A GENE CONTROLLING BELL- AND PHOTICALLY-INDUCED OVULATION IN MICE*

B. E. ELEFThERIOU AND M. B. KRISTAL†

The Jackson Laboratory, ‡ Bar Harbor, Maine 04609, U.S.A.

(Received 14th May 1973)

Summary. Virgin female mice of two unrelated, highly inbred strains, BALB/cBy and C57BL/6By, seven of their derived recombinant inbred strains, and their F₁ hybrid generations, were exposed either to an intermittently ringing bell or to a flashing, high-intensity light. The females were treated, before exposure, with 1.5 i.u. PMSG. After exposure, the number of ova per treated or per responding female and the percentage of responding females were counted. The matching strain distribution pattern and the test of a congenic line permitted characterization of a gene exerting a major effect on bell- or flash-induced ovulation. The gene is located in Chromosome 4 (LG VIII). We propose the symbol *Bfo*. There appeared to be one or more additional loci involved and possibly interaction between loci.

INTRODUCTION

Recent evidence indicates that pheromonal facilitation of PMSG-induced ovulation in mice is mediated genetically, in the male, by a single dominant gene (Eleftheriou, Bailey & Zarrow, 1972). In an attempt to clarify the mechanism of action of this phenomenon in the female mouse, a number of experimental manipulations were attempted. Based on recent findings, it appears that changes in the social or physical environment, or alterations in the sensory input of the PMSG-primed female mouse, reveal significant strain variability in the percentage of females responding and the number of ova recovered from each female (Eleftheriou, Christenson & Zarrow, 1973).

Because of these findings, we have attempted to analyse genetically the phenomenon of facilitation of ovulation of PMSG-primed female mice by exposure to an intermittently ringing bell or a flashing, high-intensity light. For this genetic analysis, we have used recently developed inbred strains of mice known as recombinant inbred (RI) strains. Such strains are derived from the cross of two unrelated, but highly inbred progenitor strains, and then maintained independently from the F₁ generation under a regimen of strict inbreeding. The procedure genetically fixes the chance recombination of genes

* Dedicated to the memory of M. X. Zarrow.

† Present address: Department of Psychology, SUNY-Buffalo, 4230 Ridgeway Road, Buffalo, New York 14226, U.S.A.

‡ The Jackson Laboratory is fully accredited by The American Association for Accreditation of Laboratory Animal Care.

in generations following the F_1 until, by about generation F_{20} , a permanent pattern is established. The resulting battery of strains can be looked upon, in one sense, as a replicable recombinant population. The utility of such strains for analysing gene systems is described elsewhere (Bailey, 1971). Details on derivation of these lines and the application of this genetic tool to endocrine system research has been presented previously (Eleftheriou & Bailey, 1972).

In this paper, we report the application of these strains in characterizing a new gene, which controls facilitation of PMSG-induced ovulation by the ringing of a bell or the flashing of a high-intensity light.

MATERIALS AND METHODS

Immature female mice of the RI strains (CXBD, CXBE, CXBG, CXGH, CXBI, CXBJ, CXBK), their progenitor strains (C57BL/6By, BALB/cBy), and their reciprocal F_1 hybrids (B6CF₁ and CB6F₁), were used. These females were weaned at 21 days of age and housed individually in a room containing animals of the same strains and sex. Food and water were supplied freely and a 12-hr light:12-hr dark cycle was maintained, with lights turned on at 06.00 hours. At 09.00 hours on Day 24 of age, each female received a subcutaneous injection of 1.5 i.u. PMSG in 0.1 ml of 0.85% NaCl solution. It had previously been shown that this dose of PMSG did not cause ovulation at 24 hr in these strains of mice (Christenson & Eleftheriou, 1972). After the injection of PMSG, females were returned to their home cage and assigned randomly to one of the following experimental manipulations. Data presented in this report were collected during September to November, 1972.

Effects of exposure to ringing bell (Exp. 1)

At 51 hr after PMSG injection (noon of the 2nd day), the females were transferred to an isolation room with optimum ventilation and environmental conditions identical to their previous quarters. These females were exposed to an auditory stimulus of 97 dB, delivered at randomized 2-sec intervals every 15 sec until the end of the daylight cycle (18.00 hours). The females were killed by cervical dislocation approximately 15 hr later (72 to 73 hr after PMSG injection) and the oviducts were removed for examination under a dissecting microscope at $\times 40$ magnification. Ova counts were made as well as observations on the state of the ova according to the criteria described by Zarrow, Caldwell, Hafez & Pincus (1958). Control animals for this experiment were females treated with PMSG, handled similarly at 51 hr as the females in the experimental groups, but not exposed to the bell.

The data were subjected to statistical treatment according to methods described by Winer (1962). An analysis of covariance was applied to the body weight and ovulation data of females injected with PMSG and those injected with PMSG and exposed to the bell to determine the relationship between the two variables. Subsequently, an analysis of variance was applied to determine the effects of treatment and strain, and a Student-Newman-Keul's Test was performed and a Tukey's W calculated to aid in the construction of a strain distribution pattern.

Effects of exposure to flashing light (Exp. 2)

In this experiment, at 51 hr after PMSG injection (noon of the 2nd day), the females were transferred to a ventilated, sound-attenuated chamber, 52 in. deep, 72½ in. wide, and 108 in. high, with white walls and ceiling. Cages were placed on two shelves along the length of one wall. The surfaces and walls of the shelves were lined with aluminum foil to a distance of 18 in. outward from the back wall. The underside of the upper shelf and the one above it were also lined with aluminium foil.

Light flashes were delivered only during the daylight segment of the light: dark cycle by means of two Honeywell Slave Strobosars (Model 200, each rated at 480 Beam Candle Power Seconds at 10 ft for 1/500 sec, with 60° coverage, and a colour temperature of approximately 6000°K). The Strobosars were positioned 52½ in. apart, 36 in. from the back wall of the shelves, 24 in. above the lower shelf (3 in. above the upper shelf) and angled approximately 45° inward from perpendicular to the shelves and approximately 15° down from horizontal. The amount of bedding was reduced to a quantity small enough to prohibit tunnelling, and the aluminum foil wall and shelf lining made it impossible for the mouse to turn in a direction that would eliminate the flash effect.

The Strobosars were triggered simultaneously by a Lehigh Valley Synchronous Film Reader on a variable interval schedule ranging from 50 to 300 sec ($\bar{X}$ = 90 sec). The programming apparatus was outside the chamber to eliminate auditory cuing of impending flash. The females were killed by cervical dislocation approximately 15 hr later (72 to 73 hr after PMSG injection), the oviducts were removed, and ova were counted as previously described. Data were also analysed statistically as outlined in Exp. 1, with the addition of an analysis of variance encompassing both the bell and the flash treatments.

Confirmation of response patterns (Exp. 3)

Based on the statistical analyses, the various strains and hybrids fell into either a high responding group (facilitation) or a low responding group (no facilitation). The pattern in which the high or low responses were distributed among the RI strains was compared to the strain distribution patterns (SDPs) already determined for a number of other loci. Matching SDPs indicate possible identity or close linkage of two genes. The SDP of high response (facilitation) after exposure to either a ringing bell or flashing, high-intensity light matched that of a newly identified histocompatibility locus, *H-18*, which has been identified recently by isolation of its allele from the BALB/cBy strain by introduction through a backcrossing and selection regimen into the C57BL/6By strain background. The new C57BL/6By congenic line, B6.C-*H-18*^c, is considered to be identical to C57BL/6By strain, except for a short segment of the BALB/cBy chromosome that includes the BALB/cBy allele at the *H-18* locus. The probability of this line carrying a BALB/cBy gene at any locus outside this segment after twelve backcross generations is ($\frac{1}{2}$)¹².

The matching SDPs of *H-18* and the gene controlling facilitation of ovulation of PMSG-primed females after exposure to either bell or photic stimulation suggested close linkage of the two genes. Proof of linkage would be

Table 1. Effects of exposure to an intermittently ringing bell or a flashing light on the number of ova per treated female or per responding female and percentage of ovulating female mice of the two progenitor strains, their reciprocal F₁ hybrids, seven RI strains, and one congenic line following treatment with 1.5 i.u. PMSG

Strain	Treatment: PMSG (1.5 i.u.)				PMSG + exposure to bell				PMSG + flashing light			
	Mean no. of ova per female*	% females ovulating†	Mean no. of ova per responding female*	No.	Mean no. of ova per female*	% females ovulating†	Mean no. of ova per responding female*	No.	Mean no. of ova per female*	% females ovulating†	Mean no. of ova per responding female*	No.
BALB/cBy	2.1 ± 0.8	41.6 ± 14.2	5.0 ± 1.2	12	11.9 ± 0.4	100.0 ± 0.0	11.9 ± 0.4	12	16.3 ± 0.8	100.0 ± 0.0	16.3 ± 0.8	12
C57BL/6By	1.8 ± 0.7	41.6 ± 14.2	4.4 ± 0.7	12	1.5 ± 0.7	33.3 ± 13.6	4.5 ± 1.1	12	0.9 ± 0.4	33.3 ± 13.6	2.7 ± 0.4	12
B6CF ₁	1.8 ± 0.9	33.3 ± 13.6	5.5 ± 1.2	20	9.2 ± 1.9	54.9 ± 11.1	16.8 ± 0.7	12	15.1 ± 1.0	100.0 ± 0.0	15.1 ± 1.0	12
CB6F ₁	0.8 ± 0.4	33.3 ± 13.6	2.5 ± 0.8	20	6.9 ± 1.6	54.9 ± 11.1	12.5 ± 1.3	11	14.8 ± 0.7	100.0 ± 0.0	14.8 ± 0.7	11
CXBD	1.6 ± 0.7	38.4 ± 13.5	4.2 ± 0.7	12	11.5 ± 0.9	100.0 ± 0.0	11.5 ± 0.9	12	12.5 ± 1.8	83.3 ± 10.7	15.1 ± 0.7	12
CXBE	1.6 ± 0.6	50.0 ± 14.4	3.3 ± 0.7	12	10.1 ± 1.0	91.6 ± 7.9	11.0 ± 0.5	12	16.1 ± 0.4	100.0 ± 0.0	16.1 ± 0.4	12
CXBG	0.6 ± 0.3	33.3 ± 13.6	2.0 ± 0.4	13	9.8 ± 1.2	92.7 ± 7.2	10.6 ± 0.9	13	16.5 ± 0.6	100.0 ± 0.0	16.5 ± 0.6	13
CXBH	1.2 ± 0.5	50.0 ± 14.4	2.5 ± 0.8	12	8.2 ± 1.5	83.3 ± 10.7	9.9 ± 1.2	13	17.3 ± 0.6	100.0 ± 0.0	17.3 ± 0.6	13
CXBI	1.0 ± 0.5	33.3 ± 13.6	3.0 ± 0.7	12	9.6 ± 1.1	91.6 ± 7.9	10.5 ± 0.7	12	17.4 ± 0.8	100.0 ± 0.0	17.4 ± 0.8	12
CXBJ	1.4 ± 0.6	41.6 ± 0.6	41.6 ± 14.2	12	1.6 ± 0.7	33.3 ± 13.6	5.0 ± 1.0	12	2.1 ± 1.3	25.0 ± 12.5	8.3 ± 3.7	12
CXBK	0.6 ± 0.3	25.0 ± 12.5	2.3 ± 0.6	12	12.7 ± 1.7	91.6 ± 7.9	13.9 ± 1.4	12	14.3 ± 1.5	91.6 ± 7.9	15.6 ± 0.8	12
B6.C-H-18 ^c	2.3 ± 0.8	41.6 ± 14.2	5.6 ± 1.2	12	14.8 ± 0.7	100.0 ± 0.0	14.8 ± 0.7	12	13.5 ± 1.2	100.0 ± 0.0	13.5 ± 1.2	12

* Mean ± standard error of the mean.

† Percentage ± standard deviation of the observed fraction.

obtained by testing the congenic line, B6.C-*H-18*^c, for its responsiveness to either of the two experimental treatments. If a significant high response, when compared to its unexposed control, was determined by a gene closely linked, or was possibly even a pleiotropic effect of *H-18*, then the congenic line should have a response similar to BALB/cBy instead of that of C57BL/6By. Thus, females of congenic line B6.C-*H-18*^c were treated either as outlined in Exp. 1 or Exp. 2, or injected with PMSG only and were not exposed to any sensory stimulus outlined in these two experiments. The time sequence and counting of ova were identical to those described previously. Statistical analysis consisted of a χ^2 test of the response of B6.C-*H-18*^c females with that of C57BL/6By (low) or BALB/cBy (high) females.

RESULTS

Table 1 summarizes the results obtained after exposure of PMSG-injected females either to an intermittently ringing bell or a flashing light as compared with a control group not so exposed. Generally, in the control group, the percentage of females ovulating, the mean number of ova per treated female, or the mean number of ova per responding female after treatment with 1.5 i.u. PMSG was not significantly different for the various strains examined. However the greatest mean number of ova per responding female was found in the F₁ hybrid, B6CF₁, and the smallest number of ova counted was found in RI strain CXBG (5.5 ± 1.2 and 2.0 ± 0.4 , respectively). The difference between these strains is significant ($P < 0.01$).

Upon exposure to the intermittently ringing bell, all strains, with the exception of C57BL/6By and CXBJ, exhibited a significant increase in the mean number of ova per treated or per responding female. There was also a significant increase in the percentage of females ovulating in all the strains except strains C57BL/6By, CXBJ and the reciprocal F₁ hybrids, B6CF₁ and CB6F₁.

Exposure of the PMSG-primed females to the flashing light resulted in significant increases in the mean number of ova per treated or per responding female, as well as in the percentage of females ovulating for all strains, except strains C57BL/6By and CXBJ.

The response of the congenic line B6.C-*H-18*^c was similar to that for BALB/cBy and those strains exhibiting increases in ova after exposure to either the intermittently ringing bell or the flashing light. For example, the mean number of ova per treated female of this strain was 2.3 ± 0.8 after PMSG, but it was 14.8 ± 0.7 after bell and 13.5 ± 1.2 after flashing light exposure. In the bell and light tests, the percentage of responding females was 100.0, indicating the uniformity of the effect of this phenomenon.

DISCUSSION

The pattern in which the enhancement or lack of enhancement of ovulation in females primed with PMSG was distributed among the RI strains, for females exposed to a bell or for those exposed to a flashing light, was compared with the SDPs already determined for a number of other loci. The SDP of

both exposure to bell or light matched that of an already identified histocompatibility locus isolated in strain B6.C-*H-18*^c (Bailey, 1971). The matching SDPs of this locus, *H-18*, and the gene controlling bell- or flash-induced facilitation of ovulation in females primed with PMSG suggested close linkage of the two genes. Results obtained by testing congenic line B6.C-*H-18*^c confirm that the gene controlling the bell-flash facilitation of ovulation is linked to (or possibly identical with) the gene *H-18*.

Since both reciprocal F₁ hybrids showed an ovulation rate response similar to BALB/cBy, after exposure to bell or flashing light, and the response of the congenic line B6.C-*H-18*^c was also similar to BALB/cBy, we conclude that this facilitatory ovulatory phenomenon is determined by a single dominant autosomal gene that is linked to the histocompatibility locus *H-18*. We suggest the symbol *Bfo* (bell-flash ovulation) be used to designate this locus, with *Bfo*^h to designate the allele determining high response, and *Bfo*^l the allele determining low response to bell-flash induced ovulation. *H-18*, and therefore *Bfo*, are located in Linkage Group VIII, Chromosome 4.

A peculiar situation exists in the reciprocal F₁ hybrids after bell exposure. Although the number of ova counted in these females is similar to the BALB/cBy strain, the percentage of females responding is significantly lower than BALB/cBy and approximately 50% of the F₁ females exhibited this phenomenon. Thus, the possibility exists that although both of these phenomena are linked to the same locus, and since only two alleles can exist using two inbred strains, the BALB/cBy allele is fully dominant, under certain experimental conditions and only partly dominant under others.

The phenomenon of facilitation of ovulation by a bell in females primed with PMSG has been reported previously (Eleftheriou *et al.*, 1973). This is the first time however, that a report has been made of facilitation of ovulation by a high-intensity flashing light in females primed with PMSG. Additionally, this is the first report of a genetic study and actual linkage of both phenomena.

Since this is the first genetic study of the phenomena of bell- or flashing-light induced facilitation of ovulation, it is difficult to make predictions regarding the physiological, biochemical or neuroendocrine origins of the differences in the mediation of these phenomena. For example, we do not know if the genetic differences in the positive or negative response of the primed females are due to accompanying differences in psychobehavioural activation, or differences in hypothalamo-hypophysial neuroendocrine responsiveness, or hypophysial-ovarian system reactivity, or even in the extent of responsiveness of the 24-day-old murine ovary. Furthermore, we do not know whether we are dealing with a tonic discharge by the hypothalamus of a number of releasing factors, or with specific neuroendocrine activation pathways for single or multiple gonadotrophin release.

The two major contributions of the present study are (1) the finding that exposure of PMSG-primed females either to an intermittently ringing bell or to a flashing high-intensity light results in an ovulatory response with large number of ova, and (2) the significance of finding that both these phenomena appear to be determined by the same locus, although they involve different sensory modalities.

ACKNOWLEDGMENTS

This research was supported in part by Research Grant HD-05860 from the Institute of Child Health and Human Development, in part by income from the Endowment Funds of the Jackson Laboratory, and in part by Postdoctoral Training Grant MH-12126 from the National Institute of Mental Health.

Special thanks are extended to Dr D. W. Bailey for supplying us with the stocks of RI lines and congenic line B6.C-*H-18*^c.

REFERENCES

- BAILEY, D. W. (1971) Recombinant-inbred strains. *Transplantation*, **11**, 325.
- CHRISTENSON, C. M. & ELEFThERIOU, B. E. (1972) Dose-dependence of superovulation in response to two injections of PMSG. *J. Reprod. Fert.*, **29**, 287.
- ELEFThERIOU, B. E. & BAILEY, D. W. (1972) Genetic analysis of plasma corticosterone levels in two inbred strains of mice. *J. Endocr.* **55**, 415.
- ELEFThERIOU, B. E., BAILEY, D. W. & ZARROW, M. X. (1972) A gene controlling male pheromonal facilitation of PMSG-induced ovulation in mice. *J. Reprod. Fert.* **31**, 155.
- ELEFThERIOU, B. E., CHRISTENSON, C. M. & ZARROW, M. X. (1973) The influence of exteroceptive stimuli on pheromonal facilitation of ovulation in different strains of mice. *J. Endocr.* **57**, 363.
- WINER, B. J. (1962) *Statistical Principles in Experimental Design*. McGraw-Hill, New York.
- ZARROW, M. X., CALDWELL, A. L., HAFEZ, E. S. E. & PINCUS, G. (1958) Superovulation in the immature rat as a possible assay for LH and HCG. *Endocrinology*, **63**, 748.