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Pressor and tachycardic responses to intrathecal administration of neuropeptide FF in anesthetized rats

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ABSTRACT

Neuropeptide FF (NPFF) belongs to a neuropeptide family including two precursors (pro-$NPFF_A$ and pro-$NPFF_B$) and two receptors ($NPFF_1$ and $NPFF_2$). NPFF and NPFF receptor mRNAs have been reported to be highly expressed and localized in the rat and human spinal cord. In the present study, the i.t. action of NPFF system on blood pressure and heart rate were examined using NPFF and two related agonists, NPVF and dNPA, which exhibit highest selectivities for $NPFF_1$ and $NPFF_2$ receptors, respectively. In urethane-anesthetized rats, NPFF and related peptides (5–40 nmol, i.t.) produced significant pressor and tachycardic responses at the spinal cord level. These effects were dose-dependent and similar with respect to time-course for the three peptides. Furthermore, i.t. injection of RF9 (20 nmol), a selective NPFF antagonist, significantly antagonized the cardiovascular responses to 20 nmol NPFF and related peptides (i.t.). Moreover, pretreatment of the rats with α-adrenoceptor antagonist phentolamine (1 mg/kg, i.v.) significantly reduced the pressor effects of NPFF. Nevertheless, pretreatment with muscarinic receptor and adrenoceptor antagonists (i.v.) could block the tachycardic effects induced by NPFF. Collectively, our results suggested that i.t. administration of NPFF and related peptides increased MAP and HR which were possibly mediated by the activation of both $NPFF_1$ and $NPFF_2$ receptors in the rat spinal cord. In addition, our results showed that the muscarinic receptor and adrenoceptor participated in the tachycardic response to i.t. NPFF, while α-adrenoceptor played an important role in the regulation of pressor effect of NPFF.

1. Introduction

Neuropeptide FF (NPFF, FLFQPQRFamide) was originally isolated from bovine brain through its cross-reaction with antibodies to the molluscan cardioexcitory peptide FMRF-NH_2, which possessed the similar C-terminal sequence [31]. Recent reports have shown that NPFF belongs to a neuropeptide family including two precursors (pro-$NPFF_A$ and pro-$NPFF_B$) and two G-protein coupled receptors ($NPFF_1$ and $NPFF_2$) [6,7,13,18,22,28]. In addition, it has been suggested that the pro-$NPFF_A$ peptides (NPFF and NPA-NPFF) and pro-$NPFF_B$ peptides (NPVF) are the preferred ligands for $NPFF_2$ and $NPFF_1$ receptors, respectively. Since the isolation of NPFF in 1985, the biological functions suggested for this neuropeptide including pain modulation, food intake, gastrointestinal and hormonal modulation, modulation of opiate tolerance and abstinence and cardiovascular actions.

Immunoreactive NPFF has been found to be present in the central nervous system including the spinal cord and the pituitary gland [4,16,19,21,25]. The highest NPFF transcripts were also detected in the spinal cord and medulla oblongata [28,32]. Additionally, the binding assays and autoradiographic studies have revealed the presence of NPFF bindings sites in rat spinal cord [1,2]. Subsequently, several studies have shown that $NPFF_2$ receptor mRNA is highly localized in superficial layer of dorsal spinal cord while $NPFF_1$ receptor is expressed abundantly in human spinal cord [6,32]. Moreover, the pharmacological studies have demonstrated involvement of spinal NPFF system in pain processing [33]. A summary of findings reported to date indicated that: NPFF induced analgesic and morphine modulating activities at the spinal level [21,25]; the spinal NPFF system is up-regulated

Abbreviations: 1DMe, D.YL(*N*-Me)FQPQRFamide; HR, heart rate; MAP, mean arterial pressure; dNPA, D.NP(*N*-Me)AFLFQPQRFamide; i.t., intrathecal; NPA-NPFF, NPAFLFQPQRFamide; NTS, the nucleus tractus solitarius; NPVF, VPNLPQRFamide; PAP, pulsatile arterial pressure.

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by peripheral inflammation in the rat [32]; i.t. infusion of NPFF analogue 1DMe produced a long lasting increase in spinal outflow of met-enkephalin-like immunoreactive materials in rats [5]. Taken together, these data strongly suggested that the spinal cord should be one of the important sites to explore the bioactivities of NPFF system.

In previous studies, both intracerebroventricular (i.c.v.) and intravenous (i.v.) administration of NPFF produced increases in blood pressure and heart rate in rats [3,14,24]. Additionally, bilateral microinjection of NPFF into the commissural NTS caused pressor and bradycardia effects [17]. It was notable that NPFF exerted different modulatory roles in nociceptive activities at the supraspinal and spinal level [21,25]. However, little if anything is known about the cardiovascular effects of NPFF system at the spinal level. Therefore, the present study was undertaken to investigate the cardiovascular responses to NPFF system following i.t. administration in urethane-anesthetized rats. Furthermore, selective agonists and antagonist for the NPFF receptors were used in an attempt to characterize the NPFF receptors subtype which mediates the spinal action of NPFF on the cardiovascular system. In addition, the effects of muscarinic receptor and adrenoreceptors in mediating cardiovascular responses to NPFF were also examined.

2. Materials and methods

2.1. Animals

Male Wistar rats were obtained from the Experimental Animal Center of Lanzhou University. All animals were cared for and experiments were carried out in accordance with the European Community guidelines for the use of experimental animals (86/609/EEC). All the protocols in this study were approved by the Ethics Committee of Lanzhou University, China.

2.2. Chemicals

NPFF, NPVF, dNPA and RF9 were synthesized on a solid support following the previous report [11]. Peptides were prepared by manual solid-phase synthesis using standard N-fluorenylmethoxycarbonyl (Fmoc) chemistry. Fmoc-protected amino acids (GL Biochem (Shanghai) Ltd.) were coupled to a Rink Amide MBHA resin (Tianjin Nankai Hecheng Science & Technology Co., Ltd, China). The following schedule was employed: (1) DMF wash (3×); (2) 20% piperidine/DMF (3×, 4 min); (3) DMF wash (3×); (4) N^{α}-Fmoc-Amino Acid (2.5 eq.)/HBTU (2.5 eq.)/HOBt (2.5 eq.)/DIPEA (5 eq.) in DMF (1×), 1 h; (5) DMF wash (3×); (6) Kaiser Test. RF9 was obtained after acylation of the N-terminus with 1-adamantanecarboxylic acid (3 eq.)/HBTU (3 eq.)/HOBt (3 eq.)/DIPEA (6 eq.) in DMF (1×), 1 h. The protected peptide-resin was treated with reagent K (TFA/H_2O/phenol/ethanedithiol/thioanisole, 82.5:5:5:2.5:5) for 2 h at room temperature. Gel filtration (Sephadex G-10) was performed to desalt the crude peptides. The desalted peptide was purified by preparative reversed-phase HPLC using a Waters Delta 600 system coupled to a UV detector. Fractions containing the purified peptides were pooled and lyophilized. The purity of the peptide was established by analytical HPLC. The molecular weight of the peptide was confirmed by an electrospray ionization mass spectrometer (Mariner ESI-TOF MS, Applied Biosystems, CA).

In addition, propranolol hydrochloride and phentolamine hydrochloride were obtained from Sigma Chemical Company (USA); atropine sulfate was obtained from Dongting Lake Drug Factory of Hunan, China. All drugs were dissolved in sterilized saline, and the solutions were divided into aliquots and stored in 2 ml tubes at −20 °C. The aliquots were thawed and used on the day of the experiment.

2.3. Cardiovascular measurement

Experiments were performed, as described earlier [8,9]. Male Wistar rats weighing 200–250 g (n = 53) were anesthetized with urethane (l.2 g/kg, i.p.). Supplemental doses of urethane were given as needed to maintain a uniform level of anesthesia. The trachea was incised to get rid of mucus. The animals spontaneously breathed room air. Polyethylene catheter was inserted into the left external jugular vein for i.v. administration of drugs. The right carotid artery was cannulated with polyethylene catheter and connected to a PT100 pressure transducer with its output connected to a recorder system (model BL-420F, Taimeng Technology Corporation of Chengdu, China). MAP and HR were measured directly from the pre-calibrated BL-420F recorder system. For i.t. administration, a third catheter (PE-10) was passed through a slit in the dura at the atlanto-occipital junction and positioned so that the inner tip lay at the selected level, at the T12-L1 vertebral level as described by the previous studies [29,30]. The animals were then allowed to recover for 30–40 min. The position of the i.t. catheter was verified at necropsy after each experiment.

2.4. Administration of peptides

For i.v. administration, drugs were injected in 200-μl volume over a period of 30 s in a random sequence. For i.t. administration, drugs were injected according to the method of Yaksh and Rudy [30] and were administered intrathecally over a period of 30 s in 20-μl volume of saline or drugs. The catheter was flushed with 5 μl of saline. PAP, MAP and HR were simultaneously measured with a pressure transducer plugged into a BL-420F recorder system and continuously recorded throughout the experiments on a recorder system.

In control experiments, 20 μl of saline replaced the drug solution. To examine the cardiovascular responses of NPFF and related peptides, after control experiments, increasing doses of NPFF (5, 10, 20, and 40 nmol) were i.t. injected in the same animals in the experiments at 35-min intervals (n = 6). Similarly, dose–response curves were obtained for the effect of 5, 10, 20, and 40 nmol of NPVF (n = 6) and dNPA (n = 7) on cardiovascular responses.

In experiments in which the effects of NPFF antagonist RF9 on responses to NPFF and related peptides (n = 16) were investigated, maximal changes in MAP and HR were compared before administration of RF9 and 10 min after administration, in a dose of 20 nmol, i.t. Furthermore, the roles of muscarinic receptor, α- and β-adrenoreceptors in mediating cardiovascular responses to NPFF were also investigated. As to muscarinic receptor antagonist atropine sulfate (2 mg/kg, i.v.), the effects of atropine (n = 6) on the cardiovascular responses to NPFF were tested before administration and 10 min after administration. α-Adrenoceptor antagonist phentolamine hydrochloride (1 mg/kg, i.v.) and β-adrenoceptor antagonist propranolol hydrochloride (2 mg/kg, i.v.), as well as atropine sulfate, were tested individually in six rats.

2.5. Statistical analysis

Data were given as means ± S.E.M. The cardiovascular responses of the peptides alone were analyzed with one-way ANOVA followed by the Dunnett's post hoc test, and paired Student's t test was used to establish the differences between the groups. A probability level of 0.05 or less was considered as statistically significant.

3. Results

3.1. Effects of i.t. administration of NPFF on MAP and HR

In the present study, the cardiovascular responses to i.t. administration of NPFF on MAP and HR have been investigated

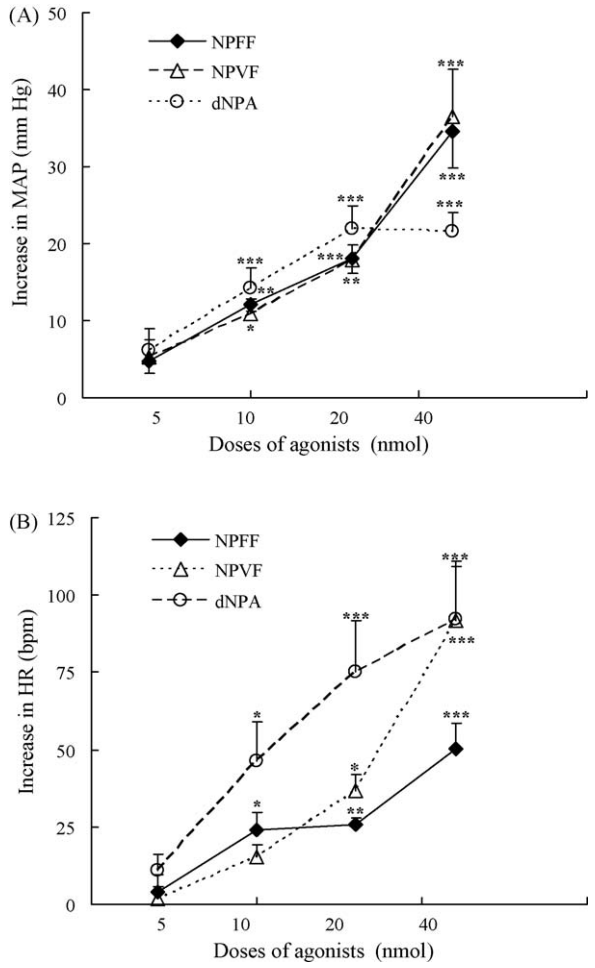


Fig. 1. Dose–response curves of i.t. NPFF and related peptides on MAP and HR in anesthetized rats. (A) Dose–response curves of NPFF (5, 10, 20 and 40 nmol, i.t., $n = 6$), NPVF (5, 10, 20 and 40 nmol, i.t., $n = 6$) and dNPA (5, 10, 20 and 40 nmol, i.t., $n = 7$) on MAP; (B) dose–response curves of NPFF (5, 10, 20 and 40 nmol, i.t., $n = 6$), NPVF (5, 10, 20 and 40 nmol, i.t., $n = 6$) and dNPA (5, 10, 20 and 40 nmol, i.t., $n = 7$) on HR. Data points represent means ± S.E.M. To establish statistical significance, the data were statistically analyzed by one-way ANOVA followed by the Dunnett's post hoc test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the vehicle control group).

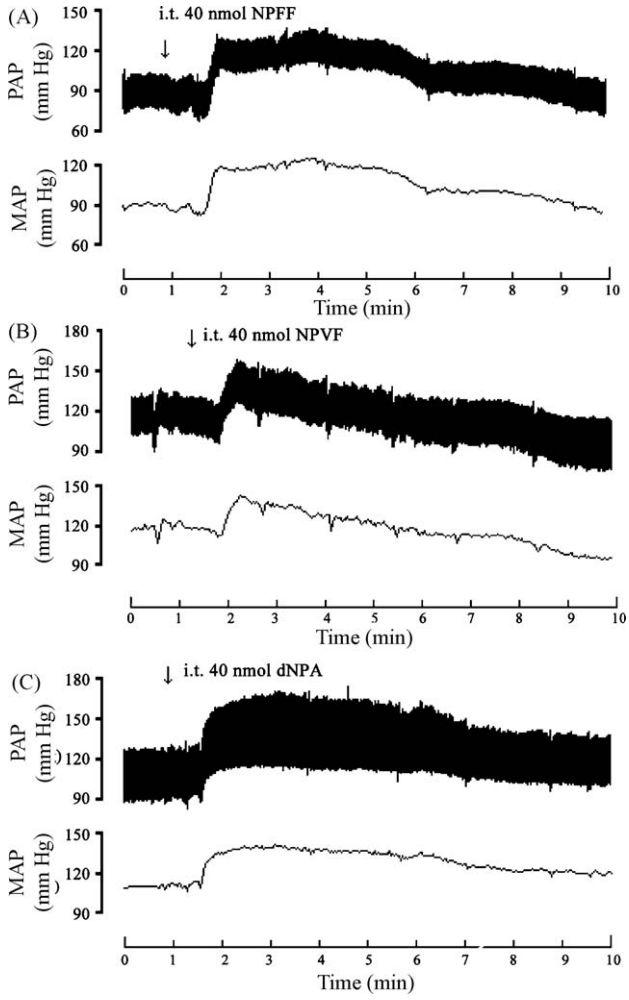


Fig. 2. Typical pressor responses to the maximum dose of NPFF (40 nmol, i.t., A), NPVF (40 nmol, i.t., B) and dNPA (40 nmol, i.t., C). Top and bottom tracings represent PAP and MAP, respectively. Arrows indicate time of injection.

in anesthetized rats. As shown in Fig. 1A, i.t. injection of NPFF (5, 10, 20 and 40 nmol) induced dose-dependent increases in MAP and HR. MAP increased 4.76 ± 1.66, 12.12 ± 0.97, 18.14 ± 2.01, 34.56 ± 4.68 mmHg from the baseline at the 5, 10, 20 and 40 nmol dose, respectively, of NPFF ($n = 6$). In addition, HR increased 3.79 ± 5.69, 23.93 ± 5.63, 25.68 ± 2.29, 50.30 ± 8.13 bpm from the baseline in the same dose range. Fig. 2A displays the records from experiments illustrating the time-course of effects of NPFF (40 nmol, i.t.) on PAP and MAP. The peak of the pressor effects was reached at approximately 1 min after i.t. injection of NPFF. The blood pressure parameter returned to the pretreatment levels within 10 min. Furthermore, control experiments with injections of the vehicle control (saline, i.t.) had no consistent effects on baseline MAP and HR in urethane-anesthetized rats (from 95.06 ± 3.46 mmHg and 396.09 ± 10.27 bpm to 94.87 ± 3.90 mmHg and 395.56 ± 9.95 bpm, respectively; $n = 6$, $P > 0.05$).

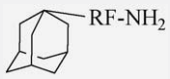
3.2. Effects of i.t. administration of NPVF and dNPA on MAP and HR

In order to characterize the NPFF receptors subtype which mediates the spinal action of NPFF on the cardiovascular system, the cardiovascular responses to NPVF and dNPA, the selective agonists for $NPFF_1$ and $NPFF_2$ receptors, respectively, were also measured [12,23,26]. The selectivity and the affinity to NPFF receptors of NPFF and related peptides used in this study are presented in Table 1 [12]. Similar to NPFF, both NPVF and dNPA significantly caused pressor and tachycardic effects when injected in doses of 5–40 nmol, i.t. (Fig. 1A and B). Interestingly, the dose–response curves of NPFF and related peptides indicated that the order of potency of these peptides to induce changes in MAP and HR were NPFF = NPVF ≥ dNPA and dNPA > NPVF ≥ NPFF, respectively (Fig. 1). Fig. 2B and C showed typical recordings of PAP and MAP before and after injections of NPVF and dNPA. The pressor responses to i.t. administration of NPFF and related peptides (40 nmol) were similar with respect to the time-course (Fig. 2).

3.3. Effects of NPFF antagonist RF9 (i.t.) on the cardiovascular responses to NPFF and related peptides (i.t.)

It is well known that the selective antagonists are required to explore the mechanism of the varied pharmacological effects induced by the G-protein coupled receptors. The effects of NPFF receptors selective antagonist RF9 (20 nmol, i.t.) on the cardiovascular responses induced by NPFF and related peptides are shown in Fig. 3 [27]. Baseline MAP has no significant changes when compared before and after i.t. administration of RF9 (from 1.29 ± 0.86 to 5.17 ± 1.73 mmHg, $n = 16$, $P > 0.05$), but a small increase in baseline HR was noted in several animals after i.t.

Table 1
Affinities (K_i) of neuropeptide FF agonists (NPFF, NPVF and dNPA) and antagonist (RF9) on human $NPFF_1$ and $NPFF_2$ receptors.

	$NPFF_1$ K_i (nM)[a]	$NPFF_2$ K_i (nM)	$S_{1/2}$[b]	Reference
NPFF, FLFQPQRF-NH_2	2.82 ± 0.06	0.21 ± 0.03	13.4	[12]
NPVF, VPNLPQRF-NH_2	0.59 ± 0.07	23.0 ± 2.1	0.026	[12]
dNPA, *D*.NP(*N-Me*)AFLFQPQRF-NH_2	2.9 ± 0.5	0.027 ± 0.001	107.4	[12]
RF9, 	75 ± 9	58 ± 5	1.29	[27]

Data are cited from the previous reports [12,27].
[a] K_i value are expressed as means ± S.E.M.
[b] $S_{1/2} = K_i$ ($NPFF_1$)/K_i ($NPFF_2$) for the selectivity index.

administration of RF9 in a dose of 20 nmol (from 1.26 ± 1.59 to 10.21 ± 3.23 bpm, $n = 16$, $P < 0.05$). The change in HR in response to RF9 returned to pre-injection values within 3 min.

At a dose of 20 nmol, NPFF and related peptides significantly induced cardiovascular responses. Subsequently, this dose was selected for evaluating the effects of RF9 and other pharmacological antagonists (atropine, phentolamine and propranolol) on the cardiovascular responses evoked by NPFF. After i.t. administration of RF9 in a dose of 20 nmol, the increases in MAP and HR in response to i.t. administration of NPFF and related peptides were almost completely antagonized (Fig. 3).

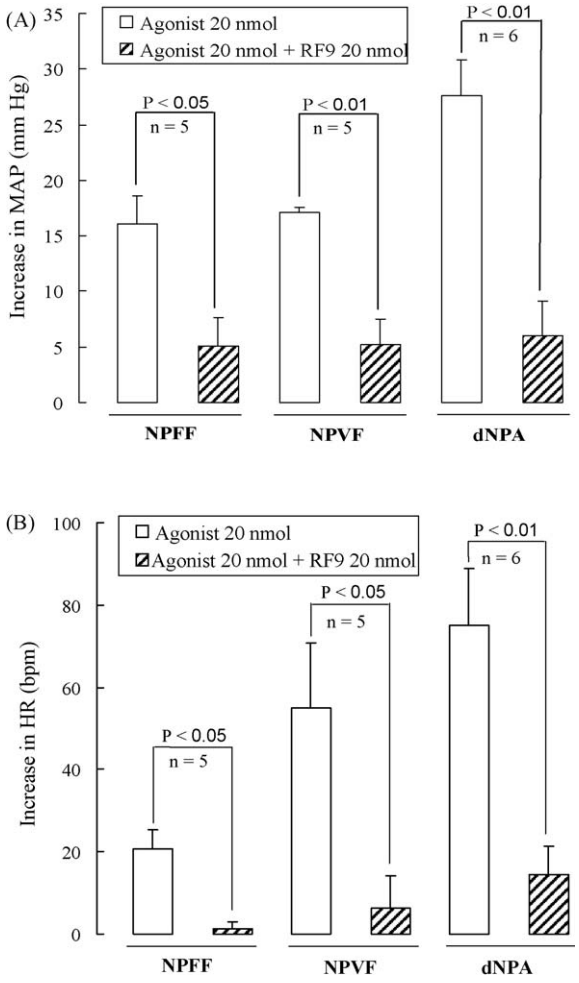


Fig. 3. Changes in MAP and HR caused by i.t. injection of NPFF and related peptides before and after the NPFF receptors antagonist RF9 (i.t., 20 nmol) in anesthetized rats. (A) NPFF, NPVF and dNPA (i.t., 20 nmol) in MAP. (B) NPFF, NPVF and dNPA (i.t., 20 nmol) in HR. Data were expressed as means ± S.E.M. "*n*" indicates the number of experiments. To establish statistical significance, the data were statistically analyzed by paired Student's *t* test ($P < 0.05$ and $P < 0.01$ indicating significant differences from the actions of agonists alone).

3.4. Effects of atropine (i.v.), phentolamine (i.v.) and propranolol (i.v.) on the cardiovascular responses to NPFF (i.t.)

Furthermore, the roles of muscarinic receptor, α- and β-adrenoreceptors in mediating cardiovascular responses to NPFF were also determined. i.v. administration of atropine induced a significant decrease in the baseline MAP and a slight increase in baseline HR (−22.10 ± 3.41 mmHg versus 1.61 ± 0.61 mmHg for saline, $P < 0.001$; 5.81 ± 7.74 bpm versus 0.22 ± 2.71 bpm for saline, $P > 0.05$, respectively; $n = 6$), which returned to pre-injection values within 3 min. Phentolamine, an α-adrenoreceptor antagonist, at a dose of 1 mg/kg caused a significant decrease in baseline MAP (−39.29 ± 5.53 mmHg versus −1.64 ± 1.15 mmHg for saline, $P < 0.01$; $n = 6$), but a slight change in baseline HR (−6.62 ± 10.83 bpm versus 2.02 ± 1.28 bpm for saline, $P > 0.05$; $n = 6$), which was consistent with the previous studies [29]. Similar to our recent report [10], basal MAP and HR were reduced considerably after i.v. administration of 2 mg/kg propranolol (−25.43 ± 3.85 mmHg versus −0.15 ± 2.00 mmHg for saline, $P < 0.01$; −72.84 ± 10.59 bpm versus 0.14 ± 0.61 bpm for saline, $P < 0.001$, respectively; $n = 6$), which returned to the pretreatment levels about 10 min after injection of this antagonist.

In our study, pretreatment of the rats with α-adrenoceptor antagonist phentolamine (1 mg/kg, i.v.) significantly reduced the cardiovascular effects of NPFF (20 nmol, i.t.). Nevertheless, pretreatment with muscarinic receptor antagonist atropine (2 mg/kg, i.v.) and β-adrenoceptor antagonist propranolol (2 mg/kg, i.v.) could only antagonized the tachycardic effects induced by NPFF (20 nmol, i.t.), but had no significant effects on pressor responses to i.t. NPFF (Table 2).

Table 2
Changes in MAP and HR caused by i.t. injection of NPFF (20 nmol) before and after i.v. administration of muscarinic and adrenergic receptor antagonists in rats.

Treatments	ΔMAP (mm Hg)	ΔHR (bpm)
NPFF (i.t.)	19.0 ± 2.5 ($n = 6$)	26.2 ± 5.1 ($n = 6$)
Atropine (2 mg/kg, i.v.) + NPFF (i.t.)	13.6 ± 1.3 ($n = 6$)	7.7 ± 1.2[a] ($n = 6$)
NPFF (i.t.)	18.1 ± 2.5 ($n = 6$)	29.6 ± 9.6 ($n = 6$)
Phentolamine (1 mg/kg, i.v.) + NPFF (i.t.)	4.7 ± 2.1[b] ($n = 6$)	7.3 ± 5.5[a] ($n = 6$)
NPFF (i.t.)	20.0 ± 2.0 ($n = 6$)	21.9 ± 6.4 ($n = 6$)
Propranolol (2 mg/kg, i.v.) + NPFF (i.t.)	21.8 ± 3.2 ($n = 6$)	2.4 ± 1.4[a] ($n = 6$)

Data were expressed as means ± S.E.M. from experiments conducted on 6 rats, respectively. To establish statistical significance, the data were statistically analyzed by paired Student's *t* test.
[a] $P < 0.05$.
[b] $P < 0.01$ (compared with the vehicle control group).

4. Discussion

Unlike other neuropeptides, the distribution of NPFF was found to be localized within the nervous and neuroendocrine systems with the highest levels in dorsal spinal cord and the posterior pituitary [4,16,19,21,25,33]. Results of the present study confirmed that i.t. administration of NPFF to the rat spinal cord evoked dose-related increases in MAP and HR. In addition, NPVF and dNPA, two NPFF agonists exhibiting different selectivities towards $NPFF_1$ and $NPFF_2$ receptors, respectively, caused pressor and tachycardic responses at the spinal level. The cardiovascular effects induced by NPFF and related peptides were almost completely antagonized by the NPFF receptors selective antagonist RF9 (i.t.) [11,15,27], indicating that the increases in MAP and HR caused by these three peptides might be linked to specific activation of NPFF receptors. Moreover, the results caused by RF9 could be explained by the activation of NPFF receptors by NPFF agonists in the spinal cord, which seem to be consistent with the fact that the mammalian spinal cord expresses abundant NPFF receptors, especially $NPFF_2$ receptor [1,2,6,32].

The previous studies using cells expressing NPFF receptors have been revealed that both $NPFF_1$ and $NPFF_2$ receptors have high affinities for NPFF [6,12]. To further explore the roles of these two types NPFF receptors in cardiovascular regulation, NPVF and dNPA, two selective agonists for $NPFF_1$ and $NPFF_2$ receptors, respectively, were used. dNPA, a stable analogue of pro-$NPFF_A$ peptide NPA-NPFF, has at least 100 times higher affinity for the $NPFF_2$ than for the $NPFF_1$ receptor [12,26], whereas the pro-$NPFF_B$ peptide NPVF has 30 times more affinity for the $NPFF_1$ than for the $NPFF_2$ receptor [12,20]. Interestingly, in our study, both NPVF and dNPA dose-dependently increased MAP and HR in a manner similar to NPFF. Therefore, our data supported the hypothesis that the activation of both $NPFF_1$ and $NPFF_2$ receptors of the rat spinal cord could produce same cardiovascular effects.

Taking into account the potencies of cardiovascular responses to i.t. administration of NPFF and related peptides, in the present study, dNPA was lest potent than NPFF and NPVF on MAP at the highest dose (40 nmol, i.t.), in contrast, dNPA displayed a higher potency to increase HR compared to NPFF and NPVF. It is difficult to explain the discrepancy at present. However, this observation might imply that i.t. administration of NPFF-related peptides induced pressor and tachycardia responses via different mechanisms. As shown in Table 1, the previous in vitro assays showed that these three peptides displayed different affinities towards $NPFF_1$ and $NPFF_2$ receptors. To our surprise, the present studies clearly indicate that the potency of NPFF agonists to evoke cardiovascular effects upon i.t. administration did not correlate with their ability to bind to the NPFF receptors. Thus, these data suggest that the potency of cardiovascular action induced by i.t. NPFF-related peptides do not depend directly or not only upon the selectivity and affinity of the compounds used. A similar hypothesis was proposed by Quelven et al. [23] in mouse tail-flick test. Taken together the above findings, both $NPFF_1$ and $NPFF_2$ receptors play important roles in the regulation of the spinal cardiovascular activities of NPFF.

In the present work, pretreatment of the rats with muscarinic receptor antagonist atropine (2 mg/kg, i.v.), α-adrenoceptor antagonist phentolamine (1 mg/kg, i.v.) and β-adrenoceptor antagonist propranolol (2 mg/kg, i.v.) significantly reduced the tachycardic responses to i.t. administration of NPFF, which indicated that muscarinic receptor and adrenoceptor played important roles in the regulation of tachycardic effect of NPFF (i.t.). In contrast, the pressor effect of i.t. NPFF could only be attenuated by pretreatment with α-adrenoceptor antagonist phentolamine. These findings agree with the above-mentioned deduction that the pressor and tachycardia responses to i.t. administration of NPFF agonists are mediated by different mechanisms. Moreover, our results showed that the muscarinic receptor and adrenoceptor participated in the cardiovascular effects induced by i.t. administration of NPFF.

In summary, the results of the present study indicated that i.t. administration of NPFF and related peptides induced significant increases in MAP and HR which were possibly mediated by the activation of $NPFF_1$ and $NPFF_2$ receptors in the rat spinal cord. Our results also showed that the muscarinic receptor and adrenoceptor participated in the tachycardic response to i.t. NPFF, while α-adrenoceptor played an important role in the regulation of pressor effect of NPFF. Collectively, these data might be helpful to further deduce the mechanisms of NPFF system in cardiovascular responses. In addition, this in vivo bioassay may be applied as a parameter to characterize the potential NPFF agonists and antagonists.

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