Oxytocic Activity in Posterior Pituitary Glands of Male Rats Measured by Two Bioassays[1] (35958)

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Oxytocic activity is generally measured in biological materials by using either the *in vivo* assay in which the intramammary pressure of guinea pigs is recorded (1) or the *in vitro* assay in which the time response for milk to be exuded from a small cube of mammary gland of a lactating rat is recorded (2). The *in vivo* assay is said to be more specific than the *in vitro* assay but less sensitive with the smallest amount of detectable oxytocin being 1×10^{-5} IU for the *in vivo* assay as opposed to 1×10^{-10} IU for the *in vitro* assay.

The purpose of this study was to compare the results of two bioassays in which similar biological material was used. Posterior pituitary glands of male rats at 4 different ages were chosen for this purpose.

Materials and Methods. Biological material selected. Sprague-Dawley-Rolfsmeyer albino rats were reared in an air-conditioned animal room at 26.5 ± 1°. The lighting was controlled with 14 hr of light from 6 a.m. to 8 p.m. Male rats were sacrificed in groups of 14 to 17 at 20, 40, 80, and 200 days. The posterior pituitaries were dissected, weighed, and stored at —20° until thawed just prior to bioassay. They were then diluted in Tyrode's solution and applied to the biological assay system at appropriate concentrations.

In vitro, *bioassay of oxytocic activity.* Lactating rats were separated from their pups on days 7 to 15 of lactation for 10 hr. The mother was then sacrificed and the mammary gland was removed and placed immediately in Tyrode's solution maintained at room temperature which was 22 ± 3°. Pieces 2 mm^3 were cut from the mammary gland and placed in Tyrode's solution. The standard and unknown preparations were made at 5 concentrations each. The standards ranged from 1×10^{-1} IU/0.1 ml to 1×10^{-9} IU/0.1 ml with 100-fold differences between doses. The unknown pituitary was diluted so that the highest concentration represented 0.01 pituitary (or 1×10^{-2} pituitary) to the lowest concentration of 1×10^{-10} pituitary.

Samples of 0.1 ml were transferred to 5 ml beakers. A piece of mammary gland of 2 mm^3 was placed into the 0.1 ml solution and a stop watch was activated. When milk was seen (with the aid of a dissecting microscope) exuding from the cut piece, the watch was stopped. The procedure was repeated in triplicate making a total of 15 readings/unknown or standard. The times in seconds were transferred to logarithms to the base 10 and concentrations were expressed in logarithms. The response followed a rectilinear pattern by this transposition. The data were then subjected to parallel line assay statistics as outlined by Finney (3). Potencies were expressed in terms of oxytocic activity in IU of oxytocin equivalent per pituitary gland.

In vivo, *bioassay of oxytocic activity.* Guinea pigs, obtained as virgins from National Laboratory Animal Company, Creve Coeur, Missouri, were used for the *in vivo* assay on days 4 or 5 of lactation. Young were removed from the mother at 10:00 p.m. on the night before the day of the oxytocin assay to insure the engorgement of the gland with milk. The procedures of the intravenous assay were identical with those outlined by Tindal and Yokoyama (1).

Comparisons of results obtained between the two assays were made using paired t test comparisons within each age (4).

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TABLE I. *In Vitro* Bioassay of Oxytocic Activity in the Posterior Pituitary Glands of Normal Male Rats at Various Stages of Development from 20 to 200 Days of Age.

Age of rats (days)	No. of rats	Body wt (g)	Oxytocic activity (mU/ posterior pituitary gland; mean ± SE)
20	10	39	9.1 ± 5.6
40	10	147	78.6 ± 32.3[a]
80	10	223	150.2 ± 74.1[a]
200	10	478	147.9 ± 62.3[a]

[a] Significantly different ($p < .05$) from 9.1 ± 5.6.

Results. A progressive increase in activity from 9.1 mU at 20 days to 150.2 mU at 80 days was observed. At 200 days, the oxytocic activity was approximately the same (147.9 mU) as it was at 80 days (Table I).

With the *in vivo* bioassay, the oxytocic activity of the posterior pituitary gland increased as the animals grew, being 18.2 mU in 20 day-old rats and progressing to 484.5 mU at 200 days (Table II).

Statistically significant differences were found between days 20 and others in the *in vitro* assay; and between days 20 and others, and 80 and 200 in the *in vivo* assay ($p<.05$). When the two assays were compared with each other, it was found that significant differences existed only between the values obtained for 200-day old male rats ($p<.05$).

TABLE II. *In Vivo* Bioassay of Oxytocic Activity in the Posterior Pituitary Glands of Normal Male Rats at Various Stages of Development from 20 to 200 Days of Age.

Age of rats (days)	No. of rats	Body wt (g)	Oxytocic activity (mU/posterior pituitary gland; mean ± SE)
20	4	38	18.2 ± 3.8[b]
40	6	149	96.7 ± 24.6[ab]
80	6	229	195.3 ± 66.2[ab]
200	7	465	484.5 ± 57.8[a]

[a] Significantly different ($p < .05$) from 18.2 ± 3.8.

[b] Significantly different ($p < .05$) from 484.5 ± 57.8.

Discussion. The *in vivo* method for measuring oxytocic activity has been preferred over the *in vitro* mammary cube method because it is more accurate. It has been felt that the lack of sensitivity for the *in vivo* assay is compensated by the great improvement in precision. Lambda values (index of precision) obtained for the *in vivo* assay were within acceptable ranges, that is, about 0.2 or less, while the lambda obtained in the *in vitro* assay ranged from 1 to 4 for our data and never approached the acceptable value of 0.2.

It has been said that the relative activity of vasopressin in stimulating contraction of myoepithelial cells in the mammary gland is about 20% of that of oxytocin. This was reported using the rabbit mammary gland assay (5). In our tests, the *in vivo* bioassay using increase in intramammary pressure of the guinea pig mammary gland indicated that vasopressin was 5 to 10% as effective as oxytocin, while the *in vitro* mammary cube assay gave responses to vasopressin that were 80% of the responses to oxytocin. By using whole posterior pituitary glands, we were actually measuring considerable amounts of vasopressin in the *in vitro* assay, yet the values obtained for the *in vitro* assay were less than those for the *in vivo* assay. The explanation for these results can only be clarified with further investigation, but it may be reasoned that the *in vivo* assay was utilizing available oxytocin more efficiently than was the *in vitro* assay at the levels measured or perhaps some molecular complex was activated metabolically by the *in vivo* system but not by the isolated mammary cube. Many different reasons may be given to explain the results obtained but the fact remains that they are in surprisingly good agreement considering the wide differences in the basic designs of the two different assays.

The present study represents the first true comparison of the *in vitro* and *in vivo* assays for oxytocic activity in similar biological material. If progress is to be made in absolutely quantifying activities of the posterior pitui-

tary neurohormones then additional studies of similar design and objective must be conducted. It would be helpful to compare levels of oxytocic activity in blood plasma of lactating cows with the two assays within a single laboratory.

Several references are available with which to compare the amount of oxytocic activities reported in this study. Dicker and Tyler (6) reported a value of 320 mU of oxytocin in the posterior pituitary gland of the adult male rat, while Lederis (7) reported values of 510 and 540 mU. Acher and Fromageot (8), using the uterine contraction assay for oxytocic activity and the blood pressure assay for vasopressin, found that normal adult female rats had 840 mU of oxytocin and 810 mU of vasopressin/posterior pituitary, while in normal male rats the levels were 830 mU and 810 mU, respectively; Young and Van Dyke (9) reported similar values. All values are near or slightly higher than those found in our study in which two assay methods for oxytocic activity are compared.

Summary. Oxytocic activity in posterior pituitary glands of male rats at 20, 40, 80, and 200 days of age was measured using two different bioassays. The first was an *in vitro* assay based on the log time response of a 2 mm^3 mammary cube in exuding milk to the log dose of oxytocin between 10^{-1} and 10^{-9} IU. The second was an *in vivo* assay based on the ability of the guinea pig mammary gland to increase intramammary pressure rectilinearly in the range of 0.25 to 1.0 mU of oxytocin. Oxytocic activities of posterior pituitaries by the *in vitro* assay were 9.1, 78.6, 150.2, and 147.9 mU at 20, 40, 80, and 200 days of age, respectively; while the *in vivo* assay gave values of 18.2, 96.7, 195.3, and 484.5 mU in the same order. Only the values obtained in 200-day-old rats were significantly different between the two assays ($p<.05$).

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