PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 302–305 (1974)

The Effects of Intraventricular Injection of Antibiotics on LH Release in the Rat (38093)

A. NEGRO-VILAR,[1] R. ORIAS,[2] AND S. M. MCCANN[3]

Department of Physiology, University of Texas, Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, Texas 75235

LH release in the rat is regulated by the hypothalamic hormone, LH-releasing factor (LRF). It appears that the hypothalamic hormone is synthesized rapidly in hypothalamus in situations calling for an increased release of LH, such as electrochemical stimulation of the anterior hypothalamic area (1) and on the afternoon of proestrus (1). In both these situations, plasma LH levels rise and this rise is preceded by an increase in LRF stored in the ventral hypothalamus (1). Since LRF appears to be a decapeptide (2), it was of interest to determine if inhibitors of RNA or protein synthesis would block the synthesis of LRF and consequently interfere with the release of LH. To investigate this possibility, antibiotics known to interfere with either RNA or protein synthesis were injected into the third ventricle of castrated female rats, and the effects on plasma LH determined. A preliminary report of this work has been presented (3).

Materials and Methods. Female rats, 200–220 g, were obtained from the Holtzman Co. (Madison, Wisconsin) and were housed in an air-conditioned room with controlled lighting (lights on from 5 AM to 7 PM).[4] They were fed Purina laboratory chow and given free access to water. The animals were castrated and used for experiments 8–12 days later. Four to five days before the experiment, third ventricular cannulae were introduced as described previously (4) while the animals were anesthetized with ether. On the day of experiment, 5 μliters of either 0.9% NaCl or the antibiotics were injected into the third ventricle. Each 5 μliters contained 30 μg of puromycin, 15 μg of cycloheximide, or 10 μg of actinomycin D dissolved in 0.9% NaCl.

Immediately prior to and at varying intervals following the third ventricular injection, the animals were anesthetized with ether and 1 ml blood samples were drawn from the jugular vein using heparinized syringes. At 2 hr after the injection of puromycin or saline, another group of animals was injected iv with 100 ng of synthetic LRF[4] and additional blood samples were obtained 10 and 20 min following injection.

In a final series of experiments, animals were sacrificed at 2 hr after injection of either puromycin or saline and acid extracts of their ventral hypothalami were prepared and assayed for LRF content by an *in vitro* assay (1). The hypothalamic tissue was extracted in a volume of 0.1 ml of 0.1 *N* HCl, centrifuged, and the clear supernatant at a dose of one hypothalamic equivalent per flask was added to incubation flasks containing six pituitary halves from donor male rats. An additional set of flasks received extract of cerebral cortical tissue to deter-

[1] Present address: Universidade de Sao Paulo, Faculdade de Medicina, Ribeirao Preto, Sao Paulo, Brasil.

[2] Present address: Departamento de Ciencias Biologicas, Universidad Nacional de Rio Cuarto, Rio Cuarto, Argentina.

[3] Supported by Grants from NIH (AM 10073 and HD 05151), the Ford Foundation, and Texas Population Research Institute.

[4] Kindly provided by Dr. John Stewart (University of Colorado Medical School, Denver, Colorado).

mine basal release of LH. Four flasks per group were used. Aliquots of medium were obtained for assay of LH content after 1 and 3 hr of incubation.

LH in plasma and media was measured by radioimmunoassay employing the method of Niswender *et al.* (5), and the results were expressed in terms of the NIH LH-S-1 standard.[5]

Results. Intraventricular injection of saline. Repeated blood sampling in the castrated animals injected with 5 μliters of 0.9% NaCl in the third ventricle resulted in a slight decrease in plasma LH apparent at 2 hr following the injection in one of two experiments (Figs. 1, 2). There was no significant change from the initial level at 1, 4, and 24 hr postinjection.

The effect of drugs which block protein synthesis on plasma LH in castrated rats. By contrast with the minimal effects of intraventricular saline, intraventricular injection of puromycin (Figs. 1, 2) or cycloheximide (Fig. 1), antibiotics which block protein synthesis, produced a dramatic decline in plasma LH apparent at 1 hr and persisting for the 24 hr duration of the experiment. To determine the specificity of this effect, a puromycin analog, puromycin aminonucleoside, which is ineffective in blocking protein synthesis, was injected at a dose of 30 μg per animal in a series of rats. It produced no significant alteration in plasma LH at any of the time intervals examined (Table I).

To determine the sensitivity of the pituitary to LRF in puromycin-treated animals, the drug was injected into the ventricle and 2 hr later the rats were given a test dose of 100 ng of synthetic LRF. The increments in plasma LH at 10 and 20 min following injection were similar in the puromycin-injected rats to the increments obtained in saline-treated controls (Fig. 2).

When the LRF content in ventral hypothalamic fragments was assayed at 2 hr following the intraventricular injection of puromycin, there was no significant difference in LRF content on comparison with that present in saline-injected animals (Fig. 3). Similar results were obtained in another experiment (not shown) in which LRF content in both groups was measured 4 hr after the intraventricular injections.

The effect of intraventricular actinomycin D on plasma LH in castrated rats. Injection of actinomycin D resulted in a decline in LH, first apparent at 1 hr and minimal LH values were attained at 2 hr following the injection (Fig. 1). The low levels were maintained throughout the 24 hr period of observation. The only difference between the response to actinomycin and that to puromycin and cycloheximide was the slightly slower decline in plasma LH observed with the inhibitor of DNA-directed RNA synthesis.

[5] We are indebted to Dr. G. Niswender (Colorado State University) for the anti-LH serum, and to Dr. Leo Reichert (Emory University) for providing LH for iodination.

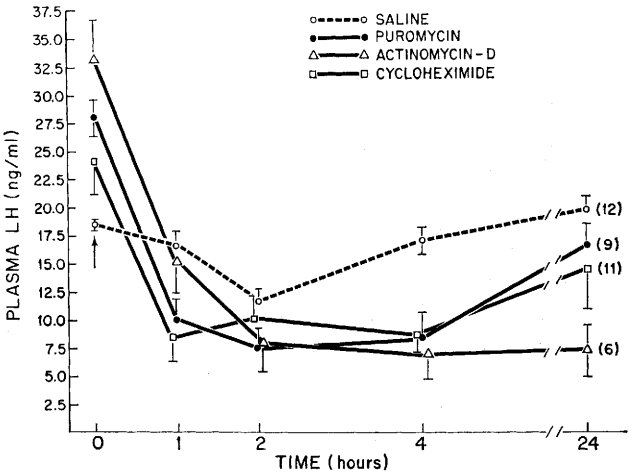

FIG. 1. Plasma LH titers just prior to and at various times after the injection of isotonic saline or the various antibiotics into the third ventricle of ovariectomized female rats. Numbers in parenthesis indicate the number of rats per group; vertical lines above and/or below the mean indicate SEM.

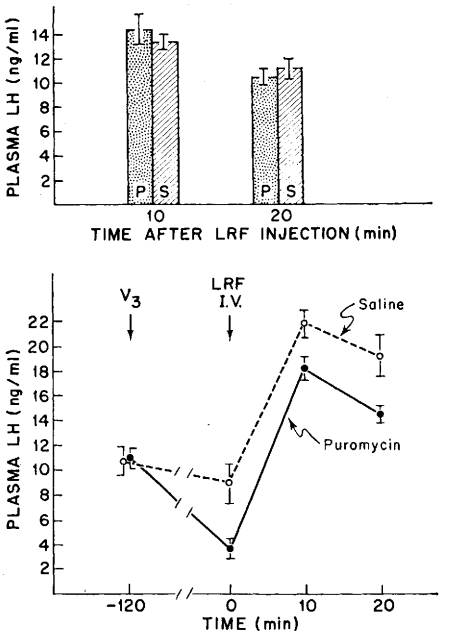

FIG. 2. Plasma LH concentrations immediately prior to and after injection of either saline or puromycin into the third ventricle of ovariectomized rats. At time 0, 100 ng of LRF was injected intravenously into both groups. The increment in plasma LH at 10 and 20 min after LRF injection is indicated by the height of the bars in the upper panel. P = puromycin; S = saline. Vertical bars indicate the SEM.

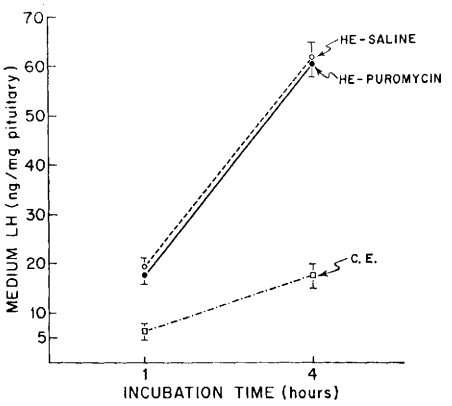

FIG. 3. LH release into the medium of pituitaries incubated *in vitro* with either cerebralcortical extract (C.E.) or hypothalamic extract (H.E.) from animals injected into the third ventricle with either saline or puromycin.

Discussion. These results demonstrate that inhibitors of either protein or RNA synthesis can produce an inhibition of LH release in castrated female rats when injected into the third ventricle. The injection into the ventricle and repeated blood sampling, on the other hand, produced only minimal changes with a slight decline in plasma LH apparent only at 2 hr in one of two experiments. The only difference between the results with the inhibitors of protein synthesis and with actinomycin was the slightly slower decline in plasma LH following actinomycin D. The specificity of the effects of the antibiotics is further suggested by the failure of the inactive analog, puromycin aminonucleoside, to lower plasma LH in castrated animals.

Our original assumption was that these antibiotics might block the synthesis of LRF and that this would then lead to a fall in hypothalamic content and a blockade of its release which would lead to a decline in plasma LH. The results of measurement of stored LRF at 2 or 4 hr after puromycin did not support this thesis since no significant declines in LRF content were observed. Thus, the mechanism by which these antibiotics block LH release remains unclear. It is still possible that they inhibit the synthesis of the releasing factor and that discharge of LRF ceases after depletion of a very small releaseable pool. If this were the case, it might not be possible to demonstrate significant decreases in LRF content. A

TABLE I. Effect of Intraventricular Injection of Puromycin Aminonucleoside (30 μg) on Plasma LH.

Initial sample	Time after injection (hr)			
	1	2	4	24
8.62 ± 2.70*	5.59 ± 2.03	5.90 ± 2.29	5.77 ± 2.70	5.43 ± 2.98

* = ng of LH/ml, ±SEM.

more likely explanation would be that the antibiotics interfere with another step in the chain of events at the hypothalamic level leading to LH release. For example, they may block synaptic transmission. Further experiments will be required to determine the exact mechanism.

It is clear that the blockade in LH release induced by puromycin was by an effect at the hypothalamic level and not by an effect after absorption of the antibiotic into portal vessels and delivery directly to the pituitary gland, since the responsiveness of the adenohypophysis to LRF was unaltered even though LH release had been largely inhibited.

It has been reported that systemic administration of these antibiotics could block both negative (6) and positive feedback (7, 8) of estrogen on LH release. Since the antibiotics blocked the release of LH *per se* in the present experiments, it is possible that the experiments of Jackson (7, 8) dealing with positive feedback may be interpreted simply in terms of the general suppression of LH release by the antibiotics.

Summary. The intraventricular injection of 5 μliters containing 30 μg of puromycin, 15 μg of cycloheximide or 10 μg of actinomycin D led to an inhibition of LH release and a fall in plasma LH levels in castrated female rats. Control injections of either saline or an analog of puromycin which lacks its ability to inhibit protein synthesis had little or no effect on the circulating levels of LH.

Since LRF content in the hypothalamus was not significantly lowered by puromycin, the cause of the inhibition of LH release produced by inhibitors of RNA or protein synthesis remains to be determined, but may involve an inhibition of the synthesis of LRF and/or other effects on hypothalamic function.

1. Kalra, S. P., Krulich, L., and McCann, S. M., Neuroendocrinology **12**, 321 (1973).

2. Matsuo, H., Arimura, A., Nair, R. M. G., and Schally, A. V., Biochem. Biophys. Res. Commun. **45**, 822 (1971).

3. Negro-Vilar, A., Orias, R., and McCann, S. M., Acta Physiol. Latinoamericana **23**, (Suppl. 3) (1973).

4. Antunes-Rodrigues, J., and McCann, S. M., Proc. Soc. Exp. Biol. Med. **133**, 1464 (1970).

5. Niswender, G. D., Midgley, A. R., Monroe, S. E., and Reichert, L. E., Proc. Soc. Exp. Biol. Med. **128**, 807 (1968).

6. Schally, A. V., Bowers, C. Y., Carter, W. H., Arimura, A., Redding, T. W., and Sato, M., Endocrinology **85**, 290 (1969).

7. Jackson, G. L., Endocrinology **90**, 874 (1972).

8. Jackson, G. L., Endocrinology **91**, 1284 (1972).

Received Jan. 28, 1974. P.S.E.B.M., 1974, Vol. 146.