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# Effects of NKH477 on renal functions and cyclic AMP production in anesthetized dogs

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**Abstract**

The present study was undertaken to evaluate the effects of an adenylate cyclase activator *N,N*-dimethyl-β-alanine[3R-(3α, 4aβ, 5β, 6β, 6aα, 10α, 10aβ, 10bα)]-5(acetyloxy)-3-ethenyldodecahydro-10, 10b-dihydroxy-3, 4a, 7, 7, 10a-pentamethyl-1-oxo-1H-naphtho [2,1-b] pyran-6-yl ester hydrochloride (NKH477) on renal functions and cyclic AMP production in the dog kidney. The intrarenal arterial infusion of NKH477 (30, 100 and 300 ng kg$^{-1}$ min$^{-1}$) increased renal blood flow, glomerular filtration rate, urine flow rate, urinary $Na^+$ and cyclic AMP excretion, fractional $Na^+$ excretion and arterial and renal venous plasma cyclic AMP concentrations in a dose-dependent manner. The intrarenal arterial infusion of rolipram (0.3 μg kg$^{-1}$ min$^{-1}$), a cyclic AMP-specific phosphodiesterase inhibitor, also caused the same renal responses as NKH477. The increasing effects of NKH477 on renal blood flow, fractional $Na^+$ excretion and renal venous plasma cyclic AMP concentration were facilitated in the presence of rolipram. NKH477 reduced glomerular filtration rate and filtration fraction in the presence of rolipram. The increasing effects of NKH477 on urine flow rate and urinary $Na^+$ excretion were not affected by rolipram. The present results suggest that NKH477 increases glomerular filtration and suppresses tubular sodium reabsorption through activation of cyclic AMP production, and thereby induces natriuresis. The results also demonstrate that renal cyclic AMP level during the activation of adenylate cyclase is regulated by phosphodiesterase IV in both the vascular and tubular sites. 

*Keywords:* NKH477; cAMP; Kidney; (Dog)

## 1. Introduction

Cyclic AMP is one of the major intracellular second messengers of various physiological responses. Many compounds which affect production or degradation of cyclic AMP have been developed for the purpose of clinical application. *N,N*-dimethyl-β-alanine[3R-(3α, 4aβ, 5β, 6β, 6aα, 10α, 10aβ, 10bα)]-5(acetyloxy)-3-ethenyldodecahydro-10, 10b-dihydroxy-3, 4a, 7, 7, 10a-pentamethyl-1-oxo-1H-naphtho [2,1-b] pyran-6-yl ester hydrochloride (NKH477) is a water-soluble derivative of an authentic adenylate cyclase activator forskolin (Toya et al., 1998). It has been reported that NKH477 directly activates adenylate cyclase to elevate cellular cyclic AMP content more potently than forskolin (Shafiq et al., 1992). NKH477 causes positive inotropic action and coronary artery vasodilation (Hosono et al., 1992; Hirasawa et al., 1993). It has been reported that NKH477 improves the propranolol- or verapamil-induced acute heart failure in dogs (Fujita et al., 1992) and the chronic heart failure in which β-adrenoceptors are down regulated in rats (Sanbe and Takeo, 1995). These pharmacological properties imply that NKH477 can be an innovative and useful drug for the treatment of heart failure.

Cyclic AMP is also involved in the control of renal functions. Although cyclic AMP is well known to mediate vasopressin-evoked antidiuresis at the collecting duct, studies in vivo have demonstrated that glucagon (Ueda et al.,

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1977) and isoproterenol (Morimoto et al., 1971), which bind with Gs protein-coupled receptors and thereby activate adenylate cyclase, increase urine flow rate in dogs. Forskolin causes renal vasodilation and natriuresis with increasing glomerular filtration rate (Tamaki et al., 1991). Therefore it would be expected that NKH477 also enhances urine formation by elevating cyclic AMP level in the kidney.

In this regard, the present study was undertaken to evaluate renal actions of NKH477 in relation to changes in renal cyclic AMP level in vivo. Changes in renal hemodynamics, urine formation and cyclic AMP release were evaluated in the dog kidney in the absence and presence of a cyclic AMP-specific phosphodiesterase inhibitor rolipram.

## 2. Materials and methods

### 2.1. Animal preparation

All animal protocols were reviewed and approved by the Animal Subjects Committee of Pharmaceutical Institute, Tohoku University. Mongrel dogs of either sex weighing 8 to 18 kg were anesthetized with sodium pentobarbital (30 mg $kg^{-1}$, i.v.) and then intubated and artificially ventilated with room air. The cephalic veins were cannulated for drug administration. Decamethonium bromide (0.25 mg $kg^{-1}$, i.v.) was given to prevent spontaneous active respiratory movement. Anesthesia was maintained by a continuous intravenous infusion of sodium pentobarbital at a rate of 6 mg $kg^{-1}$ $h^{-1}$ throughout the experiments. Inulin, dissolved in 0.45% NaCl and 2.5% dextrose, was given i.v. at a prime dose of 50 mg $kg^{-1}$ and at a maintenance dose of 1 mg $kg^{-1}$ $min^{-1}$ (0.1 ml $kg^{-1}$ $min^{-1}$). The right brachial artery was cannulated for collection of arterial blood samples and measurement of mean arterial pressure with a pressure transducer (MPU-0.5, Nihon Kohden, Tokyo, Japan) and heart rate with a cardiotachometer (RT-5, Nihon Kohden). The right and left kidneys were exposed by retroperitoneal flank incisions. Catheters for urine collection were inserted into both the right and left ureters. A curved 18-gauge needle connected to a silicone tube was inserted into the right or left renal vein to collect renal venous blood samples. All visible renal nerves were dissected away from the renal vessels and cut after ligation. Electromagnetic flow probes (2.5–3.5 mm in diameter, Nihon Kohden) were attached at the right and left renal arteries to measure renal blood flow with square-wave flowmeters (MF-27, Nihon Kohden). A curved 25-gauge needle connected to a polyethylene tube was inserted into the right or left renal artery for drug infusion. Mean arterial pressure, heart rate and renal blood flow were recorded with a polygraph system (RM-6000, Nihon Kohden). After completion of surgery, more than 90 min was allowed for stabilization.

### 2.2. Experimental protocol

When renal blood flow and urine flow rate reached constant levels for more than three consecutive monitoring periods (10 min each), urine and blood samples for basal values were obtained. Urine was collected over a 10-min period, and arterial and renal venous blood were withdrawn simultaneously at the midpoint of urine collection.

#### 2.2.1. Group 1 ($n = 6$)

NKH477 was infused into the renal artery at increasing rates of 30, 100 and 300 ng $kg^{-1}$ $min^{-1}$ for 20 min each. Beginning at 10 min after the start of infusion at each dose, the 10-min urine collection and blood sampling were performed.

#### 2.2.2. Group 2 ($n = 8$)

The NKH477 infusion and the urine and blood samplings were performed in a similar manner as in group 1 in the presence of rolipram. Rolipram (0.3 μg $kg^{-1}$ $min^{-1}$) was infused into the renal artery throughout the experiments. The infusion of NKH477 was started beginning 20 min after initiating the rolipram infusion.

### 2.3. Measurements

Blood samples were transferred into chilled tubes containing diammonium EDTA (5–10 mg $ml^{-1}$ blood) and then centrifuged to obtain plasma samples. Glomerular filtration rate was determined as inulin clearance. Inulin concentration in plasma and urine was measured by the anthrone method (Davidson and Sackner, 1963). $Na^+$ and $K^+$ were measured by flame photometry (775A, Hitachi). Plasma and urinary cyclic AMP concentration were measured by radioimmunoassay kit (Diagnostics Division, Yamasa, Tokyo, Japan).

### 2.4. Drugs

NKH477 (Nippon Kayaku, Tokyo, Japan) was dissolved in 0.9% saline. Rolipram (Meiji Seika Kaisha, Tokyo, Japan) was dissolved in a small amount of dimethyl sulfoxide and dilluted with 0.9% saline (the final concentration of dimethyl sulfoxide was less than 0.5%). We had confirmed that intrarenal arterial infusion of dimethyl sulfoxide (0.5%, 0.2 ml $min^{-1}$) did not affect renal function.

### 2.5. Data analysis

All values are expressed as means ± S.E. Renal blood flow and urine flow rate and parameters derived from them are expressed as per kidney weight (g). Overall statistical differences were evaluated by analysis of variance. Statistical differences of basal values vs. drug infusion were evaluated by student's paired *t*-test or Dunnett's test.

Differences at $p < 0.05$ were considered to be statistically significant.

## 3. Results

### 3.1. Group 1

In group 1, NKH477 was infused into the renal artery at increasing doses of 30, 100 and 300 ng kg$^{-1}$ min$^{-1}$. The infusion of NKH477 reduced mean arterial pressure and tended to increase heart rate (Table 1). The infusion of NKH477 increased renal blood flow, glomerular filtration rate, urine flow rate, urinary $Na^+$ excretion and fractional $Na^+$ excretion in a dose-dependent manner (Fig. 1). Filtration fraction remained unchanged (Fig. 1). Arterial and renal venous plasma cyclic AMP concentrations and urinary cyclic AMP excretion were increased by the infusion of NKH477 (Fig. 2). The changes in renal venous plasma cyclic AMP concentration ($108 \pm 4$, $126 \pm 7$ and $148 \pm 15$% of the basal values at 30, 100 and 300 ng kg$^{-1}$ min$^{-1}$ of NKH477 infusion, respectively) were significantly higher than the changes in arterial plasma cyclic AMP concentration ($105 \pm 3$, $114 \pm 4$ and $117 \pm 7$%, $P < 0.01$, vs. renal venous plasma cyclic AMP concentration). The values of renal parameters in the contralateral non-infused kidney did not change throughout the experiment except that a slight increase in renal blood flow was observed during the NKH477 infusion at 300 ng kg$^{-1}$ min$^{-1}$ (Table 1).

### 3.2. Group 2

In group 2, intrarenal arterial infusion of rolipram (0.3 μg kg$^{-1}$ min$^{-1}$) increased renal blood flow, glomerular

Table 1
Effects of NKH477 on systematic hemodynamics and renal hemodynamics and urine formation in the contralateral kidney (group 1)

| | Basal | NKH477 (ng kg$^{-1}$ min$^{-1}$) | | |
|---|---|---|---|---|
| | | 30 | 100 | 300 |
| MAP (mm Hg) | 112 ± 6 | 110 ± 7 | 105 ± 7 | 97 ± 8[b] |
| HR (beats min$^{-1}$) | 120 ± 13 | 119 ± 14 | 124 ± 15 | 130 ± 18 |
| *Contralateral kidney* | | | | |
| RBF (ml min$^{-1}$ g$^{-1}$) | 3.8 ± 0.3 | 3.9 ± 0.3 | 4.0 ± 0.2 | 4.1 ± 0.3[a] |
| GFR (ml min$^{-1}$ g$^{-1}$) | 0.6 ± 0.1 | 0.6 ± 0.1 | 0.7 ± 0.1 | 0.6 ± 0.1 |
| FF (%) | 32 ± 6 | 34 ± 6 | 38 ± 9 | 32 ± 6 |
| UV (μl min$^{-1}$ g$^{-1}$) | 3.8 ± 1.4 | 4.1 ± 1.4 | 4.2 ± 1.3 | 3.6 ± 1.1 |
| UNaV (μEq min$^{-1}$ g$^{-1}$) | 0.8 ± 0.3 | 0.9 ± 0.3 | 0.9 ± 0.3 | 1.0 ± 0.3 |
| FENa (%) | 0.9 ± 0.3 | 0.9 ± 0.2 | 0.8 ± 0.1 | 1.0 ± 0.2 |

Values (means ± S.E.) were obtained in the contralateral non-infused kidney before and during intrarenal arterial infusion of NKH477 at increasing doses (30, 100 and 300 ng kg$^{-1}$ min$^{-1}$). $n = 6$. MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow rate, urinary $Na^+$ excretion; FENa, fractional $Na^+$ excretion. [a]$P < 0.05$, [b]$P < 0.01$ compared with the corresponding basal values.

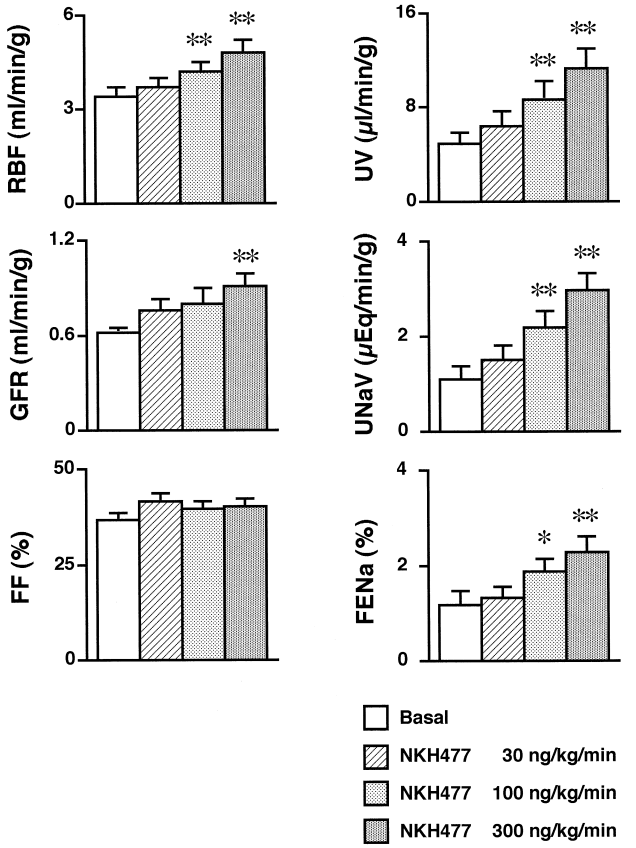


Fig. 1. Effects of NKH477 on renal hemodynamics and urine formation (group 1). RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow rate; UNaV, urinary $Na^+$ excretion; FENa, fractional $Na^+$ excretion. Values are means ± S.E. $n = 6$. NKH477 was infused into the renal artery at increasing doses of 30, 100 and 300 ng kg$^{-1}$ min$^{-1}$. *$P < 0.05$, **$P < 0.01$ compared with the corresponding basal value.

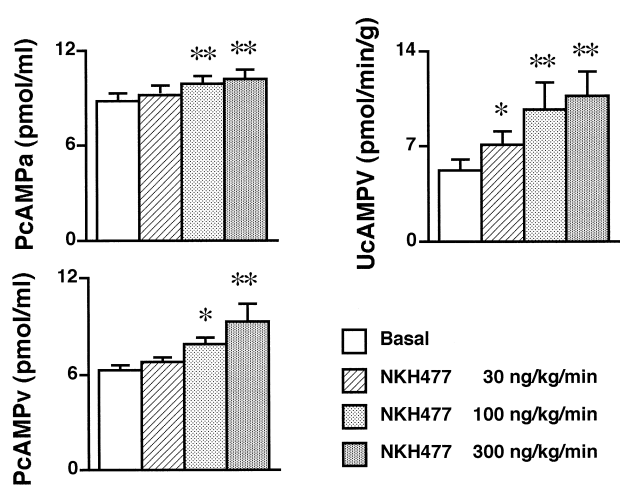


Fig. 2. Effects of NKH477 on cyclic AMP production (group 1). PcAMPa and pcAMPv, arterial and renal venous plasma cyclic AMP concentration; UcAMPV, urinary cyclic AMP excretion. Values are means ± S.E. $n = 6$. NKH477 was infused into the renal artery at increasing doses of 30, 100 and 300 ng kg$^{-1}$ min$^{-1}$. *$P < 0.05$, **$P < 0.01$ compared with the corresponding basal value.

Table 2
Effects of rolipram on renal hemodynamics, urine formation and cyclic AMP production (group 2)

| | RBF ($ml\,min^{-1}\,g^{-1}$) | GFR ($ml\,min^{-1}\,g^{-1}$) | FF (%) | UV ($\mu l\,min^{-1}\,g^{-1}$) | UNaV ($\mu Eq\,min^{-1}\,g^{-1}$) | FENa (%) | PcAMPa ($pmol\,ml^{-1}$) | PcAMPv ($pmol\,ml^{-1}$) | UcAMPV ($pmol\,min^{-1}\,g^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Basal | $3.0 \pm 0.4$ | $0.6 \pm 0.1$ | $41 \pm 10$ | $4.2 \pm 0.9$ | $0.6 \pm 0.2$ | $1.0 \pm 0.4$ | $11 \pm 3$ | $7 \pm 1$ | $5 \pm 1$ |
| Rolipram | $3.6 \pm 0.4^{b}$ | $0.8 \pm 0.1^{a}$ | $47 \pm 11$ | $6.0 \pm 0.7^{b}$ | $1.1 \pm 0.2^{b}$ | $1.2 \pm 0.4^{b}$ | $14 \pm 2$ | $14 \pm 2^{b}$ | $17 \pm 4^{a}$ |

Values means ± S.E. $n = 8$. RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow rate; UNaV, urinary $Na^+$ excretion; FENa, fractional $Na^+$ excretion; PcAMPa, arterial plasma cyclic AMP concentration; PcAMPv, renal venous plasma cyclic AMP concentration; UcAMPV, urinary cyclic AMP excretion. Rolipram was infused into the renal artery at 0.3 $\mu g\,kg^{-1}\,min^{-1}$. $^{a}P < 0.05$, $^{b}P < 0.01$ compared with the corresponding basal value.

filtration rate, urine flow rate, urinary $Na^+$ excretion, fractional $Na^+$ excretion, renal venous plasma cyclic AMP concentration and urinary cyclic AMP excretion (Table 2). The infusion of rolipram also reduced mean arterial pressure and increased heart rate (Table 3). Filtration fraction and arterial plasma cyclic AMP concentration remained unchanged (Table 2). We had previously confirmed that this dose of rolipram caused maximal natriuresis which was stable over 80 min. Figs. 3 and 4 compare the NKH477-induced renal responses (the difference between the values before and during the infusion of NKH477 at each dose) between non-treated dogs (group 1) and rolipram-treated dogs (group 2). Rolipram (0.3 $\mu g\,kg^{-1}\,min^{-1}$) significantly facilitated the NKH477-induced increases in renal blood flow and renal venous plasma cyclic AMP concentration and tended to facilitate the increase in fractional $Na^+$ excretion. However, NKH477 failed to increase glomerular filtration rate, and it reduced filtration fraction in the presence of rolipram (Figs. 3 and 4). Rolipram facilitated the NKH477-induced increase in heart rate and did not affect the hypotensive effect of NKH477 (Table 3). Effects of NKH477 on the other parameters were not affected by the infusion of rolipram (Figs. 3 and 4). The values of renal parameters in the contralateral non-infused kidney did not change throughout the experiment except that a slight increase in renal blood flow was observed during the rolipram and NKH477 infusion (Table 3).

Table 3
Effects of NKH477 in the presence of rolipram on systematic hemodynamics and renal hemodynamics and urine formation in the contralateral kidney (group 2)

| | Basal | Rolipram (0.3 $\mu g\,kg^{-1}\,min^{-1}$) | | | |
|---|---|---|---|---|---|
| | | NKH477 ($ng\,kg^{-1}\,min^{-1}$) | | | |
| | | 30 | 100 | 300 | |
| MAP (mm Hg) | $103 \pm 8$ | $102 \pm 8^{a}$ | $100 \pm 9$ | $97 \pm 9$ | $92 \pm 9$ |
| HR ($beats\,min^{-1}$) | $109 \pm 6$ | $122 \pm 6^{a}$ | $128 \pm 8$ | $134 \pm 8$ | $144 \pm 9$ |
| *Contralateral kidney* | | | | | |
| RBF ($ml\,min^{-1}\,g^{-1}$) | $3.6 \pm 0.7$ | $4.0 \pm 0.7^{a}$ | $4.3 \pm 0.7$ | $4.3 \pm 0.7$ | $4.3 \pm 0.7$ |
| GFR ($ml\,min^{-1}\,g^{-1}$) | $0.6 \pm 0.1$ | $0.7 \pm 0.1$ | $0.8 \pm 0.1$ | $0.7 \pm 0.1$ | $0.7 \pm 0.1$ |
| FF (%) | $34 \pm 7$ | $41 \pm 10$ | $41 \pm 9$ | $33 \pm 7$ | $32 \pm 9$ |
| UV ($\mu l\,min^{-1}\,g^{-1}$) | $1.9 \pm 0.3$ | $2.4 \pm 0.5$ | $3.4 \pm 0.9$ | $2.5 \pm 0.6$ | $2.1 \pm 0.7$ |
| UNaV ($\mu Eq\,min^{-1}\,g^{-1}$) | $0.3 \pm 0.1$ | $0.5 \pm 0.2$ | $0.9 \pm 0.3$ | $0.6 \pm 0.2$ | $0.6 \pm 0.2$ |
| FENa (%) | $0.3 \pm 0.1$ | $0.4 \pm 0.1$ | $0.7 \pm 0.2$ | $0.6 \pm 0.1$ | $0.6 \pm 0.2$ |

Values (means ± S.E.) were obtained in the contralateral non-infused kidney before and during intrarenal arterial infusion of NKH477 at increasing doses (30, 100 and 300 $ng\,kg^{-1}\,min^{-1}$) in the presence of rolipram (0.3 $\mu g\,kg^{-1}\,min^{-1}$). $n = 8$. MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine flow rate, urinary $Na^+$ excretion; FENa, fractional $Na^+$ excretion. $^{a}P < 0.05$, $^{b}P < 0.01$ compared with the corresponding basal values.

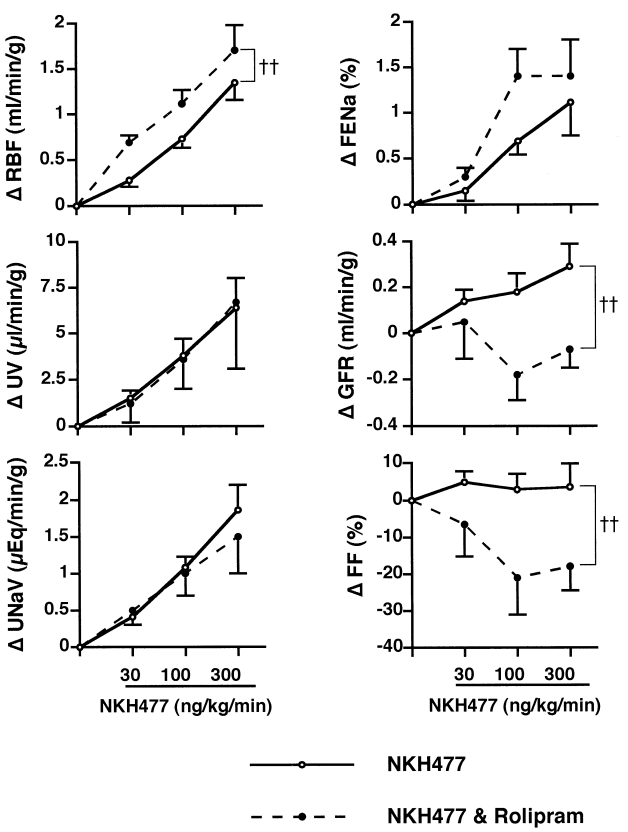


Fig. 3. Effects of NKH477 on renal hemodynamics and urine formation in the absence (group 1, $n = 6$) or in the presence (group 2, $n = 8$) of rolipram. NKH477 (30, 100 and 300 $ng\,kg^{-1}\,min^{-1}$) and rolipram (0.3 $\mu g\,kg^{-1}\,min^{-1}$) were infused into the renal artery. Δ, change from the level before NKH477 infusion. Other abbreviations are as in Fig. 1. Values are means ± S.E. ††$P < 0.01$ compared with NKH477 alone.

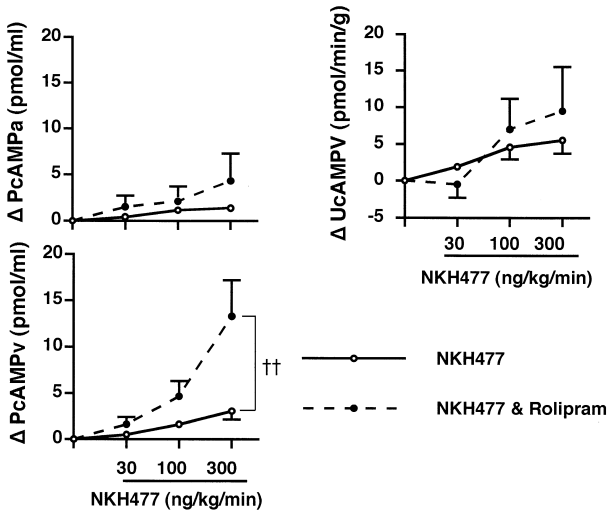


Fig. 4. Effects of NKH477 on cyclic AMP production in the absence (group 1, $n = 6$) or in the presence (group 2, $n = 8$) of rolipram. NKH477 (30, 100 and 300 ng kg$^{-1}$ min$^{-1}$) and rolipram (0.3 μg kg$^{-1}$ min$^{-1}$) were infused into the renal artery. Δ, change from the level before NKH477 infusion. Other abbreviations are as in Fig. 2. Values are means ± S.E. ††$P < 0.01$ compared with the NKH477 alone.

In both groups 1 and 2, Renal blood flow gradually increased and reached stable within 10 min during NKH477 infusion at each dose in most of all animals.

## 4. Discussion

The aim of the present study was to clarify the effects of NKH477, a direct adenylate cyclase activator, on renal cyclic AMP level, renal hemodynamics and urine formation in vivo. NKH477 was infused into the renal artery of anesthetized dogs.

It is well known that stimulation of hormone receptors coupling with adenylate cyclase causes extrusion of intracellular cyclic AMP into the extracellular space (Barber and Butcher, 1983). NKH477 is reported to facilitate acetylcholine-induced adrenal catechoalamine release with increasing cyclic AMP overflow from the adrenal gland in anesthetized dogs (Koshika et al., 1997). An authentic direct adenylate cyclase activator forskolin causes potent release of cyclic AMP from the isolated perfused rat kidney (Heuzé-Joubert et al., 1992). To estimate whether NKH477 produces cyclic AMP in the kidney in vivo, we measured arterial and renal venous plasma and urinary cyclic AMP concentrations. NKH477 at increasing doses of 30, 100 and 300 ng kg$^{-1}$ min$^{-1}$ (group 1) elevated arterial and renal venous plasma cyclic AMP concentrations in a dose-dependent manner. The elevation of cyclic AMP concentration in renal venous plasma was significantly higher than that in arterial plasma, and NKH477 also increased urinary cyclic AMP excretion. Although we have no direct evidence that these parameters correctly reflect the change in cellular cyclic AMP content, our present study demonstrates that NKH477 can produce cyclic AMP, probably through activation of adenylate cyclase, in the dog kidney in vivo.

The NKH477 infusion increased renal blood flow and glomerular filtration rate. It was reported that other stimulators of adenylate cyclase such as isoproterenol and glucagon also increased renal blood flow and glomerular filtration rate in dogs (Morimoto et al., 1971; Ueda et al., 1977). Our results are in agreement with these reports. Afferent arterioles and efferent arterioles are crucial vascular segments for the control of glomerular hemodynamics (Ichikawa and Harris, 1991). The balance of vascular tone between them critically affects glomerular filtration rate. It has been reported that forskolin stimulates the accumulation of cyclic AMP in the isolated afferent arterioles of dogs (Tamaki et al., 1989) and preferentially dilates the afferent arterioles in dogs (Tamaki et al., 1991). NKH477 may also cause the preferential dilation of afferent arterioles to elevate glomerular filtration pressure, and thereby increase glomerular filtration rate.

NKH477 increased urine flow rate. Whereas cyclic AMP acts as the second messenger of the antidiuretic hormone (Hays and Levine, 1974), the stimulators of adenylate cyclase are known to increase urine flow rate (Morimoto et al., 1971; Ueda et al., 1977). NKH477 also increased urinary $Na^+$ excretion and fractional $Na^+$ excretion. These data suggest that NKH477 directly or indirectly inhibits the tubular reabsorption of sodium. It has been reported that the stimulators of adenylate cyclase or cyclic AMP analogs inhibit $Na^+$, $K^+$-ATPase in the rat ascending limb of Henle's loop (Nishi et al., 1993), $Na^+$–$H^+$ antiporter in the rabbit tubular brush-border membrane (Weinman et al., 1987) and sodium reabsorption systems in the dog kidney (Robinson and Mirkovitch, 1980). Our present study demonstrates that NKH477 induces natriuresis, which may result from the increase in glomerular filtration rate and inhibition of sodium reabsorption.

Intracellular cyclic AMP level is suggested to be regulated by phosphodiesterase IV that specifically hydrolyzes cyclic AMP (Beavo and Reifsnyder, 1990; Nicholson et al., 1991; Beavo, 1995). The kidney is known to have high phosphodiesterase IV activity (Kariya and Dage, 1988; Masuoka et al., 1990). We have recently found that rolipram, a selective phosphodiesterase IV inhibitor (Disanto and Heaslip, 1995), caused renal vasodilation and natriuresis with increasing renal cyclic AMP release in anesthetized dogs (Tanahashi et al., 1999), suggesting that phosphodiesterase IV regulates renal cyclic AMP level to influence renal hemodynamics and urine formation. It is therefore possible that the NKH477-induced enhancement of cyclic AMP production and changes in renal functions are also regulated by phosphodiesterase IV. To elucidate whether the inhibition of renal cyclic AMP degradation affects the renal responses induced by activation of adenylate cyclase, we also examined the effects of NKH477 on renal functions in the presence of rolipram.

The intrarenal arterial infusion of rolipram at 0.3 $\mu g\,kg^{-1}\,min^{-1}$ increased renal venous plasma cyclic AMP concentration and urinary cyclic AMP excretion, and caused the same renal responses as NKH477. Rolipram enhanced the NKH477-induced increase in renal venous plasma cyclic AMP concentration. The facilitation may result from suppression of cyclic AMP degradation by the inhibitory action of rolipram on phosphodiesterase IV. It is reported that rolipram enhances the adenylate cyclase stimulator-induced cyclic AMP production in the rat aortic smooth muscle cells (Delpy et al., 1996) and the LLC-$PK_1$ cells that are derived from renal epithelial cells (Rassier et al., 1992). Expectedly, rolipram facilitated the increasing effects of NKH477 on renal blood flow and fractional $Na^+$ excretion, implying that the interaction of these drugs occurs in both the vascular and tubular sites. These results suggest that phosphodiesterase IV regulates cyclic AMP level during activation of adenylate cyclase and thereby affecting renal function.

However, glomerular filtration rate and filtration fraction were reduced by NKH477 in the presence of rolipram. Since rolipram did not affect the hypotensive effects of NKH477, the decreases in glomerular filtration rate and filtration fraction may not result from the hypotension-induced decrease in filtration pressure. It seems likely that NKH477 in the presence of rolipram increased cyclic AMP to the level which was enough to dilate both the afferent and efferent arterioles to reduce glomerular filtration pressure. Alternatively, NKH477 may reduce glomerular filtration coefficient in the presence of rolipram. It was reported that cyclic AMP reduced glomerular filtration rate with decreasing the glomerular filtration coefficient in a rat micropuncture study (Ichikawa and Brenner, 1977). These mechanisms may counteract the increasing effects of NKH477 on glomerular filtration. The increasing effects of NKH477 on urine flow rate and urinary $Na^+$ excretion were not affected by rolipram despite the facilitated increase in fractional $Na^+$ excretion, which may be due to the reduction in glomerular filtration rate.

It is also possible that humoral substances such as angiotensin II, which levels are closely related to cyclic AMP, participate in the reduction of glomerular filtration and the failure of further natriuresis during NKH477 infusion in the presence of rolipram. Angiotensin II, a vasoconstrictor peptide generated by renin and angiotensin converting enzyme, is reported to maintain the glomerular function by preferentially constricting postglomerular vessels (Schnackenberg et al., 1995), but it can also reduce glomerular filtration coefficient (Baylis et al., 1990) and evoke tubular sodium reabsorption (Alberola et al., 1994). Cyclic AMP is well known to act as a second messenger for renin release mechanisms in juxtaglomerular cells of the kidney (Hackenthal et al., 1990; Della et al., 1996). The marked increase in renal cyclic AMP level during the combined administration of NKH477 and rolipram might be able to stimulate renin release and thereby elevate circulating angiotensin II to the level that is sufficient to counteract the increasing effects of NKH477 on glomerular filtration rate and urinary $Na^+$ excretion. The present study cannot rule out this possibility.

In summary, the present study demonstrates in the dog kidney in vivo that 1) an adenylate cyclase activator NKH477 can enhance renal circulation, glomerular filtration and urinary $Na^+$ excretion with increasing renal cyclic AMP release, and 2) the inhibition of cyclic AMP degradation by a cyclic AMP-specific phosphodiesterase inhibitor rolipram enhances the NKH477-induced renal vasodilation and cAMP production, but cancels the NKH477-induced facilitation of glomerular filtration.

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