The neuroanatomical search for sexual dimorphism

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THE NEUROANATOMICAL SEARCH FOR SEXUAL DIMORPHISM

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Summary

In the rat preoptic area, there is a region of neuropil which shows anatomical differences between males and females. Destruction of this area does not prevent ovulation. Destruction of the suprachiasmatic nuclei prevents spontaneous cyclic ovulation in female rats, but ovulation can be induced by mating in such animals. The rats with suprachiasmatic lesions also showed major disturbances in the normal diurnal timing of many bodily functions. The ovulatory disturbance caused by suprachiasmatic lesions may be due to a defect in the normal timing mechanism for the facilitatory action of progesterone on LH secretion.

Zusammenfassung

Im Bereich der Area praeoptica der Ratte lassen sich morphologische Unterschiede zwischen den beiden Geschlechtern erkennen. Die Zerstörung dieses Gebietes verhindert den Ovulationsvorgang nicht. Bilaterale Zerstörung der suprachiasmatischen Kerne blockiert die spontane zyklische Ovulation bei weiblichen Ratten, jedoch kann die Reflexovulation beim Kopulationsakt noch ausgelöst werden. Ratten mit suprachiasmatischen Läsionen zeigen eine merkliche Störung der diurnalen Rhythmizität mancher Organfunktionen. Die durch suprachiasmatische Läsion erzeugte Ovulationshemmung könnte durch Ausfall des Zeitgebermechanismus bedingt sein, welcher normalerweise für die Auslösung der fördernden Wirkung des Progesterons auf die LH-Sekretion verantwortlich ist.

Introduction

Many of the functions of the central nervous system show quantitative or qualitative differences between the sexes: this is described as "sexual dimorphism". By no means all sexually dimorphic functions are involved in reproduction. Nonetheless, one of the simplest and most

frequently studied aspects of sexually dimorphic function is the ability of the brain to maintain the surges of pituitary gonadotrophic hormones required for regular cyclic ovulation in the female laboratory rat. In the intact rat there are many factors outside the nervous system which have a bearing on the final event of ovulation, and of course the presence of the gonads adds an important non-neuronal difference between males and females. To simplify the experimental approach, and at the same time to focus the observations more clearly on the nervous system, it is possible to use gonadectomised rats, and to examine the plasma levels of gonadotrophic hormones (luteinising hormone and follicle stimulating hormone) by means of radioimmunoassay. One of the most elegant paradigms is that developed by TALEISNIK and co-workers (CALIGARIS, ASTRADA and TALEISNIK, 1968) and basically involves the following: Rats are gonadectomised and their hormone levels allowed to equilibrate for at least two weeks; at this stage, the plasma levels of luteinising hormone (LH) are much higher than normal, and also do not show the four day periodic fluctuations characteristic of the intact female. At this point a single injection of oestrogen causes a depression of LH levels. Three days later, a single injection of progesterone causes a pronounced surge of LH secretion. In many ways, the induction of this surge of LH mimics the normal preovulatory surge of LH in the intact cyclic female, and involves many of the mechanisms (e.g. the steroid regulatory effects on the central nervous system) which in the intact animal are involved in ovulation. The progesterone induced LH surge in the oestrogen primed gonadectomised rat has two important properties. The first of these is that it occurs in females but not in males; the second is that it can only be induced at a fixed time of day (TALEISNIK, CALIGARIS and ASTRADA, 1971). Thus the surge shows both sexual dimorphism and a diurnal sensitivity change, both of which are also properties of the normal preovulatory surge of LH in the intact female rat.

Using this "laboratory model" of the normal rat ovulatory surge of LH, we have carried out a series of experiments to attempt to determine which parts of the central nervous system may be involved. For the purposes of this article I will refer to two series of experiments: one in which lesions were placed in a specific part of the preoptic area (POA), and one in which the suprachiasmatic nuclei (SCN) were destroyed.

Studies on the preoptic area

In our first series of experiments we started with the hypothesis that if the brain exhibits a sexually dimorphic function, and if we accept the general view that brain structure is related to function, then we might expect to find an area of the brain whose structure was diffe-

rent in the two sexes. Data was already available from a large number of experiments with different techniques and this suggested that the part of the rat brain most likely to be involved in the initiation of ovulation is the POA (e.g. see GORSKI, 1966 for a review). Thus, it had been reported that cyclic ovulation was prevented by lesions in this region or by cuts separating this region from the basal hypothalamus. Further, surgical isolation of the hypothalamus from the rest of the CNS (but leaving it in contact with the median eminence and pituitary) prevents ovulation, but if such cuts were extended rostrally so as to include the POA with the hypothalamic island, then ovulation was reported to occur (HALASZ, 1969). The lowest threshholds for induction of ovulation by electrochemical stimulation were found when the stimulating electrodes were in the POA, and the effect was prevented by cuts separating this area from the mediobasal hypothalamus (TEJASEN and EVERETT, 1967). Such observations have led to the view that somewhere in the region of the POA is located a neural mechanism which is essential for the induction of ovulation and for the facilitatory action of progesterone on LH secretion in the oestrogen primed female rat (TALEISNIK, VELASCO and ASTRADA, 1970).

While this area was regarded as essential for ovulation, several other parts of the brain, while not themeselves essential, have been reported to be able to modulate LH secretion. One of the most frequently mentioned was the amygdala (LAWTON and SAWYER, 1970), an area which itself has major fibre projections (the stria terminalis and the ventral amygdalofugal pathway) to the medial and lateral parts of the POA respectively (COWAN, RAISMAN and POWELL, 1965). VELASCO and RALEISNIK (1969) showed that stimulation of the amygdala could induce ovulation in the female rat, and the effect was prevented by section of the stria terminalis, and ARAI (1971) showed that amygdaloid stimulation caused ovulation in normal female rats but not in neonatally androgenized females or castrated males with ovarian transplants. Further evidence in favour of a role for the amygdala-stria terminalis were the autoradiographic observations (PFAFF and KEINER, 1973; STUMPF and SAR, 1971) that both the amygdaloid neurones giving origin to the strial fibres, and the neurones of the parts of the POA to which they project, concentrate exogenously administered oestrogen: it has been suggested that this may be in some way related to a role in the normal control of LH release by ovarian steroids.

Therefore we felt encouraged to make an anatomical study in rats of that part of the POA to which the amygdala projects. Specifically, by using a quantitative electron microscopic analysis of the numbers of various synaptic types, we sought to investigate the possibility that there may be an anatomical difference between male and female rat brains. Our obser-

vations (RAISMAN and FIELD, 1973) were that there was indeed a difference. Of the various categories of synapses counted, we found that the number of axo-spinous synapses per unit volume of neuropil was twice as high in females as in males. This sexual difference was found specifically in that area (the mid-dorsal part of the POA) in which the stria terminalis fibres make synaptic contacts. While at a pratical level it is not possible to carry out such observations on very many different areas of neuropil, we were able to establish that comparable sexual dimorphism did not occur in some of the immediately adjacent parts of the preoptic area, nor in that part of the ventromedial hypothalamic nucleus which also receives projections from the amygdala through the stria terminalis, nor in the lateral septal nucleus. Moreover, within the sexually dimorphic part of the preoptic neuropil, the sex difference was restricted to spine synapses - synapses on dendritic shafts were equally numerous in both sexes, and even among the axo-spinous synapses, the use of orthograde terminal degeneration techniques showed that the sexually dimorphic incidence was restricted to synapses whose axons were not in the stria terminalis.

Clearly at this stage all the observations pointed to the fact that the sexually dimorphic neuropil might be the anatomical substrate for the ovulatory mechanism. There remained only the final, crucial experiment. In regularly cycling female rats we destroyed the entire region of the mid-dorsal POA (including the whole preoptic distribution field of the stria terminalis) bilaterally (BROWN-GRANT et al., 1977). The effect of such lesions was unambiguous. After an initial temporary disturbance of cyclicity (usually a pseudopregnancy) the animals resumed regular spontaneous cyclic ovulation. Further follow up of reproductive functions revealed that mating, pregnancy, litter raising and steroid effects on LH all showed normal function. Not only did such lesions not prevent ovulation in normal females, but they also did not have the converse effect - i.e. they did not induce ovulation in neonatally androgen sterilised females.

Studies on the suprachiasmatic nuclei

Such observations do not of course mean that the sexual dimorphism in the POA has no relation to ovulation, and certainly do not exclude a role in other sexually dimorphic functions. However, since we had been primarily interested in the neural control of ovulation and LH release, we now set out to try to find which area of the brain was, in our rats, essential for spontaneous cyclic ovulation (BROWN-GRANT and RAISMAN, 1977). Here the results were clear. In agreement with the previous data of BARRACLOUGH, YRARRAZAVAL and HATTON (1964) analysis of the lesion sites in a series of rats with small bilateral lesion of va-

rious parts of the POA and anterior hypothalamus showed that the smallest area whose bilateral destruction prevented spontaneous ovulation was the area of the suprachiasmatic nuclei (SCN). In 69 rats with destruction of more than 75 % of the SCN, 56 were anovulatory, 9 showed major disturbances of ovulation, and 4 continued to ovulate. Of 41 rats where less than 25 % of the SCN were destroyed, 39 continued to ovulate regularly. In neither group was the presence or absence of ovulation correlated with damage to adjacent areas of tissue. At first sight, these observations might be taken to indicate that the sexually dimorphic mechanism resides in the SCN and not the POA. However, further analysis (BROWN- GRANT and RAISMAN, 1977) of the syndrome induced by SCN lesions precludes any such simple interpretation. If the animals rendered anovulatory by SCN lesions were allowed access to males, they mated readily, and such mating induced ovulation, followed in a number of cases by normal pregnancy, parturition and litter nursing. Thus, SCN lesions were not able to prevent induction of an effective preovulatory surge of LH provided the stimulus was adequate.

In animals rendered anovulatory by SCN lesions, plasma LH levels were examined after gonadectomy, oestrogen priming and subsequent progesterone administration. The results were puzzling. A number of the animals showed a normal LH surge, whereas the majority showed no effect.

At about this time, there became available two sets of data having relevance to these results. The first was the finding by TALEISNIK et al. (1971) that the LH surge induced by progesterone in gonadectomised oestrogen primed female rats has a diurnal rhythm. The second was the series of findings (IBUKA and KAWAMURA, 1975; MOORE and EICHLER, 1972; MOORE and KLEIN, 1974; STEPHAN and ZUCKER, 1972) that rats with lesions of the SCN showed marked disturbances in the normal diurnal timing of several functions (running, feeding, drinking, plasma corticosterone levels, pineal N-acetyl transferase activity, paradoxical and slow wave sleep). Basically the animals with lesions of the SCN showed a loss of the normal nocturnal preference for activity seen in rats raised and kept in a normal laboratory light dark cycle. These observations also seem to fit with the recent demonstration that the SCN receive a direct fibre projection from the retina (MOORE and LENN, 1972; HENDRICKSON, WAGONER and COWAN, 1972).

Combining this information with our observations of the incidence of prevention of spontaneous ovulation, we were able to show (RAISMAN and BROWN-GRANT, 1977) that in our rats suprachiasmatic lesions which blocked ovulation also caused marked disturbances of the normal nocturnal preference for eating, drinking and voluntary wheel funning, as well as

abolishing the normal diurnal variations in plasma corticosterone and pineal N-acetyl transferase activity. Conversely, in the animals where the lesions did not block ovulation, the other functions also tended to be normal. Analysis of the data from our individual rats subjected to these multiple testing procedures led us to describe these defects as a "suprachiasmatic syndrome": destruction of more than 75 % of the SCN abolishes spontaneous ovulation and causes major disturbances in the normal diurnal periodicity of several non-reproductive functions (see also STETSON and WATSON-WHITMYRE, 1976).

Such observations lead to a suggested explanation of the puzzling data on LH control found in our SCN lesioned rats. Since in the normal rat the progesterone facilitation of LH release occurs specifically in the afternoon, and since lesions of the SCN cause a disturbance in the diurnal timing of a large number of functions, the ovulation-preventing effect of such lesions could be due not to their abolition of the ability of the brain to show progesterone facilitation of LH release, but to their disruption of the normal timing of such a mechanism, so that the critical period for sensitivity to progesterone varied at random throughout the 24 hour period. Such random variation could explain why some of the rats rendered anovulatory by SCN lesions could ovulate after mating, and furthermore why some of the animals showed a progesterone induced surge of LH.

Conclusions

Returning to our original points, we can now summarise the chain of hypotheses which have been tested.

1. The normal female rat brain can mediate a progesterone induced surge of LH; the male rat brain cannot.
2. The neural mechanism for the induction of LH secretion can only be activated by progesterone at a fixed time in the 24 hour light-dark cycle.
3. That part of the POA receiving projections from the amygdala through the stria terminalis contains synapses whose incidence is sexually dimorphic.
4. Destruction of this sexually dimorphic part of the POA causes no obvious permanent disability in ovulation or any other reproductive function in female rats.
5. Destruction of the SCN prevents regular cyclic ovulation in female rats. This effect is not due to abolition of the ability to ovulate (e.g. after mating), but it is associated with marked disturbances in the diurnal periodicity of many non-reproductive functions.

At the moment therefore we must conclude that the sexually dimorphic mechanism for LH control in female rats uses a neural substrate different from that used for the maintenance of

the diurnal periodicity of LH release. Diurnal periodicity is dependent on the integrity of the SCN. Whether or not the sexually dimorphic neuropil of the POA has any role in the maintenance of the sexually dimorphic control of LH release remains unclear.

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