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The NO- and prostaglandin-independent component of the renal vasodilator effect of thimerosal is mediated by epoxyeicosatrienoic acids.

Chen, Y-J., Jiang, H. and Quilley, J.*

Department of Pharmacology, New York Medical College, Valhalla, NY 10595.

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Running title: EETs and renal vasodilation to thimerosal

Author for correspondence: J. Quilley, Ph.D

TEL. 914 594-4123

FAX 914 347-4956

E-mail John_Quilley@NYMC.edu

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Abbreviations:

Nitric Oxide (NO), Epoxyeicosatrienoic acid (EET),

Dihydroxyeicosatrienoic Acid (DHET), Arachidonic Acid (AA),

Endothelium-derived Hyperpolarizing Factor (EDHF), Cytochrome P450 (CYP),

Perfusion Pressure (PP), Nitroarginine (L-NA), Tetraethylammonium (TEA),

N-methylsulfonyl-6-(2-propargyloxyphenyl)hexamide (MS-PPOH), Cyclooxygenase

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ABSTRACT

Epoxyeicosatrienoic acids (EETs) are cytochrome P450 (CYP)-derived metabolites of arachidonic acid (AA) which elicit vasodilation via activation of K^+ channels. They have been implicated as endothelium-derived hyperpolarizing factors (EDHF), mediating the effect of some endothelium-dependent vasodilator agents such as bradykinin in some vascular tissues. We reasoned that an agent that increases the availability of free AA should also elicit CYP-dependent vasodilation that is associated with increased release of EETs and attenuated by agents that inhibit the synthesis or action of EETs. Thus, we used thimerosal as an inhibitor of reacylation of AA and determined the contribution of prostaglandins, NO and EETs to the vasodilator effect in the isolated, perfused, preconstricted kidney of the rat. Thimerosal elicited vasodilator responses that were unaffected by inhibition of cyclooxygenase (COX) with indomethacin but were reduced by the further inhibition of NO synthesis. The vasodilator activity that remained after inhibition of COX and NOS was reduced by inhibition of K^+ channels with tetraethylammonium and was associated with increased release of EETs measured by GC-MS following hydrolysis to the corresponding diols. Inhibition of CYP with miconazole or epoxygenase with MS-PPOH reduced the NO- and prostaglandin-independent vasodilator effect of thimerosal and attenuated the increase in the release of EETs. We conclude that thimerosal causes vasodilation of the isolated perfused kidney via NO-dependent and –independent mechanisms. The NO-independent component of the response involves activation of K^+ channels and is likely mediated by EETs, possibly acting as EDHF.

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Responses to endothelium-dependent vasodilator agents such as bradykinin and acetylcholine exhibit several components that involve nitric oxide (NO), prostaglandins and endothelium-derived hyperpolarizing factor (EDHF) (Vane et al., 1990; Cohen and Vanhoutte, 1995). The contribution of EDHF to the vasodilator response becomes more apparent when the synthesis of NO and prostaglandins is inhibited. Indeed, two of the criteria for EDHF-mediated responses are 1) the vasodilator activity remaining after inhibition of NO synthase (NOS) and cyclooxygenase (COX) and 2) the vasodilator activity being prevented by inhibition of K^+ channels with specific inhibitors or elevated extracellular K^+ (Campbell and Gauthier, 2002). The identity of EDHF remains to be confirmed and it is likely that several EDHFs exist, depending upon the species and vascular bed; the candidates range from a cytochrome P450-derived (CYP) metabolite of arachidonic acid (AA) (Hecker et al., 1994; Campbell et al., 1996; Fisslthaler et al. 1999) to K^+ itself (Edwards et al., 1998) as well as transfer of hyperpolarization from the endothelium to vascular smooth muscle via gap junctions (Hutcheson et al., 1999; Chaytor et al., 2001). There is considerable evidence for CYP-derived AA metabolites, namely the epoxides (EETs), as EDHFs in vascular tissues of several species including man (Campbell et al., 1996; Fisslthaler et al., 1999; Halcox et al., 2001). Moreover, a central role for cytosolic phospholipase A_2 in vasodilator responses attributed to EDHF support the role of an AA metabolite (Fulton et al., 1996: Hutcheson et al., 1999).

In addition to the standard endothelium-dependent vasodilator agents, acetylcholine and bradykinin, the acyl-CoA:lysolecithin acetyltransferase inhibitor, thimerosal has been reported to elicit endothelium-dependent vasodilation that has been attributed to NO, prostaglandins and EDHF (Forstermann et al., 1986a,b; Rosenblum et

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al., 1992; Beny, 1990). Further, low concentrations of thimerosal have been reported to enhance EDHF-mediated vasodilator responses (Mombouli et al., 1996). As an inhibitor of reacylation, thimerosal would be expected to increase intracellular levels of free AA (Burke et al., 1997), the precursor of CYP-derived EETs that have been proposed as EDHF. In addition, thimerosal has been reported to increase intracellular Ca^{2+} levels in endothelial cells (Gericke et al., 1993) and act as an IP3 receptor-sensitizing agent (Montero et al., 2001), effects that should increase the synthesis of NO, prostaglandins and EETs. Consequently, we used thimerosal as a tool to further investigate the proposition of EETs as EDHF in the rat isolated perfused kidney. Thus, we reasoned that thimerosal should produce endothelium-dependent vasodilation by stimulating the release of NO, prostaglandins and EDHF and that the residual vasodilator activity following inhibition of NOS and COX should be susceptible to inhibitors of CYP, specifically epoxygenase, and to inhibitors of K^+ channels. We tested this possibility by sequentially inhibiting COX, NOS and CYP and determining vasodilator responses to thimerosal and, in some cases, the release of EETS. The results show that both NO and NO-independent factors contribute to the vasodilator effect of thimerosal. The NO-independent component of the response which was prevented by blockade of K^+ channels and, therefore, may correspond to an EDHF, was attenuated by inhibitors of epoxygenase which abolished the thimerosal-stimulated increases in EET release. These studies indicate that EETs contribute to the renal vasodilator effect of thimerosal and provide further evidence for EETs as an integral component of EDHF-mediated responses.

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METHODS

Isolated perfused kidney. Male Wistar rats, weight 300-500g, were used for these studies in accordance with NIH guidelines. Rats were anesthetized with pentobarbitone, 65mg/kg ip, and the right kidney was prepared for perfusion as described (Fulton et al., 1992). Briefly, following a midline laparotomy, the right kidney was cannulated via the mesenteric artery to prevent interruption of blood flow and perfused with warmed (37^{o}C), oxygenated Krebs' Henseleit buffer at constant flow to obtain a baseline perfusion pressure (PP) of approximately 50-75 mmHg. The vena cava was ligated above and below the right renal vein and cut to allow exit of the perfusate and the ureter was transected. In some experiments where the perfusate was collected for the determination of EETs, the kidney was removed from the animal.

Once a stable PP was obtained, pressure was elevated with phenylephrine (0.4-1.5 x 10^{-6}M) to approximately 150-220 mmHg to amplify vasodilator responses, and vasodilator responses to bradykinin (100ng), thimerosal (1 and 10μg) and nitroprusside (1μg) were determined in the absence and presence of various pharmacological interventions. Initially, the effects of indomethacin (5.6μM) treatment on vasodilator responses were determined. Although indomethacin was without effect, it was included in the perfusate in all subsequent experiments in order to exclude any contribution of prostaglandins to vasodilator responses which were compared to those obtained in the presence of indomethacin plus L-nitroarginine (L-NA; 50μM) to inhibit NOS. In those experiments designed to address the contribution of a CYP-dependent epoxygenase to the vasodilator effect of thimerosal (which was the primary aim of this study), all experiments were conducted in the presence of indomethacin and L-NA to isolate the

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NO- and prostaglandin-independent component of the response that may be mediated by an EDHF. Tetraethylammonium (TEA; 10mM), a non-selective inhibitor of K^+ channels, was used as an indirect index of EDHF. Two inhibitors of CYP were used, miconazole (1μM) which is reported to be more specific for epoxygenase than ω-hydroxylase (Zou et al., 1994) and MS-PPOH (28μM) which is considered to be a specific epoxygenase inhibitor (Wang et al., 1998). MS-PPOH was used because of reports that the imidazole compounds may affect the activity of some types of K^+ channels (Brugnara et al., 1995) and, thereby, mask any effect on the synthesis of the putative mediator by blocking its action. The various inhibitors were added to the perfusate upon attainment of a stable basal PP. After at least 10min. exposure of the kidney to the inhibitors, phenylephrine was added to the perfusate to elevate PP. In some cases, this was not required, e.g.,L-NA plus TEA raised PP to the required level. The experiments comparing L-NA plus indomethacin to L-NA plus indomethacin plus miconazole, MS-PPOH or TEA were conducted in one series where several preparations per day were studied with one acting as a control (L-NA plus indomethacin) and the others assigned to one of the treatments. In the other series of experiments, indomethacin alone was compared to no treatment and finally indomethacin alone was compared to L-NA plus indomethacin. The data obtained for the groups treated with indomethacin alone or L-NA plus indomethacin did not differ among the different series of experiments and, therefore, the data were pooled and compared in one data set.

In all of the experiments, bradykinin was used as a positive control to assess the effectiveness of the various pharmacological interventions as our previous studies in the kidney and heart have shown that the NO- and prostaglandin-independent component of

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the vasodilator effect of bradykinin was dependent on phospholipase, CYP and K^+ channel activity (Fulton et al., 1992; Fulton et al., 1994; Fulton et al., 1995; Rapacon et al., 1996). In contrast, responses to nitroprusside were used to assess any effects of the various interventions on vasodilator mechanisms that could not be attributed to inhibition of CYP or K^+ channels. In all experiments, the sequence of administration of bradykinin, 1 and 10μg thimerosal and nitroprusside was the same. In three kidney preparations treated with L-NA and indomethacin, we found that vasodilator responses to 1 and 10μg thimerosal without the prior administration of bradykinin were not different from those we obtained in L-NA plus indomethacin-treated kidneys where bradykinin was administered before thimerosal.

Release of EETs. In some experiments, EET release from kidneys treated with indomethacin plus L-NA and those treated with the combination of indomethacin, L-NA and either miconazole or MS-PPOH was compared. Thus, 1min. perfusate collections were made immediately before and following the administration of 1 and 10ug thimerosal. The EETs were measured as their hydrolysis products, the dihydroxyeicosatrienoic acids (DHETs), by GC-MS. To 10ml. samples 3ng of a mix of deuterium-labeled EETs (8,9-, 11,12-, and 14,15-EET-d8, 1ng each) were added as internal standard. The samples were acidified to pH 4 with acetic acid and the lipids extracted with 10ml. ethyl acetate which was decanted and dried. The extract was incubated with 100μl KOH (1M) for 1h at 60^{o}C to decompose indomethacin which has a similar HPLC retention time to the DHETs. 200μl H_2SO_4 (0.5M) were added to the samples which were maintained at 60^{o}C for 30min. to convert the EETs to their respective DHETs. After adjusting to pH 4 with KOH, ethyl acetate was used to extract

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the DHETs which were dried under nitrogen and dissolved in 50µl methanol for separation by reverse phase HPLC using an HP 1050 instrument with a Beckman ODS column (25cm x 4.6mm, 5µ) and a linear gradient of 60% to 100% acetonitrile containing 0.025% acetic acid over 20min. at a flow rate of 1ml./min. The peak corresponding to authentic DHETs was collected, the sample dried and the DHETs derivatized to PFB esters and TMS ethers. PFB esters were prepared by the addition of 30µl 10% α-bromo-2,3,4,5,6-pentafluorotoluene (Aldrich) in acetonitrile and 30µl 10% N,N-diisopropylethylamine (Aldrich) in acetonitrile. After 30min. at room temperature, the samples were dried and the TMS ethers prepared by dissolving the samples in 60µl N,O-bis(trimethylsilyl)trifluoroacetamide (Sigma) and 20µl pyridine and allowing the reaction to proceed for 30min. at room temperature. The samples were dried and dissolved in 50µl isooctane and 1µl aliquots were analyzed using a HP5890 GC/MS. The GC column (DB-1; 10m, 0.25mm inner diameter, 0.25µm film thickness, Agilent) was temperature programmed from 180^{o}C to 300^{o}C at a rate of 25^{o}C/min. Methane was used as a reagent gas at a flow resulting in a source pressure of 2 torr and the MS (HP 5989A) was operated in the electron capture chemical ionization mode, monitoring ions at m/z of 481 and 489, which represented the derivatives of the unlabeled and d8-labeled DHETs. The 5,6-, 8,9-, and 11,12-DHETs exhibited the same retention time and were quantitated together, whereas 14,15-DHET which had a different retention time was quantitated separately. The amounts of DHETs in the samples were calculated by reference to a standard curve.

Removal of endothelium. In three kidneys perfused with buffer containing L-NA and indomethacin, the endothelium was removed with 0.1ml Triton X-100 (0.5%). After

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elevation of PP with phenylephrine, vasodilator responses to bradykinin, thimerosal and nitroprusside were determined.

Analysis. All data are expressed as mean±SEM. As data from different series of experiments were pooled, they were compared by ANOVA of one data set where individual comparisons made using a Bonferroni correction. A p value <0.05 was considered statistically significant.

Materials. Bradykinin, nitroprusside, TEA and L-NA were obtained from Sigma and dissolved in distilled water. Miconazole and indomethacin were also obtained from Sigma but were dissolved in ethanol and 4% sodium bicarbonate, respectively. MS-PPOH was prepared by Dr. J.R.Falck (University of Texas Southwestern Medical Center) and dissolved in ethanol. Triton X-100 was obtained from Calbiochem and diluted in distilled water.

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RESULTS

Basal PP in the untreated group (n=3) was 77±3 mmHg compared to 61±3 mmHg in the indomethacin group (n=8); 74±4mmHg for the indomethacin and L-NA group (n=11); 58±4mmHg for the indomethacin, L-NA and miconazole group (n=7); 62±5mmHg for the indomethacin, L-NA and MS-PPOH group (n=5); and 69±3 mmHg for the indomethacin, L-NA and TEA group (n=4). The elevated PP in the respective groups were 188±6, 192±7, 205±6, 173±7, 164±16, and 207±2 mmHg. In the untreated and indomethacin-treated groups, 7.5 x 10^{-7}M phenylephrine was sufficient to raise PP; this requirement was reduced to 4 x 10^{-7}M when L-NA was added and reduced to zero with the further addition of TEA. In contrast, in kidneys treated with either miconazole or MS-PPOH, the requirement for phenylephrine to elevate PP was increased, up to 1.5 x 10^{-6}M.

Inhibition of COX. Indomethacin was without effect on vasodilator responses to bradykinin, thimerosal or nitroprusside (fig. 1) when compared to the untreated group. For all the subsequent interventions, comparisons were made to the indomethacin-treated group.

Inhibition of NOS. Inclusion of L-NA in the perfusate in addition to indomethacin reduced the vasodilator responses to thimerosal without significantly affecting those to bradykinin and nitroprusside when compared to the group treated with indomethacin alone (fig. 1). The response to bradykinin was 92±13mmHg and 66±9mmHg in the absence and presence of L-NA but the difference did not achieve significance. Similarly, the response to nitroprusside was 78±10mmHg in the presence of L-NA compared to 51±4mmHg in the group treated with indomethacin alone (not significant). In contrast,

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the responses to 1 and 10μg thimerosal were reduced from 54±11 and 80±5mmHg, respectively, to 14±2 and 52±5mmHg, respectively ($p<0.05$) in the presence of L-NA.

Inhibition of epoxygenase. The addition of miconazole to indomethacin and L-NA further reduced the vasodilator effect of bradykinin to 25±6mmHg ($p<0.05$) whereas there was no significant effect on the response to nitroprusside, 59±12mmHg (not significant when compared to indomethacin alone or indomethacin and L-NA). Miconazole also caused a further reduction ($p<0.05$ when compared to indomethacin plus L-NA) in the vasodilator effects of 1 and 10μg thimerosal which reduced PP by 4±1 and 10±2mmHg, respectively (fig. 2).

Similarly, MS-PPOH significantly reduced the responses to bradykinin and 1 and 10μg thimerosal to 24±8, 5±2 and 23±13 mmHg, respectively, and insignificantly reduced the response to nitroprusside, 41±11mmHg (fig. 2).

Inhibition of K^+ channels. As expected, TEA reduced the vasodilator effect of bradykinin to 38±9mmHg without affecting the response to nitroprusside, 68±18mmHg (fig. 3). TEA also caused a significant reduction in the vasodilator effect of 1 and 10μg thimerosal, 2±2 and 16±9mmHg, respectively.

Release of EETs. Fig. 4 shows the increase in total EET release, measured as DHETs, in samples obtained 1 min. before and 1min. after challenge with 1 and 10μg thimerosal. These results are expressed as increases in EET release because of the large variations in release and relatively high basal levels; however, in all kidneys treated with indomethacin and L-NA (n=6), thimerosal produced an increase in EET release. Thus, EET release was increased by 4.9±1.8ng/min and 11.9±6.1ng/min by 1 and 10μg thimerosal, respectively. With the addition of miconazole, the increase in EET release

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was 2.0±1.9ng/min (this reflected a high value in 1 of 4 experiments) and 0.4±.0.4ng/min in response to 1 and 10μg thimerosal, respectively. Similarly, MS-PPOH (n=5) reduced the efflux of EETs from the kidney stimulated by thimerosal. Thus, in the presence of MS-PPOH, the increase in EET release in response to 1 and 10μg thimerosal was reduced to 0.8±0.6ng/min and 0.7±0.6ng/min. However, MS-PPOH failed to reduce basal EET release which was 25.2±3.9ng/min. compared to 19.7±3.7ng/min. in the indomethacin and L-NA-treated group. In contrast, basal release of EETs in the miconazole-treated group was reduced to 10.7±1.8ng/min.

Removal of endothelium. Basal PP was 70±6mmHg and was elevated to 131±8mmHg after administration of Triton X-100. After phenylephrine, PP was raised to 206±7mmHg. Under these conditions, the decrease in PP in response to bradykinin (100ng) was 5±3mmHg and that to nitroprusside, 51±13mmHg. The vasodilator effect of 1μg thimerosal was abolished, whereas 10μg thimerosal reduced PP by 4±3mmHg (in one preparation, thimerosal produced a slight increase in PP and, therefore, the vasodilator effect was taken as zero).

DISCUSSION

The results of this study show that thimerosal produces dose-dependent vasodilation of the isolated perfused kidney that is independent of prostaglandin synthesis as indomethacin was without effect. These results are in agreement with those of Forstermann et al. (1986b) and Crack and Cocks (1992) in rabbit aorta and dog coronary artery, respectively, but not those of Rosenblum et al. (1992) using mouse pial arteries, probably reflecting species and tissue differences. The prostaglandin-independent renal vasodilator effect in the presence of indomethacin consists of two components, one mediated via NO and the other via a CYP-dependent mechanism that utilizes EETs acting upon K^+ channels. Inhibition of NOS with L-NA reduced the vasodilator effect of thimerosal, showing that part of the response is mediated via NO. The NO-dependent component was more apparent with the lower dose of thimerosal as L-NA produced a greater inhibitory effect. These results are in accord with other studies that have shown vasodilator/vasorelaxant responses to thimerosal to be dependent on NO (Forstermann et al., 1986a,b). Similarly, we confirmed that the renal vasodilator effect of bradykinin is also partially dependent on NO and that when NOS is inhibited, the vasodilator effect of exogenous NO in the form of nitroprusside tends to be enhanced. The results with nitroprusside indicate that NOS was inhibited in these studies as removal of background levels of NO would be expected to increase responsiveness to administered NO. Also, the concentration of phenylephrine required to increase perfusion pressure was reduced by half in the presence of L-NA. Finally, an earlier study showed that this concentration of L-NA was sufficient to abolish the increase in cGMP release from the kidney in response to bradykinin (Cachofeiro and Nasjletti, 1991).

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We anticipated that the residual vasodilator effect of thimerosal in the presence of a NOS inhibitor would involve an EDHF as indicated by Beny (1990). Consequently, we used TEA as a non-selective K^+ channel inhibitor to support this idea. TEA, in the presence of indomethacin and L-NA, almost abolished the vasodilator effect of the lower dose of thimerosal (in 2/4 cases the vasodilator effect of thimerosal was converted to a small vasoconstrictor response in the presence of TEA), fulfilling one of the criteria for an EDHF-mediated effect. However, as these experiments were conducted in a perfused organ system where it is not possible to distinguish effects at the endothelium versus the vascular smooth muscle, we cannot exclude the possibility that TEA affected K^+ channels on the endothelium to reduce release of a vasorelaxant mediator rather than simply preventing the effect of the mediator on the smooth muscle. Thus, we and others have shown that inhibition of endothelial K^+ channels reduces EDHF- and NO-mediated responses (Doughty et al., 1999; Qiu and Quilley, 2001).

The results of these studies also support the concept of EETs as an EDHF in the rat kidney and demonstrate for the first time a CYP-dependent component to the vasodilator effect of thimerosal that also involves activation of K^+ channels. The evidence for CYP and EETs in particular is considerable. First, miconazole which is considered a relatively specific inhibitor of epoxygenase (Zou et al., 1994), greatly reduced the vasodilator effect of thimerosal. Because agents such as miconazole have been reported to influence the activity of K^+ channels, we also used another specific inhibitor of epoxygenase, MS-PPOH. This agent has been shown to inhibit the formation of EETs from AA by renal cortical microsomes with little effect on the formation of 20-HETE, an ω-hydroxylase product (Wang et al., 1998). Like miconazole, MS-PPOH

greatly reduced the vasodilator effect of thimerosal that remained following inhibition of COX and NOS and, thereby, strongly supports a role for EETs. Second, thimerosal increased the release of EETs into the renal perfusate and this stimulated release of EETs was prevented when kidneys were treated with either miconazole or MS-PPOH. We do not have an explanation for the failure of MS-PPOH to reduce basal release of EETs when miconazole caused a 50% reduction. It is possible that EETs released in response to thimerosal are derived from a different source of phospholipids than those released under basal conditions and that it is the stimulated release that is affected by MS-PPOH. Thus, EETs have been shown to be stored (Capdevila et al., 1987) although our results suggest that the EETs released in response to thimerosal are derived from CYP-dependent metabolism of AA as epoxygenase inhibitors reduced both the vasodilator effect of thimerosal and the associated increase in the release of EETs.

Thimerosal is an inhibitor of acyl transferase and as such would be expected to increase levels of free AA that would then be available for metabolic transformation by epoxygenase which is expressed principally in the endothelium, and by ω-hydroxylase which is localized to vascular smooth muscle (Roman, 2002). Thimerosal should, therefore, increase the formation of dilator EETs and constrictor 20-HETE unless it exerts a preferential effect on the endothelium. Removal of the endothelium almost abolished the vasodilator effects of thimerosal in kidneys treated with L-NA- and indomethacin. Under these conditions, the vasoconstrictor effect of 20-HETE would no longer be opposed by endothelial derived NO or EETs. Indeed, 20-HETE formation should be increased as a result of removal of an inhibitory influence in the form of NO (Oyekan et al., 1999). However, it should also be noted that indomethacin has been

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reported to inhibit the vasoconstrictor effect of 20-HETE in the rat isolated perfused kidney (Askari et al., 1997) and may mask the activity.

The effects of thimerosal in increasing EET release cannot be attributed solely to inhibition of reacylation as it has also been reported to increase the sensitivity of the IP3 receptor (Montero et al. 2001) and to increase levels of intracellular Ca^{2+} (Gericke et al. 1993)). These actions could result in activation of phospholipases to release AA as well stimulation of eNOS, a Ca^{2+}-dependent enzyme. Regardless of the mechanism by which AA is released, metabolism via COX as well as CYP could be expected unless coupling of substrate to enzyme is distinct.

We measured total EET release following their conversion to DHETs by acid hydrolysis and, therefore, we cannot attribute the vasodilator action of thimerosal to any specific regioisomer although previous studies have shown the 5,6-EET to be the most potent of the EETs. EETs have been shown to be released from the endothelium in response to some endothelium-dependent vasodilator agents and to relax vascular smooth muscle via activation of K^+ channels, thereby fulfilling the basic requirements for an EDHF (Campbell and Gauthier, 2002). In these studies, we found that removal of the endothelium abolished the vasodilator effect of thimerosal in agreement with the results of Forstermann et al. (1986a). Although the results of the present studies provide convincing evidence for a role of EETs in the NO-independent vasodilator effect of thimerosal, we cannot exclude the possibility that EETs may function as an intracellular mediator in the endothelium to activate K^+ channels and increase the influx of Ca^{2+}. However, this concept which was first suggested by Graier et al. (1995) and supported by

Rzigalinski et al. (1999), is not supported by studies showing that EETs cause vasodilation that is not dependent on an intact endothelium.

In summary, we have provided further evidence for one or more EETs acting as an EDHF in the rat kidney by showing that thimerosal, an agent that increases free AA levels, elicits endothelium-dependent vasodilation that is associated with increased release of EETs and that both effects are attenuated by inhibitors of epoxygenase.

FOOTNOTES

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Figure legends

Fig. 1.Vasodilator responses to bradykinin, thimerosal and nitroprusside in untreated kidneys (n=3), those treated with indomethacin (5.6μM; n=8) and those treated with indomethacin plus 50μM nitroarginine (n=11) *p<0.05 versus indomethacin-treated group.

Fig. 2. Effects of inhibition of cytochrome P450 with miconazole (1μM; n=7) and inhibition of epoxygenase with MS-PPOH (28μM; n=5) on NO- and prostaglandin-independent vasodilator responses (n=11) to thimerosal and bradykinin. *p<0.05 versus indomethacin plus L-NA-treated group.

Fig. 3. Effects of the K^+ channel inhibitor, TEA (10mM; n=4), on vasodilator responses to thimerosal, bradykinin and nitroprusside in kidneys treated with indomethacin and L-nitroarginine. N=11 for the indomethacin- and nitroarginine-treated group.*p<0.05 versus indomethacin plus L-NA treatment.

Fig. 4. Increases in total EET release, measured as the DHETs, in response to thimerosal in kidneys treated with indomethacin plus L-nitroarginine (n=6), indomethacin plus nitroarginine and miconazole (n=4) and indomethacin plus nitroarginine plus MS-PPOH (n=5). *p<0.05 versus indomethacin plus L-NA treatment.

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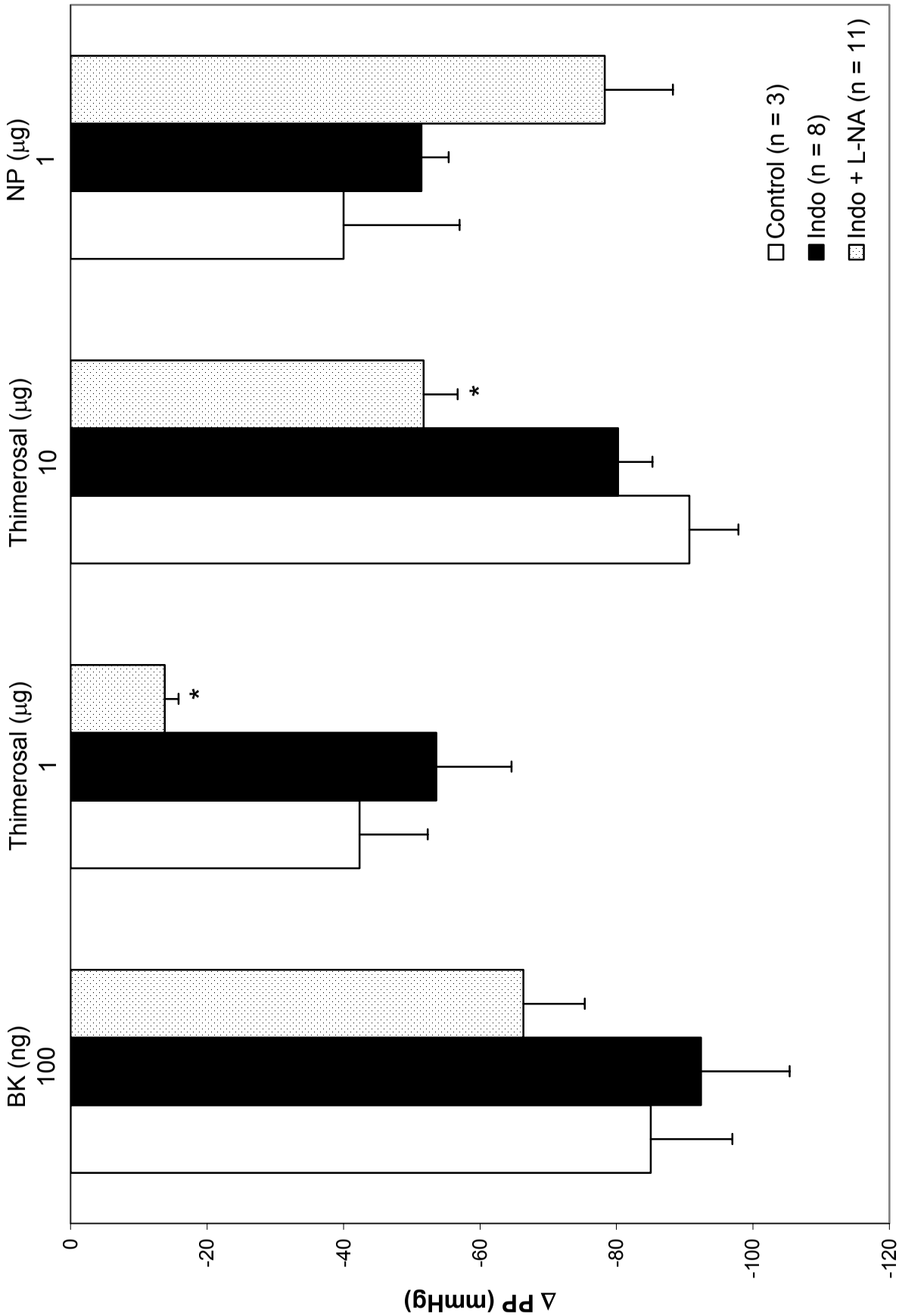

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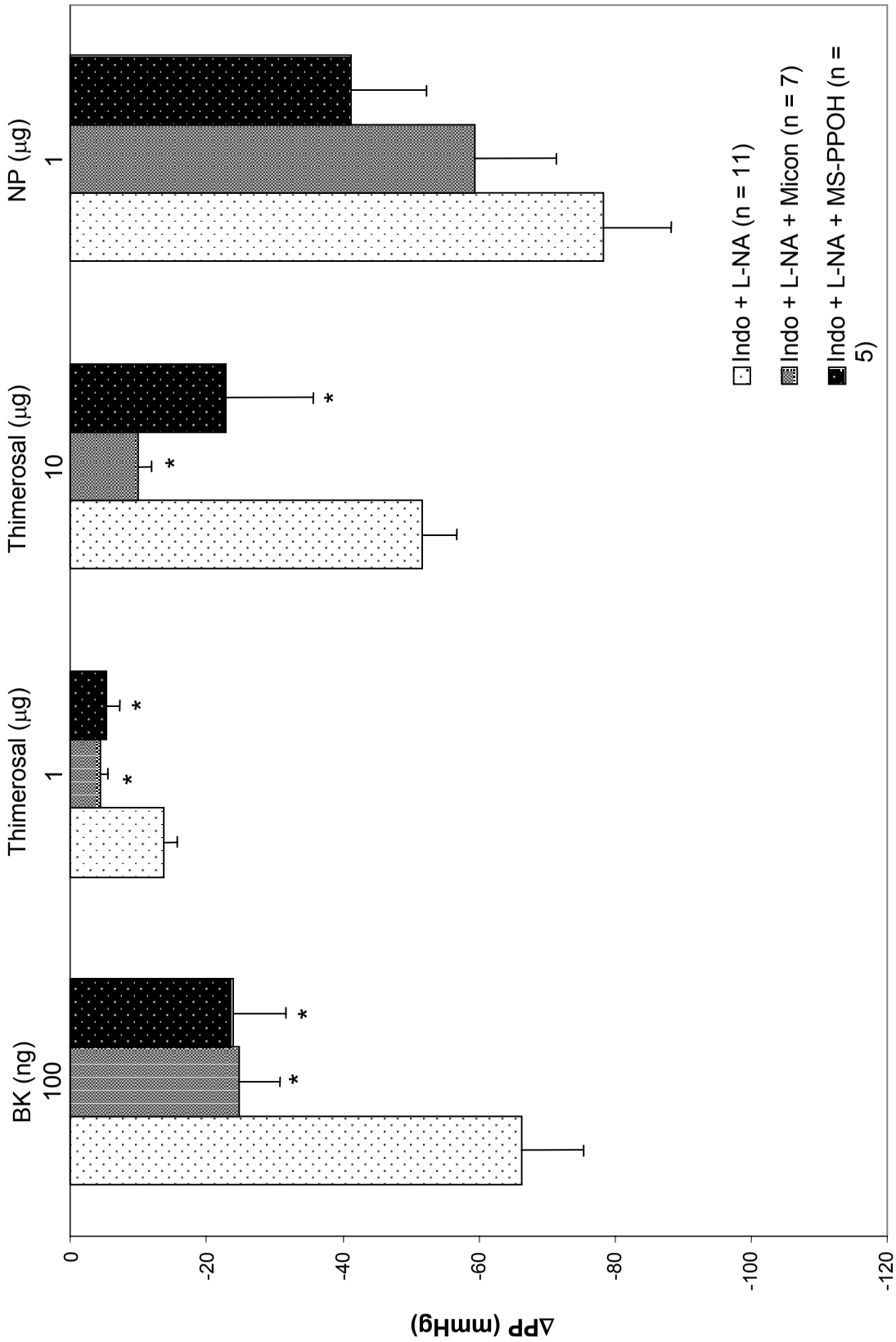

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