Autoradiographic binding studies with [3H]oestradiol and [3H]dihydrotestosterone in the autonomic genital ganglion (plexus of Frankenhaüser) of the mouse

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Abstract. Male, female and Tfm mice (testicular feminization) were injected with [3H]oestradiol or [3H]dihydrotestosterone, and autoradiograms prepared of male accessory sex organs and of the cervico-vaginal portion of the female reproductive tract. After injection of [3H]oestradiol in male, female and Tfm animals a nuclear concentration of radioactivity was found in a subpopulation - about 20–30% - of the neurons of the genital ganglion. No such concentration was seen after [3H]dihydrotestosterone. The results suggest a direct genomic effect of oestradiol on certain neurons of the autonomic genital ganglion in both sexes.

In mammals of both sexes an autonomic ganglion exists bilaterally in the subserosa of the midportion of the genital tract. In man it was described by Frankenhaüser (1867) in the female and by Reinert (1896) in the male. In the female mouse, the ganglion is located lateral to the cervix uteri in the connective tissue adjacent to the smooth muscle sheath and is called ganglion cervicale uteri (Becker 1972). In the male mouse, the ganglion is formed adjacent to the dorsal prostate and in the angle formed by the convergent seminal vesicles (Nouhouayi & Negulesco 1983).

In the guinea pig, rat and rabbit it has been shown that these ganglia contain adrenergic nerve cells, and innervate uterus, vagina and male accessory sex organs (Sjöstrand 1965; Adham & Schenk 1969; Owman et al. 1966, 1975; Owman & Sjöstrand 1965). Adham & Schenk (1969) in the female rat and Nouhouayi & Negulesco (1983) in the male mouse provided evidence also for the presence of cholinergic neurons.

Oestrogen increases the acetylcholine content of the uterus (Reynolds 1938). Similarly, the uterine norepinephrine content can be elevated by oestrogens (Owman et al. 1975), and morphological variations in the ganglion uteri cervicale of the guinea pig occur during the oestrous cycle and after ovariectomy (Coujard 1951).

In the present study thaw-mount autoradiography was used in order to investigate whether specific binding of [3H]oestradiol ([3H]E₂) and [3H]dihydrotestosterone ([3H]DHT) exists in neurons of the autonomic genital ganglion of the mouse.

Materials and Methods

Adult mice were taken from a colony kept in Tübingen, FRG, which was derived from the stock of Dr. Ohno (Duarte, University of California, USA). This study was carried out in combination with experiments on androgen and oestrogen binding (Schleicher et al. 1985) in

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Fig. 1.

Location of the genital ganglion and nerve fibre connections in the genital tract of a newborn male (a) and female (b) mouse visualized by the cholinesterase staining. In the male the ganglion is attached dorsolaterally to the dorsal prostate, in the female laterally to the utero-vaginal junction. Nerve fibres radiate into the sex organs, the bladder and the rectum. Magnification × 12.5.
normal mice and in mice hemizygous or heterozygous for X-chromosomal linked androgen-insensitive testicular feminization (Tfm) (Lyon & Hawkes 1970; Drews 1975). Therefore, males hemizygous for testicular feminization (genotype X^Tfm Y), normal intact adult females (XX) in dioestrus, and adult sex reversed males (XX-Sxr) were used. The sex reversed factor (Sxr) is a translocation of a testis determining sequence of the Y-chromosome to the X-chromosome (McLaren 1983). Sex reversed male mice are phenotypically male animals with testes (Cattanach et al. 1971).

One hour before sacrifice the mice received iv 0.5 µg/100 g body weight of [1,2,4,5,6,7,16,17-^3H]dihydrotestosterone ([^3H]DHT), specific activity 179 Ci/mmol (New England Nuclear) or [2,4,6,7,16,17-^3H]oestradiol-17β ([^3H]E2), specific activity 137 Ci/mmol (New England Nuclear), dissolved in 1:10 alcohol isotonic saline. Animals which received [^3H]DHT had been castrated for 36 h, in order to reduce endogeneous androgen levels. After decapitation, the accessory sex glands were dissected, placed on tissue holders, frozen in liquified propane and stored in liquid nitrogen. Four µm sections were cut in a wide range cryostat (Harris Mfg Co., N. Billerica MA, USA) and thaw-mounted onto emulsion coated slides (Kodak NTB 3) under safe light. The mounted slides were stored in light proof desiccator boxes for photographic exposure at −15°C. After exposure times of between 1 and 12 months, sections were histologically fixed in 4% phosphate-buffered formaldehyde (pH 7.0) and the emulsion was developed for 60 sec in Kodak D 19 and fixed for 5 min in Kodak fixer at 16°C, then stained with methylgreen pyronin (MGP) or methylene blue-basic fuchsin (MB-BF). The autoradiographic procedure has been described in detail (Stumpf & Sar 1975). The whole genital tract was stained for cholinesterase according to Bogusch (1981).

Results

The anatomical location of the genital ganglion is visible in the genital tract of newborn mice stained for cholinesterase (Fig. 1). In the male the ganglion is attached dorsolaterally to the dorsal prostate (Fig. 1a), in the female laterally to the utero-vaginal junction (Fig. 1b). Nerve fibers radiate not only to the sex organs but also to the bladder and the rectum.

After injection of [^3H]oestradiol in adult mice, in the female a concentration of radioactivity was found in the nuclei of certain neurons, about ¼ of the total population of neurons, comprising large and probably also small sized neurons. In addition a few scattered non-neuronal cells showed nuclear labelling. The nuclear accumulation of radioacti-

Fig. 2.
Autoradiogram of the genital ganglion in female (a), male (b) and X^Tfm Y mouse (c) after injection of [^3H] oestradiol-17β showing some labelled nerve cells. In the female (a) labelling of adjacent connective tissue cells is higher than labelling of the nerve cells. Magnification × 530. Exposure time 8 months, stained with methylgreen pyronin.
vity became apparent after 1 month of exposure. Fibroblasts of the adjacent lamina propria of the utero-cervical junction displayed a higher nuclear labelling when compared to the labelled cells within the ganglion (Fig. 2a).

In the male, a similar pattern of neuronal nuclear labelling as in the female existed (Fig. 2b), although in the male labelling appeared of a lower intensity, since a longer exposure time was required. In non-neuronal cells within the ganglion no clear nuclear concentration was seen.

Animals which are hemizygous for Tfm (genotype X^{Tfm}Y) do not have internal sex organs, except for small intraabdominal testes (Lyon & Hawkes 1970). The male accessory sex glands and ducts failed to develop due to androgen receptor deficiency. This deficiency is the basis for the androgen insensitivity of the Tfm mutation (Attardi & Ohno 1974; Gehring & Tomkins 1974). The female reproductive ducts are also absent, due to the testicular secretion of anti-Müllerian hormone during embryonic development. In these animals a small ganglion was found in the loose connective tissue dorso-laterally to the neck of the urinary bladder. The ganglion contained neurons with nuclear concentrations of radioactivity, similar to the male and female animals (Fig. 2c).

After injection of [{}^{3}H]dihydrotestosterone in all of the animals no nuclear labelling of radioactivity was observed in neurons, but nuclear labelling was found in nuclei of certain non-neuronal cells.

Our findings are in agreement with the results published in the literature on changes in the female autonomic ganglion caused by oestradiol. Coujard (1951) reported that after ovariectomy the volume of the nerve cells in the genital ganglion decreases and that it is restored under the influence of oestrogens. Oestrogens elevate the epinephrine content of the uterus (Owman et al. 1975). Since the adrenergic innervation of the uterus and proximal vagina in rabbit, rat and guinea pig derives exclusively from short adrenergic neurons in the genital ganglion (Owman et al. 1966), this effect must be mediated by oestrogen effects on this ganglion. However, it remains unclear whether or not oestradiol acts directly on neurons of the genital ganglion or indirectly on neuronal structures outside the ganglion. The present results provide strong evidence that a certain population of neurons of the ganglion is directly addressed genomically by oestradiol. Whether the oestrogen target neurons are peptidergic (i.e. NPY, VIP: Mattiasson et al. 1985), cholinergic or adrenergic remains to be determined by combined autoradiography and immunohistochemistry.

The presence of oestrogen target cells in the male ganglion is noteworthy and suggests that oestradiol, perhaps through aromatization of testosterone as in the brain, exerts effects not only on various cell types of the male accessory gland (Schleicher et al. 1984, 1985) but also on their innervation.

Discussion

The results of the autoradiographic studies indicate that certain neurons in the autonomic genital ganglion contain nuclear binding sites for [{}^{3}H] oestradiol. In contrast, with [{}^{3}H]dihydrotestosterone no such nuclear concentration and retention could be observed in neuronal cells. This difference between the two hormones argues for the presence of specific nuclear receptors for oestradiol. The longer photographic exposure times required in the male, when compared to the female, suggest that the number of binding sites for [{}^{3}H] oestradiol is lower in the male. Further studies are required for evaluation of possible differences in the intensity of nuclear labelling and in the number of labelled neurons between males, females and Tfm hemizygotes.

References


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