Effects of acute stress on the patterns of LH secretion in the common marmoset (Callithrix jacchus)

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ABSTRACT

Stressful stimuli associated with aggressive encounters and low social rank may affect female fertility in a variety of mammalian species. In these experiments we examined the effects of aggressive encounters and physical restraint in a primate chair on the patterns of LH secretion in ovariectomized, oestrogen-primed female marmosets. Receipt of aggression from a female conspecific, followed by physical restraint for collection of blood samples (at 10-min intervals for 4 h), resulted in marked declines in LH concentrations during oestradiol-induced LH surges in five animals (from $112 \pm 24 \mu g/l$ to $45 \pm 12 \mu g/l$; group means $\pm$ s.e.m.; $P<0.05$). This was due to reductions in LH pulse amplitude rather than to changes in pulse frequency. Decreases in plasma concentrations of LH were reversed by treating females with exogenous LH-releasing hormone (LHRH). Cortisol treatment had no effect on LH levels during oestrogen-induced LH surges. Effects of aggressive encounters and physical restraint on plasma LH were not therefore due to reduced pituitary responsiveness to LHRH or to increased plasma concentrations of cortisol. In separate experiments it was found that physical restraint alone had no effect on plasma LH in habituated subjects, and that decreases in plasma LH after receipt of aggression only occurred if animals were subsequently placed in the restraint chair. A summation of stressful effects is therefore required to produce the fall in circulating LH. A summation of social and other environmental stressors may also underlie the reduced fertility seen in free-living animals.


INTRODUCTION

In a number of primate species, subordinate females in social groups may exhibit disruption of ovarian cyclicity or reduced fertility (talapoin: Bowman, Dilley & Keverne, 1978; gelada: Dunbar, 1980; yellow baboon: Wasser & Barash, 1983; common marmoset: Abbott, 1984). In male monkeys, decreases in plasma concentrations of testosterone can occur as a result of low social rank (rhesus: Rose, Bernstein & Gordon, 1975; talapoin: Keverne, 1979). Aggressive behaviour, or the potential threat offered by higher-ranking individuals, may be important in mediating these effects on ovarian and testicular function. In some cases, low ranking individuals receive more aggression and show less sexual activity than other group members. However, there are considerable variations between primate species and more extreme effects occur in captive social groups than under natural conditions (Rowell & Dixson, 1975; Dixson & Herbert, 1977). Under stressful conditions, the plasma concentration of cortisol is often increased, and chronic increases in plasma cortisol have been implicated in the suppression of plasma luteinizing hormone (LH) in rhesus monkeys (Moberg, Watson & Hayashi, 1982; Dubey & Plant, 1985) and baboons (Sopolsky, 1985). Typically only one female breeds in captive groups of common marmosets, with the other females exhibiting varying degrees of ovarian suppression due to inadequate LH secretion (Epple, 1975; Abbott, 1984). Administration of pulses of synthetic LH-releasing hormone (LHRH) stimulates LH secretion and ovarian activity in such females, suggesting that the pituitary and ovaries retain the capacity to respond, and pointing to a central site of action to suppress reproduction (Abbott, 1987). The positive feedback effects of oestrogen on the hypothalamus and pituitary are an essential prerequisite for the LH surge and hence ovulation.

The aim of the present study was to examine effects of an acute social stress, namely receipt of aggression from a female conspecific, on patterns of LH secretion during oestradiol-induced LH surges in ovariectomized marmosets.
MATERIALS AND METHODS

Animals

Five sexually mature female marmoset monkeys were used. The monkeys were gonadectomized 4 to 48 months before the study. The animals weighed 250–400 g and full details of their management have been published previously (Hearn, Lunn, Burden & Pilcher, 1975).

Collection of blood samples

For collection of serial blood samples a polyvinyl cannula (inner diameter 0.58 mm, outer diameter 0.99 mm; Scientific Marketing Associates, London, U.K.) was chronically implanted into the internal jugular vein at least 7 days before the experiment, and the exteriorized cannulae protected in a jacket worn by the monkey (O’Byrne, 1988). Blood samples were also taken from the femoral vein by venepuncture using the method described by Hearn (1977).

Oestradiol pretreatment

The effects of receipt of aggression on LH secretion during an oestradiol-induced LH surge were examined. The monkeys were given 35 μg oestradiol benzoate (Sigma Chemical Company Ltd, Poole, Dorset, U.K.) as a single s.c. injection in 0.2 ml arachis oil to induce an LH surge (Hodges, 1978).

Behavioural observations

Twenty-four hours after receiving oestradiol benzoate, at the height of the LH surge, the monkeys were placed in the home cage of a female conspecific for 30 min, and the direction and degree of agonistic interactions recorded by two observers sitting behind a one-way mirror. Aggressive behaviours recorded from the resident monkeys included attacks, vocal threats and chases (Lipp, 1978). The responses to aggression shown by the intruder, including submissive squeals, avoidance and fleeing, were also recorded (Lipp, 1978). Three separate ovariectomized monkeys were used as resident animals for the aggression tests.

Experiments

Aggressive encounter and physical restraint

Twenty-four hours after receiving oestradiol, the monkeys were given an aggression test as described above. Immediately following the aggressive encounter the monkeys were restrained in a primate chair (O’Byrne & Morris, 1988) and serial blood samples (0.1 ml) collected at 5- to 10-min intervals for 4 h through the indwelling jugular catheter. Control experiments were carried out 24 h after administration of oestradiol, using the blood-sampling protocol described above, but in the absence of a prior aggressive encounter. The monkeys were extensively habituated to restraint in the primate chair over a period of several months before the onset of the experiment.

Receipt of aggression alone

In a second experiment the effects of receipt of aggression alone, in the absence of restraint in the primate chair, were examined. The same five monkeys were subjected to an aggressive interaction with a female conspecific, 24 h after receiving oestradiol. Blood samples (0.2 ml) were taken from the femoral vein by venepuncture 15 min before and just before the start of the aggression test and then 0, 15, 30, 45, 60 and 75 min after the 30-min aggression test. The monkeys were briefly restrained (Hearn, 1977), but not placed in the primate chair, for collection of each blood sample and returned to the home cage for periods between collections.

LHRH challenge

In order to assess pituitary responsiveness to LHRH following aggression and physical restraint, monkeys were given an LHRH challenge. Twenty-four hours after administration of oestradiol, four monkeys were subjected to the 30-min aggression test and then immediately placed in the primate chair for 4 h. Serial blood samples were collected at 10-min intervals for 1 h before and for 3 h after the i.v. administration of 200 ng synthetic LHRH (Cambridge Research Laboratories, Cambridge, U.K.) and plasma LH levels determined.

Cortisol challenge

To examine whether the stress associated with receipt of aggression and physical restraint affects LH secretion by increasing cortisol release, monkeys were treated with cortisol hemisuccinate to increase the plasma concentrations of cortisol artificially. We have previously shown that the administration of 6 mg cortisol leads to a two- to six-fold increase in circulating levels of cortisol (Dixson, 1987). Four monkeys were placed in the primate chair 24 h after receiving 35 μg oestradiol s.c. Serial blood samples were then collected at 10-min intervals for 1 h before and 3 h after i.m. administration of 6 mg cortisol hemisuccinate (Sigma Chemical Company Ltd), and plasma LH levels measured.

Measurement of plasma LH

Plasma was separated from blood by centrifugation and stored at −20°C until assayed. Plasma concentrations of LH were determined using the mouse Leydig cell bioassay as described previously (Fraser,
Abbott, Laird et al. 1986). Sensitivity of the assay was 2 µg LH/L, with inter- and intra-assay coefficients of variance of 15 and 11% respectively.

Statistics
Heterogeneity of variance in the levels of plasma LH was reduced by log transformation of the data. Differences in mean plasma LH concentrations were analysed using Student's t-test for paired observations, since each monkey was sampled either in the absence of and after receipt of aggression, or before and after drug administration. In each monkey the number of pulses of LH was calculated during the 4-h period of serial blood sampling. A pulse of LH was defined as having occurred when the hormone concentration of two consecutive samples was greater than that of the mean of the previous two samples (basal value), and the value of at least one of the peak samples exceeded the mean basal value by more than twice the coefficient of variance of the assay (Djahanbakhch, Warner, McNeilly & Baird, 1984). The amplitude of each pulse was measured by subtracting the basal from the peak value, and the mean interpulse interval was calculated for each subject.

RESULTS

Effects of agonistic interactions and physical restraint on plasma LH levels
The effects of receipt of aggression followed by physical restraint for collection of serial blood samples on the secretion of LH in the marmoset are shown in Table 1. Under these conditions, receipt of aggression results in a significant (P<0.05) lowering of mean plasma LH levels. This reduction in mean plasma LH was the result of a fall in LH pulse amplitude with no change in LH pulse frequency (Table 1). Figure 1 illustrates the plasma LH profiles for two monkeys after the 30-min aggression test, and in the absence of an aggressive encounter.

Effect of receipt of aggression alone on LH secretion
The mean plasma LH levels obtained from five monkeys immediately before an aggressive encounter with a conspecific (137.7±40.8 µg/l; n=5; group mean ± s.e.m.) were not significantly different from plasma levels of LH following an aggressive encounter in the absence of physical restraint (157.9±56.9 µg/l; n=5; group mean ± s.e.m.).

Response to the LHRH challenge
The pituitary response to LHRH following aggression and physical restraint was examined. Administration of 200 ng LHRH to stressed monkeys resulted in a prompt and dramatic increase in plasma LH levels (before treatment 75.8±20.9 µg/l, after LHRH 225.8±22.5 µg/l; group mean ± s.e.m.; P<0.05; n=4).

Response to cortisol challenge
Acute administration of 6 mg cortisol had no significant effect on mean levels of plasma LH (before treatment 126.5±46.4 µg/l, after treatment 125.0±43 µg/l; group mean ± s.e.m.; n=4).

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<tr>
<th>TABLE 1. Plasma LH parameters in the marmoset monkey following receipt of aggression and physical restraint</th>
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<td><strong>Experimental conditions</strong></td>
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<td>Mean ± s.e.m.</td>
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<td><strong>Receipt of aggression and physical restraint</strong></td>
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<td>Mean ± s.e.m.</td>
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*P<0.05 compared with physical restraint alone (paired t-test; n=5).
of serial blood samples, resulted in a profound lowering of circulating LH levels. Further, this decline in plasma concentrations of LH was the result of a reduction in LH pulse amplitude with no change in LH pulse frequency.

In a number of primate species lower ranking females receive larger amounts of aggression than higher ranking individuals (Abbott, Keverne, Moore & Yodyingyud, 1986; Vellucci, Herbert & Keverne, 1986; Wasser & Starling, 1986). Abbott et al. (1986) have provided evidence that the intensity of aggression received plays an important part in the development of an anovulatory state in subordinate female talapoin monkeys. Similarly, in yellow baboons female members of the group may join in attacking subordinates. These subordinate individuals are less fertile (Wasser & Starling, 1986). In the establishment of an all-female group of marmoset monkeys, we have also observed members of the group joining together in attacking subordinate individuals. Thus, chronic receipt, or potential threat of aggression, may be an important factor in the development of the endocrinological differences found between dominant and subordinate females. However, it is clear from the results of the second experiment, where monkeys were exposed to the same aggressive encounter but not held in a primate chair for collection of blood samples, that receipt of aggression per se was not responsible for the inhibition of LH secretion, since a reduction in plasma LH levels was not observed under these conditions. Similarly, restraint in the primate chair alone was not responsible for the inhibitory effects observed, since a decline in plasma LH levels was not seen immediately following placement of the marmoset in the primate chair (see Fig. 1). In addition, removal of the marmoset from the primate chair for 30 min in the middle of a 4-h serial blood sampling episode had no effect on subsequent LH secretion (K. T. O’Byrne, personal observation). In the ovariectomized rhesus monkey, restraint in a primate chair for collection of blood samples was also found to have no adverse effect on LH secretion (Wilson, Kesner, Kaufman et al. 1984; Gindoff & Ferin, 1987). Acute restraint stress has been shown to cause a transient stimulation of LH secretion in the male rhesus monkey (Hayashi & Moberg, 1987). However, it is interesting that restraint of intact female rhesus monkeys does result in suppression of ovarian cyclicity (E. Knobil, personal communication), thus indicating that restraint is a mild form of stress.

From our data it is difficult to identify the stressor or stressors which resulted in suppression of LH secretion in the marmoset. However, they do elude to the possibility that a number of mild or sub-threshold stressful stimuli, perhaps in this case receipt of aggression and physical restraint, summate to inhibit
gonadotrophin secretion. Similarly, in the natural environment it is probably a summation of various factors which result in reproductive suppression in primates. Thus, low ranking individuals not only receive larger amounts of aggression but suffer more competition, reduced food availability and expend more energy in obtaining food (Harcourt, 1987).

The mechanism through which stress suppresses the secretion of LH remains to be elucidated. Raised plasma concentrations of corticosteroids may be associated with reduced gonadotrophin secretion and impaired reproductive function (Baldwin, 1979; Moberg et al. 1982; Dubey & Plant, 1985; Sapolsky, 1985; Hayashi & Moberg, 1987). However, an increase in the plasma concentration of cortisol alone does not seem to be responsible for the inhibition of LH observed in the present study, since treatment with cortisol did not suppress LH secretion. Acute increases in concentrations of corticosteroids have also been shown to be without effect on LH secretion in the rhesus monkey (Hayashi & Moberg, 1987). In the present study, LHRH reversal of LH depression might indicate that stress was not mediated by a fall in pituitary responsiveness to the LHRH signal from the hypothalamus. Stress may, therefore, have acted at a suprapituitary site to inhibit LHRH release. This would be consistent with the observation in the male rhesus monkey (Hayashi & Moberg, 1987). However, in order to discount possible effects of stress at the pituitary level completely it would be necessary to conduct further experiments using a range of LHRH dosages. Since the decline in mean plasma LH following stress was not associated with a change in LH pulse frequency, it is unlikely that this effect was mediated at the level of the LHRH pulse generator. Nevertheless, the reduction in LH pulse amplitude observed may indicate that the effect of stress was mediated by activation of inhibitory pathways impinging on the LHRH nerve terminals in the median eminence, thereby reducing the amount of LHRH released to evoke each LH pulse. There is considerable evidence that endogenous opioid peptides play such a modulatory role in stress-induced effects on reproductive function (Briski, Quigley & Meites, 1984; Gilbeau & Smith, 1985; Petraglia, Vale & Rivier, 1986). Preliminary results from our laboratory support this hypothesis; the inhibition of LH secretion following receipt of aggression and physical restraint can be reversed by the opioid antagonist naloxone.

Socially mediated suppression of reproduction in the female marmoset is associated with marked reductions in tonic LH secretion as a result of suppressed LHRH release (Abbott, 1987). The demonstration that receipt of aggression and physical restraint reduced levels of plasma LH during the LH surge, without adversely affecting pituitary responsiveness to LHRH, clearly shows that acute stress can affect the positive feedback mechanism of oestrogens. It is possible, therefore, that stress may also block the preovulatory surge of LH, resulting in anovulatory cycles and infertility.

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REFERENCES


