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Endocrine modulation of neurotransmitter systems in the rat oviduct *

Modulación endocrina de los sistemas neurotransmisores del oviducto de la rata

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The presence of neurotransmitters (NTs) in the rat and rabbit oviduct and variations in their content during the sex cycle and following ovariectomy or hormonal replacement therapy are reviewed. Possible functional meaning of these changes and the mechanisms involved are discussed.

The levels of norepinephrine (NE) and of gamma amino butyric acid (GABA) in the rat oviduct varied during the estrous cycle. The lowest values for NE were found during Diestrus 2 (D_2) and the highest values for GABA during Proestrus. After ovariectomy the levels of both NTs were decreased. In ovariectomized rats treated with estradiol a recovery of NE to normal levels was observed. Ovariectomy did not modify the *in vitro* release of ^{3}H-noradrenaline (^{3}HNE) while treatment with estradiol for 7 days decreased the release. Estradiol added *in vitro* had no effect on ^{3}HNE release from the oviduct.

Progesterone (P) injected in intact rabbits increased the oviductal level of NE, the dopamine β-hydroxylase activity and the capacity to incorporate ^{3}HNE and decreased the K^+-induced release of ^{3}HNE. Progesterone added *in vitro* to the rat oviduct decreased the induced release of ^{3}HNE. In this organ the releasing process was facilitated during D_2.

The results suggest a modulatory rol of ovarian sex steroids on some NTs in the oviduct, specially NE. The effects of P differ from those of estradiol and could be the result of a direct effect of progesterone on the plasmatic membrane of nerve terminals.

INTRODUCTION

The presence of neurotransmitters (NTs) in the rat oviduct is well established through histochemical studies or NTs level measurements (1) but their functional role is poorly understood. As in other species, the levels of some NTs in the rat oviduct are also affected by the endocrine fluctuations associated with the estrous cycle or pregnancy, or by ovariectomy and/or hormonal treatments (2).

The present report will focus on the noradrenergic system of the rat oviduct during the estrous cycle as well as during ovariectomy and hormonal treatments. These results are compared with those found in oviducts of intact female rabbits treated with progesterone (3).

We have also done a restrictive comparison between the noradrenergic system of the rat oviduct and the gabaergic system recently found in this organ (4) which is described in this issue (5).

In this presentation we examine the oviductal noradrenergic system from the point of view of the neurobiology of the organ.

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MATERIALS AND METHODS

Animals

Rats. Adult female Sprague-Dawley rats weighing 240-260 g were used. They had free access to food and water and were kept with a light: dark cycle of 12:12 h. Vaginal smears were taken every morning and only rats showing more than two consecutive 4-day cycles were used for the experiments.

Animals at different stages of their estrous cycle were used to study basal and induced release of tritiated norepinephrine (^{3}HNE). Other rats were ovariectomized under nembutal-ether anaesthesia (OVX rats) and received a subcutaneous silastic implant containing 400 μg of estradiol or saline. These animals were used on the 7th day of treatment.

Blood samples obtained from intact and OVX rats were used for progesterone (P) and estradiol determinations.

Rabbits. Virgin adult female New Zealand rabbits weighing 2.5-3.5 kg were kept at constant room temperature with a dark: light cycle of 14: 10 h and free access to food and water. The rabbits were treated daily for 4, 7 and 15 days with subcutaneous injections of vegetable oil (vehicle) or 1.5 mg/kg progesterone dissolved in vehicle.

In some experiments control and progesterone treated rabbits were injected under ether anesthesia with ^{3}HNE (2.5 μCi, sp. act. 4 Ci/mmol; dissolved in 20 μl of saline with ascorbate (0.1%)) into the lumen of each oviduct 30 min before removing the organs.

^{3}HNE releasing experiments

a) *In rats.* Animals were killed by decapitation. The oviducts were removed, cleaned under dissecting microscope and placed in Tyrode solution, pH 7.4, gassed with a mixture of O_2 (95%) and CO_2 (5%). Then, they were transferred to 3.0 ml of Tyrode containing 2-3 μCi of ^{3}HNE (sp. act. 45.6 Ci/mmol; from Dupont New England Nuclear) in the presence of normetanephrine (10^{-5} M). After a washing period of 60 min, a constant spontaneous basal radioactivity outflow was obtained. Then the organs were transferred for two minutes to each of a series of 6 tubes containing 2.0 ml of normal Tyrode, except the third one containing 80 mM K^+ to assess radioactivity released under depolarizing conditions. Each series could be repeated two of three times with the same oviduct. The radioactivity was counted in a 1.0 ml sample from each tube. At the end of one experiment the organs were blotted, weighed and homogenized in 0.4 N perchloric acid. Homogenates were centrifugated and aliquots of the supernatants were used to count radioactivity.

b) *In rabbits.* When using rabbits the organs were divided in three equal length segments: ovarian, medial and uterine segment. For each segment the procedure was similar to that followed for rat oviducts.

Subcellular fractionation and sucrose gradient experiments

In order to understand the effect of progesterone on NE levels in rabbit oviducts subcellular fractionation techniques were used. Through differential centrifugation, isopycnic gradients and by using noradrenergic vesicle components as markers it was possible to study densities and chemical composition of noradrenergic vesicles and their changes after hormone treatment. The vesicles were separated by differential centrifugation and improved purification of the particles was achieved by isopycnic gradients.

The animals were killed by cervical fracture and their oviducts were homogenized in an isoosmotic sucrose solution buffered with potassium phosphate. Homogenates were fractionated and four subcellular fractions were obtained (3). Considering vesicle markers, norepinephrine (NE), dopamine-beta-hydroxylase (DBH) and ^{3}HNE distribution in tissue fractions, P3 fractions were defined as vesicle fractions. They were layered over continuous sucrose gradients and centrifugated at 201,800 x g for 15 min. Thirteen 1.0 ml fractions were collected from the gra-

dients. Density determinations in aliquots of these fractions (3) were used to check gradient linearity.

Assays

Rabbit NE was determined spectrofluorometrically after absorption on alumina, according to Campuzano *et al.* (6). NE levels in rat oviducts were determined by high performance liquid chromatography (HPLC) with electrochemical detection (7). NE levels were expressed as concentration (ng/per gram wet tissue) or content (ng per pair of oviducts) and results found were corrected for recovery as determined by using internal standard.

DBH was assayed according to the method of Belmar *et al.* (3). Proteins were determined according to Lowry *et al.* (8) using serum albumin as standard. DBH activity results were expressed as pmoles per hour per gram of wet weight and recovery of enzyme activity was checked by means of a purified preparation of DBH (SIGMA D-1893).

Plasma estradiol and progesterone were assayed by radioimmunoassay according to Forcelledo *et al.* (9).

Radioactivity was measured in total homogenates, in tissue fractions, and in gradient fractions obtained from rabbit oviducts. In releasing experiments it was measured in homogenates obtained at the end of the experiment from segments or from oviducts and also in aliquots corresponding to the effluents of the washing, basal (spontaneous) and stimulation periods. In release experiments the outflow of radioactivity was calculated and expressed as percent fractional release (percentage related to total radioactivity present in the tissue at any moment). The spontaneous outflow of radioactivity corresponds to the basal efflux. The increase of release by the effect of potassium ions was defined as induced release. Net release was calculated as the induced release minus basal release.

Radioactivity was determined by liquid scintillation spectrometry using a scintillation mixture (Toluene containing PPO, POPOP and Arcopal) as indicated elsewhere (3).

Expression of Results

All results are expressed as mean values ± standard errors. Student's t test was used to compare difference between mean values of two groups. Analysis of variance (ANOVA) followed by Duncan's test was used for multiple comparisons.

RESULTS

Some of the changes that would suggest a modulatory role of the estrous cycle or the ovarian hormones on the oviductal NTs are listed in Table 1. Changes in NTs levels, synthesis, metabolism or release should be observed during estrous cycle or after ovariectomy or hormone replacement. However, to define such a role other criteria should also be accomplished. For instance, the number and characteristics of NTs receptors should be specifically correlated with estradiol, or progesterone or both.

TABLE 1

Changes in the oviduct that would suggest endocrine modulation of its innervation

1.	Neurotransmitter levels oscillate during the sexual cycle.
2.	Neurotransmitter levels change after ovariectomy or other endocrine manipulations.
3.	Levels or activity of enzymes involved in synthesis or metabolism of neurotransmitters change during the sexual cycle or after endocrine manipulations.
4.	Uptake or release of neurotransmitters is affected by endocrine manipulations.
5.	Number or characteristics of neurotransmitter receptors is modified by excess or deficit of specific hormones.

The possible roles of NTs in the oviduct are shown in Table 2. Some of them, like the modification of the mechanical activity of the rat oviductal wall has been scarcely studied with acetylcholine (Ach) (10). The control of sphincteric regions in the mammalian oviduct by norepineprine (NE) was the first hypothesis related to a possible role for a NT in the oviduct (11).

TABLE 2

Possible roles of neurotransmitters in the oviduct

1.	Control of the tonus or rhythmic activity of the myosalpinx.
2.	Control of the ciliary activity.
3.	Control of the secretory activity.
4.	Control of the local blood circulation.
5.	Control of myosalpinx tonus at the isthmic junctions.
6.	Direct effects on gametes.

The presence of many different NTs in the oviduct suggests a complex pattern of innervation in the organ. Currently accepted NT systems in the rat oviduct are presented in Fig. 1. Besides adrenergic and cholinergic systems a peptidergic system, not shown in the figure, is also innervating the oviduct (12). A gabaergic system was proposed but as discussed elsewhere in this issue, possibly most of GABA in the oviduct should represent a non-neuronal system. The cholinergic system of the oviduct is connected mainly with the parasymphathetic sacral division and to a lesser extent with vagal fibers (13). As in other species the organization of the noradrenergic system of the rat oviduct is represented by two types of nerve-endings (14): long-axon neurons derived from the autonomic sympathetic ganglia, and short-axon neurons derived from small peripheral autonomic ganglia. It has been stated that these neurons present some properties that differenciate them from traditional neurons, for instance they can be modulated by endocrine signals (15).

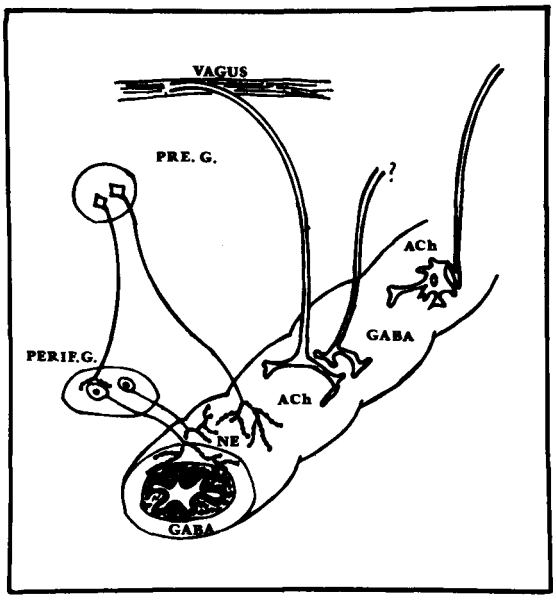

Fig. 1: Schematic diagram of the rat oviduct innervation. Indicated substances are present in the rat oviduct and, with the possible exception of GABA which is found mainly in the epithelium, play a neurotransmitter role. (NE = norepinephrine; ACh = acetyl choline; GABA = gamma amino butyric acid). Peptidergic fibers were omitted. Noradrenergic fibers are derived from a prevertebral ganglia (PRE. G.) or from a peripheral ganglia (PERIF. G.).

Endocrine modulation of oviductal neurotransmitter levels

Changes in the levels of NE and GABA were observed in the rat oviduct during the estrous cycle. The highest contents of NE were found during diestrus 1 (D_1) (44.7 ± 4.7 nmoles per gram of wet tissue, n = 6). Interestingly, for GABA the highest content and concentration (37 nmoles per mg protein, n = 4) were found during estrus, thus suggesting that endocrine signals affect them differently. The highest serum levels of progesterone were found at D_2 and those of estradiol, at proestrus. Seven days after ovariectomy a net decrease of NE concentrations was found in the rat oviduct. Treatment of animals from this group during 3 days with subcutaneous injections of estradiol (54 μg daily) or through implantation of a silastic tube containing the hormone, induced a recovery of NE levels. These observations suggest that NE availability in the rat oviduct is modulated by sex steroid hormones.

Endocrine modulation of neurotransmitter release

Other fundamental process of chemical neurotransmission that can be modulated by endocrine signals is the release of the NT. No changes were found on the K^+-induced release of ^{3}HNE recently incorporated into rat oviducts obtained 7 days after ovariectomy. In comparison with oviducts from intact rats, when these oviducts were stimulated in the presence of estradiol added to the bath (5×10^{-7} M, final concentration), again, no changes

were found on the stimulatory effect of K^+. However, when the animals were implanted with a silastic tube containing estradiol (400 μg) at the time of ovariectomy and were examined 7 days later the K^+-stimulatory effect on NE release was inhibited (Fig. 2). Plasmatic levels of estradiol in estrogen-treated animals were similar to those found during the estrous stage of the cycle.

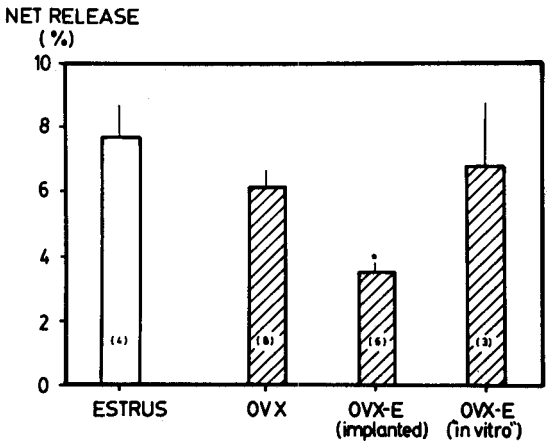

Fig. 2: Net release of ^{3}H-norepinephrine induced by K^+ (70 mM) from oviducts obtained from rats in estrus, 7-day ovariectomized rats (OVX), 7-day ovariectomized rats implanted with a silastic tube containing estradiol (E) (400 μg) at the time of ovariectomy, 7-day ovariectomized rats stimulated in the presence of estradiol *in vitro* (10^{-6} M). Bars represent mean values ± S.E.M. Number of experiments in brackets.
* $P < 0.01$ vs. estrus; two-tailed Student's t test.

During the estrous cycle of the rat K^+-induced NE release was lower during D_1 than in the other stages of the cycle. When we induced the release of radioactivity from the oviduct of estrous rats in the presence of progesterone *in vitro* (5 μM), a clear inhibition of the release was observed (Fig. 3).

Similarly K^+-induced NE release in oviducts obtained from progesterone treated rabbits was lower than that observed in control oviducts (Fig. 4).

Some of the above results found in ovariectomized rats can be explained in the light of other findings in rabbits. Kennedy and Marshall (16) demonstrated that postovariectomy NE levels in the oviduct of the rabbit can be restored by

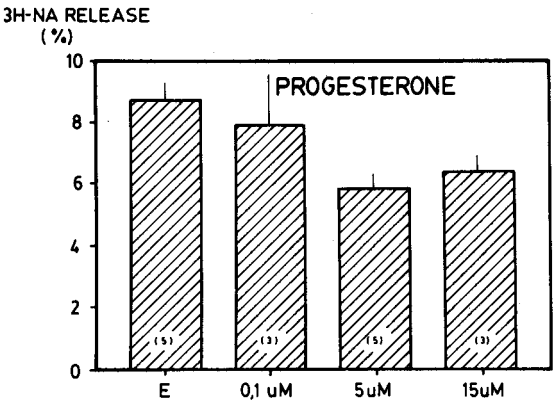

Fig. 3: Effect of progesterone *in vitro* on the induced release (70 mM, K^+) of tritiated norepinephrine (^{3}H-NA from estrous rat (E) oviducts. Each bar represents mean values ± S.E.M., the number of experiments between brackets.

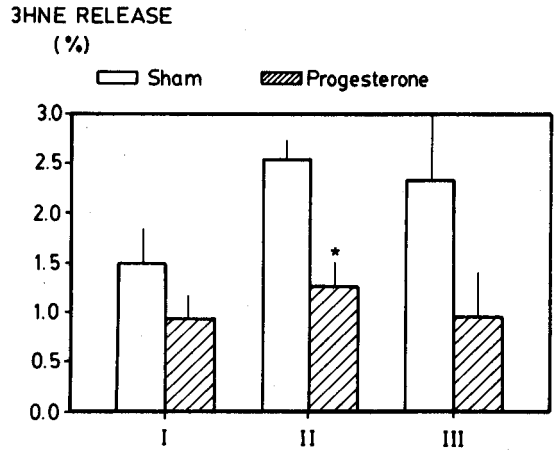

Fig. 4: Fractional induced (70 mM, K^+) release of tritiated norepinephrine (^{3}HNE) from segments of rabbit oviducts obtained after 4 days of progesterone treatment. (I, ovarian segment; II, central segment and III, uterine segment). Sham animals were injected with vehicle. Each bar represents mean values ± S.E.M. of 3-4 experiments.
*, $P < 0.02$ vs. sham group.

estrogen treatment. This was explained by the effect of estradiol on tyrosine hydroxylase (TH) activity. This enzyme regulates the rate of synthesis of NE (17) and after ovariectomy its reaction velocity (V_{max}) and also that of its pteridine cofactor were reduced. Treatment of castrated animals with estradiol-17 β (0.25 μg/kg/day) restored TH V_{max} to its normal levels. A similar mechanism may operate in the rat oviduct but this remains to be demonstrated.

In another series of experiments we studied the mechanism of endocrine induced changes of oviduct NE in the intact rabbit. Bodkhe and Harper (18) had shown that treatment of intact adult female rabbits with progesterone can increase NE levels in the distal isthmus of the oviduct. In similar experiments, we treated the animals with progesterone (1.5 mg/kg. b.w.) during 4, 7 or 15 days with daily injections. Our goal was to assess the effect of this endocrine treatment on the storage system and release of NE in the oviduct. Levels of NE, the activity of (DBH) and the incorporation and storage of ^{3}HNE injected in the lumen of the oviduct were measured (3). DBH is a structural enzyme that catalyzes the transformation of DA into NE. It has been used as a marker of vesicles which synthetize and store NE (19). Measurement of these markers was done on total homogenates of the oviducts and in tissue fractions obtained after differential centrifugation of homogenates. Aliquots of P_3 fractions were applied on continuous sucrose gradients (0.3-2.0 M) which were centrifuged at equilibrium. After centrifugation, 13 fractions were collected and marker activities were assessed in each fraction. It is assumed that marker distribution in the gradient defines the presence of subcellular particles from vesicles in the fractions. The results obtained are summarized in Figs. 5 and 6. Fig. 5 shows that after 4 days of progesterone treatment the total homogenate content of NE and DBH increased, the effect being still observed after 15 days. However after 7 days of treatment the effect of the hormone persists only on DBH. During hormone treatment the amount of ^{3}HNE in homogenates obtained from treated animals was higher than in those obtained from animals treated with vehicle. The activities of vesicle markers were specially high in P_3 and soluble tissue fractions. When aliquots of P_3 fractions obtained from progesterone and vehicle injected animals were applied on continuous sucrose gradients, the distribution of vesicle markers found after isopycnic centrifugation defined two vesicle populations (Fig. 6). DBH had a bimodal distribution in gradients obtained from control animals. A light peak was found in the density region 1.072 - 1.145 g/ml and a heavier peak in the region of 1.176 - 1.187 g/ml. In progesterone treated rabbits the light peak of DBH activity was broader, suggesting an increase in the number of less dense vesicles. Concomitantly a significant increased capacity to incorporate higher amounts of ^{3}HNE was observed in the light peaks of progesterone treated animals. Thus the increment in NE levels induced by progesterone in the intact animal could be explained by an increase of NE vesicle number. This mechanism is different to the one involved in the effect of estradiol in the oviduct of ovariectomized rabbits.

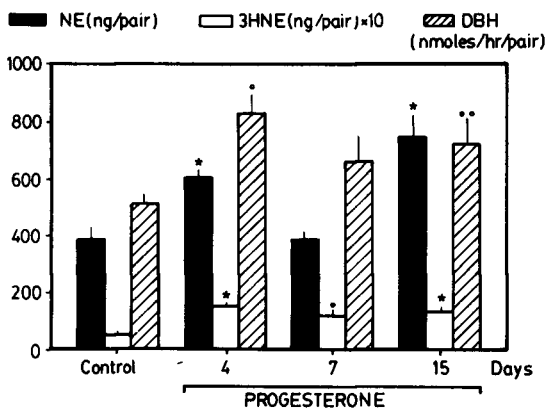

Fig. 5: Effects of progesterone on the levels of norepinephrine (NE) and activity of dopamine-beta-hydroxylase (DBH) and on the uptake of tritiated norepinephrine (^{3}HNE) of adult rabbit oviducts. Progesterone was injected daily (1.5 mg, s.c./kg b.w.) during 4, 7 or 15 days. Bars represent mean values ± S.E.M. from 8-10 experiments for NE, 3-5 experiments for ^{3}HNE and 4-6 experiments for DBH.
* $P < 0.001$ vs. control; o, $P < 0.01$ vs. control and oo, $P < 0.05$ vs. control; two-tailed Student's t test.

DISCUSSION

The results presented show that some quantitative parameters of the NT system of the rat and rabbit oviduct are affected by the endocrine condition of the animal and suggest that ovarian steroids modulate the noradrenergic system of the oviduct. However, it is difficult to define the mechanisms involved in those effects.

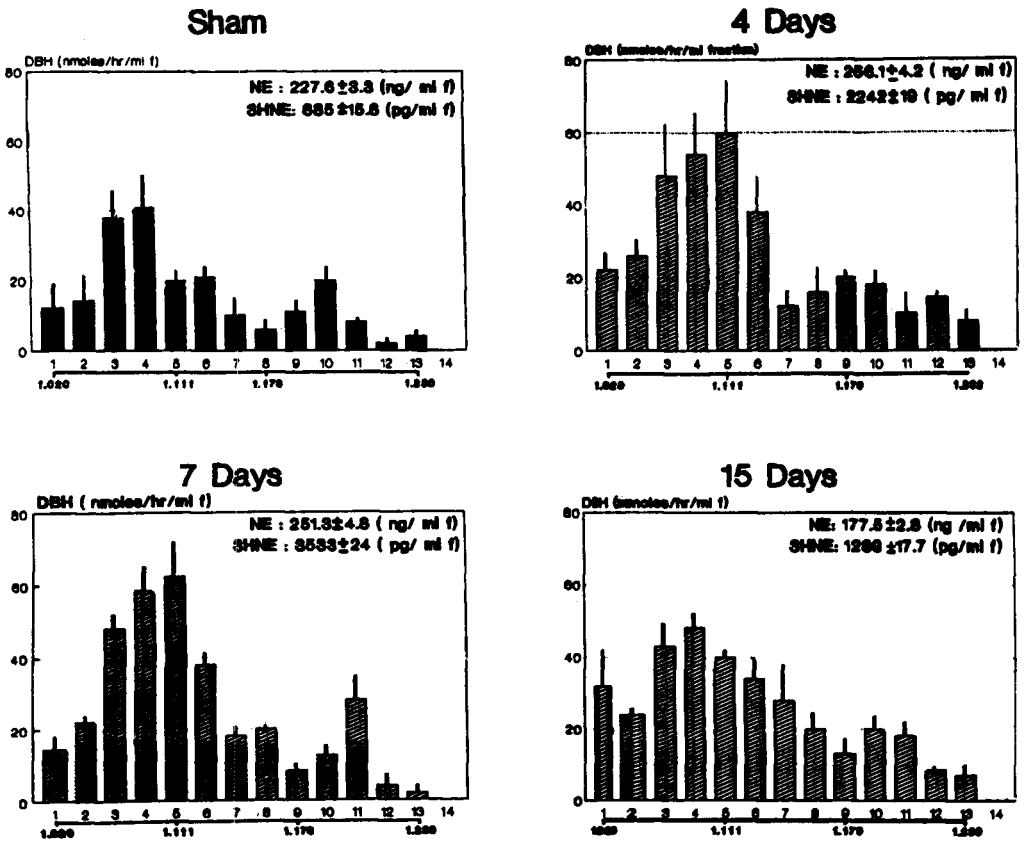

Fig. 6: Dopamine-beta-hydroxylase (DBH) activity distribution in sucrose gradient (0.3-2.0 M) fractions obtained after isopycnic centrifugation of P_3 fractions derived from the oviduct of rabbits treated with progesterone or vehicle (sham) as in Fig. 5. Each bar in the histogram represents mean values ± S.E.M. of 4-6 experiments. Bottom scales correspond to density in g/ml. Fractions collected from gradients are indicated (1-13). In each histogram the total amounts of norepinephrine (NE) and tritiated NE (^{3}HNE) present in the P_3 fraction applied on the grandient are also indicated.

Estradiol increases the level of NE both in the intact (18) and ovariectomized rabbit (16) and also, in ovariectomized rats. This effect of estradiol probably reflects changes in the synthesis of the NT and the capacity of its storage system. Our understanding of the endocrine effect on the releasing process is poor. Both estradiol and progesterone inhibited the induced release of radioactivity in the rat oviduct. However the effect of estradiol was observed only *in vivo*, a result that agrees with that of Bengtsson and Marshall (20) who found that in ovariectomized rabbits treated with estradiol the release of ^{3}HNE induced by electrical stimulus was decreased. Both in the rabbit *in vivo*, and in the rat *in vitro*, progesterone decreased the release of ^{3}HNE from the oviduct. Thus both hormones appear as inhibitory signals on induced release, the effect of progesterone being manifested both *in vitro* and *in vivo*. The variations of the induced release observed during the estrous cycle of the rat correlate with the oscillations of progesterone in serum. However, a causal relationship between them is only speculative at this time. If this hormone represents a modulatory signal on noradrenergic nerve-endings of the oviduct, the mechanism involved could be a direct effect of progesterone on the plasmatic membrane of the oviduct noradrenergic varicosities. The possibility of this nongenomic mechanism for progesterone action has been suggested also for the central nervous system (21, 22).

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REFERENCES

1. BRUNDIN, J. (9999) Adrenergic Mechanisms in ovum transport. In *Symposium on Ovum Transport and Fertility Regulation* (Harper, H.J.K.; Pauerstein, C.J.; Adams, C.E.; Coutinho, E.M.; Croxatto, H.B. and Paton, M.D., eds.). San Antonio, Texas, Scriptor, Copenhagen, pp. 243-255.
2. MARSHALL, J.M. (1981) Effect of ovarian steroids and pregnancy on adrenergic nerves of uterus and oviduct. *Amer. J. Physiol. 240:* C165-C174.
3. BELMAR, J.; LARA, H.; GALLEGUILLOS, X. (1983) Changes in noradrenergic vesicle markers of rabbit oviducts during progesterone treatment. *Biol. Reprod. 29:* 594-604.
4. DEL RIO, R.M. and CABALLERO, A.L. (1980) Presence of gamma-amino-butyric acid in rat ovary. *J. Neurochem. 34:* 1584-1586.
5. CELOTTI, F. (1991) Cholinergic and GABA systems of the oviduct. *Arch. Biol. Med. Exp. 24:* 257-268.
6. CAMPUZANO, H.C.; WEILKERSON, J.E.; JORVARTH, S.M. (1975) Fluorometric analysis of epinephrine and norepinephrine. *Anal. Biochem. 64:* 578-587.
7. OISHI, R.; MISHIMA, S.; KUNYAN, H. (1983) Determination of norepinephrine and its metabolites released from rat vas deferens using high performance liquid cromatography with electrochemical detection. *Life Sci. 32:* 933-940.
8; LOWRY, O.H.; ROSENBROUGH, N.J.; FARR, A.L.; RANDALL, K.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem. 193:* 265-276.
9. FORCELLEDO, M.L.; VERA, R. and CROXATTO, H.B. (1981) Ovum transport in pregnant and cyclic rats and its relationship to estradiol and progesterone blood levels. *Biol. Reprod. 24:* 760-765.
10. HODGSON, B.J.; EDDY, C.A. (1975) The autonomic nervous system and its relationships to tubal ovum transport- a reappraisal. *Gynecol. Invest. 6:* 162-185.
11. BRUNDIN, J. (1965) Distribution and function of adrenergic nerves in the rabbit Fallopian tube. *Acta Physiol. Scand. 66:* 5-57.
12. HELM, G.H. HAKANSON, R.; LEANDER, S.; OWMAN, C.; SJOBERG, N.-O.; SPORRONG, B. (1982) Neurogenic relaxation mediated by vasoactive intestinal polypeptide (VIP) in the isthmus of the human Fallopian tube. *Regulatory Peptides 3:* 145-153.
13. BALJET, B.; DRUKKER, J. (1980) The extrinsic innervation of the pelvic organs in the female rat. *Acta Anat. 107:* 241-267.
14. PATON, D.M.; WIDDICOMBE, J.H.; RHEAUME, D.E.; JOHNS, A. (1978) The role of the adrenergic innvervation of the oviduct in the regulation of mammalian ovum transport. *Pharmacol. Rev. 29:* 67-102.
15. OWMAN, Ch.; SJOBERG, N.-O. (1973) The effect of pregnancy and sex hormones on the transmitter level in uterine short adrenergic neurons. In: Usdin, E. and Snyder, S.H. (eds.).. *Frontiers in Catecholamine Research.* New York. Pergamon Press Inc. pp. 795-801.
16. KENNEDY, D.R.; MARSHALL, J.M. (1978) Effects of ovarian steroids on *in vitro* kinetic properties of tyrosine hydroxylase from rabbit oviducts. *Biol. Reprod. 19:* 824-829.
17. LEVITT, M.; SPECTOR, S.; SJOERDSMA, A.; UDENFRIEND, S. (1965) Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. *J. Pharmacol. Exp. Ther. 148:* 1-8.
18. BODKHE, R.R.; HARPER, J.K.M. (1972) Mechanism of eggs transport: changes in amount of adrenergic transmitters in the genital tract of normal and hormone-treated rabbit. In: Segal, S.J.; Crozier, R.; Corfman, P.A. and Condliffes, P.G. (eds.). *The regulation of the mammalian reproduction,* Charles C. Thomas Springfield: pp. 364-396.
19. VIVEROS, O.H. (1976) Dopamine-B-hydroxylase as a marker for the formation, secretion of content and fate of small and large catecholamine containing granules. In: Eranko, O. (ed.). SIF-CELLS. Washington, D.C. Fogarty International Center Proceedings N° 30: pp.89-110.
20. BENGTSSON, B.; MARSHALL, J.M. (1983) Estrogen inhibition of NA release in the rabbit oviduct. *Acta Physiol. Scand. 117:* 321-329.
21. TOWLE, A.C.; SZE, P.Y. (1983) Steroid binding to synaptic plasma membrane: differential binding of glucocorticoids and gonadal steroids. *J. Steroid Biochem. 18:* 135-143.
22. KE, F.-C.; RAMIREZ, V.D. (1990) Binding of progesterone to nerve cell membranes of rat brain using progesterone conjugated to 1251-bovine serum albumin as a ligand. *J. Neurochem. 54:* 467-472.