Effect of unilateral ureteral obstruction on renal polyamine levels in rats[1]

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Summary. Unilateral ureteral obstruction in rats, resulted in considerable alterations of polyamine levels in the obstructed kidney during a 72-h observation period, as compared with respective values for the contralateral control kidney.

The polyamines (putrescine, spermidine and spermine) are low mol. wt aliphatic compounds that play an important role in cell growth and proliferation and exhibit a wide range of biological activity[2,3]. During the past 25 years, the biosynthetic pathways have been elucidated, and many studies have been carried out that clearly indicate that the polyamines are physiologically important. Several factors - specific and nonspecific - are known to influence tissue polyamine concentrations but the regulatory mechanisms of their metabolic routes remain to be established. The purpose of the present paper is to study the renal polyamine levels following unilateral ureteral obstruction, a local mechanical stressor, when cell proliferation, increased DNA synthesis, structural and enzymic changes are also known to develop[4-9].

Materials and methods. Chemicals used: spermidine, 3HCl (Serva), spermidine, 4HCl (Serva), putrescine (tetramethylenediamine, Koch-Light Laboratories Ltd), dansyl-chloride (5-dimethylamino-1-naphthalene sulfonylchloride, Serva). All other chemicals were of reagent grade.

Female albino rats of inbred strain (CFY LATI, Hungary) ranging in weight from 200–260 g were used. Under light ether anesthesia the left ureter was completely ligated at the midportion through a midline abdominal incision. Sham-operation consisted of laparotomy. The animals had free access to water and food both before and after surgery throughout the whole observation period. At 24, 48 and 72 h after surgery the animals were bled at the same period of day (between 0900 and 1000 h), kidneys were promptly removed, and stripped of their capsules. After the contents of the renal pelvis had been drained out, the organ was blotted dry on filter paper, weighed and immediately processed for polyamine determination[10], with the exception that instead of the ninhydrine reaction the polyamines were dansylated[11]. The dansyl polyamines were subjected to TLC on pre-coated plastic silicagel sheets (Polygram Sil G, Macherey-Nagel) using an ethyl acetate + cyclohexane (2:3) solvent mixture. The fluorescent spots were identified under UV-light using polyamine reference standards run on the same sheet. The spots were cut out and extracted in 3.0 ml of a methanol + concentrated ammonia (95:5) solvent. Fluorescent measurements were done in a Locarte LFM/5 fluorescent spectrum fluorimeter equipped with a mercury arc lamp using an LF 2 primary filter (transmission for 340-380 nm), and a wedge monochromator set at 505 nm on the secondary side.

Data were analyzed for statistical significance by Student's t test.

Results. Unilateral ureteral occlusion resulted in a considerable increase in kidney fresh weight on all postoperative days tested compared with values for contralateral kidney weight (table 1). It should be noted that at 48 and 72 h values for contralateral kidney weight are somewhat higher than respective data for sham-operated control rats, but these values were not, however, statistically significant.

Data for renal polyamine levels are summarized in table 2. In the obstructed kidney, at 24 h the spermine and total polyamine concentration was reduced, whereas the putres-

Table 1. Kidney weights in rats with unilateral ureteral obstruction

Treatment	Time (h)	Fresh kidney weight (mg/100 g b.wt)	
		Control side	Occluded side
Sham-operation	24 (8)	356.4 ± 48.6	
	48 (8)	344.5 ± 22.5	
	72 (8)	350.8 ± 38.0	
Unilateral ureteral obstruction	24 (8)	334.3 ± 21.6	415.2 ± 27.4*
	48 (8)	367.3 ± 21.6	497.1 ± 38.0*
	72 (8)	380.0 ± 31.0	566.1 ± 103.4*

Data given as mean ± SD. *$p < 0.001$. Calculated by Student's t-test. Number of animals in parentheses.
Comparisons: occluded kidney versus contralateral control kidney; contralateral control kidney versus control kidney of sham-operated rats.

Table 2. Renal polyamine levels in rats with unilateral ureteral obstruction

Treatment	Time (h)	Kidney	Putrescine	Spermidine	Spermine	Total polyamines	Spermidine/spermine molar ratio
Sham-operation	24	Control side	19.1 ± 7.4 (7)	230.1 ± 34.5 (7)	286.8 ± 63.4 (7)	549.9 ± 92.8 (7)	0.784 ± 0.103 (7)
	48		18.2 ± 3.0 (6)	220.5 ± 33.9 (6)	268.7 ± 49.5 (6)	503.9 ± 78.7 (6)	0.825 ± 0.039 (6)
	72		20.4 ± 3.1 (7)	245.9 ± 47.5 (7)	330.0 ± 57.3 (7)	596.3 ± 98.3 (7)	0.747 ± 0.093 (7)
Unilateral ureteral obstruction	24	Control side	28.7 ± 7.5[a] (8)	220.1 ± 63.1 (8)	277.1 ± 61.0 (8)	516.5 ± 109.1 (8)	0.800 ± 0.145 (8)
	48		26.9 ± 9.8 (7)	229.4 ± 40.6 (8)	403.4 ± 97.3[c] (8)	645.4 ± 129.1[a] (7)	0.589 ± 0.131[c] (7)
	72		20.4 ± 7.1 (7)	253.6 ± 52.2 (8)	324.8 ± 62.0 (8)	569.6 ± 73.9 (7)	0.766 ± 0.098 (8)
	24	Occluded side	41.5 ± 10.9[b] (8)	177.9 ± 40.2 (8)	168.4 ± 57.4[c] (8)	394.1 ± 104.9[a] (8)	1.120 ± 0.235[c] (8)
	48		33.7 ± 6.9 (7)	284.8 ± 47.5[a] (7)	299.3 ± 39.3[a] (7)	615.9 ± 81.3 (7)	0.960 ± 0.113[d] (7)
	72		30.6 ± 12.4 (7)	349.6 ± 110.6[a] (7)	250.1 ± 37.6[b] (7)	641.3 ± 131.8 (7)	1.391 ± 0.465[c] (7)

Renal polyamine levels were determined at times indicated according to the method described in Materials and methods. Polyamine concentrations are expressed as nmoles/g wet tissue. Total polyamine concentration is: putrescine + spermidine + spermine in the same kidney which is also expressed in nmol/g wet tissue. Data given as mean ± SD. [a]$p < 0.05$, [b]$p < 0.02$, [c]$p < 0.01$, [d]$p < 0.001$ calculated by Student's t-test. Number of observations in parentheses.
Values compared: occluded kidney versus contralateral control kidney; contralateral control kidney versus control kidney of sham-operated rats.

cine level and spermidine/spermine ratio were elevated. At 48 h spermidine content and the spermidine/spermine ratio were enhanced, whereas the spermine concentration was lower than in the contralateral control kidney. At 72 h spermidine concentrations and spermidine/spermine ratios were further augmented. In the contralateral control kidney, the putrescine level was increased at 24 h, whereas spermine and total polyamine concentrations were elevated at 48 h after surgery, as compared with respective values of sham-operated rats.

Discussion. The enzyme ornithine decarboxylase (E.C. 4.1.1.17) is the first in the biosynthesis of polyamines, and is considered to be the site of regulation for the overall pathway. Its half-life is 11 min in the regenerating liver[12]. The increase in activity of ornithine decarboxylase seems to be part of a general response of tissues to stimulation, whether it be a stimulus to cell division or to increased activity of differentiated function[13]. Renal ornithine decarboxylase activity and polyamine levels have been shown to increase in response to various stimuli, such as after unilateral nephrectomy[14], different hormonal treatments[13,15-20] folic acid[21] suramin[22], beta endorphin or morphine[23] administration.

The results of the present study appear to demonstrate that, following unilateral ureteral obstruction, responses of polyamine metabolism are initiated rapidly in the occluded kidney. These responses are primarily characterized by a prevalance of spermidine over spermine, a condition otherwise found in rapidly proliferating tissues[24,25]. Since total polyamine concentrations were not augmented significantly in the obstructed kidney, and a redistribution occurred among polyamines, it is concluded that in addition to increased synthesis an interconversion of polyamines published for rat liver[26,27] might also contribute to the changes observed in the present study. The contralateral kidney exhibited a less apparent and a differential response-pattern. Therefore, we suggest that local factors might be involved in the polyamine alterations of the obstructed kidney, and support the view[4] that the early response of the obstructed kidney was not mediated by humoral factors.

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Effect of starvation and refeeding on catalase and superoxide dismutase activities in skeletal and cardiac muscles from 12-month-old rats[1,2]

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Summary. Catalase and superoxide dismutase (SOD) activities were determined in muscles from 12-month-old rats after severe starvation and after subsequent refeeding. Catalase increased in most muscles after starvation and decreased after refeeding, while SOD remained unchanged.

Potentially toxic levels of hydrogen peroxide (H_2O_2) and superoxide radicals are controlled in aerobic organisms by catalase and superoxide dismutase (SOD) respectively. The biological significance of the increase in catalase in the rat gastrocnemius muscle after starvation[3] remains obscure. However, catalase is cited as a marker enzyme which increases with muscle breakdown[4]. In addition, it has been suggested that evaluation of biopsy material for catalase could be used as a marker enzyme for muscle wasting[3]. There is no comparative biochemical study of the activities of catalase and SOD for different rat skeletal muscles nor are there studies to examine how these enzymes in muscles respond to refeeding after starvation. It therefore seemed of interest to establish if catalase activity differs between muscles and if different muscles respond in a similar manner after starvation and starvation-refeeding, since this would be an important consideration in choosing muscles for biopsy. Since SOD and catalase function to regulate