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The accessory olfactory system and its role in the pheromonally mediated suppression of oestrus in grouped mice

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Summary. Mice were grouped to induce suppression of oestrus and subjected to removal of the vomeronasal organs or treatment with CB 154 which lowers prolactin levels. Both treatments overcame the suppression of oestrus after 72 h. Oestrus suppression was induced in lesioned mice by haloperidol treatment which raises plasma prolactin, and oestrus returned some 72 h after withdrawal of haloperidol treatment.

Introduction

By grouping female mice it is clear that pheromonal suppression of oestrus occurs (Van der Lee & Boot, 1955; Whitten, 1959) but the neural pathways through which the olfactory cues mediate their effects and the neuroendocrine nature of these effects are not established.

In mammals there are two anatomically discrete olfactory pathways which are related to the vomeronasal organ or to the olfactory receptors in the nasal epithelium (Barber & Raisman, 1974). Whereas the main olfactory projection is via the olfactory bulb to the cortex, the vomeronasal organ projects via the accessory olfactory bulb directly to the limbic brain and is in close relationship with that part of the hypothalamus concerned with neuroendocrine regulation (Scalia & Winans, 1976). Unfortunately, removal of the olfactory bulbs damages both neural pathways. Therefore, although olfactory bulbectomy will induce a return to cycles in oestrus-suppressed females (Van der Lee & Boot, 1955; Mody, 1963), it is uncertain as to which olfactory receptors are involved. Attention has recently focused on the vomeronasal system in reproductive behaviour (Estes, 1972; Powers & Winans, 1975) and there is a suggestion that urine odours may induce reflex ovulation by a vomeronasal effect in rats (Johns, Feder, Komisaruk & Mayer, 1978). On the other hand, induction of oestrus in mice by male urine has been claimed not to be mediated by the vomeronasal system (Barber & Raisman, 1977).

Neuroendocrine changes induced by pheromones in mice have been little explored, although it has been suggested that induction of oestrus (Whitten effect) is primarily by an action on LH and then oestrogen (Bronson & Desjardins, 1969; Bronson, 1976a). However, in the pheromonal suppression of oestrous cycles it may be more pertinent to focus attention on prolactin, especially since this hormone is known to be elevated in hyperprolactinaemic amenorrhoeic women (Besser, Parke, Edwards, Forsyth & McNeilly, 1972), acyclic rats (Dickerman, Kledzik, Gelato, Chen & Meites, 1974) and ovariectomized oestrogen-treated mice maintained in groups of 6 females (Bronson, 1976b). The purpose of the present study was to investigate the role of the vomeronasal organ in mediating pheromonally induced suppression of oestrus and to examine its possible neuroendocrine basis.

Materials and General Methods

Animals

Sexually naive adult female CFLP mice weighing around 25 g were housed as groups of 8 (10 groups) or 12 (1 group) animals per cage (33·5 × 18·0 × 21·0 cm). The litter was wood

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shavings, and food and water were available *ad libitum*. The animals were kept on a controlled diurnal cycle (12 h light, 12 h dark) at a temperature of 20 ± 1°C in a room isolated from male mouse odours.

The experiments were conducted over a period of 4 months. Vaginal smears were taken from all animals at 16:00 h for the first 5 days after establishment of groups. After an interim adaptation period (of no less than 2 weeks and no more than 4 weeks), smears were again taken for 5 days or 3 successive cycles, before the start of any treatment.

The vaginal smears were taken by using a dampened spatula. This method did not cause an abnormally high incidence of cornified cells as reported by Emery & Schwabe (1936) and was used in preference to the procedure outlined by Champlin, Dorr & Gates (1973) in which the stages of the oestrous cycle were determined by the appearance of the vagina. For analysis of the vaginal smear data, the animals were divided into 2 groups, depending on whether they were cycling or in suppressed oestrus throughout the test period. If, during the test period, females showed pro-oestrous, oestrous or metoestrous smears for 2 or more successive cycles, they were classified as cycling, but persistent mucified dioestrous smears for at least 5 successive days were taken as indicative of suppressed oestrus. The data were subjected to a McNemar test for significance of changes (Siegal, 1956).

Vomeronasal organ excision

The mice were injected intraperitoneally with tribromoethanol (Avertin: 0·02 ml/g body wt) and placed supine in an adapted small-animal stereotaxic holder. The lower jaw was retracted and a small, midline incision (about 1 mm long) was made in the palate of the upper jaw 3–4 mm posterior to the upper incisor teeth. This exposed bilaterally the paramedial Jacobson's cartilages enclosing the vomeronasal neurosensory epithelium. Using a diathermic cutting tool, the entire organ on each side was cut and burnt away. The resulting gap in the upper palate was filled with polycyanoacrylate tissue cement (Simplex: Howmedica International) and the animals were allowed to recover under observation in a warm place. Histological examination of sagittal head sections showed in every case that the vomeronasal organ had been completely destroyed. Sham operations included the anaesthesia, incisions and diathermic cutting of the lower jaw in the midline.

Detailed Methods and Results

Experiment 1

Mice which had been kept singly were allocated to 2 groups of 8 each in an attempt to suppress oestrus. Vaginal smears were taken for 3 weeks before grouping and for 5 days about 3 weeks after grouping.

There was a significant ($P < 0·001$) increase in the occurrence of suppressed oestrus in the grouped females (15/16) compared with when the females were housed singly (3/16).

Experiment 2

Mice were caged in 2 groups of 8 to suppress oestrus; 15 were exhibiting suppression of oestrus when the vomeronasal organs were removed. After 2 weeks, only 3 of the 13 females (2 died) were not cyclic, while 10 had resumed oestrous cycles ($P < 0·01$). Similar treatment of 2 other groups of mice showed that the release from suppression of oestrus (14/16 before surgery) had started 72 h after the operation when 11 of the 14 had begun to cycle ($P < 0·01$). Sham operation of a fifth group had no effect on the numbers of females showing oestrous cycles (7/8 suppression before and after surgery, Table 1).

Table 1. The effects of various treatments on the suppression of oestrus in grouped mice

Exp.	Conditions	No. of females in group	No. of females in suppressed oestrus (%)		
1	Pregrouping	8	1 (12·5)		
		8	2 (25·0)		
	Grouped	8	7 (87·5)	$P < 0·001$	$P < 0·001$
		8	8 (100·0)		
		8	8 (100·0)		
		8	7 (87·5)		
2	Grouped and lesioned	6	2 (33·0)		
		7	1 (14·3)		
		8	2 (25·0)	$P < 0·01$	
		8	3 (37·5)		
	Grouped and sham lesions	8	7 (87·5)		
3	Grouped	12	10 (83·3)	$P < 0·01$	
	Grouped and CB154	12	3 (25·0)		
4	Grouped and lesioned	7	3 (43·0)		$P < 0·05$
		5	2 (40·0)		
	Grouped, lesioned and haloperidol (72 h)	7	6 (85·7)	$P < 0·01$	
		5	4 (80·0)		
	Grouped, lesioned and haloperidol withdrawn (72 h)	7	1 (14·3)		
		5	1 (20·0)		

Experiment 3

After being grouped together for 3 weeks, 10 of 12 females were not cycling when vaginal smears were taken for 5 days. At 16:00 h on the 6th day each mouse was given an intraperitoneal injection of the dopamine agonist, bromocriptine (CB 154: Sandoz Pharmaceuticals), as a dose of 5 mg/kg body weight. The vehicle was 0·5 ml saline (0·9% (w/v) NaCl).

At 72 h after the bromocriptine injection, 6 of the females were in oestrus and another 3 were in pro-oestrus, despite being grouped (Table 1). At 2 weeks after the bromocriptine treatment 10 of the females were again showing suppression of oestrus, and an injection of saline on the 6th day after 5 days of vaginal smears had no effect. All 12 females were showing suppression of oestrus 14 days later and a bromocriptine injection (same dose) again resulted in oestrus in 7 females 72 h later and in 2 more by 4 and 5 days after the single bromocriptine treatment.

Experiment 4

The vomeronasal organs were removed from 16 females and 2 weeks later vaginal smears were taken for 5 days. The dopamine antagonist, haloperidol (R1625: Searle Ltd) was then injected intramuscularly twice daily at 08:00 and 16:00 h for 4 days to the 12 surviving mice, of which 7 were exhibiting normal cycles. Each injection was of 6 μg in 0·1 ml saline and was given into alternate hind limbs to avoid trauma. Vaginal smears were taken at 16:00 h; 10 of the females showed suppression of oestrus (Table 1). When the haloperidol treatment ceased, 9 out of 11 animals (1 died) were in oestrus within 72 h. Similar twice daily injections of saline vehicle were without effect on cycles.

Discussion

Female mice when isolated were shown to have normal cycles, but when grouped, they entered a state of suppressed oestrus (Van der Lee & Boot, 1955, 1956). The introduction of male odour cues to grouped, oestrus-suppressed females reinstates their normal cycles, oestrus occurring 72 h later (Whitten, Bronson & Greenstein, 1968). In the absence of any male pheromones, normal cycles were reinstated in the present studies by bilateral removal of the vomeronasal organ, oestrus again occurring 72 h after operation. Sham-operated animals remained in a state of suppressed oestrus.

It therefore seems likely that in female mice the vomeronasal organs receive pheromonal cues from other females in a group which serve as a block to oestrus. Because of the close anatomical relationship between the vomeronasal system and hypothalamus (Scalia & Winans, 1975) such pheromones probably exert an inhibitory action on gonadotrophin release via the hypothalamus. This inhibition to gonadotrophin release can be prevented by male pheromones (Whitten, 1958; Whitten *et al.*, 1968) or, as shown here, by removal of the peripheral receptors (vomeronasal organ). The involvement of the vomeronasal system in mediating endocrine changes associated with reproduction has been suspected for some time (Estes, 1972), but has only recently been demonstrated in the rat (Johns *et al.*, 1978) in which urine odours are also associated with the timing of the oestrous cycle (Aron & Chateau, 1971). In order to demonstrate such vomeronasal effects in the rat, however, animals are first induced into persistent oestrus by continuous exposure to light before introducing the odour cue.

In the experiment reported here, the operated animals were still capable of experiencing suppression of cycles as demonstrated by the administration of the drug haloperidol, which elevates plasma prolactin (Dickerman *et al.*, 1974). When lesioned females were made anoestrous with haloperidol treatment, they nevertheless returned to oestrus 72 h after the treatment was stopped. This would suggest that the vomeronasal organ is involved in both the maintenance and establishment of the Lee–Boot effect, since lesioned females could neither enter nor sustain the suppression of oestrus when grouped. Whether female grouping has its effects on the hypothalamo–pituitary axis in a way similar to that of the drug haloperidol, i.e. by elevating prolactin, is not yet established. However, we have shown that the administration of bromocriptine, which lowers prolactin, to groups of females exhibiting suppressed oestrus, reinstated the oestrous cycle. Moreover continuous bromocriptine treatment is not required, since grouped female mice return to oestrus 72 h after a single injection of the drug. The correlative findings that suppression of oestrus can be overcome by male pheromones, or removal of the vomeronasal organ, or lowering prolactin (either by administration of bromocriptine to oestrus-suppressed females, or withdrawal of haloperidol treatment from lesioned females) with identical timing, would suggest that the pheromonal input influences the neuroendocrine mechanisms controlling oestrus by an action on prolactin. Whether prolactin is influencing the occurrence of oestrus in mice by increasing dopamine turnover and subsequently depressing LH-RH release from the median eminence, or by decreasing the sensitivity of the pituitary to LH-RH and thereby blocking positive oestrogen feedback, remains uncertain.

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