DOES SEROTONIN DEPLETION AUGMENT OR COUNTERACT THE AGGRESSION-PROVOKING EFFECT OF TESTOSTERONE IN MICE?

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INTRODUCTION

While testosterone promotes aggression, serotonin exerts the opposite effect. If testosterone influences brain serotonergic activity, one possible mechanism of action for the hormonal pro-aggressive effect would be that it might counteract an anti-aggressive effect of serotonin.

The aim of this study was to explore this possibility, following the rationale that: if testosterone does exert its pro-aggressive effect by dampening serotonergic transmission, then i) the anti-aggressive effect of anti-androgenic interventions would be counteracted by serotonin depletion, and ii) administration of testosterone would fail to cause any further increase in aggression in serotonin-depleted animals.

To examine this, we assessed if serotonin depletion, induced by administration of the tryptophan hydroxylase inhibitor p-chlorophenylalanine (p-CPA), restores aggression in gonadectomized mice and in mice displaying brain-specific knockout of androgen receptors (AR^{NesDel}), respectively, and also to what extent the pro-aggressive effect of exogenously administered testosterone in the former group is dependent on the presence of serotonin.

RESULTS

Experiment I. In testosterone treated mice, pCPA led to a significant increase in both frequency and duration of aggressive acts while it was completely void of effects in mice not treated with hormones (Fig. 1A).

Experiment II. AR^{NesDel} mice displayed low levels of aggressive behaviour in both tests and was not affected by pCPA-treatment. In the control group, although duration of aggression was robustly increased by pCPA treatment, this measure failed to reach significance (Fig 2A). However, compared to saline treated controls, there was a significantly greater number of mice attacking within 200 seconds (Fig. 2B).

COMMENT

The observation that the marked reduction in aggression exerted by gonadectomy or brain-specific androgen receptor knockout was not counteracted by serotonin depletion permits the conclusion that the pro-aggressive effect of testosterone is *not* primarily caused by an inhibition of serotonergic transmission.

Similarly, testosterone was found to exert a marked aggression-provoking effect also in the absence of this transmitter; this behavioural effect of the hormone was in fact more pronounced in pCPA-treated animals than in controls, suggesting that serotonin exerts parallel-coupled inhibitory influence on testosterone-induced aggression, causing serotonin depletion to enhance aggression in the presence but not in the absence of androgen receptor activation.

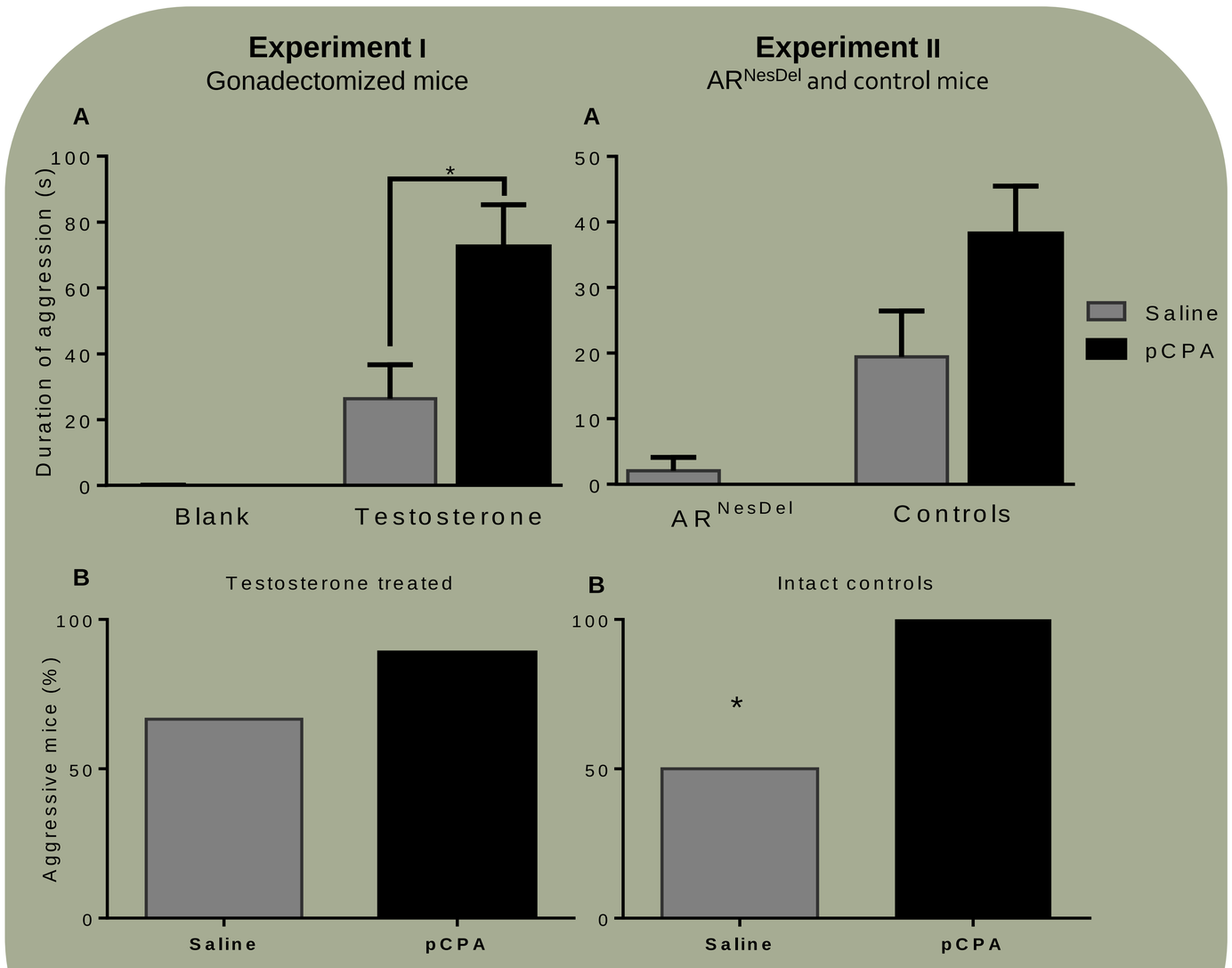

Figure 1. A) Duration of aggressive behaviour in experiment I. B) Percentage of aggressive animals in exeriment I.
* $p < 0.05$

Figure 2. A) Duration of aggressive behaviour in experiment II. B) Percentage of aggressive animals in exeriment II
* $p < 0.05$

Table I. Aggressive behaviour in experiment I. A) Results from first resident intruder test B) Results from second resident intruder test, after pCPA treatment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ against the corresponding testosterone treated mice. # $p < 0.05$ against testosterone treated mice given saline.

Experiment I				
A	**Vehicle (n=15)**		**Testosterone (n=18)**	
Number of attacks (±SEM)	0.7 (±0.7)		8.3 (±1.8)	
Time attacking (s) (±SEM)	7.0 (±7.0)***		43.9 (±9.6)	
Latency to attack (s) (±SEM)	876.2 (23.8)***		362.3 (±72.4)	
No. aggressive mice (%)	0/15 (0)*		6/18 (33)	
B	**Saline (n=8)**	**pCPA (n=7)**	**Saline (n=9)**	**pCPA (n=9)**
Number of attacks (±SEM)	0.1 (±0.1)**	0.0 (±0.0)***	8.1 (±2.8)	23.7 (±4.3)#
Time attacking (s) (±SEM)	0.1 (±0.1)***	0.0 (±0.0)***	26.4 (±10.3)	72.5 (±12.8)#
Latency to attack (s) (±SEM)	794.0 (±102.0)**	900.0 (±0.0)***	208.7 (±102.3)	65.8 (±22.0)
No. aggressive mice (%)	1/8 (12.5)*	0/7 (0)***	6/9 (67)	8/9 (89)

Table II. Aggressive behaviour in eperiment II. A) Results from first resident intruder test B) Results from second resident intruder test, after pCPA treatment. * $p < 0.05$ against AR^{NesDel} mice.# $p < 0.05$ against saline treated controls. † $p = 0.065$ against saline treated controls.

Experiment II				
A	**AR^{NesDel} (n=8)**		**Controls (n=20)**	
Number of attacks (±SEM)	3.4 (±4.9)		8.1 (±8.5)	
Time attacking (s) (±SEM)	10.8 (±7.3)		45.4 (±11.7)*	
Latency to attack (s) (±SEM)	666.4 (±110.4)		379.9 (±75.1)*	
No. aggressive mice (%)	0/8 (0)		9/20 (45)*	
B	**Saline (n=4)**	**pCPA (n=4)**	**Saline (n=10)**	**pCPA (n=9)**
Number of attacks (±SEM)	0.5 (±0.5)	0.0 (±0.0)	4.8 (±1.8)	9.1 (±2.3)*
Time attacking (s) (±SEM)	2.0 (±2.0)	0.0 (±0.0)	19.5 (±7.0)	38.2 (±7.3)*†
Latency to attack (s) (±SEM)	715.3 (±184.8)	900.0 (±0.0)	442.2 (±124.7)	90.3 (±19.5)*
No. aggressive mice (%)	1/4 (25)	0/4 (0)	5/10 (50)	9/9 (100)*#

METHODS

Experiment I. Male C57Bl/6N mice (Charles River, Denmark), were gonadectomized and implanted with slow-release pellets containing testosterone (15 mg/60days) or placebo (Innovative Research of America, USA) after 2 weeks of recovery. The dose was chosen to be in the high range of the physiological interval and have been shown to reliably increase aggression in castrated male mice (1). After 2 additional weeks, mice were housed individually for nine days before resident intruder test and pCPA treatment.

Experiment II. AR^{NesDel} mice were generated as described previously (2). Briefly, AR^{flox} female mice were bred with male Nestin-Cre mice. Intact AR^{NesDel} mice and littermate controls were housed individually for 23 days and tested in the resident intruder test.

Resident intruder test: Resident mice were tested in their home cage against an unfamiliar intruder mouse (group housed male 129/SvEv, Taconic Farms) for 15 minutes, prior to treatment with pCPA (300 mg/kg) or vehicle, once daily i.p. for 3 consecutive days, and re-tested 24 hrs after the final injection. All test were performed during the dark phase and videotaped with overhead video camera under IR illumination. Offensive biting, chasing and wrestling, when performed by the resident mouse, were scored as aggressive behaviour. Duration and frequency, as well as latency to the first instance of each behaviour, were recorded. If a behaviour could not be detected during the duration of the test, latency was set to 900 seconds. Mice attacking within 200 seconds from the start of the test was considered aggressive (3).

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