J. Pharm. Pharmacol. 1984, 36: 382–385
Received October 18, 1983

Neuronal deamination of endogenous and exogenous noradrenaline in the mesenteric artery of the spontaneously hypertensive rat

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The noradrenaline (NA) content of the mesenteric arteries from spontaneously-hypertensive rats (SHR) are greater than those in arteries from normotensive Kyoto Wistar rats (WKY). The possibility that impaired neuronal monoamine oxidase (MAO) activity in mesenteric arteries from SHR rats was responsible for the differences in NA content was explored. The in-vitro formation of dihydroxyphenylethylene glycol (DOPEG) by intact segments of mesenteric arteries was used as an index of neuronal MAO activity. There were no differences in the production of DOPEG from endogenous NA by arteries from normotensive and hypertensive rats. Moreover, the formation of DOPEG from exogenous NA was similar in arteries from SHR and WKY rats. The neuronal uptake of NA was indistinguishable between mesenteric arteries from SHR and WKY rats. The results argue against an impairment of neuronal MAO in contributing to the enhanced content of NA in the mesenteric artery of the SHR rat.

In a previous investigation, we observed a larger concentration of noradrenaline (NA) in mesenteric arteries from spontaneously hypertensive rats (SHR) than in arteries from normotensive Kyoto Wistar rats (WKY) (Head & Berkowitz 1979). One of many explanations for this phenomenon could involve an impairment of neuronal MAO activity in the mesenteric vasculature of SHR rats. The latter reasoning is based upon the observation that drug-induced inhibition of MAO is associated with an increase in the endogenous content of NA in blood vessels (Berkowitz et al 1974).

Direct measurement of MAO activity in homogenates of blood vessels will provide little insight into the functional activity of neuronal MAO. This follows from the fact that the predominant MAO activity in blood vessels is of extraneuronal origin (de la Lande et al 1970; Head et al 1977). In intact isolated segments of sympathetically innervated tissues, the predominant deaminated metabolite of NA formed in sympathetic nerves is dihydroxyphenylethylene glycol (DOPEG) (Graefe et al 1973). Moreover, DOPEG, which is lipid soluble, readily diffuses from sympathetic nerve terminals and, therefore, does not readily accumulate within terminal axons (Levin 1974; Trendelenburg et al 1980; Majewski et al 1982). In contrast, the measurement of the accumulation of DOPEG in incubation solutions is an index of neuronal MAO activity.

In the present study, we have re-examined the disposition of NA in isolated mesenteric arteries from SHR and WKY rats. Measurement of the concentrations of DOPEG formed from both endogenous and exogenous NA has enabled us to determine if there exists an impairment in neuronal MAO activity in mesenteric arteries from SHR rats.

METHODS

Male spontaneously hypertensive rats (SHR) and normotensive Kyoto Wistar rats (WKY), 16 to 20 weeks old, were purchased from Taconic Farms, Germantown, New York. Blood pressures were determined using the indirect tail cuff plethysmography procedure (Narco Biosystems, Houston, Texas) (Crabb et al 1980). The mean systolic pressure in SHR rats was 60 to 80 mm Hg higher than found in WKY rats.

Animals were decapitated and the mesenteric arteries removed, washed in ice-chilled 0·9% NaCl (saline), blotted and weighed. For experiments involving measurement of the endogeneous NA content of arteries, the tissues were homogenized in perchloric acid (400 mM) and centrifuged as described previously (Head & Berkowitz 1982). The NA contents of the acid extracts were determined using high performance liquid chromatography (hplc) in conjunction with electrochemical detection (ECD). The latter procedure is based upon that

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originally described by Kissinger et al (1973) and adopted for use with sympathetically innervated tissues (Stitzel et al 1982).

The formation of DOPEG by arteries was determined under two different experimental settings. In the first series of experiments, mesenteric arteries were incubated at 37 °C in 1·0 ml of Krebs solution aerated with 95% O_2, 5% CO_2 for 1 h. Arteries were transferred to a second series of tubes containing Krebs solution and (−)-NA ($5·9 \times 10^{-7}$ M) and incubated for 1 h. Arteries were then transferred to a final series of tubes containing Krebs solution, (−)-NA ($5·9 \times 10^{-7}$ M) and cocaine (3×10^{-5} M). Aliquots (0·1 ml) of the Krebs solution were acidified with 0·01 ml of a solution of methanol containing 10% glacial acetic acid. The DOPEG concentrations in the acidified Krebs solution were determined by a hplc-ECD procedure. The latter apparatus consisted of a solvent delivery system (Waters Associates, Model M-45), an injector (Waters Associates Model U6K), a reverse phase column (Waters Associates, C_{18} column), and an electrochemical detector (Bioanalytical Models 3A or 4A). The mobile phase comprised a solution of 0·1 M phosphate buffer titrated to pH 3·8 with 0·1 M citric acid. Ethylenediamine tetraacetic acid (100 mg litre^{-1}) (EDTA) was present in the mobile phase. Using the hplc-ECD system, DOPEG chromatographed with a retention time less than that of NA and its other major deaminated and *O*-methylated metabolites.

The formation of ^{3}H-metabolites of [^{3}H]NA (viz; normetanephrine (NM), methoxyhydroxyphenylethylene glycol (MOPEG), vanilmandelic acid (VMA) and dihydroxymandelic acid (DOMA)) by arteries was determined by the procedure of Levin (1973). Arteries were incubated in Krebs solution at 37 °C for 1 h in the presence of (−)-[^{3}H]NA (1×10^{-7} M). The Krebs solution was then acidified as described above and the ^{3}H-metabolites quantified using descending ion exchange paper chromatography (Levin 1973). For selected experiments, rats were pretreated with reserpine (2·5 mg kg^{-1} i.p.) 1·5 h before tissue removal.

The neuronal uptake of (−)-[^{3}H]NA by mesenteric arteries was determined by a procedure similar to that described by Whall et al (1980). Rats were injected with nialamide (100 mg kg^{-1} i.p.) 2 h and reserpine (2·5 mg kg^{-1} i.p.) 1·5 h before tissue removal. Arteries thus treated were incubated for 5 min in Krebs solution containing (−)-[^{3}H]NA (1×10^{-7} M) at 37 °C, then washed in amine-free Krebs solution (3 × 2 min), and homogenized in perchloric acid (400 mM). The endogenous NA content and [^{3}H]NA contents of artery extracts were determined by hplc-ECD analysis and liquid scintillation spectrometry. The degree of MAO inhibition was determined by quantifying the amount of ^{3}H-DOPEG formed during the 5 min incubation period.

Drugs used. (−)-noradrenaline bitartrate (Sigma); (−)-ascorbic acid (Fisher), EDTA (Sigma), nialamide (Pfizer), reserpine (Serpasil, Ciba Geigy), [^{3}H]NA (specific activity of 47·7 or 43·9 Ci mM; New England Nuclear). Double glass distilled or distilled deionized water was used for all agents.

Statistical analysis. All data are expressed as the mean ± the standard error of the mean (s.e.m.). The statistical significance between means was determined by the Student's *t*-test.

RESULTS

The mean concentrations of NA in mesenteric arteries from hypertensive rats (7·4 ± 1·1 μg g^{-1}, n = 9) exceeded those found in normotensive rats (4·4 ± 0·7 μg g^{-1}, n = 9) by almost 67%.

The amount of DOPEG released spontaneously from mesenteric arteries incubated in the absence of exogenous NA is shown in Table 1. The basal production of the metabolite during the 1 h incubation was similar in arteries from SHR and WKY rats. Transfer of arteries from amine-free solutions to solutions containing NA ($5·9 \times 10^{-7}$ M) was associated with a marked increase in the formation of DOPEG. The amount so produced was similar for SHR and WKY rats (Table 1). Transfer of arteries to solutions containing NA ($5·9 \times 10^{-7}$ M) and cocaine (3×10^{-5} M) reduced the formation of DOPEG to

Table 1. Formation of DOPEG from endogenous and exogenous noradrenaline (NA, [^{3}H]NA) in mesenteric arteries from SHR and WKY rats.

	DOPEG formation (nmol g^{-1} h^{-1})	
	SHR	WKY
Krebs (endogenous NA)	3·07 ± 0·51	2·18 ± 0·33
Krebs + NA ($5·9 \times 10^{-7}$ M)	6·95 ± 0·86	8·31 ± 0·96
Krebs + NA ($5·9 \times 10^{-7}$ M) + cocaine (3×10^{-5} M)	2·62 ± 0·45	2·94 ± 0·32
	[^{3}H]DOPEG formation (nmol g^{-1} h^{-1})	
Krebs + [^{3}H]NA (1×10^{-7} M)	0·30 ± 0·06	0·31 ± 0·06
Krebs + [^{3}H]NA (1×10^{-7} M) reserpine treated	0·54 ± 0·11	0·64 ± 0·04

Each value reflects the mean (± standard error of the mean) for seven or more determinations.

levels indistinguishable from those observed for its spontaneous outflow (Table 1); under these conditions the formation of DOPEG was similar in SHR and WKY arteries.

A comparison of DOPEG formation at 30 and 60 min of incubation suggested that its production was not linear. After incubation of the arteries with NA ($5{\cdot}9 \times 10^{-7}$ M) for 30 min, the mean rates of formation of the metabolite from SHR rat tissue ($5{\cdot}24 \pm 0{\cdot}72$ nmol g^{-1}) and WKY rat tissue ($5{\cdot}67 \pm 0{\cdot}68$ nmol g^{-1}) were greater than 50% of the corresponding rates of formation in arteries incubated for 1 h.

Although the amounts of DOPEG formed were similar in SHR and WKY mesenteric arteries, it was possible that the rate of production of other NA metabolites did differ. We tested this hypothesis by incubating arteries with [^{3}H]NA (1×10^{-7} M) for 1 h and then determining the contents of all ^{3}H-metabolites formed. This analysis indicated that [^{3}H]DOPEG was the major metabolite produced; the concentrations of [^{3}H]NM, [^{3}H]MOPEG, [^{3}H]VMA, and [^{3}H]DOMA were less than 10% of those of [^{3}H]DOPEG (unpublished results).

If the higher NA content found in SHR mesenteric arteries was due to the presence of greater number of noradrenergic vesicles, then differences in NA storage mechanisms could influence the amount of amine available for deamination. To examine the role of noradrenergic storage vesicles upon the neuronal deamination of [^{3}H]NA in mesenteric arteries, rats were injected with reserpine (2·5 mg kg^{-1} i.p.) 15 h before tissue removal. Amine concentrations were almost depleted by this regimen. The NA content from SHR tissue being $0{\cdot}03 \pm 0{\cdot}007$ μg g^{-1} (n = 6) and from WKY tissue, $0{\cdot}02 \pm 0{\cdot}003$ μg g^{-1} (n = 5). Although the formation of [^{3}H]DOPEG was significantly greater in the tissue from reserpine-treated compared with untreated rats (Table 1), reserpine treatment did not result in differences in the amount of [^{3}H]DOPEG formed by SHR and WKY arteries (Table 1).

Finally, experiments were conducted to ensure that the failure to detect differences in DOPEG formation in mesenteric arteries was not due to differences in the neuronal uptake of NA by the two strains of rat. If NA storage mechanisms are blocked by reserpine (2·5 mg kg^{-1} i.p.; 1·5 h before death) and MAO is inhibited by nialamide (100 mg kg^{-1} i.p. 2 h before death) then short-term incubations (5 min) with [^{3}H]NA should permit an assessment of neuronal uptake rather than total amine retention. When these experiments were performed (Table 2), the contents of [^{3}H]NA in the tissues from both strains were indistinguishable, suggesting a similarity in the uptake of NA. While pretreatment of rats with both nialamide and reserpine did markedly reduce endogenous levels of NA (Table 2), the amine contents of arteries from animals receiving the combined treatments were greater than those given only reserpine.

Table 2. Exogenous and endogenous noradrenaline (NA) contents and [^{3}H]DOPEG formation in reserpine and nialamide treated mesenteric arteries following a five minute incubation with [^{3}H]NA.

	SHR	WKY
Endogenous NA content (μg g^{-1})	$2{\cdot}14 \pm 0{\cdot}29$	$1{\cdot}79 \pm 0{\cdot}49$
[^{3}H]NA Content (nmol g^{-1} 5·0 min^{-1})	$0{\cdot}73 \pm 0{\cdot}09$	$0{\cdot}63 \pm 0{\cdot}05$
[^{3}H]DOPEG Formation (pmol g^{-1} 5·0 min^{-1})	$5{\cdot}94 \pm 1{\cdot}06$	$3{\cdot}12 \pm 0{\cdot}82$

Each value reflects the mean (± standard error of the mean) for six or more determinations.

DISCUSSION

Measurement of the NA concentrations in mesenteric arteries from SHR and WKY rats using the hplc-ECD procedure indicated that a greater concentration of NA was associated with arteries from hypertensive rats which confirms a previous observation based upon radiochemical assay (Head & Berkowitz 1979). In the present investigation, we explored the possibility that impaired neuronal MAO activity in arteries from SHR rats could be responsible for the differences in NA content in vessels from two strains of rat.

Incubation of tissue with [^{3}H]NA established that [^{3}H]DOPEG was the major metabolite generated. Thus it is a principal metabolite of NA in vascular tissue including the rat caudal artery (Zsoter et al 1982), rat heart (Fiebig & Trendelenburg 1978), rabbit heart (Majewski et al 1982), and rabbit thoracic aorta (Levin 1974). The neuronal origin of DOPEG formation in the mesenteric artery was indicated both by the marked decrease in its production when cocaine was added to the medium and by the increase in DOPEG generation when noradrenergic vesicle storage was blocked by reserpine. Evidence for the formation of DOPEG within sympathetic nerves in other tissues also is well documented (Graefe et al 1973; Majewski et al 1982).

No differences in the ability to form DOPEG from endogenous NA were found between tissue from SHR and WKY rats. It is apparent that the arteries

deaminate endogenously synthesized NA to a similar extent. The extent of formation of [^{3}H]DOPEG from exogenous [^{3}H]NA was also similar. The latter experiments were performed using a substrate concentration ($1{\cdot}0 \times 10^{-7}$ M) five-fold smaller than that used in experiments utilizing non-tritiated NA. Moreover, it was established that at this concentration, the neuronal uptake of [^{3}H]NA was similar in both strains, which argues against the possibility that differences in neuronal uptake of [^{3}H]NA masked differences in its rate of deamination. Since Rho et al (1981) observed that the incorporation of [^{3}H]NA into noradrenergic vesicles isolated from SHR mesenteric arteries was greater than that incorporated into vesicles from WKY arteries, we considered the possibility that differences in the amount of [^{3}H]NA taken up into noradrenergic vesicles may have influenced the extent of DOPEG formation in SHR and WKY arteries. Although pretreatment of the animals with reserpine was associated with a marked decrease in the endogenous NA content of the mesenteric arteries, the formation [^{3}H]DOPEG in arteries from both strains remained similar.

The results suggest a similarity in the activities of neuronal MAO in mesenteric arteries from SHR and WKY rats. The bulk of the experiments conducted in this investigation were performed under conditions in which equal concentrations of substrate were available to arteries from SHR and WKY rats. It is possible that the functional role of neuronal MAO in-vivo in arteries from hypertensive and normotensive rats may not be equivalent since plasma DOPEG concentrations are believed to be greater in SHR than in WKY rats (Vlachakis & Alexander 1981). Regardless, our failure to detect any overtly impaired activity of neuronal MAO in mesenteric arteries from SHR rats suggests that the enhanced concentrations of NA in these arteries is not a consequence of impaired neuronal metabolism of NA.

Acknowledgements

The study was supported in part by a grant from the American Heart Association West Virginia Affiliate and the Biomedical Corporation West Virginia University.

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