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ROLE OF THE AMYGDALA AND THE *ORGANUM VASCULOSUM LAMINAE TERMINALIS* IN THE CONTROL OF OVARIAN FUNCTION IN THE FEMALE RAT

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SUMMARY

Experiments have been performed to study the role of the amygdala and of the *organum vasculosum laminae terminalis* (OVLT) in the control of gonadotrophin secretion.

Amygdala. Drugs known as antagonists or agonists of the adrenergic and of the cholinergic receptors have been implanted at the level of the basomedial area of the amygdala in long-term castrated female rats. The animals were sacrificed at different time intervals after implantation, and their serum levels of LH and FSH were measured with specific radioimmunoassays and compared to those of sham-implanted animals. The results have shown that adrenergic and cholinergic receptors involved in the control of gonadotrophin secretion are present in the amygdala. In particular, the data have shown that: (1) α-adrenergic receptors of the amygdala exert an inhibitory tone on LH and FSH secretion; (2) β-adrenergic receptors seem to exert an inhibitory influence only on LH release; (3) cholinergic receptors of the muscarinic type appear to exert an inhibitory influence on LH output; (4) cholinergic receptors of the nicotinic type seem to play a role in depressing FSH secretion.

OVLT. This structure was destroyed by radiofrequency lesions in regularly cycling and in long-term castrated adult female rats. After OVLT lesion practically all cycling females (16 out of 22) entered a dioestrous status, as indicated by vaginal smears. At the moment of sacrifice (8 days after the lesion) their serum gonadotrophin levels were as low as those present in cycling animals in the phase of dioestrus. Castrated females bearing OVLT lesions had serum titres of LH and FSH as high as those of castrated controls. It is concluded that the OVLT plays a role in the control of the "cyclic" release of gonadotrophins, but is not involved in the "tonic" regulation of gonadotrophin secretion.

INTRODUCTION

This paper summarizes the data of two groups of experiments performed in this laboratory on the participation of the amygdala and of the *organum vasculosum laminae terminalis* (OVLT) in the control of gonadotrophin secretion and of ovarian function in the female rat.

I. ROLE OF THE AMYGDALA

The participation of the amygdala in the control of gonadotrophin secretion in the rat is still a controversial issue, since some reports suggest a stimulatory and others an inhibitory role of this structure on anterior pituitary function [1–9].

The possible influence of the amygdala on LH and FSH secretion has been studied through a pharmacological approach, *i.e.* by implanting bilaterally in the area of the basomedial nucleus drugs known to mimic or to antagonize adrenergic or cholinergic transmission. Such an approach is justified by the following considerations: (1) the amygdala receives afferent fibres from many areas of the brain and in particular from the brain stem [10, 11]; (2) the majority of the fibres reaching the amygdala release "traditional" neurotransmitters (noradrenaline, dopamine, acetylcholine, etc.) at their end terminals; (3) "classical" as well as "putative" neurotransmitters have been found to be present in the amygdala (*Catecholamines*: [12–14]; *Acetylcholine*: [15–19]; *Serotonin*: [20]; *GABA*: [21]; *Histamine*: [22]; *Substance P*: [23]; *Enkephalins*: [24]; *LH-RH*: [25]; (4) the amygdala may influence the hypothalamic pituitary axis through efferent impulses transported through fibres running in the stria terminalis and in the ventral amygdalofugal pathway [see 26, for references].

Adult female rats of the Sprague–Dawley strain were used in all experiments. They were castrated when they weighed 160–170 g. Castrated animals were preferred to normals to avoid interference of the spontaneous fluctuations of serum levels of gonadotrophins which occur in cycling females. Cannulae bearing crystals of the different drugs were prepared by tamping 26-gauge stainless steel tubings into a mixture of the drug to be studied and cocoa butter (ratio 1:1) as reported previously [27]. Excess material remaining on the external surface of the tubings was carefully removed. The cannulae were implanted bilaterally into the basomedial portion of the amygdala under pentobarbital anesthesia 4 weeks after castration using a Stoelting stereotaxic instrument and Pellegrino and Cushman's atlas [28]. The needles were subsequently fixed to the skull with dental cement and remained in place until the animals were sacrificed. Similar cannulae filled with cocoa butter alone were used for sham implantations. Control and experimental animals were killed with a guillotine 3,

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16, 12 and 24 h after implantation. Blood from the trunk vessels was collected at the moment of sacrifice. The sera were separated by centrifugation and stored frozen until the assays were performed. Serum LH and FSH levels were measured using, respectively, the radioimmunoassay procedures of Niswender *et al.*[29] and Daane and Parlow[30].

After sacrifice the location of the tip of the cannulae was verified histologically. Only animals in which the cannulae were correctly placed in the basomedial region of the amygdala were retained. The results have been statistically analyzed utilizing the Dunnett test [31] for multiple comparisons after one way analysis of variance.

Adrenergic antagonists

Serum levels of LH and FSH have been found to be respectively 971.14 ± 52.05 and 1938.27 ± 64.39, in a group of 35 control female Sprague–Dawley rats, which were ovariectomized at the same time as the sham-implanted and experimental animals, but which did not undergo any further surgical manipulation. Anaesthesia and sham-implantation may decrease serum levels of LH and FSH especially at the beginning of the experiment. This is evident by comparing the data of sham-implanted animals in Tables 1 and 2 with those mentioned above. This confirms a report of Borrell *et al.*[32]. Because of this, the data obtained in the experimental animals have been compared only to those found, at the corresponding intervals of time, in sham-implanted animals.

Serum levels of LH have been found to be significantly higher than in the corresponding sham-operated animals 3, 6 and 12 h after the intra-amygdalar implantation of the α-adrenergic blocker phenoxybenzamine (Table 1). Experimental and sham-implanted animals had comparable values of serum LH at 24 h after implantation. Serum levels of FSH have been found to be significantly more elevated in phenoxybenzamine implanted than in sham-operated animals 3, 12 and 24 h following placement of the drug (Table 2). At the 12 and 24 h intervals the levels of FSH in the experimental animals were significantly ($P < 0.01$) higher than those of unimplanted controls. From these results one may infer that there are α-adrenergic receptors located in the basomedial portion of the amygdala. Since the blockade of these receptors by an α-antagonist brings about stimulation of LH and of FSH release, one may argue that the physiological activation of these receptors may exert an inhibitory influence on gonadotrophin secretion.

The implantation into the amygdala of propranolol, a β-adrenergic receptor blocker, significantly increased serum LH above sham-implantation levels 12 and 24 h following placement of the drug. There were no significant differences at any other time considered (Table 1). Intra-amygdalar implants of propranolol did not affect FSH release (Table 2). These results may suggest that the basomedial region of the amygdala contains β-adrenergic receptors in addition to α-adrenergic ones. The physiological activation of these receptors appears to exert an inhibitory role on the release of LH. Apparently, β-receptors in the amygdala do not participate in the control of FSH secretion.

Cholinergic antagonism and agonists

Table 3 shows that the bilateral implantation into the basomedial region of the amygdala of the muscarinic blocker atropine significantly enhanced LH above the levels found in sham-operated animals for the whole period of observation. In particular, 12 and 24 h after implantation, operated animals had serum LH levels which were higher than those of castrated controls not submitted to stereotaxic procedures ($P < 0.01$). In the same animals, serum levels of FSH were not changed by the presence of atropine in the amygdala (Table 4). The implantation of mecamylamine, a rather specific blocker of nicotinic receptors, in the same area did not change serum LH levels. Serum FSH was significantly enhanced 6 and 24 h after implantation of this drug, and was higher than plantation of this drug, and was higher than in sham-operated animals, even if not in a significant way, at 12 h.

When the two cholinergic stimulants carbachol (which has muscarinic as well as nicotinic properties) and pilocarpine (which is an almost pure muscarinic drug) were brought to the level of the basomedial portion of the amygdala, a significant depression of

Table 1. Effects on serum LH levels of adult castrated female rats of bilateral implants in the basomedial region of the amygdala of antagonists of adrenergic receptors

Groups	Serum LH at different intervals after intra-amygdalar implantation 3 h	6 h	12 h	24 h
Sham-implanted animals	(15) 526.60 ± 30.94	(18) 563.17 ± 48.21	(19) 747.62 ± 55.64	(22) 874.44 ± 59.21
Phenoxybenzamine	(5) 800.46 ± 13.91*	(8) 772.21 ± 33.52†	(5) 1070.86 ± 143.87†	(7) 754.00 ± 100.35
Propranolol	(6) 580.77 ± 47.56	(6) 620.74 ± 53.38	(7) 1107.84 ± 81.37	(7) 1166.54 ± 141.18†

Number of animals in parentheses.
Data are expressed in terms of NIAMDD—Rat LH-RP-1.
Values are means ± SE.
Significance: *$P < 0.01$ *vs.* Sham-implanted animals. †$P < 0.05$ *vs.* Sham-implanted animals.

Table 2. Effects on serum FSH levels of adult castrated female rats of bilateral implants in the basomedial region of the amygdala of antagonists of adrenergic receptors

Groups	Serum FSH at different intervals after intra-amygdalar implantation 3 h	6 h	12 h	24 h
Sham-implanted animals	(17) 1604.59 ± 54.43	(19) 1722.71 ± 48.12	(16) 1941.50 ± 75.83	(21) 2014.38 ± 88.99
Phenoxybenzamine	(5) 2054.54 ± 116.78*	(9) 1873.04 ± 75.45	(5) 2653.80 ± 145.55*†	(4) 2812.12 ± 279.61*†
Propranolol	(7) 1545.21 ± 83.49	(6) 1527.30 ± 104.60	(6) 1724.48 ± 52.81	(7) 2176.04 ± 186.75

Number of animals in parentheses.
Data are expressed in terms of NIAMDD—Rat FSH-RP-1.
Values are means ± SE.
Significance: *$P < 0.01$ *vs.* sham-implanted animals. †$P < 0.01$ *vs.* castrated controls (1938.27 ± 64.39).

serum levels of LH at 3 h (carbachol) and at 12 h (pilocarpine) (Table 3) was observed. FSH serum titres were not affected (Table 4).

On the basis of this series of results one might postulate that cholinergic receptors involved in the control of gonadotrophin secretion are present in the basomedial region of the amygdala. Apparently, their blockade brings about a stimulation of gonadotrophin secretion which seems to depend on the pharmacological properties of the antagonist used: cholinergic inputs of the muscarinic type seem to play a rather specific role in the modulation of LH secretion, while cholinergic inputs of the nicotinic type seem to be of more importance for the regulation of FSH secretion. Stimulation of these cholinergic receptors with drugs possessing muscarinic or nicotinic activity

Table 3. Effects on serum LH levels of adult castrated female rats of bilateral implants in the basomedial region of the amygdala of antagonists and agonists of cholinergic receptors

Groups	Serum LH at different intervals after intra-amygdalar implantation 3 h	6 h	12 h	24 h
Sham-implanted animals	(24) 330.54 ± 25.09	(21) 322.20 ± 21.83	(22) 473.89 ± 26.86	(12) 576.66 ± 47.84
Atropine	(4) 474.64 ± 52.13*	(7) 708.05 ± 73.73†	(4) 798.29 ± 68.28†‡	(6) 974.25 ± 78.83†‡
Mecamylamine	(10) 324.21 ± 28.02	(5) 445.30 ± 49.86	(14) 479.95 ± 20.95	(8) 560.87 ± 58.31
Carbachol	(6) 159.03 ± 37.55†	(7) 251.31 ± 40.37	(7) 364.45 ± 45.50	(8) 633.94 ± 100.98
Pilocarpine	(7) 243.73 ± 7.82	(7) 235.51 ± 32.29	(5) 286.43 ± 7.85†	(6) 473.59 ± 70.24

Number of animals in parentheses.
Data are expressed in terms of NIAMDD—Rat LH-RP-1.
Values are means ± SE.
Significance: *$P < 0.05$ *vs.* sham-implanted animals. †$P < 0.01$ *vs.* sham-implanted animals. ‡$P < 0.01$ *vs.* castrated controls (615.01 ± 33.36).

Table 4. Effects on serum FSH levels of adult castrated female rats of bilateral implants in the basomedial region of the amygdala of antagonists and agonists of cholinergic receptors

Groups	Serum FSH at different intervals after intra-amygdalar implantation 3 h	6 h	12 h	24 h
Sham-implanted animals	(27) 1421.06 ± 72.51	(25) 1388.12 ± 45.24	(28) 1571.75 ± 52.44	(12) 1508.50 ± 104.09
Atropine	(6) 1678.85 ± 96.68	(6) 1635.82 ± 45.35	(6) 1651.87 ± 197.09	(6) 1880.70 ± 122.98
Mecamylamine	(4) 1440.38 ± 140.45	(6) 1866.74 ± 137.47*	(10) 1728.02 ± 101.44	(8) 2072.89 ± 161.73*
Carbachol	(6) 1102.12 ± 98.91	(7) 1233.93 ± 105.60	(8) 1394.26 ± 119.24	(7) 1681.87 ± 132.14
Pilocarpine	(7) 1380.49 ± 79.63	(5) 1175.62 ± 65.91	(6) 1277.02 ± 163.58	(7) 1692.21 ± 128.39

Number of animals in parentheses.
Data are expressed in terms of NIAMDD—Rat FSH-RP-1.
Values are means ± SE.
Significance: *$P < 0.01$ *vs.* sham-implanted animals.

seems to exert some inhibitory effect only on LH release.

Conclusions

It emerges from these data that the basomedial portion of the amygdala possesses several types of neurovegetative receptors, which are involved in the control of the secretion of LH and FSH.

1. α-adrenergic receptors, which seem to exert an inhibitory influence on LH and FSH release;
2. β-adrenergic receptors, which seem to exert an inhibitory influence only on LH release;
3. Cholinergic receptors of the muscarinic type which exert an inhibitory influence on LH release;
4. Cholinergic receptors of the nicotinic type which seem to be important for depressing FSH output.

The results reported here indicate that an additional "station" must be considered when interpreting the effects on gonadotrophin secretion of the systemic administration of drugs which interfere with acetylcholine and catecholamine biosynthesis, action, and catabolism. It is interesting to note that the present results showed that in several instances the secretion of LH diverges from FSH release (*e.g.* intra-amygdalar implants of propranolol increase LH release without affecting FSH release; atropine stimulates LH but not FSH secretion, etc.). These data, like others previously reported [33–39], seem to stress that the central mechanisms which control the secretion of the two gonadotrophins are substantially different. These results are difficult to reconcile with the hypothesis that a single hypothalamic releasing hormone might be responsible for the control of the two gonadotrophins [40]. On the basis of the evidence presented, it may be suggested that the amygdala represents one of the centres involved in the control of the differential secretion of the two gonadotrophins.

II. ROLE OF THE ORGANUM VASCULOSUM LAMINAE TERMINALIS

It has been recently demonstrated that LH-RH is present in several areas of the brain other than the hypothalamus. In particular, this hypothalamic hormone has been found at the level of the circumventricular organs [41]. Among these, the OVLT contains the highest amounts of LH-RH, and seems to be second only to the median eminence [42–44]. It has also been shown that, in adult female rats, castration and/or hypophysectomy bring about the disappearance of LH-RH-containing vesicles from the OVLT [45, 46].

In order to verify whether the presence of large amounts of LH-RH in the OVLT plays a physiological role in the control of gonadotrophin secretion, this structure has been destroyed by radiofrequency lesions in adult cycling and in long term castrated female rats.

For the experiments in normally cycling animals, adult female rats (initial weight 160–170 g) which had shown at least three consecutive regular 4-day oestrous cycles (as evaluated by daily vaginal smears) were subdivided into three groups: normal controls, sham-operated animals, and lesioned animals. Rats to be submitted either to sham-lesion or to OVLT destruction were mounted under pentobarbital anesthesia on a Stoelting stereotaxic instrument. The tip of a thorium electrode, connected with the Grass Lesion Maker Model LM4, was brought to the level of the OVLT (1.8 mm anterior to the bregma, 7.0 mm deep) without administering current in the case of the sham-operated animals and passing a current of 5 mAmp (voltage: 75 V; frequency: 100 kHz) for 10 s in experimental animals. Vaginal smears were taken daily after the operation. The lesioned animals which were in prolonged dioestrous (see below) were killed for blood collection at 16.00 h of the eighth day after the OVLT lesion. Normal, sham-operated controls and the few OVLT-lesioned animals which showed a pro-oestrous or an oestrous type of vaginal smears on the day of sacrifice, were bled from the retro-orbital plexus at 14.00 and at 16.00 h and killed at 18.00 h, in order to follow the pattern of the possible gonadotrophin surge. For the experiments in castrated animals, adult female rats castrated 4 weeks earlier, were subdivided into the 3 groups (controls, sham-operated animals, OVLT-lesioned animals), and sacrificed at 16.00 h, either 5 or 10 days after real or sham surgery, which was performed as described above. In all animals serum LH and FSH were measured using specific radioimmunoassay procedures [29, 30].

Non-castrated females

As shown in Table 5, the destruction of the OVLT exerted a profound influence on oestrous cyclicity. Normal controls and sham-operated animals presented respectively 5.7% and 12.5% of oestrous irregularity. On the contrary, the cycle was disrupted in 72.7% of the animals bearing OVLT lesions. Moreover, the type of alteration recorded was different in controls and sham-operated animals on one side, and in OVLT-lesioned animals on the other. The irregular cycles observed in the two groups of controls were characterized by the abnormal presence of oestrous smears, while the lesioned animals with an altered cycle appeared to have entered a status of prolonged dioestrous. OVLT lesioned animals which were in constant dioestrous (16 out of 22) had non-detectable serum levels of LH; on the contrary, FSH was within normal dioestrous ranges (135.34 ± 34.100 ng/ml). The results obtained in OVLT-lesioned animals which still showed some type of oestrous cyclicity are reported at the bottom of Figs 1 and 2. It is apparent that, in these animals, on the second day of dioestrous, serum LH levels were comparable to those found in the corresponding phase of the cycle in normally cycling and sham-operated animals. A small elevation of serum LH was observed during the afternoon of pro-oestrous, but this was considerably lower

Table 5. Effect of radiofrequency lesions of the *organum vasculosum laminae terminalis* (OVLT) on oestrous cyclicity of adult female rats

Groups	Numbers of animals non cycling/total	Percentage of non cycling animals
Normal controls	4/70	5.7
Sham-operated animals	3/24	12.5
OVLT-lesioned animals	16/22	72.7

than the peak values observed in the two control groups (note the difference in the scales). Serum LH levels were undetectable in OVLT-lesioned animals in the oestrous or dioestrous 1 phase. In the few OVLT-lesioned animals showing some type of oestrous cyclicity, FSH was low but in a physiological range during the second day of dioestrous, was undetectable during the day of pro-oestrous and exhibited an elevation in the afternoon of the day of oestrous. It appears from Figs 1 and 2 that normal controls and sham-operated rats had a pattern of serum LH and FSH levels through the cycle similar to those reported several times in the literature [see 47 for references].

Castrated females

Figures 3 and 4 show the data of the measurements of serum LH and FSH in the different groups of castrated animals. It is possible to see that 5 and 10 days after OVLT lesions the serum levels of the two hormones are identical to those found in castrated controls and in castrated sham-operated animals.

Conclusions

OVLT lesions result in the disruption of regular oestrous cyclicity in the majority of the operated animals, with the appearance of a prolonged dioestrous. In these animals, serum levels of LH are undetectable, while serum FSH is within the normal dioestrous range. In the few OVLT-lesioned animals which exhibited some sort of oestrous cyclicity, serum levels of LH showed a small sub-physiological increase at pro-oestrous; however the rise of serum LH was not accompanied by a parallel increase of serum FSH. A delayed peak of FSH during the day of oestrous was observed in these animals. LH and FSH serum titres of long-term castrated animals do not seem to be affected by OVLT destruction.

It might be concluded from these preliminary results that the OVLT is involved in the control of the "cyclic" but not of the "tonic" release of pituitary gonadotrophins. This interpretation seems to be supported by the following considerations. The OVLT is located in the so-called supraoptic recess of the third ventricle, and is situated anterior to the optic chiasm close to the preoptic area, which many results prove to be involved in the control of the cyclic release of the two gonadotrophins. It is consequently possible that the OVLT lies within the so-called preoptic tubero-infundibular pathway, and that it might be one of the centres of this region responsible for the control of the cyclic release of gonadotrophins. In support to this hypothesis one may recall that all deafferentations performed posteriorly to the optic chiasm seem to leave almost intact the ovulatory process in rodents [48, 49]. Moreover, the LH-RH content of the OVLT has been found to fluctuate during the different phases of the oestrous cycle [50]. Also the electrical activity of this structure shows oestrous-

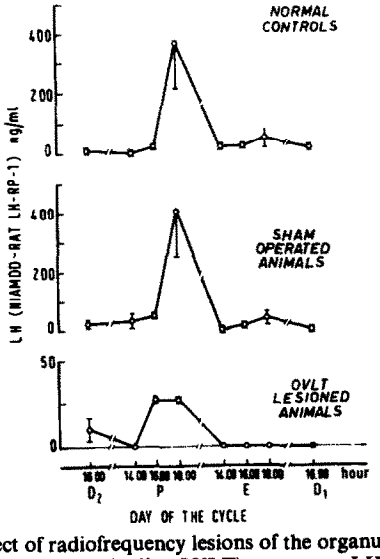


Fig. 1. Effect of radiofrequency lesions of the organum vasculosum laminae terminalis (OVLT) on serum LH levels of adult female rats sacrificed 8 days after brain surgery.

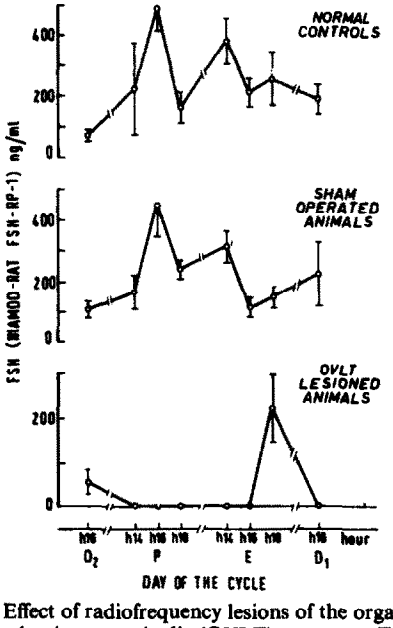


Fig. 2. Effect of radiofrequency lesions of the organum vasculosum laminae terminalis (OVLT) on serum FSH levels of adult female rats sacrificed 8 days after brain surgery.

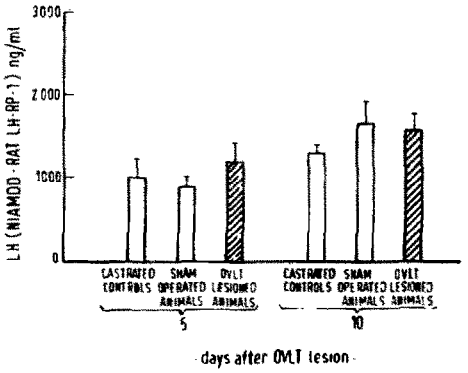


Fig. 3. Effect of radiofrequency lesions of the organum vasculosum laminae terminalis (OVLT) on serum LH levels of adult castrated female rats killed at different time intervals after brain surgery.

linked modifications [51]. Finally, the electrical stimulation of the periventricular area around the OVLT induces gonadotrophin release and ovulation in female rats [52].

The route through which LH-RH contained in the OVLT might reach the median eminence-anterior pituitary unit remains an unanswered question. The OVLT is highly vascularized and rich in tanycytes and is located in direct contact with the cerebrospinal fluid (CSF). Consequently, a transport of OVLT LH-RH toward the median eminence via the CSF cannot be disregarded. Knigge and his group have reported repeatedly that LH-RH is present in the rat CSF, and that its concentrations fluctuate according to the different phases of the oestrous cycle. Moreover LH-RH concentrations in the CSF seem to be modified by ovariectomy and by hypothalamic deafferentation [53–55]. These findings might suggest that LH-RH secreted by the OVLT and other circumventricular organs into the CSF might be picked up by tanycytes in the median eminence region and transported to the pituitary portal vessels [56, 57]. However, it must be recalled that other authors [58, 59] could not confirm under a variety of experimental conditions the presence of LH-RH in the CSF. The finding that, following OVLT lesions, the content of LH-RH decreases in the hypothalamic tissue between the OVLT and the median eminence might suggest also the transport of this principle through a nervous pathway [60].

GENERAL CONCLUSIONS

The data here presented have permitted additional information on the brain mechanisms which control gonadotrophin secretion to be obtained.

First of all the data here reported have confirmed, using a pharmacological approach, that in the female rat the amygdala is involved in the nervous processes which control gonadotrophin secretion. The experimental design selected has made it possible to elucidate that the amygdala (or at least its basomedial region) is not autonomous in exerting such a control since its function seems to depend on noradrenergic and cholinergic inputs arriving from other regions of the brain. Since a large portion of fibres reaching the amygdala originates in the brain stem [10, 26, 61–63], the data may be taken as proof of a complex interplay of three different systems in the neuroendocrine control of gonadotrophin secretion: brain stem-amygdala-hypothalamus (see, Fig. 5). Studies are presently in progress to clarify whether the implantation of the drugs used in the present study in other districts of the amygdala will also result in alterations of gonadotrophin secretion. Experiments have also been planned to elucidate the possible role of serotoninergic and histaminergic inputs arriving to the basomedial and to other zones of the amygdala in the control of LH and FSH release (Piva, Borrell and Martini, in preparation; Mas, Zancan, Piva and Martini, in preparation). Secondly, it has been shown that also the OVLT participates in the control of anterior pituitary gonadotrophins. It is possible (although not proved) that also this structure might be integrated into the brain stem-amygdala-hypothalamic circuit which has just been postulated. In this context, one may recall that amygdala efferents enter the hypothalamus mainly through its rostral part, i.e. through the preoptic area [see 26 for references], which is very close to the OVLT; it is consequently possible, even if this has not been definitely demonstrated, that

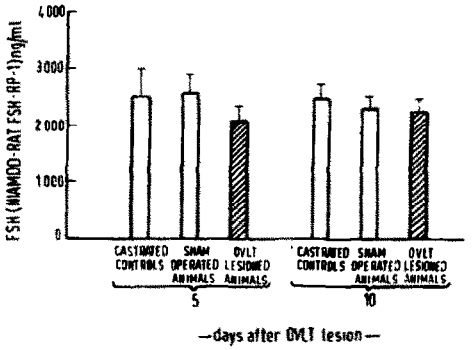
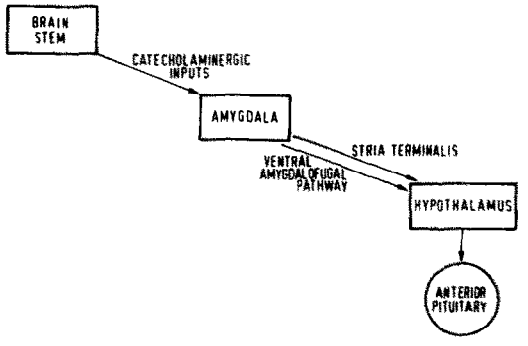


Fig. 4. Effect of radiofrequency lesions of the organum vasculosum laminae terminalis (OVLT) on serum FSH levels of adult female rats killed at different time intervals after brain surgery.



Scheme of the possible interrelationships of different nervous structures in the control of gonadotrophin secretion.

amygdalar fibres might also reach the OVLT. Moreover, the electrical stimulation of the amygdala brings about an activation of the preoptic area [64], which might be transferred to the OVLT, since it has been shown that the medial preoptic nucleus sends efferents to the periventricular area, which includes the OVLT [65, 66].

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