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Modulation of noradrenaline release from rat cortical kidney slices: effects of angiotensin I and II

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Rat kidney slices were incubated with [^{3}H]-noradrenaline and placed into a superfusion chamber between two platinum electrodes. The kidney slices accumulated and stored radioactivity. In kidney slices taken from rats whose sympathetic nerve terminals were destroyed by pretreatment with 6-hydroxydopamine accumulation of radioactivity was abolished. The α_2-adrenoceptor antagonist idazoxan (0.1–1 μM) enhanced but tetrodotoxin (TTX, 1 μM) or omission of calcium from the superfusion solution abolished the stimulation induced (S–I) outflow of radioactivity. Angiotensin (A) I (3–300 nM) and AII (1–100 nM) enhanced S–I outflow of radioactivity. The effect of AI was markedly attenuated by the angiotensin converting enzyme inhibitor captopril (3 μM) and that of AII was blocked by the AII receptor antagonist saralasin (1 μM). These results suggest that the kidney slice preparation is a valid technique to study modulation of renal noradrenaline release. Endogenous noradrenaline released from sympathetic nerves in rat kidney slices activates prejunctional α_2-adrenoceptors to inhibit its own release. AII, which can also be formed locally from AI in these kidney slices, activates prejunctional AII receptors to facilitate renal noradrenaline release.

Keywords kidney noradrenaline release prejunctional receptors angiotensin

Introduction

Sympathetic nerve terminals possess prejunctionally located receptors which when activated can modulate the amount of noradrenaline released per nerve impulse (Rand *et al.*, 1980). Prejunctional modulation of renal noradrenaline release has been mainly studied in isolated kidneys of either rat (Rump, 1987) or rabbit. However, there are drawbacks with this technique. Most of the agents tested alter basal perfusion pressure or affect pressor responses to nerve stimulation and an effect of an altered tissue perfusion on the amount of noradrenaline released cannot be excluded in every case. In rat isolated kidney AI and AII constricted the renal vasculature, enhanced pressor responses to renal nerve stimulation and facilitated noradrenaline release (Rump & Majewski, 1987). The postjunctional effects may have influenced transmitter overflow. The aim of the present study was to study renal noradrenaline release and its modulation by angiotensins by a technique which is not dependent on drug induced changes of perfusion pressure—the rat cortical kidney slice preparation.

Methods

In each experiment two cortical kidney slices (0.8 mm thick, diameter 5 mm) of Wistar rats were incubated with (−)-[2,5,6-^{3}H]-noradrenaline (20 μCi ml^{-1}, 0.5 μM) for 60 min. The slices were transferred into two superfusion chambers similar to those of Mayer *et al.* (1988). Each slice

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was held between two platinum electrodes by a polypropylene mesh and superfused at a rate of 1.7 ml min^{-1} with Krebs-Henseleit solution containing corticosterone (20 μM) for 100 min. Then the superfusate was collected in 3 min fractions and there were four electrical field stimulations (S_1–S_4), 27 min apart, each at 5 Hz for 60 s with 18 mA current strength. S_1 served as a reference stimulation. Idazoxan, AI and AII was added 12 min before S_2, S_3, S_4 in increasing concentrations. The results for each individual experiment were expressed as the S–I outflow of radioactivity in S_n (S_2, S_3, S_4) as a percentage of that in S_1. In some experiments either saralasin or captopril was present throughout the whole experiment. Drug experiments were always run in parallel with the respective control experiment. For further evaluation the S_n/S_1 ratios for drug experiments were also calculated as a percentage of the ratios obtained in the parallel control experiments. Some experiments were performed in kidney slices taken from rats pretreated with 6-hydroxydopamine (50 mg kg^{-1} i.v., 24 and 48 h previously). The Krebs-Henseleit solution had the following composition (mmol l^{-1}): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 0.45; $NaCO_3$, 25; KH_2PO_4, 1.03; D-(+)-glucose, 11.1; disodium edetate, 0.067; and ascorbic acid, 0.07. The following drugs were purchased: (−)-[ring-2,5,6-^{3}H]-noradrenaline (NEN, Dreieich, FRG). 6-hydroxydopamine hydrobromide, tetrodotoxin, corticosterone, angiotensin I acetate and Sar^1-Val^5-Ala^8 angiotensin II (saralasin) (Sigma, St Louis (MO), USA). The following drugs were a generous gift: Idazoxan-HCl (Reckitt & Colman, UK), Val^5 angiotensin II (Ciba-Geigy, Wehr, FRG) and captopril (von Heyden, München, FRG).

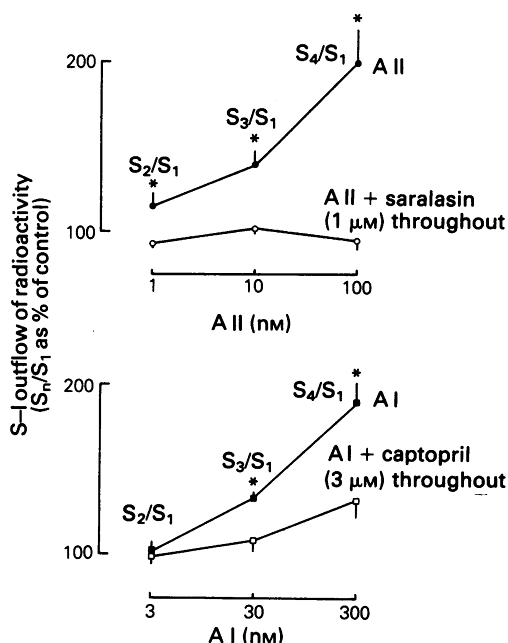

Figure 1 Effects of angiotensin (A) I and II on the S–I outflow of radioactivity from rat cortical kidney slices preincubated with [^{3}H]-noradrenaline. There were four electrical field stimulations (S_1–S_4) each at 5 Hz for 60 s. S_1 served as a reference stimulation. Either AI or AII was added 15 min after S_1 in increasing concentrations. Results are expressed as the ratios S_n/S_1 (S_2/S_1, S_3/S_1, S_4/S_1) as a percentage of the ratios of the corresponding controls. There were two sets of experiments. 1. In the absence of other drugs: AII (1–100 nM, n = 4, upper panel). In the presence of saralasin (1 μM): AII (1–100 nM, n = 5, upper panel). 2. In the absence of other drugs: AI (3–300 nM, n = 4, lower panel). In the presence of captopril (3 μM): AI (3–300 μM, n = 4, lower panel). All data are mean ± s.e. mean and were analyzed by Student's t-test. Probability levels of $P < 0.05$ were considered statistically significant. *(upper panel) indicates significant difference of AII in the presence or absence of saralasin. *(lower panel) indicates significant difference of AI in the presence or absence of captopril.

Results

The uptake of radioactivity in rat kidney slices (mean wet weight 7.7 mg, s.e. mean = 0.3, n = 36) preincubated with [^{3}H]-noradrenaline was 432220 d min^{-1} per slice (s.e. mean = 21490, n = 36). The S–I outflow of radioactivity in S_1 in the absence of other drugs was 4950 d min^{-1} (s.e. mean = 375, n = 36). Pretreatment of the rats with 6-hydroxydopamine reduced the uptake of radioactivity to 19860 d min^{-1} per kidney slice (s.e. mean = 2820, n = 4). Omission of calcium from the perfusion solution or addition of TTX (1 μM) to the perfusion solution 15 min after S_1 totally abolished the S–I outflow of radioactivity in S_2–S_4. Idazoxan (0.1–1 μM) added 15 min after S_1 in increasing concentrations dose dependently enhanced the S–I outflow of radioactivity to maximally 307.8% of control (s.e. mean = 15.7, n = 4). In the absence of other drugs either AII (1–100 nM) or AI (3–300 nM), added to the perfusion solution 15 min after S_1 in increasing concentrations, dose dependently enhanced the S–I outflow of radioactivity (Figure 1). In the presence of saralasin (1 μM) the facilitatory effect of AII was abolished and in the presence of captopril (3 μM) the facilitatory effect of AI was markedly attenuated (Figure 1).

Discussion

Rat cortical kidney slices were incubated with [^{3}H]-noradrenaline. The slices actively accumulated and stored radioactivity. In slices of rats pretreated with 6-hydroxydopamine to destroy sympathetic nerve terminals (Kostrzewa & Jacobwitz, 1974) uptake of radioactivity was almost abolished. Thus, the majority of radioactivity stored in the kidney slices was located in nerve terminals. In the superfusion chambers the slices were electrically stimulated and the S–I outflow of radioactivity in the superfusate was measured. S–I outflow of radioactivity from sympathetically innervated tissues is mainly composed of [^{3}H]-noradrenaline released from noradrenaline storage vesicles (Langer, 1970). Exocytotic release of noradrenaline is dependent on the influx of extracellular calcium (Rubin, 1970). In the present study S–I outflow of radioactivity was prevented by omission of calcium and by TTX which blocks the propagation of action potentials in axons (Narashi *et al.*, 1964). These results suggest that the S–I outflow of radioactivity from superfused cortical kidney slices can be taken as an index of noradrenaline release and this superfusion method is superior to an organ bath technique described earlier (Murphy *et al.*, 1987). Sympathetic nerve terminals possess α_2-adrenoceptors which when activated inhibit noradrenaline release (Rand *et al.*, 1980). In the rat kidney slice the α_2-adrenoceptor antagonist idazoxan markedly enhanced S–I outflow of radioactivity suggesting that under these conditions neuronally released noradrenaline inhibits its own release via inhibitory prejunctional α_2-adrenoceptors. Another prejunctional system is mediated through AII receptors. In the rat isolated kidney AII facilitated noradrenaline release and at the same time increased perfusion pressure and enhanced pressor responses to renal nerve stimulation (Rump & Majewski, 1987). The postjunctional effects of AII may have affected noradrenaline release. However, this possibility is not supported by the present study. In the rat cortical kidney slice preparation which is independent from drug induced changes in perfusion pressure AII also enhanced S–I outflow of radioactivity. This effect was blocked by the AII receptor blocking drug saralasin suggesting that facilitatory prejunctional AII receptors are present. The AII precursor AI also markedly enhanced S–I outflow of radioactivity and its effect was prevented by the angiotensin converting enzyme inhibitor captopril. This suggests that AII can be formed locally from AI in the renal cortex to activate prejunctional AII receptors. In conclusion, this kidney slice preparation is a valid technique to study modulation of noradrenaline release from sympathetic nerves in rat and possibly human kidney tissue.

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