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Gonadal hormone actions on the morphology of the vasopressinergic innervation of the adult rat brain

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Castration of adult male rats resulted in a gradual decrease in vasopressin fiber density over a period of 15 weeks to a point where hardly any fibers were found in those areas where the fibers probably are derived from the bed nucleus of the stria terminalis. The original fiber density could be restored by testosterone replacement therapy within 5 weeks. No effects of hormonal manipulations were found in the vasopressin projections of the paraventricular and the suprachiasmatic nucleus. Ovariectomy of female rats resulted in the same changes in the vasopressin fiber pathways, as did castration in males.

Besides the vasopressin (VP) fiber pathways from the hypothalamic neurosecretory nuclei to the neurohypophysis, VP fibers have been reported to innervate various other areas in the brain^{2,14}. VP probably acts as a neurotransmitter in these regions³. Part of the VP innervation appears to be sexually dimorphic, with the VP fiber density within the lateral septum (LS) being higher in male than in female rats⁸. A series of castration and testosterone supplementation experiments, followed by examination of the VP fiber network at the 26th postnatal day, revealed that the development of this sex difference depends upon the presence of androgens in the male rats⁶. Male rats which were castrated neonatally appeared to have a fiber density on the 26th day of life which was as low as in control female rats; in rats castrated at the 7th postnatal day, a fiber density intermediate to control male and female rats was detected; castration at the 14th day of life failed to prevent the development of a fiber network as dense as in control males. This suggests that around day 7, androgen exposure normally determines the density of the vasopressin fiber network as present on the 26th postnatal day. However, administration of high doses of testosterone-propionate, either to female or to neonatally castrated male rats, induced the development of a fiber network as high as in control males, while no dif-

ference in the VP fiber density was found whether the testosterone treatment took place in the first, second or third week of life. This suggests that the VP innervation of the brain can be influenced by gonadal steroids also at later stages in life.

In order to examine the possibility of gonadal hormone influences on the VP innervation in the adult rat, male and female Wistar rats were, respectively, castrated and ovariectomized, or sham-operated (controls) at 3 months of age. At 1, 3, 8 and 15 weeks after the operation 5 castrated and 5 control males were sacrificed by immersion fixation, after which 50 μ m thick transverse sections of the brain were cut. These were stained immunocytochemically for the presence of VP (for details about the fixation and staining procedure, see De Vries et al.⁸).

Changes in VP fiber density within the LS were followed by estimating the fiber density semi-quantitatively in a blind study, in which a distinction was made among 4 categories of grading densities (Table I) according to De Vries et al.⁸. Control as well as castrated male rats showed a very high VP fiber density at 1 week after operation. Three weeks later the fiber density in one of the castrated rats was still as high as in control males, but was clearly diminished in the other 4, which in fact were down at the female level. At 8 weeks after castration, 4 out of 5 rats

TABLE I

VP fiber density in the LS at several weeks after castration and subsequent testosterone supplementation in male rats, or ovariectomy in female rats

Each entry in the table represents one rat: +, almost no fibers; ++, a low fiber density; +++, a high fiber density; + + + +, a very high fiber density.

Weeks		Fiber density						
<i>Male rats</i>								
1	control	++++	++++	++++	++++	++++		
	castrated	++++	++++	++++	++++	++++		
3	control	++++	++++	++++	++++	++++		
	castrated	++++	+++	+++	+++	+++		
8	control	++++	++++	++++	++++	++++		
	castrated	+++	++	++	++	++		
15	control	++++	++++	++++	++++	++++		
	castrated	+	+	+	+	+		
25	control	++++	++++	++++	++++	++++		
	castrated	+	+	+				
	castrated plus testosterone	++++	++++	++++	++++	++++	+++	+++
<i>Female rats</i>								
25	control	+++	+++	+++	+++	+++		
	ovariectomized	+	+	+	+	+		

showed an even lower fiber density. By 15 weeks the castrated rats had reached a level at which almost no fibers could be found (Fig. 1).

In order to see whether or not this disappearance of VP fibers could be reversed by testosterone replacement therapy, 7 of the remaining castrated rats received at 20 weeks after operation an implantation of silastic tubing (Talas SR3; 1.5 mm i.d., 2.5 mm o.d.) packed with testosterone (Roucel UCLAF), while 3 other castrated rats received empty tubing. Two weeks after implantation, 1 ml blood was collected by heart puncture under ether anaesthesia and the serum testosterone level was determined by radioimmunoassay according to Verjans et al.¹⁹. In the castrated rats with testosterone implantation, levels varied between 2.4 and 3.6 ng/ml. No testosterone could be detected in the serum of the other castrated rats.

All remaining male and female rats were sacrificed by perfusion fixation 25 weeks after castration (see Table I for the number of animals per experimental group). Immunocytochemical staining for the presence of VP revealed almost no fibers in the LS of the castrated rats without testosterone supplementation.

Testosterone replacement therapy, on the other hand, was effective in restoring the fiber network, since a very high fiber density comparable to that found in control males was found in 5 out of 7 castrated males with a testosterone implantation. The remaining two rats displayed a lesser effect, the fiber density equalling that of control females. As in the castrated males, almost no VP fibers were found in the LS of ovariectomized female rats.

Similar effects of gonadal hormone manipulation were found on the VP innervation of the diagonal band of Broca, the anterior area of the amygdala, the lateral habenular nucleus, and the periventricular grey, that is, disappearance of VP fiber staining in castrated males or ovariectomized females, and re-appearance of VP fibers in castrated male rats treated with testosterone (Fig. 2). All these brain areas and also the LS probably receive their VP innervation from the BST⁷, in which recently VP neurons were demonstrated¹⁸. Since the BST contains many estrogen- and androgen concentrating cells^{10,15}, the observed effects of gonadal hormones on VP fiber density might in fact be exerted directly on the BST.

In the present study all control males displayed a

Figs. 1 and 2. Transverse sections of the rat brain, which were stained immunocytochemically for the presence of vasopressin (VP). Figs. 1A and B are photographs of dark-field illuminated sections of the lateral septum (LS), showing only very few VP fibers (arrows) in the LS at 15 weeks after castration (A), in contrast to the very high fiber density found in the LS of a control male (B); LV = lateral ventricle, bar = 150 μ m. Figs. 2A and B represent bright-field illuminated sections of the area around the third ventricle (III), showing almost no VP fibers (arrows) in the lateral habenular nucleus (LH) of a castrated rat (A) but a very high fiber density in a control rat (B). Note that this difference is not present in the periventricular nucleus (PVS). Bar = 100 μ m.

higher fiber density in the LS than did any of the control female rats, confirming previous observations⁸. This sex difference might of course be caused by the different hormonal constitution of male and female rats, but possibly also by different properties of the VP system itself. For instance, the number of VP cells projecting to the LS might differ in male and female rats.

It is of interest that no effect of castration or ovariectomy could be detected upon the VP innervation of either the dorsomedial hypothalamic or the periventricular nucleus (Fig. 2), which arises in both cases from the suprachiasmatic nucleus⁹. Neither was there any visible effect upon the VP fibers in the dorsal vagal complex or in the nucleus ambiguus, which in turn are derived from the paraventricular nucleus^{7,11-13}. These results clearly demonstrate that not only can the VP innervation of the brain be subdivided on the basis of neuronal origin but, moreover, that such subdivisions respond differentially to hormonal stimuli, and must therefore participate in distinctive ways in central functions. In this respect, the VP projections of the BST are presumably involved in sexually dimorphic functions, or at least in functions which are strongly influenced by gonadal hormones. The dynamics of the observed changes in the density of the VP network might give a clue as to what functions should be thought about. For exam-

ple, male sexual behaviour decreases with the same time-course after castration⁴. It is therefore noteworthy that VP seems to be involved in the maintenance of post-castration male copulatory behavior¹.

The changes in the VP fiber density might form an indication, that plasticity in the adult mammalian brain under influence of gonadal hormones is greater than has hitherto been suspected. Effects of testosterone and estrogens on neuritic growth could be demonstrated in tissue cultures of newborn mouse hypothalamus^{16,17}. To our knowledge, similar effects of steroids on the adult mammalian brain have as yet not been reported. That, however, gonadal hormones can also induce dendritic growth in adult brains, has been demonstrated for the song control areas of birds⁵. The present results demonstrate an influence of gonadal hormones on VP axons in the adult rat. It remains to be established, however, whether the observed changes in the VP projections of the BST represent also changes in fiber length and branching or merely differences in the state of filling of the fibers.

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