Dopamine Receptor D3 Agonist (Pramipexole) Abolishes Morphine-Induced Cardiac Fibrosis and Preserves Systolic Function in Mice

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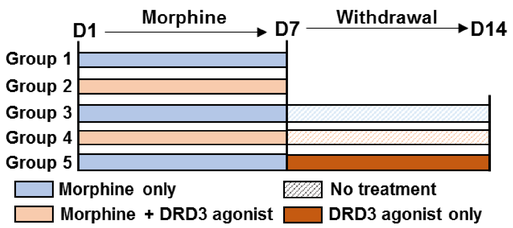
INTRODUCTION

- In addition to its common uses for the treatment of pain, morphine is used therapeutically for shortness of breath in congestive heart failure patients. However, prolonged exposure can lead to tolerance, dependence, and central nervous system (CNS) and cardiovascular (CV) alterations.
- Morphine affects the CNS and CV systems via opioid receptors.
- Morphine also modulates the response of dopamine receptors (DRD), particularly the D2-like family (DRD2 and DRD3).
- Similarities between mu-opioid receptors (mOR) and DRD3 pathways suggests synergism.
- DRD3 knockout mice show increased mOR-phosphorylation and exhibit cardiac dysfunction, myocardial fibrosis, and decreased lifespan
- The goal of this study is to determine whether the myocardial changes observed with morphine administration directly associate with a dysfunctional dopaminergic system in the heart.

MATERIALS & METHODS

STUDY DESIGN

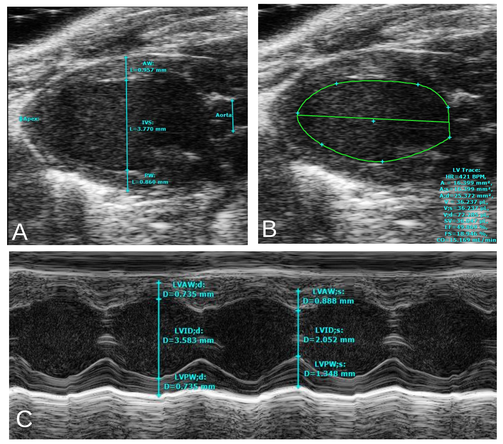
- 3 month old male C57BL/6J mice randomly assigned into 5 groups (n=6/group)
- All mice received morphine for 7 days (D7, 2 mg/kg/day)
- Separate cohorts received DRD3 agonist (pramipexole dihydrochloride, 0.5 mg/kg/day) as adjunct therapy:
  - G2 and G4, simultaneous to morphine treatment
  - G5 delivered only during the withdrawal period
- After 7 days of morphine administration, groups G1 and G2 were euthanized, while G3-G5 underwent 7 days of withdrawal from morphine (D14).



OUTPUTS

- Cardiac function by echocardiography at baseline, D7, and D14
- Picrosirius red (PSR) staining for visualization of left ventricle (LV) fibrosis
- Image Pro analysis of cross-sectional area for myocyte hypertrophy
- Immunoblot determination of protein expression; collagen 1, DRD1 and DRD3 receptors

RESULTS



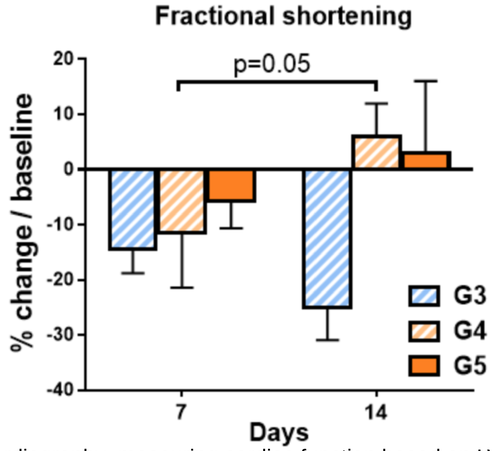


Figure 1. Morphine induced cardiac dysfunction. Echocardiography measuring cardiac function based on LV anatomical dimensions (A) and volume in long-axis (B); as well as LV myocardium anterior and posterior wall thickness in systole and diastole (C). Data showed decreased fractional shortening (FS) after 7 days of morphine treatment for all groups, compared to baseline (D0, % change=0). During the withdrawal period the FS showed a tendency to continue to decrease on G3; however, treatment with DRD3 agonist (DRD3 ag) preserved physiological function (G4 and G5) to levels comparable to baseline (mean ± SEM, n=6/group)

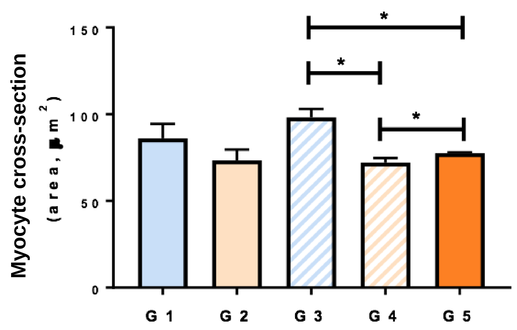


Figure 2. Cardiomyocyte cross-sectional area was used as a measure for hypertrophy. LV tissue was PSR stained and imaged at 40x magnification. Measurements were conducted using Image-Pro analysis software. which indicated significant hypertrophy in morphine-only groups compared to groups receiving DRD3 ag before or during withdrawal. Additionally, G4 shows significantly less hypertrophy than G5, indicating possible benefits to earlier administration of DRD3 ag. (values are mean ± SEM, ∗ p≤0.05).

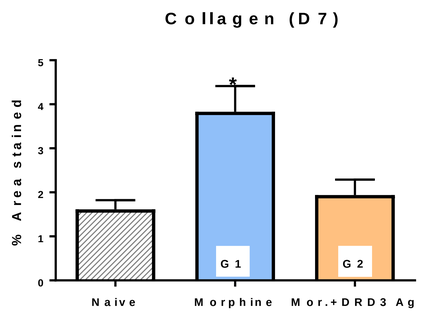


Figure 3. DRD3 ag abolished morphine-induced cardiac fibrosis. Animals received daily i.p. injections of morphine for 7 days (D7, G1) or in conjunction with DRD3 ag (G2). Values are mean +/- SEM, n=5-8/group. *p<0.05 versus all other groups.

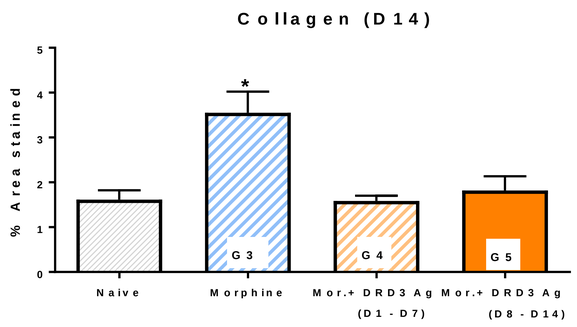


Figure 4. DRD3 ag abolished morphine induced cardiac fibrosis, independent of time intervention. Animals received daily i.p. injections of morphine for 7 days (measurement obtained at D14). G4 received DRD3 ag only during the morphine exposure period (D1-D7), while G5 received DRD3 agonist only during the withdrawal period (D8-D14). Values are mean +/- SEM, n=5-6/group. *p<0.05 versus all other groups.

RESULTS

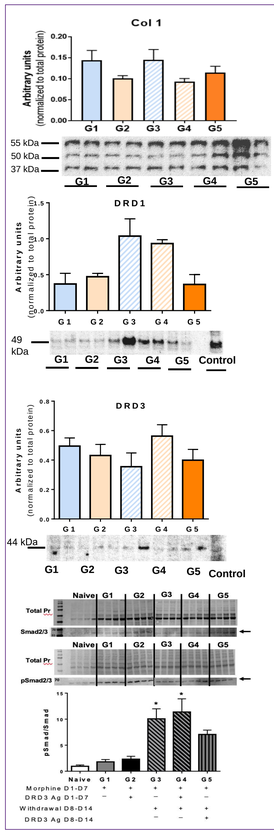


Figure 5. Collagen 1 turnover. We observed the highest levels of collagen 1 fragments with morphine treatment at D7 (G1) and after withdrawal (G3). Groups treated with DRD3 ag showed a trend towards reduced collagen fragmentation (values are mean ± SEM, n=4/group).

Figure 6. DRD1 levels seem to increase during morphine withdrawal and this was abolished by DRD3 Ag treatment during the withdrawal period. DRD1 is one of 2 dopamine receptors that mediate excitatory actions. This data suggests that DRD1 signaling mechanisms increase in the withdrawal period (mean ± SEM, n=2-3/group; control= brain lysate).

Figure 7. DRD3 ag treatment did not affect DRD3 protein levels. (mean±SEM, n=4/group).

Figure 8. Smad2/3 pathway was activated on groups G3 and G4 (withdrawal D14) compared to naïve controls. Values are mean +/- SEM, n=4/group. Arrow denotes quantified band.
*p<0.05 versus naïve.

CONCLUSION

- Our data suggest that, when used as an adjunctive therapy with morphine, DRD3 agonist (pramipexole) improves pain control, decreases morphine-induced cardiac fibrosis, decreases cardiac hypertrophy, and preserves systolic function when compared to morphine-only groups.
- Further, DRD3 agonist abolishes morphine-induced fibrosis, regardless of timing of intervention.
- Lastly, the activation of SMAD2/3 gives insight into a probable upstream pathway for the observed morphine-induced fibrosis.

FUTURE DIRECTIONS

- Some of our data showed favorable trends that were not statistically significant, perhaps as a result of the low n-value.
- Therefore, further experiments will focus on increasing sample size and investigating the signaling mechanisms responsible for DRD3 cardioprotection.

REFERENCES

1. Chang G, Chen L, Mao J: Opioid tolerance and hyperalgesia. *Med Clin North Am* 2007, 91(2):199-211.
2. Shanazari AA, Aslani Z, Ramshini E, Alaei H: Acute and chronic effects of morphine on cardiovascular system and the baroreflexes sensitivity during severe increase in blood pressure in rats. *ARYA atherosclerosis* 2011, 7(3):111-117.
3. Peacock WF, Hollander JE, Diercks DB, Lopatin M, Fonarow G, Emerman CL: Morphine and outcomes in acute decompensated heart failure: an ADHERE analysis. *Emergency medicine journal : EMJ* 2008, 25(4):205-209.
4. Flinsenberg TW, van der Leeuw J, Gafni A, van Oostrom AJ, van Wijk JP: [Is morphine indicated in patients with cardiac asthma? Indications for negative effects from the use of morphine]. *Nederlands tijdschrift voor geneeskunde* 2015, 159:A9388.
5. Drastichova Z, Skrabalova J, Jedelsky P, Neckar J, Kolar F, Novotny J: Global changes in the rat heart proteome induced by prolonged morphine treatment and withdrawal. *PLoS One* 2012, 7(10):e47167.
6. Janecka A, Fichna J, Janecki T: Opioid receptors and their ligands. *Current topics in medicinal chemistry* 2004, 4(1):1-17.
7. Corbett AD, Henderson G, McKnight AT, Paterson SJ: 75 years of opioid research: the exciting but vain quest for the Holy Grail. *British journal of pharmacology* 2006, 147 Suppl 1:S153-162.
8. Zhang Y, Zhang F, Yang C, Jin H, Yang Y, Xu M: Dopamine affects the change of pain-related electrical activity induced by morphine dependence. *Neurochemical research* 2012, 37(5):977-982.
9. Brewer KL, Baran CA, Whitfield BR, Jensen AM, Clemens S: Dopamine D3 receptor dysfunction prevents anti-nociceptive effects of morphine in the spinal cord. *Frontiers in Neural Circuits* 2014, 8.
10. Le Merec T, Marie-Claire C, Noble F, Marie N: Chronic and intermittent morphine treatment differently regulates opioid and dopamine systems: a role in locomotor sensitization. *Psychopharmacology* 2011, 216(2):297-303.
11. Pivonello R, Ferone D, Lombardi G, Colao A, Lamberts SW, Hofland LJ: Novel insights in dopamine receptor physiology. *Eur J Endocrinol* 2007, 156 Suppl 1:S13-21.
12. Neve KA, Seamans JK, Trantham-Davidson H: Dopamine receptor signaling. *Journal of receptor and signal transduction research* 2004, 24(3):165-205.
13. Sheng HY, Qu CL, Huo FQ, Du JQ, Tang JS: D2-like but not D1-like dopamine receptors are involved in the ventrolateral orbital cortex-induced antinociception: a GABAergic modulation mechanism. *Experimental neurology* 2009, 215(1):128-134.
14. Sokoloff P, Le Foll B, Perachon S, Bordet R, Ridray S, Schwartz JC: The dopamine D3 receptor and drug addiction. *Neurotoxicity research* 2001, 3(5):433-441.
15. Johnson TL, Tulis DA, Keeler BE, Virag JA, Lust RM, Clemens S: The dopamine D3 receptor knockout mouse mimics aging-related changes in autonomic function and cardiac fibrosis. *PLoS One* 2013, 8(8):e74116.
16. Yun J, Oliynyk S, Lee Y, Kim J, Yun K, Jeon R, Ryu JH, Oh S: Ajoene restored behavioral patterns and liver glutathione level in morphine treated C57BL6 mice. *Archives of pharmacal research* 2016.