EFFECT OF PREGNANCY AND SEX HORMONES ON THE TRANSMITTER LEVEL IN UTERINE SHORT ADRENERGIC NEURONS

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COMBINED fluorescence histochemistry and chemical determinations have shown that NE* in the mammalian uterus is stored exclusively in neurons (SJÖBERG, 1967). DA is found in small amounts only in the cervix region (SWEDIN and BRUNDIN, 1968) where it probably is contained in chromaffin cells (OWMAN *et al.*, 1973b). The presence of E has for a long time been controversial. There is reason to believe that the uterine E reported by some investigators is not neuronal, but derives either from the blood or from a population of chromaffin cells in or near the utero-vaginal ganglion (OWMAN *et al.*, 1973b). Thus, among uterine catecholamines, only NE is located in neurons, which supply both myometrial smooth muscle cells and uterine blood vessels. Denervation experiments have established that the rich myometrial adrenergic innervation originates in peripheral sympathetic ganglia located in the utero-vaginal junction, thus belonging to the system of "short adrenergic neurons" (OWMAN *et al.*, 1973c). The vasomotor sympathetic nerves, on the other hand, arise from the paravertebral sympathetic chain. Besides constituting a special anatomical entity, the short adrenergic neurons are unique also in a number of functional respects: they are largely unaffected by postnatal administration of nerve growth factor antiserum, they are resistant to the effect of α-methyltyrosine, reserpine, 6-OH-DA and certain sympathetic ganglion stimulants, and they are difficult to deplete by nerve stimulation (for references, see OWMAN *et al.*, 1973c). The special features of the neurotransmission mechanisms of short adrenergic neurons have been investigated in detail by SWEDIN (1971). One of the functional aspects that make the short adrenergic neurons different from classical sympathetic nerves is their susceptilibility to the influence of sex hormones, which can be elucidated by, for example, measurement of the total uterine NE content under various experimental conditions (see SJÖBERG, 1967; OWMAN *et al.*, 1973c).

A hormonal dependence can be demonstrated already in connection with the critical period of postnatal differentiation of the reproductive organs. Experiments to show this were based on the ability of a single postnatal injection of testosterone propionate to produce masculinisation of the female reproductive tract, probably by a primary effect on the hypothalamus (see JACOBSOHN, 1965). Similarly, castration of male animals shortly after birth induces a female pattern of the gonadotrophin control mechanisms (see HARRIS, 1964). These abnormalities are persistent and modify the further growth and development of the reproductive organs. For example, the cyclic events are abolished in these organs of the females which become sterile.

* Abbreviations used: DA, dopamine; E, epinephrine; HCG, human chorionic gonadotrophin; NE, norepinephrine.

and the normal androgenisation of the male is prevented. The early hormonal influence on the short adrenergic neurons (BROBERG, NYBELL, OWMAN, ROSENGREN and SJÖBERG, unpublished observations) was shown by castration of male rats immediately after birth, which markedly reduces the NE content in vas deferens (innervated by short adrenergic neurons). This reduction is more pronounced than if castration is performed on adult animals. Early androgenisation of female rats does not retard the normal increase in weight of the uterus, but the total organ content of NE is significantly reduced compared to untreated controls, both when measured in 9-week- and 13-week-old animals (Fig. 1). Thus, a single dose of testosterone propionate or postnatal castration appeared to permanently lower NE in short adrenergic neurons of uterus and vas deferens, respectively. The neurons hence seem to form a separate target system for those humoral factors which, via the early differentiation of the hypothalamus, determine the pattern of development of the reproductive tract. Hypothalamic NE does not appear to be affected by neonatal adrogenisation (or castration), whereas a significant reduction of the amine occurs in brain cortex after early androgenisation of female rats (HYYPPÄ and RINNE, 1971).

The short adrenergic neurons exhibit pronounced changes in their transmitter content also during pregnancy and after administration of female sex hormones. Such changes do not take place in organs, such as heart and ovary, supplied with the classical type of long adrenergic neurons. In a recent study, fluctuations in uterine NE were followed fluorometrically throughout pregnancy (OWMAN *et al.*, 1973a) and during the *post partum* period (GÅRDMARK *et al.*, 1971) in guinea-pigs. These animals were chosen because they often have unilateral pregnancies, which means that one of the uterine horns is not affected by the mechanical strain from the growing conceptus. In all uterine horns containing foetuses, the variation in the NE level (determined as total amine concent per uterine horn by which changes in uterine weight can be disregarded) during the gestation period were similar irrespective of whether pregnancy was bilateral or unilateral. Thus, within the first 10 days the NE content was almost doubled (Fig. 2). From the 15th day onward, the amine level showed a continuous decrease until a near-zero content was reached just before parturition, which occurs at approximately 65 days *post coitum*. A similar NE increase was found in the uterine horn devoid of foetuses from animals with unilateral pregnancy. However, this enhanced amine level remained constant until the 50th day of pregnancy, i.e. about 2 weeks before term. During these last 2 weeks, NE in the horn fell to the same minimum amounts as in the foetus-containing horns. At term, histochemically visible adrenergic nerves were present only in the cervix. Immediately after parturition (GÅRDMARK *et al.*, 1971), the NE level in the uterine horns that had contained foetuses increased transiently to have a value that was, however, still less than half of the level in nonpregnant animals. It is possible that this reflected a shortlasting rise in the level of circulating NE, resulting in an uptake into the adrenergic nerves. Uterine NE then returned to lower values, as seen one week *post partum*, after which NE started to normalise progressively. Thus, two weeks after birth, the NE content was slightly but significantly higher than the values from pregnant animals. Even one month after pregnancy, uterine NE was still about 40 per cent lower than in nonpregnant animals. The original nonpregnant level of NE in the organ was not restored until within a further 5 months. Uterine NE increased progressively during the post partum period also in the horn that had been devoid of conceptus, though the time-course

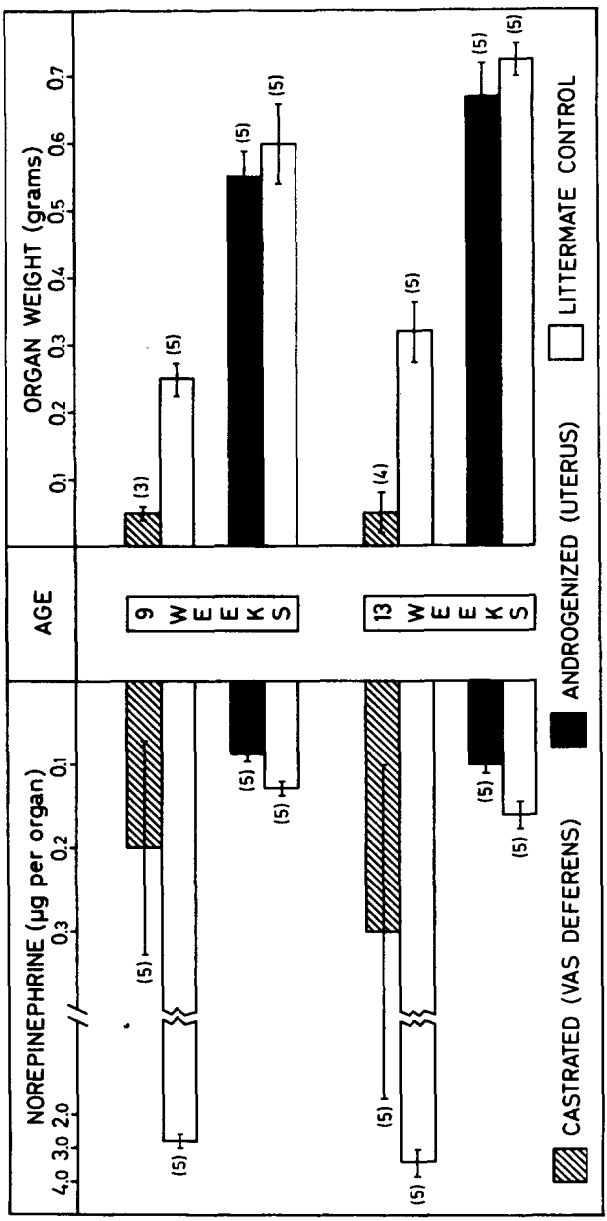

FIG. 1.—Changes in total organ content of NE and organ weight of uterus and vas deferens of 9- and 13-weeks-old rats. The females received 1·25 µg testosterone propionate 5 days after birth, the males were castrated 24 hr *post partum*. Mean ± S.E.M., number of determinations within parentheses.

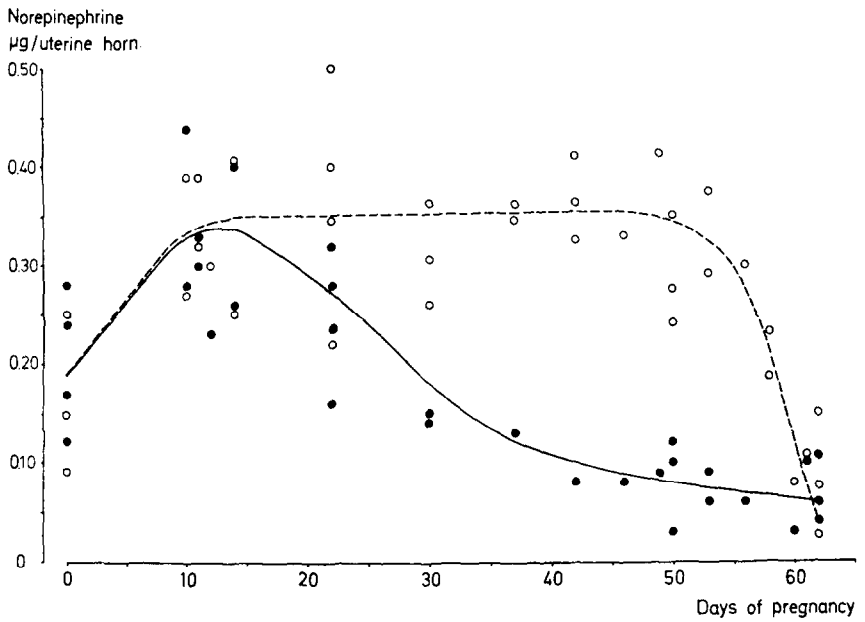

FIG. 2.—Fluorometrical determinations of total organ content of NE at various stages of unilateral pregnancy in guinea-pigs. Symbols indicate separate values from uterine horn containing foetuses (●——●) and devoid of foetuses (○- - -○).

was different from the horn which had contained foetuses: NE initially recovered more rapidly in the former horn, and during the following period there was a tendency of this horn to contain more NE than in the contralateral horn. This difference was statistically significant up to two weeks after delivery, but became less evident from 3 weeks onward.

In the fluorescence microscope (GÅRDMARK *et al.*, 1971), the high NE level at the beginning of pregnancy has been found to correspond to an increase in the total number of myometrial fluorescent nerves, whose intensity was the same or even higher than in nonpregnant animals (ROSENGREN and SJÖBERG, 1968). This picture may represent a true increase in the number of fluorescent nerve terminals and/or an increment in the transmitter content of already existing adrenergic fibres with a NE level too low for fluorescence microscopic detection. In any event, the elevation in uterine NE appears to be the result of a humoral rather than a mechanical factor because it took place in early pregnancy when the conceptus is still small, and it was equal in both horns whether containing foetuses or not. There was evidence from the measurements of the post partum recovery of NE that nerve fibres in the uterine horn which has carried foetuses were damaged and degenerated, probably as a consequence of the distension caused by the growing conceptus. This would mean that a reduction in the number of adrenergic nerves may have contributed to or, as pregnancy advanced, even been entirely responsible for the fall in the NE content found from about 10 days onwards. In the uterine horn devoid of foetuses and therefore not affected by mechanical trauma, the NE accordingly remained constantly high. The inconspicuous enlargement of this horn also at the end of pregnancy indicates that mechanical trauma is not responsible for the rapid decline of NE in the horn during the last 2 weeks before parturition. Probably, the decline is instead the result of a hormonal influence.

The assumption that the NE changes in the uterine horn lacking foetuses reflects a humoral effect on the short adrenergic neurons with little contribution from mechanical factors, is favoured by the observations that the pattern of NE changes during pregnancy was very similar in the cervix region. In the uterine horn that had been devoid of foetuses, the return of the NE was more rapid probably since it involved only a "refilling" of NE into intact adrenergic nerve fibres, whereas in the horns that had contained foetuses the recovery was slower, particularly during the first 1–3 weeks *post partum*, because it included also regenerative changes in damaged neurons.

An increase in uterine NE of the same magnitude as that seen at the beginning of pregnancy could be induced by daily treatment of normal or oophorectomised animals with 0·5 μg/kg of 17-β-estradiol benzoate. The high NE content was reached already within a week's estrogen administration (SJÖBERG, 1967; FALCK *et al.*, 1969a and b). A very characteristic finding at the microscopic level was that the total number of fluorescent myometrial adrenergic nerves had increased, and their fluorescence intensity was distinctly higher than in the controls, which sometimes gave them "clumsy" appearance. No effect was found either histochemically or chemically in ovarian or cardiac NE, whereas the catecholamine content changed in a manner resembling that of the uterus also in the vagina and oviduct (SJÖBERG, 1967; FALCK *et al.*, 1969a and b), which are supplied by short adrenergic neurons (OWMAN *et al.*, 1966). The estrogen-induced increment in uterine NE remained unaltered for more than 2 weeks following cessation of the treatment. However, when estrogen administration

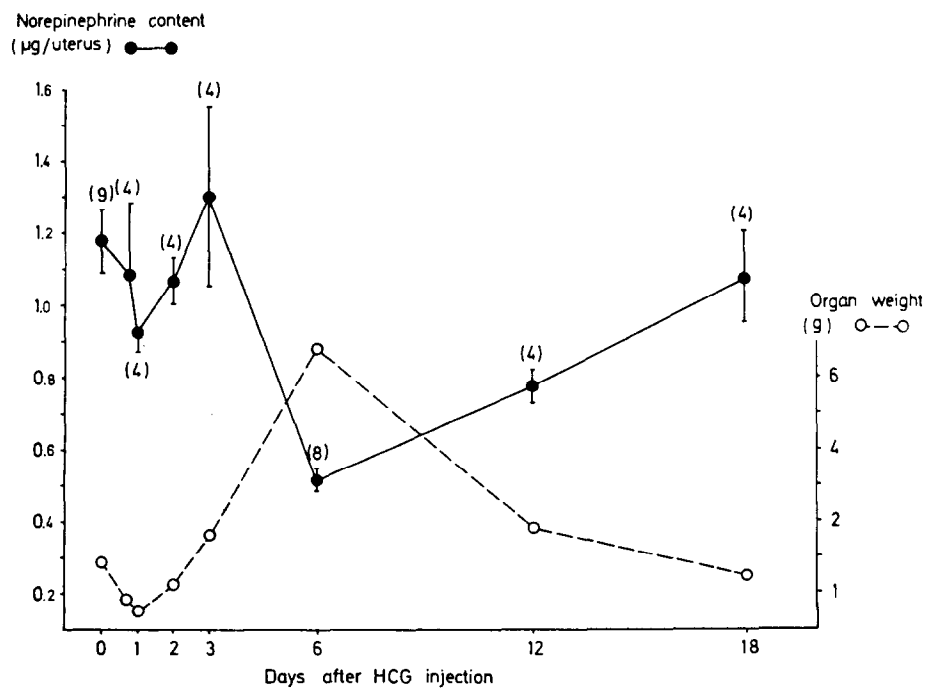

FIG. 3.—Effect of pseudopregnancy in rabbits. Total uterine content of NE (mean ± S.E.M., number of determinations within parentheses; one uterus per determination) and mean uterine weight at various time-periods after a single i.v. injection of 1500 i.u. of HCG.

was either combined with, or followed by, daily injections with 2 mg/kg of progesterone the organ content of NE returned to normal, or even subnormal, values within a week (FALCK *et al.*, 1969b). Results from two subsequent experiments offer further support for the view that uterine short adrenergic neurons are under hormonal control, and they indicate that the ovary is involved in the control mechanism. One is that HCG-induced pseudopregnancy within 3 days after the injection causes an abrupt fall in the NE content of the uterus, a mimimum amount being reached in the course of a further 3 days (Fig. 3). The amine then progressively returns to normal values, which are attained within the life-span of the progesterone-producing corpus luteum. A second

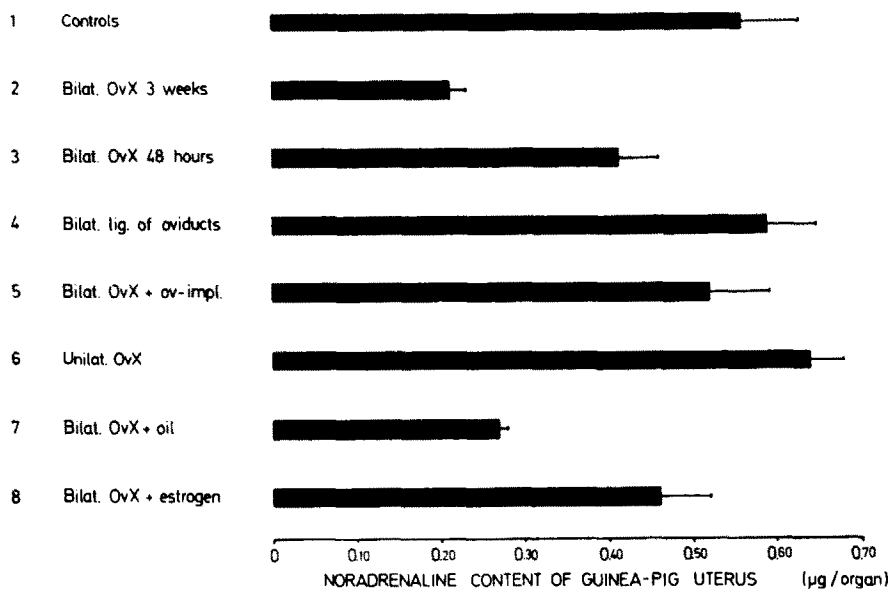

FIG. 4.—Fluorometric determinations of total uterine content of NE in guinea-pigs after bilateral or unilateral oophorectomy (OvX), with or without reimplantation of ovarian tissue, or daily injection of 0·5 μg/kg 17-β-estradiolbenzoate. Values from untreated controls, animals with bilateral ligation of the oviducts and animals treated with estrogen solvent (peanut oil) are also indicated. One uterus was used for each determination on 5–30 animals in each experiment. Mean + S.E.M.

experiment showed that the 60 per cent reduction in the total content of NE in the uterus produced by bilateral oophorectomy can be either counteracted by re-implantation of the excised ovarian tissue, or restored by estrogen administration during the third week after the oophorectomy (Fig. 4).

The female sex steroids tested caused only a slight, if any, change in the NE level of the male genital organs (OWMAN *et al.*, 1970). It may be that the hormones do not act directly on the noradrenaline metabolism of the short adrenergic neurons, but via a factor that is present only in the female. Also testosterone is without any overt effect (RYD and SJÖSTRAND, 1967; SJÖSTRAND and SWEDIN, 1970) although castration has been found to inhibit the increase in the organ content of NE normally seen with advancing age.

CONCLUSION

It is suggested that the hormonal influence on the level of neuronal NE in the anatomically and functionally unique system of short adrenergic neurons in the

reproductive tract constitutes a peripheral neuro-endocrine mechanism involved in the motor function of the oviducts and uterus.

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REFERENCES

FALCK B., OWMAN CH., ROSENGREN E. and SJÖBERG N.-O. (1969a) *Acta Endocrinol. (Kbh.)* **62,** 77–81.
FALCK B., OWMAN CH., ROSENGREN E. and SJÖBERG N.-O. (1969b) *Endocrinology* **84,** 958–959.
GÅRDMARK S., OWMAN CH. and SJÖBERG N.-O. (1971) *Am. J. Obstet. Gynecol.* **109,** 997–1002.
HARRIS G. W. (1965) *Endocrinology* **75,** 627–648.
HYYPPÄ M. and RINNE U. K. (1971) *Acta Endocrinol. (Kbh.)* **66,** 317–324.
JACOBSOHN D. (1965) *Acta Univ. Lund II* **17,** 1–19.
OWMAN CH., ROSENGREN E. and SJÖBERG N.-O. (1966) *Life Sci.* **5,** 1389–1396.
OWMAN CH., ROSENGREN E. and SJÖBERG N.-O. (1973a) *Am. J. Obstet. Gynecol.*, in press.
OWMAN CH., ROSENGREN E., SJÖBERG N.-O. and SWEDIN G. (1973b) *Acta physiol. scand.*, in press.
OWMAN CH., SJÖBERG N.-O. and SJÖSTRAND N. O. (1973c) In: *Amine Fluorescence Histochemistry.* (FUJIWARA, M., ed.). Igaku-Shoin, Tokyo. In press.
OWMAN CH., SJÖBERG N.-O., SJÖSTRAND N. O. and SWEDIN G. (1970) *Acta Endocrinol. (Kbh.)* **64,** 459–465.
ROSENGREN E. and SJÖBERG N.-O. (1968) *Acta physiol. scand.* **72,** 412–424.
RYD G. and SJÖSTRAND N. O. (1967) *Experientia* **23,** 816.
SJÖBERG N.-O. (1967) *Acta physiol. scand. Suppl.* **305,** 1–32.
SWEDIN G. (1971) *Acta physiol. scand. Suppl.* **369,** 1–34.
SWEDIN G. and BRUNDIN J. O. (1968) *Experientia* **24,** 1015–1016.