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INTERACTION BETWEEN ESTROGEN AND BIOGENIC AMINES IN THE CONTROL OF LH SECRETION

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SUMMARY

The augmentation of LH in the plasma of female 5-day cycling rats was studied after perfusion of the third ventricle with dopamine, serotonin or drugs which block its synthesis at the nerve terminals.

Dopamine induces a sharp rise of LH 4 h after infusion; this may be reduced if serotonin is given before. When serotonin is infused after dopamine it induces a fall of LH in blood but to a level still much higher than in control or in rats when dopamine was given after 5 HT.

When dopamine is destroyed in the medial basal hypothalamus by 6-hydroxy-dopamine, LH falls in plasma to a very low level. In this situation depletion of serotoninergic terminals by 5,6-dihydroxytryptamine does not significantly modify LH level in blood. In the absence of serotonin estrogen is still acting in the arcuate nucleus as an inhibitor of LH release.

These experiments support the idea that dopamine may be the mediator for the tonic positive influence on the modulatory inhibitory influence and would act together with, but independently of, estrogen. A model for such interaction is proposed.

I. INTRODUCTION

Since the experiments of Martins[1, 2], confirmed by Moore and Price[3], it became quite clear that ovarian function was controlled by the hypophysis by a mechanism of auto-regulation or feed-back. Removal of the hypophysis [4] induces gonadal atrophy and cessation of the cycles, while castration [5, 6] causes an increase in the volume of the hypophysis with the appearance of cells typical of castration. Ramirez and McCann[7] showed that hypophysial hypertrophy would correspond to the increase in gonadotrophin in the gland as well as in the circulation. Thus, in the absence of the repressive action of the gonadal steroids, the hypophysis increases not only the synthesis but also the liberation of gonadotrophin. On the other hand, injection of estrogen in castrated animals reduced the volume of the hypophysis as well as hormone levels in the gland and in the circulation [8–13]. Prolonged treatment with high doses of estrogen [14] leads to atrophy of both hypophysis and ovary in intact animals.

In 1934, Hohlweg[15] furnished the first evidence that estrogen was able to exercise a stimulatory action on the secretion of gonadotrophins. He observed that treatment with minimal doses of estrogen was able to precipitate puberty in conjunction with the appearance of corpora lutea, in immature rats. Confirmation of these results [16–18] support the assertion that estrogen is capable of causing secretion of LH by the hypophysis and inducing the formation of "corpora lutea" (for review see Flerkó[19]). On the other hand, estrogen is able to advance the ovulatory peak of LH if it is injected in the morning of diestrous in 5-day cycle rats; estrogen injections given in the afternoon have no effect [20, 21]. An extensive review [22] showed that control of the cycling of hypophyseal gonadotrophic secretion is determined by estrogen acting at critical periods controlled by the biological clock. Thus, during the morning of diestrous, the action of estrogen would be predominantly positive, thereby accelerating LH liberation and inducing the cycles. Conversely, estrogen injected at the end of estrous or metestrous would delay the following cycle, perhaps by the action of progesterone [23]. The sensitivity of the adult female to the action of estrogen is not necessarily under the exclusive control of the biological clock but may also be influenced by the particular level of estrogen. Thus, once an ovulatory response is obtained with estrogen injection, only a second LH rise was possible, even though it was diminished 6 days later. On the fourth day an inhibitory response was noted, and a significant increase of LH occurred on the eighth day [24].

Evidence has accumulated showing the participation of progesterone in the control of ovulation [22]. Its presence on the day of diestrous is indispensable for estrogen to cause the induction of the ovulatory peak of LH in the morning of proestrous [25]. On the other hand, in Rhesus monkeys, injection of progesterone during days 6–10 of the cycle blocked ovulation [26].

This review will consider the mechanism by which estrogen interferes with ovulation, and also its interaction with the biogenic amines as mediators of the neural activity and its mechanism of control. For simplicity, the participation of progesterone in the mechanism is represented only as the essential minimum.

II. NEURAL LOCALIZATION OF ESTROGEN ACTION

Flerkó and Szentagothai[27] demonstrated that a graft of a piece of ovary in the median eminence caused a reduction of gonadotrophin. If estrogen is implanted in the arcuate nucleus (AN) there is a reduction in size of the hypophysis and ovary as well as the disappearance of the cycles [28–30]. These results obtained from rats were confirmed in rabbits [31]. On the other hand, estrogen implants in the median eminence of castrated rats and in those with an ovary in the spleen caused the polyluteinic appearance to disappear and to be replaced by a polycystic one[32]. This result confirms the hypothesis that the inhibitory action of estrogen on LH secretion occurs when estrogen is administered in the arcuate nucleus. Kanematsu and Sawyer[33]; Palka, Ramirez and Sawyer[34] and Ramirez and McCann[7] showed that estrogen implanted in the median eminence reduced not only the release of LH but also its synthesis in the hypophysis.

The preoptic area (POA) has been named as the site of action of estrogen which causes positive feed-back and induction of ovulation. Its destruction induces the disappearance of cyclic activity and the maintenance of polycystic ovaries (for review see Flerkó[19]). The electrical activity of the POA varies with the cycle [35, 36] and can be altered by the systemic injection of estrogen [37]. Electrical stimulation of this region induces ovulation [38, 39] and increases LH secretion [40, 41]; there is a relation between stimulus intensity and LH secretion [42]. In males bearing an ovary in the kidney, however, stimulation of the POA does not induce ovulation, nor does it occur in females treated with testosterone during the first days of life [43]. This suggests that abolition of peaks of LH secretion and the consequent anovulatory sterility are the result of the action of testosterone on the POA to increase the threshold of positive estrogen feed-back after puberty [43, 44].

In males, cycling does not occur by elevation of the threshold of the POA sensitivity to the corresponding level of the arcuate nucleus [45]. A biochemical basis for this differentiation was given by Ladosky and Gaziri[46] and confirmed by Giulian *et al.*[47] who showed an increase of serotonin content in the hypothalamus of females on the 12th day of life; the value was reduced if the animal was treated at birth with testosterone. This variation was accompanied by reduction of monoamine oxidase content [48]. Griffiths and Hooper[49] also showed that neonatal treatment with estrogen may accompany a reduction of hypothalamic peptidases to levels comparable to males. Flerkó *et al.*[50] and Tuohimaa and Johansson[51] demonstrated a reduced capacity of the hypothalamus to fix tritiated estradiol if the animal was treated in the 1st day of life with testosterone. This was not confirmed by Maurer[52].

Table 1. Presence of corpora lutea in grafted ovaries in kidney of males after estrogen implants in the POA [32]

Estrogen	1/5	4/5
Estrogen	1/10	1/5
Estrogen	1/20	0/6
Cholesterol		0/4

If, however, the level of estrogen is increased in the POA of males to a degree which surpasses those which cause inhibitory action on the arcuate nucleus, ovulation is observed [32]. Thus, grafted ovaries in the renal capsule of male rats show corpora lutea after implantation of cannulae with estrogen in the POA. This action depends on the concentration of implanted estrogens (Table 1).

This also confirms the observation that the elevation of plasma LH to ovulatory level can be observed after strong electrical stimulation of the POA in males [53, 54].

Surgical separation of the POA from the arcuate nucleus removes the cyclic ovulatory activity from females but does not alter LH secretion in males [55, 56]. The anterior deafferentation, however, does not hinder the elevation of plasma LH levels after castration in either sex [57]. This would suggest that the arcuate nucleus is under double control: the stimulatory action of the POA and the inhibitory action of peripheral estrogen. This control would adjust the basal level of secretion since the anterior hypothalamus would give the stimulus for ovulatory peaks and so regulate cyclic activity.

Since the posterior deafferentation of the median eminence [55, 56] does not significantly alter ovulatory control, this is evidence that the amygdala is not directly involved in the control of ovulation, of sexual differentiation, and of the age of puberty (for review see Critchlow and Bar-Sela[58]). The estrogen implant in the amygdala causes an increase of LH secretion[59] while its stimulation is effective only in females [60]. A sexual dimorphism of the amygdala was also demonstrated by MacKinnon[61]. Raismann and Field[62] suggested that the connections of the amygdala are made with the POA through the preoptic component of the stria terminalis that originates from the cortical medial portion of the amygdaloid nucleus. So the limbic system may not have a direct action on the arcuate nucleus but its action could be effected through the POA. Blake *et al.*[63], however, showed that fibres which arrive at the basal medial hypothalamus by lateral tracts can influence gonadotrophin secretion by a mechanism distinct from fibres coming from an anterior portion. These fibres could be from the medial cortical hypothalamic tract, described previously by Raismann and Field[62]. Litteria and Thorner[64] reported an increased incorporation of lysine-H^3 5 weeks after castration, in the arcuate and paraventricular nuclei and a decreased one in the preoptic area, and suggested these are the places responsible for the feed-back.

Taken together, these results allow postulation of a model of interaction between the neural structures that control gonadotrophin secretion.

The POA would be stimulated by estrogen, the biological clock and the limbic system, receiving tonic inhibitory fibres from the hippocampus [42, 60]. The arcuate nucleus would receive stimulatory impulses from the POA and would have its activity inhibited by estrogen. The difference of thresholds between the POA and AN to estrogen action is that which would determine the presence or absence of gonadotrophic cyclic action [32, 45, 65]. On the other hand, the hypophysis shows a particular rhythmic sensitivity to estrogen [66, 67] and LRH [68].

III. ROLE OF BIOGENIC AMINES IN THE CONTROL OF OVULATION

The tubero-infundibular region of the hypothalamus contains the arcuate nucleus and is extremely rich in nerve endings containing dopamine (DA) and noradrenaline (NA) (for review see Hokfelt and Fuxe[69]). The small number of papers related to the existence of serotoninergic endings should be considered in the light of the technical difficulties encountered in the effort to show the presence or absence of this amine by histofluorescence (Nobin, personal communication). The presence of a high concentration of biogenic amines in the region considered as an interface between the nervous system and the hypophysis can be thought of as having a functional importance and a possible active or passive correlation with the endocrine system.

a. Catecholamines

The first evidence for the participation of catecholamines in the control of ovulation was demonstrated by Sawyer *et al.*[70], who showed that α-adrenergic blocking agents were capable of inhibiting ovulation. Tranquilizers such as reserpine and chlorpromazine that reduce the catecholamine content in the neurons may block ovulation [71], and induce pseudo pregnancy [72] and interfere in the sexual differentiation of the hypothalamus [73]. The use of the histofluorescence technique demonstrated the existence of two systems arriving in the hypothalamic region: a noradrenergic system coming from the lower brain stem [74] and dopaminergic tubero-infundibular [75]. During the estrous cycle there is a variation of the DA content in the tubero-infundibular region [76, 77, 78] and after castration there is a diminution of DA in the hypothalamus in males as well as females [79]. Also, hypothalamic DA content varies with the sexual endocrine state [80, 81, 82]. On the other hand, Kavanagh and Weisz[83] reported that in the basal hypothalamus DA is the predominant catecholamine.

Injection of dopamine in the third ventricle is capable of causing an elevation of plasma LH in males and castrated females treated with estrogen [84]. This effect is much more powerful than that shown by noradrenaline under the same conditions [85–87]. These results were confirmed by Raziano *et al.*[88] who obtained ovulation after injection of L-DOPA in the third ventricle. The amine does not have an effect on the secretion of LH when incubated *in vitro* with the hypophysis [89, 90] but an effect appears when the hypothalamus is included in the medium [91, 92]. Kordon and Glowinski[93] demonstrated that the destruction of biogenic amines in the hypothalamus blocks ovulation. Thus, it seems clear that dopamine promotes the release of LH through action on the hypothalamus.

Rubinstein and Sawyer[94] obtained a more intense response with noradrenaline and Weiner *et al.*[95] observed that in anovulatory rats after partial anterior deafferentation the basal level of NA in the medial hypothalamus is diminished but DA remained normal. NA is *diminished* only after total deafferentation accompanied by LH reduction. In conjunction, these results demonstrate that NA has a stimulatory action on the arcuate nucleus to cause increases in LH secretion and LH plasma concentration. Since this action is accentuated after stimulation of the POA but disappears after frontal deafferentation, it can be said that NA is the probable mediator between the POA and the arcuate nucleus.

b. Serotonin

The role of serotonin has been less studied than that of DA with respect to control of ovulation. There is, however, evidence demonstrating that the indolamines could have a role in LH secretion. The elevation of serotonin content in the hypothalamus by the administration of its precursor, 5-hydroxytryptophan, together with monoamine oxidase inhibitors blocks the ovulatory peaks [96–98]. The same result is obtained when serotonin is infused directly into the third ventricle [84, 99] or when it is injected systemically [100]. This last result, however, can be considered as a consequence of peripheral action [101].

The absence of serotonin in the hypothalamus after ventricular injection of 5,6-dihydroxytryptamine permits an accentuated elevation of plasma LH in castrated rats with already elevated content [57]. Treatment of castrated rats with estrogen augments the tryptophan content in the hypothalamus [102] and also in other structures not proved to be connected to the sexual endocrine control [103]. In the ewe, release of pituitary LH is dependent upon a previous reduction of serotonin but not noradrenaline in the hypophysiotropic area of the hypothalamus [104]. The conflict, however, of these data and those of Rubinstein and Sawyer[94]; Schneider and McCann[84] and Fraschini[105] who did not obtain a block in ovulation after injection of serotonin in the third ventricle has no apparent explanation.

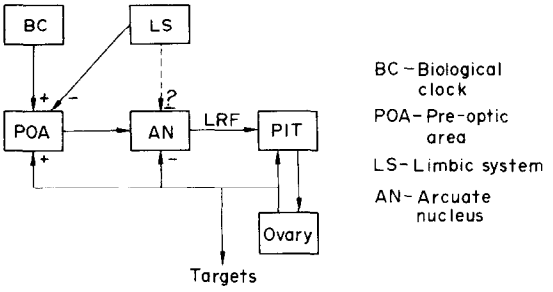

Fig. 1. Diagram for classical theory of brain–pituitary–ovary systems.

IV. MATERIALS AND METHODS

Adult female Wistar rats weighing 180–200 g showing at least three regular cycles of 5 days were used in this study.

a. Perfusion

All experiments were begun on the afternoon of proestrous when 1 ml of blood was taken from the jugular vein in animals under ether anesthesia; this sample was considered as a control. While still under anesthesia a cannula was placed stereotaxically in the third ventricle in order to permit the infusion of biogenic and synthetic amines diluted in saline. All biogenic amines were injected in 1 μg quantities diluted in 5 μl (of saline); the synthetic compounds were diluted 50 μg in 5 μl.

Depending on experimental design, the animals were again bled under anesthesia and another drug perfusion was made in the third ventricle, or a cannula containing estrogen was implanted in the arcuate nucleus. On the day of autopsy a third blood sample was taken, the ovaries and uterus recovered for histologic controls, and the brain was fixed in 10% formalin to serve as a control on the stereotaxic placement.

b. Implants

Estrogen was mixed with cholesterol, dissolved by heating, mixed, and placed in a steel cannula by capillarity. After cleaning, the cannula was placed stereotaxically in the arcuate nucleus. All implants were made 10 days after perfusion with 5,6-dihydroxytryptamine.

c. Radioimmunoassay (RIA)

RIA was carried out utilizing a Niswender–Porter standard equivalent to 0·135 NIH-LH-S1 assayed by ovarian ascorbic acid depletion.

V. RESULTS

a. Interaction between biogenic amines

The injection of dopamine in the afternoon of proestrous significantly increased plasma LH measured 4 h later (Fig. 2). The animals were then injected with serotonin. Four hours after this injection the plasma LH level was significantly decreased but remained at much higher levels than those found in the control state, pre-perfusion of dopamine. If perfusions were made in the reverse manner, dopamine elevation was impeded but not completely blocked. Resulting LH levels were much smaller than those observed following treatment with dopamine plus serotonin. These data confirm earlier results of Schneider *et al.*[89]; Kamberi *et al.*[87]; Labhsetwar[100]; Ladosky and Noronha[57] in which dopamine injected into the third ventricle significantly increased the release of LH while injections of serotonin were inhibitory. It complements also the report by Zolovick and Labhsetwar[106] who observed that after the injection of serotonin in immature rats, dopamine may induce ovulation.

The present work permits two interpretations as to the mechanism of integration between dopamine and serotonin in the control of LH secretion. The first would be that serotonin blocks dopamine penetration into the cell. Thus, it is more powerful in blocking the effect of dopamine on LH secretion when it is injected before rather than after dopamine; in the latter case dopamine could have already entered the cell. The kinetics of dopamine and serotonin after their perfusion in the third ventricle have not been studied, nor is there data that could completely justify this. An alternative explanation may be that the action of serotonin is not at the level of dopamine (penetration into the cell) but rather blocks the liberation of LH. This explanation is strengthened in the light of the fact that the high values of plasma LH obtained after serotonin perfusion subsequent to dopamine could indicate only a *decrease* of LH after serotonin injection and *not* a *blockage* of dopamine action. In this line of thought it is necessary to make more extensive studies in terms of the dynamic interrelation of the two substances.

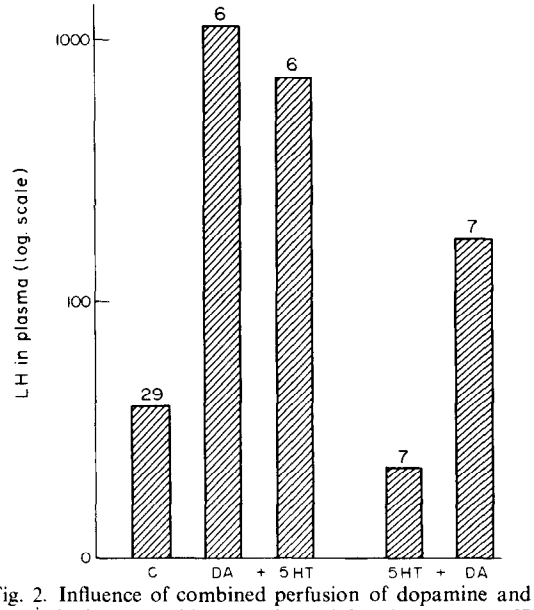

Fig. 2. Influence of combined perfusion of dopamine and serotonin into the third ventricle of female rats on LH secretion.

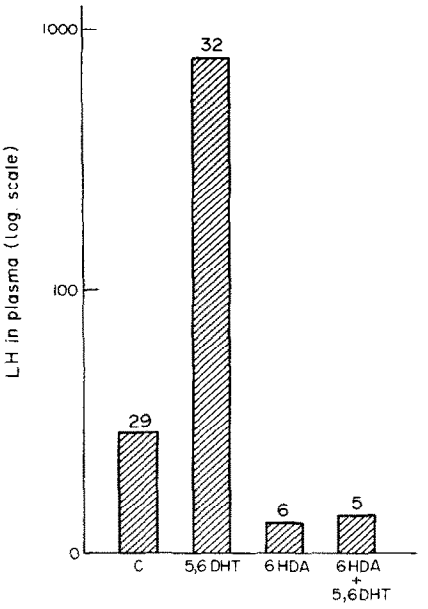

Fig. 3. LH in plasma of female rats after depletion of serotonin or dopamine in the median eminence.

The second hypothesis that might explain the mechanism is that there is a competition between serotonin and dopamine in the metabolic activity of LH–RH production in the cell. The evidence is also indirect and inconclusive. Campos and Ladosky[107] demonstrated that serotonin *in vitro* is capable of inhibiting the QO_2 of castrated, but not intact rats. This suggests interference in the energy metabolism of the hypothalamus related to the presence of sex hormones. On the other hand, Gunaga and Menon[108] in rats and Sato *et al.*[109] in pigs showed that dopamine, like other biogenic amines, increased the content of cyclic AMP in cells of the hypothalamus. When one considers that almost all biogenic amines exert this effect, it is only slightly probable that this is the modulator. This may also explain the antagonistic effects between serotonin and dopamine observed in this study.

b. Consequence of the absence of mediators in the hypothalamus

The long lasting destruction of serotonin by 5,6-dihydroxytryptamine or dopamine by 6-hydroxydopamine in the hypothalamus causes contrasting effects in plasma LH. After the destruction of dopamine there is a sharp diminution of plasma LH levels. Conversely, an elevation occurs after serotonin destruction (Fig. 3).

Since serotonin destruction in an animal whose hypothalamus is already depleted of dopamine does not cause any additional effect, it is possible to conclude that dopamine is the tonic stimulatory substance on the arcuate nucleus while serotonin is the regulator of its function.

c. Interaction between estrogen and serotonin

In an animal whose hypothalamus was previously depleted of serotonin by injection of 5,6-dihydroxytryptamine and consequently shows a high plasma LH content, the implantation of a cannula containing estrogen in the arcuate nucleus significantly reduces LH secretion (Fig. 4). The levels of plasma LH immediately after this reduction remain at levels significantly higher than in the non-implanted control animal.

These results suggest that estrogen exercises its inhibitory effect on the arcuate nucleus additively but independently of the action of serotonin since in the absence of hypothalamic serotonin LH can be reduced by the action of estrogen. As was already demonstrated by Ladosky and Noronha[57] the absence of estrogen after castration causes the secretion of plasma LH which can be further increased if serotonin is destroyed by 5,6-dihydroxytryptamine.

VI. THE MODEL

Based on results already described in the literature and mentioned earlier, and from the new data described in this work, we propose a model that justifies the integration of the action of estrogen and biogenic amines on the control of LH. As was previously mentioned, progesterone participation is not included due to the lack of experimental data to elucidate its interaction with biogenic amines in gonadotrophin control.

The key point in control of ovulation would be in the arcuate nucleus (Fig. 5) that receives the positive influence of the preoptic area transmitted by dopamine, and the negative influences of serotonin, coming perhaps from the limbic system *via* the terminal medial cortico hypothalamic tract[62] and

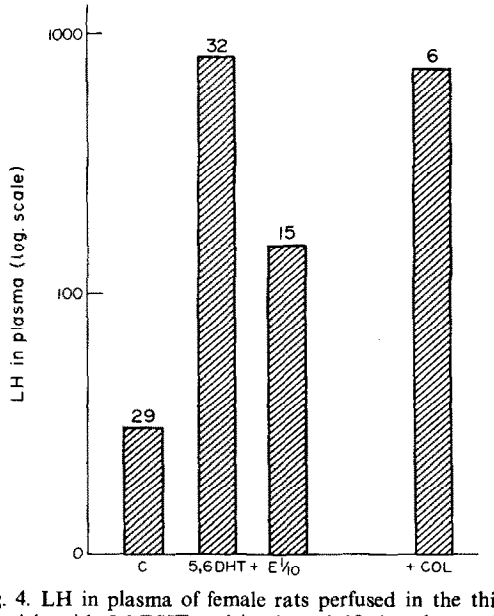

Fig. 4. LH in plasma of female rats perfused in the third ventricle with 5,6-DHT and implanted 10 days later with estrogen in the arcuate nucleus.

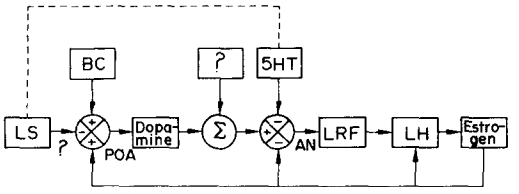

Fig. 5. Proposed block diagram for estrogen and biogenic amines interrelationship on the control of LH.

from estrogen arriving by the vascular path [110, 111]. The arcuate nucleus could also receive stimulatory fibres, probably noradrenergic, coming from the limbic system[112]. Whether these fibres have a predominant action on the secretion of LH [94] or on the action of prolactin [113] is not clear.

In the absence of a stimulatory action of the POA caused by frontal deafferentation, the arcuate nucleus receives only the tonic inhibitory stimulus of estrogen and serotonin consequently maintaining a low level of LH. This occurs in spite of some stimulatory adrenergic or dopaminergic fibres coming by the medial hypothalamic cortical tract [62]. Anterior deafferentation that blocks the ovulatory peak [56] does not reduce the content of DA[95]. These data are confirmed in the present work which shows that the destruction of catecholamines is more effective in the reduction of plasma LH levels than is frontal deafferentation. The previously mentioned controversy continues as to which catecholamines would be responsible for the stimulation of the AN since it is seen that 6-hydroxy-dopamine reduces DA and NA in the terminal portions of the neurons.

If, besides the blockage of the POA connection, the AN lacks estrogen after castration, or serotonin by the destruction of neural terminals, the LH content can rise significantly due to the absence of one of the inhibitory elements. The fact that perfusion with 6-HDA significantly diminishes LH secretion to a lower level than that observed after partial frontal deafferentation [57] suggests that not only is DA responsible for the tonic stimulus of the arcuate nucleus but also that it comes from structures other than the POA.

The preoptic area is under the tonic influence of plasma estrogen whose activity is modulated by the action of the biological clock. At this time no evidence yet exists for a mediator mechanism or how it would act on the preoptic area. Estrogen could act on the arcuate nucleus to induce activation of DNA–RNA perhaps in the same manner as in peripheral receptors. It has been seen that its action can be blocked by previous treatment with actinomycin D [114].

Velasco and Taleisnik[60] have shown that the amygdala has an inhibitory influence on the POA, but at this point there has been no identification of a biogenic amine which would be involved in the process.

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REFERENCES

1. Martins T.: *CR Soc. Biol.* **102** (1929) 605.
2. Martins T.: *CR Soc. Biol.* **104** (1930) 686.
3. Moore C. and Price D.: *Am. J. Anat.* **50** (1932) 13–72.
4. Smith P. and Engle E.: *Am. S. Anat.* **40** (1927) 159–217.
5. Fichera G.: *Archs Ital. Biol.* **43** (1905) 405–426.
6. Carmichel E. S. and Marshall F. H. A.: *J. Physiol. Lond.* **36** (1908) 431–434.
7. Ramirez V. D. and McCann S.: *Endocrinology* **72** (1963) 452–464.
8. Greep R. O.: In *Sex and Internal Secretion* (Edited by W. C. Young). William Wilkins, 3rd Edn, Vol. I (1961) 240–301.
9. NcCann S. M. and Taleisnick S.: *Endocrinology* **69** (1961) 909–914.
10. Bogdanove M.: *Vitamins Hormones* **22** (1964) 205–260.
11. Van Rees G. P.: *Major Problems in Neuroendocrinology* (Edited by E. Bajus and G. Jasmin). Karger, Basel (1964) 322–345.
12. McCann S. M. and Ramirez V. D.: *Recent Prog. Horm. Res.* **20** (1964) 131–170.
13. Parlow A. F.: *Endocrinology* **75** (1964) 1–8.
14. Flerkó B.: *Hypothalamic Control of the Anterior Pituitary* (Edited by J. Szentagothai, B. Flerkó, B. Mess and B. Halazz). Hungarian Academy of Science, Budapest (1962) 192–265.
15. Hohlweg W.: *Klin. Wochsch.* **13** (1934) 92–95.
16. Hohlweg W. and Chamorro A.: *Klin. Wochsch.* **16** (1937) 196–197.
17. Merckel C. and Nelson W. O.: *Anat. Record* **76** (1940) 391–409.
18. Hellbaum A. A. and Greep R. O.: *Proc. Soc. exp. Biol. Med.* **63** (1946) 53–56.
19. Flerkó B.: *Neuroendocrinology* (Edited by L. Martini and Ganong). Academic Press, New York (1966) 613–668.
20. Everett S. W.: *Endocrinology* **43** (1948) 389–405.
21. Brown-Grant K.: *J. Endocr.* **43** (1969) 553–562.
22. Schwartz N. B.: *Recent Prog. Horm. Res.* **25** (1969) 1–55.
23. Hobson W. C. and Hansel: *Endocrinology* **91** (1972) 185–190.
24. Weick R. F., Dierschke D. J., Karsch F. J., Yamaji T. and Knobil E.: *Endocrinology* **91** (1972) 1528–1530.
25. Mann D. R. and Baraclough C. A.: *Endocrinology* **93** (1973) 694–699.
26. Spies H. G. and Niswender G. D.: *Endocrinology* **90** (1972) 257–261.
27. Flerkó B. and Szentagothai J.: *Acta endocr., Copenh.* **26** (1957) 121–127.
28. Lisk R. D.: *J. exp. Zool.* **145** (1960) 197–208.
29. Chowers I. and McCann S. M.: *Proc. Soc. exp. Biol. Med.* **124** (1967) 260–266.
30. Chambers W. F. and Howe G.: *Proc. Soc. exp. Biol. Med.* **128** (1968) 292–294.
31. Davidson J. M. and Sawyer C. H.: *Acta endocr., Copenh.* **37** (1961) 385–393.
32. Ladosky W. and Sakata K.: *Proc. Congr. Assoc. Latinamer.* Ciencia Fisiologia (Edited by W. T. Beraldo). Belo Horizonte (1969) 69.

33. Kanematsu S. and Sawyer C. H.: *Am. J. Physiol.* **205** (1963) 1073–1076.
34. Palka Y. S., Ramirez V. D. and Sawyer C. H.: *Endocrinology* **78** (1966) 487–499.
35. Barraclough C. A. and Cross B. A.: *J. Endocr.* **26** (1963) 339–359.
36. Kalra S. P. and McCann S. M.: *Endocrinology* **93** (1973) 665–669.
37. Yagi K.: *Brain Res.* **53** (1973) 343–352.
38. Critchlow B. V.: *Am. J. Physiol.* **195** (1958) 171–174.
39. Everett S. W.: *Phys. Rev.* **44** (1964) 373–431.
40. Harris G. W. and Ruf K. B.: *J. Physiol.* **208** (1970) 243–250.
41. Clemens S. A., Shaar C. J., Kleber J. W. and Tandy W. A.: *Endocrinology* **88** (1971) 180–184.
42. Velasco M. E. and Rothchild I.: *J. Endocr.* **58** (1973) 163–176.
43. Barraclough C. A. and Gorski R. A.: *Endocrinology* **68** (1961) 68–79.
44. Barraclough C. A. and Haller E. W.: *Endocrinology* **86** (1970) 542–555.
45. Kidston A. L., Belpulsi A. and Weisz J.: *Biol. Reprod.* **9** (1973) 77.
46. Ladosky W. and Gaziri L. C. J.: *Neuroendocrinology* **6** (1970) 168–174.
47. Giulian D., Pohorecky L. A. and McEwen B. S.: *Endocrinology* **93** (1973) 1329–1335.
48. Gaziri L. C. J. and Ladosky W.: *Neuroendocrinology* **12** (1973) 249–256.
49. Griffiths E. C. and Hooper K. C.: *Acta endocr., Copenh.* **72** (1973) 9–17.
50. Flerkó B., Illei-Donhoffer A. and Mess B.: *Acta Biol. Hung.* **22** (1971) 125–130.
51. Tuohimaa P. and Johansson R.: *Endocrinology* **88** (1971) 1159–1164.
52. Maurer R. A.: *Brain Res.* **67** (1974) 175–177.
53. Quinn D. L.: *Nature* **209** (1966) 891–892.
54. Everett S. W.: *Ann. Rev. Physiol.* **31** (1969) 383–416.
55. Hallasz B. and Pupp L.: *Endocrinology* **77** (1965) 553–562.
56. Hallasz B. and Gorski R. A.: *Endocrinology* **80** (1967) 608–622.
57. Ladosky W. and Noronha J. G. L.: *J. Endocr.* **62** (1974) in press.
58. Critchlow B. V. and Bar-Sela M. E.: *Neuroendocrinology* (Edited by L. Martini and W. Ganong). Academic Press, New York, Vol. II (1967) 101–162.
59. Lawton I. E. and Sawyer C. H.: *Am. J. Physiol.* **218** (1970) 622–625.
60. Velasco M. E. and Taleisnik S.: *Endocrinology* **84** (1969) 132–139.
61. MacKinnon P. C. B.: *J. Physiol.* **210** (1970) 10–11.
62. Raisman G. and Field P. M.: *Frontier in Neuroendocrinology* (Edited by L. Martini and W. F. Ganong). Oxford (1971) 1–44.
63. Blake C. A., Weiner R. I., Gorski R. A. and Sawyer C. H.: *Endocrinology* **90** (1972) 855–861.
64. Litteria M. and Thorner M. W.: *J. Endocr.* **60** (1974) 377–378.
65. Karsch F. J., Dierschke D. J., Weick R. F., Yamasi T., Hotchkiss J. and Knobil E.: *Endocrinology* **92** (1973) 799–803.
66. Debeljuk L., Arimura A. and Schally A. V.: *Proc. Soc. exp. Biol. Med.* **143** (1973) 1164–1167.
67. Martin J. E., Tyrey L., Guerett J. W. and Fellows R. E.: *Endocrinology* **94** (1974) 556–562.
68. Negro-Vilar A., Orias R. and McCann S. M.: *Endocrinology* **92** (1973) 1680–1684.
69. Hokfelt T. and Fuxe K.: *Brain Endocrine Interaction* (Edited by Knnige and Scott). Karger, Basel (1972) 181–223.
70. Sawyer C. R., Markee J. E. and Hollinshead W. D.: *Endocrinology* **41** (1947) 395–402.
71. Barraclough C. A. and Sawyer C. H.: *Endocrinology* **57** (1955) 329–337.
72. Barraclough C. A. and Sawyer C. H.: *Endocrinology* **61** (1957) 341–351.
73. Ladosky W., Kesikowski W. M. and Gaziri I. F.: *J. Endocr.* **48** (1970) 151–158.
74. Anden H. E., Dahlstrom A., Fuxe K., Olson L. and Ungerstedt U.: *Acta physiol. scand.* **67** (1966) 313–326.
75. Fuxe K.: *Z. Zellforch* **65** (1965) 573–596.
76. Fuxe K., Hokfelt T. and Nilsson: *Life Sci.* **6** (1967) 2057–2061.
77. Fuxe K., Hokfelt T. and Nilsson: *Neuroendocrinology* **5** (1969) 257–270.
78. Ahren K., Fuxe K., Hamberder B. and Hokfelt T.: *Endocrinology* **88** (1971) 1415–1424.
79. Olson L., Fuxe K. and Hokfelt T.: *Brain Hormone Interrelationship* (Edited by K. M. Knigge and Scott). Karger, Basel (1971).
80. Stefano F. J. E. and Donoso A. O.: *Endocrinology* **81** (1967) 1405–1406.
81. Lichtensteiger W.: *J. Pharm. exp. Ther.* **165** (1969) 204–215.
82. Donoso A. O. and Moyano: *Proc. Soc. exp. Biol. Med.* **135** (1970) 633–635.
83. Kavanagh A. and Weisz J.: *Neuroendocrinology* **13** (1973) 201–212.
84. Schneider H. P. G. and McCann S. M.: *Endocrinology* **87** (1970) 249–253.
85. Kamberi I. A., Mical R. S. and Porter J. C.: *Endocrinology* **87** (1970) 1–12.
86. Kamberi I. A., Mical R. S. and Porter J. C.: *Endocrinology* **88** (1971) 1003–1011.
87. Kamberi I. A., Mical R. S. and Porter J. C.: *Endocrinology* **88** (1971) 1012–1020.
88. Raziano J., Cowchock S., Ferin and Van de Wiele R. J.: *Endocrinology* **88** (1971) 1516–1518.
89. Kamberi I. A. and McCann S. M.: *Endocrinology* **85** (1969) 815–824.
90. Schneider H. P. G. and McCann S. M.: *Endocrinology* **85** (1969) 121–132.
91. Kuhn E., Krulich L., Quijada M., Illner P., Kalra P. S. and McCann S. M.: *Proc. 52nd Meeting Endocrine Soc.* (1970) 126.
92. Kamberi I. A., Schneider H. P. G. and McCann S. M.: *Endocrinology* **86** (1970) 278.
93. Kordon C. and Glowinski J.: *Endocrinology* **85** (1969) 924–931.
94. Rubinstein L. and Sawyer C. H.: *Endocrinology* **86** (1970) 988–995.
95. Weiner R. I., Gorski R. A. and Sawyer C. H.: *Brain Endocrine Interaction* (Edited by K. Knnige and Scott). Karger, Basel (1972) 236–244.
96. Kordon C.: *Neuroendocrinology* **4** (1969) 129–138.
97. Lippman W.: *Nature* **218** (1968) 173.
98. Labhsetwar A. P.: *J. Endocr.* **54** (1972) 269–275.
99. Domawski, Przekop F. and Shubijzewski B.: *Differentiation and Neuroendocrine Regulation in the Hipothamus Hypophyseal Gonadal System* (Edited by G. Doerner) Berlin (1972).
100. Labhsetwar A. P.: *Acta endocr., Copenh.* **68** (1971) 334–344.
101. Wilson C. A. and MacDonald P. G.: *J. Endocr.* **60** (1974) 253–260.
102. Bapna J., Neff H. H. and Costa E.: *Endocrinology* **89** (1971) 1345–1349.
103. Tonge S. R. and Greengrass P. M.: *Psychopharmacologia* **21** (1971) 374–381.
104. Wheaton J. E., Martin S. K., Swanson L. V. and Stormjhak F.: *J. Anim. Sci.* **35** (1972) 801–804.
105. Fraschini F.: *Neurochemical Aspects of Hypothalamic Functions* (Edited by L. Martini and J. Meites). Academic Press, New York (1970) 141.

106. Zolovick A. and Labhsetwar A. P.: *Nature* **245** (1973) 158–159.
107. Campos D. G. J. and Ladosky W.: *Neuroendocrinology* **9** (1972) 133–141.
108. Gunaga K. P. and Menon K. M. J.: *Biochem. biophys. Res. Commun.* **54** (1973) 44.
109. Sato A., Onaya T., Kotani M., Harada A. and Yamada T.: *Endocrinology* **94** (1974) 1311–1317.
110. Lisk R. D., Ciaccio L. A. and Reutner L. A.: *Biology of Reproduction Basic and Clinical Studies* (Edited by L. T. Velardo and B. A. Karprow), III Pan American Congress of Anatomy, New Orleans (1972).
111. Lisk R. D. and Ferguson: *Neuroendocrinology* **12** (1973) 153–160.
112. Cuello A. C., Weiner R. I. and Ganong W. F.: *Brain Res.* **59** (1973) 191–200.
113. Donoso A. O. and Cukier F. O.: *Nature* **218** (1968) 969.
114. Jackson G. L.: *Endocrinology* **91** (1972) 1284–1287.