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THE ROLE OF THE ANTERIOR REGION OF THE MEDIAL BASAL HYPOTHALAMUS IN THE CONTROL OF OVULATION AND SEXUAL BEHAVIOR IN SHEEP

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Abstract. Cycling ewes with lesions placed in the anterior region of medial basal hypothalamus (MBH) showed no estrous behavior, but they all had ovulations and cyclical corpora lutea at physiological terms. Participation of the anterior region of MBH in control of sexual behavior was also supported by the experiments on castrated sheep implanted with 17-β-estradiol into the area determined previously by lesions. Some of the lesioned ewes, which were observed throughout the subsequent anestrous period, showed continued cyclical ovulations with development of cyclical corpora lutea. This phenomenon seems to suggest that in the anterior region of MBH a center of inhibitory action over the liberation of gonadotrophin releasing factors exists.

INTRODUCTION

In contrast to the confirmed role of the ventromedial hypothalamus in the control of gonadotrophin secretion and ovarian function, consisting in production and liberation of gonadotrophin releasing factors, participation of the anterior region of the hypothalamus in this control in the other species except the rat is still unclear (1,8).

Although in our previous papers on the role of the hypothalamus in the control of gonadotrophin secretion and ovulation in sheep some data concerning participation of the anterior hypothalamus in these processes were presented, this function has not been yet entirely understood. For instance, we showed that lesions of the anterior hypohalamic nuclei (suprachiasmatic, anterior, or supraoptic) do not disturb either ovulation or estrous behavior (4). On the other hand, electrostimulation of some of these nuclei (suprachiasmatic, anterior and lateral nuclei) induced ovula-

tion in anestrous ewes (5). Furthermore, implantation of crystalline 17-β-estradiol into the anterior hypothalamic area on the 6-7th day of estrous cycle evoked behavioral estrus on the 2-3rd day after the implantation only in these animals which did not have corpora lutea. However, in all the implanted ewes ovulations appeared at the next and subsequent physiological terms of the estrous cycles (4).

Since these results (obtained by use of various methods) had not fully elucidated the role of the anterior hypothalamus in the control of reproductive activity and were difficult to interpretation, further investigations on this problem were undertaken. Special attention has been paid to the role of the anterior region of the medial basal hypothalamus (MBH), localized caudally to the optic chiasm. The results of the previous experiments seemed to indicate that lesions of the anterior regions of MBH in sheep do not abolish ovulation, although they did supressed estrous behavior in the cycling ewes. Similar observations (no estrous but regular vaginal cycles) were also made by Radford (6) on few lesioned ewes.

MATERIALS AND METHODS

In the experiments on the role of the anterior region of MBH in the control of estrous cycle and ovulation carried out on six Merino ewes with regular estrous cycles, electrolytic lesions of this area were made. Simultaneously, lesions of MBH were carried out in the area of median eminence (1 sheep) dorsal medial hypothalamus (1 sheep) and anterior hypothalamic area (1 sheep). These three recently mentioned lesions have been made for control and also in order to support our previous suggestions concerning involvement of these areas of the hpothalamus in the regulation of ovulation in sheep (4).

The experiments on the participation of the anterior region of MBH in the control of estrous behavior were carried out on 17 castrated ewes by applying 17-β-estradiol implantations.

Lesions. Electrolytic lesions of hypothalamic areas were made bilaterally using a direct current of 5 ma for 1.5 min; the hypothalamic electrode as an anode and a second electrode (cathode) indwelt into the opened wound at the cranium. The electrodes were made of stainless steel wire of 0.3 mm bore and insulated in a glass capillary except for the terminal 1 mm. The implantation of the electrodes or the hormone into the hypothalamic areas was performed by means of stereotaxic technique described by Traczyk and Przekop (9) and on the basis of stereotaxic atlas of sheep hypothalamus (10).

Implantation of 17-β-estradiol. At first some preliminary experiments with estradiol implantations were carried out to define strictly the local

action of the implaned hormone. For the implantations pure 17-β-estradiol or 10% 17-β-estradiol in cholesterol were used. Pure estradiol or the mixture of estradiol in cholesterol were heated to the melting point and a tiny droplet was picked up at the tip of stainless steel tubing, care being taken to insure that some of the droplet was drawn into its lumen; the lumen of the tubing was 0.4 mm. Using this technique the tubings with pure 17-β-estradiol or with 10% 17-β-estradiol in cholesterol were prepared. According to this procedure the tubings contained hormone in their barrels as well as at their tips.

The third kind of the tubings was prepared in a little different way; the tips of the tubings with melted, pure estradiol were carefully scraped and cleaned with ethyl alcohol so that the hormone was inserted only in the barrels. Each kind of the tubings was implanted bilaterally into the area of mammillary bodies of castrated ewes and records of the occurrence of estrous behavior were kept. It should be noted that, according to our previous experiments (in which lesions were used), mammillary bodies in ewes are involved neither in the control of ovulation nor sexual behavior. Table II shows that only the tubings containing pure estradiol in their barrels could be considered as eliciting local action after the implantation, while the other kinds of tubings did not give this certainty; therefore, this kind of tubings had been used for implanations into the anterior region of MBH and into the anterior hypothalamic area.

Determination of reproductive activity and estrous behavior. The ewes were run with a ram and daily record of the occurrence of estrus or estrous behavior was kept. Function of ovaries (growth of Graafian follicles, ovulation and development of corpora lutea) was examined after laparotomy. To avoid too frequent laparotomies vaginal smears as indicative of occurrence of ovulation were taken daily. The virtual absence of leucocytes and the presence of cornified cells, regardless of the presence of other cell types in the smears had been accepted as an indication of estrus and ovulation. This acceptance was based on the experiments of Robinson and Moore (7). All lesioned ewes were observed throughout one breeding season; additionally three of them were examined throughout the subsequent quiescent sexual period. The castrated and implanted with 17-β-estradiol ewes were observed for behavioral estrus throughout 10-12 days after the implantation.

Histological examination of hypothalamic areas. At the end of the observations the animals were killed. Serial sections of the hypothalamus, 15 μ thick, made and stained according to the method of Klüver and Barrera (3) were used to reconstruct the position of lesions or estradiol implantations. The reconstructions were designed and made on the basis of stereotaxic atlas of sheep (10).

RESULTS

The effects of lesions of the hypothalamus. Table I demonstrates that all six animals with lesions placed in the anterior region of MBH at the coordinates 33.06-34.56 (in reference to frontal coordinates, see Fig. 1-4) manifested no estrous behavior while they all showed ovulations and cyclical corpora lutea at physiological terms. Furthermore, three out of

TABLE I

Locations of lesions in the hypothalamus and their effect on estrous cycles and mating behavior in sheep

Ewe	Locations of lesions in reference to frontal coordinates[a]							Reaction to lesion[b]		Period of observation (in months)
	31.56	32.12	33.06	33.81	34.56	35.12	36.04	Ovulation	Mating behavior	
166			vm	vm	vm			+	—	10
626			vm	vm	vm			+	—	5
448				vm	vm			+	—	5
33			vm	vm				+	—	5
38				vm	vm	vm		+	—	12
33/71				vm	vm	vm		+	—	10
28						ns	ns	+	+	5
20	in	in	in					—	—	5
32	pp, dm	pp, dm	pp, dm					+	+	5

a Abbreviations of lesioned nuclei: vm, ventromedial; ns, suprachiasmatic; in, infundibular; pp, periventricular; dm, dorsomedial nuclei.

b +,occurred; —, did not occur.

them (sheep 166, 38, 33/71), which were not slaughtered at the end of breeding season but observed also throughout the subsequent anestrous period, showed continued cyclical ovulations with development of cyclical corpora lutea. The other ewes out of this group, e.g., with lesion of the anterior hypothalamic area (sheep 28) or with lesion of dorsal medial hypothalamus (sheep 32) showed physiological estrous cycles; while in sheep with lesion in the area of infundibular nuclei (sheep 20) estrous cycles completely disappeared.

The effects of estradiol implantation in castrated ewes. Pure 17-β-estradiol as well as the hormone with cholesterol implanted into the mammillary bodies in the tubings containing the hormone melted at their tips in-

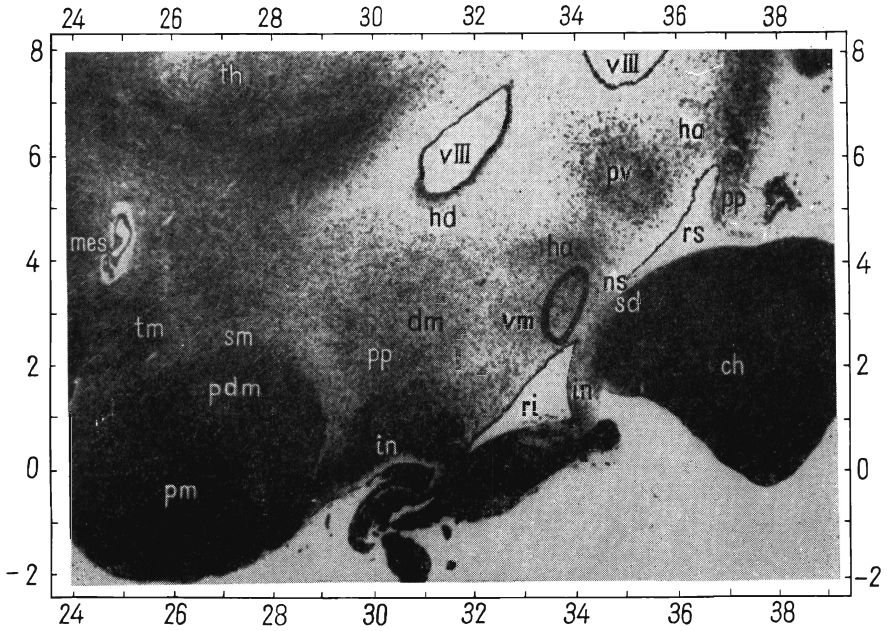

Fig. 1. Sagittal section of the sheep hypothalamus, plane 0.72; area designed in the anterior region of MBH encompasses location of lesions in sheep 166, 626, 448, 33, 38 and 33/71. Abbreviations for this and subsequent Figures: ch, chiasma opticum; ci, capsula interna; dm, nucleus dorsomedialis; fx, fornix; ha, nucleus hypothalamicus anterior; hc, hypophysis cerebri; hd, area dorsalis hypothalami; hl, area hypothalamica lateralis; in, nucleus infundibularis; mes, mesencephalon; np, nucleus hypothalamicus parvocellularis; ns, nucleus suprachiasmaticus; pdm, pars dorsomedialis nuclei mammillaris medialis; pf, nucleus perifornicalis; ph, pars horizontalis nuclei paraventricularis; pm, pars medialis nuclei mammillaris medialis; pp, nucleus periventricularis; pv, nucleus paraventricularis; ri, recessus infundibularis; rs, recessus supraopticus; sd, nucleus supraopticus diffusus; sm, nucleus supramammillaris, sop, pars posterior nuclei supraoptici; th, thalamus; tm, nucleus tuberomammillaris; to, tractus opticus; vm, nucleus ventromedialis; vIII, ventriculus tertius.

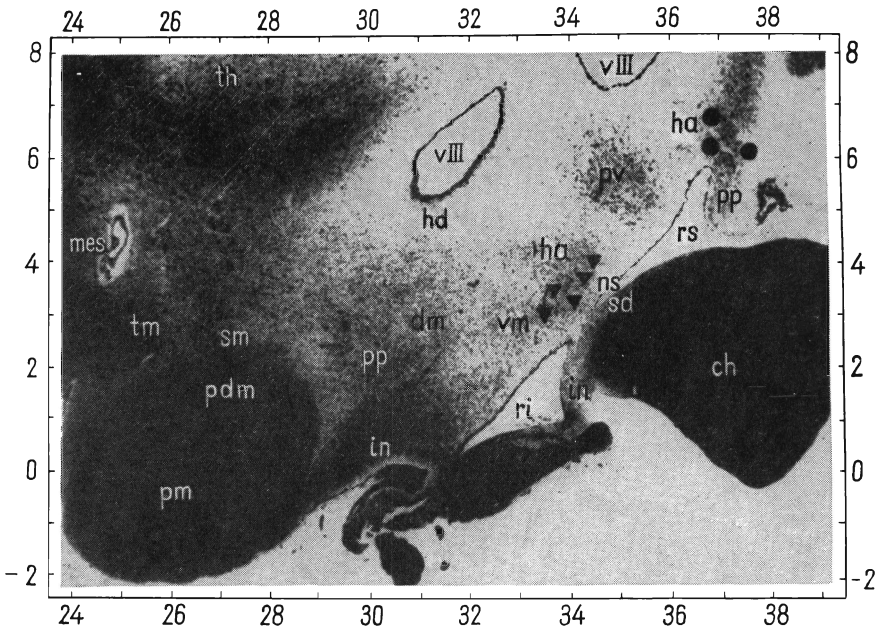

Fig. 5. Sagittal section of the sheep hypothalamus, plane 0.72; the sites of estradiol implants in ovariectomized sheep: ▲ denote the ewes which showed behavioral estrus (sheep 155, 216, 264, 87, 74), while ● the ewes which showed no reaction after estradiol implantation (sheep 67, 241, 313). Abbreviations as in Fig. 1.

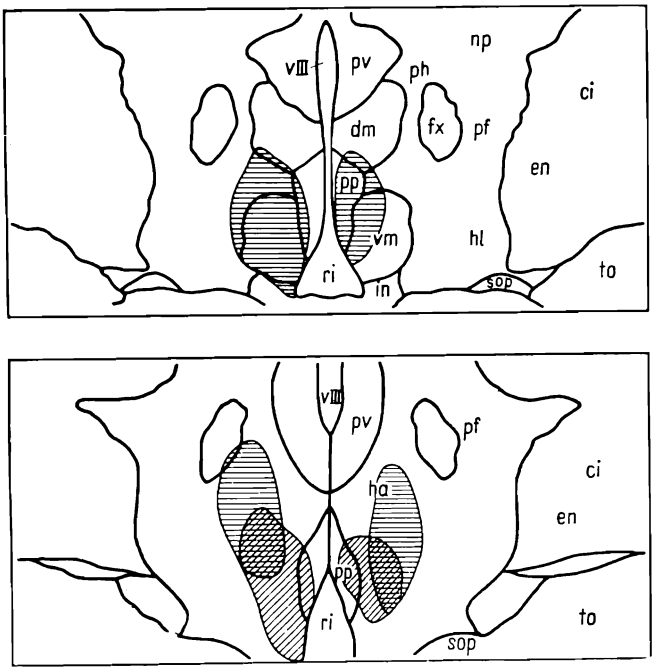

Fig. 2. Sites of lesions in sheep 166 (horizontal stripes) and sheep 626 (slantwise stripes) in frontal plane 33.06 (above) and 34.56 (below). Abbreviations as in Fig. 1.

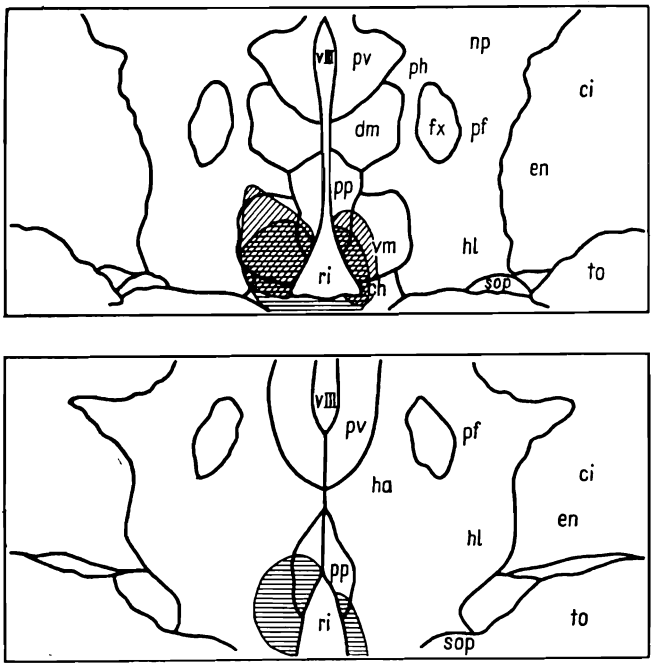

Fig. 3. Sites of lesions in sheep 33 (slantwise stripes) and sheep 448 (horizontal stripes) in frontal plane 33.06 (above) and 33.81 (below). Abbreviations as in Fig. 1.

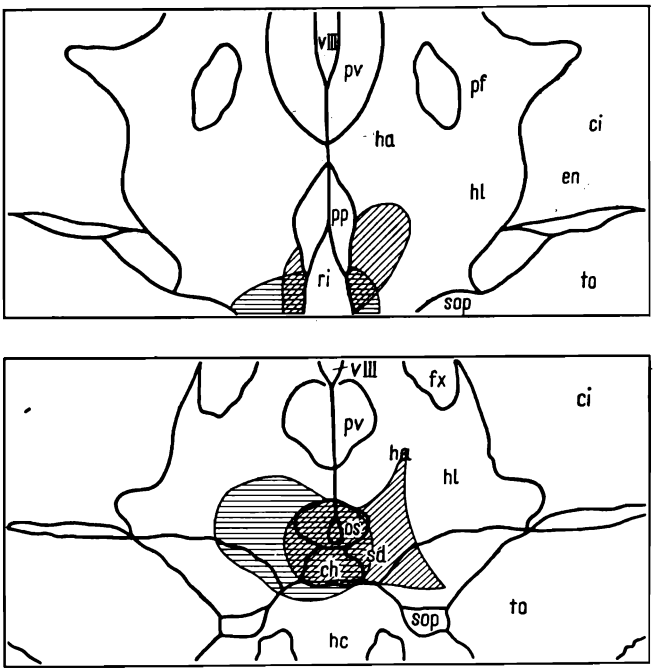

Fig. 4. Sites of lesions in sheep 38 (slantwise stripes) and sheep 33/71 (horizontal stripes) in frontal plane 33.81 (above) and 34.56 (below). Abbreviations as in Fig. 1.

TABLE II

Effect of 17-β-estradiol, implanted in different forms into the mammillary bodies, on sexual behavior (behavioral estrus) in ovariectomized ewes

Number of implanted ewes	Form in which estradiol was implanted	Reaction of animals to implantation
3	pure estradiol melted at the tip of the tubing	behavioral and vaginal estrus on the 2-5 day[a] after implantation
3	a mixture of estradiol + cholesterol (1:10) melted at the tip of the tubing	behavioral and vaginal estrus on the 2-5 day after implantation
3	pure estradiol melted only into the barrel of the tubing	neither behavioral nor vaginal estrus after implantation

a The day of implantation was a 0 day.

duced behavioral and vaginal estrus. Implantations of 17-β-estradiol into the same formation and areas in the tubings, containing the hormone only in their barrels, induced neither behavioral nor vaginal estrus (Table II). Implantations of the recently mentioned tubings into the anterior hypothalamic area also did not elicit any reaction. On the contrary these tubings implanted into the anterior region of MBH induced mating behavior (behavioral estrus) but not vaginal estrus (see Table III and Fig. 5-7).

TABLE III

Effect 17-β-estradiol implanted into the anterior region of the medial basal hypothalamus (MBH) and other parts of the hypothalamus on sexual behavior (behavioral estrus) in ovariectomized ewes

Ewe	Location of implantations in reference to frontal coordinates[a]					Reaction to implantation
	33.81	35.12	36.04	36.44	37.19	
155		ns, sd				behavioral estrus 24 hr
216		ns, ha				behavioral estrus 24 hr
264		ns, ha				behavioral estrus 24 hr
87	vm, vm					behavioral estrus 24 hr
74	vm, vm					behavioral estrus 24 hr
67				ha, ha		no reaction
241				ha, ha		no recation
313					ap, ap	no reaction

a Abbreviations of the nuclei and areas: ap, area preoptica; ha, hypothalamic anterior; ns, suprachiasmatic; sd, supraoptic diffused; vm, ventromedial nuclei.

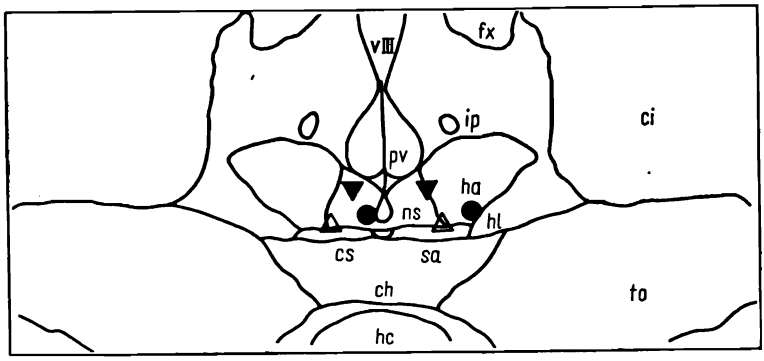

Fig. 6. Sites of estradiol implants determined by reference to frontal plane 35.00: △, sheep 155; ●, sheep 216; ▲, sheep 264. Abbreviations as in Fig. 1.

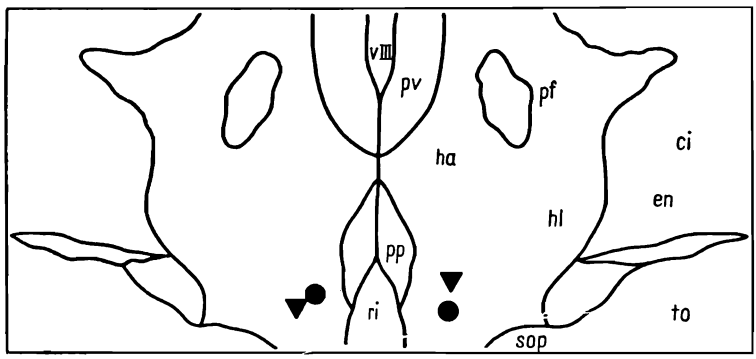

Fig. 7. Sites of estradiol implants determined by reference to frontal plane 33.81: ▲, sheep 87; ●, sheep 74. Abbreviations as in Fig. 1.

DISCUSSION

Clear cut effect of lesions of the anterior region of MBH, localized between coordinates 33.06 and 34.56 (in reference to frontal coordinates), resulting in loss of mating behavior in sheep indicates that this region is associated with the control of estrous behavior in this animal. There is a possibility that the areas of periventricular nuclei, adjacent laterally to this region are also involved in this control. The participation of the described region in the regulation of estrous behavior is in agreement with the results obtained using estradiol implantation in castrated ewes. Since the hormone implanted in the tubings, used in these experiments, was acting only locally, therefore, the behavioral estrus induced in castrated animals after the implantation into the anterior region of MBH may be taken upon as the effect of local action of 17-β-estradiol on the neurons of this region. According to the effects of lesions the area involved in this control is located more posteriorly in relation to the area determined in the experiments with implantations of 17-β-estradiol.

However, the area estimated by means of lesions seems to be more reliable than that designed with implantations of the hormone, as an error may be the result of diffusion of the hormone.

Thus, the results obtained using both methods are in complete agreement.

The negative behavioral effect of estradiol implantations into the anterior hypothalamic area observed in the present study is different from that described in our previous experiments (4). As it was mentioned above, the implantations of a crystalline estradiol into the anterior hypothalamic area in cycling ewes on the 6-7th day of estrous cycle resulted in the

occurrence of behavioral estrus only in a few animals without corpora lutea. It seems very likely, that greater solubility of the cryctalline estradiol, than that of melted one, augmented its diffusion and made possible to spread the hormone down till the anterior region of MBH, or induce behavioral estrus on general way of blood circulation; while the diffusion of the melted hormone, inserted only in the barrel of the tubing, is slown down in the time and space and so the action of the hormone is only local.

Anterior region of MBH seems to be also involved in the regulation of liberation of gonadotrophin releasing factors. This assumption is based on the fact that three ewes with lesions of the described region continued cyclical ovulations at physiological terms throughout the whole subsequent anestrous period. This phenomenon seems to suggest that there is a center in the anterior region of MBH which exerts inhibitory action over the liberation of gonadotrophin releasing factors produced by ventromedial region of this formation (2, 5). Since, this phenomenon of continued ovulations, during anestrous period, has been observed only in few cases further experiments are needed for its support.

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