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Attenuation of adrenomedullin-induced renal vasodilatation by N^G-nitro L-arginine but not glibenclamide

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1 The present study was conducted in order to elucidate the *in vivo* contribution of nitric oxide (NO) and the glibenclamide-sensitive potassium channel in the renal action of adrenomedullin in anaesthetized dogs.

2 Intrarenal arterial infusion of adrenomedullin (20 ng kg^{-1} min^{-1}) elicited a pronounced increase in renal blood flow with no changes in systemic blood pressure. The renal vasodilator action of adrenomedullin was markedly attenuated by pretreatment with N^G-nitro L-arginine (L-NOARG), but this was reversed by continuous infusion of L-arginine.

3 Pretreatment with glibenclamide almost completely blocked the renal vasodilatation induced by lemakalim, but had no effect on the renal vasodilator and diuretic action of adrenomedullin.

4 Intrarenal arterial infusion of adrenomedullin induced diuresis and natriuresis. Diuretic and natriuretic action of adrenomedullin was also attenuated by L-NOARG. L-Arginine partly reversed the effect of L-NOARG and adrenomedullin-induced diuresis and natriuresis.

5 These data indicate that the *in vivo* renal vasodilator action of adrenomedullin is mediated by the release of NO. The glibenclamide-sensitive potassium channel is not involved in the renal action of adrenomedullin, at least, not in anaesthetized dogs. Since the inhibition of L-NOARG of adrenomedullin-induced diuresis occurred concomitantly with the attenuation of the renal vasodilator action of adrenomedullin, direct involvement of NO in adrenomedullin-induced diuresis remains to be established.

Keywords: Adrenomedullin; nitric oxide; glibenclamide; N^G-nitro L-arginine; EDRF

Introduction

Adrenomedullin is a potent vasodilator peptide first found in human phaeochromocytoma (Kitamura *et al.*, 1993a). Adrenomedullin exists not only in naive adrenal medulla, but also in circulating blood and in the kidney (Kitamura *et al.*, 1993a). Messenger RNA of adrenomedullin is expressed in the kidney (Kitamura *et al.*, 1993b; Sakata *et al.*, 1993). We have shown that adrenomedullin is a potent renal vasodilator with a diuretic action (Ebara *et al.*, 1994). These data imply a possible role of adrenomedullin in the regulation of renal function. However, the mechanism(s) of action of adrenomedullin has not been elucidated. The structure of adrenomedullin has homology with those of calcitonin gene-related peptide (CGRP) and amylin, sharing a six-residue ring structure formed by a disulphide bond and the C-terminal amide structure (Kitamura *et al.*, 1993a). In addition, adrenomedullin and CGRP may share, at least in part, a common receptor, since the vasodilator action of adrenomedullin was attenuated in the presence of CGRP[8-37], an antagonist of the CGRP receptor (Nuki *et al.*, 1993). Since both adrenomedullin and CGRP elevate adenosine 3':5'-cyclic monophosphate (cyclic AMP) in cultured vascular smooth muscle cells (Kubota *et al.*, 1985; Eguchi *et al.*, 1994; Ishizaka *et al.*, 1994), it was speculated that adrenomedullin and CGRP act directly on vascular smooth muscle and elicit vasorelaxation via the accumulation of cyclic AMP. In contrast, endothelium-dependence (Brain *et al.*, 1985; Prieto *et al.*, 1991; Gray & Marshall, 1992a,b) and the possible implication of the glibenclamide-sensitive potassium channel (Nelson *et al.*, 1990) in the vasodepressor action of CGRP have been reported. Therefore, the present study was performed to elucidate the *in vivo* role of NO and the glibenclamide-sensitive potassium channel in the renal action of adrenomedullin.

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Methods

Animal preparation

Mongrel dogs of either sex weighing 8 to 19 kg were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). The anaesthetic was supplemented as required to maintain surgical anaesthesia. The dogs were ventilated artificially by use of a constant volume respirator (model 607, Harvard). The right brachial artery and vein were cannulated for blood sampling and an infusion of inulin solution (100 mg kg^{-1} bolus followed by 100 mg kg^{-1} h^{-1} in saline, 0.2 ml kg^{-1} min^{-1}), respectively. Another catheter was placed in the abdominal aorta via the right femoral artery and systemic blood pressure was monitored continuously with a pressure transducer (400T, Nihon Kohden). The left kidney was exposed through a retroperitoneal flank incision as described previously (Okumura *et al.*, 1992). All visible renal nerves were cut and 10% phenol in 70% ethanol was applied around the renal artery to facilitate observation of the direct effects of the peptide on the renal vasculature and tubular function, without the influence of changes in activity of the renal nerves. Renal blood flow was measured by an electromagnetic flow meter (MFV-1200, Nihon Kohden). A 23-gauge needle was inserted into the left renal artery proximal to the flow probe for infusion of saline or adrenomedullin solution, at the rate of 0.3 ml min^{-1}. A polyethylene catheter was inserted into the left ureter for urine collection. After the completion of surgery, the dogs were left for 60 to 90 min to allow stabilization of systemic blood pressure, renal blood flow and urine flow.

Protocols

Effects of L-NOARG on renal responses to adrenomedullin The dogs were divided into three groups.

Group I (adrenomedullin alone) After two consecutive timed urine collections with midpoint arterial blood collections (3 ml each), adrenomedullin was infused for 25 min into the renal artery, at the rate of 20 ng kg^{-1} min^{-1} in 6 dogs; 5, 10 and 20 min after the start of the peptide infusion, blood samples were obtained with timed urine collections. Additional blood samples with timed urine collections were taken at 10, 30 and 60 min after the cessation of peptide infusion.

Group II (adrenomedullin with L-NOARG) Five dogs were given L-NOARG into the renal artery at the rate of 80 μg kg^{-1} min^{-1} for 25 min. Ten min later, control blood samplings with timed urine collection were started. The remainder of the experiment was essentially the same as for group I. Our previous study has shown that renal blood flow decreased following intrarenal arterial infusion of L-NOARG at the rate of 80 μg kg^{-1} min^{-1} for 25 min and remained depressed for more than 1 h after the cessation of infusion (Yukimura *et al.*, 1992).

Group III (adrenomedullin with L-NOARG and L-arginine) Four dogs received intrarenal arterial infusion of L-arginine hydrochloride at the rate of 2 mg kg^{-1} min^{-1} during the entire period of the experiments. Thirty min after the start of L-arginine infusion, the same protocol was adopted as that used for group II.

In an additional group of experiments, the effectiveness and specificity of L-NOARG were assessed in 5 dogs. For this purpose, bolus injections of acetylcholine (20 ng kg^{-1}) and nitroglycerin (1 μg kg^{-1}) into the renal artery was made before and 30–60 min after the cessation of intrarenal arterial infusion of L-NOARG (80 μg kg^{-1} min^{-1} for 25 min). The increase in renal blood flow was assessed by comparison of the maximal increase and the area under the response curve for the renal blood flow. The latter was performed on a Macintosh IIsi computer using public domain NIH Image programme.

Effects of glibenclamide on renal vascular responses to adrenomedullin To elucidate the role of the glibenclamide-sensitive potassium channel in the renal vascular action of adrenomedullin, glibenclamide (3 mg kg^{-1}) was administered intravenously to 4 dogs. After 30 min, adrenomedullin was infused into the renal artery at the rate of 20 ng kg^{-1} min^{-1} in 4 dogs. As a corresponding control group, 4 additional dogs were given the vehicle, dimethylformamide (40 μl kg^{-1}) instead of glibenclamide, after which adrenomedullin was administered.

In a separate group of experiments ($n=3$), effective blockade of the renal vasodilator action of lemakalim by glibenclamide was assessed. Intrarenal arterial injection of lemakalim (1 μg kg^{-1}) was conducted after administration of vehicle (40 μl kg^{-1}, i.v.) and glibenclamide (3 mg kg^{-1}, i.v.), respectively.

Analytical procedures

Inulin was determined by colorimetry, as described by Walser *et al.* (1955) and glomerular filtration rate (GFR) was estimated by the inulin clearance. Urinary and plasma concentrations of sodium and potassium were measured by flame photometry (205D, Hitachi, Tokyo, Japan).

Statistical analysis

Results presented in the paper are shown as means ± s.e.mean. Intergroup differences in the data were analysed either by Student's t test or one-way analysis of variance (ANOVA). Data within the groups were analysed by a complete randomized block ANOVA. Following ANOVA, individual statistical differences were determined by Duncan's multiple range comparison test (SuperANOVA, Abacus Concepts., Berkeley, U.S.A.). When data appeared heteroscedastic, logarithmic transformation was performed before data analysis (Zar, 1984).

Drugs

The drugs used were: acetylcholine chloride, L-arginine hydrochloride (Ishizu Pharmaceutical, Osaka), nitroglycerin (Millisrol inj., Nippon Kayaku, Tokyo), sodium pentobarbitone (Tokyo Chemical Industry, Tokyo), glibenclamide, inulin (Sigma, St. Louis, U.S.A.). Human adrenomedullin and N^G-nitro L-arginine were purchased from Peptide Institute (Osaka). Lemakalim was a kind gift from SmithKline Beecham Seiyaku (Tokyo).

Results

Effects of L-NOARG on renal responses to adrenomedullin

Figure 1 shows the effects of L-NOARG and L-arginine on the renal action of adrenomedullin. As previously reported (Ebara *et al.*, 1994), intrarenal arterial infusion of adrenomedullin at the rate of 20 ng kg^{-1} min^{-1} elicited a marked increase in renal blood flow from 4.0 ± 0.3 to 5.9 ± 0.4 ml g^{-1} min^{-1} at 20 min without any changes in systemic blood pressure. GFR tended to increase from 0.97 ± 0.06 to 1.05 ± 0.05 ml g^{-1} min^{-1} at 10 min, but this change was not statistically significant. As a result, filtration fractions were decreased from 38.3 ± 3.1% to 26.7 ± 1.2% with adrenomedullin alone ($P<0.01$). Adrenomedullin increased urine flow and urinary excretion of sodium and potassium approximately two-fold.

The pre-infusion value of renal blood flow was significantly lower in group II (2.5 ± 0.2 ml g^{-1} min^{-1}) than in group I (4.0 ± 0.3 ml g^{-1} min^{-1}). This value in group III was 3.6 ± 0.6 ml g^{-1} min^{-1}, which was not statistically different either from that in group I or in group II. Following L-NOARG treatment, adrenomedullin increased renal blood flow significantly from 2.5 ± 0.2 to 3.0 ± 0.2 ml g^{-1} min^{-1} at 20 min, but the magnitude of the increase was apparently less than that observed in the absence of L-NOARG. Figure 2 summarizes the magnitude of the increases in renal blood flow elicited by adrenomedullin. Adrenomedullin alone increased renal blood flow by 1.87 ± 0.14 ml g^{-1} min^{-1} (by 48.2 ± 5.3%) at 20 min. In dogs pretreated with L-NOARG (group II), this increase in renal blood flow at 20 min after the start of adrenomedullin infusion was 0.46 ± 0.10 ml g^{-1} min^{-1} (by 18.5 ± 3.8%) which was significantly smaller than that seen with adrenomedullin alone. When dogs were given L-arginine during the entire period of the experiment, L-NOARG failed to attenuate the elevation of renal blood flow elicited by adrenomedullin (Figures 1, 2). Under this condition, adrenomedullin increased renal blood flow by 1.73 ± 0.40 ml g^{-1} min^{-1} (by 47.2 ± 7.9%) which was not statistically different from that with adrenomedullin alone.

Pre-infusion values of GFR were 0.97 ± 0.06 ml g^{-1} min^{-1} in group I 0.65 ± 0.06 ml g^{-1} min^{-1} in group II and 0.83 ± 0.11 ml g^{-1} min^{-1} in group III, respectively. There was a statistically significant difference only between those of group I and group II ($P<0.05$). Adrenomedullin did not affect the course of GFR following either L-NOARG or L-NOARG in combination with L-arginine (Figure 1). Pretreatment with L-NOARG attenuated adrenomedullin-induced reduction in filtration fraction (37.6 ± 4.8% during control and 33.6 ± 4.5% at 20 min after the start of adrenomedullin infusion, $P>0.05$). When L-arginine was given with L-NOARG, adrenomedullin elicited a reduction in filtration fraction from 38.0 ± 4.8% to 28.0 ± 3.7% ($P<0.05$).

Pre-infusion values of urine flow and urinary excretion of

sodium and potassium were significantly lower in group II (adrenomedullin with L-NOARG) and group III (adrenomedullin with L-NOARG and L-arginine) compared to group I (adrenomedullin alone) (Figure 1). Adrenomedullin alone significantly increased urine flow and urinary sodium excretion from 22.8 ± 4.3 to 52.0 ± 7.9 $\mu l\ g^{-1}\ min^{-1}$ and from 5.2 ± 1.0 to 9.6 ± 1.3 $\mu Eq\ g^{-1}\ min^{-1}$, respectively. Similarly, adrenomedullin significantly increased urine flow and urinary electrolytes excretion following treatment with L-NOARG irrespective of co-administration of L-arginine. The percentage increases in urine flow elicited by adrenomedullin were $166 \pm 63\%$ in group I (adrenomedullin alone) which was not statistically different from those in the L-NOARG group (group II, $140 \pm 56\%$) or those in the L-NOARG and L-arginine-treated group (group III, $147 \pm 52\%$). Urinary excretion of sodium increased by $111 \pm 23\%$ with adrenomedullin alone, which was statistically the same as group II ($200 \pm 106\%$) and group III ($185 \pm 30\%$). Similarly, there was no statistically significant difference in the percentage increases in urinary potassium excretion among these groups (group I, $77 \pm 23\%$, group II $85 \pm 39\%$, group III, $85 \pm 13\%$). Fractional excretion of sodium increased significantly ($P < 0.01$) in all groups from $3.4 \pm 0.6\%$ to $6.0 \pm 0.8\%$ (group I), from $1.3 \pm 0.5\%$ to $2.6 \pm 0.7\%$ (group II) and from $1.3 \pm 0.3\%$ to $3.3 \pm 0.9\%$

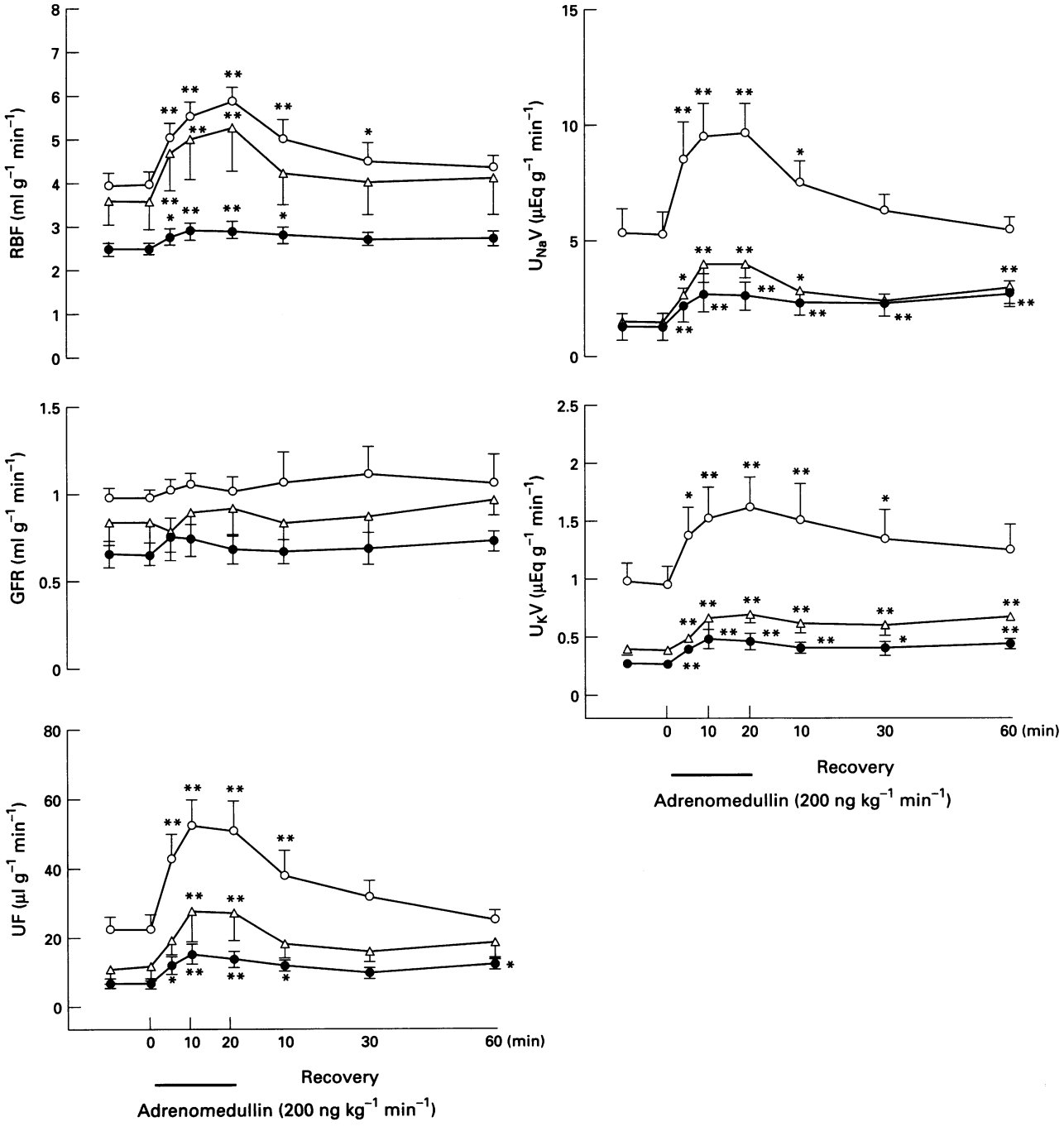

Figure 1 Effects of N^G-nitro L-arginine (L-NOARG) on the renal action of adrenomedullin. Adrenomedullin was infused into the renal artery for 25 min at the rate of $20\ ng\ kg^{-1}\ min^{-1}$. Symbols represent the following: (○), group I (adrenomedullin alone); (●) group II (adrenomedullin with L-NOARG); (△) group III (adrenomedullin with L-NOARG and L-arginine); RBF, renal blood flow; GFR, glomerular filtration rate; UF, urine flow; $U_{Na}V$, urinary excretion of sodium; U_KV, urinary excretion of potassium. Data were corrected for the kidney weight. $^*P < 0.05$ and $^{**}P < 0.01$ compared to the pre-infusion values.

(group III) after 20 min of adrenomedullin infusion, respectively. However, since basal output of fluid and electrolytes from the kidney was reduced by L-NOARG (Figure 1), pretreatment with L-NOARG significantly attenuated adrenomedullin-induced increase in urine flow and urinary excretion of sodium when increases were compared in terms of absolute value (Figure 2). The increases in urine flow and urinary sodium excretion in group II (adrenomedullin following L-NOARG treatment) were 6.9 ± 1.9 μl g^{-1} min^{-1} and 1.3 ± 0.4 μEq g^{-1} min^{-1} which were significantly lower than those with adrenomedullin alone (group I, 28.5 ± 6.4 μl g^{-1} min^{-1}, 4.5 ± 1.0 μEq g^{-1} min^{-1}). Even when L-arginine was given with L-NOARG treatment, basal output of fluid and electrolytes remained depressed (Figure 1). The increases in urine flow and urinary excretion of sodium during adrenomedullin administration in group III (adrenomedullin treatment together with L-arginine and L-NOARG) were 16.1 ± 6.3 μl g^{-1} min^{-1} and 2.6 ± 0.4 μEq g^{-1} min^{-1}, respectively. The magnitude of the adrenomedullin-induced increase in urine flow and urinary sodium excretion in group III ranged between those in group I and group II and these values were not statistically different from those in group I or group III.

As described above, basal urine flow and urinary excretion of sodium were lower in group II and group III compared to those in group I, which may suggest L-NOARG-decreased urine flow and urinary excretion of sodium was not reversed by administration of L-arginine. However, we have data showing that reduced output of fluid and sodium by L-NOARG at a

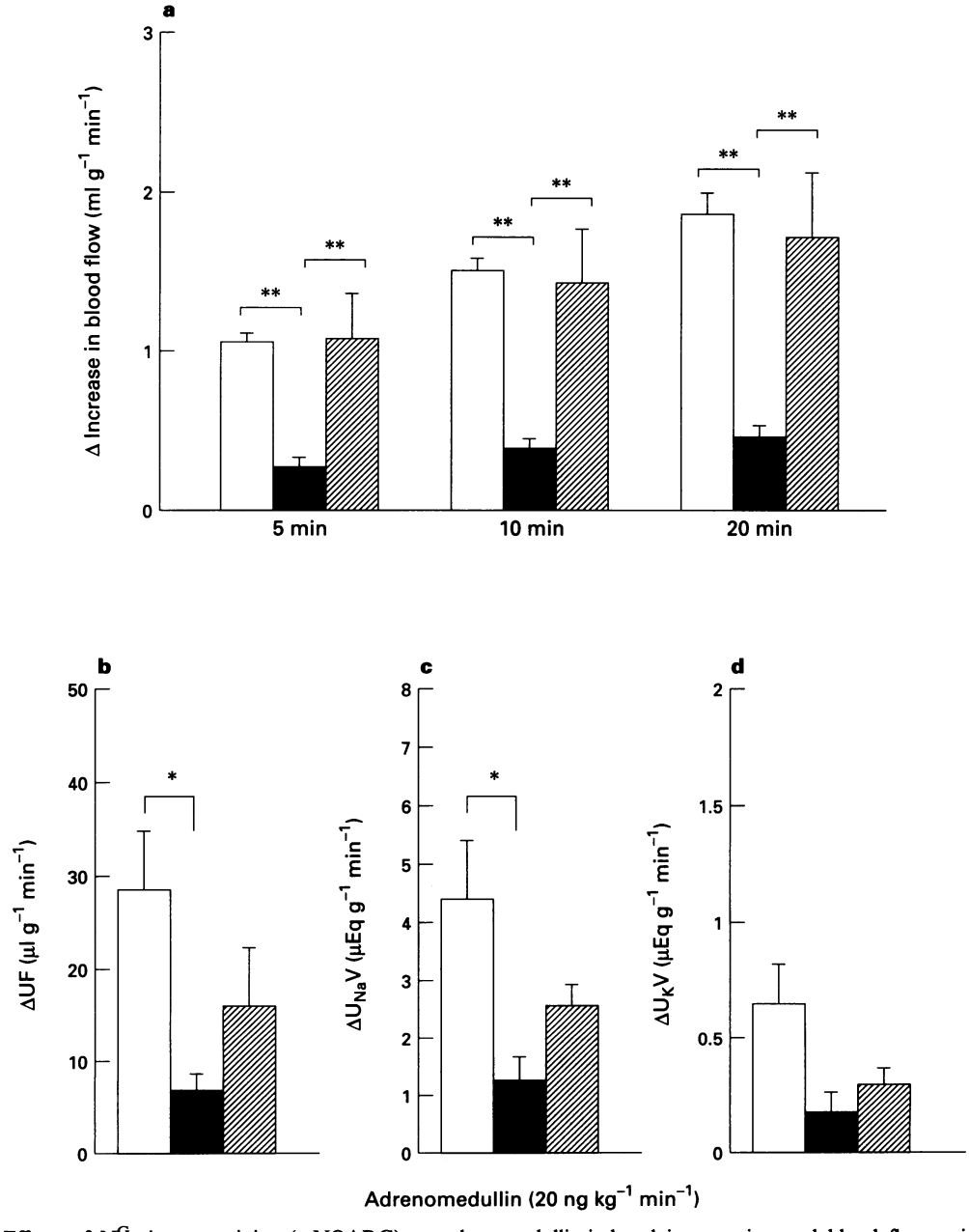

Figure 2 Effects of N^G-nitro L-arginine (L-NOARG) on adrenomedullin-induced increase in renal blood flow, urine flow and urinary electrolytes excretion. (a) Increase in renal blood flow from the level just before adrenomedullin infusion. Abscissa scale shows the time after the start of adrenomedullin infusion. Lower panels: increase from the level just before adrenomedullin infusion in urine flow (UF, b), urinary excretion of sodium ($U_{Na}V$, c) and urinary excretion of potassium (U_KV, d) at 20 min after the start of adrenomedullin infusion. Open column, group I (adrenomedullin alone); solid column, group II (adrenomedullin with L-NOARG); hatched column, group III (adrenomedullin with L-NOARG and L-arginine). $^*P < 0.05$ and $^{**}P < 0.01$ between the indicated groups.

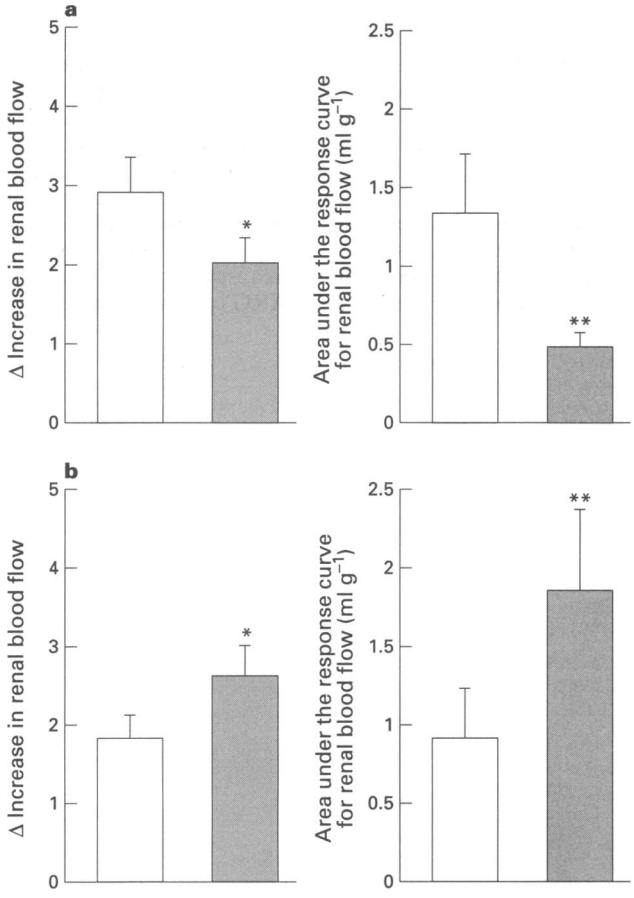

Figure 3 Effects of N^G-nitro L-arginine (L-NOARG) on the renal blood flow increase induced by intrarenal arterial injection of (a) acetylcholine and (b) nitroglycerin. Open column indicates the renal blood flow increase before L-NOARG; hatched column following L-NOARG administration. Left panel shows the increase in renal blood flow. Right panel indicates the area under the response curve for the renal blood flow. $^*P<0.05$ and $^{**}P<0.01$ compared to the values before L-NOARG administration.

dose used in the present experiments was reversed by subsequent infusion of L-arginine. Intrarenal arterial infusion of L-NOARG for 30 min at a rate of 80 μg kg^{-1} min^{-1} decreased urine flow and urinary excretion of sodium from 25.2 ± 10.0 to 8.0 ± 2.4 μl g^{-1} min^{-1} and from 3.1 ± 0.8 to 1.1 ± 0.3 μEq g^{-1} min^{-1}, respectively; these values reverted to 34.9 ± 10.2 μl g^{-1} min^{-1} and 4.7 ± 1.2 μEq g^{-1} min^{-1} as a result of subsequent infusion of L-arginine at a rate of 2 mg kg^{-1} min^{-1} ($n=5$, unpublished observation).

Figure 3 shows the effective blockade by L-NOARG of the vasodilator action of acetylcholine (ACh), a typical endothelium-dependent vasodilator. Bolus injection of ACh into the renal artery increased renal blood flow by 2.9 ± 0.5 ml g^{-1} min^{-1}. Following L-NOARG, ACh increased renal blood flow by 2.0 ± 0.3 ml g^{-1} min^{-1}, which was significantly lower than ACh alone. Increase by ACh of the area under the response curve for renal blood flow was 1.3 ± 0.4 ml g^{-1} before L-NOARG, and this was significantly attenuated following L-NOARG (0.5 ± 0.1 ml g^{-1}). In contrast, the renal vasodilator action of nitroglycerin was markedly potentiated by L-NOARG. Nitroglycerin increased renal blood flow by 1.8 ± 0.3 ml g^{-1} min^{-1} before L-NOARG, and this was significantly increased by L-NOARG treatment (2.6 ± 0.4 ml g^{-1} min^{-1}). The area under the response curve with nitroglycerin was also increased from 0.9 ± 0.3 to 1.9 ± 0.5 ml g^{-1} following L-NOARG treatment.

Effects of glibenclamide on renal vascular responses to adrenomedullin

Following vehicle (dimethylformamide) administration, intrarenal arterial injection of lemakalim increased renal blood flow by 0.77 ± 0.12 ml g^{-1} min^{-1}, which was almost completely blocked by glibenclamide administration (by 0.04 ± 0.02 ml g^{-1} min^{-1}) (Figure 4). In contrast, glibenclamide did not affect the adrenomedullin-induced increase in renal blood flow (Figure 4). In addition, adrenomedullin-induced diuresis and natriuresis were not affected by glibenclamide (Figure 4).

Discussion

The main finding of the present study was that L-NOARG markedly attenuated the renal vasodilator action of adrenomedullin. Intrarenal arterial infusion of adrenomedullin at a rate of 20 ng kg^{-1} min^{-1} induced a marked increase in renal blood flow without changes in blood pressure, indicating renal vasodilatation elicited by adrenomedullin. We previously reported that there was a dose-dependent increase in renal blood flow by adrenomedullin including the dose used in the present study (Ebara *et al.*, 1994). Following L-NOARG treatment, this renal vasodilatation was significantly attenuated. L-NOARG is a potent inhibitor of NO synthase (NOS) (Dubbin *et al.*, 1990) and endothelium-dependent vasodilatation (Moore *et al.*, 1990; Yamashita *et al.*, 1991; Okumura *et al.*, 1992). In fact, L-NOARG markedly attenuated the renal vasodilator action of ACh, a typical endothelium-dependent vasodilator, but not that of nitroglycerin (Figure 3). Furthermore, L-NOARG-induced attenuation of the renal vasodilator action of adrenomedullin was largely reversed by sustained administration of L-arginine. These data strongly suggest that arginine-derived NO is involved in the mechanism of adrenomedullin-induced renal vasodilatation. As the magnitude of the increase in renal blood flow induced by nitroglycerin was potentiated by L-NOARG, it is unlikely that L-NOARG suppressed the vasodilator action of vasoactive substances in a non-specific manner.

Eguchi *et al.* (1994) reported that specific adrenomedullin receptors exist and functionally couple to adenylate cyclase in rat cultured vascular smooth muscle cells. As cyclic AMP is an intracellular mediator of vasodilatation, they proposed that the vasorelaxant effect of adrenomedullin is mediated by the accumulation of cyclic AMP in vascular smooth muscle cells. This mechanism of vasodilator action may also function, at least in part, in the renal vasodilator action of adrenomedullin that remains following L-NOARG. Recently, it was shown that cyclic AMP induces NOS and enhances NO production in rat vascular smooth muscle cells (Imai *et al.*, 1994). However, it is unlikely that the cyclic AMP-inducible NOS is involved in the renal vasodilator action of adrenomedullin, for NOS induction requires at least 60 min (Szabo *et al.*, 1993) whereas the renal vasodilator action of adrenomedullin became manifest immediately after the start of administration.

It was shown that specific binding sites of adrenomedullin in cultured vascular smooth muscle cells also interact with CGRP (Eguchi *et al.*, 1994). CGRP[8-37], an antagonist for CGRP receptor, blocked adrenomedullin-induced cyclic AMP formation in cultured vascular smooth muscle cells (Eguchi *et al.*, 1994) and antagonized the vasodilator effect of adrenomedullin in the rat perfused mesenteric vascular bed (Nuki *et al.*, 1993). Therefore, it was speculated that adrenomedullin and CGRP may interact with the same receptor. Although CGRP also stimulates adenylate cyclase it was shown that endothelium-derived NO is involved in the vasorelaxation of isolated vascular strips (Gray & Marshall, 1992a,b). Our finding that L-NOARG attenuated the renal vasodilator action of adrenomedullin may be consistent with the hypothesis that adrenomedullin interacts with the CGRP receptor.The vasodilator action of adrenomedullin was accompanied by a sig-

nificant increase in urine flow and the urinary excretion of sodium and potassium in all groups. Since basal output of fluid and electrolytes with L-NOARG even in the presence of L-arginine (groups II and III) was lower than that in control group (group I), the interpretation of the effects of L-NOARG on adrenomedullin-induced diuresis is difficult. When adrenomedullin-induced increases in urine flow and urinary electrolytes excretion were expressed as percentage increases, there are no statistically significant effects of L-NOARG and/or L-arginine. However, significant attenuation of adrenomedullin-induced diuresis and natriuresis were found with L-NOARG treatment when increases were compared in terms of absolute change. When L-arginine was given with L-NOARG, adrenomedullin-induced increases in urine flow and urinary excretion of sodium were not statistically different from those observed with adrenomedullin alone. Therefore, it is suggested that L-arginine, partially reversed the attenuation by L-NOARG of adrenomedullin-induced diuresis and natriuresis. It is well known that NO stimulates cyclic GMP production. Atrial natriuretic peptide enhances cyclic GMP production and inhibits sodium and water transport in the tubular cells (Zeidel *et al.*, 1987). We previously mentioned that NO may be involved in the regulation of renal tubular reabsorption of sodium and water, since a small dose of L-NOARG causes antidiuresis and

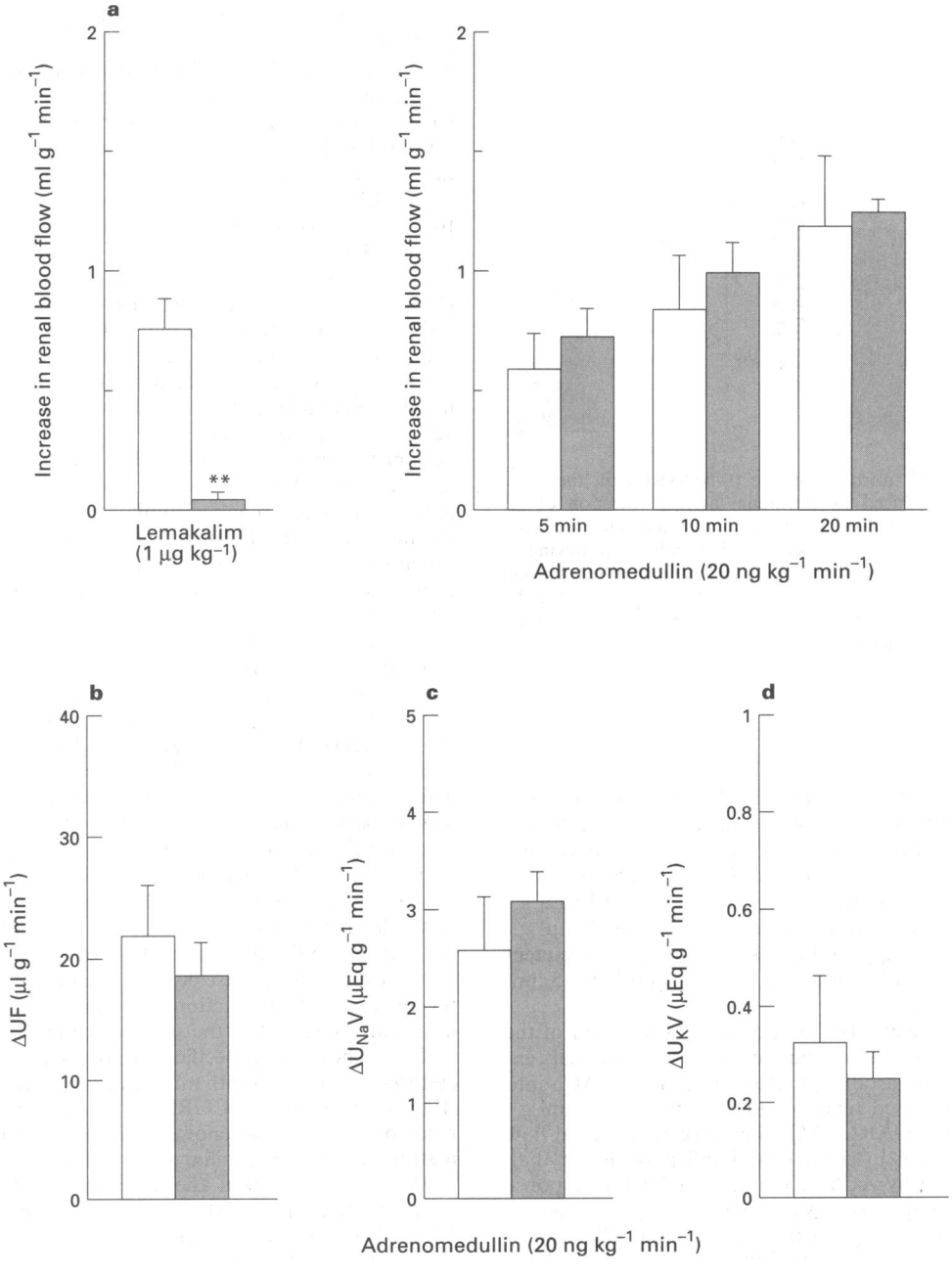

Figure 4 Effects of glibenclamide on increases in renal blood flow, urine flow and urinary excretion of electrolytes induced by intrarenal infusion of lemakalim and adrenomedullin. (a) Increase in renal blood flow by lemakalim (left panel) and by adrenomedullin. Abscissa scale shows the time after the start of adrenomedullin infusion. Lower panels: increase from the level just before adrenomedullin infusion in urine flow (UF, b), urinary excretion of sodium ($U_{Na}V$, c) and urinary excretion of potassium (U_KV, d) at 20 min after the start of adrenomedullin infusion. Open column indicates the renal blood flow increase after vehicle (dimethylformamide) administration; stippled column following glibenclamide administration. $^{**}P < 0.01$ compared to the values after vehicle administration.

antinatriuresis without affecting renal blood flow, glomerular filtration rate or systemic blood pressure (Yukimura *et al.*, 1992). Our present results together with these findings may suggest that adrenomedullin causes diuresis and natriuresis, at least in part, through enhanced production of NO.

However, there are limitations in elucidating the mechanism(s) of the diuretic action of adrenomedullin. First, the reduced basal output of fluid and electrolytes elicited by L-NOARG was not apparently reversed by L-arginine. Although we do not know the reason, we have data showing that antidiuretic action of L-NOARG could be reversed by L-arginine (for details, see results). Nevertheless, this may suggest that L-NOARG has a non-specific action in altering the intrarenal regulatory mechanisms, particularly the process of renal tubular reabsorption. Previous reports on the effects of NOS inhibition by arginine analogues gave controversial results. In dogs, NOS inhibitors caused antidiuresis (Yukimura *et al.*, 1992; Majid *et al.*, 1993). Radermacher *et al.* (1992), on the contrary, reported that inhibition of NOS resulted in attenuation of sodium reabsorption in rat isolated perfused kidney. Thus, the role of NO in the renal tubular function remains to be established. Second, whether the diuretic action of adrenomedullin is a result of direct action of adrenomedullin on the tubules is unknown at present. The diuretic action is always associated with renal haemodynamic changes (Ebara *et al.*, 1994). If the diuretic action of adrenomedullin is secondary to the haemodynamic change, attenuation of adrenomedullin-induced renal vasodilatation by L-NOARG would have resulted in blocking of the diuretic action of adrenomedullin. In this respect, it is of note that the filtration fraction was decreased with adrenomedullin alone and a reduced filtration fraction would decrease postglomerular oncotic pressure, which may lead to the inhibition of proximal tubular transport (Baylis *et al.*, 1976). Since L-NOARG blocked adrenomedullin-induced reduction in the filtration fraction, inhibition by L-NOARG of adrenomedullin-induced diuresis may be solely a result of blockade of the NO-mediated haemodynamic changes elicited by adrenomedullin. Therefore, following pretreatment with L-NOARG and L-arginine, adrenomedullin again elicited a reduction in filtration fraction and adrenomedullin-induced diuresis was recovered at least in part. Finally, in order to clarify the mechanism of action of adrenomedullin on urine formation, a more precise study, such as microperfusion of the tubular segment *in vitro* would be required.

Nelson demonstrated that activation of the potassium channel of vascular smooth muscle cells by CGRP leads to a relaxation of rabbit mesenteric artery which is blocked by glibenclamide. This fact, together with the notion of the similarity of receptors for adrenomedullin and CGRP, led us to test whether activation of the glibenclamide-sensitive potassium channel is involved in the renal action of adrenomedullin. Glibenclamide effectively blocked the renal vasodilator action of lemakalim, a typical ATP-sensitive potassium channel opener. However, glibenclamide had no effect on the adrenomedullin-induced increase in renal blood flow, urine flow and urinary excretion of electrolytes. These data indicate that the *in vivo* renal vasodilator action and diuretic action of adrenomedullin in dogs are not related to activation of the glibenclamide-sensitive K channel.

In summary, our observations suggest that adrenomedullin has an arginine-derived NO-mediated renal vasodilator action in anaesthetized dogs. The glibenclamide-sensitive potassium channel is not involved in the renal action of adrenomedullin.

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