EFFECT OF AGE, CRYPTORCHIDISM AND HYPOPHYSECTOMY ON CYCLIC AMP CONCENTRATION IN RAT TESTIS

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Testicular adenylate cyclase (AC) activity has been found in particulate fractions from dog (Pulsinelli & Eik-Nes, 1970) and rat testes (Hollinger, 1970), with no measurable activity in the soluble fraction. Testicular AC has been reported to be stimulated by gonadotrophins (Murad, Strauch & Vaughan, 1969; Kuehl, Patanelli, Tarnoff & Humes, 1970), catecholamines (Murad *et al.*, 1969; Kuehl *et al.*, 1970), ACTH (Kuehl *et al.*, 1970) and growth hormone (Casillas & Hoskins, 1971).

Although testicular AC has received some attention, few reports have dealt with the actual quantification of endogenous cyclic AMP. The effect *in vitro* of gonadotrophins on the cyclic AMP content of isolated rat seminiferous tubules has been reported to be an elevation in the level of this nucleotide (Dorrington, Vernon & Fritz, 1972). In this study, the endogenous level of cyclic AMP in the rat testis was measured under a variety of conditions including hypophysectomy, cryptorchidism and age.

Male Sprague–Dawley rats were used in all experiments and were obtained from commercial sources. Hypophysectomized and control rats which had been subjected to sham operation were also obtained commercially. Surgery was performed on these animals at 60 days of age. Artificial cryptorchidism was induced in a separate group of rats at 20 days of age as described previously (Davis, Morris & Hollinger, 1965). All animals were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care and were fed freely.

Rats were decapitated and the testes were quickly excised and placed in liquid N₂ for approximately 20 min. Immersion in liquid N₂ achieves rapid freezing (Robison, Butcher & Sutherland, 1971) and has been used in several studies (Ditzion, Paul & Pauk, 1970; Burkard, 1972). Such treatment produced only a negligible change (5% decrease) in weight of the tissue. The frozen weight of the samples was therefore used in all calculations.

The samples were homogenized in 5 ml 1% HClO₄ to which 3630 ct/min authentic [³H]cyclic AMP (Amersham/Searle; S.A. 20 Ci/mmol) had been added to assess the rate of recovery. Following removal of the testicular capsule, homogenization was carried out using a motor-driven Teflon pestle (clearance 0.1 to 0.15 mm). The samples were then boiled for 2 min and centrifuged at 600 g for 15 min. The supernates were decanted and adjusted to pH 7.0 with

KOH. The samples were then recentrifuged at 4° C for 15 min at 600 *g* and the supernates were collected. Recovery of cyclic AMP was measured by counting 1-ml aliquots in 10 ml Aquasol (New England Nuclear) using a Beckman LS-200 B liquid scintillation spectrometer. The recovery rate averaged 75 %.

Reagents for the radioimmunoassay of cyclic AMP were obtained from Collaborative Research, Inc. (Waltham, Massachusetts) and the technique was based on a method previously described (Steiner, Kipnis, Utiger & Parker, 1969; Steiner, Parker & Kipnis, 1970). The radioimmunoassay has been shown to be highly specific for cyclic AMP and eliminates the necessity of isolating the cyclic nucleotide from other tissue nucleotides. The ability of mono-, di- and triphosphate nucleotides of adenine, guanine, cytidine and uridine to cross-react is negligible. All show less than 0.005 % of the potency of cyclic AMP. Even 3',5' cyclic nucleotides of these purine and pyrimidine bases exhibit minimal cross-reactivity. This procedure has been used in several studies (Ferrendelli, Steiner, McDougall & Kipnis, 1970; Steiner, Ferrendelli & Kipnis, 1972) and shown to produce results compatible with those obtained by other methods (Burkard, 1972).

All reagents were prepared in sodium acetate buffer (0.05 M, pH 6.2). Each tube contained 0.3 ml of the unknown specimen, 0.1 ml cyclic AMP antiserum and 0.1 ml [¹²⁵I]succinyl cyclic AMP tyrosine methyl ester (SCAMP-TME). Standard curve samples were made over a range of 0.10 to 25 pmol. Standard curve tubes contained 0.1 ml authentic cyclic AMP, 0.2 ml sodium acetate buffer (0.05 M, pH 6.2), 0.1 ml cyclic AMP antiserum and 0.1 ml SCAMP-TME. The samples were allowed to incubate for 2 to 3 hr at 4° C and 0.1 ml 1:200 carrier normal rabbit serum and 0.05 ml anti-rabbit IgG were then added to each tube. The samples were incubated for 16 hr at 4° C. Sodium acetate buffer (2 ml) was then added to each tube and the samples were centrifuged at 4000 rev/min at 4° C for 20 min. The supernates were discarded and the precipitate was assayed for radioactivity using a Packard γ -spectrometer with an efficiency of 51 %.

The concentration of the nucleotide was found to decrease by approximately 50 % with advancing age, going from nearly 140 pmol/100 mg tissue at 25 days of age to 70 pmol/100 mg tissue at 220 days of age (Text-fig. 1).

The concentration of cyclic AMP was found to be elevated by 74 % ($P < 0.05$) in the atrophic cryptorchid testes (Table 1). The concentration of cyclic AMP was also found to be significantly increased in testes from hypophysectomized animals ($P < 0.05$). The activity of AC has been reported to increase fivefold in rat testis from 25 to 60 days of age (Hollinger, 1970). This elevation with age is difficult to reconcile with the decrease in cyclic AMP concentration in rat testis with age observed in the present study. Recently, it has been reported that an 'f component' isoenzyme of phosphodiesterase, specific to the testis, also increases with age in the rat. The appearance of the isoenzyme coincides with the appearance of mature spermatozoa (Monn, Desautel & Christiansen, 1972). The activity of this phosphodiesterase, which possesses a twentyfold lower K_m for cyclic AMP than the non-tissue specific 'c component' may predominate, leading to a subsequent net reduction in the level of cyclic AMP in the testis.

TEXT-FIG. 1. Effect of age on concentration of cyclic AMP in rat testis. Each point is the mean of three or four determinations. Vertical bars represent the S.E.

The increase in cyclic AMP concentration in the cryptorchid testis was unexpected and might be explained by the presence of predominantly immature cell types in the abdominal testis (Davis & Firlit, 1966). This, in a sense, would be mimicking an immature testis, which has a higher concentration of cyclic AMP than the more mature organ. An alternative explanation might be related to a compensatory increase in gonadotropin output in unilaterally cryptorchid rats (Clegg, 1965). An increase in pituitary FSH and ICSH might be expected to affect an increase in the level of cyclic AMP in the cryptorchid testis since these gonadotrophins are known to stimulate testicular AC (Murad *et al.*, 1969; Kuehl *et al.*, 1970).

On the basis of tissue water (data not shown), the concentration of cyclic AMP in the mature rat testis was calculated to be 7.2×10^{-8} M. This concentration of cyclic AMP falls within the range of 10^{-6} to 10^{-8} M reported for other tissues and fluids of the rat (Steiner *et al.*, 1970).

Table 1. Effect of cryptorchidism or hypophysectomy on the cyclic AMP content of rat testis

Testis tissue	Cyclic AMP ($\mu\text{mol}/100 \text{ mg tissue}$)	P
Scrotal	100 ± 4 (4)	
Cryptorchid	174 ± 34 (4)	< 0.05
Sham control	72 ± 6 (3)	
Hypophysectomized	136 ± 22 (4)	< 0.05

Cryptorchid animals were used 75 days after surgery and hypophysectomized animals were used 169 days after surgery. Each value is expressed as the mean $\pm$ S.E. Values in parentheses are the numbers of determinations.

The high concentration of cyclic AMP in immature or cryptorchid testes may be explained on the basis of higher content of this nucleotide in undifferentiated proliferating cell types. Cyclic AMP has been implicated in the regulation of mitotic activity in several tissues (MacManus & Whitfield, 1969; Rixon, Whitfield & MacManus, 1970) and may be intimately involved in the capacity of cells to proliferate. Since the undifferentiated cells adjacent to the basement membrane in the germinal epithelium of the testis are those which are most mitotically active, and do not possess the 'f component' isoenzyme of phosphodiesterase (Monn *et al.*, 1972), the concentration of cyclic AMP is highest in testis tissue composed primarily of these cell types.

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