Hiroshima Journal of Medical Sciences
Vol.36, No.3, 315~318, September, 1987
HIJM 36-45

A Galanin-Like Peptide in the Colon of the Golden Hamster

Shinya KISHIMOTO, Hirofumi KOBAYASHI, Norio TAKABA, Reiko KONEMORI, Satoru SHIMIZU, Masahiro YAMAMOTO, Takaji TAMARU, Goro KAJIYAMA and Akima MIYOSHI

The First Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan

(Received June 25, 1987)

Key words: Golden hamster, Galanin, Cell body, Fiber, Submucosal plexus, Myenteric plexus

ABSTRACT

Galanin-like immunoreactive peptide was studied in the colonic wall of the golden hamster using the immunoflurescent technique. Galanin-like immunoreactivity was observed mainly in cell bodies of the myenteric plexus and in fibers in the lamina propria mucosae and in the circular muscle layer. The submucosal plexus and submucosal connective tissue, however, contained very little galanin-like immunoreactive peptide. This peptide appears to be instrinsic mainly to the colon. The presence of the peptide indicates some possible biological roles in the colonic function.

A peptide containing 29 amino acid residues was recently isolated from porcine upper intestine. It was named galanin because of its N-terminal glycine and its C-terminal alanine amide residue[12]. One of actions is to cause the contraction of smooth muscle of the gastrointestinal and genitourinary tracts[5,8,12]. The only structural similarities to other peptides are present at the C-terminus, where there is a correspondense to physelamin in two, substance P in three, and gonodoliberin in four amino acids, respectively. The immunoreactivity of galanin has been reported to exist in the intestine[1,4,6], central nervous system[2,6,10,11], and genitourinary tracts of some mammals. In the present study the authors have tried to investigate the existence of histochemical immunoreactivity of galanin in the golden hamster colon.

MATERIALS AND METHODS

Male golden hamsters, weighing about 40 g, were killed and their abdomens were cut open. Then the entire colon was resected and samples (0.5 cm in length) were taken from the following four parts of the colon: ascending, transverse, descending, and rectum. Each specimen was fixed in p-bezoquinone in 0.01M phosphate buffered saline at pH 7.4 (PBS) for 3 hr at 4°C and then washed in 7% sucrose in PBS at 4°C, four to six times. The fixed samples were made into cryostat blocks by freezing them on a specimen matrix and samples of 20 μ in thickness were cut at −35°C. The sections were then placed on poly-L-lysin coated glass slides and air dried.

For evaluation of histology prior to the immunohistochemistry, tissue (0.5 × 0.5 cm in size) from each region was also fixed in Bouin's solution for 2 hr and embedded in paraffin wax. Sections 3 μ in thickness were cut on a Jung microtome, dewaxed, and stained with hematoxylin and eosin.

The antiserum against galanin (kindly gifted by Professor Julia M. Polak, Hammersmith Hospital, London) has used in this study.

The cryostat sections were studied by the indirect immunofluorescence method of Coons and his workers[3].

The control for immunostaining included preabsorption of the primary antiserum with synthetic galanin (PLI Co), and normal rabbit serum was used as the first layer in place of a

primary antiserum. In this control study the galanin first layer was also completely ommitted.

RESULTS

Conventional histopathological examinations showed that the epithelia and villi of each part of the colon were usually intact and both circular and longitudinal muscular layers were also normal (Fig. 1).

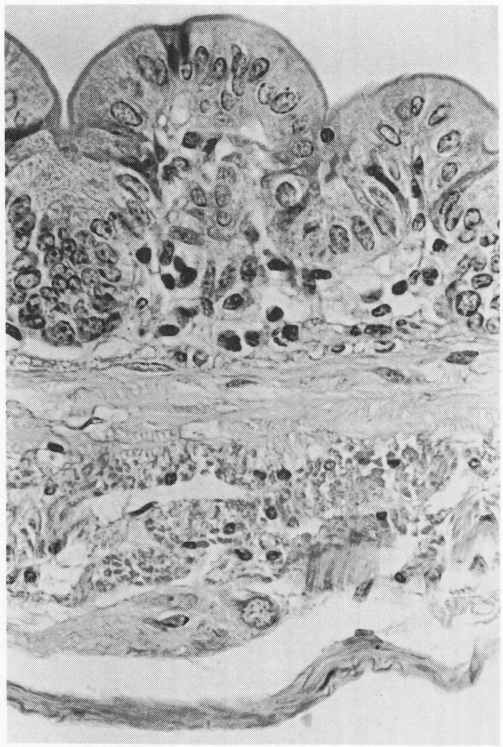

Fig. 1. Histology of the normal colon of the golden hamster (HE stain, × 100).

Galanin-like immunoreactivities exist in the nerve cell body of both the submucosal and myenteric plexuses in three parts of the colon and rectum (Fig. 2 and 3). The varicose nerve fibers with galanin-like immunoreactivities which originated from these nerve cells of the myenteric plexuses, run parallel to circular muscles (Fig. 4). It seemed that the nerve fibers end at the smooth muscle cells. The nerve fibers from the submucosal plexus were projecting upward towards the top of the mucosal surface and surrounded the epithelial cells (Fig. 5 and 6).

After absorption of galanin antiserum with galanin, none of the immunofluorescent structures described above were observed.

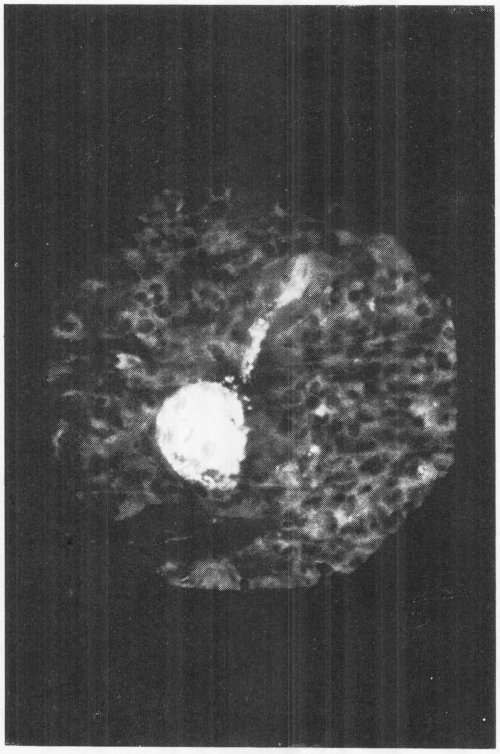

Fig. 2. A galanin-like immunoreactive cell body in the submucosal plexus of the colon (IF, × 400).

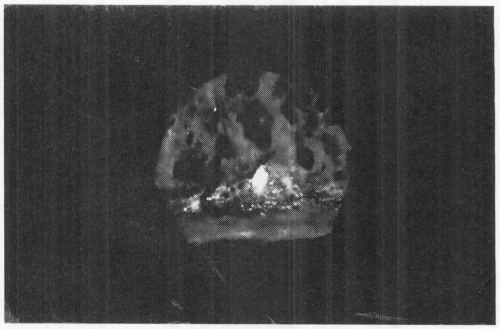

Fig. 3. Galanin-like immunoreactive cell bodies in the myenteric plexus of the colon (IF, × 200).

DISCUSSION

Galanin-like immunoreactivity has been demonstrated in the nerves of the gastrointestinal tract and/or the central nervous system of several mammals including man[1,2,4,6,10,11]. Therefore, it

Fig. 4. The varicose galanin-like immunoreactive fibers were observed in the circular muscle layer. The fibers were seen originating from the myenteric plexus and running parallel to the circular muscle (IF, × 200).

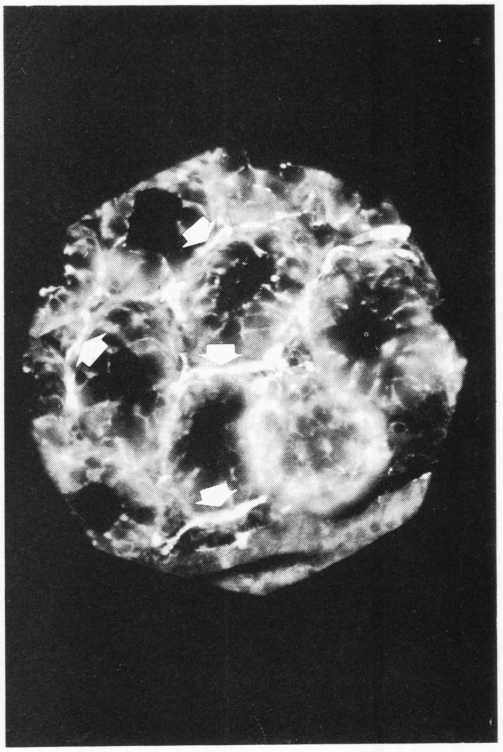

Fig. 5. These fibers were going upward the top of the mucosal surface (IF, × 200).

may be called galanin nerve[4]. In the colon of the golden hamster, however, the galanin-like immunoreactivity, galanin nerve, has been not ascertained. The present results showed that galanin nerves and their fibers also existed in the golden hamster colon. Galanin-like immunoreactivity was exclusively observed in the myenteric plexus and in the fibers of the circular layer. Galanin-like immunoreactive fibers, however, were almost negative in the longitudinal muscle layer. Furthermore, the lamina propria mucosae contained a dense network of galanin-like immunoreactive fibers surrounding colonic glands. These suggest that galanin may be involved in motility like segmentation of the colon and intestinal secretion. Only a few galanin-like immunoreactive nerves and fibers were seen in submucosal plexus and the submucosal connective tissue. Many galanin-like immunoreactive cell bodies were particulary observed in the myenteric plexus but not many were observed in the submucosal plexus.

Considerable species differences were noticed in the distribution of galanin-like immunoreactivity by Melander et al[7]. The golden hamster cell bodies in the submucosal plexus had a sparse galanin-like immunoreactivity, whereas they were numerous there in the rat, mouse, guinea-pig, and pig[7]. And further a dense innervation of the lamina propria mucosae in the golden hamster of this study was observed just as like in pig and guinea pig colon studies. However the lamina mucosae had a sparse galanin-like immunoreactive network in the rat and mouse studies. Negative galanin-like immunoreactivities, however, do not always denote the non-existence of the peptide, because it may be due to low peptide concentrations and/or to a low sensitivity of the technique, as suggested by Melander et al[7]. One must be careful in interpreting the data.

The origin of the galanin-like immunoreactive fibers in the colonic wall of the golden hamster

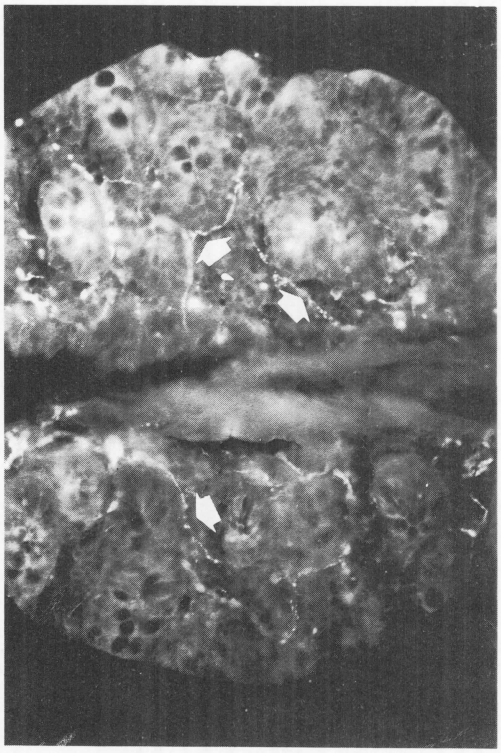

Fig. 6. The galanin-like immunoreactive fibers are also seen surrounding colonic glands (IF, × 200).

has not been established in this study. However, all cell bodies observed, contained galanin-like immunoreactivity in the colonic wall of the golden hamster, suggesting that the fibers may have an intrinsic origin.

It has been suggested that there are some structural differences in the porcine galanin between various different species[7,9]. Taking this into consideration, we may hypothesize that hamster galanin may be different in its chemical structure from porcine galanin. Further study will be necessary on this point.

REFERENCES

1. **Bauer, F.E., Adrian, T.E., Christofides, N.D., Ferri, G-L., Yanaihara, N., Polak, J.M. and Bloom, S.R.** 1986. Distribution and molecular heterogeneity of galanin in human, pig, guinea pig, and rat gastrointestinal tracts. Gastroent. **91**: 877-883.
2. **Bauer, F.E., Christofides, N.D., Hacker, G.W., Blank, M.A., Polak, J.M. and Bloom, S.R.** 1986. Distribution of galanin immunoreactivity in the genitourinary tract of man and rat. Peptides **7**: 5-10.
3. **Coons, A.H., Leduc, E.H. and Connolly, J.M.** 1955. Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application of a study of the hyperimmune rabbit. J. Exptl. Med. **162**: 49-60.
4. **Ch'ng, J.L.C., Christofides, N.D., Anand, P., Gibson, S.J., Allen, Y.S., Su, H.C., Tatemoto, K., Morrison, J.F.B., Polak, J.M. and Bloom, S.R.** 1985. Distribution of galanin immunoreactivity in the central nervous system and the response of galanin containing neuronal pathways to injury. Neurosic. **16**: 343-354.
5. **Ekbald, E., Hakanson, R., Sundler, F. and Wahlestedt, C.** 1985. Galanin: neuromodulatory and direct contractile effects on smooth muscle preparations. Br. J. Pharmacol. **86**: 241-246.
6. **Ekbald, E., Rokaeus, A., Hakanson, R. and Sundler, F.** 1985. Galanin nerve fibers in the rat gut: distribution, origin and projections. Neurosci. **16**: 355-363.
7. **Melander, T., Hokfelt, T., Rokaeus, A., Fahrenkrug, J., Tatemoto, K. and Mutt, V.** 1985. Distribution of galanin-like immunoreactivity in the gastrointestinal tract of several mammalian species. Cell Tissue Res. **239**: 253-270.
8. **Ohhashi, T. and Jacowitz, D.M.** 1985. Galanin potentiates electrical stimulation and exogenous norepinephrine-induced contractions in the rat vas deferens. Regul. Pept. **12**: 163-171.
9. **Rokaeus, A., Melander, T., Hökfelt, T., Lundberg, J., Tatemoto, K., Carquist, M. and Mutt, V.** 1984. A galanin-like peptide in the central nervous system and intestine of the rat. **47**: 161-166.
10. **Skofitsch, G. and Jacowiz, D.M.** 1985. Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. Peptides **6**: 509-546.
11. **Skofitisch, G. and Jacowiz, D.M.** 1985. Galanin-like immunoreactivity in capsaitin sensitive sensory neurons and ganglia. Brain Res. Bull. **15**: 1981-195.
12. **Tatemoto, K., Rokaeus, A., Jornvall, T.J. and Mutt, V.** 1983. Galanin a novel biologically active peptide from porcine intestine. FEBS Lett. **164**: 124-128.