Histofluorescent Study of Catecholamine-Containing Elements in Cholinergic Ganglia from the Calf and Dog Lung (37613)

DAVID JACOBOWITZ, KENNETH M. KENT, JEROME H. FLEISCH, AND THEODORE COOPER

Laboratory of Clinical Science, National Institute of Mental Health, and Cardiology Branch and Laboratory of Chemical Pharmcology, National Heart and Lung Institute, Bethesda, Maryland 20014

An extensive system of varicose adrenergic nerve terminals has been found in close proximity to sympathetic ganglion cells (1–4). Furthermore, intraganglionic adrenergic-like nerve terminals are present in some parasympathetic visceral ganglia, most notably those in cholinergic ganglia of the digestive tract (5–7) and heart (8). The present study represents a histofluorescent examination of catecholamine-containing elements in cholinergic ganglia from calf and dog lung. Calf lung cholinergic ganglia were found to contain varicose adrenergic fibers; analogous ganglia in dog lung were devoid of such elements.

Materials and Methods. Two calves and two dogs were anesthetized with 35 mg/kg sodium pentobarbital. Tissues from the hilar regions of the lungs were rapidly removed and immediately frozen in isopentane cooled by liquid nitrogen. Specimens were freeze dried *in vacuo* at −35° for 3 to 7 days and then treated with paraformaldehyde gas at 80° for 1 hr according to the method of Falck and Hillarp (9, 10). Paraffin sections were cut at 14 μm and examined for monoamine fluorescence with a Leitz fluorescence microscope. Photographs were taken using Kodak Tri K film.

Results. Clusters of cholinergic ganglion cells were readily identified in connective tissue regions lateral to the cartilage rings of the intrapulmonary bronchi. The cholinergic nature of these ganglia was apparent from their low background fluorescence, occasionally augmented by orange-yellow autofluorescent lipofuscin pigment granules in some of the cell bodies (8). Ganglia of the calf lung contained green fluorescent varicose fibers in proximity to some of the cell bodies (Fig. 1). About 10% of the cell bodies appeared to be in apposition to adrenergic-like fibers. Large green fluorescent smooth nerve trunks occasionally penetrated the ganglia. Also, many intense green fluorescent mast cells were observed in the lung; a small number of mast cells were seen within the ganglia. These cells have been reported to contain dopamine (11). In addition, clusters of intense yellow-green fluorsecent chromaffin cells were sometimes noted in the cholinergic ganglia (Fig. 2) and in connective tissue between the cartilage and the ganglia.

No fluorescent fibers were seen in proximity to the ganglion cell bodies located in the hilum region of the dog lung. As observed in the calf, only a sparse number of chromaffin cell clusters were present within the ganglion and in connective tissue between the cartilage and the ganglia (Fig. 3). A short smooth fluorescent process was observed to emanate from a chromaffin cell cluster within the ganglion.

Discussion. The present study demonstrates that an intraganglionic system of varicose fibers are situated in cholinergic ganglia of the calf lung similar to that previously observed in cholinergic ganglia of the gut (5, 6), heart (8) and sympathetic ganglia (4). On the other hand, our study did not demonstrate such elements in cholinergic ganglia in the dog lung possibly due to tissue handling, processing or species differences in lability of amine stores in these extremely delicate nerve terminals found in these ganglia.

Cholinergic ganglia in the calf and dog lung

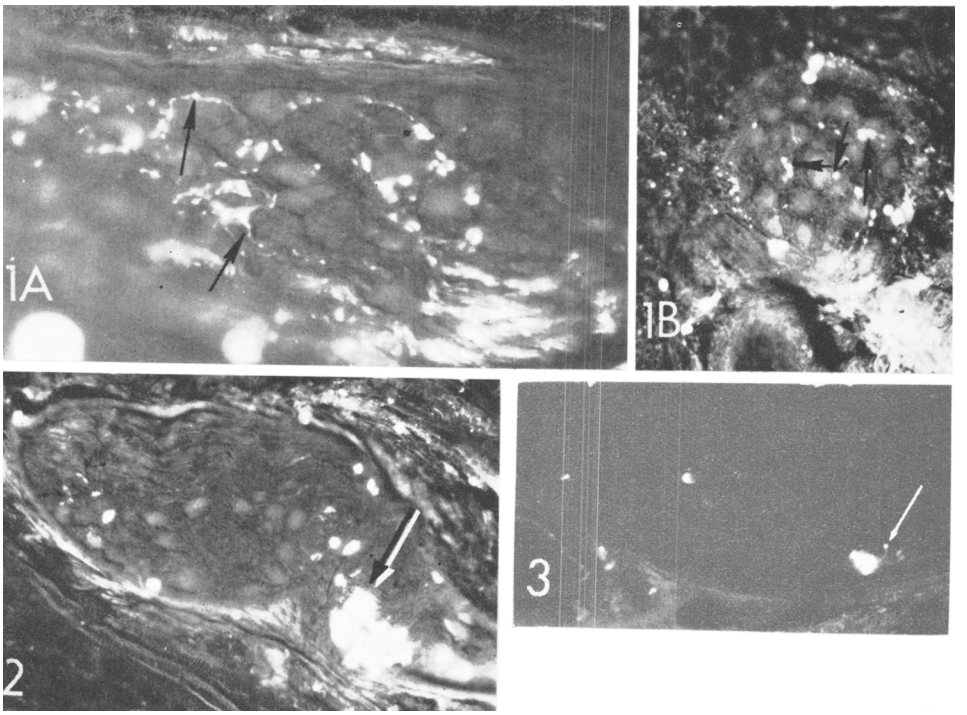

FIG. 1. Calf lung (A) fluorescent varicose fibers (arrows) in close proximity to non-fluorescent cholinergic cell bodies. Note large adrenergic nerve trunk (bottom right). (×342). (B) Another calf lung with adenergic-like varicosities (arrows) in apposition to cholinergic cell bodies. (×100).

FIG. 2. Calf lung; cholinergic ganglion with a large cluster of intense fluorescent chromaffin cells (arrow). (×100).

FIG. 3. Dog lung; cholinergic ganglion with a small cluster of chromaffiin cells. A small fluorescent process is observed to emanate from this cluster (arrow). (×145).

were found to contain a small number of chromaffin cell clusters similar to those previously observed in cholinergic ganglia of the heart (8, 12, 13) and in sympathetic ganglia (4). These small intensely fluorescent bodies were defined as chromaffin cells by histochemical criteria in that, morphologically, they are strikingly similar to adrenal chromaffin cells (4, 8). Electrophysiologic studies support the hypothesis that chromaffin cells are responsible for a postsynaptic inhibitory mechanism (15–17). The innervated chromaffin cells of the cardiac ganglia have been postulated to influence ganglionic transmission by a release of catecholamines from processes in close proximity to ganglion cell bodies and/or release of amine into the capillaries which could then permeate the ganglion (8).

The presence of the intraganglionic system of catecholamine-containing varicosities in cholinergic ganglia of calf lung suggests that the sympathetic neurotransmitter has a physiologic role in ganglionic transmission. Numerous studies have shown that catecholamines can influence functional activity of ganglia (14). The significance of our observation lies in the fact that catecholamines, in addition to their primary effects on bronchial smooth muscle (bronchodilation), may have a secondary but nevertheless important action in lung ganglia. Alpha receptor stimulation depresses ganglionic transmission whereas beta receptor stimulation facilitates transmission (18). Activation of ganglionic alpha or beta receptors could result in bronchodilation or bronchoconstriction, respectively. Depression of ganglionic transmission ,alpha receptor stimulus) would decrease the amount of acetylcholine at the end organ (bronchodila-

tion) whereas facilitation (beta receptor stimulus) would increase the quantity of acetylcholine at the bronchial smooth muscle (bronchoconstriction). The relative importance of this effect of the catecholamines in the neural regulation of bronchial smooth muscle tone is unknown.

1. Hamberger, B., Norberg, K-A., and Sjöqvist, F., Int. J. Neuropharmacol. **2**, 279 (1963).

2. Norberg, K-A., and Sjöqvist, F., Pharmacol. Rev. **18**, 743 (1966).

3. Jacobowitz, D., and Woodward, J. K., J. Pharmacol. Exp. Ther. **162**, 213 (1968).

4. Jacobowitz, D., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 1929 (1970).

5. Norberg, K-A., Int. J. Neuropharmacol. **3**, 374 (1964).

6. Jacobowitz, D., J. Pharmacol. Exp. Ther. **149**, 358 (1965).

7. Jacobowitz, D., and Nemir, P., J. Thorac. Cardiovasc. Surg. **58**, 678 (1969).

8. Jacobowitz, D., J. Pharmacol. Exp. Ther. **158**, 227 (1967).

9. Falck, B., Acta Physiol. Scand. **56**, Suppl. 197 (1962).

10. Falck, B., Hillarp, N-A., Thieme, G., and Torp, A., J. Histochem. Cytochem. **10**, 348 (1962).

11. Falck, B., Nystedt, T., Rosengren, E., and Stenflo, J., Acta Pharmacol. Toxicol. **21**, 51 (1964).

12. Friedman, W. F., Pool, P. E., Jacobowitz, D. M., Seagren, S., and Braunwald, E., Circ. Res. **29**, 26 (1968).

13. Vogel, J., Jacobowitz, D., and Chidsey, C., Circ. Res. **24**, 71 (1969).

14. Volle, R. L., Pharmacol. Rev. **18**, 839 (1966).

15. Eccles, R. M., and Libet, B., J. Physiol. (London) **157**, 484 (1961).

16. Libet, B., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 1945 (1970).

17. Kababian, J. W., and Greengard, P., Science **174**, 1346 (1971).

18. De Groat, W. C., Circ. Res. **21–22**, Suppl 3, 135 (1967).

Received April 30, 1973. P.S.E.B.M., 1973, Vol. 144.