PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 168, 330–333 (1981)

Suppression by Naloxone of Rise in Plasma Growth Hormone and Prolactin Induced by Suckling (41281)

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Abstract. The effects of three different doses of the specific opiate antagonist, naloxone (0.2, 2.0, and 5.0 mg/kg body wt), on release of growth hormone (GH) and prolactin (PRL) induced by suckling were determined in postpartum lactating rats. Blood was collected from mother rats via an indwelling atrial cannula 8 hr after separation from their pups. Suckling for 30 min induced a 3-fold increase in plasma GH and more than a 10-fold increase in plasma PRL. Injection of naloxone into mother rats just prior to suckling by their pups produced significant inhibition of both GH and PRL release. Doses of 0.2 and 5.0 mg naloxone/kg body wt almost completely inhibited GH release, whereas an intermediate dose (2.0 mg/kg body wt) was partially effective. Inhibition of PRL release by naloxone was dose related, and the highest dose (5.0 mg/kg body wt) decreased plasma PRL values by 60–80%. These results suggest that the endogenous opioid peptides are involved in release of GH and PRL induced by the suckling stimulus in the rat.

The suckling stimulus in postpartum lactating rats has been shown to produce a rapid increase in prolactin (PRL) and growth hormone (GH) release (1). The endogenous opioid peptides similarly were demonstrated to elevate PRL and GH release in rats (2–4), and therefore may be involved in the suckling-induced release of these two hormones. A preliminary report by Ferland *et al.* (5) indicated that naloxone could partially inhibit the suckling-induced surge of PRL release in rats, but the effects of naloxone on GH release have not been studied. In the present investigation, the effects of several doses of naloxone on the suckling-induced release of GH and PRL were determined in postpartum lactating rats.

Materials and Methods. Sprague–Dawley virgin rats (3 months old) were obtained from Harlan Industries (Cumberland, Ind.), and housed in a temperature-controlled room (24 ± 1°) on a 14:10-hr light:dark cycle (lights on at 0700 hr). Food (Purina Laboratory Chow, Ralston Purina Co.) and water were provided *ad libitum*. Two weeks after arrival, three female rats were housed with one male per cage to induce pregnancy, and pregnant females were handled once daily to minimize possible stress from handling. In late gestation, they were placed in individual plastic cages with nesting material. On the second day postpartum, the litters were reduced to eight pups each and the mother rats were each implanted with an indwelling silastic atrial cannula (Dow Corning, Midland, Mich.) under light ether anesthesia. The free end of the cannula was brought underneath the skin and exited 1 cm posterior to the base of the skull. The mother rats began to nurse their pups within 1 hr after recovery from surgery. Cannulas were rinsed once daily with heparinized saline.

On the fifth day postpartum, the mother rats were randomly divided into four groups

[1] Aided by National Institute of Aging Postdoctoral Fellowship AG05147.

[2] Aided by National Institute of Aging Postdoctoral Fellowship AG05208.

[3] Aided by NIH Research Grants AG00416 from the National Institute on Aging, AM04784 from the National Institute of Arthritis, Metabolism and Digestive Diseases, and CA10771 from the National Cancer Institute. Published with the approval of the Michigan Agricultural Experiment Station as journal article No. 9762.

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0037-9727/81/110330-04$01.00/0

and separated from their pups for 8 hr, beginning at 0700 hr. The pups were covered with cotton to prevent loss of body temperature. One hour before naloxone or saline injection, silastic extensions were attached to the atrial cannulae and 200 IU of heparin was injected. Immediately after basal blood samples (0.6 ml) were drawn, three different doses (0.2, 2.0, and 5.0 mg/kg body wt) of the specific opiate antagonist, naloxone hydrochloride (Endo Labs, Garden City, N.J.), were injected iv via the cannulas and the mother rats were returned to their pups. These three doses previously were reported to reduce basal serum PRL and GH in male rats (2). They were suckled for 30 min, after which time they were again separated from their pups. Control rats were injected with the vehicle used for naloxone, 0.87% NaCl. Blood samples (0.6 ml) were removed 15, 30, and 60 min after initiation of suckling and replaced with an equal volume of physiological saline.

Plasma was immediately separated and stored at −20° until assayed. Plasma PRL and GH were measured by RIAs by the double antibody methods previously described (6, 7). Plasma PRL was assayed at two dilutions, each in triplicate, and plasma GH in a single dilution, in triplicate, using materials provided by NIAMDD. Data were expressed as nanograms of rat PRL-RP-1 or rat GH-RP-1 per milliliter of plasma. Analysis of variance and Student–Newman–Keul's test for multiple comparisons among groups were used to analyze the data. The level of significance chosen was $P < 0.05$.

Results. Plasma GH in the control rats increased rapidly in response to the suckling stimulus, from baseline values of 78 ng/ml to peak values of 262 ng/ml by 15 min after initiation of suckling (Fig. 1). Plasma GH returned to basal values by 60 min. Naloxone, when injected in a dose of 0.2 or 5.0 mg/kg body wt, almost completely inhibited GH release induced by suckling, whereas the intermediate dose (2.0 mg/kg body wt) was effective only at 15 min. There were no significant differences in plasma GH at 60 min between control and naloxone-treated rats.

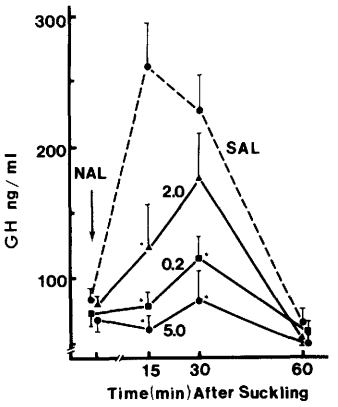

FIG. 1. Effects of saline (SAL) and naloxone (NAL; 0.2, 2.0, and 5.0 mg/kg body wt) on rise in plasma GH induced by the suckling stimulus. The values are the mean ± SEM of 15 or 16 rats, except in the group (n = 8) injected with 5.0 mg/kg body wt of NAL. The asterisk indicates significant differences from SAL-injected controls, $P < 0.05$.

Suckling also induced a significant rise in plasma PRL in the control rats as compared to the concentrations prior to suckling (Fig. 2). Plasma PRL rose for the first 30 min after the onset of suckling, reaching maximum levels of 840 ng/ml, and remained high for 60 min. Administration of naloxone prior to suckling, inhibited the suckling-

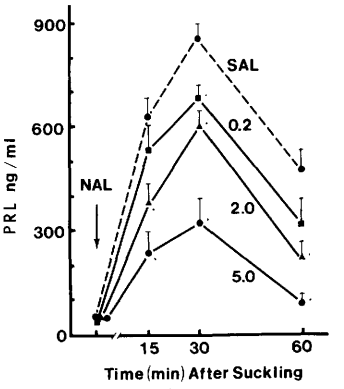

FIG. 2. Effects of saline (SAL) and naloxone (NAL; 0.2, 2.0, and 5.0 mg/kg body wt) on rise in plasma PRL induced by the suckling stimulus. The values are the mean ± SEM of 15 or 16 rats, except in the group (n = 8) injected with 5.0 mg/kg NAL. The asterisk indicates significant differences from SAL-injected controls, $P < 0.05$.

induced PRL rise in a dose-related manner. The low dose of naloxone (0.2 mg/kg body wt) only minimally inhibited PRL release, but doses of 2.0 and 5.0 mg/kg body wt reduced PRL levels significantly at all time intervals studied. The highest dose of naloxone (5.0 mg/kg body wt) reduced plasma PRL values by 60–80%.

No obvious changes were observed in suckling behavior of the pups or nursing behavior of the mother rats after injection of naloxone. The mother rats began nursing their pups within 3 to 5 min after being returned to their cages, as did the saline-injected controls.

Discussion. The rapid elevation of both plasma PRL and GH levels observed here after initiation of suckling in postpartum lactating rats is in good agreement with previous reports that suckling induces acute release of these two hormones in lactating rats (1). The present results clearly show that naloxone can inhibit the suckling-induced release of GH, as well as of PRL. This demonstrates that naloxone can inhibit GH release induced by a physiological stimulus in the rat. It is not clear why the 2.0 mg/kg body wt dose of naloxone was less effective than the 0.2 mg/kg body wt dose in preventing release of GH, but the highest dose used, 5.0 mg/kg body wt, was the most effective of all doses used and prevented any increase in plasma GH by suckling. Naloxone has been observed to inhibit GH release in response to administration of 2-deoxy-D-glucose in rats (8), and to exercise (9) or argine infusion (10) in human subjects. These reports, together with the present finding, demonstrate that naloxone can inhibit an acute increase in GH release induced by suckling and other kinds of stimuli.

The inhibition of the suckling-induced rise in plasma PRL by naloxone is in agreement with the previous observation of Ferland *et al.* (5). In addition, our results show that suppression of PRL release by naloxone is dose related, with the highest dose showing the greatest (60–80%) inhibitory effect, as was also true for GH release.

These actions of naloxone are believed to be due to a specific antagonism of the effects of endogenous brain opiates on GH and PRL release. Although actions have been attributed to naloxone other than its anti-opioid effects, including possible agonistic activity at high doses (11), our results, as well as those of others, indicate a consistent effect of opioid antagonists on modulating the release of pituitary hormones. The report by Shaar *et al.* (12) that a 0.2 mg/kg body wt dose of naloxone was optimal for inhibiting basal PRL and GH release in 29-day-old female rats, and that higher doses were ineffective, has not been duplicated in studies on adult rats (13–15). Thus, Rossier *et al.* (15) showed that even 10 mg naloxone/kg body wt depressed basal plasma PRL levels in adult male rats. Hypothalamic opiates have been shown to be reduced by the suckling stimulus in lactating rats (16, 17), which is consistent with our view that the brain opiates are involved in the suckling-induced rise in GH and PRL release.

The mechanisms by which naloxone inhibited the suckling-induced rise in GH and PRL release are not entirely clear at present, but its effects probably are mediated via hypothalamic neurotransmitters and hypophysiotropic hormones. Brain opiates have been shown to increase serotonin metabolism and to depress dopamine activity in the hypothalamus of rats (18–20). The suckling stimulus in postpartum rats has been shown to enhance hypothalamic serotonin metabolism (21–23). Since serotonin is known to increase both PRL and GH release (24), this could at least partly explain the rise in plasma PRL and GH produced by the suckling stimulus. The suckling stimulus may act first to increase hypothalamic opioid activity, resulting in a rise in serotonin metabolism, the latter in turn producing an increase in PRL and GH release. Serotonin is believed to promote PRL secretion by increasing release from the hypothalamus of a PRL-releasing factor, and may increase GH secretion either by increasing release of a GH-releasing factor or by decreasing release of somatostatin (24). The suckling-induced rise in brain opioid activity also may produce a

decrease in hypothalamic dopamine activity, which would evoke a rise in PRL but not GH release (24). Since naloxone has been consistently demonstrated to counteract the brain opiates on pituitary hormones release, its effects would be expected to be the opposite of those of the opiates and result in a reduction in GH and PRL release.

1. Meites, J., *in* "Breast Disease" (T. Marchant and N. Nyrijesy, eds.), p. 9. Raven Press, New York (1978).
2. Bruni, J. F., Van Vugt, D. A., Marshall, S., and Meites, J., Life Sci. **21**, 461 (1977).
3. Rivier, C., Vale, W., Ling, N., Brown, M., and Guillemin, R., Endocrinology **100**, 238 (1977).
4. Lien, E. L., Fenickel, R. L., Garsky, V., Sarantakis, D., and Grant, N. H., Life Sci. **19**, 837 (1976).
5. Ferland, L., Kledzik, G. S., Cusan, L., and Labrie, F., Mol. Cell. Endocrinol. **12**, 267 (1978).
6. Niswender, G. D., Chen, C. L., Midgley, A. R., Jr., Meites, J., and Ellis, C., Proc. Soc. Exp. Biol. Med. **130**: 793 (1969).
7. Sonntag, W. E., Steger, R. W., Forman, L. J., and Meites, J., Endocrinology **107**, 1875 (1980).
8. Okajima, T., Motomatsu, T., Kato, K., and Ibayashi, H., Life Sci. **27**, 755 (1980).
9. Spiler, I. J., and Molitch, M. E., J. Clin. Endocrinol. Metab. **50**, 516 (1980).
10. Morley, J. E., Baranetsky, N. G., Wingert, T. D., Carlson, H. E., Hershman, J. M., Melmed, S., Levin, S. R., Jamison, K. R., Weitzman, R., Chang, R. J., and Verner, A. A., J. Clin. Endocrinol. Metab. **50**, 251 (1980).
11. Sawynok, J., Dinsky, C., and LaBella, F. S., Life Sci. **25**, 1621 (1979).
12. Shaar, C. J., Frederickson, R. C. A., Dininger, N. B., and Jackson, L., Life Sci. **21**, 853 (1977).
13. Grandison, L., and Guidotti, A., Nature (London) **270**, 357 (1977).
14. Cicero, T. J., Schainker, B. A., and Meyer, E. R., Endocrinology **104**, 1286 (1979).
15. Rossier, J., French, E., Rivier, C., Shibasaki, T., Guillemin, R., and Bloom, F., Proc. Nat. Acad. Sci. USA **77**, 666 (1980).
16. Chen, C. L., Kumar, M. S. A., Liu, J. M., and Song, I. C., *in* "Prog. and Abstracts 62nd Annual Meeting of The Endocrine Society," p. 240, No. 663 (1980).
17. Panerai, A. E., Sawynok, J., LaBella, F. S., and Friesen, H. G., Endocrinology **106**, 1804 (1980).
18. Ferland, L., Fuxe, K., Eneroth, P., Gustafsson, J. A., and Skett, P., Eur. J. Pharmacol. **43**, 89 (1977).
19. Van Loon, G. R., and DeSouza, E. B., Life Sci. **23**, 971 (1978).
20. Meites, J., Bruni, J. F., Van Vugt, D. A., and Smith, A. F., Life Sci. **24**, 1325 (1979).
21. Kordon, C., Blake, C. A., Terkel, J., and Sawyer, C. H., Neuroendocrinology **13**, 213 (1973/74).
22. Gallo, R. V., Rabbi, J., and Moberg, G. P., Endocrinology **97**, 1096 (1975).
23. Mena, F., Enjalbert, A., Carbonell, L., Priam, M., and Kordon, C., Endocrinology **99**, 445 (1976).
24. Meites, J., and Sonntag, W. E., Annu. Rev. Pharmacol. Toxicol. **21**, 295 (1981).

Received April 15, 1981. P.S.E.B.M. 1981, Vol. 168.