Precise regulation of organ size is required to maintain organ function within an appropriate physiological range. This regulation includes compensatory growth of paired organs, in which one undergoes hypertrophy and/or hyperplasia after removal of the other. Compensatory organ growth has been demonstrated for the ovary, kidney, thyroid, and adrenal gland (16, 24, 32, 34). However, the mechanisms underlying the growth responses are not clear. A neural component to the growth response has been suggested for each of these organs (5, 10, 14, 32), with the clearest demonstration of neurally mediated compensatory growth occurring in the adrenal gland. In a series of experiments, Dallman and colleagues (8) showed that nerves, and not ACTH, are required for compensatory adrenal growth and hypothesized that a neural reflex loop with both afferent and efferent neural limbs was involved.

Primary afferent nerves include a subset of nerve fibers that are selectively sensitive to the neurotoxin capsaicin (6, 20). Capsaicin-sensitive afferent fibers are predominantly nociceptors that are classically known for the mediation of pain responses (reviewed in Refs. 12, 20). These fibers can also initiate various autonomic reflexes, including regulation of cardiovascular, respiratory, gastrointestinal, and urinary tract function (reviewed in Ref. 29). However, it has more recently been established that these fibers can also have local effector functions via the release of neurotransmitters, such as calcitonin gene-related peptide (CGRP) and substance P, from their peripheral terminals. More specifically, capsaicin-sensitive afferent fibers have been implicated in the modulation of vascular dilation and permeability, immune cell function and inflammation, nonvascular smooth muscle contractility, and autonomic ganglia neurotransmission (reviewed in Refs. 20, 29). Collectively, these studies suggest that capsaicin-sensitive nerve fibers are potential candidates for mediating compensatory organ growth on both afferent and efferent limbs of the neural reflex.

The afferent innervation of the adrenal gland includes CGRP-positive, capsaicin-sensitive nerve fibers that reach the gland via the splanchnic nerve (19, 31, 37, 38). Periaxonal application of capsaicin to peripheral nerves has been used previously to selectively eliminate capsaicin-sensitive afferent fibers from specific tissues (reviewed in Ref. 21). In the present studies, a method for the periaxonal application of capsaicin to the splanchnic nerve is developed. Periaxonal capsaicin treatment is then compared with systemic capsaicin treatment to validate its effectiveness and local site of action. Finally, periaxonal capsaicin treatment is used bilaterally and unilaterally to determine whether adrenal capsaicin-sensitive afferents mediate compensatory adrenal growth.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (200 g; Harlan, Indianapolis, IN) were used in all experiments. The animals were
housed on a 12:12-h light-dark cycle with free access to food and water. All procedures were approved by the University of Minnesota Animal Care and Use Committee and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society.

Periaxonal capsaicin treatment. Rats were anesthetized with pentobarbital sodium (60 mg/kg ip), and the thoracic splanchnic nerve was exposed just proximal to the adrenal gland, as described previously (23). Small plugs of cotton saturated with capsaicin (33 mM; Fluka Chemical, Ronkonkoma, NY), vehicle (5% Tween 80, 5% ethanol, 90% saline), or saline were applied to the nerve for 15 min; a 15-min drug exposure was chosen based on previous work (reviewed in Ref. 12). Rats were then sutured and administered antibiotic (Naxcel, 50 mg/kg im).

Systemic capsaicin treatment. Rats were administered a 4-day systemic (subcutaneous) treatment with capsaicin (day 1 = 30 mg/kg, days 2 and 3 = 60 mg/kg, day 4 = 100 mg/kg), similar to the multiday systemic treatment protocols previously used by others (reviewed in Ref. 12), or vehicle (10% Tween 80, 10% ethanol, 80% saline) under pentobarbital sodium anesthesia.

Adrenal CGRP content. At 7 days after completion of periaxonal or systemic capsaicin treatment (n = 7–10/group), rats were killed by decapitation and adrenal glands were removed, cleaned, and decapsulated. A 7-day recovery period after drug treatment was used throughout the present studies to allow ample time for degeneration of the capsaicin-sensitive fibers and recovery of the hypothalamic-pituitary-adrenal axis. The dorsal half of the lumbar spinal cord was also collected. The content of CGRP in the adrenal capsule that includes the outer zones of the cortex, the adrenal core that consists of the inner zones of the cortex and the medulla, and the dorsal lumbar spinal cord were determined by RIA as described previously (13, 39).

Compensatory adrenal growth experiments. At 7 days after periaxonal splanchnic nerve treatment (n = 7–9/group), rats were anesthetized and left adrenal glands were removed (adrenalectomy) or were visualized and not manipulated (sham adrenalectomy). After 5 days, rats were killed by decapitation and adrenal glands were collected, cleaned, and weighed. The 5-day time point was selected empirically based on a pilot experiment in which the amount of compensatory growth in positive controls at 3 days was not large enough to permit detection of manipulations that might suppress growth (data not shown). For each experiment, adrenals removed during the adrenalectomy and/or at the end of the experiment were separated into capsules and cores, and CGRP content was assessed by RIA, as described above, to verify the effectiveness of the periaxonal capsaicin treatment.

Retrograde labeling experiment. At 7 days after periaxonal splanchnic nerve treatment (n = 5/group), rats were anesthetized and retrograde tracer (5 μl of 2% Fast blue, 2% True blue in saline) was injected into the adrenal gland over 15 min. Care was taken to ensure that tracer did not leak from the gland. After 7–9 days, rats were anesthetized and transcardially perfused with saline followed by 4% paraformaldehyde. Ipsilateral dorsal root ganglia from thoracic (T) levels 7–11 were removed and sectioned (30 μm). An observer blinded to the treatment group counted the number of labeled dorsal root ganglion neurons with visible nuclei (to minimize double counting). Some contralateral dorsal root ganglia were randomly selected and examined as a negative control.

Spinal cord c-Fos experiment. At 7 days after periaxonal splanchnic nerve treatment (n = 4–5/group), rats were anesthetized and received left adrenalectomy and right sham adrenalectomy (the fat surrounding the adrenal was probed but the adrenal was not removed). The left adrenals removed during adrenalectomy were placed in Zamboni fixative for subsequent immunolabeling of adrenal nerve fibers. At 2 h

Fig. 1. Capsaicin applied periaxonally to the splanchnic nerve decreased adrenal immunolabeling for adrenal calcitonin gene-related peptide (CGRP)-positive fibers. A: adrenal capsule contained a plexus of CGRP-positive nerve fibers (arrows) in vehicle-treated controls. B: in contrast, the CGRP-positive fibers were largely eliminated from the adrenal capsule after periaxonal capsaicin treatment. C: adrenal medulla contained many CGRP-positive fibers (arrows) and chromaffin cells (*) in vehicle-treated controls. D: in contrast, CGRP-positive nerve fibers, but not CGRP-positive chromaffin cells (*), were absent from the medulla after periaxonal capsaicin treatment. n = 4/group. Bar = 200 μm.
after surgery, rats were perfused (as described above) and spinal cord segments T6–T8 were removed. Spinal cords were sectioned (30 μm) and c-Fos immunolabeling was performed on a one in five series of sections by the method of Bhatnagar and Dallman (3). The number of c-Fos-positive nuclei in the dorsal horn was counted by an observer blinded to the treatment group.

Adrenal nerve immunohistochemistry. Adrenal nerve immunohistochemistry (n = 4/group) for CGRP-, neuropeptide Y (NPY)-, vasoactive intestinal peptide (VIP)-, tyrosine hydroxylase (TH)-, neuronal nitric oxide synthase (nNOS)-, and vesicular ACh transporter (VAChT)-positive nerve fibers was performed as described previously (39).

Statistical analysis. Data are presented as means ± SE. Statistical significance was determined by ANOVA (with repeated measures when appropriate) or by t-test. When necessary, homogeneity of variance was obtained by performing ANOVA after square-root transformation. Statistical significance was taken as P < 0.05.

RESULTS

Validation of periaxonal capsaicin treatment. Periaxonal application of capsaicin decreased immunolabeling of CGRP-positive fibers in the adrenal capsule and medulla but did not decrease immunolabeling of CGRP-positive chromaffin cells (Fig. 1). Periaxonal capsaicin treatment decreased the content of CGRP in the adrenal capsule but not the core (Fig. 2A). Systemic capsaicin treatment also decreased the content of CGRP in the adrenal capsule, but it increased CGRP content in the adrenal core (Fig. 2B). CGRP content of dorsal lumbar spinal cord was decreased following systemic but not periaxonal capsaicin treatment (Fig. 2C). Moreover, periaxonal capsaicin treatment did not affect immunolabeling of TH-positive chromaffin cells or NPY-, VIP-, VAChT-, TH-, or nNOS-positive nerve fibers in the adrenal (Fig. 3). Finally, the number of cells in ipsilateral T7 and T8 dorsal root ganglia that were retrogradely labeled from the adrenal gland was reduced by periaxonal capsaicin pretreatment (Fig. 4). No retrogradely labeled cells were observed in the contralateral dorsal root ganglia that were examined as a negative control (data not shown).

Periaxonal capsaicin inhibits compensatory adrenal growth. Bilateral pretreatment of the splanchic nerve with capsaicin attenuated, but did not prevent, compensatory adrenal growth at 5 days after unilateral adrenalectomy (Fig. 5). In a second experiment comparing the effects of bilateral and unilateral periaxonal capsaicin pretreatment, bilateral pretreatment prevented compensatory adrenal growth (Fig. 6). Unilateral pretreatment to the left (afferent) splanchic nerve attenuated compensatory adrenal growth (Fig. 6). Although the differences in right adrenal weight after left adrenalectomy between bilateral vehicle and left (afferent) capsaicin pretreatment were small, the differences were statistically significant. However, unilateral left capsaicin pretreatment did not completely block compensatory adrenal growth, suggesting that the right (efferent) capsaicin treatment had an additional effect on compensatory adrenal growth (Fig. 6).

DISCUSSION

The present studies develop a method for the selective removal of capsaicin sensory fibers from the adrenal gland via periaxonal application of capsaicin, a neurotoxin specific for a subset of sensory neurons (19, 37). Periaxonal capsaicin treatment removed adrenal CGRP-positive fibers from the adrenal cortex and medulla and decreased adrenal capsular CGRP content similarly to systemic capsaicin treatment. Periaxonal
Capsaicin did not decrease CGRP content in the adrenal core, which may be due to the presence of CGRP-positive chromafﬁn cells that are not sensitive to capsaicin. Similarly, systemic capsaicin increased the CGRP content in the adrenal core, which is likely due to an increase in CGRP expression in chromafﬁn cells (31). Systemic capsaicin treatment also reduced CGRP content in the dorsal lumbar spinal cord similarly to previous reports of substance P depletion (reviewed in Ref. 12), whereas periaxonal capsaicin treatment did not, suggesting that periaxonal capsaicin had a local site of action. Importantly, periaxonal capsaicin treatment did not affect immunolabeling for TH-, NPY-, VIP-, VACHT-, or nNOS-positive nerve fibers, which are markers of the adrenal innervation from preganglionic sympathetic, postganglionic sympathetic, and/or medullary ganglion cell sources (18, 19, 27), confirming that capsaicin was selective for sensory neurons. These results suggest that periaxonal treatment of the splanchnic nerve can selectively remove adrenal capsaicin-sensitive afferent fibers as effectively as systemic treatment, while avoiding potential complications from a systemic drug treatment. Moreover, periaxonal capsaicin treatment to the thoracic splanchnic nerve reduced retrograde labeling of dorsal root ganglion neurons from the adrenal gland. The majority of retrogradely labeled dorsal root ganglion neurons were distributed in thoracic segments 7–9, consistent with earlier work (42). Importantly, many dorsal root ganglion neurons were retrogradely labeled from the adrenal gland despite periaxonal capsaicin treatment. These results suggest that the adrenal gland receives significant afferent innervation that is not sensitive to capsaicin.
In the present studies, bilateral periaxonal capsaicin treatment of the splanchnic nerve consistently inhibited compensatory adrenal growth. Moreover, unilateral periaxonal capsaicin to the afferent side alone decreased the extent of compensatory adrenal growth but did not completely block compensatory adrenal growth. These data suggest that capsaicin-sensitive fibers contribute to both the afferent and efferent limbs of a neural reflex controlling compensatory adrenal growth, although this effect is predominantly on the afferent side. Consistent with this finding, unilateral adrenalectomy increased the number of c-Fos-positive nuclei in ipsilateral dorsal spinal cord and periaxonal capsaicin pretreatment reduced c-Fos activation in response to unilateral adrenalectomy. Previously, it has not been known whether the neural signal to initiate compensatory adrenal growth consisted of an activation of an afferent neural pathway or the loss of a tonic afferent signal (8, 22). These data suggest that the afferent signal that initiates compensatory adrenal growth is conveyed, at least in part, by spinal cord neurons that are activated in response to adrenalectomy and that this activation is mediated by adrenal capsaicin-sensitive afferent nerve fibers. This idea is supported by previous work showing that treatment of the ipsilateral splanchnic nerve with lidocaine before adrenalectomy delays compensatory adrenal growth (11, 22) and that a brief pinch of the adrenal nerve and vascular pedicle can initiate transient compensatory adrenal growth of the contralateral adrenal at 12 h that is resolved by 24 h (9). Because capsaicin treatment alone would be expected to produce a transient activation of afferent fibers in the splanchnic nerve (21), local application of capsaicin may induce a similar, transient compensatory growth of the contralateral adrenal. However, if compensatory growth is induced by capsaicin, the response is resolved by 5 days, because no differences were observed in the right adrenal weight of rats that underwent capsaicin pretreatment of the left splanchnic nerve followed by sham left adrenalectomy.

On the basis of the results from the present work and from earlier studies, a model of the peripheral and central pathways mediating compensatory adrenal growth can be proposed (Fig. 8). First, adrenal capsaicin-sensitive fibers within the ipsilateral splanchnic nerve are activated in response to adrenalectomy, resulting in stimulation of second-order neurons. This activation is conveyed to the spinal cord, where it is integrated with other signals to initiate compensatory adrenal growth.

Fig. 4. Number of dorsal root ganglia neurons retrogradely labeled from the adrenal gland was reduced by periaxonal splanchnic nerve capsaicin (hatched bars) pretreatment compared with vehicle (solid bars) in segments thoracic (T) 7 and 8 but not T9–T11. n = 5/Group. *P < 0.05 vs. vehicle.

Fig. 5. Bilateral periaxonal capsaicin treatment to the splanchnic nerve attenuated compensatory adrenal growth. A: right adrenal weight at 5 days after left unilateral adrenalectomy (hatched bars) was greater than that after sham adrenalectomy (solid bars) in vehicle-pretreated or capsaicin-pretreated rats. B: however, the mean increment in adrenal weight after vehicle treatment was greater than that after capsaicin treatment, indicating that bilateral periaxonal capsaicin attenuated compensatory adrenal growth. n = 7/Group. *P < 0.05, **P < 0.01 vs. sham adrenalectomy; #P < 0.01 vs. vehicle.

Fig. 6. Unilateral periaxonal capsaicin application to the left (afferent) or right (efferent) splanchnic nerves affected compensatory adrenal growth. The right adrenal weight at 5 days after left unilateral adrenalectomy (hatched bars) was greater than that after sham adrenalectomy (solid bars) in rats pretreated with vehicle bilaterally or with capsaicin unilaterally. Bilateral capsaicin pretreatment prevented this compensatory adrenal growth. Moreover, the right adrenal weight after left unilateral adrenalectomy was reduced in rats pretreated with capsaicin bilaterally or on the left side alone. There were no differences in right adrenal weights after left sham adrenalectomy between the different drug treatments. n = 7–9/Group. *P < 0.05, **P < 0.01 vs. sham adrenalectomy; #P < 0.05 vs. adrenalectomy with bilateral vehicle.
neurons in the ipsilateral dorsal horn of the spinal cord; the second-order neurons are likely spinothalamic tract (STT), spinomesencephalic tract (SMT), spinoreticular tract (SRT), and/or spinohypothalamic tract (SHT) neurons whose projections decussate and ascend to the brain in the contralateral spinal cord (15, 40). Adrenal splanchnic capsaicin-sensitive afferents could also include fibers reaching the brain via the vagus nerve (7). Brain regions receiving afferent information from the STT, SMT, SRT, SHT, and vagus nerve include the periaqueductal gray, parabrachial nucleus, nucleus of the solitary tract, and several other reticular, pontine, thalamic, and hypothalamic nuclei (15, 40, 41). Many of these regions can then provide direct or indirect input to presympathetic regions controlling sympathetic output to the adrenal gland, such as the paraventricular nucleus of the hypothalamus, the A5 noradrenergic cell group, the caudal raphe nuclei, the rostral ventrolateral medulla, and the ventromedial medulla (30, 33). It is likely that neurons or fibers of passage in the ipsilateral ventral hypothalamus are involved, because electrolytic lesions in this region and hypothalamic hemi-islands prevent compensatory adrenal growth (10, 22). Finally, presympathetic input can descend via the contralateral spinal cord (8). The specific efferent pathway from the spinal cord to the adrenal has not been defined. However, the finding that 6-hydroxydopamine or guanethidine treatment inhibits compensatory adrenal growth (26) implicates adrenal postganglionic sympathetic innervation of the contralateral gland in the growth response. Splanchnic denervation contralateral to the side of adrenalectomy does not impair the growth response (8), consistent with the observation that the adrenal postganglionic sympathetic innervation reaches the adrenal by traveling along the blood vessels and not via the splanchnic nerve (25, 37). The adrenal postganglionic sympathetic innervation regulates the hyperplasic portion.

Fig. 7. Capsaicin applied periaxonally to the splanchnic nerve reduced activation of spinal cord dorsal horn neurons in response to unilateral adrenalectomy. A: in vehicle-treated controls, several c-Fos-positive nuclei (arrows) were present in the ipsilateral dorsal horn of thoracic spinal cord after unilateral adrenalectomy. Bar = 100 μm. B: after capsaicin treatment, few c-Fos-positive nuclei (arrows) were present in the ipsilateral dorsal horn of thoracic spinal cord after unilateral adrenalectomy. Bar = 100 μm. C: in T6 and T9 spinal cord of vehicle-treated controls, the number of c-Fos-positive dorsal horn nuclei was greater on the side ipsilateral to unilateral adrenalectomy than on the contralateral side. In contrast, after capsaicin treatment, there was no difference between the number of c-Fos-positive nuclei ipsilateral and contralateral to adrenalectomy. n = 4–5/Group. ***P < 0.01 vs. ipsilateral; ##P < 0.01 vs. vehicle; ##P < 0.05 vs. previous segment.

Fig. 8. Schematic showing potential neural pathways mediating compensatory adrenal growth. STT, spinothalamic tract; SMT, spinomesencephalic tract; SRT, spinoreticular tract; and SHT, spinohypothalamic tract.
of the compensatory growth response (26), possibly by production of trophic NH₂-terminal fragments from the ACTH precursor proopiomelanocortin (4, 28) and/or by modulation of growth factors (1, 2). The possible additional contribution of capsaicin-sensitive fibers to the efferent limb of the compensatory growth reflex suggests that neurotransmitters commonly found in these fibers, such as CGRP, substance P, and glutamate, may also be involved in modulating these processes.

**Perspectives**

On the basis of the results of the present studies, it is intriguing to speculate that capsaicin-sensitive afferent fibers may be involved in regulating compensatory growth of other organs. For instance, vagal innervation has been implicated in mediating compensatory growth of the ovary and kidney (5, 14, 36). Vagal afferent fibers include capsaicin-sensitive fibers (17, 35), suggesting that the effects of vagotomy may be due to an interruption of the capsaicin-sensitive fibers responsible for the initiation of the compensatory growth reflex. This idea is supported by the fact that compensatory growth of the ovary is inhibited by vagotomy when performed immediately after hemastracation but not when performed at 4.5 h after hematicastration (36). Additional experiments are required to evaluate the possible contribution of capsaicin-sensitive afferents to compensatory growth of other organs.

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**REFERENCES**


