CHAPTER IV

RESULTS

1. Chronotropic and inotropic effects of *Passiflora foetida* extracts on rat isolated atria

The basal contraction of right and left atria were 0.5 ± 0.05 g and the basal rate of right atria were 250 ± 10 beats/min (Figure 9). The extracts *Passiflora foetida* were freshly dissolved in DMSO 99.5% before experiment. The final concentration of DMSO was 0.25 % V/V. At this concentration, DMSO had no significant effects on the basal rate and force of contraction on the isolated heart tissue in this study (n=6, mean± SEM) (Figure 10, 11 and 12). In this study, the effects of *Passiflora foetida* extracts (fraction 002: Sub-fractions PF002-(1-4), PF002-5, and PF002-7 and fraction 003: PF003-1, PF003-2 and PF003-(3-5)) were tested for their intrinsic chronotropic and inotropic activities as follows:

1.1 Effects of *P. foetida* extracts on left atria

The inotropic effects of PF002-(1-4) PF002-(5-6) and PF003-1 were determined on left atria (Figure 13, 14, and 16). The results showed that PF002-(1-4) PF002-(5-6) and PF003-1 had no effect on inotropic response of left atria (Figure 19 and 20). Contraction profiles showed that PF002-7, PF003-2 and PF003-(3-5) had transient negative inotropic effects (Figure 14, 16 and 17). PF002-7 and PF003-2 had significantly decreased inotropic response by 18.90 ± 3.16 and 15.52 ± 1.22 at initial time (n=6, $p<0.001$) (Figure 19 and 20). However, the inotropic activities of PF003-2

inversely changed from negative effect at initial time to positive inotropic effect at 5 and 15 minutes later (Figure 17). PF003-2 at the concentration of 100 μg/ml significantly increased the contractile force of the left atria by 8.76 ± 4.01 % and 19.22 ± 4.95 % at 3 min and 15 min, respectively ($p < 0.05$ and $p<0.001$, n=6) (Figure 20).

1.2 Effects of *P. foetida* extracts on right atria

The results showed that PF002-(1-4) had no effect on inotropic and chronotropic response of the isolated right atria (Figure 21 and 23). PF002-(5-6) had a small effect on chronotropic and inotropic responses of the isolated right atria (Figure 14). Treatment of PF002-(5-6) caused slightly decreased in force contraction by 11.80 ± 4.32 % ($p<0.05$, n=6) (Figure 21). PF003-1 decreased inotropic effect by 15.71 ± 3.25 % and 18.51±4.01 % at 10 min and 15 min, respectively (n= 6, $p<0.05$) (Figure 22) and had no effect on chronotropic response of isolated right atria (Figure 24). The contraction profiles showed that PF002-7 and PF003-2 had transient negative inotropic effect followed by the chronotropic effects (Figures 15 and 17). In contrast, PF003-(3-5) had significant negative chronotropic effects with the heart rate reduction by 9.61 ± 1.53 % and 14.87 ± 1.17 % at 3 min and15 min ($p < 0.05$,n=6) (Figure 24).

2. The chronotropic and inotropic effect of *Passiflora foetida* extracts: PF003-2

Based on the inotropic activity, PF003-2 was further investigated for its potential mechanisms of actions including β-adrenoceptor, serotonin receptor, Ca^{2+} release from internal storage, and catecholamine release and reuptake.

2.1 β-adrenoceptor

At the concentration of 0.1 μM, as shown in Figure 26 and 29A, NE significantly elicited the positive inotropic and chronotropic characteristic of β-adrenoceptor agonist (Figure 26). The force of contraction on left and right atria increased by 82.41 ± 13.65 % and 48.11 ± 15.14 % (n=4, $p<0.05$), respectively (Figure 29A). In addition, the pace maker activity of the right atria increased by 34.26 ± 6.33 % (n=4, $p<0.05$) (Figure 29A). As shown in Figure 25 and 29B, propranolol (10 μM) significantly decreased chronotropic and inotropic responses of isolated rat atria. The reduction of force of isolated rat left atria caused by propranolol was 15.14 ± 3.35 % (n=6, $p<0.05$). In isolated right atria, propranolol reduced chronotropic response by 25.01±3.53 % and increased inotropic response by 14.03± 3.45 %. The presence of 10 μM propranolol suppressed both basal rate and force of contraction of left and right atria (Figure 25 and 29B). Moreover, propranolol completely blocked the positive inotropic and chronotropic effect of 0.1 μM NE (Figure 27, 30 and 31). The results showed that positive inotropic effect of PF003-2 was partially inhibited by propranolol (10 μM) ($p<0.05$, n=6) (Figure 28, 32 and 33).

2.2 Serotonin receptor

Serotonin at the concentration of 10 μM caused positive inotropic and chronotropic response of left and right atria (Figure 34). The heart rate and contractile force of atria at 15 minute increased by 9.43 ± 2.80 % and 23.32 ± 1.74 %, respectively (P<0.05, n=4) (Figure 38A). Ketanserin (10μM), a selective 5-HT_2 receptor antagonist, significantly

decreased rate and force of contraction of left and right atria at 15 minute by 12.70 ± 2.29 % and 11.54 ± 2.60 %, respectively ($P<0.05$, n=4) (Figure 35 and 35B). In this study, ketanserin (10 μM) significantly blocked the effect of serotonin (1 μM) on the heart ($p<0.05$, n=4) (Figure 36, 39 and 40). The results show that at this concentration, ketanserin significantly inhibited positive inotropic effect of PF003-2 at 3, 5, 10 and 15 minute ($P<0.05$, n=6) (Figure 37, 41 and 42).

2.4 β-adrenoceptor and serotonin receptor

As shown in Figure 43, 44 and 45, inotropic and chronotropic effect of PF003-2 was completely inhibited by propranolol (10 μM) and ketanserin (10 μM). Positive inotropic response was significantly decreased by 8.62±2.31 and 6.36± 1.64 % at 3 and 15 minute. (n=5, $p<0.05$).

2.5 Role of Calcium release from internal storage (SR)

Caffeine (10 mM) was used as a positive control to induce Ca^{2+} release from sarcoplasmic recticulum (SR). Figure 46A, 46B and 47 presented the relationship between Ti / Tss and the resting interval in the presence of caffeine. The ratio of Ti / Tss in the presence of caffeine was markedly inhibited the rested-state contraction in the time interval 10 seconds to 5 minute. Figure 46C and 48 established the relationship between Ti / Tss and the resting interval in the presence of PF003-2. The results showed that caffeine significantly inhibited the rested-state interval 10 seconds to 5 minute ($p<0.05$, n=4) (Figure 47). In contrast to caffeine, PF003-2 did not significantly inhibit the rested-state interval (Figure 48).

2.6 The storage catecholamine

In isolated preparations of left and right atria, tyramine produced an increase in heart rate and force of contraction via releasing of storage NE from adrenergic nerve (Figure 49 and 52). However, the positive chronotropic and inotropic effects of tyramine markedly decreased in the atria isolated from rat pretreated with reserpine (5 mg/kg, i.p. for 2 days) (Figure 50 and 53). These effects were due to the action of reserpine on blocking catecholamine reuptake, leading to a deprivation of presynaptic storage NE. With the use of isolated preparations from reserpinized rat, PF003-2 was able to cause the chronotropic and inotropic effects (Figure 51). In addition, the action of PF003-2 in the reserpinized tissue was not statistically different from those obtained from normal tissues (Figure 54). It is unlikely that PF003-2 exerted its action via activation of catecholamine release.

2.7 Catecholamine reuptake.

2.7.1 NE reuptake

This study was performed to examine the effect of PF003-2 (100 μg/ml) on reuptake of NE with the use of amitriptyline. As shown in figure 55 and 58, amitriptyline (10 μM) could blocked reuptake of NE (0.1 μM). Comparison to NE (Figure 57), the inotropic response of NE in the presence of amitriptyline increased from 40.62 ± 6.79 % to 63.92 ± 17.97 % (n=6) (no significant). PF003-2 was 5 minute added prior to NE (0.1 μM). The results showed that PF003-2 could not block the reuptake of NE (0.1 μM) (Figure 56 and 59).

2.7.2 Serotonin reuptake

This study was performed to examine the effect of PF003-2 (100 μg/ml) on reuptake of serotonin with the use of fluoxetine, a selective serotonin reuptake inhibitor. As shown in Figure 60 and 62, serotonin (1 μM) caused significantly positive inotropic and chronotropic response by 36.61±4.01% and 2.68 ± 0.22 %, respectively (n=5, $p<0.05$). Fluoxetine (1 μM) exerted negative inotropic and chronotropic effect (Figure 63) and could not blocked reuptake of serotonin (1 μM) (Figure 60 and 64). In addition, PF003-2 was 5 minute added prior to serotonin (1 μM). The results showed that PF003-2 could not block the reuptake of serotonin (1 μM) (Figure 61 and 65).

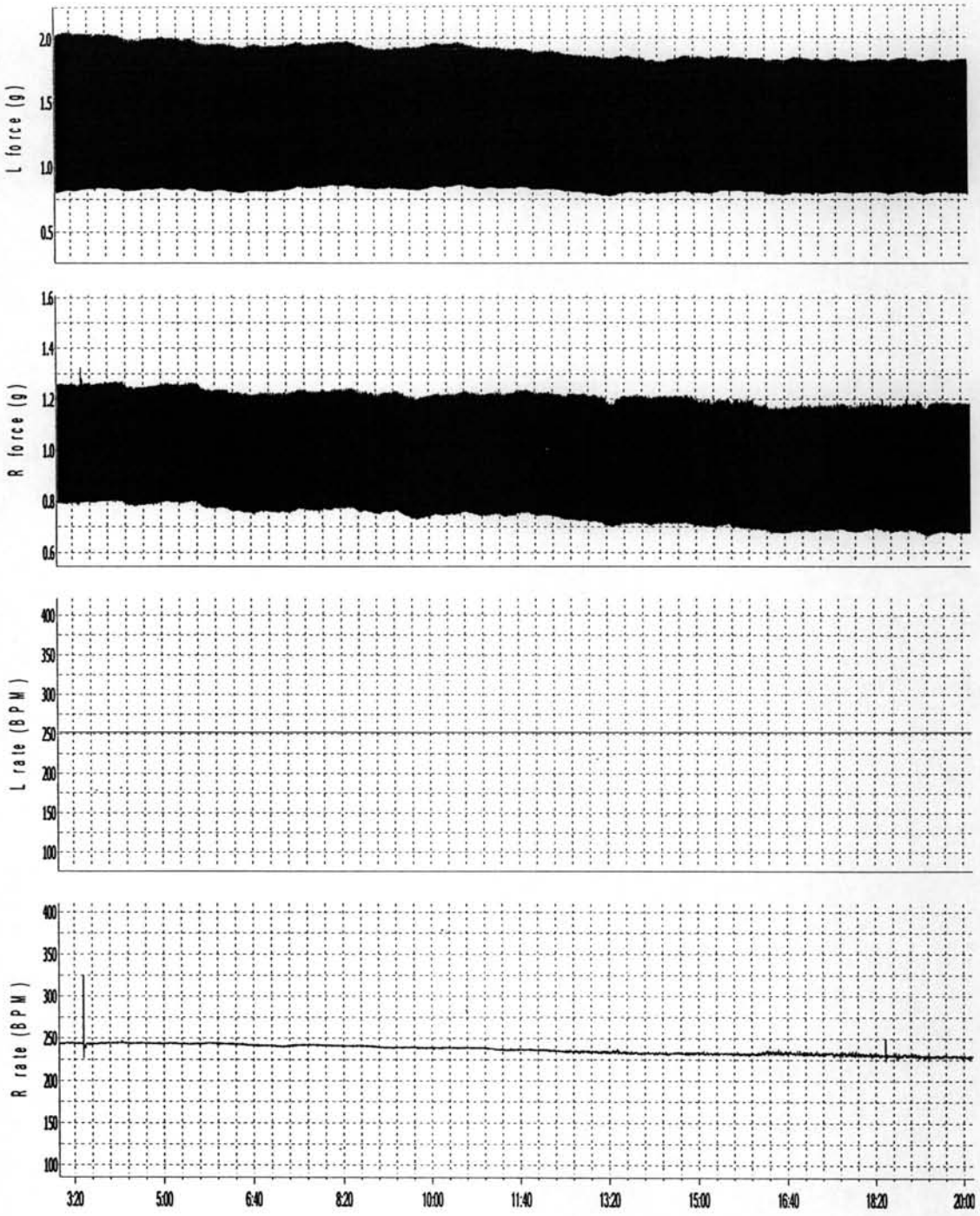


Figure 9 Chronotropic and inotropic response on the left and right atria before treating

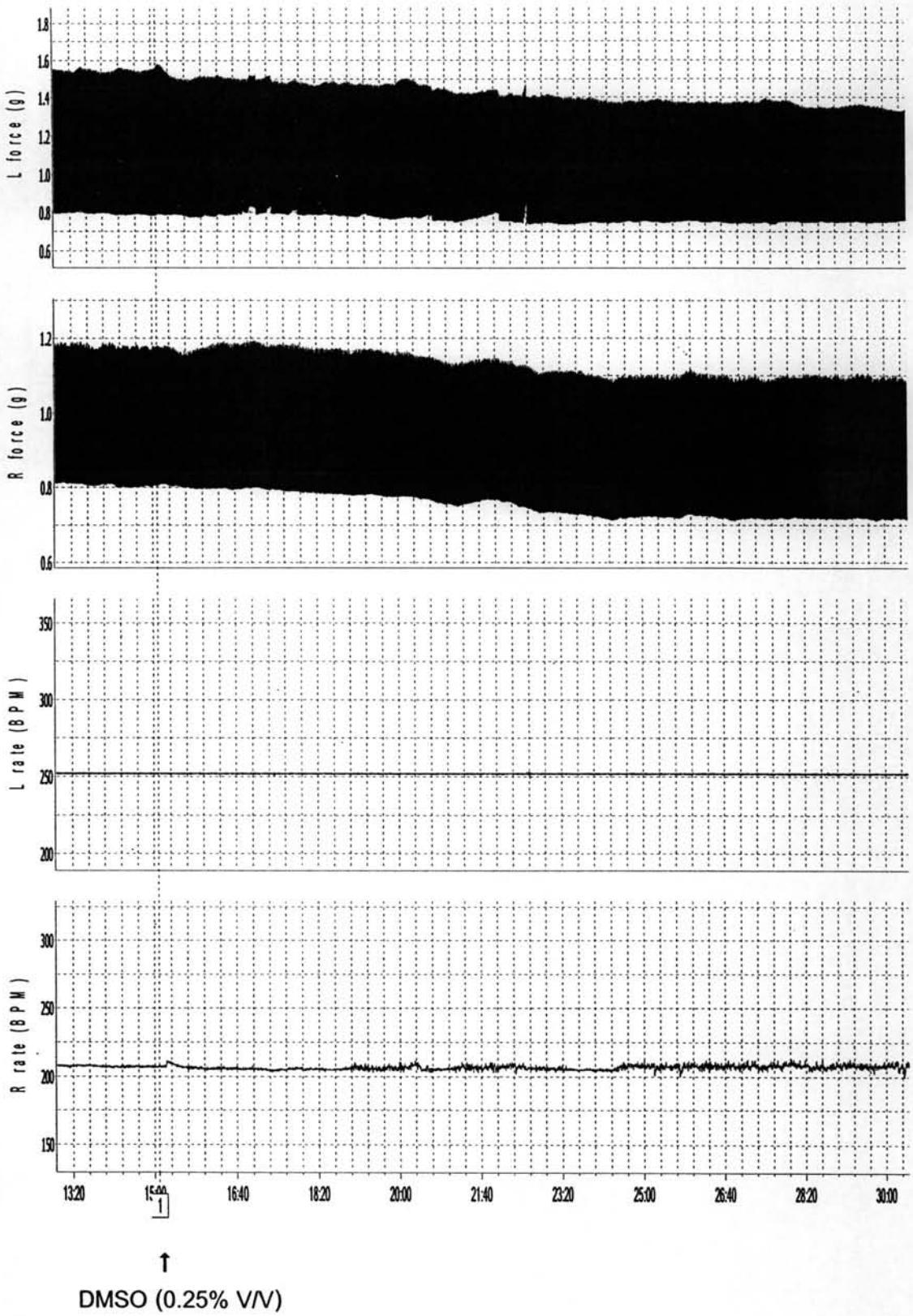


Figure 10 Chronotropic and inotropic response on the left and right atria in the presence of DMSO (0.25% V/V)

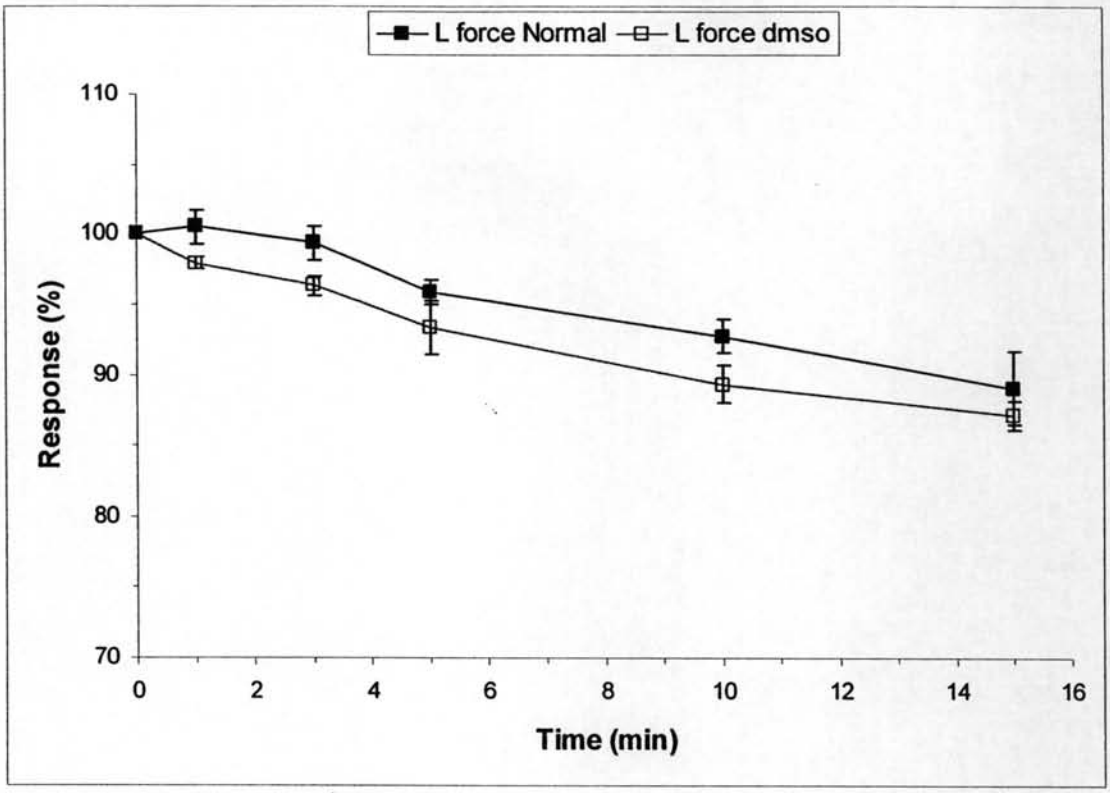
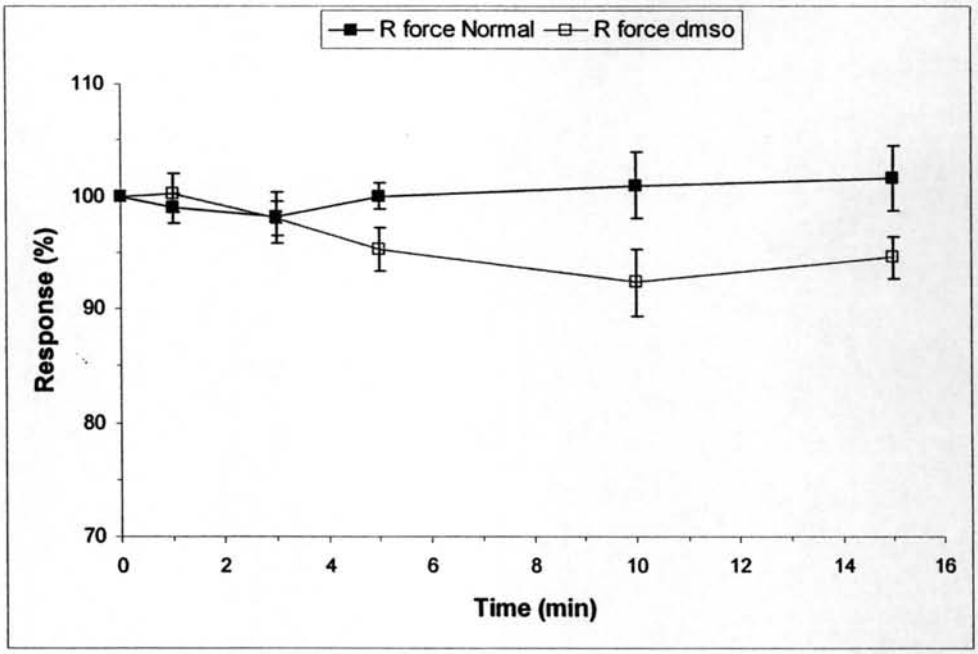


Figure 11 Inotropic response on the left atria in the absence and presence of DMSO (0.25% V/V), n=6 (mean ± SEM)

A) Right force



B) Right rate

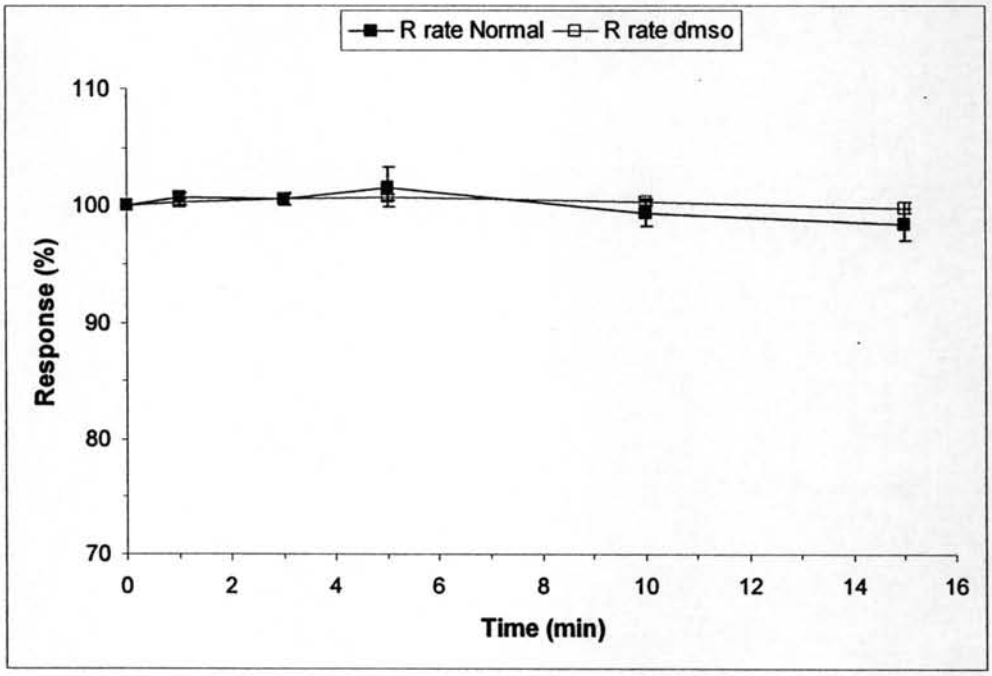


Figure 12 Inotropic (A) and chronotropic (B) response on the right in the absence and presence of DMSO (0.25% V/V), n=6 (mean ± SEM)

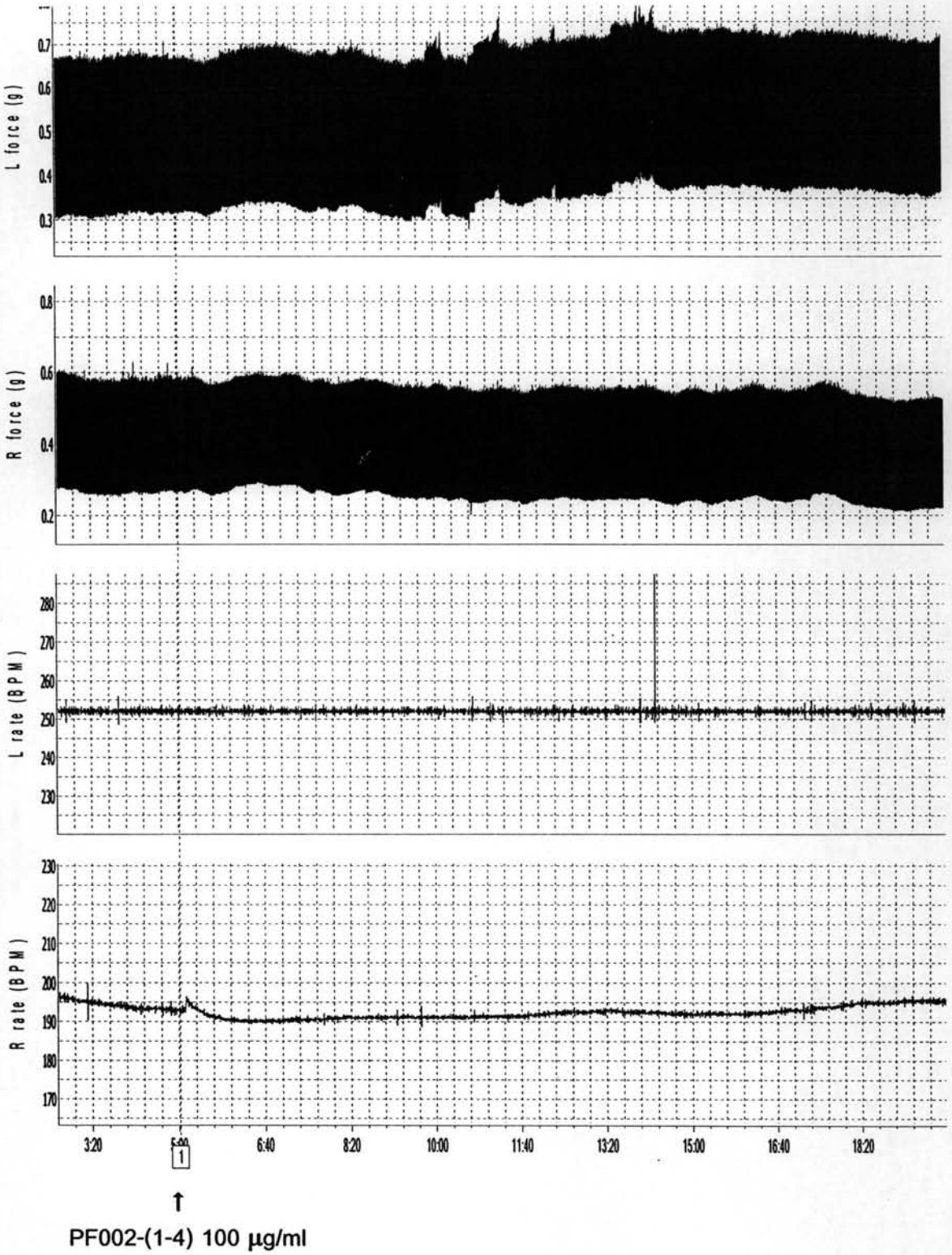


Figure 13 Chronotropic and inotropic response on the left and right atria in the presence of PF002-(1-4) (100 µg/ml)

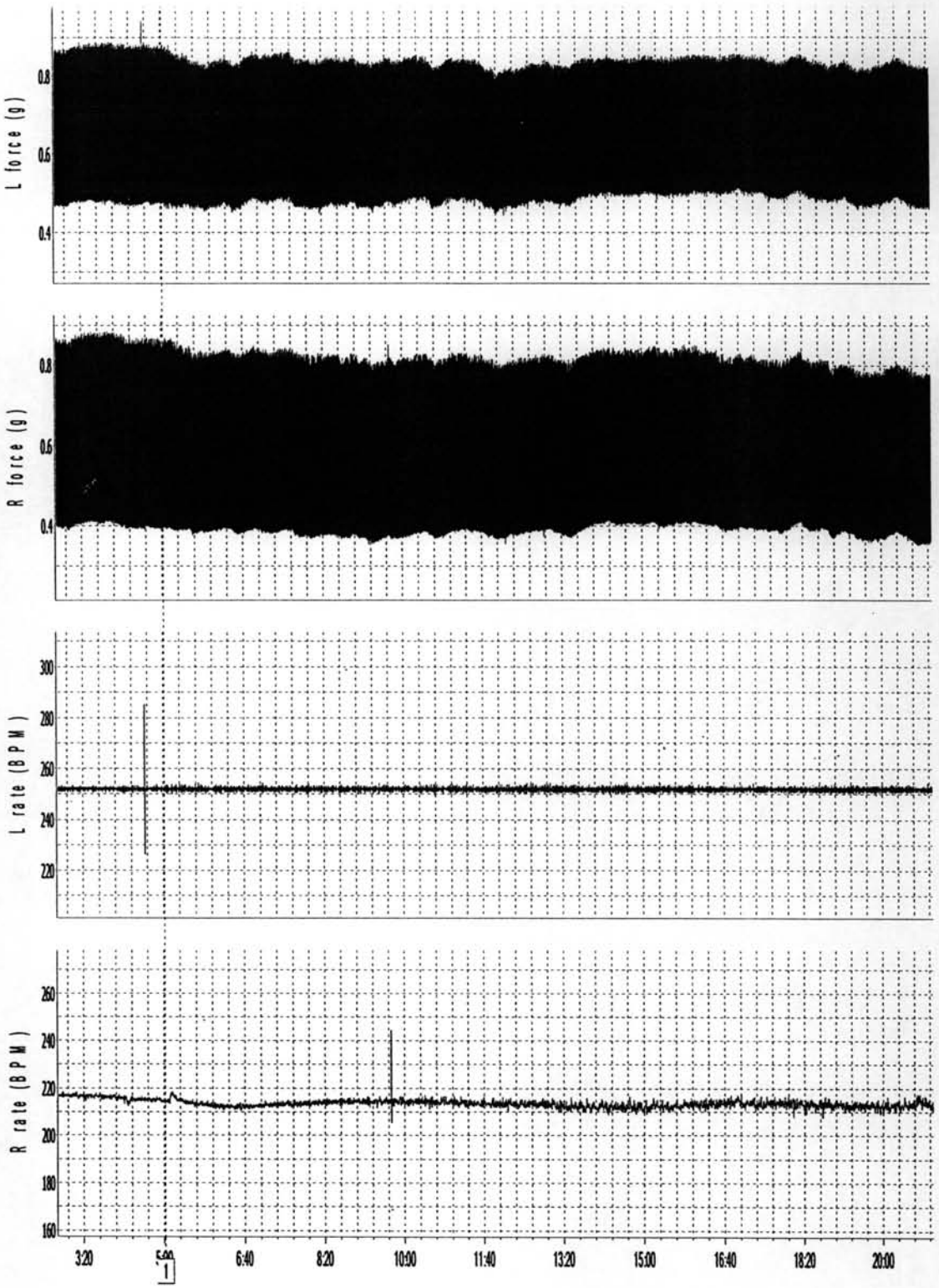


Figure 14 Chronotropic and inotropic response on the left and right atria in the presence of PF002-(5-6) (100 µg/ml)

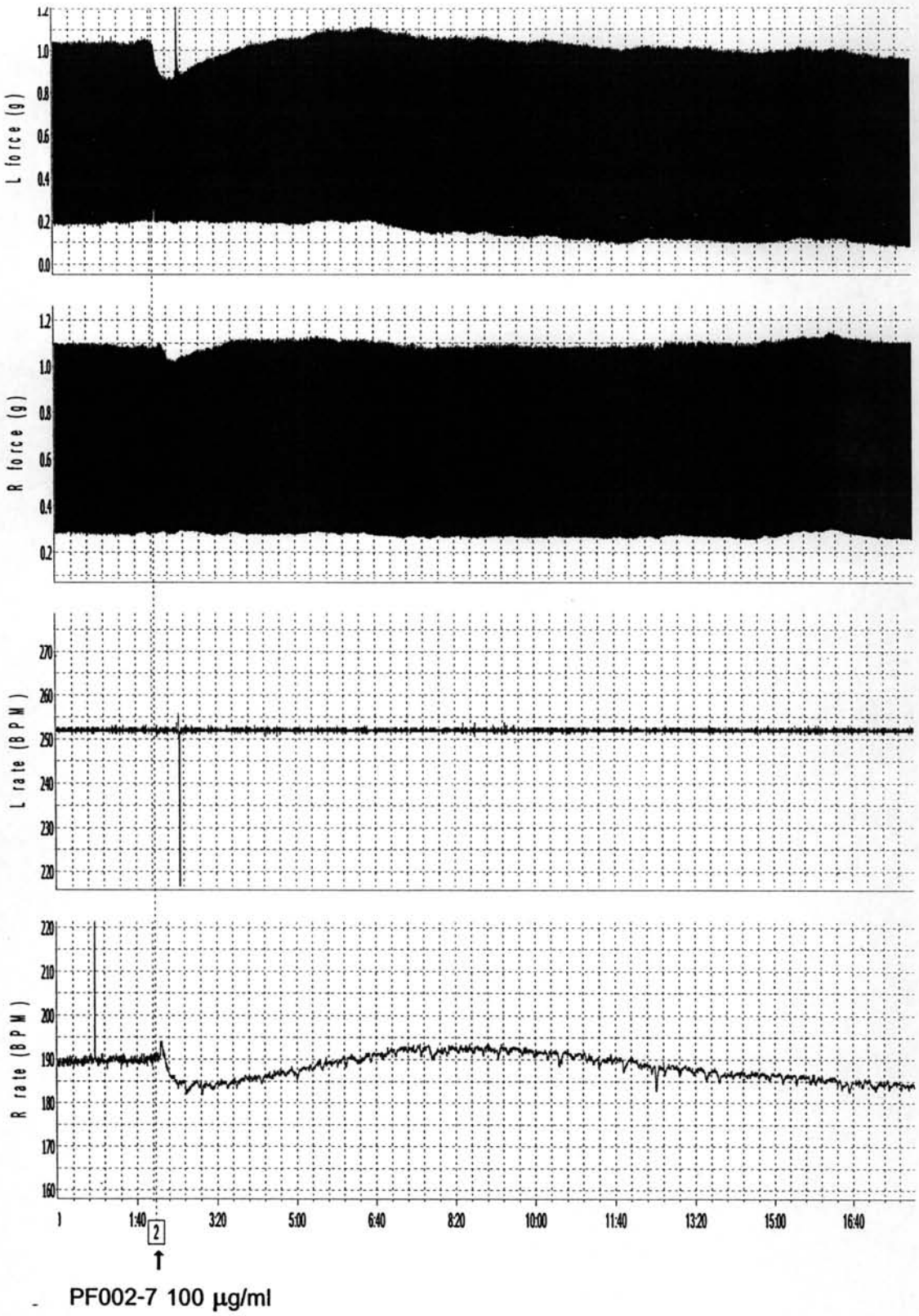


Figure 15 Chronotropic and inotropic response on the left and right atria in the presence of PF002-7 (100 µg/ml)

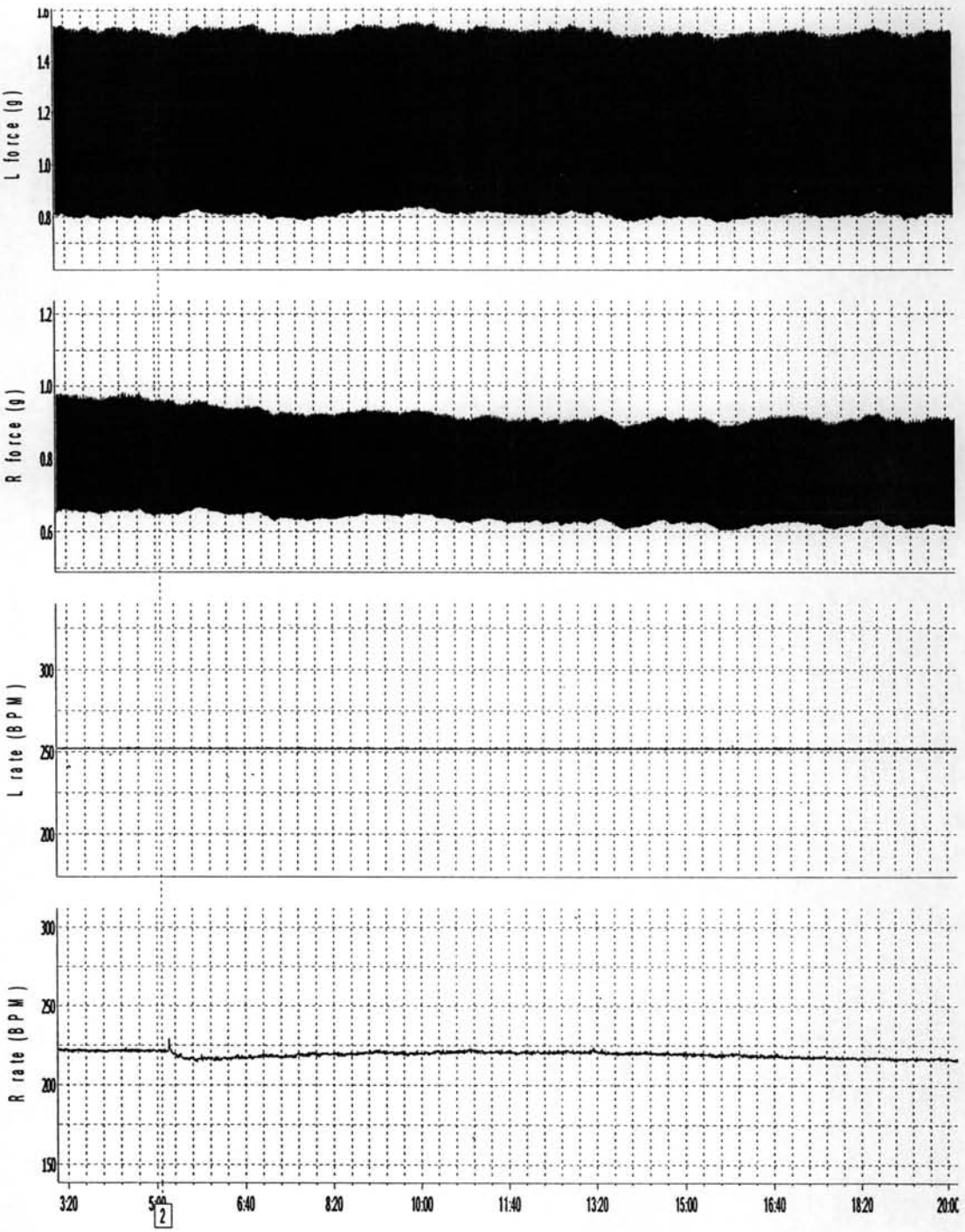


Figure 16 Chronotropic and inotropic response on the left and right atria in the presence of PF003-1 (100 µg/ml)

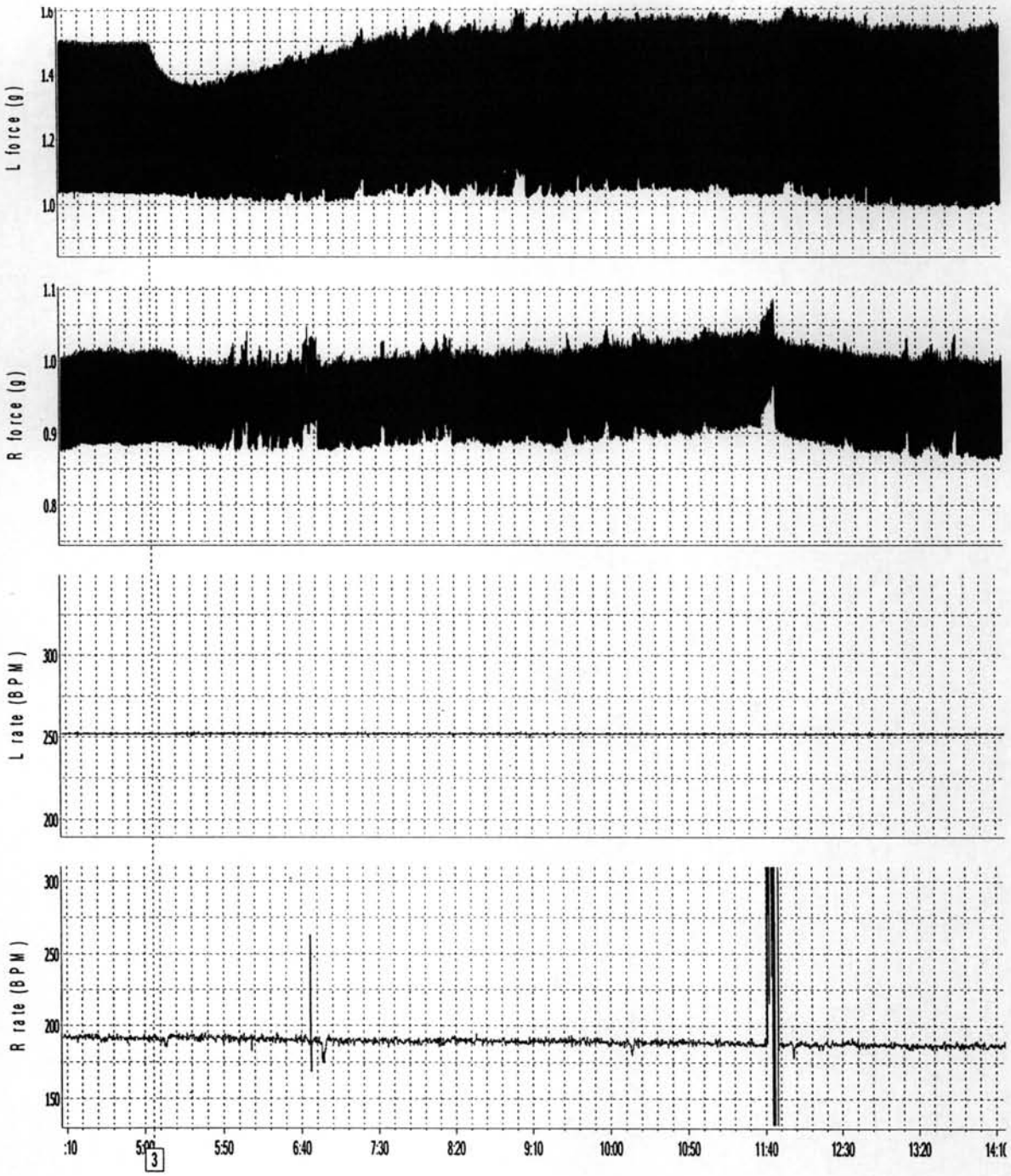


Figure 17 Chronotropic and inotropic response on the left and right atria in the presence of PF003-2 (100 μg/ml)

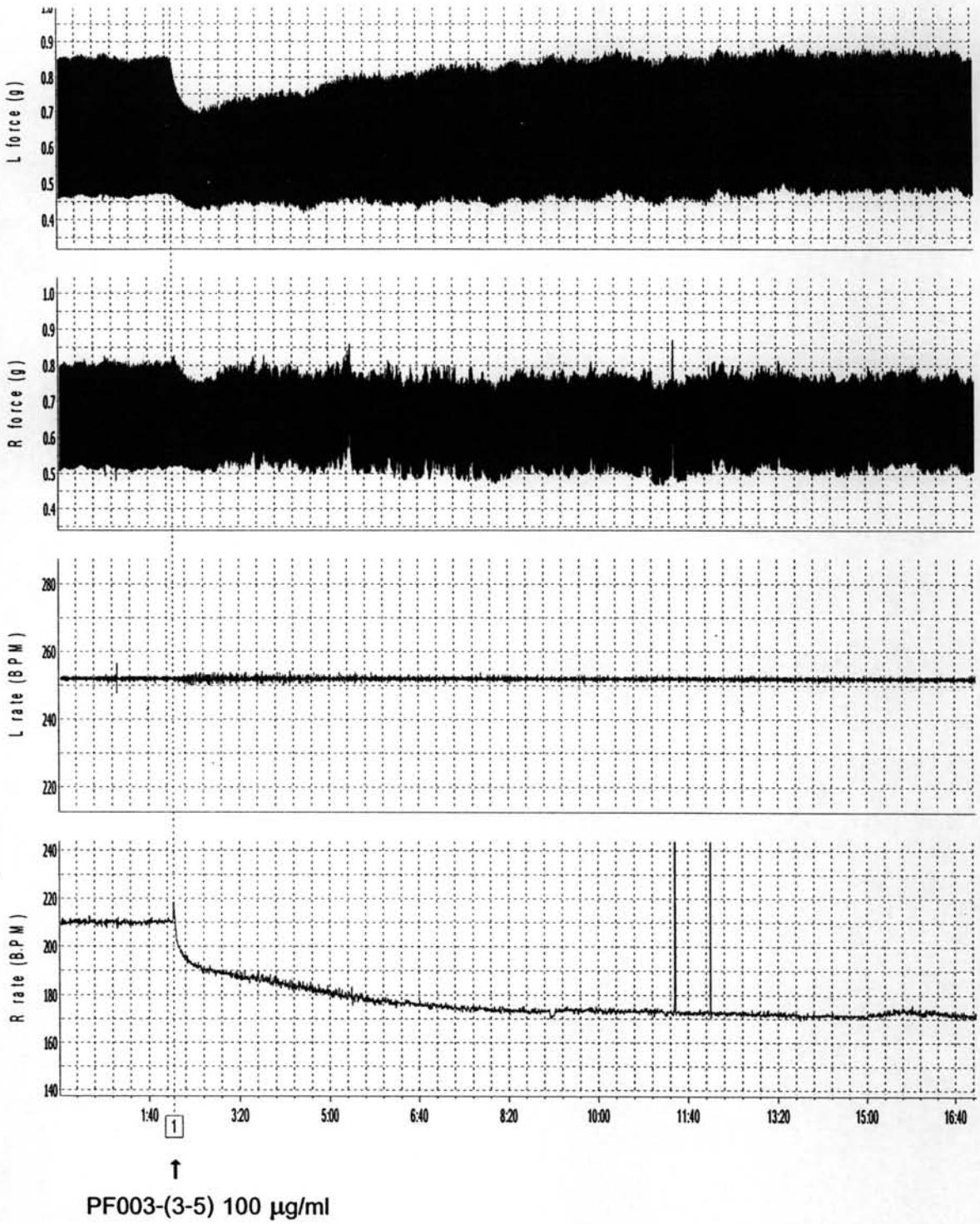


Figure 18 Chronotropic and inotropic response on the left and right atria in the presence of PF003-(3-5) (100 µg/ml)

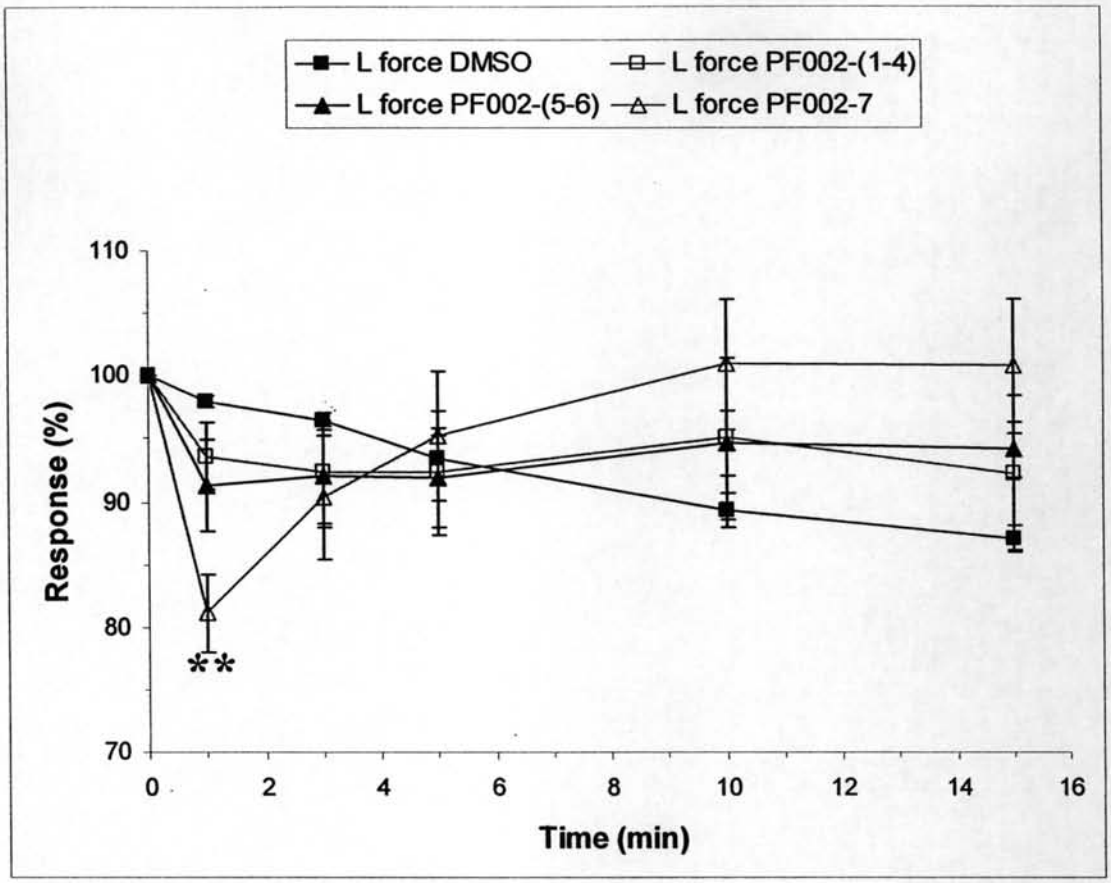


Figure 19 The inotropic reponse of the left atria in the presence of PF extract ; PF002-(1-4), PF002-(5-6) and PF002-7 100 μg/ml, n=6, mean ± SEM, *p<0.05, significantly different from DMSO 0.25% group (unpaired t-test)

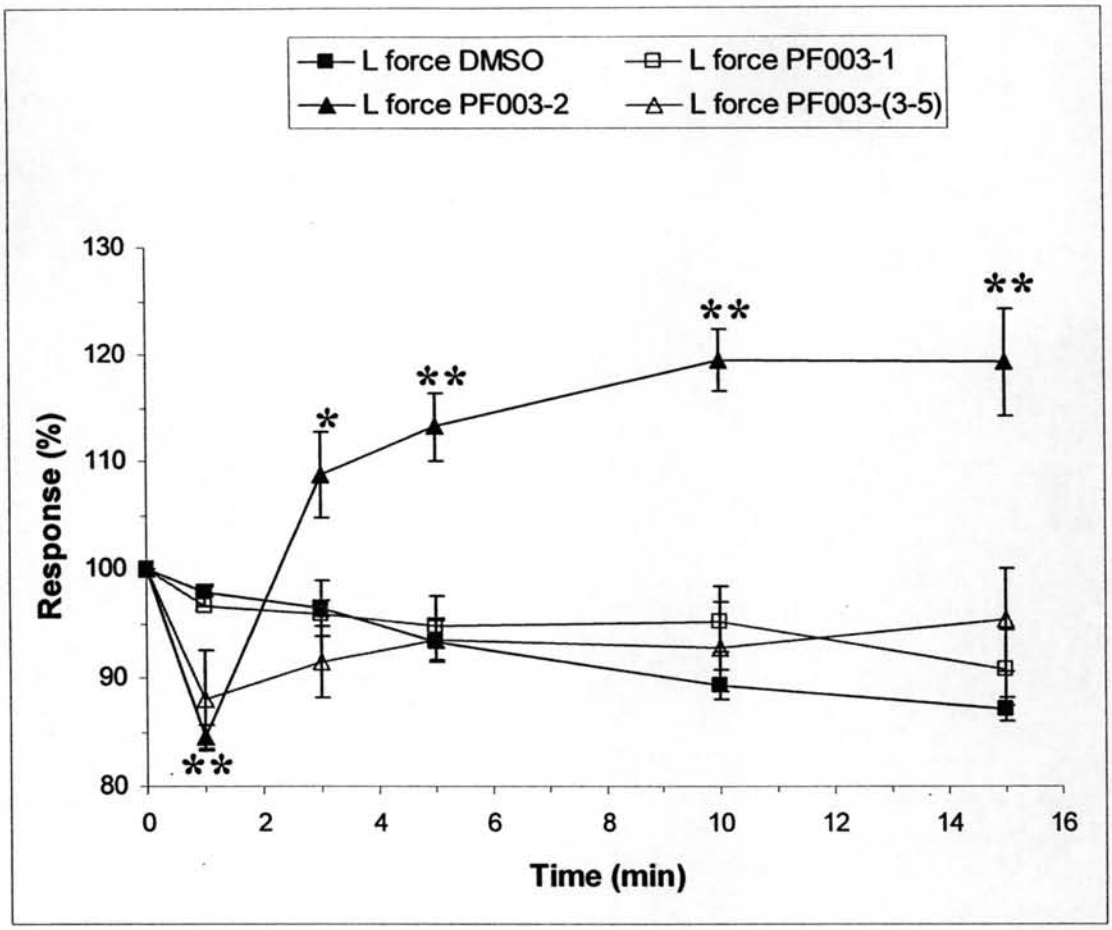


Figure 20 The inotropic reponse on the left atria in the presence of PF extract ; PF003-1, PF003-2 and PF003-(3-5) 100 μg/ml, n=6, mean ± SEM, *p<0.05, **p<0.001 significantly different from DMSO 0.25% group (unpaired t-test)

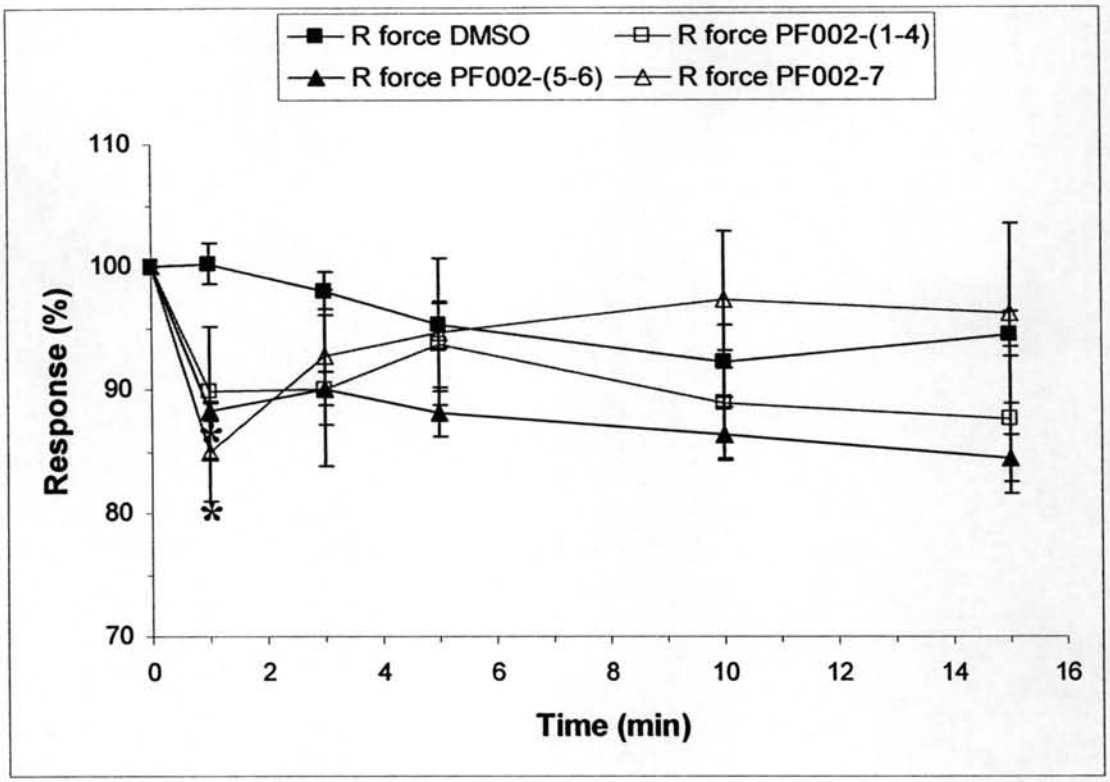


Figure 21 The inotropic reponse on the right atria in the presence of PF extract ; PF002-(1-4), PF002-(5-6) and PF002-7 100 μg/ml, n=6, mean ± SEM, *$p<0.05$, significantly different from DMSO 0.25% group (unpaired *t*-test)

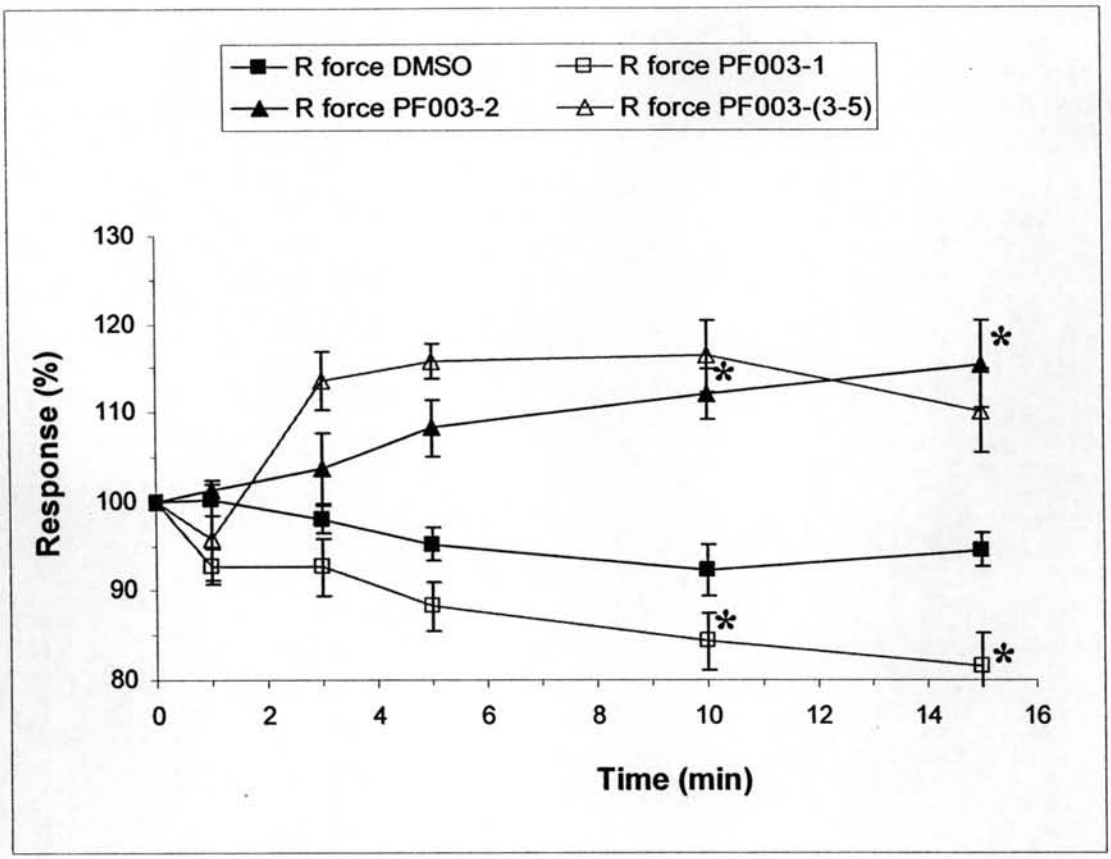


Figure 22 The chronotropic and inotropic reponse on the right atria in the presence of PF extract ; PF003-1, PF003-2 and PF003-(3-5) 100 μg/ml, n=6, mean ± SEM, *p<0.05, significantly different from DMSO 0.25% group (unpaired t-test)

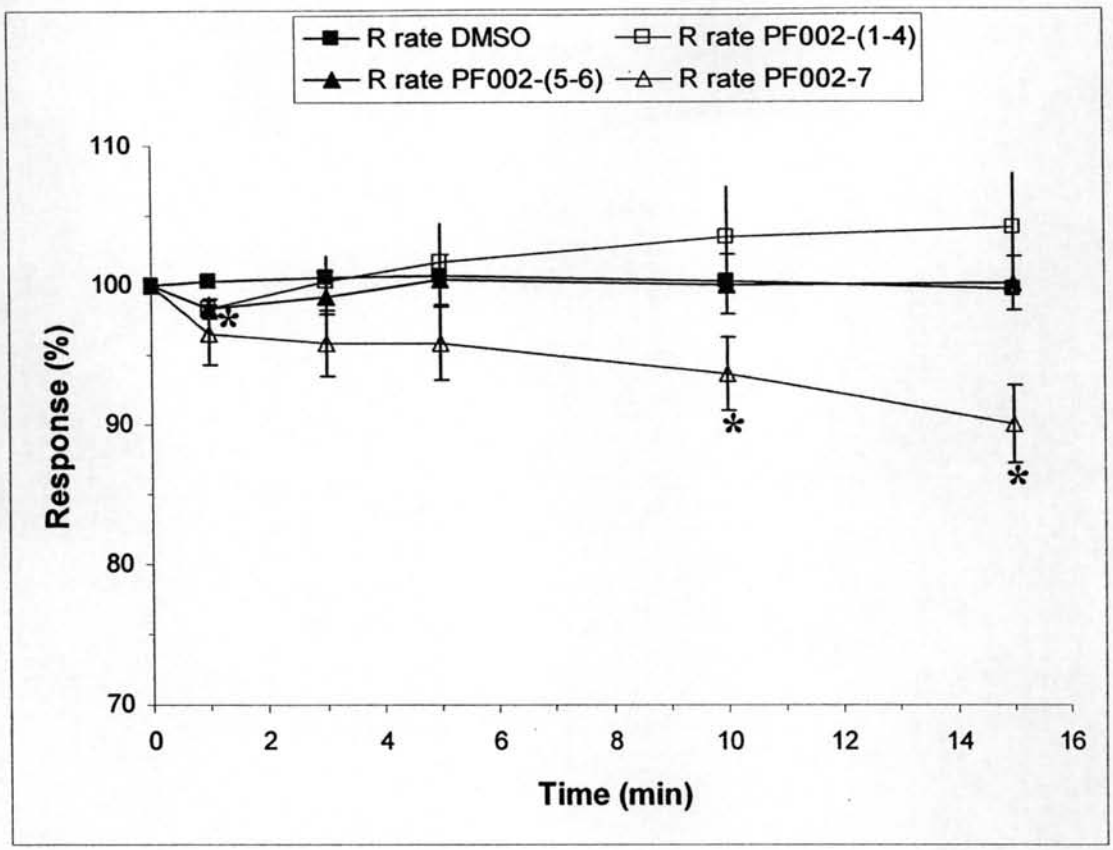


Figure 23 The chronotropic reponse on the right atria in the presence of PF extract ; PF002-(1-4), PF002-(5-6) and PF002-7 100 μg/ml, n=6, mean ± SEM, *$p<0.05$, significantly different from DMSO 0.25% group (unpaired *t*-test)

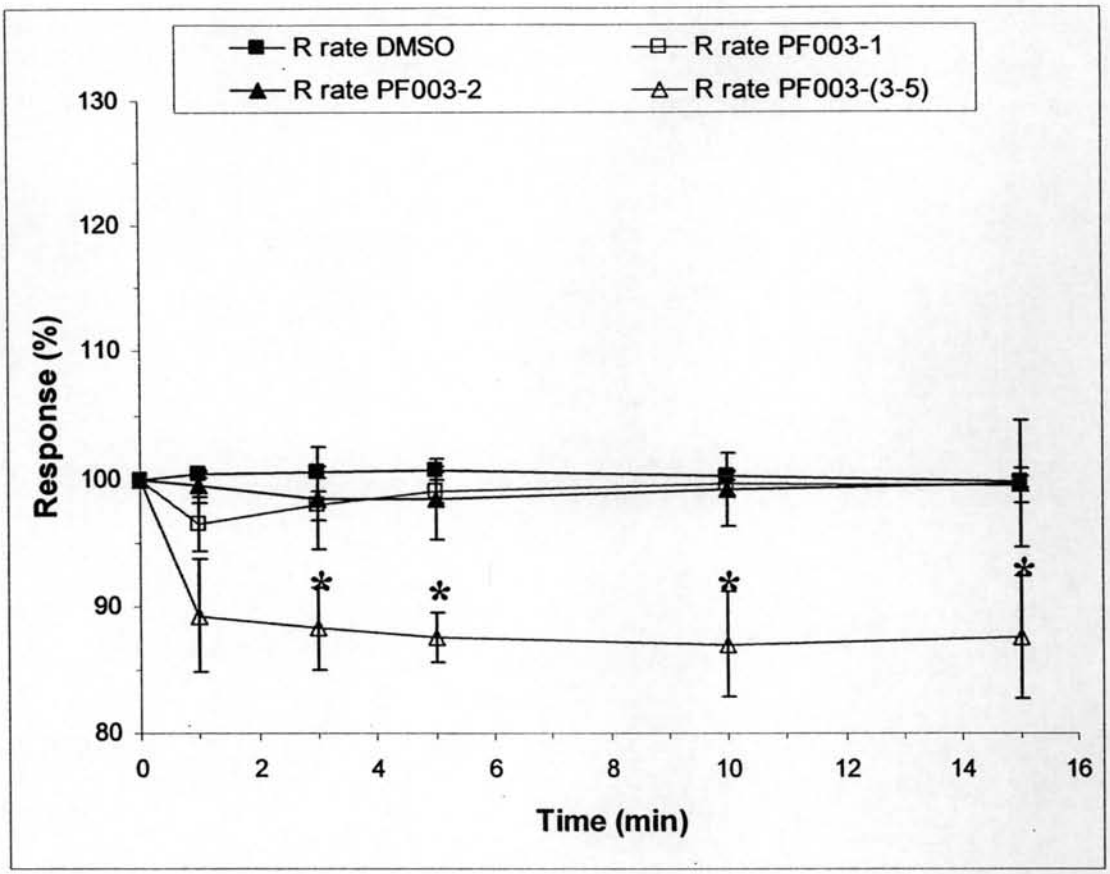


Figure 24 The chronotropic response on the right atria in the presence of PF extract ; PF003-1, PF003-2 and PF003-(3-5) 100 μg/ml, n=6, mean ± SEM, *p<0.05, significantly different from DMSO 0.25% group (unpaired *t*-test)

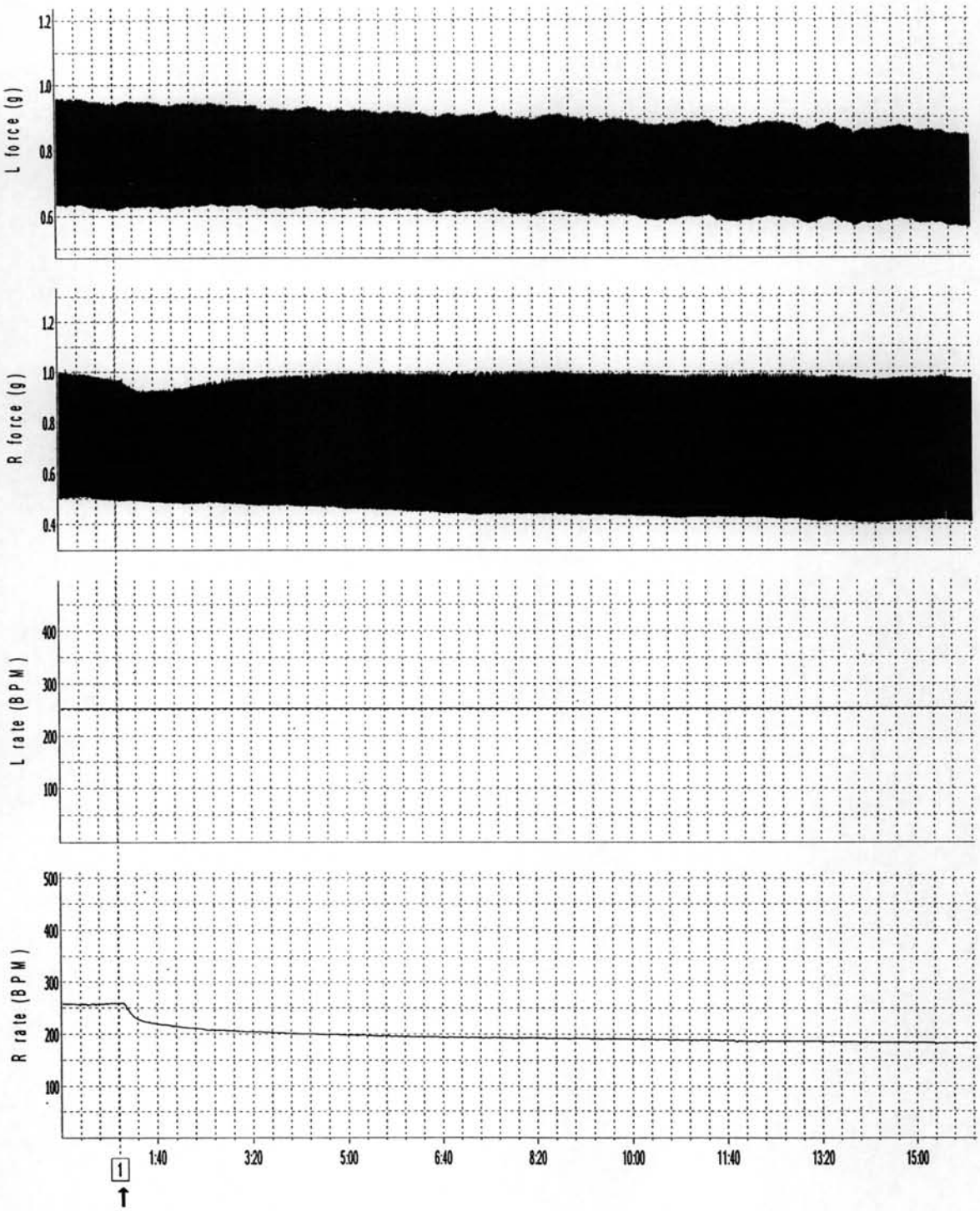


Figure 25 Chronotropic and inotropic response on the left and right atria in the presence of propranolol (10 µM)

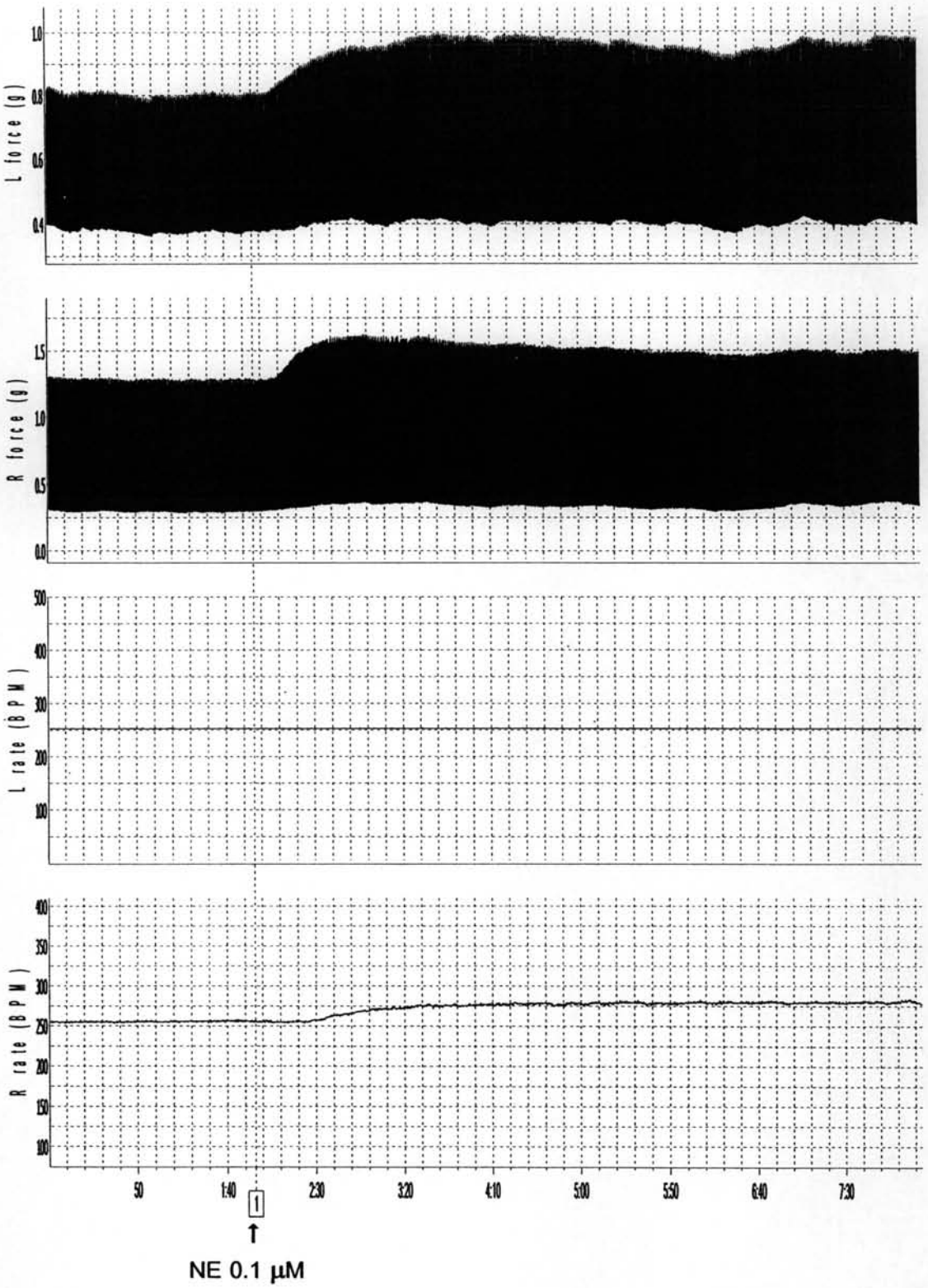


Figure 26 Chronotropic and inotropic response on the left and right atria in the presence of NE (0.1 μM)

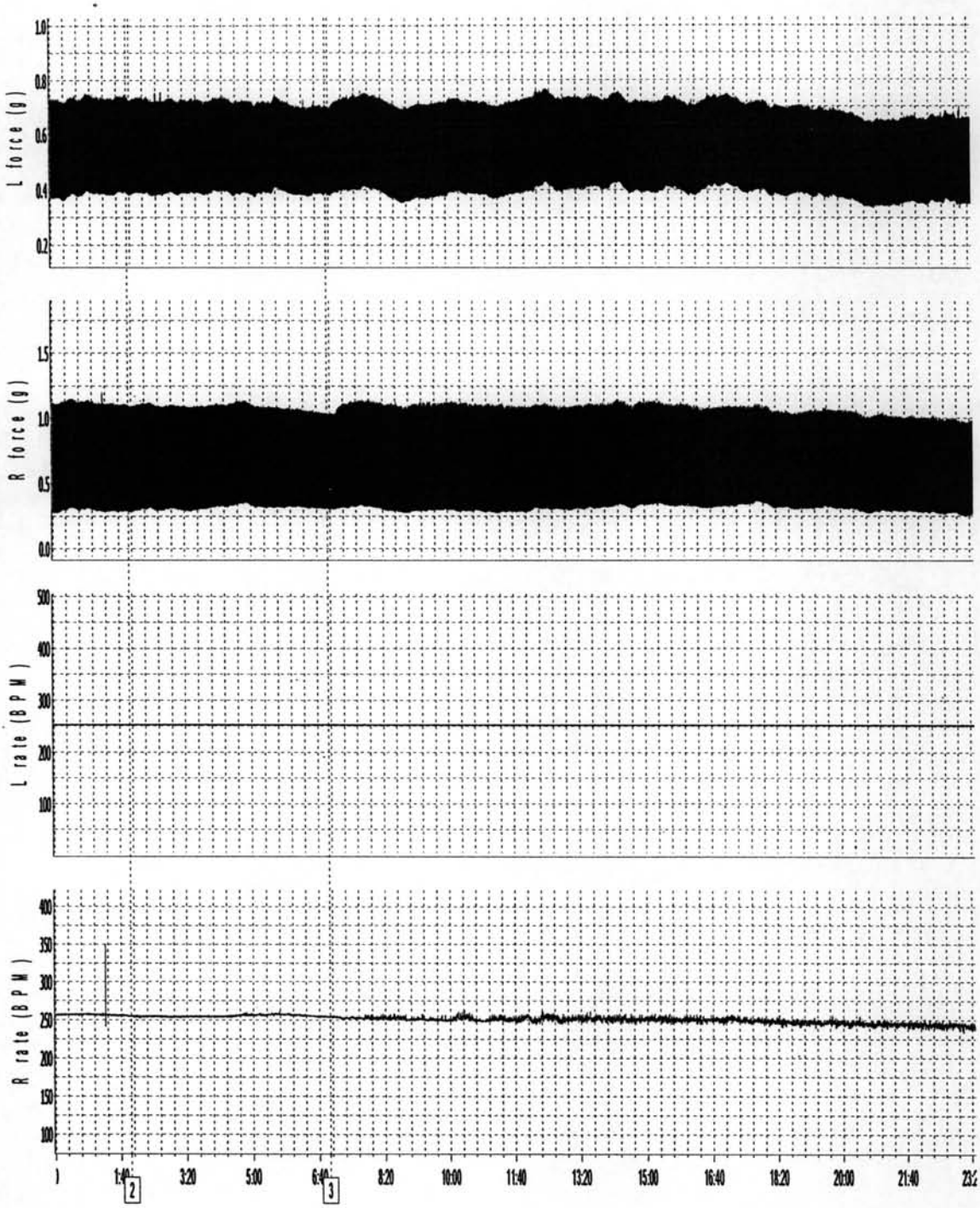


Figure 27 Chronotropic and inotropic response of NE (1 μM) on the left and right atria in the presence of propranolol (10 μM)

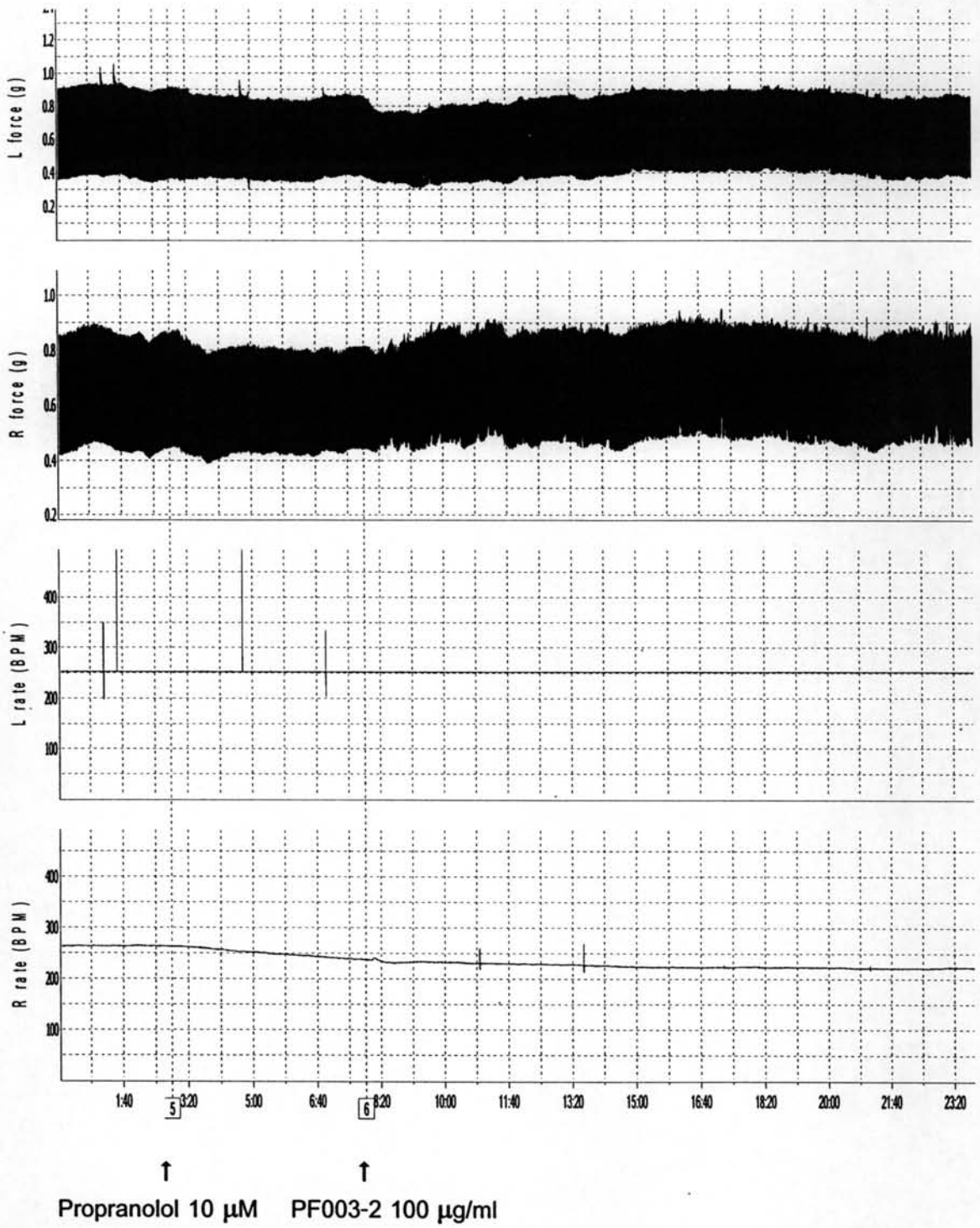
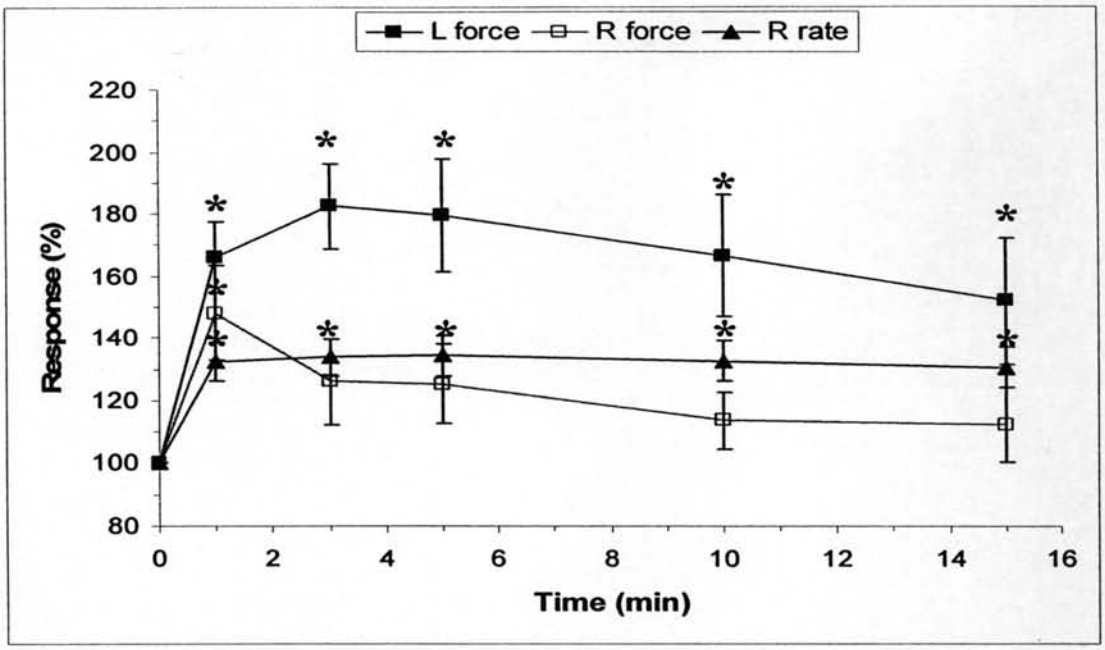


Figure 28 Chronotropic and inotropic response of PF003-2 (100 μg/ml) on the left and right atria in the presence of propranolol (10 μM)

A. Norepinephrine



B.Propranolol

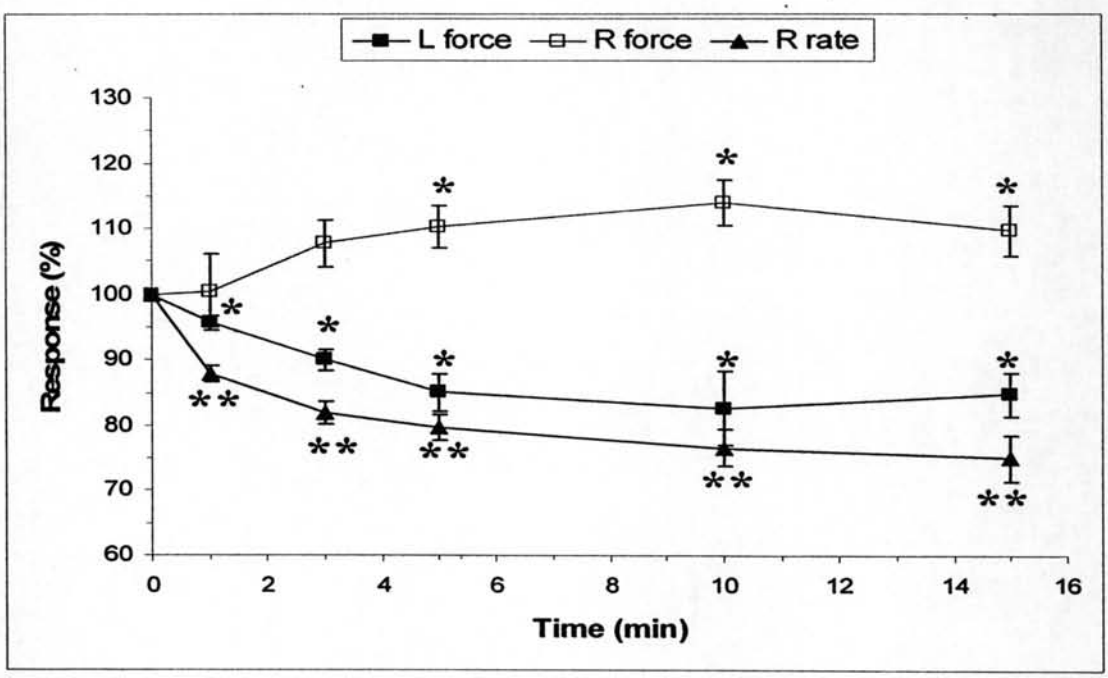


Figure 29 The chronotropic and inotropic reponse of **(A)** NE (0.1μM), n=4 and **(B)** propranolol (10 μM), n=6 on the left and right atria, mean ± SEM *p<0.05, significantly different from response at 0 min (paired t-test)

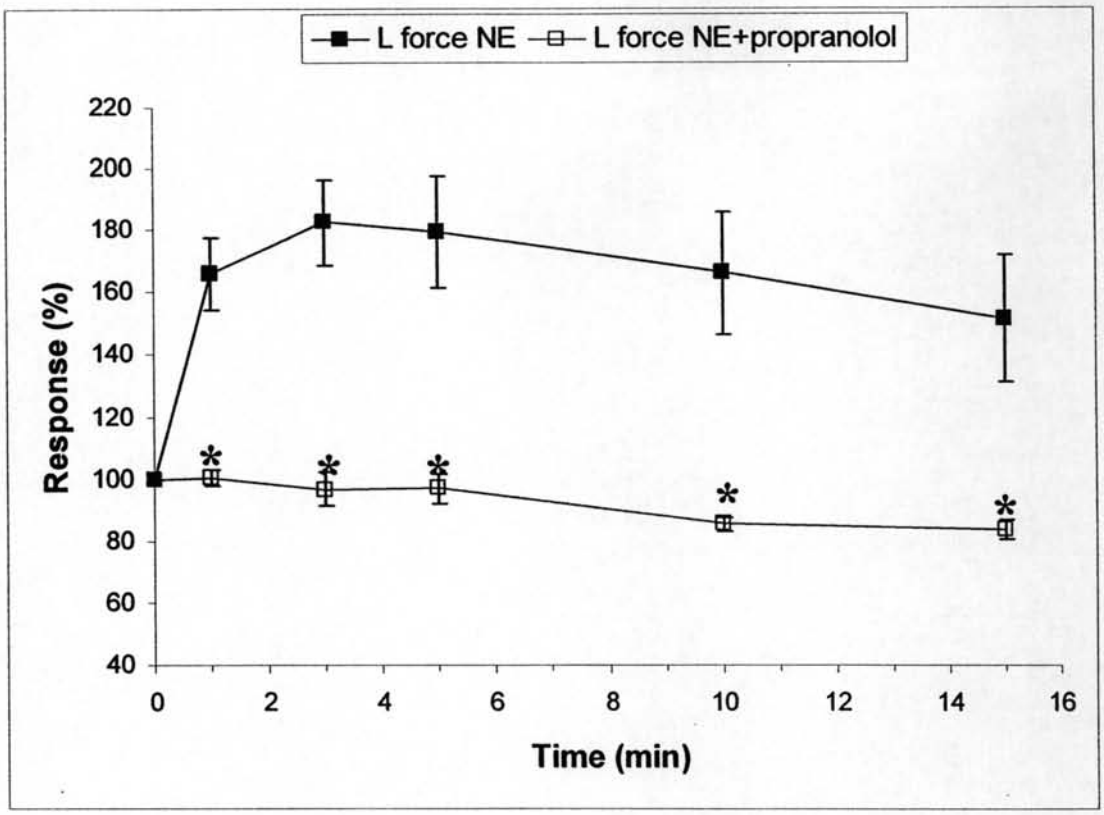
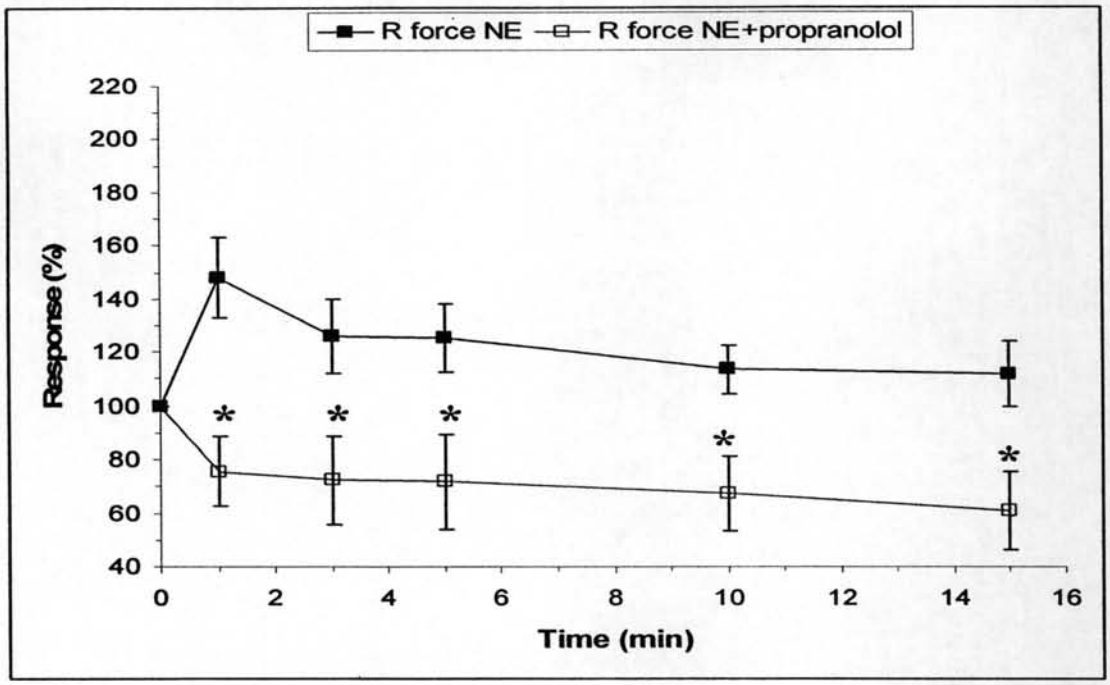


Figure 30 The inotropic response of NE (0.1 μM) on left atria in the presence of propranolol (10 μM), n=4 (mean ± SEM), *P<0.05, significant different from NE group

A) Right force



B) Right rate

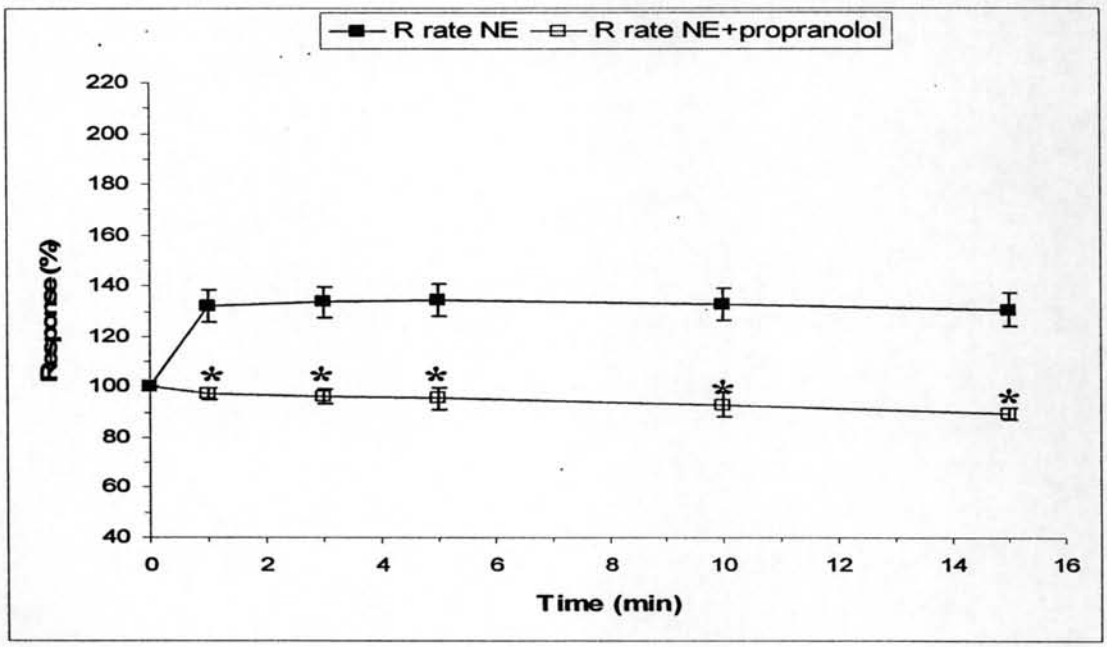


Figure 31 The inotropic and chronotropic response of NE (10 μM) on **(A)** force and **(B)** rate of contraction of right atria in the presence of propranolol (10 μM), n=4 (mean ± SEM), *P<0.05, significant different from NE group (unpaired *t*-test)

A. Left atria

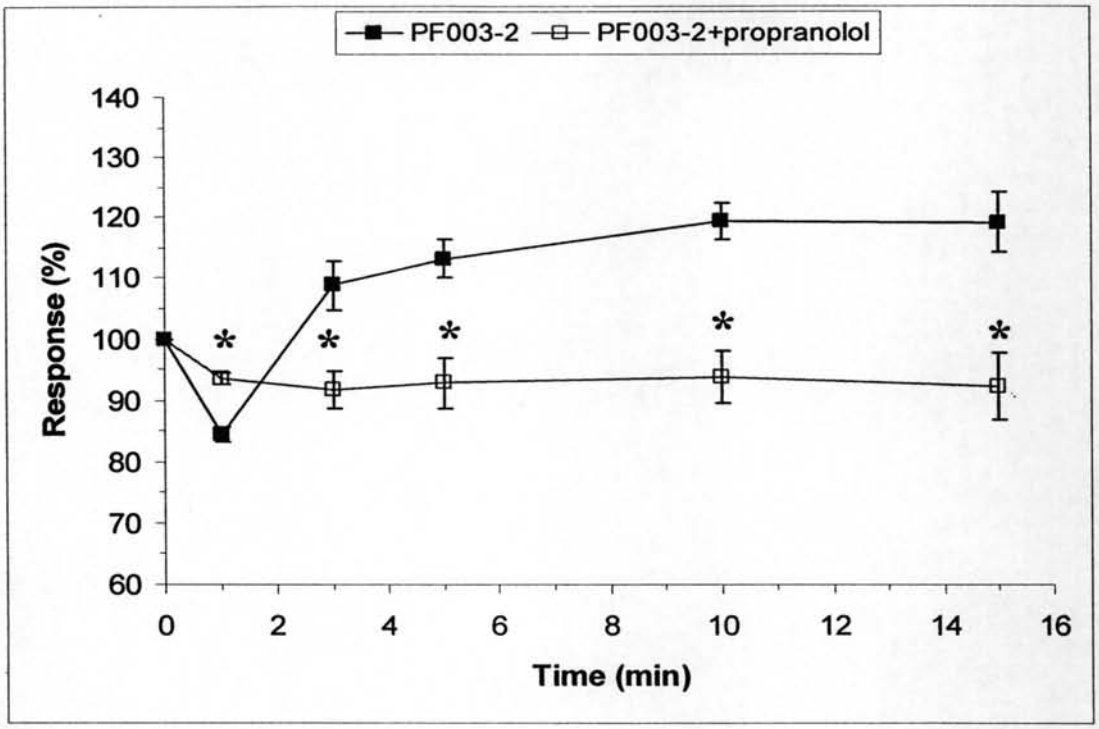
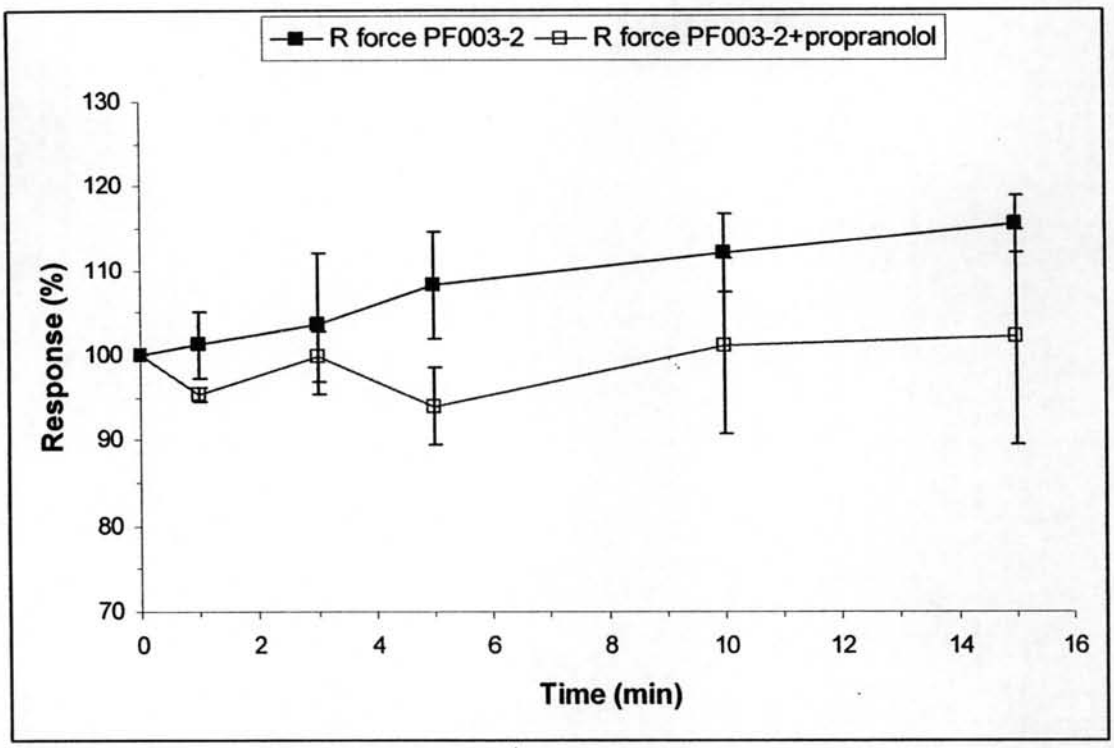


Figure 32 The inotropic response of PF003-2 (100 μg/ml) on the left atria in the presence of propranolol (10 μM), n=6 (Mean±SEM), *p<0.05, significantly different from PF003-2 group (unpaired *t*-test)

A). Right force



B) Right rate

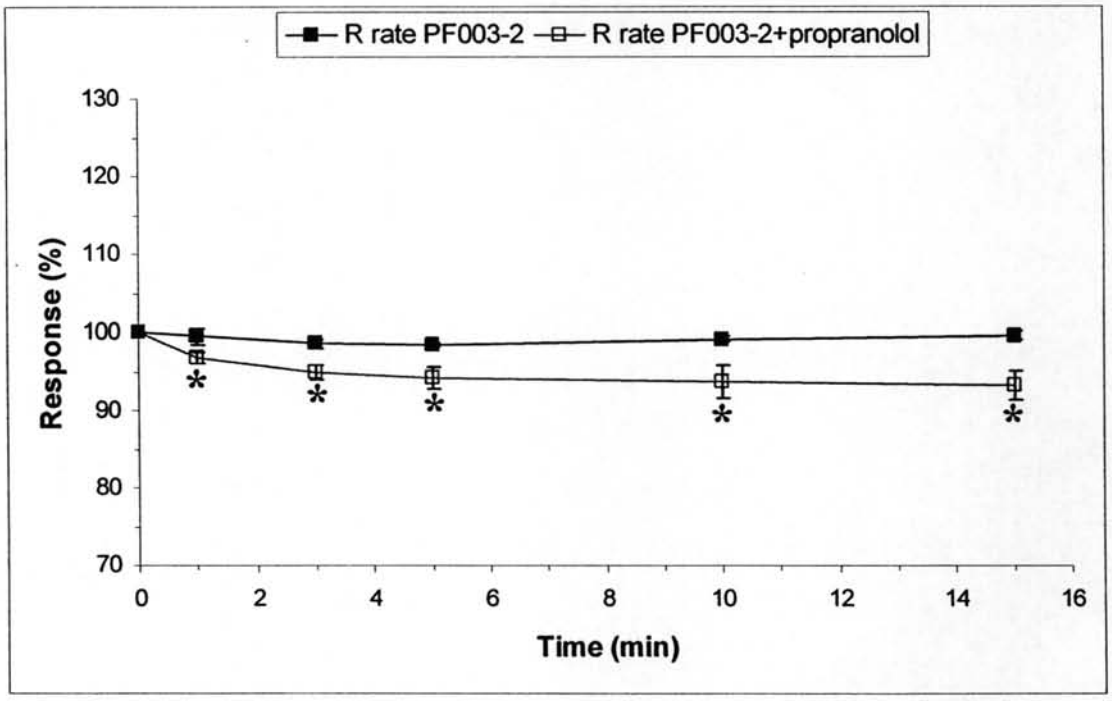


Figure 33 The A) inotropic and B) chronotropic response of PF003-2 (100 μg/ml) on the right atria in the presence of propranolol (10 μM), n=6 (Mean±SEM), *p<0.05, significantly different from PF003-2 group (unpaired t-test)

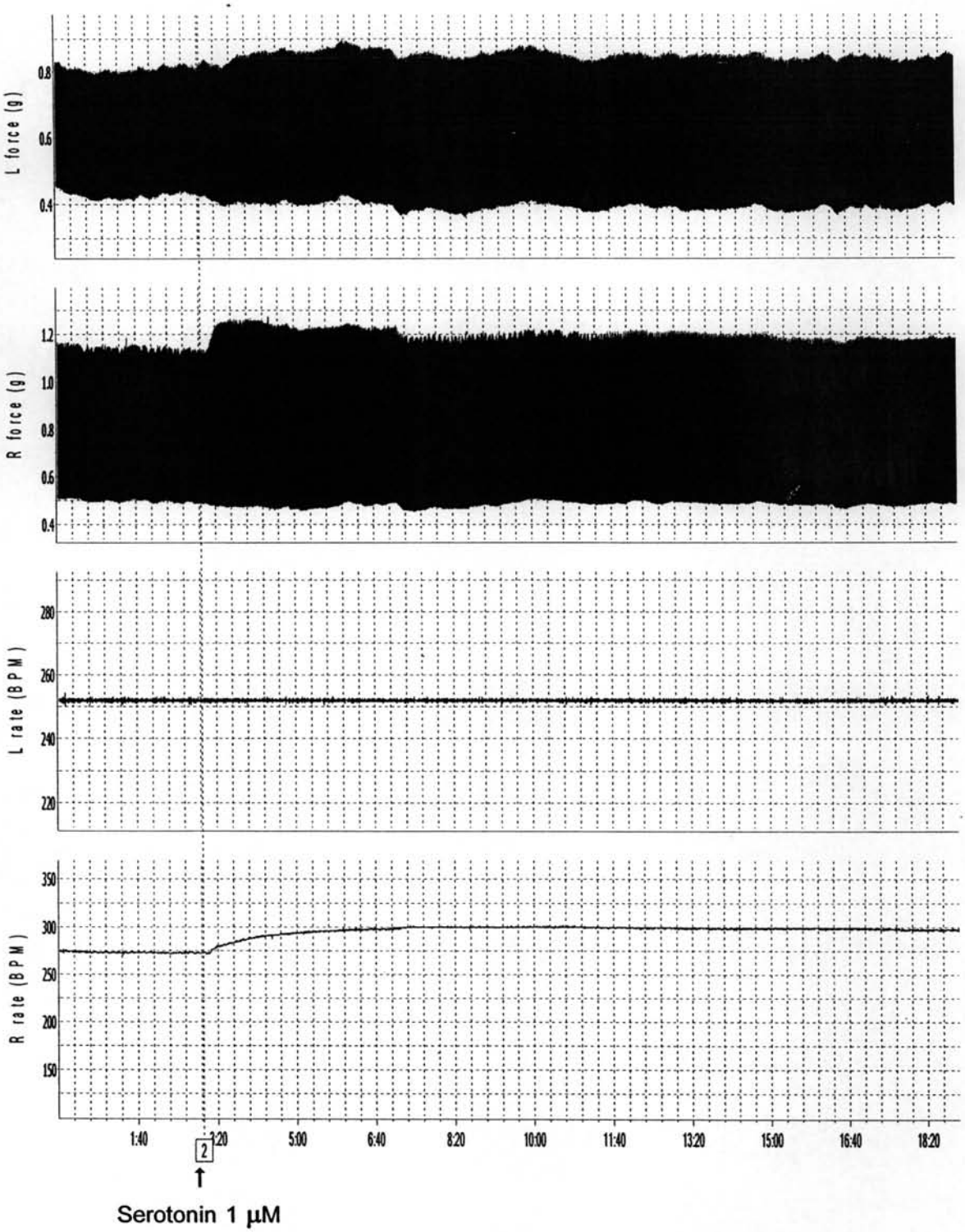


Figure 34 Chronotropic and inotropic response on the left and right atria in the presence of serotonin (1 µM)

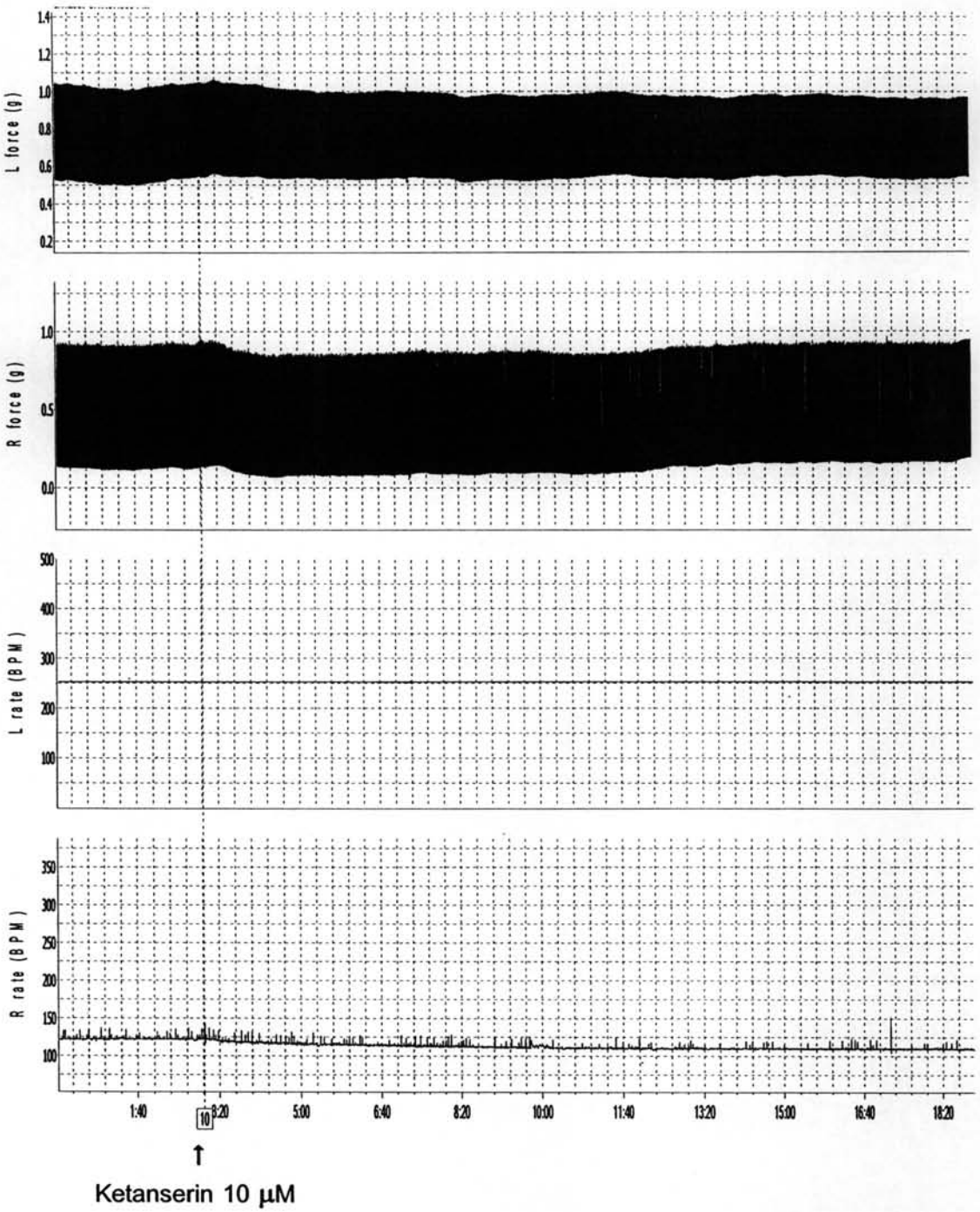


Figure 35 Chronotropic and inotropic response on the left and right atria in the presence of ketanserin (10 μM)

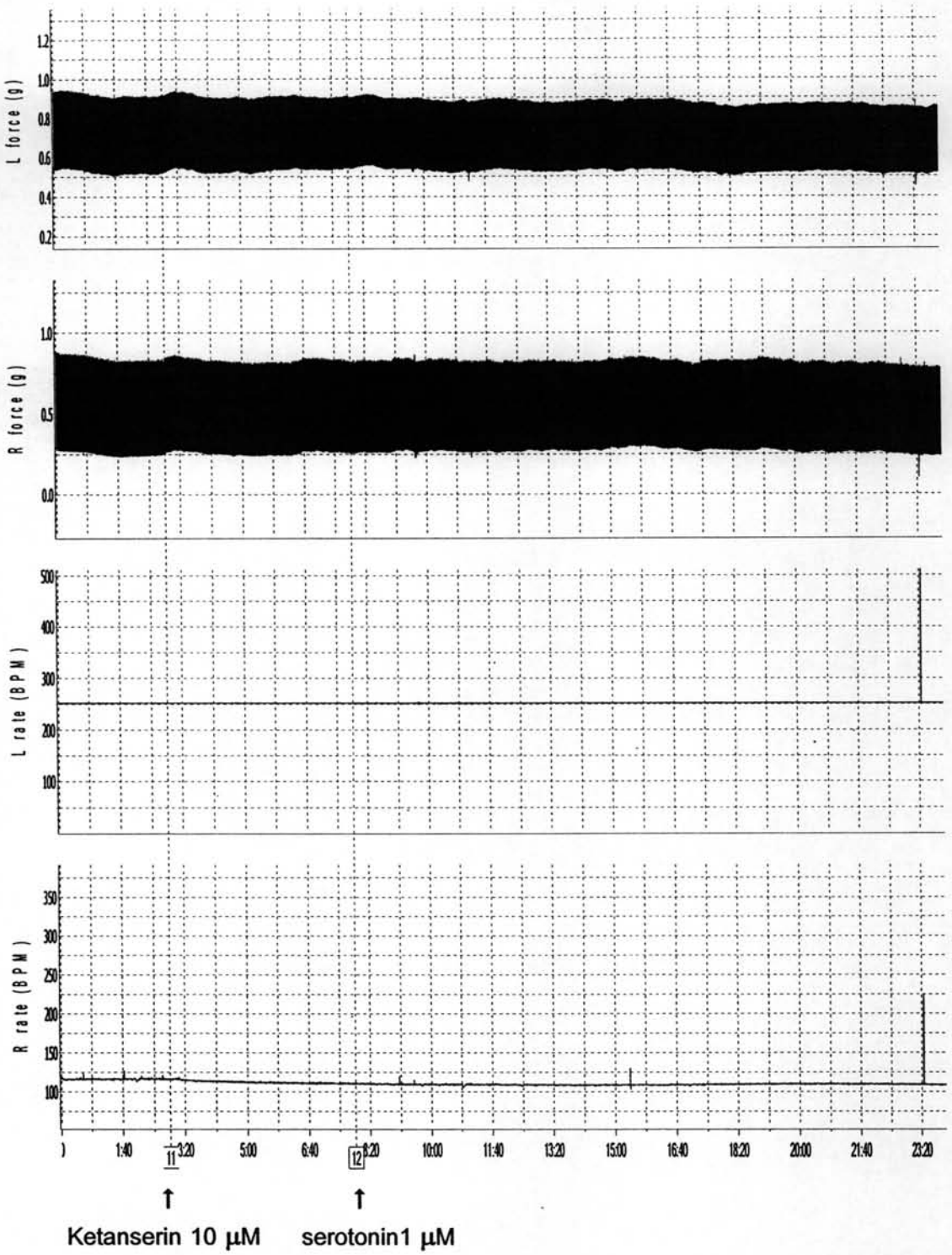


Figure 36 Chronotropic and inotropic response of serotonin (10 µM) on the left and right atria in the presence of ketanserin (1 µM)

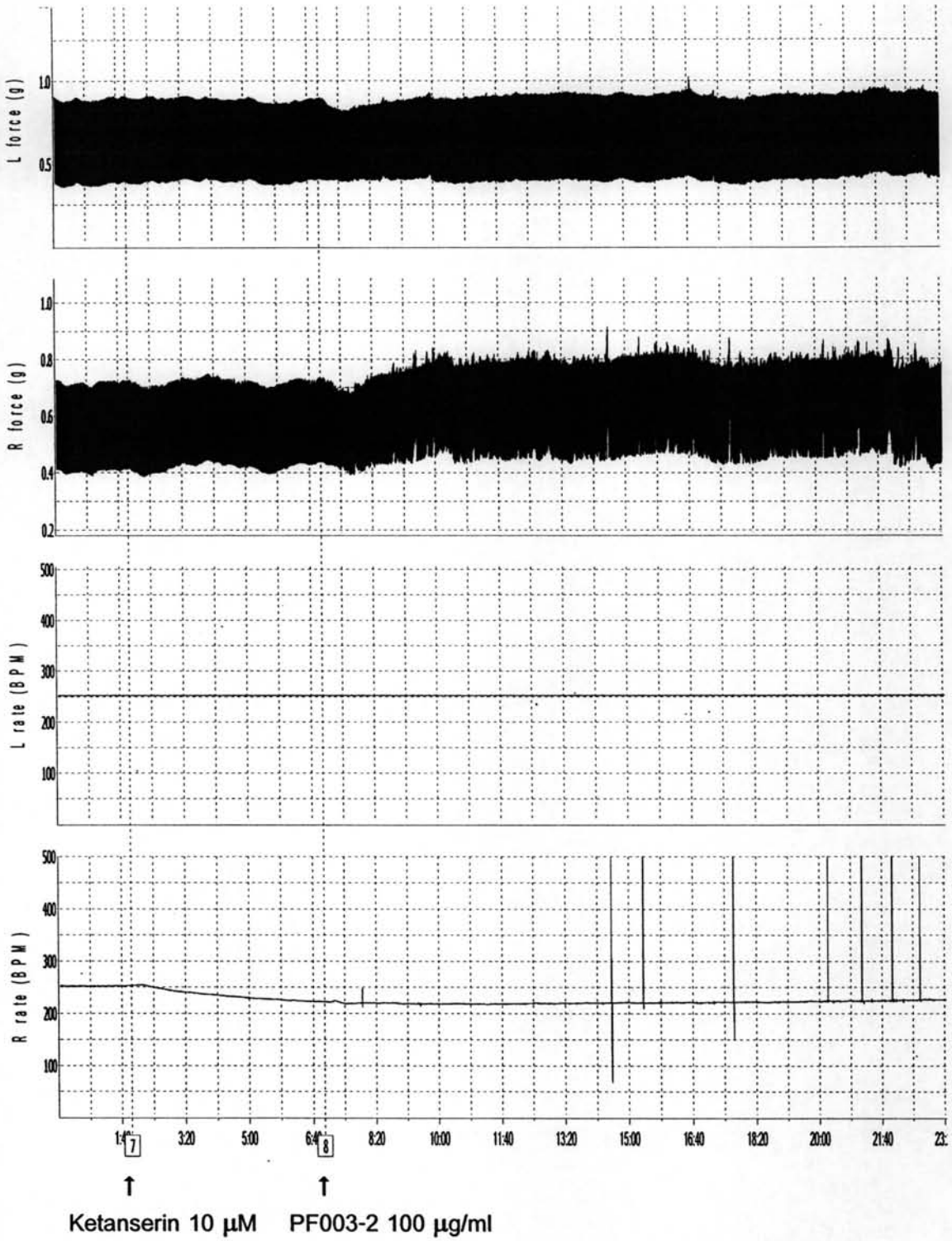
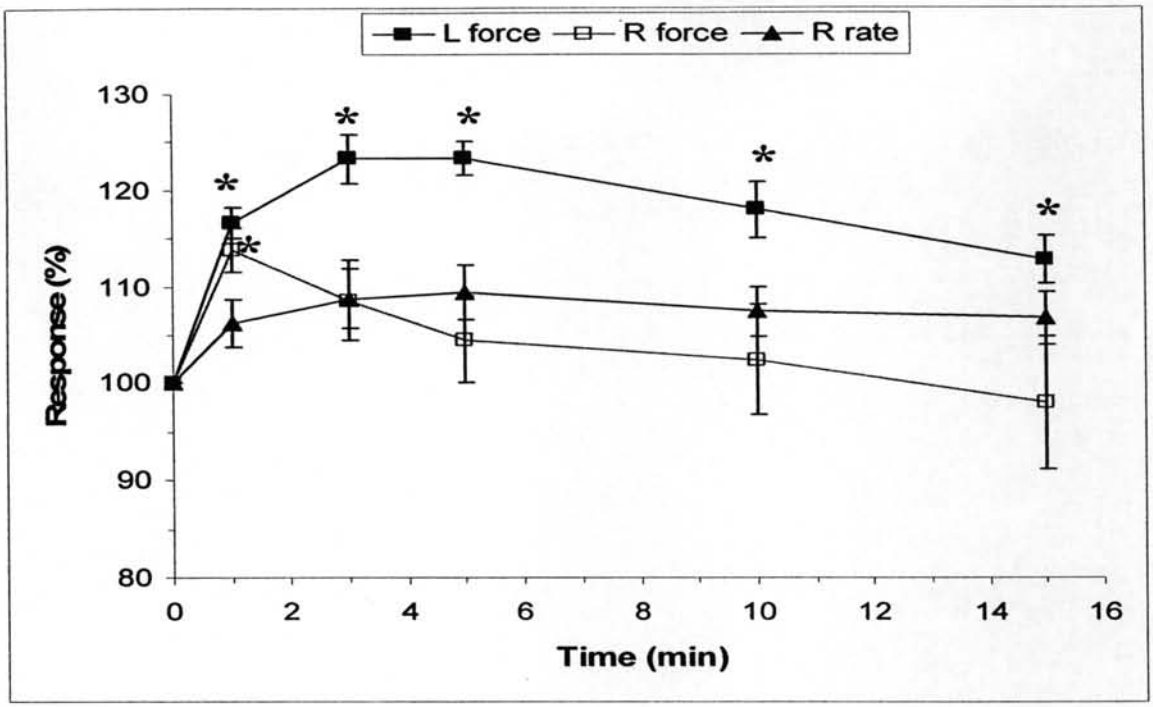


Figure 37 Chronotropic and inotropic response of PF003-2 (100 µg/ml) on the left and right atria in the presence of ketanserin (10 µM)

A. serotonin



B. Ketanserin

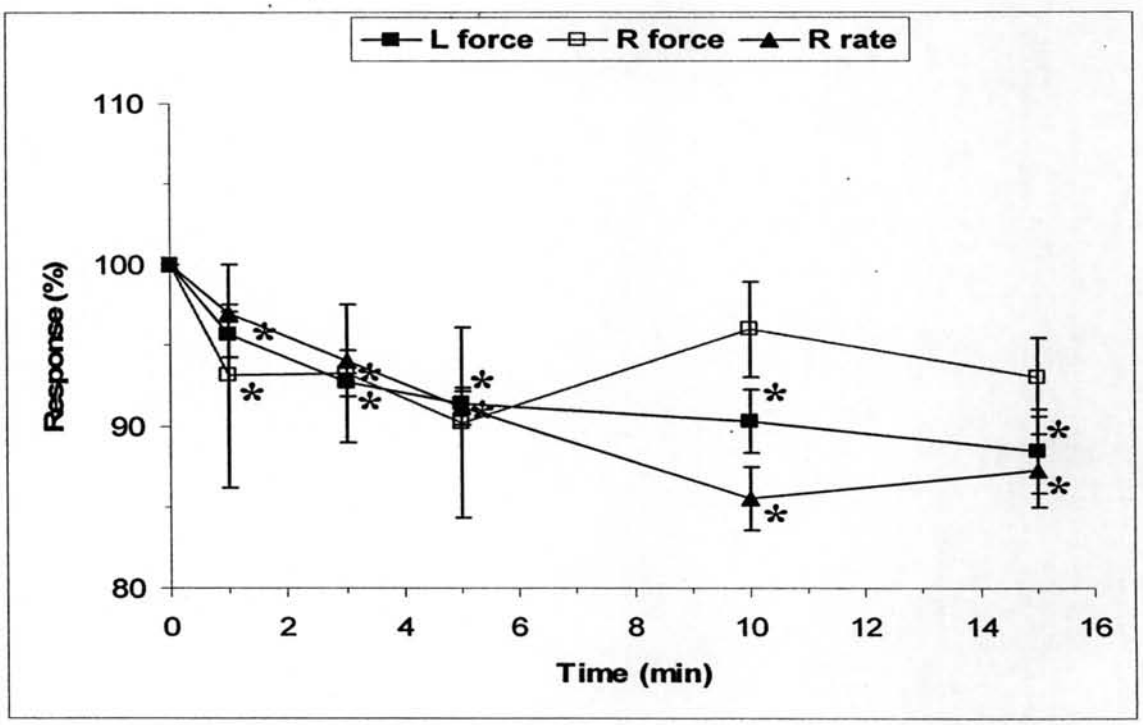


Figure 38 The chronotropic and inotropic reponse of **(A)** serotonin (1 μM) and **(B)** ketanserin (10μM) on the left and right atria, n=4 (mean ± SEM), *p<0.05, significantly different from PF003-2 group (paired t-test)

A. Left atria

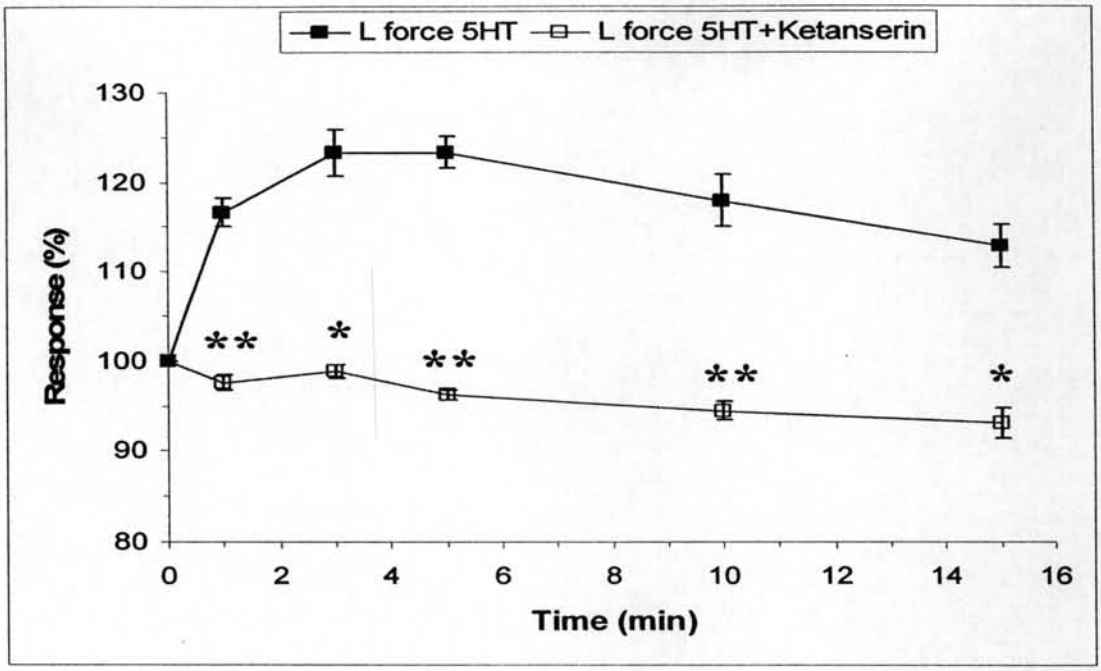
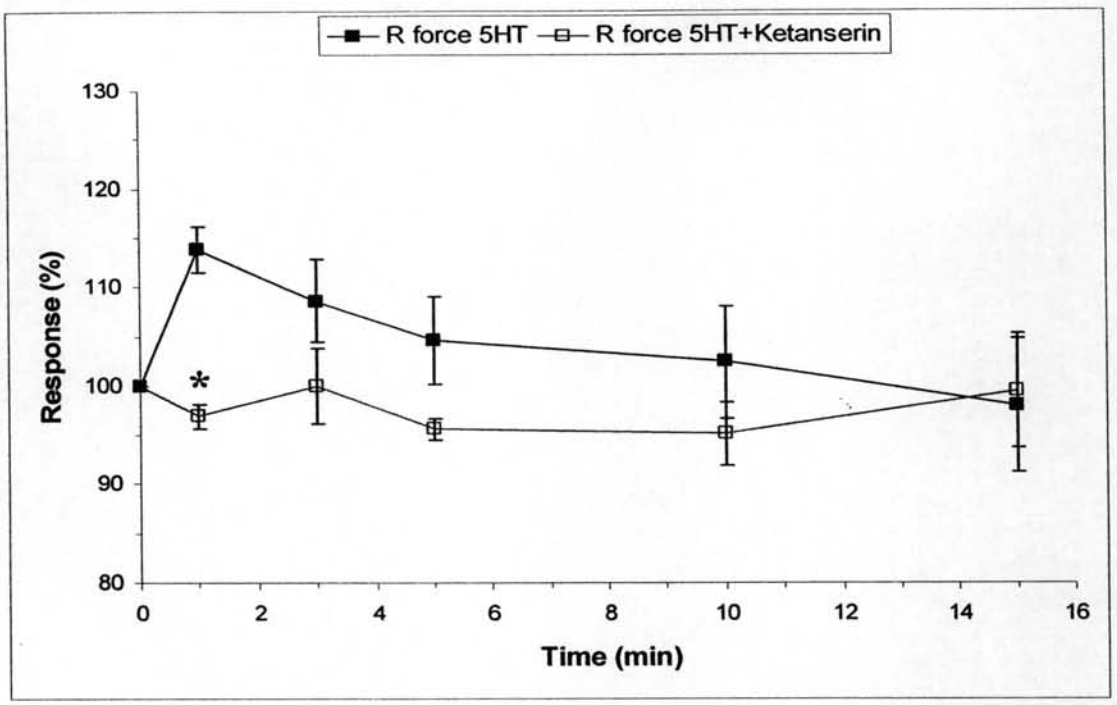


Figure 39 The inotropic response of serotonin (1 μM) on left atria in the presence of ketanserin (10 μM), n=4 (mean ± SEM) *p<0.05, **p<0.001 significant different from serotonin group (unpaired t-test)

A) Right force



B) Right rate

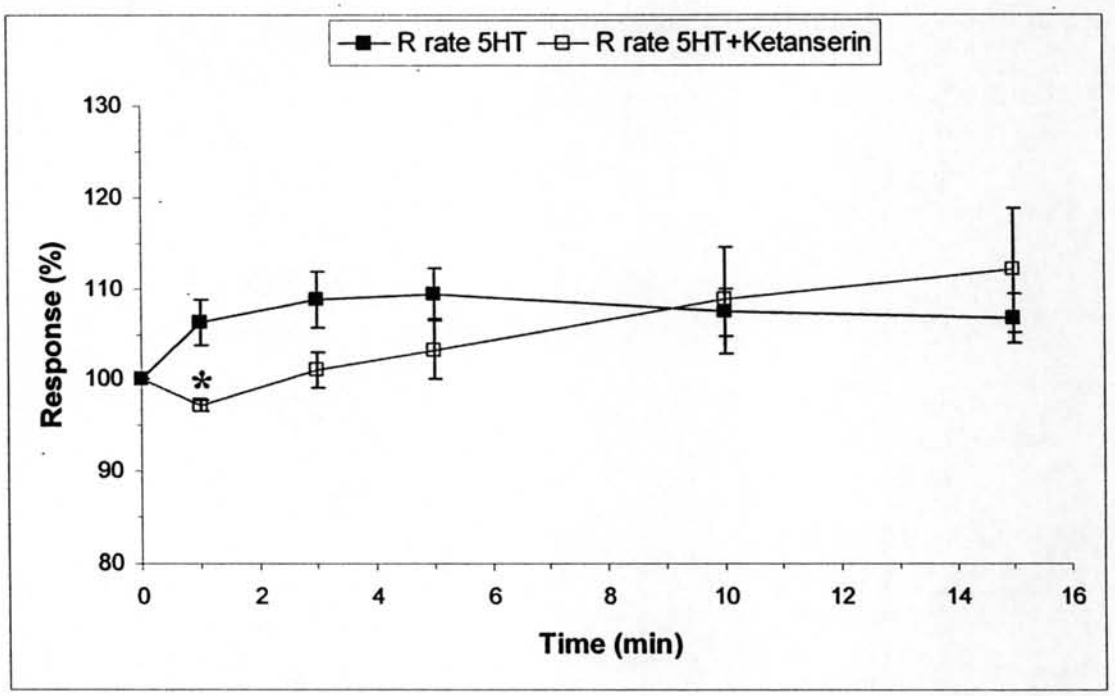


Figure 40 The **(A)** inotropic and **(B)** chronotropic response of serotonin (1 μM) on right atria in the presence of ketanserin (10 μM), n=4 (mean ± SEM) *$p<0.05$, **$p<0.001$ significant different from serotonin group (unpaired *t*-test)

A. Left atria

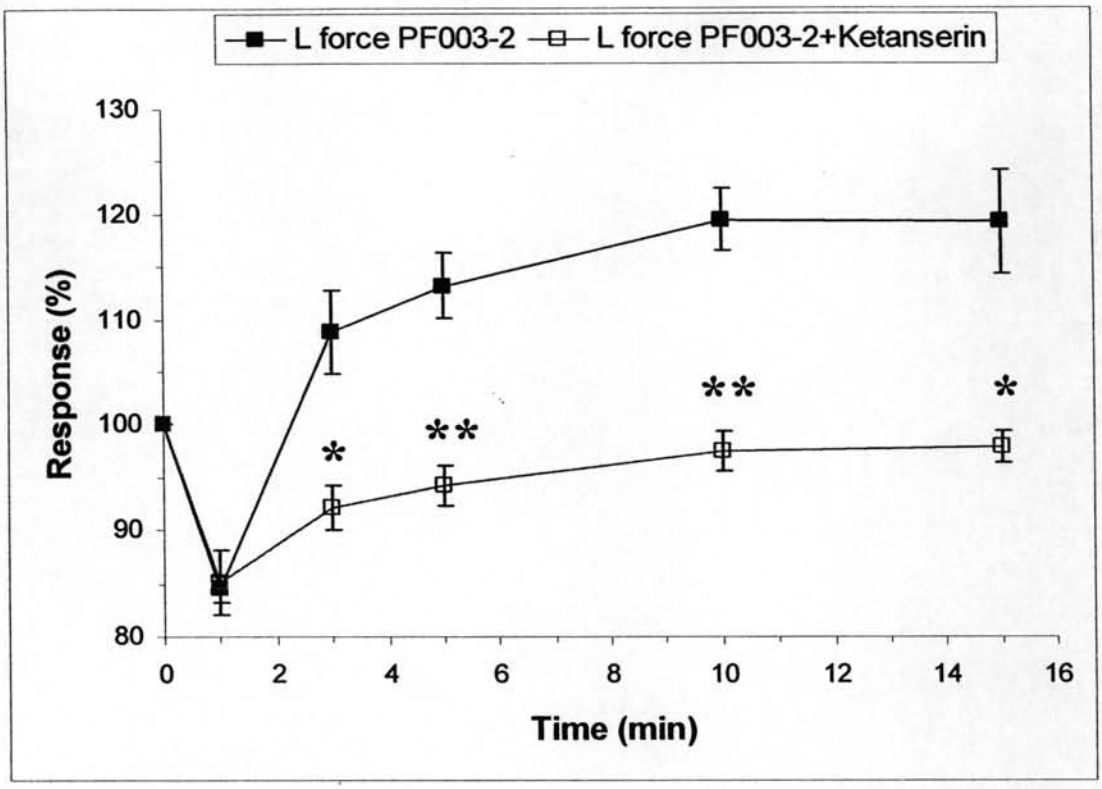
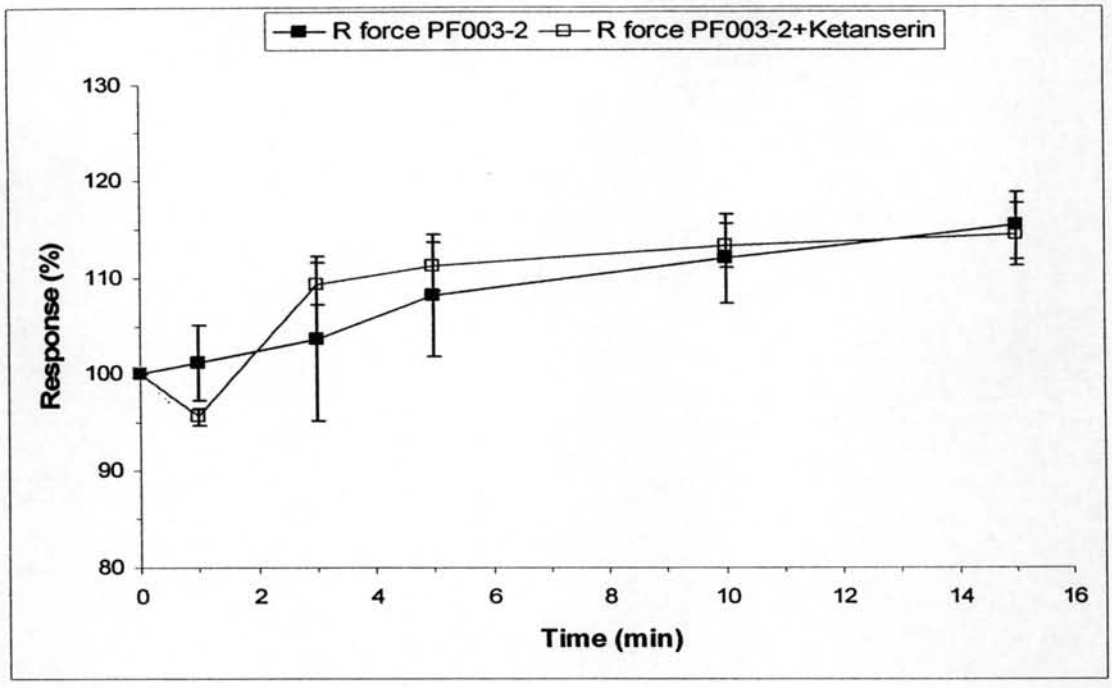


Figure 41 The inotropic response of PF003-2 (100 μg/ml) on the left atria in the presence of ketanserin (10 μM), n=6 (mean ± SEM) *p<0.05, **p<0.001 significantly different from serotonin group (unpaired t-test)

A) Right force



B) Right rate

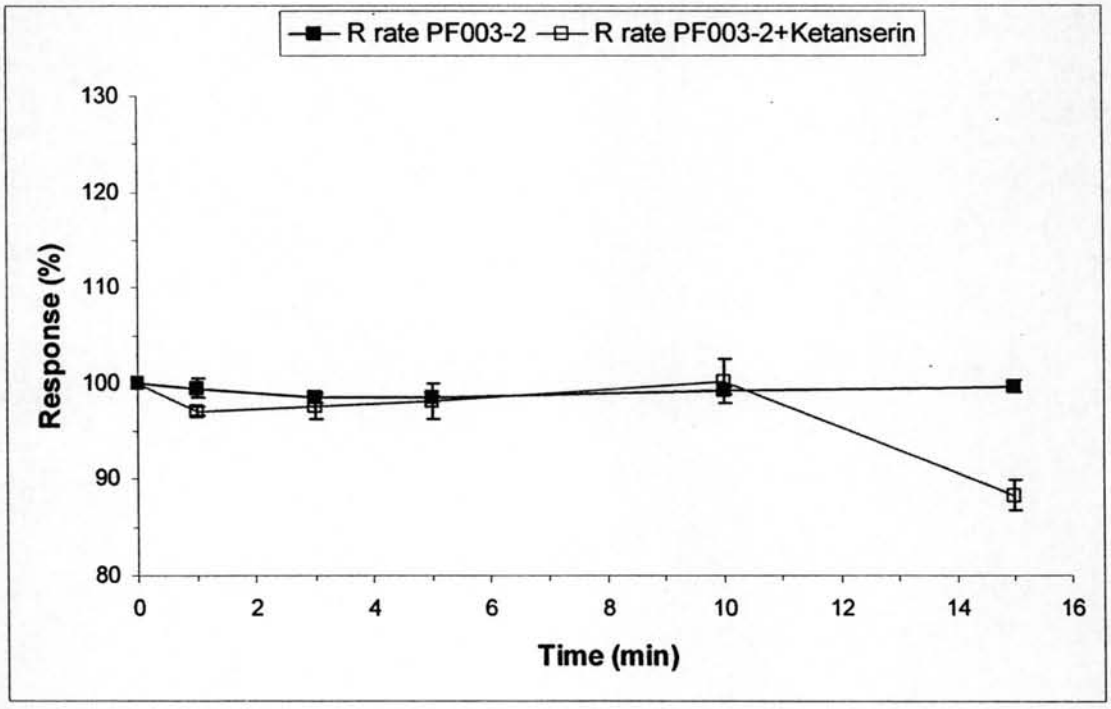


Figure 42 The **A)** inotropic and **B)** chronotropic response of PF003-2 (100 μg/ml) on the right atria in the presence of ketanserin (10 μM), n=6 (mean ± SEM) *P<0.05 significantly different from serotonin group (unpaired *t*-test)

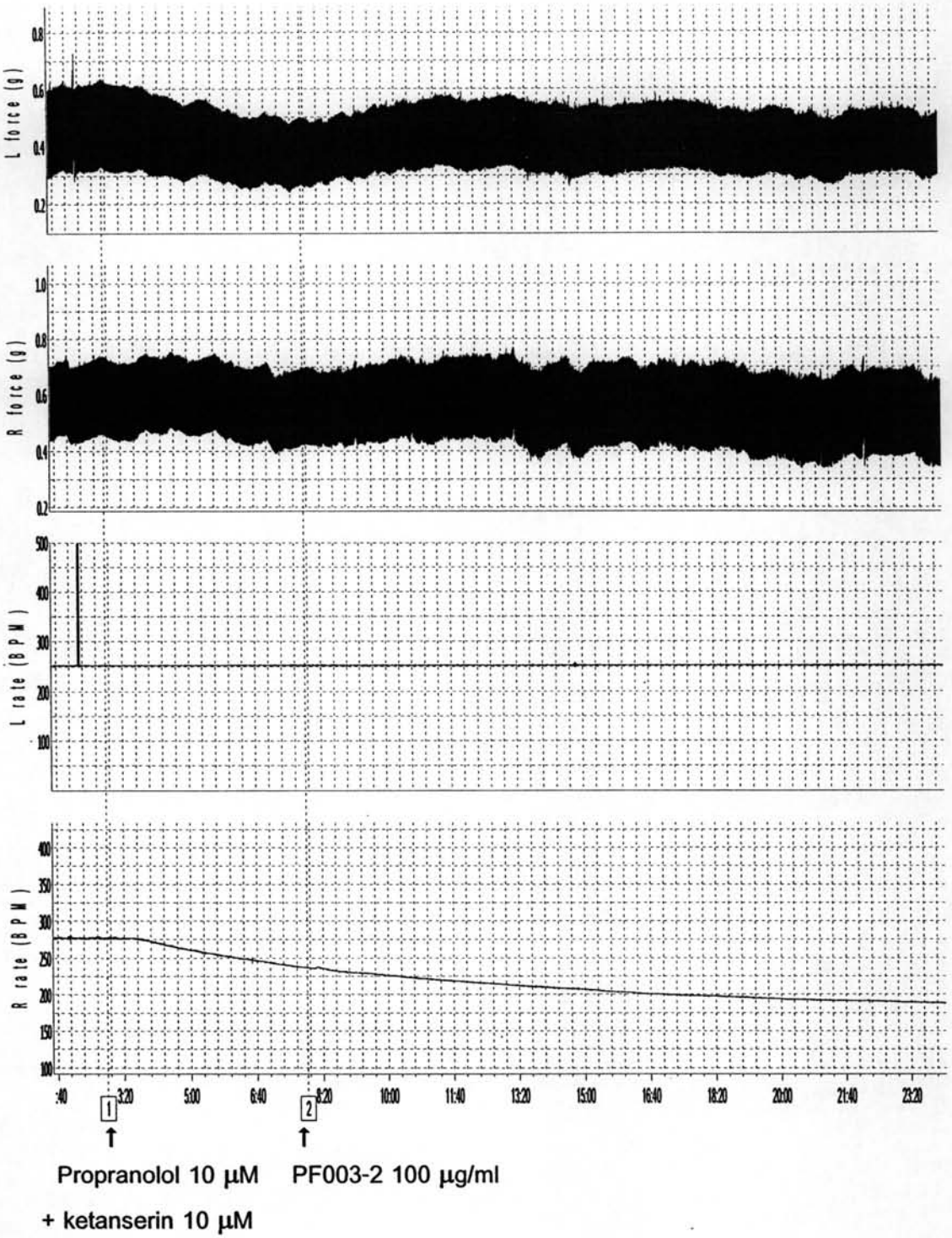


Figure 43 Chronotropic and inotropic response of PF003-2 (100 μg/ml) on the left and right atria in the presence of propranolol (10 μM) and ketanserin (10 μM)

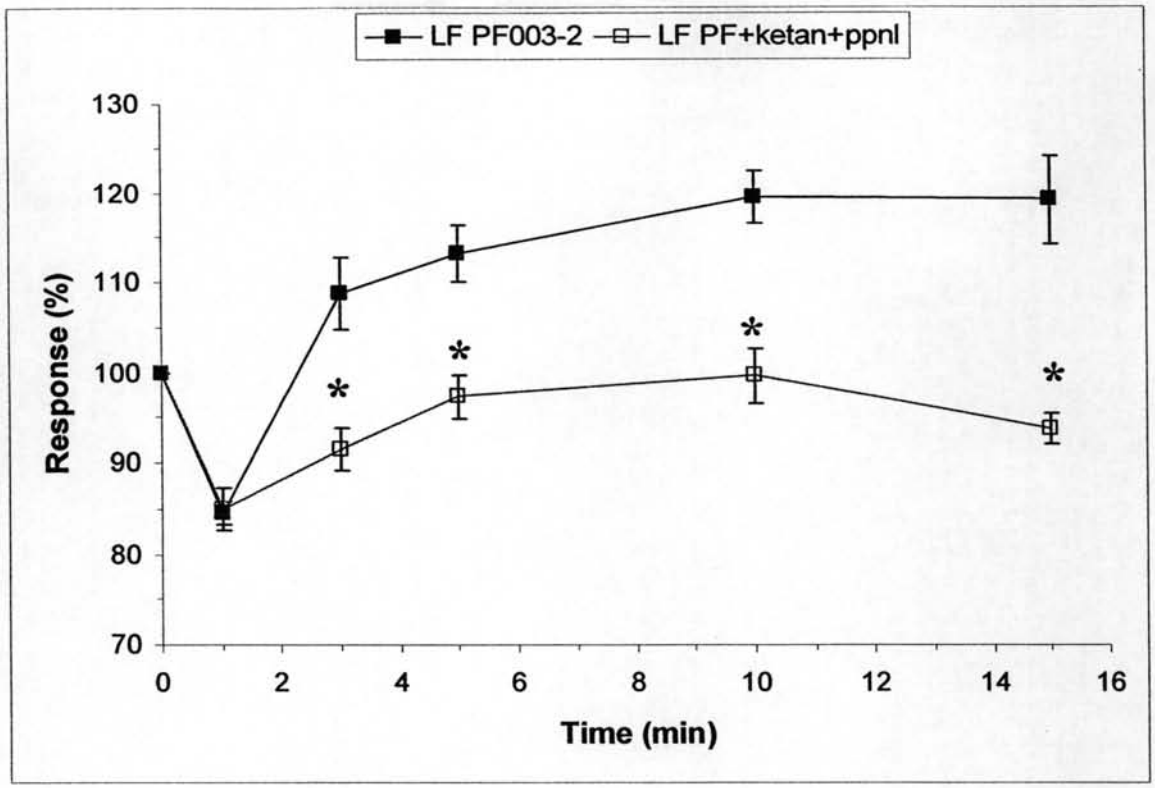
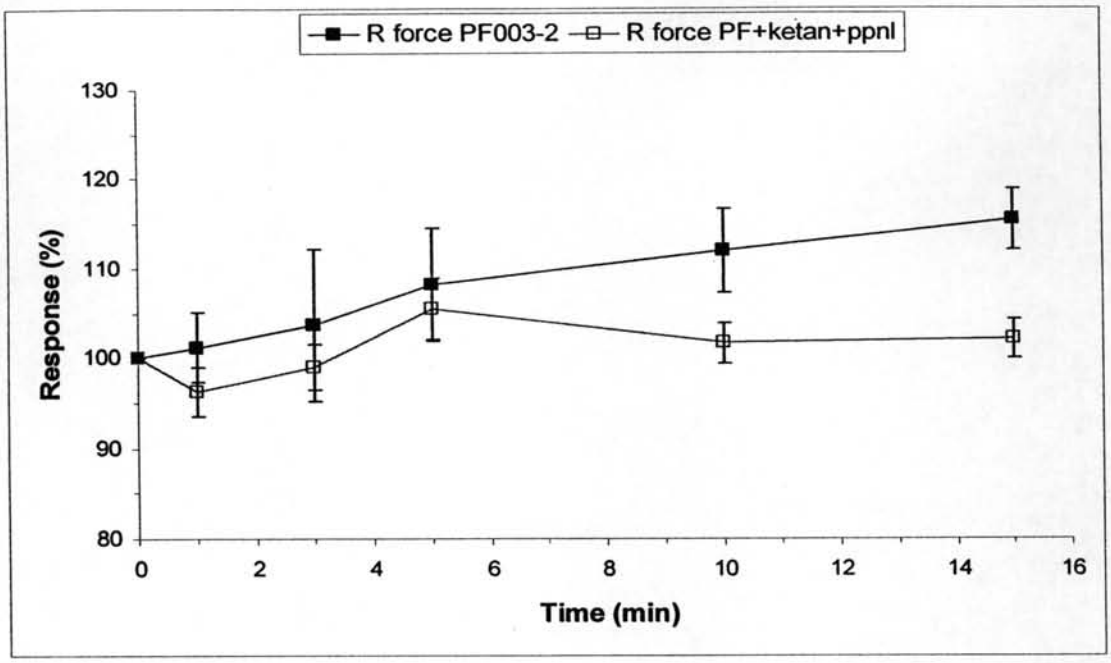


Figure 44 The inotropic response of PF003-2 (100 μg/ml) on the left atria in the presence of propranolol(10 μM) and ketanserin (10 μM), n=5 (mean ± SEM) *P<0.05 significant different from serotonin group (unpaired *t*-test)

A) Right force



B) Right rate

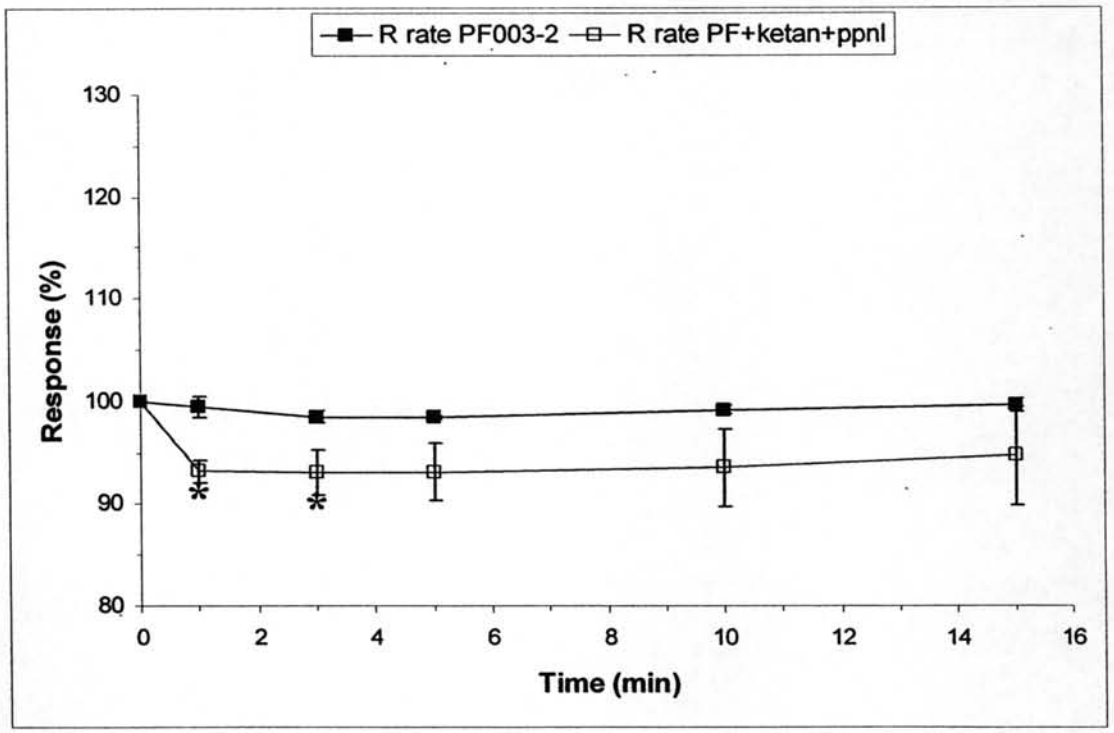


Figure 45 The A) inotropic and B) chronotropic response of PF003-2 (100 μg/ml) on the right atria in the presence of propranolol(10 μM) and ketanserin (10 μM), n=5 (mean ± SEM) *P<0.05 significant different from serotonin group (unpaired *t*-test)

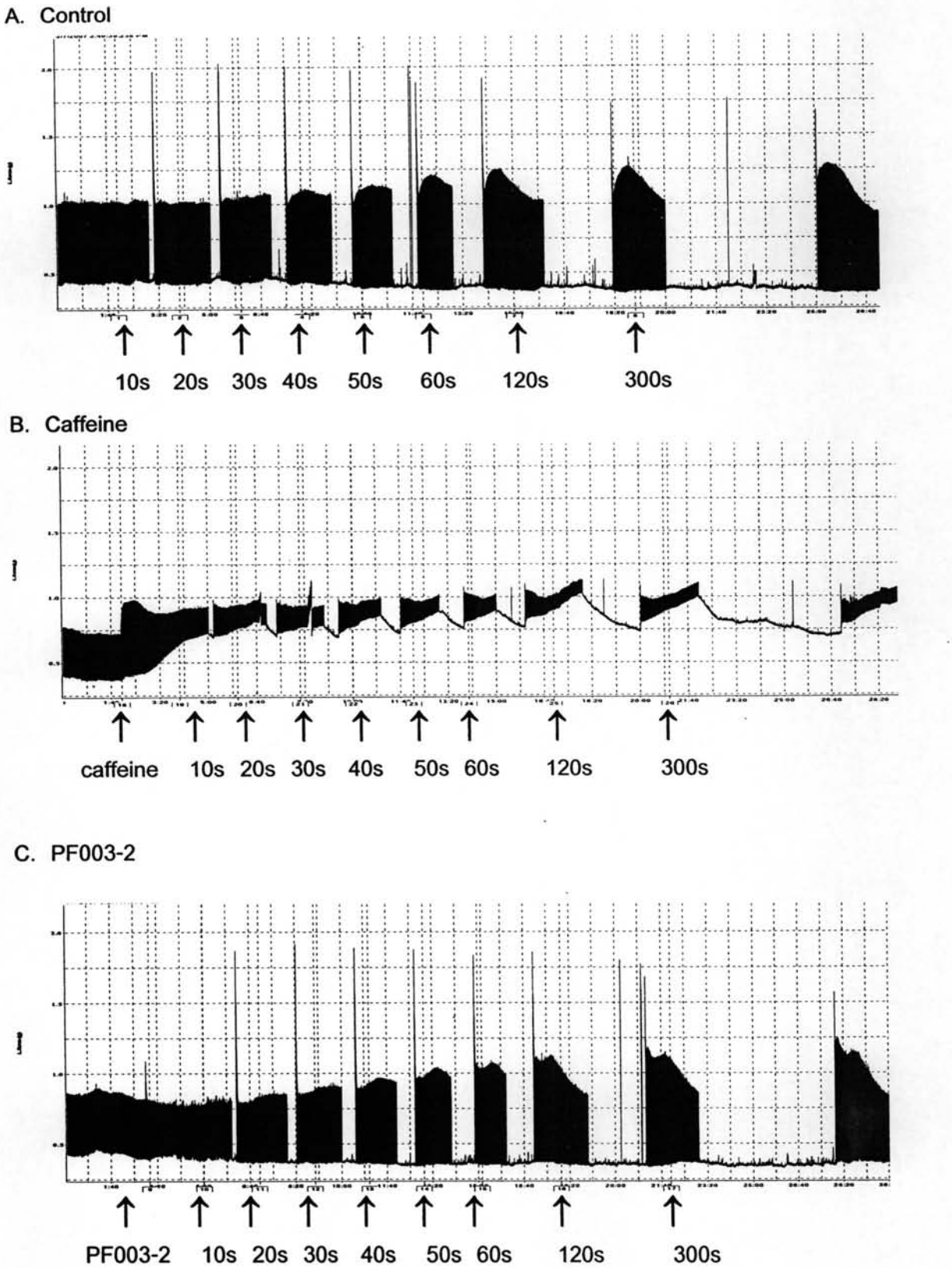


Figure 46 A representative example on inotropic response of (A) control, (B) Caffeine and (C) PF003-2 on the rest interval of the range 10 to 300 seconds on the left atria

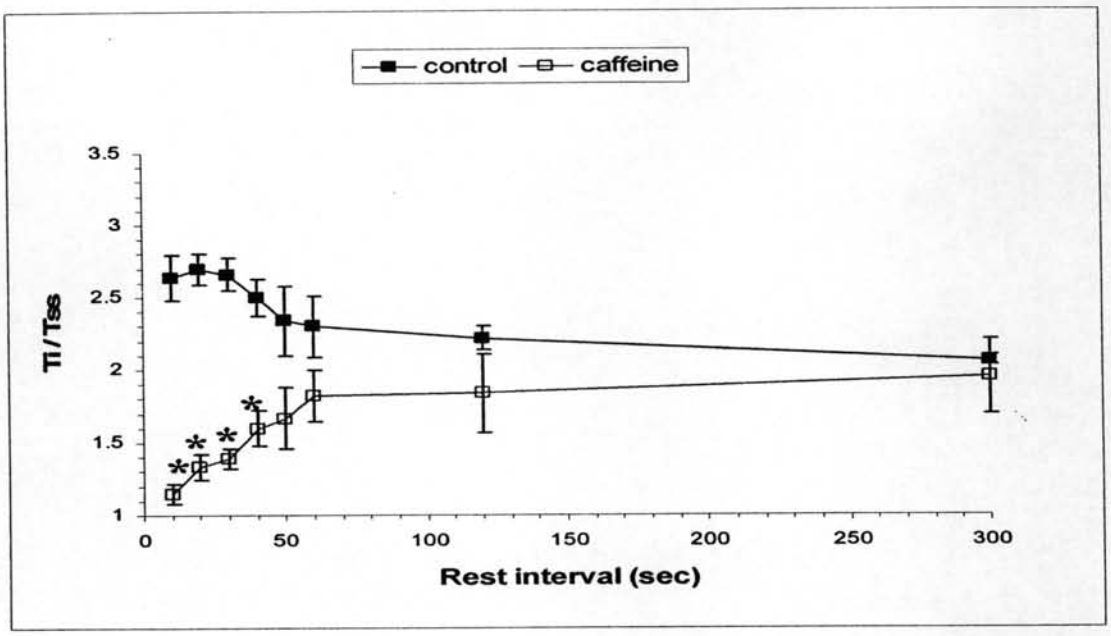


Figure 47 Effect of caffeine on the relationships between the Ti / Tss and the rest interval of range 10 to 300 seconds, n=4 (mean ± SEM), *P<0.05 significantly different from control group (paired *t*-test)

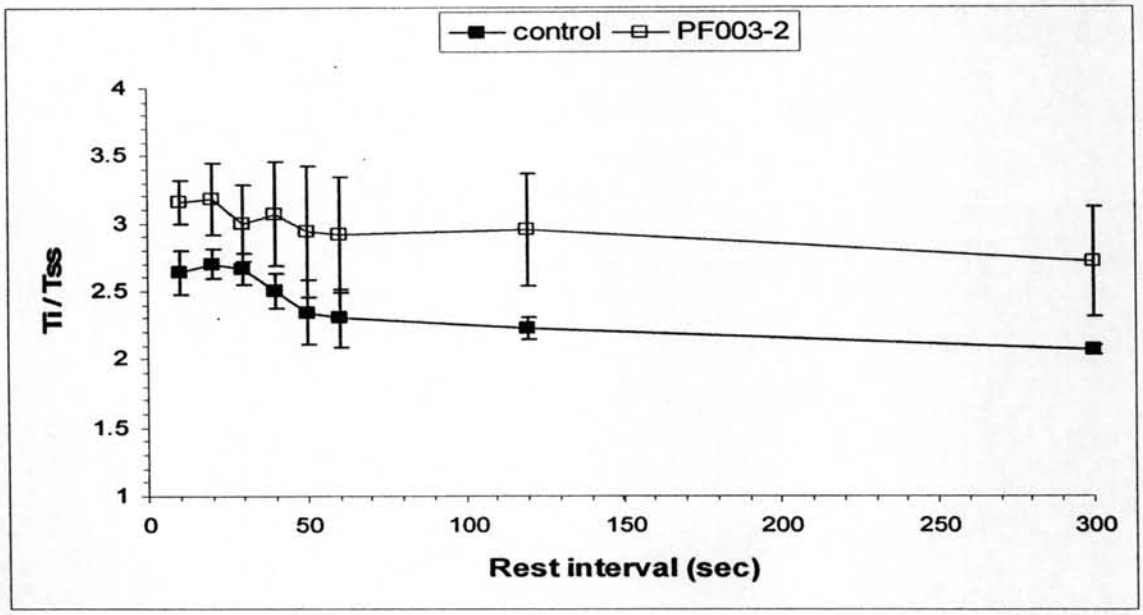


Figure 48 Effect of PF003-2 on the relationships between the Ti / Tss and the rest interval of range 10 to 300 seconds, n=4 (mean ± SEM) *P<0.05 significantly different from control group (paired *t*-test)

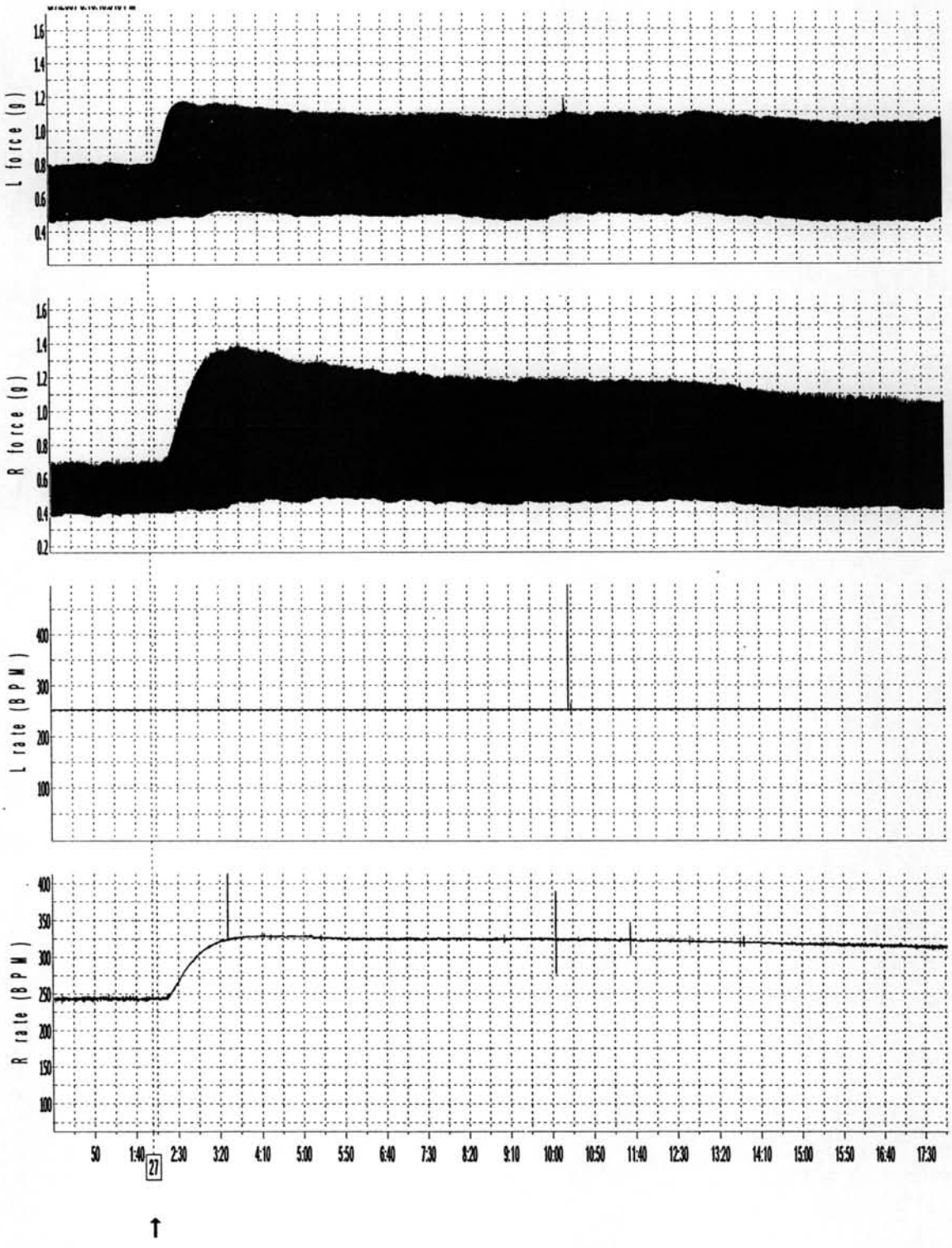


Figure 49 Chronotropic and inotropic response on the left and right atria in the presence of tyramine (10 µM)

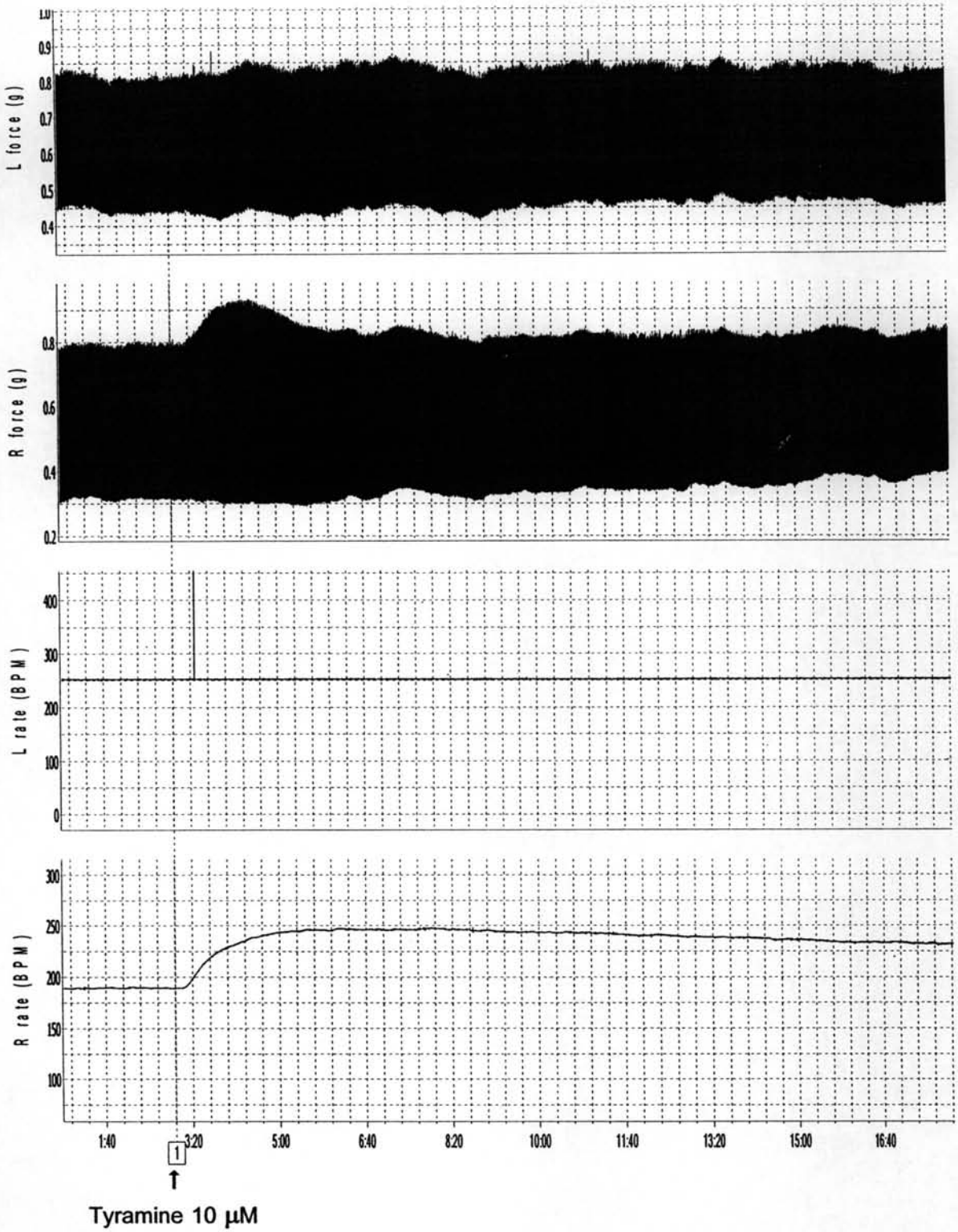


Figure 50 Chronotropic and inotropic response of tyramine (10 μM) on the left and right atria in rats pretreated with reserpine (5 mg/kg, i.p. for 2 days)

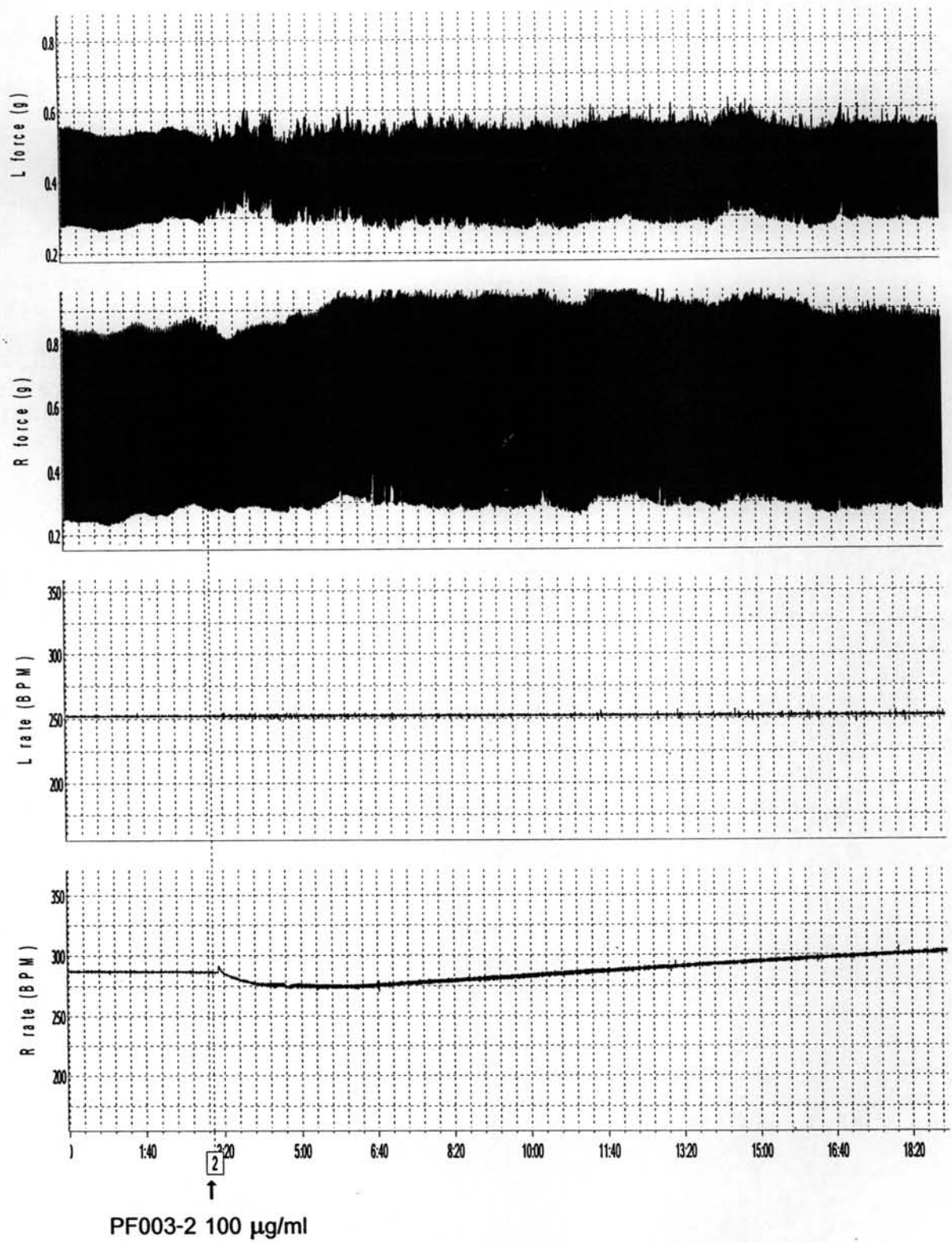


Figure 51 Chronotropic and inotropic response of PF003-2 (100 µg/ml) on the left and right atria in rats pretreated with reserpine (5 mg/kg, i.p. for 2 days)

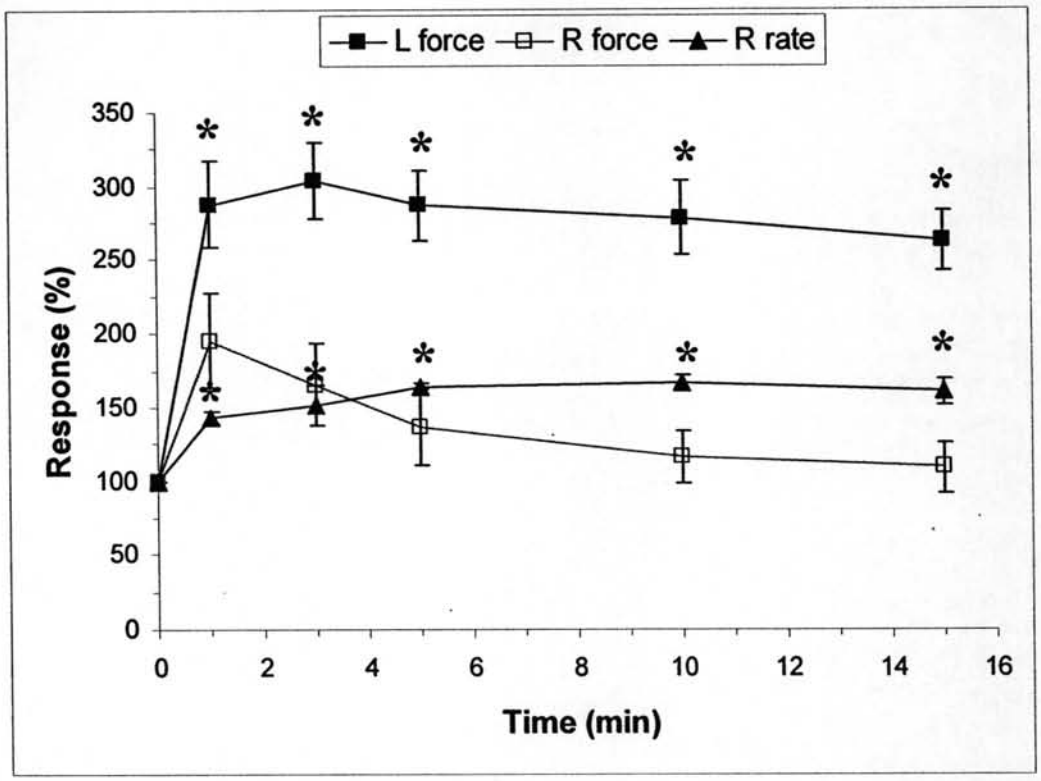
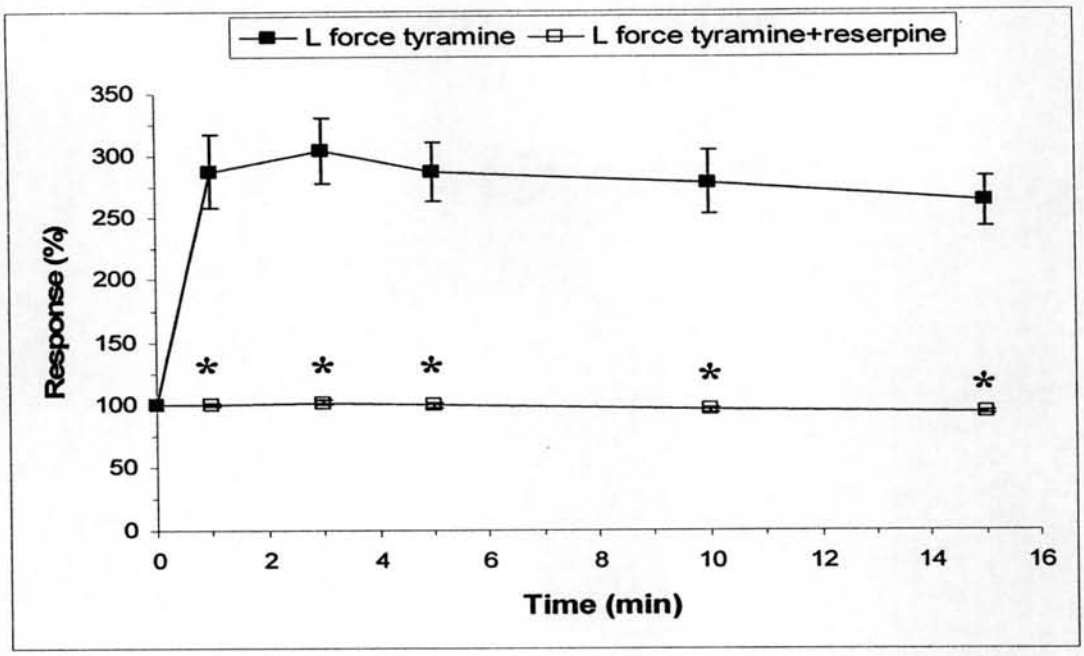


Figure 52 The chronotropic and inotropic response of tyramine (10 μM)on the left and right atria in normal rat, n=3 (mean ± SEM), *P<0.05 significantly different from response at o min (paired *t*-test)

A. Left atria



B. Right atria

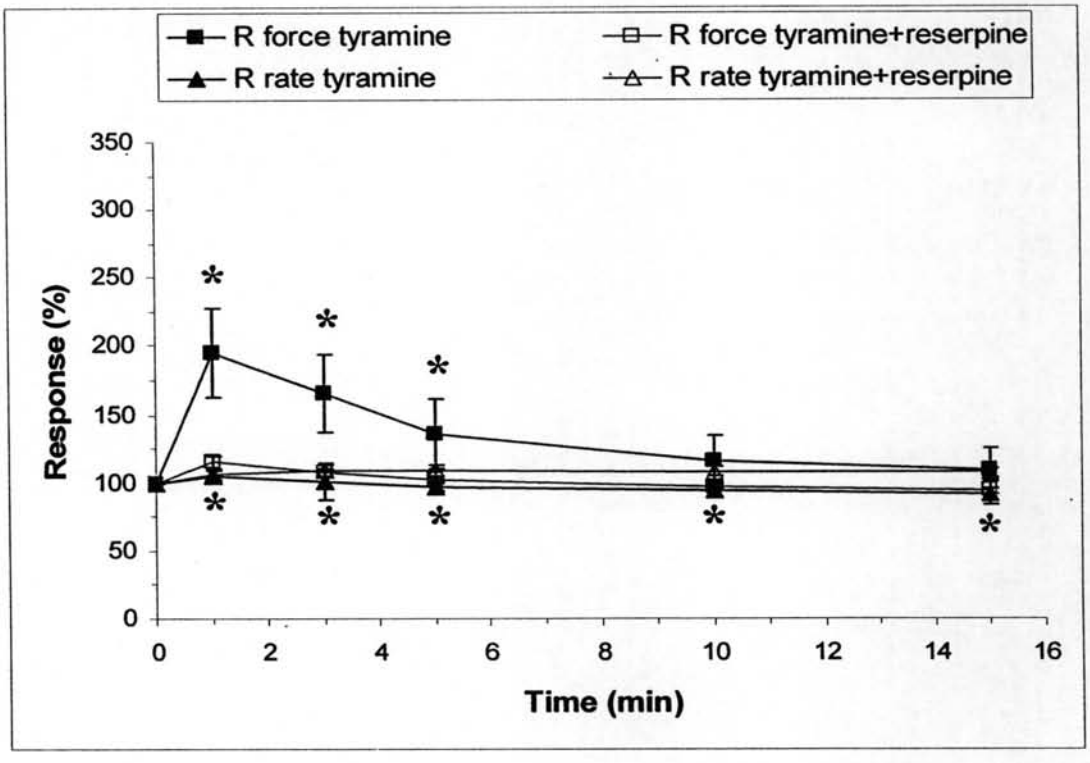
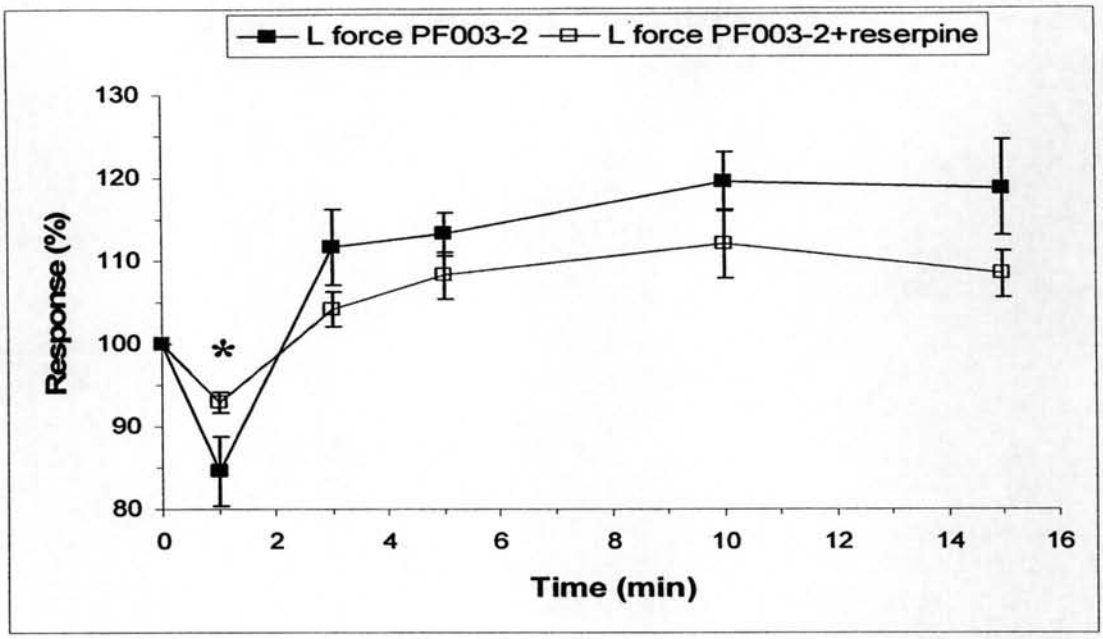


Figure 53 The chronotropic and inotropic reponse of tyramine (10 μM)on **(A)** left and **(B)** right atria in reserpinzed rat (5 mg/kg, i.p. for 2 days), n=7 (mean ± SEM), *P<0.05, significantly different from tyramine group in normal rats (unpaired *t*-test)

A. Left atria



B. Right atria

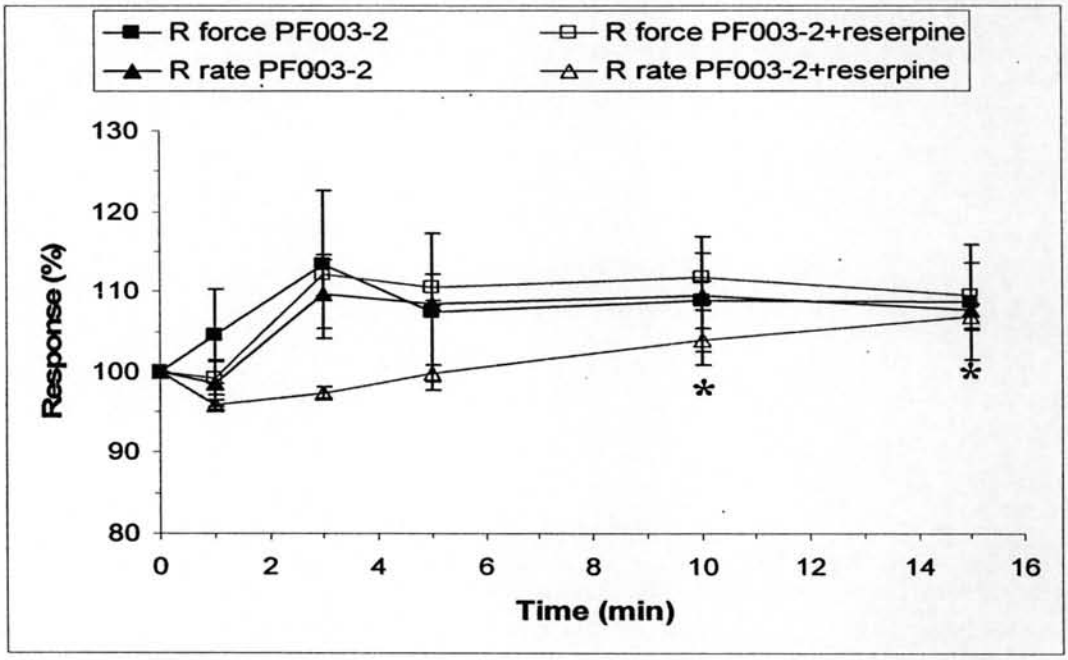


Figure 54 The chronotropic and inotropic reponse of PF003-2 (100 μg/ml) on **(A)** left and **(B)** right atria in reserpinzed rat (5 mg/kg, i.p. for 2 days), n=7 (mean ± SEM) *P<0.05, significantly different from tyramine group in normal rats (unpaired *t*-test)

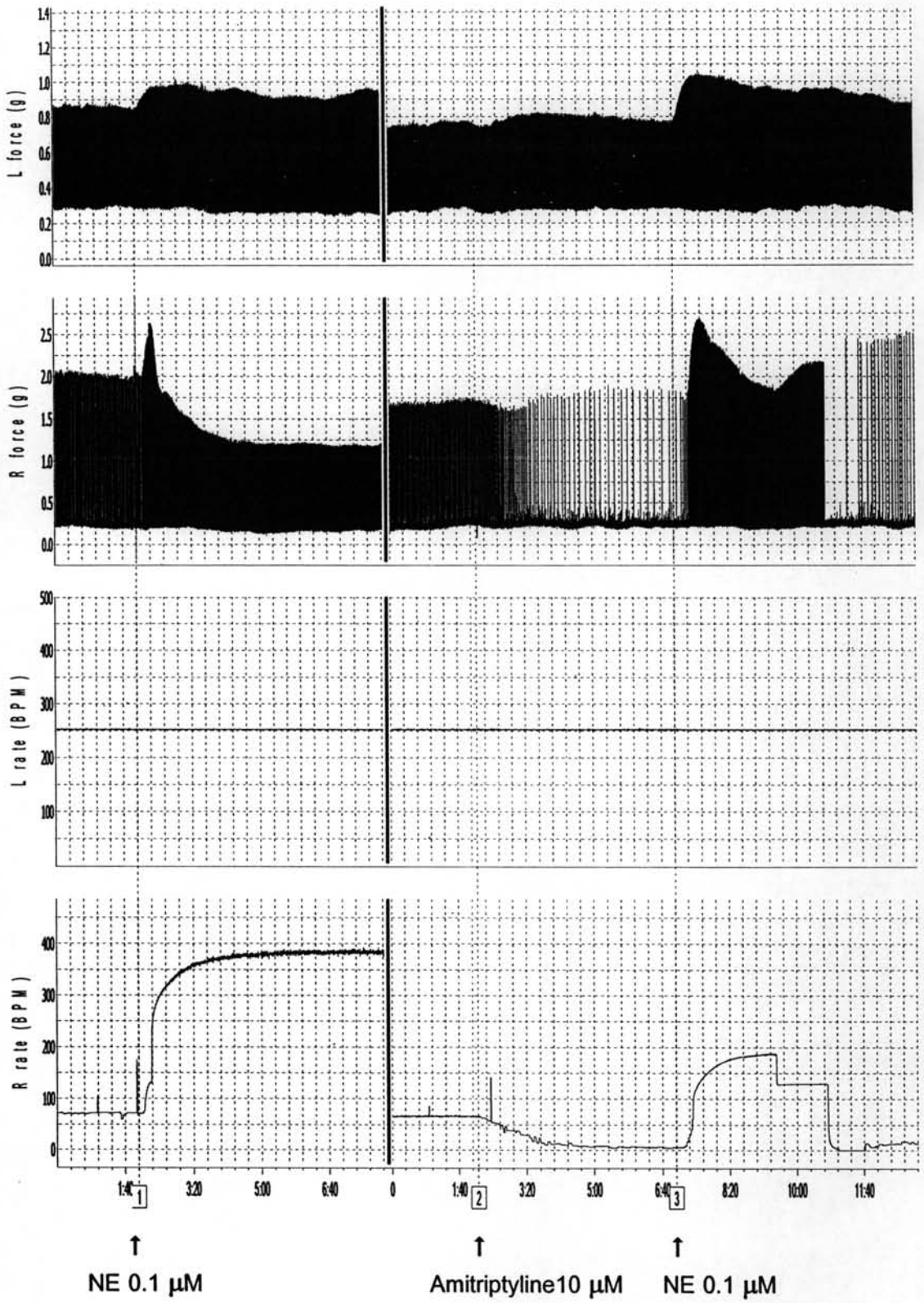


Figure 55 The chronotropic and inotropic reponse of NE (0.1 μM) and NE (0.1 μM) in presence of amitriptyline (10 μM) on the left and right atria

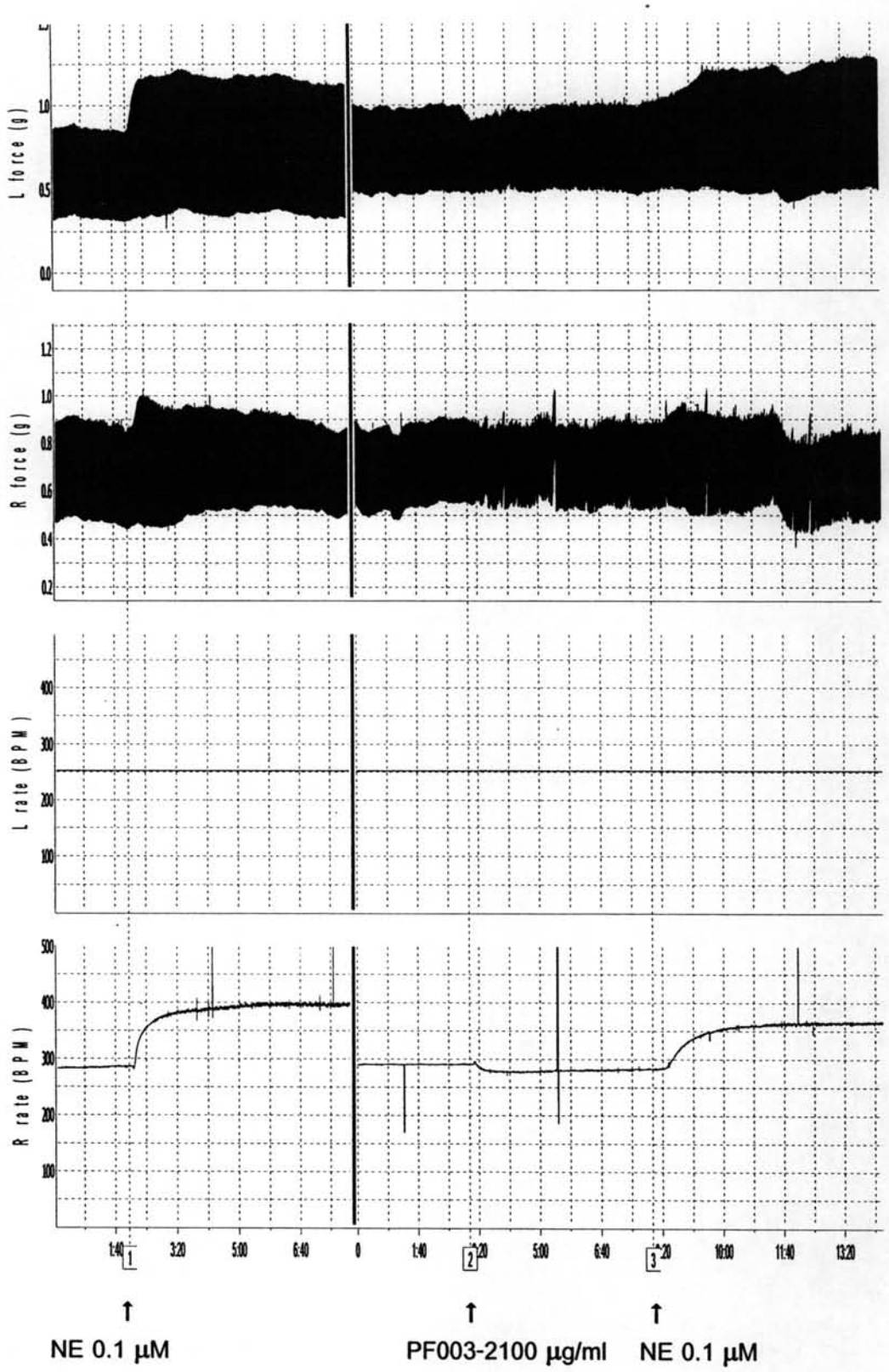


Figure 56 The chronotropic and inotropic reponse of NE (0.1 μM) and NE (0.1 μM) in presence of PF003-2 (100 μg/ml) on the left and right atria

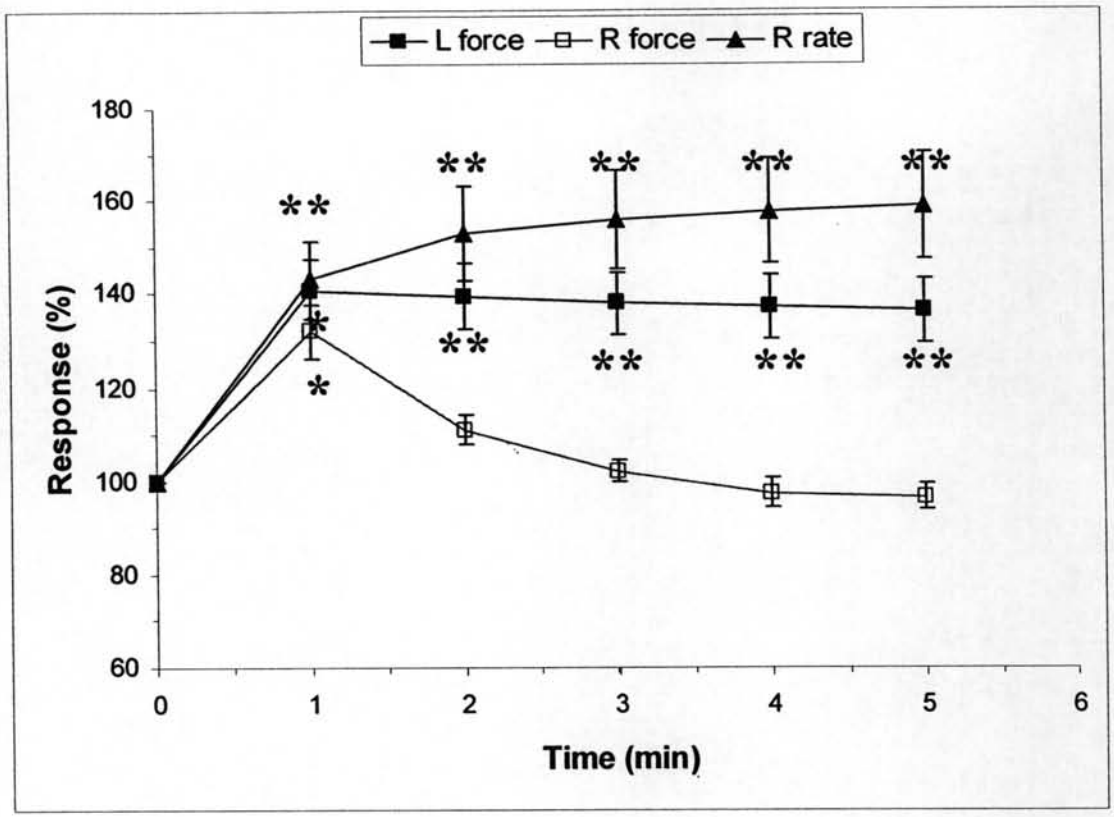
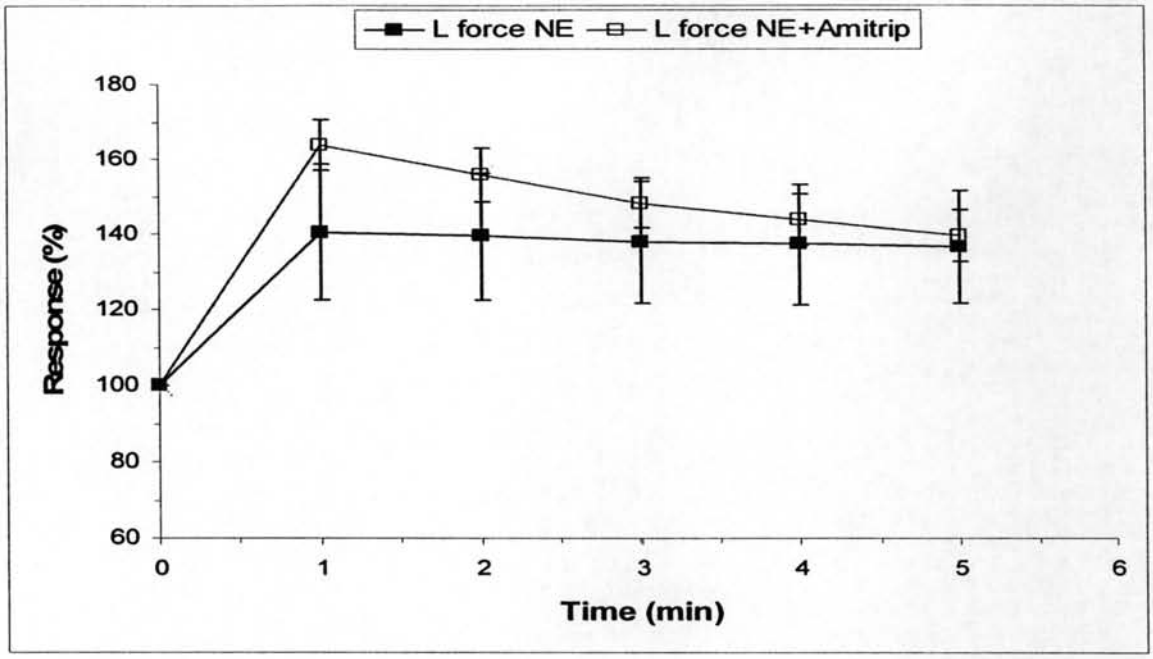


Figure 57 The chronotropic and inotropic response of NE (0.1 μM) on the left and right atria, n=6 (mean ± SEM), **P*<0.05, ***P*<0.001 significantly different from response at o min (paired *t*-test)

A. Left atria



B. Right atria

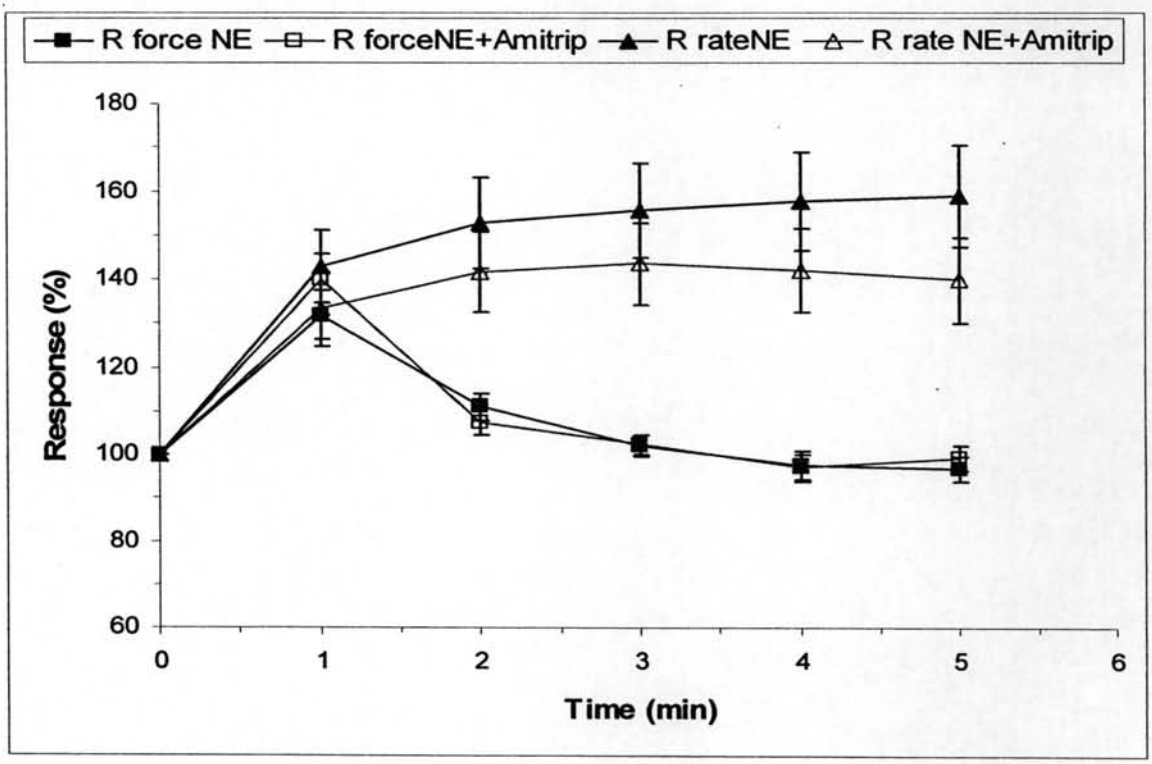
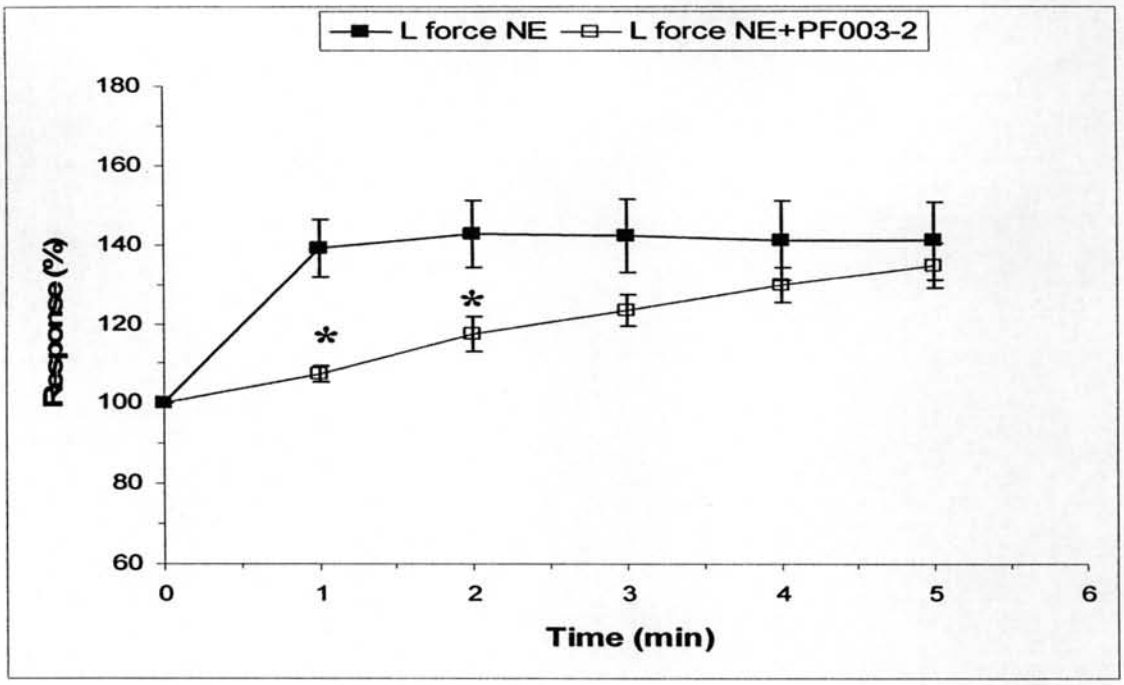


Figure 58 The chronotropic and inotropic reponse of NE (0.1 μM) and of NE (0.1 μM) in presence of amitriptyline (10 μM) on **(A)** left and **(B)** right atria, n=6 (mean ± SEM), (unpaired *t*-test)

A. Left atria



B. Right atria

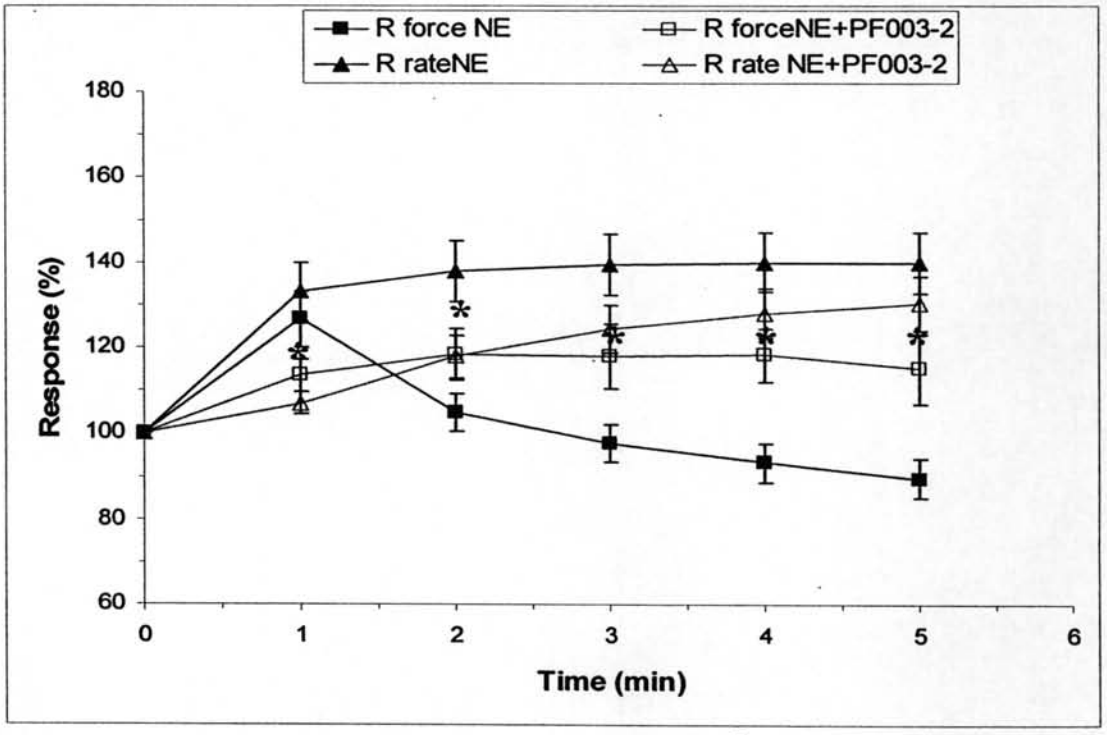


Figure 59 The chronotropic and inotropic response of NE (0.1 μM) and of NE (0.1 μM) in presence of PF003-2 (100 μg/ml) on **(A)** left and **(B)** right atria, n=6 (mean ± SEM), *P<0.05, significant different from NE group (unpaired *t*-test)

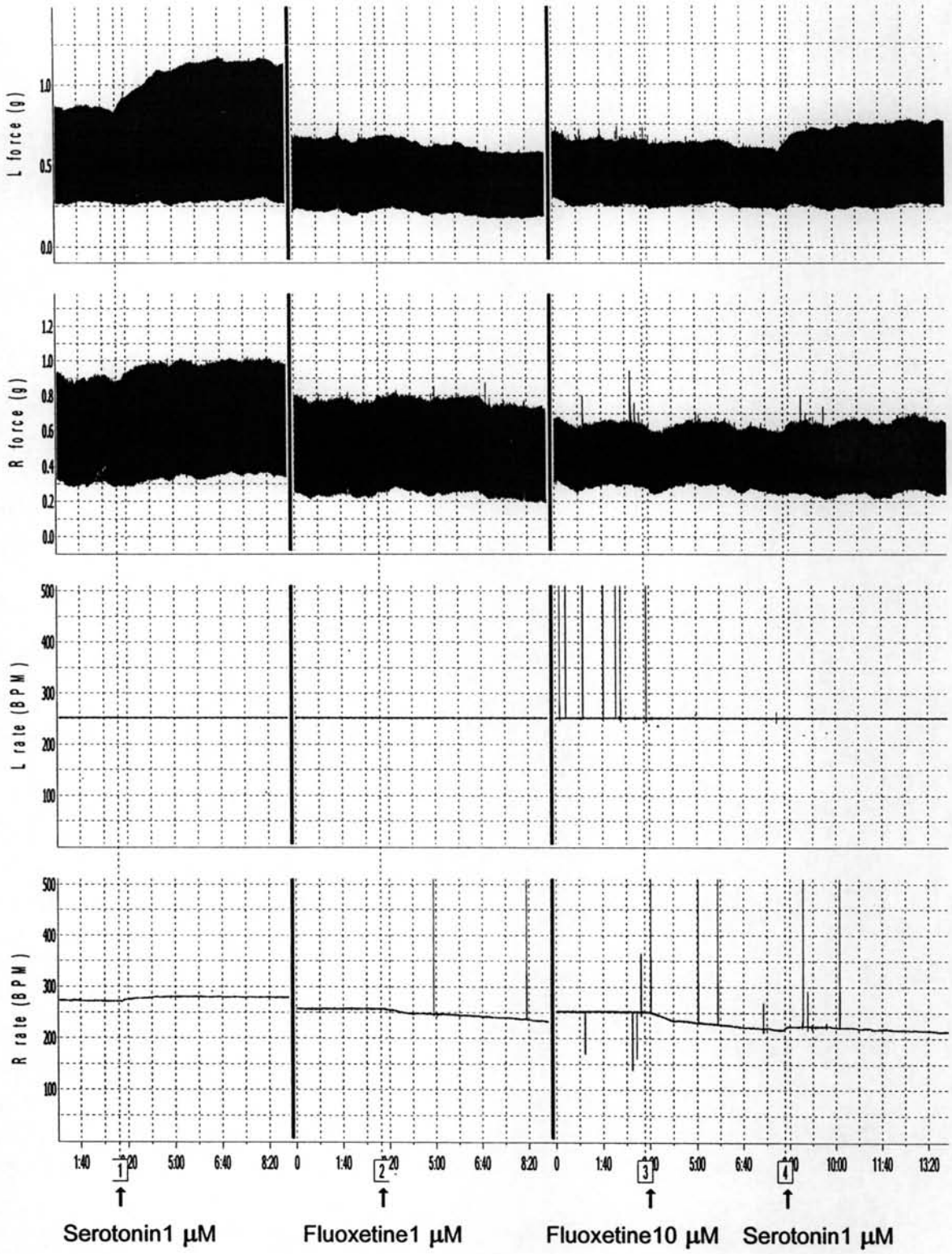


Figure 60 The chronotropic and inotropic reponse of serotonin (1 μM), fluoxetine (1μM) and serotonin (1 μM) in presence of fluoxetine (1 μM) on the left and right atria

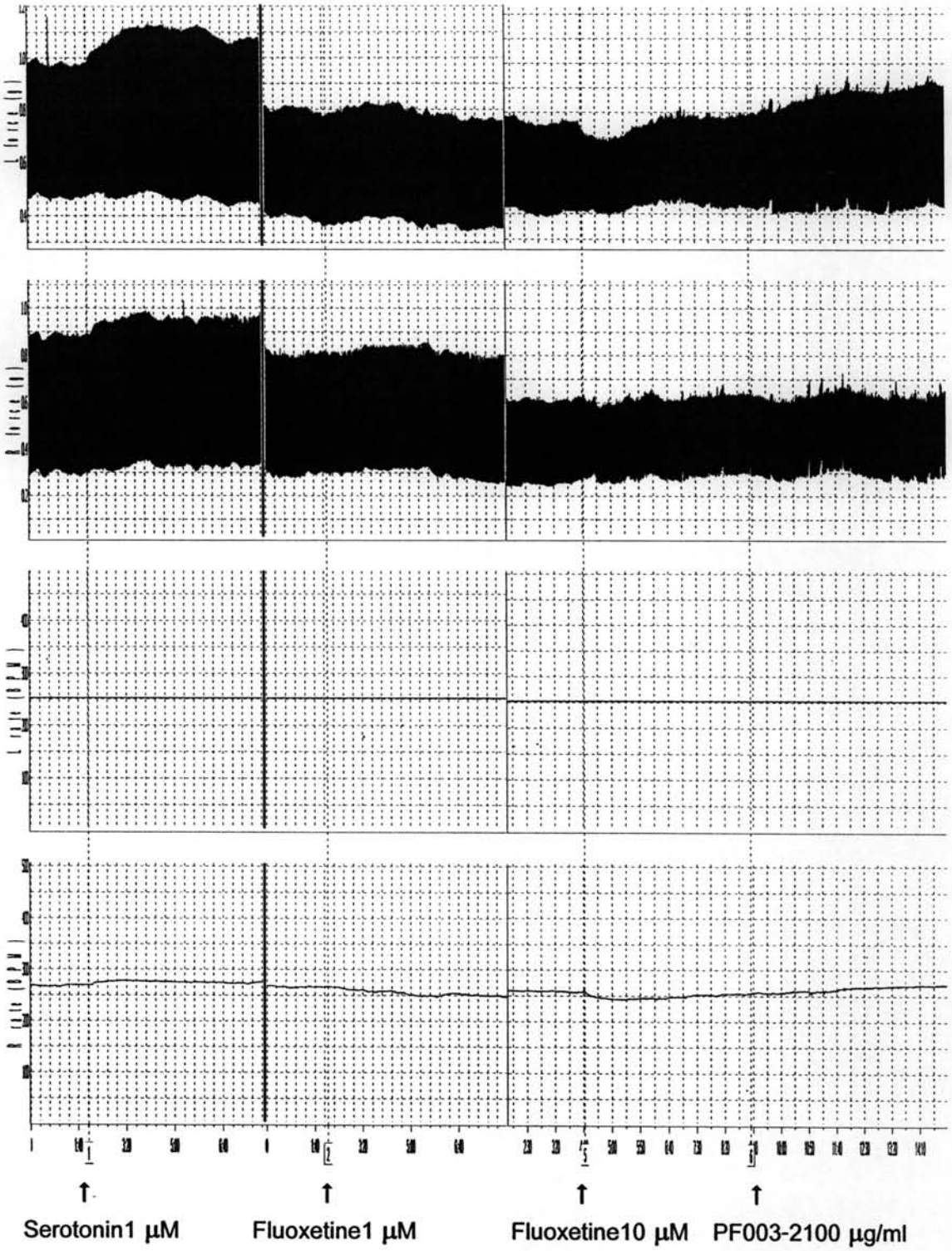


Figure 61 The chronotropic and inotropic reponse of serotonin (1 μM), fluoxetine (1μM) and serotonin (1 μM) in presence of PF003-2 (100 μg/ml) on the left and right atria

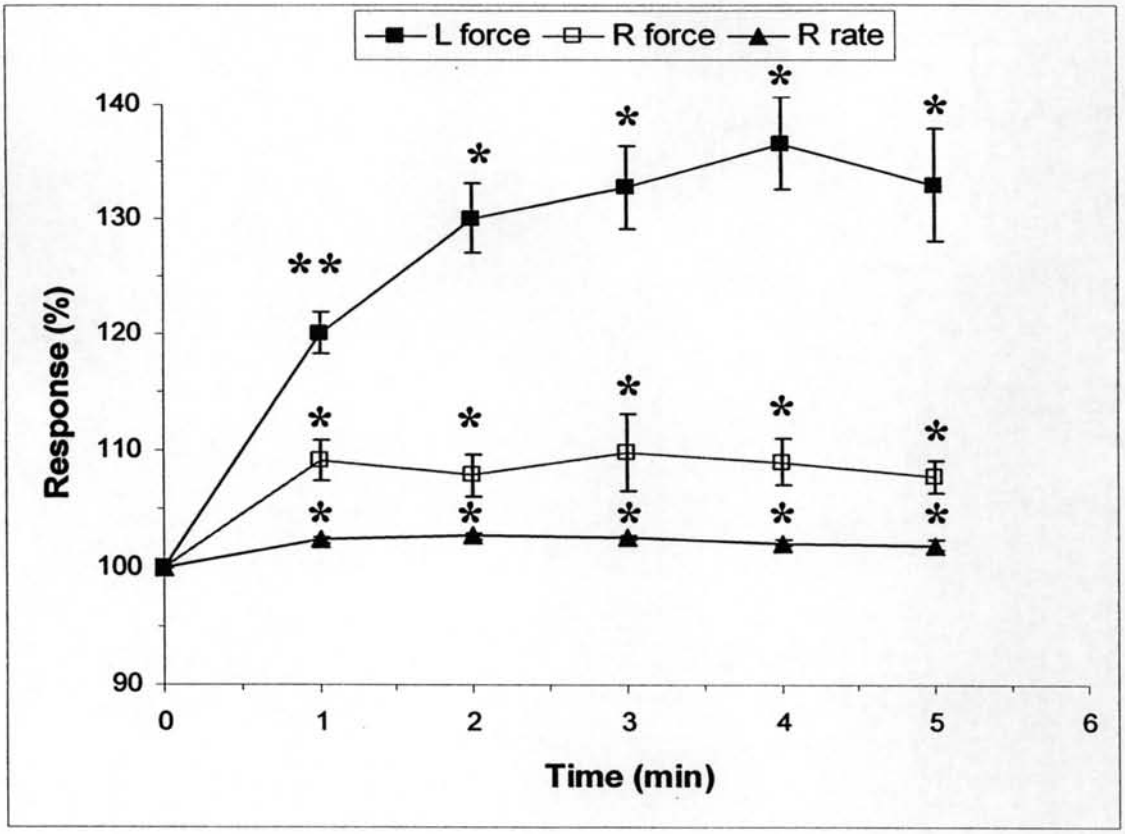


Figure 62 The chronotropic and inotropic response of serotonin (1 μM) on the left and right atria, n=4 (mean ± SEM), *P<0.05, **P<0.001 significantly different from response at 0 min group (paired *t*-test)

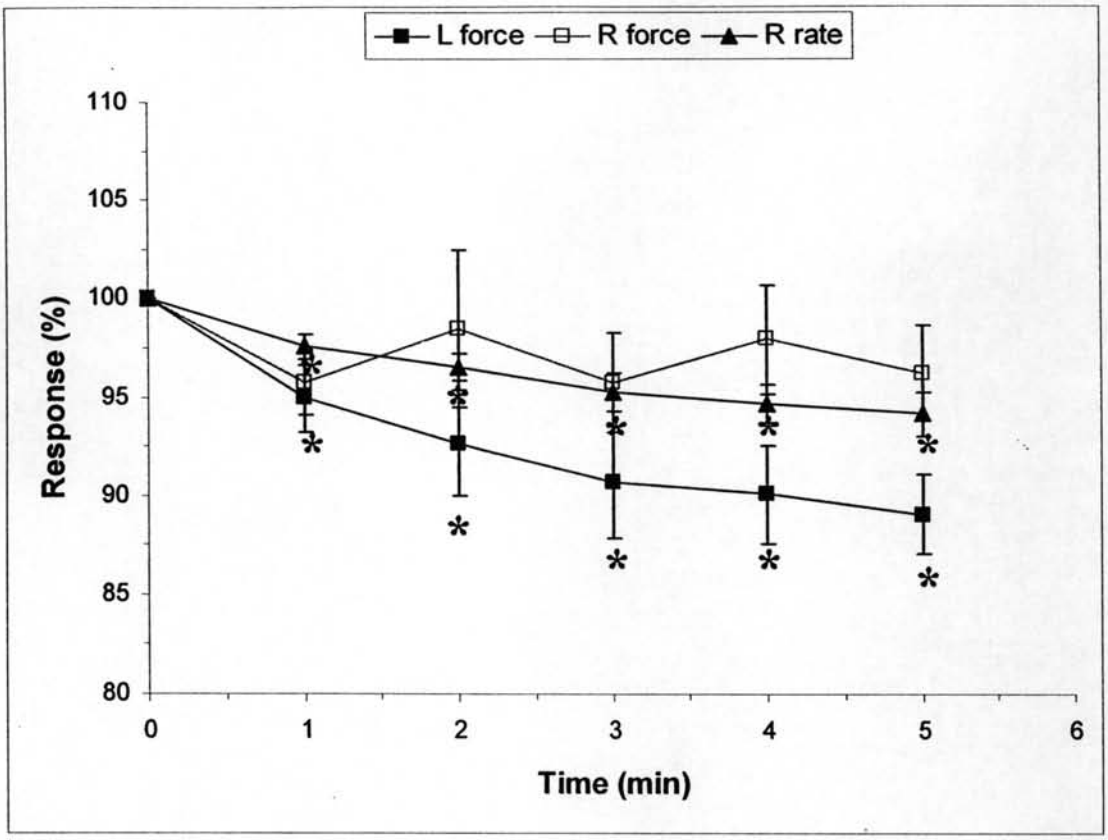
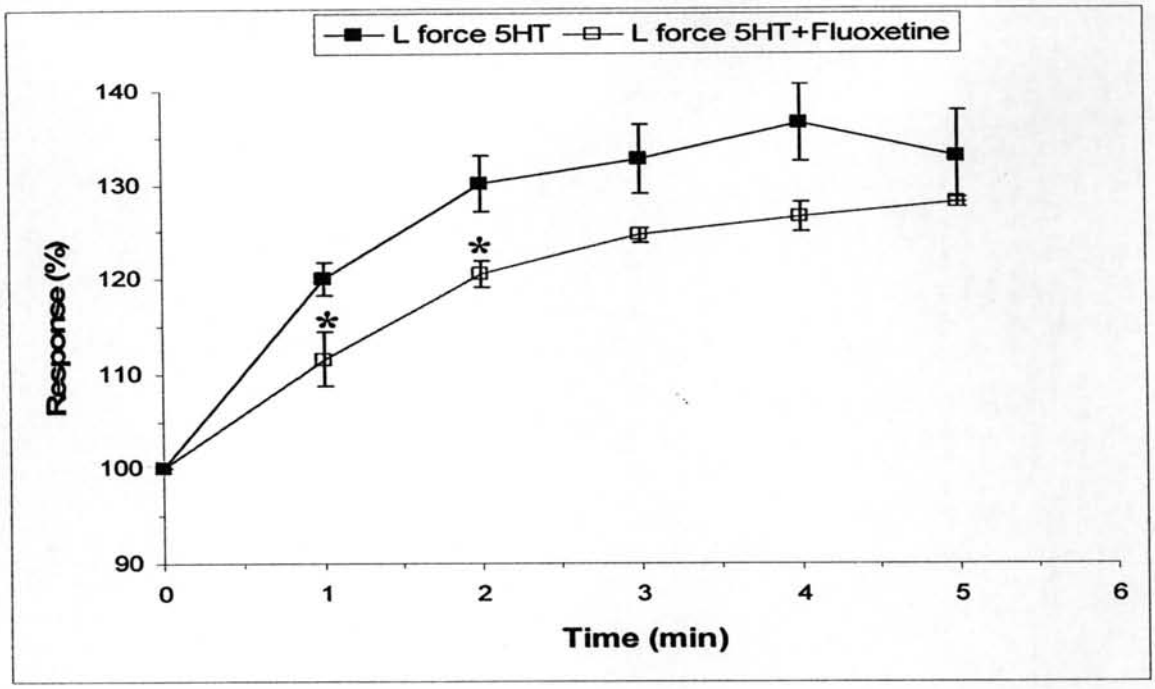


Figure 63 The chronotropic and inotropic response of fluoxetine (1 μM) on the left and right atria, n=4 (mean ± SEM), *P<0.05, significant different from response at 0 min (paired *t*-test)

A. Left atria



B. Right atria

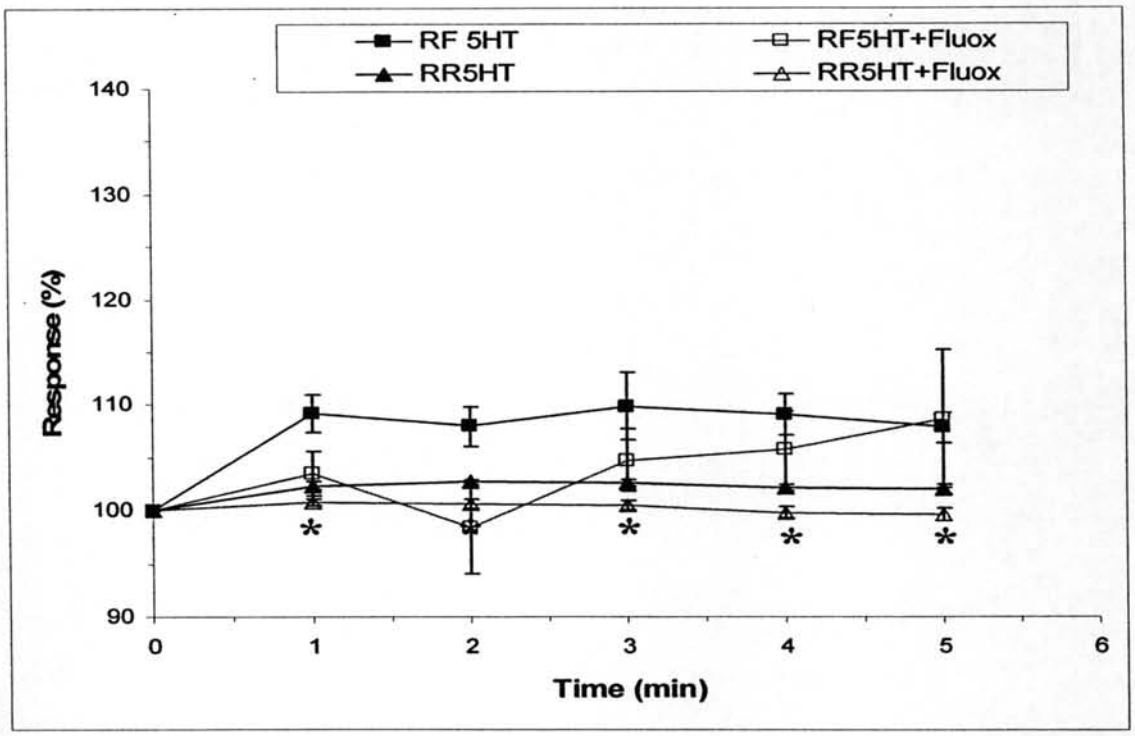
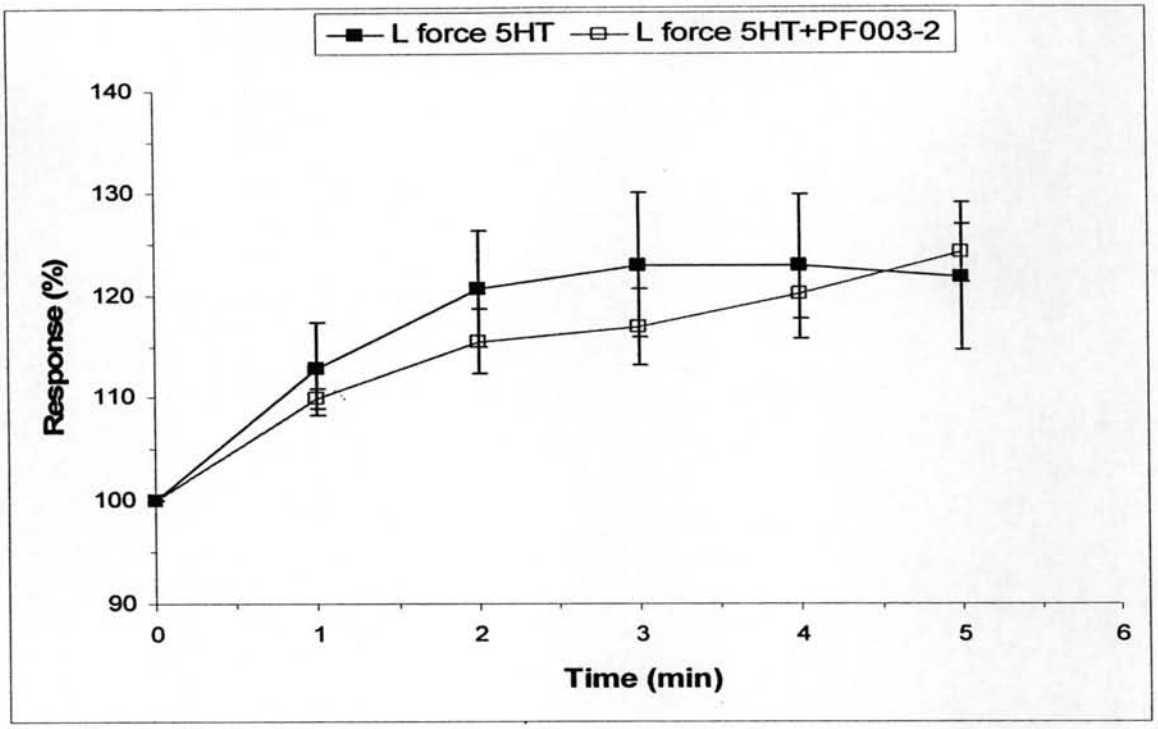


Figure 64 The chronotropic and inotropic response of serotonin (1 μM) and serotonin (1 μM) in presence of fluoxetine (10 μM) on **(A)** left and **(B)** right atria, n=4 (mean ± SEM)

*P<0.05, significantly different from serotonin group (unpaired *t*-test)

A. Left atria



B. Right atria

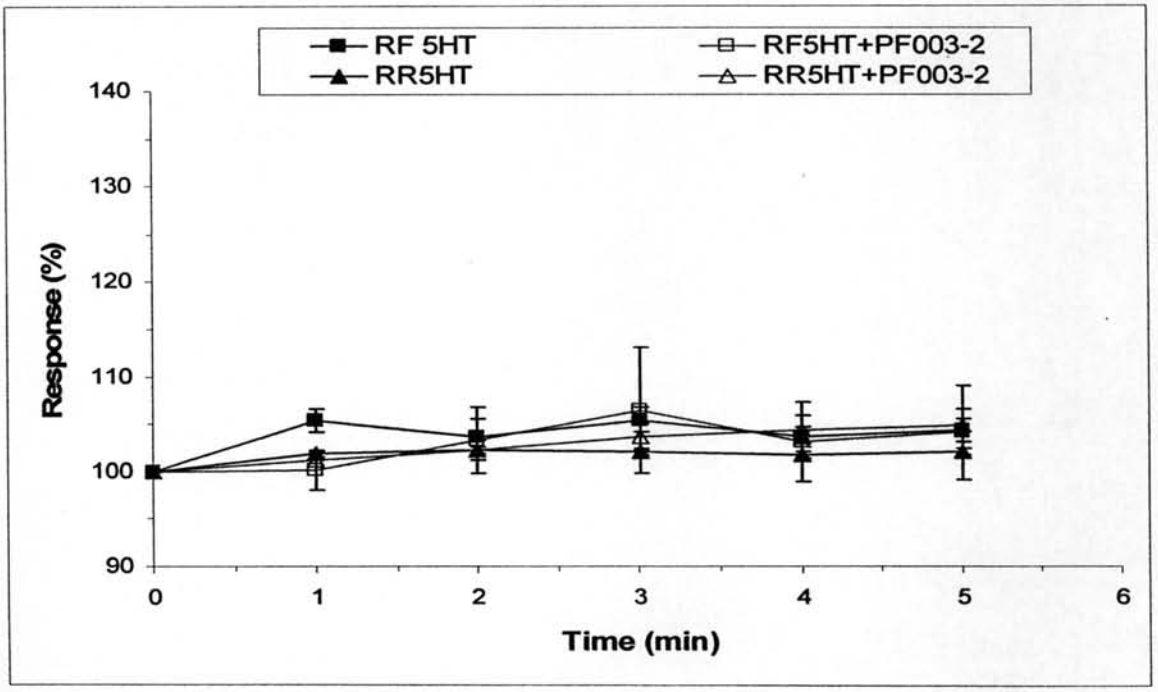


Figure 65 The chronotropic and inotropic reponse of serotonin (1 μM) and serotonin (1 μM) in presence of PF003-2 (100 μg/ml) on **(A)** left and **(B)** right atria, n=5 (mean ± SEM) (unpaired *t*-test)