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## CATECHOLAMINE-CONTAINING SYMPATHETIC SPINAL NEURONS INNERVATING

THE CAT HEART

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Intensive study of sympathetic preganglionic neurons in recent years [3-5, 8] has shown that besides the classical preganglionic neurons located in the intermediolateral nucleus of the gray matter of the spinal cord, other "untypical" neurons also exist. They may lie in the dorsomedial, lateral, and central part of the ventral horn [3-5]. Some of these cells have axons running to the heart through the stellate ganglion without interruption [3]. Reports widening our ideas on the neurotransmitters involved in the function of sympathetic preganglionic neurons have been published. They have revealed peptides (enkephalin, neurotensin, somatostatin, substance P) and serotonin [8, 9]. In the investigation described below the localization and neurotransmitter involvement of "untypical" sympathetic preganglionic neurons concerned with the regulation of cardiac activity were studied.

## EXPERIMENTAL METHOD

Experiments were carried out on 28 noninbred cats weighing 2.5-3.5 kg. Under chloralose-pentobarbital anesthesia (50 and 10 mg/kg, respectively, intraperitoneally) the right stellate ganglion of the animals was exposed through an extrapleural approach. The localization of neurons whose axons are not interrupted in the stellate ganglion was determined by retrograde axonal transport of uranyl acetate (40% aqueous solution) through the caudal anastomosis of the stellate ganglion [2]. The presence of catecholamines in the cytoplasm of the neurons was established by the reaction with glyoxylic acid [6] and glutaraldehyde [1]. Fluorescence was observed in the Lyumam I-3 luminescence microscope. Resconstruction of the sections was carried out with the aid of the N-307 graph plotter.

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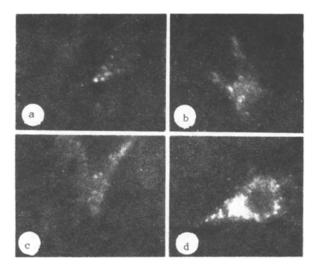


Fig. 1. Catecholamine-containing spinal cord neurons retrogradely labeled through the caudal anastomosis. Ocular 7, objective 90. a) n. intercalatus; b) lateral region of ventral horn; c) dorsomedial region of ventral horn; d) central region of ventral horn.

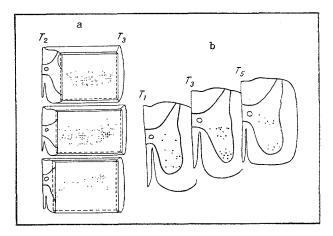


Fig. 2. Localization of catecholamine-containing neurons in spinal cord (reconstruction of sections on N-307 graph plotter). a) Longitudinal sections with a step of 200  $\mu;$  b) transverse sections through thoracic segments T1-T5 of the spinal cord; dots indicate neurons with double label; circles indicate catecholamine-containing neurons, not retrogradely labeled.

In electrophysiological experiments the white rami communicantes and the common sympathetic trunk were stimulated before they enter the stellate ganglion. Activity was derived from the caudal anastomosis [2]. It was averaged and analyzed with the F-37 signals analyzer. Regional transmission was blocked by application of a 5% solution of pentamine and 0.1% solution of atropine to the stellate ganglion [7].

## EXPERIMENTAL RESULTS

Fibers not interrupted in the stellate ganglion were shown to be axons of "untypical" preganglionic neurons, located in the lateral, dorsomedial, and central zones of the ventral horn and in n. intercalatus. Labeled neurons were clearly distinguished by the right fluorescence of greenish-golden granules in the cytoplasm. Cells discovered in the ventral horn were large and polygonal in shape, whereas cells found in n. intercalatus were small (Fig.1).

To determine the neurotransmitter nature of these neurons, retrograde labeling was combined with the reaction for catecholamines [1]. Simultaneously with fluorescence of granules of the marker, the cell cytoplasm gave a diffuse greenish luminescence due to combination of glutaraldehyde with catecholamines (Fig. 1). A positive result also was obtained in the test with glyoxylic acid.

The localization of neurons revealed by the double label was identical with that obtained previously by retrograde axonal transport (Fig. 2). As will be clear from the longitudinal sections, moreover, their predominant location was shifted from the ventral horn toward n. intercalatus, depending on the depth of the section (Fig. 2a). The largest number of cells was found in segments T3 and T4 of the spinal cord, where they were located mainly in the central part of the ventral horn. They were fewer in number in segments T1 and T5, and were located mainly in the lateral region of the ventral horn and in n. intercalatus (Fig. 2b).

To study the functional role of the neurons described above electrophysiological experiments were carried out. During stimulation of the caudal anastomosis of the stellate ganglion, B-waves with conduction velocity of between 3.6 and 17.2 m/sec  $(7.3 \pm 0.4 \text{ m/sec})$  were recorded in the white rami communicantes of T3 and T4 (antidromic stimulation). Similar waves were preserved in the caudal anastomosis in response to stimulation of the white rami communicantes and of the common sympathetic trunk after blockade of ganglionic conduction. Repetitive above-threshold stimulation of the common sympathetic trunk before its entry into the right stellate ganglion led to an increase in the heart rate on average by  $44 \pm 6$  beats/min (from 144 to 188 beats/min). Blocking ganglionic conduction reduced the observed effect to  $23 \pm 4$  beats/min (from 131 to 154 beats/min). With an increase in the strength of stimulation (up to 3-5 thresholds) the increase in the heart rate amounted to  $31 \pm 7$  beats/min (from 138 to 169 beats/min). Intravenous injection of the cardio-selective beta-adrenoblocker talindol (2 ml of a 2% solution) completely blocked the increase in heart rate in response to stimulation of the common sympathetic trunk.

The experiments thus demonstrated the existence of catecholamine-containing sympathetic preganglionic neurons whose axons run to the heart without interruption in the stellate ganglion. In their axonal conduction velocity, they can be classed as B neurons. Stimulation of their axons, in the composition of the caudal anastomosis, leads to a marked increase in heart rate.

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