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EFFECTS OF ELECTRICAL STIMULATION AND OF DIRECT CURRENT AND HIGH FREQUENCY LESIONS OF THE HYPOTHALAMUS ON GONADOTROPHIN RELEASE AND PUBERTY IN FEMALE RATS

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SUMMARY

The arcuate nucleus region of the hypothalamus of twenty-eight day old female rats was subjected to either electrical stimulation (AC), direct current (DC) lesions, high frequency (HF) lesions or to sham-procedures. Gonadotrophin levels were measured at various times after treatment. At 0-30 mn after treatment a significant rise in LH level was seen in AC, DC and HF rats, which rise was significantly higher in DC and HF rats than in AC rats. At 3 hours after treatment no differences in LH levels were found between the various groups ; FSH levels tended to be higher in stimulated and lesioned rats, but this reached significance for HF rats only. At 2 days after treatment LH as well as FSH levels were not different in experimental and control groups with the exception of lower FSH levels in the AC group. AC rats uniformly showed advancement of vaginal opening (VO), accompanied by (first) ovulation ; occurrence of these phenomena was much more variable in DC and HF rats. Measurements of gonadotrophin levels at 17.00 h on the day preceding the day of VO demonstrated peak levels of both LH and FSH in all control and AC rats. For DC and HF rats high levels of LH and FSH were encountered in those animals that had ovulated on the day of VO.

It is concluded that in the immature rat hypothalamic lesions are effective via stimulation of gonadotrophin release. Advanced first ovulation in lesioned and stimulated rats is preceded by gonadotrophin surges comparable to those at spontaneous first ovulation, while, at least under experimental conditions, VO may occur without ovulatory gonadotrophin release and ovulation.

INTRODUCTION

In immature rats interference with brain function by means of either direct current (DC) lesions, high frequency (HF) lesions or purely electrical stimulation with alternating current (AC) of the hypothalamus may advance vaginal opening (VO) and first ovulation (DONOVAN and VAN DER WERFF TEN BOSCH, 1959 ;

SCHIAVI, 1964; MEIJS-ROELOFS and MOLL, 1972; MEIJS-ROELOFS, 1972). Little is known about the hormonal chain of events which to all probability connects intervention in brain function with advancement of vaginal opening and first ovulation. This may (at least in part) explain the discrepancies in interpretation of results obtained in lesion studies: stimulation *vs* removal of inhibition as modus operandi of lesions? (see e.g. SHERWOOD and TIMIRAS, 1974; RUF, YOUNGLAI and HOLMES, 1974).

In the adult rat an acute stimulatory effect on gonadotrophin release of DC lesions (in most cases referred to as electrochemical stimulation) is well-known (see e.g. BISHOP *et al.*, 1972; CLEMENS *et al.*, 1971; KALRA *et al.*, 1971). An acute increase in ovarian oestrogen and progestin content after DC lesions in immature rats has been reported (RUF, YOUNGLAI and HOLMES, 1974). In personal studies we found a marginal elevation of FSH levels and a clear increase in LH levels within 3 hours after AC stimulation of the hypothalamus (MEIJS-ROELOFS and UILENBROEK, 1973).

The present work deals with changes in gonadotrophin levels at various times after both DC lesions, HF lesions and AC stimulation. It was hoped that comparison of these three methods of manipulation of brain function might provide a better insight in their mode of action and usefulness in research on puberty. Of particular interest seemed the question whether lesions act via removal of inhibition or by stimulation of hypothalamic drive of pituitary-ovarian function.

MATERIAL AND METHODS

Female rats of the R-Amsterdam strain, a *Wistar* substrain, were used. They were weaned at 22 days of age and kept in a controlled temperature of 22-25°C with lights on from 05.00-19.00 h. They received standard dry pellets and tap water *ad libitum*. At 28 days of age litter-mates were divided over the various experimental and control groups; body weights were 48-60 g at this age. Rats were subjected to either alternating current (AC) stimulation, direct current (DC) lesions, high frequency (HF) lesions or to sham procedures. Blood samples were taken at various times after operation for determination of serum gonadotrophin concentrations. All rats were weighed and checked for vaginal opening (VO) daily. From the day of VO vaginal smears were taken during at least 20 days; animals were killed at the vaginal metoestrous stage that followed the first day of oestrus after 20 days of smearing. If vaginal cyclicity was irregular or absent, rats were killed 30 days after VO. In one separate experiment blood samples were taken on the day preceding the expected day of VO as judged by the appearance of the vaginal area (see MEIJS-ROELOFS *et al.*, in press). In this experiment rats were killed at first metoestrus or on the day following that of VO, if a metoestrous smear was not then found. Some intact rats were bled at 33 days of age to provide an additional control group.

Brains and ovaries of operated rats were prepared for histological study of stimulation and lesion sites and for determination of presence or absence of corpora lutea.

Blood for gonadotrophin determination was obtained under light ether anaesthesia by puncture of the ophthalmic venous plexus and was allowed to clot overnight in a refrigerator before centrifugation. Serum samples were stored at — 20°C. Serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations were estimated by radioimmunoassay as described earlier (WELSCHEN *et al.*, 1975).

Stimulation and lesion procedures were performed stereotaxically under ether anaesthesia. Electrodes were inserted bilaterally 0.5 mm from the midsagittal plane and aimed at the region of the arcuate nucleus, using the stereotaxic atlas of the developing rat brain of SHERWOOD and TIMIRAS (1970). For AC stimulation bipolar stainless steel electrodes (Rhodes Medical Instruments, California, model SNE-100) with an outer diameter of 0.25 mm were used. A current of ± 350 μA peak-to-peak, consisting of trains of biphasic rectangular pulses with on/off periods of 10 sec, was passed during 60 mn, using a Grass S-8 stimulator (for further details see MEIJS-ROELOFS, 1972).

For DC and HF lesions, unipolar stainless steel electrodes (⌀ 0.2 mm ; uninsulated tip 0.5 mm) were employed. DC lesions were made with a continuous direct current of 1.5 mA during 10 sec (15 mC) ; a Grass constant current unit connected with a Grass S-8 stimulator was used. For HF lesions a radio frequency current was passed during 20 sec. Long-term and short-term sham procedures were performed : electrodes were inserted during 60 mn or a few seconds respectively and no current was passed. Since no differences were seen for any of the parameters studied, data from both types of sham-operated controls have been combined. Only rats in which lesions included (parts of) the arcuate nucleus were used. The various groups with HF and DC lesions and the individual rats in these groups differed in advancement of VO. Slight differences in the position of the lesions seemed to be related to these differences in time of VO. Lesions reaching the posterior border of the optic chiasm and causing definite damage of the median eminence appeared to be more effective in advancing VO than lesions with a slightly more posterior and slightly more dorsal position. Following DC and HF lesions anovulation occurred with varying frequency ; no association between anovulation and position of the lesions could be found in in the present animal material.

In some cases groups of rats were subdivided on the basis of presence or absence of advanced VO. Criteria were as described previously (MEIJS-ROELOFS, 1972) and involved comparison with litter-mates as well as with the pooled controls : age and body weight at VO should be lower than in all intact litter-mates and at least 3 days and/or 15 g less than those of the pooled controls for acceptance of advancement. Mean age and body weight at VO in the pooled intact and sham-operated controls were 38.3 ± 0.3 days and 92.5 ± 1.3 g ($n = 33$).

Without treatment, day of VO and day of first ovulation nearly always coincide in our rats (MEIJS-ROELOFS, 1972).

The statistical analysis of the results was carried out using Wilcoxon's two sample test. A difference was considered as significant, if the double tail probability was $\leqslant 0.05$. Values were expressed as mean ± SEM.

RESULTS

A. — In the first group of rats the effects of AC stimulation and of HF and DC lesions of the region of the arcuate nucleus on acute LH release as well as on the occurrence of VO and cyclicity were studied. Blood was taken immediately after the 60 min period of stimulation in the AC rats ; 30 minutes after treatment in the DC and HF rats and at comparable times in the respective control groups. Results are given in table 1 A and figure 1. A uniform and clear effect on VO was obtained in the AC rats : all rats showed an advancement of VO to 32 or 33 days of age with a mean body weight of 61.3 g. In the DC and HF group advancement of VO was less pronounced and less uniform : only half of the DC rats and 1/4 of the HF rats showed advancement of VO ; moreover cyclicity as judged by the presence of corpora lutea (table 1) was clearly disturbed in these two groups, whereas $\geqslant 2$ generations of corpora lutea were always present in AC rats. In the cyclic animals of all three groups the first cycle tended to be prolonged and often had a pseudopregnancy-like length.

All three treatment groups showed a significant rise in LH concentration as compared to the controls. Moreover, LH levels were significantly higher in HF and DC rats than in the AC group.

B. — The second group of AC, DC and HF rats and their controls were bled 3 hours after treatment as well as 2 days after treatment, at 15.00 h. Results are given in table 1 B and figure 1.

Again all AC rats showed advanced VO in contrast to the HF and DC rats ; the percentages of DC and HF rats with advanced VO were somewhat higher than in group A. Cyclicity, as judged by presence of $\geqslant 2$ generations of corpora lutea was less disturbed in this experiment.

At three hours after treatment LH levels did not differ between the various experimental and control groups. FSH levels at three hours after operation tended to be somewhat higher in the stimulated and lesioned animals, but this was of (borderline) significance in the HF group only.

TABLE I

Effects of AC stimulation and of DC and HF lesions of the arcuate nucleus region on vaginal opening and subsequent cycles

Experimental group	Effect on VO	N° of rats	Vaginal opening at		N° of rats with ⩾ 2 generations of corpora lutea (1)
			Age (days)	Bodyweight (g)	
AC	+	7	32.4 ± 0.2	61.3 ± 1.7	7/7
DC	+	4	33.5 ± 0.3	71.9 ± 4.4	3/4
	—	4	41.3 ± 0.5	100.8 ± 2.7	1/4
HF	+	2	33.0 ± 0.0	62.8 ± 0.3	0/2
	—	6	38.5 ± 1.0	92.0 ± 6.3	1/6
Sham	—	9	39.0 ± 0.9	95.5 ± 4.0	9/9
Intact	—	7	37.6 ± 0.3	91.4 ± 1.4	
AC	+	7	32.4 ± 0.2	64.5 ± 0.6	7/7
DC	+	6	33.8 ± 0.5	63.8 ± 1.6	5/6
	—	4	39.5 ± 0.7	86.0 ± 3.6	3/4
HF	+	4	33.5 ± 0.3	66.0 ± 1.5	2/4
	—	6	40.7 ± 0.8	90.8 ± 2.6	4/6
Sham	—	8	40.0 ± 0.7	90.6 ± 2.4	8/8
Intact	—	7	38.5 ± 0.6	95.9 ± 2.9	

(1) 20-30 days after VO.

Two days after treatment LH levels were generally low and no differences were found between any of the experimental and control groups. The same holds for the FSH levels, with the exception of the AC group where FSH tended to be lower than in the other groups of lesioned as well as control animals. The data are shown in figure 1.

C. — In this group of rats blood was taken from AC, DC and HF rats and from untreated rats at 17.00 h on the day preceding the expected day of VO. Effects of the various treatments on day of VO and on first ovulation are presented in table 2 ; LH and FSH levels are shown in figure 2.

A uniform advancement of VO and first ovulation was again found in all AC rats. In this experiment an advancement of VO, slightly smaller than that in the AC rats, was found in the majority of the DC and HF rats, but in about half of the DC and HF rats VO was not accompanied by first ovulation.

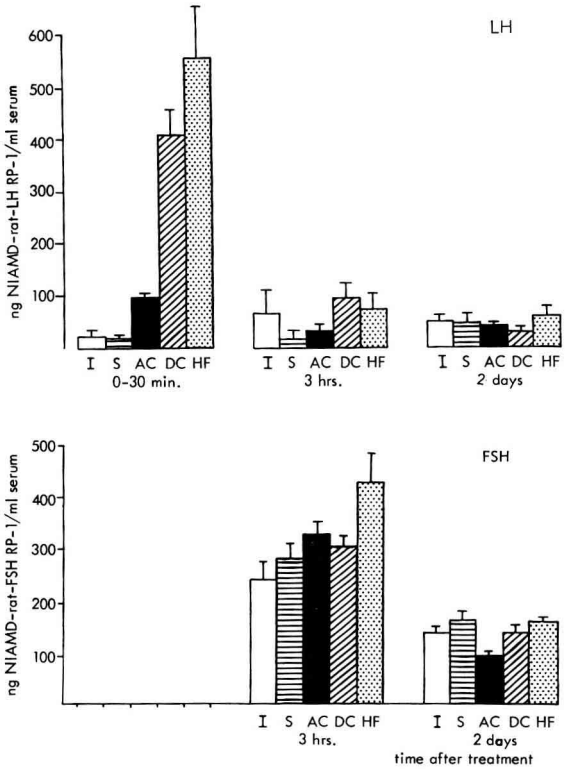

FIG. 1. — *Serum LH and FSH concentrations at various times after a direct current* (DC) *or a high frequency* (HF) *lesion or after electrical stimulation* (AC) *of the region of the arcuate nucleus.* Data on intact (I) or sham-stimulated (S) rats included. Values are given as mean ± SEM, each group consisted of 7-10 rats.

TABLE 2

Effects of AC stimulation and of DC and HF lesions of the arcuate nucleus region on vaginal opening and first ovulation

Experimental group	Effect of treatment (1)	N° of rats	Vaginal opening at		Ovulation in... rats	N° of corpora lutea
			Age (days)	Bodyweight (g)		
AC	+	9	32.3 ± 0.2	59.1 ± 0.9	9/9	8.8 ± 0.4
DC	+	12	32.2 ± 0.2	58.9 ± 1.7	6/13	8.9 ± 0.4
	—	1	39	95		
HF	+	11	33.9 ± 0.1	65.0 ± 1.8	5/13	8.5 ± 0.3
	—	2	36.5 ± 0.5	80.0 ± 2.0		
Intact	—	8	38.5 ± 0.7	88.4 ± 2.4	8/8	10.0 ± 0.4

(1) + : advanced vaginal opening.
— : no effect.

High ovulatory concentrations of both LH and FSH were found in all untreated rats (1 350 ± 105 ng/ml and 1 101 ± 66 ng/ml respectively) as well as in all AC treated rats (1 020 ± 126 *vs.* 588 ± 60 ng/ml). In the DC group high LH and FSH levels were encountered in the rats that had ovulated on the day of VO

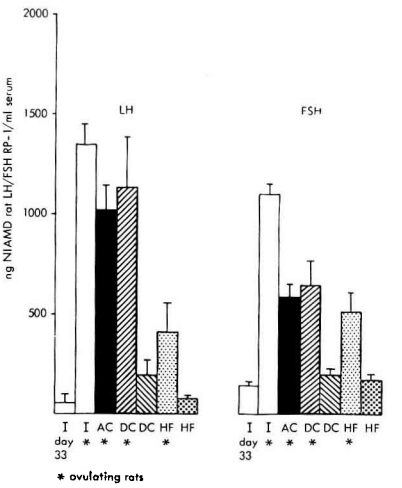

FIG. 2. — *Serum LH and FSH concentrations at 17.00 h on the day preceding the day of vaginal opening in intact rats (I) after electrical stimulation* (AC) *after a direct current* (DC) *lesion or after a high frequency* (HF) *lesion of the region of the arcuate nucleus.* Intact rats bled at 33 days of age served as additional controls. Values are given as mean ± SEM, each group consisted of 5-8 rats.

(1 138 ± 240 *vs* 639 ± 137 ng/ml), while low values were found in rats where VO was not accompanied by (first) ovulation. In HF rats also, elevated LH and FSH levels were found in rats that had ovulated one day later (410 ± 146 *vs.* 511 ± 99 ng/ml) and low levels in the non-ovulating rats. In contrast to the findings in all other groups of ovulating animals LH values in ovulating HF rats were lower than the FSH values. The LH levels of the HF rats showed, although to a minimal degree only, overlap of gonadotrophin levels of ovulating and non-ovulating rats.

Ovarian histology showed normal numbers of corpora lutea in all ovulating rats, although numbers tended to be slightly higher in the untreated controls. Partially luteinized follicles or corpora lutea with an entrapped ovum were occasionally found among the AC, DC and HF rats, but also in a single control rat. In non-ovulating DC and HF rats large atretic follicles and/or thin-walled follicular cysts were the dominating elements in the ovaries.

DISCUSSION

The reported observations show that both direct current (DC) and high frequency (HF) lesions as well as purely electrical (AC) stimulation of the area of the arcuate nucleus cause an acute increase of gonadotrophin secretion, at least of LH. This

effect was least pronounced after AC stimulation. Two days after treatment gonadotrophin levels of treated and control animals could generally not be distinguished, with the exception of AC animals that tended to have lower FSH levels.

Clearly, the acute increase in LH level, observed in all treated animals, was not uniformly related to advancement of vaginal opening (VO) and first ovulation. Gonadotrophin levels two days after treatment did not predict advancement of VO and/or ovulation either, an observation that seems to be in agreement with that reported by RAMIREZ (1974).

However, the highly constant occurrence of advanced VO and advanced first ovulation in AC rats, showing a modest, acute rise in LH and low FSH levels two days later, suggest a relationship between gonadotrophin levels and VO and ovulation at least in this group. Another difference between AC rats on the one hand and DC and HF rats on the other, is the lesser damage to brain structures that may be essential for both negative and positive feedback effects and, therefore, for processes leading to vaginal opening and first ovulation. The greater brain damage in DC and HF rats, possibly interfering to a variable degree with feedback mechanisms, might account for the more irregular advancement of VO in these animals as well as for the frequent occurrence of VO not associated with ovulation and subsequent cycles.

It seems likely that the acute increase in gonadotrophin level, presumably caused by an increase in gonadotrophin releasing factor secretion induced by a lesion or by stimulation, causes a rise in ovarian steroid production as observed by RUF *et al.* (1974) in rats with DC lesions. This may first lead to inhibition of gonadotrophin secretion (e.g. low FSH levels as observed in AC rats) and subsequently to ovulatory gonadotrophin release by positive feedback, provided negative and positive feedback centers are intact. A similar chain of events seems to occur in male induced puberty in the mouse (BRONSON and DESJARDINS, 1974). Advancement of first ovulation was indeed always associated with a preceding surge of gonadotrophin secretion, comparable to that at spontaneous first ovulation in control rats (see also MEIJS-ROELOFS *et al.*, 1975). In non-ovulating rats no gonadotrophin surge was seen on the day before VO, emphasizing that experimentally induced advanced VO does not necessarily imply advanced onset of cycling. Advanced VO not associated with a gonadotrophin surge may be brought about by an oestrogen secretion that is inadequate to elicit a positive feedback effect (*a*) or can not elicit a positive feedback because of damage to essential brain structures (*b*). Possibility (*b*) may be the case in our lesioned rats that remained a-cyclic till at least 30 days after VO.

An unusual phenomenon was the higher ovulatory elevation of FSH than of LH as observed in HF rats only. A quantitatively disturbed regulation of gonadotrophin secretion, caused by hypothalamic damage, may be the explanation.

The observed hormonal effects of interference with brain function can all be adequately described as stimulatory effects. No evidence was obtained that lesions eliminate inhibitory influences on gonadotrophin release as has often been suggested (see e.g. CRITCHLOW and BAR-SELA, 1967). The similarity between the effects of lesions and of electrical stimulation is a further argument for considering the lesions as stimulatory. It is surprising that also HF lesions result in immediate gonadotrophin release and thus act stimulatory in immature rats, whereas HF

lesions do not induce ovulation in adult rats (EVERETT, 1964). The more caudal position of the lesions in our immature rats may partly explain this discrepancy.

The present observations finally suggest that interference with brain function stimulates the hypothalamo-hypophysial-ovarian axis at a different site, but otherwise in a comparable way as treatments like administration of gonadotrophins or oestrogens, which may also induce advancement of VO and first ovulation. It seems essential that one of the elements of the hypothalamo-hypophysial-ovarian system is activated ; it does not seem to be essential which element is activated primarily. Therefore, it can in our opinion not be deduced from such experiments that either the brain, the hypophysis or the ovaries have a dominant role in sexual maturation.

The more constant results regarding VO, ovulation and cycles obtained with the AC stimulation technique indicates that AC stimulation is a better tool for further exploration of the role of the brain in sexual maturation than the lesion technique. The observation that AC stimulation is without influence on sexual maturation at an age where lesions are effective (compare HOROWITZ and VAN DER WERFF TEN BOSCH, 1962 and MEIJS-ROELOFS, 1972) may at least in part be due to the stronger acute stimulation of gonadotrophin release caused by lesions.

Sexual Maturation, 3 rd Workshop
August 31, September 3, 1975.

RÉSUMÉ

EFFET DE STIMULATIONS ÉLECTRIQUES ET DE LÉSIONS ÉLECTRIQUES OU ÉLECTROLYTIQUES DE L'HYPOTALAMUS SUR LA LIBÉRATION DES GONADOTROPINES ET LA PUBERTÉ CHEZ LA RATTE

Des rattes âgées de 28 jours ont été soumises à une lésion à courant à haute fréquence (HF), ou à une lésion électrolytique (courant continu, DC), ou à une stimulation électrique (courant alternatif et pulsatif, AC) ou à une opération-fantôme dans l'hypothalamus basal au niveau du noyau arqué. Le dosage des hormones gonadotropes sériques à divers moments après le traitement montre une augmentation immédiate (0-30 mn après l'opération) du niveau de LH dans les groupes AC, DC et HF. Cette augmentation est plus forte dans les groupes DC et HF que dans le groupe AC. Après 3 heures, les niveaux de LH sont comparables dans tous les groupes, tandis que les niveaux de FSH tendent à être plus élevés chez les animaux stimulés ou lésés, mais ce n'est significatif que dans le groupe HF. Deux jours après l'opération les niveaux en LH et FSH sont comparables dans les groupes traités ou témoins, à l'exception du groupe AC où la concentration en FSH est plus faible. L'ouverture vaginale (VO) ainsi que la première ovulation sont accélérées chez toutes les rattes AC, les résultats sont plus variables pour les rattes DC et HF. A 17 h 00 la veille de l'ouverture vaginale, la LH et la FSH présentent des pics (d'un niveau préovulatoire) chez les rattes témoins et AC. Chez les rattes DC et HF les concentrations de LH et FSH sont élevées chez celles où l'ovulation se produit le jour de l'ouverture vaginale.

Cette étude met en évidence que chez la Ratte immature une lésion hypothalamique a une action stimulante sur la sécrétion des hormones gonadotropes (1), que l'ovulation précoce est précédée d'une décharge de gonadotropines comme la première ovulation spontanée (2) et que l'ouverture vaginale dans des conditions expérimentales peut se produire sans décharge de gonadotropines et sans ovulation.

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