

Effects of Hibernation on the Enteric Nervous System of the Thirteen-lined Ground Squirrels.

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Abstract

Hibernating animals experience dramatic changes in the structure and function of their digestive system along with other body organ systems. During hibernation, the gut motility, is significantly slowed down. Gut motility is controlled by the enteric nervous system moment-by-moment. The aim of this study was to investigate the changes in the enteric nervous system during hibernation. Whole-mount myenteric and submucosal plexus preparations were dissected and used for immunohistochemical staining for various neurochemical markers for enteric neurons. The human neuronal protein HuC/D is used as a marker for all enteric neurons. Choline acetyltransferase (ChAT) is an enzyme for the synthesis of an excitatory neurotransmitter, acetylcholine. Nitric oxide synthase (NOS) is an enzyme for the synthesis of an inhibitory neurotransmitter, nitric oxide. Substance P (SP) is an excitatory neurotransmitter. Vasoactive intestinal polypeptide (VIP) is a neurotransmitter that inhibits gut motility. Segments of ileum were homogenized and used for measuring acetylcholine levels in summer active, winter torpor, and interbout arousal squirrels. The results suggested that there was no quantitative change in the total number of neurons in the enteric nervous system during winter torpor or interbout arousal. There was no significant difference in the number of neurons immunoreactive to NOS, SP, and VIP. However, there was a significant decrease in the number of neurons immunoreactive to ChAT in the myenteric plexus of the winter torpor squirrel. A significant decrease in choline content was seen in the winter torpor squirrels compared to the interbout arousal squirrels. Selective downregulation of ChAT and a decrease of choline content in the ileum may contribute to gut hypomotility during winter torpor in thirteen-lined ground squirrels.

Introduction

As an adaptation to the prolonged fasting and low body temperature, hibernating animals (e.g. thirteen-lined ground squirrels) experience dramatic changes in the structure and functions of their digestive system. During hibernation, the stomach and intestine experience atrophy (Carey and Sillis, 1996; Naya et al., 2009). The digestive enzyme activities are reduced (Balslev-Clausen et al., 2003), but the absorptive capacity of the intestine is enhanced (Carey and Sillis, 1996). The motility of the gastrointestinal tract is reduced (Studier et al., 1976; Gossling et al., 1980) and the ion secretion by intestinal epithelium is enhanced (Carey, 1990; 1992). Modulation of gastrointestinal function during hibernation helps to conserve energy and also prepare the animal for re-feeding after arousal from hibernation.

Functions of the digestive system are controlled by the enteric nervous system (ENS) in concert with the inputs from the autonomic nervous system and the central nervous system. The ENS is the intrinsic control of the digestive function. It is located within the wall of the gut. Several neurotransmitters are present within the ENS. Acetylcholine (ACh) is a major excitatory neurotransmitter that stimulates smooth muscle contraction and epithelial ion secretion. Nitric oxide (NO) is a major inhibitory neurotransmitter that suppresses gut motility. Substance P (SP) is a neurotransmitter that stimulates gastrointestinal motility (Sarna, 2010). Vasoactive intestinal polypeptide (VIP) is a neurotransmitter that suppresses gut motility (Sarna, 2010) but stimulates intestinal ion secretion. The aim of this study was to investigate the changes of these neurochemical markers in the enteric nervous system during hibernation.

Methods

Tissue harvest

In order to investigate the changes in the number of cholinergic, nitrenergic, SP, and VIP neurons in the ENS, summer active, winter torpor, and winter interbout arousal squirrels were used in the study. Summer active and interbout arousal squirrels were euthanized by CO₂ inhalation, and winter torpor squirrels were euthanized by cervical dislocation. The ileum of the small intestine was removed and fixed in Zamboni's fixative.

Immunohistochemical staining of enteric neurons

Whole-mount myenteric and submucosal plexus preparations were dissected and used for immunohistochemical staining for human neuronal protein HuC/D, ChAT, NOS, SP, and VIP. Thirty random ganglia throughout the myenteric and submucosal plexuses were chosen, and the number of immunoreactive neurons was counted. A one way analysis of variance (ANOVA) was used to determine statistical significance and a Tukey's HSD post-hoc test was used to determine individual differences between group means. $P < 0.05$ was considered statistically significant.

Acetylcholine assay

In order to quantify the amount of acetylcholine present in the ileum, a commercially available Choline/Acetylcholine Assay Kit (cat #MAK056, Sigma-Aldrich) was used. A 1-cm segment of ileum was collected from the summer active, winter torpor, and winter interbout arousal squirrels and homogenized with choline assay buffer. The solution was centrifuged and the supernatant was separated and used for acetylcholine assay according to the manufacturer's instruction. The acetylcholine level from each animal was normalized with its tissue weight.

Data Analysis

Data are expressed as means \pm SEM with n values representing the numbers of animals in each group. One-way ANOVA was used to determine statistical significance among different groups. Tukey's post-hoc HSD tests were performed for comparison of groups with significant difference. $P < 0.05$ was considered statistically significant

Results

There was no significant difference in the number of HuC/D, NOS, SP, and VIP-immunoreactive neurons in the myenteric and submucosal plexuses among summer active, winter torpor, and interbout arousal squirrels (Figures 1,3,4,5). A significant decrease in ChAT immunoreactive neurons in the myenteric plexus of the winter torpor squirrels compared to the summer active and interbout arousal squirrels was seen. No significant change was seen in the submucosal plexus (Figure 2). NOS was not present in the submucosal plexus of the squirrel ileum (Figure 3).

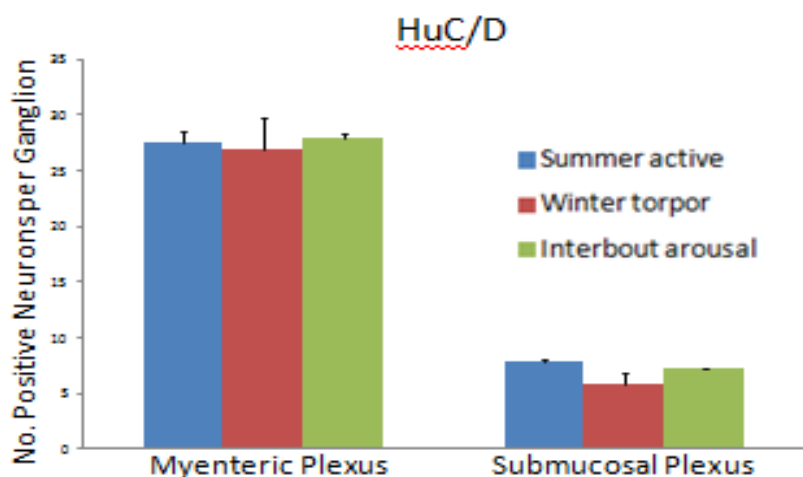


Figure 1. There was no significant difference in the number of HuC/D-IR neurons in the myenteric and submucosal plexuses among summer active, winter torpor, and interbout arousal squirrels.

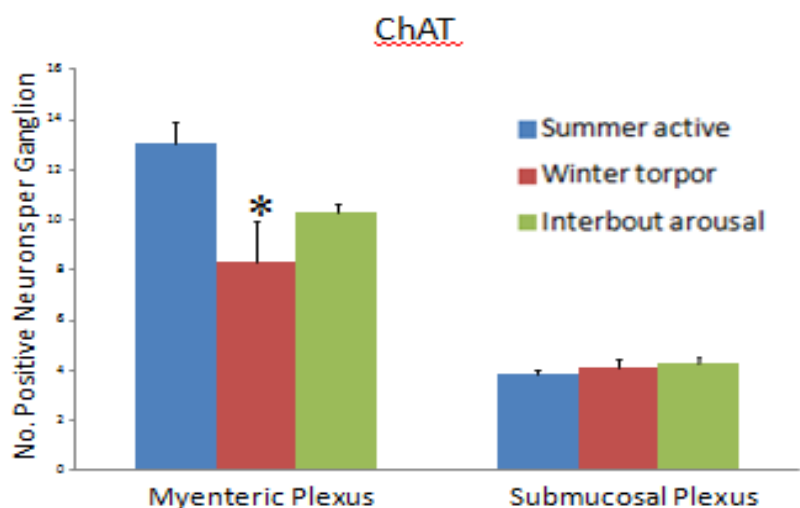


Figure 2. The number of ChAT-IR neurons was significantly decreased in the myenteric plexus during winter torpor (* $P < 0.05$), however, the number of ChAT-IR neurons in the submucosal plexus remained unchanged

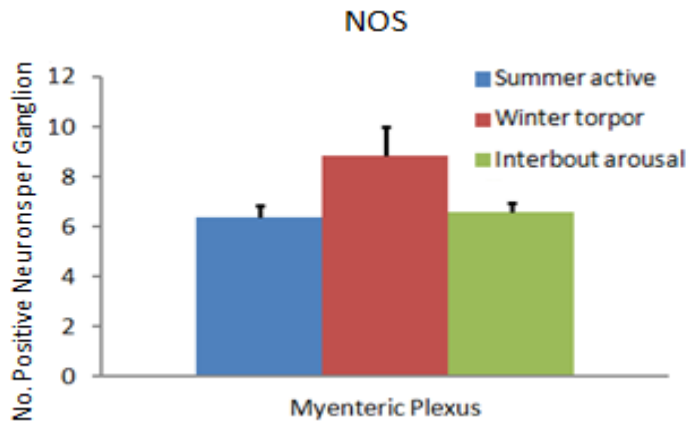


Figure 3. There was no significant change in NOS-IR neurons in the myenteric plexus of summer active, winter torpor and interbout arousal squirrels.

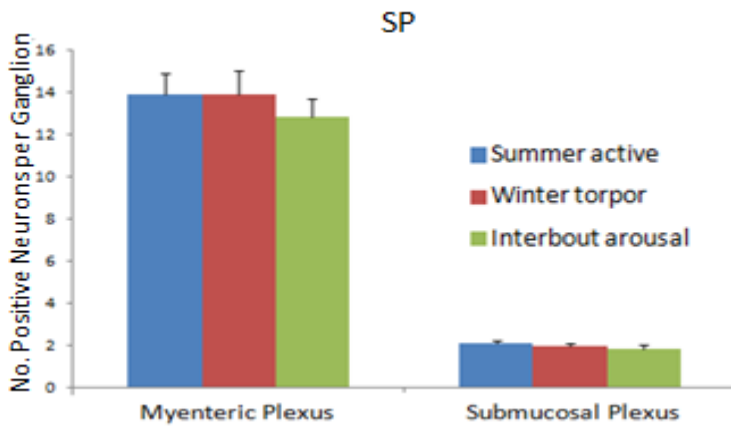


Figure 4. There was no significant difference in the number of SP-IR neurons in the myenteric and submucosal plexuses in summer active, winter torpor, and interbout arousal squirrels.

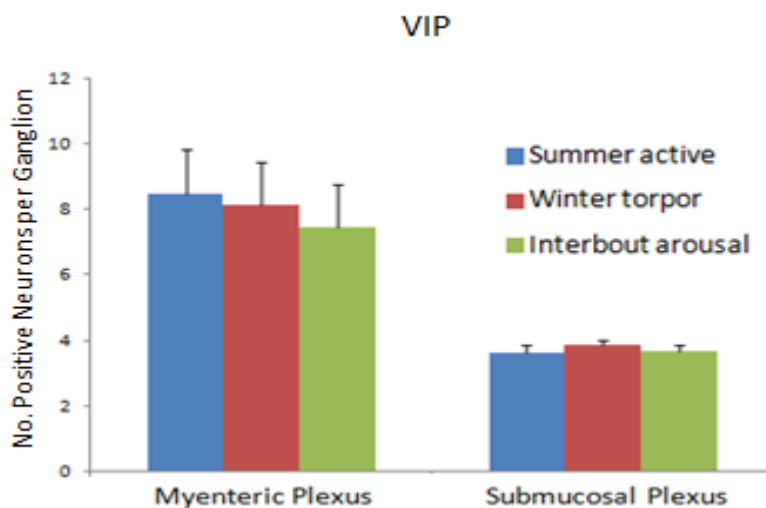


Figure 5. There was no significant difference in the number of VIP-IR neurons in the myenteric and submucosal plexuses in summer active, winter torpor, and interbout arousal squirrels.

The amount of choline in the ileum was measured in the summer active, winter torpor, and interbout arousal squirrels. A significant decrease in the total choline concentration was seen in winter torpor squirrels comparing to the interbout arousal squirrels (0.052 ± 0.07 ng/ μ l/mg tissue vs. 1.20 ± 0.17 ng/ μ l/mg tissue; $P < 0.05$), however, there was no significant difference between winter torpor and summer active squirrels (0.052 ± 0.07 ng/ μ l/mg tissue vs. 0.84 ± 0.16 ng/ μ l/mg tissue; $P > 0.05$).

Discussion

The results demonstrated that there was no quantitative change in the total number of neurons in the enteric nervous system during winter torpor or interbout arousal. No significant changes in the numbers of neurons immunoreactive to NOS, SP, and VIP was found. However, there was a significant decrease in the number of neurons immunoreactive to ChAT in the myenteric plexus during winter torpor.

The results from the acetylcholine assay showed the quantitative decrease in the total choline concentration between the winter torpor squirrels and the interbout arousal squirrels. There was no significant difference in the total choline concentration between the summer active and interbout arousal squirrels or the summer active and winter torpor squirrels. These results suggested that selective downregulation of choline acetyltransferase in myenteric neurons during winter torpor would lead to the reduced synthesis of acetylcholine and slowed gastrointestinal motility in the hibernating animals.

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