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Disinhibitory behavior and $GABA_A$ receptor function in serotonin-depleted adult male rats are reduced by gonadectomy

Anders I. Svensson*, Anders Berntsson, Jörgen A. Engel, Bo Söderpalm

Department of Pharmacology, Institute of Physiology and Pharmacology, University of Göteborg, Box 431, SE 405 30 Gothenburg, Sweden

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Abstract

Impulsive and aggressive behaviors in, e.g., personality or substance abuse disorders in man and corresponding behaviors in rats may involve serotonin (5-HT), γ-amino-butyric $acid_A$/benzodiazepine receptor complexes ($GABA_A$/BDZ-RC) and steroid hormones, e.g., testosterone. Here, we studied the effect of gonadectomy on disinhibitory behavior in individually housed 5-HT-depleted rats and on $GABA_A$/BDZ-RC function in vitro, in corticohippocampal synaptoneurosomes prepared from the brain of these animals. 5-HT depletion by intracerebroventricular 5,7-dihydroxytryptamine (5,7-DHT)-induced disinhibitory behavior in a shock-induced behavioral inhibition model (punished conflict model) 14 days after operation. Gonadectomy in connection with the 5-HT depletion reduced the disinhibitory behavior and testosterone substitution prevented this effect. Shock threshold and drinking motivation were not affected by gonadectomy and/or 5-HT depletion. The relative epididymides weight was increased in 5-HT-depleted as compared to sham-operated rats. However, the serum concentrations of testosterone and the relative testes weights were not different in 5-HT-depleted rats as compared to controls. GABA-induced (30, 100, 300 μM) $^{36}Cl^-$-uptake into synaptoneurosomes was lower in 5,7-DHT + gonadectomized rats compared to the control group. This effect was reversed by substitution with testosterone. These results demonstrate that gonadectomy reduces disinhibitory behavior in 5-HT-depleted rats and that $GABA_A$/BDZ-RC may be involved in this effect. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Conflict behavior; Disinhibition; HPG axis; Impulsivity; Testosterone; Testis; Shock-induced behavioral inhibition; Rat

1. Introduction

Several psychiatric disorders characterized by a poor impulse control are associated with signs of a decreased central serotonergic neurotransmission, for instance with low concentrations of the main serotonin (5-HT) metabolite 5-HIAA in the cerebrospinal fluid [4,7,26–28,47,48]. These disorders include violent suicide, impulsive behavior, aggressive behavior, and alcohol dependence. Also in the rat, depletion of 5-HT by, for instance, selective 5-HT neurotoxins produce impulsive behavior [43], aggressive behavior (cf. Refs. [10,42]), disinhibited behavior in conflict models (cf. Refs. [17,36,43]), and an increased alcohol intake in the choice between alcohol and water (cf. Refs. [16,20,25]). Thus, there are obvious similarities between the behaviors observed in low-serotonergic humans and low-serotonergic rats.

Disinhibited behaviors in conflict models, e.g., in Vogel's conflict model that involves shock-induced behavioral inhibition are commonly interpreted to reflect anxiolytic-like effects. However, as suggested by Soubrié [43], these behaviors may also reflect impulsive behavior, at least after certain manipulations, e.g., after 5-HT depletion (see also Ref. [42]).

We have previously reported that depletion of 5-HT by the 5-HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) produces behavioral disinhibition in a modified Vogel's conflict test and that this disinhibited behavior was antagonized by the $GABA_A$-antagonists picrotoxin and bicuculline and by the inverse BDZ agonist Ro 15-4513 [41], as well as by the weak $GABA_A$-antagonist naloxone [42,45]. These findings indicate that the disinhibitory behavior observed after 5-HT depletion may involve enhanced endogenous activity at brain $GABA_A$/benzodiazepine chloride ionophore receptor complexes ($GABA_A$/BDZ-RC).

Interestingly, evidence supports a link also between testosterone and disinhibited/impulsive behavior both in

* Corresponding author. Tel.: +46-31-773-3418; fax: +46-31-773-3284.

E-mail address: anders.i.svensson@pharm.gu.se (A.I. Svensson).

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man and in the rat. Accordingly, clinical studies and case reports indicate a connection between the abuse of anabolic steroids and impulsive and aggressive behavior (e.g., Ref. [9]). Furthermore, women with bulimia nervosa, a disorder to some extent characterized by a poor impulse control, display significantly higher serum levels of free testosterone compared to controls [44]. Data also indicates a connection between testosterone and disinhibited and aggressive behavior in the rat. Accordingly, aggression in male rats is proportional to serum testosterone concentration [3]. Furthermore, testosterone treatment produces behavioral disinhibition in some studies [5,6]. However, other workers have failed to observe disinhibited or impulsive-like behavior after testosterone treatment [18].

Interestingly testosterone may influence both 5-HT neurotransmission and activity at the $GABA_A$/BDZ-RC. Thus, testosterone in supraphysiological levels may decrease brain 5-HT tissue levels [30], whereas gonadectomy may produce an increase in 5-HT levels [15]. Furthermore, the reduced testosterone metabolites androsterone and α-androstanediol are positive modulators of the $GABA_A$/BDZ-RC (e.g., Ref. [38]). As mentioned earlier, both 5-HT and GABAergic mechanisms have been implicated in the regulation of disinhibited behavior.

Here we studied the effect of gonadectomy on disinhibited behavior in individually housed 5-HT-depleted rats by using a punished conflict model, as well as the effect of gonadectomy on $GABA_A$/BDZ-RC function in vitro in synaptoneurosomes prepared from these rats.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (B&K, Universal, Sollentuna, Sweden) weighing 270–350 g were used. The animals were kept under controlled light–dark conditions (light on at 5:00 A.M. and off at 7:00 P.M.) and at constant cage temperature (25°C) and cage humidity (65%). A 7-day adaptation period to the animal maintenance facilities of the department was allowed prior to the start of the experiments. The animals had free access to standard laboratory chow and water when not participating in Vogel's drinking conflict test, drinking motivation or shock threshold experiments. All animal procedures were approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden.

2.2. Surgery

2.2.1. 5,7-DHT lesion

All animals were given 25 mg/kg, ip, desipramine 30 min before being anaesthetized with chloral hydrate 400 mg/kg, ip. Approximately 30 min later they were placed in a stereotaxic instrument (Kopf), with the tooth bar at -3 mm. The skull was exposed and two holes were drilled cranial to the lateral ventricles (anterior–posterior: 1.0 mm behind bregma; medial–lateral: ± 1.5 mm from midsagittal suture). A syringe was slowly lowered into the left and the right ventricle, respectively (3.7 mm, below the skull surface). 5,7-DHT (225 μg in 9 μl of 0.9% NaCl with ascorbic acid 0.1 mg/ml) was slowly infused into each ventricle during approximately 2 min and the syringe was left in place for another 5 min. Sham-lesioned animals received an equivalent volume of the vehicle instead. The syringe was carefully removed and the skin was closed with three to four clips (Michel 7.5×1.8 mm).

2.2.2. Gonadectomy

Immediately after the 5,7-DHT (or sham) lesion, during the same anesthesia as above, scrotal incisions were performed and the main arteries and veins as well as the ductus deferens were located and ligated after which the testes and epididymides were removed. Sham-gonadectomized rats were exposed to similar scrotal incisions.

2.2.3. Capsule implantation

Immediately after the gonadectomy (or sham-operation), during the same anesthesia as above, three Silastic capsules containing testosterone or empty capsules were implanted subcutaneously in the flank.

All the rats weighed at least 275 g when operated upon. After surgery, the animals were transferred to single cages and left to recover for 2 weeks before being used in the shock-induced behavioral inhibition test.

2.3. Shock-induced behavioral inhibition

A modified Vogel's drinking conflict model was used. On the first day of the experiment, the animals were adapted for 10 min to a Plexiglas box (inner-dimensions $30 \times 24 \times 20$ cm) enclosed in a sound-proof cage and equipped with a grid-floor of stainless steel bars and a drinking bottle containing a 5.5% (w/v) glucose solution. After a 24-h period of water deprivation, the animals were adapted to the same test-chamber for a further 5 min. During this period, the animals had free access to the glucose solution. After a further 24 h of water deprivation, the animals were returned to the Plexiglas box. When approaching the drinking spout (usually within 20 s) the animal was allowed to drink for 30 s, after which the first electric shock (0.16 mA for 2 s) was administered between the spout of the drinking bottle and the grid-floor. Upon every further attempt to drink, an electric shock was administered. The number of shocks accepted during a 10-min session was recorded. All experiments were carried out between 10 A.M. and 4 P.M. The animals were used only once in this test.

2.4. Shock threshold and drinking motivation tests

In order to obtain conformity with Vogel's conflict test the animals were treated identically in the shock threshold

test including water deprivation for 48 h. Each rat was placed in the Plexiglas box previously described. The shock threshold was determined step-wise by manually increasing the current delivered through the grid-floor (0.05, 0.06, 0.08, 0.10, 0.13, 0.16, 0.20, 0.25, 0.3, 0.4, 0.5, 0.6 mA) until the rat showed an avoidance reaction to the electrical stimulus (jump, jerk, or similar) as judged by an assistant that was blind to the treatment and the shock level applied. There was a 15-s shock-free interval between each step. The current amplitude threshold was recorded. Immediately after the shock threshold had been determined (see above) each rat was placed in its individual home cage and this was supplied with a drinking bottle containing 50 ml of a 5,5% (w/v) glucose solution. The total amount of liquid (g) consumed during 10 min was recorded for every rat.

2.5. Preparation of synaptoneurosomes

Synaptoneurosomes were prepared essentially according to the method of Hollingsworth et al. [21]. Rats were decapitated and their brains rapidly taken out and placed in ice-cold preparation buffer (PB = NaCl 118.5 mM, KCl 4.7 mM, $MgSO_4$ 1.18 mM, $CaCl_2$ 2.5 mM, HEPES (2-(4-(2-hydroxyethyl)-1-piperazinyl)-ethansulfonic acid) 20 mM, and Tris–base 9 mM). The cerebral cortices and hippocampi were dissected free from white matter, and the dura and pia mater were removed. The cortices and hippocampi from one animal from each treatment group (vide infra) were placed in ice-cold PB. The tissue from each animal was homogenized in 7 ml of PB in a glass–Teflon-homogenizer. The homogenate was gravity filtered through two layers of nylon filter (160 μM) in a Sweenex filter holder. The resultant material was filtered once more over a 10-μm Millipore filter. The filtrate was centrifuged at $1000 \times g$ for 15 min and the supernatant was decanted. The pellet was resuspended in 2 ml of PB in a glass–Teflon-homogenizer, and was thereafter diluted with 30 ml of PB and centrifuged once more at $1000 \times g$ for 15 min. The supernatant was again decanted and the remaining pellet weighed and diluted with PB, to yield a final protein concentration of approximately 10 mg/ml. The resulting material after a similar cortical preparation has been described in detail and is considered to mainly be made up of sealed neuronal membranes, often arranged in a "snowman"-like fashion ("synaptoneurosomes"), with a limited number of other "cells" (a "cell-free" preparation) [29,37]. In the present study, we added hippocampus to the preparation, since this brain region shows a high density of $GABA_A$/BDZ-RC, and since this procedure increases the tissue yield from each animal.

2.6. $^{36}Cl^-$-uptake in synaptoneurosomes

Assay tubes containing 300 μl of PB (with pH 7.4 at 30°C) were prewarmed in a water bath (30°C), before addition of 100 μl of the synaptoneurosomal suspension. The suspension was left to incubate for 20 min before the addition of 50 μl GABA (or PB) together with 0.5 μCi of $^{36}Cl^-$ (50 μl). The mixture was rapidly vortexed and the flux of $^{36}Cl^-$ terminated 5 s later by the addition of 5 ml ice-cold PB containing 100 μM picrotoxin. The mixture was then immediately vacuum-filtered (Schleicher & Scheull filters, GF31, 24 mm) and the tube and the filter were rinsed twice more with 5 ml of the picrotoxin-containing buffer. The filters were placed into scintillation vials and 4.5 ml of scintillation fluid (Beckmann Ready-Safe) were added. Radioactivity was counted over night (DPM), using conventional liquid scintillation techniques. Data are expressed as percent stimulation of baseline $^{36}Cl^-$ accumulation (5 s in the absence of GABA).

2.7. Serum concentration of testosterone

The serum concentration of testosterone in trunk blood was estimated by using a ^{125}I testosterone DA kit (ICN, 07-189102). This method has been used earlier to measure testosterone in adult male rats [14].

2.8. Experimental designs

Rats were randomly, during the same anesthesia, 5,7-DHT-lesioned (5,7-DHT) or sham-lesioned (sham), gonadectomized (gon), or sham-gonadectomized (sham) and implanted with testosterone-filled (test) or empty capsules (sham). This procedure yielded the following treatment groups: (1) sham/sham/sham; (2) sham/gon/sham (3) 5,7-DHT/sham/sham; (4) 5,7-DHT/gon/sham; (5) 5,7-DHT/gon/test; and (6) 5,7-DHT/sham/test.

Rats from groups (1)–(6) were tested in the shock-induced behavioral inhibition test and in the shock threshold and drinking motivation tests (separate experiments).

To compare the amount of GABA-induced $^{36}Cl^-$-uptake in the treatment groups, synaptoneurosomes from rats in group (1) to (5) were prepared (between 12 A.M. and 2 P.M.) 1 day after the shock-induced behavioral inhibition test.

Testosterone was estimated in serum taken from trunk blood between 12 A.M. and 2 P.M. the day after the shock-induced behavioral inhibition test was performed. The rats were killed by decapitation. Epididymides and testes from treatment groups (1) and (3) were dissected and weighed and expressed as the relative organ weight ± S.E.M. Relative organ weight is here the total weight of both the left and right organ divided by rat weight (mg/100 g).

2.9. Drugs

Desipramine HCl (Sigma, St. Louis, MO) was dissolved in distilled water and administered intraperitoneally 2.0 ml/kg. Chloral hydrate (Merck) was dissolved in 0.9% NaCl and given intraperitoneally 5.0 ml/kg. 5,7-DHT (Sigma) was dissolved 0.9% NaCl with ascorbic acid 0.1 mg/ml and given intracerebroventricularly (2×9 μl). GABA (Sigma) and picrotoxin (Sigma) were dissolved in assay buffer.

Picrotoxin was light-protected throughout the experiment. Silastic capsules (length: 50 mm, inner diameter: 1.57 mm, SIKEMA) were filled with crystalline testosterone (4-androsten-17β-ol-3-one, Sigma) as described by Damassa et al. [11]. All capsules were incubated in 0.9% NaCl at 1 day before use and the capsules were washed in 70% ethanol for 30 min and thereafter in saline 30 min before implantation.

2.10. Statistics

A Kruskal–Wallis analysis followed by Mann–Whitney *U* test was used for evaluation of the differences between treatment groups in the shock-induced behavioral inhibition test, the shock threshold test, in the drinking motivation test and with respect to serum concentrations of testosterone. Mann–Whitney *U* test was used for evaluation of the differences between treatment groups in mean relative epididymides, mean relative testes weights, and in rat weights. Correlation between shock-induced behavioral inhibition and shock threshold was evaluated with the paired correlation analysis followed by Fisher's *r* to *z* test. A repeated measures analysis of variance (ANOVA), followed by Fisher PLSD [12,49] implemented for comparisons with unequal *n*:*s* [1], was used to evaluate the difference in GABA-induced $^{36}Cl^-$-uptake between treatment groups. A *P* value $\leq .05$ was considered to be statistically significant. When appropriate, multiple comparisons were corrected for using Holm's procedure, a weighted improvement of the Bonferroni procedure [22].

3. Results

3.1. Shock-induced behavioral inhibition

The results shown in Fig. 1 were pooled from three independent experiments and are expressed as percent of controls (= sham-operated animals), which accepted approximately 20 shocks/10 min in absolute terms. Treatment with the 5-HT neurotoxin 5,7-DHT produced behavioral disinhibition by significantly increasing the number of shocks accepted in the punished conflict test as compared to controls. Rats that were both 5,7-DHT-lesioned and gonadectomized accepted a significantly lower number of shocks than rats that were 5,7-DHT-lesioned and sham-gonadectomized and than gonadectomized, 5,7-DHT-lesioned animals that received subcutaneous testosterone substitution by means of three testosterone-filled capsules. This dose of testosterone was used since previous dose-finding experiments had indicated that gonadectomized, 5,7-DHT-lesioned rats treated with three, but not one or two, capsules accepted significantly more shocks than gonadectomized, 5,7-DHT-lesioned animals that were not substituted (data not shown).

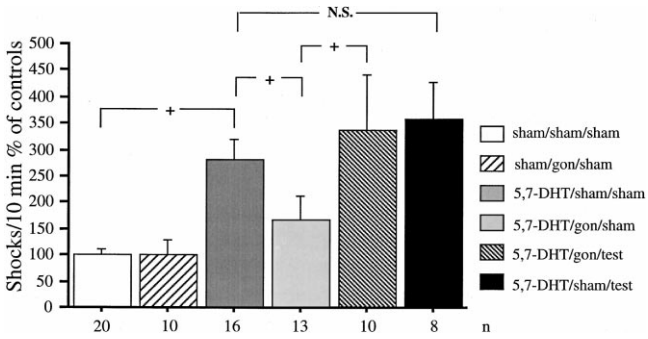

Fig. 1. Effect of 5,7-DHT-lesion, and/or gonadectomy (gon) with or without testosterone substitution (test) on the number of shocks accepted during a 10-min test in the shock-induced behavioral inhibition model (expressed in % of sham-operated controls). Shown are the means ± S.E.M. *Statistics*: Kruskal–Wallis ANOVA $H = 30.4$, $P < .0001$, followed by Mann–Whitney *U* test. The multiple comparisons were corrected for using Holm's procedure, $^{+}P < .05$, NS (non-significant) = $P > .05$.

Table 1
Effect of 5,7-DHT-lesion, and/or gonadectomy (gon) with or without testosterone substitution (test) on the shock threshold and on drinking motivation — the amount of 5.5% glucose solution consumed during 10 min after a 48-h period of water deprivation. Shown are the means ± S.E.M. of 6–17 observations

Treatment	*n*	Shock threshold (mA)	Liquid consumption (g)
Sham/sham/sham	17	0.12 ± 0.01	14.51 ± 0.87
Sham/gon/sham	9/10	0.09 ± 0.01 ($n = 10$)	12.62 ± 0.80 ($n = 9$)
5,7-DHT/sham/sham	15	0.16 ± 0.02	14.12 ± 0.87
5,7-DHT/gon/sham	13	0.12 ± 0.02	12.92 ± 0.96
5,7-DHT/gon/test	6	0.15 ± 0.03	14.50 ± 1.29
5,7-DHT/sham/test	8	0.13 ± 0.02	12.63 ± 1.46

Statistics: Shock threshold data: Kruskal–Wallis ANOVA $H = 16.0$, $P = .0067$, followed by Mann–Whitney *U* test. The multiple comparisons were corrected for using Holm's procedure. *Drinking motivation data:* Kruskal–Wallis ANOVA $H = 4.616$, $P = .4645$. There were no statistically significant differences in shock threshold or drinking motivation between the different treatment groups.

3.2. Shock threshold and drinking motivation tests

None of the treatments statistically significantly altered the shock threshold or drinking motivation (Table 1).

3.3. Correlation between shock-induced behavioral inhibition and shock threshold

There was no correlation between the number of shocks accepted in the punished conflict model and the shock threshold neither in all treatment groups together nor in each treatment group (all treatment groups: correlation = – .052, $P = .680$, paired correlation analysis followed by Fisher's *r* to *z*).

3.4. GABA-induced $^{36}Cl^-$-uptake into synaptoneurosomes

GABA-induced $^{36}Cl^-$-uptake was significantly lower in rats that were both 5-HT-depleted and gonadectomized

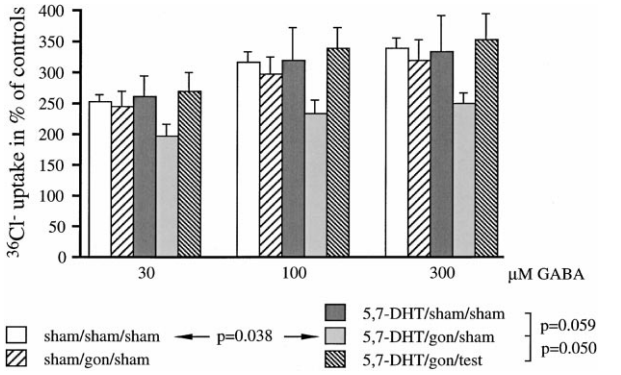

Fig. 2. Effect of GABA (30, 100, and 300 μM) on $^{36}Cl^-$-uptake in corticohippocampal synaptoneurosomes from 5,7-DHT-lesioned, and/or gonadectomized (gon) rats with or without testosterone substitution (test). Shown are the means + S.E.M. of three to nine experiments performed in triplicates. *Statistics*: Repeated measures ANOVA. GABA effect: $F(2,40) = 60.5$, $P < .0001$ (group effects are shown).

(5,7-DHT/gon/sham) as compared to controls (sham/sham/sham, $P < .05$, Fig. 2). Testosterone substitution (5,7-DHT/gon/test) prevented this effect ($P = .050$) of gonadectomy (5,7-DHT/gon/sham). Furthermore, there was a tendency for a lower GABA-induced $^{36}Cl^-$-uptake in rats that were both 5-HT-depleted and gonadectomized (5,7-DHT/gon/sham), as compared to rats that were only 5-HT-lesioned (5,7-DHT/sham/sham, $P = .059$).

3.5. Serum testosterone levels

Serum concentration of testosterone was significantly lower in sham-lesioned gonadectomized rats as compared to control rats. There were, however, no other statistically significant differences between the treatment groups with respect to serum levels of testosterone (Table 2).

3.6. Epididymides, testes, and rat weight

The relative epididymides weight, but not relative testes weight, was significantly higher in 5-HT-lesioned as compared to sham-lesioned rats. Moreover, the rat weight was significantly lower in 5-HT-lesioned as compared to sham-lesioned rats (Table 2).

4. Discussion

Depletion of 5-HT by 5,7-DHT treatment produced a pronounced disinhibitory behavior in a model involving shock-induced behavioral inhibition. This effect has been documented previously by several different investigators (cf. Refs. [17,23,24,39]). The disinhibitory behavior of the 5-HT-depleted rats was in turn reduced by gonadectomy, and testosterone substitution, in a dose that did not significantly alter the behavior of 5-HT-depleted, non-gonadectomized rats, prevented this effect of gonadectomy. The lack of effect of testosterone treatment on the behavior in 5-HT-depleted, non-gonadectomized rats can probably not be explained by a ceiling effect, since it previously has been demonstrated that further significant behavioral disinhibition can indeed be obtained by pharmacological manipulations in these rats [39]. None of the experimental manipulations applied significantly influenced shock thresholds or drinking motivation and there was no correlation between shock-induced behavioral inhibition and shock threshold levels. Hence, in all probability, the behavioral alterations observed are not secondary to unspecific effects on shock threshold and/or drinking motivation.

Interestingly, gonadectomy did not affect behavior in sham-lesioned rats. This lack of effect can probably not be explained by a low baseline behavioral performance in non-lesioned rats, since it previously has been demonstrated that further significant behavioral inhibition can indeed be

Table 2
Effect of 5,7-DHT and/or gonadectomy (gon) with or without testosterone substitution (test) on serum concentration of testosterone (S-testosterone), relative epididymides and testes weight, and on rat weight

Treatment	S-testosterone (nmol/l)	Relative epididymides weight (mg/100 g)	Relative testes weight (mg/100 g)	Rat weight (g)
Sham/sham/sham	3.34 ± 0.93	92 ± 4	767 ± 36	366 ± 5
n	11	6	6	6
Sham/gon/sham	0.01 ± 0.00[†]	ND	ND	ND
n	4			
5,7-DHT/sham/sham	1.45 ± 0.45[a]	104 ± 2[†]	839 ± 25[a]	335 ± 5[††]
n	10	6	6	6
5,7-DHT/gon/sham	2.32 ± 1.96[a,b]	ND	ND	ND
n	6			

Shown are the means ± S.E.M. and *n* values. ND = not determined. *Statistics: S-testosterone:* Kruskal–Wallis ANOVA $H = 10.4$, $P = .015$, followed by Mann–Whitney *U* test. The multiple comparisons were corrected for using Holm's procedure. *Relative epididymides weight, relative testes weight, and rat weight:* Mann–Whitney *U* test.

[a] Non-significant compared to sham/sham/sham.

[b] Non-significant compared to 5,7-DHT/sham/sham.

[†] $P < .05$.

[††] $P < .01$.

obtained at the present base-line level (cf. Ref. [41]). We have earlier reported that gonadectomy enhances shock-induced behavioral inhibition in group-housed rats [46]. Thus, the negative result here of gonadectomy on shock-induced behavioral inhibition could possibly be due to the fact that the rats were housed individually after operation in the present experiment. Indeed, it has earlier been reported that individual housing of rats perturbs the androgen hormone system by, e.g., increasing plasma testosterone levels [13]. Needless to say, further studies are required to explain why shock-induced behavioral inhibition is unaffected by gonadectomy in individually housed rats and enhanced by gonadectomy in group-housed rats.

Interestingly, earlier investigators have reported results indicating that 5-HT inhibits the hypothalamus–pituitary–gonad (HPG) axis. Accordingly, in vitro, in tissue obtained from the mediobasal hypothalamus in adult male rats, 5-HT has been demonstrated to inhibit release of the luteinizing hormone (LH)-releasing hormone [8]. Furthermore, inhibition of 5-HT synthesis by parachlorophenylalanine (pCPA) resulted in an enhanced release of LH in vivo in adult male rats [32]. 5-HT also appears to regulate central negative feedback mechanisms of the HPG axis. pCPA administration to hemicastrated rats, as compared to controls, produced a significantly higher compensatory rise of testosterone in peripheral blood [33].

From these above referred findings the hypothesis was raised that 5-HT depletion in our experiments increases the activity of the HPG axis. This hypothesis is supported in our experiments by the following. First, 5-HT-depleted rats display an increased relative epididymides weight as compared to sham-depleted rats. This finding could not be explained by a decreased rat weight since the relative testes weight was not affected by 5-HT depletion. Epididymis weight has been reported to increase after treatment with testosterone and to decrease after gonadectomy [31,34,35]. Secondly, 5-HT-depleted but not sham-depleted rats were sensitive to the effect of gonadectomy on shock-induced behavioral inhibition.

However, serum testosterone levels did not statistically significantly differ between 5-HT-depleted rats and controls. This negative finding may be explained by a high intra- and inter-individual variation over time in serum concentrations of testosterone in adult male rats [19]. Accordingly, there is a high variation also in our serum concentration data of testosterone. It is not possible to disclose tentative differences in testosterone release by examining a single time-point. To address this issue, experiments with cannulated animals, allowing multiple blood sampling, will have to be performed. It should be noted that while gonadectomy, as expected, completely abolished serum testosterone levels in sham-lesioned animals, it did not in 5,7-DHT-lesioned rats. The reason for this peculiar finding remains unclear but could be speculated to involve a 5-HT regulated, e.g., via LH release (see above), androgen production from, e.g., aberrant testes tissue and/or the adrenal glands.

Regarding the mechanism(s) underlying the effect of gonadectomy on 5,7-DHT-induced disinhibition it may be speculated that the hypothesized increase in the activity of the HPG axis is involved. First, gonadectomy reduced 5,7-DHT-induced disinhibitory behavior and testosterone prevented this effect indicating that activity at the gonadal level of the HPG axis is of importance. Secondly, a higher dose of testosterone (three capsules) was needed in this experiment to reverse the enhanced shock-induced behavioral inhibition after gonadectomy in 5-HT-lesioned rats as compared to the earlier reported testosterone dose needed (one capsule) to reverse the enhanced shock-induced behavioral inhibition after gonadectomy in non-lesioned, group-housed rats [46].

As mentioned in the Introduction, gonadectomy may produce an increase in brain 5-HT levels [15]. Moreover, an increase in brain 5-HT levels by intracerebroventricular administration of 5-HT enhanced shock-induced behavioral inhibition in 5,7-DHT-treated rats [41]. Thus, it may be speculated that the serotonergic system is involved in the mechanisms underlying the effect of gonadectomy on 5,7-DHT-induced disinhibition. This is however unlikely, since the 5-HT depletion was performed according to a protocol that causes a severe general and selective depletion of brain 5-HT [41]. Needless to say, it is not possible to totally rule out the possibility that serotonergic system is involved in the mechanisms underlying the effect of gonadectomy on 5,7-DHT-induced disinhibition, since it could be speculated that the very low brain 5-HT levels in the 5,7-DHT-treated rat could be affected by gonadectomy. However, as mentioned in the Introduction, testosterone may also influence the GABAergic system (among other systems).

Therefore, we examined the function of the $GABA_A$/BDZ-RC in vitro by estimating GABA-induced $^{36}Cl^-$-uptake into synaptoneurosomes in the different treatment groups. GABA-induced $^{36}Cl^-$-uptake most likely reflects increased $^{36}Cl^-$ flux across $GABA_A$/BDZ-RC, since it previously has been demonstrated to be antagonized by the $GABA_A$/benzodiazepine chloride ionophore blocker picrotoxin [40]. Interestingly, we found that the GABA-induced $^{36}Cl^-$-uptake was lower in rats that were both 5-HT depleted and gonadectomized as compared to controls. Interestingly, testosterone, just as was the case with the behavioral effect, prevented the effect of gonadectomy in 5-HT-depleted rats. In line with these findings, it has earlier been demonstrated that an anabolic–androgenic steroid increases GABA-induced $^{36}Cl^-$-uptake [6]. Furthermore, also other adrenocortical hormones, possibly corticosterone, have been demonstrated to regulate disinhibitory behavior and GABA-induced $^{36}Cl^-$-uptake in 5-HT-depleted rats [39].

However, 5-HT depletion or gonadectomy alone did not alter the GABA-induced $^{36}Cl^-$-uptake. In contrast to these findings, one earlier study has indicated that 5-HT depletion alone induces a lower GABA-induced $^{36}Cl^-$-uptake than controls. The GABA-induced $^{36}Cl^-$-uptake was, however, estimated 4 weeks after 5,7-DHT treatment

[40] as compared to 2 weeks after 5,7-DHT treatment in the present study.

In conclusion, gonadectomy reduced disinhibitory behavior in 5-HT-depleted rats and $GABA_A$/BDZ-RC may be involved in this effect. These results may be of clinical importance since our results indicate that the HPG axis and $GABA_A$/BDZ-RC may be involved in the mechanisms underlying the link between low brain 5-HT neurotransmission and dysfunctional impulsive behavior in patients. Interestingly, there is support from other investigators for an association with a more labile regulation of testosterone and emotional lability in healthy men [2]. Thus, clinical studies are warranted to investigate whether a drug that decreases the activity of the HPG axis also decreases disinhibitory/impulsive behavior in patients with or without signs of a lowered serotonergic neurotransmission in brain.

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