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Effects of the unilateral implant of haloperidol at the preoptic-anterior hypothalamic area, on ovulation

José Luis Morán & Roberto Domínguez

Biology of Reproduction Research Unit, FES-Zaragoza, UNAM AP 9-020, CP1500, México

In order to analyse whether the participation of the dopaminergic system of the preoptic-anterior hypothalamic area (POA-AHA) in the regulation of ovulation is asymmetric and varies during the estrous cycle of the rat, as it occurs with the cholinergic system, the effects of a unilateral implant of haloperidol were studied. Cyclic fourth-day rats with a permanent cannula directed to the right or left side of the POA-AHA, at 13:00 h of estrous (E), diestrous 1 or 2 (D1, D2) or proestrus (P), received an implant of haloperidol (10.0 ± 3.0 μg) or cholesterol (8.0 ± 2.0 μg). The animals were killed at the morning of the next expected day of estrous. In comparison with cholesterol-implanted animals, the ovulation rate was reduced by haloperidol implantation made on E or D1 on either side of POA-AHA (Cholesterol: 26/50 *vs* Haloperidol: 1/35, $P<0.05$). The implantation of haloperidol on D2 or P did not affect ovulation (Cholesterol: 30/37 *vs* Haloperidol:23/34, ns). The injection of gonadotropin-releasing hormone (3.7 μg/kg) sc on the afternoon of P to rats with an implant of haloperidol made on E or D1 on either side of POA-AHA, restored ovulation (19/22 *vs* 1/35, $P<0.01$). The injection of estradiol benzoate (10 μg) at 13:00 h on D2, to D1-haloperidol-implanted rats restored ovulation. The same treatment to E-haloperidol-implanted animals restored ovulation when the haloperidol was placed into the left side of POA-AHA (5/6), and it was ineffective when haloperidol was in the right side (0/8). These results suggest that the participation of the POA-AHA-dopaminergic system on the neuroendocrine mechanisms controlling the release of GnRH on the afternoon of proestrus and ovulation, varies during the estrous cycle, and is necessary at the beginning of the estrous cycle for normal ovulation. The positive feedback of estrogens on D2 needs the integrity of the right side of POA-AHA.

Keywords: ovulation; POA-AHA; dopaminergic system; haloperidol; unilateral; implant

Introduction

The participation of the dopaminergic system in the regulation of the gonadotropin secretion and ovulatory process is controversial since both stimulatory, inhibitory and neutral roles of dopamine have been described (Weiner & Ganong, 1978; Sawyer & Klifton, 1980; Tasaka *et al.*, 1985). Previous results of our laboratory has shown that the effects of the blockade of the dopaminergic system on ovulation varies during the estrous cycle and presents some type of circadian variations (Domínguez *et al.*, 1987).

The effects of unilateral blockade or stimulation of the cholinergic system at the preoptic-anterior hypothalamic areas (POA-AHA), provoked by the unilateral implant of atropine or pilocarpine, on spontaneous ovulation, is asymmetric and varies during the estrous cycle (Cruz *et al.*, 1989; Cruz *et al.*, 1992; López *et al.*, 1992).

In those animals which the cholinergic system of POA-AHA was blocked or stimulated, the estrogen stimulatory feedback resulting on ovulation was working only when the implant of atropine or pilocarpine was done on the left side of POA-AHA (Cruz *et al.*, 1992; López *et al.*, 1992).

To continue with the study of the laterality and asymmetry on the hypothalamic areas regulating gonadotropin release and ovulation, we decided to analyse whether the participation of the dopaminergic system of POA-AHA on these mechanisms, is also asymmetric and lateralized. To analyse this hypothesis, we studied the effects on spontaneous ovulation of a unilateral implant of haloperidol in POA-AHA, performed on each day of estrous cycle of the rat.

Results

Since the unilateral implantation of cholesterol reduced the ovulation rate, without affecting the number of ova shed, nor the weight of the ovaries and uterus, the results of the implantation of haloperidol on ovulation, number of ova shed, weight of the ovaries and uterus were compared with those in the cholesterol-implanted animals.

The effects of haloperidol implant on ovulation varied according with the day of the cycle when the implant was done. The implants made on estrus or diestrus 1 resulted in the blockade of ovulation, while when the implants were done on diestrus-2 or proestrus, ovulation was not affected. Differences on the implant on the left or right side affecting ovulation were not observed. In comparison with the cholesterol-implanted animals, the weight of the uterus was lower when haloperidol was implanted in the left side of POA-AHA on estrus, and was increased by same implant made on diestrus-1 (Table 1).

The injection of GnRH on the expected day of proestrus to rats with a unilateral implant of haloperidol made on estrus or diestrus-1, induced ovulation in 86% of the animals. In comparison with ovulating cholesterol-implanted rats, the number of ova shed by those animals implanted with haloperidol on estrus and treated with GnRH, was significative lower (Cholesterol 11.9 ± 0.5 *vs* Haloperidol + GnRH: 7.6 ± 1.9, $P<0.05$) (Table 2).

The animals with an implant of haloperidol in the right side of POA-AHA, made on estrus, receiving estradiol benzoate on diestrus-2 did not ovulated, while those with the implant in the left side did. Seventy-five percent of haloperidol-implanted rats on diestrus-1 ovulated after estradiol benzoate administration on diestrus-2 (Table 3).

Discussion

Present results suggest that the preovulatory release of GnRH on the day of proestrus resulting in ovulation, depends on the function of the dopaminergic system of both sides of the POA-AHA region at the beginning of the cycle. Because no differences on the ovulation blockade provoked by the implants placed in the right or left side of POA-AHA on estrus or diestrus-1 were observed, the asymmetry observed in the cholinergic system does not occur with the dopaminergic system at POA-AHA.

Correspondence: J.M. Morán
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Table 1 Ovulation rate and mean ± SEM of the number of ova shed and weight of the ovaries and uterus in intact rats with a unilateral implant of cholesterol or haloperidol in the right or left side of the preoptic-anterior hypothalamic area (POA-AHA) performed on each day of the estrous cycle. The animals were sacrificed on the next expected estrus day

Day/Group	*Side*	*Ovulation rate*	*Number of Ova Shed*	*Ovarian weight*	*Uterus weight*
Estrus					
Cholesterol	Right	8/15	11.9 ± 0.6	29.7 ± 1.3	192 ± 7
Haloperidol	Right	1/11	12	29.0 ± 0.9	187 ± 7
Cholesterol	Left	6/15	12.0 ± 1.0	29.6 ± 0.9	217 ± 9
Haloperidol	Left	0/10	0	28.0 ± 1.2	174 ± 11**
Diestrus-1					
Cholesterol	Right	6/10	10.7 ± 1.9	27.7 ± 0.5	214 ± 22
Haloperidol	Right	0/7*	0	27.3 ± 0.9	177 ± 10
Cholesterol	Left	6/10	11.3 ± 0.3	28.7 ± 1.7	180 ± 11
Haloperidol	Left	0/7*	0	24.6 ± 1.6	224 ± 17**
Diestrus-2					
Cholesterol	Right	6/7	13.0 ± 0.5	30.1 ± 1.1	210 ± 31
Haloperidol	Right	4/7	13.0 ± 0.0	27.5 ± 1.3	179 ± 7
Cholesterol	Left	4/10	12.5 ± 0.5	29.6 ± 3.2	191 ± 9
Haloperidol	Left	4/12	12.5 ± 1.0	31.5 ± 1.1	191 ± 10
Proestrus					
Cholesterol	Right	10/10	11.1 ± 1.0	27.7 ± 1.5	193 ± 9
Haloperidol	Right	7/7	11.5 ± 0.8	26.8 ± 1.5	187 ± 5
Cholesterol	Left	10/10	12.2 ± 0.2	27.6 ± 1.3	186 ± 7
Haloperidol	Left	8/8	11.2 ± 1.0	26.6 ± 1.1	207 ± 2

*$P < 0.05$ Chi Square test; **$P < 0.05$ Student's t test.

Table 2 Ovulation rate and mean ± SEM of the number of ova shed and weight of the ovaries and uterus in intact rats with a unilateral implant of haloperidol in the right or left side of the preoptic-anterior hypothalamic area (POA-AHA) performed estrous or diestrus-1. The animals received 3.7 μg/kg of gonadotropin releasing hormone (GnRH) at 13:00 h on the expected day of proestrus and were sacrificed in next morning

Day/Group	*Side*	*Ovulation rate*	*Number of Ova Shed*	*Ovarian weight*	*Uterus weight*
Estrus					
Haloperidol	Right	1/11	12	29.0 ± 0.9	187 ± 7
Haloperidol + GnRH	Right	4/5	6.8 ± 2.8	29.1 ± 1.7	200 ± 9
Haloperidol	Left	0/10	0	28.0 ± 1.2	174 ± 11
Haloperidol + GnRH	Left	3/5	8.7 ± 3.0	33.5 ± 1.6**	220 ± 14**
Diestrus-1					
Haloperidol	Right	0/7	0	27.3 ± 0.9	177 ± 10
Heloperidol + GnRH	Right	6/6*	11.2 ± 0.7	29.6 ± 1.4	221 ± 19**
Haloperidol	Left	0/7	0	24.6 ± 1.6	225 ± 17
Haloperidol + GnRH	Left	6/6*	12.5 ± 0.8	29.8 ± 2.3	208 ± 14

*$P < 0.05$ Chi Square test; **$P < 0.05$ Student's t test.

Table 3 Ovulation rate and mean ± SEM of the number of ova shed and weight of the ovaries and uterus in intact rats with a unilateral implant of haloperidol in the right or left side of the preoptic-anterior hypothalamic area (POA-AHA) performed on estrous or diestrus-1. The animals received 10 μg/kg of estradiol benzoate (EB) at 13:00 h of the diestrus day 2 and were sacrificed 44 h later

Day/Group	*Side*	*Ovulation rate*	*Number of Ova Shed*	*Ovarian weight*	*Uterus weight*
Estrus					
Haloperidol	Right	1/11	12	29.0 ± 0.9	187 ± 7
Haloperidol + EB	Right	0/8	0	32.6 ± 1.8	236 ± 16**
Haloperidol	Left	1/10	0	28.0 ± 1.2	174 ± 11
Haloperidol + EB	Left	5/6*	6.0 ± 1.2	32.0 ± 1.5	235 ± 9**
Diestrus-1					
Haloperidol	Right	0/7	0	27.3 ± 0.9	177 ± 10
Heloperidol + EB	Right	5/6*	8.6 ± 0.8	29.2 ± 2.1	250 ± 9**
Haloperidol	Left	0/7	0	24.6 ± 1.6	225 ± 17
Haloperidol + EB	Left	4/6*	7.8 ± 2.2	30.7 ± 2.3**	232 ± 7

*$P < 0.05$ Fisher's exact probability test; **$P < 0.05$ Student's t test.

When haloperidol was injected subcutaneously on diestrus-2 or proestrus, 50% of animals did not ovulate (Dominguez *et al.*, 1987).

We have reported haloperidol injected on the day of diestrus-2 or proestrus partially blocked ovulation (Dominguez *et al.*, 1987). The amount of haloperidol used in this study was higher than in present one (2.5 mg/k). Then, we can assume that other dopaminergic system(s) plays a stimulating role on the regulation of gonadotropin secretion related to ovulation, since according with Kordon *et al.* (1994) 'the possibility exists that DA may act through D1 rather than D2 receptors possibly in the median eminence via receptors or nerve terminals. Therefore the A12 neurosecretory neuronal system which is tonically active in the suppression of PRL secretion may also be involved in the regulation of LH'.

The positive feedback effects of estrogen on diestrus-2 needs the functional integrity of the dopaminergic and cholinergic systems at the right side of POA-AHA. In all experiments, estrogen administration to rats with an implant of haloperidol, atropine (Cruz *et al.*, 1992), or pilocarpine (López *et al.*, 1992), induced ovulation only when the drugs were implanted on the left side of POA-AHA, and was ineffective when the implant was in the right side.

On the other hand, the estradiol-stimulating feedback resulting in ovulation was not present in rats with a unilateral lesion on the left side of the anterior hypothalamic area (Morán *et al.*, 1994). Such differences can be explained based on the time between the implant and the injection of estrogen (48 h) and the lesion and the injection (>25 days). To explain such discrepancies, we have previously proposed 'during this time period a compensatory neural mechanism (neuronal plasticity) developed' (Morán *et al.*, 1994).

Materials and methods

Adult (190–250 g) virgin rats of the C II Z-V strain, from our own stock were maintained under conditions of controlled lighting (lights on from 05:00 to 19:00 h) with free access to food and tap water. A cannula (20 gauge outer diameter) was implanted stereotaxically under anaesthesia induced with sodium pentobarbital (35 mg/kg) (Anestesal, Smith Kline Norden de México, México), following the method previously described (Cruz *et al.*, 1989). Estrous cycles were monitored by daily vaginal smears and only animals showing three consecutive 4 day cycles were used.

Unilateral implants haloperidol in POA-AHA

After the rats with an outer cannula implanted showed three consecutive 4 day cycles, an inner cannula (25 gauge) with a 10.0 ± 3.0 μg crystals of haloperidol (Sigma Chemical Co, St. Louis MS, USA) or 8.0 ± 2.0 μg of cholesterol (Sigma) at the tip was inserted at 13:00 h on estrous, diestrous 1, diestrous 2 or proestrus day and the pellet ejected. The animals were autopsied on the morning of the expected day of estrous after unilateral implantation.

Hormonal replacement

Groups of animals with an unilateral implant of haloperidol performed on estrus or diestrus 1, at 13:00 h on the expected day of proestrus were injected subcutaneously (sc) with 3.7 μg/kg of gonadotropin releasing hormone (GnRH) (synthetic luteinizing hormone releasing hormone, Sigma) and sacrificed the next morning. Such dose of GnRH induced ovulation in rats with unilateral blockade or stimulation of the POA-AHA cholinergic system (Cruz *et al.*, 1992; López *et al.*, 1992).

Another group of rats with a unilateral implant of haloperidol on estrus or diestrus 1, on the second day of diestrus received 10 μg of estradiol benzoate sc (Sigma) and were killed 44 h later.

Autopsy procedure and statistical analysis

The animals were killed by decapitation, the oviducts dissected and ova counted using a dissecting microscope. The ovaries and uterus were dissected and weighed in a precision balance. The brain, fixed in formaldehyde 10%, was cut serially at 80 μm and stained with cresyl violet. The stereotaxic atlas of König & Klippel (1963) was used to place the implants. Those animals with the implant outside of the POA-AHA were discarded from analysis (two rats).

Data of the weight of the ovaries and uterus were analysed by multifactorial analysis of variance (MANOVA), followed by Tukey's test or by Student's *t* test; ovulation rate (number of ovulating/number of rats treated) was analysed by a Chi square or Fisher's exact probability test; the number of ova shed by ovulating animal by the Kruskal-Walis test, followed by U-Mann Whitney test. A probability value equal or less than 5% was admitted as significant.

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