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Effects of short- and long-term sympathectomy on vasoconstrictor responses of the rat mesenteric arterial bed

[1]Vera Ralevic & Geoffrey Burnstock

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT

1 The effects of short- and long-term sympathectomy were evaluated on vasoconstrictor function of constantly perfused mesenteric arterial beds isolated from rats: the effects of short-term sympathectomy were assessed at 3 and 8 days after 6-hydroxydopamine (6-OHDA) treatment of adult rats; the effects of long-term sympathectomy were assessed in adult rats treated at youth with guanethidine.

2 The relative degree of residual sympathetic innervation of the mesenteric arterial preparations was assessed by responses to electrical field stimulation (EFS; 16 Hz, 1 ms, 90 V, 30 s). Control responses were 95.6 ± 3.9 mmHg ($n=35$). Responses after sympathectomy were: 3 days after 6-OHDA, 2.9 ± 0.9 mmHg ($n=15$) <8 days after 6-OHDA, 14.1 ± 2.1 mmHg ($n=14$) <guanethidine, 21.1 ± 4.1 mmHg ($n=16$).

3 Three days after 6-OHDA treatment there was an increase in the sensitivities of response to vasopressin and endothelin, producing leftward shifts of the dose-response curves of 0.66 ± 0.11 and 0.88 ± 0.13 log units respectively ($n=7-11$), and a small increase in sensitivity of responses to noradrenaline (NA) and ATP. The maximal response to 5-hydroxytryptamine (5-HT) was increased. In contrast, there was a decrease in maximal constriction to NA and to the α_1-adrenoceptor agonist methoxamine. The α_2-adrenoceptor agonist clonidine did not elicit vasoconstriction at basal tone. There was no difference in vasodilator responses to the β-adrenoceptor agonist isoprenaline in preparations with tone raised with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 0.1-0.3 μM).

4 Eight days after 6-OHDA sympathectomy there was no significant difference in sensitivities or maximal responses to ATP, vasopressin and endothelin, but a small increase in the sensitivity of responses to 5-HT. Maximal responses to NA and methoxamine were significantly lower than the controls, but sensitivities were similar. There was no significant difference in vasodilator responses to isoprenaline in $PGF_{2\alpha}$-raised tone preparations.

5 After long-term guanethidine sympathectomy maximal responses to 5-HT and NA were significantly reduced. Responses to ATP, vasopressin and endothelin were unchanged.

6 In mesenteric arterial preparations from untreated rats, ouabain (0.1 mM), a blocker of the Na^+/K^+ pump, significantly augmented the sensitivity and maximal responses to EFS, NA, methoxamine and 5-HT. Responses to ATP, vasopressin and endothelin were unaffected.

7 It is concluded that in the rat mesenteric arterial bed, short-term sympathectomy, where only 3% of the sympathetic nerve-mediated response remained, results in non-uniform changes in sensitivity and maximal responses to different vasoconstrictors, which cannot be entirely explained by changes in the Na^+/K^+ pump. Most of these changes disappeared at 8 days after 6-OHDA treatment, when nerve-mediated responses had partially returned. After long-term guanethidine sympathectomy, there was little change in responses to vasoconstrictors, and nerve-mediated responses were reduced to 22%. Although the variable factors are complex, it appears that in general, changes in responses of smooth muscle to vasoconstrictor substances after sympathetic denervation only occur if there is near-complete loss of nerve-mediated responses.

Keywords: Denervation; guanethidine; 6-hydroxydopamine; noradrenaline; rat mesenteric arteries; sympathectomy

Introduction

The trophic relationship between perivascular nerves and blood vessel structure and function has been the subject of considerable interest for many years. Denervation, widely used to study this relationship, has been shown to have different consequences according to whether it is carried out in the adult or the growing animal. An increase in vascular DNA synthetic activity and wall cross-sectional area has been shown after denervation of adults, in contrast to the decrease observed when denervation is carried out in the growing animal (Bevan, 1975; Bevan & Tsuru, 1981; Lee *et al.*, 1987; Sarmento *et al.*, 1987). Sympathectomy of the young, but not of the adult rabbit has been shown to produce an increase in collagen with corresponding increase in stiffness of the vascular wall and decrease in maximal contractility (Bevan, 1989).

The functional consequences of sympathetic denervation are typically a non-selective increase in sensitivity, but not maximal responsiveness of the smooth muscle to compounds, although heterogeneous changes in responsiveness to agents and selective receptor upregulation have also been described (Watanabe *et al.*, 1982; Lacroix & Lundberg, 1989). Denervation supersensitivity can be prejunctional, resulting from an increase in the concentration of transmitter at the postjunctional site due to a loss of uptake of neurotransmitter into the nerve terminal (Trendelenburg, 1966). Partial depolarization of the smooth muscle has been suggested to account for non-specific postjunctional denervation supersensitivity (Aprigliano & Hermsmeyer, 1977; Abel *et al.*, 1981) and may be related to a decrease in Na^+/K^+ pump activity as well as to a change in permeability or binding to calcium (Carrier & Hester, 1976; Fleming, 1987). In the guinea-pig vas deferens (Fleming, 1987) and rabbit ear artery (Bevan, 1989) inhibition of the Na^+/K^+ pump with ouabain *in vitro* caused depolarization of the cell membrane and was able to mimic the effects

[1] Author for correspondence.

of denervation. Responses in the innervated but not in the denervated rabbit ear artery were augmented by ouabain (Bevan, 1989).

Rat mesenteric arteries are richly innervated and thus provide a suitable model to study the relationship between perivascular nerves and smooth muscle. Sympathetic denervation of rats with reserpine or 6-OHDA produced an increase in affinity of mesenteric arterial α_1-adrenoceptors, but no change in the number for up to 7 days after treatment (Colucci *et al.*, 1981). Long-term guanethidine sympathectomy of rats has been shown to produce a decrease in mesenteric smooth muscle cell layers and in maximal responses to noradrenaline (NA) (Lee *et al.*, 1987). Long-term 6-OHDA sympathectomy causes reduced media : lumen ratio of rat mesenteric resistance vessels and reduced calcium sensitivity (Nyborg *et al.*, 1986).

The main aim of the present study was to examine the effects of short- and long-term sympathectomy on rat mesenteric arterial constrictor function. Short-term sympathectomy was assessed at 3 and 8 days after treatment of adult rats with 6-hydroxydopamine (6-OHDA). Long-term sympathectomy was assessed in adult rats having undergone guanethidine sympathectomy at youth. Postjunctional effects of NA, methoxamine (α_1-adrenoceptor agonist), clonidine (α_2-adrenoceptor agonist), isoprenaline (β-adrenoceptor agonist), ATP and 5-hydroxytryptamine (5-HT), endothelin and vasopressin, were tested. In addition, the possibility that the Na^+/K^+ pump could have a role in denervation supersensitivity was examined.

Methods

Guanethidine sympathectomy

Matched litters of male Sprague-Dawley rats were given subcutaneous (s.c.) injections of either guanethidine sulphate (Ismelin) 50 mg kg^{-1}, or saline from day 8 after birth for 3 weeks, 5 days per week (Johnsson *et al.*, 1976; Aberdeen *et al.*, 1990). Rats were killed at 12–14 weeks of age by asphyxiation with ether.

6-Hydroxydopamine sympathectomy

Male Sprague-Dawley rats, age-matched with those used for experiments with guanethidine sympathectomy, were injected (i.p.) with 6-OHDA dissolved in sterile saline containing 1 mg ml^{-1} (0.1%) ascorbic acid to retard oxidization of the drug. The injection regime was 100 mg kg^{-1} on day 1 of treatment, 250 mg kg^{-1} on day 2 (Aberdeen *et al.*, 1990), and on day 3 or on day 8 the rats were killed by asphyxiation with ether.

Isolated mesenteric arterial bed preparation

Mesenteric beds were isolated and set up for perfusion by the method of McGregor (1965) as described previously (Ralevic *et al.*, 1994). The abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was severed, the gut dissected away and the preparation mounted on a stainless steel grid (7 × 5 cm) in a humid chamber (custom made at University College London). Preparations were perfused at a constant flow rate of 5 ml min^{-1} with a peristaltic pump (model 7554-30, Cole-Parmer Instrument Co., Chicago, Illinois). The perfusate was Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2–5% CO_2 and maintained at 37°C. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (model P23XL, Viggo-Spectramed, Oxnard, CA) on a side arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass Instrument Co., Quincy, Mass). Preparations were allowed to equilibrate for 30 min before experimentation.

The degree of sympathectomy was estimated by assessing responses to electrical field stimulation (EFS), produced by passing a current at mid-range frequency (16 Hz, 90 V, 1 ms, 30 s) across each preparation from the metal needle and the wire grid on which the preparation rested (which acted as two electrodes). Vasoconstrictor responses of preparations to increasing doses (50 μl bolus injections) of the vasoconstrictor agents were then assessed. Individual doses were applied at intervals of at least 2 min, but as much as 30 min, depending on the agonist and the time it took for the tone to return to baseline. The intervals between injections were consistent for a particular agonist and hence between control and treated preparations; 10–15 min was allowed between consecutive dose-response curves. Two groups of 3- and 8-day 6-OHDA-treated rats and their respective controls were used. In one group responses to ATP, 5-HT, vasopressin and endothelin were determined. In the second group responses to NA, methoxamine and clonidine at basal tone, and to isoprenaline and clonidine at raised tone (tone raised with prostaglandin $F_{2\alpha}$) were examined. Guanethidine-treated animals were also divided into two groups: in one group responses to NA and ATP were tested; in the other group responses to 5-HT, vasopressin and endothelin were tested. The responses to a single dose of potassium chloride (KCl) (0.15 μmol) was tested at the end of each experiment.

In a separate group of untreated animals responses to EFS, NA, ATP, methoxamine, KCl, vasopressin and endothelin were tested in the absence and presence of ouabain (0.1 mM), added to the perfusate at the start of the period of equilibration.

Drugs used

All drugs were applied as 50 μl bolus injections into a rubber septum proximal to the preparation. Drug dilutions were made up daily from stock solutions of 10 or 100 mM (concentrates stored frozen) in distilled water, except for NA and 5-HT, which were made up daily as stock solutions in 0.1 mM ascorbic acid and diluted in distilled water. The following drugs were obtained from Sigma Chemical Co. (St. Louis, MO): clonidine hydrochloride, noradrenaline bitartrate, adenosine 5'-triphosphate (disodium salt), 5-hydroxytryptamine (creatinine sulphate), isoprenaline bitartrate, methoxamine (hydrochloride), 9,11-dideoxy-11α,9α-epoxy-methano prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and ouabain. Vasopressin and endothelin were obtained from Cambridge Research Biochemicals Ltd. (Northwich, Cheshire, U.K.).

Data analysis

All data are presented as means ± s.e. pD_2 values were calculated as the negative log of the dose required to produce a half-maximal response. Responses to ATP and to endothelin did not reach a maximum, hence pD_{20}, pD_{30} or pD_{60} values (negative log of the dose required to produce vasoconstriction of 20, 30 or 60 mmHg, respectively) were calculated where appropriate, as indicated. Vasodilator responses were evaluated as a percentage of the $PGF_{2\alpha}$-induced increase in tone above baseline. Statistical analysis was performed by analysis of variance (ANOVA) or ANOVA with repeated measures as appropriate. A probability (P) < 0.05 was taken as significant. Post hoc analysis was performed by Student's t test with Bonferroni correction.

Results

6-OHDA treated animals weighed significantly less than the controls. Rat weights were: 344.1 ± 6.4 g ($n = 22$), 286.6 ± 3.7 g ($n = 15$) ($P < 0.001$) and 313.6 ± 7.3 g ($n = 14$) ($P < 0.01$) in controls, 3 days after 6-OHDA treatment and 8 days after 6-OHDA treatment, respectively. There was no significant difference between the weights of the control and guanethidine treated rats: 447.3 ± 5.6 g ($n = 15$) and 431.9 ± 7.5 g ($n = 16$), respectively.

Short-term sympathectomy: 3 days after 6-OHDA treatment

There was no significant difference between the basal perfusion pressures of the preparations: controls, 25.6±2.2 mmHg (n=22); 3 days after 6-OHDA, 27.6±3.0 mmHg (n=15); 8 days after 6-OHDA, 27.1±2.1 mmHg (n=14). Vasoconstrictor responses to EFS (16 Hz, 90 V, 1 ms, for 30 s) were reduced from 96.8±4.8 mmHg (n=22) in controls to 2.9±0.9 mmHg (n=15) after 6-OHDA treatment.

After 6-OHDA treatment there was a significant increase in the sensitivity of responses to NA (Table 1). However, maximum responses to NA and to methoxamine were significantly reduced (Figure 1a, b). Maximum responses to NA were 187.6±19.0 mmHg (n=9) in controls and 103.0±14.1 mmHg (n=6) after 6-OHDA. Maximum responses to methoxamine were 96.4±5.3 mmHg (n=11) in controls and 30.8±8.7 mmHg (n=8) after 6-OHDA. Clonidine (5–5000 nmol) had no vasoconstrictor effects at basal tone. In preparations with tone raised with $PGF_{2\alpha}$ (0.1–0.3 μM), clonidine generally elicited small constrictions, particularly at higher doses, although weak and inconsistent relaxations were observed in some preparations

Table 1 pD_2 values for NA, methoxamine, 5-HT and vasopressin; $pD_{20/30}$ values for ATP and pD_{60} values for endothelin after guanethidine and 6-OHDA treatment

	NA	*Methoxamine*	*5-HT*	*ATP*	*Vasopressin*	*Endothelin*
Control	7.35±0.10 (9)	6.55±0.14 (11)	8.31±0.05 (11)	6.08±0.06 (11)	10.20±0.05 (11)	9.72±0.24 (7)
3-Day 6-OHDA	7.79±0.09 (6)*	6.45±0.14 (8)	8.41±0.08 (7)	6.49±0.08 (7)*	10.86±0.10 (7)*	10.60±0.08 (7)*
8-Day 6-OHDA	7.67±0.09 (6)	6.73±0.04 (6)	8.76±0.03 (8)*	6.22±0.22 (8)	10.33±0.09 (8)	9.93±0.06 (8)
Control	7.77±0.06 (7)	NT	8.04±0.10 (6)	5.87±0.29 (6)	10.25±0.16 (6)	10.28±0.11 (5)
Guanethidine	7.75±0.05 (7)	NT	8.28±0.12 (7)	6.11±0.14 (7)	10.12±0.11 (7)	10.12±0.14 (7)

For ATP pD_{30} values are given in the 6-OHDA group and the respective controls; in guanethidine treated preparations and their respective controls pD_{20} values were calculated for ATP since not all responses reached 30 mmHg. NT=not tested. Number of animals given in parentheses. *Denotes significant difference from the controls (P<0.01).

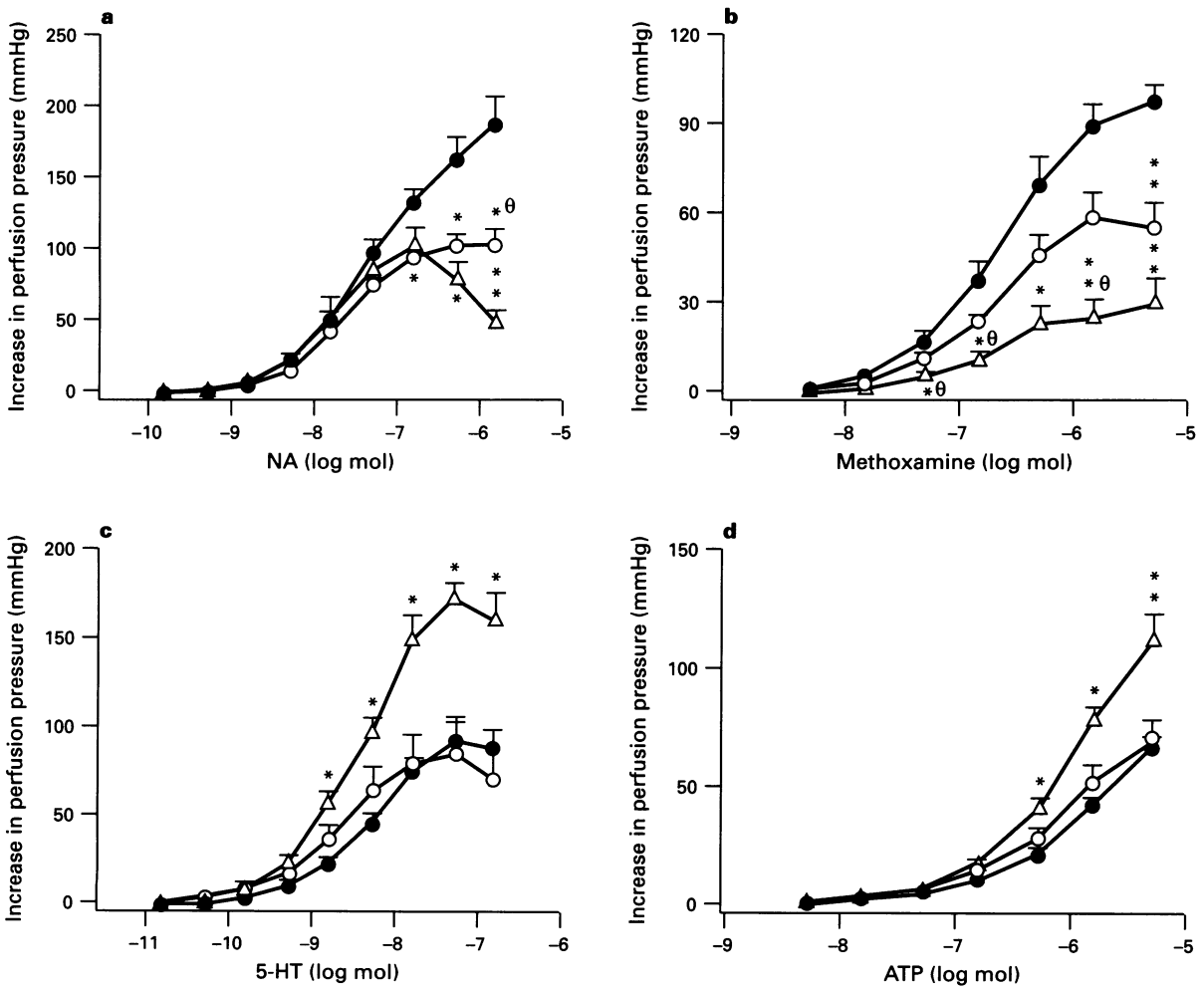

Figure 1 Vasoconstrictor responses to (a) noradrenaline (NA), (b) methoxamine, (c) 5-hydroxytryptamine (5-HT) and (d) adenosine 5′-triphosphate (ATP). Mesenteric arterial preparations were from control rats (●, n=7–11) and rats treated with 6-OHDA commencing 3 days previously (△, n=5–7) and 8 days previously (○, n=8). *P<0.01 and **P<0.001 denote significant difference between treated and control groups. θ Denotes significant difference between the two 6-OHDA-treated groups. Vertical lines show s.e.mean.

(data not shown). Isoprenaline (0.005–50 nmol) elicited dose-dependent relaxation which was not significantly different between the groups: pD_2 value of control was 9.39±0.10 ($n=11$), pD_2 value after 6-OHDA was 9.32±0.37 ($n=8$). Maximal relaxations were 33.85±3.16% and 43.73±8.95% in controls ($n=11$) and after 6-OHDA ($n=8$), respectively.

Marked supersensitivity was seen for both vasopressin (Figure 2a, Table 1) and endothelin (Figure 2b, Table 1); there was a shift to the left of the dose-response curves of 0.66±0.11 and 0.88±0.10 log units, respectively, without a change in the maximum. The sensitivity of responses to ATP (pD_{20} values) was increased (Figure 1d, Table 1). The maximum response to 5-HT was significantly increased, but sensitivity was unchanged (Figure 1c, Table 1). There was no difference in responsiveness between the groups to KCl; responses were 73.5±4.5 mmHg ($n=11$) in controls and 72.1±5.9 mmHg ($n=7$) in 6-OHDA-treated preparations.

Short-term sympathectomy: 8 days after 6-OHDA treatment

There was no significant difference in basal perfusion pressure between control preparations, 25.6±2.1 mmHg ($n=22$), and preparations from 6-OHDA treated rats, 27.1±2.1 mmHg ($n=14$). Vasoconstrictor responses to EFS (16 Hz, 90 V, 1 ms, 30 s) were reduced from 96.8±4.8 mmHg ($n=22$) to 14.08±2.1 mmHg ($n=14$).

After 6-OHDA treatment maximal responses to NA and methoxamine were significantly reduced, although sensitivities were similar (Figure 1a, b and Table 1). Maximum responses to NA were 187.6±19.0 mmHg ($n=9$) in controls, and 105.3±7.9 mmHg ($n=6$) after 6-OHDA. Maximum responses to methoxamine were 96.4±5.3 mmHg ($n=11$) in controls, and 59.2±8.2 mmHg ($n=6$) after 6-OHDA. Clonidine (5–5000 nmol) had no effect at basal tone. At raised tone ($PGF_{2\alpha}$; 0.1–0.3 μM) clonidine generally elicited small but variable contractile effects, although weak relaxations were seen in some preparations at lower doses. Responses to isoprenaline (0.005–50 nmol) were similar in controls and after 6-OHDA treatment: pD_2 values were 9.39±0.10 ($n=11$) and 9.25±0.15 ($n=6$), respectively. Maximal relaxations were 33.85±3.16% ($n=11$) and 29.19±5.14% ($n=6$) in controls and after 6-OHDA, respectively.

After 6-OHDA treatment there was a significant increase in the sensitivity of constrictor responses to 5-HT, but no change in the maximum response (Figure 1c and Table 1). There was no change in responses to ATP, vasopressin and endothelin (Figures 1 and 2, Table 1). Responses to KCl were also similar between the two groups: 73.5±4.5 mmHg ($n=11$) in controls and 69.9±8.2 mmHg ($n=8$) after 6-OHDA treatment.

Long-term sympathectomy: guanethidine treatment

There was no difference in basal perfusion pressure between the preparations: 28.1±1.4 mmHg ($n=15$) and 30.3±1.8 mmHg ($n=16$) for control and guanethidine treated preparations, respectively. Vasoconstrictor responses to EFS (16 Hz, 90 V, 1 ms, 30 s) were significantly attenuated after sympathectomy: 93.6±6.7 mmHg ($n=13$) in controls and 21.1±4.1 mmHg ($n=16$) after guanethidine.

Maximum constriction to NA (at 0.5 μmol) was smaller after guanethidine treatment although at other doses responses were similar (Figure 3a). Vasoconstrictor responses to 5-HT were significantly smaller after guanethidine treatment, the maximum response being reduced by approximately 60%, from 150.3±13.7 mmHg ($n=6$) to 57.0±5.2 mmHg ($n=6$) (Figure 3b). Responses to 5-HT between the 6-OHDA and guanethidine control groups were different for reasons which are not apparent; however, the meaningful comparisons were those made within each group, between control and treated preparations run in parallel. There was no difference between the response curves to ATP (ANOVA); these did not reach a maximum, thus pD_{20} values are presented in Table 1.

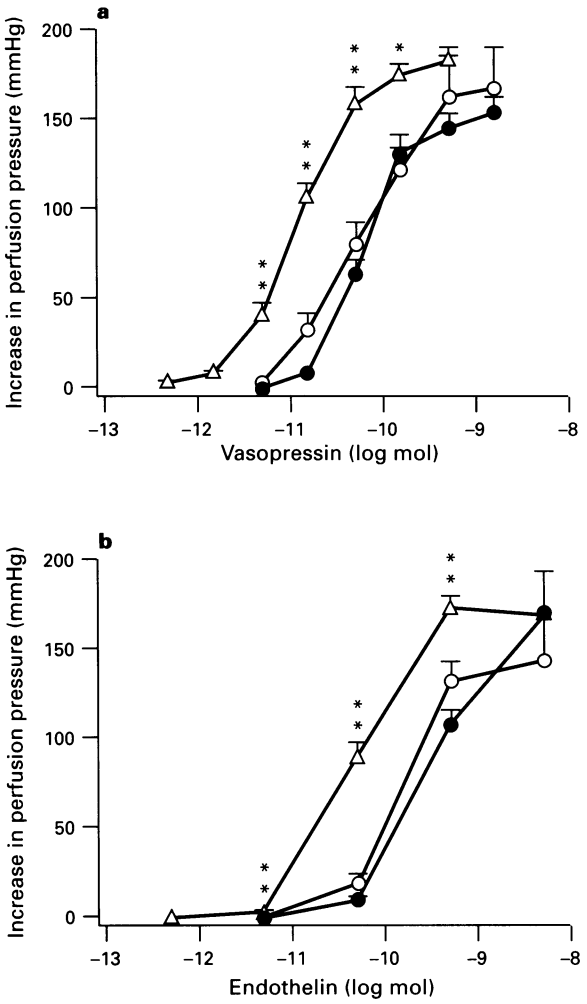

Figure 2 Vasoconstrictor responses to (a) vasopressin and (b) endothelin. Mesenteric arterial preparations were from control rats (●, $n=11$) and rats treated with 6-OHDA commencing 3 days previously (△, $n=7$) and 8 days previously (○, $n=8$). *$P<0.01$ and **$P<0.001$ denote significant differences between the groups. Vertical lines show s.e.mean.

Vasoconstrictor responses to vasopressin (Figure 3c) and endothelin (Figure 3d) were similar in control and guanethidine-treated groups. There was no significant difference in vasoconstrictor responses to KCl (0.15 μmol); responses were 69.9±28.9 mmHg ($n=13$) in controls and 72.0±7.6 mmHg ($n=14$) after guanethidine treatment.

Effects of ouabain

Ouabain (0.1 mM) had no significant effect on the tone of the preparations. The effects of ouabain on vasoconstrictor responses of the mesenteric preparations to EFS and to the various agonists are shown in Figures 4, 5 and 6, and pD_2 values are given in Table 2. In the presence of ouabain there was an increase in sensitivity and maximal responsiveness to EFS (Figure 4), NA, methoxamine and 5-HT (Figure 5). Responses to ATP (Figure 5), vasopressin and endothelin (Figure 6) were unchanged.

Discussion

Two different sympatholytic agents were required to induce short- and long-term sympathectomy respectively because of the time constraints associated with their injection protocols.

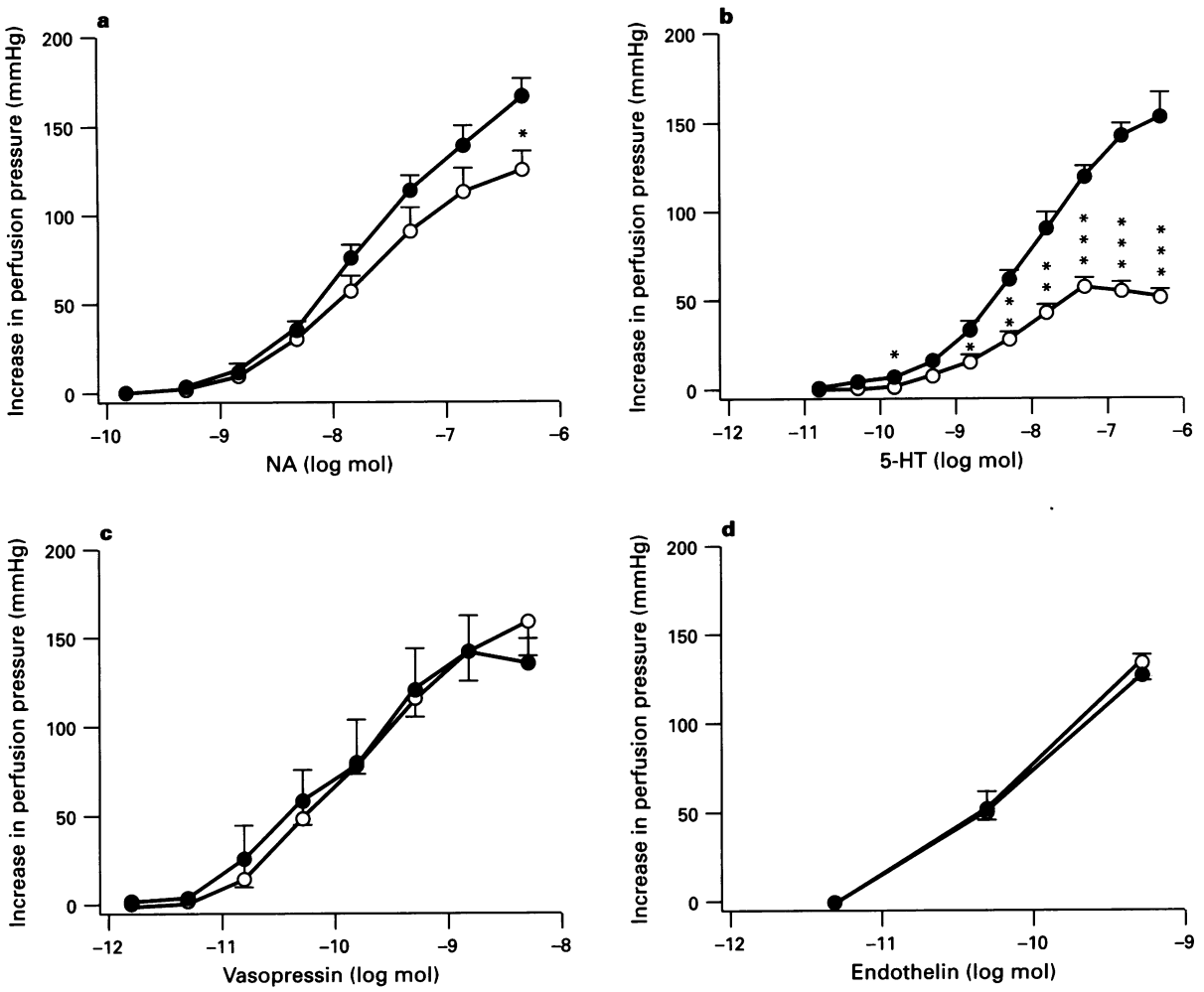

Figure 3 Vasoconstrictor responses to (a) noradrenaline (NA), (b) 5-hydroxytryptamine (5-HT), (c) vasopressin and (d) endothelin, in mesenteric arterial preparations from control rats (●, $n=5-7$) and after long-term guanethidine treatment (○, $n=6-8$). $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ denote significant differences between the groups. Vertical lines show s.e.mean.

Table 2 pD_2 values for NA, methoxamine, 5-HT and vasopressin; pD_{30} values for ATP and pD_{60} values for endothelin in the absence and presence of ouabain (0.1 mM)

	NA	*Methoxamine*	*5-HT*	*ATP*	*Vasopressin*	*Endothelin*
Control	7.61±0.09 (8)	6.54±0.09 (6)	8.41±0.08 (6)	5.89±0.09 (8)	10.22±0.08 (7)	10.01±0.05 (8)
Ouabain	7.92±0.11 (8)*	6.96±0.15 (5)*	8.82±0.08 (5)**	6.19±0.11 (7)	10.30±0.06 (6)	10.46±0.10 (7)**

Number of animals given in parentheses. $^{*}P<0.05$ and $^{**}P<0.01$ denote significant difference from the control

6-OHDA produces rapid sympathectomy following uptake into and destruction of sympathetic nerve terminals (Thoenen & Tranzer, 1968). However, cell bodies of adult animals are not affected by selective doses of 6-OHDA, therefore nerve terminals regenerate within a few weeks. In the present study reductions of rat mesenteric sympathetic nerve-mediated responses of approximately 97% and 85% were seen at 3 and 8 days after 6-OHDA treatment, respectively. Chronic guanethidine treatment is highly selective for sympathetic nerves in rats: it destroys the cell bodies of sympathetic ganglia and produces a near complete long-lasting sympathectomy (Heath & Burnstock, 1977; Aberdeen *et al.*, 1990). However, guanethidine injection typically takes place over a number of weeks and thus is not suitable for short-term sympathectomy. The protocol we adopted has been shown to produce a decrease in NA levels of the rat mesenteric vein of approximately 87% at 6 weeks after treatment, and 98% at 20 weeks (Aberdeen *et al.*, 1990). In the present study, at 12–14 weeks after guanethidine treatment there was an approximately 77% reduction in sympathetic vasoconstriction of the mesenteric arterial bed. Thus, the functional studies indicate that residual innervation of the rat mesenteric arterial bed after sympathectomy increased in the order: 3 days after 6-OHDA < 8 days after 6-OHDA < guanethidine. Duration of sympathectomy and treatment are additional variables.

The present results demonstrate that in the rat mesenteric arterial bed sympathectomy is associated with differential changes in responses to different constrictor agents. Based on the main effects observed for changes in sensitivities and maximal responses three profiles appear: for vasopressin and endothelin; for NA and methoxamine; and for 5-HT and ATP. At 3 days after 6-OHDA sympathectomy there was an increase in sensitivity to

vasoconstrictors, with the exception of 5-HT and methoxamine. Differential supersensitivity after sympathectomy has been demonstrated in the rabbit ear artery, where pronounced supersensitivity to 5-HT but no change in responsiveness to NA was suggested to be due to a greater influence of the activity of the Na^+/K^+ pump on responses to 5-HT (Bevan, 1989). A shift to the left of the dose-response curve without a change in the maximum, as seen in the present study for vasopressin and endothelin, is the most consistent finding in non-specific denervation hypersensitivity and is believed to be due to partial depolarization of smooth muscle cells (Aprigliano & Hermsmeyer, 1977; Goto *et al.*, 1978; Abel *et al.*, 1981). Potentiation of responses of the mesenteric vasculature to 5-HT, methoxamine and NA by ouabain is consistent with a role for membrane depolarization and a decrease in the activity of the Na^+/K^+ pump in denervation supersensitivity; however, absent or weak augmentation by ouabain of responses to ATP, vasopressin and endothelin is in contrast to the marked denervation supersensitivity to these agents, suggesting that other factors may be involved. Changes in permeability or binding of calcium have been suggested to occur after denervation (Carrier & Hester, 1976; Fleming, 1987).

The lack of change in sensitivity of responses to methoxamine is in line with the smaller increase in sensitivity to NA compared to the other agents. The difference between these two agents may be partly due to pre-junctional supersensitivity. Unlike NA, methoxamine is not a substrate for the neuronal transport system (Trendelenburg *et al.*, 1970) and therefore is unaffected by reduced adrenergic neuronal uptake, resulting in a relatively greater concentration of applied NA than of methoxamine at the postjunctional site. In the rat vas deferens 6-OHDA treatment caused an approximately 5 fold greater

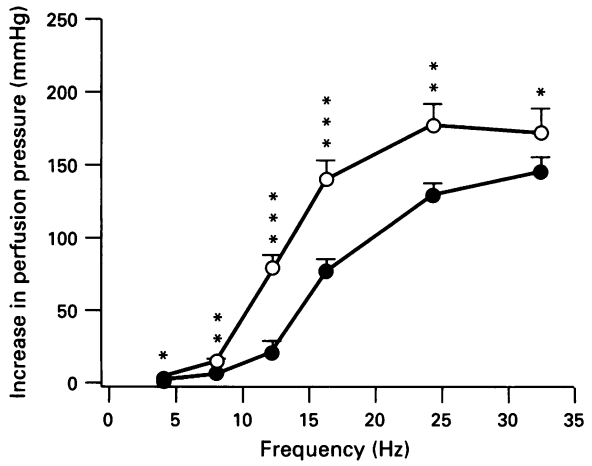

Figure 4 Vasoconstrictor responses of the rat isolated perfused mesenteric arterial bed to electrical field stimulation (4–32 Hz, 1 ms, 90 V, 30 s) in the absence (●, $n=8$) and presence (○, $n=8$) of ouabain (0.1 mM). Significant differences are denoted by $^*P<0.05$, $^{**}P<0.01$ $^{***}P<0.001$. Vertical lines show s.e.mean.

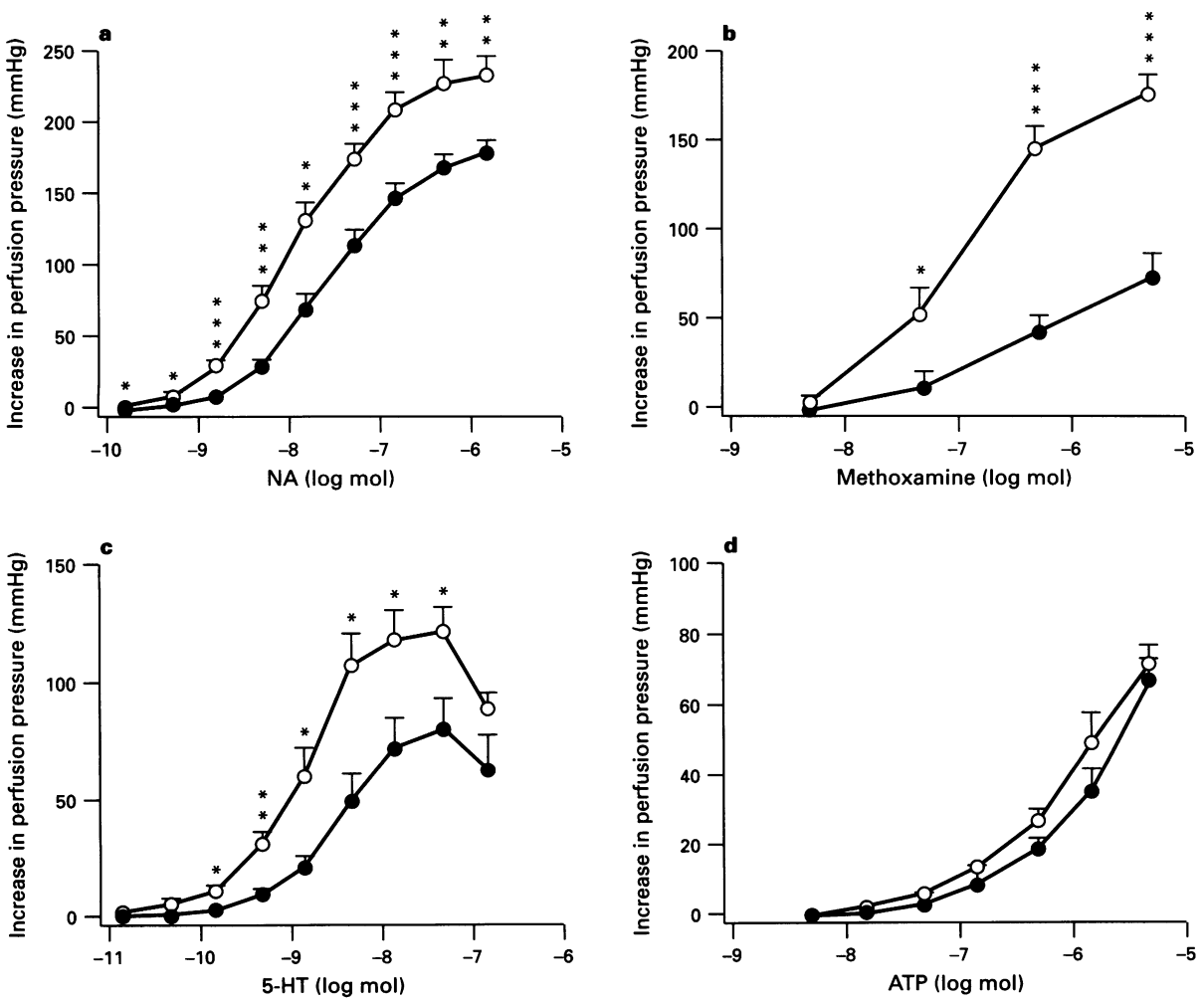

Figure 5 Vasoconstrictor responses of the rat isolated perfused mesenteric arterial bed in the absence (●) and presence (○) of ouabain (0.1 mM) to (a) noradrenaline (NA) ($n=8$), (b) methoxamine ($n=5$), (c) 5-hydroxytryptamine (5-HT) ($n=5-6$) and (d) adenosine 5'-triphosphate (ATP) ($n=7-8$). $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ denote significant difference from control. Vertical lines show s.e.mean.

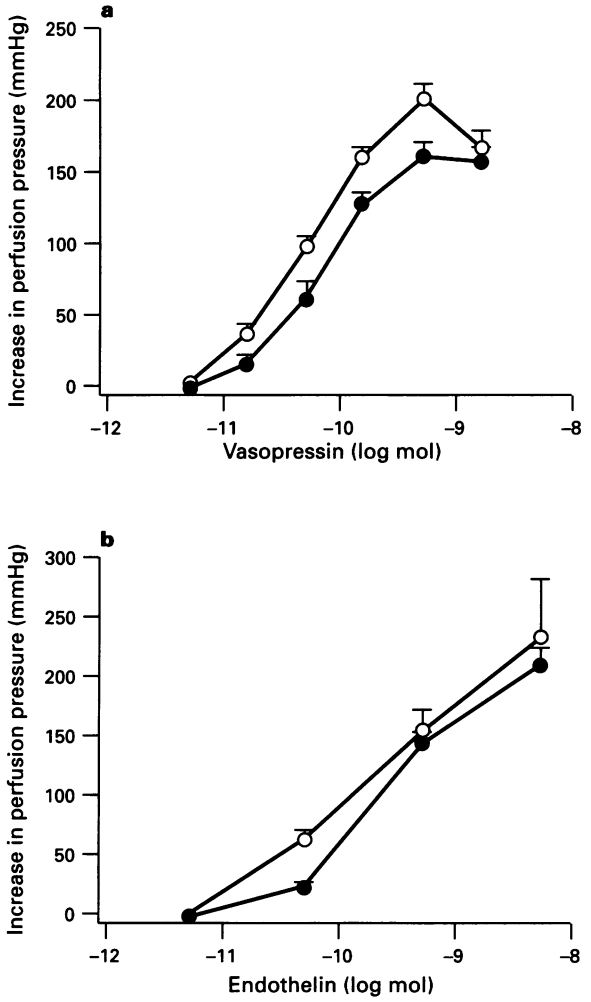

Figure 6 Vasoconstrictor responses of the rat isolated perfused mesenteric arterial bed in the absence (●) and presence (○) of ouabain (0.1 mM) to (a) vasopressin ($n=6-7$) and (b) endothelin ($n=6-8$). Vertical lines show s.e.mean.

increase in sensitivity to NA compared to methoxamine, suggested to be due to pre- as well as postjunctional supersensitivity to NA (Westfall & Fedan, 1975). It is also possible that sympathectomy results in differential regulation of subtypes of α_1-adrenoceptors and that methoxamine and NA are subtype-selective for these receptors. The α_2-adrenoceptor agonist clonidine had no contractile effects at basal tone, confirming previous findings that the excitatory adrenoceptors in rat mesenteric arteries are predominantly α_1-adrenoceptors (Pipili, 1986).

Maximal responses to NA and methoxamine, but not to the other vasoconstrictors, were reduced at 3 days after 6-OHDA sympathectomy. On the other hand, binding studies have shown no change in the number (but an increase in the affinity) of α_1-adrenoceptors in rat mesenteric arteries at up to 48 h after 6-OHDA denervation (Colucci *et al.*, 1981). Vasodilator α_2-adrenoceptors have been shown to be present on endothelial cells in some vessels (Angus & Cocks, 1989); however, weak and inconsistent relaxations to clonidine at raised tone suggest that these may not have functional significance in rat mesenteric arteries. Endothelial β_1-adrenoceptors have been described in rat mesenteric arteries (Graves and Poston, 1993); however, vasodilator effects of isoprenaline were similar before and after sympathectomy (3 and 8 days after 6-OHDA). Subtype-specific changes after sympathectomy have been described in other tissues: supersensitivity of α_2- but not α_1-adrenoceptors in pig nasal mucosa (Lacroix & Lundberg, 1989), and an increase in α_2, but not α_1-adrenoceptor binding sites in rat vas deferens (Watanabe *et al.*, 1982). It is not clear why maximal responses to 5-HT were augmented. Structural changes do not generally occur after adult sympathectomy (Bevan, 1989), and are unlikely to explain the different patterns of changes in contractile responsiveness because of their heterogeneity.

At eight days after 6-OHDA sympathectomy the changes seen at 3 days partially or wholly disappeared. A significant increase in the contractile response to EFS suggested that there was some sympathetic reinnervation, which may have allowed normalization of maximal responses and reversal of supersensitivity. An implication of these findings is that only a small degree/short duration of sympathetic innervation is required to prevent adaptive changes in rat mesenteric arteries.

Long-term guanethidine sympathectomy was associated with relatively few changes in response, there being a decrease in maximal constriction to NA and to 5-HT, but no change in responses to ATP, endothelin and vasopressin. The result with NA is consistent with a previous finding of a decrease in the maximum contractile response to NA in the rat mesenteric arterial bed after neonatal guanethidine sympathectomy (although the sensitivity of responses was increased) (Lee *et al.*, 1987). Structural changes found after long-term sympathectomy include a reduction in smooth muscle cells layers of large and small mesenteric arteries (Lee *et al.*, 1987) and a reduced media : lumen ratio and calcium sensitivity of mesenteric resistance vessels (Nyborg *et al.*, 1986). However, if structural changes do occur, this cannot account for the lack of uniformity of changes in responses to the various agonists. Compensatory changes involving other systems should also be considered. For instance, after sympathectomy the 5-HT content of several organs in rats and mice increases (Thoenen, 1972) and there is an augmented delivery of catecholamines from the adrenal medulla into the circulation (Lee *et al.*, 1991).

In conclusion, the results of this study show that in the rat mesenteric arterial bed sympathectomy is associated with different changes in responses to different vasoconstrictors. Supersensitivity and changes in maximal responses after short-term sympathectomy cannot be explained solely by changes in membrane potential and the Na^+/K^+ pump. Changes after short-term sympathectomy largely disappear at 8 days after 6-OHDA sympathectomy, concomittant with partial recovery of sympathetic nerve-mediated responses. In general, few changes are seen after long-term guanethidine sympathectomy, associated with a greater degree of sympathetic function. Thus, although there are other variables it appears that a crucial relationship exists between the degree of sympathetic innervation and smooth muscle contractile responsiveness, such that near-complete sympathectomy is required for changes in responses to vasoconstrictor agents.

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