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The role of nephrectomy and proadifen in blood pressure homeostasis following an acute kinin-induced hypotension in normotensive rats

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1 We have, in the present work, studied the importance of the kidneys and the renal hypotensive agent, medullipin, in modulating the blood pressure (BP) response to bradykinin, as well as their ability to influence the balance between the NO- and the adrenergic systems superimposed on a bradykinin-induced hypotension.

2 The rats were pretreated with the NO-synthase inhibitor, N^{ω}-nitro-L-arginine methyl esther (L-NAME) (0.3 g kg^{-1}), proadifen (50 mg kg^{-1}), an inhibitor of the medullipin-system, and nephrectomy (24 h) (Nx) alone, and L-NAME in combination with proadifen, Nx or phentolamine (2 mg kg^{-1}). Subsequent injections of bradykinin (3, 6, 15, 30 μg kg^{-1}) induced an acute hypotensive response. The fall in BP was dose-dependent in all groups ($P<0.01$), except in the Nx/L-NAME group. No differences in the fall in BP were observed between the groups.

3 The duration of the hypotensive response was abbreviated after L-NAME-treatment ($P<0.05$). Proadifen-treatment and Nx had no significant effect on the duration of the hypotension in control rats or in L-NAME-treated rats. Pretreatment with phentolamine prevented the L-NAME-induced rapid restoration of BP ($P<0.001$).

4 In L-NAME-treated rats a transient hypertension followed the bradykinin-induced hypotensive response. This hypertensive response was not observed after Nx or proadifen-treatment alone, and addition of Nx or proadifen to L-NAME treatment did not alter the hypertensive response as compared to L-NAME alone. Phentolamine, however, abolished the L-NAME-induced hypertension ($P<0.05$).

5 In conclusion, the present results do not support the involvement of the medullipin-system or other hypotensive systems localized in the kidneys, in modulating and counteracting the compensatory adrenergic response following an acute bradykinin-induced hypotension. A hampering modulating effect of the NO-system on this compensatory adrenergic response was confirmed, indicating a close relationship between these two systems in BP homeostasis.

Keywords: Bradykinin; cytochrome P-450; medullipin; nephrectomy; NO; NO-synthase antagonist; blood pressure; homeostasis; sympathetic nervous system

Introduction

The maintenance of a stable blood pressure (BP) is dependent on the interaction of hypertensive and hypotensive forces (Dazu, 1989; Burnstock, 1990). By manipulation of one or more of these forces, and measurement of the resulting changes in blood pressure, suggestions can be made regarding the importance of the various systems in BP homeostasis. After an acute hypotensive response induced by bradykinin, Bjørnstad-Østensen & Berg (1994) observed a decrease in the duration of the hypotension as well as a subsequent hypertensive response in rats where NO-synthesis had been blocked. Since both observations were prevented by α-adrenoceptor blockade, it was concluded that the NO-system played a role in modulating an adrenergic response, compensating for the acute fall in BP. This effect appeared to be more prominent in binephrectomized (Nx) rats than in non-Nx rats, suggesting the presence of another hypotensive modulating system localized in the kidneys, such as for instance the medullipin-system. Medullipin I is an arachidonic acid metabolite secreted from renomedullary interstitial cells (Muirhead *et al.*, 1977; Muirhead, 1988). Medullipin I is conveyed to the liver where it is converted to the active vasodilator compound, medullipin II, by the cytochrome P-450 enzyme system (Muirhead *et al.*, 1989b). Furthermore, renal medullipin I-secretion is increased when renal perfusion pressure is increased (Karlström & Göthberg, 1987; Karlström *et al.*, 1988). Thus, activation of medullipin-secretion may be expected when BP is elevated by NO-synthase inhibition. In N^{ω}-nitro-L-arginine methyl esther (L-NAME)-treated rats, it is therefore possible that removal of the kidneys and thus also the medullipin-system, will influence the counterbalancing of the adrenergic compensatory response following a bradykinin-induced hypotension.

In the present study the role of Nx on BP homeostasis during the hypotensive response to bradykinin was studied in normotensive Wistar rats. Moreover, the cytochrome P-450 blocker, proadifen, was used to evaluate the importance of the medullipin-system in normal non-Nx rats. The role of the NO-system was established by the use of the NO-synthase inhibitor, L-NAME. The present results demonstrated the close counterbalance between the NO- and the adrenergic systems superimposed on acute BP-changes induced by bradykinin, whereas the kidneys or the medullipin-system were not observed to influence this homeostatic balance.

Methods

Surgical procedures

Male Wistar rats (250–300 g) were fed on a normal salt (0.7% NaCl) diet. The animals were allowed food and water *ad lib* until the day of the experiment. The rats were then anaesthetized with pentobarbitone (70 mg kg^{-1}, *i.p.*, unless otherwise indicated) and tracheotomized. Catheters were inserted into the femoral artery and vein. The artery catheter was

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connected to a Statham pressure transducer and a Hewlett Packard 7402A recorder, and arterial BP was recorded continously. All drugs were administered through the vein catheter as bolus injections (0.15 ml, 15 s) unless otherwise indicated. After each injection, the catheter was flushed with 0.1 ml phosphate-buffered saline (PBS; 0.01 M Na-phosphate, pH 7.4, 0.14 M NaCl).

Nephrectomy (Nx) was performed 24 h prior to BP experiments. The animals were anaesthetized (136 mg kg^{-1} chloralhydrate and 30 mg kg^{-1} pentobarbitone, *i.p.*), flank incisions were made, and both kidneys were removed. The rats were allowed food and water over night, and 24 h later they were anaesthetized with pentobarbitone (50 mg kg^{-1}, *i.p.*) and prepared as described above. The 24 h Nx also removed the renal renin-angiotensin system since such animals did not respond to converting enzyme inhibitor (Bjørnstad-Østensen & Berg, 1994).

Experimental design

After a control period of 5 min, the animals were divided into seven groups which were pretreated with two injections 15 min apart, containing: PBS and PBS in the control group (group 1, $n=7$); L-NAME (0.3 g kg^{-1}) and PBS in group 2 ($n=9$); and PBS followed by proadifen (50 mg kg^{-1}, *i.p.*) in group 3 ($n=6$). Group 4 ($n=6$) was treated as group 1, but the rats were Nx 24 h prior to the experiment. Group 5 ($n=6$) was given L-NAME and proadifen. In group 6 ($n=8$) Nx was performed as in group 4, but the first injection of PBS was replaced by L-NAME. Group 7 ($n=6$) received L-NAME followed by phentolamine (2 mg kg^{-1}). Thirty minutes after the second pretreatment injection, all rats were given four doses of bradykinin (3, 6, 15, 30 μg kg^{-1}) 5 min apart. The dose of L-NAME and phentolamine was the same as in previous studies in which a hypertensive and hypotensive effect on BP had been established in normotensive non-Nx rats (Berg *et al.*, 1989; Bjørnstad-Østensen & Berg, 1994). The dose of proadifen was the same as that used in studies by Muirhead *et al.* (1989a) where its ability to block the medullipin-system was established.

The BP nadir and the fall in BP (ΔBP_{min}) after each injection of bradykinin were determined. As a measure of the duration of the hypotensive response, the time when BP showed a 3/4 recovery, calculated in relation to the preinjection value, was recorded. Maximum BP (BP_{max}) following recovery was determined, and ΔBP_{max} was calculated as the difference between BP_{max} and the BP just prior to the administration of bradykinin.

Materials

The following chemicals were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.: bradykinin acetate salt, N^{ω}-nitro-L-arginine methyl esther (L-NAME), and proadifen (SKF 525A). Heparin was obtained from Nycomed (Olso, Norway), pentobarbitone from The National hospital (Oslo, Norway), and Regitin (phentolamine) from Ciba-Geigy (Basel, Switzerland).

Statistics

The results are given as mean ± s.e.mean. A concentration-response relationship for the mean values for ΔBP_{min}, duration of the hypotensive response, and ΔBP_{max} (curve slopes) was tested for each group by two-way analysis of variance (ANOVA). The same parameters were used to analyse for differences between groups by Analysis of Variance and Covariance with Repeated Measures. Differences in BP prior to administration of bradykinin were tested by two sample *t* tests. Values of $P<0.05$ were considered significant.

Results

As shown in Table 1, the basal BP after L-NAME-treatment (group 2) was significantly higher, and after Nx (group 4) significantly lower, than in control rats (group 1), whereas proadifen (group 3) had no effect on basal BP. The basal BP after L-NAME-treatment was not significantly altered by addition of Nx, proadifen, or phentolamine (groups 5–7).

Injection of bradykinin caused an immediate fall in BP followed by a rapid return to approximately the preinjection level. Typical recordings of the haemodynamic response in the different groups are shown in Figure 1. The fall in BP (ΔBP_{min})

Table 1 Basal blood pressure (BP) prior to administration of bradykinin

	BP (mmHg)		
Group 1 PBS/PBS	111±6		
Group 2 L-NAME/PBS	149±4	***	
Group 3 PBS/proadifen	111±5	NS	
Group 4 Nx/PBS	80±5	**	
Group 5 L-NAME/proadifen	147±14	NS	NS^{+}
Group 6 Nx/L-NAME	126±11	NS	NS^{+}
Group 7 L-NAME/Phent	142±8	*	NS^{+}

*$P<0.05$; **$P<0.01$; ***$P<0.001$; NS, $P>0.05$ when compared to the control group (group 1), and NS^{+}, $P>0.05$ compared to the L-NAME-treated group (group 2). Phent–phentolamine.

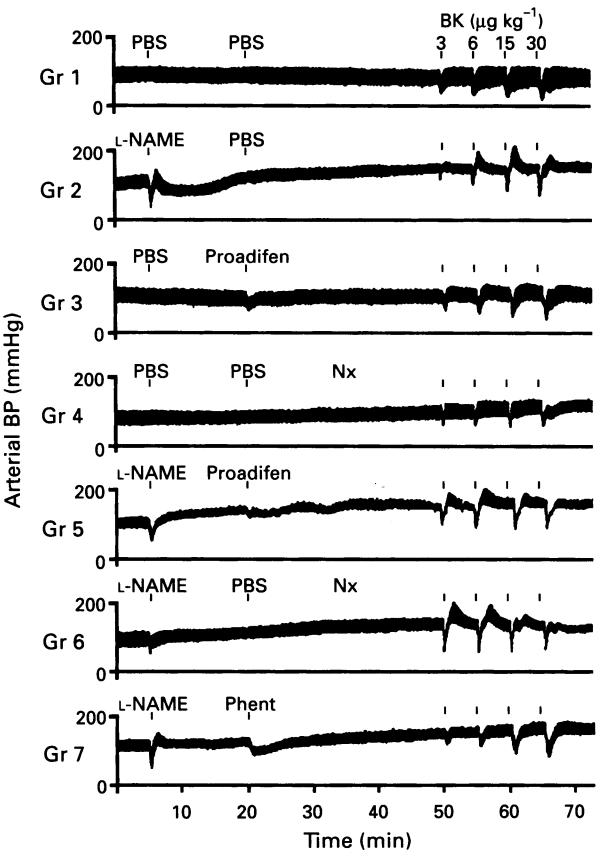

Figure 1 Typical recordings showing the BP responses to bradykinin (BK) in a control rat (group 1), in rats pre-treated with L-NAME (group 2), proadifen (group 3), by nephrectomy (Nx) (group 4), with L-NAME and proadifen (group 5), Nx and L-NAME (group 6), or L-NAME and phentolamine (group 7). In all rats given L-NAME a decrease in the duration of the hypotensive response was observed, as well as a marked hypertensive response following the BK-induced hypotension. These L-NAME-induced responses were abolished by phentolamine. The BK-induced BP-responses were not altered by proadifen or Nx in either control rats or L-NAME-treated rats. Phent, phentolamine.

was dose-dependent in all groups ($P<0.01$), except in the L-NAME/Nx-group (Figure 2) where a maximum fall in BP was already obtained after the first dose of bradykinin. No differences in ΔBP_{min} were observed between the groups. Thus, L-NAME, proadifen, and Nx had no effect on the acute fall in BP compared to control rats, and addition of proadifen, Nx or phentolamine to L-NAME-treatment did not alter the BP-response as compared to L-NAME alone.

The duration of the hypotensive response was significantly shorter in L-NAME-treated rats (group 2) than in control rats (group 1) ($P<0.05$). Proadifen (group 3) had no effect on the duration of the hypotensive response (Figure 3a). In Nx rats (group 4) a tendency towards an abbreviated hypotension was observed for the lower doses of bradykinin; however, the total dose-response curve was not significantly different from that of the control rats (group 1) (NS) (Figure 3a). When rats in addition to L-NAME were treated with proadifen (group 5) or Nx (group 6), the duration of the hypotensive response was not different from rats treated with L-NAME alone (Figure 3b). However, pretreatment with phentolamine prevented the L-NAME-induced rapid return in BP ($P<0.001$, compared to group 2) (Figure 3b), and the duration of the hypotensive response was not significantly different from that of the control rats (group 1) (NS). A dose-dependency was observed for all groups ($P<0.006$), except for the Nx/L-NAME group. These results indicate that L-NAME induced an abbreviation of the

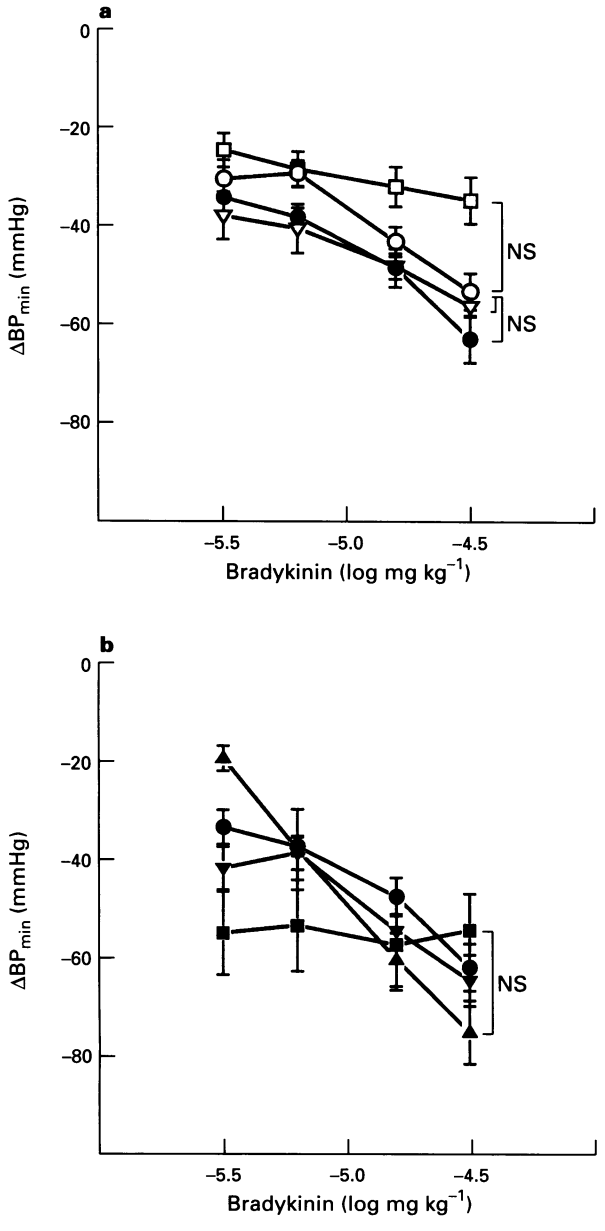

Figure 2 Fall in mean arterial blood pressure (ΔBP_{min}) immediately after administration of bradykinin. In (a), control rats (group 1) (○) ($n=7$) are compared to L-NAME-treated rats (group 2) (●) ($n=9$), proadifen-treated rats (group 3) (▽) ($n=6$), and Nx rats (group 4) (□) ($n=6$). In (b), L-NAME-only-treated rats (group 2) (●) are compared to rats treated with L-NAME together with proadifen (group 5) (▼) ($n=6$), Nx (group 6) (■) ($n=7$), and phentolamine (group 7) (▲) ($n=6$)). Analysis of Variance and Covariance with Repeated Measures revealed no differences between groups (NS, $P>0.05$) as indicated on the figure.

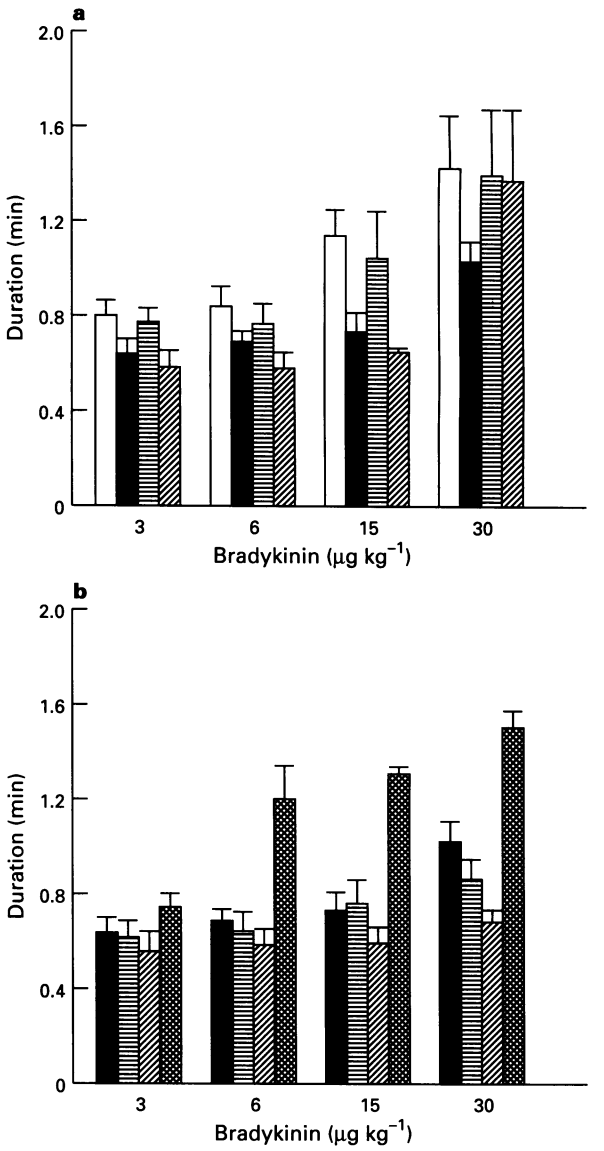

Figure 3 Duration of the hypotensive response induced by bradykinin: in (a), control rats (group 1) (open columns) ($n=7$) are compared to rats treated with L-NAME (group 2) (solid columns) ($n=9$), proadifen (group 3) (striped columns) ($n=6$), and Nx (group 4) (hatched columns) ($n=7$). In (b), L-NAME-treated rats (solid columns) are compared to L-NAME and proadifen-treated rats (group 5) (striped columns) ($n=6$), Nx and L-NAME-treated rats (group 6) (hatched columns) ($n=7$), and L-NAME and phentolamine-treated rats (group 7) (cross-hatched columns) ($n=6$). L-NAME reduced the duration of the bradykinin-induced hypotensive response ($P<0.05$), whereas proadifen and Nx did not (NS) (a). Addition of proadifen or Nx to L-NAME-treatment did not alter the duration of the hypotensive response as compared to L-NAME only (NS), whereas phentolamine abolished the L-NAME-induced abbreviation ($P<0.001$) (b).

bradykinin-induced hypotension because NO no longer hampered the adrenergic reponse compensating for the acute fall in BP, whereas other hypotensive agents, produced in the kidney, did not influence this homeostatic balance.

After the bradykinin-induced hypotension, BP stabilized at about preinjection levels in the control rats (group 1) (Figure 1a), whereas in L-NAME-treated rats (group 2) the hypotensive response was followed by a transient hypertensive response (Figure 1b). This hypertensive response was not observed in rats pre-treated with proadifen (group 3) or in Nx rats (group 4) (NS compared to group 1) (Figure 4a). In rats treated with proadifen or Nx in addition to L-NAME (group 5 and 6, respectively), ΔBP_{max} was significantly higher than the control group (group 1) ($P<0.001$ and $P<0.05$, respectively) but was not different from that seen in rats treated with L-NAME only (group 2) (NS) (Figure 4). However, the late L-NAME-dependent hypertension was totally abolished by addition of phentolamine (group 7) ($P<0.05$, compared to group 2) (Figure 4b). These results indicate that although renal release of hypotensive agents such as medullipin may be incresed after elevation of basal BP with L-NAME, removal of such agents did not influence the later hypertension following the acute fall in BP induced by bradykinin, but was caused only by an adrenergic activation which in L-NAME-treated rats was no longer hampered by a continuous NO-production.

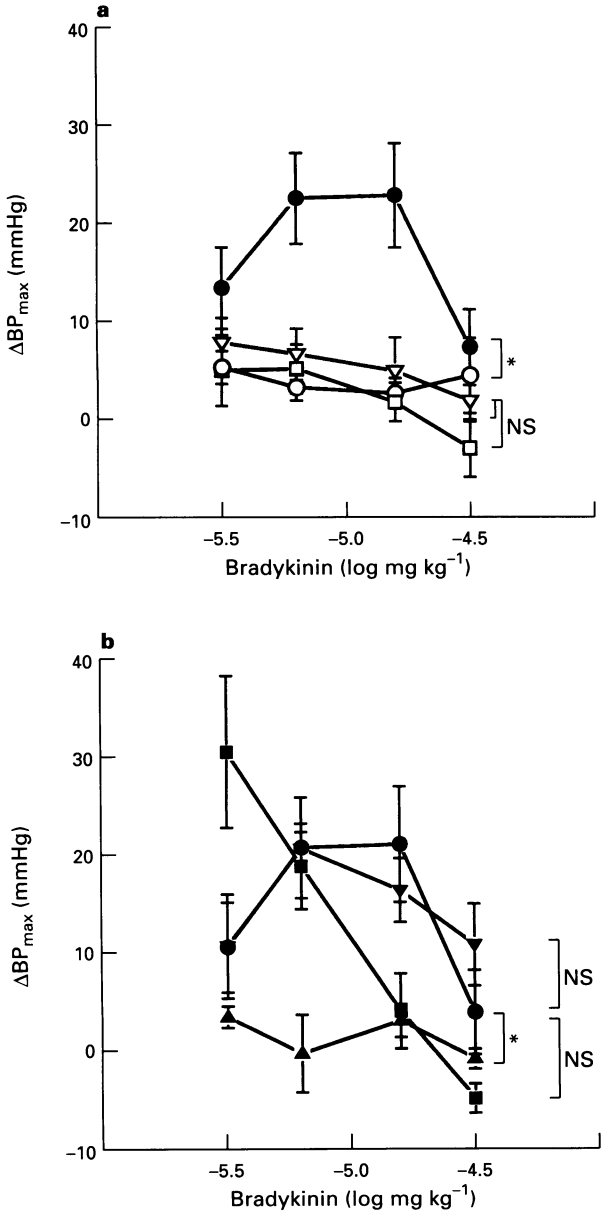

Figure 4 Hypertensive response (ΔBP_{max}) following the restoration of BP after bradykinin-induced hypotension. In (a) the control rats (group 1) (○) ($n=7$) are compared to L-NAME-treated rats (group 2) (●) ($n=9$), proadifen-treated rats (group 3) (▽) ($n=6$), and Nx rats (group 4) (□) ($n=6$). In (b), the L-NAME-treated group (●) is shown in comparison to the L-NAME and proadifen group (group 5) (▼) ($n=6$), the Nx and L-NAME group (group 6) (■) ($n=7$), and the L-NAME and phentolamine group (group 7) (▲) ($n=6$). A late hypertension was observed in rats treated with L-NAME. This hypertensive response was not altered by either proadifen or Nx, but was totally abolished by phentolamine. Differences between the groups tested by Analysis of Variance and Covariance with Repeated Measures are indicated on the figure. (*$P<0.05$; NS, $P>0.05$).

Discussion

The present study was based on the previous observation (Bjørnstad-Østensen & Berg, 1994) that pretreatment with the NO-synthase blocker, L-NAME, shortened the duration of an acute bradykinin-induced hypotension, and induced a subsequent, transient hypertensive response, both of which were blocked by α-adrenoceptor blockade. These observations were interpreted as indicating that the NO-system functions as a continuous hypotensive force, which when removed, allows the compensating hypertensive adrenergic-system to produce a faster and overshooting return in BP. The L-NAME-induced late hypertensive response appeared to be more prominent in Nx than in non-Nx rats, possibly suggesting the presence of a renal hypotensive system also participating in the modulation of the adrenergic hypertensive response. In the present study, the role of the kidneys in modulating acute BP changes was systematically investigated. Although the role of the NO-system as a modulator counteracting the adrenergic-system was confirmed, the present results do not support the involvement of a potential hypotensive system localised in the kidneys. This was concluded from the observation that the ΔBP dose-response curve from Nx rats was not significantly different from that of control rats, although a statistically nonsignificant tendency towards a shortening of the duration of the hypotensive response was observed with Nx alone for the low doses of bradykinin. This observed lack of renal influence was further substantiated by the fact that no late hypertensive response was observed in Nx rats, whereas a late hypertension was observed in all groups receiving L-NAME. Furthermore, no difference in either the duration of the hypotensive response or the late hypertensive response was observed when Nx was added to the L-NAME-treatment. Moreover, pretreatment with proadifen, a blocker of the cytochrome P-450 system which is involved in both the synthesis and secretion of medullipin I by the renomedullary cells, as well as its conversion to medullipin II in the liver (Muirhead *et al.*, 1989a, b), was used to inhibit the hypotensive medullipin system. In accordance with observations made by Muirhead *et al.* (1989a) injection of proadifen caused only a minor, transient hypotension and unsteadiness in BP. Furthermore, we did not observe any effect of proadifen on BP return after bradykinin-induced hypotension in either control rats or L-NAME-treated rats, in spite of which L-NAME significantly increased basal BP and thus would be expected to activate medullipin I-secretion (Karlström & Göthberg, 1987; Karlström *et al.*, 1988). These results indicate that the medullipin-system did not play a role in maintenance of basal BP or in the BP homeostasis after an acute kinin-induced fall in BP.

The only group not showing a dose-dependency in the hypotension induced by bradykinin, was the Nx/L-NAME-treated group where a maximal ΔBP_{min} was observed already after the lowest dose of bradykinin. A lack of dose-dependency was not observed for either Nx or L-NAME alone. We have no explanation for why Nx in combination with L-NAME should alter the ΔBP_{min} concentration-response curve to bradykinin.

In Nx rats, basal BP was lower than in non-Nx rats. This is in agreement with previous observations in anaesthetized animals (T. Berg, unpublished observations). The reason for this has not been established, but removal of the renal renin-angiotensin system may be one possible explanation.

In conclusion, the present results do not support the involvement of the medullipin-system or other potential hypotensive systems localized in the kidneys in modulating and counteracting the compensatory adrenergic response following a bradykinin-induced acute hypotension. A hampering, modulating effect of the NO-system on the compensatory adrenergic response was confirmed, indicating a close relationship between these two systems in BP homeostasis.

References

BERG, T., CARRETERO, O.A., SCICLI, A.G., TILLEY, B. & STEWART, J. (1989). Role of kinin in regulation of rat submandibular gland blood flow. *Hypertension*, **14**, 73–80.

BJØRNSTAD-ØSTENSEN, A. & BERG, T. (1994). The role of nitric oxide, adrenergic activation and kinin-degradation in blood pressure homeostasis following an acute kinin-induced hypotension. *Br. J. Pharmacol.*, **113**, 1567–1573.

BURNSTOCK, G. (1990). Local mechanisms of blood flow control by perivascular nerves and endothelium. *J. Hypertens.*, **8** (suppl 7), 95–106.

DZAU, V.J. (1989). Multiple pathways of angiotensin production in the blood vessel wall: evidence, possibilities and hypotheses. *J. Hypertens.*, **7**, 933–936.

KARLSTRÖM, G., ARNMAN, V., FOLKOW, B. & GÖTHBERG, G. (1988). Activation of the humoral antihypertensive system of the kidney increases diuresis. *Hypertension*, **11**, 597–601.

KARLSTRÖM, G. & GÖTHBERG, G. (1987). The humorally mediated antihypertensive system of the rat kidney. *J. Hypertens.*, **5** (suppl 5), 591–597.

MUIRHEAD, E.E. (1988). The medullary system of blood pressure control. *Am. J. Med. Sci.*, **295**, 231–233.

MUIRHEAD, E.E., BYERS, L.W., BROOKS, B., BROWN, P. & PITCOCK, J.A. (1989a). Biologic contrasts between medullipin I and vasoactive glycerol compounds. *Am. J. Med. Sci.*, **298**, 93–103.

MUIRHEAD, E.E., BYERS, L.W., CAPDEVILA, J., BROOKS, B., PITCOCK, J.A. & BROWN, P.S. (1989b). The renal anti-hypertensive endocrine function: its relation to cytochrome P-450. *J. Hypertens.*, **7**, 361–369.

MUIRHEAD, E.E., RIGHTSEL, W.A., LEACH, B.E., BYERS, L.W., PITCOCK, J.A. & BROOKS, B. (1977). Reversal of hypertension by transplants and lipid extracts of cultured renomedullary interstitial cells. *Lab. Invest.*, **25**, 162–172.

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