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ELEVATION OF ARTERIAL PRESSURE IN RATS BY TWO NEW VERTEBRATE PEPTIDES: FLFQPQRF-NH₂ AND AGEGLSSPFWSLAAPQRF-NH₂ WHICH ARE IMMUNOREACTIVE TO FMRF-NH₂ ANTISERUM

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ABSTRACT

We found that two recently characterized neuropeptides Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH₂ (F-8-F-NH₂) and Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH₂ (A-18-F-NH₂) elevate mean arterial blood pressure (MAP) in conscious, unrestrained rats. The pressor activities of both agents were attenuated, but not abolished, by prior treatment with guanethidine or prazosin. These results suggest that F-8-F-NH₂ and A-18-F-NH₂ elevate MAP in rats by potentiating the release of catecholamines and by mechanisms independent of catecholamine release.

INTRODUCTION

The cardioexcitatory peptide, Phe-Met-Arg-Phe-NH₂ (FMRF-NH₂) was first isolated and characterized by Price and Greenberg from ganglia of the clam *Macrocallista nimbosa* (1). Subsequently, FMRF-NH₂-like peptides which are structurally distinct from FMRF-NH₂ were detected in CNS neurons of many mammalian species using antiserum raised against FMRF-NH₂ (2, 7, 9, 10). Recently, three of these FMRF-NH₂-like peptides were isolated and chemically characterized to be Leu-Pro-Leu-Arg-Phe-NH₂ in chicken brain (3); Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH₂ (F-8-F-NH₂) and Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH₂ (A-18-F-NH₂) in bovine brain (11). F-8-F-NH₂ and A-18-F-NH₂ were found to be unevenly distributed in CNS with the highest concentrations in dorsal spinal cord, periaqueductal grey and medulla pons and the lowest concentrations in cortex, striatum and cerebellum (5). Although intraventricularly injected F-8-F-NH₂ was found to attenuate morphine induced analgesia (11), the physiological role of F-8-F-NH₂ and A-18-F-NH₂ still remains to be ascertained. Both FMRF-NH₂ and Leu-Pro-Leu-Arg-Phe-

NH₂, which share the same C-terminal dipeptide amide (Arg-Phe-NH₂) with F-8-F-NH₂ and A-18-F-NH₂, are cardioactive (1). Thus, we have decided to investigate the peripheral cardiovascular properties of these two novel neuropeptides F-8-F-NH₂ and A-18-F-NH₂.

We here report that both A-18-F-NH₂ and F-8-F-NH₂ have substantial ability to elevate mean arterial blood pressure (MAP) in conscious, unrestrained rats. Furthermore, the ability of both neuropeptides to elevate MAP is partially, but not completely, attenuated by prior treatment with guanethidine or prazosin. The results suggest that both neuropeptides may have roles in the regulation and maintenance of blood pressure by release of catecholamines as well as actions that do not require catecholamine release.

MATERIALS AND METHODS

Materials Male Sprague-Dawley rats (200-300 g) were obtained from Taconic Farms, MD. A-18-F-NH₂ and F-8-F-NH₂ were custom synthesized by Peninsula Laboratories, Inc. (Belmont, CA) and purified by reverse-phase high pressure liquid chromatography. Prazosin was a gift of Darwin Cheney, (Ciba-Geigy); guanethidine was from Ciba-Geigy.

Animals Animals were fasted overnight and then anesthetized with halothane and implanted with external jugular vein and internal carotid artery catheters (polyethylene, PE 50) as previously detailed (4). The animals were housed in individual cages, allowed to recover from anesthesia and heart rate (HR) and MAP determined (4).

Dose Response Studies Peptides were dissolved in saline and injected via external jugular catheters with MAP and HR being continuously recorded. Each dose (0.1-0.4 ml) was followed by an 0.3 ml flush of sterile saline. For pre-treatment studies, prazosin (100 µg/kg iv via slow infusion) or guanethidine (15 mg/kg; s.c.) were given and single dose studies (100 µg/kg each of F-8-F-NH₂ and A-18-F-NH₂) performed after the MAP had stabilized.

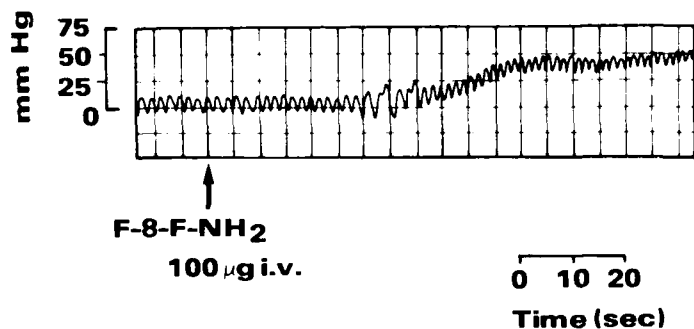


Figure 1. Typical Pressor Response to a Maximum Dose of F-8-F-NH₂. Rats were treated as in Method with 100 µg F-8-F-NH₂ and MAP and HR continuously monitored. A typical pressor response is shown. The mean arterial pressure of saline treated rats was 114 ± 3.4 mm Hg; this basal level was set as 0.



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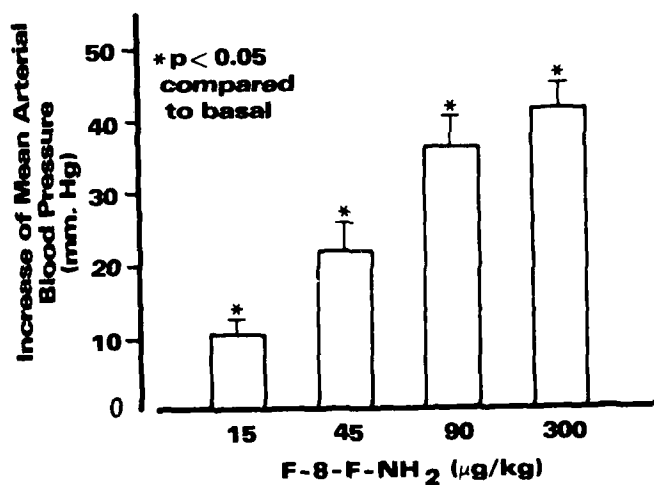
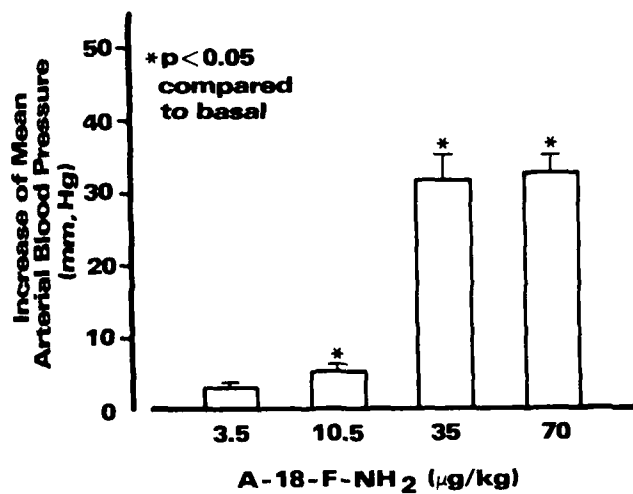


Figure 2. Dose-Response to A-18-F-NH₂ and F-8-F-NH₂ in Conscious Rats. Dose response studies were performed for A-18-F-NH₂ (Top) and F-8-F-NH₂ (Bottom) on MAP in conscious, unrestrained rats. Data represent mean \pm SEM of 4-6 separate determination. (*p 0.05 vs saline infusion; t-test). The mean arterial pressure of saline treated rats was 114 ± 3.4 mm Hg; this basal level was set as 0.

RESULTS

A-18-F-NH₂ and F-8-F-NH₂ elevate MAP. Fig. 1 shows a typical response to injection of 100 µg/kg (92.3 nmole/kg) F-8-F-NH₂. As is seen, the effect of A-18-F-NH₂ was not immediate and was sustained for 30-60 sec. Dose response studies for A-18-F-NH₂ and F-8-F-NH₂ revealed that both neuropeptides caused an approximate 40 mm Hg increase in MAP, but that considerably higher doses of F-8-F-NH₂ were required both on a molar as well as a µg/kg basis (Fig. 2). A threshold dose of 10.5 µg/kg (5.5 nmole/kg) was obtained for A-18-F-NH₂ while F-8-F-NH₂ required 15 µg/kg (13.9 nmole/kg). Maximal elevations of MAP were obtained with 35 µg/kg A-18-F-NH₂ (18.2 nmole/kg) and 300 µg/kg F-8-F-NH₂ (277 nmole/kg).

TABLE I

Effects of Prazosin and Guanethidine Pre-treatment
on the Pressor Effects of A-18-F-NH₂ and F-8-F-NH₂

Peptide	Percent Maximal Response	
	Prazosin	Guanethidine
A-18-F-NH ₂ (100 µg/kg)	29.2 ± 3.4*	45.2 ± 1.4*
F-8-F-NH ₂ (100 µg/kg)	44.0 ± 5.8*	65.7 ± 5.53*

*p 0.05 vs control (pre-treatment response).

Effects of Prazosin and Guanethidine Table I shows that prazosin inhibited by 71% the rise in MAP due to a maximum dose of A-18-F-NH₂ and inhibited by 56% the rise in MAP due to F-8-F-NH₂.

Table I also shows the effect of guanethidine pre-treatment on the pressor response to both peptides. As was seen with prazosin, guanethidine pre-treatment more effectively inhibited the ability of A-18-F-NH₂ to raise MAP and had lesser effects on F-8-F-NH₂. In both cases guanethidine pre-treatment did not abolish the effects of A-18-F-NH₂ or F-8-F-NH₂ on MAP.

DISCUSSION

It is now well known that in mammalian CNS, there is a group of peptides which is immunoreactive to antiserum raised against the molluscan cardioexcitatory neuropeptide FMRF-NH₂. Recently, two of such peptides, octapeptide (F-8-F-NH₂)

and octadecapeptide (A-18-F-NH₂), were isolated from bovine brain and chemically characterized (11). These two peptides are not N-terminally extended forms of FMRF-NH₂ and their biological activities remain to be established.

In the present study, F-8-F-NH₂ and A-18-F-NH₂ were found to elevate mean arterial pressure in conscious, unrestrained rats. Previously, using anesthetized rats, FMRF-NH₂ and LPLRF-NH₂ (a FMRF-NH₂ like peptide of chicken brain) were found to evoke pressor responses when injected i.v. or intracisternally (1). FMRF-NH₂, LPLRF-NH₂, F-8-F-NH₂ and A-18-F-NH₂ are structurally quite different, however, they all have the same Arg-Phe-NH₂ at their C-termini. Although the structure-activity relationship of the peptide is not studied here, Arg-Phe-NH₂ may be the molecular feature responsible for the vasopressor responses observed for FMRF-NH₂, LPLRF-NH₂, F-8-F-NH₂ and A-18-F-NH₂. In fact, using FMRF-NH₂ analogues on anesthetized rats, it was found that carboxy terminal Arg-Phe configuration was essential for the pressor activity (6).

The pressor effect of FMRF-NH₂ and LPLRF-NH₂ in the anesthetized rat was suggested to be mediated by release of catecholamines and stimulation of α -adrenergic receptors because the effect of these two peptides was nearly completely blocked by guanethidine and phentolamine (1). In contrast, the pressor effect of F-8-F-NH₂ and A-18-F-NH₂ in the conscious rat was attenuated but not completely blocked by guanethidine or prazosin. Thus, although a portion of activity of F-8-F-NH₂ and A-18-F-NH₂ may be related to release of catecholamines, these two peptides may have actions independent of catecholamine release as well. However, it should be noted that FMRF-NH₂ and LPLRF-NH₂ were tested in anesthetized rats (1) while F-8-F-NH₂ and A-18-F-NH₂ were tested in conscious rats in this study.

These studies indicate that two neuropeptides, A-18-F-NH₂ and F-8-F-NH₂, can elevate MAP in conscious rats; whether these two peptides have physiological roles related to the regulation and maintenance of blood pressure remains to be established.

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The experiments reported herein were conducted according to the principles set forth in the guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHEW, publication No. (NIH) 85.23.

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