Seizure-induced delay of puberty in female rats: effects of age, stress and opioid antagonists

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ABSTRACT

We have shown that pre- and post-pubertal female rats are sensitive to seizures. For example, daily convulsions commencing at 24 days of age delay puberty. Here we examine the effect of seizures at various ages. In addition, because opioid peptides are implicated in regulating the onset of puberty and are activated by convulsions, we also investigate the effect of opioid antagonists in the seizure-induced delay of puberty.

A single daily electroconvulsive shock (ECS) was given for 10 days to neonatal (days 2–11), infantile (days 15–24) and juvenile (days 22–31) rats. The treatment delayed vaginal opening (VO) in juvenile rats. Neonatal and infantile rats were unaffected. VO was also delayed by daily ECS for only 5 days in the late juvenile (days 27–31) period. The opioid receptor antagonists naltrexone, nalmefene and injected before and after single daily ECS were unable to block this effect of ECS on VO. To examine whether the effect of ECS is related to stress, we examined several stressors known to induce opioid-mediated alterations in gonadotrophin secretion. Footshock, immobilization and ether stress administered in the juvenile period (days 27–31) did not affect the timing of VO. In addition, rats anaesthetized with halothane, and then given ECS, still showed a delay of VO. These data demonstrate that rats in the late juvenile stage of development are most sensitive to convulsions. We also suggest that opioids are not critical to the mechanism by which the ECS disturbs puberty, and that ECS elicits its effect seemingly independently of the convulsive stress.

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INTRODUCTION

Clinical studies reveal that epilepsy, in its various forms, is associated with reproductive neuroendocrine abnormalities. For example, both men and women with partial seizures of temporal lobe epilepsy exhibit an array of symptoms including hypogonadotrophic hypogonadism, hyperprolactinaemia and polycystic ovarian syndrome (Dana-Haeri, Trimble & Oxley, 1983; Pritchard, Wannamaker, Sagel et al. 1983; Herzog, Seibel, Schomer et al. 1986). Temporal lobe epilepsy is also common in children and frequently results from earlier attacks of status epilepticus (O’Donahoe, 1979). Evidence that infantile seizures may also be associated with neuroendocrine problems is somewhat limited. Several authors have drawn attention to the incidence of precocious puberty and epilepsy (Elian, 1970; Konar & Basak, 1977; Pennfold, Manson & Caldicott, 1978; Williams, Schitt & Savage, 1978). McGowan (1983) has reported that adult epileptics appear, in general, to be smaller than normal. These reports suggest that the influence of neonatal or infantile seizures on puberty as well as adult reproductive function is worthy of further study.

Previous work in our laboratory has shown that the female rat reproductive system is sensitive to artificially-induced seizures both pre- and post-pubertally (Wilkinson, Bhanot, Pincock & Donald, 1982; Bhanot & Wilkinson, 1982; 1984). Daily convulsions, induced with fluorothyl or electroconvulsive shock (ECS), commencing at 24 days of age, delay vaginal opening (VO) which occurs at approximately day 34 in our population. This delay in VO may occur via a derangement of the luteinizing hormone (LH) surge mechanism (Bhanot & Wilkinson, 1984). The effect occurs despite an ECS-induced state of hyperprolactinaemia, a condition known to advance VO and first ovulation (Ojeda, Urbanski & Ahmed, 1986).
The neonatal rat brain appears to be particularly sensitive to seizures. Brain development (Wasterlain, 1976), ontogeny of reflexes (Wasterlain, 1977) and brain protein synthesis (Fando, Conn & Wasterlain, 1979) are all compromised by the induction of seizures in newborn pups. We have, therefore, extended our previous work to an examination of whether neonatal seizures also affect the onset of puberty. In addition, we have investigated the possibility that seizure-induced delays in sexual maturation might be mediated through the release of endogenous opioid peptides (EOP). These neuropeptides have been implicated in regulating the onset of puberty (Wilkinson & Landymore, 1989) and seizures are known to activate central opioid systems (Hitzemann, Hitzemann, Blatt et al. 1987; Lasón, Przewlocki & Przewlocki, 1987).

A further mechanistic possibility is that seizures may delay VO through a stress response (Rameley, 1974). Acute and chronic stress have been shown to influence gonadotrophin secretion significantly in rats of various ages (Briski, Quigley & Meites, 1984; Briski & Sylvester, 1987; Restrepo & Armario, 1987). Recent evidence suggests that such altered gonadotrophin secretion is hypothalamically mediated via corticotrophin-releasing factor (CRF) and EOP in both rats (Petraglia, Vale & Rivier, 1986; Adams, Andrews, Hillier et al. 1987; Almeida, Nikolakis & Herz, 1988) and monkeys (Gindoff & Ferin, 1987). Here we explore the hypothesis that the etiology of ECS-induced neuroendocrine abnormalities and delayed puberty may be associated with post-convulsion stress.

MATERIALS AND METHODS

Animals

Female Sprague–Dawley rats were obtained from Canadian Hybrid Farms, Halls Harbour, Nova Scotia, Canada at various ages. Preweaning pups were shipped in litters of eight and, where applicable, weaned at 21 days of age. Older rats were housed four to six per cage in light (light on 07.00–21.00 h) and temperature (21 °C) controlled rooms. Food and water were available ab libitum.

Neonatal convulsions

Electroconvulsive shock was induced by passing current (120 V, 60 Hz, 60 nA) for 1 s through two steel electrodes held bilaterally, just medial to the developing ears. Control pups were touched with the electrodes, but no current was passed. All animals were immediately returned to the home cage with their dam.

A single ECS was induced daily for 10 days (day 2 through to day 11 of life) between 10.00 and 11.00 h. Administration of current in this fashion produced a full clonic-tonic seizure lasting between 30 s and 1 min. The mortality rate was zero.

Pups were housed with their dams until weaning at 21 days of age. Body weight data was collected every other day until VO. Animals were then killed by decapitation for determination of ovarian and uterine weight as well as examination for the presence of corpora lutea.

Infantile convulsions

Electroconvulsive shock was induced in the same fashion as described above except that steel ear clips were used. Female Sprague–Dawley rats arrived at 10 days of age and were housed as described. A single daily ECS was administered for 10 days between 10.00 and 11.00 h, commencing on day 15 and terminating on day 24. Body weight was monitored until VO occurred, at which point animals were killed and examined as described above.

Juvenile convulsions

A series of four experiments was conducted at varying time-points within the juvenile period (approximately days 21–31 of life). Seizures were induced as described. Animals aged 21 days were obtained and housed as before. Body weight was measured every other day until VO.

In the first of four experiments, animals were given a single daily ECS between 10.00 and 11.00 h, commencing on day 22 and ending on day 31. In a separate experiment, shocks were administered over 5-day periods, rather than 10 days, i.e. one group from day 22 to day 26 and another from day 27 to day 31. In a third experiment, ECS was administered daily from day 26 to day 30 in either the morning (10.00–11.00 h) or afternoon (16.00–17.00 h). The experimental series was concluded with an experiment involving a single shock in the morning (10.00–11.00 h) of day 31.

Juvenile convulsions and oestrous cyclicity

Rat pups aged 20 days on arrival were weaned and housed as described above. A single daily electroconvulsion was administered to animals beginning at age 27 days and ending at age 31 days, between 10.00 and 11.00 h. Control rats received ear clips only. Body weight was recorded every other day until VO. On the day of VO, and on a daily basis thereafter, rats underwent daily vaginal smears between 16.00 and 17.00 h. Smears were taken from control rats each day for 3 weeks, while shocked rats were examined for 2 weeks followed by 1 week of rest and an additional 2 weeks in which smears were taken. Data on oestrous cyclicity were charted on an individual basis.
Effect of opioid antagonists

The opioid antagonists naloxone, nalmefene and naltrexone were administered (via s.c. injection) to juvenile rats. Beginning at age 27 days, one of three groups received two daily injections of 2.5 mg naloxone or nalmefene/kg, or 50.0 mg naltrexone/kg separated by 30 min. This treatment was continued until VO. A second group received naloxone, nalmefene or naltrexone injections separated by 30 min together with a single ECS, given 10 min after the first injection. A third group received only a single ECS. Groups 2 and 3 were treated only from day 27 to day 31. All treatments were administered between 09.30 and 11.00 h. Body weight was recorded every 2 days until VO. Rats were then killed and the ovaries examined for the presence of eggs or corpora lutea.

Effects of stress

Footshock

Animals aged 21 days were obtained and housed as described above. Footshock apparatus consisted of a homemade wooden chamber (inside dimensions approximately 25 x 25 x 25 cm) with a hinged plexiglass lid and 18 metal bars comprising the floor. A Grason-Stadler 700 Shock Generator was used to pass 1 min of continuous scrambled shocks of 2.5 mA intensity through the metal bars. Animals were placed in the apparatus two at a time. One group of ten animals received treatment in the morning for 5 days beginning on day 27, while another group of ten received one treatment on the morning of day 31. Control animals were exposed to 1 min in the apparatus without shock for 5 days from day 27 to day 31. Body weight was monitored until VO at which time animals were killed and examined as described.

Restraint stress

Restraint stress was effected by placing rats in ventilated plexiglass cubes of 7.5 x 4.5 x 5 cm inside diameter. Animals could not change position during treatment. In the first of two experiments, 19-day-old animals with dams were obtained as described. They were weaned at 21 days of age and immobilized for 15 min once per day in the morning (between 09.00 and 10.30 h) for 5 days, from day 27 to day 31. In a second experiment, 21-day-old animals were obtained and housed as described. Beginning on day 27, they received 30 min of immobilization stress between 09.00 and 10.30 h. Body weight was recorded every second day until VO when animals were decapitated and examined as described.

Ether stress

A series of three experiments was conducted using rats aged 27–31 days. Animals were placed in a vapour-soaked jar (4.5 litres) two at a time until they were fully anaesthetized (approximately 1–2 min). Unconsciousness was maintained by placing a 25 ml beaker containing an ether-soaked gauze pad on its side in front of the animal. This light anaesthesia was maintained for 9 min. Rats were then allowed to recover and returned to the home cage. Total time anaesthetized was approximately 15 ± 2 min. In each of two experiments, eight animals were treated between 15.00 and 17.00 h for 5 days from day 27 to day 31. In a third experiment, ether stress was administered to ten animals between 09.00 and 10.30 h over the same ages. In all experiments, control animals were handled briefly during the time of treatment. The mortality rate due to ether anesthesia was approximately 25%. Animals were weighed every other day until VO when they were killed and the ovaries examined.

Anaesthesia and juvenile convulsions

Halothane anaesthesia was administered concomitant with a single daily ECS between 09.30 and 11.00 h from day 27 to day 31 of life. Animals aged 20 or 21 days were obtained and housed as described above. Halothane was administered through a Fluotec 18438A vaporizer at a rate of 1.5 litres/min for 30 s followed by 3 litres/min until 60 s after cessation of movement, and was mixed with oxygen at a rate of 2 litres/min. As soon as the rats were fully unconscious, they received a single ECS and were returned to their home cage to recover. Anaesthetized control animals received ear clips only. The mortality rate due to halothane anaesthesia was zero. Body weight was recorded every other day until VO when animals were killed.

Statistical analysis

Sample means were compared using Student’s t-test. A value of P < 0.05 was considered to indicate a significant difference.

Drugs

Naloxone and naltrexone were purchased from Sigma (St Louis, MO, U.S.A.). Nalmefene was a generous gift from the Schering-Plough Corp. (Kenilworth, NJ, U.S.A.).

RESULTS

Effects of ECS on age at VO

Data compiled from twelve experiments demonstrate that the late juvenile to peripubertal stage is more sensitive to the delaying effects of ECS than any other prepubertal time-period (Figs 1 and 2). The mean age at VO (± s.e.m.) of rats convulsed daily in the late juvenile stage was 37.1 ± 0.3 days while that for
controls was 33.9 ± 0.3 days (P < 0.005; n = 4 experiments). The delaying effect of ECS on age at VO in this time-period was slightly, but not significantly, more effective than that seen when convulsions were administered over the entire juvenile period (days 22–31). Here, convulsed rats underwent VO at 38.0 ± 0.2 days while controls opened at 35.2 ± 0.4 days of age (P < 0.005; Fig. 1, see also Fig. 2). Convulsions induced daily in the early juvenile period (days 22–26) did not cause a delay in VO (Fig. 1). In shocked animals, mean age at VO was 35.8 ± 0.3 days, while control VO occurred at 34.8 ± 0.4 days.

**Effects of ECS on body weight at VO**

That the late juvenile period is the most sensitive to the puberty-delaying capacity of ECS was confirmed by examination of mean body weight at VO. Seizures induced in either the day 27–day 31, or day 22–day 26 time-period were associated with higher mean weight at VO. Both groups were significantly (P < 0.005) heavier than controls. For example, convulsed rats (days 27–31) weighed 125.6 ± 1.9 g, whereas control rats were 111.2 ± 1.6 g. ECS administered over the entire juvenile period (days 22–31) also effected an increase in body weight at VO.

Seizures induced daily in either the morning or the afternoon during the late juvenile period produced the same effect on mean weight at VO. While there was no significant difference in body weight between rats convulsed in the morning or afternoon, both groups were significantly heavier than controls at VO.

**Effects of ECS on number of corpora lutea and ovarian and uterine weights**

Repeated daily ECS failed to produce any consistent effect on the number of corpora lutea present in the ovaries on the day of first ovulation. Ovarian and uterine weights were similarly inconsistently affected by exposure to ECS-induced seizures.

**Effects of ECS on oestrous cyclicity**

Administration of repeated daily ECS from age 27 days to age 31 days interfered with oestrous cyclicity following VO. The average length of the first complete oestrous cycle in shocked rats was 7.0 ± 0.8 days, while cycle length in controls was 4.8 ± 0.2 days (P < 0.01; n = 12 rats/group, data not shown). First cycle length in all shocked rats was 5 days or greater and 25% showed a cycle length greater than 8 days. This ECS-induced increase in cycle length had disappeared by approximately 55 days when cycle length was 4.3 ± 0.1 days for both shocked and non-shocked animals.

**Effects of opioid antagonists and juvenile convulsions**

The EOP antagonists naloxone, nalmefene and naltrexone were unable to block the delay in puberty induced by administration of ECS in the late juvenile period, although nalmefene has a slight but not

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[Image of graph showing mean age at VO for different age ranges at ECS treatment (days).]
significant tendency to attenuate the delay. These data are expressed as cumulative % VO graphs in Fig. 3a–c. This method of data presentation permits ready visual assessment of the age at VO in control and treated groups, providing information not available in mean comparisons. As in Fig. 2, curves shifted to the right or left of controls represent delayed or advanced puberty respectively. Natural variation in the timing of VO does occur in individual experiments (Badawi & Wilkinson, 1988). The increased age at VO in both the ECS and ECS plus opioid antagonist groups was associated with increased body weight at VO. No consistent changes in the number of corpora lutea or uterine and ovarian weight were present. Treatment of rats with EOP antagonists alone produced a slight though non-significant advance in VO with respect to both age (Fig. 3d) and body weight. Again, number of corpora lutea and reproductive organ weights were not affected, indicating appropriate onset of puberty in all groups.

Effects of footshock, restraint and ether stress

Footshock, restraint and ether stress had no effect on mean age at sexual maturation. Mean age and weight at VO for control and animals exposed to footshock, immobilization or ether vapour were not different. These data indicate no significant advance or delay
in puberty in treated groups compared with their respective controls. The lack of effect of these stressors is substantiated by examination of control and treated ovarian and uterine weights, as well as the number of corpora lutea at VO. This inspection demonstrated that all animals reached sexual maturation normally (results not shown).

**Effects of anaesthesia and juvenile convulsions**

A further attempt to elucidate a possible role for post-convulsion stress with respect to the etiology of ECS led us to examine the effect on puberty of ECS administered to anaesthetized animals. Halothane anaesthesia failed to block the delay in puberty caused by ECS administered in the late juvenile period (Fig. 4). Both treated and control groups had normal reproductive organ weights and a normal number of corpora lutea, indicating that sexual maturation occurred appropriately (even though delayed in the halothane- and halothane plus ECS-treated groups) (data not shown).

**DISCUSSION**

We have previously demonstrated that ECS has a disruptive effect on the onset of sexual maturation in
the female rat. Further, this disruption is probably located within the hypothalamus and is conspicuously associated with the absence of LH surge activity after repeated seizures (Bhanot & Wilkinson, 1984). In the present study, we isolated the prepubertal period most sensitive to the delaying effect of seizures and examined the mechanism of action of ECS during that period. Our data demonstrate that during the late juvenile stage of development (days 27–31 postnatal) the female rat hypothalamo-pituitary-ovarian axis is exquisitely sensitive to electroseizures. Convulsed animals were both older (Fig. 1) and heavier at VO, indicating a true delay in sexual maturation. This delay occurred whether shocks were administered in the morning or afternoon, and was not associated with significant changes in reproductive organ weight.

Our observation that both neonatal and infanitle rats were refractory to the delaying effects of ECS suggests that the hypothalamic neuroendocrine environment required for first ovulation was either not altered by these early convulsions or that it recovered to allow first ovulation to occur. This is surprising in the light of data demonstrating that neonatal ECS retards whole brain development and brain protein synthesis (Wasterlain, 1976; Fando et al. 1979), and that endocrine events critical to sexual maturation occur during the infantile period (Ojeda et al. 1986). This recovery capacity of the reproductive axis was reduced in the early juvenile period (during which ECS only slightly delays puberty) and ten convulsions administered over the entire juvenile period were no more potent than five seizures over the late juvenile period only (Fig. 1). Conversely, a single ECS administered at the end of the late juvenile period had no effect on age at VO. Having thus clearly established the specific sensitivity of ECS in the late juvenile period, we then demonstrated that the defect(s) induced by ECS over this period continued to affect the reproductive axis after first ovulation by preventing normal oestrous cyclicity following VO (i.e. in the absence of ECS). The effect was transient however, and disappeared by 55 days. These data indicate that following the cessation of electroconvulsive insult, spontaneous recovery is possible and that no permanent defect due to ECS has occurred.

What is the mechanism by which ECS disrupts the timing of VO? Those monoamines which have been implicated in the regulation of pubertal onset (Advis, Simpkins, Chen & Meites, 1978; Wuttke, Honma, Lamberts & Höhn, 1980), may also be responsible for mediating the antidepressant effect of ECS (Grahame-Smith, Green & Costain, 1978; Green & Deakin, 1980), and it has been demonstrated that repeated ECS decreases a2-adrenoceptor density in the hypothalamus (Stanford & Nutt, 1982). These data, together with evidence of altered prolactin secretion following ECS in rats (Bhanot & Wilkinson, 1984) and man (Arato & Bagdy, 1982; Whalley, Dick, Watts et al. 1982) strongly suggest that the hypothalamic monoaminergic system is disrupted and hence interference in reproductive hormone secretion is involved in the ECS-induced delay in puberty. However, electroconvulsions are also associated with a plethora of neurochemical changes, and the possibility of a role...
for other systems, such as the EOP, in the efficacy of this effect cannot be excluded. The EOP are known to regulate gonadotrophins and gonadotrophin-releasing hormone secretion during the onset of puberty (Wilkinson & Landymore, 1989). They are also activated centrally by seizures (Holladay, Hitzemann, Curell et al. 1982; Sarne, Weissman & Urca, 1982; Hitzemann et al. 1987; Lasón et al. 1987). The present studies have attempted to determine the significance of EOP activation with respect to ECS-induced delays in sexual maturation by injection of three non-selective EOP antagonists to block EOP receptors both 10 min before and 20 min after daily late juvenile administration of ECS. The failure of naloxone, nalmefene and naltrexone treatment to prevent the delay in puberty (Fig. 3) may be explained in two ways. The first and most obvious of these is that opioid peptides are simply not involved in the etiology of ECS-induced delay of VO. Alternatively, the opioid blockade elicited by the daily antagonist challenge that we have employed may not be rigorous enough to prevent the effect of ECS-related opioid activation. This seems unlikely since we have previously shown (Landymore & Wilkinson, 1988) that a single injection of naltrexone (50 mg/kg) occupies hypothalamic μ-opioid binding sites for long periods (50% occupancy at 10 h post-injection; complete blockade up to 3 h). The lower dose of naloxone (2.5 mg/kg) would also block receptors over the period used in the present experiments (i.e. 10 min before and 20 min after ECS). Our unpublished results for nalmefene (2.5 mg/kg) indicate complete blockade for 1–2 h. We can conclude, therefore, that ECS is unlikely to delay the timing of VO via an opioidergic mechanism, although an involvement of other opioid receptor subtypes cannot yet be excluded.

At this point, the influence of opioid antagonism on sexual maturation deserves comment. Previous work by several groups has suggested that prepubertal opioid blockade may induce some degree of sexual precocity, at least in a subgroup of rats (Sirinatshinghi, Motta & Martini, 1985; Landymore & Wilkinson, 1988; Meijis-Roelofs & Kramer, 1988). The present data also show that the three antagonists used induce a small shift to the left of the age-at-VO curves which would appear to confirm that opioid blockade, in the juvenile-peripubertal period, can induce precocious puberty.

Finally, we have considered the possibility that ECS-associated stress may be at least partially responsible for the delay in VO. However, repetitive acute administration of footshock, restraint or ether stress during the late juvenile period is devoid of any effect on sexual maturation. Restraint stress (Keim & Sigg, 1976) and footshock (Terman, Lewis & Liebeskind, 1986) in adult males, and ether stress (Johnston & Negro-Vilar, 1986) in adults and prepubertal males are all associated with stress-related hypothalamic defects. Nevertheless, we conclude that the endogenous drive to initiate cyclicity overrides any stress-induced pathology. Thus post-convulsion stress, and any related compromises, are probably not causal to the delay in puberty associated with ECS. Additional data in support of this conclusion were obtained from anaesthetized rats given ECS. As an alternative approach to the question of stress involvement in ECS-induced delay of puberty, we hypothesized that anaesthesia should block or attenuate stress-increased CRF secretion (Plotsky & Vale, 1984). After receiving ECS while under halothane anaesthesia, rats displayed almost no tonic or clonic reaction, and puberty was delayed in a similar manner to that in non-anaesthetized convulsed rats (Fig. 4). Indeed, halothane is routinely used as a preconvulsion treatment in adult rats, and appears to have no interaction with ECS-induced neurochemical alterations (see Costain, Green & Grahame-Smith, 1979). These data suggest that the hypothalamic defect(s) induced in late juvenile female rats by repetitive ECS administration is a direct result of the electroshock and is not contingent upon the resulting convulsion.

In conclusion, the drive to attain first ovulation is most susceptible to electroconvulsions during the period when adult pattern biorhythmicity is initiated, just before pubertal onset (Ramaley, 1979). Electrical induction of seizures before this time is inconsequential to the timing of puberty, despite the presence of observable neurochemical and anatomical defects. Although clearly activated by seizures, opioid peptides are not critical to the mechanism by which ECS delays puberty. That ECS can delay puberty even when the convulsion itself is blocked by anaesthetic suggests a central and specific nature for the mechanism by which this pathology is produced. A clue to the identity of the neurochemical system responsible for this specificity may lie in the recent observation that excitatory amino acid antagonists can block electroconvulsions via the N-methyl-d-aspartate receptor complex and that pretreatment with naloxone fails to antagonize this seizure blockade (Tortella, Ferkany & Pontecorvo, 1988). Preliminary experiments with non-competitive N-methyl-d-aspartate antagonists such as dextorphan are currently underway in our laboratory.

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