Effects of variations in sodium intake on the acute vasodepressor response to kininase II inhibition in rats with mild two-kidney, one-clip hypertension

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Summary

1. Unilateral renal artery constriction in rats maintained on a sodium-deplete, but not sodium-replete, diet induced an augmented acute vasodepressor response to kininase II inhibition produced by an intravenous injection of the dipeptidyl carboxypeptidase inhibitor captopril (250 μg) during continuous saralasin-induced angiotensin II blockade (10 μg/min). Dietary sodium restriction alone in sham-operated rats had no effect.

2. Acute bilateral adrenalectomy (18-24 h) did not preclude the demonstration of an augmented response to kininase II inhibition in sodium-depleted rats with benign two-kidney, one-clip hypertension. Neither did chronic administration of deoxycorticosterone acetate in intact rats elicit an augmented response.

3. The augmented acute vasodepressor response to kininase II inhibition in sodium-depleted rats with benign two-kidney, one-clip hypertension is probably due to bradykinin potentiation and secondary to an increased activity of the kallikrein–kinin system. The mechanism responsible for this apparent increase is not known, but neither hyperangiotensinemia nor hyperaldosteronism seems to play a role.

Key words: captopril, kininase II, mineralocorticoids, saralasin, sodium.

Introduction

We previously demonstrated that conscious rats with benign, but not malignant, two-kidney, one-clip hypertension exhibit an augmented vasodepressor response to kininase II inhibition produced by an acute intravenous injection of the dipeptidyl carboxypeptidase inhibitor captopril during continuous saralasin-induced angiotensin II blockade (Lee, Kushiro, Gassia, Girolami, Lupu & Maxwell, 1979). These studies were conducted in rats subjected to prior dietary sodium restriction to challenge the kallikrein–kinin system (Wong, Talamo, Williams & Colman, 1975). It was also observed that although some of the rats did not develop significant hypertension, despite having a constricting clip (0.25 mm internal diameter) on one renal artery, they nonetheless exhibited a similarly augmented response to kininase II inhibition, suggesting the possibility that an augmented kallikrein–kinin system may have contributed to the defence of normotension.

As a logical sequel, experiments were therefore undertaken to investigate whether mild or marginal hypertension produced by a lesser degree of unilateral renal artery constriction may be consistently associated with an augmented response to kininase II inhibition and whether variations in dietary sodium intake would affect this response. In addition, since mineralocorticoid excess increases and adrenalectomy decreases renal kallikrein excretion in rats (Geller, Margolius, Pisano & Keiser, 1972), the effects of kininase II inhibition on the blood pressure of rats treated with deoxycorticosterone acetate and of sodium-depleted, two-kidney, one-clip hypertensive rats subjected to acute bilateral adrenalectomy were also studied to assess whether secondary hyperaldosteronism may be responsible for the augmented vasodepressor response to kininase II inhibition seen in sodium-depleted rats with benign two-kidney, one-clip hypertension.

Methods

Male Sprague–Dawley rats with initial body weights of 170–200 g were used. They were
maintained either on a low sodium diet (Lonalac, Mead Johnson) or on a normal sodium diet of standard rat chow (Purina). All rats were studied at 4–5 weeks postoperatively. The groups are as follows.

Group 1. Two groups of rats were subjected to sham operation and two groups to unilateral renal artery constriction by use of solid silver clips of 0.30 mm internal diameter. One group of each was maintained on the low sodium diet and the other on standard rat chow.

Group 2. One group of rats was subjected to unilateral renal artery constriction with clips of 0.25 mm internal diameter and maintained on the low sodium diet. Bilateral adrenalectomy was performed at 18–24 h before study.

Group 3. One group of rats received subcutaneous implants of deoxycorticosterone acetate (DOCA, 125 mg pellet) and another was subjected to the operative procedure only. Both were maintained on standard rat chow.

On the day before study, one cannula in the carotid artery and two cannulae in the same jugular vein were implanted and exteriorized between the scapulae, respectively, for measuring mean arterial pressure and for facilitating the administration of drugs with minimal disturbance of the animal. All operative procedures were performed under ether anaesthesia and the studies were conducted with the animals in the conscious, unrestrained state.

Kininase I inhibition was produced as previously described (Lee et al., 1979), by injecting an intravenous bolus of 250 μg of SQ 14 225 (captopril, Squibb) in 0.25 ml of 5% glucose solution after stabilization of blood pressure during continuous angiotensin II blockade produced by saralasin infusion (10 μg/min). This dose of SQ 14 225, when superimposed on an intravenous infusion of bradykinin (4 μg/min), markedly potentiated the vasodepressor effects of the infused bradykinin (–15 ± 5.0 vs –88 ± 15.2 mmHg, n = 6), and the infusion dose of saralasin completely prevented the pressor response to 360 ng of angiotensin II.

Results

Effect of variations in dietary sodium intake in 0.30 mm-clipped rats (Fig. 1)

Unilateral renal artery constriction with clips of 0.30 mm internal diameter produced modest increases in mean arterial pressure, but did not alter the vasodepressor response to saralasin-induced angiotensin II blockade in rats maintained on a normal sodium diet, nor did it effect a greater response to saralasin in rats maintained on a low sodium diet than that effected by dietary sodium restriction alone in sham-operated rats. However, although neither dietary sodium restriction nor unilateral renal artery constriction alone had any effect, both together induced an augmented vasodepressor response to kininase II inhibition.

Effect of adrenalectomy in 0.25-clipped rats subjected to dietary sodium restriction

Bilateral adrenalectomy caused resting mean arterial pressure (93.3 ± 7.4 mmHg, n = 6) to fall toward hypotensive levels, but did not preclude the demonstration of an augmented vasodepressor response to kininase II inhibition. The magnitude of this response (–13.7 ± 4.7 mmHg) is similar to that previously seen in hypertensive rats with an unilateral 0.25 mm-clip and maintained on the same sodium-depleted diet (Lee et al., 1979).

Effect of DOCA treatment in intact rats

Saralasin infusion produced pressor responses in non-treated (n = 11) as well as in DOCA-treated (n = 12) rats and was therefore not used. However, although DOCA-treatment resulted in hypertension (121.8 ± 5.5 vs 97.6 ± 2.9 mmHg,
Although neither dietary sodium restriction nor unilateral renal artery constriction alone affected the refractoriness of blood pressure to kininase II inhibition, the augmented vasodepressor response produced by their combined effects probably reflects an increased activity of the kallikrein-kinin system and represents potentiation of bradykinin, either in the circulation or at the level of the vascular receptor. Evidence in support of this interpretation that the kallikrein-kinin system may be most likely responsible is provided by our recent observations that the vasopressor response to infusions of the estero-protease and kallikrein inhibitor aprotinin (Vogel, 1979) is also augmented in rats at an early phase of benign two-kidney, one-clip hypertension and that both of the augmented responses to kininase II inhibition and aprotinin are abolishable by acute contralateral nephrectomy (T. C. Lee, T. Kushiro, J. P. Gassia & M. H. Maxwell, unpublished work). In addition we previously reported that when rats in the malignant phase of two-kidney, one-clip hypertension failed to respond to kininase II inhibition, they also failed to respond to aprotinin infusion (Lee et al., 1979). This simultaneous lack of an effect by both procedures in non-responsive animals, as well as the renal dependency of their opposite but parallel effects in responsive animals, indicate therefore that the effects of kininase II inhibition and aprotinin on blood pressure are most probably commonly mediated via their actions on the kallikrein-kinin system. The mechanism(s) responsible for the apparent increase in the activity of the kallikrein-kinin system observed in the present study is not known, but neither hyperangiotensinaemia nor hyperaldosteronism seems to play a role, although plasma bradykinin levels have been shown to correlate positively with plasma renin activity and the level of sodium-retaining steroid (Wong et al., 1975). An augmented vasodepressor response to kininase II inhibition may reflect an increase in kinin generation, or a decrease in kininase I activity or an increase in kininase II activity. However, an augmented response to aprotinin, mediated presumably via kallikrein inhibition, would not be consistent with an increase in kininase II activity unless total kininase activity is decreased.

Regardless of the precise mechanism involved, our results are consistent with the hypothesis that the kallikrein-kinin system could exert an important antihypertensive function in two-kidney, one-clip hypertension. This function may be particularly important during dietary sodium restriction.

Dietary sodium restriction has been shown to increase plasma bradykinin concentration in normal human subjects (Wong et al., 1975; Vinci, Zusman, Izzo, Bowden, Horwitz, Pisano & Keiser, 1979). If a similar increase occurs in intact rats, it apparently was insufficient to be assessed indirectly by kininase II inhibition under the conditions of our study. That unilateral renal artery constriction had an apparently additive effect with dietary sodium restriction to result in an augmented vasodepressor response suggests the possibility that any increase in kinins produced by the mild degree of constriction used might have also been insufficient to be demonstrable in the sodium-replete state in 0-30 mm-clipped rats. Thurston & Swales (1978) reported that kininase II inhibition in pentobarbital-injected, sodium-depleted rats produced a greater fall in blood pressure than in pentobarbital-injected, sodium-loaded rats. The difference between this observation and ours could have been due to an iatrogenic effect of recent surgery and pentobarbital anaesthesia (Lee et al., 1979), but their finding is consistent with an increase in plasma bradykinin concentration which has been found during dietary sodium restriction in man (Wong et al., 1975; Vinci et al., 1979).

Urinary kallikrein excretion in two-kidney, one-clip hypertensive rats has variously been reported to be increased (Johnston, Matthews & Dax, 1976), decreased (Carretero & Sicilii, 1978; Keiser, Geller, Margullus & Pisano, 1976b) or unchanged up to 5 weeks postoperatively (Albertini, Rosas, Croxatto & Roblero, 1978). However, urinary kallikrein excretion may not necessarily or invariably reflect intrarenal kallikrein-kinin activity or secretion of renal kinins into circulation. It has been shown that enhancement of intrarenal kinin generation by renal venous constriction in dogs was accompanied by reduced urinary kallikrein excretion (Olsen, 1978) and that mineralocorticoid-induced increases in the latter had no effect on plasma bradykinin concentration (Vinci et al., 1979). Renal venous kinin output in man (Hultén, Lecerof & Hökfelt, 1977) and ureteral kallikrein excretion in dogs (Keiser, Andrews, Guyton, Margolius & Pisano, 1976a) with chronic unilateral renal artery stenosis have been found to be manyfold greater from the contralateral kidney than from the stenotic kidney, but whether these

\[ P < 0.025 \] and hypokalaemia (2.4 ± 0.2 vs 3.6 ± 0.1 mmol/l, \( P < 0.025 \)), it did not effect a greater response to captopril in treated rats (−3.6 ± 1.7 vs −1.5 ± 2.1 mmHg for DOCA-treated and non-treated groups respectively).
rates of secretion and excretion reflect relative reductions in the stenotic kidney or represent compensatory increases which might result in a net increase for the total system are not known. In rabbits, Nekrasova, Chernova, Sharapov & Kovaleva (1970) found increased kallikrein excretion from the contralateral kidney. Although the augmented effects of kininase II inhibition and aprotinin on the blood pressure of sodium-depleted rats with two-kidney, one-clip hypertension depend on the presence of an intact contralateral kidney, an augmented kallikrein–kinin system in the non-stenotic kidney would be consistent but may not be necessary.

In apparent contrast to our findings, Thurston & Swales (1978) reported that the vasodepressor response to kininase II inhibition was reduced in sodium-depleted rats with two-kidney, one-clip hypertension. However, using a 0.20 mm clip as did these investigators we recently found that the responses both to kininase II inhibition and to aprotinin fell progressively from initial augmented levels at 2 weeks to insignificant levels at 4 weeks postoperatively (T. Kushiro, T. C. Lee, J. P. Girolami & M. H. Maxwell, unpublished work), when malignant hypertension developed (Lee et al., 1979). An unknown protein found in a purified fraction of rat kidney extract and capable of stimulating kallikrein excretion was reported by Croxatto, Silva & Boric (1979). The physiology of this protein is not yet known. However, since increased kallikrein excretion by the non-stenotic kidney has been reported (Nekrasova et al., 1970), the possibility that this protein may serve as a humoral signal to the contralateral kidney after unilateral renal artery constriction is interesting.

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