

linked to the pyrrolic nitrogen was established taking into account the characteristic fragmentation of N-alkylpyrroles⁶ and the signals in the NMR-spectra. In fact the fragment ion at $M^+ - 1$ and the 3H broad singlet at δ 2.90 are diagnostic for a N-methylpyrrole unit in **V**. Likewise the fragment ions at m/e 423 and 408, together with the signals at δ 6.98–7.34, 2.98 and 3.94 are indicative for a $C_6H_5CH_2CH_2$ -unit linked to the nitrogen atom in **IV**. The structures of **IV** and **V** were definitively established by their partial synthesis.

The reaction of scalaradial⁷ (**VI**) with β -phenylethylamine hydrochloride and methylamine hydrochloride in ethanol at 60 °C for 20 min yielded **IV** and **V**, respectively (identified by comparison of their chromatographic and spectral properties).

The chains linked to the pyrrole ring in **IV** and **V** can be considered to derive from phenylalanine and glycine. This further supports the hypothesis previously reported²⁻⁴ for the biogenetic origin of pyrroloterpenes from mevalonate and an amino acid precursor.

- 1 This work has been carried out in the frame of the 'Progetto Finalizzato per l'Oceanografia e i Fondi Marini', C.N.R., Roma.
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Ontogenetic changes in relative levels of cyclic AMP-dependent and cyclic GMP-dependent protein kinases in prostates, epididymides and testes from guinea-pigs

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Summary. Changes in relative levels of cyclic AMP-dependent protein kinase (A-PK) and cyclic GMP-dependent protein kinase (G-PK) in prostates, epididymides and testes from guinea-pigs were examined at 3 different ages. During postnatal development, a decrease in the ratio of the 2 classes of protein kinases was seen in prostates, whereas increases of the ratios of the enzymes were found in epididymides and testes.

Several lines of recent evidence have suggested that adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP) may independently mediate opposing effects of certain physiological agents in the lungs³ and other tissues⁴, presumably via the phosphorylation activity of cyclic AMP-dependent and cyclic GMP-dependent protein kinases respectively^{5,6}. Kuo has shown that cyclic GMP-dependent protein kinase exists in high levels in some mammalian tissues⁷, and that the changes in relative levels of the 2 classes of protein kinases occur in the lung, heart and brain of developing guinea-pigs⁸. Here we report our observations on the changes of these 2 kinases in male reproductive system during the course of postnatal sexual maturation.

Materials and methods. (γ -³²P) ATP was purchased from New England Nuclear. Cyclic GMP and cyclic AMP were obtained from Boehringer, Mannheim (BRD); arginine-rich histone (HA) was from Worthington.

Male guinea-pigs of 3 different ages (7 days old, weighing 80–150 g; 30 days old, weighing 400–550 g; and 100 days old, weighing 950 to 1200 g), were used. Testes, epididymides and prostates were dissected immediately after decapitation of the animals and homogenized in 3 volumes of 50 mM potassium phosphate buffer (pH 7.0) with glass-Teflon homogenizers. The homogenates were centrifuged for 20 min at 30,000 \times g, and the supernatant solutions were used as the sources of the cytosol enzymes. The pellets were resuspended in the original volume of the same buffer. To

Age-related changes in the estimated ratios of cyclic AMP-dependent to cyclic GMP-dependent protein kinase activity in cytosols and particulates of prostates, epididymides and testes from guinea-pigs

Age (days)	(A-PK/G-PK) ratio					
	Cytosol Prostate	Epididymis	Testis	Particulate Prostate	Epididymis	Testis
7	1.53 \pm 0.14	2.89 \pm 0.24	4.26 \pm 0.61	2.34 \pm 0.26	4.02 \pm 0.75	3.93 \pm 1.02
30	1.02 \pm 0.08 ^a	4.02 \pm 0.26 ^b	9.46 \pm 1.10 ^c	1.82 \pm 0.13	5.78 \pm 0.41	6.66 \pm 0.85
100	0.81 \pm 0.04 ^c	9.11 \pm 0.58 ^c	14.52 \pm 1.67 ^c	1.22 \pm 0.16 ^a	8.29 \pm 0.94 ^b	7.98 \pm 0.89 ^a

Assay conditions were as described in the text. The (A-PK/G-PK) ratio is defined as: (activity in the presence of cyclic AMP – basal activity): (activity in the presence of cyclic GMP – basal activity). The means (\pm SE) of the values obtained from 3 to 4 animals for each group were shown. Significantly different from the 7-day-old animals. ^a $p < 0.05$; ^b $p < 0.02$; ^c $p < 0.01$.

each suspension Triton X-100 was added to make a final concentration of 0.2%

Crude protein kinase modulators from rat liver was prepared through the steps of boiling the extract and precipitation of the factors from 5% of trichloroacetic acid⁹⁻¹¹.

The standard assay system⁷ for protein kinase activity contained, in a final volume of 0.2 ml, potassium phosphate buffer, pH 7.0, 10 μ moles; theophylline, 0.5 μ moles; arginine-rich histone, 40 μ g; $MgCl_2$, 2 μ moles; (γ -³²P) ATP, 1 nmole, containing about 1.2×10^6 CPM; crude protein kinase modulator, containing 50 μ g protein; with or without cyclic AMP or cyclic GMP, 60 pmole; and appropriate amounts (4-30 μ g) of protein kinase preparations. The reaction was carried out for 10 min at 30°C. 1 unit of enzyme activity is defined as that amount of enzyme which transfer 1 pmole of ³²P from (γ -³²P) ATP in recovered histone under the assay conditions.

Results. Protein kinase activities in cytosols of guinea-pig testes and prostates at 3 different ages, assayed in the presence and absence of cyclic AMP or cyclic GMP, were proportional to increasing amount of enzyme protein added to the incubation system under the assay conditions (figure). Furthermore, the cyclic nucleotide-stimulated protein kinase activities increased after the age of 30 days (figure). In prostate cytosols, the cyclic AMP-stimulated protein kinase activity, compared to the cyclic GMP-stimulated enzyme activity, was much higher in the 7-day-old animal, only slightly higher in the 30-day-old animal, and conversely lower in the 100-day-old animal. In testes, however, the levels of A-PK remained higher throughout the course of development (figure). Increases in the protein

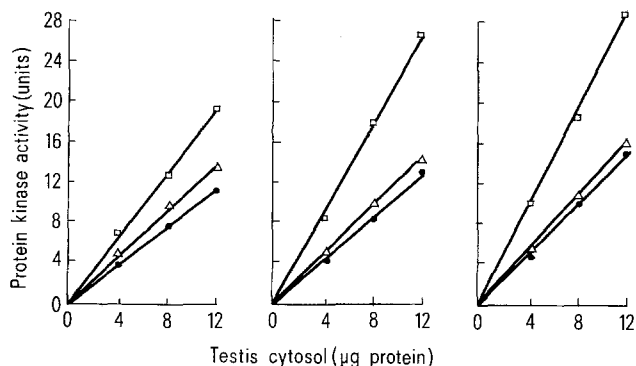
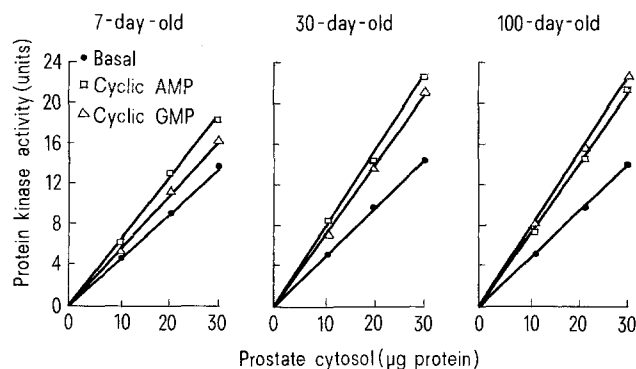
kinase activities similar to those of testes were also found in the cytosols of epididymides during the course of sexual maturation (data not shown). Moreover, we noted that the ontogenetic changes in protein kinases in the particulate fractions of these 3 tissues were essentially the same as those in the cytosol fractions (data not shown).

The relative activity of the enzyme stimulated by cyclic AMP and cyclic GMP was different in these 3 groups of animals. The age-related changes in the ratios of levels of cyclic-AMP-dependent protein kinase (A-PK) to cyclic GMP-dependent protein kinase (G-PK) in both cytosols and particulates are compared in the table. As the epididymis and the testis developed, the (A-PK/G-PK) ratio increased. In the prostate, however, the ratio decreased during development.

Discussion. The adult prostate from 100-day-old adult guinea-pigs is a unique tissue in which the cyclic GMP-stimulated protein kinase activity was higher than the cyclic AMP-stimulated enzyme activity. This observation suggests that physiological events mediated by the cyclic GMP-dependent protein kinase may be particularly critical in the adult prostate while those mediated by the cyclic AMP-dependent enzyme may be more important in the 7-day-old neonate. The higher (A-PK/G-PK) ratio in the adult testis and epididymis, on the other hand, reflects that the cyclic AMP-dependent protein kinase may be involved in the processes of spermatogenesis and steroidogenesis as animals reach sexual maturity.

Interestingly, decreases of basal enzyme activity in these 3 tissues, decreases of (A-PK/G-PK) ratio in epididymides and testes, and an increase of the ratio in prostates in 900-day-old animals, compared to 100-day-old animals, were noted¹². It remains to be seen whether there is a correlation existing between the decrease in protein kinase activities and the decline of sexual functions in the aged (900-day-old) animals.

It would be of great interest to explore other cyclic nucleotide-related parameters, such as the contents and ratios of cyclic AMP to cyclic GMP, and those of cyclic AMP phosphodiesterase to cyclic GMP phosphodiesterase¹³ during development and aging. It was also observed that the progressively decreased activities of both inhibitory modulator of cyclic AMP-dependent protein kinase and stimulatory modulator of cyclic GMP-dependent enzyme in testes during development¹², again suggesting a significant role of the cyclic AMP-dependent enzyme in maturation of testes.



Protein kinase activity in cytosols of the prostate and the testis from developing guinea-pigs as a function of the amount of enzyme protein. Assay conditions were as described in the text except for the various amounts of enzymes used, as indicated. If present, the concentration of either cyclic nucleotide was 0.3 μ M.

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