Autoradiographic studies of efferent nerves from the celiac ganglion in the cat

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Summary. Postganglionic sympathetic fibers originating in the celiac ganglion can be traced by autoradiography to the stomach, spleen, liver and pancreas.

Autoradiography is a method widely used for tracing pathways in the CNS but its use in the peripheral nervous system has been more limited[1-5]. Recently Kelts[6] has reported using this method to trace efferent fibers from the left celiac ganglion of cats to the contralateral celiac ganglion, satellite ganglia between the 2 celiac ganglia, and the adrenal gland. The present study followed the postganglionic sympathetic fibers originating in the left celiac ganglion of cats as they extended further to the stomach, spleen, liver and pancreas.

Cats were anesthetized with Ketaset and a ventral midline incision was made in order to locate the ganglion. From 5 to 15 µl of tritium-labeled leucine (1 mCi/ml) was injected into the ganglion by means of a Hamilton syringe. Post injection periods from 48 to 98 h allowed for diffusion and anterograde axonal transport of the amino acid. The animals were then anesthetized and transcardially perfused with 10% buffered neutral formalin. A portion of the abdominal aorta containing the celiac trunk and the superior mesenteric artery with the celiac and superior mesenteric ganglia intact was removed. Branches of the celiac artery along with their accompanying nerves were traced to the organs studied. Sections of the celiac artery with its surrounding tissue were excised. Tissue from the region of the greater curvature of the stomach, from the superior pole of spleen, from the posterior lobe of the liver and from the omental area of the pancreas about 3 cm from the spleen was removed. All tissue was embedded in paraffin and serially sectioned. The sections were mounted on glass slides and dipped in Kodak NTB 2 emulsion. After a 3-week incubation period the sections were developed in D19 and fixed with Kodak Ektaflo. Ganglionic tissue was stained with 1% cresyl violet, the other tissue with hematoxylin and eosin. All slides were studied using light and darkfield optics.

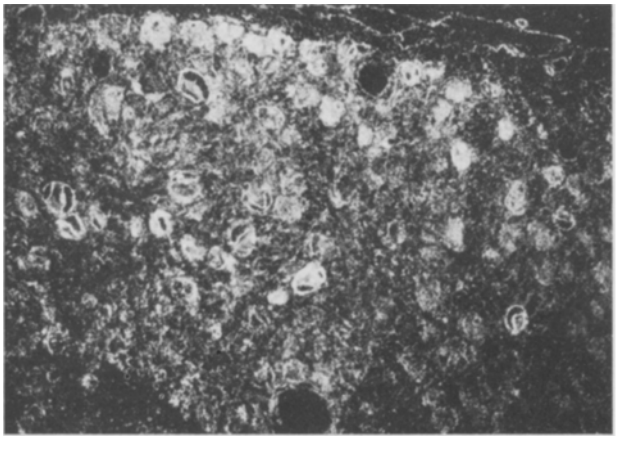

Figure 1. Left celiac ganglion shows dense concentrations of silver grains. Darkfield × 200.

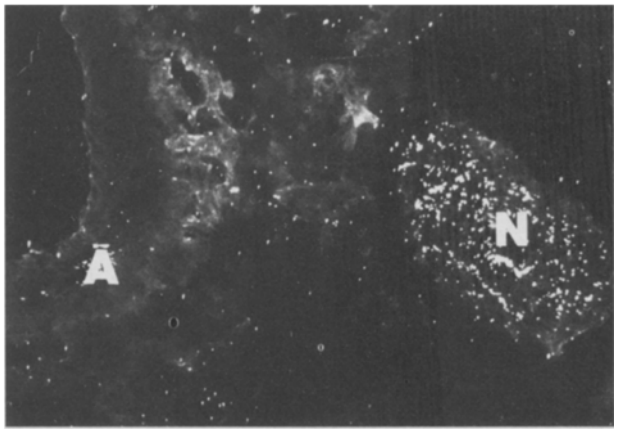

Figure 3. Small labeled nerve (N) near an artery (A). Darkfield × 500.

Figure 2. Large heavily labeled nerve shortly after emerging from the celiac ganglion. Darkfield × 475.

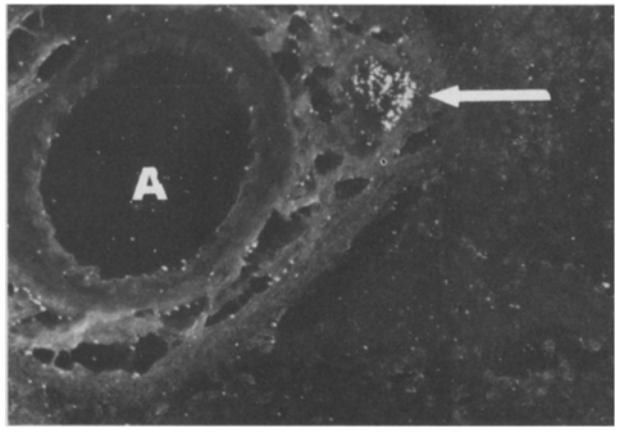

Figure 4. Small labeled nerve (arrow) close to a trabecular artery (A) in the spleen. Darkfield × 620.

In all ganglia studied, ^{3}H-leucine had infiltrated the neurons. Outlines of cell bodies could easily be seen due to dense concentrations of the silver grains (fig. 1). This heavy labeling could readily be traced from the cell bodies into the nerves leaving the ganglia (fig. 2). These large nerves branched repeatedly forming a plexus of smaller labeled nerves which accompanied small arteries (fig. 3). This network of small nerves and blood vessels could be followed to the organs being studied. In addition small labeled nerves in close proximity to blood vessels were found within the stomach, spleen and pancreas (fig. 4). No apparent differences were observed between 48 and 98 h postinjection survival periods.

This study demonstrates autoradiographically that nerve cell bodies in the celiac ganglion have postganglionic sympathetic fibers which innervate the spleen, stomach, pancreas and liver.

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Tumor-promoting phorbol esters and mezerein induce contraction in hydra

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Summary. 12-*O*-tetradecanoyl phorbol-13-acetate (TPA, 1–100 ng/ml) induced a reversible contraction in the hydra, *Hydra japonica*. Another tumor-promoting phorbol ester, phorbol-12,13-didecanoate (PDD), and mezerein also induced contraction, but non-tumor-promoting derivatives such as 4*a*-PDD and phorbol did not.

Tumor-promoting phorbol esters, as represented by 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), are known to produce various biological and biochemical effects when administered to cultured cells[2]. They enhance cell transformation[3,4], modulate cell differentiation[5–7], enhance cellular biochemical activities[2,6], and alter membrane properties[2,6]. These compounds exert their effects on cells of different tissue or species origin, as well as on normal and tumorgenic cells. More recent studies suggest that they also interfere with early development in lower animals such as sea urchins[8] and nematodes[9]. Although interaction with cell surface membranes is thought to be essential for their action[2,6], the mechanism of the interaction leading to these pleiotropic effects remains ill-defined.

In this study, we have examined the effects of these tumor-promoting phorbol esters on another lower animal form, the coelenterate, *Hydra japonica*. Interestingly, these agents induce a marked and sustained contraction in the hydra, whereas non-tumor-promoting derivatives do not.

Materials and methods. *Hydra japonica*[10] was cultured in a medium containing 1.0 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.1 mM KCl and 2.0 mM $NaHCO_3$ (pH 7.8)[11]. The hydras were fed daily with *Moina macropopa* and were starved 1 or 2 days before experiments. 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), phorbol-12,13-didecanoate (PDD), 4*a*-PDD, phorbol, and mezerein were kindly donated by Dr H. Yamasaki of International Agency for Research on Cancer. The compounds were dissolved in dimethyl sulfoxide to a concentration of 0.1%.

Results and discussion. In control experiments, hydras were sucked up into pipettes and dropped into 2 ml of culture medium. Irritation from pipetting caused the hydras to contract, but the contracted hydras extended immediately and remained so in the absence of mechanical or light stimulation. When a contracted hydra was dropped into a TPA-containing solution, it extended first and then contracted markedly, forming a tightly-contracted ball. The tentacles contracted more rapidly than the body column. At a low concentration of TPA (1 ng/ml), numerous contraction-extension movements were observed before the final prolonged contraction. Therefore, the TPA-induced contraction was scored as positive only when a contracted hydra remained like a ball with markedly contracted tentacles for at least 30 min. The table shows the average time required by various phorbol esters to induce the contraction. In the presence of 10 ng/ml TPA, the tentacles, but not the body column, of a contracted hydra were partially dissociated after 24 h. In the presence of 1 ng/ml TPA, a slight extension of the body column was observed after marked contraction, and the contracted hydra gradually extended during the following 12 h. The hydras remained alive during the 2 weeks of treatment with 1 ng/ml TPA, and feeding responses were observed.

Another tumor-promoting phorbol ester, PDD, and mezerein (both at 100 ng/ml) also induced marked contraction, whereas the non-tumor promoting phorbol derivatives, phorbol and 4*a*-PDD, produced no contraction, even at 100 ng/ml.

Detached tentacles with hypostome contracted in TPA (100 ng/ml) with a delay of 14.3 min (n = 14), whereas isolated body columns had a delay of 34.9 min (n = 15). Thus TPA acts on the tentacles more rapidly than on the body column. A preferential effect on tentacles has also been observed with glutathione[12].

Since biological effects of TPA are reversible both in vivo and in vitro[2,5,7], the reversibility of the TPA effect on the

Average time required for contraction induction in hydra

Compound		Time (min) required for contraction after addition of test compounds
TPA	100 ng/ml	9.5 ± 4.2* (n = 22)
	10 ng/ml	28.5 ± 18.5 (n = 20)
	1 ng/ml	160.4 ± 43.3 (n = 16)
Mezerein	100 ng/ml	8.9 ± 2.5 (n = 21)
PDD	100 ng/ml	111.3 ± 48.8 (n = 19)
4*a*-PDD	100 ng/ml	> 720** (n = 16)
Phorbol	100 ng/ml	> 720** (n = 20)

* Mean ± SD. ** No contraction within 12 h. See text for further details.