Inhibition of Bradykinin-Induced Plasma Extravasation Produced by Noxious Cutaneous and Visceral Stimuli and Its Modulation by Vagal Activity

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Miao, Frederick Jia-Pei, Wilfrid Jänig, Paul G. Green, and Jon D. Levine. 1997a). However, they are not affected by acute interruption or decentralization ( interruption of the preganglionic axons ) of the lumbar sympathetic innervation of the knee joint nor by epinephrine or another substance released from the adrenal medulla (Green et al. 1995; Miao et al. 1996a,c). Thus reduction of BK-induced PE, generated by both intrathecal nicotine as well as by transcutaneous electrical stimulation of unmyelinated afferents, appears to be mediated by ascending tracts in the spinal cord and by activation of the hypothalamic-pituitary-adrenal (HPA) axis. The depression of BK-induced PE produced by intrathecal nicotine is potentiated by several orders of magnitude after subdiaphragmatic vagotomy, which is reversed by electrical stimulation of the central stumps of the cut vagus nerve (Miao et al. 1994).

These results are compatible with the view that intrathecal nicotine activates neurons in the spinal cord associated with the nociceptive system or central projections of these nociceptive primary afferent neurons. Indeed nicotinic receptors are found in the cord at this level (Boyd et al. 1991; Khan et al. 1994b). Furthermore, previous experiments in which the abdominal vagal afferents were interrupted or stimulated suggest that activity in these visceral afferents regulate the suppression of BK-induced PE by central inhibitory actions (Miao et al. 1994). To further characterize these phenomena, we tested whether graded stimulation of unmyelinated cutaneous afferents, of spinal peritoneal afferents from the abdominal cavity and of spinal visceral afferents from the urinary bladder, lead to a graded inhibition of BK-induced PE and whether this inhibition is potentiated after interruption of the subdiaphragmatic vagus. In these experiments, the cutaneous afferents were excited by transcutaneous electrical stimulation at a strength that was suprathreshold for unmyelinated fibers and at a variable frequency; the visceral afferents were stimulated with capsaicin, over a range of doses.

INTRODUCTION

Synovial plasma extravasation induced experimentally by intra-articular infusion of bradykinin (BK-induced PE) is dose dependently decreased by nicotine injected intrathecally at the level of the lumbar spinal cord (Miao et al. 1994). BK-induced PE is also depressed by transcutaneous electrical stimulation of unmyelinated afferent nerve fibers (Green et al. 1995, 1997). Both of these effects are abolished after transection of the spinal cord at the thoracic level of T₁/T₂, after hypophysectomy, or after blockade of synthesis of corticosterone (Green et al. 1995; Miao et al. 1994, 1996c, 1997a). However, they are not affected by acute interruption or decentralization ( interruption of the preganglionic axons ) of the lumbar sympathetic innervation of the knee joint nor by epinephrine or another substance released from the adrenal medulla (Green et al. 1995; Miao et al. 1996a,c). Thus reduction of BK-induced PE, generated by both intrathecal nicotine as well as by transcutaneous electrical stimulation of unmyelinated afferents, appears to be mediated by ascending tracts in the spinal cord and by activation of the hypothalamic-pituitary-adrenal (HPA) axis. The depression of BK-induced PE produced by intrathecal nicotine is potentiated by several orders of magnitude after subdiaphragmatic vagotomy, which is reversed by electrical stimulation of the central stumps of the cut vagus nerve (Miao et al. 1994).

METHODS

The experiments were performed on male Sprague-Dawley rats except for those experiments in which the spinal afferents from the urinary bladder were stimulated. In these experiments female Sprague-Dawley rats were used (300–400 g; Bantin and Kingman, Fremont, CA). Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (65 mg/kg, Anthony Products, Arcadia, CA). Animal care and use conformed to the guidelines of the National Institutes of Health for the care and use of experimental...
Perfusion of the knee joint

Knee joint perfusion was performed as previously described (Coderre et al. 1989; Miao et al. 1996b). In brief, after incision of the skin and connective tissue overlying the anterior aspect of the knee and the saphenous vein, Evans blue dye (50 mg/kg) was administered intravenously in the saphenous vein. Ten minutes after injection of the dye, a 30-gauge needle was inserted into the cavity of the knee joint for the infusion of fluid (250 µl/min, controlled by a syringe pump from Sage Instruments, Model 351, Cambridge, MA). After infusion of an initial volume of 100–200 µl of vehicle, a second needle (25-gauge) was inserted into the knee joint, ~3 mm from the inflow needle. This second needle served as an outflow cannula. Fluid was withdrawn from the joint through the outflow cannula using a second syringe pump. The fluid was infused and withdrawn at a constant rate of 250 µl/min. Perfusate samples were collected every 5 min for periods of ~185 min. Samples were analyzed for the amount of Evans blue dye by spectrophotometric measurement of absorbance at 620 nm. Absorbance at this wavelength is linearly related to the dye concentration (Carr and Wilhelm, 1964).

After a 15-min baseline perfusion period with vehicle (normal saline), plasma extravasation into the knee joint was stimulated by adding bradykinin (BK, 160 ng/ml, i.e., 1.5 × 10⁻⁷ M) to the perfusion fluid. Both knee joints in the same rat were perfused simultaneously.

Stimulation of spinal visceral afferents by capsaicin

Spinal visceral afferents from the peritoneal cavity and from the urinary bladder were excited by capsaicin. Capsaicin was injected into the peritoneal cavity in progressively higher doses (10⁻⁶ to 1 mg/kg) at 20-min intervals.

Because of their short and relatively straight urethra, female rats were used in the experiments in which visceral afferents from the urinary bladder were stimulated; it is extremely difficult to cannulate the urethra of male rats. A PE-50 polyethylene cannula was placed into the urinary bladder through the urethra without causing trauma. Starting 30 min after beginning the bradykinin infusion into the knee joint cavity, solutions of capsaicin (1 ml) in increasing concentrations (10⁻¹⁰ to 10⁻⁴ M) were injected into the urinary bladder. Each solution was left in the bladder for 20 min, and the bladder then was emptied before a solution with the next higher concentration of capsaicin was injected. An age-matched group of female rats was used as controls in which neither the vagus nerve was cut nor spinal visceral afferents stimulated.

The level of basal synovial plasma extravasation as well as the maximal level of BK-induced PE were smaller in female than in male rats (see Table 1). The reason for this gender difference is probably related to the differences in sex hormones (unpublished observations).

Electrical stimulation of the hindpaw

Two stainless steel electrodes were placed transversely in the plantar surface of the hindpaw contralateral to the perfused knee joint (~10 mm apart). Afferent fibers were excited by a square wave stimulus at an intensity of 25 mA (0.25-ms pulse duration). This stimulus intensity was found to reliably excite C fibers (Green et al. 1995). The stimulus frequencies were doubled progressively, starting with 0.0625 on up to 1 Hz. A stimulation period at a given frequency lasted for 20 min.

Subdiaphragmatic and cervical vagotomy

The vagus nerves were dissected bilaterally at the subdiaphragmatic level, as described previously (Miao and Lee 1989). The dose that produced 50% of the maximum inhibition (ED₅₀ values) were determined for each joint. From these values, the geometric means for ED₅₀ with 95% confidence intervals were calculated (Fleming et al. 1972; Miao and Lee 1989).

Dose-response relationships

Dose-response relationships for capsaicin effects on the inhibition of BK-induced PE were obtained by a cumulative dose method (Miao and Lee 1989). The doses that produced 50% of the maximum inhibition (ED₅₀ values) were determined for each joint. From these values, the geometric means for ED₅₀ with 95% confidence intervals were calculated (Fleming et al. 1972; Miao and Lee 1989).

Materials

Bradykinin acetate, 8-methyl-N-vanillyl-6-nonenamide (capsaicin), and polyoxyethylene sorbitan mono-oleate (Tween 80) were purchased from Sigma Chemical (St. Louis, MO). Capsaicin was first dissolved in a solution mixed with ethanol and Tween 80 (1:1 ratio) and then diluted in normal saline (Travenol Laboratories, Deerfield, IL.). The final concentration of ethanol was <0.1%.

Experimental procedure and statistics

Results are based on different experimental interventions, each conducted on at least eight knee joints (for data summary, see Table 1).
FIG. 1. Effect of intraperitoneal capsaicin on bradykinin-induced plasma extravasation (BK-induced PE) in sham surgery control and in acute subdiaphragmatic vagotomized rats. After baseline plasma extravasation in the first 3 samples, bradykinin (BK; 160 ng/ml) was added to perfusion fluid, and for remainder of the experiment, was the only substance in the perfusion fluid. In control group, rats were with intact vagus nerves and did not receive any injection of capsaicin (●, n = 8). In sham control group, the abdomen was only opened to expose subdiaphragmatic vagus nerves, without cutting them, and then closed again (○, n = 8). Bilateral subdiaphragmatic vagus nerves of rats in vagotomy group were cut 1 h before knee joint perfusion experiment (Δ, n = 8). In sham and vagotomy groups, capsaicin was injected in cumulative doses into the peritoneal cavity (10⁻⁶ to 1 mg/kg). In this and subsequent figures, data are presented as means ± SE. Time-effect curve of sham control group was significantly different from that of acute vagotomy group (F = 32.619, P < 0.01). Both curves of sham and vagotomy groups were significantly different from that in control group in which no capsaicin was injected (F = 49.681 and 170.970, respectively, both P < 0.01).

Table 1). Data are presented as means ± SE; significant differences between pairs of time-effect curves were determined by two-way (group × time) repeated measures analysis of variance (ANOVA). Significant differences between pairs of dose-response curves were determined by two-way (group × dose) repeated measures ANOVA. Differences were considered statistically significant at P < 0.05.

RESULTS

Effects of graded stimulation of spinal visceral afferents by capsaicin

STIMULATION BY INTRAPERITONEAL APPLICATION OF CAPSAICIN. Stimulation of visceral afferents by capsaicin injected intraperitoneally dose dependently inhibited BK-induced PE in the knee joint. This inhibition started at 10⁻⁴ mg/kg and was maximal at ~10⁻¹ mg/kg (● in Fig. 1 and ○ in Fig. 2). Acute subdiaphragmatic or cervical vagotomy significantly potentiated the inhibition of BK-induced PE, which was generated by intraperitoneal capsaicin (subdiaphragmatic vagotomy: ● in Fig. 1 and ● in Fig. 2; cervical vagotomy: × in Fig. 2). After vagotomy, inhibition is now detectable from 10⁻⁶ mg/kg capsaicin and maximal at ~10⁻² mg/kg. The ED₅₀ for inhibition in the sham surgery group was significantly larger than those of subdiaphragmatic vagotomy as well as cervical vagotomy [1.9 × 10⁻⁶ mg/kg (subdiaphragmatic vagotomy) and 1.1 × 10⁻⁶ mg/kg (cervical vagotomy) vs. 3.6 × 10⁻⁴ mg/kg (sham surgery), all P < 0.01; Table 1]. There was no significant difference in the dose-response curves of the inhibition of BK-induced PE between both types of vagotomy (Fig. 2 and Table 1).

STIMULATION OF AFFERENTS OF THE URINARY BLADDER BY INTRAVESICAL INJECTION OF CAPSAICIN. Stimulation of visceral afferents from the urinary bladder by intravesical instillation of capsaicin solution dose dependently inhibited BK-induced PE in sham-vagotomized animals. The inhibition started at ~10⁻⁸ M and was maximal at 10⁻⁴ M capsaicin. Acute subdiaphragmatic vagotomy significantly potentiated this inhibition (● vs. ○ in Fig. 3). The inhibition now started at ~10⁻¹₀ M, and the ED₅₀ of the inhibition after vagotomy was significantly smaller than the ED₅₀ before vagotomy (6.9 × 10⁻¹₀ M vs. 1.6 × 10⁻⁷ M, P < 0.01; Table 1).

Effects of graded stimulation of cutaneous afferents by transcutaneous electrical stimulation of the plantar skin

BK-induced PE was depressed powerfully by transcutaneous electrical stimulation of C fibers as described previously (Green et al. 1995, 1997). The depression of BK-induced PE was graded when the frequency of the electrical stimulus at C-fiber strength (25 mA) was varied from 0.0625 to 1 Hz being maximal at 1 Hz stimulation (● in Fig. 4 and ○ in Fig. 5). After acute subdiaphragmatic vagotomy, low frequency stimulation at 0.0625 Hz, which did not affect BK-induced PE in sham control rats, already produced a depression of BK-induced PE (● in Fig. 4 and ● in Fig.
transcutaneous electrical stimulation led to a graded depression of BK-induced PE and, second, that vagotomy potentiated depression produced by both stimuli. Based on these results, we hypothesize that intrathecal nicotine does excite neurons of the nociceptive pathway. The arguments in favor of this hypothesis are as follows: 1) the nicotinic action was mimicked by stimulation of somatic and visceral nociceptive afferents; 2) vagotomy potentiated the inhibition elicited by both stimulation of nociceptive afferents and intrathecal nicotine (Miao et al. 1994, 1997b); 3) hypophysectomy attenuated both (Green et al. 1995; Miao et al. 1996c); and 4) blockade of glucocorticoid receptors attenuated both (Green et al. 1995; Miao et al. 1996c). These observations suggest a common modulatory mechanism shared by functionally different types of somatic and visceral nociceptive afferent input systems and that a nociceptive-neuroendocrine negative feedback loop, which controls neurogenic inflammatory processes in the synovia of the rat knee joint, is inhibited continuously by activity in vagal afferents and released after vagotomy.

We also found that acute vagotomy does not influence basal synovial plasma extravasation but increases maximal BK-induced PE (see Table 1). At present we have no expla-

DISCUSSION

Previously we have shown that BK-induced PE in the rat knee joint is inhibited dose dependently by intrathecal applied nicotine and by electrical stimulation of cutaneous C but not A fibers. It furthermore was shown that both depressions were mediated by the HPA axis but not by the sympathetic outflow to the knee joint or by the sympatho-adrenal system (Green et al. 1995; Miao et al. 1996c). In the present study, we have shown first that graded stimulation of peritoneal afferents and visceral afferents from the urinary bladder by capsaicin and of cutaneous C-fiber afferents by
FIG. 4. Effect of subdiaphragmatic vagotomy on depression of BK-induced PE into knee joint generated by continuous transcutaneous electrical stimulation of unmyelinated afferents (25 mA, 0.0625 ± 1 Hz, of 0.5 ms duration). After establishment of baseline PE in first 3 samples, BK (160 ng/ml) was added to perfusion fluid and, for remainder of the experiment, was the only substance in perfusion fluid (○, n = 8). In a second group of rats with intact vagus nerves (●, n = 8), C fibers were stimulated electrically, via electrodes in 1 hindpaw, starting 40 min after onset of perfusion of BK, whereas in a third group of animals (ª, n = 8) cutaneous C fibers also were stimulated, but these animals were vagotomized subdiaphragmatically immediately before the experiments. Frequency of stimuli varied from 0.0625 to 1 Hz. Time-effect curve in acute vagotomy group was significantly different from that in sham surgery group (ª vs. ●, F = 20.085, P < 0.01). Curves of both the acute vagotomy and sham surgery groups were significantly different from that of no-stimulation control group (ª vs. ○, F = 349.166, ● vs. ○, F = 25.756, both P < 0.01).

nation for this latter change. If ongoing activity in abdominal vagal afferents continuously activates the HPA axis leading to some depression of BK-induced PE, one would expect an enhancement of BK-induced PE after acute vagotomy.

Figure 6 outlines the putative central neuronal and neuroendocrine circuits that might mediate the vagal modulation of the depression of BK-induced PE generated by noxious stimulation of skin as well as by stimulation of spinal visceral afferents. Afferent activity from skin, viscera, and probably the deep somatic domain is proposed to converge on the same spinal ascending tract neurons that project via the brain stem to the hypothalamus. The nature of this ascending tract is unknown. However, it is clear from our data that this central neural pathway is under powerful inhibitory control from abdominal vagal afferents acting via the nucleus of the solitary tract (NTS) either at the level of the spinal cord, the brain stem and/or the hypothalamus. The final efferent signal derives from the adrenal cortex and is possibly a glucocorticoid (Green et al. 1995, 1997). The target cells mediating the inflammatory process upon which this endocrine signal acts, leading to depression of BK-induced PE, are unclear. They generally are assumed to be cells belonging to the immune system (e.g., macrophages, lymphocytes, and polymorphonuclear leukocytes) or the microvascular endothelium. However, this signal from the adrenal cortex also could act primarily on sympathetic postganglionic terminals because these are necessary to mediate a large part of the BK-induced PE (Green et al. 1997; Miao et al. 1996b,d).

Although the spinal mechanism by which intrathecal nicotine inhibits BK-induced PE is not known, several lines of evidence suggest that it is through spinal nociceptive neurons because there are nicotinic receptors on nociceptive sensory neurons (Boyd et al. 1991; Khan et al. 1994b). Given that the nociceptive neurons can be activated by nicotine via these nicotinic cholinergic receptors (Renshaw et al. 1993; Steen and Reeh 1993), nicotine also should stimulate the central terminals of these afferent neurons. Although some investigators have shown that intrathecal nicotine generates nociceptive behavior in rats (Khan et al. 1994a–c) others have elicited antinociceptive effects by these nicotinic stimuli (Gillberg et al. 1990; Rogers and Iwamoto 1993). The reason for these differences are unknown but may be related to the fact that nicotinic receptors are not only on the central terminals of nociceptive afferent neurons but also on other neurons in the spinal cord (Hösl and Hösl 1994; Khan et al. 1994d).

Intraperitoneal capsaicin probably produces depression of BK-induced PE through stimulation of two sets of afferents. First, it may stimulate spinal afferents from the visceral peritoneal lining covering abdominal and pelvic organs that project through different splanchnic nerves to the thoracic and upper lumbar spinal cord (Jänig and Morrison 1986). Most of these afferents are mechanosensitive and chemo-sensitive (i.e., responding to inflammatory agents, ischemia, etc.) (for review, see Jänig 1996). They are involved in visceral nociception and visceral pain (see Cervero and Morrison 1986; Gebhart 1995), visceral protective reflexes (Jänig 1996;
tory responses via the HPA axis is in agreement with reports that indicate that activity in the vagus nerve inhibits this axis (Bueno et al. 1989; Gonzalez-Fernandez and Gonzalo-Sanz 1987) and, therefore, the effects of stimuli that activate the HPA axis. Because activation of the HPA axis produces a potent anti-inflammatory effect, an enhanced activity of the HPA axis after removal of the vagal afferent activity would be expected to potentiate the anti-inflammatory effect of intrathecal nicotine, noxious visceral stimuli, or noxious cutaneous stimuli in reducing synovial plasma extravasation. An interesting corollary of the present study is that spinal visceral afferents and vagal visceral afferents have functionally opposite reflex effects on the neurogenic inflammatory process via the HPA axis.

Recently it was shown in experiments in monkeys and rats that vagal afferents are involved in control of nociception and pain. In rats, cervical vagotomy reduces stress-induced analgesia generated by intermittent foot shocks (Maixner and Randich 1984; Maixner et al. 1982). Furthermore, stimulation of volume receptors from the right atrium produces inhibition of tail flick reflex to noxious radiant heat (Maixner and Randich 1984). In monkeys, electrical stimulation of cervical vagal afferents suppresses transmission of impulse activity in spino-thalamic relay neurons with nociceptive function at all levels of the spinal cord. Electrical stimulation of subdiaphragmatic vagal afferents has no effect on the spino-thalamic relay neurons in this species. These

Jäning and Häbler (1995) and possibly regulations of visceral organs. Second, it also may excite afferents innervating the parietal peritoneum. Little is known about the functional properties of these afferents, but they also may respond to local mechanical and chemical stimuli (Bahns et al. 1986), these afferents may belong to the so-called “silent afferents”, which are only activated under special extreme conditions (Michaelis et al. 1996). They are not visceral in the true sense but deep somatic afferents (see Lewis 1942). Finally, intraperitoneal capsaicin may stimulate vagal afferents that project to the nucleus of the solitary tract (Ritter and Dinh 1988). The functional types of vagal afferents excited in this way are unknown.

Capsaicin injected into the urinary bladder excites sacral as well as lumbar visceral afferents (vagal visceral afferents should not be involved because there is no vagal innervation to the urinary bladder) (Jäning 1996; Jäning and Morrison 1986). Both sets of afferents are involved in visceral nociception of the urinary bladder (Jäning and Koltzenburg 1993); both also may be involved in inhibitory control of BK-induced PE. The magnitude of the vagotomy-induced leftward shift for the dose-response curve and the maximal depression of BK-induced PE generated by the three stimulus paradigms were similar in degree (Table 1).

The hypothesis of vagal afferent modulation of inflammatory responses via the HPA axis is in agreement with reports that indicate that activity in the vagus nerve inhibits this axis (Bueno et al. 1989; Gonzalez-Fernandez and Gonzalo-Sanz 1987) and, therefore, the effects of stimuli that activate the HPA axis. Because activation of the HPA axis produces a potent anti-inflammatory effect, an enhanced activity of the HPA axis after removal of the vagal afferent activity would be expected to potentiate the anti-inflammatory effect of intrathecal nicotine, noxious visceral stimuli, or noxious cutaneous stimuli in reducing synovial plasma extravasation. An interesting corollary of the present study is that spinal visceral afferents and vagal visceral afferents have functionally opposite reflex effects on the neurogenic inflammatory process via the HPA axis.

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observations are in favor of the notion that particularly cardio-pulmonary afferents are involved in this inhibitory control. The central pathways that mediate this effect are neurons in the subcerebro-parabrachial complex (noradrenergic) and neurons in the nucleus raphe magnus of the rostroventral medulla (serotonergic) that project to the dorsal horn (for review, see Foreman 1989). Similar results were obtained in the rat by Gebhart and coworkers. Here transmission of nociceptive impulses from skin and colon in the dorsal horn and the tail-flick-reflex elicited bynoxious heat stimulation of the tail were enhanced by electrical stimulation of myelinated vagal afferents and depressed by electrical stimulation of unmyelinated vagal afferents. The subdiaphragmatic vagus afferents were particularly powerful in eliciting these modulatory effects. As in the monkey, the inhibitory effects were generated via descending systems from the subcerebro-parabrachial complex and from the rostroventral medulla. The facilitatory effect was mediated by suprapontine pathways (see Gebhart and Randich 1992; Randich and Gebhart 1992).

The present study, together with our previous reports (Green et al. 1995; Miao et al. 1994, 1996c), has demonstrated a novel nociceptive-neuroendocrine negative feedback mechanism that regulates microvascular permeability in the knee joint of the rat. It is hypothesized that excitation of the spinal ascending neurons, generated by intrathecal nicotine, cutaneous, or visceral noxious stimuli, decreases synovial plasma extravasation via activating the HPA axis and that activity of vagal afferents from abdominal visceral organs modulates this negative-feedback circuit, leading to its attenuation when the level of activity in vagal afferents increases and to its potentiation when the level of vagal activity decreases. Vagal afferents from the abdominal organs project organotopically to the NTS (Ritter et al. 1992).

The second-order neurons in the nucleus of the solitary tract interact with neurons of the ascending system to the hypothalamus; the hypothalamic neurons are activated by the nociceptive visceral and cutaneous inputs. It is unclear at which site this interaction occurs, but central pathways mediating these inhibitory vagal effects may be the same as those that are involved in inhibitory control of nociceptive and pain from the visceral domain (Foreman 1989; Gebhart and Randich 1992; Randich and Gebhart 1992) (Fig. 6). This feedback system may play an important role in modulating somatic inflammatory responses under physiological (e.g., by changes of motility and chemical content of the duodenum and small intestine after a meal) and pathophysiological conditions (e.g., by intoxication and inflammation of the small intestine).

The functional type(s) of the vagal afferents involved in this modulation of the negative feedback neuroendocrine mechanism that controls BK-induced PE are unknown. The subdiaphragmatic vagus nerves in the rat are composed of five major branches projecting to the stomach, to the liver, and, via the coeliac ganglion, particularly to the small intestine (Precht and Powlley 1985; Ritter et al. 1992). Recent studies in our laboratory suggest that afferents in the coeliac and accessory coeliac branches are important in this inhibitory modulation but not afferents in the hepatic and gastric branches (Miao et al. 1997b).

In summary, noxious stimulation of skin and viscera produces depression of BK-induced PE, and this depression is potentiated after subdiaphragmatic vagotomy as is the suppression of BK-induced PE by intrathecal nicotine. The results imply that intrathecal nicotine may excite spinal nociceptive neurons and/or axons. Further elucidation of the underlying mechanisms should provide details on important intrinsic neural pathways for modulation of the inflammatory response.

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