Mechanisms of angiotensin II chronotropic effect in anaesthetized dogs

C. Lambert

Faculty of Medicine, Department of Pharmacology, Université de Montréal, C.P. 6128, Succursale Centre-Ville, Montréal, Québec, Canada H3C 3J7

1 The chronotropic effect of angiotensin II (5 µg in 1 ml of Tyrode solution), injected directly into the sinus node artery of 24 anaesthetized and vagotomized dogs pretreated with a β-adrenoceptor antagonist, was evaluated before and after the administration of: (a) an angiotensin II AT₁ receptor antagonist (losartan, 50 µg kg⁻¹ min⁻¹ infused i.v. for 120 min), (b) an α-adrenoceptor antagonist (prazosin, 1 mg kg⁻¹ i.v. bolus injected), (c) a Ca²⁺ channel blocker (nifedipine 50, 100 and 200 µg kg⁻¹ i.v. bolus injected) and (d) a protein kinase inhibitor (staurosporine, 800 nM infused via the sinus node artery at 0.6 ml min⁻¹ for 15 min).

2 Losartan and staurosporine by themselves had no effect on basal systemic arterial pressure and heart rate, whereas prazosin and nifedipine caused significant diminutions of both parameters.

3 Angiotensin II induced significant increases in heart rate, the mean augmentations being 29 ± 2 beats min⁻¹. Losartan, nifedipine and staurosporine significantly decreased the chronotropic effect of angiotensin II, the mean respective diminutions being 65 ± 8, 40 ± 9 and 64 ± 10%, whereas prazosin had no effect.

4 This work has demonstrated that angiotensin II exerts *in vivo* a significant positive chronotropic effect that is mediated via AT₁ receptors located in the region of the sinoatrial node. This effect is independent of the adrenergic system. It is decreased by the inhibition of the production of protein kinases, most probably of protein kinase C, and by the blockade of the voltage-sensitive L-type Ca²⁺ channels. Other studies are obviously needed to ascertain the role of angiotensin II in the control of heart rate and/or the genesis of arrhythmias.

Keywords: Angiotensin II; chronotropic effect; AT₁ receptors; losartan; α-adrenoceptors; prazosin; Ca²⁺ channels; nifedipine; protein kinase C; staurosporine

Introduction

Recent biochemical and functional evidence strongly suggests the presence of a renin-angiotensin system, as well as, the synthesis of angiotensin II in the heart (Lindpainter & Ganten, 1988). It has been suggested that angiotensin II may, in the normal heart, modulate coronary blood flow, inotropy, and chronotropy, whereas under pathological conditions, the cardiac renin-angiotensin system may influence or contribute to ventricular growth, postinfarction ventricular remodelling and myocardial metabolism. It may also generate ventricular arrhythmias during ischaemia and reperfusion-induced myocardial injury (Dostal & Baker, 1993).

It has been shown by this laboratory that angiotensin II has a significant positive chronotropic effect, when injected into the sinus node artery of dogs (Lambert *et al.*, 1991). It has also been reported that the stimulatory effects of angiotensin II on the contractile frequency of spontaneously beating cardiomyocytes of neonatal rats are blocked by specific angiotensin II receptor antagonists (Rogers *et al.*, 1986). These results indicate that angiotensin II can increase heart rate. Furthermore, they are in agreement with the findings that high-affinity angiotensin II binding sites are present in heart membrane preparations, as well as, in the specialized conduction system of the rat heart, where the highest concentrations were found in the sinoatrial but mainly in the atrioventricular nodes (Saito *et al.*, 1987; Sechi *et al.*, 1992).

It has been demonstrated that the binding of angiotensin II to its specific G-protein-coupled AT₁ receptors can stimulate phospholipase C in the majority of its target tissues. In turn, this results in the hydrolysis of phosphoinositides and the production of two intracellular second messengers, inositoltriphosphate and diacylglycerol. Inositoltriphosphate releases Ca²⁺ from intracellular stores and diacylglycerol increases the affinity of protein kinase C for Ca²⁺. It has also been shown that the activation of angiotensin II receptors

promotes Ca²⁺ influx either by increasing the activity of receptor-operated channels or by modulating the activity of voltage-sensitive Ca²⁺ channels (Catt *et al.*, 1993; Edwards & Ruffolo, 1994). The mechanisms implicated in the angiotensin II chronotropic effect are still unknown. The aim of the present study was to evaluate the roles of angiotensin II AT₁ receptors, of extracellular Ca²⁺ influx and of protein kinase C, in this effect. To do so, the chronotropic effect of angiotensin, injected directly into the canine sinus node artery, was evaluated before and after the administration of a specific angiotensin II AT₁ receptor antagonist (losartan), of a Ca²⁺ channel blocker (nifedipine) and of a protein kinase inhibitor (staurosporine). Since it is well recognized that an interaction exists between the renin-angiotensin system and the sympathetic nervous system, the influence of α-adrenoceptors on the angiotensin II chronotropic effect was investigated by studying the chronotropic effect of angiotensin II before and after the administration of a specific α-adrenoceptor antagonist (prazosin).

Methods

Animal preparation

Adult mongrel dogs (*n* = 24) of either sex, weighing 20 to 30 kg, were anaesthetized with sodium thiopentone (25 mg kg⁻¹, i.v.) and α-chloralose (80 mg kg⁻¹ followed by 300 mg i.v. hourly) and heparinized (200 iu kg⁻¹, i.v.). Artificial respiration was maintained with room air through an endotracheal tube by means of a Harvard pump (model 607). A right thoracotomy was performed at the level of the fourth intercostal space, the pericardium was incised and the heart was suspended in a pericardial cradle. The main sinus node artery was cannulated with a polyethylene catheter connected

to a 3-way stopcock. The sinus node arterial flow, evaluated retrogradely in all the preparations, ranged from 0.35 to 1.00 ml min⁻¹. The sinus node artery was perfused with fresh blood, withdrawn from the femoral vein, at an individualized constant flow (mean value of 0.55 ± 0.05 ml min⁻¹) using a Harvard pump (model 22) until the end of the experiment. This technical procedure has no effect on sinus node function because of extensive arterial anastomoses in the region of the canine sinus node (Nadeau & James, 1966). The left femoral artery and vein were cannulated for pressure measurements and drug injections, respectively. The animals were submitted to a bilateral cervical vagotomy and administered propranolol (1 mg kg⁻¹, i.v.). The blockade of β -adrenoceptors was ascertained in 4 dogs by the pre- and post-propranolol administration of isoprenaline (0.01 μ g kg⁻¹, i.v.). Isoprenaline produced mean increases of 40 ± 22 beats min⁻¹ before propranolol, whereas it had no effect (0 ± 0 beats min⁻¹) after administration of propranolol.

Experimental protocol

After the completion of all the surgical preparations and a 30 min period of stabilization, Tyrode solution (2 × 1 ml) was bolus injected (1 min) into the sinus node artery with an intervening 30 min interval. These control injections were followed by 2 injections of angiotensin II (5 μ g in 1 ml of Tyrode solution) also at 30 min intervals. Systolic and diastolic blood pressures and heart rate were determined.

Following these 4 baseline determinations, the animals were separated into 4 groups ($n=6$ per group) and received either: (1) losartan (DuP 753) 50 μ g kg⁻¹ min⁻¹ infused for 120 min via the femoral vein at 0.4 ml min⁻¹ (Timmermans *et al.*, 1993); (2) prazosin 1 mg kg⁻¹ bolus injected via the femoral vein (Stanaszek *et al.*, 1983); (3) nifedipine 50, 100 and 200 μ g kg⁻¹ bolus injected via the femoral vein at 30-min intervals (Talajic & Nattel, 1986); or (4) staurosporine 800 nM infused for 15 min via the sinus node artery at 0.6 ml min⁻¹ (Matsui *et al.*, 1991).

The blockade of α -adrenoceptors was ascertained in 6 dogs by the pre- and post-prazosin administration of phenylephrine (1.0 μ g kg⁻¹, i.v.). Phenylephrine produced significant mean increases in blood pressure of 21 ± 3 mmHg before prazosin, whereas it had only a slight effect (3 ± 2 mmHg) after administration of prazosin. The doses of nifedipine were based on the results of Talajic & Nattel (1986) who demonstrated that these dosages produce blood concentrations that significantly decrease arterial blood pressure and significantly increase AV conduction time, effective refractory period and Wenckebach cycle length in anaesthetized dogs. The concentration of staurosporine was based on a previous report by Tamaoki *et al.* (1986) who demonstrated *in vitro* that nanomolar concentrations of staurosporine significantly inhibit protein kinase C extracted from rat brain. However, as suggested by Matsui *et al.* (1991), in our study the concentration of staurosporine was increased to counteract the poor intracellular penetration of the drug when administered *in vivo*. Direct sinus node artery injections of angiotensin II were repeated: (1) 60 and 120 min following the beginning of losartan infusion; (2) 15 and 45 min following the bolus injection of prazosin; (3) 15 min after each bolus injection of nifedipine; and (4) immediately and 30 min after the end of staurosporine infusion. Systolic and diastolic arterial blood pressures and heart rate derived from an electrocardiogram (lead II) were recorded on a polygraph system (Ealing Scientific Ltd., model 50-9927).

Angiotensin II was injected into the sinus node artery at 30 min intervals to avoid tachyphylaxis. A previous study in this laboratory, using the same experimental model, demonstrated that under these conditions, the chronotropic effect of angiotensin I or II, is maintained for at least 150 min (Lambert *et al.*, 1991). Since the present protocols never exceeded 150 min, it was assumed that the responses to angiotensin II remained constant with the different time cycles that were used.

All the procedures for animal experimentation were done in accordance with the guidelines of the Canadian Council for Animal Care and monitored by an institutional animal care committee.

Drugs

Angiotensin II acetate salt (Sigma Chemical Co., St-Louis, Mo, U.S.A.) was dissolved in Tyrode solution. The solutions of angiotensin II were kept on ice to assure stability and warmed to 37°C immediately prior to injection. Losartan (DuP 753) potassium salt (Du Pont Medical Products, Billerica, Ma., U.S.A.) was dissolved in NaCl 0.9%. Prazosin hydrochloride (Sigma Chemical Co., St-Louis, Mo., U.S.A.) was dissolved in dimethylsulphoxide (DMSO):NaCl 0.9% (5:35 ml) immediately before use. Nifedipine (Sigma Chemical Co., St-Louis, Mo., U.S.A.) was dissolved in 2 ml of ethanol 90%. Staurosporine (Boehringer Mannheim Biochemica, Laval, Canada) was dissolved in DMSO:Tyrode solution (10 μ l:25 ml). ($\pm$)-Propranolol hydrochloride, ($\pm$)-isoprenaline hydrochloride and (-)-phenylephrine hydrochloride (Sigma Chemical Co., St-Louis, Mo, U.S.A.) were dissolved in NaCl 0.9%. DMSO and all the components of the Tyrode solution were purchased from Fischer Scientific (Montréal, Canada).

Statistics

Data are presented as the mean ± s.e.mean. Results were evaluated by Student's paired *t* tests or one-way repeated measures analysis of variance (ANOVA) and Bonferroni *t* tests. The critical level of significance was set at $P \leq 0.05$.

Results

Effects of bilateral vagotomy and blockade of β -adrenoceptors

Bilateral vagotomy and blockade of β -adrenoceptors caused a significant decrease (7 ± 3%) in basal heart rate, which remained relatively constant throughout the control period, the mean value being 115 ± 4 beats min⁻¹. Basal mean systemic arterial pressure was not affected by these manipulations, the mean value being 104 ± 4 mmHg.

Effects of Tyrode solution and angiotensin II

The injection of Tyrode solution (1 ml) into the sinus node artery of the 24 dogs caused a slight increase in heart rate with a mean augmentation of 6 ± 1 beats min⁻¹, whereas it had no effect on mean systemic arterial pressure.

The injection of angiotensin II (5 μ g in 1 ml of Tyrode solution) into the sinus node artery of the 24 dogs caused significant increases in heart rate and mean systemic arterial pressure with mean augmentations of 29 ± 2 beats min⁻¹ and 44 ± 2 mmHg, respectively.

Effects of angiotensin II AT₁ receptor antagonist, losartan

Lambert *et al.* (1994) reported, using the same experimental model in 2 dogs, that a slight increase (3 and 5%) in heart rate and a decrease (26 and 8%) in mean systemic arterial pressure were noted following an infusion of NaCl 0.9% at 2 ml min⁻¹ for 180 min. In the present study, losartan was dissolved in NaCl 0.9% and infused i.v. at 0.4 ml min⁻¹ for 120 min. Losartan had no significant chronotropic or pressor effects, the mean values being 117 ± 7, 109 ± 7 and 104 ± 7 beats min⁻¹ and, 85 ± 9, 64 ± 12 and 65 ± 6 mmHg at baseline as well as after 60 and 120 min of infusion. However, the chronotropic effect of angiotensin II was diminished (37 ± 16%) and significantly reduced (65 ± 8%) following the 60 and 120 min

Figure 1 Effects on heart rate and mean systemic arterial pressure of Tyrode solution (1 ml) and angiotensin II (AII) ($5 \mu\text{g}$ in 1 ml of Tyrode solution) bolus injected directly into the sinus node artery of anaesthetized and vagotomized dogs pretreated with a β -adrenoceptor antagonist, before as well as after the i.v. infusion of losartan ($50 \mu\text{g kg}^{-1} \text{ min}^{-1}$ for 120 min). Data are shown as mean $\pm$ s.e.mean ($n=6$). Results were evaluated by one-way repeated measures analysis of variance (ANOVA) and Bonferroni t tests. Asterisks (*) indicate significant differences from the baseline response to AII.

Figure 2 Effects on heart rate and mean systemic arterial pressure of Tyrode solution (1 ml) and angiotensin II (AII) ($5 \mu\text{g}$ in 1 ml of Tyrode solution) bolus injected directly into the sinus node artery of anaesthetized and vagotomized dogs pretreated with a β -adrenoceptor antagonist, before as well as 15 and 45 min after a bolus i.v. injection of prazosin (1 mg kg^{-1}). Data are shown as mean $\pm$ s.e.mean ($n=6$). Results were evaluated by one-way repeated measures analysis of variance (ANOVA) and Bonferroni t tests.

infusion of losartan. The systemic arterial pressor effect of angiotensin II was also affected with significant decreases of 70 ± 4 and $74 \pm 4\%$ after 60 and 120 min of losartan, respectively (Figure 1).

Effects of α -adrenoceptor antagonist, prazosin

Prazosin was dissolved in DMSO: NaCl 0.9% (5:35 ml). Preliminary studies in 4 dogs have determined that the injection of the vehicle alone had no chronotropic (131 ± 13 vs 131 ± 14 beats min^{-1}) or pressor (116 ± 4 vs 112 ± 5 mmHg) effect in our experimental model. Nor did it have any effect on the chronotropic (130 ± 3 vs 128 ± 4 beats min^{-1}) or pressor (135 ± 1 vs 134 ± 2 mmHg) effect of angiotensin II. Prazosin caused significant decreases in heart rate and mean systemic arterial pressure with mean values of 108 ± 8 and 102 ± 6 beats min^{-1} and, 104 ± 8 and 90 ± 4 mmHg before and after the bolus injection, respectively. However, as depicted in Figure 2, the α -blockade had no influence on the chronotropic or pressor effect of angiotensin II.

Effects of Ca^{2+} channel blocker, nifedipine

Nifedipine was dissolved in ethanol 90% (2 ml). Preliminary studies in 4 dogs have determined that the injection of the vehicle alone had no chronotropic (114 ± 12 vs 114 ± 12 beats min^{-1}) or pressor (78 ± 18 vs 74 ± 19 mmHg) effect in our experimental model. Nor did it have any effect on the chronotropic (136 ± 10 vs 136 ± 10 beats min^{-1}) or pressor (155 ± 9 vs 146 ± 5 mmHg) effect of angiotensin II. The bolus injections of nifedipine caused significant dose-dependent decreases in heart rate and systemic arterial pressure, the mean maximal reductions being 27 ± 11 and $30 \pm 7\%$, respectively (Table 1). As depicted in Figure 3, the Ca^{2+} channel blockade significantly reduced the chronotropic and pressor effect of angiotensin II, the mean maximal reductions being 40 ± 9 and $68 \pm 5\%$, respectively. These decreases were both dose-related.

Effects of protein kinase inhibition by staurosporine

Staurosporine was dissolved in DMSO: Tyrode solution ($10 \mu\text{l}:25 \text{ ml}$). Preliminary studies have determined that the injection of the vehicle alone, directly into the sinus node artery for 15 min at 0.6 ml min^{-1} , had no chronotropic or pressor effect, nor any effect on the chronotropic or pressor effect of angiotensin II in our experimental model. Staurosporine caused no significant effect in heart rate and mean systemic arterial pressure with mean values of 114 ± 10 and 115 ± 12 beats min^{-1} and, 111 ± 14 and 116 ± 12 mmHg before and after the 15 min infusion. The chronotropic effect of angiotensin II was significantly reduced ($64 \pm 10\%$) immediately

Table 1 Effects on heart rate and mean systemic arterial pressure of i.v. injections of nifedipine at 30 min intervals, to anaesthetized and vagotomized dogs pretreated with a β -adrenoceptor antagonist

	Baseline	50	Nifedipine ($\mu\text{g kg}^{-1}$)	
			100	200
Heart rate (beats min^{-1})	125 ± 11	119 ± 11	108 ± 12	$87 \pm 11^*$
Mean arterial systemic pressure (mmHg)	93 ± 11	$70 \pm 12^*$	$61 \pm 9^*$	$63 \pm 9^*$

Data are shown as mean $\pm$ s.e.mean ($n=6$). Results were evaluated by one-way repeated measures analysis of variance (ANOVA) and Bonferroni t tests. Asterisks (*) indicate significant differences from the baseline values.

Figure 3 Effects on heart rate and mean systemic arterial pressure of Tyrode solution (1 ml) and angiotensin II (AII) (5 µg in 1 ml of Tyrode solution) bolus injected directly into the sinus node artery of anaesthetized and vagotomized dogs pretreated with a β -adrenoceptor antagonist, before as well as immediately and 30 min after bolus i.v. injections of nifedipine (50, 100 and 200 µg kg⁻¹) at 30 min intervals. Data are shown as mean $\pm$ s.e. mean ($n=6$). Results were evaluated by one-way repeated measures analysis of variance (ANOVA) and Bonferroni t tests. Asterisks (*) indicate significant differences from the baseline response to AII.

Figure 4 Effects on heart rate and mean systemic arterial pressure of Tyrode solution (1 ml) and angiotensin II (AII) (5 µg in 1 ml of Tyrode solution) bolus injected directly into the sinus node artery of anaesthetized and vagotomized dogs pretreated with a β -adrenoceptor antagonist, before as well as immediately and 30 min after the direct infusion into the sinus node artery of staurosporine (800 nM at 0.6 ml min⁻¹ for 15 min). Data are shown as mean $\pm$ s.e. mean ($n=6$). Results were evaluated by one-way repeated measures analysis of variance (ANOVA) and Bonferroni t tests. Asterisk (*) indicates significant differences from the baseline response to AII.

after the infusion of staurosporine, but it had returned to baseline values 30 min later. The pressor effect of angiotensin II was not affected by the infusion of staurosporine (Figure 4).

Discussion

The dose of angiotensin II injected in our model (5 µg) was selected to produce a significant and reproducible positive chronotropic effect. It is similar to the doses used by other investigators in the dog (Doursout *et al.*, 1988), rat (Knappe & van Zwieten, 1988), and sheep (Lee *et al.*, 1980). The cardiac concentrations obtained after the injection of this dose of angiotensin II into the sinus node artery, are certainly many times higher than the normal endogenous plasma concentrations. Nevertheless, we believe that our findings are compatible with physiopathological roles of angiotensin II since following the discovery of local renin-angiotensin systems, it has been suggested by Dzau (1987) that tissue angiotensin concentrations might exceed those of circulating plasma and by Dostal & Baker (1993) that in the presence of the appropriate stimuli, high concentrations of angiotensin II might be achieved in the heart, which could influence cardiac function. In agreement with these propositions, Lambert *et al.* (1991) have previously reported that the injection of 5 µg of angiotensin I into the canine sinus node artery produced an increase in heart rate similar to the augmentation related to the same dose of angiotensin II. This effect of angiotensin I was almost completely blocked by the administration of captopril, an angiotensin converting enzyme inhibitor, suggesting the presence of a converting enzyme system in the region of the sinus node and moreover, that the tissue concentrations of angiotensin I obtained were in a physiopathological range.

Losartan is a potent, non-agonistic, orally active, selective and competitive angiotensin receptor antagonist with a long duration of action (Timmermans *et al.*, 1991). It shows a very high selectivity for the AT₁ receptor subtype (30,000 more than for AT₂) (Chiu *et al.*, 1990). Its effects have been studied in numerous conscious and anaesthetized, as well as, normotensive and hypertensive experimental dog models. It has been demonstrated that an i.v. infusion of 5 µg kg⁻¹ min⁻¹ of losartan does not lower blood pressure in anaesthetized dogs, but that it does block the pressor response to an i.v. bolus of angiotensin II (Chan *et al.*, 1991; Timmermans *et al.*, 1993). Our results are in agreement with these findings since, in our model, the 120 min i.v. infusion of losartan had no effect on basal heart rate, caused only a non-significant diminution in basal mean systemic arterial pressure and, significantly decreased the chronotropic and pressor effects induced by angiotensin II injected directly into the sinus node artery. In our study, the pressor effect induced by angiotensin II was affected first by losartan before the chronotropic effect. As already discussed, when angiotensin II is injected locally, its concentrations in the area of the sinus node are certainly many times higher than its systemic and vascular concentrations. Losartan is a competitive antagonist of angiotensin II receptors. Our results suggest that a significant degree of cardiac accumulation of losartan was required before it could inhibit the chronotropic effect related to the high cardiac concentrations of angiotensin II that were obtained. Our findings also strongly suggest that the activity of angiotensin II on heart rate is mediated through the AT₁ receptors. A recent report by Saavedra *et al.* (1993) supports this concept. Using quantitative autoradiography in rat heart sections, they demonstrated that binding could be totally displaced by losartan in both the sinoatrial and atrioventricular nodes, whereas it was in-

sensitive to a specific AT₂ receptor antagonist (PD 123177), again suggesting that angiotensin II receptors in the conduction system belong to the AT₁ subtype.

It is well established that the sympathetic nervous system has an important role in the control of myocardial function. For many years, β -adrenoceptors have been considered to be the exclusive adrenoceptor population through which catecholamines exert their actions on cardiac muscle. In our experiment, blockade of the β -adrenoceptors was accomplished with propranolol so that these receptors could not be implicated as mediators of any of the effects that we observed on heart rate. On the other hand, during the last 20 years, α_1 -adrenoceptors have also been identified in myocardial extracts. Furthermore, it is now recognised that these receptors could participate in the regulation of the cardiac rhythm, conduction and force of contraction by affecting different electromechanical processes under either physiological or pathological conditions (Terzic *et al.*, 1993). Even if usually, in normal adult hearts, α_1 -adrenoceptor agonists induce no effect on sinus node automaticity (Hewett & Rosen, 1985), since the α -adrenoceptors were not blocked in our study, the possibility that a direct or reflex mediated stimulation of these receptors might have contributed to or modulated the angiotensin II-induced chronotropic effect was not totally precluded.

To examine this possibility, the chronotropic effect of angiotensin II was evaluated before and after the acute i.v. administration of prazosin, a potent competitive postsynaptic α_1 -adrenoceptor antagonist (Stanaszek *et al.*, 1983). In this study, prazosin significantly decreased both basal mean systemic arterial pressure and heart rate. This is in agreement with the observation of other investigators who have reported that the arteriolar and venous vasodilatation produced by prazosin is not consistently associated with reflex tachycardia (Brogden *et al.*, 1977) and that prazosin exerts a direct negative chronotropic effect on the canine sinus node (Schwartz *et al.*, 1982). In the present experimental model, the blockade of the α_1 -adrenoceptors had no influence on angiotensin II-induced increase in heart rate, suggesting that these receptors do not directly or indirectly participate or contribute to the chronotropic effect of angiotensin II.

The chronotropic effect of angiotensin II was evaluated before and after the administration of increasing doses of nifedipine. As expected, nifedipine by itself caused dose-related significant decreases in basal mean systemic arterial pressure and heart rate. It also significantly reduced both the chronotropic and pressor effect induced by angiotensin II. It is obvious that the interaction between angiotensin II and nifedipine might be an entirely non-specific relation between a positive and a negative chronotropic agent. In the case of specific interaction, our results suggest that angiotensin II produces its effect on heart rate, at least in part, through the activation of voltage-sensitive Ca²⁺ channels of the L-type. Other studies are obviously needed to investigate the possibility that angiotensin II modulates cardiac voltage-sensitive Ca²⁺ channels of the T-type and that this modulation participates in its chronotropic effect.

The chronotropic effect of angiotensin II was also evaluated before and after the administration of staurosporine a highly potent, nonselective, inhibitor of several kinases (Xu *et al.*,

1993). Staurosporine was selected because it is the most potent inhibitor of protein kinase C available to date (Takenaka *et al.*, 1993). It was injected directly into the sinus node artery to assure that maximal concentrations were obtained at this site of action. Staurosporine had no significant effect on basal mean systemic arterial pressure and heart rate nor did it have any influence on the pressor effect induced by angiotensin II. The lack of effect of staurosporine on angiotensin II induced-increase in blood pressure was certainly related to low systemic concentrations of staurosporine due to its general blood and tissue distribution, generating non-effective or non-significant inhibitory concentrations in the vascular cells, the site of action of angiotensin II pressor effect. On the other hand, staurosporine significantly decreased the chronotropic effect generated by angiotensin II. The inhibitory effect of staurosporine was totally reversible, since the angiotensin II effect on heart rate was fully restored 30 min following the end of the administration of staurosporine. The results suggest that protein kinase C participated in the activation of Ca²⁺ channels stimulated by angiotensin II and that this second messenger had a crucial role in the mediation of its chronotropic effect. This observation is in agreement with the results of Xu *et al.* (1993) who, using neonatal rat cardiomyocytes, demonstrated a diminution in cytoplasmic Ca²⁺ concentration in response to angiotensin II in cells pretreated with protein kinase C inhibitors (staurosporine and NPC 15437). This is also consistent with the report of Chardonnens *et al.* (1990) who found that tumour promoting phorbol ester, a potent stimulator of protein kinase C, was able to modulate the cellular action of several vasoactive hormones, including angiotensin II. Nevertheless, since it is well established that staurosporine has only a limited selectivity for protein kinase C and that it strongly inhibits other protein kinases such as protein kinase A and p60^{src} tyrosine kinase (Tamaoki, 1991; Matsui *et al.*, 1991), other studies are obviously needed to confirm the specific and exclusive participation of protein kinase C in the chronotropic effect of angiotensin II.

In summary, it has been demonstrated that angiotensin II exerts *in vivo* a significant positive chronotropic effect that is mediated via AT₁ receptors located in the region of the sinoatrial node. This effect is independent of the adrenergic system. It is decreased by the inhibition of the production of protein kinases, most probably of protein kinase C, and by the blockade of the voltage-sensitive L-type Ca²⁺ channels. It is beyond the scope of this study to extrapolate the physiological and/or pathological roles of the chronotropic effect induced by angiotensin II. Further studies are obviously needed to ascertain this. However, as in many other investigations, it is very tempting to postulate that the renin-angiotensin system, via angiotensin II, is implicated in the control of heart rate and/or the genesis of arrhythmias.

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