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Apomorphine-Induced Inhibition of Episodic LH Release in Ovariectomized Rats with Complete Hypothalamic Deafferentation[1] (40296)

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Our laboratory recently reported that apomorphine, a drug that stimulates dopamine receptors, caused a transient (50–60 min) but marked inhibition of the episodic pattern of LH release normally observed in ovariectomized rats (1, 2). This effect is mediated by activation of dopamine receptors since pimozide and d-butaclamol, agents which block these receptors, prevent the inhibitory effect (1, 2). The present study was designed to determine if this inhibition is mediated by an initial activation of dopamine receptors within the hypothalamic-pituitary unit (3, 4), or outside of it in some other region of the brain with a significant dopaminergic input such as the neostriatum (3). Therefore, the effects of apomorphine on episodic LH release were determined in ovariectomized rats previously subjected to complete hypothalamic deafferentation in order to isolate the medial basal hypothalamus (MBH)-pituitary unit from the rest of the brain.

Materials and methods. Adult female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) weighing 260–280 g were maintained on a lighting schedule of 14 hr light: 10 hr darkness (light on 0500–1900 hr) and fed lab chow and water *ad libitum.* Daily vaginal smears were taken and only those rats showing two or more consecutive 4-day estrous cycles were used for experimentation.

Deafferentation of the MBH was performed with a small double-edged Halasz-type knife (5) of bayonet shape (dimensions: vertical 2.0 mm, radius 1.6 mm). Under sodium pentobarbital anesthesia (35 mg/kg bw), the animal's head was placed in a stereotaxic instrument with the ear bars 2.4 mm above the level of the tooth bar. After drilling a hole in the skull, the knife was lowered through the superior sagittal sinus to the base of the skull 8.3 mm anterior to the interaural line. The knife was first rotated to the right 90°, and then 180° to the left (to maximize the probability for completeness of the anterior section of the cut). The blade was next stereotaxically moved 3 mm posteriorly, and then rotated 180° to the right. It was then moved anteriorly 3.3 mm (to assure completeness). Finally, the blade was rotated 90° toward the starting position, and removed from the brain at the point of entry. Following deafferentation, vaginal smears were taken for 3 to 6 weeks after which time only those rats having shown either constant vaginal estrous or diestrous smear patterns for three weeks or more were ovariectomized.

Six weeks following ovariectomy a polyethylene cannula was inserted into the external jugular vein and used for collecting blood samples the following day. An additional cannula was placed subcutaneously in the animal's back for later drug administration. The next day, after an iv injection of 200 units heparin, unanesthetized, unrestrained animals were bled continuously through a piece of flexible tubing, one end of which was connected to the animal's cannula and the other end through a peristaltic pump to a microliter syringe kept on ice for the collection of blood samples. Fifty or 100 μl whole blood were collected every 5 or 10 min, respectively, and added directly to assay tubes (kept in an ice bath) containing 400 or 450 μl of phosphate buffered saline with 0.1% gelatin. After collecting blood samples for a 1½- to 2 hr-control period, animals were injected with apomorphine hydrochloride (a selective stimulator of dopamine receptors (6, 7), Merck Chem., Rahway, NJ, 1.5 mg/kg in saline) through the indwelling sc cannula. Bleeding was then continued for an additional 1 to 1½-hr period. Whole blood sam-

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ples were analyzed for LH by a slight modification (8) of the ovine-ovine rat LH double antibody radioimmunoassay of Niswender *et al.* (9). LH values (ng/ml whole blood) are expressed in terms of the NIAMDD Rat LH-RP-1 preparation which has a biological potency equivalent to 0.03 × NIH-LH-S1.

Following experimentation, rats were perfused with 10% formalin plus 1% calcium chloride. The extent of hypothalamic deafferentation was determined both by visual examination of the cut at the base of the brain as well as by close histological examination after sectioning brains at 50 μm in the transverse plane and staining with Nissls stain using basic fuchsin.

Results. Forty-five of 53 animals (85%) showed persistently leucocytic (constant diestrous) vaginal smear patterns for at least 3 weeks following hypothalamic surgery. The remaining eight rats (15%) exhibited persistent vaginal cornification (constant estrous) during this same period of time. No hypothalamic necrosis was observed in the great majority of animals subjected to deafferentation and later used for experimentation. The necrosis that was seen in a few rats involved only the extreme rostral or caudal sections of the deafferented tissue and never involved the arcuate nucleus-median eminence region. The pituitary glands of all experimental animals were not damaged by the knife. Additionally, no apparent histological differences with regard to the extent of deafferentation were discernible between constant estrous and constant diestrous animals (see Fig. 1). The deafferented tissue included all of the arcuate nucleus and median eminence, much of the ventromedial nucleus, and variable amounts of the dorsomedial nucleus. The posterior part of the suprachiasmatic nucleus was included within one side of the hypothalamic island in 2 of 8 constant estrous and 3 of 12 constant diestrous rats.

Twelve of the 45 rats displaying a persistently leucocytic smear pattern following hypothalamic deafferentation were randomly selected for bleeding 6 weeks after ovariectomy. In all 12 animals pulsatile LH release was absent and LH levels were very low (<28 to <110 ng/ml). The rat in constant diestrus, depicted in Fig. 1, had <28 ng LH/ml whole blood during a 3 hr bleeding period. Of the eight completely deafferented, constant estrous animals bled 6 weeks after ovariectomy, five exhibited pulsatile LH release during a 1½- to 2 hr-control period of bleeding (Fig. 2), though at somewhat reduced levels when compared with pulsatile LH release normally seen in ovariectomized rats. In the remaining three rats, problems occurred during the bleeding procedure in one, while the other two animals displayed either nonepisodic, low blood LH levels or only one LH pulse in the control period.

Apomorphine caused a stereotyped gnawing behavior pattern in ovariectomized rats with complete hypothalamic deafferentation, much as it does in intact or ovariectomized animals not subjected to hypothalamic surgery (1, 2, 6). This agent was administered to eight rats with complete hypothalamic deafferentation which previously had shown constant vaginal estrous smear patterns before ovariectomy. In the five rats having well defined episodic LH release patterns during the control period, apomorphine caused an inhibition (four rats) or reduction (one rat) of pulsatile LH secretion lasting at least 40–90 min. Three examples are given in Fig. 2. The extent of the cut in the middle animal represented in Fig. 2 is shown in the top of Fig. 1. The response to apomorphine could not be determined in the remaining 3 rats because of the reasons cited above.

Discussion. This study demonstrates that apomorphine, a specific dopamine receptor stimulating agent (6, 7), can exert an inhibitory effect on episodic LH release in ovariectomized rats previously subjected to complete hypothalamic deafferentation. We have previously observed this inhibition in ovariectomized animals not subjected to complete hypothalamic deafferentation (1, 2) and have shown that the sc injection of saline (1) or distilled water (2) into ovariectomized rats had no effect on episodic LH release. Furthermore, the sc injection of apomorphine into animals with hypothalamic deafferentation was accomplished through the use of an indwelling sc cannula connected to a sufficient length of flexible tubing to extend out of the animal's cage. Thus, the animals were unaware of any injection procedure. It appears from these and our previous data that the inhibition of episodic LH release caused

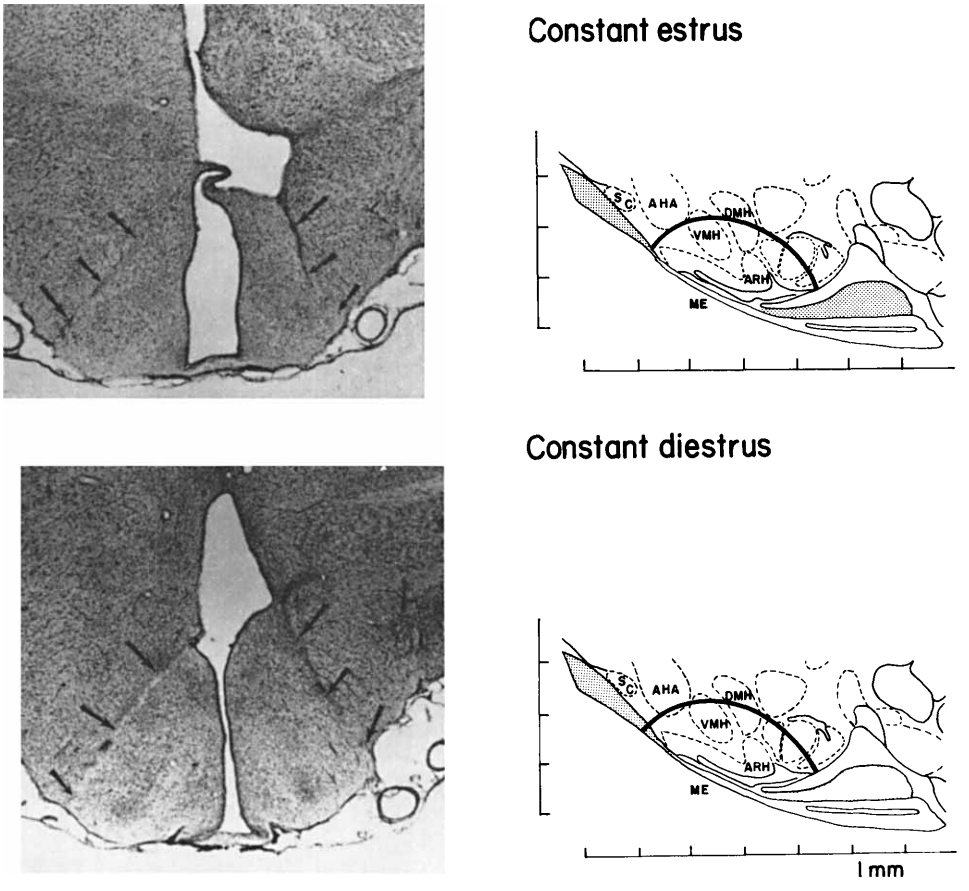

FIG. 1. Representative sagittal reconstructions indicating the extent of complete hypothalamic deafferentation in rats subsequently showing constant estrous or constant diestrous vaginal smear patterns. Actual brain cross sections for each of these animals are shown to the left. The arrows indicate the location of the knife cut.

by apomorphine is a result of activation of dopamine receptors within the medial basal hypothalamus (MBH) and/or pituitary gland, and not outside this region.

The postsynaptic dopamine receptors responsible for inhibition of episodic LH release are probably associated with neurons innervated either by dopaminergic neurons originating in the arcuate nucleus or within the substantia nigra, and both these areas send axonal projections to the median eminence (10–13). In this regard, the median eminence contains high concentrations of LHRH (14, 15), apparently within the terminals of LHRH neurons. It is possible that activation of dopamine receptors on these LHRH neurons may result in an inhibition of LHRH release. A hypothalamic site of action for apomorphine is suggested by the evidence that portal vein infusion of dopamine had no effect of LH release (16), while the in vitro pituitary secretion of LH was inhibited by dopamine only when the median eminence was included in the incubation (17). Alternatively, a pituitary site of action cannot be ruled out since dopamine receptors are present there (4).

It should be emphasized that the inhibition of episodic LH release by apomorphine could only be tested in those few hypothalamic-deafferented animals showing a constant vaginal estrous smear pattern, since only in these rats was episodic LH release present after ovariectomy. The vast majority of deafferented rats (85%) exhibited a constant vaginal diestrous smear pattern and in this type animal LH levels were very low and nonpulsatile after ovariectomy. Blake and Sawyer (18)

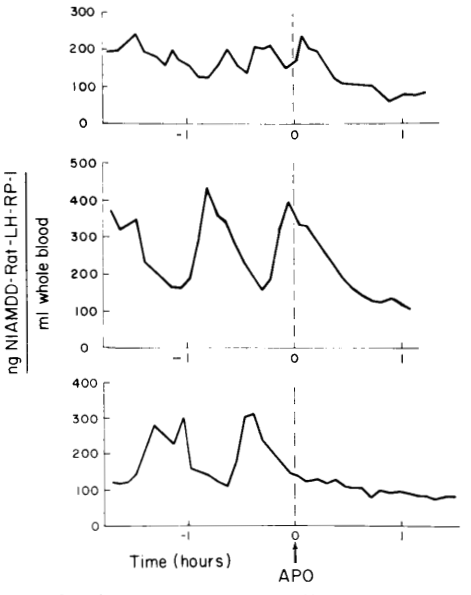

FIG. 2. Three examples of the effect of apomorphine (APO; 1.5 mg/kg sc) on episodic LH release in ovariectomized rats with complete deafferentation of the medial basal hypothalamus.

indicated that 5 of 11 animals subjected to complete deafferentation of the MBH had constant vaginal estrous smear patterns and episodic LH release after ovariectomy. These authors suggested on the basis of these animals that pulsatile LH secretion may possibly be inherent to the MBH-pituitary unit. In only a small percentage of the rats in the present report was the deafferented hypothalamic tissue capable of maintaining episodic LH secretion. In agreement with Blake and Sawyer (18), complete MBH deafferentation also had produced constant vaginal cornification in these rats. Inclusion of the suprachiasmatic nucleus within the deafferented region has been suggested to account for the persistence of LH secretion and this constant vaginal estrous smear pattern (19). In the present study the suprachiasmatic nucleus was anterior to, or destroyed by the knife cut in the large majority of rats in both groups. Moreover, even when a portion of this nucleus was included within the deafferented hypothalamic tissue in a few constant diestrous rats, very low, nonepisodic blood LH levels still resulted. Thus, the reason why some rats should continue to show episodic LH release while others do not, when the extent of hypothalamic deafferentation appears similar in both groups, is not clear at present. The absence of pulsatile LH secretion following MBH deafferentation may be due to severing the axons of LHRH neurons whose cell bodies lie outside the MBH and/or interrupting fibers stimulating LHRH synthesis and/or release. Complete deafferentation results in a large decrease in LHRH content in the rat MBH (20, 21). Furthermore, norepinephrine has been suggested to play an excitatory role in the regulation of LH secretion (1, 22–25) and the content of norepinephrine in the MBH is totally depleted by deafferentation (26). Nevertheless, afferent input to the MBH seems to be required in the great majority of rats to sustain episodic LH secretion. Moreover, dopamine receptors within the MBH-pituitary region seem responsible for mediating the inhibitory effect of apomorphine on pulsatile LH secretion.

Summary. Complete neural deafferentation of the MBH in 53 rats resulted in a constant vaginal diestrous smear pattern in 85% of the rats, and in this type animal very low blood LH levels and absence of episodic LH release followed ovariectomy. The remaining 15% had a constant vaginal estrous smear pattern, and most demonstrated pulsatile LH secretion following ovariectomy. Thus, afferent input to the MBH seems to be required in most rats to sustain episodic LH secretion. Administration of apomorphine, a dopamine receptor stimulator, to animals which were in constant estrus before ovariectomy, resulted in inhibition of pulsatile LH secretion, suggesting that this apomorphine-induced inhibition is a result of activation of dopamine receptors within, rather than outside, the MBH-pituitary unit.

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1. Drouva, S. V., and Gallo, R. V., Endocrinology **99,** 651 (1976).

2. Drouva, S. V., and Gallo, R. V., Endocrinology **100,** 792 (1977).
3. Fields, J. Z., Reisine, T. D., and Yamamura, H. I., Brain Res. **136,** 578 (1977).
4. Cronin, M., Roberts, J., and Weiner, R., Fed. Proc. **36,** 278 (1977).
5. Halasz, B., and L. Pupp, Endocrinology **77,** 553 (1965).
6. Anden, N. E., Rubenson, A., Fuxe, K., and Hokfelt, T., J. Pharmacol. **19,** 627 (1967).
7. Ernst, A. M., Psychopharmacologia **10,** 316 (1967).
8. Osland, R. B., Gallo, R. V., and Williams, J. A., Endocrinology **96,** 1210 (1975).
9. Niswender, G. D., Midgley, A. R., Jr., Monroe, S. E., and Reichert, L. E., Jr., Proc. Soc. Exp. Biol. Med. **128,** 807 (1968).
10. Fuxe, K., Acta, Physiol. Scand. **64** (Suppl.) 247, 37 (1965).
11. Bjorklund, A., Moore, R. Y., Nobin, A., and Stenevi, U., Brain Res. **51,** 171 (1973).
12. Palkovits, M., Brownstein, M., Saavedra, J. M., and Axelrod, J., Brain Res. **77,** 137 (1974).
13. Kizer, J. S., Palkovits, M., and Brownstein, M. J., Brain Res. **108,** 363 (1976).
14. Palkovits, M., Arimura, A., Brownstein, M., Schally, A. V., and Saavedra, J. M., Endocrinology **95,** 554 (1974).
15. Wheaton, J. E., Krulich, L., and McCann, S. M., Endocrinology **97,** 30 (1975).
16. Kamberi, I. A., Mical, R. S., and Porter, J. C., Endocrinology **87,** 1 (1970).
17. Miyachi, Y., Mecklenburg, R. S., and Lipsett, M. B., Endocrinology **93,** 492 (1973).
18. Blake, C. A., and Sawyer, C. H., Endocrinology **94,** 730 (1974).
19. Hayashi, S., Mennin, S. P., and Gorski, R. A., Neuroendocrinology **14,** 321 (1975).
20. Weiner, R. I., Pattou, E., Kerdelhue, B., and Kordon, C., Endocrinology **97,** 1597 (1975).
21. Brownstein, M. J., Arimura, A., Schally, A. V., Palkovits, M., and Kizer, J. S., Endocrinology **98,** 662 (1976).
22. Sawyer, C. H., Markee, J. E., and Hollinshead, W. H., Endocrinology **41,** 395 (1947).
23. Ojeda, S. R., and McCann, S. M., Neuroendocrinology **12,** 295 (1973).
24. Kalra, S. P., and McCann, S. M., Neuroendocrinology **15,** 79 (1974).
25. Krieg, R. J., and Sawyer, C. H., Endocrinology **99,** 411 (1976).
26. Weiner, R. I., Shryne, J. E., Gorski, R. A., and Sawyer, C. H., Endocrinology **90,** 867 (1972).

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