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Short communication

Involvement of muscarinic receptors in the control of female puberty in the rat

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Abstract

Immature female rats (21 days of age) were chronically intraperitoneally treated with guanethidine or muscarinic agents. The effects on the timing of puberty and ovarian wet weight, protein and total RNA and DNA contents were studied. While guanethidine (20.0 mg/kg/day) was ineffective, trihexyphenidyl and especially propantheline (15.0 mg/kg/day) delayed vaginal opening (by 23%) and the first vaginal oestrus (by 28%), and lowered ovarian weight (by 37%) and other ovarian growth parameters. Carbachol (0.2 mg/kg/day) reversed the effects of propantheline. Thus, in contrast to the adrenergic system, the cholinergic system appears to substantially contribute to the accurate onset of female puberty and ovarian growth in the rat.

Keywords: Ovary; Puberty, female; Muscarinic receptor; Cholinergic system

1. Introduction

The control of female puberty includes not only hormonal mechanisms, but also the influences of ovarian innervation (Ojeda et al., 1983a). Adrenergic innervation has been most extensively studied, but it is unclear whether or not it affects the timing of puberty and ovarian growth (Aguado and Ojeda, 1984; Lara et al., 1990; Flores and Dominguez, 1992). On the other hand, the involvement of the cholinergic system in the timing of female puberty has not yet been investigated.

Here we demonstrate that, in contrast to the poor adrenergic influence, the cholinergic system, through muscarinic receptors, substantially contributes to the accurate timing of female puberty and ovarian growth in the rat.

2. Materials and methods

From the beginning of the juvenile period (day 21) (Ojeda et al., 1983a), until death (day 65) Wistar female rats (Department of Pharmacology) were intraperitoneally treated with: guanethidine sulphate (Sigma Co., USA) (20.0 mg/kg/day, single daily injection) (Flores and Dominguez, 1992); trihexyphenidyl hydrochloride (Lederle, USA) (2 × 1.0 mg/kg/day, injections every 12 h) (elimination half-life 10–12 h) (Cedarbaum and Schleifer, 1990); propantheline bromide (Searle Co., Germany) (3 × 5.0 mg/kg/day, injections every 8 h) (duration of activity 6 h) (Brown, 1990); carbamylcholine chloride (carbachol) (Sigma) (2 × 0.1 mg/kg/day, injections every 12 h) (Goodman and Gilman, 1955); or carbachol + propantheline. In a dose-finding experiment, the animals received 0.6, 3.0, 15.0, or 45.0 mg/kg/day of propantheline (in three daily doses). All drugs were dissolved in saline. Control animals always received saline, thrice daily. Guanethidine-treated animals were also injected thrice daily (guanethidine in the morning). Single doses were adjusted by 0.01 ml for every 2.0 g change of body weight. The animals were kept under a 12/12 h light/dark schedule (light from 07:00–19:00 h), at 24 ± 1°C, with free access to food and water, and were weighed daily to the nearest gram, checked for vaginal opening, and vaginal smears were taken to record the first vaginal oestrus (the day of first ovulation), defined in accordance with Aguado and Ojeda (1984). Control animals showed vaginal opening at the age of 36.3 ± 0.9 and

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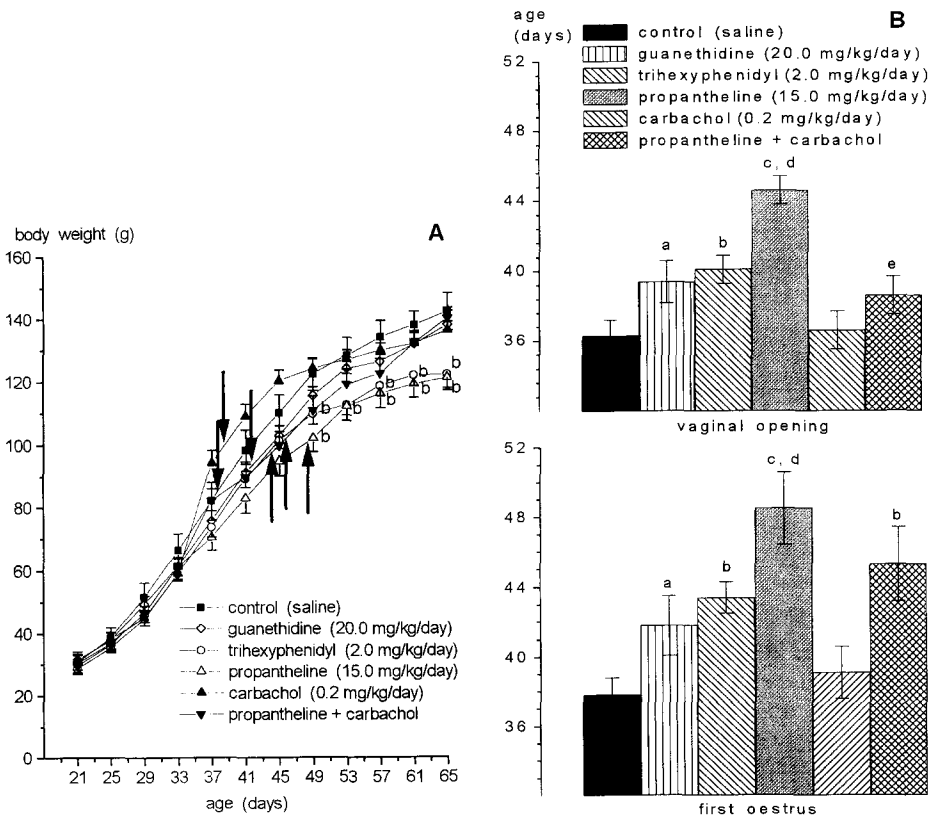

Fig. 1. Effects of guanethidine and muscarinic drugs on the body weight increase (A, left) and the timing of female puberty (B, right). The drugs were administered intraperitoneally, from day 21 to day 65. A: Arrows pinpoint the mean body weight at first vaginal oestrus. Values are expressed as the means ± S.E.M. of 12 animals per group. [a] $0.05 < P < 0.1$, [b] $P < 0.05$ and [c] $P < 0.001$ vs. control (ANOVA and Dunnett's test). [d] $P < 0.1$ vs. guanethidine (ANOVA and Scheffe's test). [e] $P < 0.05$ vs. propantheline (ANOVA and Scheffe's test).

34.0 ± 0.3 (in the dose-finding experiment) days, and the first vaginal oestrus at the age of 37.8 ± 1.0 and 36.5 ± 1.0 (in the dose-finding experiment) days. After the animals were killed, the ovarian weight, protein (Lowry et al., 1951) and total RNA and DNA (Munro and Fleck, 1966) contents were measured. Since an effect of a manipulation on ovarian weight may be masked if determined at a specific gynaecological age (Ojeda et al., 1983b), the animals were killed at a specific age (day 65) at which all had attained first ovulation, but were not fully mature (controls showed about 6% of pseudodioestrus days during the last 10 days before death). Absolute measured values (mg, μg) were compared (Ojeda et al., 1983b). Data were anal-

Table 1
Effects of guanethidine and muscarinic drugs on ovarian growth during puberty

Treatment group	Ovarian weight (mg/pair)	Ovarian protein content (mg/pair)	Ovarian total RNA content (μg/pair)	Ovarian total DNA content (μg/pair)	Body weight (g)
Saline (control)	52.6 ± 3.6	1.35 ± 0.04	75.57 ± 2.27	48.26 ± 1.97	142.1 ± 6.0
Guanethidine (20.0 mg/kg/day)	51.0 ± 3.0	1.39 ± 0.03	71.62 ± 3.24	45.30 ± 2.13	137.8 ± 4.7
Trihexyphenidyl (2.0 mg/kg/day)	44.6 ± 2.8	1.27 ± 0.04	63.60 ± 2.61 [a]	41.76 ± 1.20	122.1 ± 4.2 [a]
Propantheline (15.0 mg/kg/day)	33.4 ± 3.3 [b,c]	1.17 ± 0.05 [a,c]	49.60 ± 4.44 [b,c]	36.40 ± 1.72 [a,d]	121.0 ± 3.8 [a]
Carbachol (0.2 mg/kg/day)	51.8 ± 2.6	1.34 ± 0.04	72.84 ± 2.58	46.24 ± 2.48	136.0 ± 3.0
Carbachol + propantheline	45.5 ± 2.6 [e]	1.32 ± 0.04 [e]	69.40 ± 2.65 [e]	40.47 ± 1.85 [a]	140.0 ± 3.5 [e]

From the age of 21 days, the animals were given chronic intraperitoneal drug treatments, and were killed at the age of 65 days. Ovarian wet weight, and protein and total RNA and DNA contents were determined. Each group consisted of 12 animals. Values are expressed as the means ± S.E.M. Since no difference was observed between the left and right ovary, values per pair of ovaries are shown. [a] $P < 0.05$ and [b] $P < 0.001$ vs. control (ANOVA and Dunnett's test). [c] $P < 0.05$ and [d] $P < 0.1$ vs. guanethidine (ANOVA and Scheffe's test). [e] $P < 0.05$ vs. propantheline (ANOVA and Scheffe's test).

ysed by analysis of variance (ANOVA) and Dunnett's or Scheffe's test. Significance was determined at the levels of 0.05, 0.01, 0.001 or 0.1 for Scheffe's test.

3. Results

From day 49 onward, the animals receiving trihexyphenidyl or 15.0 mg/kg/day of propantheline weighed somewhat less than the controls. None of the other treatment groups differed from the controls at any age. None of the treatments affected body weights at first vaginal oestrus (Fig. 1A).

Guanethidine had no significant effect on any of the parameters measured (Fig. 1B and Table 1). In contrast, trihexyphenidyl delayed the onset of vaginal opening and first vaginal oestrus and lowered the ovarian total RNA content (Fig. 1B and Table 1). Propantheline (15.0 mg/kg/day) markedly delayed the onset of puberty (Fig. 1B) and lowered all the ovarian growth parameters (Table 1). In the dose-finding experiment, lower doses of propantheline (0.6 and 3.0 mg/kg/day) were ineffective, whereas 45.0 mg/kg/day further delayed the onset of vaginal opening (age 59.8 ± 2.4 days) and first vaginal oestrus (age 61.3 ± 1.8 days), and additionally lowered the ovarian growth parameters (results not shown). Carbachol alone had no effect, but it reversed the effects of propantheline (15.0 mg/kg/day) (Fig. 1B and Table 1).

4. Discussion

The timing of vaginal opening and first vaginal oestrus in the control animals was consistent with data in the literature (Ojeda et al., 1983a), and showed negligible interexperimental variation. The effect of guanethidine on the timing of puberty and ovarian growth was not significant (Fig. 1B and Table 1), possibly because we applied the drug at later stages of life (day 21 to 65) than other authors did (day 1 to 21) (Lara et al., 1990; Flores and Dominguez, 1992).

In order to completely block muscarinic receptors throughout the experiments, doses somewhat higher than therapeutic doses in humans were used (trihexyphenidyl 2.0 mg or 6 μmol/kg/day, $\approx$ 10-fold antiparkinsonian dose, and propantheline 15.0 mg or 33 μmol/kg/day, $\approx$ 15-fold anti-ulcer dose). Under such conditions, in contrast to guanethidine, muscarinic antagonists, especially propantheline, clearly delayed the onset of puberty and inhibited the ovarian growth (Fig. 1B and Table 1). According to body weights, the drugs caused no generalized debilitation. The trihexyphenidyl-treated and propantheline-treated animals differed from the controls only at the time points at which they were of significantly younger gynaecological age, and the effect was reversed by carbachol (Fig. 1A). Except for carbachol-induced peristalsis, no other drug effects on food and water consumption or on motor activity could be detected by routine daily inspections.

The propantheline (15.0 mg/kg/day)-induced delay of vaginal opening (by 23%) and the first vaginal oestrus (by 28%) and the decrease in ovarian weight (by 37%), protein (14%), total RNA (35%) and total DNA contents (25%) by far exceeded not only the previously reported effects of guanethidine (vaginal opening delayed by 6% and the first vaginal oestrus by 13%) (Lara et al., 1990), but also the effects of other procedures that have been used to study the neural mechanisms regulating female sexual development, such as bilateral abdominal vagotomy (Ojeda et al., 1983b), hypothalamic lesions (Ojeda et al., 1983a), temporal lobe deafferentation (Gerendai et al., 1993) and lesions of dorsal raphe nuclei (Ayala et al., 1994). Furthermore, the effect of a higher dose of propantheline, used in the dose-finding experiment (45.0 mg/kg/day), was dramatic (the onset of vaginal opening was postponed by 76% and the first vaginal oestrus by 68%).

The highly similar effects of the two antimuscarinic drugs and the antagonism of propantheline and carbachol (Fig. 1 and Table 1) suggest a specific muscarinic control of female puberty. The more pronounced effects of propantheline than of trihexyphenidyl could be due to dose differences. The lack of the effect of carbachol, when administered alone, may indicate that the cholinergic influence was at a high level of tonic activity.

A single subcutaneous injection of atropine (Dominguez et al., 1982) or intrahypothalamic atropine implants (Cruz et al., 1992) have been shown to diminish ovulation in mature rats, and both effects are reversed by gonadotropin releasing hormone, gonadotropins or oestradiol (Dominguez et al., 1982; Cruz et al., 1992). Accordingly, the effects of drugs observed in the present experiments could have been mediated through hypothalamic muscarinic receptors. However, since propantheline was effective in an approximately 5 times lower dose than subcutaneous atropine (148 μmol/kg) (Dominguez et al., 1982), the involvement of peripheral muscarinic receptors could not be excluded. The presence of muscarinic receptors in human ovaries (Batra et al., 1993), and the observation that muscarinic receptors mediate steroid secretion and proliferation of ovarian tumour cells in vitro (Luck, 1990; Popper and Batra, 1994) support such a possibility.

In conclusion, we demonstrated that antimuscarinic drugs, administered during the juvenile and peripubertal period, postponed the onset of puberty and inhibited ovarian growth in the rat, to an extent which in humans would require medical attention. Thus, it seems

that, in contrast to the poor adrenergic influence, tonic activity of the cholinergic system, exerted through muscarinic receptors, is necessary for the accurate timing of female puberty and ovarian growth during puberty.

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