Biochemical Pharmacology, Vol. 36, No. 14, pp. 2331–2335, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

EFFECTS OF ANGIOTENSIN I CONVERTING ENZYME INHIBITION AND CALCIUM CHANNEL BLOCKADE ON PLASMA LEVELS OF ACTIVE AND INACTIVE RENIN IN CONSCIOUS RABBITS

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(*Received* 3 *October* 1985; *accepted* 10 *February* 1987)

Abstract—The interplay of juxtaglomerular (jg) calcium fluxes and exposure to AII in the regulation of jg renin secretion, was examined *in vivo*. An inhibitor of angiotensin I converting enzyme (captopril), a blocker of calcium channels (verapamil) and AII amide were infused, singly or in combination, into the ear vein of conscious rabbits. The effects on arterial pressure, and on levels of active and inactive plasma renin were monitored. Captopril ($50\ \mu g \cdot min^{-1} \cdot kg^{-1}$) produced a greater percentage increase in renin secretion than did verapamil ($20\ \mu g \cdot min^{-1} \cdot kg^{-1}$), whilst the percentage fall in arterial pressure was similar. AII amide counteracted more effectively the actions of captopril than those of verapamil. When captopril was infused first, addition of verapamil did not enhance renin secretion ($P > 0.2$). When verapamil was infused first, addition of captopril greatly enhanced renin secretion ($P < 0.01$). However, when captopril was infused first, and its actions then suppressed by AII amide, addition of verapamil led to extremely high rates of renin secretion.

The findings suggest the following: (1) the short loop negative feedback plays *in vivo* an important role in the rapid modulation of jg renin secretion and the action of AII may involve up- and down-regulation at the receptor and/or post-receptor level; (2) infused agents have rapid access to the critical sites of jg cells; (3) exposure to raised concentrations to AII not only reduces the effectiveness of AII, but also enhances jg secretory responses to lack of AII, as well as to calcium channel blockade. Thus, at least some of the jg calcium channels appear to respond both to AII and to blockers; (4) extreme changes in the levels of active renin are possible without changes of inactive renin levels. Secretion of the latter may be under separate control, or its secretion rate parallelled by the rate of its activation.

Angiotensin II (AII) depresses renin (EC 3.4.23.15) secretion by raising systemic arterial pressure and renal perfusion pressure, by expanding the extracellular fluid, and by acting directly on the juxtaglomerular (jg) cells. The depressant effects may be modulated by other actions of AII, such as facilitation of sympathetic neurotransmission [1], actions on sodium and/or chloride flux across tubular cells [2, 3], and enhanced release of eicosanoids [3]. Numerous studies in isolated kidneys and renal tissues [3–8] suggest that among the AII effects on renin secretion, the direct action on the jg cells is the dominant component, at least in the minute to minute control of the secretion rate. Interaction of AII with its receptors on jg cells depresses renin secretion by increasing the stretch of the walls of afferent arterioles, and by enhancing calcium influx through the cell membrane [3–8]. The association of raised calcium in the jg cytosol with reduced secretory activity is a rare pattern in stimulus-secretion coupling, which jg cells share with parathyroid cells. It is not yet certain whether the post-receptor pathways, such as phosphoinositide turnover, which respond to AII in other cells [9, 10], are also stimulated in jg cells.

Conflicting views have been expressed as to the type of channel through which AII influences calcium flux. Churchill [6, 7] postulates that AII raises jg cytosol calcium by a mechanism independent of the voltage-operated channels affected by verapamil-type blockers. However, Fray *et al.* [5] found that these blockers effectively counteracted AII and concluded that both agents acted on the same channels.

The direct actions of AII on jg renin secretion are usually referred to as the short loop negative feedback (SLNF) because it is postulated that they are mainly due to locally formed AII. Juxtaglomerular cells contain significant concentrations not only of renin, but also of its substrate, of angiotensin I converting enzyme (ACE) and of angiotensin II itself [8]. Therefore, the short loop may be even shorter in that the crucial AII may originate and act in the cells themselves. This may be important because jg cells appear to secrete much more renin into the surrounding interstitium than into the afferent arteriole so that the bulk of renin enters the vascular system only at the level of the peritubular capillaries [8]. Furthermore, jg cells not only secrete renin, but also take it up—a process which may inhibit renin secretion [11]. Such feedback inhibition by renin itself would, of course, supplement the depressant action of AII.

We report *in vivo* observations on the interplay between jg calcium flux and jg exposure to AII in

the functioning of the SLNF. To study these links, the following agents were infused, singly or in combination, into the ear vein of conscious rabbits: (a) captopril which inhibits ACE and thereby induces high concentrations of plasma renin and low or absent levels of AII; (b) verapamil which blocks the influx of calcium into cells and thereby raises both plasma renin and AII; (c) angiotensin II amide. Their actions were assessed by monitoring the arterial pressure, and the plasma concentrations of active and inactive renin, and of ACE.

Some of the present results were reported at two international congresses [12, 13].

MATERIALS AND METHODS

The following materials were used: angiotensin I (kindly supplied by Dr D. R. Bangham, National Institute for Biological Standards, London NW3 6RB, U.K.); ^{125}I-labelled angiotensin I (Centre National de Transfusion Sanguine, Paris, Ets Orsay Les Ullis, France); angiotensin II amide (Hypertensin, Ciba); captopril (pure substance, kindly given by E. R. Squibb & Sons, Princeton, NJ); verapamil (ampoules Cordilox, Abbott Laboratories); angiotensinogen-rich plasma from nephrectomized sheep was prepared by Dr Fiona Broughton-Pipkin.

Conscious rabbits were fasted overnight. They were lightly tranquillized with diazepam ($0.2\,mg \cdot kg^{-1}$ i.m.), and placed unrestrained into open cardboard boxes with access to lettuce and water. Small areas of skin were anaesthetized with lignocaine, and plastic cannulae (outer diameter 0.63 or 0.75 mm) inserted through injection cannulae into the ear vein and artery for infusion of drugs, sampling of blood and monitoring of blood pressure. After a dose of $300\,U \cdot kg^{-1}$ of heparin i.v., $170\,\mu l \cdot min^{-1}$ of saline plus $0.6\,U \cdot kg^{-1} \cdot min^{-1}$ of heparin was infused. Drugs were added to this infusion as specified. Infusions of captopril were initiated by a loading dose of $1\,mg \cdot kg^{-1}$, and of verapamil by a loading dose of $70\,\mu g \cdot kg^{-1}$. Plasma samples were stored at −120° for assays of ACE, and at −20° for all other assays. Plasma renin activity (PRA; rate of AI formation by the endogenous plasma renin and angiotensinogen) was measured by a miniature version of a radioimmunoassay (RIA) of generated AI [14]. AI formation was linear over 2 hr with PRA values of $1–70\,pmol \cdot hr^{-1} \cdot ml^{-1}$. The mean intra-assay coefficient of variation (CV) was 6.6 and 5.2% for PRA measurements of 4 and $28.5\,pmole \cdot hr^{-1} \cdot ml^{-1}$, respectively (both N = 5). Plasma renin concentration (PRC) was measured in the same way as PRA, but in the presence of sheep angiotensinogen (1.4 nmole per ml of rabbit plasma). To measure total renin concentration (TRC), the inactive renin in rabbit plasma was activated as described by Osmond and Cooper [15], but exposure to trypsin was increased until maximal activation was obtained. This was achieved by incubating with trypsin ($1\,mg \cdot ml^{-1}$) at 25° for 30 min, and then adding $1\,mg \cdot ml^{-1}$ of soya bean trypsin inhibitor. TRC was then measured as described for PRC. AI formation was linear over 2 hr in PRC and TRC assays ranging from 7 to $150\,pmole \cdot hr^{-1} \cdot ml^{-1}$. The CV of PRC was 7.3%, and of TRC 5.8% (N = 24). Inactive plasma renin concentration (IPRC) was calculated as TRC − PRC. All RIA of AI were carried out with duplicate aliquots and analysed with a computer program developed by Dr G. Malan at this Medical School, which analyses the results by constructing a model based on the established error-profile of the assay. ACE activity was measured as rate of hydrolysis of hippuryl-L-histidyl-L-leucine [16]. Arterial pressure at 45 min of an infusion period was calculated as the average of the pressure at 42.5, 45 and 47.5 min.

Assays were carried out on blood samples collected at 40 min and at the end (50 min) of an infusion period, except when stated otherwise. During infusions of captopril and/or verapamil, PRA and PRC levels were usually still rising between 40 and 50 min, but values differed by less than 15%. Results at 40 and 50 min were therefore combined and their average used. To minimize the blood volume required, some assays were not carried out in all samples. The volume of fluid infused in the course of one experiment, exceeded the volume of blood collected by about 30%; the haematocrit fell by 5–10%. The limitations imposed by the use of non-anaesthetized animals were accepted to avoid the potent effects of anaesthetics on renin secretion. The significance of differences was estimated by Student's *t*-test for paired or unpaired samples as appropriate.

RESULTS

Captopril ($50\,\mu g \cdot min^{-1} \cdot kg^{-1}$) promptly inhibited ACE. After 5 min of infusion, no ACE activity was detectable in serum by fluorimetry and extremely high bolus doses of AI (up to $10\,\mu g \cdot kg^{-1}$ i.v.) had absolutely no effect on the blood pressure. This indicated that both serum and cell-bound ACE were effectively inhibited by the infused captopril and that circulating AII was very low or absent. The increase in PRA was highly significant ($P < 0.005$). It was effectively suppressed by infused AII amide, $60\,ng \cdot min^{-1}$ clearly ($P < 0.01$) reducing the raised PRA (Fig. 1a). The changes in PRC closely resembled those of PRA. The low IPRC ($3–10\,pmole \cdot ml^{-1}$) did not change significantly. The reduction in mean arterial pressure (MAP) after 45 min of captopril infusion was 17.8 ± 8.3 mm Hg.

When renin secretion is stimulated by blockade of calcium channels, the enhanced formation of AI and its conversion to AII proceeds without interference and high circulating AII levels must be assumed. The rise in PRA (Fig. 1a) produced by verapamil was less pronounced ($P < 0.05$) than that seen with captopril. MAP was reduced by 21.4 ± 5.4 mm Hg. Exogenous AII amide reversed the actions of verapamil less effectively than those of captopril (Fig. 1a); the raised PRA was not significantly reduced by 60, 120 or $180\,ng \cdot min^{-1}$ ($P > 0.1$).

The difference between the actions of captopril and verapamil emerged more clearly when the individual changes in each rabbit were considered. Captopril increased PRA by 918 ± 350% (mean ± SD) above the control value in the same rabbit, and verapamil by 456 ± 252%; the difference was significant ($P < 0.05$). No such difference was seen

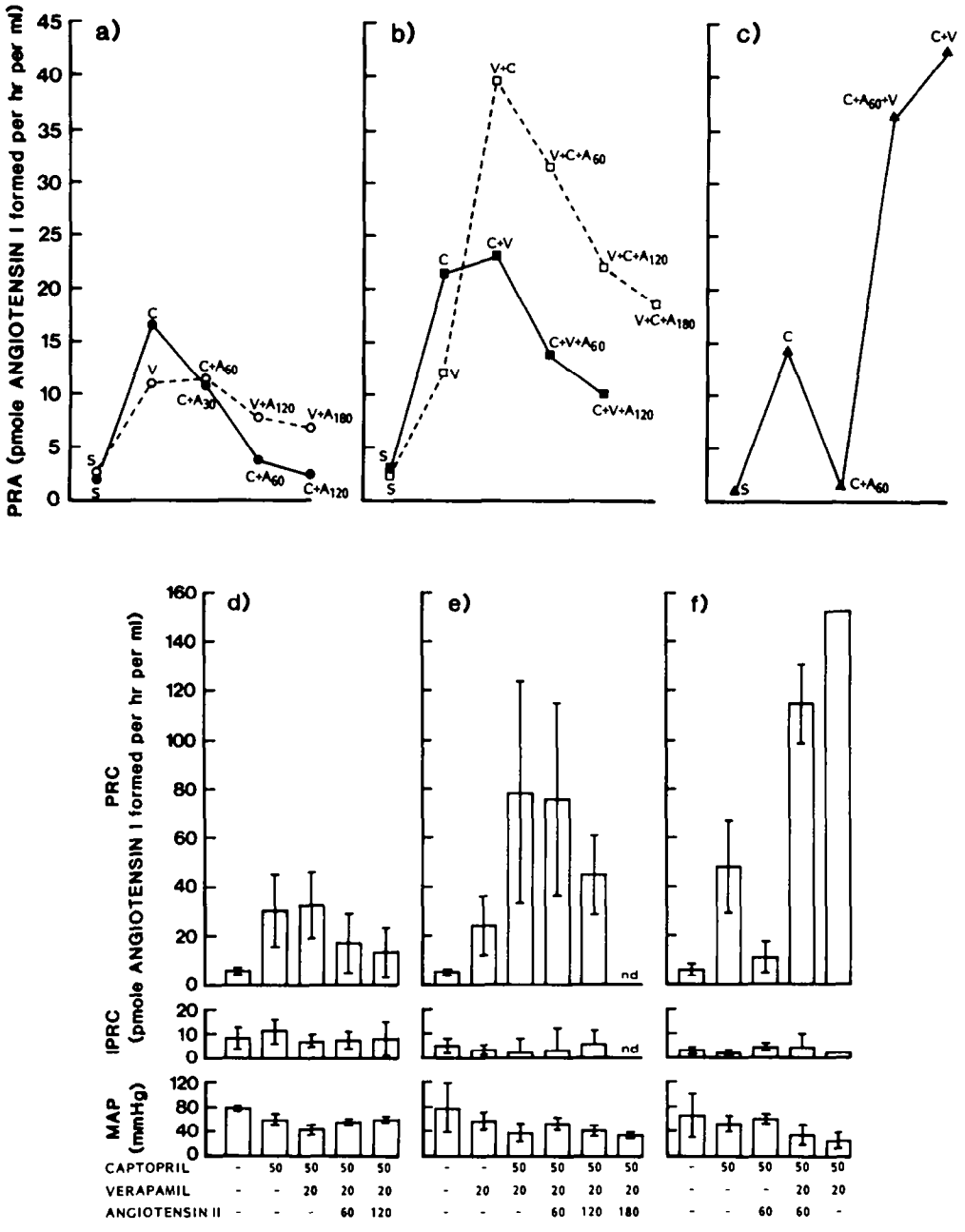

Fig. 1. Effect of captopril (C), verapamil (V) and angiotensin II amide (A) on plasma renin and blood pressure.

(a, b, c) C (50 $\mu g \cdot min^{-1} \cdot kg^{-1}$), V (20 $\mu g \cdot min^{-1} \cdot kg^{-1}$) and A (subscript gives dose in $ng \cdot min^{-1}$) were infused singly or in combination. Each symbol gives the mean plasma renin activity (PRA) averaged from the values at 40 min and at the end (50 min) of an infusion period (see Methods). C, V and A are shown in the order in which they were added to the saline (S) infusion. Number of rabbits N = 6 in (a), 6 in (b) and 4 in (c).

(d, e, f) Concentrations of active and inactive plasma renin (PRC and IPRC): each column shows mean ± SD of values found at 40 and 50 min of an infusion period, as described for PRA in (a, b, c). Mean arterial pressure (MAP): mean ± SD of combined measurements at 42.5, 45 and 47.5 min of an infusion period. The doses of C and V are given in $\mu g \cdot min^{-1} \cdot kg^{-1}$ and A in $ng \cdot min^{-1}$. SD was not calculated when N of blood samples was less than 4. Nd = not done.

(a) ●——●, infusion of C, then addition of rising concentrations of A; ○- - - -○, infusion of V, then addition of rising concentrations of A. (b) ■——■, infusion of C to which first V, and then rising concentrations of A were added. PRC, IPRC and MAP are shown in (d); □- - - -□, infusion of V to which first C, and then rising concentrations of A were added. PRC, IPRC and MAP are shown in (e). (c) ▲——▲, infusion of C to which first A (60 $ng \cdot min^{-1}$) and then V was added. Finally, A was omitted. PRC, IPRC and MAP are shown in (f).

in the reduction of the MAP (24 ± 8% and 27 ± 8%, respectively; $P > 0.1$).

Since both captopril and verapamil increase PRA and PRC, but have opposite effects on plasma AII, combined administration was used to examine, *in vivo*, whether even short-lasting changes in circulating AII influenced the secretory responsiveness of jg cells. When captopril was infused first and verapamil added later (Fig. 1b, solid line; Fig. 1d), AII formation was inhibited from the beginning. The combination captopril + verapamil therefore acted after and during a phase of low or absent circulating AII. As Figs 1b and 1d show, the addition of verapamil did not further increase the PRA and PRC levels ($P > 0.2$) initially raised by captopril.

When the initial hyperreninaemia was produced by verapamil (Fig. 1b, broken line; Fig. 1e), it would have been associated with high circulating levels of AII. When the endogenous AII formation was then inhibited by adding captopril to the infusion, PRA and PRC increased clearly ($P < 0.01$), and exceeded the PRA and PRC produced by the same combination of drugs, but infused in the order captopril first, and then added verapamil. The difference was significant for PRA ($P < 0.05$) and for PRC ($P < 0.01$). In contrast, the fall in MAP produced by the addition of the second drug was similar ($P > 0.1$) with either order of drug administration (Figs 1d and 1e).

Infused AII amide depressed both the greater and the smaller secretory responses induced by captopril plus verapamil, by a similar amount; the percentage reduction of the lower secretory response (captopril first, and then added verapamil) was therefore far greater.

The differences in PRA and PRC observed when captopril and verapamil were infused in different orders suggested that *in vivo* a short exposure of jg cells to high concentrations of AII may enhance subsequent secretory responses. To test this possibility, the experiment shown in Fig. 1b (solid line) and Fig. 1d was modified. Endogenous AII was abolished by captopril as before, but a dose of AII amide was then infused which almost restored PRA, PRC and blood pressure to pre-captopril levels (Figs 1c and 1f). Calcium channels were now blocked with verapamil which dramatically raised PRA and PRC to levels above those produced by captopril alone ($P < 0.01$). This was in clear contrast to the lack of enhancement seen on addition of verapamil in Fig. 1b (solid line) and Fig. 1d; moreover, the absolute values of PRA and PRC were much higher ($P < 0.05$ and $P < 0.01$, respectively) in the modified experiment, despite the continued infusion of AII amide. Discontinuation of AII amide appeared to raise PRA and PRC even further.

The levels of IPRC associated with the very high PRA and PRC were low, but the apparent fall in IPRC was not significant ($P > 0.1$).

DISCUSSION

The present evidence that infused AII amide promptly inhibits renin secretion and infused captopril and verapamil promptly impair the short loop negative feedback, suggests that agents arriving in the renal artery have ready access to the critical sites in the jg apparatus. However, the part played by AII formed inside jg cells [8] requires further study.

Some of the systemic and local factors (see Introduction) which could contribute to the observed rapid changes in the secretory responsiveness of jg cells, are difficult to assess in experiments in conscious rabbits, and the present study allows only limited conclusions as to their relative importance. The role of systemic arterial pressure appears to be subject to modulation by other influences. This was suggested by the finding that doses of captopril and verapamil which produced similar falls in blood pressure led to very different secretory responses. As systemic blood pressure is an important determinant of renal perfusion pressure, the role of the latter may also be subordinate to other regulatory mechanisms. An important pointer to the mechanisms involved may be seen in the finding that changes in jg responsiveness induced by AII outlasted the actual presence of AII: periods during which the endogenous circulating AII ($t_{\frac{1}{2}}$ only 1–4 min) was raised by verapamil were followed by phases of enhanced secretory responses to lack of AII; infusion of exogenous AII amide enhanced the response to calcium channel blockade (Figs 1c and 1f). This extension of AII effects beyond the actual exposure to AII suggested that actions on AII receptors and/or post-receptor mechanisms contributed to the observed changes in jg responsiveness. Although only much slower changes in jg responsiveness have been reported *in vivo* [17], reductions in AII binding capacity of cultured rat artery muscle cells have been found after only 5 min of exposure to AII [18]. Reduced responses of blood vessels after exposure to AII have been attributed to prior occupancy of a proportion of the receptors and/or to a decrease in the affinity or number of AII receptors [19–21]. No distinction between these possibilities can be made on the basis of the present *in vivo* experiments, even though it appears plausible that AII actions at the receptor or post-receptor level contributed to the results. If the observed changes in jg responsiveness are due to modulation of AII receptors, the latter are likely to be located—like most receptors for peptides—on the cell surface, and AII is probably able to change their number or affinity without transduction by altered calcium fluxes or by other second messenger mechanisms. The nature of the post-receptor processes which may have contributed to the observed changes in jg responsiveness, requires further study. Changes in cytosol calcium levels are unlikely to be the responsible factor: enhanced secretory responses were observed at a time when plasma AII and its promotion of calcium flux into jg cells, would have returned to low values. Moreover, jg responses were increased after either verapamil or exogenous AII amide, despite the opposite effects of these drugs on the free calcium in the cytosol.

The observation that after transient exposure to AII, jg secretion was enhanced not only in response to converting enzyme inhibition, but also to blockade of voltage-operated calcium channels, indicates that AII may induce a more general increase in jg reactivity. However, as verapamil is a use-dependent

blocker and more effective on open channels [22], its greater effectiveness after AII amide could partly be due to its facilitated access to channels opened by AII. Whatever the mechanism by which AII affects the action of verapamil, the present observations support the view that at least some calcium channels respond both to AII and to blockers, as postulated by Fray [5]. Actions on such a common channel may also account for the observation that AII was more effective against captopril than against verapamil (Fig. 1a). A model of a calcium channel which is linked to both a voltage gate and a receptor gate, has been proposed by Glossmann *et al.* [23]. The increased effectiveness of verapamil after jg exposure to AII also suggests that verapamil influences jg secretion mainly by actions on calcium channels, and that its effects on other receptors, on eicosanoid production and on sodium channels [24] play only a minor role.

Extreme rises in active plasma renin occurred without accompanying increases in inactive renin, or definite evidence of its enhanced activation. This appears to support the view [25] that active and inactive renin are released under separate control. Our findings would only be compatible with a common control, if changes in the rate of release of inactive renin were co-ordinated with a precisely parallel change in the rate of its activation.

Acknowledgements—This work was supported by the Medical Research Council of Great Britain. We thank the Special Trustees of the Middlesex Hospital for laboratory facilities.

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