Inhibitory Effects of Trifluoperazine and Chlorpromazine, Calmodulin Inhibitor, on Autoregulation of Renal Blood Flow

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Abstract—An intra-arterial infusion of a calmodulin inhibitor trifluoperazine (3 mg/min) or chlorpromazine (5 mg/min) caused obvious inhibition of autoregulation of renal blood flow in dogs. Simultaneous infusion of CaCl₂ (30 mg/min) or Ca channel activator BAY K 8644 (5 μg/min) with these calmodulin inhibitors reduced renal blood flow, but could not block the inhibitory activity of calmodulin inhibitors on the autoregulation. The present experiment shows the contribution of the Ca-calmodulin system to the mechanism of autoregulation of renal blood flow.

The renal blood flow is maintained remarkably constant by autoregulation over a wide range of perfusion pressures. As for the mechanism, presently it is accepted that the principal process is the myogenic response of the vascular smooth muscle automatically adjusting tone to the change of perfusion pressure. We have demonstrated a chain of evidence that autoregulation of renal blood flow is directly dependent on transmembrane influx of Ca²⁺ through Ca channels; the abolition of autoregulation caused by verapamil, nifedipine and diltiazem was antagonized by CaCl₂ (1, 2) or BAY K 8644, a Ca channel activator (3, 4). Also we have reported that EDTA abolished renal autoregulation, and this effect of EDTA was counteracted by simultaneous infusion of CaCl₂, but not by that of BAY K 8644 (4).

Several investigators have shown that the Ca-calmodulin system is involved in the vascular smooth muscle activity (5, 6). Therefore, the mechanism of renal blood flow autoregulation performed by the myogenic response, presumably activated by the Ca²⁺ influx through Ca channels induced by the change of perfusion pressure may be dependent on calmodulin.

The present experiment was designed to test the above hypothesis. The pressure-flow relation was examined before and during infusion of a calmodulin inhibitor, trifluoperazine or chlorpromazine (7) in the kidney of anesthetized dog. Furthermore, the effect of simultaneous infusion of CaCl₂ or BAY K 8644 with these calmodulin inhibitors was also examined.

Ten mongrel dogs of either sex, weighing 12–20 kg, were anesthetized with α-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 an electric manometer (transducer: 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 of α-chloralose and urethane were supplemented when necessary, and sodium heparin was supplemented constantly by 100 U/kg/hr. 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).

The experimental protocol consisted of 4 periods. The first period was for the control, and the perfusion pressure was changed between 60 and 200 mmHg to examine the autoregulatory response to the renal vasculature. Then, in the second period, trifluoperazine (3 mg/min) or chlorpromazine (5 mg/min) was infused into the renal artery, and autoregulation was examined again. In the third period, simultaneous infusion of CaCl$_2$ (30 mg/min) with trifluoperazine or chlorpromazine was performed, and the autoregulation was examined. In the forth period, BAY K 8644 (5 μg/min) was infused with trifluoperazine or chlorpromazine. The changing maneuver of the perfusion pressure was performed 10 min after the onset of each infusion. Doses of trifluoperazine and chlorpromazine were determined to cause adequate inhibition of the renal autoregulation by a preliminary search.

BAY K 8644 was donated by Bayer AG. Trifluoperazine dihydrochloride and chlorpromazine hydrochloride were purchased from Funakoshi and Wako, respectively. BAY K 8644 was dissolved in 99.5% ethanol to a concentration of 1 mg/ml. This stock solution was diluted to a desired concentration with 0.9% saline. The dose of drugs is expressed as the weight of the base.

The efficiency index of autoregulation (ARI) was calculated according to the formula of Semple and DeWardener (8):

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ARI = \frac{(RBF_2 - RBF_1)/RBF_1}{(P_{RA2} - P_{RA1})/P_{RA1}}
\]

where the renal blood flow changes RBF$_2$ from the initial value of RBF$_1$ when renal perfusion pressure is altered to P$_{RA2}$ from the initial value of P$_{RA1}$.

Differences of means were analyzed using the paired t-test and were considered significant when P<0.05. Data will be presented as means±S.E.

Control observations show a complete autoregulation which maintained renal blood flow constant and an ARI value less than 0.1 in the range of 120–200 mmHg. Partial autoregulation was also shown between 100 and 120 mmHg (Figs. 1 and 2).

The intra-arterial infusion of trifluoperazine increased renal blood flow significantly at a perfusion pressure above 120 mmHg and
caused a pressure-dependence of renal blood flow and ARI higher than that of the control period in all perfusion pressure ranges (Fig. 1). Mean systemic blood pressure decreased significantly from 116±6 to 110±4 mmHg (P<0.05) at 10 min after the start of trifluoperazine infusion. Simultaneous infusion of CaCl₂ (30 mg/min) or BAY K 8644 (5 μg/min) with trifluoperazine decreased renal blood flow and shifted the pressure-flow curve parallel to the right, but the autoregulation was still impaired. Consequently, the blood flow rate was significantly lower than the control rate at perfusion pressures below 120 mmHg (Fig. 1).

The infusion of chlorpromazine (5 mg/min) also caused a pressure-dependence of renal blood flow in all perfusion pressure ranges; i.e., chlorpromazine impaired the autoregulation (Fig. 2). ARI between 120 and 200 mmHg was higher than that of the control period (Fig. 2). Simultaneous infusion of CaCl₂ (30 mg/min) or BAY K 8644 (5 μg/min) with chlorpromazine decreased renal blood flow to a level significantly lower than that of the control for perfusion pressures below 120 mmHg (Fig. 2). ARI during simultaneous infusion of CaCl₂ or BAY K 8644 with chlorpromazine was even higher than during the infusion of chlorpromazine alone (Fig. 2).

Calmodulin has been considered to be an important regulator in the mechanism of vascular smooth muscle contraction (5, 6). Calmodulin inhibitor W-7 (5) produced relaxation of rabbit aortic strips with various agonist, and these agonist-induced contractions were inhibited in a noncompetitive fashion. W-7 also shifted the dose-response curve for CaCl₂ both rightward and downward (5, 6). This inhibitory effect of W-7 was obviously different from those of Ca antagonists such as verapamil and nifedipine (6). Thus, these observations suggested that calmodulin contributed to a common process in the course of smooth muscle contraction.

Previously, one of the authors reported that verapamil and nifedipine inhibited renal blood flow autoregulation, but these inhibitory effects were antagonized by simultaneous infusion of CaCl₂ with Ca antagonists in dog kidney (1). Furthermore, our recent study showed that the inhibitory effects of verapamil and nifedipine in renal autoregulation were antagonized by simultaneous infusion of the Ca channel activator BAY K 8644 (3, 4). Therefore, it is considered that the mechanism establishing auto-
regulatory vascular response involves, at least partly, the process of Ca\(^{2+}\) entry through Ca channels over changes of perfusion pressure.

The present study showed that calmodulin inhibitor, such as trifluoperazine and chlorpromazine, abolished renal blood flow autoregulation. Kanamori et al. (6) reported that chlorpromazine was a more effective Ca\(^{2+}\) influx inhibitor than a calmodulin inhibitor, in lower concentrations. However, we also observed that the abolition of renal autoregulation by trifluoperazine and chlorpromazine was not antagonized by simultaneous infusion of CaCl\(_2\) or BAY K 8644. Therefore, the doses of trifluoperazine and chlorpromazine used in the present study seem to have inhibited calmodulin. Since Cooper and Malik (9) also reported that norepinephrine-induced renal vasoconstriction was inhibited by calmodulin inhibitors such as W-7, trifluoperazine and calmidazolium in rats, calmodulin may play an important role in an intracellular mechanism contributing renal vascular responses to the various agonists and to the change of perfusion pressure.

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