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Differential effect of ω-conotoxin on release of the adrenergic transmitter and the vasoconstrictor response to noradrenaline in the rat isolated kidney

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1 The effects of the Ca^{2+} channel blockers ω-conotoxin (ω-CgTx), nifedipine and diltiazem, on the increase in tritium overflow and perfusion pressure elicited by renal nerve stimulation (RNS), veratridine (Vt) and potassium chloride (KCl), and on the vasoconstrictor response produced by noradrenaline (NA) were investigated in the isolated kidney of the rat perfused with Tyrode solution and prelabelled with [^{3}H]-noradrenaline ([^{3}H]-NA).

2 RNS (1–4 Hz), Vt (15–90 nmol) and KCl (150–500 μmol) produced renal vasoconstriction and enhanced the tritium overflow in a frequency- and concentration-dependent manner, respectively.

3 Administration of ω-CgTx (50 nM) inhibited RNS-, Vt- and KCl-induced overflow of tritium; the associated renal vasoconstriction produced by RNS or Vt but not by KCl was inhibited. In contrast, ω-CgTx failed to alter the vasoconstrictor response elicited by exogenous NA.

4 Infusion of nifedipine (10 μM) enhanced the tritium overflow elicited by RNS and KCl but not by Vt; a low dose of nifedipine (1.4 μM) enhanced the tritium overflow elicited by all these stimuli. Low doses of diltiazem (6 μM) failed to alter the tritium overflow produced by these stimuli. However, higher doses of diltiazem (60 μM) reduced the tritium overflow elicted by RNS or Vt but enhanced that caused by KCl. The renal vasoconstriction produced by RNS, Vt and KCl as well as by exogenous NA was inhibited by low and high doses of nifedipine and diltiazem.

5 These data suggest that (a) RNS, Vt and KCl enhance the release of adrenergic transmitter by promoting the influx of Ca^{2+} into the nerve terminal through specific Ca^{2+} channels, probably N-type Ca^{2+} channels that are distinct from those located in the vascular smooth muscle and (b) ω-CgTx could be a useful tool to differentiate between Ca^{2+} channels at the adrenergic nerve terminal and vascular smooth muscle.

Introduction

Depolarization of adrenergic nerve terminals by various stimuli including electrical stimulation of nerve fibres, veratridine (Vt) and potassium chloride (KCl) promotes release of noradrenaline (NA) in several tissues by increasing the influx of extracellular calcium (Ca^{2+}) into the nerve fibre (Hukovic & Muscholl, 1962; Kirpekar & Wakade, 1968; Kirpekar & Prat, 1979; Palaty, 1982). The pivotal role of Ca^{2+} in promoting transmitter release from adrenergic nerve terminals during depolarization has been well established (see reviews by Katz, 1969; Llinás & Heuser, 1977). The demonstration that high doses of nifedipine, verapamil, D600 or diltiazem, agents known to block slow inward Ca^{2+} current in the cardiac and vascular smooth muscles (Fleckenstein, 1977), inhibited the release of NA elicited by electrical stimulation, KCl or activation of nicotinic receptors in the rabbit isolated heart or pulmonary artery, suggests that Ca^{2+} enters the nerve terminal via voltage-sensitive Ca^{2+} channels (Starke & Schümann, 1973; Göthert *et al.*, 1979; Zelis *et al.*, 1985). However, there are several reports indicating that these agents and other Ca^{2+} channel antagonists were either ineffective or enhanced the release of NA elicited by electrical stimulation or KCl in the cat isolated heart (Haeusler, 1972), rabbit aorta (Karaki *et al.*, 1984) and ear artery (Steinsland *et al.*, 1985), and rat and guinea-pig arteries and vas deferens

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(Zsotér *et al.*, 1984; Wolchinsky & Zsotér, 1985). These observations together with the finding that high-affinity binding of [^{3}H]-nitrendipine is directly linked to inhibition of voltage-dependent Ca^{2+} uptake in smooth muscle (Bolger *et al.*, 1983), but not in rat cortical synaptosomes (Wei & Chiang, 1985), suggest that Ca^{2+} channels at adrenergic nerve terminal probably differ in various tissues and species and are also distinct from those located in cardiac and smooth muscle. Recent electrophysiological studies in the neurones of chick dorsal root ganglion and the rat superior cervical ganglion have suggested the existence of three types of voltage-sensitive Ca^{2+} channels, L, T and N-type which differ in their conductance and rate of activation and inactivation (Nowycky *et al.*, 1985; Hirning *et al.*, 1986). Moreover, L- but not T- and N-type Ca^{2+} channels are sensitive to dihydropyridines and L- and N- but not T-type Ca^{2+} channels are blocked by ω-conotoxin (ω-CgTx), a 27-amino acid peptide neurotoxin fraction isolated from *Conus geographus* venom (Nowycky *et al.*, 1985; McCleskey *et al.*, 1986). The purpose of this study was to determine the characteristics of voltage-dependent Ca^{2+} channels involved in the depolarization-induced release of adrenergic transmitter and to test the hypothesis that Ca^{2+} channels at the adrenergic nerve terminal are distinct from those located in the vascular smooth muscle. The effects of ω-CgTx and low and high concentrations of nifedipine and diltiazem on NA release elicited by renal nerve stimulation (RNS), Vt and KCl and on the renal vasoconstriction elicited by these stimuli and exogenous NA were investigated in the rat isolated kidney perfused with Tyrode solution.

Methods

All the experiments in this study were performed on male Sprague-Dawley (Harlan) rats weighing 300–350 g. Rats were anaesthetized with ether, the abdomen opened by a midline incision, and the kidney was isolated according to the procedure described by Böke & Malik (1983). In brief, the left renal artery with its sympathetic nerve plexus was carefully dissected free from the connective tissue. The aorta was ligated below and above the renal artery, and a catheter was inserted into the renal artery. The kidney was flushed with heparinized saline (100 u ml^{-1}), isolated and immediately placed in a warmed plexiglass box. The kidney was perfused with Tyrode solution at a constant flow rate of 5 ml min^{-1} by use of a Harvard peristaltic pump. The temperature of the perfusion fluid was maintained at 37°C and was bubbled with a mixture of 95% O_2/5% CO_2. The composition of the Tyrode solution (mM) was: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 12, NaH_2PO_4 0.42 and α-D(+)-glucose 5.6. Changes in the perfusion pressure were measured with a pressure transducer (Statham P_{23} ID) and recorded on a physiograph (Esterline Angus). Since the flow rate was kept constant, changes in perfusion pressure reflect alterations in renal vascular resistance.

After a 20-min stabilization period, 25 μCi of tritiated noradrenaline ([^{3}H]-NA, 1.67 pmol, 14.9 Ci mmol^{-1}) dissolved in 5 ml Tyrode solution, containing 2 mg of ascorbic acid, was infused into the renal artery over a period of 25 min with an infusion pump (Braun-Melsungen) to label the endogenous stores of the neurotransmitter. After the [^{3}H]-NA infusion and before beginning the experimental protocol, the kidney was perfused for 1 h with [^{3}H]-NA-free Tyrode solution to wash the radioactivity from the extraneural spaces. A bipolar platinum electrode was placed around the renal artery to stimulate the periarterial nerve plexus with rectangular pulses at 2 Hz (supramaximal voltage, 1 ms duration) for 1 min periods at 10 min intervals. Tritium overflow was determined by mixing 1 ml of the renal perfusate with 10 ml of Insta-gel emulsifier (Packard) and measuring the radioactivity in a liquid scintillation counter (Searle Mark III 6880).

Experimental protocol

The following three series of experiments were performed.

Series 1 This series of experiments was conducted to determine the effect of electrical stimulation of periarterial renal nerves, Vt or KCl on tritium overflow and on the vascular tone in kidneys perfused with normal Tyrode solution. Renal nerves were stimulated for 1 min at 1, 2, 3 and 4 Hz at 10 min intervals in a random order with supramaximal biphasic rectangular pulses (30–40 V) of 1 ms duration delivered by a Grass Stimulator (Model S_{44}). In another two groups of kidneys, Vt (15–90 nmol) or KCl (150–500 μmol) or their respective vehicles was injected as a bolus at 10 min intervals in a random order into the side port of the tubing leading to the renal artery. Samples of the renal perfusate were collected for 1 min periods immediately before and during RNS at each frequency of stimulation or for 2 min periods before and immediately after the administration of each dose of Vt, KCl or their respective vehicles. In an additional series of experiments, perfusate samples were collected immediately before and during RNS (2 Hz) or after administration of Vt (90 nmol) or KCl (500 μmol); [^{3}H]-NA and its tritiated metabolites were separated by alumina and ion exchange column chromatography as described by Graefe *et al.* (1973). Changes in the basal pressure

as well as the rise in perfusion pressure and tritium overflow elicited by RNS, Vt and KCl were determined.

Series 2 The purpose of this series of experiments was to examine the effects of the Ca^{2+} channel blockers, ω-CgTx, nifedipine and diltiazem on tritium overflow and vascular responses elicited by RNS, Vt and KCl. The experimental protocol consisted of two periods separated by a 10 min interval. In the first period (P_1) the kidney was perfused with normal Tyrode solution. During the second period (P_2), ω-CgTx (50 nM), nifedipine (1.4 and 10 μM), diltiazem (6 and 60 μM) or their respective vehicles was infused into the kidney after the collection of perfusate samples in P_1 and the infusion of these agents was maintained during P_2. Renal nerves were stimulated (2 Hz) or Vt (45 nmol), KCl (250 μmol) or their respective vehicles was injected into the renal arterial circuit during both periods. Changes in perfusion pressure were monitored and the renal perfusate samples were collected during both periods (P_1 and P_2) prior to and during RNS or after administration of Vt or KCl. Alterations in the basal and stimulation-induced rise in perfusion pressure and tritium overflow produced by Ca^{2+} channel blockers during the second period were expressed as a ratio of the corresponding values obtained during the first period in the absence of the drug and compared to the corresponding ratio obtained during the infusion of the vehicles of Ca^{2+} channel blockers.

Series 3 This series of experiments was performed to investigate the effects of the Ca^{2+} channel blockers, ω-CgTx (50 nM), nifedipine (10 μM), diltiazem (60 μM) and their respective vehicles on the renal vasoconstriction produced by exogenously administered noradrenaline (NA). After the stabilization period, NA (0.9 nmol) was injected as a bolus at 10 min intervals into the renal arterial circuit prior to and 10 min after infusion of Ca^{2+} channel blockers or their vehicles. Changes in perfusion pressure were recorded as in Experimental series 1.

Drugs

The following drugs used in this study were purchased: tritiated noradrenaline, (−)-[-7-^{3}H(N)]-NA (14.9 Ci mmol^{-1}, New England Nuclear, Boston, MA, U.S.A.), veratridine (Sigma Chemical Co., St. Louis, MO, U.S.A.), noradrenaline bitartrate (Levophed, Winthrop Lab., Inc., New York, NY, U.S.A.) and ω-conotoxin (Peninsula Lab., Inc., Belmont, CA, U.S.A.). The following drugs were gifts: diltiazem (Marion Lab., Kansas City, MO, U.S.A.) and nifedipine (Pfizer, Inc., New York, NY, U.S.A.). Veratridine was dissolved in dimethylsulphoxide (4 mg ml^{-1}) to prepare stock solution and stored in small aliquots at −20°C and diluted with saline just before use. A refrigerated stock solution of NA (1 mg ml^{-1}) was diluted with saline on the day of the experiment. ω-conotoxin was dissolved in sterile water (1 mg ml^{-1}) to prepare stock solution and stored in small aliquots at −20°C. Diltiazem was dissolved in small volumes of Tyrode solution and added to the perfusion fluid to obtain the final concentration. Nifedipine was dissolved in dimethylsulphoxide (2.5 mg ml^{-1}) and diluted with Tyrode solution just before infusion into the arterial circuit. Because nifedipine is light-sensitive, the solution and perfusion apparatus were covered with aluminium foil during its use.

Analysis of data

The basal tritium efflux represents the amount of tritium in the samples collected for 1 or 2 min periods immediately before RNS or administration of Vt or KCl. The increase in tritium output and perfusion pressure produced by various stimuli was calculated by subtracting the basal tritium efflux and the basal perfusion pressure from that obtained during RNS or immediately after the administration of Vt or KCl. The results are presented as mean ± s.e. mean. The data were analyzed by one-way analysis of variance. Student's *t* test for paired and unpaired observations was used to determine the difference between groups. Differences between means were considered to be significant if the probability (*P*) of the null hypothesis being true was less than 0.05.

Results

Effect of renal nerve stimulation, veratridine and KCl on tritium overflow and vascular tone in the rat isolated kidney perfused with Tyrode solution and prelabelled with [^{3}H]-NA

The rat isolated kidney perfused with normal Tyrode solution maintained a steady basal perfusion pressure for periods up to 4 h. During this period the weight of the kidney, which averaged 1.6 g, remained unchanged. Stimulation of renal nerves at 1–4 Hz increased tritium overflow and perfusion pressure. The increase in tritium overflow and perfusion pressure was related to the frequency of stimulation (Figure 1). Administration of Vt (15–90 nmol) or KCl (150–500 μmol) into the renal arterial circuit increased the tritium overflow in a dose-dependent manner (Figure 1). Vt also produced vasoconstriction and a dose-related increase in perfusion pressure; the rise in perfusion pressure elicited by Vt

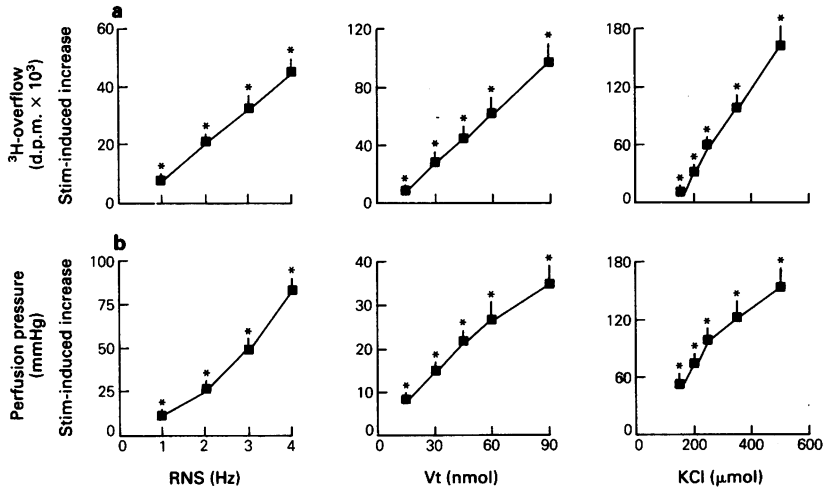

Figure 1 Effect of renal nerve stimulation (RNS), veratridine (Vt) and potassium chloride (KCl) on the tritium overflow (a) and on the perfusion pressure (b) in the rat isolated kidney perfused with Tyrode solution and prelabelled with [^{3}H]-noradrenaline. Bars represent s.e.mean. * Value significantly different from the basal: $P < 0.05$. $n = 6$ in all experimental groups.

peaked in 30–40 s and returned to basal level within 5 min. On the other hand, KCl in doses of 150–200 μmol produced a biphasic effect on perfusion pressure, i.e., an initial rapid transient decrease (10–15 mmHg) probably due to hyperpolarization lasting for 10 s (not shown in Figure 1) followed by an increase in perfusion pressure, as a result of depolarization which returned to basal level within 4 min. Higher doses of KCl (250–500 μmol) produced a rise in perfusion pressure which peaked in 30–40 s and returned to basal level within 4 min. Injections of the corresponding vehicles into the kidney did not alter the tritium overflow or perfusion pressure. Stimulation of renal nerves at 2 Hz or administration of Vt (45 nmol) and KCl (250 μmol) at 10 min intervals consistently increased perfusion pressure and the overflow of tritium; the increment in tritium efflux was less during P_2 than in P_1. Analysis of perfusate samples by column chromatography revealed that the increase in tritium overflow elicited by RNS, Vt and KCl consisted primarily of intact [^{3}H]-NA (79 ± 4, 77 ± 6 and 70 ± 4%, respectively, $n = 6$).

Effect of Ca^{2+} channel blockers on the increase in tritium overflow and perfusion pressure elicited by renal nerve stimulation, veratridine and KCl in the rat isolated kidney

In kidneys perfused with normal Tyrode solution and prelabelled with [^{3}H]-NA, infusion of ω-CgTx (50 nM) inhibited tritium overflow elicited by RNS (2 Hz), Vt (45 nmol) and KCl (250 μmol) (Figure 2). The rise in perfusion pressure produced by RNS or Vt but not that caused by KCl was inhibited by ω-CgTx (Figure 2). In contrast, nifedipine (10 μM) enhanced the overflow of tritium elicited by RNS and KCl but failed to alter that elicited by Vt (Figure 3). A low concentration of nifedipine (1.4 μM) enhanced the tritium overflow elicited by RNS, Vt and KCl by about 40 to 80% of the control (data not shown). Addition of diltiazem (6 μM) to the perfusion medium failed to alter the tritium overflow elicited by any of these stimuli. However, higher doses of diltiazem (60 μM) inhibited the overflow of tritium elicited by RNS and Vt but enhanced that elicited by KCl (Figure 4). The rise in perfusion pressure produced by RNS, Vt or KCl was inhibited by lower as well as by higher concentrations of nifedipine and diltiazem. The reduction in the vasoconstrictor response to these stimuli produced by lower concentrations of diltiazem and nifedipine was similar to that produced by higher concentrations of these agents (75 to 95% of the control) (Figures 3 and 4). The basal tritium efflux or perfusion pressure was not altered by ω-CgTx, nifedipine or the lower concentration of diltiazem, whereas the basal tritium efflux but not the perfusion pressure was enhanced by the higher concentration of diltiazem (Figures 2–4).

Effect of Ca^{2+} channel blockers on the rise in perfusion pressure produced by exogenous NA in the rat isolated kidney

Administration of nifedipine (10 μM), diltiazem (60 μM) but not ω-CgTx (50 nM) inhibited the rise in

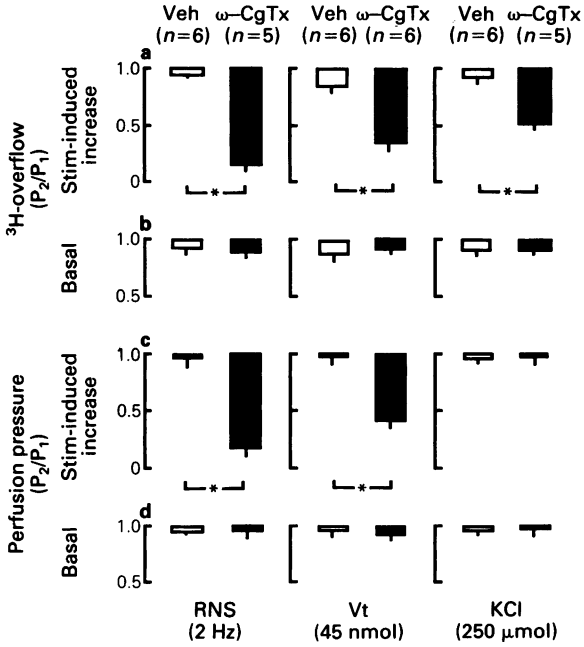

Figure 2 Effect of Ca^{2+} channel blocker, ω-conotoxin (ω-CgTx, 50 nM), or its vehicle (Veh) on the basal and on the increase in tritium overflow (a, b) and perfusion pressure (c, d) elicited by renal nerve stimulation (RNS, 2 Hz), veratridine (Vt, 45 nmol) and potassium chloride (KCl, 250 μmol) in the isolated perfused kidney of the rat prelabelled with [^{3}H]-noradrenaline. P_1 and P_2 represent the first and second periods of collection of the perfusate, respectively. In the first period (P_1), the kidney was perfused with normal Tyrode solution, whereas, in the second period (P_2) ω-CgTx or its vehicle was also infused. * Value significantly different from the corresponding value during vehicle treatment: $P < 0.05$. n as indicated in figure.

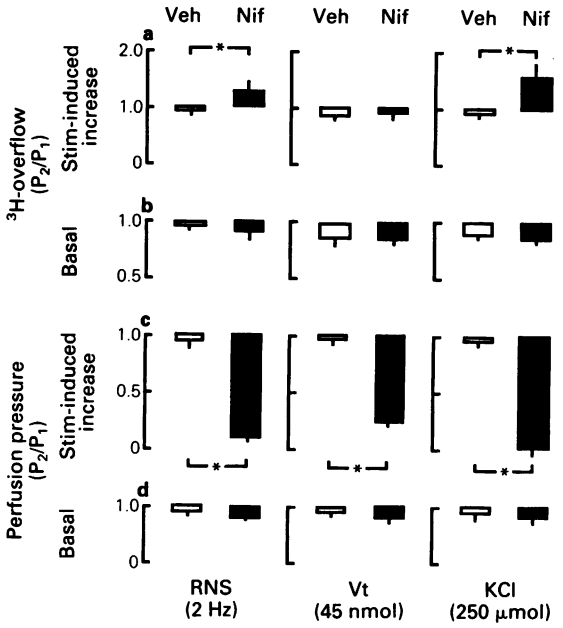

Figure 3 Effect of Ca^{2+} channel blocker, nifedipine (Nif, 10 μM), or its vehicle (Veh) on the basal and on the increase in tritium overflow (a, b) and perfusion pressure (c, d) elicited by renal nerve stimulation (RNS, 2 Hz), veratridine (Vt, 45 nmol) and potassium chloride (KCl, 250 μmol) in the rat isolated perfused kidney prelabelled with [^{3}H]-noradrenaline. P_1 and P_2 are the same as in Figure 2. Nif or its vehicle was infused in the second period (P_2). * Value significantly different from the corresponding value during vehicle treatment: $P < 0.05$. $n = 6$ in each experimental group.

perfusion pressure produced by injected NA (0.9 nmol, Figure 5). Infusion of the corresponding vehicles had no effect on the vasoconstrictor response to NA (Figure 5). The basal perfusion pressure was not altered by any of these Ca^{2+} channel blockers.

Discussion

The results of the present study in the isolated kidney of the rat perfused with Tyrode solution are consistent with the hypothesis that RNS, Vt and KCl promote the release of adrenergic transmitter by enhancing the influx of Ca^{2+} into the nerve terminal through specific voltage-dependent Ca^{2+} channels, probably N-type Ca^{2+} channels that are distinct from those present in the vascular smooth muscle. Moreover, the results suggest that ω-CgTx could be a useful tool to differentiate between Ca^{2+} channels at the nerve terminal and in the vascular smooth muscle.

In the rat isolated kidney perfused with Tyrode solution and prelabelled with [^{3}H]-NA, either RNS, Vt or KCl can increase the overflow of tritium and produce renal vasoconstriction in a frequency- or dose-related manner, respectively. It is well established that release of adrenergic transmitter in response to electrical stimulation of nerve fibres, Vt or KCl requires the influx of extracellular Ca^{2+} into the nerve terminal (Huković & Muscholl, 1962; Kirpekar & Prat, 1979; Kirpekar & Wakade, 1968; Palaty, 1982). However, the mechanism by which Ca^{2+} enters the nerve terminal in response to these stimuli is not yet established. It has been postulated that Ca^{2+} enters the nerve terminal through voltage-dependent Ca^{2+} channels since, in the rabbit isolated heart or pulmonary artery, nifedipine, diltiazem and verapamil were shown to inhibit release of NA elicited by various depolarizing stimuli (Starke &

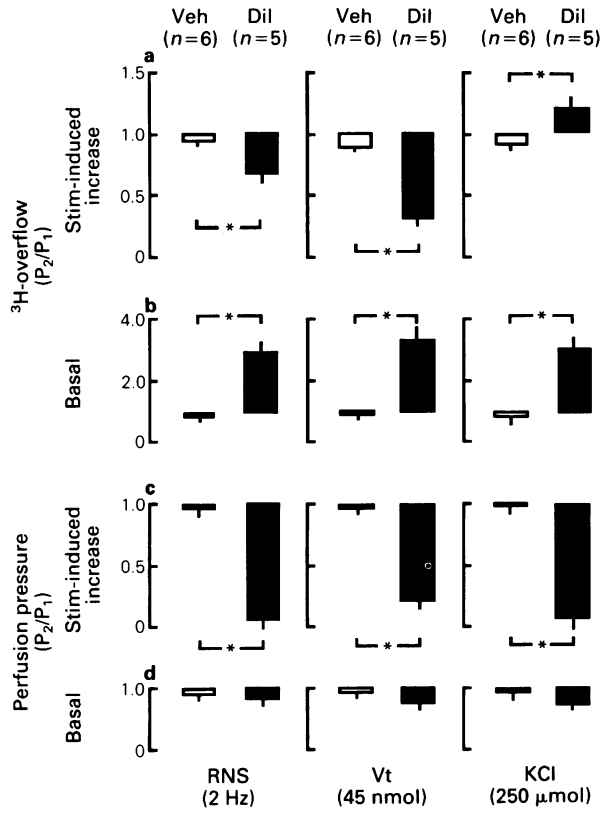

Figure 4 Effect of Ca^{2+} channel blocker, diltiazem (Dil, 60 μM), or its vehicle (Veh) on the basal and on the increase in tritium overflow (a, b) and perfusion pressure (c, d) elicited by renal nerve stimulation (RNS, 2 Hz), veratridine (Vt, 45 nmol) and potassium chloride (KCl, 250 μmol) in the rat isolated perfused kidney prelabelled with [^{3}H]-noradrenaline. P_1 and P_2 are the same as in Figure 2. Dil or its vehicle was infused in the second period (P_2). * Value significantly different from the corresponding value during vehicle treatment: $P < 0.05$. n as shown in figure.

Schümann, 1973; Göthert *et al.*, 1979; Zelis *et al.*, 1985). However, in these studies, the concentrations of Ca^{2+} channel antagonists used were much higher than those required to block Ca^{2+} channels in the vascular smooth muscle or cardiac muscle, indicating that the neurones are relatively resistant to these Ca^{2+} channel blockers. An important finding in the present study was that ω-CgTx, a neurotoxin that has been suggested to block the hypothetical L- and N- but not T-type Ca^{2+} channels in chicken sensory and rat sympathetic neurones (McCleskey *et al.*, 1986; Hirning *et al.*, 1986), inhibited tritium overflow elicited by RNS, Vt and KCl but failed to alter the vascular response to exogenous NA. The observation, taken together with our demonstration that nifedipine, a dihydropyridine Ca^{2+} channel antagonist that has been shown to inhibit L- but not N- and T-type Ca^{2+} channels (Nowycky *et al.*, 1985; Hirning *et al.*, 1986), enhanced tritium overflow elicited by RNS, Vt and KCl but reduced the vasoconstrictor response to exogenous NA, suggests that the release of transmitter noradrenaline in the rat kidney is promoted by the influx of Ca^{2+} through N-type Ca^{2+} channels, that are distinct from those present in the vascular smooth muscle. Supporting this conclusion were the findings that nitrendipine, which inhibits L-type Ca^{2+} current, had little effect on KCl-evoked NA release; however, ω-CgTx, which largely inhibits N-type current reduced NA release in the neurones of rat superior cervical ganglion (Hirning *et al*, 1986) and in brain synaptosomes (Reynolds *et al.*, 1986). A corollary of this conclusion is that L-type Ca^{2+} channels probably located at the postjunctional sites are sensitive to blockade by nifedipine and diltiazem, whereas N-type Ca^{2+} channels located at the prejunctional sites are sensitive to blockade by ω-CgTx. In view of the electrophysiological evidence that nifedipine and ω-CgTx are capable of blocking L-type Ca^{2+} channels in the neurones (Nowycky *et al.*, 1985; McCleskey *et al.*, 1986), our findings that diltiazem and nifedipine but not ω-CgTx attenuated the renal vasoconstriction elicited by KCl as well as exogenous NA suggest that 'L-type Ca^{2+} channels' located in the renal vascular smooth muscle are different from L-type Ca^{2+} channels present in the neurones. The ability of ω-CgTx to inhibit the renal vasoconstriction produced by RNS and Vt in our study appears to be due to its presynaptic inhibitory effect on the release of NA and not due to blockade of Ca^{2+} channels at the postsynaptic sites in the smooth muscle because the renal vasoconstrictor response produced by exogenous NA was not altered by the toxin. The demonstration that ω-CgTx inhibited KCl-evoked tritium overflow but did not alter the associated renal vasoconstriction suggests that the vasoconstrictor response produced by KCl in the rat kidney is independent of its facilitatory effect on the release of adrenergic transmitter at the nerve terminal.

That Ca^{2+} channels at the adrenergic nerve terminal are distinct from those located at the postjunctional sites in the vascular smooth muscle was supported by our demonstration that the lower concentration of diltiazem which inhibited the vasoconstrictor response to RNS, Vt and KCl, failed to alter the overflow of tritium elicited by these stimuli. The inability of nifedipine and the lower concentration of diltiazem to inhibit the overflow of tritium in our study was not due to inability of these agents to reach an effective concentration at the adrenergic neuroeffector junction because the renal vasocons-

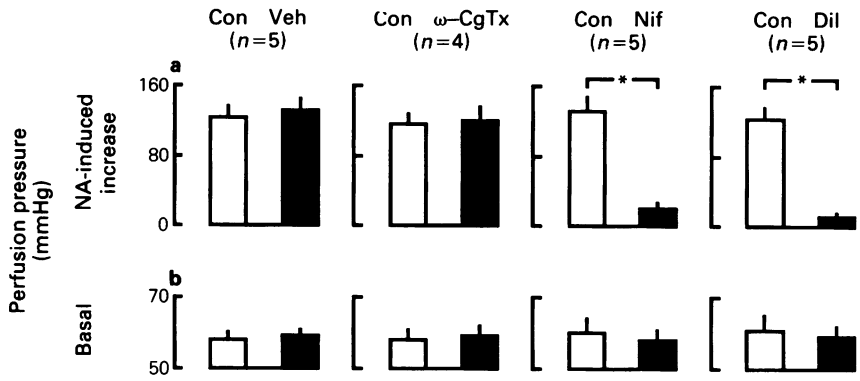

Figure 5 Effect of Ca^{2+} channel blockers, ω-conotoxin (ω-CgTx, 50 nM); nifedipine (Nif, 10 μM) and diltiazem (Dil, 60 μM), or their vehicle (Veh) on the basal (b) and on the increase (a) in the perfusion pressure produced by noradrenaline (NA, 0.9 nmol) in the rat isolated perfused kidney. The Ca^{2+} channel blockers or their vehicle were infused after the control (Con) response to NA. * Value significantly different from the control: $P < 0.05$. n as shown in figure.

trictor response produced by RNS, Vt and KCl was inhibited by these agents. On the other hand, the higher concentration of diltiazem inhibited tritium overflow elicited by RNS and Vt but enhanced that elicited by KCl. Although diltiazem inhibited RNS- or Vt-induced tritium overflow, it produced an increase in the basal efflux of tritium. The decrease in RNS- or Vt-induced tritium overflow produced by diltiazem was not the result of a large increase in the basal tritium efflux, because KCl-induced tritium overflow was not reduced but rather enhanced by this dose of diltiazem. The mechanism of increase in basal tritium efflux produced by diltiazem, which could be due to alterations in intracellular Ca^{2+} metabolism or inhibition of storage and/or uptake of neurotransmitter, is not known. Moreover, the mechanism by which diltiazem inhibited RNS- and Vt- but not KCl-induced tritium overflow remains to be determined. It has been postulated that diltiazem, by blocking voltage-dependent Ca^{2+} channels, inhibited electrically- and KCl-evoked release of NA in the rabbit pulmonary artery (Zelis *et al.*, 1985) and release of dopamine and acetylcholine elicited by KCl in rabbit caudate slices (Starke *et al.*, 1984). However, our finding that diltiazem enhanced KCl-evoked tritium overflow suggests that a mechanism other than blockade of Ca^{2+} channels is responsible for the reduction of RNS- and Vt-induced transmitter release by diltiazem. Since the release of transmitter noradrenaline elicited by electrical stimulation of nerve fibres or administration of Vt requires the entry of Na^+ through tetrodotoxin-sensitive channels as well as the presence of Ca^{2+} in the perfusion medium (Kirpekar & Misu, 1967; Kirpekar & Prat, 1979), whereas KCl-evoked release is also Ca^{2+}-dependent but not affected by tetrodotoxin (Kirpekar & Wakade, 1968), the mechanism by which diltiazem inhibited RNS- and Vt- but not KCl-induced tritium overflow probably involves interference with Na^+ channels rather than Ca^{2+} channels (Starke *et al.*, 1984).

In conclusion, the present study suggests that RNS, Vt and KCl promote the release of adrenergic transmitter in the rat kidney by enhancing the influx of Ca^{2+} through a specific type of voltage-dependent Ca^{2+} channel, probably N-type Ca^{2+} channels, that are distinct from those located in the vascular smooth muscle. Moreover, ω-CgTx could be a useful tool to differentiate between Ca^{2+} channels at the adrenergic nerve terminal and the vascular smooth muscle.

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