Nitric Oxide Synthase Inhibitor L-NAME has No Effect on ^{86}Rb Accumulation in Rat Renal Cortical Slices

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Abstract. The aim of present study was to investigate the effect of the nitric oxide synthase inhibitor L-NAME on the ^{86}Rb uptake in rat renal cortical slices. Rats were divided into three groups: 1. Control. 2. Acute: L-NAME (10 mg/kg i.v.) as a bolus 15 min before the excision of the kidneys. 3. Sub-chronic: L-NAME (10 mg/kg/day) *per os* for 4 days. Renal cortical slices were incubated for 10, 20, 30, 60, 90, 180 seconds in Krebs-Ringer solution containing 50 kBq ^{86}Rb/100 ml ($T = 37$°C, $P_{O_2} \sim 159$ mm Hg). ^{86}Rb accumulation (S/M) was calculated as the ratio of the radioactivity of the cortical slices (S) and the radioactivity of the incubating medium (M).

The S/M ratio can be described as a function of time by the following equations. Control: $y = 0.265 \ln(x) - 0.220$, $r_{xy} = 0.886$; acute L-NAME: $y = 0.224 \ln(x) - 0.171$, $r_{xy} = 0.921$; sub-chronic L-NAME: $y = 0.331 \ln(x) - 0.496$, $r_{xy} = 0.942$. ($y = \mathrm{S/M}$, $x = t$). $p < 0.001$ in all of the groups, but there is no difference between the groups.

In conclusion, L-NAME administered *in vivo* failed to influence the *in vitro* ^{86}Rb accumulation in rat renal cortical slices.

Key words: L-NAME — ^{86}Rb-uptake — Renal cortical slices — Rat

Introduction

Excretory function of the kidney is the only regulated point of the sodium and water output in the organism. As the renal function is highly influenced by blood flow the study of renal blood flow (RBF) has great importance. For this reason RBF has been widely investigated.

One of the methods for determining blood flow of different organs is based on Sapirstein's technique (Sapirstein 1958). The principle of this technique is that the content of indicator in a given organ is directly proportional to both the blood flow and the organ's ability to extract the indicator provided that the indicator was

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injected into the left ventricle and mixed perfectly. This technique was adopted for RBF determination by Hársing and Pelley (1965). According to it, the cardiac output and the renal fraction of cardiac output are determined and the RBF is calculated as the product of cardiac output and the renal fraction of cardiac output.

One of the indicators for determining RBF is ^{86}Rb isotope. ^{86}Rb is transported into the cells by the Na^+, K^+-ATPase in the same way as K^+ (Bartha and Wüstenberg 1975; Kasalická et al. 1983). The accumulated amount of ^{86}Rb isotope depends on the blood flow and the ^{86}Rb extraction of the organ. The suitability of ^{86}Rb accumulation technique for determining RBF was proved (Hársing et al. 1975). ^{86}Rb is even convenient for determining regional blood flow in the different layers of the kidney (Rosivall et al. 1979; Young et al. 1990).

Using ^{86}Rb accumulation technique for determining RBF the possible effects of the applied drugs on the ^{86}Rb uptake must be taken into consideration. In past decade, the renal effects of nitric oxide have been widely investigated. In those experiments, the nitric oxide synthase inhibitor L-NAME was observed to increase the blood pressure and decrease RBF (Baylis and Qiu 1996; Yamada et al. 1996; Tost et al. 2000). L-NAME is a substituted L-arginine analogue, which inhibits NO production (Collier and Vallance 1989). In our previous experiments renal haemodynamic effects of L-NAME have been estimated by ^{86}Rb accumulation technique (Hably et al. 1998; Tost et al. 2000); for this reason we wanted to know whether the L-NAME has a direct inhibitory effect on ^{86}Rb uptake or the decreased ^{86}Rb content in the kidney is really the result of the decreased RBF. Hence, the aim of present study was to determine the effect of L-NAME on the ^{86}Rb accumulation in renal cortical slices *in vitro*, where the ^{86}Rb uptake of the cells is independent from haemodynamic effects. In this case the ^{86}Rb content in the renal cortical slices depends only on their ^{86}Rb extraction ability.

According to previous results haemodynamic effects of a single intravenous injection of L-NAME reach a maximum 5 min after the administration and last at least 50 min (Alemayehu et al. 1994). In order to assure the maximum effect of L-NAME, ^{86}Rb accumulation was investigated 15 min after a single intravenous injection of L-NAME and following 4 day *per os* administration of L-NAME, respectively.

Materials and Methods

Experiments were carried out on renal cortical slices originating from female Wistar rats weighing 200–220 g. Rats were maintained on standard rat chow with free access to water prior to the experiments.

Animals were divided in three groups:

I. Control: ($n = 12$) No intervention before the determination of ^{86}Rb accumulation had occurred.

II. Acute L-NAME administration: ($n = 12$) In anaesthesia L-NAME (10 mg/kg

b.m. = 3.71×10^{-5} mol/kg b.m.) was given intravenously as a bolus into a tail vein. 15 minutes were waited before the excision of the kidneys.

III. Sub-chronic L-NAME pretreatment: ($n = 12$) Rats received L-NAME (10 mg/kg/day) for the four last consecutive days before the determination of the ^{86}Rb accumulation. L-NAME was dissolved in the drinking water (3.71×10^{-4} mol/l).

Preparation of renal cortical slices

In sodium pentobarbital (60 mg/kg i.p.) anaesthesia kidneys were removed through median laparotomy, then rats were sacrificed by decapitation. Kidneys were immediately cooled down to a temperature of +2 °C in Krebs-Ringer solution. Decapsulation and dissection of kidneys was done at +2 °C. Cortical slices with a thickness of 0.3 mm were dissected and pooled samples weighing 180–220 mg were placed into the incubating solution. The time elapsing since the excision of the kidneys was 3–6 min until incubation commenced.

Investigation of ^{86}Rb accumulation

Krebs-Ringer solution was used as incubating solution at a temperature of 37 °C. Solution was saturated and continuously bubbled through by atmospheric air ($P_{O_2} \sim$ 159 mm Hg). ^{86}Rb isotope was dissolved in the medium in a concentration of 50 kBq/100 ml. As in haemodynamic studies 90 s are left for the ^{86}Rb accumulation (Hably et al. 2001) in present *in vitro* experiments the incubation periods were chosen around this point of time: renal cortical slices were incubated in the medium for 10, 20, 30, 60, 90 or 180 s. Finishing the incubation, cortical slices were rapidly removed from the incubation medium, blotted on filter paper carefully and pitted in NaOH solution (16 %) at 60 °C. The radioactivity of both the specimens and the incubating solution was measured (Gamma-counter, Wizard, Wallac).

^{86}Rb accumulation (S/M) was calculated as the ratio of the radioactivity of 1 g renal cortical tissue (S) and 1 ml incubating solution (M).

Statistical analysis was performed by regression and unpaired t-test.

Results

The S/M ratio of the specimens has been plotted against the incubation time (Fig. 1). The ^{86}Rb accumulation of renal cortical slices reaches a nearly constant level at 90 s. Using statistical computer program (Microsoft Excel 97) graphs were fitted to the measured points and the correlation coefficients were estimated. The value of the correlation coefficients is nearly 1.00 (control: $r_{xy} = 0.886$; acute L-NAME: $r_{xy} = 0.921$; sub-chronic L-NAME: $r_{xy} = 0.942$) which shows a very good correlation between the fitted graphs and the measured points. The connection between the ^{86}Rb accumulation and the given time can be described by logarithmic equation (Fig. 1).

Graphs were converted by logarithmic transformation and analysis of regression was conducted. According to the analysis of regression there is a positive correlation between the incubation time and the ^{86}Rb accumulation (Table 1).

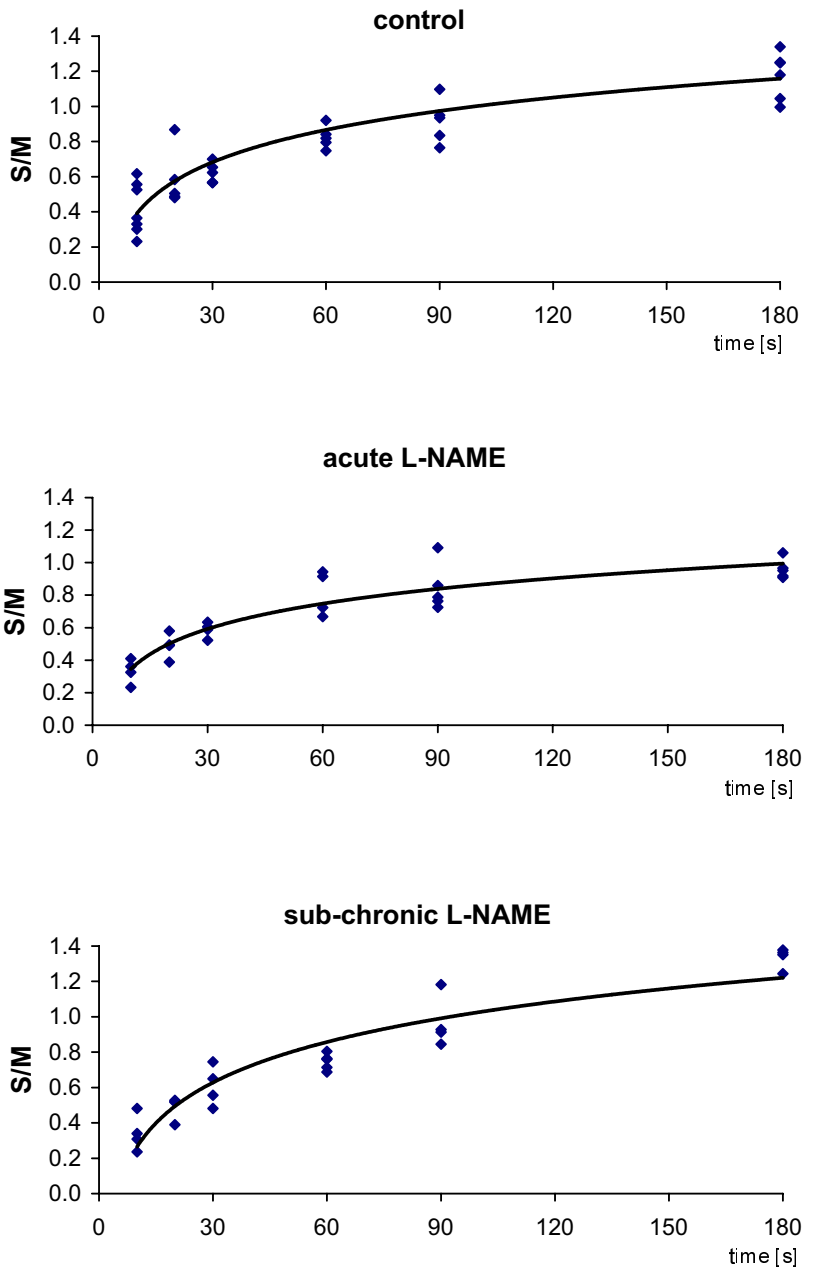

Figure 1. ^{86}Rb accumulation of rat renal cortical slices as a function of time. Each point on the graphs is the S/M ratio in one specimen. Control : $y = 0.265\ln(x) - 0.220$, $r_{xy} = 0.886$, $p < 0.001$; acute L-NAME: $y = 0.224\ln(x) - 0.171$, $r_{xy} = 0.921$, $p < 0.001$; sub-chronic L-NAME: $y = 0.331\ln(x) - 0.496$, $r_{xy} = 0.942$, $p < 0.001$.

Table 1. The equations of the graphs following logarithmic transformation

	equations	r_{xy}	r^2
Control	$y = 0.610 \log(x) - 0.219$ *	0.886^+	0.784
Acute L-NAME	$y = 0.516 \log(x) - 0.170$ *	0.921^+	0.848
Sub-chronic L-NAME	$y = 0.760 \log(x) - 0.495$ *	0.942^+	0.888

r_{xy}, correlation coefficient; r^2, coefficient of determination; * significance of the slopes of the lines, $p < 0.001$; $^+$ significance of the correlation coefficient, $p < 0.001$. There is no significant difference between the L-NAME treated and control groups.

However, neither the graphs nor the equations with or without L-NAME administration differ from each other, i.e. the nitric oxide synthase inhibitor L-NAME failed to influence the ^{86}Rb uptake of renal cortical slices.

Discussion

In previous studies ^{86}Rb accumulation in different cells was investigated and it was concluded that ^{86}Rb accumulation is a sensitive marker for active potassium uptake (Henriksson et al. 1990). It was proved that ^{86}Rb is transported by Na^+, K^+-ATPase (Anner and Haupert 1993). This way ^{86}Rb accumulation is a parameter which reflects Na^+, K^+-ATPase pumping activity.

Drugs which influence the Na^+, K^+-ATPase activity may alter the ^{86}Rb uptake (Hajnóczky et al. 1992). Although the effect of some drugs on Na^+, K^+-ATPase activity had been investigated no data were found on the effect of L-NAME in the kidney.

In present experiments we investigated the *in vivo* effects of L-NAME on the ^{86}Rb uptake of renal cortical slices *in vitro* and pointed out that L-NAME does not influence the ^{86}Rb accumulation of renal cortical slices. On the base of these results we suppose that following an i.v. bolus of ^{86}Rb *in vivo* the decreased ^{86}Rb content in the kidney after nitric oxide synthase inhibition is really the result of the decreased blood flow.

The method applied in our present experiments, namely investigation of some functions *in vitro* following pretreatment with L-NAME *in vivo* is widely used. In previous experiments, in the guinea pig isolated aorta, the relaxation to bradykinin was abolished by *in vivo* treatment with L-NAME (Corriu et al. 1998). Chronic L-NAME treatment resulted in significant reduction of endothelium-dependent relaxation to acetylcholine in isolated rat arteries (Zanchi et al. 1995; Holécyová et al. 1996). According to these results the blockade develops *in vivo* and is still effective *in vitro*. As in our experiments there is no difference in ^{86}Rb content of renal cortical slices originating from control or L-NAME treated rats we suppose that neither acute nor sub-chronic *in vivo* L-NAME treatment has an effect on net ^{86}Rb transport in rat renal cortical slices.

In previous experiments NOS blockade has been reported not to affect Na^+, K^+-ATPase activity in isolated rat arteries (Adeagbo et al. 1994). NO donor sodium nitroprusside (Redondo et al. 1995) or NO precursor L-arginine (Battle and Chan 1988) failed to have any effect on Na^+, K^+-ATPase activity either. These observations are in good agreement with our results.

However, in other studies decreasing effect of NO on Na^+, K^+-ATPase activity has been observed in porcine aortic endothelial cells, while nitric oxide synthase blockade by L-NAME increased Na^+, K^+-ATPase activity (Gruwel and Williams 1998). In contrary, nitric oxide or nitric oxide donor sodium nitroprusside activates Na^+, K^+-ATPase pumping activity (Gupta et al. 1994b), while nitric oxide synthase inhibitor L-NMMA decreases it (Gupta et al. 1994a) in isolated rabbit aortic rings. The differences could be explained by species differences.

In summary, both acute and sub-chronic *in vivo* administration of the nitric oxide synthase inhibitor L-NAME failed to influence the *in vitro* ^{86}Rb accumulation. We conclude that L-NAME has no effect on net ^{86}Rb transport of rat renal cortical slices.

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