that increase in intracellular Ca^{2+} by CGRP is dependent on the activation of L-type Ca^{2+} channel. This finding is consistent with a previous report that CGRP causes a voltage-dependent elevation of intracellular Ca^{2+} in nonbeating guinea pig ventricular myocytes (12). The magnitude of CGRP-induced Ca^{2+} influx and sarcoplasmic reticulum (SR) Ca^{2+} release in beating myocytes was determined in the presence of 15 mM of caffeine, a concentration that could quickly deplete SR Ca^{2+} store. After SR Ca^{2+} depletion by caffeine, depolarization induced a 20 ± 3% increase in $[Ca^{2+}]$ transient above baseline, presumably reflecting fractional Ca^{2+} influx during electrical pacing (Fig. 4). Reapplication of CGRP to the cells in the presence of caffeine induced a 38 ± 4% increase in $[Ca^{2+}]$ transient above baseline, indicating an additional 18 ± 2% increase in Ca^{2+} influx by CGRP compared with pacing-induced $[Ca^{2+}]$ transient alone (Fig. 4), a finding consistent with the reports that CGRP increases Ca^{2+} current in atrial myocytes (21, 22). Accordingly, the fractional SR Ca^{2+} release without and with CGRP was 45 ± 5 and 69 ± 6%, respectively, after subtracting their Ca^{2+} influx amount (Fig. 4). Thus CGRP induced an additional 21 ± 6% SR Ca^{2+} release compared with pacing-induced SR Ca^{2+} release alone. Evidently, the enhanced Ca^{2+} release from SR is induced by enhanced Ca^{2+} influx after CGRP stimulation. In addition to its effect on increasing intracellular Ca^{2+} release during cell contraction, CGRP also accelerated myoplasmic Ca^{2+} uptake during cell relaxation, because the half-maximum Ca^{2+} uptake time was shortened by 30% after CGRP stimulation. It is known that the rate of relaxation of $[Ca^{2+}]$ transient is dependent on the systolic Ca^{2+} levels in cardiac myocytes (3). Thus the enhanced myoplasmic Ca^{2+} uptake during cell relaxation is likely due to the fact of increased systolic intracellular Ca^{2+} after CGRP stimulation. Although CGRP significantly shortened myoplasmic Ca^{2+} uptake time during cell relaxation, it did not do so in the presence of L-type Ca^{2+} channel blockade by nifedipine. The signal of the $[Ca^{2+}]$ transient is usually small in the presence of L-type Ca^{2+} channel blockade. The resolution of the Ca^{2+} uptake slope of the small $[Ca^{2+}]$ transient can be confused with background noise level. This interference due to a small signal-to-noise ratio may reduce our software detection capacity for Ca^{2+} uptake slope assessment.

It is well established that phosphorylation of voltage-dependent L-type Ca^{2+} channels by protein kinase A increases transsarcolemmal Ca^{2+} influx and Ca^{2+}-induced Ca^{2+} release (27). Recent biochemical evidence indicates that $CGRP_1$ receptors belong to a G protein-coupled receptor superfamily, its activation resulting in increased cAMP production (1). In agreement with the biochemical evidence, our results indicate that stimulation of $CGRP_1$ receptors in rat ventricular myocytes leads to the activation of protein kinase A, because the inhibition of protein kinase A by Rp-cAMPS eliminates CGRP-induced increase in $[Ca^{2+}]$ transients (Fig. 5). Protein kinase A phosphorylates voltage-dependent L-type Ca^{2+} channels, which leads to increased open probability of Ca^{2+} channel and enhanced Ca^{2+} influx (27). With the use of a modified buffer solution, a previous study reported that CGRP-induced cell shortening in rat ventricular myocytes was independent of activation of L-type Ca^{2+} channels and protein kinase A (2). The discrepancy between those results (2) and the results reported in this study and by others (12) is not understood. Perhaps the different experimental conditions may account, in part, for the different results. In conclusion, CGRP exerts a potent positive inotropic effect on adult rodent ventricular myocytes. This effect is mediated through the stimulation of $CGRP_1$ receptors. Activation of ventricular $CGRP_1$ receptors leads to Ca^{2+}-induced Ca^{2+} release during contraction and facilitated Ca^{2+} uptake during relaxation. The mechanism for CGRP signal transduction involves the activation of protein kinase A in ventricular myocytes.

Perspectives

Stimulation of $CGRP_1$ receptor induces potent positive inotropic effect on rodent ventricular myocytes, as determined by cell-shortening velocity and intracellular $[Ca^{2+}]$ transient measurements. This positive inotropic effect is mediated through the activation of sarcolemmal L-type Ca^{2+} channels, which leads to Ca^{2+}-induced Ca^{2+} release. In addition, CGRP increases the rate of SR Ca^{2+} uptake during relaxation. The cellular mechanism involved in CGRP signaling transduction requires the activation of cAMP-dependent protein kinase. Substantial evidence supports the view that in many forms of heart failure there is an abnormality of excitation-contraction coupling, which reduces the delivery of Ca^{2+} to the contraction sites, thereby impairing cardiac performance. A reduction of Ca^{2+} release from SR during contraction can cause systolic heart failure, and reduced Ca^{2+} uptake by SR may impair myocardial relaxation and contribute to the development of diastolic heart failure. The effects of CGRP in improving Ca^{2+} handling during contraction and relaxation in ventricular myocytes may account for the clinical observation that CGRP improves ventricular contractile function in congestive heart failure patients. Furthermore, it becomes evident that the sensory nervous system may play an important role in cardiovascular regulation.

We thank Bruce Davison for excellent technical support and Dr. Barbara E. Shykoff for assisting with the data analysis.

This study was supported by Grant FDT-00088905 from a Pilot Clinical Pharmacology Training Program and by the Department of Anesthesiology, the State University of New York at Buffalo.

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Received 19 March 1998; accepted in final form 2 October 1998.

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