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Release of Norepinephrine from the Cat Ovary: Changes after Ovulation[1]

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ABSTRACT

The distribution of intraneuronal constituents involved in norepinephrine (NE) storage, uptake, and release were used to estimate changes in NE secretion from the cat ovary after ovulation induced with eCG plus hCG. The content of NE and ATP, which are principally stored in small noradrenergic vesicles (isolated at a density of 1.041 g/ml in Percoll gradient), decreased after ovulation. However, the activity of dopamine β-hydroxylase, which is principally associated with large noradrenergic vesicles (isolated at a density of 1.033 g/ml in Percoll gradient), was only slightly decreased. Mg^{2+}-dependent ATPase, located in both large and small storage vesicles, decreased only in the small storage vesicles, suggesting that preferential secretion from small noradrenergic vesicles occurred. The hormonal treatment also affected the functional capacity of the vesicles, as evidenced by the decrease in uptake and storage capacity as well as the decrease in the stimulated release of ^{3}H-NE observed after ovulation. The aforementioned changes are characteristically seen after a sympathetic discharge; thus they strongly support the notion that ovarian sympathetic activity increases during the ovulatory process, resulting in the postovulatory decrease in both the size and functional capacity of the intraneuronal compartment where NE is stored.

INTRODUCTION

The mammalian ovary has a well-defined sympathetic innervation [1, 2]. Increasing evidence suggests that this innervation plays an important role in regulating ovarian functions such as follicular maturation, steroid secretion, and ovulation [3–5]. Catecholamines, and more specifically norepinephrine (NE), appear to exert their effects by acting on specific receptors located on thecal and/or granulosa cells [6, 7].

Whereas ovarian NE content has been found to decrease during ovulation [8, 9], NE content in follicular fluid of preovulatory follicles increases during the preovulatory period [10]. This suggests that changes in the secretory activity of intraovarian noradrenergic nerve terminals occurs during the periovulatory period. In support of this notion, Wolf et al. [11], using an in vivo-perfused rat ovary preparation, have reported a preovulatory increase of NE released to the perfusate in the afternoon of proestrus. These findings, however, must be interpreted with caution because the push-pull cannula used is freely permeant to plasma constituents and the changes observed may have resulted from the elevation in plasma NE levels described to occur in the afternoon of proestrus [12].

To circumvent this problem, we induced ovulation in cats and assessed the biochemical changes that occurred in ovarian noradrenergic nerve terminals and NE storage vesicles by measuring both NE levels and dopamine β-hydroxylase (DβH) activity. NE and DβH (the enzyme that catalyzes the conversion of dopamine to NE) have been widely used as specific vesicular markers (13–15). As additional vesicular markers, we measured ATP (which is costored with NE) and Mg^{2+}-dependent ATPase (enzyme bound to the membrane of NE storage vesicles). To determine the functional significance of the changes observed, we studied the uptake of [^{3}H]norepinephrine (^{3}H-NE) by the storage vesicles and the subsequent release of incorporated ^{3}H-NE induced by a depolarizing stimulus. We chose the cat ovary because of its dense sympathetic innervation [1] and the changes in the intensity of NE-fluorescent nerves occurring between animals in estrus and anestrus [16].

MATERIALS AND METHODS

Materials

Coomassie Brilliant blue dye G-250 and eCG were from Calbiochem-Behring Corp. (San Diego, CA). Percoll was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). ^{3}H-NE (sp.act. 18.8 Ci/mmol) and [^{3}H]tyramine (sp.act. 26 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA). Other reagents were from Sigma Chemical Co. (St. Louis, MO).

Animals

Adult female cats weighing 2.0–2.5 kg were used. Animals were maintained with free access to food and water

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in rooms (2 m × 3 m) on a natural L:D cycle. Room temperature was 22°C. To induce estrus or ovulation, cats were treated with eCG or a combination of eCG and hCG, as described by Wildt et al. [17] with some minor modifications. For the control group (estrous), estrus was induced with a single intramuscular dose of 750 IU of eCG dissolved in saline. After 3 days, the animals were allowed to interact freely with a vasectomized male; when lordosis occurred, they were immediately separated from the male and used for experiments. For the ovulating group (n = 18), ovulation was induced with the same dose of eCG (750 IU) followed by 2 intramuscular doses of hCG (500 IU) 6 and 7 days after the eCG injection. Three days after the last injection, the animals were used for experiments. With this procedure, ovulation was induced in 100% (18 of 18) of the cats treated with eCG and hCG but did not occur in any of the animals treated with only eCG (6 of 6). To evaluate the effect of eCG alone, NE levels and DβH total activity were measured 10 days after the eCG injection (eCG, 10 days).

The day of the experiments, animals were anesthetized with sodium pentobarbital (30 mg/kg i.p.). The ovaries were removed through an abdominal midline incision, cleaned, inspected for the presence of mature and/or ruptured follicles (corpora hemorrhagica), and used for the experiments. In ovulating cats, each ovary exhibited 4–7 ruptured follicles.

Preparation and Purification of Noradrenergic Vesicles by Differential Centrifugation and Percoll Gradient Centrifugation

Differential centrifugation procedures to obtain tissue fractions and Percoll gradients to obtain storage vesicle fractions were performed as previously described [18].

In each experiment, ovaries from two cats were used. They were minced with scissors and homogenized in 10 vol cold (4°C) 0.25 M sucrose/1 mM phosphate buffer, pH 7.4, by 3 strokes of a conical glass-glass homogenizer (clearance 0.11–0.15 mm). The homogenate was centrifuged at 1 000 × g for 10 min to remove cell debris and nuclei (P_1). The sediment was suspended in 1 ml of buffer and centrifuged as above. The supernatants were pooled and centrifuged at 12 000 × g for 20 min to obtain a mitochondrial fraction (P_2). The supernatant was finally centrifuged at 105 000 × g for 60 min to obtain a microsomal pellet (P_3, vesicular fraction) and a final supernatant (Sf). One milliliter of the resuspended P_3 fraction was mixed with 11 ml of 15% (vol/vol) of Percoll made isosmotic with 0.25 M sucrose/1 mM phosphate buffer, pH 7.4. The suspension was centrifuged at 78 000 × g for 30 min in a Type 65 angle head rotor of a Beckman L5-65 ultracentrifuge (Beckman Instruments, Palo Alto, CA). At the end of the run, 6 fractions of 2 ml each were collected from the bottom of the tube by means of a piercing needle. Density of the fractions was determined by refractometry.

Uptake of ^{3}H-NE by the Storage Vesicles

Uptake of ^{3}H-NE was carried out according to Fried [19] with minor modification. Aliquots (0.34 ml) of the resuspended P_3 fraction (0.10–0.16 mg protein/ml) were incubated at 30°C for 20 min with 0.06 ml of the following medium (final concentration): 0.2 μM ^{3}H-NE; 15 mM ATP; 5 mM $MgCl_2$; 5 mM Tris/HCl, pH 7.4; 20 mM NaCl; and 5–40 μM NE. This mixture was incubated for 20 min at 30°C in a shaking thermoregulated bath. Blanks were samples incubated at 4°C. To stop the reaction, 2 ml of cold (4°C) 0.25 M sucrose/1 mM phosphate buffer, pH 7.4, was added, and the suspension was vacuum-filtered through Millipore (Type EG, 0.2 μm) filters (Millipore Corp., Bedford, MA) and washed twice with 1 ml of 0.25 M buffered sucrose. Radioactivity retained in the filter was determined by liquid scintillation counting. To identify the population of vesicles that took up ^{3}H-NE, some samples of the P_3 fraction, which had been incubated with 5 μM NE for 30 min, were centrifuged at 105 000 × g for 60 min. The pellet was washed, suspended in 0.25 M buffered sucrose and centrifuged in Percoll gradients to separate storage vesicles as described above.

Both NE and ^{3}H-NE were measured and the distribution of each was precisely determined in each of the gradient fractions.

Release of ^{3}H-NE

Ovaries from estrous or ovulating cats were cut into 0.5-mm slices, preincubated for 20 min in Krebs-bicarbonate buffer solution, pH 7.4, gassed with O_2 (95%)/CO_2 (5%), and incubated for 30 min at 37°C with 2 μCi of ^{3}H-NE. The 65 mM KCl-induced release and the spontaneous release of the neurotransmitter were studied as previously described [20]. Results are expressed as percentages of fractional release.

Assays

NE. Tissue and gradient fractions (0.5 ml) obtained for the NE assay were precipitated with perchloric acid to a 0.4 M final concentration. After centrifugation, the supernatant was purified by adsorption chromatography on alumina and assayed spectrofluorimetrically for NE as previously described [15, 20].

DβH. DβH activity in tissue and gradient fractions was determined by the enzyme-catalyzed hydroxylation of [^{3}H]tyramine to [^{3}H]octopamine as previously described [14, 15]. This product was chemically oxidized to ^{3}H-*p*-hydroxy-benzaldehyde and selectively extracted in toluene. Aliquots (0.2 ml) of the sample fractions obtained for the assay were incubated for 60 min at 37°C with an incubation mixture containing optimal concentrations of $CuSO_4$ (1–5 μM) and *p*-hydroxy-mercury-benzoate (0.05–2.0 mM) to inactivate endogenous inhibitors [18]. Samples obtained from Percoll gradients required only $CuSO_4$.

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Adenosine triphosphate. The assay was carried out according to the firefly method of Stanley and Williams [21]. The emission of light by the firefly-luciferin-luciferase system was measured in a Nuclear Chicago scintillation counter (Searle Instr., Oakville, ON) with a double photomultiplier detector used out of coincidence. When ATP was to be measured, samples were deproteinized with 0.4 M perchloric acid and centrifuged at 15 000 × g for 10 min. Before the assay, 0.1 ml of the acid supernatant was neutralized with KOH.

Mg^{2+}-dependent ATPase. Activity was assayed according to Johnson et al. [22] with some modifications. We used 5 mM HEPES/Li^+, pH 7.4, and 10 μg/ml oligomicine to inhibit the mitochondrial enzyme [23].

Proteins. Proteins were determined by the method of Bradford [24] using BSA as a standard.

Statistics

All the results are expressed as mean ± SEM. Statistical differences between two groups were assessed with the two-tailed Student's t-test with a significance level of $p < 0.05$.

RESULTS

NE, DβH, and Protein Concentrations in Homogenates and Tissue Fractions Obtained by Differential Centrifugation

The ovarian weight and the concentration of NE and DβH activity in total homogenates of ovaries from estrous (eCG-treated) and ovulating cats are shown in Table 1. After ovulation, there was a 93% decrease in NE concentration and a 90% decrease in DβH activity. This decrease was still apparent when the results were expressed per total ovary rather than per gram of tissue (Table 1), indicating that ovulation was followed by a decline in absolute NE levels and DβH activity values. In contrast, the decline in NE concentration and DβH activity 10 days after administration of eCG was no longer evident when the results were expressed per total ovary (Table 1). In fact, both NE content and DβH activity per ovary were 1.5- and 2-fold greater than in estrous control ovaries, indicating that the large 4-fold increase in ovarian weight induced by eCG was accompanied by a concomitant, though less pronounced, increase in catecholaminergic activity. Although the concentrations of NE and DβH activity decreased after ovulation (especially NE), their distribution in tissue fractions obtained by differential centrifugation remained unchanged (Fig. 1). In both estrous and ovulating cats, the highest concentrations for ovarian NE were observed in the P_3 and Sf fractions, and for DβH activity in the P_3 fraction. The highest protein concentration was found in the Sf fraction. Since the highest specific activities (amount/mg protein) of both NE and DβH were present in the P_3 fraction, this was considered the vesicular fraction.

Isoosmotic Percoll Gradient Distribution of NE, DβH, Mg^{2+}-dependent ATPase, and ATP Derived from the Vesicular Fraction

Aliquots of the resuspended vesicular fraction from the ovaries of estrous and ovulating cats were mixed with Percoll to obtain a 15% solution and centrifuged at 78 000 × g for 30 min. Both NE and DβH activity presented a unimodal distribution, but the main activities equilibrated at different densities (1.041 g/ml for NE and 1.033 g/ml for DβH, Fig. 2). The P_3 fraction from ovulating cats exhibited a 90% decrease in NE content over the entire gradient and a decrease (50%) in DβH activity at a density of 1.033 g/ml.

ATPase activity (Fig. 3) showed a bimodal pattern of distribution with two peaks (densities of 1.041 g/ml and 1.033 g/ml) that corresponded to those of NE and DβH activity respectively. After ovulation, there was a decrease in the ATPase activity corresponding to the NE peak and a slight displacement (to a lower-density region) of the peak corresponding to that of DβH activity. In estrous animals, the distribution of ATP was very similar to that of NE, namely

TABLE 1. NE content and DβH activity in total ovarian homogenates from estrous, eCG (10 days after)-treated, and ovulating cats.*

	Estrous	eCG (10 days after)	Ovulating
Ovarian weight (mg/pair)	305 ± 26	1 268 ± 348[f]	1 385 ± 251[b]
NE			
ng/g tissue	3 446 ± 217	1 598 ± 245[f]	304 ± 39[b,d]
ng/pair ovaries	1 009 ± 73	1 569 ± 436	387 ± 57[b,c]
DβH			
nmol/h/g tissue	9.56 ± 0.94	4.4 ± 0.20[f]	1.25 ± 0.18[b,e]
nmol/h/pair ovaries	2.38 ± 0.25	5.5 ± 1.86[f]	1.61 ± 0.30[a,d]
Protein			
mg/g tissue	91.1 ± 2.6	68.6 ± 9.9	87.6 ± 1.7
mg/pair ovaries	27.9 ± 2.4	90.0 ± 19.2[f]	120.3 ± 21.3[b]

*Results correspond to the mean ± SEM from 3–8 animals.
[a]$p < 0.02$, [b]$p < 0.001$ vs. estrous; [c]$p < 0.02$, [d]$p < 0.005$, [e]$p < 0.001$ vs. eCG; [f]$p < 0.001$ vs. estrous.

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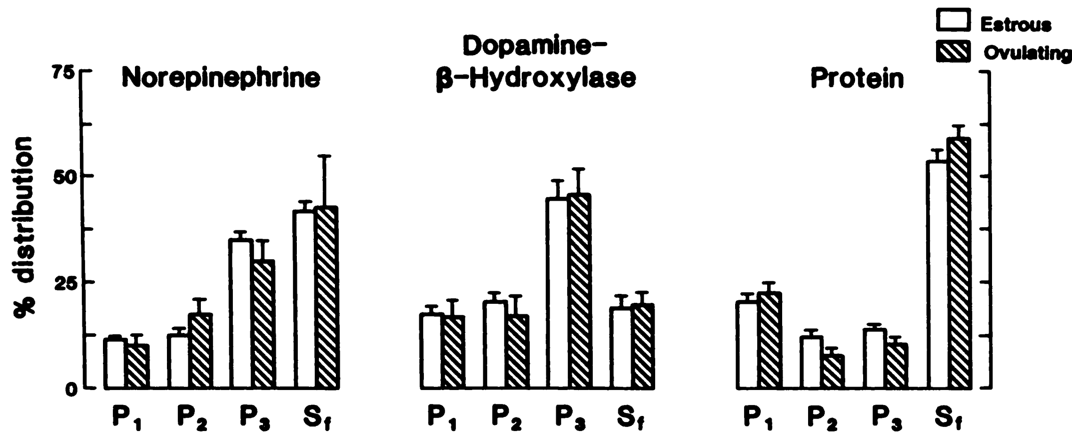

FIG. 1. Distribution of vesicle markers (NE, DβH) and protein in tissue fractions obtained by differential centrifugation of ovaries from estrous and ovulating cats. Results are expressed as percentage of distribution of the markers ± SEM from 10 individual experiments for estrous and 6 for ovulating cats.

a unimodal distribution with the principal peak located between 1.055 and 1.041 g/ml. After ovulation, there was a dramatic decrease in the amount of the nucleotide to values below the limit of detection of our assay (<2 pmol/assay).

Uptake of ^{3}H-NE into the Vesicular Fraction from the Ovaries of Estrous and Ovulating Cats

Aliquots of the resuspended vesicular fraction (P_3) were incubated with increasing ^{3}H-NE concentrations to characterize the kinetics of uptake of the neurotransmitter (Fig. 4A). Estrous ovaries showed the highest capacity for NE uptake (V_{max}, 3.17 ± 0.5 pmol/mg protein/min). After ovulation, the uptake capacity was reduced to 10% of that in controls. A 3-fold increase in the K_m value (concentration of NE that resulted in 50% of the maximal uptake capacity) was also evident. The distribution in Percoll gradients of the ^{3}H-NE taken up by the vesicles is shown in Figures 4B and 4C. ^{3}H-NE distribution obtained in estrous animals was very similar to that of endogenous NE (compare Fig. 4C with Fig. 2). After ovulation, the total amount of ^{3}H-NE incorporated and retained by the vesicles decreased to 10% of control values and preferentially accumulated in the light-density region of the gradient (Fig. 4B).

Release of ^{3}H-NE from the Ovaries of Estrous and Ovulating Cats

K^+ depolarization produced a 10-fold increase in the release of recently incorporated ^{3}H-NE into estrous ovaries (Fig. 5). After ovulation, K^+-induced release was decreased by 80% (i.e., 1.7-fold only). The radioactivity released in 8 min was 26% of that in estrous ovaries (2.75 ± 0.73% for estrous and 0.72 ± 0.16% for ovulating ovaries n = 3, $p <$ 0.001). After ovulation, the spontaneous efflux of radioactivity increased almost 4-fold (from 0.26 ± 0.05% in estrous ovaries to 0.83 ± 0.04 in ovulating ovaries, n = 3, $p <$ 0.001). The total uptake of ^{3}H-NE by ovaries from ovulating animals was 47% of that of ovaries from estrous animals (19.1 ± 2.6% for estrous and 9.02 ± 1.7% for ovulating, n = 3; $p < 0.001$).

DISCUSSION

The results of this study suggest that the changes of NE content associated with ovulation are due to activation of the sympathetic nerves innervating the cat ovary. It is well known that the cat ovary is densely innervated by sympathetic fibers [1, 25, 26], some of which are closely associated with primordial and developing follicles [2].

The method of Wildt et al. [17] to induce ovulation was successful in all the cats used. The dose of eCG, however, was probably supraphysiological as shown by the large increase in ovarian weight found in cats treated with eCG plus hCG or eCG alone in comparison to cats undergoing natural estrus. This effect of eCG on ovarian weight has been previously demonstrated in cats [17], rats [27], and rabbits [28]. We did not observe a decrease in NE content after eCG as described in the rat by Bahr and Ben-Jonathan [27]. In contrast, 10 days after eCG injection, both NE content and DβH activity increased, only to decline (below estrous levels) after ovulation had been induced with hCG. These results are thus similar to those obtained in the rat ovary by Morimoto et al. [9], who found a gradual increase in NE content after eCG treatment and depletion after hCG injection. The results also demonstrate that, although the cat is an induced ovulator, its ovarian sympathetic nerves respond as does the rat ovary (a cyclic ovulator) to eCG plus hCG treatment.

Since, as shown here, the cat's ovary contains very high levels of NE and also exhibits readily measurable DβH activity, it provides an attractive model for studying the changes that may occur in the nerve terminal after release of NE. If we accept that NE is released by an exocytotic-like process

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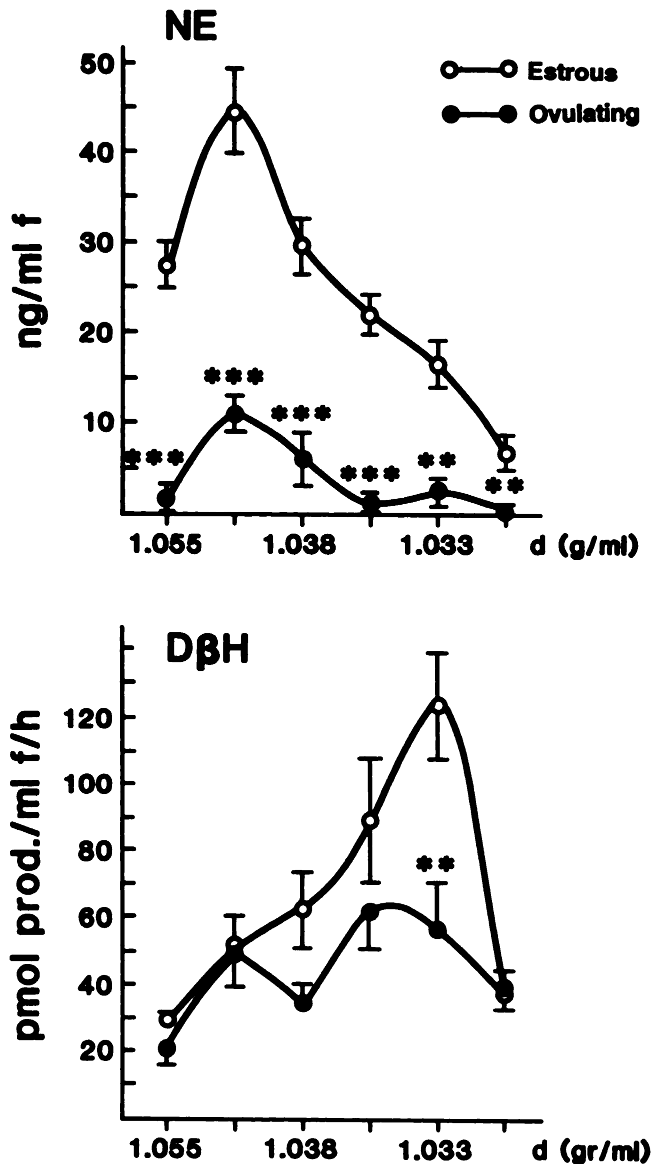

FIG. 2. NE (ng/ml of gradient fraction) and DβH (pmol product/ml of gradient fraction/h) distribution obtained after centrifugation of the vesicular fraction (P_3) in isoosmotic Percoll gradient. Values are expressed as mean ± SEM of 6 experiments for each experimental group: estrous cats (o—o); and ovulating cats (●—●). The abscissa represents density of the fractions. *** = $p < 0.001$ vs. estrous. ** = $p < 0.02$ vs. estrous.

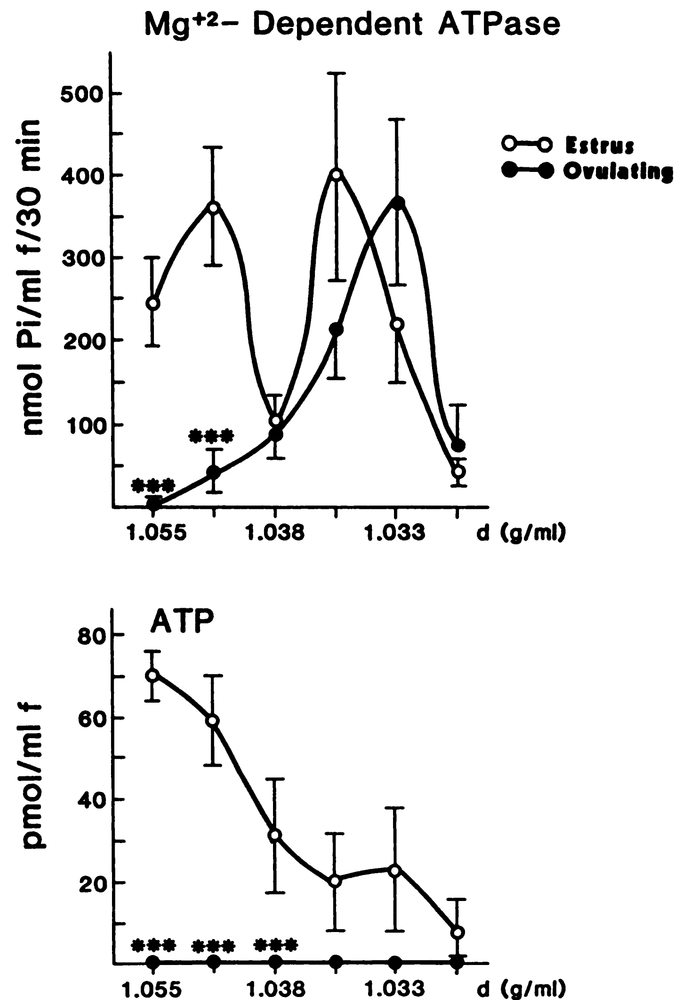

FIG. 3. Mg^{2+}-dependent ATPase (nmol Pi formed/ml of gradient fraction/30 min) and ATP (pmol/ml of gradient fraction) distribution obtained after centrifugation of the vesicular fraction (P_3) in isoosmotic Percoll gradient. Values represent mean ± SEM of 4 experiments in each experimental group: estrous cats (o—o); ovulating cats (●—●). Abscissa represents density of the fractions. *** = $p < 0.001$ vs. estrous.

[29], the release process should be accompanied by a decrease of the intravesicular constituents of the NE storage vesicles with minor changes in the membrane constituents of the vesicles [13]. In our in vitro preparation, these changes occurred independently of any changes in plasma NE levels.

In spite of the decrease in NE levels observed after ovulation, the distribution of catecholamine in the tissue fractions obtained by differential centrifugation remained unchanged. This proportional decrease of NE in all subcellular fractions suggests that NE is preferentially located and released from one intracellular compartment and that at the time studied after ovulation it had diffused from the tissue. Since the decrease in NE levels is greater than that of DβH, it is possible that NE is released from a compartment that has minimal DβH activity or that DβH activity is located in other intracellular organelles. DβH has been described as a constituent of the inner face of the membrane of NE storage vesicles isolated from the splenic nerve and chromaffin granules [30, 31]. DβH has also been described as a partially soluble constituent of these catecholamine-storing vesicles. Therefore, in a secretory process mediated by exocytosis, DβH activity would either decrease partially or not change at all depending on its relative distribution between intravesicular (soluble) and membrane-bound enzyme.

To obtain additional information regarding the intracellular compartment from which NE is released, we studied

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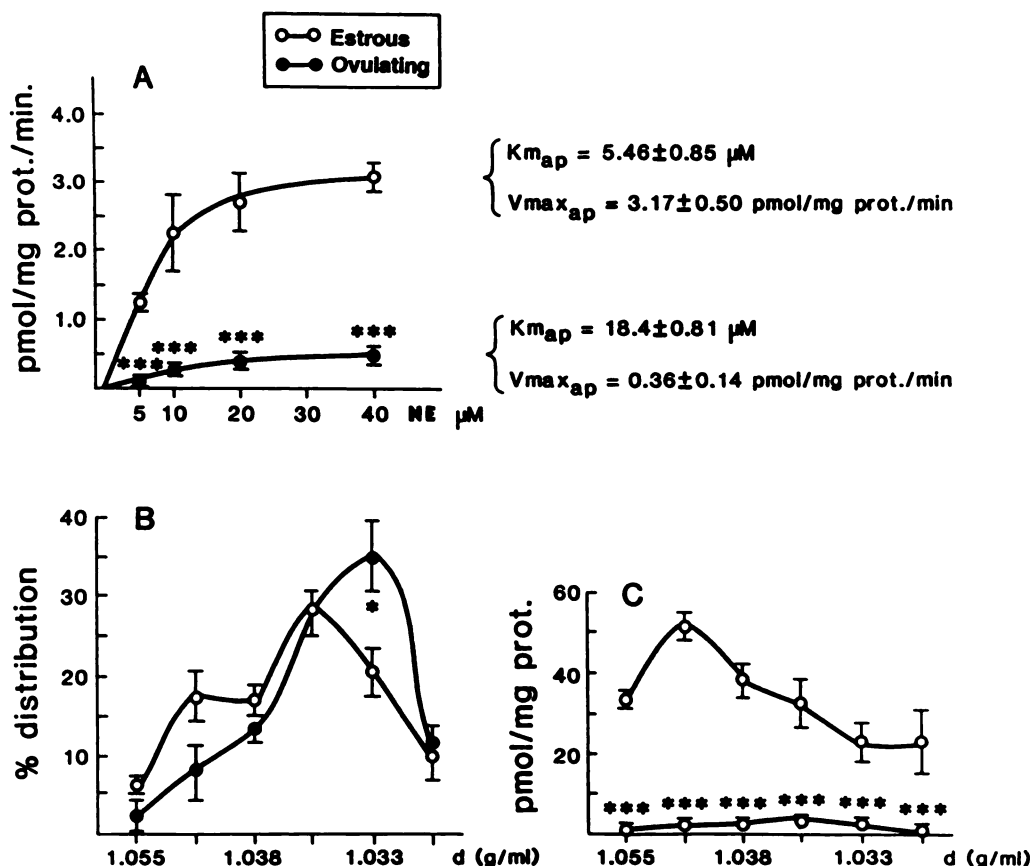

FIG. 4. A: Effect of ovulation on NE uptake by noradrenergic storage vesicles from vesicular fraction (P_3). Aliquots of P_3 fractions were incubated for 20 min with increasing amounts of NE as described in *Materials and Methods*. After vacuum filtration, NE was determined by scintillation counting. B and C: Effect of ovulation on the percentage of distribution (B) and total amount of ^{3}H-NE (C) retained by vesicles isolated on Percoll gradients. Aliquots of P_3 fractions were incubated with 5 μM NE for 30 min and centrifuged in Percoll gradients as described in *Materials and Methods*. The amount of ^{3}H-NE was determined by scintillation counting and spectrofluorimetry. Results are expressed as mean ± SEM of 3 individual experiments in each experimental group. * = $p < 0.05$ vs. estrous. *** = $p < 0.001$ vs. estrous.

the distribution of NE and DβH activity on Percoll density gradients. As we described previously [18], two zones of the gradient containing NE or DβH activity can be observed. At higher density (d = 1.041 g/ml), a small-vesicle fraction that contains more than 80% of NE and less than 15% of DβH is present; a lower-density fraction (d = 1.033 g/ml) that corresponds to large vesicles contains more than 70% of DβH and less than 10% of NE. Willems and De Potter [32] also demonstrated that small vesicles in the rat vas deferens that contain principally NE are almost devoid of DβH activity, whereas large vesicles have almost all DβH activity and less NE. The presence of large and small storage vesicles of NE in sympathetic nerve terminals of the cat's ovary provides a regulatory mechanism for release of NE. Large vesicles secrete NE only at a high firing rate of the neurons [33]. That this mechanism is also operative in the cat's ovary was demonstrated from the analysis of the population of vesicles involved in the release of NE. If NE content and DβH activity are considered biochemical markers for the presence of small and large vesicles in the sympathetic nerve terminals of the cat ovary, the differential decrease of NE content and DβH activity after ovulation can be explained as a preferential participation of small vesicles in the release of NE.

To further clarify this issue, we studied the distribution of ATP (a soluble constituent of the vesicles). ATP distribution was the same as NE distribution, suggesting that the nucleotide is co-stored with NE in small vesicles, as has been described in other sympathetically innervated organs [30–32]. The depletion of ATP in the small-vesicle fraction of ovaries from ovulating cats supports the view that ATP is co-released with NE. In this context, ATP on its own might act as a neuromodulator of ovarian function or be metabolized to original adenosine to interact with gonadotropins in their effects on oocyte maturation [34–36].

Although the similarity in the changes of NE and ATP levels found after ovulation permits us to suggest that NE is preferentially secreted from small vesicles, we were not

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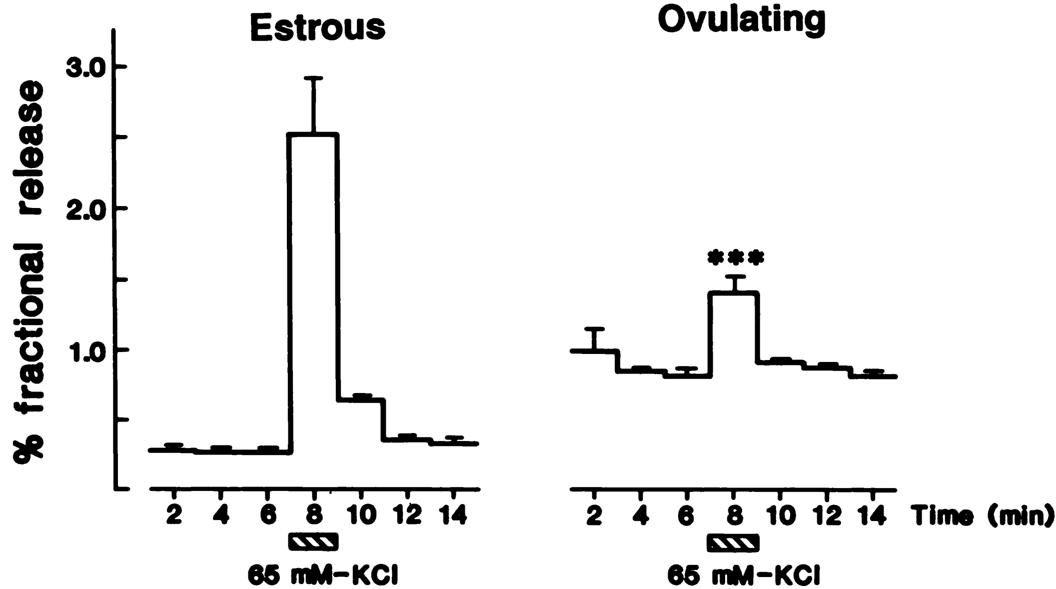

FIG. 5. Effect of ovulation on K^+-induced release of ^{3}H-NE recently taken up. Depolarization was produced by exposure of the tissue to 65 mM KCl for 2 min. Before depolarization, spontaneous efflux of radioactivy was also measured. Results are expressed as mean ± SEM of 3 individual experiments for each group. *** = $p < 0.001$ vs. estrous.

able to determine whether large vesicles are also involved in the secretory process. This issue was clarified, however, by the examination of changes in Mg^{2+}-dependent ATPase distribution. In estrous animals, this enzyme presented a bimodal distribution with peaks of activity at the same density as NE and DβH (namely, small and large vesicles, respectively). After ovulation, ATPase activity associated with NE-storing vesicles (small vesicles) practically disappeared, but the activity associated with DβH (large vesicles) was only slightly modified. ATPase plays a fundamental role in the generation of the electrochemical H^+ gradient across the vesicle membrane needed for neurotransmitter uptake [22, 23]. Its presence in both populations of storage vesicles in the estrous cat ovary suggests that all vesicles are functionally active in the uptake of NE; but, according to the NE distribution, only the small vesicles are able to accumulate NE. The presence of ATPase in DβH-containing vesicles may be related to the uptake of dopamine, the physiologic substrate of DβH to form NE. On functional grounds, the decreased ATPase activity in small vesicles makes these vesicles unable to take up NE from the cytoplasm. This was clearly demonstrated by the striking decrease in ^{3}H-NE uptake capacity of the vesicle fraction from ovulating cats. In addition, the 3-fold increase in the K_m indicated a decreased affinity of NE for its transmembrane carrier. Indeed, the only region of the gradient able to take up ^{3}H-NE was that in which ATPase was present (Fig. 4B).

The decrease in NE content after ovulation and the finding that this decrease is associated with depletion of the catecholamine from the storage vesicle give strong support to the notion that the decrease in ovarian NE concentration induced by gonadotropin is due to NE secretion from the small-vesicle fraction of sympathetic nerve terminals. The minor decrease of DβH activity is probably due to release of soluble DβH from large storage vesicles that may participate in the secretory process. The decrease in ATPase activity concomitant with the decline in uptake capacity of ^{3}H-NE suggests that there is a period after ovulation when the nerve terminals are depleted of functionally active storage vesicles. This possibility was experimentally confirmed in our study because the release of ^{3}H-NE recently taken up was decreased after ovulation. The observed decrease in ^{3}H-NE uptake and the increase in the spontaneous release of ^{3}H-NE further indicate that the number of vesicles able to take up the neurotransmitter is decreased and that a preferential efflux of NE from the cytoplasm occurs.

The results of this study demonstrate for the first time that the changes in ovarian NE content observed after gonadotropic stimulation of the cat's ovary are tightly related to the changes in the subcellular organization of ovarian sympathetic nerve terminals. The nature of these changes indicates that gonadotropins enhance the release of NE from the small storage vesicles of the sympathetic nerves of the cat ovary. Whether gonadotropins act directly on the perikarya of the sympathetic neurons that project to the ovary to regulate their firing rate or at the presynaptic membrane to modify release of the neurotransmitter remains to be established.

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