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Inhibition of Ornithine Decarboxylase Activity in Mouse Kidney by Administration of Diamines

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On testosterone administration the hypertrophied kidneys of castrated mice exhibited a striking increase in the activity of ornithine decarboxylase (EC 4.1.1.17) and an accumulation of polyamines. The effect on ornithine decarboxylase activity could be inhibited by injections of putrescine or its homologues, 1,3-diaminopropane and 1,5-diaminopentane (cadaverine). Most effective was 1,3-diaminopropane which also restrained the accumulation of polyamines.

In growing tissues the capacity of enzymes to form diamines and polyamines is elevated and the amines accumulate in the tissues (for a review see Ref. 1). Because these amines exert an unusually wide scope of biological activities considerable interest has been aroused around their function(s). Information on their physiological significance is likely to come forth if means to inhibit the endogenous formation of the amines were discovered. Putrescine and spermidine, ubiquitously present in mammalian tissues, have been shown to inhibit the activity of ornithine decarboxylase *in vivo*.[2,3] The close homologues of putrescine, *i.e.* 1,3-diaminopropane and 1,5-diaminopentane (cadaverine) appear in mammalian tissues in low concentrations, when present. These "unphysiological" amines have been shown to be strong inhibitors of ornithine decarboxylase activity *in vivo*.[4,5] 1,3-Diaminopropane cannot compensate for putrescine in the synthesis of spermidine and spermine.[6] Injections of 1,3-diaminopropane into partially hepatectomized rats not only inhibit the ornithine decarboxylase activity and prevent the accumulation of putrescine and spermidine but also restrain the stimulation of DNA synthesis in the regenerating rat liver.[7]

In castrated mice daily injections of testosterone propionate resulted in a striking increase in kidney weight and renal RNA but only in minor change in DNA.[8] During the development of this hypertrophy renal putrescine rose sharply and the ornithine decarboxylase activity was enhanced to values more than 1000 times the control level. This report describes the inhibitory effects of some diamines on polyamine metabolism in the mouse kidney stimulated to growth by administration of testosterone.

METHODS

White male mice (NMRI strain) were gonadectomized at the age of 8 weeks. Three weeks later testosterone propionate (British Drug House Ltd., Poole, England), 200 μg in 50 μl of arachis oil, was injected subcutaneously daily for 3 days. Castrated controls were given arachis oil only. Putrescine dihydrochloride and cadaverine dihydrochloride were purchased from Sigma Chem. Comp., St Louis, Missouri, and 1,3-diaminopropane from Fluka A.G., Buchs SG, Switzerland. The diamines were dissolved in 0.9 % NaCl solution, neutralized and injected (150 μmol/100 g body wt.) intraperitoneally in small volumes (0.10 – 0.12 ml). Controls were given 0.9% NaCl solution only.

For preparation of enzyme extracts the mice were stunned and exsanguinated. The kidneys were removed and immediately homogenized in 7 volumes of cold 0.1 M sodium phosphate buffer (pH 7.2) containing 10^{-4} M EDTA, 5×10^{-4} M dithiothreitol and 0.2 % (w/v) glucose. The homogenate was centrifuged at 20 000 *g* for 20 min at 4 °C. The supernatant was decanted and used for determining the ornithine decarboxylase activity. This was

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Table 1. Effect of some aliphatic diamines on ornithine decarboxylase activity in the kidney of testosterone-treated castrated mice. Values are means ± S.E. of the mean, $n = 6$.

Treatment	Ornithine decarboxylase activity (nmol/mg soluble protein/h)	Percentage
Castrated controls	0.01 ± 0.003	< 0.01
Testosterone	205 ± 21.2	100
Testosterone + cadaverine	140 ± 10.9	68
Testosterone + putrescine	67 ± 10.2	33
Testosterone + 1,3-diaminopropane	2 ± 0.5	1

done by measuring the $^{14}CO_2$ released from the carboxyl-labelled ^{14}C-ornithine as previously described.[8] All incubations were performed in a total volume of 1.0 ml. The amount of tissue in the incubation mixture was 2.5 mg except at the incubation of kidneys of castrated controls where the tissue amount was 62.5 mg, as it was known that the enzyme activity in kidneys of castrated controls is low in comparison to that of testosterone-treated ones. For the same reason the ^{14}C-carboxyl labelled DL-ornithine monohydrochloride (final concentration 10^{-4} M) had a higher specific activity when kidneys of untreated castrated mice were incubated: 5 mCi/mmol instead of 0.5 mCi/mmol. The incubation time was 60 min and 30 min, respectively, at 37 °C.

Extracts for determination of amine contents were prepared by homogenizing the minced kidneys in 9 volumes of a solution of 4 % sulfosalicylic acid and 0.04 % EDTA. The extract was heated in a boiling water-bath for 30 min whereupon the mixture was chilled and centrifuged. The sample was adjusted to pH 2.0 – 2.5 and filtered. Chromatographic separation and quantitative estimation of the amines in the extract were carried out by high resolution liquid chromatography using an automatic amino acid analyzer. To make it possible to separate diaminopropane and putrescine we used a modified method by Kremzner:[9] Three buffers with pH 5.25 and 5 % 1-propanol were pumped at 60 °C through a column (0.9 × 6.3 cm) of Durrum DC 6A sequently at a flow-rate of 70 ml/h for different elution times: Buffer I: 0.12 M sodium citrate, 20 min; buffer II: 0.2 M sodium citrate plus 1.30 M sodium chloride, 30 min; buffer III: 0.12 M sodium citrate plus 2.45 M sodium chloride, 44 min.

Protein was measured by the method of Lowry *et al.*[10] with bovine serum albumin as standard.

RESULTS

Effects of some aliphatic diamines on ornithine decarboxylase activity in the testosterone-stimulated mouse kidney. Mice were injected for 3 days with testosterone propionate in order to stimulate ornithine decarboxylase activity in the kidneys. Testosterone administration has previously been reported to abruptly elevate renal ornithine decarboxylase. Daily injections of 200 μg testosterone propionate enhanced ornithine decarboxylase activity to a plateau level after 3 days. This level was rather stable during the remainder of the three-week experimental period.[8] As shown in Table 1, in the present study testosterone administration

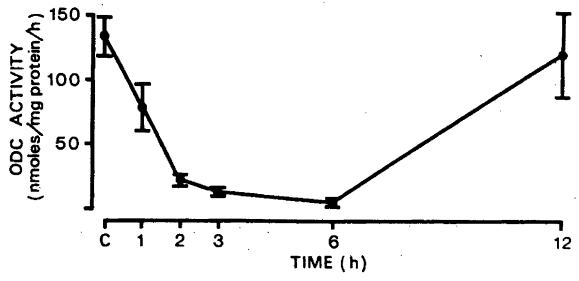

Fig. 1. Time course of inhibition of testosterone-activated ornithine decarboxylase activity [nmol/(mg soluble protein/h)] in mouse kidney after a single injection of 1,3-diaminopropane (150 μmol/100 g body wt.). Each point represents the mean ± S.E. of the mean of six observations except for the point at 3 h which stands for the mean of five observations.

Table 2. Effect of 1,3-diaminopropane on the concentrations (nmol/g) of 1,3-diaminopropane, putrescine, spermidine and spermine in the kidney of testosterone-treated castrated mice. Values are means ± S.E. of the mean, $n=6$. * Sign stands for degree of significance of the difference between the groups receiving testosterone alone and when combined with 1,3-diaminopropane (**, $p<0.01$; ***, $p<0.001$).

Treatment	1,3-Diamino-propane	Putrescine	Spermidine	Spermine
Castrated controls	10 ± 2.4	16 ± 2.0	351 ± 10.2	713 ± 9.9
Testosterone	15 ± 8.6	208 ± 11.6	458 ± 7.7	906 ± 23.7
Testosterone + 1,3-diamino-propane	186 ± 30.8 ***	74 ± 15.4 ***	348 ± 16.0 ***	795 ± 22.6 **

similarly led to a marked increase in renal ornithine decarboxylase activity. The activity increased to a level above ten thousand times that of castrated controls: *i.e.* from 0.01 nmol/(mg soluble protein/h) to 205 nmol/(mg soluble protein/h). To testosterone-treated mice cadaverine, putrescine or 1,3-diaminopropane was given (in a dose of 150 μmol/100 g body wt.) 6 h and 3 h before the animals were killed. All three amines significantly reduced the enzyme activity, 1,3-diaminopropane being the most potent, reducing it by 99 % (Table 1).

Fig. 1 shows the time course of inhibition by 1,3-diaminopropane on ornithine decarboxylase activity in the kidney. Again, before the actual experiments, the mice were treated for three days with testosterone propionate. A single dose of 1,3-diaminopropane was injected and the enzyme activity was assayed at the hours indicated in Fig. 1. Already 1 h after the injection of the inhibitor the enzyme activity was significantly reduced and the maximum effect was observed 6 h after the injection. After 12 h the ornithine decarboxylase activity had returned to normal. In this connection it should be mentioned that 1,3-diaminopropane (10^{-3} M) did not directly influence the ornithine decarboxylase activity of the kidney when added to the incubate *in vitro* (results not shown).

Effects of 1,3-diaminopropane on polyamine levels in the testosterone-stimulated mouse kidney. From an earlier investigation [8] it was known that administration of testosterone propionate for 3 days caused a marked increase in the renal content of putrescine. The levels of spermidine and spermine were also elevated. These effects of testosterone were confirmed (Table 2). As also shown in Table 2 a single injection of 1,3-diaminopropane to testosterone-treated mice caused a decrease in the renal concentration of putrescine 6 h later. Furthermore, injection of 1,3-diaminopropane significantly decreased the concentrations of spermidine and spermine. Concerning the renal content of 1,3-diaminopropane only small peaks were found at the place of this amine on the chromatograms from castrated and testosterone-treated mice, whereas peaks representing nearly 200 nmol/g were found from mice treated with both testosterone and 1,3-diaminopropane. As a result of the low amounts of endogenous 1,3-diaminopropane we have not been able to isolate and hence identify the amine with other established procedures.

DISCUSSION

The three diamines investigated are all capable of reducing ornithine decarboxylase activity in the mouse kidney undergoing hypertrophy. In rat liver, at regeneration after partial hepatectomy [4] or growth hormone administration,[11] in Ehrlich ascites carcinoma cells,[12] and in rat ovary stimulated by human chorionic gonadotrophin [13] the diamines have been shown to inhibit the enzyme activity. In the present study, as well as in the studies of hepatic ornithine decarboxylase activity, 1,3-diaminopropane was shown to be the most effective inhibitor. In contrast, using Ehrlich ascites tumor cells or rat ovary 1,5-diaminopentane was more effective in diminishing ornithine decarboxylase activity. Variations in the inhibition by different diamines can be explained by differences in the cellular capacity

to take up the actual diamines but also in differences in the capability to retain and catabolize the amine. As to the catabolism it is known that putrescine and cadaverine are better substrates of diamine oxidase than 1,3-diaminopropane.[14] Aminoguanidine is an efficient inhibitor of diamine oxidase activity. Investigation of the ability of different diamines to reduce ornithine decarboxylase activity when simultaneously diamine oxidase activity is blocked by aminoguanidine administration might expose further details of the regulation of putrescine formation.

As to the regulation of ornithine decarboxylase activity by diamines it has been shown that, in some biological systems, this can be achieved, at least partly, by promoting the synthesis of a protein inhibitor termed antizyme.[15–17] We have searched for antizyme activity in the kidneys after 1,3-diaminopropane administration without success (results not shown). These negative results appear explicable by the fact that free antizyme activity cannot exist as long as ornithine decarboxylase activity persists.

The concentrations of putrescine and spermidine were profoundly reduced within 6 h after the injection of the amine. Putrescine turnover in tissues is known to be high. In regenerating liver the half-life of putrescine has been calculated to 2 h while spermidine turned over with a half-life of 2 days or more.[18,19] If the turnover of these amines is the same in the hypertrophic mouse kidney, putrescine contents should be expected to fall drastically but not that of spermidine when the synthesis of the amines was inhibited for only 6 h as in our study. There may be other explanations for the rapid reduction in spermidine concentration. A tentative hypothesis is that the 1,3-diaminopropane caused a release of spermidine. In preliminary studies we have found an elevated urinary excretion of spermidine after injections of 1,3-diaminopropane.[20] It should be mentioned that the diamine histamine is known to be released by basic compounds (see Ref. 21).

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