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Persistent release of noradrenaline caused by anticancer drug 4′-epidoxorubicin in rat tail artery in vitro

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Abstract

Anthracycline derivatives including 4′-epidoxorubicin are known to cause cardiovascular side effects. In this study we examined the effects of 4′-epidoxorubicin on sympathetic nerves of rat tail artery in vitro. Treatment with 4′-epidoxorubicin at concentrations higher than 10 μM gradually increased the resting tension of the arterial strips, an effect which was greatly enhanced by subsequent addition of 10 μM cocaine. This increase of the resting tension by 4′-epidoxorubicin was prevented by prazosin, suppressed in the arterial strips of reserpine-pretreated rats, and reduced by superoxide dismutase. However, tetrodotoxin and histamine receptor antagonists (diphenhydramine and cimetidine) failed to influence it. The contractile response to electrical sympathetic stimulation was slightly attenuated by 30 μM 4′-epidoxorubicin. 4′-Epidoxorubicin did not shift the concentration–response curve for noradrenaline. In the superfusion experiments, the basal release of noradrenaline was increased approximately five-fold by 30 μM 4′-epidoxorubicin, and this increase was not inhibited by 0.1 μM prazosin, 0.5 μM tetrodotoxin, 10 μM cocaine or Ca^{2+}-free medium. Noradrenaline release evoked by electrical stimulation was gradually suppressed by 30 μM 4′-epidoxorubicin treatment. These results suggest that 4′-epidoxorubicin directly acts on the sympathetic nerve to cause persistent release of noradrenaline in rat tail artery.

Keywords: Noradrenaline; Anthracycline; Sympathetic nerve; Artery; (Rat)

1. Introduction

Anthracycline derivatives, such as 4′-epidoxorubicin, show a potent antitumor activity against a wide range of malignancies (Wall et al., 1996; Prozato et al., 1997). However, these drugs have some adverse effects which limit their clinical use (Lenaz and Page, 1976). The cardiotoxicity of the anthracyclines is a well-known side-effect and is manifest as left ventricular dysfunction, arrhythmias and cardiomyopathy. Arterial occlusion or stenosis is also often observed after transarterial chemotherapy, a method which has been recently developed for the treatment of hepatic malignancies (Seki et al., 1996). Several mechanisms for the cardiovascular toxicity of anthracyclines have been suggested. Anthracyclines may alter mitochondrial functions, intracellular electrolyte balance and Na^+, K^+-dependent ATPase activity, effects which are considered to be mediated by free radicals and/or inhibition of protein synthesis (Lenaz and Page, 1976; Ferrans, 1978). Inhibition of constitutive nitric oxide (NO) synthase or suppression of induction of inducible NO synthase has also been implicated in the cardiovascular toxicity (Dasan and Steven, 1994; Sakai et al., 1996). In addition, anthracycline treatment has been reported to raise the plasma level of vasoactive substances like catecholamines and histamine (Bristow et al., 1980, 1981). However, the details of this release are not known (Chen et al., 1987).

In this study, we examined the effects of 4′-epidoxorubicin on the sympathetic nerves and the smooth muscle of the rat tail artery. The results show that 4′-epidoxorubicin directly acts on the sympathetic nerves releasing noradrenaline.

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2. Materials and methods

Male Wistar rats (250–350 g) were used in the present experiment. Immediately after the animals were killed under pentobarbital anaesthesia, the tail artery was removed and placed in modified Krebs–Henseleit solution (composition in μM: NaCl 118, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2, and glucose 10; pH 7.4).

2.1. Functional experiments

The helical strips of tail artery were carefully prepared under a dissecting microscope and the endothelium was removed by rubbing with filter paper to avoid the possible involvement of endothelium-derived relaxing factors. The strips were mounted vertically in an organ chamber containing 20 ml of the modified Krebs–Henseleit solution bubbled with 95% O_2–5% CO_2 at 37°C. Tension changes were recorded isometrically. A resting tension of 0.5 g was applied during the 1-h equilibration period. Some strips were suspended between a pair of parallel platinum wire electrodes, through which electrical transmural stimulation was applied. Stimulus parameters were a duration of 0.3 ms and 10 Hz frequency and supramaximal voltage (10 V) for 5 s. The strips were electrically stimulated every 15 min. Cumulative concentration–response curves for noradrenaline were determined by increasing the concentrations of noradrenaline in a stepwise manner. 4′-Epidoxorubicin, tetrodotoxin, cocaine, prazosin, diphenhydramine, cimetidine, and superoxide dismutase were added directly to the organ chamber. Reserpine (0.25 mg/kg/day) was intraperitoneally administered for 14 days to several rats.

2.2. Measurement of endogenous noradrenaline release

The tail artery strips were suspended between a pair of parallel platinum wire electrodes and superfused with the modified Krebs–Henseleit solution, using a peristaltic pump at a flow rate of 1 ml/min (Muramatsu et al., 1983). The samples of superfusate were collected every 3 min and were immediately acidified to pH 2 with 0.6 ml of 0.4 M perchloric acid containing 1.3 mM Na_2 EDTA and 5.3 mM $Na_2S_2O_5$. Electrical stimulation was applied through a pair of electrodes. Stimulus parameters were a duration of 0.3 ms, 5 Hz frequency and supramaximal voltage (8 V) for 30 s. For the treatment with 4′-epidoxorubicin, cocaine, prazosin, and/or tetrodotoxin, the artery strips were superfused with Krebs–Henseleit solution containing these drugs. Ca^{2+}-free medium (composition in mM: NaCl 118, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10, and EGTA 0.5; pH 7.4) was also used for several release experiments.

Catecholamines in the samples were isolated using batch alumina chromatography and analyzed by using high-performance liquid chromatography (HPLC) with electrochemical detection (Ishii et al., 1995). Specifically, 33 mg of alumina was added to each 3.6 ml acidified sample. The pH was then adjusted to 8.6 with 0.5 ml of 1.5 M Tris–HCl (pH 9.0) containing 50 mM Na_2EDTA. Samples were vortexed for 30 s and placed on a rotator in the cold for 15 min. The supernatant was discarded, the alumina was washed twice with 4 ml of double-distilled water and catecholamines were eluted with 125 μl of 0.1 M perchloric acid. The HPLC-electrochemical detector was set at 0.7 V, which provided a limit of detection of approximately 10 fmol of noradrenaline. The amount of noradrenaline present in each sample was calculated by using peak area ratios relative to the internal standard 3,4-dihydroxybenzylamine. Noradrenaline release is expressed as fmol/mg wet weight of tissue.

2.3. Drugs

The following drugs were used: 4′-epidoxorubicin (Farmitalia Carlo Erba, Milan, Italy), cocaine hydrochloride (Takeda, Osaka, Japan), (−)-noradrenaline bitartrate, prazosin hydrochloride, superoxide dismutase from bovine erythrocyte, diphenhydramine hydrochloride, cimetidine crystalline (Sigma, St. Louis, U.S.A.), reserpine (Daiichiseiyaku, Tokyo, Japan), and tetrodotoxin (Sankyo, Tokyo, Japan). Drugs except reserpine were dissolved and diluted in distilled water.

2.4. Statistical analysis

All values are reported as the means ± S.E. The data were evaluated for statistical significance by using Student's *t*-test.

3. Results

3.1. Effects on the resting tension and response to electrical transmural stimulation

Electrical transmural stimulation at 10 Hz for 5 s evoked a transient contraction in isolated rat tail artery. This contraction was completely inhibited by 0.1 μM prazosin ($n = 5$) or 0.5 μM tetrodotoxin ($n = 5$). Fig. 1 shows representative effects of 30 μM 4′-epidoxorubicin on the contractile responses to electrical transmural stimulation and on the resting tension. After 4′-epidoxorubicin treatment, the resting tension rose slightly and the contractile responses to electrical stimulation were prolonged with an attenuation of the peak amplitude. Such effects developed within 1 h after addition of 4′-epidoxorubicin and lasted for at least 2 h. Controls did not demonstrate any change in resting tension or in the contractile response to electrical transmural stimulation for 2 h (data not shown). After treatment with 30 μM 4′-epidoxorubicin for 2 h, addition

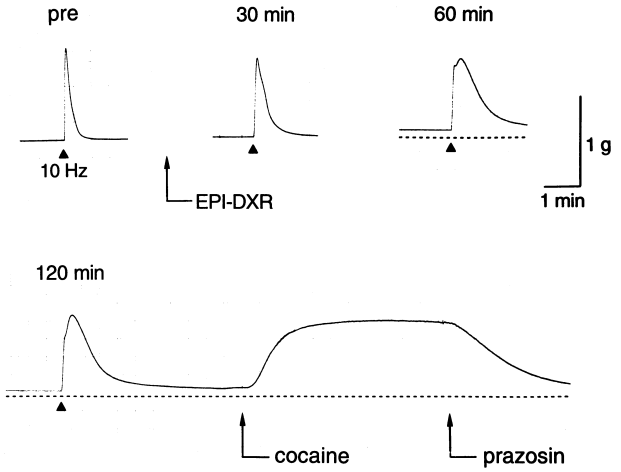

Fig. 1. Effects of 4′-epidoxorubicin (EPI-DXR) on the tonus of isolated rat tail artery. The arterial strip was electrically stimulated (10 Hz, for 5 s) every 30 min. Times represented above the traces indicate the treatment with EPI-DXR. EPI-DXR (30 μM), cocaine (10 μM) and prazosin (0.1 μM) were added cumulatively. The dashed line indicates the level of original resting tension.

of 10 μM cocaine produced a rapid elevation of the resting tension, which was suppressed by subsequent treatment with 0.1 μM prazosin (Fig. 1).

Cocaine (10 μM) produced an enhanced contractile response to electrical stimulation without a significant change in the resting tension (Fig. 2). Under such conditions, addition of 30 μM 4′-epidoxorubicin raised the resting tension and prolonged the duration of contractile responses to electrical stimulation (Fig. 2). However, the peak amplitudes of the contractile responses to electrical stimulation were gradually reduced. Prazosin (0.1 μM) but not tetrodotoxin (0.5 μM) returned the elevated tension to the original level.

The elevation of resting tension caused by 4′-epidoxorubicin was concentration dependent (Fig. 3). Treat-

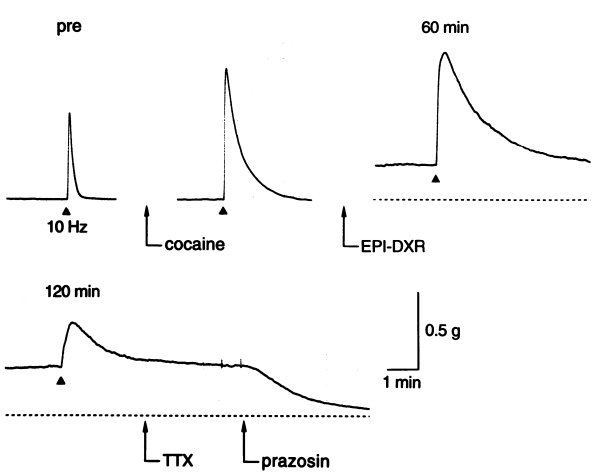

Fig. 2. Effects of cocaine and 4′-epidoxorubicin (EPI-DXR) on the tonus of isolated rat tail artery. TTX: 0.5 μM tetrodotoxin. The other experimental conditions and drug concentrations used are the same as those in Fig. 1.

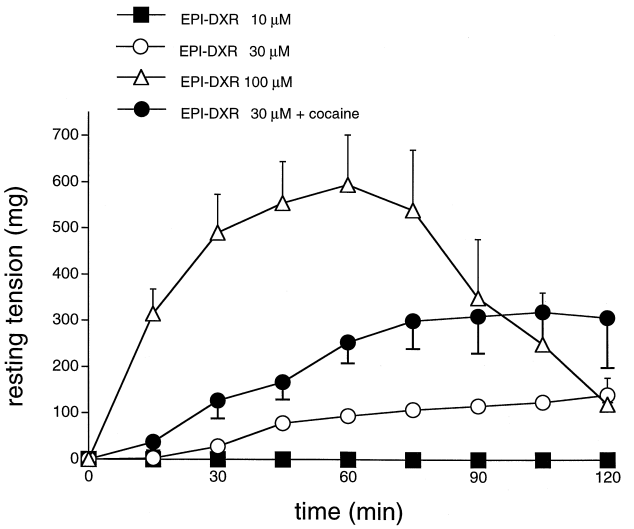

Fig. 3. Chronological changes in the tonus of isolated rat tail arteries treated with 4′-epidoxorubicin (EPI-DXR). closed square: treatment with EPI-DXR (10 μM); open circle: EPI-DXR (30 μM); open triangle: EPI-DXR (100 μM); closed circle: cotreatment with EPI-DXR (30 μM) and cocaine (10 μM). Mean ± S.E. of 3–6 experiments.

ment with 100 μM 4′-epidoxorubicin raised the resting tension more rapidly and significantly than 30 μM 4′-epidoxorubicin treatment. Treatment with 10 μM 4′-epidoxorubicin had no effect on the resting tension but the subsequent addition of 10 μM cocaine produced an elevation of resting tension (92.0 ± 18.9 mg, $n = 6$). The eleva-

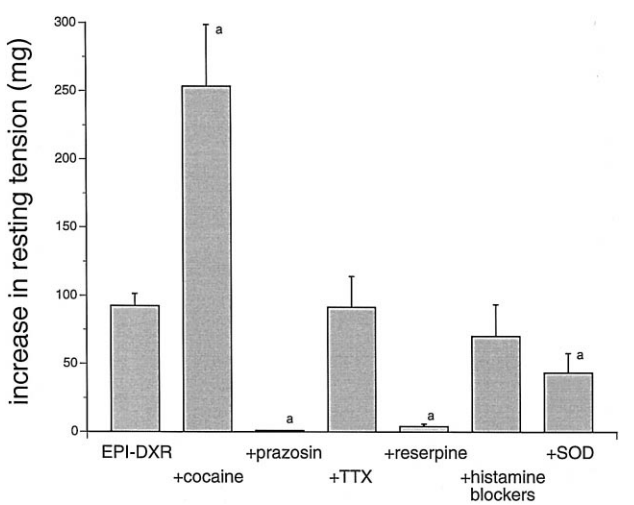

Fig. 4. Effects of various treatments on the 4′-epidoxorubicin (EPI-DXR)-induced increase in resting tension of rat tail arteries. The strips were treated with EPI-DXR (30 μM) alone or EPI-DXR (30 μM) + cocaine (10 μM), prazosin (0.1 μM), tetrodotoxin (TTX, 0.5 μM), histamine blockers (0.1 μM diphenhydramine and 0.1 μM cimetidine), or superoxide dismutase (SOD, 200 U/ml) for 60 min. Reserpine was administered intraperitoneally (0.25 mg/kg/day) for 14 days before the animals were killed. SOD at 100 U/ml was twice applied every 30 min (total 200 U/ml). The ordinate indicates the actual values for resting tension above the original level. Mean ± S.E. of 3–6 experiments. (a) Significantly different from EPI-DXR alone ($P < 0.05$).

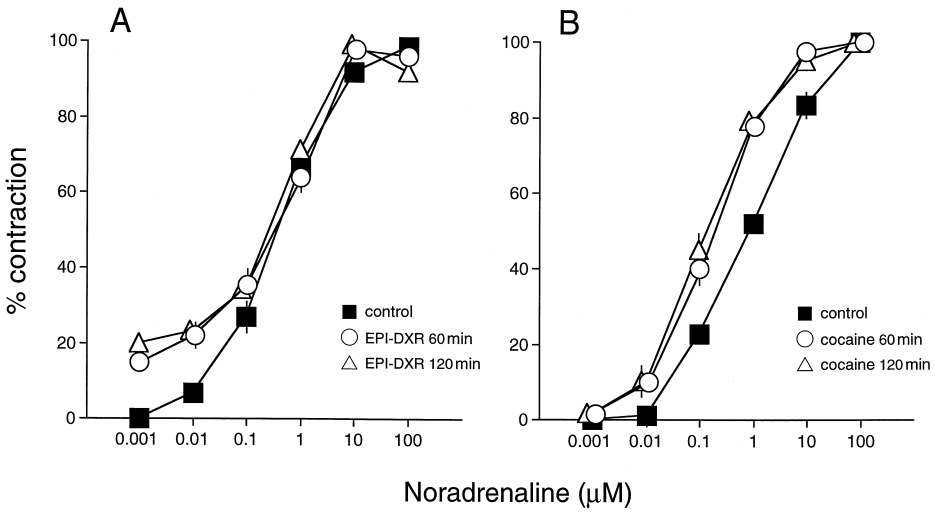

Fig. 5. Effects of 4′-epidoxorubicin (EPI-DXR) and cocaine on the concentration–response curves for noradrenaline in rat tail artery. EPI-DXR (30 μM) or cocaine (10 μM) was added for 60 and 120 min. Control: the responses before treatment with EPI-DXR or cocaine. Mean ± S.E. of four experiments.

tion of resting tension caused by 4′-epidoxorubicin was suppressed by prazosin (0.1 μM) and in the strips isolated from rats pretreated with reserpine (0.25 mg/kg/day, 14 days), and was partially inhibited by superoxide dismutase (200 u/ml), but was not suppressed by treatment with tetrodotoxin (0.5 μM) or histamine receptor antagonists (0.1 μM diphenhydramine and 0.1 μM cimetidine) (Fig. 4).

3.2. Effects on the contractile response to exogenous noradrenaline

Noradrenaline (0.01–100 μM) caused a concentration-dependent contraction in the rat tail artery. Treatment with 4′-epidoxorubicin (30 μM) for 1 and 2 h raised the resting tension, but did not alter the concentration–response curves for noradrenaline at concentrations higher than 0.1 μM (Fig. 5A). Cocaine (10 μM) shifted the concentration–response curve for noradrenaline leftwards (Fig. 5B).

3.3. Effects on noradrenaline release

The basal release of endogenous noradrenaline from rat tail artery strips was 14.8 ± 0.5 fmol/mg/min ($n = 9$). Superfusion of 30 μM 4′-epidoxorubicin for 30 or 60 min increased the basal release of noradrenaline five-fold, which was not significantly changed by subsequent treatment with 10 μM cocaine, 0.1 μM prazosin, 0.5 μM

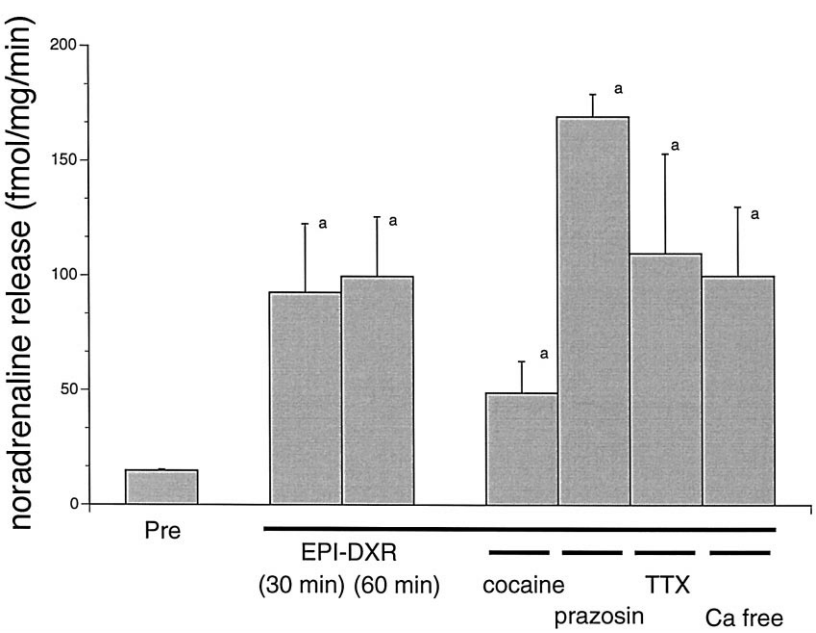

Fig. 6. Effects of 4′-epidoxorubicin (EPI-DXR) on spontaneous noradrenaline release from superfused tail arteries of rats. pre: before superfusion of EPI-DXR. After superfusion of 30 μM EPI-DXR for 60 min, the strips were treated with cocaine (10 μM), prazosin (0.1 μM), tetrodotoxin (TTX, 0.5 μM) or Ca^{2+}-free medium in the presence of EPI-DXR. Mean ± S.E. of 3–5 experiments. (a) Significantly different from pre-value ($P < 0.05$).

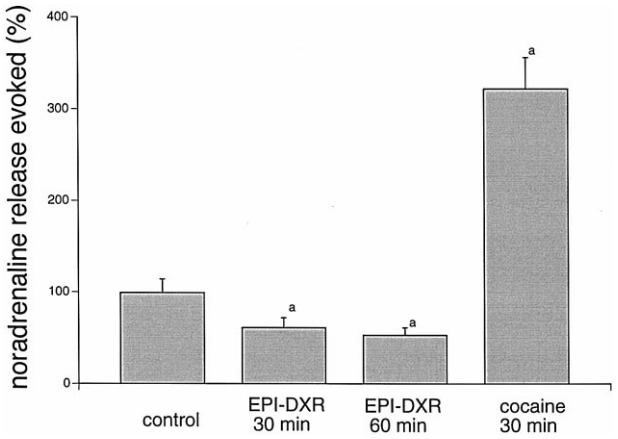

Fig. 7. Noradrenaline release evoked by electrical transmural stimulation in rat tail artery strips. The arterial strips were superfused and electrically stimulated every 30 min at 5 Hz for 30 s. The net release above basal level is represented as a percentage relative to the first stimulation. Control: the evoked release 30 and 60 min after superfusion without 4′-epidoxorubicin (EPI-DXR, 30 μM) and cocaine (10 μM). Mean ± S.E. of 3–5 experiments. (a) Significantly different from control ($P < 0.05$).

tetrodotoxin or by the elimination of Ca^{2+} from the medium (Fig. 6).

Electrical stimulation (at 5 Hz for 30 s) evoked a significant and reproducible increase in noradrenaline release. The net amount of noradrenaline released above the basal level was 97 ± 16 fmol/mg upon the first stimulation ($n = 5$), an effect which was abolished in Ca^{2+}-free medium ($n = 3$). Superfusion of 30 μM 4′-epidoxorubicin for 60 min decreased the evoked release, but cocaine (10 μM) increased the evoked release approximately three-fold (Fig. 7).

4. Discussion

In the present study, treatment with 4′-epidoxorubicin produced diverse effects on isolated rat tail artery: it raised the resting tension of arterial strips and prolonged the duration and attenuated the peak amplitude of the sympathetic contractions evoked by electrical stimulation. All these effects were abolished after treatment with prazosin, an α_1 adrenoceptor antagonist, indicating that α_1 adrenoceptors are involved in the responses to 4′-epidoxorubicin.

The effect of 4′-epidoxorubicin on resting tension was greatly potentiated by cocaine, an inhibitor of noradrenaline uptake by the sympathetic nerve terminals. This augmented response was also abolished by subsequent treatment with prazosin. 4′-Epidoxorubicin failed to raise the resting tension in tail artery isolated from reserpine-pretreated rats. These results suggest that 4′-epidoxorubicin may directly act on sympathetic nerves to release noradrenaline. In fact, superfusion experiments clearly demonstrated an increase in endogenous noradrenaline release after 4′-epidoxorubicin treatment.

The inability of tetrodotoxin to inhibit the augmented release of noradrenaline suggests that Na^+ channel-mediated processes are not involved in the augmented release. Rather, since anthracyclines are known to alter mitochondrial functions, intracellular electrolyte balance and Na^+, K^+-ATPase activity (Lenaz and Page, 1976; Ferrans, 1978), persistent depolarization in sympathetic nerve terminals might be produced by 4′-epidoxorubicin, which in turn causes an increase in the spontaneous release of noradrenaline and gradually attenuates the evoked release in response to electrical stimulation. Alternatively, because anthracyclines are known to produce free radicals, which result in the adverse effects of anthracyclines (Olson et al., 1981; Hüsken et al., 1995), the 4′-epidoxorubicin-derived free radicals may cause the release of noradrenaline. Preliminary experiments showed a partial inhibition by superoxide dismutase of the elevation of resting tension induced by 4′-epidoxorubicin (Fig. 4). The persistent release of noradrenaline in Ca^{2+}-free medium (Fig. 6) further suggests that noradrenaline release evoked by 4′-epidoxorubicin is not mediated through physiological processes.

In vivo, it has been reported that treatment with anthracyclines raises the plasma level of catecholamines and that this effect is inhibited by histamine receptor antagonists (Bristow et al., 1980, 1981). This evidence may be related to the observed effect of 4′-epidoxorubicin on sympathetic nerves. However, cimetidine and diphenhydramine failed to inhibit the elevation of resting tension caused by 4′-epidoxorubicin in the present study.

Cocaine produced a great enhancement of contraction in 4′-epidoxorubicin-treated artery (Fig. 1), whereas it did not cause a parallel increase in noradrenaline release (Fig. 6). While this discrepancy may be accounted for by a supersensitive action of cocaine on postjunctional α_1 adrenoceptors (Fleming, 1975), the contractile responses to non-uptake$_1$ agonists (phenylephrine or methoxamine) were not potentiated by cocaine (data not shown). Therefore, the dissociation between the contractile response and noradrenaline release in the presence of 4′-epidoxorubicin and cocaine cannot be explained and should be investigated in the future.

The sustained release of noradrenaline would promote the adverse effects of anthracyclines on the heart such as ventricular failure, arhythmias and cardiomyopathy (Rona, 1985; Schömig et al., 1995). More recently, anthracyclines have been used for the transarterial chemotherapy of hepatic tumors (Uchida et al., 1990). With this treatment a high bolus dose of anthracyclines (more than 1 mM) is directly injected into the hepatic artery, which often produces hepatic arterial spasm during and after the transarterial infusion of the drugs (Seki et al., 1996). The persistent release of noradrenaline observed in the present study may be one of the causes for the arterial spasm or occlusion and liver damage (Iwai and Shimizu, 1996).

5. Conclusion

In conclusion, the present study shows that anthracycline 4′-epidoxorubicin directly acts on the sympathetic nerve (terminals) to enhance the basal release of noradrenaline, suggesting a possible relation to the cardiovascular toxicity of this drug.

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