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The role of nitric oxide, adrenergic activation and kinin-degradation in blood pressure homeostasis following an acute kinin-induced hypotension

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1 Nitric oxide (NO) has been suggested as the mediator of the vascular response to bradykinin. In the present study, we found that NO did not mediate the hypotensive response to bradykinin. In addition, the significance of kininase II in terminating a kinin-induced hypotension and the role of the adrenergic system in compensating for the acute fall in blood pressure (BP) was established.

2 In normal rats, the NO-synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) induced a rise in basal BP ($\Delta BP = 40 \pm 6$ mmHg, $P < 0.0014$) which was not altered by pretreatment with phentolamine ($\Delta BP = 50 \pm 6$ mmHg, NS). L-NAME did not attenuate the acute fall in BP in response to bradykinin (3–30 $\mu g\,kg^{-1}$) or kallikrein (6–300 $\mu g\,kg^{-1}$). However, a significant decrease was observed in the duration of the hypotensive response ($P < 0.027$). This shorter duration was not observed after pretreatment with phentolamine in addition to L-NAME. Phentolamine alone prolonged the hypotensive response to bradykinin ($P < 0.04$). These experiments confirm the role of NO-formation as a hypotensive component in BP homeostasis but not the role of NO as a mediator in kinin-induced hypotension. It further shows that the continuous NO-release also impedes the compensatory adrenergic hypertensive response following the acute fall in BP induced by bradykinin.

3 The hypertensive respose to intravenously administered phenylephrine was found to be unchanged by preadministration of L-NAME (NS) thus showing that L-NAME did not change the sensitivity to the adrenergic response. In a separate protocol on L-NAME-treated rats we found no difference in heart rate (NS) during the recovery period following bradykinin before as compared to after administration of phentolamine. It was therefore concluded that the observed alterations in the duration of the hypotensive response were most probably due to changes in peripheral vascular resistance.

4 To confirm further that NO is not a mediator in kinin-induced hypotension, we used an experimental model where the response to bradykinin was prolonged by preventing kinin degradation by kininase II-converting enzyme inhibitor (CEI). To produce a hypotensive response purely dependent on kinin, the studies were performed after removal of the renin-angiotensin system by nephrectomy (Nx). In this model, bradykinin (6 $\mu g\,kg^{-1}$, i.v.) induced a prolonged hypotensive response. Pretreatment with L-NAME did not alter the magnitude or the progression of the hypotensive response to bradykinin, thus confirming that NO was not a mediator in BK-induced hypotension.

5 To study the mechanisms involved in terminating the hypotensive response to bradykinin, the results from the Nx CEI-treated rats were compared with Nx animals not treated with CEI. In the latter group, bradykinin induced a short hypotensive response, i.e. 0.5 ± 0.1 min as compared to the 17 ± 1 min after CEI ($P < 0.003$). After kininase II-inhibition (and L-NAME), BP recovery was totally dependent on the adrenergic system, since phentolamine prevented a recovery in BP during the experimental period ($P < 0.01$, compared to the CEI/L-NAME group). These results demonstrate the importance of kininase II as the major agent in terminating a bradykinin-induced hypotension, whereas the adrenergic system plays a small, although significant role in compensating for the fall in BP. The continuous release of NO therefore not only lowers basal BP but also impedes the compensatory adrenergic response.

Keywords: Bradykinin; kallikrein; EDRF; NO, NO-synthase antagonist; sympathetic nervous system; blood pressure regulation; converting enzyme inhibitor; kininase II

Introduction

Bradykinin is a potent vasodilator peptide released by kallikrein (E.C. 3.4.21.35) from the precursor kininogen. Recent studies strongly indicate that kinin participates in the control of blood pressure (BP) (Kiowski *et al.*, 1992; Cachofeiro *et al.*, 1992; Pellacani *et al.*, 1992). It has been suggested that the hypotensive effect of bradykinin is mediated through the release of endothelium-derived relaxing factor (EDRF) or nitric oxide (NO): bradykinin has been shown to stimulate the release of NO from bovine pulmonary artery endothelial cells (O'Shaughnessy *et al.*, 1992) and from guinea-pig isolated hearts (Kelm & Schrader, 1988). Moreover, experiments in rats demonstrated a shorter hypotensive response to bradykinin after treatment with NO-synthase inhibitor, thus indicating a role of NO in the late phase of bradykinin-induced hypotension (Rees *et al.*, 1990). However, similar results were not obtained by O'Shaughnessy *et al.* (1992), thus questioning the role of NO as a mediator in bradykinin-induced hypotension.

Since BP is the result of the balance between hypotensive and hypertensive systems (Dzau, 1989; Burnstock, 1990), any change in BP may be counteracted and modulated by opposing systems. It is therefore possible that the observed shortening of the hypotensive response to bradykinin in rats treated with NO-synthase inhibitor may be explained by a hampered NO-modulation of a compensatory hypertensive response following an acute fall in BP. In the present work, the role of NO-formation in hypotension induced by bradykinin and

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kallikrein was studied in protocols which in addition allowed us to study adrenergic involvement in the BP regulation. The results do not support the concept that bradykinin- and kallikrein-induced hypotension is mediated through NO-formation but indicate that an abbreviated kinin-induced hypotensive response after inhibition of NO-formation is due to the fact that the adrenergic response following the acute fall in blood pressure is no longer impeded by the vasodilator NO-system. Furthermore, the mechanisms involved in terminating the bradykinin-induced hypotension were elucidated.

Methods

Surgical procedures

Male Wistar rats (250–300 g) were anaesthetized with pentobarbitone (70 mg kg^{-1}, i.p., unless otherwise indicated) and tracheotomized. Arterial BP was recorded continuously through a catheter in the femoral artery connected to a Statham strain-gauge transducer and a Hewlett Packard 7402 A recorder. All drugs were administered as bolus injections (0.15 ml, 20 s) through a catheter in the femoral vein, 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).

Experimental design

Protocol 1: The effect of L-NAME on bradykinin- and kallikrein-induced hypotension with and without pretreatment with phentolamine The rats were prepared as described above and divided into four groups: The control group ($n = 6$) received two sham injections of PBS 5 min apart; the second group ($n = 6$) received PBS and L-NAME (0.3 g kg^{-1}) at the same time intervals; the third group ($n = 6$), the α-adrenoceptor antagonist, phentolamine (2 mg kg^{-1}) and L-NAME (0.3 g kg^{-1}); and the fourth group ($n = 6$) received phentolamine (2 mg kg^{-1}) followed by PBS; 15 min later, all animals were given bradykinin (3, 6 and 30 μg kg^{-1}) and kallikrein (6, 30, 60 and 300 μg kg^{-1}) in consecutive injections administered 5 min apart. As a measure of the duration of the hypotensive response to bradykinin and kallikrein, the time when BP had returned to 75% of the maximum fall was recorded.

Protocol 2: The effect of L-NAME on the sensitivity to phenylephrine An experimental group ($n = 8$) and a control group ($n = 8$) received L-NAME (0.3 g kg^{-1}) or a sham injection with PBS, respectively; 15 min later, all rats received phenylephrine (0.3, 3, 13, 25, 50 and 150 μg kg^{-1}, 0.1 ml) injected 5 min apart, and the hypertensive response was recorded for each dose.

Protocol 3: The effect of bradykinin on heart rate before and after administration of phentolamine in the presence of L-NAME All rats ($n = 6$) were given an initial injection of L-NAME (0.3 g kg^{-1}); 15 min later, they received two injections of bradykinin (6 and 30 μg kg^{-1}) 5 min apart. After another 5 min, the rats were given phentolamine (2 mg kg^{-1}), and after 5 min, the injections of bradykinin were repeated. Heart rate was recorded before bradykinin and during the BP recovery period.

Protocol 4: The effect of L-NAME and phentolamine on bradykinin-induced hypotension in nephrectomized (Nx) rats pretreated with CEI All animals were nephrectomized 24 h prior to the experiment to prevent a hypotensive response due to the renal renin-angiotensin system when the CEI was administered. Binephrectomy was performed through flank incisions during anaesthesia (136 mg kg^{-1} chloralhydrate and 30 mg kg^{-1} pentobarbitone, i.p.). The following day the rats were anaesthetized with pentobarbitone (50 mg kg^{-1}), prepared as described above, and given an initial injection of CEI (10 mg in 0.5 ml 0.45% NaCl). The rats were divided into three groups; the control group ($n = 6$) was given two sham injections with PBS 5 and 20 min after CEI; the second group ($n = 6$) L-NAME (0.3 g kg^{-1}) followed by PBS; and the third group ($n = 6$), L-NAME followed by phentolamine (2 mg kg^{-1}) at the same time intervals. Five minutes later, all rats were given one dose of bradykinin (6 μg kg^{-1}). BP was recorded for another period of 30 min. The order in which L-NAME and phentolamine was administered was altered from that in protocol 1 because the initial BP was lower in Nx than in normal rats. If phentolamine was given first, BP in preliminary experiments was found to drop so low that a proper response to L-NAME and subsequently bradykinin could not be recorded.

Protocol 5: The effect of L-NAME on bradykinin- and kallikrein-induced hypotension with and without pretreatment with phentolamine in Nx rats All animals were nephrectomized 24 h prior to the experiment. On the day of the experiment, the rats were prepared as described above and treated as outlined in protocol 1, except that L-NAME was administered prior to phentolamine in group 3 for the same reasons as given above for protocol 4. Also for the same reasons, the fourth group (phentolamine and PBS) was omitted from this protocol. To determine the hypertensive response which in Nx rats followed the hypotension, the area exceeding the BP level before each injection of bradykinin and kallikrein was determined by computerized planimetry.

Materials

Heparin was obtained from Nycomed, Oslo, Norway; pentobarbitone from The National Hospital, Oslo, Norway; bradykinin acetate salt, N^{ω}-nitro-L-arginine methyl ester (L-NAME), and L-phenylephrine hydrochloride were from Sigma Chemical Co., St. Louis, MO, U.S.A.; Regitin (phentolamine) from Ciba-Geigy, Basel, Switzerland; and captopril (converting enzyme inhibitor, CEI) from Squibb, Princeton, NJ, U.S.A. Kallikrein was purified from the rat submandibular gland as previously described (Johansen *et al.*, 1987).

Statistics

The results are expressed as mean ± s.e.mean. Dose-response relationships were tested by two-way analysis of variance (ANOVA). One-way ANOVA was used to test for differences between the groups in protocol 1 and 5. When differences were observed, Student's two-sample t test were used to determine differences between groups at a particular dose. Student's two-sample t-test was also used to determine differences in heart rate in protocol 3. To analyse for differences between groups in protocol 2 and 4, Analysis of Variance and Covariance with Repeated Measures (BMDP) were used. A P value less than 0.05 was considered significant.

Results

Protocol 1: The effect of L-NAME on bradykinin- and kallikrein-induced hypotension with and without pretreatment with phentolamine

Bradykinin and kallikrein induced an immediate fall in BP which shortly (after 0.5 ± 0.1 min) returned to preinjection levels (Figure 1a). The immediate drop in BP was found to be dose-dependent ($P < 0.01$) and did not differ among the groups with the exception of the phentolamine-only group where the fall in BP in response to bradykinin was significantly less than the other three groups ($P < 0.02$) due to the lower starting BP (Figure 2). A dose-dependency was also

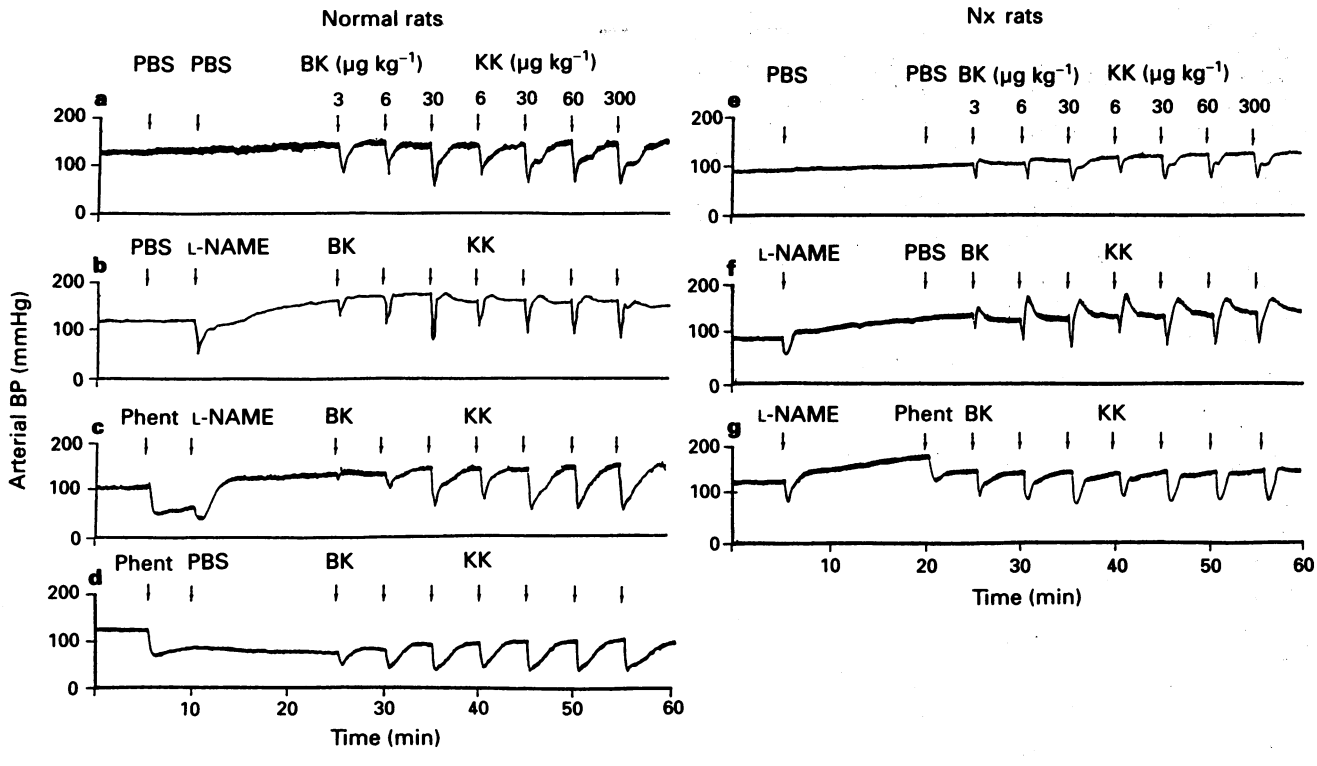

Figure 1 Typical recordings of the haemodynamic response in normal rats (a–d) and nephrectomized (Nx) rats (e–g) to bradykinin (BK) and kallikrein (KK) in a control rat (a and e), N^{ω}-nitro-L-arginine methyl ester (L-NAME)-treated rat (b and f), a rat pretreated with phentolamine (Phent) in addition to L-NAME (c and g), and a rat pretreated with phentolamine only (Phent) (d). L-NAME caused a decrease in the duration of the hypotensive response both in normal and nephrectomized rats, which was abolished by preadministration of phentolamine. In Nx L-NAME rats, hypotension was followed by hypertension which was also abolished by phentolamine.

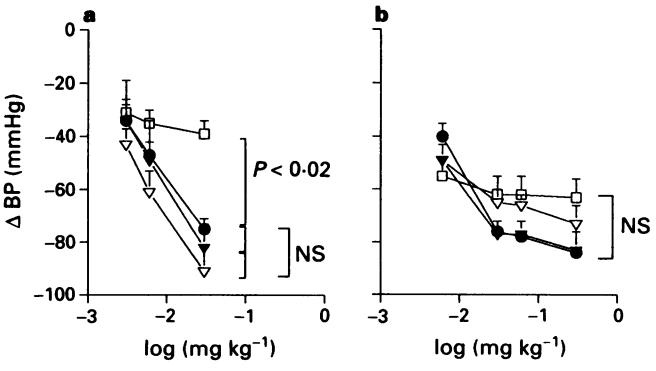

Figure 2 Graph showing the fall in mean arterial blood pressure (ΔBP) in normal rats immediately after administration of bradykinin (3, 6, 30 μg kg^{-1}) (a) or kallikrein (6, 3, 60, 300 μg kg^{-1}) (b) in the control group (●) ($n = 6$), in N^{ω}-nitro-L-arginine methyl ester (L-NAME)-treated rats (▽) ($n = 6$), in rats pretreated with phentolamine in addition to L-NAME (▼) ($n = 6$), and after pretreatment with phentolamine only (□) ($n = 6$). A dose-dependency was observed for all groups ($P < 0.01$). Differences between groups are indicated on the figure.

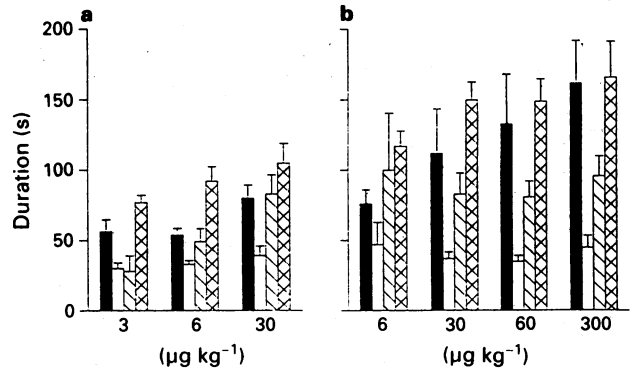

Figure 3 The duration of the hypotensive response in non-nephrectomized animals induced by bradykinin (3, 6, 30 μg kg^{-1}) (a) and kallikrein (6, 30, 60, 300 μg kg^{-1}) (b) in control rats (solid columns) ($n = 6$), in rats treated with N^{ω}-nitro-L-arginine methyl ester (L-NAME) (open columns) ($n = 6$), in rats treated with phentolamine in addition to L-NAME (hatched columns) ($n = 6$), and in rats treated with phentolamine (cross-hatched columns) ($n = 6$). As a measure of the duration of the hypotensive response to bradykinin and kallikrein, the time when BP had returned to 75% of the maximum fall, was recorded. There was a significant decrease in the duration of the hypotensive response in the presence of L-NAME ($P < 0.003$ and $P < 0.027$ respectively), whereas the duration in rats treated with phentolamine and L-NAME was not significantly different from that in the control group (NS). After pretreatment with phentolamine alone there was a significant increase in the duration of the hypotensive response after bradykinin compared to control rats and phentolamine/NAME-treated rats ($P < 0.04$). The same tendency was observed also for kallikrein although the differences were not statistically significant.

observed for the duration of the hypotensive response ($P < 0.03$) (Figure 3). In L-NAME-treated animals, the duration of the bradykinin-induced ($P < 0.003$) and the kallikrein-induced ($P < 0.027$) hypotensive response was significantly shorter than in the control group. However, in the group given phentolamine in addition to L-NAME no significant

difference (NS) was observed compared to the control group (Figure 3). In the fourth group after pretreatment with phentolamine alone, the duration of the hypotensive response to bradykinin was significantly prolonged compared to the control as well as the phentolamine/NAME-treated group ($P<0.04$). The same tendency was observed also after kallikrein although this difference was not statistically significant.

After the administration of L-NAME (group 2), BP increased from 119 ± 4 to 159 ± 5 mmHg ($P<0.001$). Injection of phentolamine (group 3) resulted in an initial fall in BP of 49 ± 6 mmHg ($P<0.0004$), but did not alter the increase in BP induced by the subsequent administration of L-NAME (from 95 ± 5 just prior to and 145 ± 7 mmHg 15 min after L-NAME (NS) compared to the group pretreated with L-NAME only).

Protocol 2: The effect of L-NAME on the sensitivity to phenylephrine

L-NAME did not alter the sensitivity to phenylephrine since there was no significant difference (NS) in the hypertensive response to increasing doses of phenylephrine in rats pretreated with L-NAME as compared to control animals (Figure 4).

Protocol 3: The effect of bradykinin on heart rate before and after administration of phentolamine in the presence of L-NAME

The change in heart rate from before to after injection of bradykinin was not altered by administration of phentolamine in L-NAME-treated rats: The change in heart rate following the first dose of bradykinin was -5 ± 4 strokes min^{-1} before compared to -7 ± 4 strokes min^{-1} after administration of phentolamine, and -4 ± 8 strokes min^{-1} compared to -5 ± 6 strokes min^{-1} for the second bradykinin dose (NS).

Protocol 4: The effect of L-NAME and phentolamine on bradykinin-induced hypotension in Nx rats pretreated with CEI

CEI itself induced a minor fall in BP (-4 ± 3, -2 ± 1 and -3 ± 2 mmHg in the three groups, respectively) (NS) indicating that the renin-angiotensin system had been successfully eliminated. In the control group, injection of bradykinin caused a fall in BP, which returned to pre-bradykinin levels after 17 ± 1 min (Figure 5). Administration of L-NAME induced an elevation of BP from 86 ± 7 to 125 ± 13 mmHg ($P<0.0018$), but the fall in BP in response to bradykinin equalled that of the control rats (NS) (Figure 5). In rats pretreated with phentolamine in addition to L-NAME, BP prior to administration of bradykinin equalled that of control rats (82 ± 8 and 90 ± 3 mmHg, respectively, NS). The immediate drop in BP following bradykinin was equal to that in control rats, whereas, in these rats, BP remained at the minimum value throughout the experimental period, and was in this respect significantly different from both the control and the L-NAME-treated animals ($P<0.01$) (Figure 5).

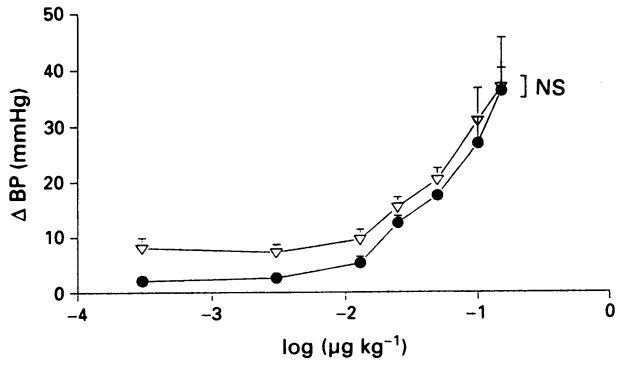

Figure 4 The hypertensive response to increasing doses of phenylephrine in control rats (●) ($n=8$) and N^{ω}-nitro-L-arginine methyl ester (L-NAME)-treated rats (▽) ($n=8$). No significant difference (NS) was observed between the two groups indicating that L-NAME did not change the sensitivity to phenylephrine.

Protocol 5: The effect of L-NAME on bradykinin- and kallikrein-induced hypotension with and without pretreatment with phentolamine in Nx rats

To determine the role of kininase II in terminating a bradykinin-induced hypotension, the response to bradykinin after CEI (protocol 4) was compared to that of the corresponding dose of bradykinin in Nx rats not treated with CEI (protocol 5). The duration of the hypotensive response was clearly longer lasting when kininase II was inhibited by CEI (17 ± 1 min) than without CEI (0.5 ± 0.1 min) ($P<0.003$) (Figures 5 and 1g).

The response pattern in Nx rats not treated with CEI (protocol 5) resembled that of non-Nx rats (protocol 1). In the untreated control group, bradykinin and kallikrein caused a dose-dependent fall in BP ($P<0.001$) and in the duration of the hypotensive response ($P<0.0003$) (Figure 6). However, the duration of the hypotensive response was significantly shorter than in non-Nx rats for bradykinin ($P<0.01$) (Figures 3 and 6). This was also observed for kallikrein, although the difference was not statistically significant. As in non-Nx rats L-NAME-treatment resulted in a shortening of the hypotensive period following kallikrein ($P<0.005$), whereas for bradykinin the same tendency was observed, although the difference was not significantly different from the control group (Figure 6). After administration of phentolamine in addition to L-NAME, we observed a significant prolongation of the hypotensive response as compared to that in the L-NAME-treated animals as well as the control animals ($P<0.017$) (Figure 6). On the other hand, in Nx rats, the hypotension was followed by a short period of hypertension which was enhanced by L-NAME ($P<0.025$) (Figures 1f and 7). This hypertensive response, which was barely observed in normal rats (Figure 1b), was almost totally abolished by phentolamine ($P<0.026$) (Figures 1f and 7).

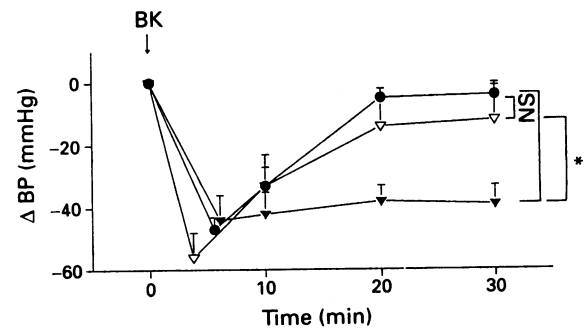

Figure 5 The fall in BP after an intravenous injection of bradykinin ($6\ \mu g\ kg^{-1}$) in nephrectomized rats pretreated with converting enzyme inhibitor (CEI) after a sham injection with vehicle (●) ($n=6$), after L-NAME (▽) ($n=6$), and after L-NAME and phentolamine (▼) ($n=6$). BP prior to administration of bradykinin did not differ significantly in control rats and rats treated with L-NAME and phentolamine, whereas BP in rats treated only with L-NAME was significantly higher ($P<0.02$). In the L-NAME-treated group, the fall in BP to bradykinin paralleled that in control rats (NS). In the group treated with L-NAME and phentolamine, the hypotensive response to bradykinin showed a significantly different development over time from the control group as well as from the L-NAME-treated group (*$P<0.01$). BP in this group remained at the minimum levels throughout the experimental period.

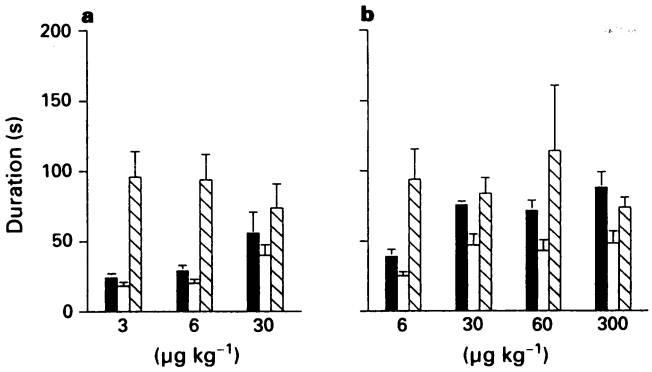

Figure 6 The duration of the hypotensive response in nephrectomized rats induced by bradykinin (3, 6, 30 μg kg^{-1}) and kallikrein (6, 30, 60, 300 μg kg^{-1}) in control rats (solid columns) ($n = 6$), in rats treated with N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) (open columns) ($n = 6$), and in rats treated with phentolamine in addition to L-NAME (hatched columns) ($n = 6$). As a measure of the duration of the hypotensive response to bradykinin and kallikrein, the time when BP had returned to 75% of maximum fall, was recorded. There was a significant decrease in the duration of the hypotensive response in the presence of L-NAME after injection of kallikrein ($P < 0.005$). This tendency was also observed after injection of bradykinin, although the change was not statistically significant. There was a significant increase in the duration of the hypotensive response after pretreatment with phentolamine in addition to L-NAME compared to both control and L-NAME-treated rats ($P < 0.017$).

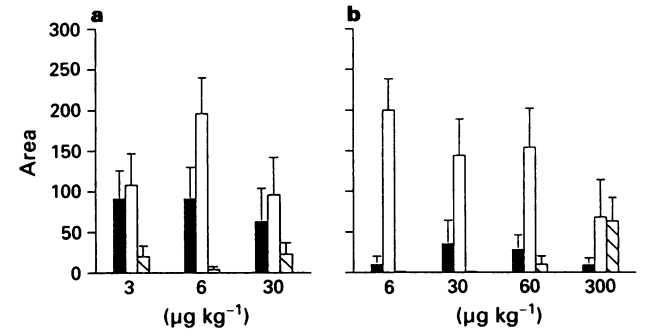

Figure 7 The hypertension following acute hypotension in nephrectomized rats in response to bradykinin (3, 6, 30 μg kg^{-1}) (a) and kallikrein (6, 30, 60, 300 μg kg^{-1}) (b) in control rats (solid columns) ($n = 6$), in rats treated with N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) (open columns) ($n = 6$), and rats treated with phentolamine in addition to L-NAME (hatched columns) ($n = 6$). The area under the BP curve exceeding the BP level before each injection of bradykinin and kallikrein was measured and determined by computerized planimetry. There was a significant increase in the hypertension following kallikrein-induced hypotension in L-NAME-treated rats compared to control rats ($P < 0.025$). After injection of bradykinin the same tendency was observed, although not statistically significant. Phentolamine almost abolished the hypertensive response ($P < 0.026$).

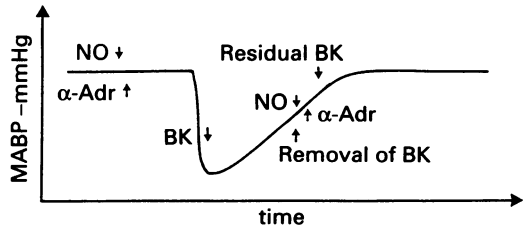

Figure 8 Illustration of the balance between hypotensive and hypertensive systems in the regulation of BP. The present results can easily be explained if the hypotensive response to bradykinin and kallikrein is divided into two phases; a first period where hypotensive systems predominate, in this case by a high concentration of plasma kinin, which is followed by a second period of recovery where hypertensive components, in this case adrenergic activation, takes over while plasma kinin is being removed. The NO system functions as a continuous hypotensive force, and the release of NO is not altered by either the kinin or the adrenergic system.

Discussion

In contrast to previous studies in whole animals, (Whittle *et al.*, 1989; Rees *et al.*, 1990) and what may be deduced from studies on cell cultures (Palmer *et al.*, 1988; Bogle *et al.*, 1991; O'Shaughnessy *et al.*, 1992) and isolated organs (Kelm & Schrader, 1988), the present results do not support a role for NO-formation in bradykinin- or kallikrein-induced hypotension. In studies on acetylcholine- and bradykinin-induced hypotension in rats (Rees *et al.*, 1990) and acetylcholine-induced hypotension in guinea-pig (Aisaka *et al.*, 1989), it was suggested that the hypotension could be divided into two phases, an immediate fall in BP which was resistant to NO-synthase blocker, and a late hypotensive phase which was sensitive to NO-synthase blocker. Although we, like Rees *et al.* (1990) did observe an abbreviation of the hypotensive response after injection of bradykinin in rats treated with L-NAME, we found this effect to be abolished by α-adrenoceptor blockade, thus indicating that NO-release normally modulates the adrenergic recovery response following an acute fall in BP. Furthermore, when the effect of the adrenergic system was removed by phentolamine but with the NO-system intact, a prolonged hypotensive response to bradykinin was observed. The present results can easily be explained if the hypotensive response to bradykinin and kallikrein is not interpreted as two phases of hypotension, but as a first period where hypotensive systems predominate, in this case by a high concentration of plasma kinin, which is followed by a second period of recovery where hypertensive components, in this case adrenergic activation, take over while plasma kinin is being removed (Figure 8). In this balance, the NO-system functions as a continuous hypotensive force not altered by either the kinin or the adrenergic system (Figure 8), and impedes the compensatory adrenergic response following the acute fall in BP. A NO-independent bradykinin-induced relaxation has been demonstrated in isolated coronary arteries of the pig (Kauser & Rubanyi, 1992), which is in agreement with the results of the present study.

To confirm our results that bradykinin-induced hypotension is not mediated through NO-release, we also used an experimental model known to represent a longer-lasting kinin-dependent hypotension, i.e. prevention of degradation of kinin by kininase II (angiotensin converting enzyme) inhibition. To make this a pure kinin-dependent response the influence of the renin-angiotensin sysem was excluded by nephrectomy (Ørstavik *et al.*, 1982). Also in this model, NO-synthase inhibition had no effect on the hypotensive response to bradykinin.

In non-Nx rats, the effect of phentolamine declined (although it was not abolished) by increasing doses of kallikrein but not bradykinin. This observation may be explained by a continuous release of kinin when kallikein was present in the circulation in unphysiologically high concentrations. This would probably result in a prolonged period of high plasma kinin concentration, thus counterbalancing the compensatory hypertensive response for a longer period of time.

It could be argued that the fall and return of BP following an acute injection of bradykinin may be altered by differences in starting BP induced by L-NAME. In this study, the difference in starting BP was eliminated by administration of the α-adrenoceptor blocker, phentolamine in addition to L-NAME, instead of increasing basal BP in the control group by adrenaline (see Rees *et al.*, 1990). If NO were a mediator in bradykinin-induced hypotension, an abbreviated hypotension should also have been expected to occur in this situation, which it did not. Furthermore, it is possible that the discrepancy between the *in vitro* studies showing bradykinin-dependent release of NO in cell cultures (Palmer *et al.*, 1988) and isolated organs (Kelm & Schrader, 1988) and our *in vivo* studies could have been explained by an enhanced adrenergic response due to an increased sensitivity to phenylephrine by L-NAME. However, this possibility was excluded since the

dose-response curve to phenylephrine was not altered by administration of L-NAME.

The present study did not allow us to conclude that the changes in BP were due to alterations in peripheral vascular resistance, since we did not measure cardiac output. However, phentolamine did not result in any change in heart rate during the recovery phase following bradykinin in L-NAME-treated non-nephrectomized rats, and it is therefore likely that the observed changes in BP were indeed a result of altered adrenergic control of peripheral vascular resistance.

Since bradykinin has been shown to be a potent inducer of adrenal gland medullary hormone release (Feldberg & Lewis, 1964), the increased adrenergic activity in L-NAME-treated rats may have been a result of a specific adrenaline release from the adrenal glands and not to adrenal adrenaline or peripheral nerve noradrenaline release induced by the fall in blood pressure as such. However, the recovery in L-NAME-treated rats occurred within 30 s after the injection of bradykinin or kallikrein. It therefore seems likely that the compensatory hypertensive response was induced by noradrenaline released from sympathetic nerves innervating resistance vessels in response to the acute fall in BP, and that this hypertensive response became apparent when it was no longer impeded by NO release. Similar studies with another hypotensive agent such as acetylcholine to test this hypothesis could not be done since the hypotensive effect of acetylcholine is mediated through NO synthesis (Furchgott & Zawadzki, 1980).

These studies also allowed us to draw some conclusions on the mechanisms involved in BP-recovery after a kinin-induced hypotension. In normal rats, the recovery of BP was rapid and only slightly dependent on adrenergic activation. However, BP-recovery was heavily dependent on removal of bradykinin by kininase II/converting enzyme as demonstrated by the markedly prolonged hypotensive response after administration of CEI. This was not due to the use of Nx rats in the latter protocol since the rapid return in BP was also observed in untreated Nx rats. On the other hand, when kininase II was inhibited by CEI in Nx rats, BP-recovery was totally dependent on the adrenergic system. Since a short duration pattern was observed in both Nx and non-Nx rats not treated with CEI, it could be concluded that the kidney was of little importance in the removal of kinin, even though the kidney is one of the richest sources of kininase II (Erdös, 1979). This is in accordance with studies by Ishida *et al.* (1989) showing that nephrectomy did not affect the concentration of endogenous plasma kinins. These results demonstrate that an acute bradykinin-induced hypotension is normally terminated by a rapid removal of the hypotensive agent by kininase II, and that this recovery response is to a small but significant extent enhanced by the adrenergic system (Figure 8).

To our surprise we found that Nx rats (not treated with CEI) seemed to have a stronger adrenergic response than non-Nx rats. This was concluded from the observed shorter duration of the hypotensive response and the subsequent period of overshoot of hypertension in Nx rats; both responses being α-adrenergically dependent since they were blocked by phentolamine. This enhanced adrenergic compensation in Nx rats seemed normally to be modulated and well balanced by the NO-system, since this observation was apparent only after NO-synthase inhibition. This was a side-observation arising from the need of a proper protocol for determining the role of kininase II in BP-recovery, and we have no explanation for why Nx rats should be more adrenergically active than non-Nx rats. However, it is possible that by removing the kidneys, we also removed a hypotensive agent which normally would balance the adrenergic compensatory response. Medullipin, an arachidonic acid metabolite released by the kidney and which functions through the release of NO (Gothberg & Karlstrom, 1991), may be the responsible agent.

As in previous studies (Rees *et al.*, 1990; O'Shaughnessy *et al.*, 1992), the present study showed that inhibition of NO-formation resulted in an elevated basal BP. It has therefore been suggested that basal release of NO may play an important role in BP regulation as a hypotensive system, similar to the role of the sympathetic nervous system in maintaining a vasoconstrictor tone (Figure 8). Since the response to L-NAME was not altered by pretreatment with phentolamine as demonstrated in the present study, the two systems under basal conditions seem to play a role in BP homeostasis independent of each other.

The acute hypotensive effect of bradykinin has also been reported to be mediated through synthesis of prostaglandin E_2 (PGE_2) and the duration of the hypotensive response by PGI_2 synthesis (Türker *et al.*, 1982). However, our experimental setup was identical to that of Rees *et al.* (1990), except that in their studies all rats were pretreated with indomethacin, and the hypotensive response to bradykinin as observed by Rees *et al.* (1990) was similar to that observed by us. Prostaglandins are therefore not likely to play a role in the observed hypotensive effect of bradykinin.

In conclusion, in spite of previous suggestions, the present results clearly demonstrate that NO is not a mediator of kinin-induced hypotension. Thus the mediator for the hypotensive action of kinins remains unknown. It further shows the importance of NO-formation in impeding the small, although significant, compensatory adrenergic hypertensive response following the acute fall in BP. The present study also demonstrates the importance of converting enzyme/kininase II as the major agent in terminating bradykinin-induced hypotension.

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References

AISAKA, K., GROSS, S.S., GRIFFITH, O.W. & LEVI, R. (1989). L-arginine availability determines the duration of acetylcholine-induced systemic vasodilatation in vivo. *Biochem. Biophys. Res. Commun.*, **163**, 710–717.

BOGLE, R.G., COADE, S.B., MONCADA, S., PEARSON, J.D. & MANN, G.E. (1991). Bradykinin and ATP stimulate L-arginine uptake and nitric oxide release in vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **180**, 926–932.

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

CACHOFEIRO, V., SAKAKIBARA, T. & NASJLETTI, A. (1992). Kinin, nitric oxide, and the hypotensive effect of Captopril and Ramiprilat in hypertension. *Hypertension*, **19**, 138–145.

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

ERDÖS, E.G. (1979). Bradykinin, kallidin and kallikrein. In *Handbook of Experimental Pharmacology* ed. 2, Vol. 25, pp. 427–448. Berlin: Springer-Verlag.

FELDBERG, W. & LEWIS, G.P. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. *J. Physiol.*, **171**, 98–108.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

GOTHBERG, G. & KARLSTROM, G. (1991). Physiological effects of the humoral renomedullary antihypertensive system. *Am. J. Hypertens.*, **4**, (10 Pt 2), 569S–574S.

ISHIDA, H., SCILI, A.G. & CARRETERO, O.A. (1989). Role of angiotensin converting enzyme and other peptidases in vivo metabolism of kinins. *Hypertension*, **14**, 322–327.

JOHANSEN, L., BERGUNDHAUGEN, H. & BERG, T. (1987). Rapid purification of tonin, esterase B, antigen gamma and kallikrein from rat submandibular gland by fast protein liquid chromatography. *J. Chromatog.*, **387**, 347–359.

KAUSER, K. & RUBANYI, G.M. (1992). Bradykinin-induced, N^{A}-nitro-L-arginine-insensitive endothelium-dependent relaxation of porcine coronary arteries is not mediated by bioassayable relaxing substances. *J. Cardiovasc. Pharmacol.*, **20** (Suppl. 12), 101–104.

KELM, M. & SCHRADER, J. (1988). Nitric oxide release from the isolated guinea pig heart. *Eur. J. Pharmacol.*, **155**, 317–321.

KIOWSKI, W., LINDER, L., KLEINBLOESEM, C., VAN BRUMMELEN, P. & BÜHLER, F.R. (1992). Blood pressure control by the renin-angiotensin system in normotensive subjects. Assessment by angiotensin converting enzyme and renin inhibition. *Circulation*, **85**, 1–8.

ØRSTAVIK, T.B., CARRETERO, O.A., JOHANSEN, L. & SCICLI, A.G. (1982). Role of kallikrein in the hypotensive effect of captopril after sympathetic stimulation of the rat submandibular gland. *Circ. Res.*, **51**, 385–390.

O'SHAUGHNESSY, K.M., NEWMAN, C.M. & WARREN, J.B. (1992). Inhibition in the rat of nitric oxide synthesis in vivo does not attenuate the hypotensive action of acetylcholine, ATP or bradykinin. *Exp. Physiol.*, **77**, 285–292.

PALMER, R.M.Jan., ASHTON, D.S. & MONCADA, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **333**, 664–666.

PELLACANI, A., BRUNNER, H.R. & NUSSBERGER, J. (1992). Antagonizing and measurement: Approaches to understanding of hemodynamic effects of kinins. *J. Cardiovasc. Pharmacol.*, **20** (Suppl. 9), 28–34.

REES, D.D., PALMER, R.M.J., SHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746–752.

TÜRKER, R.K., ERCAN, Z.S., ERSOY, A. & ZENGIL, H. (1982). Inhibition by nicotine of the vasodilator effect of bradykinin: evidence for a prostacyclin-dependent mechanism. *Arch. Int. Pharmacodyn.*, **257**, 94–103.

WHITTLE, B.J.R., LOPEZ-BELMONTE, J. & REES, D.D. (1989). Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. *Br. J. Pharmacol.*, **98**, 646–652.

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