Vagal Influence on Compensatory Ovarian Growth Is Important Only Briefly after Hemicastration

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Hemicastration induces growth of the remnant ovary in the rat. As evidenced by the effects of total abdominal vagotomy, vagal innervation markedly influences this compensatory ovarian growth. In the present experiments, vagotomy inhibited compensatory ovarian growth when performed immediately after hemicastration, but not when delayed until 4.5 hr after hemicastration. Brief exposure of subdiaphragmal portion of the vagi nerves to 2% lidocaine shortly before hemicastration also inhibited compensatory growth. Fifteen minutes after hemicastration, markedly elevated tissue concentrations of cyclic adenosine monophosphate (cAMP) were recorded in the remnant ovaries This accumulation of cAMP was inhibited by vagotomy that preceded hemicastration, as well as by lidocaine pretreatment of the vagi nerves, and partly by vagotomy that followed 10 min after hemicastration. At 5 hr after hemicastration, tissue cAMP concentrations in the remnant ovaries were not elevated and were not affected by vagotomy. The present results suggest that vagal influence on the compensatory ovarian growth is important only during a short period of time after hemicastration (apparently shorter than 4.5 hr), and that it, at least briefly after hemicastration, includes neural input to the ovary.

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As first described by Hatai (1), hemicastration (unilateral ovariectomy) induces enlargement of the remnant ovary in the rat. This compensatory growth is due to follicular activation aimed at compensating for the loss of hormonal and ovulatory function (2), and it critically depends on gonadotropins (3). However, the rat ovary is richly innervated (4) and innervation apparently influences both the prepubertal and mature ovaries (5–7). Based on the effects of various surgical dennervation procedures, it has been shown that ovarian innervation is involved in the compensatory growth, as well (8, 9). A part of the innervation is vagal in origin—vagal afferents supply the ovary and there is a pathway connecting it with vagal nuclei in the brainstem (10, 11). Total (bilateral) abdominal vagotomy inhibits compensatory ovarian growth, clearly indicating involvement of the vagi nerves in this process (8, 12). The results presented here suggest that vagal influence is important only during the first few hours after hemicastration, and that it, apparently, includes neural input to the ovary.

Materials and Methods

Experimental Design. Female mature Wistar rats (at least two consecutive 4- to 5-day estrus cycles as estimated by vaginal smears) were housed (4–5 per cage) at 22° ±2°C, under a 12:12-hr light:dark schedule (light from 0700 hr), and were given pelleted food and water *ad libitum*. At inclusion, animals weighed between 140 and 200 g.

In the first series of experiments, compensatory ovarian growth was studied. 1) Animals were hemicastrated and immediately submitted to bilateral abdominal vagotomy. 2) Animals were vagotomized 4.5 hr after hemicastration. 3) The abdominal vagi nerves were treated locally with 2% lidocaine for 10 min and hemicastration was performed 30 min later. Compensatory ovarian growth was always estimated 10 days after hemicastration.

In the second series of experiments, the ovarian tissue cAMP concentrations were measured. Animals were vagotomized immediately before or 10 min after hemicastration. The abdominal vagi nerves were treated locally with lidocaine and hemicastration followed after 30 min. In both cases, the remnant ovaries were removed 15 min after hemicastration. Animals were vagotomized 4.5 hr after hemicastration, and the remnant ovaries were removed 30 min later.

Hemicastration. We always removed the right ovary, and always between 0900 and 1200 hr on day of estrus in anesthetized rats (chloral hydrate 300 mg/kg, intraperitoneally, Merck Co., Germany) (12). Briefly, the

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ovary was exposed through a dorsolateral incision, ligated in the hilar area, and removed. For sham surgery, the ovary was only exposed and left in place (12). The incision was closed with sutures using sterile catgut.

Total Abdominal (Subdiaphragmal) Vagotomy and Sham Vagotomy. These procedures were performed as described by Burden and coworkers (8, 12). Briefly, a midventral incision was made through the upper abdominal wall along the linea alba. The stomach was pulled caudally, the ligaments connecting the liver and the stomach were cut, and the liver was reflected. The esophagus was exposed and the anterior and posterior vagal trunks were separated from it under a magnifying glass and cut with fine scissors immediately below the diaphragm. For sham vagotomy, the nerves were exposed and left intact. The incision was closed with sutures using sterile catgut. Successful vagotomy was confirmed by the presence of an enlarged, distended stomach at the autopsy and by decreased body weight gain over a 10-day period (8, 12, 13).

Local Application of Lidocaine to the Abdominal Vagi Nerves. The esophagus and the abdominal vagi nerves were exposed as described above and were underlain with a plastic gutter made from a micropipette tip and coated with a few threads of cotton. Twenty-five microliters of 2% lidocaine (Lidokain 2%, Krka, Slovenija) was dripped on the exposed structures. A few threads of cotton were placed on the ventral side of the structures and another 25 μl of lidocaine was dripped. The cotton threads and plastic gutter were removed after 10 min. For sham treatment, a total of 50 μl of saline was applied. Testing with methylene blue revealed no leakage in the abdominal cavity during a 10-min exposure. Unlike vagotomy, this procedure did not affect the stomach size or the body weight gain over a 10-day period.

Assessment of Ovarian Growth. The remnant ovaries were collected on ice, cleaned under a magnifying glass, weighed, and kept at −22°C until biochemical analysis. Ovarian wet weight, protein content (14), and total RNA and total DNA (15) contents were measured as parameters of growth. Both absolute (milligram and microgram) (12) and relative (per 100 g body weight) (8, 9) values were considered.

cAMP Measurement. Ovaries were removed, quickly cleaned on ice under a magnifying glass, weighed, and put in cold Hank's buffer (pH 7.4; 20 μl/mg tissue) containing 1 mM isobutyl-methylxanthine (IBMX, Sigma Co., St. Louis, MO) for 5 min with gentle shaking. Ovaries were then homogenized in 1 ml of 10% trichloroacetic acid (w/v; TCA, Merck Co.) in a Potter-Elvehjem glass homogenizer. The supernatant obtained by centrifugation of the homogenate (2000*g*, 5 min at 4°C) was washed four times with 4 ml of water-saturated ether (10% H_2O, v/v) to remove the acid. To assure that the samples aimed for the assay would not be acidic, 100 μl of Tris EDTA (4 mM EDTA) buffer, pH 7.5 (constituent of the assay kit), was added to 200-μl aliquots (in duplicate) of the washed extract. These samples were then assayed for cAMP in accordance with the instructions for use of [^{3}H] cAMP Biotrak assay kit (TRK 432, Amersham, Buckinghamshire, UK). A Beckman LS 1701 scintillation counter was used (Beckman Instruments, Fullerton, CA).

Statistics. Data were analyzed by ANOVA and Newman-Keuls' test at 95% significance level using STATISTICA for Windows software.

The experiments complied with the Croatian Law on Animal Well-being (Zakon o dobrobiti životinja) and were approved by the Ethical Committee at the School of Medicine, Zagreb University.

Results

Effects of Vagal Manipulations on the Body Weight Gain. Abdominal vagotomy decreased the body weight gain. The mean change in body weight during the observed 10 days after the surgery was, in vagotomized animals hemicastrated or not, between −6.6 ± 5.7 g and −2.3 ± 7.2 g, while in sham-vagotomized animals, this change was between 18.9 ± 3.6 g and 24.1 ± 4.2 g. In contrast, local application of lidocaine to the vagi nerves had no effect on the body weight gain—the mean change in body weight over the observed 10 days was similar in all four experimental groups (saline or lidocaine locally + hemicastration or sham surgery), and was within the range of 19 to 25 g. Vagal manipulations had no effect on body weight in the acute experiments (15–30 min) in which ovarian cAMP was measured.

Effects of Vagal Manipulations on the Compensatory Ovarian Growth. As shown in Figures 1 and 2, the remnant ovaries in hemicastrated + sham-vagotomized or saline-treated animals were markedly heavier 10 days after the surgery (by 40%–66%), and contained more proteins, more total RNA, and more total DNA (by 33%–67%) than the control ones ($P < 0.05$). Figure 1A shows that abdominal vagotomy performed immediately after hemicastration inhibited this compensatory growth—ovarian parameters in vagotomized animals did not differ from the control values. In contrast, Figure 1B shows that vagotomy was ineffective when delayed until 4.5 hr after hemicastration and the compensatory growth was similar to that observed after hemicastration alone. Figure 2 shows that local application of lidocaine to the abdominal vagi nerves before hemicastration also inhibited compensatory growth. Ovarian parameters in hemicastrated rats treated with lidocaine were still higher (14%–35%; $P < 0.05$, except for total RNA) than in controls, but also significantly lower ($P < 0.05$) than in hemicastrated animals treated with saline. None of the manipulations affected significantly the ovarian growth parameters in sham hemicastrated animals.

Effects of Hemicastration and Vagal Manipulations on the Ovarian Tissue cAMP Concentration. As shown in Figure 3, tissue cAMP levels in remnant ovaries in hemicastrated + sham-vagotomized or saline-treated rats were 2- to 3-fold ($P < 0.05$) control values 15 min after

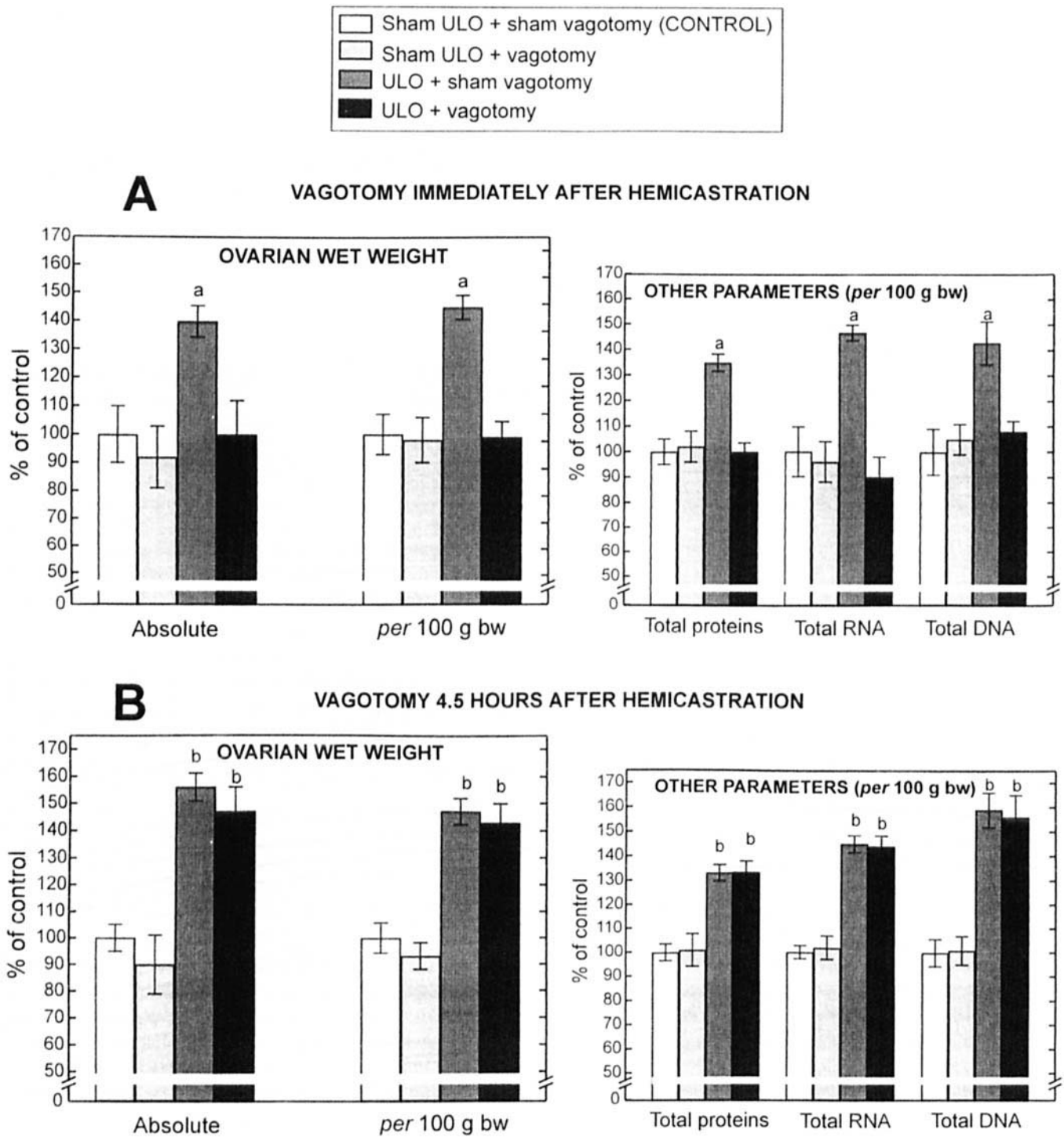

Figure 1. Effect of total abdominal vagotomy on compensatory ovarian growth. (A) Vagotomy immediately after hemicastration (unilateral ovariectomy, ULO). (B) Vagotomy 4.5 hr after hemicastration. Mature rats underwent (right) hemicastration (or sham surgery) on estrus and were vagotomized (or sham-vagotomized) (A) immediately after hemicastration or (B) 4.5 hr after hemicastration. The remnant (left) ovaries were removed ten days after the surgery and organ wet weight, protein content, and total RNA and total DNA contents were determined. Ovarian growth parameters were analyzed by ANOVA and Newman-Keuls' test. Identical patterns of changes were observed regardless of whether absolute of relative (per 100 g body weight) values had been used. Therefore, both types of data are shown only for the ovarian wet weight. Results are presented as the percentage of control (means ± SEM, 9–10 animals per group), where 100% = mean value for the control group. The control values were: absolute wet weight (milligrams) (A) 30.4, (B) 33.4; relative wet weight (mg/100 g body weight) (A) 18.7, (B) 19.5; total protein content (mg/100 g body weight) (A) 0.78, (B) 0.89; total RNA content (μg/100 g body weight) (A) 19.2, (B) 19.3; and total DNA content (μg/100 g body weight) (A) 22.4, (B) 22.2. $^{a}P < 0.05$ vs all other groups, $^{b}P < 0.05$ vs control and vs sham ULO + vagotomy.

the surgery. Figure 3A shows that this early hemicastration-induced cAMP accumulation was inhibited by vagotomy that preceded hemicastration and to a lesser extent by vagotomy that followed 10 min after hemicastration. In the latter case, ovarian tissue cAMP concentration was lower ($P < 0.05$) than that measured after hemicastration alone, but

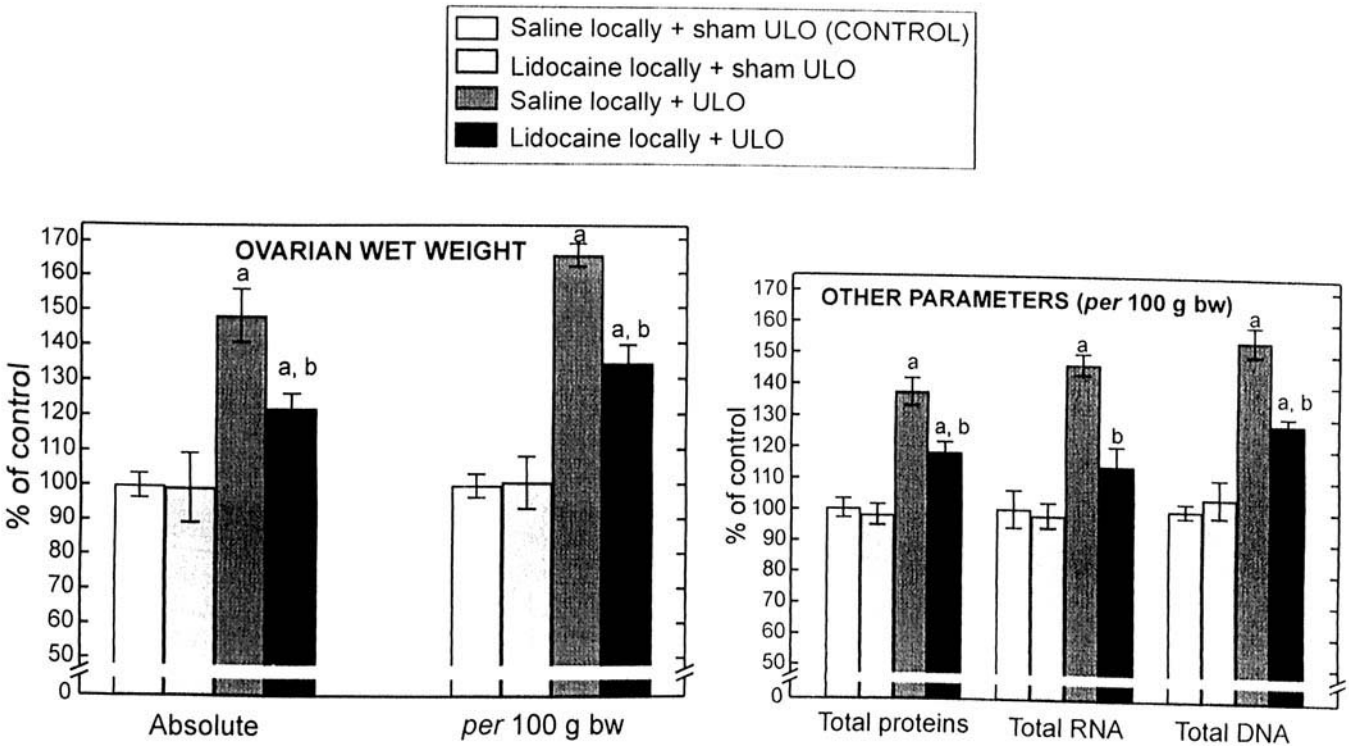

Figure 2. Effect of 2% lidocaine applied locally to the abdominal vagi on the compensatory ovarian growth. Subdiaphragmal vagi nerves in mature rats were treated locally with 2% lidocaine (or saline) for 10 min and (right) hemicastration (unilateral ovariectomy, ULO) or sham surgery was performed 30 min later. Animals were treated on estrus. The remnant (left) ovaries were removed 10 days after the surgery and organ wet weight, protein content, and total RNA and total DNA contents were determined. Ovarian growth parameters were analyzed by ANOVA and Newman-Keuls' test. Identical patterns of changes were observed regardless of whether absolute of relative (per 100 g body weight) values had been used. Therefore, both types of data are shown only for the ovarian wet weight. Results are presented as the percentage of control (means ± SEM, 10 animals per group), where 100% = mean value for the control group. The control values were: absolute wet weight, 34.1 mg; relative wet weight, 20.7 mg/100 g body weight; total protein content, 0.91 mg/100 g body weight; total RNA content, 20.5 μg/100 g body weight; and total DNA content, 23.6 μg/100 g body weight. $^{a}P < 0.05$ vs control and vs lidocaine locally + sham ULO, $^{b}P < 0.05$ vs saline locally + ULO.

still higher than values in animals vagotomized before hemicastration ($P < 0.05$) and higher than control values (with borderline significance, $P = 0.052$). Figure 3B shows that pretreatment of the nerves with 2% lidocaine also inhibited the hemicastration-induced cAMP accumulation in the remnant ovary. As shown in Figure 3C, at 5 hr after hemicastration, the ovarian cAMP levels were neither elevated nor affected by vagotomy performed 30 min earlier. None of the treatments affected ovarian cAMP levels in sham-hemicastrated rats.

Discussion

The amount of compensatory ovarian growth observed in the present experiments was consistent and comparable to the literature data (8, 12). Vagotomy clearly inhibited compensatory growth when performed immediately after hemicastration, while it was clearly ineffective when delayed until 4.5 hr after hemicastration. Brief application of 2% lidocaine locally to the abdominal vagi nerves shortly before hemicastration also inhibited compensatory growth. This last effect was not as pronounced as the effect of the "early" vagotomy, but it could be estimated as 50% reduction in compensatory growth. Local treatment of the nerves with lidocaine and "delayed" vagotomy apparently differed in their effects on compensatory ovarian growth (inhibition by lidocaine, no effect of delayed vagotomy) and body weight gain (no effect of lidocaine, reduced by delayed vagotomy). These observations support the view that the effect of vagal disruption on the compensatory growth is, at least partly, attributable to interruption of specific vagal-ovarian innervation and not to nonspecific mechanisms such as disturbed alimentary and/or metabolic processes (12). Regarding the different effects of the early and late vagotomy, and the fact that lidocaine most probably acted for no longer than a few hours (16), vagal influence on compensatory ovarian growth seems to be important only during a brief period of time (apparently shorter than 4.5 hr) after hemicastration.

The hemicastration-induced accumulation of cAMP in the remnant ovary recorded 15 min after the surgery suggests the existence of signals reaching the remnant ovary soon after hemicastration. Considering that cAMP accumulation was completely inhibited by preoperative vagotomy or lidocaine pretreatment of the vagi nerves, and partly by vagotomy performed 10 min after hemicastration, vagal involvement in transmission of these signals seems obvious. Their nature, however, remains unclear. Gonadotropins, which are of critical importance for compensatory ovarian

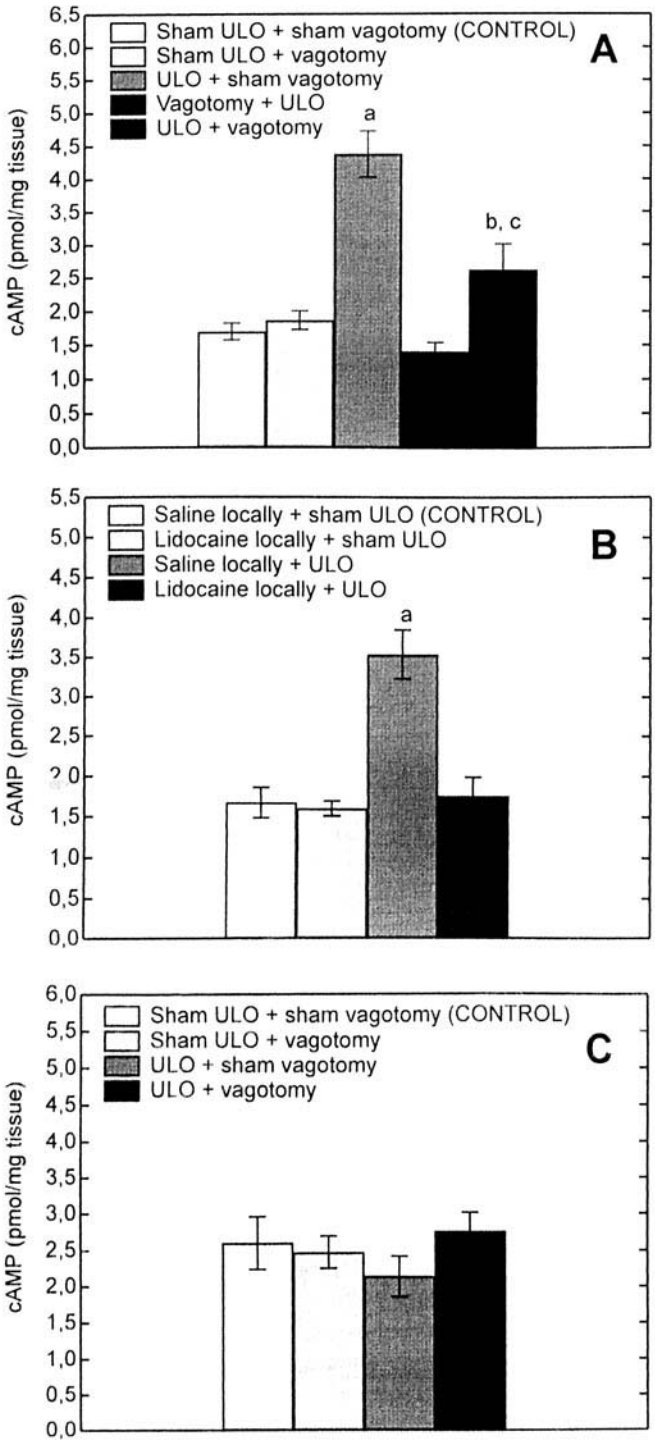

Figure 3. Effects of hemicastration (unilateral ovariectomy, ULO) and vagal manipulations on ovarian tissue cAMP levels. (A) Mature rats were vagotomized (or sham vagotomized) before or 10 min after (right) hemicastration (or sham surgery). (B) 2% lidocaine (or saline) was applied locally to abdominal vagi nerves for 10 min, and hemicastration followed 30 min later. In both cases, the remnant (left) ovaries were removed 15 min after hemicastration and tissue cAMP was measured. (C) Animals were vagotomized 4.5 hr after hemicastration, and remnant ovaries were removed 30 min later for tissue cAMP measurement. Animals were treated on estrus. Each bar = means ± SEM, 7 to 10 animals per group. Data were analyzed by ANOVA and Newman-Keuls' test. $^{a}P < 0.05$ vs all other groups, $^{b}P < 0.05$ vs vagotomy + ULO, $^{c}P = 0.052$ vs control.

growth, utilize the intracellular cAMP signaling (17). Hemicastration induces a (transient) rise in serum gonadotropin levels (18, 19), which is inhibited by abdominal vagotomy (8). Yet, the increase in serum gonadotropins has been repeatedly reported to occur several hours after hemicastration (18–20). According to Welschen and Dullaart (19), the first change to be detected is a rise in serum follicle stimulating hormone (FSH) levels that occurs at 5 hr after the surgery in rats hemicastrated on estrus or diestrus 2. In contrast, in the present experiments, cAMP accumulation in the remnant ovary was observed as early as 15 min after the surgery. Furthermore, at 5 hr after hemicastration, cAMP levels in the remnant ovary were neither elevated nor affected by abdominal vagotomy performed 30 min earlier (4.5 hr after hemicastration). Therefore, it is unlikely that cAMP accumulation was mediated through vagal-gonadotropin interactions. Involvement of some other "non-neural" extraovarian mediator acting at the ovarian level cannot be ruled out. The time course of events, however, suggests that cAMP accumulation was induced by neural signals, either directly or through some intraovarian factor. The input might have originated from vagal neurons ("visceromotor" or sensory) or from other neurons innervating the ovary, and vagus could have been the afferent or the efferent arm, or both, of a multisynaptic reflex mechanism. Recent morphological studies (11) have outlined the anatomical basis for such complex neural interactions.

In the present experiments, permanent (vagotomy) and transient (local lidocaine treatment) disruption of vagal function inhibited both the early hemicastration-induced ovarian cAMP accumulation and the compensatory ovarian growth. The nature of this association needs to be further investigated. Mayerhofer and coworkers (21) have demonstrated that vasoactive intestinal polypeptide (VIP), a neurotransmitter present in both autonomic and sensory neurons (including vagus) and abundantly present in the rat ovary, *in vitro* stimulates cAMP accumulation and FSH receptor expression in primary follicles of the immature rat ovary. Exposure of VIP-primed follicles to FSH results in follicular growth. It seems plausible that the early vagus-dependent signals inducing ovarian cAMP accumulation in the present experiments might have been involved in the early phases of compensatory growth in a similar way.

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