INTRODUCTION

Studies indicate a modest positive association between testosterone and aggression (see Archer, 1991 for review). In general, this positive association has been based on group-comparison studies, correlation studies or treatment and/or intervention studies. In group-comparison studies men and women selected for atypical violence (such as prison inmates) have been found to possess higher testosterone levels than nonviolent controls (Kreuz and Rose, 1972; Dabbbs et al., 1987; Dabbbs and Hargrove, 1997). Elevated testosterone levels have also been found in violent, antisocial, alcoholic offenders (Bergman and Brismar, 1994; Virkkunen et al., 1994). In correlation studies a significant link between testosterone and increased self-rated aggression was found in offenders (Bergman and Brismar, 1994; Virkkunen et al., 1997). In another double-blind placebo-controlled crossover trial of low- and high dose methyltestosterone, increased irritability, hostility and violent feelings were reported in normal male volunteers (Su et al., 1993). The absence of increased aggression by exogenous testosterone has been reported by Wu et al. (1982), O’Carroll et al. (1985) and Anderson et al. (1992).

In comparison to androgens, much less attention has been paid to oestrogens in male aggressive behaviour. Oestrogens have been related to aggression in animals (Simon and Whalen, 1986; Hilakivi-Clarke et al., 1997), while human studies on oestrogen and aggressive behaviour have been sparse. Oestrogens have been used with some success to suppress violent sexual aggression (see review by Bradford, 1983). Oestrogen administration has also been shown to attenuate dementia-related physical and sexual aggression in elderly men, while verbal aggression has remained unchanged (Kyomen et al., 1991; Kay et al., 1995; Shelton and Brooks, 1999).

Whereas steroid hormones may be intrinsic factors involved in aggression, alcohol is clearly an important external factor associated with the expression of human aggression (Chermack and Giancola, 1997; Brismar and Bergman, 1998). Alcohol-mediated steroid hormone changes may also affect underlying relationships between hormones and aggression, as shown in male mice (Hilakivi-Clarke et al., 1997). The aim of the present study was to explore the relationship between aggression and endogenous testosterone and oestradiol in men with a history of alcohol-related aggression and in an age-matched male control population. Interpersonal partner violence, as assessed by the revised Conflict Tactics Scale 2 (Straus et al., 1996), was chosen as the target behaviour.

SUBJECTS AND METHODS

Participants and procedure

Male volunteers were recruited through advertisements, which were placed in two major national newspapers, soliciting men with a history of alcohol-related aggression (AGG+). The potential subjects were instructed to contact the National Public Health Institute by phone or mail. All prospective subjects received a letter by mail further describing the study, an informed consent form, the questionnaires and a stamped envelope for completed questionnaires. Seventy-three men responded by calling or writing and 59 (72.6%) returned completed questionnaires. Within 2 weeks after receipt of the completed questionnaires, each participant was given a laboratory appointment for the collection of a blood sample. The subjects were told not to
Blood samples, taken from median cubital vein, were voluntarily obtained from 46 of the 59 AGG+ men between 07.00 and 09.30 hours (mean 08.05 hours).

For the control sample (AGG−), 300 male residents of Helsinki aged 20–50 years were randomly drawn from the official Finnish Population Register. A letter was sent to all potential control subjects explaining the purpose of the study and how the subjects had been drawn from the Finnish Population Register, together with questionnaires identical to those sent to the AGG+ men. The final control sample of 60 men completed the questionnaires and 45 of the controls eventually gave a morning blood sample, as described for the AGG+ men.

In the AGG+ group, two participants were excluded as drugs or anabolic steroids had been used in the previous 3 months, and in one subject ethanol was found in the blood sample. Three subjects were excluded based on negative responses on all of the six different aggression measures in the questionnaires. In the AGG− group one subject was excluded because ethanol was detected in the blood sample. Thirteen men reported use of sedatives or sleeping pills in the previous 12 months, but this was not considered a sufficient cause for exclusion. The final sample size consisted of 40 AGG+ men (age: 42.9 ± 1.8 years) and 44 AGG− men (age: 39.7 ± 1.5 years) for whom complete data sets were available. All subjects gave informed consent and the National Public Health Institute’s Ethical Committee approved the study.

**Questionnaires**

The Revised Conflict Tactics Scale (CTS2) by Straus et al. (1996) was used for the estimation of the subjects’ interpersonal aggression during the preceding year. The CTS2, as revised from the widely used CTS1 (Straus, 1979), consists of items describing emotional and cognitive negotiation (six items), minor and severe psychological aggression (eight items), minor and severe physical assault (12 items), minor and severe sexual coercion (seven items), and minor and severe injury (six items). In the present study, the two negotiation scales representing qualitatively different issues were separately analysed. The emotional negotiation scale was determined by the questions of how frequently ‘I showed my partner I cared even though we disagreed’, ‘showed respect for my partner’s feelings about an issue’, ‘said I was sure we could work out a problem’. The cognitive negotiation scale was assessed by the questions of how frequently ‘explained my side of a disagreement to my partner’, ‘suggested a compromise to a disagreement’, and ‘agreed to try a solution to a disagreement my partner suggested’. For the other items, the minor and severe scales representing quantitatively different issues were combined. The reliability analyses by Cronbach’s alpha indicated good internal consistency for the overall scales (negotiation: 0.83; psychological aggression: 0.77; physical assault: 0.93; injury: 0.80) except for sexual coercion (0.67), which was lower than the original standardization (Straus et al., 1996). The level of sexual coercion was too low to be reliably analysed in the present population and, consequently, this scale was excluded from further analyses.

In addition, all subjects completed questionnaires on demographic background, marital status, level of education, alcohol-related questions, the extent of spouse abuse in current relationship, drug and steroid use, as well as information about medication, smoking and eating habits and other life-style factors. The six negative responses, which were used as exclusion criteria for the AGG+ men, involved the following questions. (1) Have you got into fights when drinking? (MAST; Selzer, 1971). (2) Have you or someone else been injured as a result of your drinking? (AUDIT; Saunders et al., 1993). (3) Alcohol increases feelings of arousal and aggression? (AEQ; Brown et al., 1987). (4) Do you have aggressive and/or violent personality? (our question). (5) Do you become aggressive when you drink? (our question). (6) Have you hurt or injured others during alcohol drinking during the last 12 months? (our question).

For assessment of alcohol abuse Selzer’s (1971) revised Michigan Alcoholism Screening Test (MAST) was used, which is an alcoholism-screening device with a weighted scoring system consisting of 24 items and a score rate from 0 to 53. The MAST, with associated results from the present population, has been previously described in more detail (von der Pahlen et al., 2002b).

**Radioimmunoassays**

Hormone measurements were performed by radioimmunoassay techniques in venous plasma samples stored at −70 °C until determinations. Testosterone levels were determined with standard reagent sets from Orion Diagnostica (Finland). Free testosterone levels were determined with a reagent set from Diagnostic Products (Los Angles, CA, USA). Testosterone within-assay variability (CV%) was 6.6%, between-assay variability was 7.0% at 0.96 nmol/l (n = 10), and the detection limit was 0.1 nmol/l. Free testosterone within-assay variability was 4.3% and between-assay variability was 5.5% at 4.6 pmol/l (n = 10) and the detection limit was 0.5 pmol/l. Oestradiol levels (within-assay variability 9.0%, n = 12; between-assay variability 4.7%, n = 4, at approx. 40 pmol/l; detection limit 18 pmol/l) were determined by a standard radioimmunoassay reagent set (Oestradiol-2) from DiaSorin (Italy). Ethanol levels were determined by headspace gas chromatography (Sigma 2000; Perkin Elmer). The within- and between-assay coefficients of variation were 4.0 and 5.1%, respectively, at 1.5 nmol/l (n = 10).

**Statistical analyses**

The present data did not display normal distribution and thus all statistical analyses (Mann–Whitney U, chi-squared and Spearman’s rho) were non-parametric. Two-tailed analyses were used for the calculations of significance. SPSS (10th edn), was used for the estimation of the regression line and the statistics. Group data are expressed as means ± SEM. Adjustment procedures were applied for the specific analysis of a hormone–behaviour relationship independent of another hormone’s variation. Thus, for example, adjusted testosterone values were created by changing the constants of the regression equation of the testosterone–oestradiol function so that the ‘normalized’ testosterone values displayed no correlation with oestradiol.
RESULTS

On the CTS2, the AGG+ group scored significantly higher than did the AGG− group on psychological aggression, physical assault and injury (Table 1), while no significant difference was detected in either emotional or cognitive negotiations. The self-rated alcohol misuse in the AGG+ group (MAST = 21.7 ± 1.9) was significantly higher than in the AGG− group (10.0 ± 1.4; P < 0.001), as previously reported (von der Pahlen et al., 2002b). No significant differences were observed with body weight and height (83.8 ± 2.0 and 181.9 ± 1.8 kg; 181.3 ± 0.9 and 180.3 ± 0.9 cm, AGG− and AGG+, respectively). The AGG+ men tended to be less educated than the AGG− subjects (<13 years of education: 47.4 vs 31.4%). Also, the AGG+ men were significantly (P < 0.01) more frequently unemployed than were the AGG− group (35.0 vs 9.1%).

The correlation between hormones and behaviour indicated a positive association between oestradiol and emotional negotiation scale (Fig. 1a), which was significant in the AGG− group (Table 2) as well as in the combined population (r = 0.332; P < 0.01). Because of the positive overall correlation between oestradiol and testosterone levels (Fig. 2a), adjustments for testosterone variation were also applied, resulting in minor changes to the correlation between oestradiol and emotional negotiation (Table 2). However, as shown in Table 2, the adjustment for oestradiol abolished the trends for non-adjusted correlation between testosterone and emotional negotiation.

Both with and without the adjustments for testosterone, oestradiol was associated with the cognitive negotiation and psychological aggression (Fig. 1b), being more apparent in the AGG− group (significance in the combined population for both behavioural scales: P < 0.01). No significant correlation was found between testosterone and cognitive negotiation and psychological aggression scales.

Also, testosterone was found to correlate positively with both physical assault and the injury scales in the AGG+ group (see Table 1 and the averaged function for injury in Fig. 1c).

Table 1. Conflict Tactics Scale-2 (CTS2) scores of interpersonal aggression (means ± SEM) and the significance of the differences between controls (AGG−) and men selected for alcohol-related aggression (AGG+) in a study on oestradiol and human male alcohol-related aggression

<table>
<thead>
<tr>
<th>CTS2 scores</th>
<th>AGG−</th>
<th>AGG+</th>
<th>Significance (P-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negotiation (total)</td>
<td>57.8 ± 5.8</td>
<td>56.3 ± 6.3</td>
<td>0.8666</td>
</tr>
<tr>
<td>Emotional</td>
<td>33.3 ± 3.4</td>
<td>33.2 ± 3.6</td>
<td>0.984</td>
</tr>
<tr>
<td>Cognitive</td>
<td>23.1 ± 3.0</td>
<td>24.7 ± 3.2</td>
<td>0.723</td>
</tr>
<tr>
<td>Psychological (total)</td>
<td>15.8 ± 2.7</td>
<td>33.6 ± 5.4</td>
<td>0.002**</td>
</tr>
<tr>
<td>Minor</td>
<td>14.1 ± 2.5</td>
<td>29.6 ± 4.1</td>
<td>0.002**</td>
</tr>
<tr>
<td>Severe</td>
<td>1.6 ± 0.7</td>
<td>4.6 ± 1.3</td>
<td>0.037*</td>
</tr>
<tr>
<td>Physical (total)</td>
<td>1.4 ± 0.5</td>
<td>11.6 ± 3.1</td>
<td>0.001***</td>
</tr>
<tr>
<td>Minor</td>
<td>1.1 ± 0.3</td>
<td>8.8 ± 2.3</td>
<td>0.001***</td>
</tr>
<tr>
<td>Severe</td>
<td>0.3 ± 0.2</td>
<td>3.1 ± 0.9</td>
<td>0.002**</td>
</tr>
<tr>
<td>Injury (total)</td>
<td>0.9 ± 0.0</td>
<td>1.6 ± 0.6</td>
<td>0.002**</td>
</tr>
<tr>
<td>Minor</td>
<td>0.9 ± 0.0</td>
<td>1.3 ± 0.4</td>
<td>0.072</td>
</tr>
<tr>
<td>Severe</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.2</td>
<td>0.005**</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001 (Mann–Whitney U).

Fig. 1. Running averages for the relationship between behaviour and hormones in a study on oestradiol and human male alcohol-related aggression. (a) Emotional negotiation to oestradiol; (b) psychological aggression to oestradiol; (c) injury to free testosterone. Open circles (controls: AGG−) and closed circles (men selected for alcohol-related aggression: AGG+) show the mean individual values measured with the revised conflict tactics scale (CTS2) for 10 (AGG+) or 11 (AGG−) consecutive ascending plasma hormone levels. The diagram is arranged so that each circle shares nine (AGG+) or 10 (AGG−) data points with the circles adjacent to it, eight or nine with the circles one place removed, seven or eight with the circles two places removed etc., and none with circles 10 or 11 (or more places) away from it. Four of these values for each group also mark the means for the quartiles and are encircled with their SEM displayed. Group differences (corresponding quartiles) are indicated by

*P < 0.05 and **P < 0.01 (Mann–Whitney U).
The adjustment for oestradiol made no difference (Table 2). No significant correlation was observed in the AGG− men, merely because of the lack of physical assault and injury in this present control population.

In Fig. 2a the overall positive correlation between individual oestradiol and testosterone levels are presented. The chi-squared analysis revealed significant distribution differences with the AGG+ individuals inflicting injury (CTS2 score >1) being more prevalent below the regression line than those AGG+ men who did not report marked injury behaviour ($\chi^2 = 8.87; P < 0.01$) and the AGG− individuals ($\chi^2 = 13.91; P < 0.001$). A further analysis was performed on those individuals who were within the injury-related testosterone range of the injury-prone AGG+ individuals (hatched in Fig. 2a). Here, significantly lower oestradiol levels, despite no differences in testosterone levels, were found in the injury-prone AGG+ individuals, than in the AGG− group (Fig. 2b).

The preceding results are based on plasma free testosterone and oestradiol levels adjusted for free testosterone variation. In most cases the corresponding correlation between total testosterone and physical assault in the AGG+ men was indicated (original $\rho = 0.365; P < 0.05$; oestradiol-adjusted $\rho = 0.260; P = 0.088$). In contrast to free testosterone (Table 2), no significant relation was observed between total testosterone and physical assault in the AGG+ men (original $\rho = 0.178; P = 0.272$; oestradiol-adjusted $\rho = 0.212; P = 0.188$).

Age did not significantly correlate with any of the aggression parameters and the adjustment for age did not change the significance between the hormones and the aggression parameters. Age-adjusted MAST scores correlated positively with psychological aggression ($\rho = 0.354; P < 0.05$) and tended to correlate with the injury parameter ($\rho = 0.280; P = 0.085$) in the AGG+ men. However, little change was observed when the correlation between hormones and CTS2 parameters was adjusted for MAST.

### DISCUSSION

The present results, showing positive associations between testosterone and physical assault and injury in the AGG+ men, are in line with earlier findings (Kreuz and Rose, 1972; Archer, 1991; Dabbs and Dabbs, 2000). However, testosterone, although correlating with the injury scale, cannot explain the higher aggression scores in the AGG+ than in the AGG− men (Fig. 1c). Many AGG− men had free testosterone levels in the >60 pmol/l range that was associated with high injury score in the AGG+ men, but the AGG− did not show any increase in injury. It is possible that aggression itself is caused by factors other than testosterone, but that the underlying strength by which the aggression is expressed is related to testosterone when the social control of these other factors breaks down. Most probably alcohol, which reduces the cognitive control of behaviour, is one of the main players, as is suggested by our present findings. These considerations may explain some of the controversy in the literature regarding the relationship between androgens and different features of aggression (Albert et al., 1993).

The present findings show, for the first time, that oestradiol rather than testosterone is positively related with less violent forms of human male aggression. From this it follows that earlier findings of testosterone association with, for example, anger (Gray et al., 1991; Suarez et al., 1998; van Honk et al., 1999) and verbal aggression (Oliveira et al., 1980; Gladue, 1991a; Soler et al., 2000), could be re-evaluated with respect to the potential role of oestradiol. It is quite possible that the less-violent forms of aggression were linked directly to elevated oestradiol and that the correlations with testosterone were secondary, being caused by the correlation of testosterone to oestradiol. This notion is supported by one of the earlier studies, which shows a positive correlation between verbal aggression and both oestradiol and testosterone (Gladue, 1991b). Quantitative and qualitative differences in the various inventories of physical aggression may explain...
some support for the preceding conclusions can also be found from experimental animal data. investigations on male mice (Brain and Bowden, 1979; Haug et al., 1986; Hilakivi-Clarke, 1996; Cologer-Clifford et al., 1999), rats (Christie and Barfield, 1979; Hilakivi-Clarke et al., 1996) and birds (Schlinger and Callard, 1990; Wingfield et al., 2001), show that oestradiol itself, in addition to testosterone, may produce a variety of aggressive behaviours. that oestrogen mechanisms are needed for the development of the male aggression potential in mice has been shown in a recent knockout genetic study (Toda et al., 2001). Here, the disruption of the CYP19 gene (coding for the aromatase that converts testosterone to oestradiol) resulted in a complete loss of the development of aggressive behaviour. Two studies have dealt with the aspect of oestriadiol and alcohol-related aggression in male mice (Hilakivi-Clarke, 1996; Hilakivi-Clarke et al., 1997). Interestingly, it was show that oestradiol treatment increased both alcohol drinking and alcohol-related aggression but that mice, which exhibit a paradoxical decrease in serum oestradiol levels by alcohol, may be particularly prone to alcohol-induced aggression. However, regarding instinctive aggressive behaviours, it is not possible to compare animal data with human data such as that derived from the present study, as the human data was based on psychosocial questionnaires. the development of constructs particularly designed to assess the biological components of human aggression will be a good target for future research on aggressive behaviour.

the experimental animal data as well as our present results demonstrate a need for the re-evaluation of intervention studies in which testosterone has been administered in order to study behavioural effects in men (see review by Zitzmann and Nieschlag, 2001). the interpretation of experiments with testosterone administration may be complicated by the aromatization to oestradiol and its subsequent effects. further studies in this field would require recognition of the oestradiol effect. to the best of our knowledge, our study is the first to report that endogenous oestradiol may in fact counteract testosterone-related physical aggression with inflicted injury. the lower oestradiol:testosterone ratio, despite more excessive alcohol drinking in the injury-inflicting AGG+ men than in the controls, may even be more meaningful than is shown by our present results. This is due to the oestradiol-elevating and testosterone-lowering effects of chronic alcohol intake (Burra et al., 1992), which could mask even greater differences in the original oestradiol:testosterone ratio. Our results support the hypothesis that regulation of human violent behaviour may involve the deliberate balance between male androgens and female oestrogens. further support for the hypothesis is provided by our finding of the positive correlation between testosterone-independent oestradiol and the emotional negotiation subscale, which measures self-rated feelings of care and respect for the partner during aggressive conflict situations. the present finding of oestradiol-associated empathic behaviour in men may also relate to a recent observation of elevated oestradiol levels in men becoming fathers (Berg and Wynne-Edward, 2001).

both empathy and aggression are complex behaviours that include a number of regulatory factors. that oestradiol may be involved in both behaviours is perhaps not so surprising when one considers the multiple actions of steroid hormones.

earlier discrepancies in the relationships between testosterone and physical aggression. the dissection of aggression into component parts, and the use of oestradiol as a covariate, will be helpful in producing reliable future studies on the relationship between testosterone and aggression.

fig. 2. low oestradiol levels in men with high injury scores in a study on oestradiol and human male alcohol-related aggression. (a) correlation between individual plasma oestradiol and free testosterone levels in controls (AGG−; open circles), men selected for alcohol-related aggression (AGG+) having a low injury score (<1; closed filled circles) and AGG+ men having a high injury score (>1; big hatched circles). spearman’s rho is 0.395 (p < 0.01) for the overall correlation. the diagonal line marks the linear regression line without the constant term. the hatched area marks the free testosterone range of the AGG+ men with high testosterone-dependent injury score (selected from the ascending phase of the injury-testosterone function, see fig. 1c). (b) Oestradiol and (c) free testosterone means ± SEM in men with testosterone levels in the hatched area (Fig. 2a) related to injury. Open bars: AGG− (n = 14); hatched bars: AGG+ men with high injury scores (n = 9). **p < 0.01 (Mann–Whitney U).
However, little is known about the possible mechanism for such actions by oestradiol. Oestrogen-mediated reduction of aggressive effects, by suppressing the anterior pituitary gonadotrophic function in men (Bell, 1978; Barfield, 1984), may play a role in antagonizing physical and violent aggression. On the behavioural level, verbal skills could be a common denominator for psychological aggression and negotiation capacities. However, there are no previous data on an oestradiol-mediated increase in the verbal skills of men. If anything, a recent study showed that oestradiol administration to men caused impairment in verbal memory (Cherrier et al., 2002). Empathic skills should counteract especially physical and violent aggression. However, the CTS2 questionnaire has some limitations for assessing further mechanisms. The emotional negotiation questions involve an empathic component, but the scale measures the frequency of conflicts indirectly. This 'limitation' may explain why the testosterone-adjusted oestradiol correlation with injury did not reach significant negative values and why the negotiation scales did not differ between the AGG− and the AGG+ groups. Another limitation, especially with regard to the generality of our findings, is that our selection method could well have included an over-representation of individuals who acknowledged concern about their behaviour. The present data, using the available instruments for recording behaviour, are limited for assessing more closely the role of sex hormones in behaviour.

The effect of alcohol on steroid hormones may be a complicating factor in understanding the relationship between these hormones and aggressive behaviour during alcohol intake. Especially in women, alcohol has been observed to elevate both testosterone (Eriksson et al., 1994; Sarkola et al., 2001) and oestradiol (Välimäki et al., 1983; Mendelson et al., 1988) levels, which could explain part of their alcohol-mediated pronouncement of aggressive behaviour. A testosterone-mediated alcohol effect may also be possible in men, as suggested by a recent finding of testosterone elevations caused by acute alcohol intake in men (Sarkola and Eriksson, 2003). However, excessive chronic alcohol intake is usually associated with decreased testosterone and elevated oestradiol levels (Burra et al., 1992). Although our data are too limited to draw firm conclusions, it is interesting to note that the three possible outliers (with the highest AGG+ oestradiol values seen in Fig. 2a) had lower than average testosterone levels, high MAST scores (mean = 23), and low scores on physical aggression (mean = 1). Thus, alcohol misuse may in some situations create 'chemical castration' reducing injury-inflicting aggressive behaviour in men.

Another complicating factor regarding the alcohol effects is that the current CTS2 questionnaire does not assess to what degree the self-reported aggression is occurring during the influence of alcohol. We suggest that future studies would benefit from using questionnaires specifying both sober state aggression and aggression under the influence of alcohol.

The present findings show that testosterone is positively related to severe and violent expression of physical alcohol-related aggression and that oestradiol is positively associated with psychological aggression in men. Together with our novel finding that oestradiol, also, is positively related to emotional empathic behaviour that may even counteract the physical aggression, the broader aspect of sex is raised. The present results might provide new insight into the biological basis for the current view that differences between the sexes in aggression mainly concern physical aggression associated with injury (Archer, 2000). It should also be noted that androgens and oestrogens are responsible for most of the observed differences between men and women in brain structure and function (Rubinow and Schmidt, 1996). Far fewer or no differences have been reported between sexes for less violent forms of aggression such as anger, verbal aggression and physical aggression without injury (Gladue, 1991a; Archer et al., 1995; Harris and Knight-Bohnhoff, 1996; Harris et al., 1996; Archer, 2000). Moreover, empathic behaviour has been reported to be more prevalent in women than in men (Rushton et al., 1986; Eisenberg et al., 1989; Cohn, 1991; Harris et al., 1996; Jaffee and Hyde, 2000). Our present results suggest that oestradiol–testosterone-related regulation may not only explain individual differences in men, but may also explain some of the broader differences between sexes regarding empathic and aggressive behaviour.

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