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Critical Time Period for the Vagino-cervical Stimulus to Induce Pseudopregnancy in the Dioestrous Rat

M. J. Soares[A] *and W. A. Chamley*[B]

[A] Department of Anatomy and Reproductive Biology, University of Hawaii, Honolulu, Hawaii 96822.
[B] Pacific Biomedical Research Centre, University of Hawaii, Honolulu, Hawaii 96822; present address: Animal Research Institute, Werribee, Vic. 3030.

Abstract

Groups of rats experienced mechanical stimulation of the vagino-cervical region to induce pseudopregnancy at various intervals from 0900 h on dioestrus − 1 (D−1) to 1800 h on D−2. Twenty-four of 26 rats which were stimulated on D−1 experienced immediate pseudopregnancy whereas when the stimulus was given on D−2, 39 of 45 rats continued to cycle and the latest time when such mechanical stimulation would reliably induce immediate pseudopregnancy was 2400 h on D−1. Abolition of the dark phase between 1800 h D−1 and 0600 h D−2 did not alter this critical time period (2400 h on D−1). Stimulation as late as 1200 h on D−2 caused alterations in the normal pattern of 4-day cyclicity in 8 of 11 rats.

Introduction

The rat displays differential responsiveness to vagino-cervical stimulation during the oestrous cycle. Mechanical stimulation of the vagino-cervical region during the morning of pro-oestrus, oestrus, or dioestrus − 1 results in the induction of direct pseudopregnancy (Staples 1965), whereas stimulation on the morning of dioestrus − 2 results in a low percentage of immediate pseudopregnancies, a higher percentage of pseudopregnancies which are delayed until after the subsequent ovulation and uninterrupted oestrous cycles (DeFeo 1963; Castro-Vasquez and McCann 1979). To date, changes in pseudopregnancy induction following vagino-cervical stimulation during the oestrous cycle have only been evaluated at 24-h intervals. The purpose of the present study was to define more precisely the temporal changes in responsiveness to vagino-cervical stimulation during the dioestrous period of the 4-day cycling rat.

Materials and Methods

Series 1

In the first series of experiments, 61 virgin female Wistar rats (200–280 g) were used. These females were housed in pairs and maintained under controlled lighting (12 h light and 12 h dark, lights on at 0600 h). Purina rat chow and water were freely available throughout the study. Vaginal smears were taken daily between 0800 and 1000 h. Only females displaying at least two consecutive 4-day oestrous cycles were used. The rat 4-day oestrous cycle was typified by vaginal smears of: nucleated epithelial cells, pro-oestrus; cornified cells, oestrus; leucocytes, dioestrus−1 (D−1) and dioestrus−2 (D−2). Vagino-cervical stimulation consisted of mechanical vibration of the vagina and cervix for 30 s with a brass rod (5 mm diameter) attached to a vibrator (DeFeo 1966). In this series of experiments prolongation of leucocytic vaginal smears was used as the criterion for successful pseudopregnancy induction and this has been shown to correlate with a prolonged

phase of progesterone secretion in the pseudopregnant rat with plasma levels becoming higher than in cycling rats (de Greef and Zeilmaker 1974; Pepe and Rothchild 1974). The presence of cornified cells in the vaginal smear was taken to indicate the end of a pseudopregnancy. This type of smear is only seen in the pseudopregnant rat when plasma levels of progesterone have returned to basal values (de Greef and Zeilmaker 1974).

Groups of female rats were stimulated at either 0900 h D−1, 1500 h D−1, 2100 h D−1, 2400 h D−1, 0300 h D−2, 0600 h D−2, 0900 h D−2, 1200 h D−2, 1500 h D−2 or 1800 h D−2. Some females provided data about response to stimulation at more than one stimulation time and where this was the case, such an animal was required to display at least two successive 4-day oestrous cycles before being re-used.

Series 2

Since the series 1 experiment indicated that there was an abrupt alteration in response to vagino-cervical stimulation occurring between 2400 h D−1 and 0300 h D−2, a second series of experiments was conducted to evaluate the role of the light–dark cycle in relation to this changed response. In these experiments, the dark phase of D−1 was eliminated and lights were not turned off until 1800 h on D−2. Female rats were stimulated at either 2400 h D−1 (n = 8) or 0300 h D−2 (n=7).

Table 1. Induction of pseudopregnancy following vagino-cervical stimulation during dioestrus in the rat under controlled lighting and after delaying onset of darkness

Stimulation time	No. of rats	No. of pseudopregnant rats Direct	Delayed	Length (h) of direct or delayed pseudopregnancy (mean ± s.e.m.)
		Controlled lighting		
0900 h D−1	6	6	0	14·1±1·7
1500 h D−1	6	5	0	13·7±1·3
2100 h D−1	7	7	0	14·4±1·3
2400 h D−1	7	6	0	12·8±0·7
0300 h D−2	6	1	0	12·0
0600 h D−2	8	0	1	21·0
0900 h D−2	8	0	0	
1200 h D−2	11	0	3	14·0±1·5
1500 h D−2	6	0	0	
1800 h D−2	6	0	1	15·0
		Onset of darkness delayed		
2400 h D−1	8	7	0	14·0±0·49
0300 h D−2	7	0	1	15·0

Results

Series 1

The results of these experiments are shown in Table 1. When vagino-cervical stimulation was carried out on D−1, 24 out of 26 rats (92%) experienced an immediate pseudopregnancy. When stimulation was carried out early on D−2 (0300–0900 h), 20 out of 22 rats (91%) continued to show regular, 4-day oestrous cycles. Stimulation at 1200 h on day 2 resulted in altered cyclicity in 8 out of 11 rats (73%) with three of these females experiencing a delayed pseudopregnancy and the remaining five having irregular oestrous cycles. There were no significant differences (as tested by Student's t-test) in the duration of pseudopregnancy for females which were stimulated at different times during dioestrus.

Series 2

Omission of a dark phase between 1800 h on D−1 and 1800 h on D−2 had no significant effect upon the response to vagino-cervical stimulation given at either 2400 h D−1 (7 out of 8 rats had direct pseudopregnancies) or 0300 h D−2 (6 out of 7 rats continued to cycle) (Table 1).

Discussion

Our results indicate that in the cycling rat, the last time that the neuroendocrine mechanisms associated with the initiation of an immediate pseudopregnancy can be activated by mechanical stimulation of the vagino-cervical region is 2400 h D−1. Furthermore, acute disruption of the light–dark rhythm between D−1 and D−2 does not appear to influence this temporal pattern of responsiveness to vagino-cervical stimulation. However, stimulation at 1200 h D−2 does affect the normality of subsequent oestrous cyclicity but the mechanisms which underlie this latter effect are as yet unknown.

It is known that prolactin is an initial luteotrophic stimulus for pseudopregnancy in the rat (Freeman *et al.* 1974; Smith *et al.* 1975, 1976). When cervical stimulation was given at 1900 h pro-oestrus (12 h light and 12 h dark, lights on at 0600 h), there was a consequential, twice daily release of prolactin (Freeman *et al.* 1974): a nocturnal release occurred between 0100 and 0900 h and a diurnal release occurred between 1500 and 2100 h (Smith *et al.* 1976), and, during every day of the pseudopregnancy, the nocturnal surge was the greater of the two (Freeman and Neill 1972).

Although previous studies have shown that vagino-cervical stimulation on D−2 can result in delayed pseudopregnancy (Greep and Hisaw 1938; Beach *et al.* 1975; Castro-Vasquez and McCann 1979), our data indicate that during most of D−2 this method of inducing pseudopregnancy does not work. There are a number of possible explanations for this change in responsiveness to cervical stimulation: (1) failure of afferent impulses resulting from cervical stimulation to be transmitted; (2) alteration in luteal sensitivity to prolactin pulses; (3) a change in pituitary response to hypothalamic regulation; or (4) a change in hypothalamic response to afferent impulses triggered by cervical stimulation.

Freeman *et al.* (1974) suggested that the reflex was preserved in some mnemonic system because nocturnal surges of prolactin which lasted 6–8 days could be induced in long-term ovariectomized rats given cervical stimulation. However, recent experiments by Murakami *et al.* (1979) have indicated that in the intact rat, production of prolactin surges early in pseudopregnancy was not continued because of a 'memory' and became dependent upon elevated levels of progesterone.

In the experiments which have been reported in this paper, a major change in the type of response to cervical stimulation was seen between rats stimulated at 2400 h on D−1 and 0300 h on D−2, i.e. at about the time when plasma progesterone levels begin to decline precipitously (Smith *et al.* 1975). Progesterone does appear to be essential for the maintenance of prolactin surges (de Greef and Zeilmaker 1978; Murakami *et al.* 1979; Takahashi *et al.* 1980) and may be important for the establishment of the initial mnemonic system (Everett 1963). This does not mean, however, that the other factors which have been mentioned were not implicated in the overall failure of response to cervical stimulation after 2400 h on D−1.

Our results therefore indicate that induction of a direct pseudopregnancy in the dioestrum is possible provided that the D−2 nocturnal surge of prolactin can be activated. Indeed, activation of this surge by giving a vagino-cervical stimulation at 2400 h D−1 must have been immediate. This view is supported by the work of Smith and Neill (1976), who found that the rates of change for serum prolactin levels increased sharply as the stimulation time moved closer towards 0100 h. In addition, other workers have found that a pseudopregnancy can be initiated during the afternoon of D−2 by treating female rats with methods designed to achieve sustained prolactin output (Nikitovitch-Winer and Everett 1958; Schwartz 1969).

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