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Delta-Opioid Augments Cardiac Contraction through β -Adrenergic and CGRP-Receptor Co-signaling

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Abstract

Cardiac epinephrine and calcitonin gene-related peptide (CGRP) are produced by intrinsic cardiac adrenergic cells (ICA cells) residing in human and animal hearts. ICA cells are neuromodulatory cells expressing δ -opioid receptors (DOR). We hypothesized that δ -opioid stimulation of ICA cells enhances epinephrine and CGRP release, which results in the augmentation of heart contraction.

Methods—Rats were injected with DOR-agonist DPDPE (100 μ g/kg) with or without 10-min pretreatment with either β -adrenergic receptor (β -AR) blocker propranolol (2 mg/kg) or CGRP-receptor (CGRP-R) blocker CGRP₈₋₃₇ (300 μ g/kg), or their combination. Hemodynamics were monitored with echocardiogram and systolic blood pressure (SBP) was monitored via a tail arterial catheter.

Results—Changes in left ventricular fraction-shortening (LVFS) and heart rate (HR) were observed at 5-min after DPDPE infusion. At 5-min DPDPE induced a $36 \pm 18\%$ ($p < 0.001$) increase of the LVFS, which continues to increase to $51 \pm 24\%$ ($p < 0.0001$) by 10 min, and $68 \pm 19\%$ ($p < 0.001$) by 20 min. The increase in LVFS was accompanied by the decrease of HR by $9 \pm 5\%$ ($p < 0.01$) by 5 min and $11 \pm 6\%$ ($p < 0.001$) by 15 min post DPDPE infusion. This magnitude of HR reduction was observed for the remainder of the 20 min. Despite the HR-reduction, cardiac output was increased by $17 \pm 8\%$ ($p < 0.05$) and $28 \pm 5\%$ ($p < 0.001$) by 5- and 20-min post DPDPE administration, respectively. There was a modest ($9 \pm 9\%$, $p = 0.03$) decrease in SBP that was not apparent until 20 min post DPDPE infusion. The positive inotropism of DPDPE was abrogated in animals pretreated with propranolol, CGRP₈₋₃₇, or combined propranolol+CGRP₈₋₃₇. Furthermore, in whole animal and cardiomyocyte cell culture preparations, DPDPE induced myocardial protein-kinase A (PKA) activation which was abrogated in the animals pretreated with propranolol+CGRP₈₋₃₇.

Conclusion—DOR agonists augment myocardial contraction through enhanced β -AR and CGRP-R co-signaling.

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Introduction

Opioid receptors are G-protein coupled receptors that have a wide range of tissue distribution and are composed of multiple variants of mu, kappa and δ -opioid receptors (DOR) (30). In the rat heart, DOR is the predominant isoform, and its activation can protect myocytes from ischemia/reperfusion injury (2, 33). Until recently, the source of cardiac DOR remained elusive, until recently it was found, that in the ventricle, DOR is expressed exclusively on intrinsic cardiac adrenergic (ICA) cells in human and rat hearts (12, 14, 15). Conversely, in the atria, DOR was exclusively expressed by the postganglionic parasympathetic nerves (5, 26). As of yet, there has not been a study to demonstrate whether cardiac muscle cells express DOR immunoreactivity. However, in the absence of DOR expression in myocytes, δ -opioid agonist-enhanced myocyte contractility (21) and intracellular Ca^{2+} mobilization (11, 34) in cardiomyocyte cultures strongly indicating that DOR agonist mediates indirect cardiac inotropism via the activation of ICA cells in ventricular myocardium. The ICA cell was discovered by Huang *et al* in 1996 (12) and confirmed by Ebert & Thompson (6). Subsequent studies have established that ICA cells are unique cardiac neuroparacrine cells which play a critical role in cardiac development and adult heart function (7, 12, 14, 15). ICA cells release epinephrine and CGRP independent of cardiac autonomic innervation (14–16). Both endogenous epinephrine and CGRP are involved in δ -opioid regulated myocardial protection against ischemia/reperfusion (14, 15). Like epinephrine, CGRP is a potent neurohormone exerting positive cardiac inotropic effects. Treatment of porcine ventricular myocardial strip with CGRP increases its contraction (25, 38), and intravenous administration of CGRP in heart failure patient drastically improves cardiac systolic function (10). The mechanism of CGRP-enhanced cardiac contractility is mediated via the L-type calcium channel by way of PKA activation (13). Preliminary studies showed that DOR stimulation of ICA cells enhances left ventricular systolic function in the rat heart (14). The detailed hemodynamic effects and mechanism underlying cardiac DOR stimulation have not yet been fully characterized. In the present study, we investigated whether δ -opioid agonists can enhance inotropic function of the rat heart and the mechanism of such activity. The aims of this study are to assess the effects of DPDPE in the presence or absence of β -AR/CGRP_R blockade on (i) LV systolic function by evaluating LV fraction shortening (LVFS); (ii) on HR and systolic blood pressure (SBP); (iii) cardiac output; (iv) δ -opioid-enhanced β -AR/CGRP_R co-signaling activation of PKA.

Materials and Methods

[d-Pen²,d-Pen⁵]-Enkephalin (DPDPE), CGRP_R antagonist CGRP₈₋₃₇ was obtained from Sigma-Aldrich (St Louis, MO). The administration of intravenous drugs was carried out as a 10-minute injection via the tail vein. The investigation conformed to the principles outlined in the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health Publication No. 85-23, revised 1996. The protocols for using animal tissue samples were approved by the Institutional Review Board and the Institutional Animal Care and the Use Committee of the University of Texas Medical Branch and University of Houston and Baylor College of Medicine.

Effect of δ -opioid agonist on LV systolic function

To assess the effects of δ -opioid agonism on LV systolic function, transthoracic echocardiogram (echo) was performed using the Acuson X300 (Siemens Medical Solutions) on adult Sprague-Dawley rats (n=8/group). Rats were initially anesthetized with intraperitoneal injection of xylazine and ketamine cocktail. Initial baseline echo was performed then followed serially after the intravenous administration of DPDPE (100 $\mu\text{g}/\text{kg}$). LVFS was calculated as [(LV end-diastolic dimension – LV end-systolic dimension)/

LV end-diastolic dimension] measured in M-mode of the short axis at the papillary muscle level. HR was calculated in M-mode with the included software on the Acuson X300. Cardiac output (CO) was derived from 2-mm pulse wave Doppler interrogating the proximal right ventricular outflow tract (RVOT) obtained in the short axis at the aortic valve level timed cross sectional area (CSA) of RVOT then multiplied by the HR. The calculation was as followed: $[(TVI_t * HR_t * CSA) - (TVI_0 * HR_0 * CSA)] / (TVI_0 * HR_0 * CSA)$, where t = indicated time and 0=acquisition obtained just prior to the administration of DPDPE. Echo derived CO correlates well with invasive thermodilution data (24). SBP was monitored invasively via an indwelling tail arterial catheter system (iWokx, Dover, NH).

Effects of β -AR and CGRPR antagonists on δ -opioid mediated LV augmentation

Rats (n=6/group) were pretreated with non-selective β -AR antagonist propranolol (2 mg/kg) and selective CGRPR antagonist CGRP₈₋₃₇ (300 μ g/kg) 10 minutes prior to administration of DPDPE (100 μ g/kg). To assess the effect of individual β -AR and CGRPR blockade on δ -opioid-induced LV inotropism, the effect of DPDPE on LV inotropism was determined in two separate groups of animals which had been pretreated with propranolol or CGRP₈₋₃₇ 10 minutes before DPDPE administration (n=6/group). Echo parameters were monitored as described above.

Delta-opioid agonist induced PKA activation

Mixed cardiac culture (n=4) isolated from adult rat as previously described (12, 14, 15). The isolated cardiocytes were cultured for 7 days then treated with DPDPE (0.1 μ M), then PKA activity was determined after indicated times of stimulation. For blockade studies, the mixed cardiac cultured cells were treated with DPDPE (0.1 μ M) in the presence (20 min pretreatment) or absence of propranolol (1 μ M) and CGRP₈₋₃₇ (10 nM) then PKA activity was determined after 8 min of DPDPE stimulation. For *in vivo* assessment of DPDPE-induced PKA activities, anesthetized adult rats (n=5) were injected with DPDPE (100 μ g/kg) in the presence (20 min pretreatment) or absence of propranolol (2 mg/kg) and CGRP₈₋₃₇ (300 μ g/kg). The total DPDPE stimulation time was 10 min. The whole heart was then explanted and immediately snapped frozen in liquid nitrogen. PKA activities were determined by using PKA assessment kit according to the manufacturer instruction (Promega, Madison, WI).

Statistical Analysis

The data was analyzed and expressed as the percentage of time 0, which is just prior to the administration of DPDPE, expressed as mean $\pm$ SD. The comparison to baseline indicated a Dunnett multiple comparison adjustment. The post baseline comparisons were adjusted using the Tukey Kramer adjustment. All computations were conducted using SAS v. 9.2. A p-value of less than 0.05 is taken to indicate significance.

Results

Effect of δ -opioid agonist on LV systolic function

Compared with the baseline, LVFS increased by $36 \pm 18\%$ ($p < 0.001$) at 5 min post DPDPE infusion. LVEF increased by $63 \pm 16\%$, compared with baseline ($p < 0.001$) at 15 min, which is an additional increase compared with that at 5 min ($p < 0.01$; Fig. 1). Associated with this increased LVFS, there was a modest decrease in HR ($-9 \pm 5\%$, $p < 0.05$; Fig. 2B). A modest reduction in SBP ($9 \pm 9\%$, $p = 0.030$) was seen at 20 min post DPDPE injection (Fig. 2C). Myocardial O₂ consumption can be estimated by the $HR \times SBP$, an index termed as a double product. A smaller index indicates less O₂ consumption (20). The double product decreased by $22 \pm 5\%$ ($p < 0.05$) following DPDPE infusion. Despite decreased HR, CO was

increased by $17 \pm 8\%$ ($p < 0.05$) by 5 min with continuous increase to $28 \pm 6\%$ ($p < 0.01$) by 10 min maintaining at this levels for at least 20 min (Fig. 3B).

CGRPR and β -AR antagonists blunted the cardio-augmentative effect of δ -opioid agonist

Since DPDPE enhances the secretion of cardiac epinephrine and CGRP from ICA cells (14, 15), we determined whether blocking of β -AR and CRPRR could abrogate neurohormonal effect on δ -opioid-elicited LV augmentation. In the animals pretreated with propranolol +CGRP₈₋₃₇, DPDPE conferred no augmentation of LVEF (Fig. 4). We further assessed individual contribution of β -AR or CRPRR signaling on DPDPE-elicited LV augmentation. In the animals pretreated with propranolol alone, DPDPE exerted no significant effect on LVFS ($n=6$, Fig. 4). In those animals pretreated with CGRP₈₋₃₇ alone, DPDPE had no effect on LVFS ($n=6$, Fig. 4).

δ -opioid agonist induces PKA activation via β -AR and CGRPR co-stimulation

Time course of DPDPE-induced PKA activation *in vitro* showed that PKA activity peaks at ~6–8 min post treatment ($n=4$; Fig. 5A). *In vitro* stimulation of mixed cardiomyocytes ($n=4$) with DPDPE (0.1 μ M) for 8 min leads to more than 15.5 ± 1.5 fold ($p < 0.001$) enhancement of PKA activity compared with saline treated ones (Fig. 5B). This DPDPE-induced PKA activity was attenuated by $94 \pm 1\%$ ($p < 0.001$) when pretreated with propranolol+CGRP₈₋₃₇ (Fig. 5C). A similar effect was observed with *in vivo* model, but to a lesser extent. DPDPE induced $21 \pm 13\%$ ($p < 0.05$) increase of PKA activity, which was abrogated in the animals pretreated with propranolol+CGRP₈₋₃₇ (Fig. 5C).

Discussion

The present study demonstrated that the specific DOR agonist DPDPE increases LV inotropism in intact adult rats. This effect was achieved by δ -opioid stimulation of ICA cells which release endogenous epinephrine and CGRP. The ICA cell is a novel type of cardiac neuroparacrine cell that has a critical role in cardiac development and adult heart function (6, 12, 14, 15). ICA cells release epinephrine and CGRP in a regulatory fashion which is independent of autonomic innervation (14–16). ICA cell-derived epinephrine and CGRP are involved in δ -opioid regulated myocardial protection against ischemia/reperfusion injury (14, 15). Therefore, it is conceivable that δ -opioid-evoked release of epinephrine and CGRP can interact with their respective G-protein coupled receptors on the myocytes to activate adenylate cyclase and subsequent PKA activation (Fig. 6)(1, 4, 28, 36).

The involvement of β -AR and CGRPR was evident by the abrogation of the DPDPE-induced LV augmentation and PKA activation in the presence of β -AR and CGRPR blockade. This finding indicates that epinephrine and CGRP are the facilitators of DPDPE-enhanced LV augmentation. This functional evidence is consistent with previous biochemical assays that showed that DPDPE markedly enhances the release of endogenous epinephrine and CGRP from ICA cells (14, 15). It is interesting to note that, in the presence cardiac β -AR blockade alone, δ -opioid-evoked CGRP release failed to augment LVFS (Fig. 4). This finding is in agreement with a previous *in vivo* canine study by Katori et al (19) who demonstrated that CGRP-enhanced LV augmentation was dependent of intact myocardial β -AR receptor signaling. Furthermore, in the presence of β -AR antagonist, CGRP-mediated LV augmentation is abrogated (19). These data indicate that constitutive intrinsic cardiac epinephrine- β -AR signaling is required for CGRP-mediated LV augmentation. Additionally, it was equally interesting to detect that δ -opioid-activation of ICA cells conferred no LV augmentation in the presence of cardiac CGRPR blockade (Fig. 4). To the best of our knowledge, this is the first time to have demonstrated that endogenous epinephrine- β -AR-mediated LV augmentation is dependent on intact myocardial CGRPR

signaling. If confirmed in future study, this constitutive cardiac CGRP signaling may exert a “permissive” role for β -AR-mediated LV augmentation. Our data suggests that β -AR and CGRPR can cross talk in a reciprocally reinforcing (or mutually permissive) manner achieving optimal cardiac regulation. Blunting either limb of these adrenoceptor pathways can counter balance its counterpart signaling profoundly.

The timing of PKA activation correlates with the timing of enhanced LV contractility observed with echo (Figs. 1C & 5). Activation of both β -AR and CGRPR has been shown to regulate cardiac contractility in a PKA dependent manner (13, 28, 31, 36). PKA activation is unlikely to be directly mediated by δ -opioid agonist as DOR agonists are known to reduce the intracellular accumulation of cAMP, which would lead to suppression of PKA activation (30, 32). There was a marked difference in the degree of PKA activation between *in vitro* and *in vivo* experiments (Fig. 5). This was likely due to the high background of constitutively activated PKA secondary to heightened endogenous adrenoceptor factors in conscious animals. Despite the differences in the magnitudes, the data are well correlated providing a proof-of-concept to demonstrate the applicability of δ -opioid treatment in whole animal.

There is a modest 9% SBP decrease following DPDPE infusion (Fig. 2C). This most likely resulted from the spillover of cardiac CGRP into systemic circulation following δ -opioid stimulation of ICA cells (14, 16). In consistent with previous studies, δ -opioid agonist can decrease HR (Fig. 2). This reduction is most likely vagally mediated (8, 29) due to δ -opioid stimulation of cardiac parasympathetic neurons which express DOR (7, 28). DPDPE increased CO by as much as 28% (Fig. 3C). The increase in CO without concomitant HR acceleration reveals a favorable hemodynamic profile for potential δ -opioid-based therapy. Despite the decrease in HR, the DPDPE increased CO by as much as 28% (Fig. 3C). The reduced HR-SBP double product associated with δ -opioid therapy indicated that increased CO following DPDPE infusion is achieved at no increase in myocardial oxygenation consumption. This salient feature may offer a therapeutic potential, if such an effect can be replicated in ischemic cardiomyopathy.

Study limitation

The role of DOR agonists in cardiac function has been a matter of contentious debate. There are studies showing that DOR agonist depressed cardiac function in Bio 14.6 hamster (3), while others demonstrated the escalation of cardiac contractility (9, 22). There is also a report showing that both DOR agonists and antagonists have negative inotropism (23). These conflicting results may have been from the use of different animal models and/or experimental conditions. The present study was performed in rats with normal hearts. Animal with heart failure may respond differently due to changes in the expression of receptor profile or modification of the underlying signaling pathways. There are also multiple etiologies that lead to heart failure, and each subgroup may also response differently to δ -opioid agonist. The finding presented here is not necessarily applied to any diseased heart. Furthermore, the present study did not explore the cellular mechanisms underlying myocardial β -AR and CGRPR cross-talking. Therefore, additional investigation is required.

In summary, δ -opioid agonist DPDPE increases LV contractile function, cardiac output with the concomitant decrement of HR and double products. The augmentation of LV systolic function by δ -opioid agonist is mediated through the co-activation of myocardial β -AR and CGRPR and with subsequent enhancement of PKA activities. Active cross-talking exists between cardiac β AR and CGRPR signaling in a manner of mutually permissive reinforcement.

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Highlights

Recently, we discovered that the intrinsic cardiac adrenergic cell (ICA cell) expresses myocardial δ -opioid receptors. The present study demonstrated that activation of ICA cells with δ -opioid receptor agonist augments myocardial contraction through enhanced myocardial beta-adrenergic and CGRP receptor co-signaling. This adreno-peptidergic receptor signaling is coupled with cAMP-dependent PKA pathway.

Figure 1. Effect DPDPE on LVFS. (A & B). An example of increased LVFS after 20 min of DPDPE infusion (B) compared with baseline (A). Images are derived from M-mode data of the LV (short-axis view) at the papillary muscle level. (C). The percent changes of LVFS at indicated times after the infusion of DPDPE (n=8/time point). ***: $p < 0.001$.

Figure 2.
Effect of DPDPE on heart rate (A,B), systolic blood pressure (C) and double product (D).
(n=8)

Effect of DPDPE on HR, SBP and double product. (A). An example of HR measurement showing cardiac cycle duration was 292 msec translated into 205 bpm. Image is derived from M-mode of LV short-axis view at the papillary muscle. The percentage changes of HR (B), SBP (C) and double product (D) are plotted at indicated times after the infusion of DPDPE (n=8/time point). *: p<0.05; **: p<0.01.

Figure 3. Effect of delta-opioid antagonist on cardiac output (n=5)
 Effect of DPDPE on cardiac output. (A). An example of TVI acquisition. Image is derived from 2D echocardiography of the LV in short-axis view at the level of the aortic valve and 2-mm pulse wave Doppler acquisition at the RVOT (A). The Doppler signal was inverted to be upright. (B) Percent changes of CO at indicated time after administration of DPDPE (n=8/group). *p<0.05; **p<0.01; ***p<0.001.

Figure 4. Effects DPDPE on LVFS in the absence or presence of propranolol, CGRP₈₋₃₇, or propranolol+CGRP₈₋₃₇ (n=6/group). **: p<0.001; ns=non-significant.

Figure 5. DPDPE activation of PKA is mediated via β_2 -AR/CGRPR. (A) Cardiocyte culture (n=4) were stimulated with DPDPE (0.1 μ M), then PKA activity was determined at indicated times. (B) Mixed cardiocyte cultures were stimulated with saline or DPDPE (0.1 μ M) for 8 min in the presence (20 min pretreatment) or absence of propranolol (1 μ M)+CGRP₈₋₃₇ (10 nM). (n=4). (B) Adult rats were injected with saline or DPDPE (10 μ g/kg/min) for 10 min in the presence (20 min pretreatment) or absence of propranolol (2 mg/kg) and CGRP₈₋₃₇ (300 μ g/kg). (n=5). *: p<0.05; **: p<0.01; #: p<0.001.