

Molecular Basis for Autonomic Remodeling in the Ventricular Tachypacing Canine Model of Congestive Heart Failure

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Disclosures and Conflicts of Interest

The authors have no disclosures
or conflicts of interest to report
related to this project



Background

- Atrial fibrillation (AF) is the most common clinical dysrhythmia.
 - AF prevalence increases with age (**Thom *et al.*, 2006**)
 - Clinical and public health burden of AF will continue to rise (**Lloyd-Jones *et al.*, 2004; Go *et al.*, 2004**)
- AF is commonly associated with congestive heart failure (CHF). (**Wang *et al.*, 2003**)
 - Nearly one-half of all patients with AF have CHF.
 - The two are frequently comorbid.
- The autonomic nervous system undergoes salient changes in CHF.
 - Increased sympathetic nervous system activity and signaling (**Francis, 1986; Meredith *et al.*, 1993; Hasking *et al.*, 1986**)
 - Altered molecular parasympathetic signaling (i.e. G α i) (**Bohm *et al.*, 1992, 1994**)



Background

- The autonomic nervous system plays a key role in the genesis and maintenance of AF.
 - In experimental models and humans, prolonged parasympathetic stimulation promotes AF. (**Page *et al.*, 1991**; **Haj-Darwish *et al.*, 1993**; **Dobrev *et al.*, 2001**)
 - Increased atrial sympathetic innervation in AF. (**Yang and Levy, 1984**; **Gould *et al.*, 2006**)
 - Parasympathetic and sympathetic nervous systems work in tandem to promote AF. (**Sharifov *et al.*, 2004**)
 - Autonomic innervation to the left atrium is heterogeneous (i.e. pulmonary veins (PVs) and posterior left atrium (PLA) manifest a unique autonomic profile). (**Liu *et al.*, 1997**; **Arora *et al.*, 2007**; **Tan *et al.*, 2007**)



Background

- The autonomic nervous system appears involved in AF pathogenesis in the setting of CHF.
 - Increased sympathetic and parasympathetic nerve discharges prior to paroxysmal atrial tachycardia in the setting of CHF. (**Ogawa *et al.*, 2007**)
 - As shown earlier, there is an increase in autonomic innervation in the posterior left atrium in canine CHF (**Villuendas *et al.*, AHA 2007**).
- However, the role that molecular autonomic nervous system remodeling plays in the genesis of AF has not been thoroughly examined in structural heart disease (particularly in CHF).



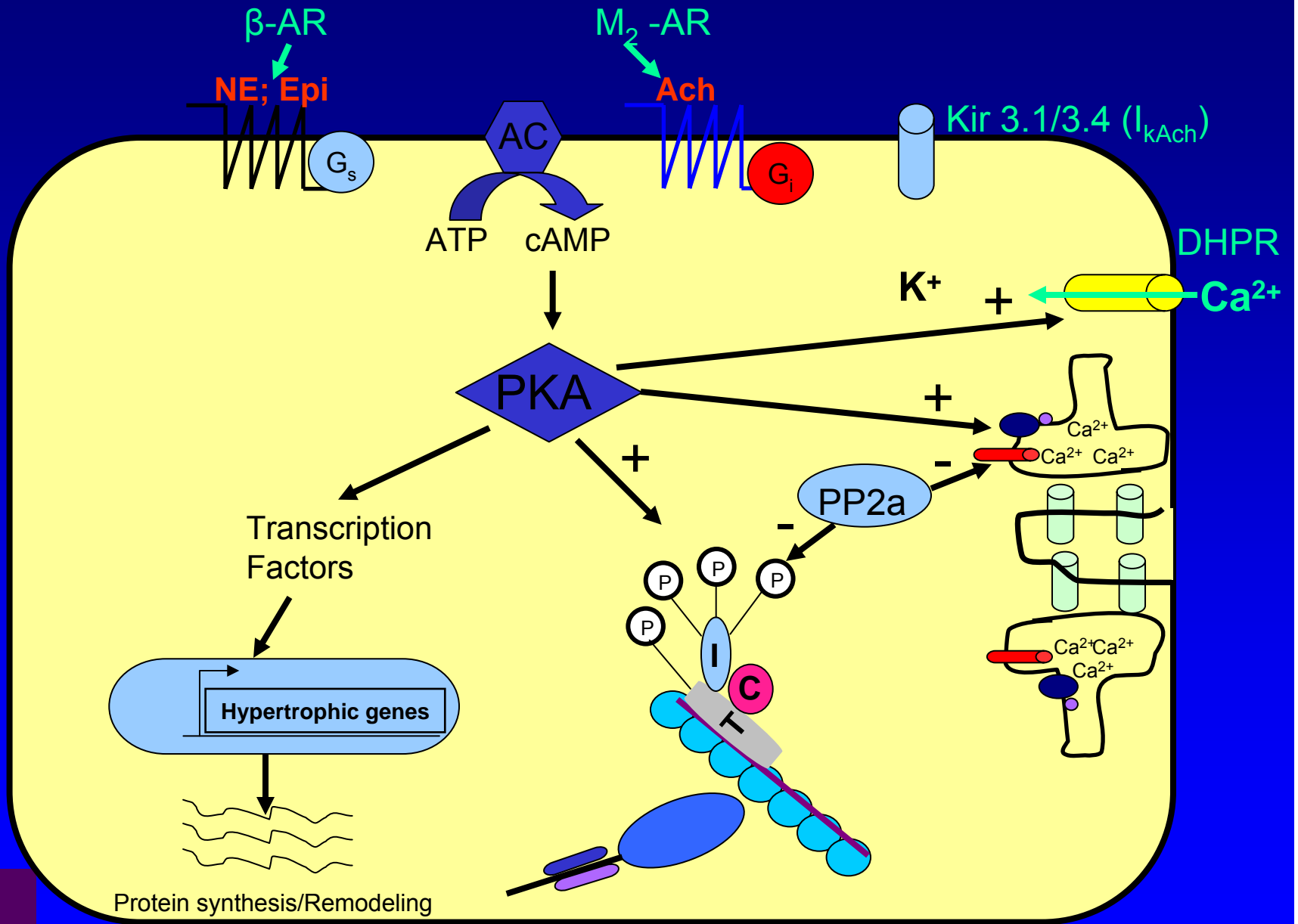
Research Hypotheses

Chronic atrial stretch secondary to pacing-induced CHF results in:

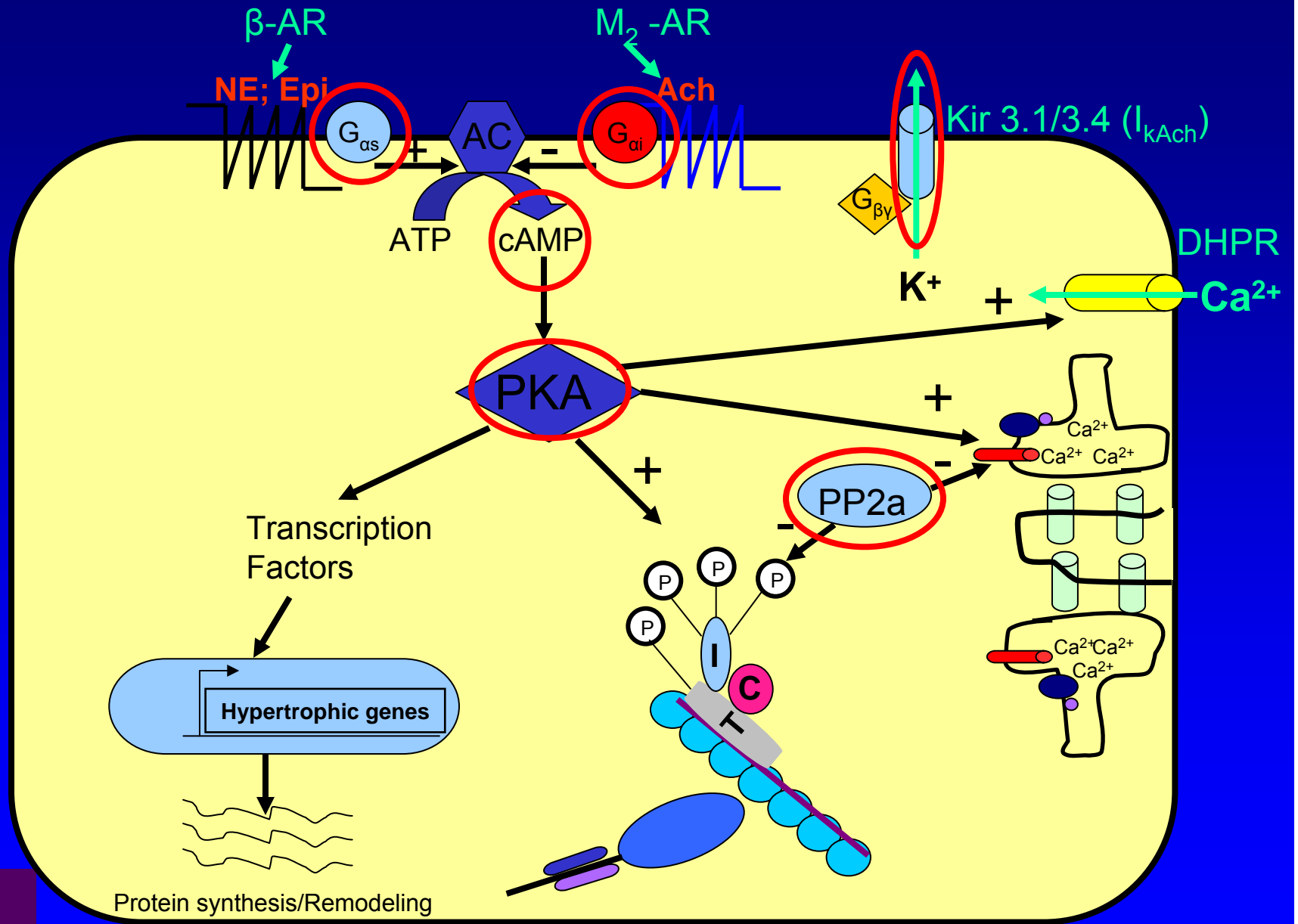
- 1) Upregulation of sympathetic and parasympathetic signaling pathways in the left atrium
- 2) Molecular autonomic remodeling in the left atrium is heterogeneous, being more pronounced in the PLA relative to other regions



Atrial Autonomic Remodeling in Experimental CHF



Atrial Autonomic Remodeling in Experimental CHF



Objectives

- To examine the expression and activation of the following key autonomic signaling molecules in the left atrium and pulmonary veins in a CHF model of AF
 - **Sympathetic signaling:** $G_{\alpha s}$
 - **Parasympathetic signaling:** $G_{\alpha i}$ ($G_{\alpha i2}$, $G_{\alpha i3}$), IKAch (Kir3.1)
 - **Downstream effectors:** cAMP, protein kinase A (expression, activity), protein phosphatase type 2
- To compare the relative expression of the above listed signaling molecules in the PVs, PLA, and left atrial appendage (LAA)



Methods

- CHF induced by RV pacing in 8 dogs at 240 beats/min for 3-4 weeks; 8 control (age-matched) dogs also used
- Atria and PVs obtained both CHF and control dogs
- Frozen PLA, PVs and LAA subjected to western blotting for protein expression of:
 - **G α s**
 - **G α i (G α i2, G α i3)**
 - **IKAch (Kir3.1)**
 - **PKA_{cat}**
- PLA, PVs and LAA subjected to ELISA for:
 - **cAMP accumulation/concentration**
 - **Protein kinase A (PKA) activity**
 - **Protein phosphatase Type 2a (PP2a) activity**

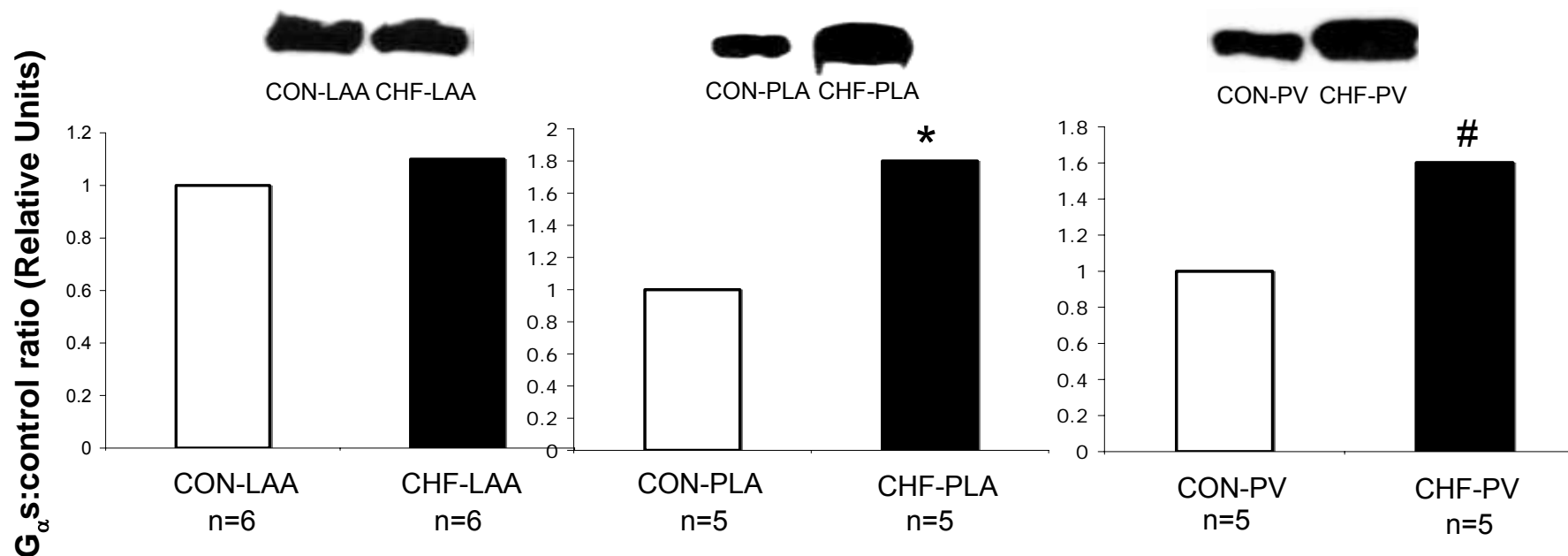


Results

Sympathetic signaling pathway



Intra-regional G_{α} s-protein expression

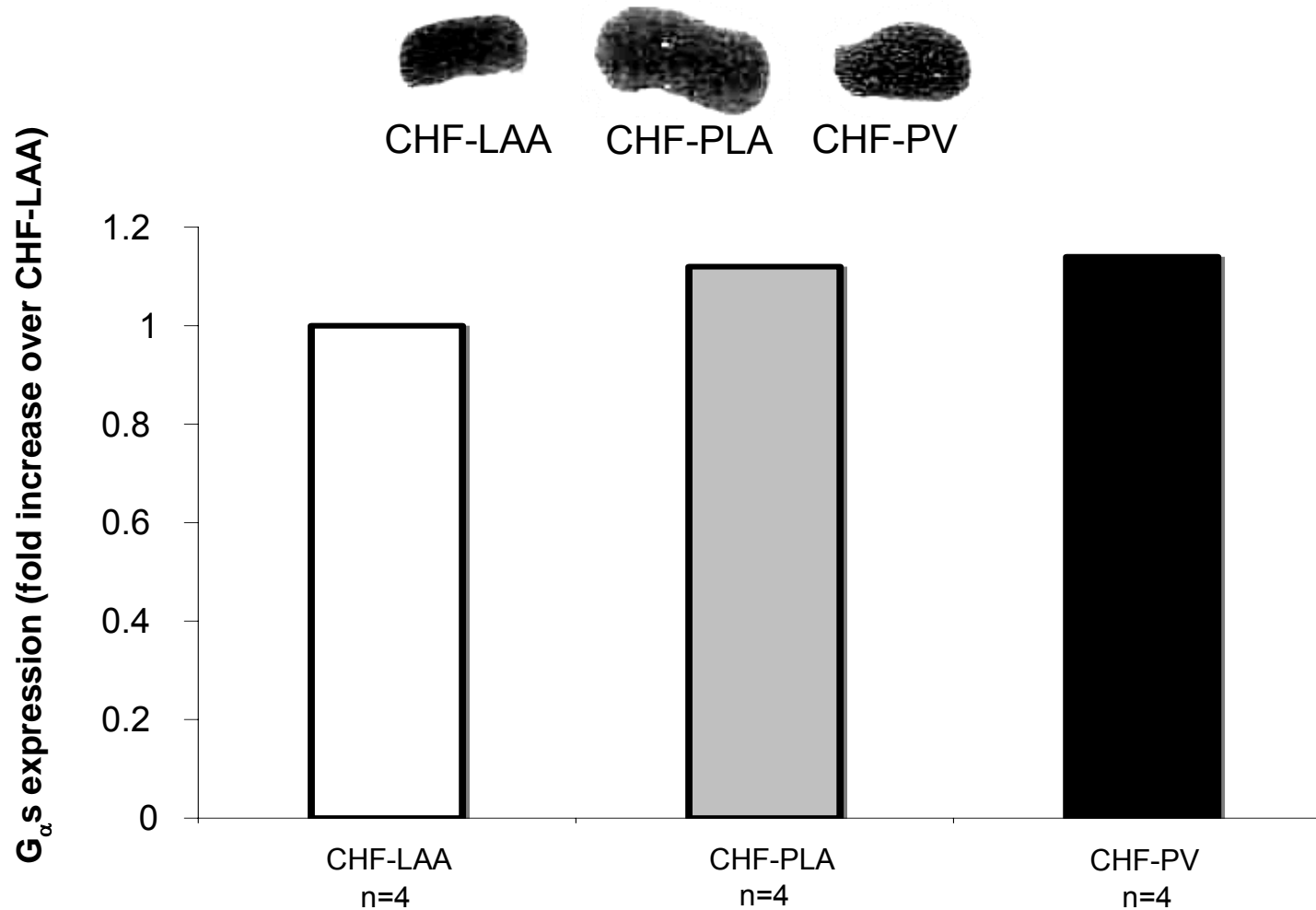


Sample size (n) denotes number of atrial muscles utilized. CON indicates control (white bars) and CHF indicates congestive heart failure (black bars)

*considered significant vs. CON-PLA ($P < 0.05$)

#considered significant vs. CON-PV ($P < 0.05$)

Inter-regional $G_{\alpha s}$ -protein expression in failing atria



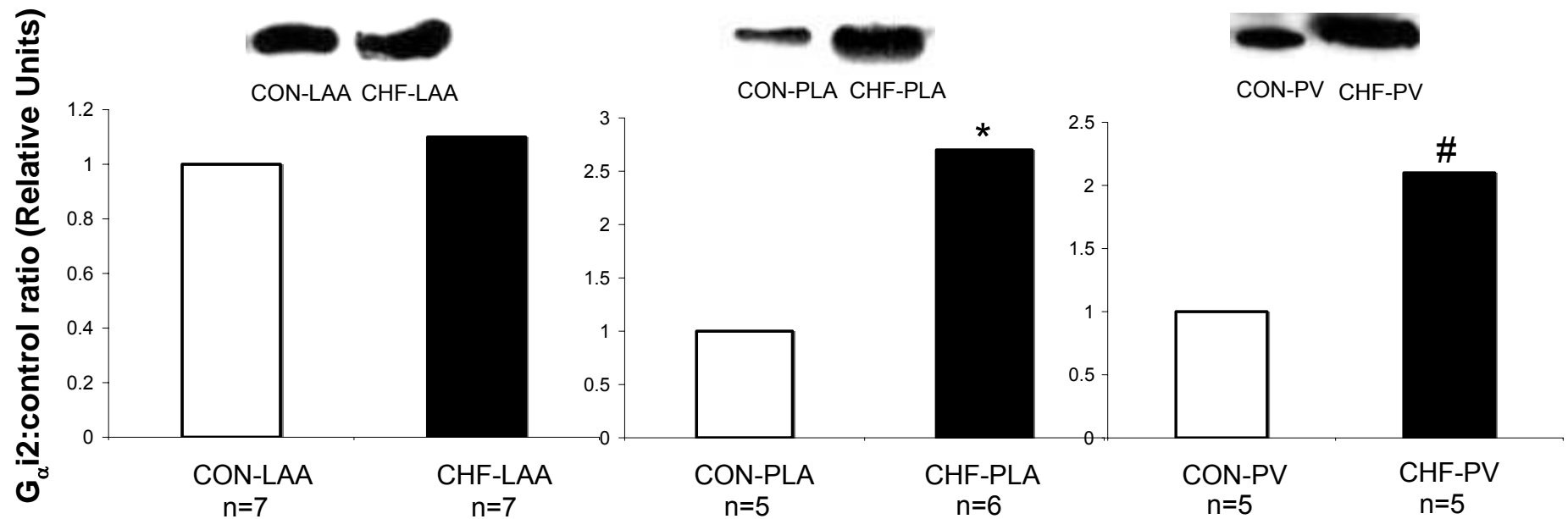
Sample size (n) denotes number of atrial muscles utilized.

Results

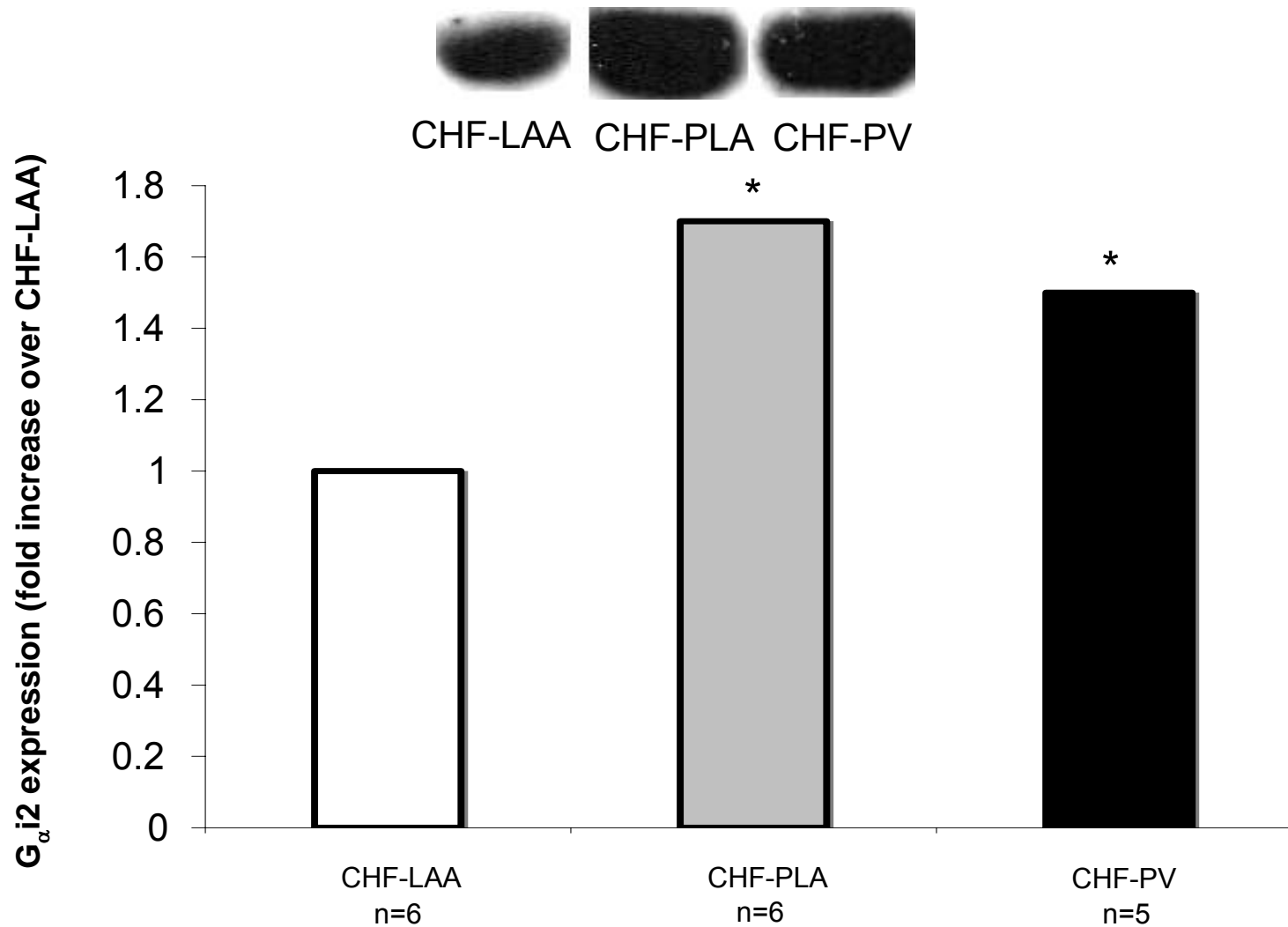
Parasympathetic signaling pathway



Intra-regional $G_{\alpha i2}$ -protein expression



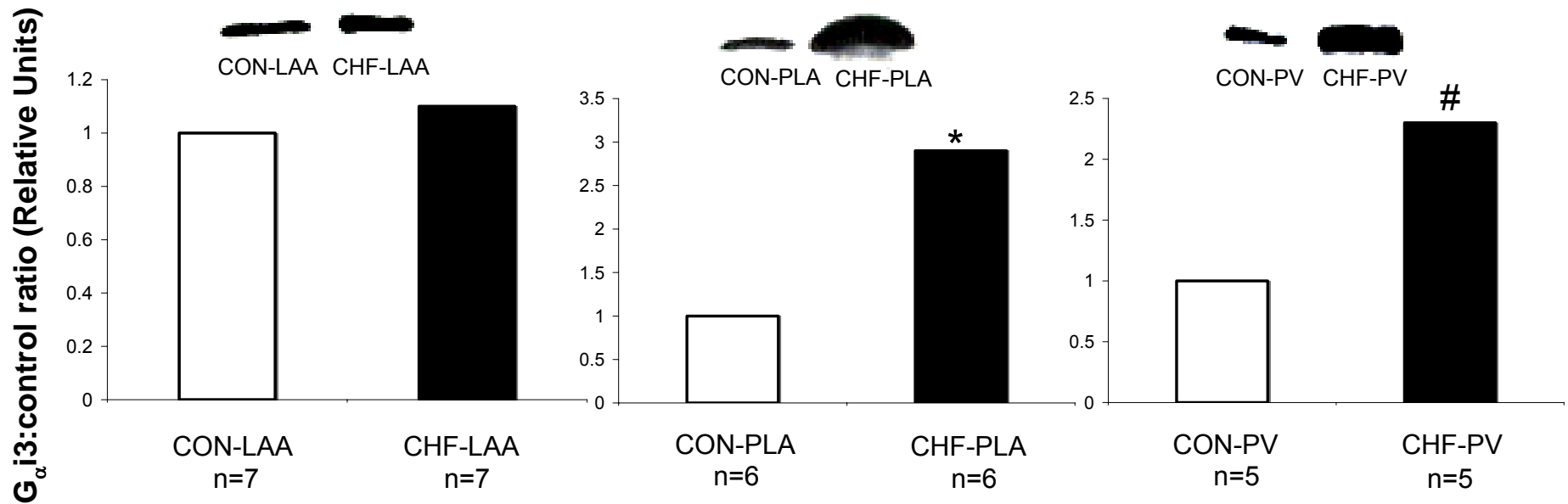
Inter-regional $G_{\alpha i2}$ -protein expression in failing atria



Sample size (n) denotes number of atrial muscles utilized.

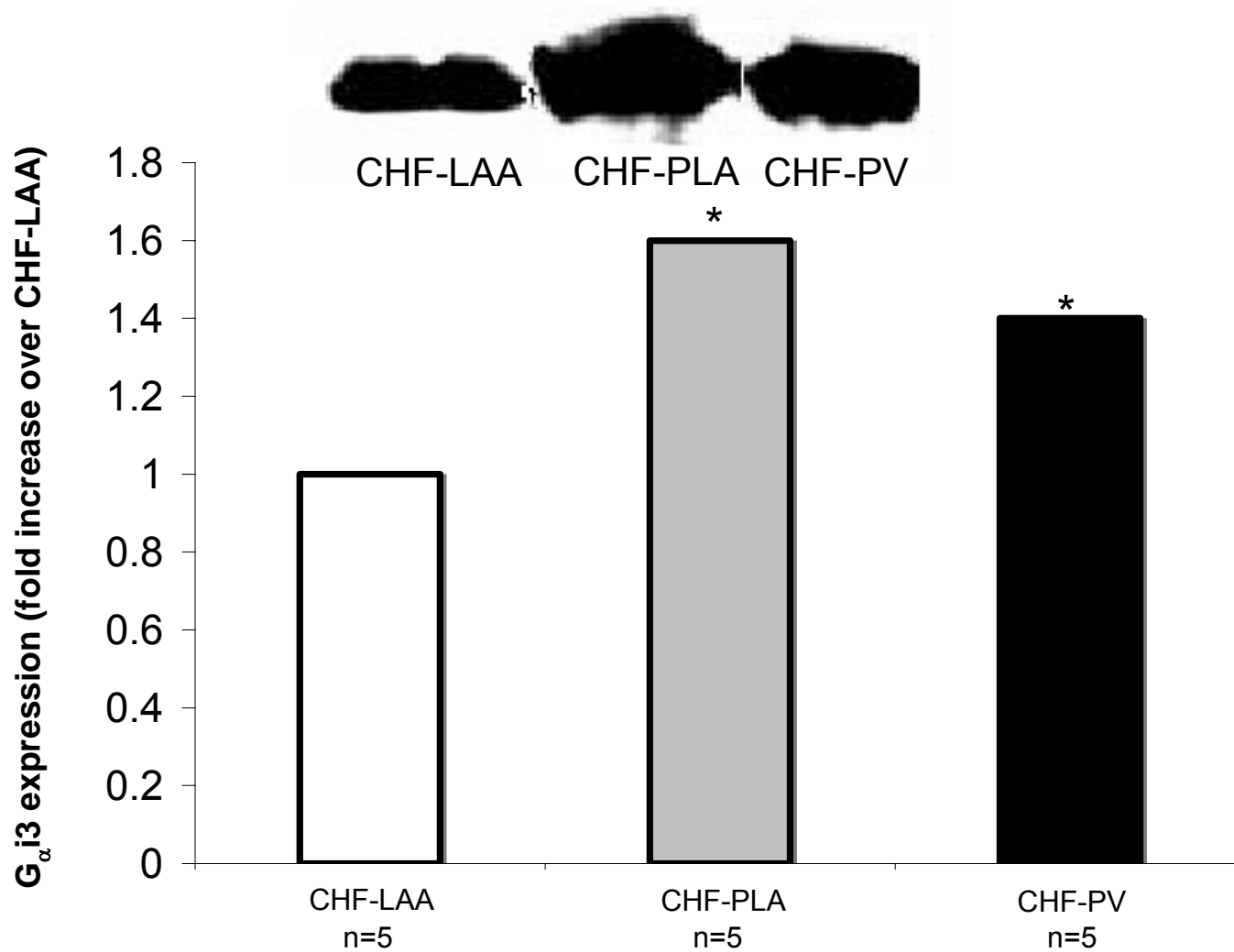
*considered significant vs. CHF-LAA ($P < 0.05$)

Intra-regional $G_{\alpha}i3$ -protein expression



Sample size (n) denotes number of atrial muscles utilized.
*considered significant vs. CON-PLA ($P < 0.05$)
#considered significant vs. CON-PV ($P < 0.05$)

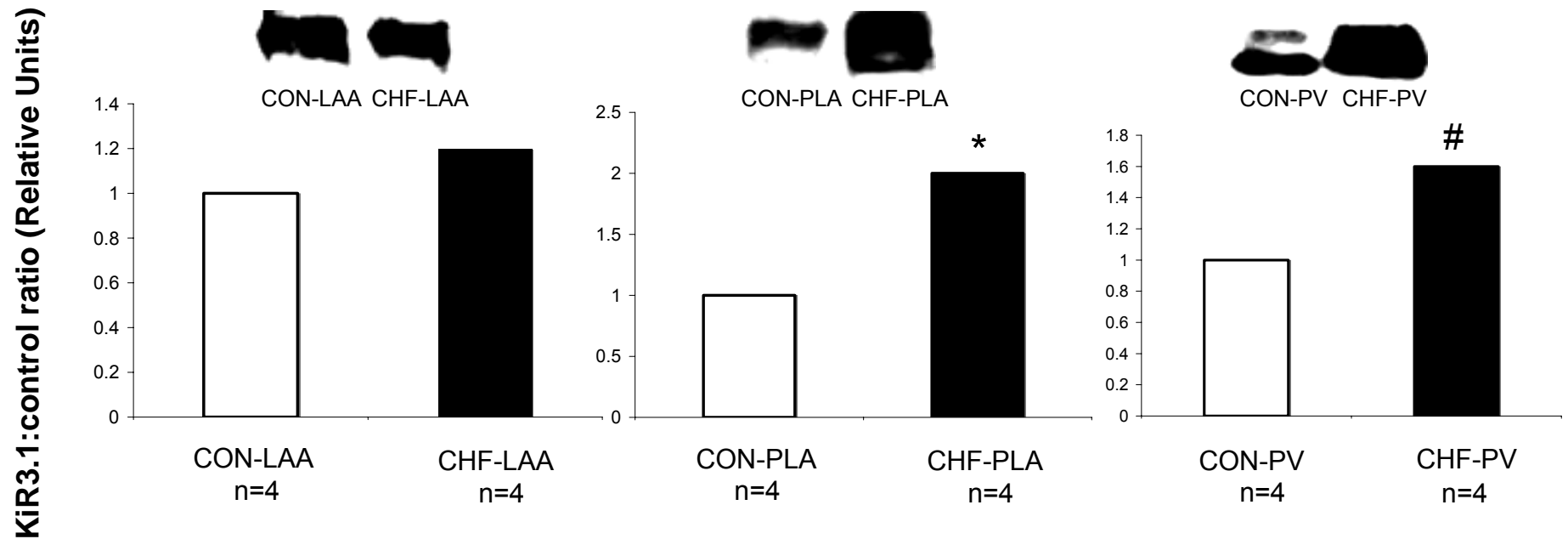
Inter-regional $G_{\alpha i3}$ -protein expression in failing atria



Sample size (n) denotes number of atrial muscles utilized.

*considered significant vs. CHF-LAA ($P < 0.05$)

Intra-regional KiR 3.1 protein expression

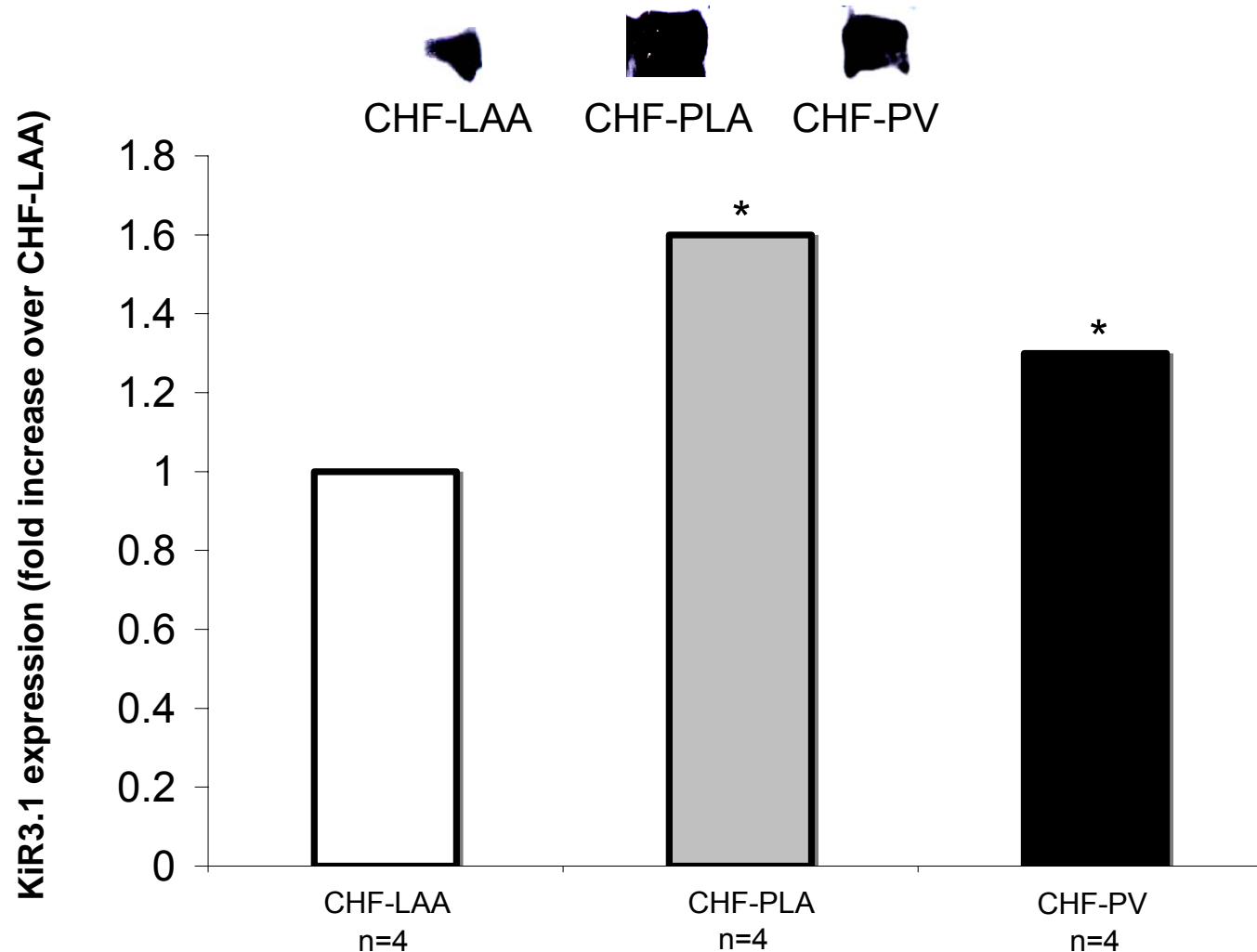


Sample size (n) denotes number of atrial muscles utilized.

*considered significant vs. CON-PLA ($P < 0.05$)

#considered significant vs. CON-PV ($P < 0.05$)

Inter-regional KiR-3.1-protein expression in failing atria



Sample size (n) denotes number of atrial muscles utilized.

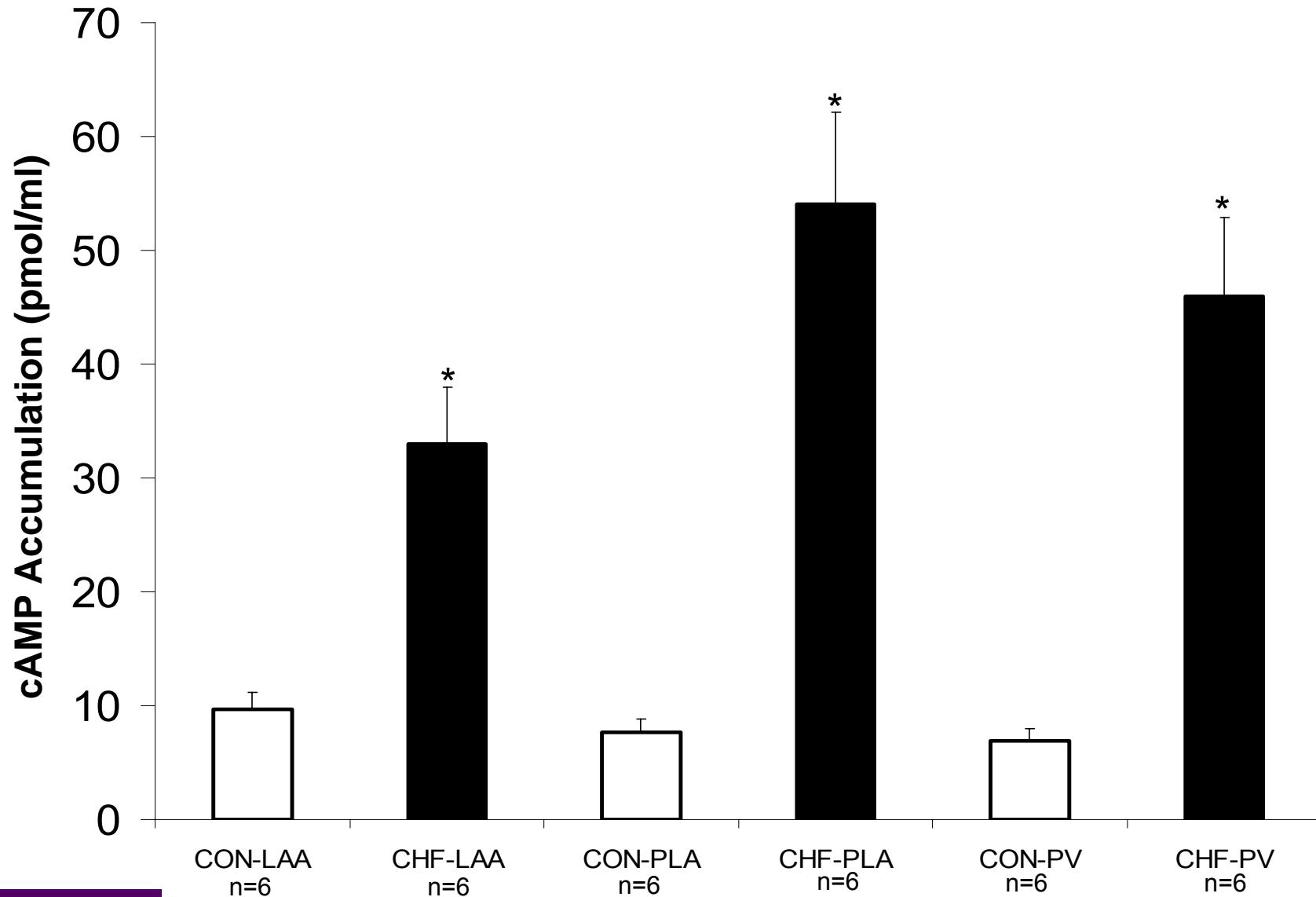
*considered significant vs. CHF-LAA ($P < 0.05$)

Results

Downstream signalling effectors

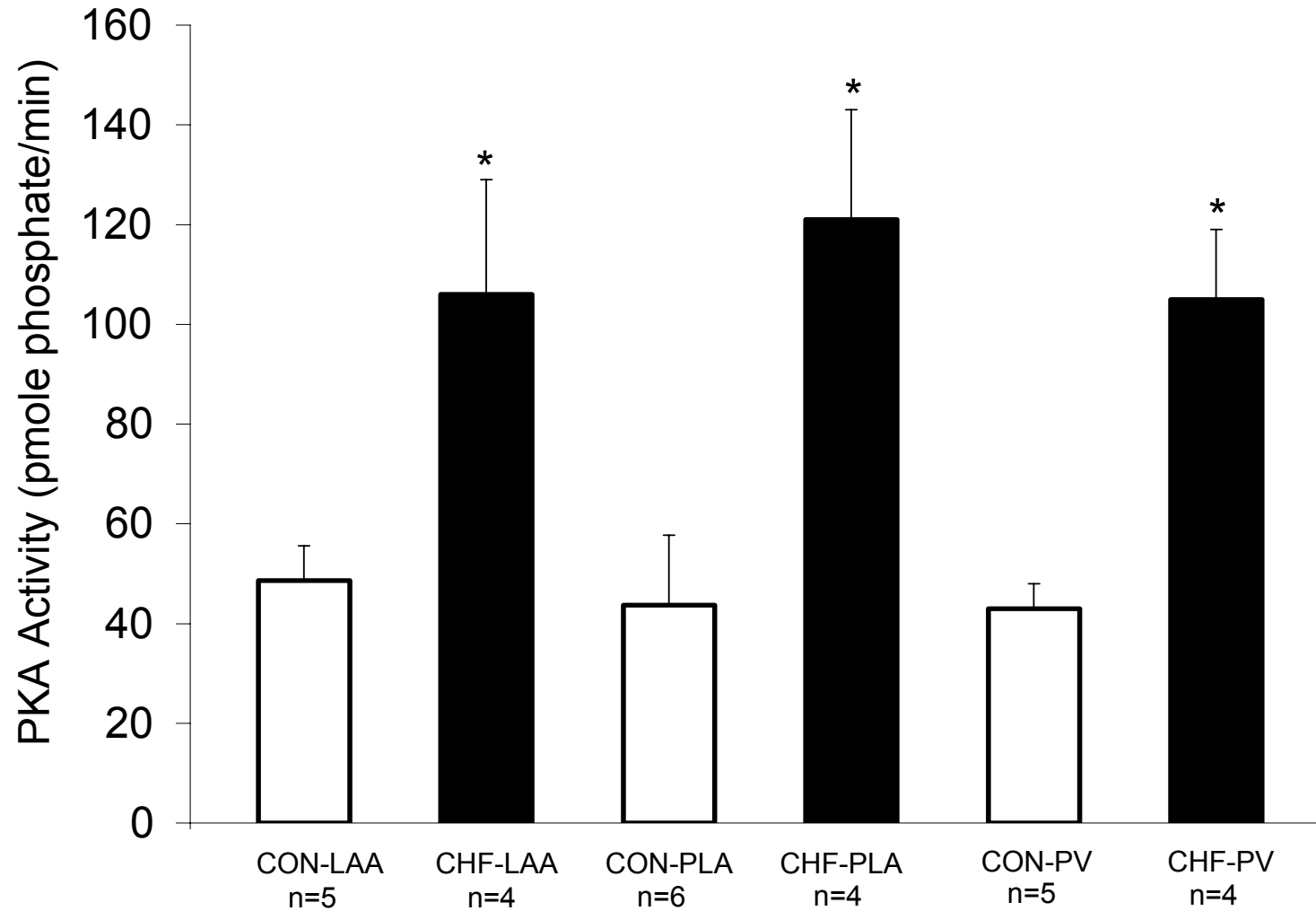


cAMP Accumulation



Sample size (n) denotes number of atrial muscles utilized.
*considered significant vs. respective controls
($P < 0.05$) for all groups

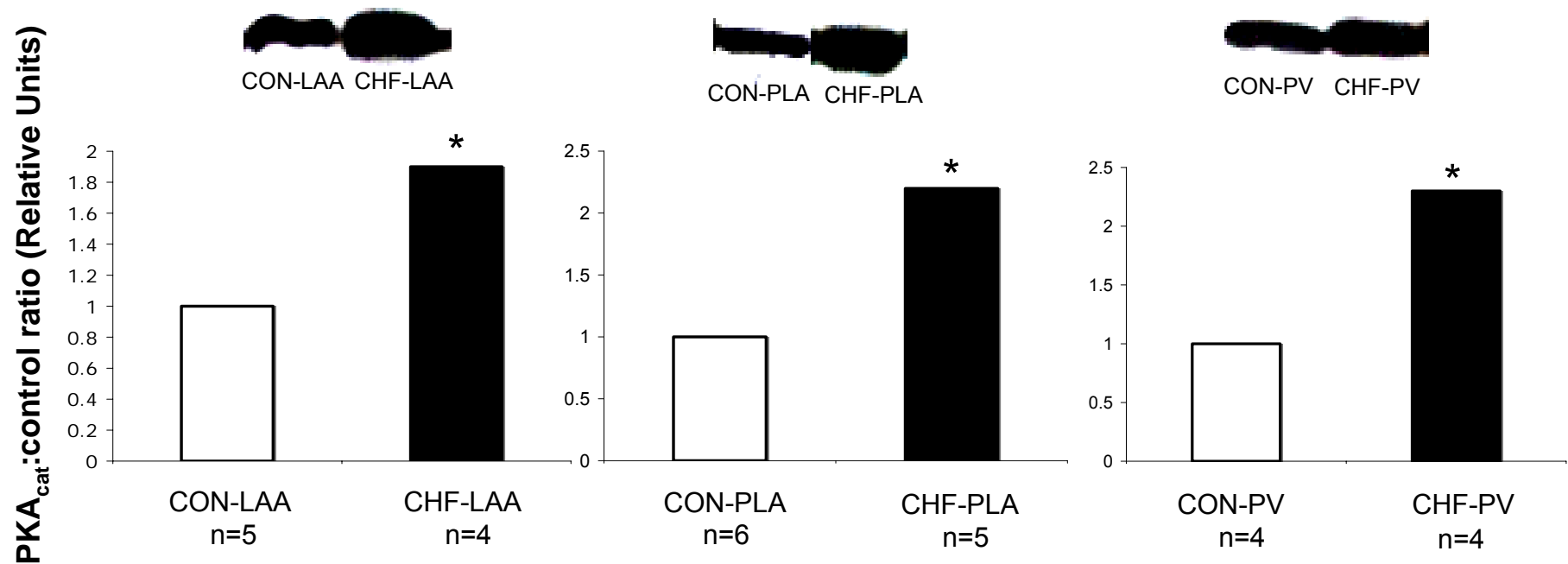
PKA Catalytic Activity



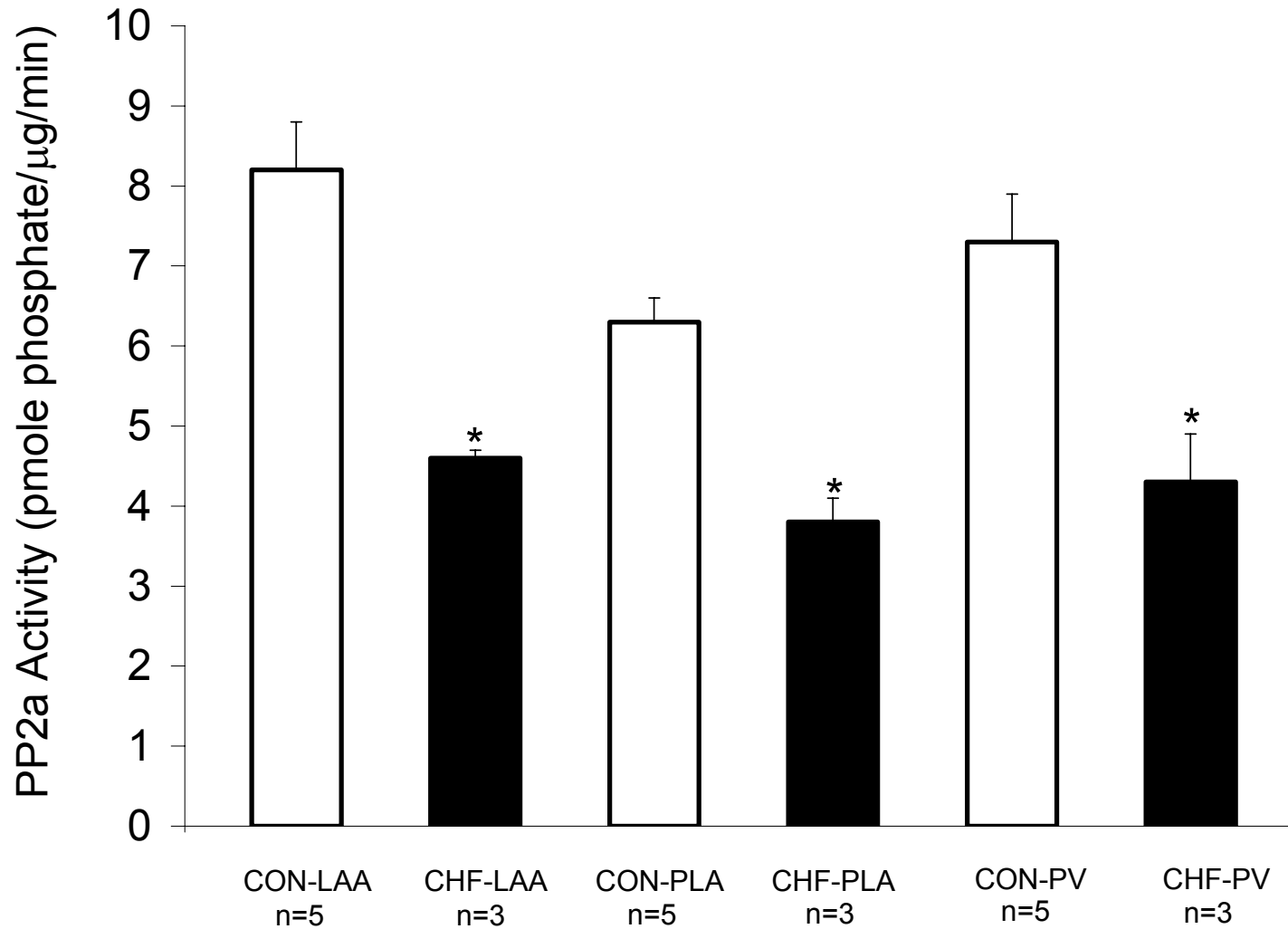
Sample size (n) denotes number of atrial muscles utilized.

*considered significant relative to respective control ($P < 0.05$).

Intra-regional PKA_{cat}-protein expression



PP2a Catalytic Activity



Sample size (n) denotes number of atrial muscles utilized.

*considered significant vs. respective controls
($P < 0.05$) for all groups

Data Summary

- Canine CHF secondary to ventricular tachypacing results in alterations in:

1. Sympathetic signaling

- $G_{\alpha s}$ expression is increased in the PLA and PVs (PLA=PV>LAA).

2. Parasympathetic signaling

- $G_{\alpha i2}$, $G_{\alpha i3}$, Kir 3.1 expression are increased in the PLA and PV (PLA≥PV>>LAA).

3. Downstream effector molecules

- cAMP levels are increased in the LAA, PLA, and PVs.
- PKA catalytic activity is increased in the LAA, PLA, and PVs.
- PKA_{cat} expression is increased in the LAA, PLA, and PVs.
- PP2a catalytic activity is decreased in the LAA, PLA, and PVs.



Conclusions

- CHF results in marked remodeling of parasympathetic and sympathetic signaling pathways in the posterior left atrium and pulmonary veins.
- Molecular autonomic remodeling is heterogeneous, with signaling pathways more significantly affected in the PLA (and to a lesser extent in the PVs) than in the rest of the left atrium.
- The alterations in parasympathetic and sympathetic signaling in CHF correspond to altered nerve expression and innervation of the left atrium.
- Altered expression and activity of downstream effectors of the autonomic signaling systems occurs independent of atrial region.



Implications

- Small G-proteins may be plausible targets for modulation of the arrhythmogenic substrate arising from autonomic system activation in fibrillating atria in CHF.
- The preferential increase in expression of sympathetic as well as parasympathetic signaling components in the PLA in the setting of CHF makes this region a suitable target for regionally directed therapies for AF.

