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Effects of hypoxia on contractile responses of rabbit aortic strips to transmural electrical stimulation *

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Summary. To clarify the effects of hypoxia on adrenergic transmission, we examined the contractile responses of isolated rabbit aortic strips to electrical stimulation, the concentration-response relationships for noradrenaline and KCl, and the electrical stimulation-evoked overflows of total [^{3}H] and [^{3}H]noradrenaline from strips preloaded with [^{3}H]noradrenaline in media equilibrated with gas mixtures containing various concentrations of O_2. Contractile responses to electrical stimulation were completely inhibited by tetrodotoxin and α-adrenoceptor antagonists such as phentolamine and phenoxybenzamine, but were not affected by indomethacin. When the concentration of O_2 in the gas mixture was decreased from 95% to 20%, the contractile responses to electrical stimulation remained unchanged, but as the concentration of O_2 was further decreased, the responses were inhibited concentration-dependently. At 0% O_2, the response was inhibited by about 80% when compared with control values obtained at 95% O_2, and the electrical stimulation-evoked overflows of total [^{3}H] and [^{3}H]noradrenaline into the superfusates were decreased by about 55%. At 0% O_2, the concentration-response curve for exogenous noradrenaline was shifted to the right about 50-fold and the maximum response was decreased by 25%. The maximum contractile responses of aortic strips from animals pretreated with reserpine or 6-hydroxydopamine to high KCl were decreased slightly (about 15%). These results suggest that inhibition of adrenergic transmission under hypoxic conditions is mainly the result of a decrease in the stimulus-evoked release of noradrenaline and of a decrease in the affinity of α-adrenoceptor for noradrenaline and/or inhibition of signal transduction mechanisms, although hypoxia also causes a slight decrease in the contractility of vascular smooth muscle.

Key words: Hypoxia – Aortic strips – Transmural electrical stimulation – Noradrenaline release – α-Adrenoceptor

Introduction

Recently, we have shown that the effects of hypoxia on the turnover rates of noradrenaline and adrenaline in the sympatho-adrenal system, as an index of the functional activities of neurons involved, differed depending on the organ studied: turnover rates were increased in the heart and the adrenal gland, decreased in the submaxillary gland and unchanged in the stomach (Lee et al. 1987). However, since changes in turnover rates induced by hypoxia were virtually abolished following treatment with a ganglion blocker or transection of the spinal cord at C_{5-6} level, we concluded that these effects originated mainly in the brain (Lee et al. 1987). Other investigators have also reported that the changes in both cardiac function and vascular resistance observed under hypoxic conditions are chiefly due to indirect effects of hypoxia, resulting from a direct effects of hypoxia on the brain (Downing et al. 1963; DeGeest et al. 1965) and from peripheral chemoreceptor-mediated or pulmonary inflation-mediated brain reflexes (Daly and Scott 1958, 1959, 1963; Kontos et al. 1970; Vatner and Rutherford 1978; Rutherford and Vatner 1978). Previous investigators who examined the direct effects of hypoxia on peripheral sympathetic neurons and their effectors were exclusively concerned with the contractile responses of isolated vascular smooth muscle to exogenous noradrenaline or high KCl. They indicated that the contractile responses to only one or two concentrations of noradrenaline (or adrenaline) or to high KCl were considerably decreased under hypoxic conditions and concluded that the decrease was mainly a result of the direct inhibitory effects of hypoxia on the contractile machinery of the vascular smooth muscle (Coret and Hughes 1964; Shibata and Briggs 1967; Detar and Bohr 1968; Hughes and Coret 1969; Detar and Gellai 1971; Namm and Zucker 1973; Vanhoutte 1976; Coburn et al. 1979; Ebeigbe et al. 1980). A few investigators noted, however, that hypoxia had a lesser effect on contractile responses to KCl than on those to noradrenaline (Shibata and Briggs 1967; Coburn et al. 1979). Thus, at present, direct effects of hypoxia on adrenergic neurotransmission, i.e., on noradrenaline release from nerve terminals and on the interaction between noradrenaline and its receptors, are more or less unknown.

In the present study, we examined the effects of hypoxia on the contractile responses of rabbit aortic strips to transmural electrical stimulation, the concentration-response curves for noradrenaline and KCl, and the electrical stimulation-evoked overflows of total [^{3}H] and [^{3}H]noradrenaline from the strips preloaded with [^{3}H]noradrenaline.

Materials and methods

Tissue preparation and measurement of mechanical activity. Thoracic aortae were removed from exsanguinated rabbits

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(2–3 kg) and helically cut into strips of approximately 4 by 20 mm in size. The helical strips were mounted under a resting tension of 1.5 g in a 10-ml tissue bath containing Krebs' solution of the following composition (in mmol/l): NaCl 120.7; KCl 5.9; $CaCl_2$ 2.5; $MgCl_2$ 1.2; $NaHCO_3$ 25.0; NaH_2PO_4 1.2; glucose 11.5; $CaNa_2EDTA$ 0.026; and ascorbic acid 0.113. The medium was maintained at $37 \pm 0.5°C$ and continuously bubbled with a gas mixture of 95% O_2 and 5% CO_2 before and during control experiments. Strips were allowed to equilibrate in the solution for at least 2 h before experiments were begun. The upper end of the strip was connected to the lever of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo, Japan) and isometric contractions were recorded on a heat-writing recorder (Nihonkoden Kogyo).

For transmural electrical stimulation, the strips were placed between a pair of stimulating electrodes and unless specified otherwise, a train of 0.3-ms-square pulses of 50 V and 40 Hz was delivered from an electronic stimulator (Nihonkoden Kogyo) for a period of 10 s as described by Toda (1971).

To obtain cumulative concentration-response curves for noradrenaline and KCl, various amounts of noradrenaline and KCl (dissolved in 100 µl of Krebs' solution) were cumulatively added to the tissue bath until a maximum response was observed. Osmotic adjustment was not made when KCl was added.

After responses to electrical stimulation, noradrenaline or KCl had been recorded in the control medium, O_2 concentrations were changed by bubbling the Krebs' solution with gas mixtures containing various percentages of O_2 and 5% CO_2 in N_2. Preliminary experiments have shown that after changing the mixture from 95% O_2 to 0% O_2, it took about 40 min for the contractile responses to electrical stimulation to reach steady-state levels. Therefore, we waited at least 50 min after changing the gas mixture before attempting to determine the effects of hypoxia on the contractile responses. At the end of each experiment, we reexamined the contractile responses in a medium equilibrated with 95% O_2 and confirmed that the effects of hypoxia were reversible.

Superfusion experiments. Superfusions of aortic strips were carried out according to methods described previously (Su and Bevan 1970; Nedergard and Schrold 1973; Steinsland et al. 1973) with a slight modification. The helical strips were preincubated with 1.0 µmol/l [^{3}H]noradrenaline (specific activity, 1.62 GBq/mol) in 5 ml of Krebs' solution equilibrated with a gas mixture of 95% O_2 and 5% CO_2 at 37°C for 60 min. They were then washed three times with [^{3}H]noradrenaline-free medium, transferred to 2-ml superfusion chambers, and superfused with [^{3}H]noradrenaline-free Krebs' solution at 37°C at a rate of 2 ml/min. The Krebs' solution in the superfusion chamber and the reservoir was bubbled with a gas mixture of 95% O_2 and 5% CO_2. To remove the oxygen, the gas mixture was replaced by 95% N_2 and 5% CO_2 60 min after the onset of superfusion. Starting 110 min after the onset of superfusion, 4 ml fractions of superfusate were collected every 2 min into tubes containing 10 mg/ml sodium bisulfite, 10 mmol/l EDTA and 100 ng noradrenaline as a carrier in a total of 0.4 ml of 1 mol/l perchloric acid. After collecting the first three fractions for determination of the spontaneous overflows of total [^{3}H] and [^{3}H]noradrenaline, the strips were electrically stimulated for 20 s and the collection of the superfusate was continued. At the end of the superfusion, the strips were blotted slightly, weighed and homogenized in 3 ml of 0.1 mol/l perchloric acid containing 1 mg/ml of sodium bisulfite, 1 mmol/l EDTA and 100 ng of 3,4-dihydroxybenzylamine as an internal standard. After centrifugation at $15{,}000 \times g$ for 15 min, aliquots of the supernatant were used for the determination of total [^{3}H] and [^{3}H]noradrenaline remaining in the tissue.

The release of [^{3}H] radioactivity was calculated as fractional release: total [^{3}H] or [^{3}H]noradrenaline released in each sample expressed as a fraction of the total radioactivity present in the tissue at the time of sampling. The overflow of radioactivity evoked by electrical stimulation was calculated by subtracting from the total [^{3}H] or [^{3}H]noradrenaline overflow the spontaneous overflow, which is assumed to be unchanged during and after the period of nerve stimulation.

Assay of [^{3}H]noradrenaline. The [^{3}H]noradrenaline released into the superfusate and remaining in the tissue was extracted by the alumina batch method. The superfusate and the acid-deproteinized tissue extract were adjusted to pH 8.3–8.4 with 3 ml of 1 mol/l Tris-HCl (pH 8.6) and noradrenaline was adsorbed onto 25 mg of acid-washed alumina. After repeated washings with cold redistilled water, noradrenaline was eluted from the alumina with 200 µl of 0.1 mol/l HCl and the eluate was analyzed for noradrenaline by a high-performance liquid chromatograph (HPLC) with an electrochemical detector and a $5C_{18}$ reverse-phase column (4.6×150 mm) as described in detail elsewhere (Miwa et al. 1986a, b). The radioactivity in the noradrenaline peak was determined after collection of the peak into glass scintillation vials. The radioactivity in the peak was corrected for recovery through extraction, which was calculated by comparing the peak height of carrier noradrenaline for superfusates and 3,4-dihydroxybenzylamine for the tissue extract with that of known amounts of authentic standards.

Radioactivity measurement. One-milliliter samples of the superfusate or of the HPLC fractions or 100 µl samples of acid-deproteinized tissue extract were added to 10 ml of scintillator containing 8.0 g of 2,5-diphenyloxazole (PPO), 0.6 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP), and 540 ml of Triton-X 100 per liter of toluene, and the radioactivity was measured in a Packard Tricarb liquid scintillation spectrometer (Model 4430, Downers Grove, IL, USA). Corrections for quenching were made with the external standard method.

Depletion of noradrenaline by chemical denervation with 6-hydroxydopamine (6-OHDA) or by pretreatment with reserpine. To obviate the effects of endogenous noradrenaline which is released during stimulation of aortic strips with high KCl, we attempted to deplete endogenous noradrenaline by chemical denervation with 6-OHDA or by pretreatment with reserpine. One group of rabbits received repeated injections of 6-OHDA according to previously recommended schedules (Thoenen and Tranzer 1968; Maling et al. 1970). That is, the animals received two doses of 6-OHDA (25 mg/kg, i.v.) 24 h apart, two additional doses (50 mg/kg, i.v.) on 7 and 8 days and then were killed between 1 and 2 weeks later. The other group of rabbits received two injections of reserpine (3 mg/kg, i.p., each) 24 h apart and were killed

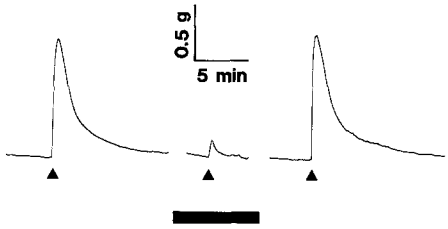

Fig. 1. Typical tracings showing the effects of hypoxia on contractile responses of rabbit aortic strips to transmural electrical stimulation. Rabbit thoracic aortic strips were mounted in the tissue bath of 10-ml capacity containing Krebs' solution. The solution was bubbled with a gas mixture of 95% O_2/5% CO_2 or 95% N_2/5% CO_2 (indicated by a *bar*). Contractile responses of the strips were evoked by transmural electrical stimulation (50 V, 40 Hz of 0.3-ms-square pulses for 10 s) (indicated by *arrowheads*) and the developed tension was isometrically recorded

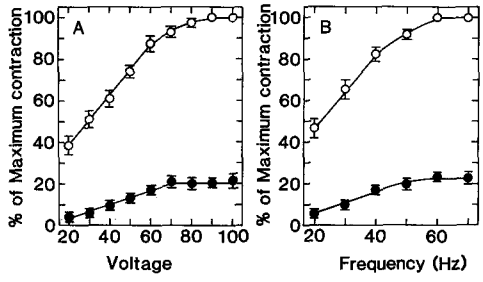

Fig. 2. Contractile responses of rabbit aortic strips to transmural electrical stimulation at various voltages (**A**) and frequencies (**B**) under normal (○——○) and hypoxic conditions (●——●). The strips were mounted in a tissue bath containing Krebs' solution equilibrated with a gas mixture of 95% O_2/5% CO_2 and transmurally stimulated. After control responses were recorded, the gas mixture was replaced by 95% N_2/5% CO_2 and the contractile responses were recorded after equilibration. The voltage (given in V) of the transmural electrical stimulation was varied with the frequency fixed at 40 Hz (**A**) or the frequency was varied with the voltage fixed at 50 V (**B**). Each point is expressed as a percentage of the maximum responses (A: 1.15 ± 0.15 g, $n = 12$; B: 0.937 ± 0.13 g, $n = 12$) obtained in medium equilibrated with 95% O_2/5% CO_2 and is the mean ± SEM of twelve determinations

24 h after the second dose (Maling et al. 1970; Wakade and Krusz 1972). The effectiveness of the treatments was confirmed by measuring tissue noradrenaline content, using HPLC with electrochemical detection as described above.

Chemicals. Chemicals were obtained from the following sources: (−)-[ring-2,5,6-^{3}H]noradrenaline, from New England Nuclear, Boston, MA, USA; noradrenaline bitartrate, phenoxybenzamine hydrochloride, 6-OHDA hydrochloride, 3,4-dihydroxybenzylamine hydrobromide, reserpine and indomethacin, from Sigma, St. Louis, MO, USA; activated alumina, from Wako Pure Chemical Industries, Ltd., Osaka, Japan; phentolamine mesylate, a gift from Ciba-Geigy Japan, Tokyo, Japan; tetrodotoxin, from Sankyo Co., Ltd., Tokyo, Japan.

Data analysis. All results were expressed as means ± SEM. EC_{50} values were defined as the concentration of exogenous noradrenaline or KCl which produced 50% of the maximal response and were calculated according to Fleming et al. (1972). The data were subjected to a two-way analysis of variance, and when significant F values were encountered, Neuman-Keuls' multiple range test was used to test for significant differences between treatment means (Steel and Torrie 1960). A probability level of $P < 0.05$ was considered statistically significant.

Results

Figure 1 shows typical tracings of contractile responses of rabbit thoracic aortic strips to transmural electrical stimulation (50 V, 40 Hz of 0.3-ms-square pulses for 10 s) under normal and hypoxic conditions. After changing a gas mixture from 95% O_2/5% CO_2 to 95% N_2/5% CO_2, contractile responses of aortic strips to electrical stimulation decreased to about 20% of the control value. When the tissue bath was reoxygenated with a mixture of 95% O_2/5% CO_2, the contractile responses rapidly returned to the control values.

The pH of the medium equilibrated with 95% N_2/5% CO_2 was 7.39 ± 0.01 ($n = 8$), which was not significantly different from the value observed during control experiments (7.40 ± 0.01, $n = 8$).

Figure 2 shows the contractile response of rabbit aortic strips to transmural electrical stimulation applied at various voltages (A) and frequencies (B) under both normal and hypoxic conditions. At a fixed frequency of 40 Hz, the contractile responses increased linearly with increasing voltage up to 60 V and reached a plateau at about 70 V under both normal and hypoxic conditions (Fig. 2A). With the voltage fixed at 50 V, the contractile responses increased linearly with increasing frequency of stimulation up to 40 Hz and reached a plateau at about 50 Hz under both conditions. In the following experiments, electrical stimulation was applied at 50 V and 40 Hz with 0.3-ms-square pulses for 10 s, which was not a supramaximal but a submaximal stimulus, in order to detect accurately either increases or decreases in contractile responses under both normal and hypoxic conditions.

As shown in Table 1, the contractile responses of aortic strips to transmural electrical stimulation were completely blocked by tetrodotoxin (3×10^{-7} mol/l) under both normal and hypoxic conditions. The responses were inhibited in a concentration-dependent manner by α-adrenoceptor antagonists such as phentolamine and phenoxybenzamine under both normal and hypoxic conditions and were completely blocked at 10^{-6} mol/l. The contractile responses were unaffected by indomethacin (10^{-5} mol/l), an inhibitor of cyclooxygenase, under either condition (data not shown).

Figure 3 shows the effects of various concentrations of O_2 on the contractile responses of aortic strips to transmural electrical stimulation. Contractile responses at 20% O_2 were not significantly different from control values obtained at 95% O_2. As the concentration of oxygen was decreased even further, contractile responses were inhibited concentration-dependently. At 0% O_2, the responses were about 20% of the control value.

Figure 4 and Table 2 show the effects of hypoxia on spontaneous and electrical stimulation-evoked overflows of total [^{3}H] and [^{3}H]noradrenaline from aortic strips prelabelled with [^{3}H]noradrenaline. Under hypoxic conditions, in which the medium had been equilibrated with 95% N_2/5% CO_2, spontaneous and electrical stimulation-evoked overflows of total [^{3}H] were inhibited by 40% and 53%,

Table 1. Effects of tetrodotoxin, phentolamine or phenoxybenzamine on contractile responses to transmural electrical stimulation under either normal or hypoxic conditions

Drugs and gas phase	Electrical stimulation-induced contractions (% of control)					
	No drug (tension, g)	10^{-8} (mol/l)	3×10^{-8} (mol/l)	10^{-7} (mol/l)	3×10^{-7} (mol/l)	10^{-6} (mol/l)
Tetrodotoxin						
95% O_2/5% CO_2	100 (1.08 ± 0.08)	N.D.	N.D.	N.D.	0	N.D.
95% N_2/5% CO_2	100 (0.16 ± 0.07)	N.D.	N.D.	N.D.	0	N.D.
Phentolamine						
95% O_2/5% CO_2	100 (0.97 ± 0.11)	98.3 ± 0.7	86.6 ± 0.9	38.1 ± 2.5	18.0 ± 3.8	0
95% N_2/5% CO_2	100 (0.14 ± 0.05)	96.7 ± 1.1	82.2 ± 1.1	22.7 ± 1.6	8.2 ± 0.85	0
Phenoxybenzamine						
95% O_2/5% CO_2	100 (0.86 ± 0.14)	69.6 ± 0.88	38.1 ± 1.3	14.3 ± 3.5	5.4 ± 2.8	0
95% N_2/5% CO_2	100 (0.10 ± 0.11)	72.2 ± 1.4	40.6 ± 2.7	16.9 ± 2.2	4.6 ± 1.9	0

Contractile responses to transmural electrical stimulation (50 V, 0.3 ms, 40 Hz for 10 s) were recorded in the absence of drugs under either normal (95% O_2/5% CO_2) or hypoxic (95% N_2/5% CO_2) conditions. Thereafter, drugs were cumulatively added to the incubation-medium and the responses were recorded under either conditions. Each value is expressed as a percentage of control responses obtained in the medium equilibrated with either 95% O_2/5% CO_2 or 95% N_2/5% CO_2 without drugs and is the mean ± SEM of eight determinations. N.D.; not determined

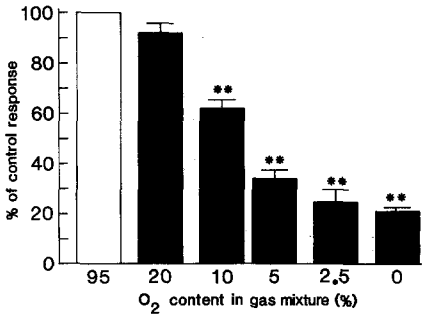

Fig. 3. Effects of various concentrations of O_2 on the contractile responses of aortic strips to transmural electrical stimulation. The strips were stimulated (50 V, 40 Hz of 0.3-ms-square pulses for 10 s) in the tissue bath of 10-ml capacity containing Krebs' solution and isometric tension was recorded. After control responses were recorded in the medium equilibrated with 95% O_2/5% CO_2, the oxygen concentration in the medium was decreased stepwise by bubbling with gas mixtures containing various concentrations of O_2 (20%, 10%, 5%, 2.5% and 0%). Each value is expressed as a percentage of the control response (0.824 ± 0.070 g, n = 12) obtained in the medium equilibrated with 95% O_2/5% CO_2 and is the mean ± SEM of twelve determinations. $^{**}P < 0.01$; significantly different from the control value

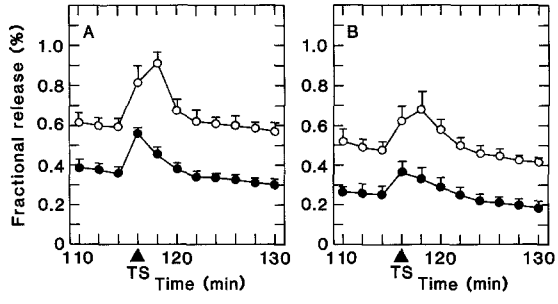

Fig. 4. Effects of hypoxia on fractional release of total [^{3}H] (**A**) and [^{3}H]noradrenaline (**B**) from rabbit thoracic aortic strips prelabelled with [^{3}H]noradrenaline. Rabbit aortic strips were preincubated with [^{3}H]noradrenaline in Krebs' solution equilibrated with 95% O_2/5% CO_2 for 60 min at 37° C. After preincubation, the strips were superfused with [^{3}H]noradrenaline-free Krebs' solution at a rate of 2 ml/min. For hypoxic experiments (●——●), the gas mixture bubbling the medium in the perfusion chamber of reservoir was replaced with a mixture of 95% N_2/5% CO_2 after the initial washout period of 60 min from the onset of superfusion. For control experiments (○——○), the medium was continued to be bubbled with 95% O_2/5% CO_2. After a further washout period of 50 min, fractions of the superfusate were collected every 2 min. Transmural electrical stimulation (50 V, 40 Hz of 0.3-ms-square pulses for 10 s) was applied immediately after collection of the first three samples (▲). Each point represents the mean ± SEM (n = 8)

respectively (Fig. 4A and Table 2). Similarly spontaneous and electrical stimulation-evoked overflows of [^{3}H]noradrenaline were inhibited by 42% and 57%, respectively (Fig. 4B and Table 2). The amounts of noradrenaline, expressed as a percentage of electrical stimulation-evoked total [^{3}H] overflow, are not significantly different from one another normal or hypoxic conditions (62.0 ± 9.1% vs. 57.3 ± 12.3%, n = 8) (Table 2).

Figure 5 shows the effects of various concentrations of O_2 on the concentration-response curves for exogenous noradrenaline. When the concentration of oxygen was decreased from 95% to 20%, the concentration-response curves for noradrenaline were unchanged: the EC_{50} values were $0.979 \pm 0.17 \times 10^{-7}$ and $1.07 \pm 0.15 \times 10^{-7}$ mol/l (n = 8), respectively, under 95% O_2 and 20% O_2. However, with a further decrease in the concentration of O_2, the concentration-response curves were shifted to the right and the maximum response was decreased. At 0% O_2, the EC_{50} value for exogenous noradrenaline (51.6 ± 15.3×10^{-7} mol/l, n = 8) was about 50 times as large as that observed at 95% O_2 ($0.979 \pm 0.17 \times 10^{-7}$ mol/l, n = 8) and the maximum contractile response was 75% of the control value.

Figure 6 shows the effects of hypoxia on KCl-induced contractions of aortic strips of the rabbits pretreated with reserpine (A) or 6-OHDA (B). Following the pretreatments, the content of noradrenaline in the aorta decreased to about 1% of control: control group, 913.9 ± 29.4 ng/g tissue, n = 4; reserpine treated group, 6.8 ± 6.8, n = 3; 6-OHDA

Table 2. Effects of hypoxia on spontaneous and electrical stimulation-evoked overflows of total [^{3}H] and [^{3}H]noradrenaline from rabbit thoracic aortic strips prelabelled with [^{3}H]noradrenaline

	Fractional overflow (%)	
	Gas phase	
	95% O_2/5% CO_2	95% N_2/5% CO_2
Total [^{3}H] overflow		
Spontaneous[a]	0.586 ± 0.064	0.352 ± 0.035**
Evoked[b]	0.758 ± 0.059	0.356 ± 0.034**
[^{3}H]noradrenaline overflow		
Spontaneous[a]	0.477 ± 0.045	0.277 ± 0.042**
Evoked[b]	0.470 ± 0.069	0.204 ± 0.044**

For details of experimental procedures, see Legend to Fig. 4
[a] Spontaneous overflow is the mean of fractional release of [^{3}H]radioactivity in three fractions just before the electrical stimulation. [b] Stimulation evoked-overflow is the total increase in the overflow of total [^{3}H] or [^{3}H]noradrenaline above the spontaneous overflow. The values are means ± SEM of eight determinations. ** $P < 0.01$; significantly different from control values obtained in the medium equilibrated with 95% O_2/5% CO_2

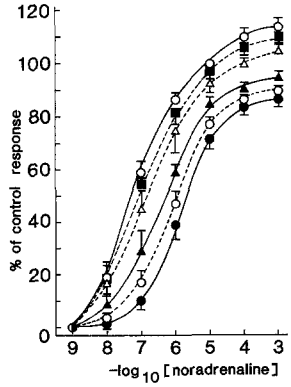

Fig. 5. Effects of various concentrations of O_2 on concentration-response curves for exogenous noradrenaline. Rabbit aortic strips were mounted in a tissue bath of 10-ml capacity and contractile responses of the strips to various concentrations of noradrenaline were isometrically recorded in the medium equilibrated with gas mixtures containing various concentrations of O_2. ○——○, 95% O_2; ■----■, 20% O_2; △----△, 10% O_2; ▲——▲, 5% O_2; ○——○, 2.5% O_2; ●——●, 0% O_2. Each point is expressed as a percentage of the control response (4.33 ± 0.16 g, $n = 8$) evoked by 10^{-5} mol/l noradrenaline in the medium equilibrated with 95% O_2/5% CO_2 and is the mean ± SEM of eight determinations

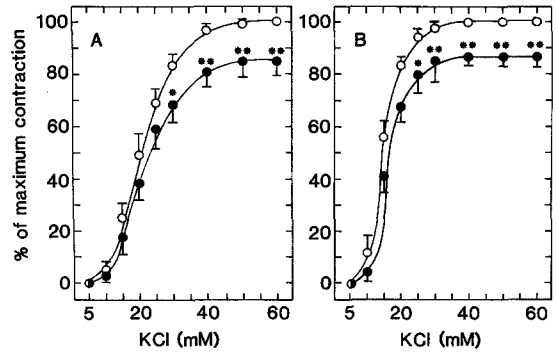

Fig. 6. Effects of hypoxia on contractile responses to KCl of aortic strips from rabbits pretreated with reserpine (**A**) or 6-OHDA (**B**). Contractile responses of the aortic strips to various concentrations of KCl (5 mmol/l – 60 mmol/l) were isometrically recorded in Krebs' solution bubbled with a gas mixture of 95% O_2/5% CO_2 (○——○) or 95% N_2/5% CO_2 (●——●). Each point is expressed as a percentage of the control responses (A: 4.54 ± 0.79 g, $n = 6$; B: 4.58 ± 0.28 g, $n = 6$) evoked by 60 mmol/l KCl in medium equilibrated with 95% O_2/5% CO_2 and is the mean ± SEM of six determinations. * $P < 0.05$; ** $P < 0.01$; significantly different from control values

treated group, 10.5 ± 6.5, $n = 3$. Under hypoxic conditions (95% N_2/5% CO_2), the maximum contractile response of reserpine- or 6-OHDA-pretreated aortic strips to high KCl was inhibited slightly but significantly (by about 15%), whereas EC_{50} values for KCl were unaffected: the EC_{50} values under normal and hypoxic conditions were 21.4 ± 1.3 mmol/l and 20.9 ± 1.4 mmol/l, respectively, for the reserpine group ($n = 8$), and 15.2 ± 0.34 mmol/l and 16 ± 0.54 mmol/l, respectively, for the 6-OHDA group ($n = 8$).

Discussion

In the present investigation, contractile responses of rabbit thoracic aortic strips to transmural electrical stimulation were completely blocked by tetrodotoxin, a specific blocker of Na channels in membranes of neuronal tissues (Agnew et al. 1986; Ulbricht et al. 1986), and by α-adrenoceptor antagonist such as phentolamine and phenoxybenzamine (Table 1). These results indicate that the contractile responses to transmural electrical stimulation are the result of α-adrenoceptor stimulation by noradrenaline which is released from adrenergic nerve terminals present in the strips. Contractile responses were not affected by indomethacin, an inhibitor of cyclooxygenase (Higgs and Vane 1983). These results indicate that products of the cyclooxygenase pathway are not involved in the contractile responses of aortic strips to electrical stimulation.

Severe hypoxia inhibited the contractile responses of aortic strips to electrical stimulation by 80% (Figs. 1, 2 and 3). It is believed that contractile responses to transmural electrical stimulation consist of the following processes: noradrenaline release from nerve terminals, binding of released noradrenaline to α-adrenoceptors, signal transduction, followed by contraction of the vascular smooth muscle (Somlyo and Somlyo 1968; Kamm and Stull 1985). We attempted to determine which of these processes is the most susceptible to O_2 deficiency.

The overflow of [^{3}H]noradrenaline evoked by transmural electrical stimulation was markedly decreased by hypoxia (Fig. 4 and Table 2). It is generally accepted that most of noradrenaline released into junctional clefts is removed by neuronal and extraneuronal uptake mechanisms (Vanhoutte et al. 1981). Therefore, the overflow of [^{3}H]noradrenaline is considered to represent that portion of noradrenaline released into junctional clefts which overflows into the superfusion medium without being taken up. It follows, therefore, that the decrease in the overflow of [^{3}H]noradrenaline observed during hypoxia is the result of either a decrease in noradrenaline release or an increase in noradrenaline uptake. The latter is improbable, since uptake mechanisms of noradrenaline in isolated brain synaptosomes (Pastuszko et al. 1982) and isolated pulmonary

arteries (Rorie and Tyce 1983) have been reported to be susceptible to hypoxia. Thus, our results strongly suggest that noradrenaline release from nerve terminals is being inhibited under hypoxic conditions. The effects of hypoxia on noradrenaline release and on neuronal and extraneuronal uptake mechanisms are the subject of a forthcoming paper.

The maximum KCl-evoked contractile responses of reserpine-or 6-OHDA-pretreated strips were slightly (about 15%) inhibited by hypoxia, whereas EC_{50} values were unaffected (Fig. 6). These results are essentially similar to those presented in previous reports (Shibata and Briggs 1967; Coburn et al. 1979) and suggest that the contractile mechanism of vascular smooth muscle is only slightly inhibited by hypoxia.

Previous investigators demonstrated that when only one or two concentrations of noradrenaline or adrenaline were used, the contractile responses to these agonists in vascular strips were considerably decreased under hypoxic conditions. They concluded that the decrease was a result of direct depressant effects of hypoxia on the contractile machinery of vascular smooth muscle (Coret and Hughes 1964; Detar and Bohr 1968; Hughes and Coret 1969; Detar and Gellai 1971; Namm and Zucker 1973; Vanhoutte 1976; Coburn et al. 1979; Ebeigbe et al. 1980). However, the present investigation showed that under hypoxic conditions, the concentration-response curves for exogenous noradrenaline were considerably shifted to the right (the EC_{50} value for noradrenaline was increased by a factor of about 50), whereas the decrease in the maximum response was small. These results taken together with the effects of hypoxia on KCl-induced contractile responses suggest that the decrease in the contractile responses to exogenous noradrenaline observed under hypoxic conditions is mainly due to the shift of the concentration-response curve to the right.

The contraction of rabbit aortae of noradrenaline is considered to be mediated predominantly by α_1-adrenoceptor (Docherty et al. 1981). Activation of α_1-adrenoceptor is reported to activate signal transduction mechanisms such as Ca^{2+}-influx through Ca^{2+} channels (Dipolo and Beaugé 1983) and hydrolysis of phosphoinositides (Berridge 1984). The hydrolysis of phosphoinositides has been hypothesized to result in the formation of two intracellular messengers, inositol-1,4,5-triphosphate, which mobilizes Ca^{2+} from the intracellular Ca^{2+} pools (Streb et al. 1983; Berridge and Irvine 1984; Spät et al. 1986), and 1,2-diacylglycerol, which activates the Ca^{2+}-sensitive, phospholipid-dependent protein kinase C (Nishizuka 1984). Thus, the shift to the right of the concentration-response curves for noradrenaline could be induced by a decrease in the affinity of α_1-adrenoceptor for noradrenaline and/or by inhibition of the signal transduction mechanisms. The mechanisms by which the concentration-response curves for noradrenaline are shifted to the right are now under investigation in our laboratory.

In summary, the present investigation shows that 80% of the electrical stimulation-induced contraction, as a model for adrenergic neurotransmission, was abolished by hypoxia. This 80% loss may be made up of decreased noradrenaline release (to at least 43% of control), a shift of the concentration-response curve for noradrenaline to the right (to 50-fold of control) and decreased contractility of vascular smooth muscle (to 85% of control). The significance of the shift of the concentration-response curve for noradrenaline to the right in the case of electrical stimulation-induced contraction is unclear, because the concentration of noradrenaline in the vicinity of α_1-adrenoceptor is unknown. However, when the tension developed by transmural electrical stimulation is compared with the concentration-response curve for exogenous noradrenaline under control conditions, the concentration of noradrenaline in the vicinity of α_1-adrenoceptor can be estimated to be around 10^{-8} mol/l. It appears that at this concentration range, the concentration-response curve under hypoxic conditions is markedly displaced downward (Fig. 5). These results taken together indicate that a shift of the noradrenaline concentration-response curve to the right plays a critical role in the hypoxia-induced inhibition of contraction in response to electrical stimulation. Thus, the present investigation indicates that the inhibition of adrenergic neurotransmission by hypoxia is primarily a result of a decrease in stimulus-evoked noradrenaline release and of a decrease in the affinity of α_1-adrenoceptor for noradrenaline and/or inhibition of signal transduction mechanisms.

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