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Effects of hypoxia on noradrenaline release and neuronal reuptake in isolated rabbit thoracic aortic strips*

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Summary. To clarify the effects of hypoxia on stimulus-evoked noradrenaline release and on neuronal reuptake of the released noradrenaline, we examined the effects of hypoxia on contraction responses of rabbit thoracic aortic strips to transmural electrical stimulation and on the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline from the strips prelabelled with [^{3}H]noradrenaline. This was done in the presence or absence of an inhibitor of neuronal uptake (cocaine). In a medium equilibrated with a gas mixture of 95% O_2/5% CO_2 (control), cocaine doubled the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline; there was a concomitant increase (130%) in contractions to electrical stimulation. At 0% O_2 (95% N_2/5% CO_2, hypoxia), cocaine had no significant effects on either the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline or contractions. In the absence of the drug, hypoxia decreased the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline to 47% and 43%, respectively, of the control values, whereas these values were 31% and 28%, respectively, after exposure to cocaine. The inhibition by hypoxia of contraction responses to electrical stimulation was greater in the presence of cocaine than in its absence. These results show that hypoxia inhibits both noradrenaline release evoked by a given stimulus and neuronal uptake.

Key words: Hypoxia – Aortic strips – Noradrenaline release – Neuronal uptake – Rabbit

Introduction

In our recent work (Lee et al. 1988), using isolated rabbit thoracic aortic strips as a model of a neuroeffector system, we showed that contraction responses to transmural electrical stimulation were inhibitied by about 80% under hypoxic conditions, in which the incubation medium was equilibrated with 95% N_2/5% CO_2, compared with responses obtained in medium equilibrated with 95% O_2/5% CO_2. The stimulation-evoked overflow of [^{3}H]noradrenaline was inhibited by about 55% and the concentration-response curve for exogenous noradrenaline was shifted to the right fiftyfold. On the other hand, contraction responses to high KCl, which provide an index of the contractility of the vascular smooth muscle, were affected only slightly. From these data, we concluded that the decrease in contraction responses of the strips to electrical stimulation observed under hypoxic conditions was the result, mainly, of a decrease in the stimulation-evoked overflow of noradrenaline and of a decrease in the affinity of α-adrenoceptors for noradrenaline and/or inhibition of signal transduction mechanisms. It is generally accepted that most of the noradrenaline released into the junctional clefts of the blood vessel wall is removed by neuronal reuptake (Vanhoutte et al. 1981). Thus, the [^{3}H]noradrenaline overflow monitored by the superfusion technique is considered to respresent that portion of the released noradrenaline which appears in the superfusate without being retaken up. Therefore, the decreased overflow of [^{3}H]noradrenaline observed under hypoxic conditions could result from either a decreased release or an increased reuptake of noradrenaline.

Therefore, to directly determine the effects of hypoxia on stimulus-evoked noradrenaline release from, and reuptake into nerve terminals, we examined the effects of hypoxia on contraction responses of rabbit aortic strips to electrical stimulation and on the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline from strips prelabelled with [^{3}H]noradrenaline, both in the absence and presence of an inhibitor of neuronal uptake (cocaine).

Materials and methods

Tissue preparation and measurement of mechanical activity. Thoracic aortae were removed from exsanguinated rabbits (2–3 kg) and cut into helical strips of approximately 4 by 20 mm in size. The strips were mounted in a tissue bath of 10-ml capacity containing Krebs' solution composed of (in mmol/l): NaCl, 120.7; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.5; $CaNa_2EDTA$, 0.026; and ascorbic acid, 0.113. The solution was gassed with 95% O_2/5% CO_2, and maintained at 37° C. The strips were maintained at a resting tension of 1.5 g for a 1-h equilibration period, after which electrical stimulation was given every 10 min by means of a pair of platinum electrodes. Stimulus parameters used were 0.3-ms square wave pulses of 50 V and 40 Hz for a period of 10 s. The voltage and the frequency were submaximal, as described recently (Lee et al. 1988). The pulse train was delivered from an electronic stimulator (Nihonkoden Kogyo Co., Tokyo, Japan), and the isometric contractions of the strips were recorded using a force-displacement transducer (Nihonkoden Kogyo Co.).

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In order to obtain results consistent with those from superfusion experiments, an estimate of the contraction response of each specimen was obtained by integrating the area under the response curve. Each value was expressed as a percentage of control responses seen in the medium equilibrated with 95% O_2/5% CO_2 in the absence of cocaine.

Protocols of contraction experiments. To examine the effects of hypoxia on contraction responses of the strips to electrical stimulation in the presence and absence of cocaine, experiments were carried out using single muscle strips subjected, in succession, to the following experiments:

1) electrical stimulation in a normoxic medium (equilibrated with 95% O_2/5% CO_2),

2) electrical stimulation in a hypoxic medium (equilibrated with 95% N_2/5% CO_2),

3) reoxygenation and electrical stimulation,

4) addition of cocaine (final concentration, 1×10^{-5} mol/l) to the incubation medium,

5) electrical stimulation in the normoxic medium containing cocaine,

6) electrical stimulation in the hypoxic medium containing cocaine,

7) reoxygenation in the presence of cocaine and electrical stimulation.

Measurement of O_2 concentrations. The actual O_2 concentrations in the incubation medium were determined by means of an oxygen electrode (Model 53, Yellow Springs Instruments, Yellow Springs, OH, USA) equipped with a TR 8652 digital electrometer (Advantest Co., Tokyo, Japan). The potential applied to the oxygen electrode was −0.6 V. The level of oxygen concentration detected after $Na_2S_2O_4$ was added to eliminate dissolved oxygen was used as a zero level (Schömig et al. 1987). As described by Chance and Williams (1955) and Chappell (1964), the oxygen concentration in the medium was calculated by comparing the observed value with that in the medium equilibrated with air at 37° C (217 μmol/l) and expressed in terms of molar concentrations.

Superfusion experiment. Helical strips were preincubated with 1.0 μmol/l [^{3}H]noradrenaline (final specific activity, 185 MBq/mol) in Krebs' solution equilibrated with a gas mixture of 95% O_2/5% 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 equipped with platinum electrodes, and superfused with [^{3}H]noradrenaline-free medium at 37° C at a rate of 2 ml/min. For control experiments, the Krebs' solution in the superfusion chamber and reservoir was bubbled with a gas mixture of 95% O_2/5% CO_2 throughout the experiment. In experiments under hypoxic conditions, a fresh set of aortic strips was used and the gas mixture was replaced by 95% N_2/5% CO_2 min after the onset of superfusion. In both cases (normoxic and hypoxic), 4-ml fractions were collected every 2 min starting 110 min after the onset of superfusion. The fractions of the superfusate were collected into tubes containing 10 mg/ml sodium bisulfite, 10 mmol/l EDTA, and 100 ng of noradrenaline as a carrier, in a total of 0.4 ml of 1 mol/l perchloric acid. After collecting the first three fractions for the determination of spontaneous overflow, the strips were electrically stimulated with 0.3-ms square wave pulses of 40 Hz for 20 s (50 V), and 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 sodium bisulfite and 1 mmol/l EDTA. After centrifugation at $15{,}000 \times g$ for 15 min, 1-ml aliquots of the supernatant were used for the determination of total [^{3}H] remaining in the tissue.

When we examined the effects of hypoxia on overflow of total [^{3}H] and [^{3}H]noradrenaline in the presence of cocaine, we used separate sets of strips. After the strips were labelled with [^{3}H]noradrenaline, then washed with [^{3}H]noradrenaline-free medium, they were superfused with [^{3}H]noradrenaline-free medium equilibrated with 95% O_2/5% CO_2 as described above. Fifty-six minutes after the beginning of the superfusion, cocaine was added to the superfusing medium at a final concentration of 1×10^{-5} mol/l. In the experiments under normoxic conditions, the strips were superfused with the cocaine-containing medium which was equilibrated with 95% O_2/5% CO_2 throughout the experiment. In the experiments under hypoxic conditions, the gas mixture bubbling the superfusion chamber and the reservoir was replaced by 95% N_2/5% CO_2 60 min after the beginning of superfusion. In these superfusion experiments in the presence of cocaine, collection of superfusates and electrical stimulation were carried out according to the same experimental protocol as described for the superfusion experiments in the absence of cocaine.

Assay of [^{3}H]noradrenaline in the superfusate. The [^{3}H]noradrenaline released into the superfusate was extracted by the alumina batch method as described in detail elsewhere (Miwa et al. 1986; Lee et al. 1987). The superfusate was adjusted to pH 8.3−8.4 with (1 mol/l Tris-HCl buffer (pH 8.6) and the noradrenaline was adsorbed onto 25 mg of acid-washed Al_2O_3. 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 HPLC with an electrochemical detector and a $5C_{18}$ reverse-phase column (4.6 × 150 mm). 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 a carrier noradrenaline with that of a known amount of an authentic standard.

In a separate experiment, we determined the absolute amount of noradrenaline released into the superfusates using the strips prelabelled with [^{3}H]noradrenaline as described above. This experiment showed that, under normoxic conditions in the absence of cocaine, the spontaneous and the maximum noradrenaline overflow after electrical stimulation in 2-min fraction were 2.12 ± 0.09 ng and 2.77 ± 0.25 ng ($n=6$), respectively, while under normoxic conditions in the presence of cocaine, the values were 2.18 ± 0.09 ng and 3.55 ± 0.13 ng ($n=6$), respectively. These values are negligible in comparison with the amount (100 ng) of noradrenaline which was added as a carrier to the superfusate in each 2-min fraction. Thus, the fluctuation in the amount of noradrenaline overflow from the strips was considered not to affect the recovery of a carrier noradrenaline.

Radioactivity measurement. 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.

The overflow of [^{3}H] radioactivity and [^{3}H]noradrenaline was calculated as percent release: total [^{3}H] or [^{3}H]noradrenaline collected in each fraction expressed in percent 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 the spontaneous overflow from the total [^{3}H] or [^{3}H]noradrenaline overflow after electrical stimulation and summing the difference. The spontaneous overflow was assumed not to change with time both during and after period of nerve stimulation.

Chemicals. Chemicals were obtained from the following sources: (−)-[ring-2,5,6-^{3}H]noradrenaline (specific activity, 1.62 GBq/mmol), from New England Nuclear, Boston, MA, USA; noradrenaline bitartrate, 3,4-dihydroxybenzylamine hydrobromide, from Sigma, St. Louis, MO, USA; cocaine hydrochloride, from Takeda Chemical Industries, Ltd., Osaka, Japan; activated alumina, from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Statistical analysis. All results were expressed as means ± SEM. 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 (Steel and Torrie 1960). A probability level of $P < 0.05$ was considered statistically significant.

Results

As described in "Materials and methods", we attempted to examine the effects of hypoxia on contraction responses of aortic strips to electrical stimulation in the absence and presence of cocaine using the same strip throughout. Thus, it usually took 11 h, to carry out one series of experiments. Therefore, to confirm that contraction responses to electrical stimulation do not change significantly over time, we carried out the following control experiments.

Control experiment 1 (Fig. 1A): After a 1-h equilibration period in the cocaine-free normoxic medium (equilibrated with 95% O_2/5% CO_2), aortic strips were electrically stimulated every 10 min for the next 10 h in the same medium. The contraction responses to electrical stimulation in the normoxic medium were found to be stable throughout the experimental period.

Control experiment 2 (Fig. 1B): Following a 1-h equilibration period in the cocaine-free normoxic medium, aortic strips were electrically stimulated every 10 min for 5 h. Then, cocaine was added to the incubation medium to a final concentration of 1×10^{-5} mol/l, and electrical stimulation was continued for an additional 5 h (Fig. 1B). After addition of cocaine, contraction responses to the stimulation, which were expressed in terms of integral of the responses, gradually increased and reached a plateau within 50 min (data not shown). Thereafter, the responses were stable throughout the experimental period. In a steady-state, the responses observed after addition of cocaine were 231 ± 3.3% of the values observed before its addition ($n = 10$).

Hypoxia-reoxygenation experiment (Fig. 1C): After a 1-h equilibration period in the cocaine-free normoxic medium, aortic strips were electrically stimulated every 10 min for the first 100 min in the same medium. Then the gas

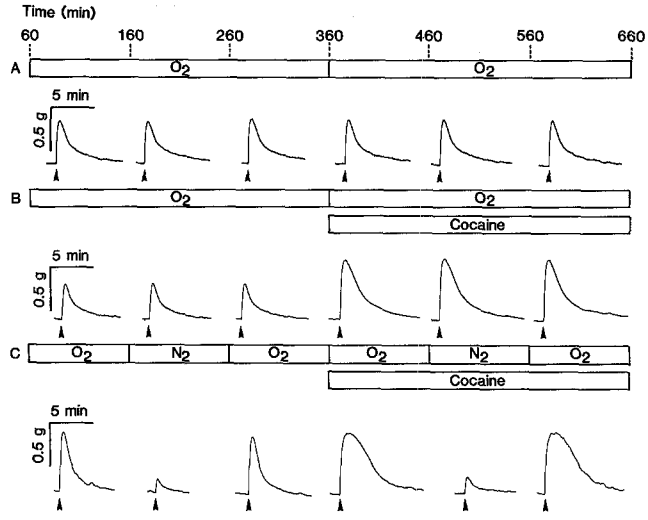

Fig. 1. Typical tracings showing the effects of cocaine and/or hypoxia on the contraction responses of rabbit aortic strips to transmural electrical stimulation. Helical strips of rabbit thoracic aortae were prepared and mounted in a tissue bath. All strips were maintained at a resting tension of 1.5 g in Krebs' solution equilibrated with 95% O_2/5% CO_2 (normoxic medium) for a 1-h equilibration period. After the equilibration period, one group of the strips was electrically stimulated every 10 min (indicated by *arrowheads*) for 10 h in the same normoxic medium (**A**). Another set of strips was stimulated every 10 min for 5 h in the normoxic medium and the stimulation was continued for 5 h more following the addition of cocaine (final concentration, 1×10^{-5} mol/l) (**B**). The other set of the strips, in normoxic medium, was electrically stimulated every 10 min for 100 min. Then the gas mixture bubbling the incubation medium was changed from 95% O_2/5% CO_2 ("O_2") to 95% N_2/5% CO_2 (hypoxic medium: "N_2") and electrical stimulation was continued for another 100 min. After incubation with hypoxic medium, the medium was reoxygenated with 95% O_2/5% CO_2 and the stimulation was continued for another 100 min. Then cocaine was added to the incubation medium (final concentration, 1×10^{-5} mol/l) and the experiments were repeated (**C**). Note that the time scale of the record of contraction responses is different from the scale of the bars, which represent the conditions of the incubation medium. In this figure, only contraction responses in the steady-state under various conditions are shown

mixture was changed from 95% O_2/5% CO_2 to 95% N_2/5% CO_2 and the strips were stimulated for further 100 min. The contraction responses decreased gradually and reached a steady-state within 50 min (data not shown). Thereafter, the responses remained stable: the responses were about 20% of the values before the gas change. When the strips were then reoxygenated with 95% O_2/5% CO_2 after the 100-min incubation in the hypoxic medium, contraction responses returned to control levels. One hundred minutes after reoxygenation, cocaine was added to the incubation medium to a final concentration of 1×10^{-5} mol/l, and the strips were stimulated for another 100 min. The responses gradually increased and reached a plateau within 50 min, after which the responses were stable at a level 234 ± 5.7% of the values seen in the original cocaine-free normoxic medium (Fig. 1C and Table 1). The responses were not significantly different from the values seen in the absence of exposure to hypoxia (control experiment 2). When the strips were made hypoxic once again, contraction responses gradually decreased, levelled off within 50 min and remained constant up to 100 min after the gas change. In a steady-state,

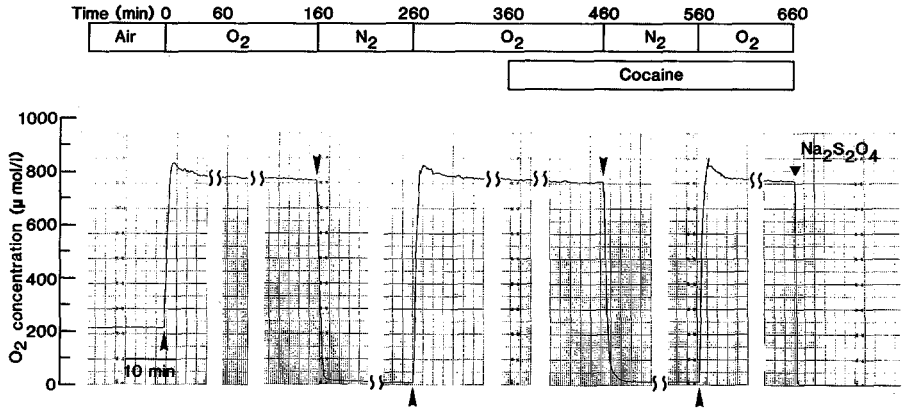

Fig. 2. Typical tracings of the changes in the oxygen concentration in the incubation medium. Using the same experimental setup and time table as in Fig. 1C, the oygen concentration in the incubation medium was measured using an oxygen electrode. The level of oxygen concentration detected after $Na_2S_2O_4$ (indicated by "$Na_2S_2O_4$") was added to eliminate dissolved oxygen was used as a zero level. The actual oxygen concentration in the medium was calculated by comparing the observed value with the value in the medium equilibrated with air (indicated by "Air")

the responses were $14.9 \pm 2.8\%$ of the values seen in the cocaine-free normoxic medium. When the strips were reoxygenated following a 100-min incubation in the hypoxic medium, the responses returned to the prehypoxic levels.

These results show that (1) contraction responses to electrical stimulation do not change during our experimental period regardless of the absence or presence of cocaine, (2) the effects of hypoxia on contraction responses of the strips are completely reversible in the absence or presence of cocaine.

Therefore, in the following set of experiments, we decided to use the values found in the normoxic medium with and without cocaine as controls for the respective values obtained in the hypoxic medium.

Figure 2 shows typical tracings of the changes in the oxygen concentration following replacement of the 95% O_2/5% CO_2 gas mixture by 95% N_2/5% CO_2 and vice versa, in both the absence and presence of cocaine. In the normoxic medium, the O_2 concentration was 795 ± 3.6 μmol/l ($n = 8$). After changing the mixture from 95% O_2/5% CO_2 to 95% N_2/5% CO_2, the O_2 concentration rapidly decreased and reached a steady-state (12.5 ± 1.1 μmol/l, $n = 8$) within 10 min. When the incubation medium was reoxygenated with 95% O_2/5% CO_2, the oxygen concentration of the medium rapidly returned to the control level observed in the normoxic medium. After cocaine was added to the incubation medium, essentially similar results were obtained.

Table 1 summarizes the effects of hypoxia on contraction responses of rabbit aortic strips to electrical stimulation in the absence or presence of cocaine. As previously reported (Langer and Enero 1974; Wyce 1974; Brandão et al. 1980; Rorie et al. 1980), cocaine considerably augmented contraction responses of the strips to electrical stimulation under normoxic conditons (Column 1 in Table 1). Under hypoxic conditions, cocaine had no significant effects on contraction responses (Column 2 in Table 1). Hypoxia, in the absence of cocaine, reduced contraction responses to about 17% of the control responses observed under normoxic conditions. In the presence of cocaine, the apparent inhibition of responses by hypoxia was even greater, because of the 130% increase caused by cocaine under normoxic conditions (Column 3 in Table 1).

Table 1. Effects of hypoxia on contraction responses of rabbit aortic strips to transmural electrical stimulation in the absence or presence of cocaine

	Contraction response (% of control) gas phase		
	95% O_2/5% CO_2 (A)	95% N_2/5% CO_2 (B)	B/A (%)
No drug	100	17.3 ± 1.8**	17.3 ± 1.8
Cocaine	230.6 ± 3.9*	14.9 ± 2.8**	6.4 ± 1.2*

The effects of hypoxia on contraction responses to electrical stimulation in the absence or presence of cocaine were determined using single muscle strips as described in the legend to Fig. 1C. Each value in the first two columns is expressed as percentage of the control value obtained in medium equilibrated with 95% O_2/5% CO_2 (normoxic conditions) in the absence of cocaine and is the mean ± SEM of 8–15 specimens

* $P < 0.01$; significantly different from no drug group in each column; ** $P < 0.01$; significantly different from respective control values obtained under normoxic conditions

Figure 3, Table 2 and Table 3 show the effects of hypoxia on spontaneous and electrical stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline from aortic strips superfused in the absence or presence of cocaine. Cocaine produced no detectable changes in the spontaneous overflow of total [^{3}H] and [^{3}H]noradrenaline under either normoxic (Fig. 3 and Column 1 in Table 2) or hypoxic conditions (Fig. 3 and Column 2 in Table 2). The spontaneous overflow of total [^{3}H] and [^{3}H]noradrenaline was decreased by hypoxia to about 70% and 80%, respectively, of the corresponding control values obtained under normoxic conditions (Fig. 3 and Column 3 in Table 2).

Under normoxic conditions, cocaine increased the electrial stimulation-evoked overflow of total [^{3}H] and of [^{3}H]noradrenaline by about 90% and 100%, respectively (Fig. 3 and Column 1 in Table 3). In contrast, under hypoxic conditions, cocaine had no significant effect on the electrical stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline (Fig. 3 and Column 2 in Table 3). In the absence of

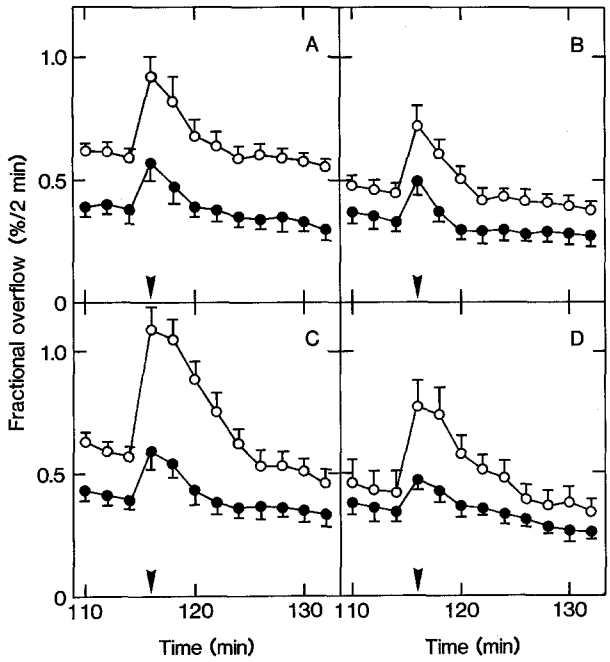

Fig. 3. Effects of hypoxia on spontaneous and electrical stimulation-evoked overflows of total [^{3}H] (*left panel*; **A** and **C**) and [^{3}H]noradrenaline (*right panel*; **B** and **D**) from rabbit aortic strips in the absence (*upper panels*) or presence of cocaine (*lower panels*). The strips were preincubated with [^{3}H]noradrenaline in a Krebs' solution equilibrated with a gas mixture of 95% O_2/5% CO_2. After washing with a Krebs' solution, the strips were superfused with [^{3}H]noradrenaline-free medium at a rate of 2 ml/min. In control experiments (○——○), the superfusing medium was bubbled with 95% O_2/5% CO_2 throughout the experiments. In experiments under hypoxic conditons (●——●), the gas mixture bubbling the superfusing medium was replaced by 95% N_2/5% CO_2 60 min after the onset of superfusion. Starting 110 min after the onset of superfusion, 4-ml fractions of the superfusate were collected every 2 min. After collection of three fractions for the determination of spontaneous overflow, transmural electrical stimulation (*arrowhead*) was applied for 20 s and collection of the superfusate was continued. To examine overflow of total [^{3}H] or [^{3}H]noradrenaline in the presence of cocaine, cocaine was added to the superfusing medium (final concentration, 1×10^{-5} mol/l) 56 min after the onset of superfusion and the superfusion experiments were carried out according to the same experimental protocol as in the absence of cocaine. Each point is expressed as the fractional overflow per 2-min sample and is the mean ± SEM of 8–12 specimens

Table 2. Effects of hypoxia on the spontaneous overflow of total [^{3}H] and [^{3}H]noradrenaline from rabbit aortic strips prelabelled with [^{3}H]noradrenaline in the absence or presence of cocaine

	Spontaneous overflow[a] (%/2 min) gas phase		
	95% O_2/5% CO_2 (A)	95% N_2/5% CO_2 (B)	B/A (%)
Total [^{3}H]			
No drug	0.586 ± 0.064	0.394 ± 0.085	70.2 ± 5.19
Cocaine	0.617 ± 0.085	0.353 ± 0.095	70.8 ± 7.25
[^{3}H]Noradrenaline			
No drug	0.477 ± 0.045	0.346 ± 0.086	78.8 ± 6.29
Cocaine	0.432 ± 0.03	0.325 ± 0.067	78.7 ± 5.58

For experimental details, see legend to Fig. 3

[a] Spontaneous overflow is the mean fractional overflow of [^{3}H] radioactivity in three fractions just before the electrical stimulation. Each value represents the mean ± SEM of 8–12 specimens

Table 3. Effects of hypoxia on the overflow of total [^{3}H] and [^{3}H]noradrenaline evoked by electrical stimulation of rabbit aortic strips in the absence or presence of cocaine

	Electrical stimulation-evoked overflow[a] (%) gas phase		
	95% O_2/5% CO_2 (A)	95 N_2/5% CO_2 (B)	B/A × 100 (%)
Total [^{3}H]			
No drug	0.758 ± 0.059	0.356 ± 0.034***	46.9 ± 4.4
Cocaine	1.463 ± 0.177**	0.454 ± 0.028***	31.0 ± 1.9**
[^{3}H]Noradrenaline			
No drug	0.470 ± 0.069	0.204 ± 0.044***	43.4 ± 9.3
Cocaine	0.950 ± 0.121**	0.268 ± 0.037***	28.2 ± 3.9*

For experimental details, see legend to Fig. 3

[a] Electrical stimulation-evoked overflow is the sum of the increase in the overflow of total [^{3}H] or [^{3}H]noradrenaline above the spontaneous overflow in fractions after electrical stimulation. Values are expressed in terms of fractional overflow as defined in "Materials and methods" and represent means ± SEM of 8–12 specimens

* $P < 0.05$; ** $P < 0.01$; significantly different from "no drug" group in each column; *** $P < 0.01$; significantly different from respective controls obtained in the medium equilibrated with 95% O_2/5% CO_2

cocaine, hypoxia reduced the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline to about 47% and 43%, respectively, of the control values obtained under normoxic conditions. In the presence of cocaine, the inhibition by hypoxia of the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline was greater than the inhibition seen in the absence of the drug, because the cocaine-induced increase in electrical stimulation-evoked overflow occurred only under normoxic conditions. In the presence of cocaine, hypoxia inhibited the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline to 31% and 28%, respectively, of the values observed under normoxic conditons. These values are significantly lower than the values (47% and 43%) seen in the absence of the drug (Column 3 in Table 3).

Discussion

In the present investigation, cocaine, which inhibits the neuronal noradrenaline reuptake mechanism (Iversen 1963), increased the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline from rabbit aortic strips (Fig. 3 and Column 1 in Table 3), with a concomitant increase in the contraction responses of the strips to electrical stimulation (Fig. 1 and Column 1 in Table 1). These results are in good agreement with previous reports (Langer and Enero 1974; Brandão et al. 1980; Rorie et al. 1980) and suggest that, in the presence of normal oxygen levels, the neuronal reuptake mechanism plays an important role in the inactivation of released noradrenaline. In contrast, under hypoxic conditions, cocaine had no significant effects either on the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline from the strips (Fig. 3 and Column 2 in Table 3) or on

contraction responses of the strips to electrical stimulation (Fig. 1C and Column 2 in Table 1). Taken together, these results suggest that the neuronal reuptake mechanism for the noradrenaline released into junctional clefts is almost completely inhibited under hypoxic conditions.

As reported recently (Lee et al. 1988), hypoxia inhibited the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline from aortic strips prelabelled with [^{3}H]noradrenaline in the absence of cocaine. The present results are inconsistent with those of Dart et al. (1987), who reported that electrical stimulation-evoked noradrenaline overflow from perfused hearts of rats was not affected by hypoxia. This discrepancy may be due to species- and/or tissue-differences.

After inhibition of neuronal uptake by cocaine, hypoxia further inhibited the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline (Column 3 in Table 3 and Fig. 3). Since the stimulation-evoked overflow of total [^{3}H] and [^{3}H]noradrenaline after inhibition of neuronal uptake is considered to reflect mainly noradrenaline release, these results suggest that hypoxia severely inhibits the noradrenaline release evoked by electrical stimulation.

Thus, we have shown that hypoxia inhibits not only stimulation-evoked noradrenaline release but also neuronal reuptake.

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