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ANF-LIKE PEPTIDE(S) IN THE PERIPHERAL AUTONOMIC NERVOUS SYSTEM

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SUMMARY: The recent demonstration of the atrial natriuretic factor (ANF) within the brain has been extended in the present study by the additional localization of ANF-like activity in the peripheral nervous structures. Using a sensitive radioimmunoassay, it was possible to detect ANF-like immunoreactive peptide(s) in crude and chromatographically separated extracts of parasympathetic rat ganglia. The partially purified ANF-like peptide exhibited a biological action similar to cardiac ANF. This finding supports a possible involvement of ANF in the regulation of both, central and peripheral neuronal activities.

A new hormonal peptide, atrial natriuretic factor (ANF), is produced in mammalian atria, where it can be found in relatively large quantities (1,2,3). Recent studies have also demonstrated the presence of ANF-like immunoreactive peptides in tissues other than the heart (4,5,6). Of great interest and possible importance is the finding that such peptides are present in different anatomical and functional regions of the brain (6,7). This suggests a local intraneuronal production of ANF and a possible involvement of ANF in processes of neurotransmission and/or neuromodulation, the former being supported by the demonstration in the brain of mRNA coding for ANF (8) and the latter by the demonstration of ANF receptors in these regions of the brain (9). By analogy with other neuropeptides such as the vasoactive

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ABBREVIATIONS:

AA: Amino-acid; ACN: Acetonitrile; ANF-IR: ANF-like immunoreactive peptide; AcOH: Acetic acid; ACTH: Adrenocorticotropin 1-24; Arg: Arginine; BSA: Bovine serum albumin; DNA-ase: Deoxyrybonucleic acid hydrolase; EDTA: Disodium ethylendiamine tetraacetate; KRBB: Krebs-Ringer bicarbonate buffer; PMSF: Phenylmethylsulfonyl fluoride; RIA: Radioimmunoassay; RP-HPLC: Reverse-phase, high performance liquid chromatography; Ser: Serine; TFA: Trifluoroacetic acid; Tyr: Tyrosine.

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intestinal polypeptide, substance P and several others (10,11,12), one might anticipate that ANF would also be present and fulfill a function in the peripheral autonomic nervous system. Here we present evidence that ANF-like immunoreactivity is not restricted to the central nervous system, but can also be found in the autonomic ganglia of the rat.

MATERIALS AND METHODS

1. Extraction procedure. 220 female Sprague-Dawley rats weighing 170-400 g allowed free access to food and water maintained under controlled conditions of light, heat and humidity, were used in this study. Both ganglia nodosa of the vagus system (usually 40 ganglions were collected/1 ml of acid) were removed from the decapitated animals, blotted dry and placed in glass tubes containing 1 ml of 0.1 M AcOH and inhibitors of endogenous proteases in the following final concentrations: Pepstatin A 5 µM, PMSF 10 µM and EDTA 27 µM. After homogenization in a Polytron homogeniser for 30 sec. at high speed, the homogenates were centrifuged for 20 minutes at 10,000 x g at 4°C.

Activated Vycor glass beads (Corning Glassware, Corning N.Y.) were used for extraction of ANF-IR, as previously described (13). The lyophilized samples were stored frozen at -20°C until further processing.

2. RP-HPLC. The lyophilized ganglion extract was dissolved in a mobile phase composed of 15% ACN in 0.1% TFA and injected onto a C_{18} µ Bondapack column (0.39 X 30 cm) in a LKB-HPLC system. The sample was eluted with a linear gradient (15% to 50%) of ACN at a flow rate of 1 ml/min. Two ml fractions were collected and assayed separately for ANF immunoreactivity. Fractions 14 to 22 (28-44 min) corresponding to the lower molecular weight peptides (25-30 AA) were rechromatographed on RP-HPLC under the same conditions after concentration in a Speed-Vac.

3. Radioimmunoassay for ANF. This was performed essentially as previously described (13). Using the second antibody precipitation for separation of free from antibody-bound tracer, the sensitivity of the method was 0.39 pg.

4. Zona glomerulosa cell suspension. The suspension of rat adrenocortical cells was prepared as previously published (14, 15). After the adrenal capsules were digested with collagenase and DNA-ase, the dispersed cells were centrifuged, washed, and resuspended in KRBB containing 0.2% BSA to give 2 X 10^5 cells per 0.5 ml of medium. After evaporation to 25% of the original volume, 50 µl of individual fractions from HPLC were added to the cell suspension prior to the addition of ACTH (10^{-8} M). All assays were carried out in quadruplicate. After a 2 h incubation at 37°C in 5% CO_2 in air, the cells were centrifuged and the medium frozen at -20°C until assayed for aldosterone by RIA.

RESULTS

The radioimmunoassay employed in the present study is specific and sensitive using antibodies which have been demonstrated not to crossreact with several non-ANF-related peptides (16). As seen in Figure 1, the ANF-IR in ganglion homogenates from one series of 40 rats gave a competition curve parallel to that of synthetic 26 AA ANF (Arg 101-Tyr 126) in the radio-

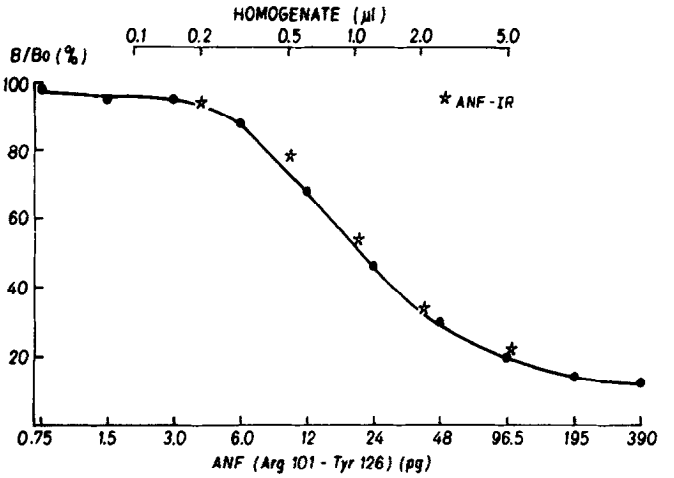

Figure 1:

Standard curve for synthetic ANF (Arg 101-Tyr 126) and ANF immunoreactivity in homogenate of the ganglia nodosa. Scale of the abscissa in Ln.

immunoassay system. For the second purification by RP-HPLC (illustrated in Figure 2) only a small part of the original ANF-IR was recovered from fractions where lower molecular forms of ANF are found. Three distinct immunoreactive peaks were obtained with the main ANF-IR peak eluting with 33% acetonitrile; this corresponds to the elution conditions of the synthetic 28 AA peptide (Ser 99 - Tyr 126). In order to investigate whether this extracted ANF-like substance also shares a common biological activity with ANF of cardiac origin, we tested the inhibition of the ACTH-stimulated aldosterone release from zona glomerulosa cells (17). The basal aldosterone

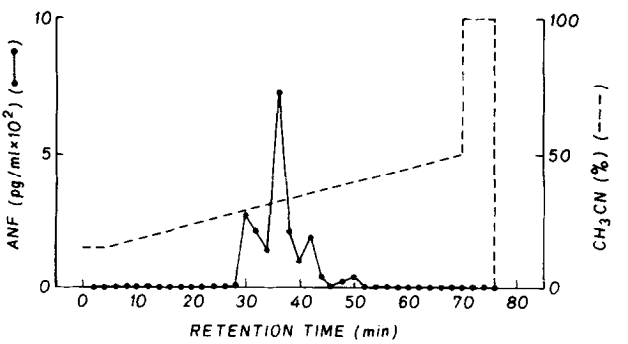

Figure 2:

Elution pattern of the ANF immunoreactivity from ganglia nodosa rechromatographed on RP-HPLC.

production was 1.1 ± 0.1 ng/2 X 10^5 cells (mean ± SEM) and in the presence of 10^{-8}M ACTH, it increased to 34.8 ± 2.0 ng/2 X 10^5 cells. The extract from fraction 18 (36 min.) (Figure 2) was able to inhibit the stimulatory effect of ACTH on aldosterone release by 26% (to 25.9 ± 2.6 ng/2 X 10^5 cells). The fractions which did not contain ANF-IR did not alter the stimulated aldosterone secretion of dispersed cells. The limited quantity of the studied substance(s) did not allow us to measure the influence of higher concentrations on aldosterone release under basal and stimulated conditions.

DISCUSSION

ANF belongs to a newly discovered family of peptides with predominantly natriuretic, diuretic and vasodilating actions (18). The synthetic forms of several atrial peptides are already available and a specific, sensitive radioimmunoassay has been developed (13). ANF-like material has been demonstrated in histological sections of brain and salivary gland (4, 6, 7) and can also be detected by RIA in chromatographically separated fractions of the former (5). This indicates that ANF is present and perhaps synthesized in tissues other than the atria. In the present study we have demonstrated the existence of ANF-IR in ganglia nodosa of the rat. To the best of our knowledge, this is the first report of an ANF-like peptide in the peripheral autonomic nervous system. During the isolation procedure we found that among lower molecular forms of ANF-IR, the predominant compound eluted from HPLC behaved similarly to synthetic ANF composed of 28 AA (Ser 99-Tyr 126). Interestingly, the ANF-IR appears to be recognized not only by the antibodies against synthetic ANF, but also by one of the target cells of ANF, zona glomerulosa cells of rat adrenals. Despite the large number of rats employed, only limited amount of the peptide was available in our study; nevertheless, the results suggest a high degree of similarity between cardiac (synthetic) and ganglionic ANF.

It should be kept in mind that the vagus nerve is composed in large majority of sensory fibers (19). It conducts impulses via an afferent

pathway from the cardio-pulmonary area, including reflexes from cardiac baroreceptors (20). Interestingly, it has been shown that ANF itself is capable of activating those sensory receptors (21). The present study indicates that ANF may be present even in the neurons of the afferent pathway. It is conceivable that ANF can be released from the nerve endings of such neurons and modulate the threshold of its own nerve endings (if they exist) as has been proposed for sensory neurons containing substance P (22). Albeit attractive, this possibility remains to be proved. Currently, studies are underway which aim to specifically localize ANF in peripheral neuronal tissues and to evaluate the biochemical and biological properties of extracted ANF-IR. Preliminary results indicate the presence of ANF-IR at the sympathetic side of the peripheral autonomic nervous system in even higher concentrations than those found in the ganglia nodosa.

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