Fluorescent Retrograde Labeling of Serotonergic (5HT) Neurons in the Central Nervous System (CNS) of the Rat

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Central 5HT projections to the cerebral cortex, cerebellum and spinal cord were studied by using histofluorescence method for monoamines combined with retrograde fluorescent dye technique.

Dye injections were made with two sets in individual animals; A) PI into precingular cortex, and DAPI into cerebellum. B) DAPI into cerebellum and NY into lumbar spinal cord. The 5HT cells labeled by these dyes were easily differentiated from non-labeled 5HT cells under the fluorescence microscope.

Result; 1) Cerebral 5HT fibers were mainly derived from the dorsal raphe (B7), median raphe (B8) and B9. 2) Cerebellar 5HT fibers were mainly derived from B7 and B8. 3) Spinal cord 5HT fibers were mainly derived from the raphe pallidus (B1), raphe obscurus (B2) and raphe magnus (B3). 4) A small number of cells in B7 and B8 sent divergent axons to both the cerebrum and cerebellum. However, we failed to find any divergent axons projecting to both the cerebellum and spinal cord. In addition, we can easily find out DAPI labeled cells in B3, but majority of them did not show 5HT fluorescence.

PI: propidium iodide DAPI: 4,6-diamidino-2-phenylindol HCl NY: nuclear yellow

Fluorescence histochemical and biochemical studies on monoamines in rat neostriatum

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The dopamine (DA) and 5-hydroxytryptamine (5-HT) characteristics of rat neostriatal fluorogenic compound were investigated by quantitative microfluorimetry in combination with biochemical analysis of monoamines.

Wistar male rats (250-300g) were used. p-Chlorophenylalanine (PCPA, 300mg/kg) and alpha-methylparatyrosine (alpha-MT, 250 mg/kg) were injected i.p. 24-72 h and 4 h before sacrifice, respectively. Formaldehyde induced fluorescence microscopy was applied to the neostriatum. The specimens were exposed to formaldehyde of 75% relative humidity at 80°C for 1 h. The fluorescence intensity of the neostriatum was examined with a Zeiss fluorescence microscope (MPM01 system). Simultaneous assay of DA and 5-HT in each tissue sample was performed by a high performance liquid chromatograph (Yanagimoto, L-2000L).

The fluorescence intensity reduction in the neostriatum of the rats treated with PCPA and alpha-MT was about 20 % of alpha-MT alone. The administration of PCPA caused the marked decrease in 5-HT alone.

Adrenergic and Cholinergic Innervation of Bovine Mesenteric Lymphatics

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Physiological and pharmacological studies of innervation of isolated bovine mesenteric lymphatics suggest that both postganglionic sympathetic nerves and non-adrenergic inhibitory nerves are distributed in the lymphatic wall (Ohhashi and Roddie, Am. J. Physiol., 240: H498-H504, 1981). We have carried out a histochemical demonstration of both aminergic and cholinergic nerves of the lymphatics by the consecutive use of the glyoxylic method and Karnovsky's technique on the whole mount preparation and the transverse specimen cut by a cryostat. They were observed and photographed under a fluorescence and a tungsten light microscope, respectively. Aminergic and cholinergic nerves were distributed densely within the smooth muscle layers as well as in the adventitia of the lymphatic wall. Secificity of cholinergic fibers was confirmed by eliminating the pseudocholine esterase activity using 10^{-4}M iso-OMPA solution added to the incubation medium.

A STABLE AND SIMPLE METHOD OF "FAGLUPAGAS FIXATION" FOR CATECHOLAMINES IN AIM OF ROUTINE EXAMINATION

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The wet fluorescence histochemistry using GA or Faglu with cryostat sections is highly sensitive for detecting catecholamines with simple procedures. The GA cryostat method is rapid and sensitive than FA cryostat method. In the present study, we used various organs of animal and human immersed overnight in FA GLUPAGAS. The tissue pieces were then frozen sectioned. Cryostat sections were immersed in FAGLUPAGAS. Then these sections were immersed in FAGLUGAS which was omitted PA. After completely washing out yellow color of PA, the sections were mounted on glass slides, air dried and finally embedded in Entellan. This method possessed a number of attractive features. It had a high sensitivity for CA neurons, and the procedure was relatively simple and stable. This FAGLUPAGAS method allowed us to observe CA fluorescence in various organs by immersion fixation. For experiments, the technique can be combined with various methods, such as retrograde axonal transport of fluorescent dyes or AChE histochemistry. Thus, this technique seems suitable for routine examination of human materials in clinical pathological diagnosis.

FA: 4% paraformaldehyde, GLU: 0.5% glutaraldehyde, PA: 0.2% picric acid, GA: 2% glyoxylic acid, S: 15% sucrose.