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Effect of sympathectomy and demedullation on increased myenteric and dorsal vagal complex Fos-like immunoreactivity by cholecystokinin-8

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Abstract

Chemical sympathectomy with daily, intraperitoneal (IP) injections of guanethidine sulfate to adult rats, attenuated myenteric, but not dorsal vagal complex (DVC) Fos-like immunoreactivity (Fos-LI) by cholecystokinin-8 (CCK). This technique destroys only 60–70% of the sympathetic neurons, and spares the hormonal source of catecholamines, the adrenal medulla.

The goal of the current study is to evaluate the effect of complete sympathectomy or destroying 100% of the sympathetic neurons by injecting guanethidine to 1-day-old pups (40 mg/kg daily for 5 weeks), and surgically removing the adrenal medulla.

In the DVC, demedullation and sympathectomy–demedullation increased Fos-LI by CCK in the area postrema and nucleus of the solitary tract, but sympathectomy–demedullation increased it only in the area postrema. In the myenteric plexus, sympathectomy increased this response in the duodenum, and demedullation increased it in the duodenum and jejunum. On the other hand, sympathectomy–demedullation attenuated myenteric Fos-LI in the jejunum.

These results indicate that catecholamines may play an inhibitory role on the activation of the DVC neurons by CCK. In the myenteric neurons, however, catecholamines may have both inhibitory and excitatory roles depending on the level of the intestine e.g., duodenum vs. jejunum. This may also indicate that CCK activates the enteric neurons by different mechanisms or through different pathways.

Keywords: Cholecystokinin; Guanethidine sulfate; Myenteric plexus; Fos; Dorsal vagal complex; Sympathectomy; Catecholamines; Adrenal medulla

1. Introduction

The functions of the gastrointestinal (GI) system are controlled by hormonal and neuronal systems located inside and outside the gut. One of the early discovered gut hormones that evokes numerous GI-related responses such as contraction of smooth muscles and gall bladder is cholecystokinin (CCK, reviewed recently in Ref. [14]). These, and many other responses are mediated through interactions with two G-protein coupled receptors, CCK_1 and CCK_2.

The enteric nervous system, or the ENS, coordinates most of the internal functions of the gut. This system consists mainly of two nerve plexuses, submucosal located under the submucosa, and myenteric located between the outer longitudinal and the inner circular muscle layers of the gut wall. The ENS is also connected to the parasympathetic nervous system (mainly vagus nerve), the sympathetic nervous system (celiac, cranial and caudal mesenteric ganglia by the mesenteric and splanchnic nerves) and spinal afferents [2].

Our previous published work on the possible role of the ENS on CCK-gut related functions, shows activation of this system by CCK [3,5,6,12–21]. CCK, given intraperitoneally (IP), and oleate, a long chain fatty acid that stimulates the release of endogenous CCK, increased Fos-like immunoreactivity (Fos-LI), a marker for neuronal activation [11], in the dorsal vagal complex (DVC) of the brainstem and in specific neurons in the myenteric plexus. This activation was dependent on CCK_1 receptors. Finally, subdiaphragmatic

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vagotomy abolished DVC Fos-LI by CCK, whereas myenteric Fos-LI by CCK was only attenuated by guanethidine-induced sympathectomy. The previous results demostrated that activation of the DVC by CCK was dependent on a parasympathetic pathway, namely vagal, but the myenteric activation was dependent on a sympathetic route [3,5,6,12–21]. On the other hand, the remaining myenteric activation was attributed to the sympathetic neurons spared by the guanethidine treatment. Daily IP injections of guanethidine sulfate in adult rats, which we utilized in our previous work [3,5,6,12–21], destroy only 60–70% of the sympathetic neurons and spare the hormonal source of catecholamines, the adrenal medulla [7,8].

Therefore, the goal of the current experiment is to examine the role of complete sympathectomy in increased DVC and myenteric Fos-LI in response to CCK, by removing both the neuronal and the hormonal sources of catecholamines. The only known protocol of chemical sympathectomy that destroys 100% of the sympathetic neurons is by treating 1-day-old pups with daily IP injections of guanethidine sulfate (40 mg/kg) for five weeks [7]. However, this technique, similar to our previous one [3], spares the hormonal source of catecholamines, the adrenal medulla. Therefore, in addition to complete chemical, neuronal sympathectomy, we also performed demedullation of the adrenal gland, to eliminate the hormonal source of catecholamines.

2. Materials and methods

2.1. Animals

The Tuskegee University Animal Care and Use Committee approved the experiments. We used 60, 1-day-old male Sprague Dawley pups (*Harlan, IN*). The animals were housed with the dams until they reached adulthood (21 days), they were moved to single wire-mesh cages with a controlled environment (lights were on from 0600 to 1800 and temperature was maintained at 21.5 °C). Pups had ad libitum access to water and pelleted rodent chow (*Teklad, WI*). To enhance adaptation to the laboratory, we handled each pup for 10 min/day for the first seven days, and each pup was given a daily IP injection of 0.5 ml saline. Injections of CCK-8 started when the pups reached 12–17 weeks of age, and 280–320 g in weight.

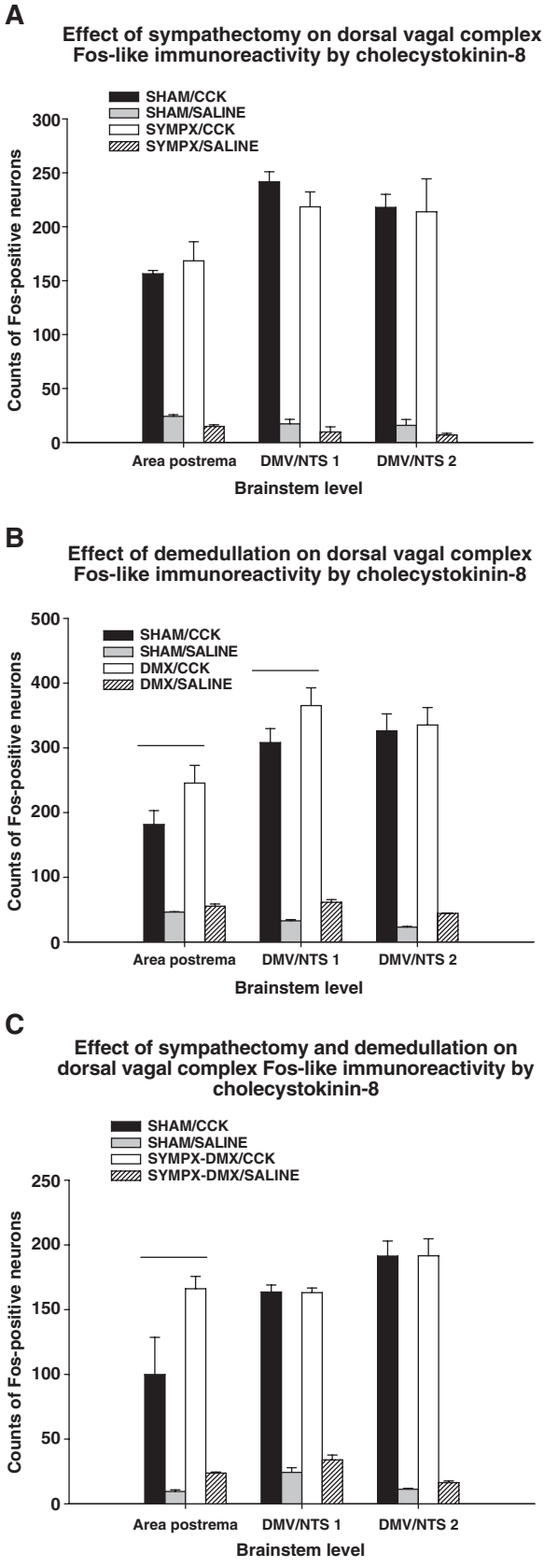

Fig. 1. Mean±SEM of Fos-LI counts in three areas of the dorsal vagal complex of the brainstem according to the rat brain atlas [9]. Three groups of rats ($n=5$ rats/group) received CCK-8 (40 μg/kg intraperitoneally (IP)) or saline after one of the following treatments: A: daily injection of guanethidine sulfate for five weeks (40 mg/kg, IP) starting at 1 day of age to destroy the sympathetic nervous system. B: surgical removal of the adrenal medulla. C: surgical removal of the adrenal medulla in rats treated daily with guanethidine sulfate for 5 weeks starting at 1 day of age. CCK increased Fos-LI in all of the sham groups (black bars) compared to control (gray bars). CCK also increased Fos-LI in the treated or surgically manipulated rats (white bars) compared to sham treated or operated rats (striped bars). The lines above the bars represent significant difference between the white and the black bars. Abbreviations and levels: Area postrema at −4.8 mm caudal to the interaural plan NTS/DMV1 = nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus (DMV) at −4.3 mm caudal to the interaural plan. NTS/DMV2 = NTS and DMV at −4.8 mm caudal to the interaural plan.