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24. Steroid Action in CNS and Anterior Pituitary—I

STEROID HORMONE TARGET CELLS IN THE EXTRAHYPOTHALAMIC BRAIN STEM AND CERVICAL SPINAL CORD: NEUROENDOCRINE SIGNIFICANCE

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SUMMARY

Maps of selected cross sections of rat midbrain, pons, medulla oblongata, cerebellum and spinal cord are presented showing the distribution of target neurons with nuclear concentration of radioactivity as obtained in thaw-mount autoradiograms after injection of [^{3}H]-oestradiol, [^{3}H]-dihydrotestosterone or [^{3}H]-corticosterone. The results indicate a wide distribution of steroid hormone receptors in the midbrain, hindbrain and spinal cord and suggest an important activational role of steroid hormones on extrahypothalamic neuroendocrine, autonomic, sensory and motor systems.

INTRODUCTION

Extrahypothalamic structures have received little attention in neuroendocrinology during the past four decades. This is in contrast to the hypothalamus and median eminence. The regulation of the pituitary was, and still is, considered largely from aspects of "hypothalamic control", through "hypothalamic releasing factors", and the "hypophyseotrophic area", a region claimed as being capable of maintaining near normal functions of gonads, adrenals and the thyroid [1]. No direct neural input to the median eminence from outside the hypophyseotrophic area [2] and maximal extractability of releasing factors from the "median eminence" were adduced to support this concept. Although the validity of many of these above observations and the concepts derived from them have been challenged [3] and releasing factors have been localized outside of the "hypophyseotrophic area", in the past, little attention was given to data that did not fit, including those that implement the lower brain stem. For instance, in 1968, Grino *et al.*[4] reported the development of "castration-like cells" in the pituitary after lesioning of the midbrain central grey. Taleisnik and Beltramino[5] provided electrophysiological evidence for circuitry that includes midbrain structures in the regulation of gonadotropin secretion. Earlier results from our laboratory suggested involvement of the central grey of the midbrain and hindbrain as well as the nucleus tractus solitarii in oestrogen feedback [6], with many more sites included in extended studies [7, 8]. Oestrogen, androgen, glucocorticoid and mineralcorticoid target cells are extensively distributed throughout the lower brain stem. In contrast to extrahypothalamic sites, no or only low levels of corticosteroid receptors could be demonstrated in the hypothalamus [9]. A brief review of the topography of oestrogen, androgen and corticosteroid target neurons in the midbrain, pons, medulla and cervical spinal cord is presented here.

MATERIALS AND METHODS

Sprague–Dawley rats, 5–6 rats for each hormone, were injected intravenously under ether anesthesia, 48 h after gonadectomy or andrenalectomy, with 0.5–1.0 μg/100 g body weight (b.w.) of [^{3}H]-oestradiol-17β (S.A. 95–100 Ci/mmol); [^{3}H]-dihydrotestosterone (S.A. 44 Ci/mmol) or [^{3}H]-corticosterone (S.A. 55–80 Ci/mmol), dissolved in absolute alcohol–isotonic saline. In addition, competition studies were conducted in order to establish specificity of the radioactivity that concentrates in nuclei of certain cells, by preinjecting 100× excess of unlabelled hormone of the same or different kind as the subsequently injected labelled hormone. 0.5, 1 or 3 h afterwards, the animals were decapitated and the brain removed. Autoradiograms were prepared according to the thaw–mount technique of Stumpf and Roth [10]. The autoradiographic exposure times ranged between 6 to 12 months.

RESULTS

With all of the three hormones studied, a typical nuclear concentration of radioactivity in certain cells (target cells) is seen, with varying intensity, and an anatomical distribution of target cells characteristic for each class of hormones is observed. Examples of autoradiograms have been published elsewhere [11, 12]. Figures 1–12 schematically depict the anatomical distribution of target neurons obtained after a single injection of [^{3}H]-oestradiol (Figs 1, 4, 7, 10), [^{3}H]-dihydrotestosterone (Figs 2, 5, 8, 11) or [^{3}H]-corticosterone (Figs 3, 6, 9, 12). Radioactively labelled cells other than neurons are not indicated here. As can be recognized in the midbrain at the level of the inferior colliculus (Figs 1–3), oestrogen-specific radioactivity (E_2) is concentrated in neurons of the central grey, similar to androgen (DHT), but different from the corticosteroid (Cost). In contrast to oestradiol,

DHT [13] and Cost [9] are concentrated in motor neurons. At the level of the pons (Figs 4–6), similarly, DHT and Cost target neurons are accumulated in motor nuclei and areas of the reticular formation. E_2 concentrates mainly in the dorsal region, including the nucleus (n.) parabrachialis, locus ceruleus and central grey, with some overlap with the DHT distribution, while with Cost the above cell groups appear to be spared. In the cerebellar cortex, after [^{3}H]-oestradiol Golgi-type II cells are labelled, while after [^{3}H]-dihydrotestosterone a weak nuclear concentration is seen in Purkinje cells [13]. Also after [^{3}H]-corticosterone a weak nuclear uptake of radioactivity is seen in Purkinje cells, but not as distinct as after [^{3}H]-dihydrotestosterone. In addition, labelled neurons are observed in cerebellar nuclei (not depicted here) with all of the hormones studied.

In the medulla oblongata (Figs 7–9), similar to the other regions, androgen and glucocorticoid target cells are most strongly present in motor nuclei, such as the n. nervi trigemini, n. nervi facialis, n. nervi hypoglossi and n. ambiguus. The area posterema and the n. tractus solitarii contain target cells for oestrogen and androgen. With the glucocorticoid no such concentration is seen in neurons of the n. tractus solitarii and the area postrema, although with [^{3}H]-corticosterone glial cells show weak labelling.

In the spinal cord (Figs 10–12) oestrogen target cells exist mainly in laminae I and II, while androgen and glucocorticoid target cells are found scattered in most regions with the strongest nuclear concentration in the motor neurons of laminae VIII and IX [13, 9].

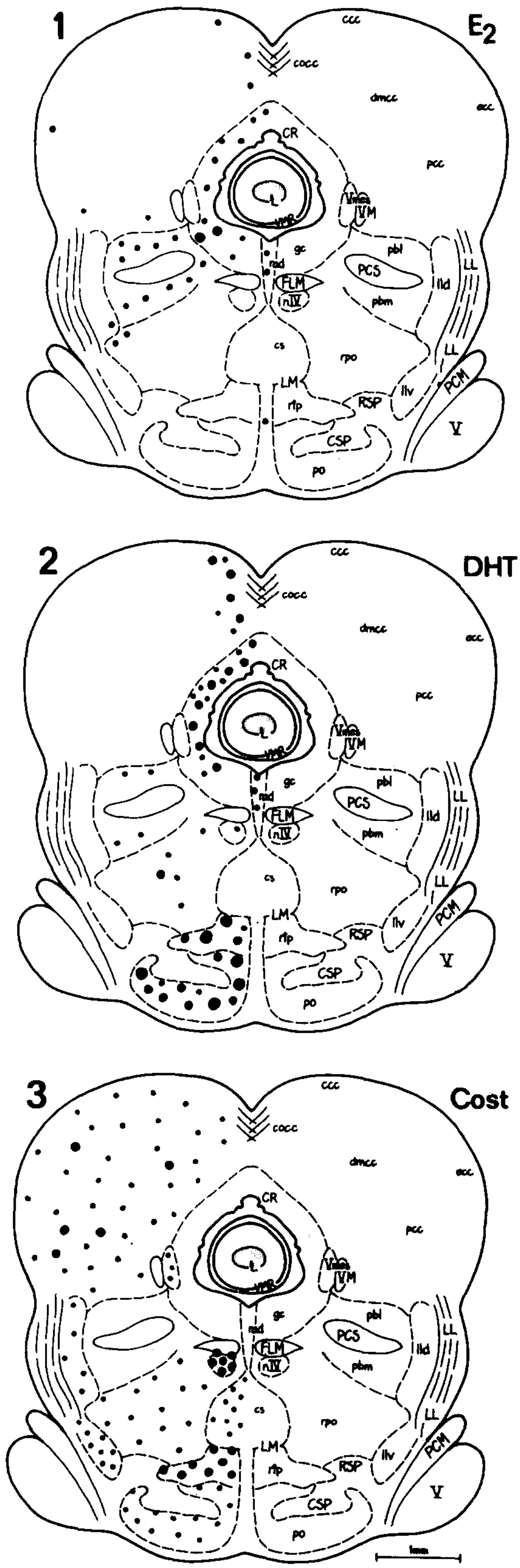
DISCUSSION

The autoradiographic results indicate a widespread presence of steroid hormone target neurons in the lower brain stem. This wide distribution is unexpected from the viewpoint of contemporary "hypothalamic" neuroendocrinology and feedback. The neuroendocrine and other significance of these observations remains to be clarified. Some conclusions may be drawn, however, from the anatomical distribution and associated histochemical observations. Oestrogen target neurons are aggregated most strongly in the dorsal portions that are derived from the alar plate and contain structures that are largely related to sensory functions [14]. With androgen strongest representation of target neurons exists in the ventral portions of the lower brain stem and spinal cord that are derived from the floor plate and contain mostly motor-related structures [14]. There is, though, some overlap and additional special groupings of target cells found with either sex hormone are, for instance, in the reticular formation. Glucocorticosteroids, as well as mineralcorticosteroids [9], appear to address the largest population of cells in the central nervous system, with the exception of the diencephalon, when compared to the sex steroids. In the lower brain stem and spinal cord many regions that are known to be involved with sensory as well as motor functions contain target neurons for corticosteroids. Similar to androgen, highest nuclear concentration is seen in the large motor neurons after [^{3}H]-corticosterone.

Evidence presented here and elsewhere in the literature support our view that steroid hormones are activators of select structures in the brain and peripheral tissues. For instance, in the preoptic-septal region, the localization of oestrogen target sites corresponds with the sites where gonadotrophin releasing factor producing cells have been located [15]. In this region oestrogen implants resulted in stimulation of LH secretion while lesions eliminated this effect [16]. Using a combined immuno-autoradiography technique, [^{3}H]-oestradiol and neurophysin antibodies have been localized in identical cells in the supraoptic nucleus [17]. Oestradiol is known to stimulate oxytocin production and secretion [18]. With a combined formaldehyde induced fluorescence-autoradiography technique, nuclear concentration of [^{3}H]-oestradiol and cytoplasmic fluorescence have been demonstrated to exist in identical neurons in the hypothalamus [19, 20], midbrain, pons, and medulla oblongata [21]. Oestradiol is known to stimulate catecholamine turnover [22]. With the same combination technique, [^{3}H]-dihydrotestosterone derived radioactivity and formaldehyde induced fluorescence have been localized in identical neurons in the lower brain stem [23]. This evidence and other more functional data published in the literature strongly support our view that gonadal and adrenal steroids are genomic activators of various specific systems in the brain and spinal cord. Thus, probably, many of the events observed in the "hypothalamus" and related to the modulation of pituitary functions and behaviour are influenced or governed by some of the labelled cell groups in the lower brain stem. Connections between lower brain stem target cell groups and the hypothalamus, as well as other forebrain regions, have been recently confirmed or newly established [24, 25, 26, 27].

Our observation that steroid hormones or metabolites of them concentrate at different intensities in different cell groups or individual cells probably is im-

Figs 1–12. Topographical distribution of steroid hormone target neurons in caudal midbrain (Figs 1–3), pons (Figs 4–6), medulla oblongata (Figs 7–9), and cervical spinal cord (Figs 10–12) after injection of [^{3}H]oestradiol (E_2), [^{3}H]-dihydrotestosterone (DHT) or [^{3}H]-corticosterone (Cost). Schematic drawings of frontal cross sections prepared from thaw-mount 'autoradiograms. The size and frequency of the dots (left half) indicate the intensity of nuclear uptake of radioactivity and frequency of target neurons. Designation of structures on right side. For explanation of abbreviations see *Anatomical Neuroendocrinology* [11].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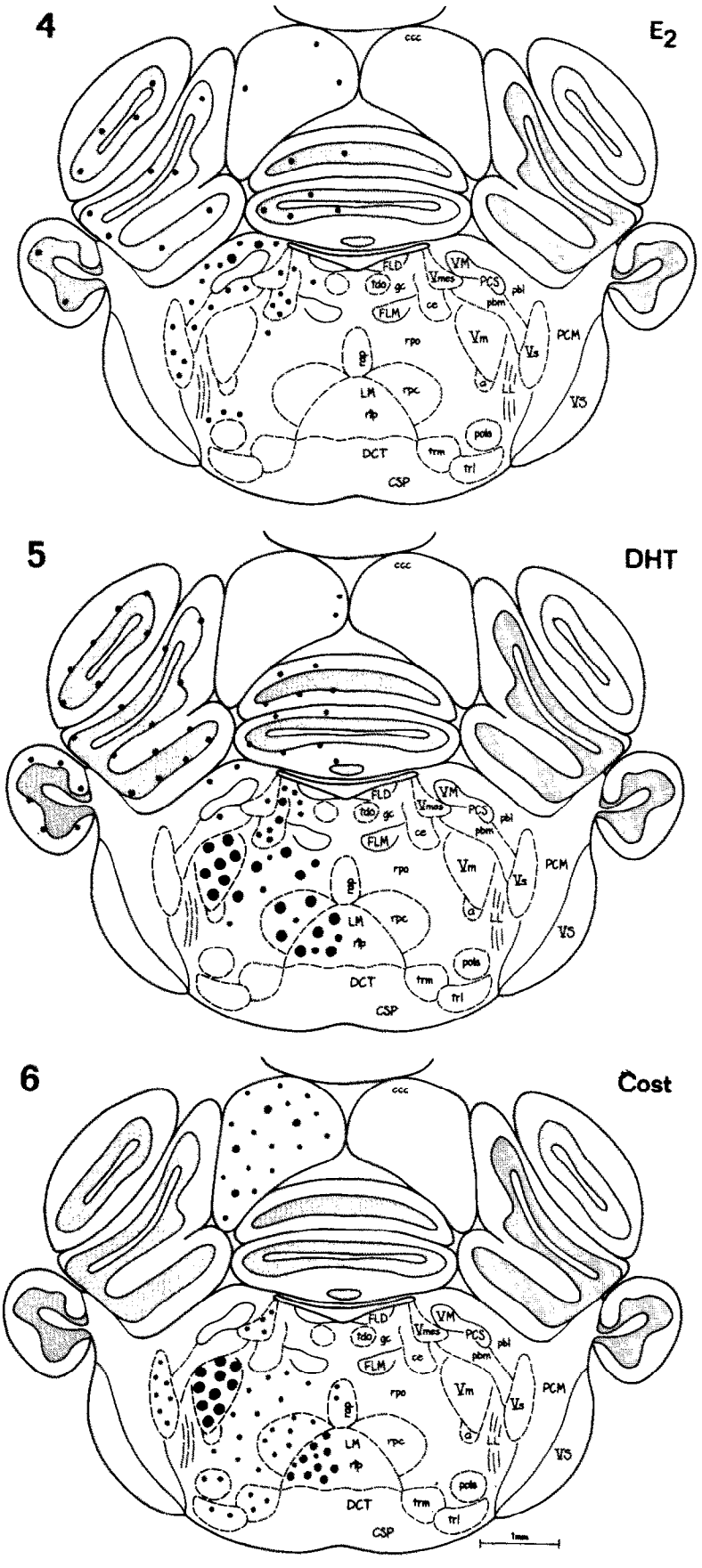
Figs. 1–3.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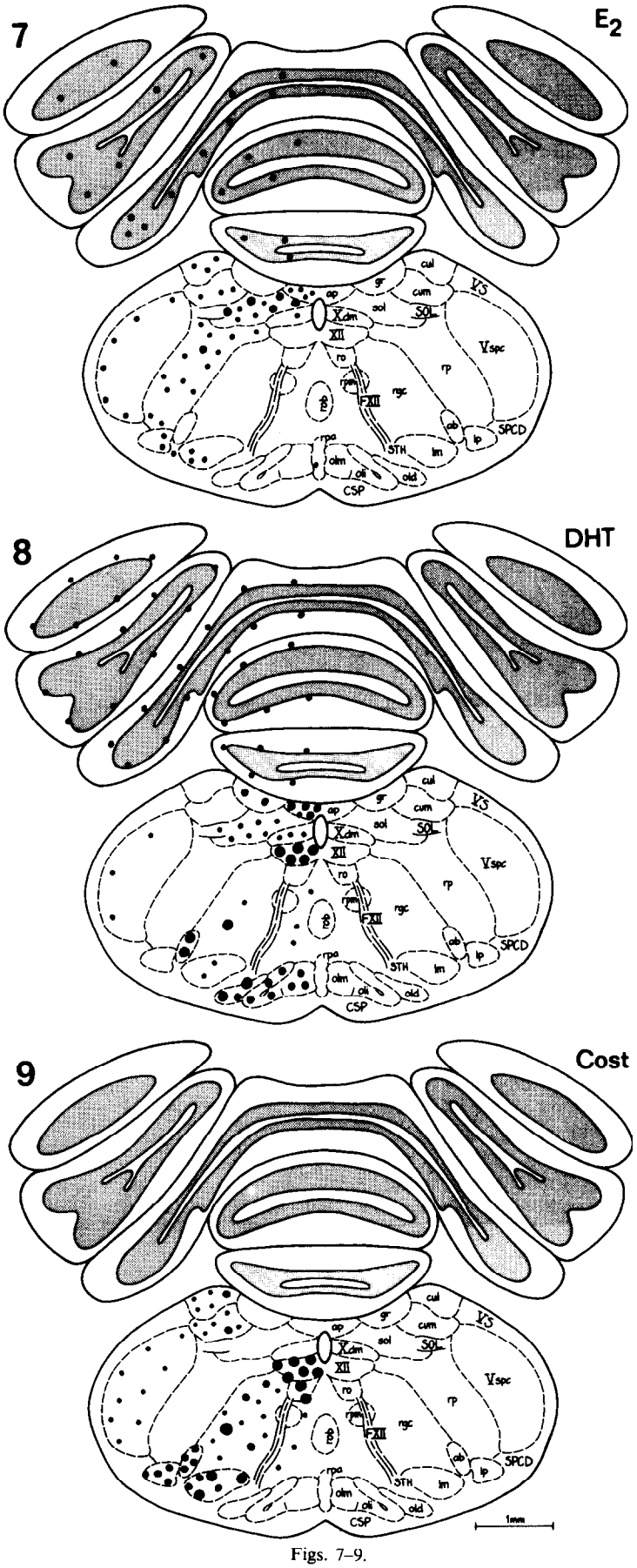
Figs. 4–6.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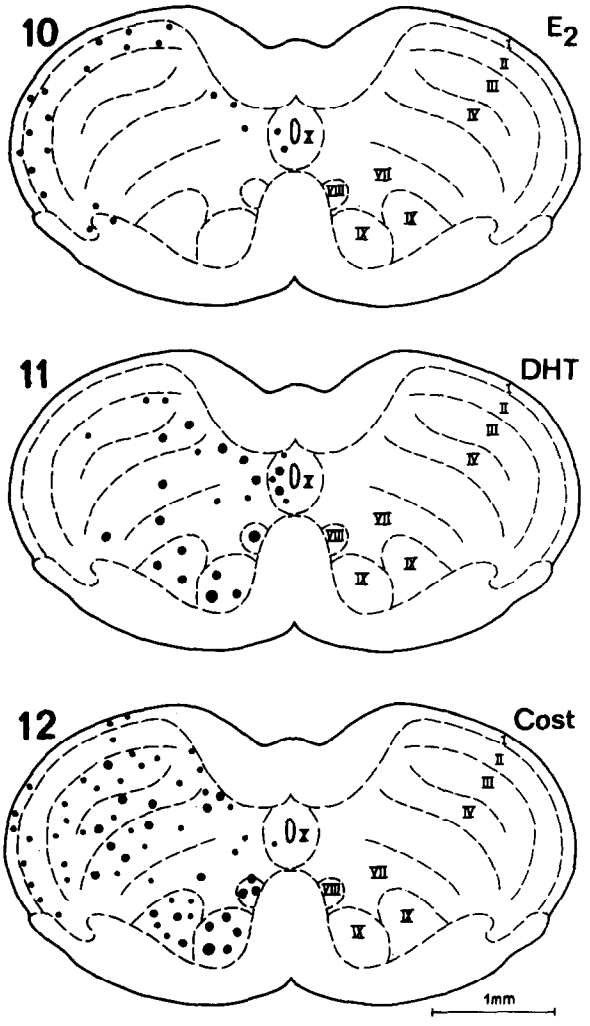
Figs. 7–9.



Figs. 10–12.

portant for a functional threshold or a graded response, that is, a dependency of steroidal genomic activation on the number of receptors as well as hormone levels in the tissue and blood. Since the presence of receptors is believed to be essential for actions of the hormones, cells with a high number of hormone binding sites (receptors) probably respond stronger than those with a low number of receptors at a given blood level. The cells with a higher number of receptors may show a response at relatively low blood levels, while under the same conditions cells with a lower number of receptors may show a corresponding lower response or no measurable response at all. The autoradiographic observations presented here open up new areas of research and demand further detailed studies of midbrain, pons, medulla oblongata, cerebellum and spinal cord, in order to substantiate the neuroendocrine, behavioral, sensory, motor and autonomic relationships of steroid hormone activation.

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