LONG LASTING CHANGES IN RNA SYNTHESIS IN THE FOREBRAINS OF FEMALE RATS TREATED WITH TESTOSTERONE SOON AFTER BIRTH*

Hiraku Shimada and Aubrey Gorbman
Department of Zoology, University of Washington
Seattle, Washington

Received November 25, 1969

Considerable interest has become focussed on the permanent effects of brief periods of hormone treatment of newborn rats. This action, which has been called "induction" and "differentiation", is well illustrated in experiments in which neonatal females are treated with androgens. Such treatment is followed, in adulthood, by abnormally modified sexual behavior (Gray et al., 1965; Levine and Mullins, 1966), by sterility due to permanently altered hypothalamic control over gonadotropin secretion (Barraclough and Gorski, 1961; Petrusz and Flerko, 1965; Yazaki, 1966), and even by a modified type of steroid hormone metabolism, presumably by the liver (Kraulis and Clayton, 1968). Where an applied chemical stimulus has produced a permanent developmental change, it is natural to question whether there is a qualitative change in RNA synthesis in the affected tissues. By use of the DNA-RNA hybridization technique, originally described by Gillespie and Spiegelman (1965), it has been shown by Whiteley et al. (1966) that progressive changes in RNA transcription take place during development of sea urchin embryos, yielding successively different populations of RNA. Although a considerable volume of research has shown that there are quantitative changes in RNA transcription in rat brains during early postnatal development (Dellweg et al., 1968; Itoh and Quastel, 1969), there is very little information available concerning qualitative changes of the type found by Whiteley et al. (1966) in whole embryos.

*This research was supported by grant #AM10902 from the U. S. National Institute of Health.

We report here some evidence for the production of new and characteristic species of RNA in the brains of 47 day old female rats which were given testosterone propionate when 2 days old.

MATERIALS AND METHODS: Pups in one group of 2 day old Sprague-Dawley female rats received a single subcutaneous injection of 1 mg of testosterone propionate (TP) in 0.03 ml sesame oil, while pups of a second group received 0.03 ml of sesame oil given in the same way. Forty five days after hormone treatment, and two hours before death, three rats from each group received an intracervical injection of 1 mc of ^{3}H-uridine (0.01 ml, 25-29 c/mM) under light ether anesthesia. The two hour labeling period was selected after prior testing to yield RNA with specific radioactivity high enough for the competitive hybridization experiment; this labeling period is longer than that recommended by Bondy et al. (1968) for the specific labeling of m-RNA in the rat brain. Rats were killed by exsanguination through decapitation during ether anesthesia. The brain was rapidly removed, cut into two parts (forebrain and midbrain-hindbrain) and frozen on dry ice. The forebrain sample contains diencephalon as well as cerebrum. RNA was extracted from each part of the brain by the method of Scherrer and Darnell (1962). The RNA extract was treated with DNase and Pronase, purified by use of cetyltrimethyl ammonium bromide according to Ralph and Bellamy (1964) and passed through a Sephadex G-50 column.

RESULTS AND DISCUSSION: The single neonatal administration of TP consistently decreased slightly the rate of RNA synthesis in the 47-day old rat brain. This decrease was 12% to 29% in forebrain and 10% to 38% in the midbrain-hindbrain). Since it seems reasonable to assume that most or all of the TP administered neonatally was metabolised during the 45-day period, this consistent result suggests that TP produced a long-lasting change of some kind in the RNA synthesizing mechanism in the rat's brain.

For the competitive DNA-RNA hybridization experiments, DNA was prepared

from rat liver according to Church and McCarthy (1968) and immobilized in single strand form (alkaline denatured) on nitrocellulose membrane filters by the method of Gillespie and Spiegelman (1965). Non-labeled brain RNA was prepared in the same way as described above separately from the forebrain and midbrain-hindbrain of control and TP-treated rats. As a check for the specificity of DNA-RNA hybridization, ^{3}H-RNA from whole brain was competed for hybridization to DNA with the RNA from liver. As shown in Fig. 1, non-labeled RNA from liver competed less effectively with labeled RNA from brain than does non-labeled RNA from brain, indicating that the RNA's extracted from liver and brain are in some degree detectable by this technique different and organ-specific, as pointed out previously by McCarthy and Hoyer (1964) and by Sullivan (1968).

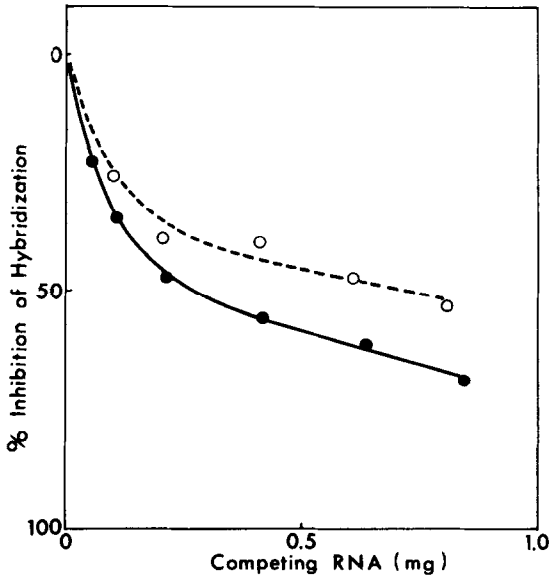

Figure 1

Uridine-labelled rat (47-day old female) brain RNA (15 µg) was incubated in a series of tubes, each containing 15 µg DNA on a membrane filter as well as varying amounts of "cold" RNA. The upper curve (open circles) shows the progressive reduction in DNA-binding of the labeled brain RNA by "competition" with cold rat liver RNA. In the lower curve (solid circles) the effect of cold brain RNA was tested in a similar way.

To learn whether testosterone injection evokes transcription of new species of RNA in rat brain, the competitive DNA-RNA hybridization experiments were conducted between the brain RNA from control female rats and that from TP-treated female rats of the same age.

Unlabeled RNA from the forebrains of controls was a less effective competitor than RNA from the forebrains of TP-treated females (Fig. 2B) when competing with H^3-RNA from TP-treated females. This indicates that RNA of the forebrain of TP-treated females contains species of RNA which do not occur in the forebrains of control female rats. In the reciprocal experiment (Fig. 2A) in which DNA-binding of ^3H-RNA from the forebrains of control rats was measured in competition with non-labeled RNA from the forebrains of TP-treated or from controls, both non-labeled RNA's were equal in competitive ability. The results illustrated in Fig. 2 show that administration of testosterone propionate to newborn rats evokes the later production (at 47 days) of new species of RNA in the forebrains of "pubertal" female rats. Since the overall rate of synthesis of RNA in the forebrain is reduced in TP-treated rats compared with normal control rats, and since, within the limits detected by this technique, the forebrains of TP-treated rats have all the species of RNA that are in the forebrains of control rats, it is likely that neonatal administration of TP causes the reduction of the rate of the synthesis of all RNA species common with normal female rats' forebrains, or a selective reduction in some forebrain RNA species, at the same time it evokes the synthesis of some new RNA species which are not characteristic of the forebrain of normal females.

This is apparently different from the effects of steroid hormones in other tissues. Administration of estrogen provokes an increased rate of RNA synthesis in chicken oviduct, and at the same time, the production of new species of RNA (Hahn et al., 1968, O'Malley et al., 1968). Prednisolone causes a reduction of the RNA synthesis in the rat thymus, but it does not evoke production of new species of RNA (Drews, 1969).

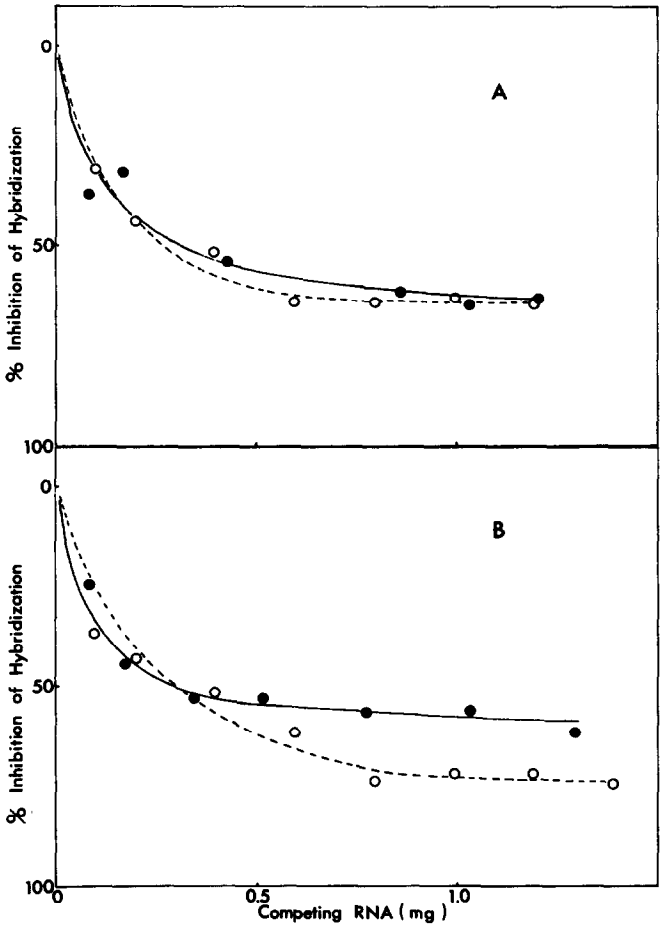

Figure 2

Radioactive uridine labeled rat forebrain RNA (15 µg) was incubated in a series of tubes, each containing DNA on a membrane filter and varying amounts of cold RNA competing with the labeled RNA for binding with the DNA.

In 2A radioactive uridine-labeled forebrain RNA from 47-day old females was competed with increasing amounts of normal 47-day female forebrain RNA (closed circles), or with forebrain RNA from 47-day old testosterone-treated females (open circles).

In 2B the labeled uridine was from testosterone-treated female, and its DNA-binding was competed, as in the complementary experiment above, with forebrain RNA from normal (closed circles) or testosterone-treated (open circles) 47-day old females.

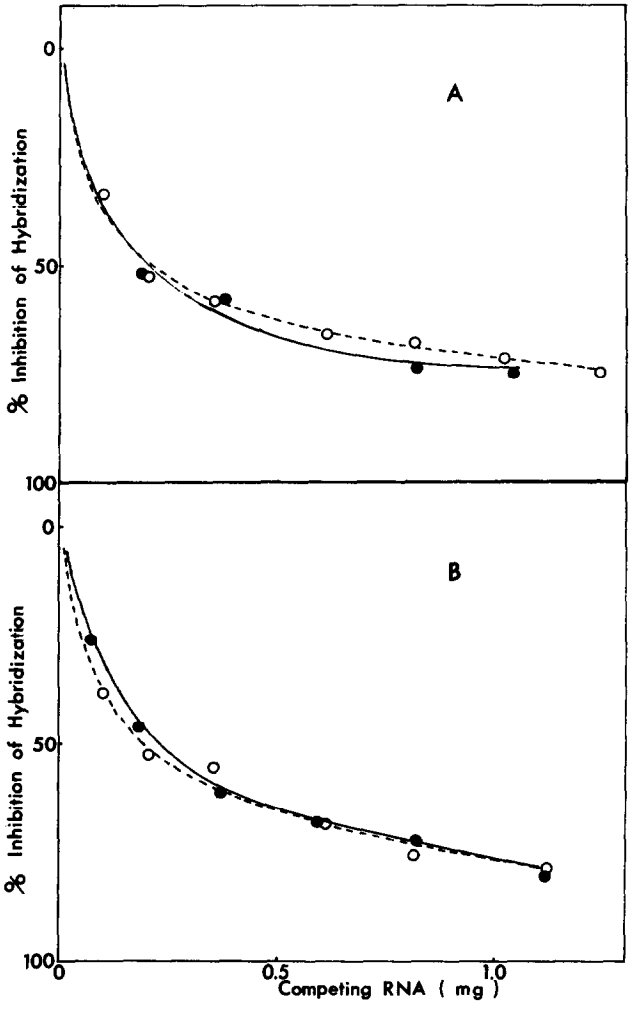

Figure 3

Radioactive uridine-labeled 47-day old female rat hindbrain RNA was incubated in a series of tubes, each containing DNA on a membrane filter and varying amounts of cold RNA competing with the labeled RNA for binding with the DNA.

In 3A the DNA-binding of the radioactive uridine-labeled RNA from hindbrains of of 47-day old control females competed with varying amounts of cold hindbrain RNA from control 47-day old females (closed circles) or TP-treated females (open circles).

In 3B the labeled hindbrain RNA was from TP-treated 47-day old females, and its DNA-binding was competed, as in the complementary experiment above, with hindbrain RNA from normal (closed circles) or TP-treated (open circles) 47-day old females.

It is clear, as shown in Fig. 3, that non-labeled RNA from hindbrains of both normal and TP-treated females competed equally well with the labeled RNA from the hindbrains of either control or TP-treated rats; thus, there is no demonstrable qualitative difference among the RNA species tested. Accordingly, though TP has an inhibitory effect on the RNA synthesis both in the forebrain as well as the hindbrain, synthesis of new species of RNA is demonstrable only in the forebrain.

These results provide a basis for interpreting the inhibition of ovulation and the behavioral masculinization of female rats which follows the neonatal administration of TP. The apparently permanent effects on brain function are paralleled by changes in RNA transcription, and hence, may be an example of hormonally evoked stable differentiation of RNA synthesis. Although it is probable that brain cells are thus responding directly to the hormone, these data do not establish the actual initial site of action of the hormone. The testosterone stimulated reduction in rate of RNA synthesis in the forebrain as well as in the hindbrain may be interpreted, in the light of prior observations by others (Dellweg et al., 1968; Itoh and Quastel, 1969) that RNA synthesis in the brain of the rat progressively decreases with age. Thus, the testosterone appeared to evoke in a permanent or definitive way this phase of RNA metabolism in the young rat's brain, in addition to its qualitative effect.

REFERENCES

Barraclough, C. A., and Gorski, R. A., Endocrinol. 68, 68 (1961).

Bondy, S. C., and Roberts, S., Biochem. Jour. 109, 533 (1968).

Church, R., and McCarthy, B. J., Biochem. Genetics 2, 55 (1968).

Dellweg, H., Gerner, R., and Wacker, A., Jour. Neurochem. 15, 1109 (1968).

Drews, J., European J. Biochem. 7, 200 (1969).

Gillespie, D., and Spiegelman, S. A., Jour. Mol. Biol. 12, 829 (1965).

Gray, J. A., Levine, S., Broadhurst, P. L., Animal Behaviour 13, 33 (1965).

Hahn, W. E., Church, R. B., Gorbman, A., and Wilmot, L., Gen. Comp. Endocrinol. 10, 438 (1968).

Itoh, T., and Quastel, J. H., Science 164, 79 (1969).

Kraulis, I., and Clayton, R. B., Jour. Biol. Chem. 243, 3546 (1968).

Levine, S., and Mullins, R. F., Science 152, 1585 (1966).

McCarthy, B. J., and Hoyer, B. H., Proc. Nat. Acad. Sci. 49, 729 (1964).

O'Malley, B. W., McGuire, W. L., and Middleton, P. A., Nature 218, 1249 (1968).

Petrusz, P., Flerko, B., Acta. Biol. Hung. 16, 169 (1965).

Ralph, R. K., and Bellamy, A. R., Biochem. Biophys. Acta. 87, 9 (1964).

Scherrer, K., and Darnell, J. E., Biochem Biophys. Res. Communic. 7, 486 (1962).

Sullivan, D. T., Proc. Nat. Acad. Sci. 59, 846 (1968).

Whiteley, A. H., McCarthy, B. J., and Whiteley, H. R., Proc. Nat. Acad. Sci. 55, 519 (1966).

Yazaki, I., Annot. Zool. Japon. 39, 71 (1966).