Endurance exercise potentiates the stimulatory influence of oestrogen–progesterone on LH and FSH release in the rat*

E. C. Todoroff†, M. Wilkinson‡§ and A. Bonen†‡

†Division of Kinesiology and Departments of ‡Physiology and Biophysics and §Obstetrics and Gynecology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 3J5

Summary. Ovariectomized rats were treated with oestradiol benzoate and progesterone or GnRH. Prolonged exercise (running 4 days per week for 6 weeks) markedly potentiated the oestrogen/progesterone-induced release of LH and FSH, but the pituitary response to an injection of GnRH was unaffected. In contrast, at 24 h after a single exercise bout there was no apparent effect on steroid and GnRH stimulated LH and FSH responses although an acute exercise session given on the day of the LH surge inhibited steroid-induced LH release in some rats. We conclude that strenuous, prolonged exercise-training in the ovariectomized rat seems to modify the ability of the hypothalamus to release GnRH. The results were not attributable to a single bout of exercise since the gonadotrophin responses immediately or 24 h after such exercise did not parallel the results observed in the trained rats.

Introduction

Endurance exercise is frequently associated with delayed menarche (Warren, 1980; Frisch *et al.*, 1980; Malina, 1983) and alterations in normal human menstrual function (Speroff & Redwine, 1980; McArthur *et al.*, 1980; Bonen *et al.*, 1981; Schwartz *et al.*, 1981). These abnormalities include decreases in luteinizing hormone (LH) and oestradiol concentrations in blood (Dale *et al.*, 1979; Warren, 1980; Baker *et al.*, 1981; Boyden *et al.*, 1983; Cumming *et al.*, 1985a), low concentrations of follicle-stimulating hormone (FSH) (Warren, 1980), decreases in prolactin release (Shangold *et al.*, 1979; Shangold & Levine, 1982; Boyden *et al.*, 1982) and an abnormally short luteal phase (Shangold *et al.*, 1979; Bonen *et al.*, 1981). Cumming *et al.* (1985b) describe an exercise-induced release of LH but a significant decline in LH pulse frequency. A prospective study by Boyden *et al.* (1984) involving women undergoing marathon training reported a progressive decline in gonadotrophin response to synthetic GnRH as training was increased. Substantial evidence therefore suggests that a hypothalamic–pituitary dysfunction with exercise and training exists. However, the evidence cannot distinguish between a primary pituitary defect and/or a hypothalamic dysfunction.

An animal model of exercise-induced reproductive abnormalities would be of great value. Few studies have been performed. Carlberg & Fregley (1985) have reported that oestrous cycles are abnormal after endurance exercise training in a pattern similar to that for menstrual cycles in women. There are reports that exercise provoked a fall in basal plasma LH and prolactin concentrations (Tache *et al.*, 1978) and ovarian hypo-activity in trained rats (Chandra *et al.*, 1978).

The present study was designed to determine the effect of endurance training on the ability of oestrogen/progesterone to induce a surge of gonadotrophins in the ovariectomized rat.

*Reprint requests to Dr A. Bonen, Division of Kinesiology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 3J5.

Materials and Methods

Animals and methods

Sprague-Dawley rats (Canadian Breeding Farm and Laboratories, St Constant, Quebec) were maintained under standard lighting conditions (12 h light:12 h dark; lights on 07:00 h) and constant temperature (22–24°C). Food and water were supplied *ad libitum*. Upon arrival, age and weight were 41–47 days and 125–150 g respectively.

Ovariectomy. Bilateral ovariectomy was performed under ether anaesthesia 10 days after arrival.

Training protocol. At 2 weeks after surgery, animals were randomly assigned to a control or an experimental group. Experimental and control animals were familiarized with the exercise conditions by running on a motor-driven rodent treadmill 5–10 min per day at a speed of 12–15 m/min for 5 days before the beginning of the training programme. Experimental rats were exercised on the treadmill at 15 m/min for 30 min set at a 9% incline on the first day of training. Thereafter, time, speed and incline were gradually increased each week for 6 weeks. During the final week, rats were running 20 m/min at 15% incline for 60 min/day, 4 days per week. Control rats remained sedentary throughout the experimental period. Body weight was recorded weekly throughout training.

We examined the effects of this 6-week training period on LH and FSH surges induced by an appropriate schedule of steroid injections (Caligaris *et al.*, 1968), and by administration of gonadotrophin releasing hormone (GnRH) (Wilkinson & Bhanot, 1985) as described below. Since it was important to dissociate the effects of training from the effects of the last exercise bout, additional exercise groups were included in the present study. In one group the effects of the steroid and GnRH treatments on LH and FSH surges were examined 24 h after a single exercise bout. This provided a direct comparison with the trained animals which were also investigated 24 h after their last exercise session. In another group the steroid and GnRH effects were examined immediately after a single exercise bout. This then permitted an assessment of the effects of exercise on LH and FSH responses to the stimuli provided.

Experimental treatments

Trained animals. Gonadotrophin surges were elicited in ovariectomized rats during the final week of training using the method of Caligaris *et al.* (1968). Rats were injected with oestradiol benzoate at 12:00 h (Day 1; 20 µg oestradiol benzoate per animal s.c. in 0.1 ml sesame oil). This was followed 72 h later with an injection of progesterone (Day 4; 1.0 mg per animal s.c. in 0.1 ml sesame oil). A surge of LH and FSH occurs 5 h later, at which time the animals were decapitated (i.e. 17:00 h on Day 4). In addition to the group of animals receiving the steroid injections, two control groups were included (oil + oil; oestradiol + oil). Animals were exercised on Days 1, 2 and 3 (i.e. not on the day of progesterone injection).

During the final week of the training period animals were injected with oestradiol benzoate at 12:00 h (Day 1; 20 µg per animal s.c. in 0.1 ml sesame oil). This was followed ~77 h later with a s.c. injection of GnRH (Day 4; 1 µg/kg body weight). Rats were killed by decapitation 15 min later (see Wilkinson & Bhanot, 1985). GnRH was injected during the final week of training and experimental animals continued to train on Days 1, 2 and 3 (i.e. not on the day of GnRH injection). Saline-injected animals served as controls and also received oestradiol benzoate treatment as described for the trained animals.

Acute exercise bout

Delayed effects of exercise. To establish that any effects were due to the training programme and not to the last single exercise bout, an acute exercise experiment was conducted. Animals were familiarized with treadmill running for 5 consecutive days. The steroid and GnRH administration schedules remained identical to those described for the trained rodents. However, the acute exercise occurred 24 h before GnRH or progesterone injection.

Immediate effects of exercise. After 5 consecutive days of treadmill familiarization, animals ran at treadmill speed and incline identical to that for Week 3 of training (15 m/min at 9% incline for 60 min). Experimental treatments and dosages (i.e. steroids or GnRH) were identical to those in the training study. Animals (N = 8) received an injection of oestradiol benzoate on Day 1 followed 72 h later by progesterone administration at 12:00 h. A surge would then be expected at 17:00 h. Therefore, animals were exercised in the last hour (i.e. 16:00–17:00 h) immediately before the expected LH and FSH surges.

To investigate the immediate effects of exercise on GnRH-induced and LH and FSH surges another group of ovariectomized rats received oestradiol on Day 1. They were then exercised on Day 4. Since GnRH-induced effects on LH and FSH are studied 15 min after GnRH administration (Wilkinson & Bhanot, 1985), this was injected immediately after 60 min of exercise and the animals were killed 15 min later. Control animals remained sedentary and were given hormone treatments identical to those of the exercising rats.

Assays and analyses

Hormone assay. Trunk blood collected after decapitation was allowed to clot and then centrifuged (4°C) to obtain serum. Samples were stored at –70°C. LH and FSH were determined by double-antibody radioimmunoassay using reagents supplied by NIAMDD and expressed in terms of LH/FSH RP-1 standards. The assay sensitivities were

1.95 ng (LH) and 7.8 ng (FSH). Intra-assay co-efficients of variation were 5% (LH) and 7.6% (FSH). All samples were assayed together to avoid inter-assay variation. Results are expressed as mean hormone concentrations (ng/ml) $\pm$ s.e.m.

Enzyme analysis. Ten trained and 10 control rats were assigned to subgroups for determination of succinate dehydrogenase (SDH). After completion of the training period the hindlimb muscles were rapidly excised, frozen and stored in liquid nitrogen (-79°C). SDH activity (Costill *et al.*, 1979) was measured in the soleus muscle to document changes in the oxidative capacity of skeletal muscle after training.

Statistical analysis. Data were analysed using Student's *t* test. Level of significance was chosen as $P < 0.05$.

Drugs. Oestradiol benzoate and progesterone were obtained from Sigma (St Louis, MO, U.S.A.). Gonadotrophin-releasing hormone was a gift from Dr S. Raiti, National Pituitary Agency (210 W. Lafayette St., Baltimore, MD, U.S.A.)

Results

Effects of an endurance training programme

Enzyme analysis. Succinate dehydrogenase activity (SDH) of the soleus muscle was increased 77% in the endurance-trained animals ($9.48 \mu\text{mol/g/min}^{-1}$) when compared with sedentary control animals ($5.35 \mu\text{mol/g/min}^{-1}$).

Organ and body weights. Adrenal gland, anterior pituitary gland and uterine weights were not affected by the training programme (Table 1; row A). Uterine weights were markedly increased after the steroid treatments ($P < 0.05$) but there was no difference between control and trained animals. The mean of the body weights of all the endurance-trained animals ($300 \pm 3 \text{ g}$) was slightly but significantly ($P < 0.05$) lower than that of the control animals ($321 \pm 2 \text{ g}$).

Steroid hormone treatment and GnRH treatment. There was no difference in basal (unstimulated) secretion of gonadotrophins between trained ($816 \pm 71 \text{ ng LH/ml}$; $1425 \pm 91 \text{ ng FSH/ml}$) and control ($861 \pm 81 \text{ ng LH/ml}$; $1648 \pm 134 \text{ ng FSH/ml}$) rats (5/group). As expected, oestradiol benzoate treatment alone decreased LH and FSH concentrations similarly for sedentary ($N = 5$) and trained ($N = 6$) animals (control $317 \pm 46 \text{ ng LH/ml}$; $1030 \pm 85 \text{ ng FSH/ml}$; trained $402 \pm 31 \text{ ng LH/ml}$; $1009 \pm 161 \text{ ng FSH/ml}$). However, steroid-stimulated gonadotrophin surges were significantly higher in trained animals when compared to control animals (Fig. 1). The responsiveness of the pituitary to stimulation with GnRH did not change after the endurance training programme (control, $N = 14$; $2029 \pm 192 \text{ ng LH/ml}$; $1335 \pm 46 \text{ ng FSH/ml}$; trained, $N = 12$; $2840 \pm 369 \text{ ng LH/ml}$; $1344 \pm 82 \text{ ng FSH/ml}$).

Table 1. Organ and body weights of endurance-trained and sedentary control animals after treatment with oestradiol benzoate (OB) and progesterone (P) or GnRH

Treatment	Group	No. of rats	Body weight (g)	Wt of anterior pituitary (mg)	Wt of uterus (mg)	Wt of paired adrenal glands (mg)
Oil + oil	Control	5	324 ± 14	17 ± 2	125 ± 8	36 ± 2
	Trained	5	306 ± 20	19 ± 2	136 ± 10	39 ± 2
OB + oil	Control	5	329 ± 7	21 ± 4	347 ± 47	50 ± 2
	Trained	6	292 ± 11	29 ± 5	329 ± 16	44 ± 2
OB + P	Control	16	316 ± 4	25 ± 1	323 ± 15	43 ± 2
	Trained	18	310 ± 4	23 ± 1	311 ± 8	45 ± 1
OB + saline	Control	4	319 ± 13	15 ± 1	287 ± 13	40 ± 2
	Trained	5	299 ± 6	16 ± 2	288 ± 24	42 ± 2
OB + GnRH	Control	14	321 ± 6	23 ± 1	283 ± 8	39 ± 1
	Trained	12	293 ± 8	20 ± 1	328 ± 23	44 ± 2

Values are means $\pm$ s.e.m.

Fig. 1. Effects of an endurance training programme on LH and FSH secretion after treatment of ovariectomized rats with oil, oestradiol benzoate (OB) or OB and progesterone (P). Values are mean $\pm$ s.e.m. for the numbers of animals in parentheses. * $P < 0.005$ compared with control value.

Fig. 2. Effect of a single exercise bout on the ovulatory surge of LH and FSH secretion induced by oestradiol benzoate (OB) plus progesterone (P). CTL: secretion in non-exercised controls; T = endurance-trained rats; A: surge generated 24 h after a single exercise bout; B and C: rats were given a single exercise bout 4 h after the injection of progesterone; two responses were seen, a normal surge (B) and an abnormal surge (C). Values are mean $\pm$ s.e.m. for the number of rats in parentheses. * $P < 0.005$, T vs CTL and B vs C.

Effects of an acute exercise bout

The immediate effect of an acute exercise bout on gonadotrophin surges was opposite to that of the training programme (B and C in Fig. 2). Only 4 out of the 8 animals exhibited an LH surge. Similarly, the steroid-induced surge of FSH occurred in only 5 out of 8 animals. Those animals that exhibited a hormone surge had hormone values similar to those of sedentary control animals for both LH and FSH. In contrast, the effect of an acute exercise bout was not present 24 h after exercise (A in Fig. 2). The stimulatory effect of oestrogen and progesterone was not potentiated in animals killed 24 h after exercise.

The acute exercise bout did not alter the responsiveness of the pituitary to GnRH stimulation (control, $N = 14$: 2829 ± 192 ng LH/ml; 1335 ± 46 ng FSH/ml; acute, $N = 8$: 3095 ± 331 ng LH/ml; 1602 ± 168 ng FSH/ml). Similarly, at 24 h after an acute exercise bout, the responsiveness of the pituitary to GnRH stimulation ($N = 5$: 2639 ± 1042 ng LH/ml; 1980 ± 258 ng FSH/ml) did not differ in control and endurance-trained animals.

Discussion

Previous studies have reported a relatively high incidence of menstrual cycle abnormalities in endurance-trained athletes (for review, see Loucks & Horvath, 1985). However, it is difficult to determine the specific location of the changes in the hypothalamo-pituitary-ovarian system.

The present results for rats demonstrate that endurance exercise produces an impairment of the surge mechanism that is probably located within the hypothalamus. The steroid-induced gonadotrophin surge was enhanced in the endurance-trained rats. However, the pituitary gland response to endogenous GnRH was similar in trained and sedentary animals. Therefore, any increase in gonadotrophin surge height with steroid treatments is likely to be of hypothalamic origin. Determination of a full dose response to GnRH is clearly desirable, but one explanation for the large LH/FSH surge in endurance-trained animals is that the training regimen increases the releasable pool of hypothalamic GnRH. For example, GnRH release into the portal circulation may be inhibited or the biosynthesis of this factor is increased. That this is due to chronic training is illustrated by the results of the acute experiments. At 24 h after a single exercise bout there was no influence on steroid or GnRH-stimulated LH and FSH responses. In addition, an acute exercise bout does not immediately increase LH/FSH release and in some rats the secretion is actually abolished. These results strongly suggest that endurance training is involved in the hyperresponsiveness to oestradiol/progesterone feedback. The stressful nature of a single, acute exercise session may be responsible for the inhibition of the LH/FSH surge. A previous study has noted that steroid-induced LH release is sensitive to an acute stressor such as a generalized convulsion (Wilkinson *et al.*, 1982).

The mechanism by which chronic exercise modifies hypothalamic GnRH output is unclear. It has been suggested that endogenous opiate peptides might play a significant role in the reproductive hormonal response to chronic exercise (for review, see Harber & Sutton, 1984). For example, both acute and sustained exercise are reported to elevate blood concentrations of β -endorphin. Recent work by Blake *et al.* (1984) reported that running rats to exhaustion induced changes in β -endorphin and Leu-enkephalin levels in some brain areas, such as the nucleus accumbens, but not in the hypothalamus. This would not, however, exclude possible changes in sub-hypothalamic nuclei. Since this type of endogenous opiate is known to exert inhibitory control of GnRH release (Kalra & Kalra, 1983) it is possible that central opiates may also be affected by exercise.

It remains to be determined whether oestrogen stimulatory feedback is deranged in exercising women, but this and the possible role of opiates is amenable to experimentation.

Financial support for this work was obtained from NSERC (A6449) (A.B.) and the Canadian Medical Research Council (M.W.). We thank Susan McIlquham and Joan Livingston for secretarial assistance.

References

- Baker, E.R., Mathur, R.S., Kirk, R.F. & Williamson, H.O. (1981) Female runners and secondary amenorrhea: correlation with age, parity, mileage and plasma hormonal and sex-hormone-binding globulin concentrations. *Fert. Steril.* **36**, 183–187.
- Blake, M.J., Stein, E.A. & Vomachka, A.J. (1984) Effects of exercise training on brain opioid peptides and serum LH in female rats. *Peptides* **5**, 953–958.
- Bonen, A., Belcastro, A.N., Ling, W.Y. & Simpson, A.A. (1981) Profiles of selected hormones during menstrual cycles of teenage athletes. *J. appl. Physiol.* **50**, 545–551.
- Boyden, T.M., Pamenter, R.W., Stanforth, P.R., Rotkis, T. & Wilmore, J.M. (1982) Prolactin responses, menstrual cycles and body composition of women runners. *J. clin. Endocr. Metab.* **54**, 711–714.
- Boyden, T.M., Pamenter, R.W., Grosso, D., Stanforth, P., Rotkis, T. & Wilmore, J.H. (1983) Sex steroids and endurance running in women. *Fert. Steril.* **39**, 629–632.
- Boyden, T.M., Pamenter, R.W., Stanforth, P.R., Rotkis, T. & Wilmore, J.H. (1984) Impaired gonadotrophin-releasing hormone stimulation in endurance-trained women. *Fert. Steril.* **41**, 359–363.
- Caligaris, L., Astrada, J.J. & Taleisnik, S. (1968) Stimulating and inhibiting effects of progesterone on the release of luteinizing hormone. *Acta endocr., Copenh.* **59**, 177–185.
- Carlberg, K.A. & Fregley, M.J. (1985) Disruption of estrous cycles in exercise-trained rats. *Proc. Soc. exp. Biol. Med.* **179**, 21–24.
- Chandra, A.M., Patra, P.B., Chatterjee, P. & Deb, C. (1978) Effect of long-term treadmill running on gonadal activity in female rats. *Endokrinologie* **72**, 299–303.
- Costill, D.L., Fink, W.J., Vanhandel, P.J., Miller, J.M. & Sherman, M.A. (1979) Muscle fiber composition and enzyme activities of elite distance runners. *Med. Sci. Sports Exercise* **8**, 96–100.
- Cumming, D.C., Vickovic, M.M., Wall, S.R. & Fluker, M.R. (1985a) Defects in pulsatile LH release in normally menstruating runners. *J. clin. Endocr. Metab.* **60**, 810–812.
- Cumming, D.C., Vickovic, M.M., Wall, S.R., Fluker, M.R. & Belcastro, A.N. (1985b) The effect of acute exercise on pulsatile release of LH in women runners. *Am. J. Obstet. Gynec.* **153**, 482–485.
- Dale, E., Gerlach, D.H. & Wilhite, A.L. (1979) Menstrual dysfunction in distance runners. *Obstet. Gynecol.* **54**, 47–53.
- Frisch, R.E., Wyshak, G. & Vincent, L. (1980) Delayed menarche and amenorrhea in ballet dancers. *New Engl. J. Med.* **303**, 17–19.
- Harber, V.J. & Sutton, J.R. (1984) Endorphins and exercise. *Sports Medicine* **1**, 154–171.
- Kalra, S.P. & Kalra, P.S. (1983) Neural regulation of luteinizing hormone secretion in the rat. *Endocr. Reviews* **116**, 311–351.
- Loucks, A.B. & Horvath, S.M. (1985) Athletic amenorrhea: a review. *Med. Sci. Sports Exercise* **17**, 56–72.
- Malina, R.M. (1983) Menarche in athletes: a synthesis and hypothesis. *Annl. hum. Biol.* **10**, 1–24.
- McArthur, J.W., Buller, B.A., Beitins, I.Z., Panage, M., Badger, T.M. & Klibanski, A. (1980) Hypothalamic amenorrhea in runners of normal body composition. *Endocr. Res. Commun.* **7**, 13–25.
- Schwartz, B., Cumming, D.C., Riordan, E., Selye, M., Yen, S.S.C. & Rebar, R.W. (1981) Exercise-associated amenorrhea: a distinct entity. *Am. J. Obstet. Gynec.* **141**, 662–670.
- Shangold, M. & Levine, H.S. (1982) The effect of marathon training upon menstrual function. *Am. J. Obstet. Gynec.* **146**, 862–869.
- Shangold, M., Freeman, R., Thyssen, B. & Gatz, M. (1979) The relationship between long distance running, plasma progesterone and luteal phase length. *Fert. Steril.* **31**, 130–133.
- Speroff, L. & Redwine, D.B. (1980) Exercise and menstrual function. *Physician and Sports Medicine* **5**, 42–45.
- Tache, Y., DuRuisseau, P., Ducharme, J.R. & Collu, R. (1978) Pattern of adenohipophyseal hormone changes in male rats following chronic stress. *Neuroendocrinology* **26**, 208–219.
- Warren, M.P. (1980) The effects of exercise on pubertal progression and reproductive function in girls. *J. clin. Endocr. Metab.* **51**, 1150–1157.
- Wilkinson, M. & Bhanot, R. (1985) Infantile ovariectomy potentiates the stimulatory effect of oestrogen/progesterone on LH secretion in the rat. *J. Endocr.* **106**, 133–139.
- Wilkinson, M., Bhanot, R., Pincock, J.A. & Donald, L. (1982) Flurothyl-induced convulsions delay the onset of sexual maturation in the female rat. *J. Endocr.* **95**, 37–41.

Received 22 July 1986