Opioid Peptide Gene Expression in the Primary Hereditary Cardiomyopathy of the Syrian Hamster

III. AUTOCRINE STIMULATION OF PRODYNORPHIN GENE EXPRESSION BY DYNORPHIN B*

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Prodynorphin mRNA and dynorphin B expression have been previously shown to be greatly increased in cardiac myocytes of BIO 14.6 cardiomyopathic hamsters. Here we report that exogenous dynorphin B induced a dose-dependent increase in prodynorphin mRNA levels and stimulated prodynorphin gene transcription in normal hamster myocytes. Similar responses were elicited by the synthetic selective κ opioid receptor agonist U-50,488H. These effects were counteracted by the κ opioid receptor antagonist Mr-1452 and were not observed in the presence of chelerythrine or calphostin C, two specific protein kinase C (PKC) inhibitors. Treatment of cardiomyopathic cells with Mr-1452 significantly decreased both prodynorphin mRNA levels and prodynorphin gene transcription. In control myocytes, dynorphin B induced the translocation of PKC-α to the nucleus and increased nuclear PKC activity without affecting the expression of PKC-β₁, -ε, or -ζ. Acute release of either U-50,488H or dyn B over single normal or cardiomyopathic cells transiently increased the cytosolic Ca²⁺ level and on the releasable sarcoplasmic reticulum in both groups of cells. The possibility that prodynorphin gene expression may affect the function of the cardiomyopathic cell through an autocrine mechanism is discussed.

Dynorphin B (dyn B)¹ is a biologically active end product of the prodynorphin gene acting as a selective κ opioid receptor agonist (1, 2). In rat ventricular myocytes, dyn B appears to be constitutively released shortly after synthesis, as indicated by the observation that the levels of secreted dyn B significantly exceeded those of the intracellular peptide (3, 4). The finding that the myocardial cell expresses κ opioid receptors (5, 6) and that the stimulation of these receptors affects the cytosolic Ca²⁺ and pH homeostasis as well as the inotropic state in isolated cardiac myocytes (4, 7, 8) suggests that prodynorphin mRNA translation into dyn B may be part of an autocrine mechanism of regulation of the myocyte function. Furthermore, the observation that κ opioid receptor stimulation is coupled to protein kinase C (PKC) (8) and that PKC is involved in the regulation of prodynorphin gene expression (4) raises the possibility that the gene itself may be regulated in an autocrine fashion by one of its peptide products. In this regard, it may be conceivable that dyn B would affect prodynorphin gene expression in pathological conditions characterized by an increase in the synthesis and release of this opioid peptide from the myocardial cell. In companion studies (50, 51), we have shown that the expression of both prodynorphin mRNA and dyn B is markedly enhanced in cardiac myocytes isolated from BIO 14.6 cardiomyopathic Syrian hamsters compared with cells obtained from normal hamster hearts and that PKC activation and/or intracellular Ca²⁺ loading may be involved in the regulation of prodynorphin gene expression throughout the cardiomyopathic process.

In this study, we aimed at investigating whether the stimulation of κ opioid receptors by dyn B or by U-50,488H, a synthetic selective κ opioid receptor agonist (9), may affect prodynorphin mRNA expression or the rate of transcription of the prodynorphin gene in cardiac myocytes isolated either from normal or from cardiomyopathic hamsters. In attempting to verify whether endogenously synthesized κ opioid receptor ligands may regulate prodynorphin gene expression, we also assessed prodynorphin mRNA levels and prodynorphin gene transcription in cardiomyopathic myocytes that have been treated in the presence of Mr-1452, a selective antagonist of κ opioid receptors (10, 11). Finally, the possible consequences of κ opioid receptor agonism were further investigated in normal and cardiomyopathic cells by examining the effects produced by a short- or long-term exposure to U-50,488H or dyn B on the cytosolic Ca²⁺ level and on the releasable sarcoplasmic reticular Ca²⁺ pool.

MATERIALS AND METHODS

Dyn B was purchased from NeoSystem Laboratoire (Strasbourg, France). Certified peptide purity was 98% and was confirmed in our laboratory by reverse-phase high performance liquid chromatography. Dyn B was received as a lyophilized water-soluble peptide and was dissolved immediately before use in the same medium in which cardiac myocytes were resuspended, containing (mM): 116.4 NaCl, 5.4 KCl, 1.6 MgSO₄, 26.2 NaHCO₃, 1.0 NaH₂PO₄, 5.6 d-glucose, 1.0 CaCl₂ (pH 7.38 ± 0.05 in the presence of 95% O₂, 5% CO₂).

(Trans-dl)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)ethyl]cyclohexyl]benzeneacetamide/methanesulfonate hydrate (U-50,488H) was purchased from The Upjohn Co. (N-[(3-Furylmethyl)-α-metoxazocine methanesulfonate (Mr-1452) was a gift from Boehringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, CO). Caffeine was purchased from Sigma. Ryanodine, purified, was from BIOMOL Res. Labs., Inc. (Plymouth Meeting, PA). Animals and all the other chemicals were from the sources listed in the first article of our series of studies (50).

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¹ This abbreviation is used for dynorphin B and PKC, protein kinase C; MARCKS, myristoylated alanine-rich PKC substrate; U-50,488H, (trans-dl)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)ethyloxethyl]benzeneacetamide/methanesulfonate hydrate; Mr-1452, (d/-)-N-(3-furylmethyl)-α-metoxazocine methanesulfonate; SR, sarcoplasmic reticulum.
FIG. 1. Effects of dyn B or U-50,488H on prodynorphin mRNA expression in isolated cardiac myocytes from normal hamsters. The myocardial cells were isolated from 60-day-old F1B control animals. The upper panel shows representative autoradiograms of the ribonuclease protection analysis of myocardial prodynorphin mRNA. Autoradiographic exposure was for 2 days on Kodak X-Omat film with an intensifying screen. The bar indicates the position of a 400-base pair radiolabeled DNA marker, showing that the single protected fragment migrates with a molecular size of 400 bases, corresponding to prodynorphin mRNA. A, untreated myocytes; B, C, D, E, and F, 4 h of exposure to 0.1, 0.2, 0.5, 1.0, and 10 μM dyn B, respectively; G, 4 h of treatment with 1 μM U-50,488H; H, 4 h of treatment with 1 μM dyn B in the presence of 1 μM Mr-1452; I, 4 h of exposure to 1 μM U-50,488H in the presence of 1 μM Mr-1452. The lower panel shows the levels of prodynophin mRNA determined in each experimental condition. The data are expressed as mean values ± S.E. (n = 6). * significantly different from the control value; significant differences were observed throughout groups B–E, but not through groups E–G (one-way analysis of variance, Newman Keul’s test).

Cardiac myocytes were isolated from 60-day-old control (F1B) or cardiomyopathic (BIO 14.6) hamsters by using the procedure described in the first study of this series (50). The extraction of RNA, the determination of prodynorphin mRNA, the isolation of myocardial nuclei, the assessment of purity of the nuclear fraction and the nuclear run-off transcription assay were all performed as described (in the first study of this series (50)), as were the identification of dynorphin B-like material, the immunoblotting analysis and the quantitative immunonautoradiography of PKC isozymes, the measurement of PKC activity, and the estimation of cytosolic calcium in single myocardial cells.

Acute Release of Dyn B, U-50,488H, or Caffeine over Single Cardiac Myocytes—Each agent was rapidly “puffed” from a micropipette positioned directly above a single resting myocyte (the concentrations of dyn B or U-50,488H in the pipette were 10 or 100 μM, respectively, while the concentration of caffeine was 10 mM). Pressure pulses of 20 p.s.i. were applied to the pipette with a picospritzer II (General Valve Corp., Fairfield, NJ). The duration of these pulses was 2 s for the experiments with dyn B or U-50,488H and 200 ms for the studies in the presence of caffeine.

Data Analysis—The statistical analysis of the data was performed by using a one-way analysis of variance, followed by Newman Keul’s test and assuming a p value less than 0.05 as the limit of significance.

RESULTS

Using the same methodology outlined in our companion studies (50, 51), we have examined whether dyn B or the synthetic selective κ opioid receptor agonist U-50,488H may affect the levels of prodynorphin mRNA in hamster ventricular myocytes. A 4-h incubation of myocytes isolated from 60-day-old control animals in the presence of increasing concentrations of dyn B produced a dose-dependent stimulation of prodynorphin mRNA expression (Fig. 1). This effect was evident at a concentration as low as 0.1 μM and reached a plateau when the myocytes were incubated in the presence of concentrations of dyn B ranging between 1 and 10 μM (Fig. 1). Similar to dyn B, U-50,488H (1 μM) markedly increased prodynorphin mRNA levels in control cells (Fig. 1). Cell treatment with the specific κ opioid receptor antagonist Mr-1452 completely antagonized the effects produced by dyn B or U-50,488H (Fig. 1). Dyn B or U-50,488H also failed to affect prodynorphin mRNA levels in control myocytes that were treated with 5 μM chelerythrine or 1 μM calphostin C, two highly selective PKC inhibitors (12–15) (Fig. 2). In companion studies (50, 51), we have shown that prodynorphin mRNA was significantly more expressed in myocytes isolated from cardiomyopathic hamsters than in myocardial cells from control animals. In the present study, the incubation for 4 h of cardiomyopathic myocytes in the presence of 1 μM Mr-1452 markedly down-regulated the expression of prodynorphin mRNA. Although, mRNA levels remained higher than the levels observed in control cells or in cardiomyopathic myocytes that were treated either with chelerythrine or with calphostin C (Fig. 3). Similar effects were observed when cardiomyopathic myocytes were exposed for 4 h to 5 or 10 μM Mr-1452 (not shown). The incubation of cardiomyopathic myocytes with 1 μM dyn B elicited a further slight increase in the expression of prodynorphin mRNA (Fig. 3). Similar results were observed when cardiomyopathic cells were incubated for 4 h in the presence of 1 μM U-50,488H (not shown).

We have previously shown that the increase in prodynorphin mRNA levels observed in cardiomyopathic myocytes was attributable to an increase in the transcription of the prodynorphin gene (50, 51). Here we performed additional run-off experiments in isolated myocardial nuclei to verify whether the stimulation of prodynorphin mRNA expression elicited by dyn B or U-50,488H may also occur at the transcriptional level. The incubation of control myocytes in the presence of 1 μM dyn B or U-50,488H was able to induce a marked increase in prodynorphin gene transcription that was completely antagonized by 1 μM Mr-1452 or by myocyte treatment with 5 μM chelerythrine or 1 μM calphostin C (Fig. 4). Exposure of myocytes isolated from cardiomyopathic animals to the opioid receptor antagonist resulted in a marked decrease in the transcription rate of the prodynorphin gene which remained above that observed in nuclei that have been isolated from control cells (Fig. 4).
The cardiac cells were isolated from 60-day-old BIO 14.6 hamsters. Representative autoradiograms of the ribonuclease protection analysis of myocardial prodynorphin mRNA are shown in the upper panel. Autoradiographic exposure was performed as described in Fig. 1. A, untreated control cells; B, untreated cardiomyopathic myocytes; C, 4 h of treatment of cardiomyopathic cells with 1 μM Mr-1452; D and E, 4 h of treatment of cardiomyopathic cells with 5 μM chelerythrine or 1 μM calphostin C, respectively; F, 4 h of exposure of cardiomyopathic myocytes to 1 μM dyn B; G, 4 h of exposure of cardiomyopathic cells to 1 μM dyn B in the presence of 1 μM Mr-1452; H and I, 4 h of treatment of cardiomyopathic myocytes with 1 μM dyn B in the presence of 5 μM chelerythrine or 1 μM calphostin C, respectively. Averaged values of prodynorphin mRNA levels are reported in the lower panel. The data are expressed as mean values ± S.E. (n = 6). #, significantly different from the control value; **#, significant difference between two groups (one-way analysis of variance, Newman Keul’s test).

The exposure of control myocytes to 1 μM dyn B for 30 min increased the nuclear expression of PKC-α (Figs. 5 and 6). A concomitant reduction in the amount of PKC-α was observed in the cytosolic fraction from dyn B-treated control cells (Figs. 5 and 6). The treatment with dyn B did not apparently affect the expression of PKC-δ, -ε, or -ζ (Figs. 5 and 6). The phosphorylation of the acrylodan-labeled myristoylated alanine-rich PKC substrate (MARCKS) peptide, a high affinity fluorescent substrate for PKC (16–19), occurred at a higher rate in the presence of dyn B. Equal amounts of protein (20 μg) from each sample were subjected to 8% SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting as described in the first article of our series of studies (50). Autoradiograms are representative of six separate experiments. The arrows to the left of each panel indicate PKC immunoreactivity as confirmed in peptide antigen competition experiments (results not shown). The numbers to the right of each panel refer to the molecular mass (kilodaltons) of marker proteins. Lanes 1, 3, and 5 correspond, respectively, to total cell lysates, cytosolic, or nuclear fractions isolated from untreated cells; lanes 2, 4, and 6 correspond, respectively, to total cell lysates, cytosolic, or nuclear fractions isolated from dyn B-treated myocytes.

**Fig. 3.** Effect of α opioid receptor antagonist on prodynorphin mRNA expression in isolated cardiomyopathic myocytes treated in the absence or presence of dyn B or PKC inhibitors.

**Fig. 4.** Effect of α opioid receptor stimulation on the rate of transcription of the prodynorphin gene in isolated myocardial nuclei. Myocardial nuclei were prepared from myocytes that have been isolated from the heart of 60-day-old control or cardiomyopathic hamsters and the nuclear run-off assay was performed as described in the first report in our series of studies (50). Autoradiograms are representative of six separate experiments. 1, transcription of the prodynorphin gene; 2, cyclophilin mRNA. Autoradiographic exposure was for 2 days on Kodak X-Omat film with an intensifying screen. The bars on the right indicate the position of 400- or 220-base pair radiolabeled DNA markers, showing that the single protected fragments migrated with a molecular size of 400 or 270 bases, corresponding to prodynorphin or cyclophilin mRNA, respectively. A, nuclei isolated from untreated control cells; B, nuclei isolated from control myocytes exposed for 4 h to 1 μM dyn B; C, nuclei isolated from control cells exposed for 4 h to 1 μM U-50,488H; D, nuclei isolated from control myocytes incubated for 4 h in the presence of 1 μM dyn B and 1 μM Mr-1452; E and F, nuclei isolated from control cells that have been treated for 4 h with 1 μM dyn B in the presence of 5 μM chelerythrine or 1 μM calphostin C, respectively; G, nuclei isolated from untreated cardiomyopathic cells; H, nuclei isolated from cardiomyopathic myocytes that have been exposed for 4 h to 1 μM Mr-1452.

**Fig. 5.** Effect of dyn B on the subcellular distribution of PKC isozymes in normal myocytes. Total cell lysates, cytosolic and nuclear fractions were prepared from myocytes that have been isolated from 60-day-old control hamsters and then incubated for 30 min in the absence or presence of 1 μM dyn B. Equal amounts of protein (20 μg) from each sample were subjected to 8% SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting as described in the first article of our series of studies (50). Autoradiograms are representative of six separate experiments. The arrows to the left of each panel indicate PKC immunoreactivity as confirmed in peptide antigen competition experiments (results not shown). The numbers to the right of each panel refer to the molecular mass (kilodaltons) of marker proteins. Lanes 1, 3, and 5 correspond, respectively, to total cell lysates, cytosolic, or nuclear fractions isolated from untreated cells; lanes 2, 4, and 6 correspond, respectively, to total cell lysates, cytosolic, or nuclear fractions isolated from dyn B-treated myocytes.

**Fig. 6.** Quantitative analysis of the subcellular distribution of PKC isozymes in normal myocytes treated in the absence or presence of dyn B. Data are expressed as percentage changes in the intensity of autoradiographic bands of total extracts (T), cytosolic (C), or nuclear (N) fractions from dyn B-treated myocytes (hatched bars) relative to the intensity in the autoradiograms of the corresponding samples from untreated cells (white bars, 100%). The data are expressed as mean values ± S.E. (n = 6). *, significantly different from the control value.
Effects of dyn B on nuclear PKC activity. Cells were dissociated from the heart of 60-day-old control or cardiomyopathic hamsters. Nuclear PKC activity was measured in the presence of the acrylodon-labeled MARCKS peptide, according to the method described in the first article in our series of studies (50). The reaction mixture contained, in a final volume of 1 ml, 10 mM Tris/HCl, pH 7.0, 90 mM KCl, 3 mM MgCl₂, 0.3 mM CaCl₂, 0.1 mM EGTA, 100 µM ATF, 10% ethylene glycol, 0.5 µg phosphatidylserine, 0.1 µg 1,2-dioctanoyl-sn-glycero-7-phosphate, and 75 mM acrylodon-labeled MARCKS peptide. Peptide phosphorylation was started by the addition of 10 µg of nuclear protein (arrow) and was followed at 37 °C. As the acrylodon-peptide becomes phosphorylated, it undergoes a time-dependent decrease in its fluorescence at 480 nm.  ●, nuclei were isolated from untreated control cells; ●, nuclei were isolated from control cardiomyopathic cells; ●, nuclei were isolated from dyn B-treated control cells were preincubated for 30 min with 5 µM chelerythrine or 1 µM calphostin C, respectively, before being added to the reaction mixture. The time course of the fluorescence of the acrylodon-peptide alone (●) is also reported. The data are expressed as mean values ± S.E. (n = 6). From 600 to 1200 s, ●, ●, or ● were significantly different from ●, ●, or ●, from 600 to 800 s, ● was significantly different from ●, from 500 to 700 s, ● was significantly different from ●, no significant difference was observed between ● or ● and (one-way analysis of variance, Newman Keul’s test).

Axonterminal release of dyn B (arrow) from a micropipette over a single resting control (panel A) or cardiomyopathic (panel B) myocyte, superfused in the absence (left tracings) or in the presence (right tracings) of the selective κ opioid receptor antagonist Mr-1452 (1 µM in the bathing fluid). The concentration of U-50,488H or dyn B in the pipette was 100 or 10 µM, respectively. In each panel, an electrically driven (Grass stimulator, model SD 9, Grass Instrument Co., Quincy, MA) cytosolic Ca²⁺ transient (●) is also shown for comparison. Each tracing is representative of 8 separate experiments.

Discussion

The data presented in this report show that the exposure of control hamster ventricular myocytes to dyn B resulted in a dose-dependent stimulation in the expression of prodynorphin mRNA that dynorphin mRNA levels were increased by both acute and chronic administration of U-50,488H. These effects appear to be mediated by the interaction of the opioid agonists with κ opioid receptors, since they were prevented by the specific κ opioid receptor antagonist Mr-1452, and occurred at the transcriptional level, as indicated by the results in nuclear run-off experiments. Interestingly, Mr-1452 markedly reduced prodynorphin gene expression in cardiomyopathic myocytes, suggesting an autocrine function of endogenously synthesized κ opioid receptor ligands. The obser-
viation that the opioid antagonist was not able to affect basal prodynorphin mRNA in control cells suggests, that in cardiomyopathic myocytes due to the marked increase in the synthesis and release of endogenous dyn B, the amount of peptide in the medium might have been raised above a critical concentration, acting in an autocrine fashion at the level of κ opioid receptors to elicit a tonic feed-forward stimulation of prodynorphin gene expression. Since we observed only a slight further increase in prodynorphin mRNA levels following the exposure of cardiomyopathic myocytes to dyn B or to U-50,488H, we cannot exclude that the amount of dyn B being released by cardiomyopathic cells might have approached the maximal effect of the opioid ligand on the expression of the prodynorphin gene. Subsequently, addition of exogenous dyn B or of the synthetic ligand to cardiomyopathic myocytes would only produce minimal additive effects. In companion studies (50, 51), we have shown that PKC is involved in mediating the increase in prodynorphin gene expression observed in cardiomyopathic cells. On the other hand, we have also previously shown that, in the myocardial cell, κ opioid receptors are coupled to PKC (7). In the present study, the finding that dyn B or U-50,488H failed to affect prodynorphin mRNA levels and prodynorphin gene transcription in control myocytes that have been treated with chelerythrine or calphostin C indicates that κ opioid receptor stimulation may have increased the expression of the prodynorphin gene through a PKC dependent pathway. Such a possibility appears to be confirmed by the finding that the treatment of control myocytes with dyn B induced the translocation of PKC-α to the nucleus and increased nuclear PKC activity. Although Mr-1452 significantly down-regulated prodynorphin gene expression in cardiomyopathic myocytes, the levels of prodynorphin mRNA in cardiomyopathic cells exposed to the opioid antagonist remained higher than those detected in cardiomyopathic myocytes treated in the presence of PKC inhibitors. This might be due to the fact that autocrine stimulation of κ opioid receptors, by increasing the nuclear expression of PKC-α without affecting the expression of PKC-δ, -ε, and -ζ, may only have elicited the activation of part of the PKC isozymes available in the myocardial cell. In this regard, the expression of both PKC-δ and PKC-ε, besides that of PKC-α, were found to be increased in nuclei isolated from cardiomyopathic myocytes and, in the presence of these nuclei, the phosphorylation of the MARCKS peptide occurred at a higher rate than that observed in the presence of nuclei isolated from dyn B-treated control cells. Therefore, the total amount of activated PKC which contributes to stimulate the expression of the prodynorphin gene in cardiomyopathic myocytes may result from a number of different stimuli that may share PKC activation as a common regulatory mechanism of gene expression. This possibility is supported by the observation that, in the myocardial cell, different receptor systems are coupled to PKC, including muscarinic, α1-adrenergic, adenosine, angiotensin II, and endothelin-1 receptors, as well as poorly characterized stretching sensitive “mechanoceptors” (23–27). Further support is the finding that PKC activation by agonists of these receptors results in common downstream consequences, including the stimulation of gene expression and the hypertrophic growth (28–31).

Here we show that acute release of both dyn B or U-50,488H over single normal or cardiomyopathic myocytes elicited a transient increase in [Ca2+]i of similar magnitude in both groups of cells. Such a response appeared to be mediated by κ opioid receptors and to involve the release of Ca2+ from an intracellular storage site since it was completely abolished by a specific κ opioid receptor antagonist and was preserved in a Ca2+-free buffer. It may be of interest that this effect was observed following a direct local exposure to the opioid agonists, as it might occur when endogenously synthesized κ opioid receptor ligands are secreted from the myocardial cell. The results ob-

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**Fig. 9.** Changes in [Ca]i occurring in normal or cardiomyopathic myocytes following a prolonged exposure to U-50,488 or dyn B. The cardiac myocytes were isolated from 60-day-old control or cardiomyopathic hamsters and superfused in a buffer of the same composition as that reported in the legend of Fig. 8. In each single control or cardiomyopathic cell, the releasable SR Ca2+ pool was assessed at rest by a rapid “puff” (arrow) of caffeine (10 mM in a micropipette positioned over a single myocyte) before and after the superfusion in the presence of the indicated concentrations of each agent. Each tracing is representative of 8 separate experiments.
tained following a prolonged exposure of normal or cardiomyopathic myocytes to dyn B or U-50,488H show that these opioid agonists ultimately led to a depletion of Ca\(^{2+}\) from an intracellular pool in both groups of cells. In previous studies we have shown that \(\kappa\) opioid receptor stimulation released Ca\(^{2+}\) from the SR in rat cardiac myocytes (3, 7, 8) and from an intracellular pool in neuroblastoma-2 a cells (7). The receptor activation depleted these Ca\(^{2+}\) storage sites in both cell types and was coupled with a rapid and sustained increase in phosphoinositide turnover (7, 32). These observations suggest that the opioid-induced effects on cytosolic Ca\(^{2+}\) homeostasis observed in the present study may represent a general mechanism for the action of \(\kappa\) opioid receptor agonists. The present data also show that the time course of [Ca\(^{2+}\)], increase in response to a sustained exposure to the opioid agonists was significantly more prolonged in cardiomyopathic than in normal cells. This difference may be due to the presence of altered Ca\(^{2+}\) efflux rates in cardiomyopathic cells compared with normal myocytes, as suggested by the finding that the sarcolemmal Ca\(^{2+}\) ATPase activity and gene expression were both reduced in myocardial cells from BIO 14.6 cardiomyopathic hamsters (33). Therefore, due to the abnormal [Ca\(^{2+}\)], observed at rest in cardiomyopathic myocytes, a further Ca\(^{2+}\) loading due the opioid-mediated Ca\(^{2+}\) release from the SR may require a more prolonged time for Ca\(^{2+}\) extrusion through the sarcolemma in cardiomyopathic myocytes compared with normal cells. On the other hand, we cannot exclude that the difference in the time course of [Ca\(^{2+}\)], increase observed among cardiomyopathic and normal cells in response to \(\kappa\) opioid receptor stimulation may reflect the presence of abnormalities in Ca\(^{2+}\) sequestration and/or release at the level of the SR. Supporting such a hypothesis are the observation that the SR Ca\(^{2+}\) ATPase activity and gene expression are also inhibited in cardiomyopathic hamster hearts (33) and the finding that the number of ryanodine-binding sites is increased in cardiac membrane preparations from BIO 14.6 hamsters, suggesting a defect in the function of the ryanodine-sensitive SR calcium release channel (34, 35).

The possible consequences of the present results, suggesting that dyn B may be involved in an autocrine feed-back loop regulating prodynorphin gene expression, remain to be elucidated. We may only speculate that tonic release of dyn B, by depleting the SR releasable Ca\(^{2+}\) pool, may contribute to elicit the decrease in the amplitudes of the cytosolic Ca\(^{2+}\) transient and of the associated contraction previously observed in isolated cardiomyopathic cells (36). Moreover, it is now clear that opioid peptides also act as growth regulators in many normal and malignant tissues (37–43). Recently, tonic release of opioid peptides has been implicated in the regulation of neuroblastoma proliferation through the activation of specific opioid receptors (44). Interestingly, accumulating evidence show that opioid peptides may be produced in an autocrine and, probably, paracrine manner (44–46) and may influence proliferation and differentiation in a wide variety of cells and tissues, including neurons and glia in the nervous system (47) and myocardial and epicardial cells in the neonatal heart (48). These findings have led to the consideration of opioid peptides as growth factors which act by regulating cell proliferation and are also able to alter cell migration and the orchestration of cells into a specific architecture (49). We cannot exclude that these “ trophic” effects of opioid peptides might also apply to primary hypertrophic cardiomyopathies, a number of diseases which, besides showing an impairment of myocyte contractility and cytosolic Ca\(^{2+}\) handling, also exhibit substantial alterations in processes related to the growth, differentiation and architectural assembly of cardiac myocytes. However, it must be emphasized that we have not yet demonstrated whether endog-
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