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UNILATERAL PARAMEDIAN-SAGITTAL BRAIN CUT EXTENDING FROM THE LEVEL OF THE ANTERIOR COMMISSURE TO THE MIDLEVEL OF THE THIRD VENTRICLE ABOVE THE AMYGDALA AFFECTS GONADAL FUNCTION IN MALE RAT: A LATERALIZED EFFECT

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The aim of the present investigations was to study involvement of fiber systems to and from the insular cortex above the amygdala in the neural control of the hypophysio-testicular axis in male rats. Animals were subjected to a unilateral paramedian-sagittal brain cut above the amygdala, extending from the level of the anterior commissure to the midlevel of the third ventricle and causing among others partial deafferentation of the insular cortex. Right-sided cut induced a significant rise in basal testosterone secretion *in vitro* of both testes as compared to intact or sham-operated controls without affecting serum testosterone level. By contrast, left-sided cut slightly suppressed testicular steroidogenesis and significantly decreased serum testosterone concentration. In animals underwent sham or actual cut on either side, serum luteinizing hormone levels were similar, but significantly lower than those in intact controls. No change was observed in serum FSH concentration of any experimental group. The results indicate that afferent and efferent connections of the partially deafferented cortical regions including among others the insular cortex are involved in the control of testosterone secretion. The data further suggest functional laterality of the interrupted structures.

Keywords: Testosterone – LH – insular cortex – deafferentation – laterality

INTRODUCTION

Increasing number of data indicate that a pituitary-independent mechanism exists in the control of gonadal functions, i.e. a direct neural pathway between these glands and the brain is involved in this control. Early studies have provided indirect evidence on the existence of a direct neural pathway between the gonads and the brain [see for review 8]. These data include unilateral changes in the metabolic activity and gonadotrop hormone-releasing hormone content of the hypothalamus following hemicastration [10, 15] and the effects of unilateral lesion of cerebral structures on the gonadal functions following hemicastration. Early studies have indicated that in male rats unilateral deafferentation of the hypothalamus prevents the hemicastration-induced FSH rise only if right-sided deafferentation is combined with hemicastration on the right side [18]. Similarly, in adult females right-sided lesion of the retrochias-

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matic area with the neurotoxin kainic acid has been reported to prevent the development of compensatory ovarian hypertrophy following ovariectomy ipsilateral to brain intervention [17]. Furthermore, right-sided lesion of the preoptic area on the day of estrus induced a significant decrease in the ovulation rate [16].

Our recent neuromorphological studies using the viral transneuronal tracing technique have proved the existence of this long, multisynaptic pathway and allowed us to map cerebral structrures transneuronally connected with the gonads [11, 12]. Following inoculation of neurotropic virus into the testis, besides the infection of preganglionic sympathetic neurons in thoraco-lumbar spinal cord segments and in the sacral parasympathetic nucleus, several cerebral structures exhibited retrogradely labeled perikarya: among others the caudal raphe nuclei, the nucleus of the solitary tract, the locus coeruleus, the periaqueductal gray of the mesencephalon, the hypothalamic paraventricular nucleus and the lateral hypothalamus. Telencephalic structures containing retrogradely labeled neurons were the bed nucleus of the stria terminalis, the central amygdala and the insular cortex. The infected neurons in the insular cortex (granular, dysgranular, and agranular insular cortex and lower part of the secondary somatosensory area) occupied a well-described region and were almost exclusively pyramidal cells.

In further studies we investigated whether the area of the insular cortex transneuronally connected with the testis is involved or not in the control of testicular functions. We found that right- but not left-sided thermocoagulation of the insular cortex suppressed testicular steroidogenesis. At the same time both right- and left-sided lesion induced a significant increase in serum luteinizing hormone (LH) concentration and no change in FSH secretion [3]. These observations indicated the involvement of the insular cortex in the neural control of testicular functions and suggested that the right insular cortex may play a predominant role in the regulation of male endocrine reproductive processes.

The aim of the present study was to investigate the effect of partial deafferentation of the insular cortex on the gonadotrop-testicular axis. The brain surgery consisted of a unilateral, paramedian-sagittal brain cut above the amygdala extending from the anterior commissure to the midlevel of the third ventricle interrupting among others fibers to and from the insular cortex. The reason of this kind of experimental design was to avoid the damage of the amygdala, since previous studies indicated the role of the amygdala in the control of gonadal functions [6, 7, 13].

MATERIALS AND METHODS

Animals

Adult male rats of Sprague-Dawley strain, weighing 420 ± 20 g, were used. Animals were kept under standard laboratory conditions (room temperature 22 ± 1 °C, with a 12-hour light-day cycle, with lights on at 06:00 h). Regular rat chow and tap water were provided *ad libitum*. The experimental procedures were approved by the Local Animal Use Committee.

Surgery

The animals were anesthesized with Hexobarbital (50 mg/kg body weight intraperitoneal) and put into a stereotaxic frame (Stoelting stereotaxic instrument) in a position that the head was in a 3.3 mm dorsoventral nose-down position relative to the interauricular line [19]. The scalp was cut by a parieto-temporal sagittal incision, the skull was cleaned. According to the location and size of the knife-cut an opening was drilled to accomodate the knife (length of knife blade in sagittal: 2.5 mm), then the knife was inserted into the brain. The knife was made from stainless steel rod, flattened and sharpened to form a blade. The parameters of the insertion of the knife: anteroposterior direction = rostral end of the knife –0.5 mm, posterior end of the knife –3.0 mm posterior to the bregma; lateral direction = 4.5 mm lateral to the midline; vertical direction = 7.1 mm inferior to the surface of the skull. After intervention the skin was sutured. Sham surgery composed of anesthesia and opening of the skin and the skull. The location of the knife-cut is shown schematically in Fig. 1A and B.

Animals were sacrificed 7 days postsurgery.

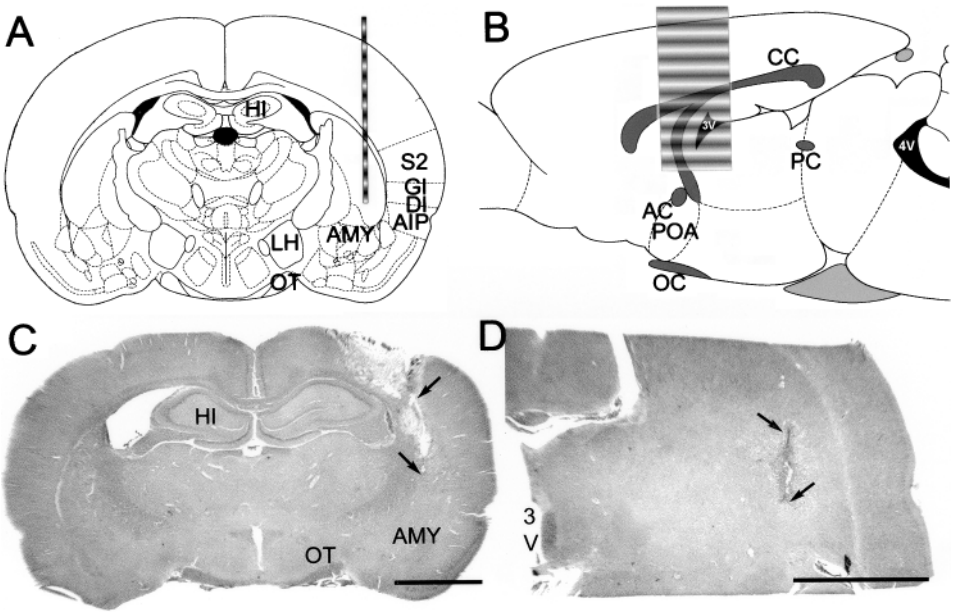

Fig. 1. Schematic illustration of the knife cut (A and B). A shows the vertical extension of the cut in the coronal plane (striped bar). B illustrates the anteroposterior extent of the cut (striped plate) projected to the mid-line (sagittal section). The microphotos illustrate the knife cut (arrows) in the coronal (C) and in the horizontal plane (D). Abbreviations: 3V: third ventricle; AC: anterior commissure; AIP: agranular insular cortex; AMY: amygdala; CC: corpus callosum; DI: dysgranular insular cortex; GI: granular insular cortex; HI: hippocampus; LH: lateral hypothalamus; OC: optic chiasm; OT: optic tract; PC: posterior commissure; POA: preoptic area; S2: secondary somatosensory cortex. Scale bar: 200 μm

Sample collection

Animals were sacrificed by decapitation, and trunk blood was collected. Blood was allowed to clot, and serum was separated by centrifugation and was stored at –20 °C until assayed for testosterone, LH and FSH.

Testicular incubation

When animals were killed, testes were immediately removed, weighed, then decapsulated and incubated in 3 ml medium 199 containing 25 mM Hepes and 0.1% BSA in a metabolic shaker at 35 °C for 2 hours. After the incubation media were transferred to tubes and stored at –20 °C until assayed for testosterone. The results are expressed as testosterone produced per testis (nmol/testis) during the incubation period.

Assays

Testosterone concentrations of serum or tissue culture medium (Medium) were determined by radioimmunoassay (RIA) as previously described in detail [5].

Briefly, 20 µl serum was extracted with 2 ml ether. The dried extract was dissolved in 500 µl assay buffer (ASB, 0.5 M phosphate-buffered saline with 1 g/l gelatin, pH 7.4). For standard, Calbiochem testosterone for high-performance liquid chromatography standard was used in a nine-step series ranging 3.5–1000 fmol/tube. From 20 µl Medium samples, direct determination was made. In this case, to each standard tube 21 µl unused Medium was added. The RIA tubes contained the samples (in duplicates) or standards, 7 nl/tube antibody (CV-RT 17, 1 : 100,000 final dilution) and 12,000 cpm ^{3}H-labeled testosterone (100 fmol, The Radiochemical Center, Amersham) in a total volume of 0.7 ml ASB. The cross-reactions of the antibody: 5α-dihydrotestosterone (DHT) 45%, 5β-DHT 9%, and androstendione 2%. With other 27 natural and synthetic steroids examined, the antibody showed less than 0.05% cross-reaction. After an overnight incubation at 4 °C, the bound and free steroids were separated by dextran-coated charcoal. The radioactivity was measured in a two-phase liquid scintillation system. The sensitivity limit of the assay is 3 fmol/tube. The inter- and intra-assay coefficients for variation were 9.8% and 5.9%, respectively.

LH and FSH levels from the rat sera were determined by RIA utilizing a National Hormone and Pituitary Program kit. For reference, rLH-RP-3 and rFSH-RP-2 preparations were used. The inter- and intra-assay coefficients of variation were 7–9% and 4–6%, respectively.

Statistical analysis

Results were analyzed by ANOVA followed by Student-Newman-Keuls multiple comparison methods. Results were considered significant if $p < 0.05$.

Histology

The brains were fixed in 10% formalin and embedded in paraffin. Brains were cut in serial sections of 7 μm in the coronal or in the horizontal plane, and every tenth section was mounted on gelatin-coated slides. Sections were stained with hematoxylin-eosin and coverslipped.

RESULTS

Body and testis weight

During the postoperative period there was no increase in body weight in any experimental group. Similarly, there was no difference in testis weight between groups, and there was no difference between the weight of the right and left testis within groups (data not shown).

Basal testosterone secretion in vitro

Basal testosterone secretion *in vitro* of intact controls and animals subjected to right- or left-sided sham cut was similar. A remarkable difference ($p < 0.01$) in steroidogenesis was evident between rats with right- and left-sided deafferentation (Fig. 2). Right-sided cut resulted in a significant increase in steroidogenesis of both testes as compared to intact controls and to animals subjected to sham operation on the right side ($p < 0.05$). In contrast, following left-sided cut basal testosterone secretion decreased significantly ($p < 0.05$ vs. intact controls and left-sided sham operated group).

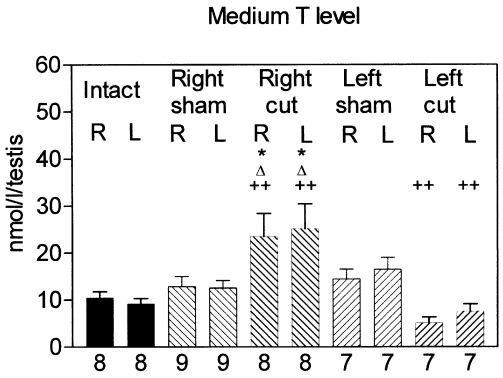

Fig. 2. Effect of right- or left-sided knife cut on basal testosterone (T) secretion *in vitro* of the right and the left testis. The data are mean ±SEM. The numbers below the columns indicate the number of animals. Asterisks indicate significant difference ($p < 0.05$) from the intact control group, Δ significant difference from animals subjected to sham operation on the right side ($p < 0.05$), ++ significant difference between right- and left-sided cut ($p < 0.01$)

Serum testosterone concentration

In animals subjected to right-sided cut, serum testosterone concentration was similar to that observed in sham operated rats. By contrast, left-sided cut resulted in a significant ($p < 0.05$) decrease in serum testosterone level as compared either to sham-operated animals or the group in which right-sided cut was performed (Fig. 3).

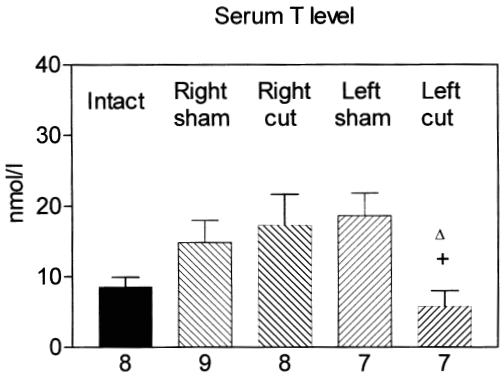

Fig. 3. Effect of right- or left-sided knife cut on serum testosterone (T) concentration. The data are mean ± SEM. The numbers below the columns indicate the number of animals. Δ and + indicate significant difference ($p < 0.05$) from the sham operated groups and from animals subjected to right-sided cut, respectively

Serum LH and FSH concentration

Both in sham-operated controls and in animals subjected to either right- or left-sided intervention serum LH concentration decreased significantly ($p < 0.05$) (Fig. 4). Neither right- or left-sided cut interfered with serum FSH levels (data not shown).

Localization of the lesion

Schematic drawing (Fig. 1A and B) and coronal and horizontal section of the brain illustrate the location and extent of the intervention (Fig. 1C and D). In anteroposterior direction the extension of the deafferented area (projected to the median-sagittal surface of the brain) is the following: the anterior edge of the knife was at the level of the rostral border of the anterior commissure; posteriorly, the cut ended at the middle level of the third ventricle. In vertical direction, the cut ended above the amygdala. No perceptible damage was present in the amygdala, the hippocampus, and hypothalamic structures.

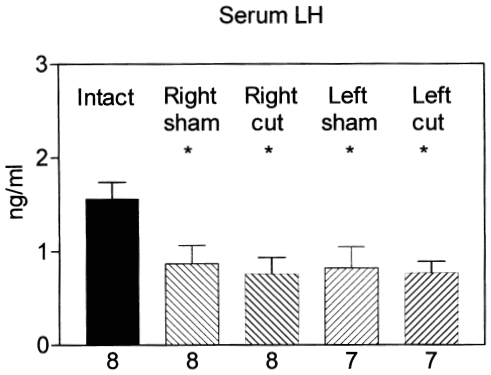

Fig. 4. Effect of right- or left-sided knife cut on serum LH concentration. The data are mean ± SEM. The numbers below the columns indicate the number of animals. Asterisk indicates significant difference ($p < 0.05$) compared to intact controls

DISCUSSION

The results of the present study indicate that a unilateral paramedian sagittal brain cut above the amygdala either on the right or on the left side causing among others partial deafferentation of the insular cortex alters testicular steroid secretion with no change in serum LH concentration. Since the effect of the cut on testicular steroidogenesis was independent of LH secretion, the observations suggest that the information carried by the interrupted fibers could be of physiological significance in the pituitary-independent, direct neural control of testosterone secretion.

The insular cortex has extensive reciprocal neural connections with several brain areas including the thalamus, the lateral hypothalamus, the bed nucleus of the stria terminalis, and the amygdala [4, 20, 21, 22]. Our previous studies indicated that the lateral hypothalamus, the bed nucleus of the stria terminalis and the amygdala exhibited infected neurons following virus inoculation into the testis. Since the amygdala and at least the majority of its innervation, was not affected by the present intervention, it could be assumed that fibers responsible for the effect observed following partial deafferentation of the insular cortex might be those which connect the lateral hypothalamus and/or the bed nucleus of the stria terminalis with the insular cortex.

It is of note that in the present investigations the knife cuts above the amygdala on the right- or on the left-hand side, resulted in changes in testicular steroidogenesis different from the ones observed following unilateral lesion of the insular cortex [3]. The latter intervention on the right side induced suppression of testosterone secretion with enhanced LH secretion while left-sided surgery did not interfere with steroidogenesis but resulted in a slight but significant increase in serum LH concentration. The apparent discrepancy is probably due to the fact that in the present study the insular cortex was only partially deafferented, and the major part of fibers to and from the amygdala was saved, and the deafferented region was not limited to the

insular cortex but was more extended. As mentioned in the introduction, both human and experimental data indicate the role of the amygdala in the neural control of testicular functions [7, 13]. Our previous studies indicated that left-sided deafferentation of the mediobasal portion of the temporal lobe (the deafferented area included the amygdala) in rats hemiorchidectomized on the left side suppressed steroidogenesis of the remaining testis [7]. Therefore, it is reasonable to suppose that fibers to and from the insular cortex involved in the control of testicular steroidogenesis include both direct input and output towards the hypothalamus and other brain structures and innervation via the amygdala.

The observation that the knife cut on the right- and left-hand side influences testicular steroidogenesis in a different way is consistent with the view that cerebral structures controlling gonadal functions are lateralized [see for review 8, 9]. Previous studies have revealed morphological and biochemical asymmetry in hypothalamic distribution of gonadotrop hormone-releasing hormone (GnRH) [1, 14]. Both the number of GnRH neurons and the neurohormone content of the hypothalamus was found significantly higher on the right than of the left side. In addition, unilateral deafferentation of the hypothalamus prevented the hemicastration-induced FSH rise only if right-sided deafferentation was combined with hemicastration on the right side [18]. These observations strongly suggest right-sided dominance of the hypothalamus in neuroendocrine control of endocrine reproductive functions. As mentioned above, lesion studies have revealed also right-sided predominance of the insular cortex [3]. By contrast, the effect of deafferentation of the amygdala [7] and the results of the present study indicate that interruption of certain telencephalic fiber systems influences testicular functions if the deafferentation is on the left side. These observations might suggest that integrity of all neuronal circuitries of both hemispheres is required for the full control of endocrine reproductive processes. This assumption is supported also by our previous results which indicate that in hemicastrated animals transection of the corpus callosum alters testicular steroidogenesis and serum FSH concentration [2].

In conclusion, the present report suggests that efferent and/or afferent connections of the cerebral cortex above the amygdala play a modulatory role in the control of testicular testosterone secretion by a lateralized neural mechanism. On the basis of the present results, however, no information can be provided about the origin of the pathway(s) responsible for the effect. Even with this shortcoming, the data presented here should be a useful guide for future studies investigating the specific cerebral structures involved in the regulation of testicular functions.

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