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Individual Differences in Sexual Responsiveness to Estrogen and Progesterone in Ovariectomized Rats[1]

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POWERS, J. B. AND E. S. VALENSTEIN. *Individual differences in sexual responsiveness to estrogen and progesterone in ovariectomized rats.* PHYSIOL. BEHAV. 8 (4) 673–676, 1972.—Individual differences among 83 ovariectomized rats in behavioral responsiveness to estrogen were measured by scoring the quality of sexual receptivity induced by injections of estradiol benzoate (EB) and progesterone (P). The P dose remained constant but the quantity of EB administered was systematically reduced over successive weeks until lordosis behavior could no longer be elicited. This EB dose was considered threshold. This sequence of weekly hormone injections and receptivity tests was repeated to assess the reliability of our procedures. Animals had thresholds of either 2.0, 1.0 or 0.5 μg/kg EB on both tests; the correlation between threshold values on the two tests was high ($r=0.66$; $p<0.001$). Sixty-two females were used to determine the facilitating effects of various quantities of P following EB treatment. Subgroups were tested after the E alone and again after one of 6 P doses. Zero, 20, 50 and 100 μg P failed to elevate receptivity scores significantly; both 250 and 500 μg P had significant facilitating effects. The results demonstrated that individual differences in EB sensitivity can be measured reliably, and a further analysis also suggests similar individual differences in P responsiveness. Our threshold determination procedures provide a useful technique for measuring the effects of various experimental manipulations on the hormone sensitivity of brain mechanisms which regulate estrous behavior.

Estrogen Progesterone Sexual receptivity Hormone sensitivity Individual differences

IN MOST MAMMALIAN species the expression of sexual behavior in both males and females is to some extent dependent upon the actions of gonadal hormones [27]. Although little is known about the mechanisms by which these hormones activate patterns of reproductive behavior, considerable attention has been paid to the variables which seem important in determining responsiveness to hormonal stimulation after puberty. The notion has developed that sexual behavior in adulthood is more dependent on the sensitivity of tissues affected by gonadal hormones than by the quantity of hormones stimulating these tissues [27.] This hypothesis has been more extensively investigated in males [2, 14, 15, 23] but some evidence suggests this principle can be extended to females as well [13, 28].

In female rats and perhaps all rodents, sexual receptivity is induced by the sequential actions of estrogen and progesterone but different species appear to vary in sensitivity to these hormones [27]. Most investigators interested in the variables which affect the expression of sexual receptivity have customarily utilized suprathreshold quantities of estrogen and progesterone to assure optimal behavior responsiveness. To our knowledge there has been little interest in establishing the sensitivity of individual animals to ovarian hormones [4, 5, 16, 17], although a variety of attempts have been made to obtain dose-response relations for groups of animals [3, 8, 18, 22, 25]. This seems somewhat surprising particularly in view of much recent evidence which indicates that the responsiveness of adult female rodents to ovarian hormones can be dramatically reduced by exposure to androgens during a critical period of development [12, 24]. This effect could involve reduced sensitivity to estrogen [9, 11], to progesterone [6, 7], or to both hormones [19, 26].

The present study was undertaken for the purpose of developing a standard method for assessing individual differences in behavioral responsiveness to gonadal steroids and for evaluating hormone sensitivity changes following various experimental interventions. The procedures we have developed yield estrogen thresholds with greater homogeneity across animals than initially anticipated, but the differences which do exist remain highly consistent over successive tests.

EXPERIMENT 1

Method

Eighty-three Long–Evans females were obtained from Simonsen Laboratories, Inc., Gilroy, California, and housed individually with food and water available ad lib under a

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partially reversed light cycle (colony illuminated between midnight and noon). Approximately 10 days after their arrival in the laboratory, all rats were ovariectomized under Equithesin (Jensen-Salsbery Laboratories, Kansas City, Mo.) anesthesia and hormonal replacement therapy with behavioral testing was begun. The general objective of the injection and testing procedure was to assess behavioral sensitivity to estrogen by measuring the intensity of receptivity following estrogen and progesterone injections when the quantity of estrogen was systematically reduced over successive weeks until estrous behavior could no longer be elicited.

Varying amounts of estradiol benzoate (EB) and a constant 0.5 mg quantity of progesterone (P) were injected subcutaneously (sc) 48 and 6 hr, respectively, before behavior tests were conducted. (Progynon Benzoate and Proluton were generously supplied by the Schering Corporation, Bloomfield, N.J.). Both hormones were dissolved in sesame oil. EB injection volume (ml) was 0.5 × body weight in kg. The P volume was a constant 0.1 ml per animal.

Sexual receptivity was measured by scoring the lordosis responses elicited by vigorous Long–Evans male rats previously adapted to semicircular mating arenas (76 cm dia and 40 cm in height and width). The quality of each lordosis was rated on a scale from 0–3: 0=no concave arching of the back, 1, 2 and 3=slight, moderate and full arching, respectively. Other behaviors occasionally displayed by estrous females such as darting, hopping and head-shaking were not quantified. Receptivity tests were terminated after response scores to 10 adequate mounts by the male had been obtained. Testing was also discontinued if the male ejaculated on or after the fifth response. If ejaculation occurred before this, testing was resumed in 5–10 min. For each behavior test, the mean response score was used as the receptivity index (RI).

On the day of ovariectomy all females were injected with 40 μg/kg EB followed 42 hr later by the standard 0.5 mg P treatment but receptivity was not assessed. Seven days after this initial priming sequence, the single weekly injection of EB was reduced to 4 μg/kg and over successive weeks was further reduced by one-half. The quantity of P given 6 hr before each behavior test was always 0.5 mg. Weekly injections and tests were continued until a threshold criterion had been met. An EB dose was considered threshold when the receptivity index (RI) was 0.2 or below. However, if a score of 0.2 or below was obtained when the preceding week's RI was 1.0 or greater, the test was repeated the following week at the same EB dose. If the RI was again 0.2 or below, this quantity of estrogen was considered a valid threshold; if the score was above this level the dose was reduced the following week. One week after threshold was reached, the identical injection (starting with 40 μg/kg EB) and testing procedures were repeated until a second threshold was obtained. After completion of the second threshold series, sexual receptivity following a large quantity of estrogen in the absence of progesterone was determined for 19 females selected arbitrarily from the 83 animals used in the threshold tests. An EB dose of 40 μg/kg and 0.1 cc sesame oil were injected sc 48 and 6 hr, respectively, before conducting a standard receptivity test. These procedures were separated from the second threshold series by 1 week.

Results

Behavioral responsiveness to exogenous estrogen varied among individual animals but the correlation between first and second thresholds was high ($r=0.66$; $p<0.001$). Thresholds obtained on both tests were either 2.0, 1.0 or 0.5 μg/kg. Figure 1 presents the distribution of these values and indicates the reliability of our testing procedures for obtaining consistent threshold measures. The mean values of Test 1 and 2 were 1.22 and 1.28 μg/kg respectively; 45 of 83 females gave identical thresholds on both tests. Among the remaining animals, equal numbers (N=19) either increased or decreased their thresholds by 1 EB step; in no case was a 2-step shift observed, i.e., either from 2.0 to 0.5 μg/kg or vice versa.

Nineteen of the 83 females were tested for receptivity following injection of 40 μg/kg EB. The mean RI equalled 0.50; 11 animals failed to respond even though this large quantity of estrogen was over 30 times the mean threshold values of Tests 1 and 2. When the response scores were analyzed separately for the females having thresholds on the second estrogen-progesterone test of either 2.0 μg/kg or 1.0 μg/kg, there was some tendency for the more sensitive animals (threshold at 1.0 μg/kg) to show higher RI's to the 40 μg/kg EB alone although the difference between the two subgroups was not significant (mean RI's equalled 0.76 and 0.28, respectively).

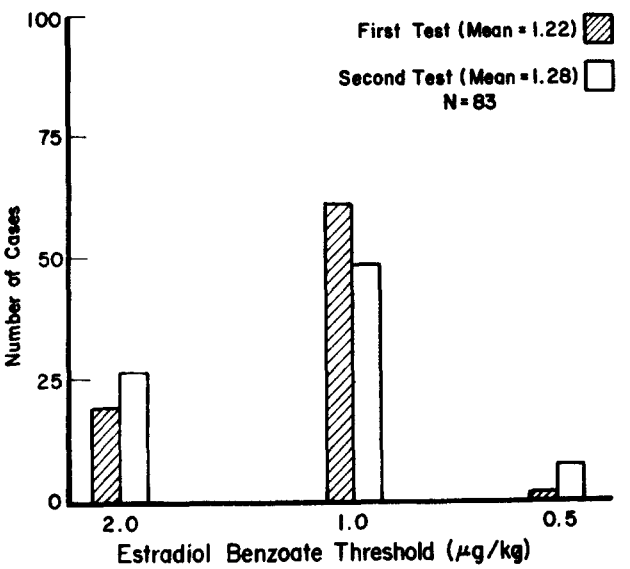

FIG. 1. Distribution of EB thresholds in ovariectomized rats on 2 successive threshold tests. For details of threshold criteria and testing procedures, cf. text.

EXPERIMENT 2

Method

Sixty-two of the females tested in Experiment 1 were used to assess behavioral responsiveness to varying amounts of progesterone following a constant estrogen injection. Females began this injection series two weeks after completing Test 2 of Experiment 1; none had been used in the tests with 40 μg/kg EB alone. The behavior-facilitating effects of a range of progesterone quantities was assessed by using individual females in only one of six dosage groups. This design was chosen rather than one which necessitated the testing of each female with all progesterone doses in order to minimize the occurrence of significant lordosis responding following estrogen alone which frequently occurs when this hormone

is injected in quantities close to threshold levels over several successive weeks.

Forty-two hr after receiving 10 μg/kg EB, all animals were pre-tested for receptivity by the standard procedures described above. Immediately after this test, each female was injected with one of the following P doses: sesame oil vehicle (N=11); 20 μg (N=11); 50 μg (N=10); 100 μg (N=10); 250 μg (N=10) and 500 μg (N=10). Six hours later a second receptivity test was given.

Results

Mean and median pre- and post-progesterone response scores are shown in Fig. 2. In none of the dosage groups did the mean pre-test RI exceed 0.25 thus confirming the results obtained in Experiment 1 that an estrogen amount considerably above threshold does not induce behavioral estrus in the absence of progesterone treatment. Sexual responsiveness was significantly facilitated by 250 and 500 μg of progesterone ($p<0.005$), Wilcoxon test) whereas the increase in receptivity following both 50 μg and 100 μg was minimal in the majority of animals as can be seen by inspection of the median scores. The absence of facilitation 6 hr after either oil or 20 μg treatments indicates that under our conditions stimulation resulting from copulation during the pre-test contributed very little to the elevated response scores observed after the higher progesterone doses.

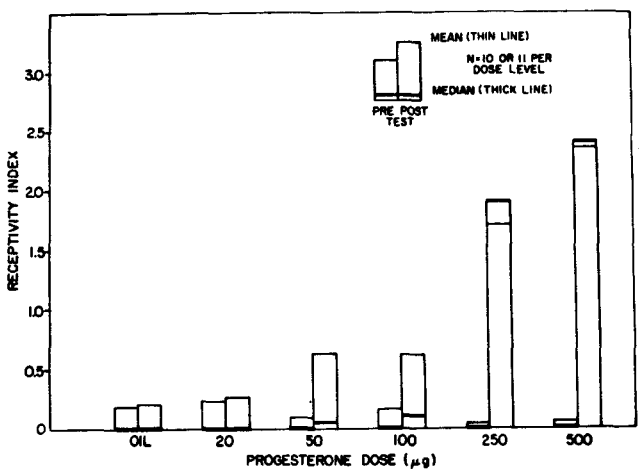

FIG. 2. Facilitation of sexual receptivity in ovariectomized rats by varying doses of progesterone (P) following a single injection of 10 μg/kg estradiol benzoate. Pre- and post-test scores were obtained immediately before and 6 hours after the injection of P, respectively. Individual animals were tested in only one P dose condition.

DISCUSSION

Ovariectomized Long–Evans rats differ in their behavioral responsiveness to estrogen and the differences are in general replicated on a second test (Fig. 1). The range over which estrogen thresholds vary is not large; not a single threshold fell above 2.0 μg/kg or below 0.5 μg/kg.

Meyerson [18] has obtained dose-response curves for varying quantities of EB administered on a body weight basis using ovariectomized Sprague–Dawley females, but the procedures used did not allow determination of individual thresholds. However, the results appear reasonably consistent with the threshold values found in this study. At 2.5 μg/kg approximately 60% of Meyerson's animals failed to display lordosis. The fact that fewer animals responded at this dose than responded at 2.0 μg/kg in our threshold tests could be due to the former animals having received a random sequence of EB quantities over successive test periods or to differences between Sprague–Dawley and Long–Evans females in behavioral responsiveness to estrogen.

This relatively small range of threshold values obtained in the present study may to some extent be due to the particular sequence of EB doses chosen. The first injection of 40 μg/kg was administered to assure that all animals would initially be exposed to a quantity of EB substantially above threshold. This injection was given on the day of ovariectomy to circumvent the tendency for ovariectomized rats to become less responsive to exogenous ovarian hormones the longer they have been without hormonal stimulation. It is possible that this relatively large amount of estrogen enhanced lordosis responding the following week when 4 μg/kg EB was given. If such an effect did occur it would tend to impose more homogeneity on the obtained threshold values than actually exists. That is, if the initial EB dose had been considerably less than 40 μg/kg with presumably less carry-over effect, more animals might have reached thresholds above 2 μg/kg. Although we cannot rule out this possible carry-over effect a more detailed analysis of the test scores at the 4.0 μg/kg EB dose indicates that this was not a major contributing factor. The highest RI scores at the 4.0 μg/kg dose test were obtained by females whose subsequent thresholds were the lowest (0.5 μg/kg) and the lowest mean RI was shown by the animals with highest thresholds (2.0 μg/kg). Thus, any possible carry-over effect from the 40 μg/kg treatment did not completely mask differential response intensities the following week.

Our decision to decrease the weekly dose of EB by one-half beginning with 4 μg/kg also may have contributed to the reliability of the results. Had we reduced the size of the EB steps, a more sensitive titration of thresholds may have been obtained. Under such a testing regimen a smaller percentage of animals might have achieved identical scores on the two tests, but we would expect the correlation to be of the same order.

The results of Experiment 2 (Fig. 2) provide additional quantification to the demonstration that progesterone, in appropriate doses, facilitates sexual receptivity in estrogen-primed females [1, 3, 6, 10, 20]. Some enhancement of lordosis responding occurred following the 50 and 100 μg P treatments, but over half the animals in both these groups had RI's of 0.1 or less. It is clear that a dose between 100 and 250 μg represents threshold for the majority of animals tested. This figure is consistent with earlier findings [3] but is somewhat higher than the values determined by Clemens, Hiroi and Gorski [6] with a different dosage and sequence of EB injections.

Our procedures do not allow a determination of progesterone sensitivity for individual animals. The variability among females within each P group, reflected to some extent in Fig. 2 by the difference between mean and median scores, suggests that individual differences in sensitivity to progesterone as well as to estrogen do indeed exist. It is not known to what extent the progesterone sensitivity function is dependent upon the intensity or completeness of prior estrogen conditioning processes. Although the quantity of EB injected (10 μg/kg) did not induce appreciable levels of receptivity prior to P injection, it was approximately 8 times

the mean EB thresholds in Experiment 1. It is clear that the EB level could have been reduced considerably and still effectively conditioned females to display lordosis. It might be argued that 10 μg/kg EB, even though suprathreshold when followed by optimal quantities of progesterone, could differentially condition animals to the facilitating actions of P depending upon individual EB thresholds. The results do not support such a view because at the suboptimal progesterone doses, the subjects showing the greatest facilitation of receptivity were not those most sensitive to EB as indicated by their thresholds in Experiment 1, but rather had thresholds distributed over the entire 0.5–2.0 μg/kg range. Thus the facilitation of receptivity observed in some animals at the low progesterone doses most likely represents a high responsiveness to progesterone rather than to differential estrogen-sensitivity effects.

During the normal estrous cycle of intact female rats, the display of sexual receptivity requires the sequential action of estrogen and progesterone since the estrogen secreted prior to the progesterone surge is not by itself sufficient to induce behavioral estrus [20]. Clearly, the quantities of estrogen needed to effectively condition the appropriate neural systems so that progesterone may facilitate receptivity are much less than the quantities needed to assure full receptivity without progesterone. Of the 81 animals which were tested in our experiments after estrogen alone (62 in the P study at 10 μg/kg and 19 in a separate test at 40 μg/kg), over 75% failed to display lordosis responses when mounted by vigorous males. Considerable variability in response intensity characterized the remaining animals, particularly those receiving 40 μg/kg EB. Response scores ranged from 0.1–2.6. Had we determined thresholds for estrogen alone, the values most likely would have covered a much wider range than the one we found in Tests 1 and 2 using both estrogen and progesterone.

Recently we have utilized the EB threshold technique described here to demonstrate that lesions restricted to the medial preoptic area dramatically reduce the dose of estrogen necessary to induce receptivity [21]. It was noted at that time that the EB threshold technique does not clearly separate changes in sensitivity to EB and P. Any experimental treatment which affects the sensitivity of one hormone system may also affect the other, either directly or indirectly. Although these interactive effects of estrogen and progesterone undoubtedly complicate any attempt to understand the neural substrates mediating sexual behavior, the use of both tests described here should make it possible to determine the hormonal system principally modified.

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