Right occipital cortex suppresses male rat testosterone secretion by a pituitary-independent mechanism

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Abstract

OBJECTIVES: In addition to being regulated by the hypothalamo-hypophyseal system, testosterone (T) secretion is influenced by a number of less understood mechanisms. The aim of the present study was to examine whether defined areas of the right cerebral cortex could modulate T production.

METHODS: In adult male Wistar rats right frontal or occipital decortication, anterior or posterior callosotomy and corresponding sham-operations were performed. After 7-day survival time, T secretion *in vitro*, serum T and LH concentrations were measured by RIA.

RESULTS: Right occipital decortication and posterior callosotomy resulted in an increase in T secretion *in vitro* when compared to the corresponding sham-operated controls. In contrast, right frontal decortication or anterior callosotomy did not interfere with steroidogenesis. Serum LH concentration was not altered by any interventions.

CONCLUSION: The right occipital but not the right frontal cortex is involved in the control of T secretion. The caudal part of the corpus callosum accommodating the fibers originating from the occipital cortex might have a similar function. The fact that LH remained unchanged in all experimental groups suggests that the right occipital cortex and the caudal part of the corpus callosum influence testicular steroidogenesis by a pituitary-independent mechanism.

ORIGINAL ARTICLE

Introduction

It is well established that gonadal functions are mainly governed by the hypothalamo-hypophyseal system. Gonadal steroidogenesis, however, is also influenced by less understood, so-called fine-tuning mechanisms including recently described pure neural links between the nervous system and the gonads [12, 14, 15]. The role of innervation in the control of gonadal functions is indicated by studies in which transection or lesion of the peripheral or the central nervous system were performed [3,10].

Transection of the superior spermatic nerve in the rat induced a significant decrease in testicular serotonin content [6], attenuation of serum androgen levels and a decrease in testicular gonadotropin receptors [5]. Vasectomy which includes the interruption of the inferior spermatic nerve, has been reported to modify testicular functions both in mature and neonatal rats [13]. Moreover, in hemicastrated peripubertal animals right-sided vagotomy induced a significant decrease in serum LH and FSH concentrations and suppressed the basal T secretion *in vitro* of the remaining testis [10]. In addition, a number of brain areas, such as the caudal raphe nuclei, locus coeruleus, periaqueductal grey, dorsal hypothalamus, and the hypothalamic paraventricular nucleus have been reported to be transneuronally connected with the gonads [14, 15] and have been suggested to be involved in the direct neural control of gonadal functions. This view is supported by data which indicate that right-sided lesion of the insular cortex resulted in a decrease in serum and basal T secretion *in vitro* with no change in LH level [11]. Furthermore, complete transection of the corpus callosum led to an increase in T secretion of hemiorchidectomized rats [2], suggesting that the callosotomy-induced upregulation of testicular steroidogenesis has been ascertained by a pituary-independent mechanism.

The aim of the present study was to investigate whether the frontal and the occipital cortex are involved in the control of testicular steroidogenesis. Since discrete segments of the corpus callosum receive fibers from different cortical areas [9, 16], in addition to unilateral cortical lesion, partial (anterior and posterior) callosotomy was also performed. For the unilateral cortical lesion, the right hemisphere has been selected since the majority of observations suggest that in both male and female rats there is a predominance of the right-sided brain structures involved in the control of gonadal functions [12].

Material and Methods

All the experiments here described were performed according to the principles set out in the Declaration of Helsinki and approved by the Bioethics Committee of the University of Sassari, Italy. Adult male Wistar rats weighing 340±20g were used. Animals were housed in a temperature-controlled room (22°±2 °C) with a 12-hr light/dark cycle and fed on a standard diet and water. The animals were divided into 8 groups (10 subjects each) and underwent the following procedures: i. right-sided frontal decortication, ii. right-sided frontal sham decortication, iii. right-sided occipital decortication, iv. right-sided occipital sham decortication, v. anterior callosotomy, vi. posterior callosotomy, vii. sham callosotomy and viii. intact animals.

Rats were anesthetized with zolazepam and tiletamine (Zoletil, *Virbac*, 50mg/kg) and put into a stereotaxic frame in a position with the head in a 3.3 mm dorsoventral nose-down position relative to the interauricular line [17].

To perform lesions in the right frontal cortex, the scalp was cut along the midline and a hole (3 mm lateral to the midline, 3 mm anterior to the bregma and 4 mm long) was drilled in the right frontal bone. Similar procedures were performed for the right occipital lesions, but the 4 mm long hole was drilled 3 mm lateral to the midline and 3 mm posterior to the lambda. Ablation of the two cortical areas was made by a 2 mm deep suction. In case of callosotomy, a unilateral hole was drilled with a center 0.4 mm lateral to the bregma. To perform anterior or posterior callosotomy, the corpus callosum was transected according to the method previously described in the mouse by Schalomon and Wahlstein and modified by Banczerowski et al. [2] for the rat. Coordinates of the anterior and posterior callosotomy were as follows: anterior callosotomy: 6 mm posterior and 0.4 mm lateral to the bregma; angle 44.5° from vertical; depth of the incision 7.8 mm; posterior callosotomy: 6 mm posterior and 0.4 mm lateral to the bregma; angle 0° from vertical; depth of the incision 2.9 mm. Additional groups of animals (ii, iv and vii) underwent sham brain operation which included anaesthesia, immobilization of the head in the stereotaxic frame as well as opening of the skull and the dura mater.

Rats were killed by decapitation 7 days after surgery. Trunk blood was collected and serum was separated by centrifugation and stored at –20 °C until T and LH titration. Testes were removed immediately, weighed, decapsulated and incubated in 3ml M199 tissue culture medium containing 25 mM HEPES and 0.1% bovine serum albumin in a metabolic shaker at 35 °C for 2h. Afterwards, the medium was stored at –20 °C for hormone determination. In order to check the location and extent of callosotomy, brains were fixed in 10% formaldehyde, dehydrated in alcohol and paraffin embedded. Serial sections, 5μm thick, were stained according to Nissl's method.

T concentrations in serum and tissue culture medium were determined by radioimmunoassay (RIA) as previously described in detail [7]. Briefly, to 20μl medium or ether extract of 20μl serum, 7nl/tube antibody (CV-RT 17, 1:100,000 final dilution) and 12,000 cpm ^{3}H-labelled T (100fmol, Radiochemical Center, Amersham) were added in a total volume of 0.7ml ASB.

After an overnight incubation at 4°C and separation with dextran-coated charcoal, the radioactivity of the bound fraction was measured in a two-phase liquid scintillation system. The sensitivity limit of the assay is 3fmol/tube. The inter- and intra-assay coefficients for variation were 9.8 and 5.9%, respectively. LH levels from the rat sera were determined by RIA utilizing a National Hormone and Pituitary Program kit. For reference, rLH-RP-3 preparation was used. The inter- and intra-assay coefficients of variation were 7–9 and 4–6%, respectively.

Results were analysed by ANOVA and considered significant when $p< 0.05$.

Results

During the postsurgical period, animals recovered well showing neither clinical signs nor changes in body weight. There was no difference in testis weight among groups as well as between left and right testis (data not shown). Sham-operated animals showed a significant decrease in T release compared to intact animals (Figures 1, 2, 3, 4). Therefore, the results of the actual surgery are compared to those obtained following the relevant sham operation.

Serum T concentration

Lesion of the right-sided occipital cortex resulted in a significant increase ($p< 0.05$) in serum T concentration. Following ablation of the frontal cortex on the right side, only a slight, not significant rise in serum T

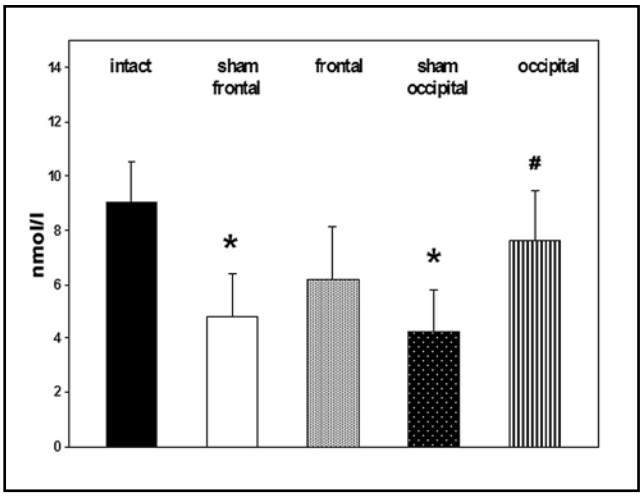

Figure 1. Serum T levels after cortical lesions.
* = significant difference in comparison with intact rats;
# = significant difference in comparison with rats underwent sham occipital cortical lesion. Data are expressed as mean ±SEM, $p< 0.05$.

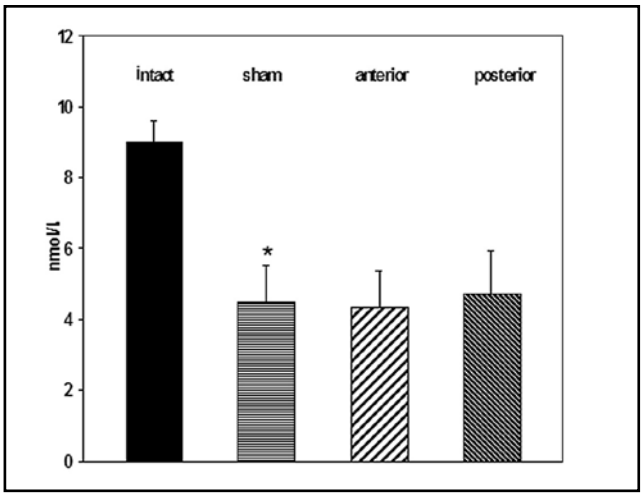

Figure 2. Serum T levels after callosotomy.
* = significant difference in comparison with intact rats. Data are expressed as mean ±SEM, $p< 0.05$.

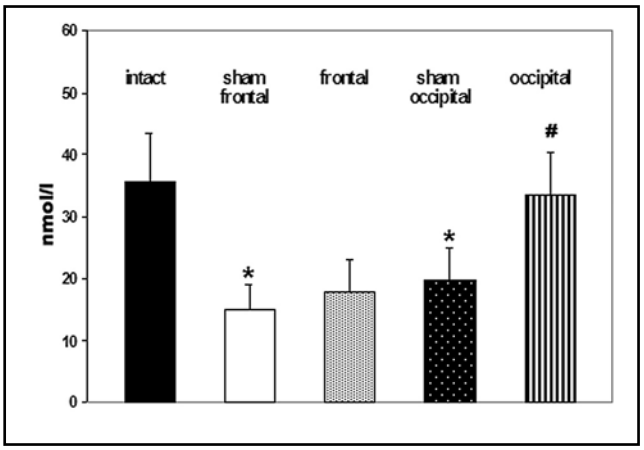

Figure 3. Levels of basal T secretion *in vitro* after cortical lesions.
* = significant difference in comparison with intact rats;
# = significant difference in comparison with rats underwent sham occipital cortical lesion. Data are expressed as mean ±SEM, $p< 0.05$.

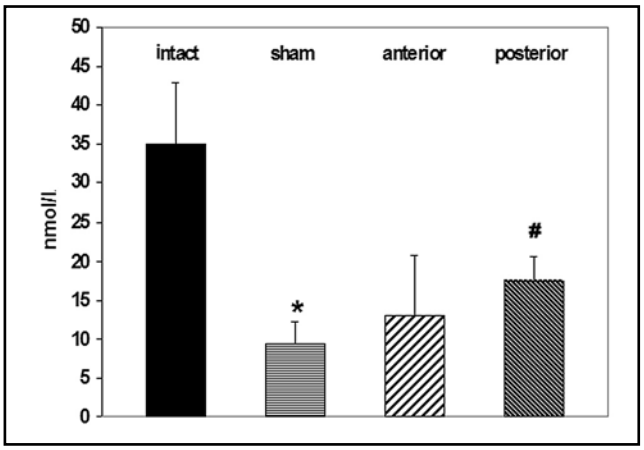

Figure 4. Levels of basal T secretion *in vitro* after callosotomy.
* = significant difference in comparison with intact rats;
# = significant difference in comparison with sham callosotomized rats. Data are expressed as mean ±SEM, $p< 0.05$.

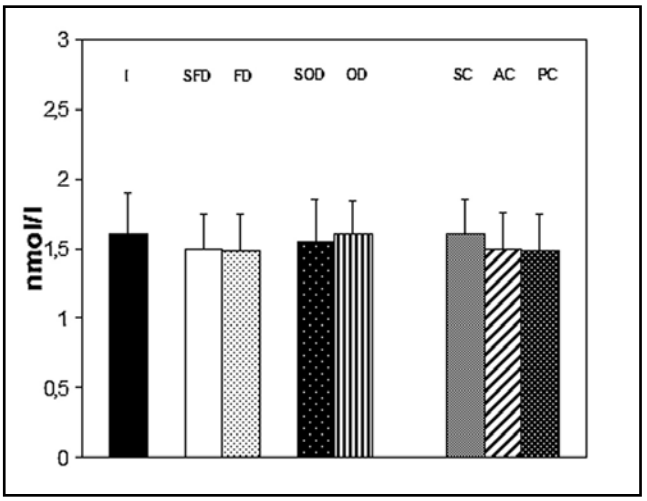

Figure 5 (above). Serum LH concentration after cortical lesion and callosotomy. **I**= intact; **SFD**= sham frontal decortication; **FD**= frontal decortication; **SOD**= sham occipital decortication; **OD**= occipital decortication; **SC**= sham callosotomy; **AC**= anterior callosotomy; **PC**= posterior callosotomy. Data are expressed as mean ±SEM, p< 0.05.

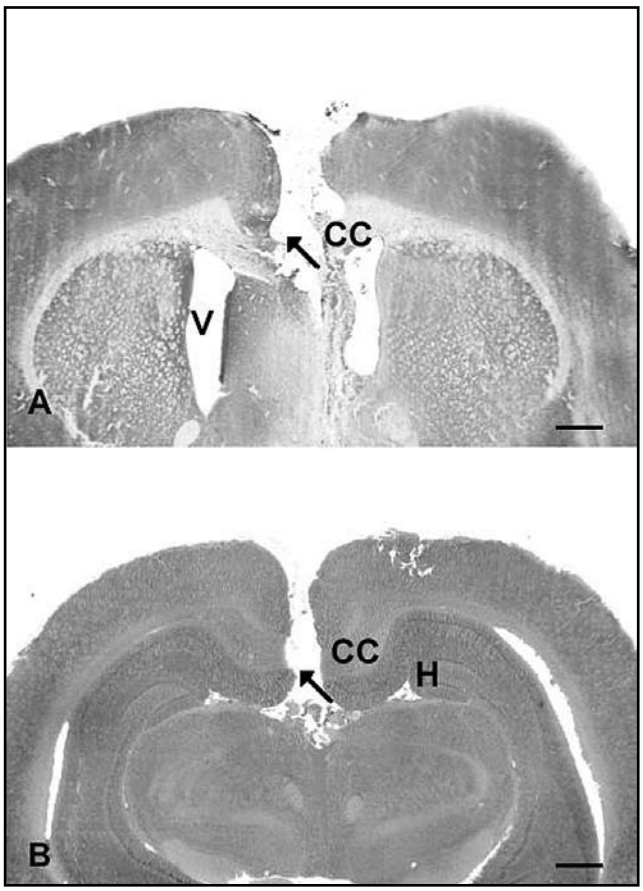

Figure 6 (right). Coronal section illustrating the anterior (A) and posterior (B) callosotomy 7 days post-surgery. The lesions produced a limited inflammatory process in the surrounding cortical areas. Nissl's staining. **V** = lateral ventricle; **CC** = corpus callosum; **H** = hippocampus. Arrows indicate the cut of the corpus callosum. Bar = 1mm.

level could be observed (Figure 1). Serum T concentration was not altered either by anterior or posterior callosotomy (Figure 2).

Basal T secretion in vitro

Occipital cortical ablation resulted in a significant rise in basal T secretion *in vitro* (p< 0.05). By contrast, frontal cortical lesion did not interfere with this parameter (Figure 3). Posterior callosotomy induced a significant increase in T release (p< 0.05), while anterior transection of the corpus callosum did not determine any significant effect (Figure 4).

Serum LH concentration

Neither cortical ablation (frontal or occipital) nor partial callosotomy (anterior or posterior) altered serum LH levels (Figure 5).

Brain histology

The histological examination of the site of the surgical lesions showed that both anterior and posterior transection of the corpus callosum were performed according to the scheduled protocol and involved the whole thickness of the corpus callosum. The lesions produced a limited extracallosal inflammation (Figure 6).

Discussion

The results of the present study demonstrate for the first time an inhibitory effect of the right occipital cortex on testicular steroidogenesis. Since it is well known that fibers from different cortical areas run in discrete segments of the corpus callosum [9,16], the increase in basal T levels after right occipital decortication and posterior callosotomy suggests, on the one hand that these structures are involved in the control of testicular steroidogenesis, and on the other hand, confirms that fibers from the occipital cortex are located in the splenium of the corpus callosum.

Our results suggest that commissural fibers from the right occipital area may exert an inhibitory effect on testicular steroidogenesis. This specific action does not seem to be pituitary-mediated, since serum concentration of LH, the main endocrine signal controlling T secretion, did not change after callosotomy and decortication. Temporolimbic structures such as the insular cortex and amygdala, [3,15] as well as the hypothalamic paraventricular nucleus [18] have been reported to exert a regulatory action on T secretion by a pituitary-independent, pure neural mechanism.

On the basis of the present results, it can be assumed that the occipital cortex and fibers of the posterior corpus callosum could be components of brain areas

controlling endocrine functions of the testis via a neural route. It can be supposed that the occipital cortex through temporolimbic structures is interconnected with hypothalamic centers and brain stem nuclei [4] projecting to the spinal cord. From these cell groups neural signals may reach the testis via parasympathetic fibers of the vagus nerve and through efferent fibers originating from the sympathetic and parasympathetic preganglionic neurons of the spinal cord [14].

The decrease in T levels in all sham operated rats, as compared to intact animals, might be due to the stress conditions occurring during the surgical period. Pre- and postoperative handling, such as restrain, anaesthesia and the healing of the surgical wound should be considered as sources of stress. It is well established that physical stressors resulting in an increase in corticosterone levels reduce both serum and intra-testicular T content in rodents [8]. Other stressing factors including cerebral injuries, isolation, low temperatures, electrical stimuli also induce a decrease in T production [1].

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