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Contribution of endogenous opioids and nitric oxide to papillary muscle contractile impairment in cholestatic rats

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Abstract

Attenuated responsiveness to adrenoceptor stimulation has been proposed as an important factor underlying cardiovascular complications of cholestasis. We examined isolated papillary muscle responsiveness to α (phenylephrine) and β-adrenoceptor (isoproterenol) agonists in 7-day bile duct-ligated rats. We investigated the role of nitric oxide (NO) and endogenous opioids in papillary muscle hyporesponsiveness to isoproterenol stimulation. In order to evaluate the effect of NO and endogenous opioids, animals were treated with chronic subcutaneous injections of N (ω)-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg/day) or naltrexone (20 mg/kg/day), or isolated papillary muscles were exposed acutely to the same drugs (10^{-4} and 10^{-6} M, respectively) in an organ bath. The basal contractile force of papillary muscle, $+dT/dt_{max}$ and $-dT/dt_{max}$, was significantly decreased in bile duct-ligated rats compared to sham-operated ones ($P<0.05$, for each value). The concentration–response curve for phenylephrine and isoproterenol demonstrated a reduced maximum effect in bile duct-ligated rats compared to the sham-operated group ($P<0.01$ and 0.05, respectively). Basal contractile abnormalities of bile duct-ligated rats were corrected by L-NAME or naltrexone treatment, either acute or chronic. While chronic L-NAME treatment resulted in a left-ward shift ($P<0.05$), it had no effect on the maximum effect in bile duct-ligated rats. Acute L-NAME treatment did not influence isoproterenol responsiveness. Acute and chronic naltrexone treatment resulted in partial and complete correction of the hyporesponsiveness of bile duct-ligated rats, respectively ($P<0.05$). This investigation demonstrates that the papillary muscles of 7-day bile duct ligated-rats have an impaired basal contractility and hyporesponsiveness to both α and β-adrenoceptor stimulation. It also provides evidence for the involvement of increased opioidergic tone and NO overproduction in cholestasis-induced cardiac impairment.

Keywords: Adrenoceptor; Cardiomyopathy; Cholestasis; Endogenous opioid peptide; NO (Nitric oxide); Papillary muscle

1. Introduction

Cholestatic liver disease is associated with widespread derangements in cardiovascular (Bomzon, 1986; Green et al., 1986; Lumlertgul et al., 1991; Tajuddin et al., 1990) and renal (Better and Bomzon, 1998; Heidenreich et al., 1987) systems. The association between obstructive jaundice and post-operative acute renal failure and shock, as the most life-threatening complications, is a well-established clinical phenomenon (Better, 1986; Green and Better, 1994). Although various pathophysiological mechanisms have been proposed, recent research has focused on an insufficient response of the cardiovascular system to stimulating agents, such as adrenoceptor agonists, in stressful conditions (Jacob et al., 1993; Mani et al., 2002; Namiranian et al., 2001). Consistent with this hypothesis, hyporesponsiveness to chronotropic (Gaskari et al., 2002; Mani et al., 2002; Nahavandi et al., 2001), inotropic (Binah et al., 1985, 1987; Jacob et al., 1993) and vasoactive (Namiranian et al., 2001; Tajuddin et al., 1990) agents has been reported in cholestatic patients or animals.

Various mechanisms have been suggested to explain the hyporesponsiveness of the cardiovascular system to adrenoceptor agonists. Nitric oxide (NO) and endogenous opioid

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peptides modulate cardiovascular responsiveness to endogenous and exogenous stimuli in normal and pathological states (Chowdhary et al., 2002; Massion et al., 2003; Paton et al., 2002). NO overproduction (Hajrasouliha et al., 2004; Mani et al., 2002; Nahavandi et al., 2001; Namiranian et al., 2001) and the accumulation of endogenous opioid peptides (Gaskari et al., 2002; Hajrasouliha et al., 2004; Namiranian et al., 2001; Swain et al., 1992) have been suggested to be involved in the cardiovascular complications of cholestasis.

Previous experiments have been mostly focused on the abnormal responsiveness to β-adrenoceptor stimulation of cardiac tissues from cholestatic subject. However, it is not yet clear whether this abnormality is specific and limited to β-adrenoceptors or includes other receptors such as β-adrenoceptors. Therefore, we evaluated papillary muscle responsiveness to both α and β-adrenoceptor stimulation in isolated papillary muscles of 7-day bile duct-ligated rats. However, considering the dominant modulatory role of β-adrenoceptors in cardiac tissue, we investigated the role of NO and endogenous opioid peptides in the hyporesponsiveness to β-adrenoceptor stimulation.

2. Materials and methods

2.1. Animal manipulation

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85–23, revised 1985). Male Sprague–Dawley rats, weighing 200–250 g, were used in the experiments. Animals were housed in groups of 3–4 in a room controlled at 22±1 °C and maintained under a 12-h light/12-h dark cycle, and were given free access to food and water. Laparotomy was performed under general anesthesia, induced with an intraperitoneal (i.p.) injection of ketamine HCl (50 mg/kg; Gedoon Richter, Budapest, Hungary) and chlorpromazine HCl (10 mg/kg; Daroupakhsh, Tehran, Iran). In bile duct-ligated rats, the bile duct was isolated and doubly ligated, as previously described (Hajrasouliha et al., 2004; Mani et al., 2002; Nahavandi et al., 2001; Namiranian et al., 2001). Sham-operated, age-matched rats served as controls. Sham operation consisted of laparotomy, bile duct identification and manipulation. One untied loose tie was left in place.

2.2. Drug administration

The animals were randomly divided into six groups of sham-operated and six groups of bile duct-ligated rats. Two additional groups of control rats were also studied in order to evaluate the effect of sham operation on papillary muscle responsiveness to phenylephrine and isoproterenol stimulation. Each group consisted of 6 animals. The first and the second groups of sham-operated and bile duct-ligated rats were treated with daily subcutaneous administration of isotonic sterile saline solution (normal saline, 1 ml/kg/day, s.c.) before stimulation with phenylephrine and isoproterenol. The third group of sham-operated and bile duct-ligated rats was treated with daily subcutaneous injections of N(ω)-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg; Sigma, St Louis, MO, USA), a non-selective NO synthase inhibitor. The fourth group of sham-operated and bile duct-ligated rats was treated with daily subcutaneous injections of naltrexone HCl (20 mg/kg, Iran Darou, Tehran, Iran). These rats received 6 doses of the drugs for 6 consecutive days (chronic treatment) before isoproterenol stimulation. The first dose was injected the day after surgery and the last dose was injected 24 h before papillary muscle isolation, so little drug or drug action was expected to exist in tissues at the time of the experiment (Namiranian et al., 2001). The papillary muscles of the fifth and sixth groups of sham-operated and bile duct-ligated rats were incubated with L-NAME, 10^{-4} M, and naltrexone, 10^{-6} M, in an organ bath, 40 min before stimulation with isoproterenol (acute treatment). Treatment regimens were selected based on previous studies and were shown to be able to effectively reduce NO production, antagonize the effects of accumulated endogenous opioids, and reverse the complications of cholestasis (Gaskari et al., 2002; Liu et al., 2000; Moezi et al., 2004; Nahavandi et al., 2001).

2.3. Left ventricular papillary muscle contractile study

One week after bile duct ligation or sham-operation, rats were anesthetized with i.p. injection of ketamine HCl (50 mg/kg) and chlorpromazine HCl (10 mg/kg). Then the hearts were removed. Left ventricular papillary muscles were excised and isolated in a modified Tyrode's buffer aerated with 95% O_2 and 5% CO_2. The composition of modified Tyrode's buffer in mM was as follows: NaCl, 122.5; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.1; $NaHCO_3$, 24; Glucose, 10 (pH=7.4). Papillary muscles were attached vertically to an isometric force transducer (MLT 1030/D, ADI instruments, PowerLab, Spain) under a tension of 500 mg in a 25-ml glass chamber in an organ bath (ADI Instruments, PowerLab, Spain). The temperature of the bathing buffer was 33 °C. Papillary muscles were equilibrated in the organ bath for 90 min. After equilibration, the muscles were subjected to electrical-field stimulation at 1 Hz and 30 V, which is about 20% higher than the threshold. Basal contractility was defined as the stable baseline contractile force of papillary muscles before the addition of the stimulating agents. To assess papillary muscle responsiveness to α-adrenoceptor stimulation, the papillary muscles were equilibrated with propranolol (5×10^{-6} M) (Skomedal et al., 1982), 15 min before stimulation with cumulative concentrations of phenylephrine (10^{-9} to 10^{-4} M). To obtain a β-adrenoceptor concentration–response curve, the papillary muscles were stimulated by cumulative concentrations of isoproterenol (10^{-9} to 10^{-5} M). Maximal effect (E_{max}) was defined as the contractile force after addition of the highest concentration of isoproterenol (10^{-5} M) or phenylephrine (10^{-4} M). For each concentration of isoproterenol or phenylephrine, the increase in recorded contractile force is expressed as a percentage of the basal contraction. Maximal time derivatives for the development ($+dT/dt_{max}$) and the dissipation ($-dT/dt_{max}$) of papillary muscle tension were calculated in response to different concentrations of isoproterenol or phenylephrine, and expressed as a percentage of baseline.

2.4. Statistical analysis

Data are expressed as means ± S.E.M. Statistical evaluation of the data was performed with an analysis of variance (ANOVA), followed by the Newman–Keuls test for multiple comparisons. Half-maximal effective concentrations (EC_{50}) of isoproterenol or phenylephrine were defined as the concentration that causes 50% of E_{max}, and was calculated by Prism (GraphPad software, version 4). A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Basal cardiac contractility

Basal contractile parameters are shown in Fig. 1. The basal contractile force, $+dT/dt_{max}$ and $-dT/dt_{max}$ of sham-operated rats were not significantly different from those of the control group ($P>0.05$). However, these parameters were significantly reduced in bile duct-ligated rats compared to both sham-operated and control groups ($P><0.05$, for each variable). Addition of

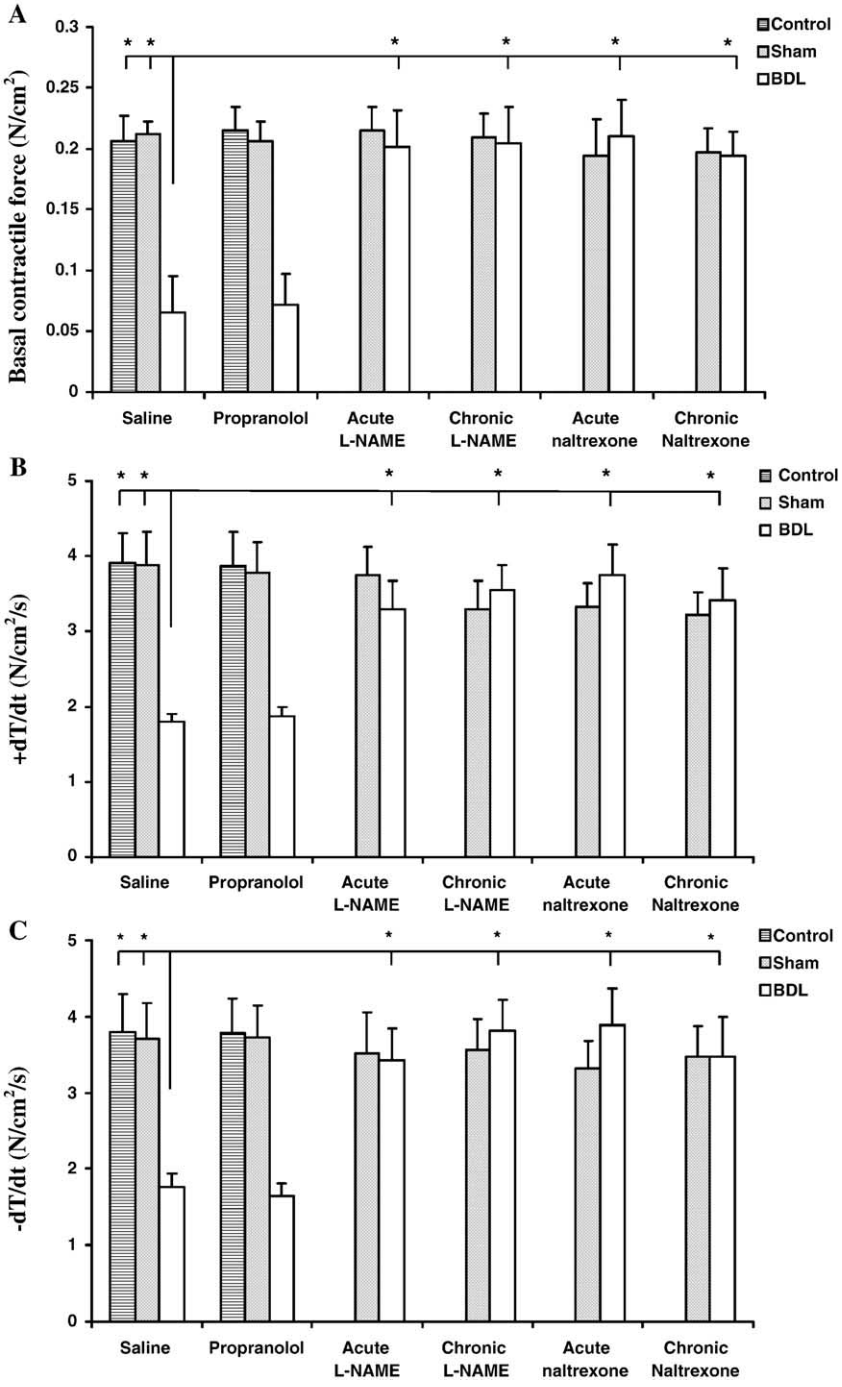

Fig. 1. Comparison of (A) basal contractile force, (B) $+dT/dt$ and (C) $-dT/dt$ of papillary muscles from control, bile duct-ligated (BDL) and sham-operated groups, which were treated with normal saline, propranolol, N(ω)-nitro-L-arginine methyl ester (L-NAME) or naltrexone. *$P<0.05$ compared to the saline-treated BDL group.

propranolol had no significant effect on the basal contractile parameters of control, sham-operated or bile duct-ligated groups. L-NAME and naltrexone treatment, either acute or chronic, corrected the basal contractile abnormalities ($P<0.05$, for each variable) in the bile duct-ligated animals, but had no significant effect on the contractile abnormalities in the sham-operated rats.

3.2. Phenylephrine and isoproterenol-stimulated cardiac contractility

Phenylephrine and isoproterenol induced a concentration-dependent increase in contractile force, $+dT/dt_{max}$ and $-dT/dt_{max}$. The E_{max} of contractile force, $+dT/dt_{max}$ and $-dT/dt_{max}$ in response to phenylephrine and isoproterenol were achieved at 10^{-4} and 10^{-5} M, respectively, and no further increase was observed with higher concentrations. The E_{max} of contractile force, $+dT/dt_{max}$ and $-dT/dt_{max}$ of the sham-operated group in response to phenylephrine or isoproterenol were not significantly different from those of the control groups (Fig. 2). A significant reduction in these parameters and also a right-ward shift of the concentration–response curve for phenylephrine and isoproterenol were observed in the bile duct-ligated group compared with the sham-operated group (for phenylephrine concentration–response curve: -5.5 ± 0.06 vs. -5.9 ± 0.05, for

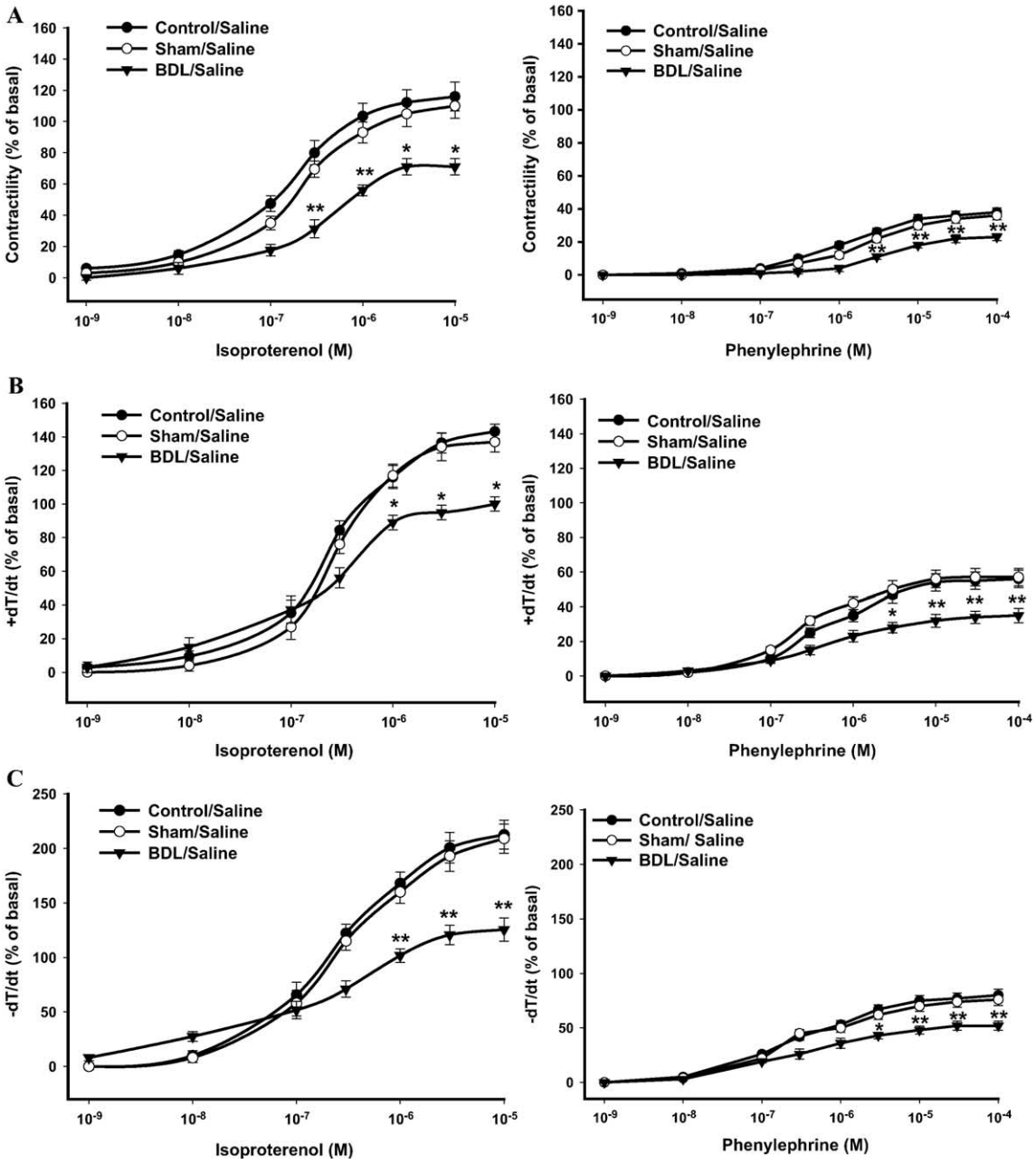

Fig. 2. Cumulative concentration–response curve for isoproterenol (left) and phenylephrine (right) in isolated left ventricular papillary muscles from control, sham-operated and 7-day bile duct-ligated (BDL) rats ($n=6$ for each group). (A) % increase in contractile force; (B) % increase in $+dT/dt$; (C) % increase in $-dT/dt$. *$P<0.05$, **$P<0.01$ compared to the sham-operated group.

isoproterenol concentration–response curve: −6.5±0.06 vs. −6.8±0.06, values are expressed as Log EC_{50}, $P<0.05$) (Fig. 2).

L-NAME treatment: As seen in Figs. 3 and 4, L-NAME treatment, either acute or chronic, did not induce a significant change in the E_{max} of contractile force, $+dT/dt_{max}$ and $-dT/dt_{max}$ of the sham-operated or bile duct-ligated groups in response to isoproterenol stimulation. However, chronic L-NAME treatment resulted in a significant decrease in the EC_{50} of the concentration–contraction curve for isoproterenol compared with that of the bile duct-ligated rats (−6.9±0.09 vs. −6.5±0.06, values are expressed as Log EC_{50}, $P<0.05$). Acute L-NAME treatment had no significant effect on isoproterenol responsiveness in bile duct-ligated rats (Fig. 4).

Naltrexone treatment: Acute or chronic naltrexone treatment did not significantly change isoproterenol responsiveness in sham-operated rats (Figs. 5 and 6). However, chronic naltrexone treatment restored to normal the responsiveness to isoproterenol stimulation in bile duct-ligated rats. Acute naltrexone treatment

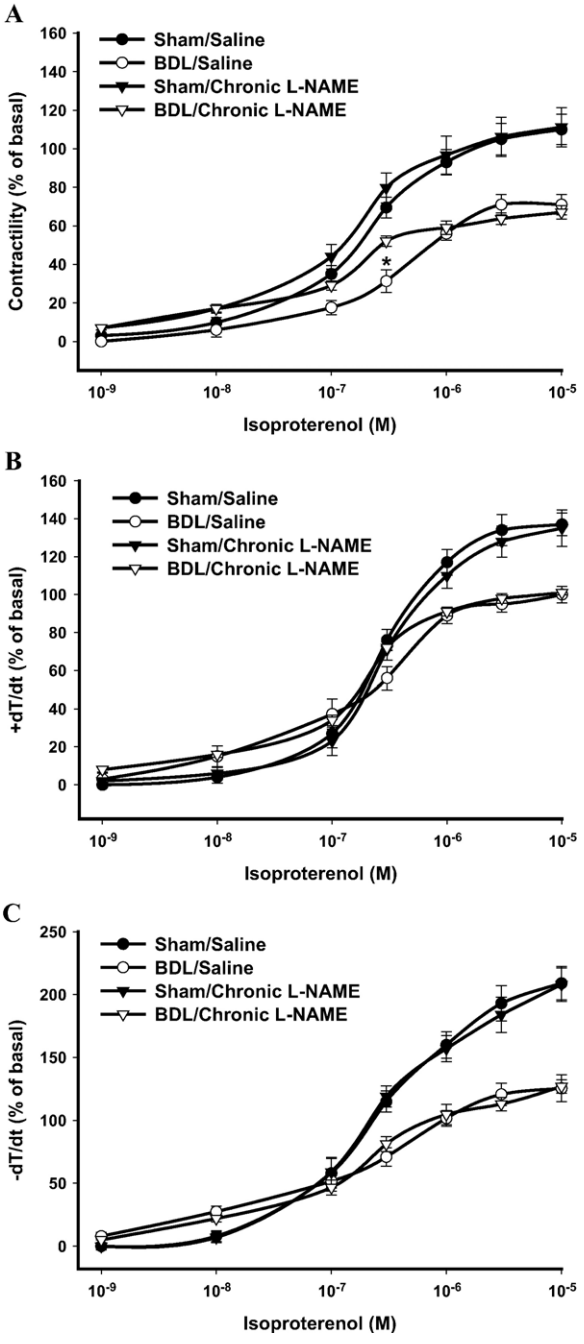

Fig. 3. Cumulative concentration–response curve for isoproterenol in isolated left ventricular papillary muscles from sham-operated and 7-day bile duct-ligated (BDL) rats, which were chronically treated with saline or L-NAME ($n=6$ for each group). (A) % increase in contractile force; (B) % increase in $+dT/dt$; (C) % increase in $-dT/dt$. *$P<0.05$ compared to the saline-treated BDL group.

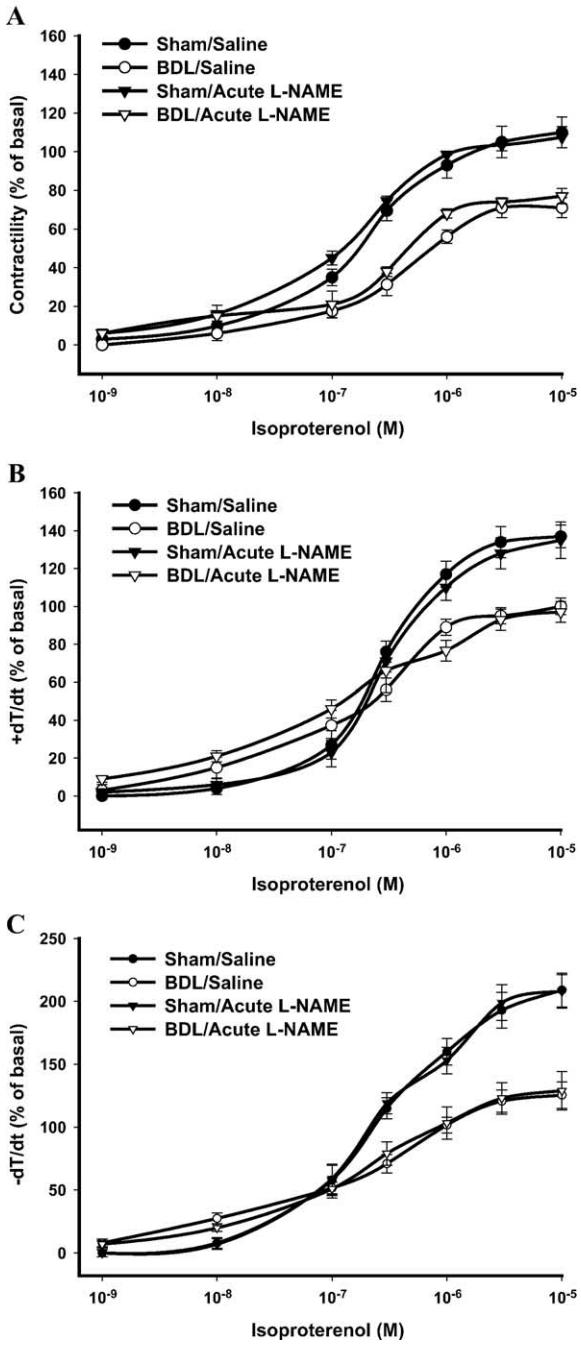

Fig. 4. Cumulative concentration–response curve for isoproterenol in isolated left ventricular papillary muscles from sham-operated and 7-day bile duct-ligated (BDL) rats, which were acutely treated with L-NAME ($n=6$ for each group). (A) % increase in contractile force; (B) % increase in $+dT/dt$; (C) % increase in $-dT/dt$.

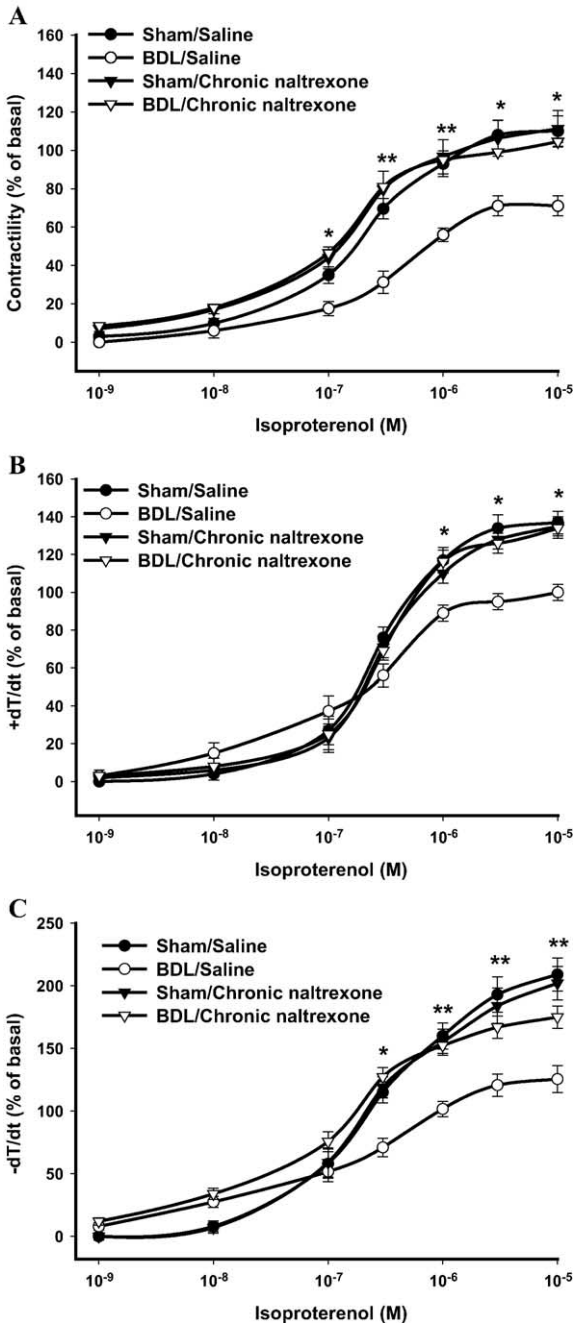

Fig. 5. Cumulative concentration–response curve for isoproterenol in isolated left ventricular papillary muscles from sham-operated and 7-day bile duct ligated (BDL) rats, which were chronically treated with naltrexone ($n=6$ for each group). (A) % increase in contractile force; (B) % increase in $+dT/dt$; (C) % increase in $-dT/dt$. $^{*}P<0.05$, $^{**}P<0.01$ compared to the saline-treated BDL group.

corrected the hyporesponsiveness of bile duct ligated-rats to isoproterenol only partially.

4. Discussion

In this study, the basal contractility of papillary muscle was significantly impaired in bile duct-ligated rats compared to control and sham-operated ones. This finding is in agreement with the in vivo and in vitro findings of Jacob et al. (1993) for 3-day bile duct-ligated rats, which showed a significant reduction in left ventricular pressure and maximal time derivative for the development of ventricular contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$).

This study showed attenuation of the E_{max} of contractile force, $+dT/dt_{max}$ and $-dT/dt_{max}$ to both α and β-adrenoceptor stimulation in 7-day bile duct-ligated rats compared to sham-

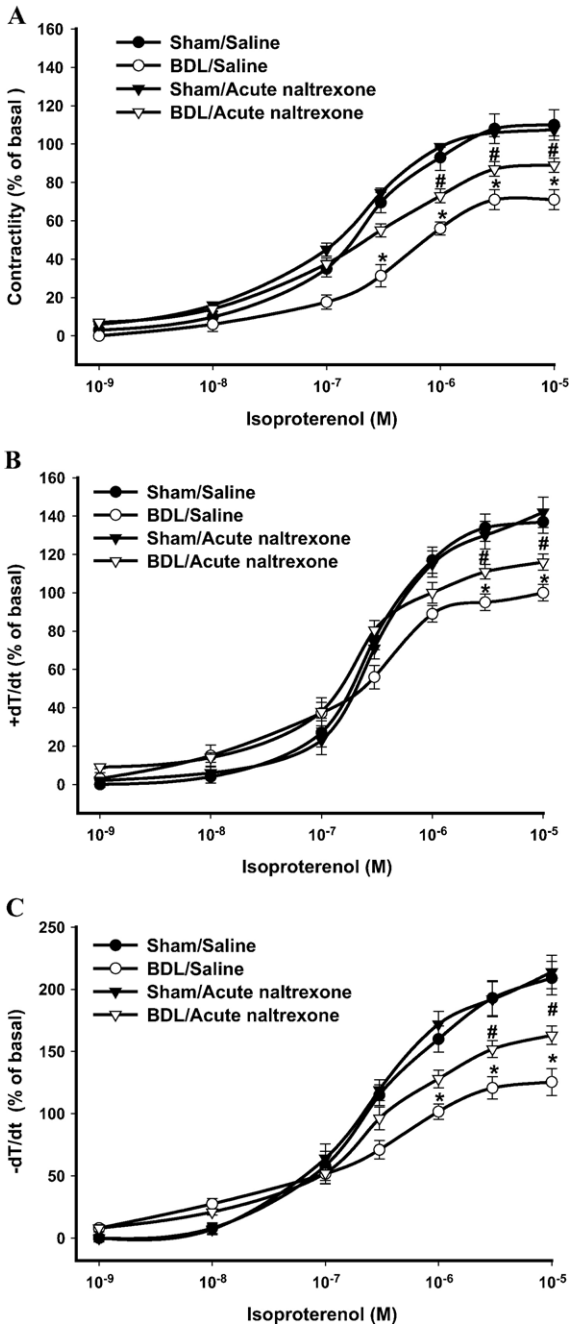

Fig. 6. Cumulative concentration–response curve for isoproterenol in isolated left ventricular papillary muscles from sham-operated and 7-day bile duct ligated (BDL) rats, which were acutely treated with naltrexone ($n=6$ for each group). (A) % increase in contractile force; (B) % increase in $+dT/dt$; (C) % increase in $-dT/dt$. $^{*}P<0.05$ compared to the saline-treated BDL group. $^{\#}P<0.01$ compared to the saline-treated sham group.

operated animals. Therefore, it seems that this hyporesponsiveness is not limited to β-adrenoceptor stimulation. Attenuated papillary muscle responsiveness to phenylephrine stimulation was consistent with previous findings of vascular hyporesponsiveness to α-adrenergic stimulation in cholestatic animals (Namiranian et al., 2001). Because of the dominant role of β-adrenoceptors in the regulation of myocardial contractility, we mainly focus on their role in the following sections.

Our findings of β-adrenoceptor hyporesponsiveness were in contrast with the normal affinity and number of cardiac β-adrenoceptors, accompanied by an intact responsiveness to β-adrenoceptor stimulation, reported by Jacob et al. (1993) for 3-day bile duct-ligated rats. The longer duration of bile duct ligation may be the basis for this discrepancy. Blunted inotropic (Lumlertgul et al., 1991), and chronotropic (Gaskari et al., 2002; Mani et al., 2002; Nahavandi et al., 2001) responsiveness has also been observed in cholestatic patients and animals.

Propranolol at the concentration of 5×10^{-6} M had no effect on the reduced baseline contractility, while it could block papillary muscle responsiveness to β-adrenoceptor stimulation (Skomedal et al., 1982). Therefore, it seems that an attenuated sensitivity of β-adrenoceptors cannot explain the reduction in baseline contractility.

4.1. Role of NO

NO contributes to the regulation of the cardiovascular system, through modulation of the adrenergic system (Chowdhary et al., 2002; Massion et al., 2003; Paton et al., 2002; Pugsley, 2002). It has also been suggested to contribute to bradycardia (Mani et al., 2002; Nahavandi et al., 2001), hypotension (Hajrasouliha et al., 2004), and hyporesponsiveness of isolated atria (Mani et al., 2002; Nahavandi et al., 2001) and vascular beds (Namiranian et al., 2001) to adrenoceptor stimulation in cholestatic liver disease. We have also recently reported NO-dependent resistance of bile duct-ligated rats to epinephrine-induced arrhythmia (Hajrasouliha et al., 2004).

According to our results, acute or chronic L-NAME administration corrects papillary muscle basal contractile abnormalities in bile duct-ligated rats. Although NO synthase inhibition was not able to restore E_{max}, it could decrease the EC_{50} of the contractile response of papillary muscles to β-adrenoceptor stimulation in bile duct-ligated rats. These results provide evidence for the role of NO in decreasing the sensitivity of papillary muscles to β-adrenoceptor stimulation following bile duct ligation. The inability of acute L-NAME treatment to decrease the EC_{50} of the contractile response suggests that chronic exposure to NO overproduction in bile duct-ligated rats induces changes which are not reversible by acute NO synthase inhibition. This is consistent with the role of NO in protecting of bile duct-ligated rats against epinephrine-induced arrhythmia (Hajrasouliha et al., 2004). The inability of acute L-NAME treatment to restore β-adrenoceptor hyporesponsiveness, despite correction of the baseline contractile impairment, suggests that the reduction of baseline contractility cannot explain the attenuated β-adrenoceptor responsiveness in bile duct-ligated rats.

4.2. Role of endogenous opioids

It was previously shown that cholestatic liver disease is associated with increased plasma levels of opioid peptides (Dehpour et al., 2000a; Swain et al., 1992). Observations compatible with this phenomenon include precipitation of an opioid withdrawal-like syndrome in cholestatic patients, as well as in the mouse model of cholestasis, after administration of an opioid antagonist (Dehpour et al., 1998, 2000b; Ghafourifar et al., 1997). The precise reason for the increased opioid activity in cholestasis is not yet completely understood, but it is likely that both overproduction of endogenous opioids and protection of these peptides from degradation may contribute to the elevation of total opioid activity (Swain et al., 1992; Thornton and Losowsky, 1988a,b).

It is well known that endogenous opioid peptides are involved in the regulation of the cardiovascular system through both central and peripheral receptors (Schadt, 1989; Szabo et al., 1987). Endogenous opioid peptides have been shown to decrease heart rate, cardiac output, peripheral vascular resistance (Champion and Kadowitz, 1998) and to modulate the autonomic nervous system (Szabo et al., 1987). Abnormalities of this system have been reported in several pathophysiologic conditions in both human and animal models of cardiovascular diseases (Pugsley, 2002). We have recently shown that administration of opioid antagonists corrects the bradycardia and hyporesponsiveness of isolated atria in response to adrenoceptor stimulation in bile duct-ligated rats (Gaskari et al., 2002), as well as the hyporesponsiveness of the vascular bed (Namiranian et al., 2001).

This study demonstrates that naltrexone administration corrects papillary muscle basal contractile abnormalities in bile duct-ligated rats. Naltrexone also improves responsiveness to β-adrenoceptor stimulation, which in the case of acute treatment manifests as a partial correction, and in the case of chronic treatment as complete normalization, of the isoproterenol concentration–response curve. Therefore, this study provides evidence for the role of endogenous opioid peptides in the contractile impairment and hyporesponsiveness to β-adrenoceptor stimulation seen in acute cholestasis.

5. Conclusion

In conclusion, this study demonstrates that papillary muscles of 7-day bile duct-ligated rats have an impaired basal contractility and responsiveness to both α and β-adrenoceptor stimulation. It also suggests that endogenous opioid peptides and NO contribute to the pathophysiology of these abnormalities. This study was complementary to our previous experiments, which have also shown the role of NO and endogenous opioid peptides in other aspects of cholestatic cardiomyopathy, such as chronotropic and electrophysiologic responses to adrenoceptor stimulation. We believe that further studies should be done to clarify how these substances contribute to the development of different aspects of cholestatic cardiomyopathy.

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