Regulation of L-Ornithine Decarboxylase and S-Adenosyl-L-methionine Decarboxylase in Rat Ventral Prostate and Seminal Vesicle

By KIRSTI PIIK, PIRKKO RAJAMÄKI, SUJIT K. GUHA and JUHANI JÄNNE
Department of Biochemistry, University of Helsinki, SF-00170 Helsinki 17, Finland

(Received 23 May 1977)

1. The activities of L-ornithine decarboxylase (EC 4.1.1.17) and S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) were dramatically enhanced in both the ventral prostate and the seminal vesicle of castrated rats in response to androgenic stimulation. The time course of the stimulation of ornithine decarboxylase together with the quantitatively different response of adenosylmethionine decarboxylase to testosterone treatment in the prostate gland and seminal vesicle indicated that the enhancement in polyamine synthesis in the ventral prostate may reflect both cellular proliferation and the restoration of the secretory functions of the organ. In the seminal vesicle, however, the stimulation of the polyamine-biosynthetic pathway more closely resembled the pattern found in other rat tissues, such as regenerating liver, undergoing compensatory growth. 2. Ornithine decarboxylase activity in the ventral prostate and especially in the seminal vesicle of sexually mature rat was diminished *in vivo* by various short-chain diamines such as 1,2-diaminoethane, 1,3-diaminopropane and putrescine (1,4-diaminobutane). These diamines had no direct effect on the enzyme activity *in vitro*. 3. In contrast with the marginal decrease in ornithine decarboxylase activity produced by diaminoethane in the ventral prostate of non-castrated animals, repeated injections of the latter amine completely prevented the intense stimulation of the enzyme activity in the ventral prostate and seminal vesicle of castrated rats at 24 h after the commencement of testosterone treatment. 4. The decrease in ornithine decarboxylase activity observed after injections of diamines (putrescine) in the ventral prostate was apparently associated with a similar decrease in the amount of immunoreactive protein as revealed by immunotitration of the enzyme with antiserum to rat ornithine decarboxylase.

Ventral prostate is one of the richest sources of natural polyamines (putrescine, spermidine and spermine) (Mann, 1964; Williams-Ashman *et al.*, 1969; Williams-Ashman & Lockwood, 1970; Williams-Ashman *et al.*, 1972) and of their biosynthetic enzymes among rat tissues (Williams-Ashman *et al.*, 1972; Raina *et al.*, 1976).

Being an androgen-responsive organ, rat prostate has been widely used as a model tissue not only for hormone-induced growth in general but also for studies on the possible physiological functions of the natural polyamines. Thus the rapid involution of the prostate gland after castration is associated with a remarkably swift depletion of the polyamines and with an almost complete disappearance of ornithine decarboxylase and adenosylmethionine decarboxylase activities (Pegg & Williams-Ashman, 1968; Moulton & Leonard, 1969; Williams-Ashman *et al.*, 1969; Pegg *et al.*, 1970; Fuller *et al.*, 1975; Takyi *et al.*, 1977). The restoration of growth of the ventral prostate of castrated rats in response to androgen administration equally rapidly results in a marked stimulation of the synthesis of putrescine and sper-

midine and a gradual normalization of the polyamine content toward values found in sexually mature rats (Moulton & Leonard, 1969; Pegg *et al.*, 1970; Fuller *et al.*, 1975; Takyi *et al.*, 1977). The enhancement in the decarboxylases responsible for the biosynthesis of the polyamines on androgenic stimulation appears to be a direct consequence of the binding of 5 α -dihydro-testosterone in the prostate gland and is probably not mediated by any second messengers, at least not by cyclic AMP (Mangan *et al.*, 1973).

Although rat ventral prostate in many ways seems to provide an excellent experimental model for studying the role of polyamines in androgen-induced tissue proliferation, this system unfortunately is complicated by the fact that the ventral lobe of the prostate also secretes polyamines into seminal fluid (Mann, 1964; Williams-Ashman & Lockwood, 1970). In other words, the enhancement in the synthesis and accumulation of polyamines in response to androgenic stimulation might conceivably represent both the proliferative process of the tissue itself as well as the restoration of its secretory activity.

In contrast with rat ventral prostate, considerably

less polyamines are found in the dorsolateral lobe of the gland, and the polyamine content of the seminal vesicle, another androgen-dependent organ, falls in the range found in resting rat tissues in general (Rhodes & Williams-Ashman, 1964; Williams-Ashman & Lockwood, 1970). Unlike the ventral prostate, the seminal vesicle does not appear to secrete any polyamines either (Rhodes & Williams-Ashman, 1964; Williams-Ashman & Lockwood, 1970). The seminal vesicle thus provides a useful experimental approach for comparative studies on androgen-induced and purely proliferative changes of polyamine metabolism during tissue growth.

On the basis of observed differences of the response of ornithine decarboxylase and adenosylmethionine decarboxylase to androgen treatment in the ventral prostate and seminal vesicle, it appears to us that the changes in polyamine synthesis in the ventral prostate may partly reflect the restoration of the functional activity of this gland. We have likewise found that both in the ventral prostate and in the seminal vesicle ornithine decarboxylase was repressible by exogenously injected diamines such as 1,2-diaminoethane, 1,3-diaminopropane and putrescine, the first of which also completely prevented the stimulation of the enzyme activity elicited by testosterone.

Materials and Methods

Materials

L-[1-¹⁴C]Ornithine (sp. radioactivity 59 mCi/mmol) was purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). S-Adenosyl-L-[1-¹⁴C]-methionine was enzymically prepared from L-[¹⁴C]-methionine (The Radiochemical Centre), by the method of Pegg & Williams-Ashman (1969). 1,2-Diaminoethane (BDH Chemicals, Poole, Dorset, U.K.), 1,3-diaminopropane (Fluka A.G., Buchs SG, Switzerland) and putrescine (Calbiochem, San Diego, CA, U.S.A.) were dissolved in water, neutralized and injected intraperitoneally. Testosterone propionate (Calbiochem) was given subcutaneously in olive oil.

Preparation of animal tissues

Male Wistar rats (350–450 g) were used in all experiments. Castration was performed via the scrotal route under light ether anaesthesia. Animals were killed by decapitation, the ventral prostate and seminal vesicles were dissected, freed from any extraneous tissue and homogenized with 6 or 5 vol. of Tris/HCl buffer (pH 7.1) containing 5 mM-dithiothreitol and 0.1 mM-EDTA. The homogenates were centrifuged at 100000g_{max} for 30 min in a Spinco ultracentrifuge. The resultant cytosol fractions were used as the source of the enzymes,

Analytical methods

The activities of ornithine decarboxylase (Jänne & Williams-Ashman, 1971a) and adenosylmethionine decarboxylase (Jänne & Williams-Ashman, 1971b) were assayed by routine methods used in this laboratory.

One unit of either ornithine decarboxylase or adenosylmethionine decarboxylase activity is defined as the amount of the enzyme catalysing the formation of 1 nmol of CO₂/30 min.

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Partial purification of ornithine decarboxylase and immunization of rabbits

Ornithine decarboxylase was partially purified from livers of thioacetamide-treated rats essentially as described by Hölttä (1975). Partially purified enzyme preparation (specific activity 400–800 units/mg of protein) was used for the immunization of female rabbits as described by Kallio *et al.* (1977c). The antiserum inhibited equally well ornithine decarboxylase from regenerating rat liver or from the ventral prostate. On immunodiffusion, the antiserum showed a single precipitation line when tested against cytosol fraction of the ventral prostate.

Immunotitration of ornithine decarboxylase

The amount of immunoreactive ornithine decarboxylase was titrated with a constant amount of antiserum by adding an increasing concentration of the enzyme. Cytosol fraction of rat ventral prostate was incubated with the antiserum for 3 h at 4°C and the residual ornithine decarboxylase activity was measured.

Results

Effect of castration and androgen treatment on ornithine decarboxylase and adenosylmethionine decarboxylase activities in ventral prostate and seminal vesicle

Castration of the rat resulted in a profound decrease in the activities of ornithine decarboxylase and adenosylmethionine decarboxylase in the ventral prostate as well as in the seminal vesicle. The activity of prostatic ornithine decarboxylase decreased, in fact, to less than 2% of that found in the normal gland within 1 week after the orchidectomy, whereas the activity of the enzyme in the seminal vesicle declined to 7% of precastration values (Figs. 1a and 1b). The activity of adenosylmethionine decarboxylase likewise declined in the ventral prostate by more than 90% over the 7-day period after the castration, but

Fig. 1. Effect of testosterone on ornithine decarboxylase and adenosylmethionine decarboxylase activities in the ventral prostate and seminal vesicle of castrated rats

The rats were castrated 8 days earlier and received a subcutaneous injection of testosterone propionate (2.5 mg/animal) once daily. The enzyme activities were assayed at the time points indicated. The vertical bars represent $\pm$ S.D. of the means. (a) Ventral prostate; (b) seminal vesicle. The upper horizontal lines in both (a) and (b) show the pre-castration value of ornithine decarboxylase activity (○) and the lower lines that of adenosylmethionine decarboxylase activity (●). There were three rats in each group.

much less in the seminal vesicle, i.e. by only about 50% (Figs. 1a and 1b). The administration of testosterone propionate rapidly restored prostatic ornithine decarboxylase to values approaching the pre-castration value at 24 h and clearly exceeded that value at 48 h after the commencement of daily androgen injections (Fig. 1a). In contrast with the ventral prostate, the pre-castration ornithine decarboxylase activity was achieved in the seminal vesicle already between 8 and 12 h, and the maximum activity, about 4 times higher than that found in non-castrated animals, was reached 24 h after testosterone injection, after which no further increases occurred (Fig. 1b). The activity of prostatic adenosylmethionine decarboxylase likewise swiftly responded to the androgenic stimulation, the enhancement of the enzyme activity being more than 8-fold 48 h after the start of the treatment with testosterone (Fig. 1a); however, activity at

that time was still slightly below the pre-castration value. The changes in adenosylmethionine decarboxylase activity in seminal vesicle were not nearly as dramatic as those seen in the ventral prostate. The moderate decrease in enzyme activity resulting from the orchidectomy was corrected to normal values 12–24 h after the injection of testosterone (Fig. 1b).

There are thus at least two differences between the responses of the decarboxylases synthesizing the polyamines to castration and to subsequent androgenic stimulation in the ventral prostate and seminal vesicle: (i) the maximum ornithine decarboxylase activity was reached more rapidly in the seminal vesicle than in the ventral lobe of the prostate; (ii) the changes in adenosylmethionine decarboxylase activity, both in response to castration and to the androgen treatment, were much greater in the ventral prostate than in the seminal vesicle.

Table 1. *Effect of diamines on ornithine decarboxylase and adenosylmethionine decarboxylase activities in the ventral prostate and seminal vesicle of non-castrated rats in vivo*

Neutralized diamines (200 µmol/100g body wt.) were injected intraperitoneally 2 and 4 h before death. The enzyme activities are expressed as units (±s.d.)/mg of protein. There were three animals in each group. The significance of the differences (as compared with the group receiving no treatment) was, by Student's *t* test: **P*<0.05, ***P*<0.01, ****P*<0.001.

Treatment	Ventral prostate		Seminal vesicle	
	Ornithine decarboxylase	Adenosylmethionine decarboxylase	Ornithine decarboxylase	Adenosylmethionine decarboxylase
None	5.20 ± 2.15	2.49 ± 0.73	0.80 ± 0.20	0.31 ± 0.03
Diaminoethane	3.33 ± 0.96	1.77 ± 0.52	0.16 ± 0.06**	0.17 ± 0.09*
Diaminopropane	1.52 ± 0.83*	1.49 ± 0.50	0.12 ± 0.02***	0.05 ± 0.01***
Putrescine	2.18 ± 0.16*	1.63 ± 0.21	0.17 ± 0.03**	0.13 ± 0.04***

Table 2. *Effect of chronic diaminoethane treatment on ornithine decarboxylase and adenosylmethionine decarboxylase activities in the ventral prostate and seminal vesicle of castrated rats injected with testosterone*

The rats were castrated 9 days earlier and received a single injection of 2.5 mg of testosterone propionate 24 h before death. The rats received either NaCl or 1,2-diaminoethane (200 µmol/100g body wt.) every 3 h starting simultaneously with the testosterone injection. The enzyme activities are expressed as nmol of CO₂ (±s.d.)/30 min per mg of protein. There were three rats in each group. The significance of the differences produced by diaminoethane was **P*<0.025 by Student's *t* test.

Treatment	Ventral prostate		Seminal vesicle	
	Ornithine decarboxylase	Adenosylmethionine decarboxylase	Ornithine decarboxylase	Adenosylmethionine decarboxylase
None	0.03 ± 0.02	0.21 ± 0.09	0.07 ± 0.01	0.11 ± 0.02
Testosterone	1.84 ± 1.04	0.74 ± 0.33	0.98 ± 0.59	0.27 ± 0.12
Testosterone + diaminoethane	0.02 ± 0.01*	0.67 ± 0.21	0.03 ± 0.02*	0.13 ± 0.05

Inhibition of ornithine decarboxylase and adenosylmethionine decarboxylase by diamines in androgen-responsive tissues in vivo

Injection of diamines (1,3-diaminopropane, putrescine, cadaverine, 1,6-diaminohexane) into rats results in a remarkably swift disappearance of ornithine decarboxylase activity in a variety of animal tissues, including regenerating rat liver (Pösö & Jänne, 1976), rat kidney (Pösö *et al.*, 1977), rat ovary (Guha & Jänne, 1977) and Ehrlich ascites cells grown in the peritoneal cavity of mice (Kallio *et al.*, 1977b).

As shown in Table 1, two injections (2 and 4 h before death) of 1,2-diaminoethane, 1,3-diaminopropane or putrescine (200 µmol/100g body wt. each time) resulted in a moderate inhibition of ornithine decarboxylase activity in the ventral prostate of non-castrated rats, whereas the inhibition of the enzyme activity in the seminal vesicle was much more pronounced and statistically significant in each case (Table 1). Although prostatic adenosylmethionine decarboxylase only exhibited insignificant changes after the amine treatments, diaminopropane and putrescine also significantly decreased the activity of adenosylmethionine decarboxylase in the seminal vesicle (Table 1). Even though prostatic ornithine decarb-

oxylase of normal rats appeared to be rather insensitive to the diamines, a continuous treatment of castrated rats with diaminoethane completely abolished the marked stimulation of ornithine decarboxylase activity elicited by testosterone at 24 h after the injection (Table 2). The androgen-induced enhancement in ornithine decarboxylase activity in the seminal vesicle was likewise prevented by the treatment with diaminoethane (Table 2). Although the treatment with the amine appeared to result in lower adenosylmethionine decarboxylase activities in both tissues, especially in the seminal vesicle, these inhibitions were not statistically significant (Table 2).

The decrease in ornithine decarboxylase activity by the diamines most probably involved a diminution in the accumulation of the enzyme protein, since none of the compounds used showed any inhibitory effect whatsoever on prostatic ornithine decarboxylase activity *in vitro* (Table 3).

Effect of putrescine treatment on the amount of immunoreactive ornithine decarboxylase

The idea that the treatment with the amines caused an inhibition of the synthesis of the enzyme protein was further supported by findings indicating that the

Table 3. *Effect of different diamines on prostatic ornithine decarboxylase activity in vitro*

Ornithine decarboxylase was assayed in the presence of 2 mM-L-ornithine by using cytosol fraction of the ventral prostate of non-castrated adult rats as described in the text.

Additions (mM)	Ornithine decarboxylase (units)
None (—)	3.36
Diaminoethane (2)	3.43
Diaminopropane (2)	3.52
Putrescine (2)	3.60

Fig. 2. *Effect of putrescine treatment on the amount of immunoreactive ornithine decarboxylase in rat ventral prostate*

Pooled cytosol fractions were prepared from the ventral prostate of non-castrated rats (three animals) receiving no treatment (□) or injected twice (2 and 4 h before death) with putrescine (200 μ mol/100 g body wt.) (■; three animals). The control cytosol fraction was diluted to contain approximately the same ornithine decarboxylase activity per ml as the cytosol fraction from putrescine-treated rats. Both preparations were then titrated with a constant amount of antiserum as described in the Materials and Methods section.

the presence of a constant amount of antiserum) occurred in a similar way in both preparations. Apparently the diminution in ornithine decarboxylase activity after the injection of putrescine was due to a decrease in the amount of immunoreactive protein, most probably because of a direct inhibition of the enzyme synthesis.

Discussion

The extreme dependence of prostatic polyamine synthesis on a continuous supply of androgens obviously offers a convenient model for studies on the role of polyamines in hormone-induced proliferation of this accessory sexual gland. Although the stimulation of polyamine formation by testosterone in the ventral prostate of castrated rats is one of the earliest biochemical events recorded after the administration of the latter hormone, there is very little, if any, evidence to support the view that polyamines mediate the androgen action in the gland, or are causally related, for example, to the early increases in the synthesis of RNA (Liao *et al.*, 1965; Pegg *et al.*, 1970; Williams-Ashman & Lockwood, 1970). Further, Fuller *et al.* (1975) concluded, from their studies on the relation of ornithine decarboxylase activity to the incorporation of [125 I]iododeoxyuridine into DNA, that in the ventral prostate the activity of ornithine decarboxylase is merely a parameter of secretory activity rather than proliferation of the gland. A high spermidine/spermine ratio, which generally indicates rapid growth in rat tissues (Jänne *et al.*, 1964), likewise appeared to correlate with the hypertrophy and secretion in the ventral prostate of androgen-stimulated castrated rats (Takyi *et al.*, 1977). In summary, any conclusions drawn as to a possible relationship between polyamine accumulation and tissue growth in rat ventral prostate must take into consideration the fact that this part of the gland also secretes polyamines and is thus not directly comparable with other tissues undergoing rapid cellular proliferation.

In fact, Fuller *et al.* (1975) reported that although changes of ornithine decarboxylase activity in the ventral prostate paralleled the secretory function, the enzyme activity in the dorsolateral lobe of the gland (containing less polyamines and probably not secreting them) was correlated more closely with the proliferative activity of the tissue, as judged by the rate of DNA synthesis.

Rat seminal vesicle, which also is stringently dependent on circulating androgens, only contains moderate concentrations of natural polyamines with relatively low activities of their biosynthetic enzymes and is not engaged with an active secretion of the polyamines, appears to respond in a different way to androgenic stimulation. The present results tend to support the idea that polyamine synthesis in the

decrease in ornithine decarboxylase activity after putrescine treatment was accompanied by a similar decrease in the amount of immunoreactive protein. Non-castrated rats were injected twice with putrescine as in Table 1, which resulted in about 60% decrease in ornithine decarboxylase activity in the ventral prostate. The cytosol fractions from untreated rats and from rats receiving putrescine were titrated with specific antiserum raised in rabbits against rat liver ornithine decarboxylase. Fig. 2 illustrates that the inhibition of prostatic ornithine decarboxylase by the antiserum was constant irrespective of the source of the enzyme, and the increase in the residual enzyme activity on increasing the amount of the enzyme (in

seminal vesicle of testosterone-treated castrated rats more closely resembles the changes found in other tissues after application of growth-promoting stimuli, i.e. there was an early and intense stimulation of ornithine decarboxylase activity with a distinct maximum and only a moderate or slight enhancement in the activity of adenosylmethionine decarboxylase. The change in the activity of the latter enzyme is similar to that found in regenerating rat liver (Hannonen *et al.*, 1972).

During the last few years an increasing number of reports has appeared describing regulation of ornithine decarboxylase by physiological (Kay & Lindsay, 1973; Clark, 1974; Jänne & Hölttä, 1974) as well as by unphysiological (Pösö & Jänne, 1976; Pösö, 1977; Guha & Jänne, 1977; Kallio *et al.*, 1977a,b) amines in cell cultures and in rat liver. As shown here, ornithine decarboxylase in male accessory glands likewise is sensitive to this 'amine repression'. The inhibition of ornithine decarboxylase could be achieved not only with putrescine, the natural product of the enzyme, but also with some short-chain diamines (diaminoethane and diaminopropane) not normally found in animal tissues. In non-castrated rats ornithine decarboxylase in the seminal vesicle appeared to be more sensitive to the diamines than the prostate enzyme. This could reflect a different penetration of the amines into these tissues. Nevertheless diaminoethane, which only marginally inhibited ornithine decarboxylase in the ventral prostate of non-castrated rats, equally completely prevented the large stimulations of ornithine decarboxylase activity evoked by testosterone, both in ventral prostate and seminal vesicle (Table 2).

The exact mechanism of the action of the amines on ornithine decarboxylase remains to be established. The decrease in ornithine decarboxylase activity is apparently not the result of any direct inhibition of the enzyme activity, since these amines at concentrations that probably never would be reached *in vivo* did not have any influence on the enzyme activity *in vitro* (Table 3).

A direct post-transcriptional inhibition of enzyme synthesis has been suggested to explain the mechanism(s) of diamine action (Jänne & Hölttä, 1974; Clark & Fuller, 1975; Kallio *et al.*, 1977a,c). An alternative, fundamentally different, concept has been proposed by Canellakis and his co-workers (Fong *et al.*, 1976; Heller *et al.*, 1976). The latter investigators discovered that the administration of putrescine resulted in formation of macromolecular (protein?) inhibitors of ornithine decarboxylase. This finding, confirmed by Clark & Fuller (1976), however, apparently does not explain the very rapid action of diamines on ornithine decarboxylase activity. In fact, the action of diamines has been shown to be practically as rapid as that resulting from the use of inhibitors of protein synthesis (Jänne & Hölttä, 1974; Pösö, 1977; Kallio *et al.*, 1977a).

In disagreement with the 'anti-enzyme concept' proposed by Heller *et al.* (1976) as the sole explanation for the inhibition of ornithine decarboxylase by diamines *in vivo*, we found that the decrease in ornithine decarboxylase activity produced by two injections of putrescine was accompanied by a similar decrease in the amount of immunoreactive enzyme protein, as determined with the aid of a specific antiserum to rat ornithine decarboxylase (Fig. 2).

In the light of the present findings it becomes apparent that a long-acting and minimally toxic amine would be an invaluable reagent for studying the consequences of a specific inhibition of polyamine synthesis during androgen-induced growth.

This work received financial support from the Ford Foundation. Dr. S. K. Guha is recipient of a research fellowship from Sigröd Jusélius Foundation.

References

- Clark, J. L. (1974) *Biochemistry* **13**, 4668–4674
- Clark, J. L. & Fuller, J. L. (1975) *Biochemistry* **14**, 4403–4409
- Clark, J. L. & Fuller, J. L. (1976) *Biochem. Biophys. Res. Commun.* **73**, 785–790
- Fong, W. F., Heller, J. S. & Canellakis, E. S. (1976) *Biochim. Biophys. Acta* **428**, 456–465
- Fuller, D. J. M., Donaldson, L. J. & Thomas, G. H. (1975) *Biochem. J.* **150**, 557–559
- Guha, S. K. & Jänne, J. (1977) *Biochem. Biophys. Res. Commun.* **75**, 136–142
- Hannonen, P., Raina, A. & Jänne, J. (1972) *Biochim. Biophys. Acta* **273**, 84–90
- Heller, J. S., Fong, W. F. & Canellakis, E. S. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1858–1862
- Hölttä, E. (1975) *Biochim. Biophys. Acta* **399**, 420–427
- Jänne, J. & Hölttä, E. (1974) *Biochem. Biophys. Res. Commun.* **61**, 446–456
- Jänne, J. & Williams-Ashman, H. G. (1971a) *J. Biol. Chem.* **246**, 1725–1732
- Jänne, J. & Williams-Ashman, H. G. (1971b) *Biochem. Biophys. Res. Commun.* **42**, 222–229
- Jänne, J., Raina, A. & Siimes, M. (1964) *Acta Physiol. Scand.* **62**, 352–358
- Kallio, A., Pösö, H., Scalabrino, G. & Jänne, J. (1977a) *FEBS Lett.* **73**, 229–234
- Kallio, A., Pösö, H., Guha, S. K. & Jänne, J. (1977b) *Biochem. J.* **166**, 89–94
- Kallio, A., Löfman, M., Pösö, H. & Jänne, J. (1977c) *FEBS Lett.* in the press
- Kay, J. E. & Lindsay, V. J. (1973) *Biochem. J.* **132**, 791–796
- Liao, S., Leininger, K. R., Sagher, D. & Barton, R. W. (1965) *Endocrinology* **77**, 763–765
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mangan, F. R., Pegg, A. E. & Mainwaring, W. I. P. (1973) *Biochem. J.* **134**, 129–142
- Mann, T. (1964) *The Biochemistry of Semen and of the Male Reproductive Tract*, pp. 194–220, Methuen, London

- Moulton, B. C. & Leonard, S. L. (1969) *Endocrinology* **84**, 1461–1465
- Pegg, A. E. & Williams-Ashman, H. G. (1968) *Biochem. J.* **108**, 533–539
- Pegg, A. E. & Williams-Ashman, H. G. (1969) *J. Biol. Chem.* **244**, 682–693
- Pegg, A. E., Lockwood, D. H. & Williams-Ashman, H. G. (1970) *Biochem. J.* **117**, 17–31
- Pösö, H. (1977) *Acta Chem. Scand. Ser. B* **31**, 71–76
- Pösö, H. & Jänne, J. (1976) *Biochem. Biophys. Res. Commun.* **69**, 885–893
- Pösö, H., Kallio, A., Scalabrino, G. & Jänne, J. (1977) *Biochim. Biophys. Acta* **497**, 288–297
- Raina, A., Pajula, R.-L. & Eloranta, T. (1976) *FEBS Lett.* **67**, 252–255
- Rhodes, J. B. & Williams-Ashman, H. G. (1964) *Med. Exp.* **10**, 281–285
- Takyi, E. E. K., Fuller, D. J. M., Donaldson, L. J. & Thomas, G. H. (1977) *Biochem. J.* **162**, 87–97
- Williams-Ashman, H. G. & Lockwood, D. H. (1970) *Ann. N.Y. Acad. Sci.* **171**, 882–894
- Williams-Ashman, H. G., Pegg, A. E. & Lockwood, D. H. (1969) *Adv. Enzyme Regul.* **7**, 291–323
- Williams-Ashman, H. G., Jänne, J., Coppoc, G. L., Geroch, M. E. & Schenone, A. (1972) *Adv. Enzyme Regul.* **10**, 225–245