Vol. 122, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 18, 1984 Pages 381-387

CORTICOTROPIN-RELEASING FACTOR IS CONTAINED WITHIN PERIKARYA AND NERVE FIBRES OF RAT DUODENUM

Hermann J. Wolter

Universität Ulm, Abteilung Anatomie, 7900 Ulm, Oberer Eselsberg, FRG

Received June 11, 1984

SUMMARY: Immunofluorescence microscopic studies revealed a corticotropin-releasing factor (CRF) staining within both myenteric plexus perikarya and nerve fibres of the rat duodenum. A CRF-immunofluorescence could be visualized also within nerve fibres close associated with myenteric and submucous blood vessels. Even the lamina propria contained CRF-immunoreactive nerve fibres, which were obviously often localized near the basal lamina.

Recently, Vale et al. isolated and characterized the 41-amino acid residue CRF from ovine hypothalami (1). It has an amino acid sequence nearly homologous to rat and human CRF (2-4) and is cleaved by proteolytic processing from its precursor molecule, the prepro-CRF (29). In light microscopic studies a CRF immunostaining has been demonstrated mainly within the parvocellular perikarya subpopulation of the rat hypothalamic paraventricular nucleus, but also within neuronal cell bodies lying scattered through both the rat preoptic and sheep arcuate nucleus (5-9,18). CRF immunoreactive nerve fibres were seen abundantly in the median eminence where they were often in close contact with capillaries of the primary portal plexus. Neurons and nerve fibres stained for CRF could be demonstrated also in the rat spinal cord and medulla oblongata (10,11).

In the rat fetus a CRF immunostaining was detected within paraventricular nucleus perikarya as well as within nerve fibres and terminals in the median eminence (12).

Numerous mucosal cells stained for CRF have been visualized in the human gastric antrum. However, no CRF immunoreactivity occured within structures of the enteric nervous system (13).

This study was conducted to examine CRF immunofluorescence within the rat enteric nervous system. These

0006-291X/84 $1.50

findings are discussed in regard to those found in CRF stimulation tests on the hypothalamo-hypophysial axis.

MATERIALS and METHODS

Tissue preparation. Male, colchicine-untreated Wistar rats (200-300 g) were anesthetized with Nembutal (60 mg/kg), the duodenum dissected and cutted immediately into 10 µm-sections on a cryostat microtome (-25°C). The sections were subsequently immersed in freshly depolimerized 4 % paraformaldehyde (in 100 mM phosphate-buffered saline, PBS; pH 7.4) for 90 min, 4°C, rinsed several times in the same buffer and stored overnight in PBS containing 5 % sucrose, 4 % normal swine serum. Thereafter, the sections were processed for immunofluorescence microscopy as previously reported (14,15).

Tissue staining. The CRF-antiserum, raised against synthetic ovine CRF (Immuno Nucl. Corp.), was used at a dilution of 1:100 (in PBS, pH 7.4 containing 0.3 % Triton X-100, 4 % normal swine serum). The sections were incubated for 16 hr followed by incubation with fluorescein-isothiocyanate (FITC-)conjungated swine anti-rabbit IgG (Dakopatts) (1:40 in PBS, 0.3 % Triton X-100, 4 % normal swine serum) for 30 min at room temperature. Then, the sections were washed extensively in PBS, examined and photographed with a Leitz Orthoplan microscope fitted with an epi-fluorescence attachment.

Specificity controls. The CRF-antiserum was preincubated with synthetic CRF-related (ovine CRF, sauvagine, renin) and CRF-unrelated (melanotropin, α-MSH; β-endorphin, β-END; Met-enkephalin, and adrenocorticotropin, ACTH) peptides (1-10 µM). Some sections were also incubated with normal swine serum in place of CRF-antibodies.

Only pre-absorption of the CRF-antiserum with synthetic ovine CRF could prevent CRF staining. Sections incubated with this pre-absorpted antiserum revealed only autofluorescent, orange-coloured, cells in the lamina propria and submucosa of the rat duodenum.

RESULTS and DISCUSSION

Using a specific CRF-antiserum directed against synthetic ovine CRF it was possible to see CRF immunofluorescence within both neuronal cell bodies and nerve fibres of the rat myenteric plexus. Some CRF-positive perikarya were localized intramurally in the longitudinal and circular muscle layer. Only a few myenteric perikarya stained by the CRF-antiserum could be visualized in the rat duodenum (Fig. 1A and 1B). Here and there, CRF-positive nerve fibres could be seen in close contact with submucosal and myenteric blood vessels, probably arterioles (Fig. 1C and 1D). Immunoreactive nerve

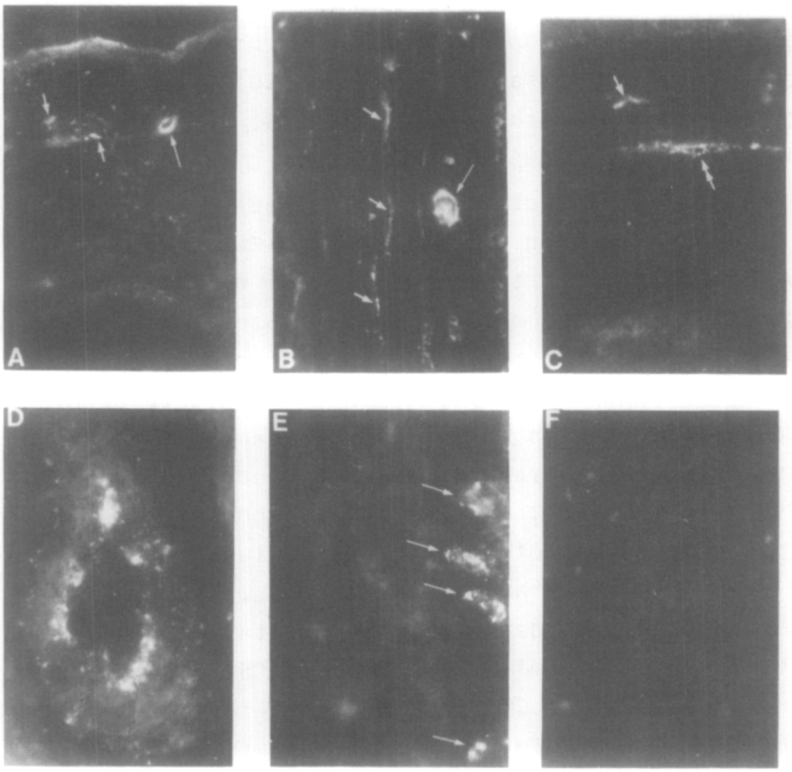

Fig. 1. Corticotropin-releasing factor (CRF) immunofluorescence in the nervous system of the rat duodenum. CRF-stained perikarya (long arrows) lying in the myenteric plexus (Fig. 1A) and intramurally (Fig. 1B) as well as beaded nerve fibres (short arrows) running through the longitudinal (Fig. 1A) and circular muscle layer (Fig. 1B) are clearly recognized. CRF-immunofluorescent nerve fibres are in close contact with myenteric and submucosal blood vessels, probably arterioles (Fig. 1C and 1D). The lamina propria of the rat duodenum contains numerous heavily fluorescent CRF-stained nerve fibres (long arrows) lying nearly the villious basal lamina (Fig. 1E). Autofluorescent, orange-coloured, cells are to reveal occassionally in the submucosa and lamina propria on adjacent serial sections incubated with CRF-antiserum (pre-absorbed with 10 µM synthetic ovine CRF).At this neither CRF-immunofluorescent perikarya nor nerve fibres are to see (Fig. 1E).

fibres located in the lamina propria were obviously often associated with the villious basal lamina (Fig. 1E). No CRF-immunofluorescent neuronal cell bodies or nerve fibres were revealed in the submucous plexus of the rat duodenum.

In blocking tests the pre-absorption of the CRF-antiserum with synthetic ovine CRF prevented all CRF-stainings and resulted in only autofluorescent, orange-coloured cells lying scattered in the submucosa and lamina propria (Fig. 1E).

The fact that no CRF immunostaining was found in the enteric nervous system of the human gastrointestinal tract

(13) might be due to inappropriate tissue preparation and staining techniques, but also to the possibility that CRF is really not present within the nerve tissue of the human intestine.

In previously conducted immunofluorescence microscopic studies an immunostaining for α-MSH and ß-END as well as ACTH was demonstrated in the mammalian gut within myenteric plexus perikarya (14,15) and its nerve fibres (14-16). These peptides are cleaved enzymatically from a common precursor molecule, the pro-opiomelanocortin (POMC) (17), to be processed to bioactive forms during axonal transport to nerve terminals. Various authors have demonstrated in biochemical studies that α-MSH (30), ß-END (31-34), and ACTH are present in distinct cell groups of both the mammalian pituitary and brain in their bioactive and inactive forms. The release of these POMC-derived peptides from rat pituitary cells is regulated by the hypothalamic CRF-secretion. Stimulation tests with the synthetic ovine CRF led to a dose-related increase of α-MSH, ß-END, and ACTH from rat pituitary cells *in vivo* and *in vitro* (1,19-21,26). These CRF effects could be abolished by both immunoneutralization with specific CRF-antibodies and prior treatment with corticosterone (1). Stress, on the other hand, results in increased α-MSH, ß-END, and ACTH plasma levels via stimulation of the hypothalamic CRF-release (22-25,27,28).

The immunohistochemical findings presented now raise three important questions: first, is the CRF-material found in the enteric nervous system of the rat duodenum of intrinsic origin, second, does this duodenal CRF regulate the synthesis and release of α-MSH, ACTH, and ß-END in the same manner as the hypothalamic CRF regulates the hypophysial peptides and, third, does stress provoke a simultaneously release of both the hypothalamic and duodenal CRF from neuronal cell bodies?

It is well-known that stress and long-term administration of adrenocorticosteroids and ACTH result in ulceration of the human stomach and duodenum (gastroduodenal ulcera) (35,36). Intracisternal injection of ovine CRF, on the other hand, results in a dose-related inhibition of gastric acid secretion mediated through centrally adrenal and vagal mechanisms (37). This CRF effect is not gastrin-dependent, because intracisternal injection of CRF elevates plasma

gastrin, which is a potent gastric acid stimulating peptide (38). Both CRF effects are blocked by adrenalectomy (37,38). These neurophysiological findings may indicate that the hypothalamic as well as the duodenal CRF are involved in the genesis of stress- and chemically induced gastrointestinal ulcera.

ACKNOWLEDGEMENTS

I wish to thank Professor M. Herrmann for the valuable help.

REFERENCES

1. Vale, W., Spiess, J., Rivier, C. and Rivier, J. (1981) Science 213, 1394-1397.

2. Rivier, J., Spiess, J. and Vale, W. (1983) Proc. Natl. Acad. Sci. USA 80, 4851-4855.

3. Spiess, J., Rivier, J., Rivier, C. and Vale, W. (1981) Proc. Natl. Acad. Sci. USA 78, 6517-6521.

4. Shibahara, S., Morimoto, Y., Furutani, Y., Notake, M., Takahashi, H., Shimizu, S., Horikawa, S, and Numa, S. (1983) J. EMBO 2, 775-779.

5. Bugnon, C., Fellmann, D., Gouget, A. and Cardot, J. (1982) Neurosci. Lett. 30, 25-30.

6. Bloom, F.E., Battenberg, E.L.F., Rivier, J. and Vale, W. (1982) Regul. Peptides 4, 43-48.

7. Swanson, L.W., Sawchenko, P.E., Rivier, J. and Vale, W. (1983) Neuroendocrinology 36, 165-186.

8. Antoni, F.A., Palkovits, M., Makara, G.B., Linton, E.A., Lowry, P. and Kiss, J.Z. (1983) Neuroendocrinology 36, 415-423.

9. Palkovits, M., Brownstein, M. and Vale, W. (1983) Neuroendocrinology 37, 302-305.

10. Schipper, J., Steinbusch, H.W.M., Vermes, I. and Tilders, F.J.H. (1983) Brain Res. 267, 145-150.

11. Merchenthaler, I., Hynes, M.A., Vigh, S., Schally A.V. and Petrusz, P. (1983) Brain Res. 275, 373-377.

12. Bugnon, C., Fellmann, D., Gouget, A. and Cardot, J. (1982) Nature 298, 159-161.

13. Nieuwenhuijzen Kruseman, A.C., Linton, E., Lowry, P.J., Rees, L.H. and Besser, G.M. (1982) Lancet 8310 ii, 1245-1246.

14. Wolter, H.J. (1983) Biochem. Biophys. Res. Commun. 117, 568-573.

15. Wolter, H.J. (1984) Brain Res. 295, 378-384.

16. Sundler, F., Alumets, J., Ekman, R., Hakanson, R. and Van Wimersma Greidanus, T.B. (1981) J. Histochem. Cytochem. 29, 1328-1335.

17. Nakanishi, S., Inoue, A., Kita, T., Nakamura, M., Chang, A.C.Y., Cohen, S.N. and Numa, S. (1979) Nature 278, 423-427.

18. Bugnon, C., Fellmann, D., Gouget, A. and Cardot, J. (1982) C.R. Hebd. Seances Acad. Sci. Ser. D 294, 279-284.

19. Proulex-Ferland, L., Labrie, F., Dumont, D., Côté, J., Coy, D.H. and Sveiraf, J. (1982) Science 217, 62-63.

20. Meunier, H., Lefevre, G., Dumont, D. and Labrie, F. (1982) Life Sci. 31, 2129-2135.

21. Rivier, C., Brownstein, M., Spiess, J., Rivier, J. and Vale, W. (1982) Endocrinology 110, 272-278.

22. Brown, M.B., Fisher, L.A., Spiess, J., Rivier, C., Rivier, J. and Vale, W. (1982) Endocrinology 111, 928-931.

23. Sutton, r.E., Koob, G.F., Le Moal, M., Rivier, J. and Vale, W. (1982) Nature 297, 331-333.

24. Britton, D.R., Koob, G.F., Rivier, J. and Vale, W. (1982) Life Sci. 31, 363-368.

25. Fisher, L.A., Rivier, J., Rivier, C., Spiess, J., Vale, W. and Brown, M.R. (1982) Endocrinology 110, 2222-2224.

26. Baird, A., Wehrenberg, W.B., Shibasaki, T., Benoit, R., Chong-Li, Z., Esch, F. and Ling, N. (1982) Biochem. Biophys. Res. Commun. 108, 959-964.

27. Rivier, C. and Vale, W. (1983) Nature 305, 325-327.

28. Akil, H., Watson, S.J., Barchas, J.D. and Li, C.H. (1979) Life Sci. 24, 1659-1665.

29. Furutani, Y., Morimoto, Y., Shibahara, S., Noda, M., Takahashi, H., Hirose, T., Asai, M., Inayama, S., Hayashida, H., Miyata, T. and Numa, S. (1983) Nature 301, 537-540.

30. Evans, C.J., Lorenz, r., Weber, E. and Barchas, J.D. (1982) Biochem. Biophys. Res. Commun. 106, 910-919.

31. Weber, E., Evans, c.J. and Barchas, J.D. (1981) Biochem. Biophys. Res. Commun. 103, 982-989.

32. Smyth, D.G. and Zakarian, S. (1980) Nature 288, 613-615.

33. Zakarian, S. and Smyth, D.G. (1982) Nature 296, 250-252.

34. Zakarian, S. and Smyth, D.G. (1979) Proc. Natl. Acad. Sci. USA 76, 5972-5976.

35. Messer, J., Reitman, D., Sacks, H.S., Smith, H. and Calmers, T.C. (1983) N. Engl. J. Med. 309, 21-24.

36. Spiro, H.M. (1983) N. Engl. J. Med. 309, 45-47.

37. Taché, Y., Goto, Y., Gunion, M.W., Vale, W., Rivier, J. and Brown, M. (1983) Science 222, 935-937.

38. Taché, Y. and Rivier, J. (1982) Soc. Neurosci. Abstr. 8, 287.