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Prostaglandin $F_{2\alpha}$ Prostaglandin E_2, Progesterone, 20α-dihydroprogesterone and Ovarian 20α Hydroxysteroid Dehydrogenase Activity in Preparturient Pelvic Neurectomized Rats (40262)

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Several authors (1–3) have shown that bilateral pelvic neurectomy (PN) blocks parturition. Pelvic neurectomy in rats also prevents luteal activation normally induced as a result of mechanical or coital stimulation (1, 2). Neither pregnancy nor pseudopregnancy in PN rats occurs unless progesterone or prolactin (1, 3) is administered. Mechanical stimulation of the cervix provokes changes in electrical activity in the hypothalamus and the pelvic nerves are pathways involved in this response (4). Furthermore, there is evidence that the release of oxytocin subsequent to cervical stretching or stimulation (Ferguson reflex) occurs in rats (5, 6). Recently, prostaglandin $F_{2}\alpha$ (PGF) has been associated with this reflex by Flint *et al.* (7). These investigators showed that vaginal distention in pregnant sheep caused elevated uterine venous prostaglandin F (7). It has also been reported that vaginal distention results in a surge of oxytocin which precedes the elevation of PGF (8). Since the rat shows a drop in progesterone and a rise in PGF and 20α-dihydroprogesterone (20α-ol) on day 22 of pregnancy (9), and since preliminary data from our laboratory showed that ten pelvic neurectomized rats (5 autopsied on day 24 and 5 autopsied on day 26) had full term fetuses which were dead *in utero* with the mothers in dystocia, we hypothesized that afferent neural stimuli from the vagina and cervix uteri during the last days of pregnancy may play an important role in the initiation of endocrine events leading to normal parturition in the rat. Therefore, the purpose of this study was to examine the effects of PN on utero-ovarian vein (UOV) prostaglandin, progesterone and 20α-ol concentrations. We also studied the ovarian capacity to catabolize progesterone to 20α-ol by examining ovarian 20α-hydroxysteroid dehydrogenase (20α-HSD) activity in sham (S) versus PN rats.

Materials and methods. Female, nullipa-rous, Sprague-Dawley rats weighing 175–225g were housed under standard laboratory conditions on a 14- to 10-hr light dark cycle. Females were placed with males of proven fertility. The morning on which sperm or vaginal plug was noted was designated as day 1 of pregnancy.

On day 8–10 of pregnancy, the animal was laparotomized and pregnancy confirmed by decidual swellings. Sham surgery or bilateral PN was performed under ether anesthesia using the anatomical approach described by Carlson and De Feo (2). In the sham group, the pelvic nerves were exposed and touched. Complete PN was evidenced by the rat's inability to void an overly distended urinary bladder without manual assistance. When PN was observed as incomplete, animals were excluded from further study.

Pelvic neurectomized and sham-operated pregnant females were laparotomized on days 20, 21 and 22 between 900 to 1200 hr. An additional 11 PN females were laparotomized on days 23 and 24 of pregnancy.

In all rats, utero-ovarian vein (UOV) blood (5 ml) was collected into chilled heparinized tubes containing at least 80 μg of indomethacin. The tubes were placed on ice and centrifuged within 5 min. The plasma was then stored at $-60°$ until assayed.

All reagents used in the radioimmunoassay of progesterone, 20α-ol, PGF and PGE have been described previously (10–15). The only exception, in the case of PGE, was that a highly specific antibody to PGE_2 was obtained from Dr. F. Dray, Pasteur Institute, Paris (14). This antibody has been tested for specificity in our laboratory and agrees with already published results by Dr. Dray (14). Solvent blank values for progesterone, 20α-ol, PGF and PGE were consistently less than 5 pg per assay tube respectively and were not significantly different from zero. The inner assay recovery of progesterone, 20α-ol, PGF

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and PGE, of 500 pg/ml of blood obtained from bilaterally gonadectomized male rats was 442 ± 51 pg/ml, 526 ± 52 pg/ml, 496 ± 35 pg/ml and 481 ± 30 pg/ml, respectively (mean ± SD, $N = 5$). The combined inter and intra assay coefficients of variation for progesterone, 20 α-ol, PGF and PGE averaged 12% and 20%, respectively.

Ovarian 20α-HSD activity was visualized with the histochemical technique of Balogh (16) using 20α dihydroprogesterone as substrate.

Numerical data were analyzed by one-way analysis of variance and grouped comparisons were made by the Newman-Keuls test (17).

Results. Pregnancy, in 10 sham-treated animals which had been allowed to go to parturition, averaged 22 days. Parturition had not occurred in 5 PN rats autopsied on day 24 and 5 PN rats autopsied on day 26. On days 24 and 26, we found the PN animals had, predominantly, full term, dead fetuses, some of which had died in the birth canal. We also found a wide range of fetal weight in the PN animals 5.76 g ± 1.23 g, ($N = 34$, mean ± SEM). In one case, after local anesthesia and caesarean section on day 24, we delivered 10 live animals which soon succumbed to apparent respiratory distress. Pelvic neurectomized females had a reduced number of live fetuses on days 20, 21 and 22. In PN animals there were 8.50 ± 0.538 fetuses/mother, ($N = 28$); 9.52 ± 0.311 fetuses/mother, ($N = 24$, $P < 0.08$) in S animals. There was also a wider range in fetal numbers (3–14 in PN, *vs.* 8–13 in S) per litter.

In S animals, progesterone values dropped significantly ($P < 0.01$) on day 22 (Fig. 1). In contrast, progesterone values were unchanged in PN animals on days 20, 21 and 22 (Fig. 1), but had declined by day 23/24.

There was no change in PGE concentration in either the S or PN group on the days studied. Values ranged from 0.795 ± 0.23 to 1.18 ± 0.19 ng/ml, and were not significantly different either by treatment or by day when tested by analysis of variance and the SNK test. On the other hand, PGF in S animals was significantly elevated ($P < 0.05$) (Fig. 2). In contrast, PGF in PN rats was unchanged on days 20, 21, 22 and 23/24. (Fig. 2).

The results of the 20α-HSD histochemistry correlated well with the 20α-ol radioimmunoassay values (Fig. 3). Low 20α-HSD activity reflected by reduced intensity and density of diformazan reaction product, was characteristic of both S and PN rats on days 20 and 21 (Fig. 4a). In contrast, high 20α-HSD activity, indicated by increased intensity and density of reaction product, was noted in S animals on day 22 (Fig. 4b) and PN animals on days 22 (Fig. 4c), 23 (Fig. 4d) and 24.

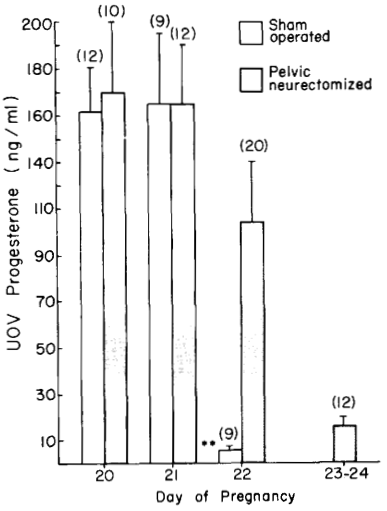

FIG. 1. Utero-ovarian vein (UOV) progesterone in sham operated or pelvic neurectomized rats. The column represents average ± standard error of the mean. The number in parenthesis represents animals in each group. ** $P < 0.01$.

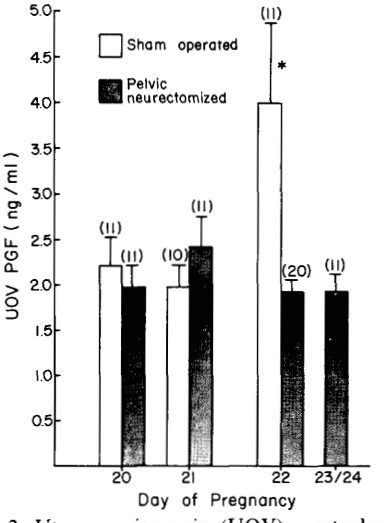

FIG. 2. Utero-ovarian vein (UOV) prostaglandin F in sham operated or pelvic neurectomized rats. The column represents average ± SE of the mean. The number in parenthesis represents animals in each group. * = $P < 0.05$.

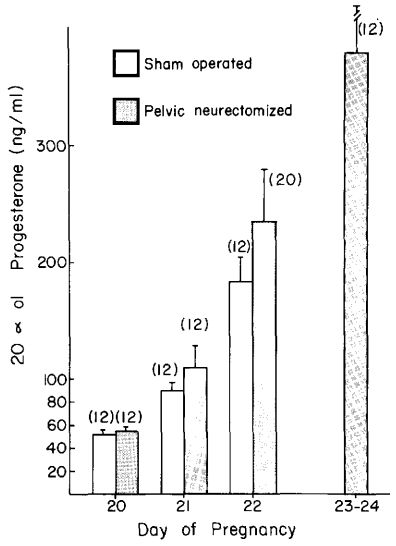

FIG. 3. Utero-ovarian vein (UOV) 20α-ol progesterone in sham operated or pelvic neurectomized rats. The column represents average 1 standard error of the mean. The number in parenthesis represents animals in each group.

Discussion. Previous papers have reported that pregnancy was prevented in female rats when the pelvic nerves were bilaterally sectioned prior to mating with fertile males (1–3). However, Spies *et al.* (3) showed that when bilateral neurectomy was performed 30 min after coitus normal pregnancies resulted, although parturition was blocked. In our studies, we confirmed that parturition was blocked at least until days 24 and 26 of pregnancy in animals that had been pelvic neurectomized on day 8 of pregnancy. This appears to be in agreement with previous papers (1–3). This block in parturition supports the hypothesis that there is a neuroendocrine reflex associated with initiation of parturition (3). Stimulation of the genital area, including the vagina and cervix uteri, results in impulses which are conveyed via the pelvic nerves into the central nervous system (CNS). These impulses terminate on hypothalamic neurons (18, 19) and presumably effect the release of gonadotrophins and oxytocin (6–9).

Earlier workers have suggested that prolonged and delayed parturition in PN rats could be attributed to delayed implantation (1). The results of the present study seem to rule out this possibility since implantation was well established, as evidenced by prominent decidual swellings when neurectomy was performed.

While there is no direct evidence presented in this paper that oxytocin release is blocked, others have shown a positive relationship between increased oxytocin and PGF in the preparturient period, with the oxytocin surge preceding the rise in PGF (8). Reflex secretion of oxytocin after vaginal distention (5) is likely to be most important in parturition, when the fetal head and forelimbs distend the vagina in the second stage of labor. In view of the endocrinological interrelationships in the preparturient period, we hypothesized that pelvic neurectomy blocks oxytocin release, which in turn, blocked the normal rise in PGF. It is interesting that PGF values do not rise even on days 23 and 24 in PN animals, when progesterone has declined. We originally hypothesized that the low levels of PGF in PN animals on day 22 were insufficient to produce a drop in progesterone on day 22. However, this hypothesis does not explain the reduced progesterone values in UOV blood on days 23 and 24, in the face of continued low levels of PGF. These observations prompt the suggestion that factors in addition to PGF may contribute to luteolysis and the initiation of parturition in the rat.

In normal pregnancy, there is a dramatic increase in luteal 20α-HSD activity in the preparturient period (20). This enzyme converts progesterone to 20α dihydroxyprogesterone and, thus, decreases the levels of circulating progesterone at this time. We originally hypothesized that the sustained progesterone levels in PN rats on day 22 may have resulted from a failure of induction of ovarian 20α-HSD in these animals. This did not prove to be the case since both S and PN rats (Fig. 2) showed a concurrent progessive increase in UOV 20α-ol and ovarian 20α-HSD activity on day 22.

It is difficult to reconcile these data with the conclusions of Wiest *et al.* (20) that progesterone catabolism to 20α-ol constitutes a normal regulatory mechanism for reducing progesterone levels prior to parturition. Recent evidence has shown that in the rat, uterine myometrium gap junction formation is a necessary prerequisite for successful parturition (21). Cutting the pelvic nerve may prevent the formation of these gap junctions. In any case, it appears that the pelvic nerve

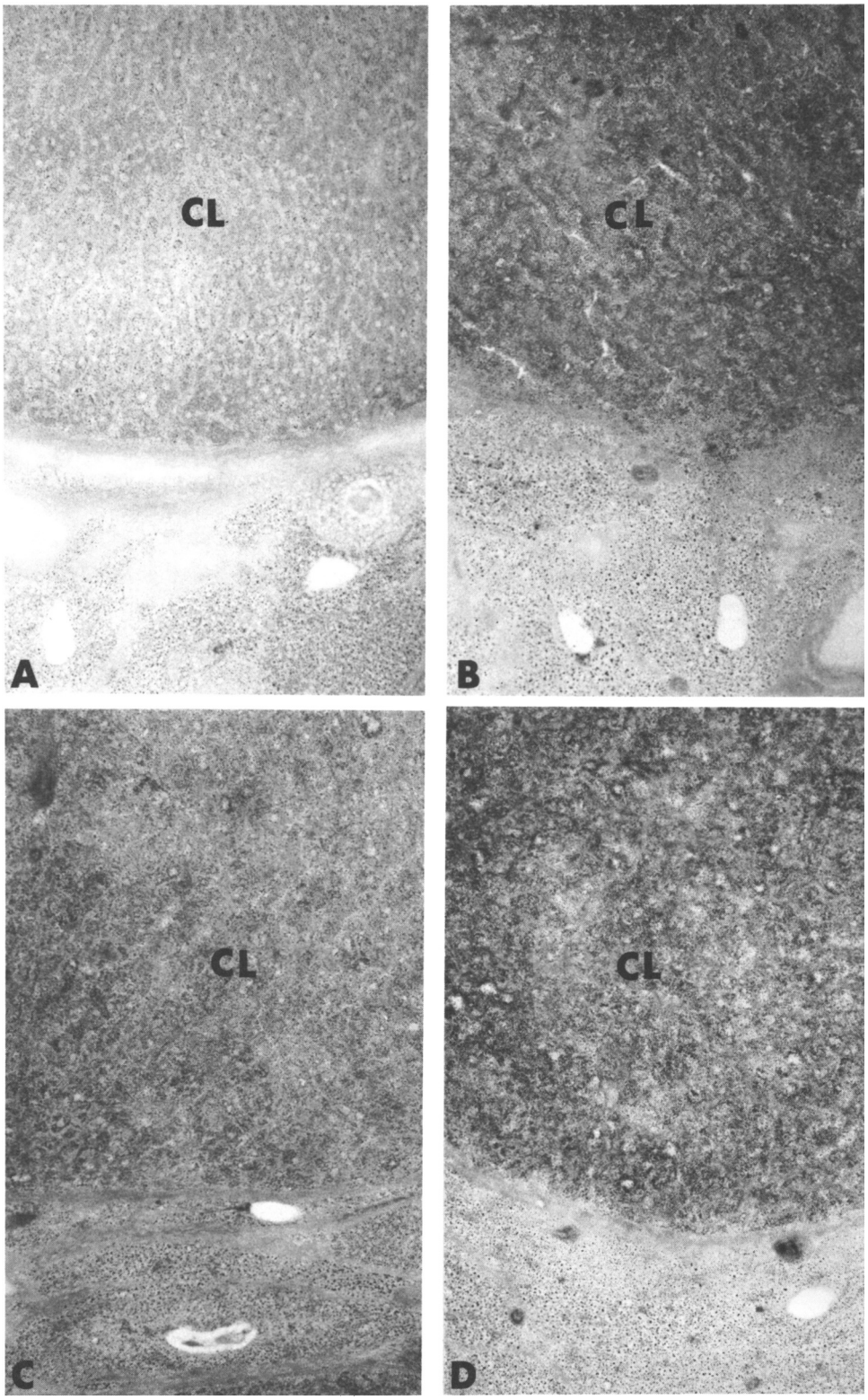

FIG. 4. Photomicrograph of 20α-hydroxysteroid dehydrogense activity with 20α-dihydroprogesterone substrate in sections of rat ovary. Note the density of diformazan reaction product in the corpus luteum (CL) × 120. A. Sham operated, day 21 of pregnancy. B. Sham operated, day 22 of pregnancy. C. Pelvic neurectomized, day 22 of pregnancy. D. Pelvic neurectomized day 23 of pregnancy.

orchestrates the endocrine and uterine events leading to parturition in the rat.

While our finding of an increase in UOV PGF is in basic agreement with another report indicating an increase in peripheral plasma $PGF_{2\alpha}$ (22) on day 22, we did not find a change in UOV PGE_2 in either sham or PN rats. We had expected to find an increase in PGE_2 since others had shown high concentrations of PGE_2 (23, 24) in both rats and humans at parturition and since one study in women had shown an increase with advancing cervical dilation (23). Ideally, we would have expected PGE_2 to rise in the sham but not the PN rats but this was not the case. We have no good explanation for this.

In conclusion, the results of this study provide evidence for a neuro-humoral reflex which is mediated via the pelvic nerves during the preparturient period in the rat. This reflex has an influence, ultimately, on utero-ovarian progesterone and PGF concentrations. Further studies are now being carried out in our laboratory to determine the mechanism(s) by which pelvic neurectomy blocks parturition.

Summary. Several workers have reported that section of the pelvic parasympathetic nerves [pelvic neurectomy (PN)] in pregnant rats is compatible with pregnancy, but parturition is blocked. The cause of this blocked parturition remains unexplained. To determine if PN had a possible action on progesterone and prostaglandin, two endocrine mediators of labor, we neurectomized (PN) or sham-operated (S) rats on days 8–10 of pregnancy. Then on days 20, 21, 22, 23 and 24 (PN only) rats were lightly etherized and utero-ovarian vein (UOV) blood was collected, centrifuged and plasma frozen and stored at −60° until radioimmunoassay for progesterone, 20α-dihydroprogesterone (20α-ol), prostaglandin $F_2\alpha$ (PGF), and prostaglandin E_2 (PGE). One ovary from each rat was immersed in a BEEM capsule containing OCT compound, frozen and stored at −20° for 20α-hydroxysteroid dehydrogenase (20α-HSD) histochemistry.

Analysis of variance indicated a significant ($P < 0.01$) decline in plasma progesterone in the S animals on the days studied but the PN animals remained unchanged through day 22. Student's Newman-Keuls (SNK) analysis for multiple critical values indicated that plasma progesterone on days 20 and 21 was not different in the S animals but declined ($P < 0.01$) on day 22. Progesterone also declined on days 23 and 24 in PN animals. There was a significant increase ($P < 0.05$) between day 20, 21 and 22 plasma PGF in the S but no change in the plasma PGF in the PN animals on any of these days or on days 23 and 24. SNK analysis showed increased PGF ($P < 0.05$) on day 22 in the S animals. But UOV PGE did not change in the PN or S groups. The density and intensity of ovarian 20α-HSD reaction product was low in both S and PN rats on days 20 and 21. Increased density and intensity of ovarian 20α-HSD reaction product was characteristic of five animals on day 22 and PN rats on days 22, 23 and 24. Collectively, these results indicate that the pelvic nerves participate in the orchestration of endocrine and uterine events in the preparturient and parturient periods in the rat.

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1. Kollar, E. J., Anat. Rec. **115**, 641 (1953).
2. Carlson, R. R., and V. J. DeFeo; Endocrinology **77**, 1014 (1965).
3. Spies, H. G., Forbes, Y. M., and Clegg, M. T., Proc. Soc. Exp. Biol. Med. **138**, 470 (1971).
4. Barraclough, C. A., and Cross, B. A., J. Endocrinology **26**, 339 (1963).
5. Ferugson, J. K. W., Surgery Gynec. Obstet. **73**, 359 (1941).
6. Moos, F., et Richard, Ph., J. Physiol. Paris **70**, (1975).
7. Flint, A. P. F., Anderson, A. B. M., Patten, P. T., and Turnbull, A. C., J. Endocrinology. **63**, 67 (1974).
8. Labhsetwar, A. P., and Watson, D. J., Biol. Reprod. **10**, 103 (1974).
9. Flint, A. P. F., Forsling, M. L., Mitchell, M. D., and Turnbull, A. C., J. Reprod. Fert. **43**, 551 (1975).
10. Challis, J. R. G., Davis, I. J., and Ryan, K. J., Prostaglandins **4**, 509 (1973).
11. Mitchell, M. D., Flint, A. P. F., and Turnbull, A. C., Prostaglandins **11**, 319 (1976).
12. Louis, T. M., Parry, D. M., Robinson, J. S., Thorburn, G. D., and Challis, J. R. G., J. Endocrinol. **73**, 427 (1977).
13. Challis, J. R., G., Dilley, S. R., Robinson, J. S., Thorburn, G. D., Prostaglandins **11**, 1041 (1976).
14. Dray, F., Charbonnel, B., and Maclouf, J., Eur. J.

Clin. Invest. **5,** 311 (1975).
15. Torjesen, P. A., Aadvaag, A., Acta Endocrinologica **86,** 162 (1977).
16. Balogh, K., J. Histochem. Cytochem. **12,** 670 (1964).
17. Weiner, B. J., Statistical Principles in Experimental Design 2nd. ed. pp. 185 New York: McGraw (1971).
18. Everett, J. W., and Quinn, D. L., Endocrinology **78,** 141 (1966).
19. Kawakami, M., and Kubo, K., Neuroendocrinology **7,** 65 (1971).
20. Wiest, W. G., and Kidwell, W. R. *in* "The Gonads" (K. W. McKerns, ed.), pp. 295 Appleton-Century-Crofts, New York (1969).
21. Garfield, R. E., Sims, S., Daniel, E. E., Science **198,** 958 (1977).
22. Shaikh, A. A., Nagvi, R. H., and Saksena, S. K., Prostaglandins **13,** 311 (1977).
23. Keirse, M. J. N. C. and A. C. Turnbull; J. Obs Gynec. B. & Comm. **80,** 970 (1973).
24. Dray, F., and Frydeman, R., Amer. J. Obstet. Gynecol. **126,** 73 (1976).

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