Different effects of noradrenaline, angiotensin II and BAY K 8644 on the abolition of autoregulation of renal blood flow by verapamil

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Summary. To examine the role of Ca channels in autoregulation of renal blood flow in response to changes of perfusion pressure, experiments were performed with perfused kidney in anesthetized dogs using a Ca channel activator, BAY K 8644, and vasoconstrictors such as noradrenaline and angiotensin II. Control observations usually showed excellent autoregulation of renal blood flow at pressures between 120 and 200 mm Hg, the autoregulatory index being less than 0.2. Verapamil (50 µg/min, i.a. infusion) obviously inhibited the renal autoregulation. Simultaneous infusion of less than 0.2 µg/min of BAY K 8644 with verapamil dose-dependently reduced renal blood flow, these drugs could not antagonize the inhibitory effect of verapamil on autoregulation. The present experiments show that Ca channels play an important role in establishing renal autoregulation, and that a mere vasoconstriction by noradrenaline and angiotensin II is distinguished from autoregulatory performance.

Key words: Autoregulation — Verapamil — BAY K 8644 — Noradrenaline — Angiotensin II

Introduction

It is well known that the kidney has a remarkable ability to maintain blood flow at a certain level in spite of changes in renal perfusion pressure. We have shown that this renal autoregulation is abolished by Ca antagonists, such as verapamil, nifedipine (Ono et al. 1974) and diltiazem (Ogawa and Ono 1986a), and that simultaneous infusion of CaCl₂ counteracted the inhibitory effects of Ca antagonists on renal autoregulation. Thus, it is considered that the mechanical response to autoregulation of renal blood flow involves influx of extracellular Ca into the vascular smooth muscle cell through Ca channels.

Recently, new dihydropyridine compounds that possess positive inotropic and vasoconstrictor properties have been synthesized: YC-170 (Takanaka and Maeno 1982) and BAY K 8644 (Schramm et al. 1983). Studies on receptor binding have shown competition between nitrendipine and [³H]-BAY K 8644 indicating a common high affinity binding site for Ca channel activator and antagonist (Janis et al. 1984). It is therefore interesting to study the interaction of Ca channel activator with Ca antagonists on renal autoregulation. We studies whether the inhibitory effect of verapamil on renal autoregulation was antagonized by BAY K 8644 in the dog kidney and, in addition, the effects of noradrenaline and angiotensin II were also studied.

Materials and methods

General. Sixteen mongrel dogs of either sex, weighing 11-23 kg, were anesthetized with z-chloralose (40 mg/kg) and urethane (400 mg/kg) intravenously, preceded by sedation with morphine hydrochloride (2 mg/kg, s.c.). The left renal artery was exposed retroperitoneally, cannulated and perfused with blood conducted from the carotid artery by means of a Harvard peristaltic pump (Model 1215). An initial dose of 500 U/kg of sodium heparin was given as anticoagulant. Perfusion pressure was regulated by the use of Starling's pneumatic resistance through which excess blood was conducted to the left jugular vein. A desired level of perfusion pressure was obtained by changing the pressure of the pneumatic resistance. Perfusion pressure and systemic blood pressure in the femoral artery were measured with electric manometers (transducers: Statham P23Db and carrier amplifiers: San-ei 1206B). Renal blood flow was measured by an electromagnetic flowmeter (Narco RT-500). These parameters were recorded on an ink-writing oscillograph (San-ei 8S-53).

Smaller doses z-chloralose and urethane were supplemented when necessary, and sodium heparin was supplemented constantly by 100 U/kg/h. A drug solution was infused into a rubber tube connected close to the shank of the renal arterial cannula by the aid of an infusion pump (Harvard Model 901).

Experimental protocol. The experimental protocol consisted of 3 or 4 periods. The first period a control and the perfusion pressure was changed between 60 and 200 mm Hg to examine the autoregulatory response of the renal vasculature. Then in the second period, verapamil was infused into the renal artery at the rate of 50 µg/min, and autoregulation was examined again.

In the third period, simultaneous infusion of BAY K 8644 (5 µg/min), noradrenaline (1 µg/min) or angiotensin II (0.1 µg/min) with verapamil (50 µg/min) was performed, and the autoregulation was examined. In the forth period, doses were increased and noradrenaline (3 µg/min) or angiotensin II (0.3 µg/min) was infused with verapamil (50 µg/min). The
changing of the perfusion pressure was performed stepwise, starting 10 min after the onset of an infusion. Dose of verapamil was determined to cause adequate inhibition of the renal autoregulation from the results of the previous study (Ono et al. 1974). Dose of BAY K 8644 was adjusted by a preliminary search to counteracted the vasodilation of above dose of verapamil. Doses of noradrenaline and angiotension II were initially selected the equipotent vasoconstrictor doses in the renal vasculature.

**Drugs and data analysis.** BAY K 8644 was donated by Bayer AG, and verapamil by Eisai Co. Angiotensin II was purchased from Osaka Protein Research Foundation, and I-noradrenaline from Fluka. BAY K 8644 was dissolved in 99.5% ethanol to a concentration of 1 mg/ml. I-noradrenaline was dissolved in 0.1 N HCl to a concentration of 1 mg/ml. These stock solutions were diluted to a desired concentration with 0.9% saline.

Renal vascular resistance (RVR) was calculated as

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RVR = \frac{\text{Renal perfusion pressure (RPP)}}{\text{Renal blood flow (RBF)}}
\]

The efficiency index of autoregulation (ARI) was calculated according to the formula of Semple and DeWardener (1959):

\[
ARI = \frac{(\text{RBF}_2 - \text{RBF}_1)/\text{RBF}_1}{(\text{PRA}_2 - \text{PRA}_1)/\text{PRA}_1}
\]

where the renal blood flow changes to RBF₂ from the initial value of RBF₁ when renal arterial (perfusion) pressure is altered to PRA₂ from the initial value of PRA₁.

Differences of mean were analyzed using paired t-test and were considered significant when \( P < 0.05 \). Data will be presented as means ± SE.

**Results**

Renal blood flow was allowed to stabilize initially for 30 min at the basal perfusion pressure of 100 mm Hg, then perfusion pressure was changed stepwise between 60 and 200 mm Hg. In the control period for each animal, renal blood flow remained nearly constant in the autoregulatory range of 120–200 mm Hg (Figs. 1A, 2A, 3A), and autoregulatory index was less than 0.2 showing excellent autoregulation (Figs. 4–6). Renal vascular resistance rose sharply in response to increasing perfusion pressure over the autoregulatory range of 120–200 mm Hg (Figs. 1B, 2B, 3B). Autoregulation was not present between 60 and 120 mm Hg, autoregulatory index being more than 0.8 (Figs. 4–6).

Renal blood flow increased by intra-arterial infusion of verapamil (50 μg/min) from 3.16 ± 0.17 to 3.48 ± 0.19 ml/g kidney weight/min \(( P < 0.001)\) at 100 mmHg of basal perfusion pressure in all 16 experiments. Systemic blood pressure did not change with this dose of verapamil. The infusion of verapamil made the renal blood flow markedly pressure-dependent at all perfusion pressures (Figs. 1A, 2A, 3A), i.e., verapamil impaired the autoregulation. Autoregulatory index were higher than those of the control period for all range of perfusion pressure (Figs. 4–6).

Simultaneous infusion of BAY K 8644 (5 μg/min) with verapamil (50 μg/min) caused vasoconstriction so that it decreased renal blood flow significantly in comparison to the corresponding control values for perfusion pressure between 80 and 120 mm Hg, and it restored the autoregulation impaired by verapamil (Fig. 1A). The curve for renal vascular resistance in relation to perfusion pressure during the simultaneous infusion of BAY K 8644 and verapamil matched closely that of the control period (Fig. 1B). Autoregulatory index recovered also to control values both for 60–120 and for 120–200 mmHg of perfusion pressure (Fig. 4).

Infusion of 1 μg/min of noradrenaline simultaneously with verapamil decreased renal blood flow to the control level at perfusion pressure below 120 mm Hg, but it could not restore autoregulation so that renal blood flow exceeded the control value at perfusion pressure above 140 mm Hg (Fig. 2A). Furthermore, simultaneous infusion of 3 μg/min of noradrenaline decreased renal blood flow significantly below the control level for perfusion pressure below 120 mm Hg, but again it did not antagonize the inhibitory effect of verapamil on the autoregulation (Fig. 2A). The relationship between renal vascular resistance and perfusion pressure during simultaneous infusion of 1 μg/min of noradrenaline with verapamil matched that during