STUDIES ON A NEW 1,5-BENZOTHIAZEPINE DERIVATIVE (CRD-401)

VI. EFFECTS ON RENAL BLOOD FLOW AND RENAL FUNCTION

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Abstract - Effects of a new coronary vasodilator, CRD-401, on renal blood flow and renal function were investigated in anesthetized dogs. Renal arterial or systemic administration of the compound produced an increase in renal blood flow. The increase in renal blood flow induced by CRD-401 was not affected by pre-treatment with propranolol, atropine or dephenhydramine. Renal arterial administration of CRD-401 exhibited an antagonistic effect on the vasoconstricting action of angiotensin-II, whereas it had no effect on that of epinephrine. When infused continuously into the renal artery, CRD-401 increased the urine flow and sodium excretion as well as renal blood flow in all the conditions of fluid loading tested. The glomerular filtration rate was enhanced under saline loading but not under water diuresis. CRD-401 caused an increase in free water clearance. When the renal blood flow was kept constant with an aortic clamp during CRD-401 infusion, sodium excretion was significantly increased but no change was observed in glomerular filtration rate and PAH clearance. The results show that CRD-401 induced natriuresis was not entirely dependent upon the renal hemodynamic changes caused by the compound. By the stop-flow method, it was shown that the ratio of [urine sodium to plasma sodium] to [urine creatinine to plasma creatinine] increased in the distal portion of the nephron by CRD-401 infusion. The present results indicate that the natriuretic action of CRD-401 is not only due to the changes in renal hemodynamics but it also may be ascribable to a direct effect of CRD-410 on the sodium reabsorption in the distal part of the nephron.

A new benzothiazepine derivative, d-3-acetoxy-cis-2, 3-dihydro-5-[(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1, 5-benzothiazepin-4(5H)-one hydrochloride (CRD-401) (1), has been shown to have a vasodilating action and to produce an increase in coronary blood flow without augmenting myocardial oxygen consumption (2). The vasodilating effect of the drug is assumed to be direct action on coronary and femoral blood vessels (3). In addition to the coronary vasodilating activity, it was also reported briefly that the compound produced an increase in renal blood flow and exerted influence upon renal function (2, 4–6).

In the present experiments, the renal action of CRD-401 was studied in detail in anesthetized dogs. Firstly, the renal vasodilating action was investigated using electromagnetic flowmeter and secondly, effects on renal function and electrolyte excretion were examined by clearance technique. The mechanism of diuretic action of the compound was also investigated.
MATERIALS AND METHODS

1. Renal vasodilating action

Male mongrel dogs weighing 15 to 18 kg were anesthetized with sodium pentobarbital (P.B.) (35 mg kg, i.v.). The left renal artery was exposed through a flank incision and a probe connected to a squarewave electromagnetic flowmeter (Nihon Kohden, MF-25) was placed around the renal artery. Blood pressure was monitored from the femoral artery with a pressure transducer. Both renal blood flow (RBF) and blood pressure were recorded simultaneously on an ink-writing oscillograph. The renal vasodilating actions of drugs were estimated by measuring the maximal increase in the blood flow measured from the pre-administration level. Drugs were administered into the renal artery through a catheter that terminated with a 22 gauge needle inserted into the artery, or into the cannulated femoral vein.

In order to investigate the mechanism of vasodilation by CRD-401, an inhibitor such as adrenergic β blocker (propranolol), anticholinergics (atropine) or antihistamine (diphenhydramine) was injected into the femoral vein through a cannula 3 to 10 min prior to administration of CRD-401.

In experiments where interaction of CRD-401 with a vasoconstrictor such as angiotensin-II or epinephrine was studied, the vasoconstrictor was injected into the renal artery before and during the continuous infusion of CRD-401.

2. Renal function

(I) Clearance experiments

Male mongrel dogs weighing 16 to 20 kg were anesthetized with P.B. (35 mg kg, i.v.). The trachea was cannulated and the animal was ventilated by an artificial respirator with room air. Both ureters were exposed through a small abdominal midline incision and catheters were introduced into the renal pelvis. The femoral artery and vein were cannulated for sampling blood and loading saline or glucose solution, respectively. The left renal artery was exposed through a flank incision and a probe was placed around the artery. RBF and blood pressure were measured by the methods mentioned above. A 22 gauge needle connected to a polyethylene catheter was inserted into the renal artery through which saline was infused continuously with an infusion pump at a rate of 0.3 ml/min. Drugs were dissolved into saline. Clearance studies were performed under one of three conditions of fluid load: a) a priming load of 200 ml of isotonic saline and the continuous infusion of saline at a rate of 2 ml/min (small saline load), b) a priming load of 400 ml of 6% dextran and a constant infusion of 2.5% glucose solution at a rate of 9.5 ml/min (water diuresis) and c) an initial load of 400 ml of isotonic saline and a continuous infusion of isotonic saline at a rate of 5.5 ml/min (mild saline load). Glomerular filtration rate (GFR) was determined by measuring inulin or creatinine clearance. Clearance of para-aminobipiric acid (PAH) was measured simultaneously with RBF, determined by an electromagnetic flowmeter. Inulin, or creatinine and PAH were dissolved in the infusion solutions mentioned above and given into the femoral vein. Each priming and sustaining dose was 1.8 g/10 min and 20.25 mg/min for inulin, 2.5 g/10 min and 15-20 mg/min for
creatinine, and 200 mg/10 min and 2.3 mg/min for PAH, respectively. After urine flow had become stabilized, periodic collections of urine and blood were started. Several urine samples serving as controls were collected during saline infusion into the renal artery. The saline infusion was then replaced with infusion of a saline containing CRD-401 and urine samples were collected. Each collection period was 5 or 10 min. Blood samples were drawn at the mid-point of each urine collection period.

(2) Aortic clamp experiments
In order to examine the effect of increased RBF produced by CRD-401 on the natriuretic response, urinary sodium excretion rate was determined under conditions where the change in RBF caused by the continuous infusion of CRD-401 was prevented by means of an aortic clamp (7). A variable resistant clamp was placed around the aorta just proximal to the left renal artery of the anesthetized dogs. The renal perfusion pressure was estimated through the catheter in the left femoral artery and the systemic blood pressure was monitored from the carotid artery. A mild saline load was performed as mentioned previously. Isotonic saline was infused continuously into the renal artery at a rate of 0.3 ml/min throughout the experiment and CRD-401 administration was performed by adding the drugs to the infusate (10 μg/kg/min). When the rate of urine flow became constant, two 10 min collections of urine were carried out and blood samples were drawn at the mid-point of each urine collection period. Following collection of control samples of urine and blood, CRD-401 was infused into the renal artery and the renal perfusion pressure was reduced by way of the aortic clamp so as to eliminate the increment in RBF caused by CRD-401 and to keep it constant at the control level before CRD-401 administration. Under these conditions, two to three samples of urine were collected in 5 or 10 min periods and blood samples were drawn at the mid-point of each period. Control experiments were run for each animal by clamping the aorta to give the same decrement in renal perfusion pressure as observed during CRD-401 infusion. Urine and blood samples were collected as described above.

(3) Stop-flow experiments
The site of action of CRD-401 diuresis along the nephron was examined in dogs by stop-flow technique (8). Osmotic diuresis was established by an intravenous infusion of 20% mannitol in isotonic saline at a rate of 9.5 ml/min. Creatinine and PAH were added to the infusion solution. Experiments were divided into two periods: an initial control period and subsequent experimental period during the continuous infusion of CRD-401 into the renal artery. After a high and stable rate of urine flow (5 to 10 ml/min) had been obtained, urine samples were collected in two 5 min periods and blood samples were drawn at the mid-point of each urine collection. A clamp was used to block off the ureter for 4 min, during which time a blood sample was drawn. After the release of the clamp, 30 serial urine sample of 0.5 to 0.8 ml in volume were collected. The constant infusion of CRD-401 into the left renal artery was then started and the same procedures were repeated.

3. Analysis
Sodium and potassium concentrations in plasma and urine were determined using an
Evans flame photometer. Concentrations of inulin, creatinine and PAH in plasma and urine were determined by the methods of Davison et al. (9), Peters (10) and Hamburger et al. (11) respectively. Osmolality of plasma and urine was measured cryoscopically by Ramsay’s method (12).

4. Drugs

The drugs used in the present experiments were as follows: papaverine hydrochloride (Iwaki Seiyaku), propranolol (I.C.I.), atropine sulfate (Tanabe Seiyaku), diphenhydramine hydrochloride (Tanabe Seiyaku), angiotensin-II (Institute for Protein Research, Osaka Univ.), epinephrine hydrochloride (Sankyo Seiyaku). CRD-401 was synthesized by Kugita et al. (1) of Organic Chemistry Research Laboratory of Tanabe Seiyaku Co., Ltd..

RESULTS

1. Renal vasodilating effect

(1) Effect on renal blood flow

CRD-401, when injected into the renal artery, produced an increase in RBF. With increasing doses of the drug, greater increase in RBF was obtained, while the systemic blood pressure remained unaltered (Fig. 1-A). When papaverine was injected into the renal artery, it also produced a dose-related increase in RBF. However, the time course of the change in RBF produced by papaverine was somewhat different from that produced by CRD-401 (Fig. 1-B). CRD-401 caused only an increase in the blood flow, while papaverine, in most cases, gave a biphasic response, i.e., an increase followed by a decrease. From the dose-response curve in Fig. 1-A, it was concluded that renal vasodilating potency of

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**Fig. 1.** Effect of CRD-401 and papaverine on renal blood flow in anesthetized dogs.

A: Dose-response curves for CRD-401 and papaverine injected into the renal artery.

B: Flow-response patterns elicited by the injection of CRD-401 and papaverine.

\( \Delta \text{RBF} \): increase in renal blood flow, \( \text{RBF} \): renal blood flow.

Each point represents the mean \( \pm \) SE of 6 experiments.

CRD-401, \( \circ \circ \); papaverine, \( \bullet \bullet \).
CRD-401 was more than three times that of papaverine. CRD-401 also produced about 10% increase in RBF, when given intravenously at a dose of 100 μg/kg (Fig. 2).

(2) Effect of inhibitors on vasodilating action of CRD-401

The increase in RBF after the renal arterial administration of CRD-401 was not affected by prior injection of propranolol (0.5 mg/kg), atropine (1 mg/kg) or diphenhydramine (5 mg/kg). Each inhibitor alone also exerted no effect upon the RBF and blood pressure.

(3) Effect of CRD-401 on the vasoconstricting action of angiotensin-II and epinephrine

Angiotensin-II and epinephrine were injected into the renal artery before and during the renal arterial infusion of CRD-401 (10 μg/kg/min). It can be seen in Fig. 3 that the vasoconstricting action of angiotensin-II was significantly suppressed by CRD-401, whereas that of epinephrine was not.

Fig. 2. Effect of i.v. injection of CRD-401 on renal blood flow. BP - blood pressure, RBF - renal blood flow.

Fig. 3. Effect of CRD-401 on the vasoconstrictor action of angiotensin-II and epinephrine in anesthetized dogs.

Each vasoconstrictor was injected into the renal artery during the renal arterial infusion of CRD-401 (10 μg/kg/min).

ΔRBF - change in renal blood flow.

Each point represents the mean ± SE of 5 experiments.
2. Renal function
   (1) Clearance experiments

   Effects of CRD-401 on renal function and urine formation were studied by the continuous infusion technique under a mild saline load. The results are shown in Fig. 4 and

   ![Graph showing dose-response relationship between the dose of CRD-401 and various parameters of the renal function.]

   **Fig. 4.** Dose-response relationship between the dose of CRD-401 and various parameters of the renal function.

   CRD-401 was infused continuously into the renal artery.

   - UV: increase in urine flow, \( \Delta U_{NaV} \): increase in sodium excretion, \( \Delta U_{Kv} \): increase in potassium excretion, \( \Delta C_{\text{in}} \): increase in inulin clearance, \( \Delta C_{\text{PAH}} \): increase in PAH clearance, \( \Delta \text{RBF} \): increase in renal blood flow.

   Each column represents the mean SE during the infusion of CRD-401.

   **Table 1.** Effect of CRD-401 on renal function.

   | Dose | UV (ml/min) | \( C_{\text{in}} \) (ml/min) | \( C_{\text{PAH}} \) (ml/min) | RBF (lEq/min) | \( U_{NaV} \) (lEq/min) | \( U_{Kv} \) (lEq/min) | BP (mmHg) |
   |------|-------------|----------------|----------------|-------------|----------------|----------------|---------|
   | Control | 0.76 | 37 | 100 | 189 | 151 | 24 | 142 |
   | 1 mg kg min | 1.56 | 36 | 98 | 196 | 231 | 27 | 143 |
   | Difference ± SE | 0.8 ± 0.3 | 1.2 | -2.5 | 7.4 | 80.27 | 3 ± 4 | 1.2 |
   | Control | 0.78 | 35 | 104 | 193 | 149 | 25 | 144 |
   | 10 mg kg min | 2.72 | 39 | 121 | 225 | 387 | 48 | 142 |
   | Difference ± SE | 2.0 ± 0.4* | 1.2 | 7.7* | 32.8* | 238.53* | 22.6* | 2.2 |
   | Control | 0.78 | 37 | 103 | 187 | 133 | 29 | 147 |
   | 30 mg kg min | 4.01 | 43 | 131 | 242 | 488 | 53 | 139 |
   | Difference ± SE | 3.2 ± 0.9* | 7.1* | 9.6* | 55.12* | 355.88* | 24.6* | 8.2 |

   CRD-401 was infused into the renal artery of anesthetized dogs under a mild saline load.

   UV=urine flow, \( C_{\text{in}} \): inulin clearance, \( C_{\text{PAH}} \): PAH clearance, RBF: renal blood flow, \( U_{NaV} \): sodium excretion, \( U_{Kv} \): potassium excretion, BP: blood pressure.

   \( N = 4 \)  *: \( P < 0.05 \)
Table 1. When CRD-401 was infused at a rate of 1 μg/kg/min, it did not produce any significant change in all the parameters measured, although urine flow and sodium excretion tended to increase. At a rate of 10 μg/kg/min, however, it caused significant increase in all the parameters. Average increases in urine flow, and in sodium and potassium excretion rates were 3.5, 2.6 and 1.9 fold of the respective control values. Both RBF and PAH clearance were increased by 16%. The increase in inulin clearance was 11.3%. At the highest dose (30 μg/kg/min) tested, CRD-401 caused further increases in urine flow and sodium excretion, i.e., 5.1 and 3.7 fold of the control respectively, although the blood pressure was slightly decreased. Clearances of inulin and PAH, and RBF were also increased by 16.3, 27.2 and 29.5% respectively.

The effects of CRD-401 on renal function were compared with those of papaverine under a small saline load. The data are shown in Table 2. CRD-401, infused into the renal artery at a rate of 10 μg/kg/min, caused statistically significant increases in urine flow, sodium excretion, clearances of inulin and PAH, and RBF. On the other hand, the papaverine infusion at a rate of 100 μg/kg/min caused smaller increases in urine flow, sodium excretion and PAH clearance than CRD-401. The change in RBF was hardly discernible, whereas inulin clearance was decreased, though the degree was small but statistically significant, by papaverine infusion.

The effect of CRD-401 (10 μg/kg/min) on free water clearance was determined with

| Left renal artery infusion | UV (ml/min) | C_{in} (ml/min) | C_{PAH} (ml/min) | RBF (ml/min) | U_{Na}V (μEq/min) |
|----------------------------|-------------|----------------|-----------------|--------------|-------------------|
| Control                    | 0.18        | 40             | 113             | 183          | 28                |
| CRD-401 10 μg kg min       | 0.78        | 43             | 133             | 220          | 147               |
| Difference ± SE            | 0.59 ± 0.12*| 3.4 ± 0.4*     | 20.4 ± 4.1*     | 36.8 ± 3.4*  | 118.8 ± 20.0*     |
| Control                    | 0.19        | 44             | 113             | 190          | 33                |
| Papaverine 100 μg kg min   | 0.32        | 41             | 121             | 199          | 59                |
| Difference ± SE            | 0.14 ± 0.05*| -3.0 ± 0.9*    | 7.8 ± 0.6*      | 9.6 ± 3.8    | 26.0 ± 6.8*       |

CRD-401 and papaverine were infused into the renal artery of anesthetized dogs under a small saline load. (See legend for Table 1)

N=6  *: P<0.05

Table 3. Effect of CRD-401 on renal function under water diuresis.

| Left renal artery infusion | UV (ml/min) | C_{in} (ml/min) | C_{PAH} (ml/min) | C_{Cr} (ml/min) | U_{Na}V (μEq/min) | RBF (ml/min) |
|----------------------------|-------------|----------------|-----------------|----------------|-------------------|--------------|
| Control                    | 3.96        | 0.77           | 3.29            | 40.7           | 8.9               | 218          |
| CRD-401 10 μg kg min       | 5.51        | 1.11           | 4.47            | 42.5           | 46.8              | 253          |
| Difference ± SE            | 1.55 ± 0.39*| 0.35 ± 0.10*   | 1.19 ± 0.36*    | 1.80 ± 1.13    | 37.9 ± 10.51      | 35 ± 11.8*   |

Dogs were given i.v. 400 ml of 6% dextran, followed by continuous infusion of 2.5% glucose at a rate of 9.5 ml/min.

UV: urine flow, C_{in}: inulin clearance, C_{PAH}: free water clearance, C_{Cr}: creatinine clearance, U_{Na}V: sodium excretion, RBF: renal blood flow.

N=5  *P<0.05
water diuresis. Results are summarized in Table 3. CRD-401 produced prompt increases in urine flow, sodium excretion, osmolar clearance and free water clearance. RBF and PAH clearance were also increased significantly, but creatinine clearance was not.

(2) Aortic clamp experiments

Aortic clamp experiments were done on 5 mongrel dogs under a mild saline load. Fig. 5 shows a representative record of the experiments in which CRD-401 (10 μg/kg/min) was infused into the renal artery while the RBF was kept constant by an aortic clamp. A typical record obtained from control experiment in the presence of the aortic clamp is also shown in Fig. 6. Table 4 summarizes the effects of reduction in renal perfusion pressure on renal function with and without CRD-401 infusion. To keep RBF constant during CRD-401 infusion, the renal perfusion pressure was reduced by approx. 25 mmHg by means of the aortic clamp. Under these conditions, inulin and PAH clearances and sodium excretion were not altered significantly (Table 4-B). When the perfusion pressure was reduced in the absence of CRD-401 by about 25 mmHg, which corresponds to the magnitude necessary for preventing the increased blood flow caused by CRD-401, RBF

![Graph](image-url)

**Fig. 5.** Prevention of CRD-401-induced increase in renal blood flow by clamping the aorta just above the renal artery.

CBP = carotid blood pressure, RPP = renal perfusion pressure, RBF = renal blood flow.

![Graph](image-url)

**Fig. 6.** Effect of the reduction in perfusion pressure upon renal blood flow in the absence of CRD-401 following aortic clamping. (See legend for Fig. 5)
and the clearances of inulin and PAH were not altered, but sodium excretion was reduced significantly (Table 4-C). Table 5 shows various parameters obtained from the aortic clamp experiments with and without CRD-401. These values were derived from Table 4. A paired comparison between these values indicated that despite the absence of a significant difference in RBF, clearances of inulin and PAH, and perfusion pressure, the amount of sodium excreted during CRD-401 infusion was significantly greater than that excreted in the control.

(3) Stop-flow experiments

Fig. 7 shows the urinary stop-flow patterns for the change in concentration of sodium, PAH and creatinine during the infusion of saline (control) or CRD-401 (10 µg/kg/min). The ratio of urine to plasma concentrations of sodium, potassium and PAH were all cor-

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**Table 4. Effect of reduction in renal perfusion pressure on renal function with and without CRD-401.**

|                | UNV (µEq/min) | C_{IN} (ml/min) | C_{PAH} (ml/min) | RBF (ml/min) | RPP (mmHg) |
|----------------|----------------|-----------------|-----------------|--------------|-------------|
| **A) Control** |                |                 |                 |              |             |
| CRD-401       | 160            | 42              | 112             | 215          | 149         |
| Difference ± SE | 226±41*        | 6±2             | 18±4*           | 35±5*        | -4±3        |
| **B) Control** |                |                 |                 |              |             |
| CRD-401 + RPP reduced | 206           | 42              | 110             | 222          | 122         |
| Difference ± SE | 40±27          | 2±1             | 4±5             | -5±10        | 25±5*       |
| **C) Control** |                |                 |                 |              |             |
| Control + RPP reduced | 120           | 46              | 119             | 214          | 125         |
| Difference ± SE | -68±18*        | 1±2             | 6±8             | -1±8         | 23±4*       |

Control experiments were carried out with saline (0.3 ml/min) infusion into the renal artery at a rate of 10 µg/kg/min.

A: during CRD-401 infusion without aortic clamp (normal positive control).
B: during CRD-401 infusion with aortic clamp.
C: control with aortic clamp.

UNV = sodium excretion, C_{IN} = inulin clearance, C_{PAH} = PAH clearance, RBF = renal blood flow, RPP = renal perfusion pressure, which was equivalent to the femoral blood pressure distal to the clamp.

N=5 * : P<0.05

**Table 5. Effect of CRD-401 (10 µg/kg/min) on sodium excretion in the absence of the change in renal hemodynamics.**

|                | UNV (µEq/min) | C_{IN} (ml/min) | C_{PAH} (ml/min) | RBF (ml/min) | RPP (mmHg) |
|----------------|----------------|-----------------|-----------------|--------------|-------------|
| 1) Control + RPP reduced | 120           | 46              | 119             | 214          | 125         |
| 2) CRD-401 + RPP reduced | 206           | 42              | 110             | 222          | 122         |
| Difference ± SE | 86±21*        | -4±2            | -9±8            | 8±14         | -3±3        |

1) Data taken from Table 4-C.
2) Data taken from Table 4-B. (See legend for Table 4)
N=5 * : P<0.05
rected by dividing by the ratio of urine to plasma concentrations of creatinine. As can be seen, the sodium pattern was elevated by CRD-401 over the region corresponding to the distal segment of the nephron, while the potassium pattern was elevated over the entire nephron.

**DISCUSSION**

In the present experiments, effects of CRD-401 on renal blood flow and renal function were examined in anesthetized dogs.

When injected into the renal artery, CRD-401 caused a dose-related increase in RBF without affecting the systemic blood pressure. This renal vasodilating action was not affected by the pre-treatment with inhibitors such as propranolol, atropine or diphenhydra-mine. This fact suggests that the vasodilating action is due to its direct action on the renal blood vessels, and this conclusion is compatible with the results obtained in other vessels (3).

When compared with the directly acting vasodilator, papaverine, CRD-401 exhibited a longer-lasting renal vasodilation than papaverine and did not produce such a biphasic
change in the blood flow as papaverine. Furthermore, CRD-401 increased GFR, while with papaverine it was decreased. The decrease in GFR caused by papaverine has been reported by Baer et al (13). These facts suggest that there are differences in vasodilating response between CRD-401 and papaverine.

In the renal artery, it was observed that CRD-401 suppressed the vasoconstriction produced by angiotensin-II but not by epinephrine, although the compound antagonized non-competitively against the constriction caused by various kinds of spasmogens in vitro (14).

The renal arterial infusion of CRD-401 resulted in an increase in urine flow, sodium excretion and RBF under all the fluid loading conditions. However, the GFR was not altered by CRD-401 in water diuresis, while it was increased significantly in saline loading. These facts indicate that the diuretic and natriuretic actions of CRD-401 were not entirely dependent upon the increase in GFR.

A variety of vasodilators have been shown to cause natriuresis associated with the simultaneous increase in RBF when administered into the renal artery (15-19). Concerning the mechanism of the diuresis produced by renal arterial infusion of vasodilators, Early and Friedler (20) have suggested that vasodilation reduces precapillary resistance and allows more complete transmission of an existing perfusion pressure to the capillary circulation. As a result of this increase in capillary perfusion pressure, the cortical volume is increased. This increased interstitial cortical volume is believed to increase the pressure upon the tubules and results in decreased sodium reabsorption. Since the natriuresis produced by CRD-401 was accompanied with an increase in RBF, changes in renal hemodynamics were likely responsible for the natriuresis, as suggested by Early and Friedler. In fact, the increase in free water clearance during CRD-401 infusion supports the possibility that CRD-401 induced natriuresis is based upon the change in renal hemodynamics (21).

Willis et al. investigated the correlation between the natriuresis and the vasodilator-induced changes in renal hemodynamics by placing a clamp around the aorta and thus preventing the drug-induced increase in RBF (7, 21-23). They reported that the natriuresis induced by acetylcholine (7), bradykinin (21) or histamine (22) was entirely dependent upon the increase in RBF, while that induced by aminophylline (23) was partly due to its direct action on renal tubules. To determine whether or not the natriuresis produced by renal arterial administration of CRD-401 was dependent upon the increase in RBF, a variable resistant clamp was placed around the aorta proximal to the renal artery and tightened enough to prevent the drug-induced increase in RBF. When the RBF was kept at the pre-treatment level after administration of CRD-401, there was a statistically significant increase in sodium excretion despite the absence of the effect on any other parameter measured (Table 5). These facts indicate that the natriuresis produced by CRD-401 was not entirely dependent upon the changes in renal hemodynamics and suggest that CRD-401 possesses a direct effect on tubular sodium reabsorption.

The result of the stop-flow experiments indicates that CRD-401 suppressed the sodium
reabsorption in the distal nephron. A similar result was also obtained by Abe et al (5). If changes in renal hemodynamics affect only proximal sodium reabsorption (24, 25), suppression of sodium reabsorption in the distal nephron by CRD-401 observed in the stop-flow experiments may be the result of the direct effect of CRD-401 on the renal tubules as indicated by the aortic clamp experiments. Thus, the natriuretic action of CRD-401 is not only due to the changes in renal hemodynamics, but also may be ascribable to the direct effect of CRD-401 on the sodium reabsorption in the distal part of the nephron.

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