Role of propylamine transferases in hormone-induced stimulation of polyamine biosynthesis

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The effect of various hormones on the activities of the four enzymes engaged with the biosynthesis of the polyamines has been investigated in the rat. Human chorionic gonadotropin induced a dramatic, yet transient, stimulation of L-ornithine decarboxylase (EC 4.1.1.17) activity in rat ovary, with no or only marginal changes in the activities of S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50), spermidine synthase (aminopropyltransferase; EC 2.5.1.16) or spermine synthase. A single injection of oestradiol into immature rats maximally induced uterine ornithine decarboxylase at 4 h after the injection. This early stimulation of ornithine decarboxylase activity was accompanied by a distinct enhancement of adenosylmethionine decarboxylase activity and a decrease in the activities of spermidine synthase and spermine synthase. In the seminal vesicle of castrated rats, testosterone treatment elicited a striking and persistent stimulation of ornithine decarboxylase and adenosylmethionine decarboxylase activities. The activity of spermidine synthase likewise rapidly increased between the first and the second day after the commencement of the hormone treatment, whereas the activity of spermine synthase remained virtually unchanged during the whole period of observation. Testosterone-induced changes in polyamine formation in the ventral prostate were comparable with those found in the seminal vesicle, with the possible exception of a more pronounced stimulation of spermidine synthase activity. It thus appears that an enhancement in one or both of the propylamine transferase (aminopropyltransferase) activities in response to hormone administration is an indicator of hormone-dependent growth (uterus and the male accessory sexual glands), and is not necessarily associated with non-proliferative hormonal responses, such as gonadotropin-induced luteinization of the ovarian tissue.

In contrast with ornithine decarboxylase and adenosylmethionine decarboxylases, the remaining polyamine-biosynthetic enzymes, which catalyse the transfer of the propylamine group from decarboxylated adenosylmethionine to putrescine (spermidine synthase) or to spermidine (spermine synthase), are stable enzymes with long molecular half-lives (for references, see Raina & Jänne, 1975). In most instances the induction of ornithine decarboxylase with concurrent tissue accumulation of putrescine greatly enhances the activity of pre-existing adenosylmethionine decarboxylase (Pegg & Williams-Ashman, 1969), which is the rate-controlling enzyme in the synthesis of spermidine from its immediate precursors (Hölttä & Jänne, 1972; Hannonen *et al.*, 1972).

Under most experimental conditions characterized by rapid formation of spermidine, an increase in the amount of adenosylmethionine decarboxylase or

spermidine synthase is not necessary, since the activation of adenosylmethionine decarboxylase by increased tissue concentrations of putrescine can alone account for the enhanced spermidine formation. This view is supported by a number of findings indicating that increases in ornithine decarboxylase activity in response to a variety of stimuli are much more striking than those found in the activity of adenosylmethionine decarboxylase (Morris & Fillingame, 1974).

The behaviour of spermidine synthase and spermine synthase during accelerated accumulation of polyamines appears to be investigated only in a few selected cases. In regenerating rat liver (Hannonen *et al.*, 1972), the activities of spermidine synthase and spermine synthase clearly increased, yet distinctly after the apparent activation of spermidine synthesis *in vivo* (Raina *et al.*, 1966) or *in vitro* (Hannonen *et al.*, 1972). Similarly, the

activities of propylamine transferases only displayed minor changes in comparison with ornithine decarboxylase, during the development of Ehrlich ascites tumours in the mouse (Kallio *et al.*, 1977).

However, Oka *et al.* (1977) more recently reported that the activity of spermidine synthase is rapidly increased in an inducible manner in mouse mammary epithelium *in vitro*. This induction could be prevented by actinomycin D and by cycloheximide (Oka *et al.*, 1977).

In the present paper we have studied the effects of various hormones on the activities of all the polyamine-biosynthetic enzymes. It appears to us that a growth-promoting action of a hormone is accompanied by a distinct, yet relatively late, stimulation of either one (spermidine synthase) or both propylamine transferase (aminopropyltransferase) activities.

Experimental

Animals and hormone treatments

Male and female rats of the Wistar or Sprague-Dawley strains were used in all experiments. Castration was performed through the scrotal route under light ether anaesthesia. Human chorionic gonadotropin (Pregnyl, Organon; 25 i.u. in 0.9% NaCl) and oestradiol (5 µg in 5% ethanol) were injected intraperitoneally into a rat in 0.5 ml of the vehicle. Testosterone propionate was injected subcutaneously (2.5 mg/rat in 0.5 ml of olive oil). The control animals received the vehicle only.

Preparation of tissue extracts

After the rats had been decapitated, the tissues were freed from any fat and homogenized in 25 mM-Tris/HCl buffer, pH 7.4, containing 0.1 mM-EDTA and 5 mM-dithiothreitol. The homogenates were centrifuged for 20 min at 100 000 *g*_{max}, and the resulting supernatant fractions were used as the source of the enzymes.

Chemicals

S-Adenosyl-L-[1-¹⁴C]methionine and *S*-adenosyl-L-[2-¹⁴C]methionine were prepared enzymically from L-[1-¹⁴C]methionine (sp. radioactivity 60 mCi/mmol) (The Radiochemical Centre, Amersham, Bucks., U.K.) and L-[2-¹⁴C]methionine (sp. radioactivity 46 mCi/mmol) (New England Nuclear Corp., Boston, MA, U.S.A.) by the method of Pegg & Williams-Ashman (1969). Decarboxylated adenosyl[¹⁴C]methionine (labelled at the C-2 of the original methionine moiety) was prepared, isolated and purified by the method of Ps *et al.* (1976). L-[1-¹⁴C]Ornithine (sp. radioactivity 58 Ci/mol) and [1-4-¹⁴C]putrescine (sp. radioactivity 116 Ci/mol) were purchased from The Radiochemical Centre. 1,3-Diaminopropane, 1,3-diaminopropan-2-

ol, 1,2-diaminoethane, cadaverine and 1,6-diaminohexane were obtained from Fluka A.G. (Buchs SG, Switzerland) and *trans*-1,4-diaminobut-2-ene was the product of K & K Laboratories (Plainview, NY, U.S.A.) The purity of the diamines was checked by paper electrophoresis and ninhydrin staining.

Enzyme assays

The activities of ornithine decarboxylase (Jnne & Williams-Ashman, 1971a) adenosylmethionine decarboxylase (Jnne & Williams-Ashman, 1971b) and spermidine and spermine synthases (Raina *et al.*, 1976) were assayed by published methods. Protein was measured by the method of Lowry *et al.* (1951).

Fig. 1. Effect of human chorionic gonadotropin on the enzymes of polyamine biosynthesis in immature rat ovary

Immature female rats (30 days old) were injected with 25 i.u. of human chorionic gonadotropin and killed at the times indicated. Ornithine decarboxylase (●) and adenosylmethionine decarboxylase (○) activities (a) are expressed as nmol of CO₂ liberated/h per mg of protein ($\pm$ S.E.M.), and spermidine synthase (▲) and spermine synthase (△) activities (b) as nmol of spermidine or spermine formed/h per mg of protein ($\pm$ S.E.M.). Each time point represents the mean result for four animals.

Results

Hormonal stimulation of ovary and uterus

A single injection of human choriogonadotropin elicited a dramatic stimulation of ovarian ornithine decarboxylase activity (Fig. 1a), which was accompanied by only marginal changes in the activity of adenosylmethionine decarboxylase (Fig. 1a). The activities of ovarian spermidine and spermine synthases (Fig. 1b) likewise remained unaltered during the whole period of observation (48h).

In contrast with the situation in ovary, oestradiol-induced stimulation of uterine ornithine decarboxylase was accompanied by a biphasic enhancement of adenosylmethionine decarboxylase activity, the first peak coinciding with that of ornithine decarboxylase at 4h and the second occurring 24h after the hormone injection (Fig. 2a). Interestingly, the early hormone-induced stimulation of the two decarboxylases was associated with a distinct decrease in propylamine transferase activities (Fig. 2b). After this initial decrease the activity

of spermidine synthase rapidly rose to reach a peak at 24h (Fig. 2b). Although gradually increasing, the response of spermine synthase to the hormone injection was clearly slower (Fig. 2b).

Hormonal stimulation of male accessory sexual glands

Castration of sexually mature male rats rapidly depletes cellular polyamines and decreases ornithine decarboxylase and adenosylmethionine decarboxylase to almost undetectable activities in rat ventral prostate (Pegg & Williams-Ashman, 1968; Williams-Ashman *et al.*, 1969) and in seminal vesicle (Piik *et al.*, 1977). The concentrations of polyamines as well as the activities of their biosynthetic decarboxylases are rapidly normalized on administration of testosterone.

The specific activities of ornithine decarboxylase and adenosylmethionine decarboxylase in normal rat ventral prostates are about 6 and 4 nmol/h per mg of protein respectively, and in seminal vesicles are

Fig. 2. Effect of oestradiol on the enzymes of polyamine biosynthesis in immature rat uterus

Immature rats were injected with 5µg of oestradiol at zero time. The results are expressed as in Fig. 1.

Fig. 3. Effect of testosterone on the enzymes of polyamine biosynthesis in castrated-rat ventral prostate. The rats (castrated at 8 days) were injected daily with 2.5 mg of testosterone propionate. The enzyme activities are expressed as in Fig. 1.

Fig. 4. Effect of testosterone on the enzymes of polyamine biosynthesis in castrated-rat seminal vesicle. The experimental details are as for Fig. 3.

both about 0.4 nmol/h per mg of protein. After castration the activity of ornithine decarboxylase decreases in 1 week to 2 and 7% in ventral prostate and seminal vesicle respectively, and the post-castration activities of adenosylmethionine decarboxylase are less than 10% (prostate) and about 50% (seminal vesicle) of the 'non-castrated' values (Piik *et al.*, 1977).

As shown in Fig. 3, the activities of ornithine decarboxylase, adenosylmethionine decarboxylase and spermidine synthase markedly increased in castrated-rat ventral prostate in response to daily testosterone injections, whereas the activity of spermine synthase remained at the low 'castration' value.

The response of polyamine-synthesizing enzyme activities to the androgen treatment was similar in seminal vesicle of castrated rats (Figs. 4a and 4b). The activity of spermine synthase remained virtually unchanged, whereas the activities of the remaining three enzymes were rapidly enhanced by testosterone.

Discussion

Dramatic stimulations of ornithine decarboxylase by a variety of hormones, such as gonadotropins in ovary (Kobayashi *et al.*, 1971; Williams-Ashman *et al.*, 1972; Maudsley & Kobayashi, 1974), oestradiol in rat uterus (Cohen *et al.*, 1970; Kaye *et al.*, 1971) and testosterone in male accessory glands of castrated rats (Williams-Ashman *et al.*, 1969; Piik *et al.*, 1977) are well documented. Considerable increases of adenosylmethionine decarboxylase activity have also been observed after hormonal treatment in male accessory glands (Piik *et al.*, 1977) and in uterus (Kaye *et al.*, 1971; Russell & Taylor, 1971), whereas the ovarian enzyme activity appears to be unaffected (Maudsley & Kobayashi, 1974). With the possible exception of mouse mammary-gland epithelium, where spermidine synthase is rapidly induced by a hormone combination (Oka *et al.*, 1977), the two propylamine transferases appear to behave as constitutive enzymes with long molecular half-lives. In addition to their less striking inducibility, mammalian spermidine synthase and spermine synthase occur in great excess in comparison with the much lower amounts of the biosynthetic decarboxylases (Raina *et al.*, 1976; Kallio *et al.*, 1977). Raina *et al.* (1976) have completed a survey of polyamine-synthesizing enzymes in different rat tissues. Although there appeared to be no correlation between individual enzyme activities in a given tissue, it is noteworthy that organs having a secretory function, such as ventral prostate and pancreas, exhibited much higher spermidine synthase activities than did other tissues. In addition to prostate and pancreas, seminal vesicle and uterus, which likewise are engaged with protein secretion, also contained an unusually high spermidine synthase activity, as shown in the present results (Figs. 2b and 4b). Relevant to this view are the findings of Oka and his associates indicating that spermidine is a mediator of glucocorticoid action on milk-protein synthesis in mouse mammary epithelium *in vitro* (Oka & Perry, 1974) and that spermidine synthase activity was rapidly enhanced during the induction of milk-protein synthesis by hormones in mammary epithelium (Oka *et al.*, 1977). Spermidine thus may have some special functions in protein secretion, which are possibly reflected as unusually high spermidine synthase activities in appropriate tissues. In the majority of cells and tissues the activity of spermine synthase is much lower than that of spermidine synthase (Raina *et al.*, 1976) and, as seen in the present results, its inducibility is less striking than that of the remaining three enzymes.

In all of the hormone-responsive tissues studied here, the unstimulated activity of ornithine decarboxylase was the lowest among the four enzymes

involved in the synthesis of polyamines. It thus seems reasonable to conclude that the activity of ornithine decarboxylase controls the rate of polyamine formation. However, during an intense stimulation of ornithine decarboxylase, such as occurring in ovary after gonadotropin treatment, the situation may be completely changed. Owing to the striking stimulation of ornithine decarboxylase activity, its specific activity reached that of spermidine synthase (Figs. 1a and 1b) and clearly exceeded those of adenosylmethionine decarboxylase and spermine synthase. A similar phenomenon could be seen in the male accessory sexual glands after testosterone treatment (Figs. 3 and 4). Thus, provided that the data obtained from the enzyme assays *in vitro* are applicable to the conditions *in vivo*, it can be inferred that, in these hormone-stimulated tissues, the rate of spermine synthesis is determined by the activities of spermine synthase and/or adenosylmethionine decarboxylase. A differing situation is encountered in the uterus (Fig. 2), where ornithine decarboxylase remained as the rate-controlling enzyme even during hormonal stimulation.

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