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Neuroeffector actions of thromboxane B_2 in dog isolated mesenteric arteries

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1 Thromboxane (TX) B_2 and epithiomethano ($sTXA_2$), in concentrations that were insufficient to alter the basal tone, potentiated contractile responses of helical strips of dog mesenteric arteries to transmural electrical stimulation. The potentiating effect of TXB_2 (up to 10^{-6} M) was not abolished by diphloretin phosphate (DPP), a prostaglandin antagonist, whereas the potentiation by $sTXA_2$ was abolished by the antagonist.

2 $sTXA_2$ and TXB_2 (3×10^{-6} M or higher) potentiated the responses to noradrenaline, the potentiation being antagonized by DPP.

3 ^{3}H-overflow evoked by transmural stimulation in superfused arterial strips previously soaked in medium containing [^{3}H]-noradrenaline was increased by TXB_2, but not altered by $sTXA_2$.

4 TXB_2 in low concentrations potentiated the contractile response to adrenergic nerve stimulation, possibly by increasing the release of noradrenaline, while the potentiation by the TXA_2 analogue appears to be due to increased sensitivity of the arteries to noradrenaline. Prejunctional effects of TXB_2 may be mediated by receptor sites functionally different from those located postjunctionally.

Introduction

Thromboxane (TX) A_2, synthesized mainly in platelets from arachidonic acid by prostaglandin H_2 (PGH_2) synthetase and TX synthetase, is a potent vasoconstrictor and a platelet aggregator, and may be responsible for vascular disorders such as vasospasm and atherosclerosis. On the other hand, TXB_2, a stable metabolite of TXA_2 (Hamberg *et al.*, 1975), has been reported to show only slight effects on blood vessels (Friedman *et al.*, 1979; Fitzpatrick *et al.*, 1980) and other tissues (Wasserman & Griffin, 1977; Crawford *et al.*, 1978). In addition to direct actions on vascular smooth muscle, prostaglandins and TX modify the contractile response to adrenergic nerve stimulation by acting on prejunctional or postjunctional sites (Brody & Kadowitz, 1974; Greenburg *et al.*, 1974; Malik *et al.*, 1976; Herman *et al.*, 1978). Our previous papers have shown that PGD_2, $PGF_{2\alpha}$ and carbocyclic TXA_2 ($cTXA_2$) produce vasoconstriction indirectly via actions on the neuroeffector junction in concentrations appreciably lower than those which directly contract smooth muscle (Nakajima & Toda, 1984; 1986). However, no detailed information is available concerning modulation of the vascular neuroeffector function by TXB_2.

The present study was therefore undertaken to establish the action of TXB_2 on the contractile response of dog isolated mesenteric arteries to adrenergic nerve stimulation and noradrenaline, and on the stimulation-evoked ^{3}H-overflow in superfused mesenteric arteries previously soaked in a [^{3}H]-noradrenaline-containing medium. Experiments were also performed to determine the mechanism of action of TXB_2 by the use of prostaglandin receptor antagonists, diphloretin phosphate (DPP) (Sanner, 1974) and ONO3708 (Toda *et al.*, 1986). Epithiomethano TXA_2 ($sTXA_2$), a stable analogue of TXA_2, was used for comparison.

Methods

Preparation

Mongrel dogs of either sex, weighing 7–14 kg, were anaesthetized with intravenous injections of sodium pentobarbitone (30 mg kg^{-1}) and killed by bleeding from the common carotid arteries. Distal portions of the superior mesenteric artery (0.6–0.8 mm o.d.) were quickly removed. The arteries were cut helically into strips approximately 20 mm long. The strips were fixed vertically between hooks in a muscle bath of 20 ml capacity containing the modified Ringer-Locke solu-

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tion, which was maintained at 37 ± 0.3°C and aerated with a mixture of 95% O_2 and 5% CO_2. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Nihonkohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 1.5 g, which is optimal for inducing maximum contractions (Toda *et al.*, 1978). The solution had the following composition (mM): NaCl 120, KCl 5.4, $NaHCO_3$ 25.0, $CaCl_2$ 2.2, $MgCl_2$ 1.0 and dextrose 5.6. The pH of the solution was 7.3–7.4. Before the start of experiments, the arterial strips were allowed to equilibrate for 60 to 90 min in control media, during which time the solutions were replaced every 10 to 15 min.

Transmural stimulation of adrenergic nerves

Mesenteric arterial strips were placed between a pair of stimulating electrodes made of platinum plate, 5 × 15 mm in size and approximately 2 mm apart from each other (Toda, 1971). The gap between the strip and the electrodes was wide enough to allow undisturbed contractions, and yet sufficiently narrow to permit stimulation of intramural nerve terminals effectively. The strips were transmurally stimulated by a train of 0.3 ms square pulses of supramaximum intensity (approximately 10 V), at frequencies of 2, 5 and 20 Hz for periods of 100, 40 and 10 s, respectively. Stimulations at 5 Hz were applied repeatedly with an interval of 10 min until steady state responses were obtained.

Recording

Isometric contractions were recorded on an ink-writing oscillograph (Nihonkohden Kogyo Co.). Contractile responses to 30 mM K^+ were first obtained, then the preparations were washed three times with control media and equilibrated for 40–50 min. Cumulative concentration-response curves for noradrenaline were obtained by adding the amine directly to the bathing media. Preparations were treated for 30 min with diphloretin phosphate (DPP) or ONO3708 and for 20 min with other agents, before the response to transmural stimulation or noradrenaline was obtained.

Experiments on tritium overflow

Isotope experiments were carried out on helical strips of mesenteric arteries of the dog, as previously described (Nakajima & Toda, 1984). Briefly, the tissue was preincubated for 60 min at 37°C with 0.5 μM [^{3}H]-noradrenaline (sp. act., 43.9 Ci mmol^{-1}). It was then superfused with the modified Ringer-Locke solution containing cocaine (3×10^{-5} M) and corticosterone (4×10^{-5} M) at a rate of 1 ml min^{-1}. The preincubated strips were stimulated electrically five times for 3 min at a frequency of 5 Hz. Stimulation periods started after 126 (S_1), 144 (S_2), 162 (S_3), 180 (S_4) and 198 min (S_5) of superfusion. The stimulation-evoked overflow of total tritium was calculated by subtraction of basal overflow. The drug was added 12 min before S_4. The drug effect on the stimulation-evoked ^{3}H-overflow was expressed as the ratio between the overflow evoked by S_4 or S_5 and that evoked by S_3. The ratios were compared with those obtained in the absence of treatment with drugs.

Statistics and drugs

Results shown in the text and figures are expressed as mean ± s.e.mean. Statistical analyses were made using Student's paired and unpaired *t* test and Tukey's method after one-way analysis of variance. Drugs used were TXB_2, $sTXA_2$ (9,11-epithio-11,12-methano TXA_2) and ONO3708((9,11),(11,12) -dideoxa-9α,11α-dimethyl-methano-11,12-methano-13,14 - dihydro - 13 -azo-14-oxo-15-cyclopentanyl-16, 17, 18, 19, 20-pentanor-15-epi- TXA_2) (Ono Pharmaceutical Co., Osaka, Japan), diphloretin phosphate (Leo Co., Helsingborg, Sweden), (±)-noradrenaline hydrochloride (Sankyo Co., Tokyo), papaverine hydrochloride (Dainippon Pharmaceutical Co., Osaka), cocaine hydrochloride and corticosterone (Sigma, St. Louis, MO, U.S.A.), (−)-[ring-2,5,6-^{3}H]-noradrenaline (NEN, Boston, MA, U.S.A.) and sodium pentobarbitone (Abbott Lab., North Chicago, IL, U.S.A.).

Results

Modification by thromboxane B_2 of the contractile responses of mesenteric arteries to transmural stimulation and noradrenaline

The addition of TXB_2 in concentrations lower than 3×10^{-6} M did not alter the arterial tension, but, at 10^{-5}, 3×10^{-5} and 10^{-4} M it caused contractions dose-dependently (6.1 ± 1.7, 34.1 ± 5.9 and 95.6 ± 19.1%, $n = 7$, relative to 30 mM K^+-induced contractions, respectively). The contraction was suppressed by treatment with ONO3708 (10^{-8} M); contractions induced by TXB_2 (3×10^{-5} M) before and after the treatment were 33.6 ± 8.8 and 0.8 ± 0.5%, respectively ($n = 4$).

Transmural electrical stimulation applied at frequencies of 2, 5 and 20 Hz for periods of 100, 40 and 10 s, respectively, produced a frequency-dependent contraction, which was abolished by tetrodotoxin (3×10^{-7} M) and suppressed by prazosin (10^{-8} M). Treatment with TXB_2 in concentrations of 10^{-7} to 10^{-6} M potentiated the contractile response to transmural stimulation. The concentration-related poten-

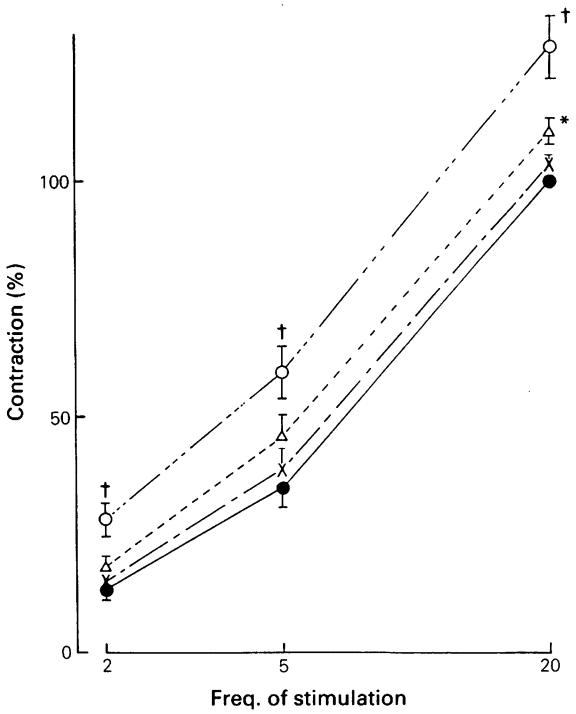

Figure 1 Modification by thromboxane B_2 (TXB_2) in concentrations of 10^{-7} (X, $n = 11$), 3×10^{-7} (Δ, $n = 11$) and 10^{-6} M (O, $n = 11$) of the contractile response of mesenteric arterial strips to transmural stimulation. Contractions induced by the stimulation at 20 Hz in control media (●, $n = 11$) were taken as 100%. The mean (± s.e.mean) absolute value was 1368 ± 121 mg ($n = 11$). Significantly different from controls; $*P < 0.05$; $\dagger P < 0.01$. Vertical bars represent s.e.mean.

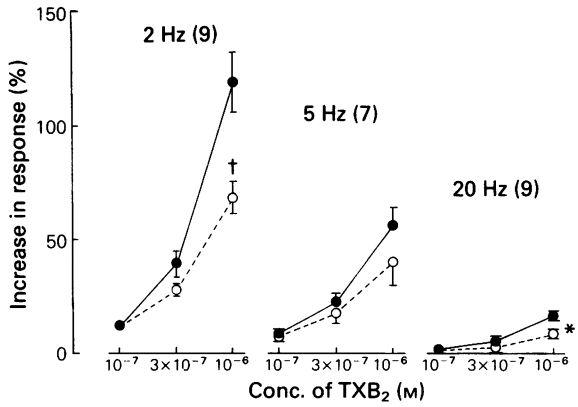

Figure 2 Potentiation by thromboxane B_2 (TXB_2) of the contractile response to transmural stimulation at frequencies of 2, 5 and 20 Hz in the absence (●) and presence (O) of 10^{-5} M diphloretin phosphate. Significantly different from controls; $*P < 0.05$; $\dagger P < 0.01$. Numbers in parentheses indicate the number of preparations used.

tiation is shown in Figure 1. The potentiation was inversely related to the frequency of stimulation applied; the increases caused by TXB_2 (3×10^{-7} M) in the response to transmural stimulation at 2, 5 and 20 Hz were 39.3 ± 6.1% ($n = 9$), 22.9 ± 3.6% ($n = 7$) and 5.5 ± 1.5% ($n = 9$), respectively. The potentiating effect of TXB_2 (10^{-7} and 3×10^{-7} M) was not significantly attenuated by treatment with diphloretin phosphate (DPP) in a concentration (10^{-5} M) that is sufficient to suppress markedly the contractions induced by prostaglandins and carbocyclic TXA_2 (Toda, 1984). On the other hand, potentiation by TXB_2 10^{-6} M was partially inhibited by treatment with this concentration of DPP (Figure 2). The response to transmural stimulation was not significantly altered by treatment with DPP alone.

Contractile responses to noradrenaline were potentiated by treatment with TXB_2 (Figure 3a); the increments caused by 3×10^{-6} and 10^{-5} M TXB_2 in the response to noradrenaline at 10^{-7} M were 141.6 ± 31.7% ($n = 15$) and 348.0 ± 53.3% ($n = 7$), respectively. Such a potentiation was abolished by treatment with DPP 10^{-5} M (Figure 3b).

Modification by epithiomethano thromboxane A_2 of the contractile responses of mesenteric arteries to transmural stimulation and noradrenaline

The addition of $sTXA_2$ in concentrations lower than 10^{-10} M did not contract the arterial strips, but contractions (5 to 40% of the 30 mM K^+-induced contractions) were observed on 3 out of the 13 arterial strips at 3×10^{-10} M and on 6 out of the 13 arterial strips at 10^{-9} M. Therefore, the preparations showing contractions in response to $sTXA_2$ under resting conditions were excluded from the further experiments. Treatment with $sTXA_2$ (10^{-10} to 10^{-9} M) potentiated the contractile response to transmural stimulation in a concentration-related manner (Figure 4). The potentiation by 3×10^{-10} and 10^{-9} M $sTXA_2$ was abolished almost completely by treatment with DPP 10^{-5} M (Figure 5).

Contractile responses to noradrenaline were potentiated by treatment with $sTXA_2$ 3×10^{-10} M (Figure 6, a). Treatment with DPP (10^{-5} M) abolished the potentiation (Figure 6b).

Effects of ONO3708, in a concentration (10^{-7} M) sufficient to suppress the contractile response to TXA_2 analogues and prostaglandins (Toda *et al.*, 1986), on the $sTXA_2$- and TXB_2-induced potentiations of contractile response to transmural stimulation were also examined. Treatment with ONO3708 at 10^{-7} M aboli-

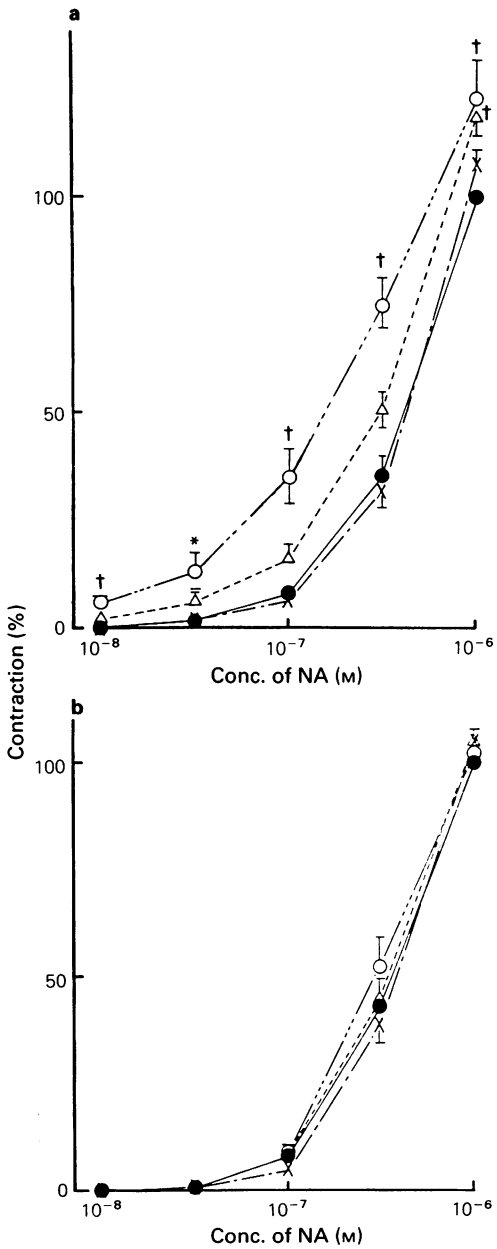

Figure 3 Modification by thromboxane B_2 (TXB_2) in concentrations of 10^{-6} (X, $n = 5$), 3×10^{-6} (Δ, $n = 15$) and 10^{-5} M (O, $n = 7$) of the contractile response to noradrenaline (NA) in the absence (a) and presence (b) of 10^{-5} M diphloretin phosphate (DPP). Contractions induced by 10^{-6} M noradrenaline in control media (a, ●, $n = 15$) and in DPP-containing media (b, ●, $n = 15$) were taken as 100%; the mean (± s.e.mean) absolute values were 2904 ± 227 mg ($n = 15$) and 3224 ± 290 mg ($n = 15$), respectively. Significantly different from controls; $*P < 0.05$; $\dagger P < 0.01$.

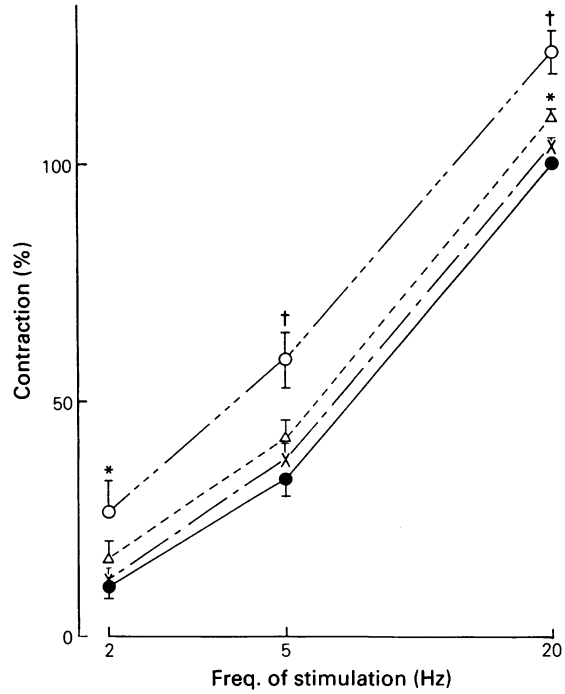

Figure 4 Modification by epithiomethano thromboxane A_2 ($sTXA_2$) in concentrations of 10^{-10} (X, $n = 7$), 3×10^{-10} (Δ, $n = 7$) and 10^{-9} M (O, $n = 7$) of the contractile response to transmural stimulation. Contractions induced by the stimulation at 20 Hz in control media (●, $n = 7$) were taken as 100%. The mean (± s.e.mean) absolute value was 1655 ± 233 mg ($n = 7$). Significantly different from controls; $*P < 0.05$; $\dagger P = 0.01$.

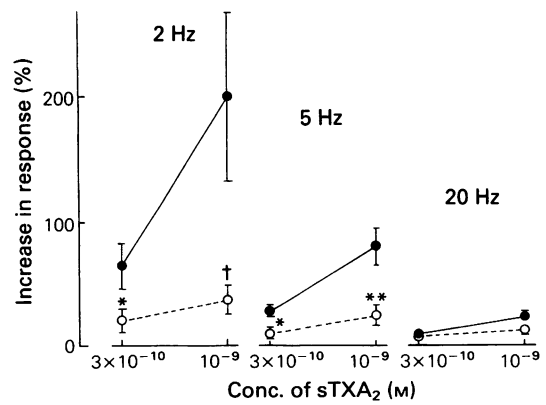

Figure 5 Potentiation by epithiomethano thromboxane A_2 ($sTXA_2$) of the contractile response to transmural stimulation in the absence (●, $n = 7$) and presence (O, $n = 13$) of 10^{-5} M diphloretin phosphate. Significantly different from controls; $*P < 0.05$; $\dagger P < 0.01$; $**P < 0.005$.

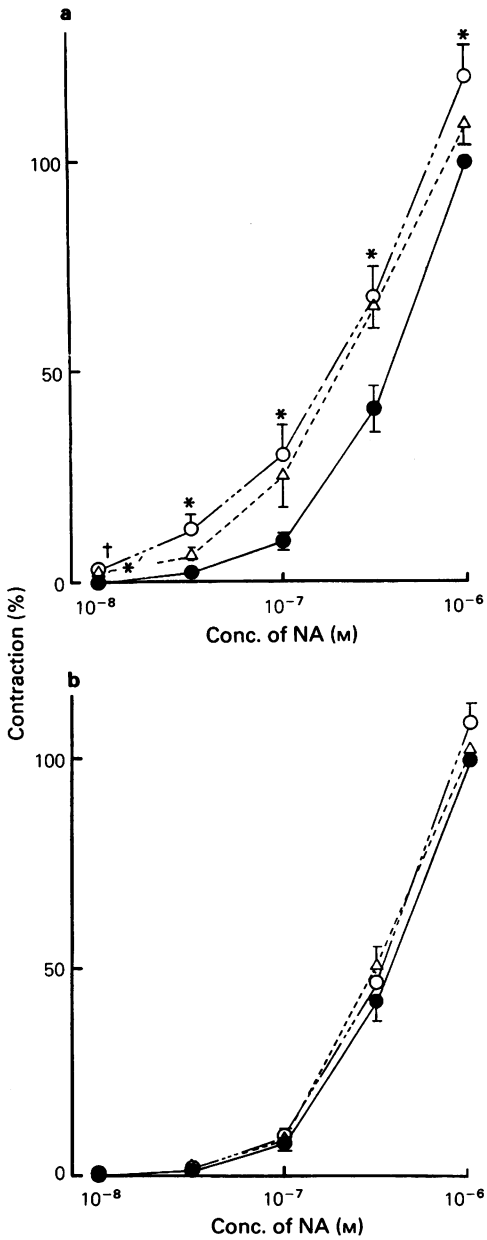

Figure 6 Modification by epithiomethano thromboxane A_2 ($sTXA_2$) in concentrations of 10^{-10} (Δ, $n = 4$) and 3×10^{-10} M (○, $n = 10$) of the contractile response to noradrenaline (NA) in the absence (a) and presence (b) of 10^{-5} M diphloretin phosphate (DPP). Contractions induced by 10^{-6} M noradrenaline in control media (a, ●, $n = 10$) and in DPP-containing media (b, ●, $n = 10$) were taken as 100%; the mean (± s.e.mean) absolute values were 3304 ± 257 mg ($n = 10$) and 3810 ± 402 mg ($n = 10$), respectively. Significantly different from controls; $*P < 0.05$; $\dagger P < 0.01$.

shed the potentiation by $sTXA_2$ of the contractile response to 5 Hz transmural stimulation, whereas the prostaglandin antagonist did not significantly affect the potentiation caused by TXB_2 3×10^{-7} M, but partially suppressed that caused by 10^{-6} M (Figure 7).

^{3}H-overflow from superfused mesenteric arteries in response to transmural stimulation

Transmural electrical stimulation was applied at a frequency of 5 Hz for 3 min to mesenteric arterial strips previously exposed for 60 min in a bathing medium containing [^{3}H]-noradrenaline (5×10^{-7} M) and superfused for 2 h in control medium. The ^{3}H-overflow and the arterial contractions were abolished by treatment with tetrodotoxin (3×10^{-7} M). The ratio of ^{3}H overflow (S4/S3) was significantly increased by treatment with TXB_2 (10^{-6} to 10^{-5} M), but not by $sTXA_2$ (3×10^{-10} and 10^{-9} M) (Figure 8); these concentrations were sufficient to potentiate the contractile response to transmural electrical stimulation (Figure 4). Typical recordings before and after treatment with TXB_2 (10^{-5} M) are shown in Figure 9. The spontaneous ^{3}H-overflow in the absence of transmural stimulation was not influenced by this concentration of TXB_2, but the stimulation-evoked ^{3}H-overflow and the contraction were appreciably increased. The increase by TXB_2 of the ^{3}H-overflow ratio was not significantly attenuated by treatment for 60 min with DPP 10^{-5} M (Figure 8).

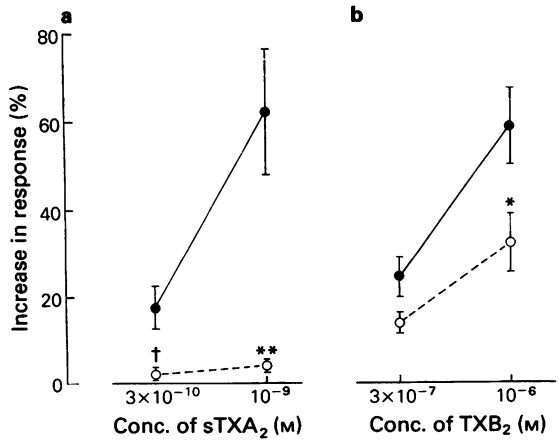

Figure 7 Potentiation by epithiomethano thromboxane A_2 ($sTXA_2$) and thromboxane B_2 (TXB_2) of the contractile response to transmural stimulation at 5 Hz for 40 s in the absence (●) and presence (○) of 10^{-7} M ONO3708. Significantly different from controls; $*P < 0.05$; $\dagger P < 0.025$; $**P < 0.005$.

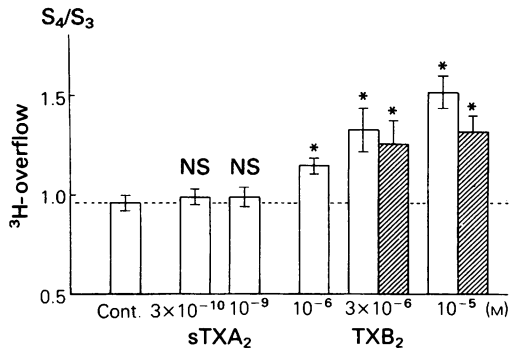

Figure 8 The ratio of ^{3}H-overflow evoked by transmural stimulation (5 Hz, 3 min) in control arteries and those treated with epithiomethano thromboxane A_2 ($sTXA_2$), thromboxane B_2 (TXB_2) and diphloretin phosphate (DPP) + TXB_2. Hatched columns represent the data from arteries treated for 60 min with 10^{-5} M DPP and for 12 min with TXB_2 before the application of transmural stimulation (S4). Significantly different from controls; $*P<0.05$.

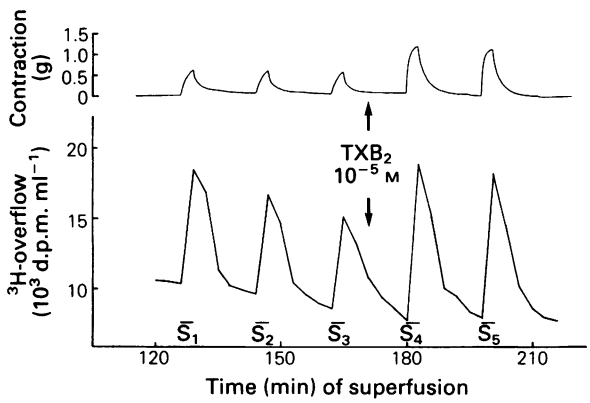

Figure 9 ^{3}H-overflow and contraction induced by transmural stimulation in a superfused mesenteric arterial strip before and after treatment with 10^{-5} M thromboxane B_2 (TXB_2). The strip was exposed for 60 min to 5×10^{-7} M [^{3}H]-noradrenaline before superfusion. Transmural stimulation at a frequency of 5 Hz for 3 min was applied five times at intervals of 18 min to the strip superfused for 126 min.

Discussion

$sTXA_2$ is the most potent vasoconstrictor of all the agents tested in dog isolated cerebral, coronary, renal and mesenteric arteries (Toda *et al.*, 1986). The present study has demonstrated that treatment with $sTXA_2$, in concentrations that were insufficient to alter the basal tone, potentiated the contractile response of dog isolated mesenteric arteries to electrical stimulation of adrenergic nerve and to noradrenaline. On the other hand, the ^{3}H-overflow evoked by transmural stimulation in superfused mesenteric arterial strips previously soaked in [^{3}H]-noradrenaline-containing medium was not increased by the TXA_2 analogue. Neuronal and extraneuronal uptake of noradrenaline does not appear to interfere with the ^{3}H-overflow, since the superfusate contained high concentrations of cocaine and corticosterone. Therefore, it may be concluded that $sTXA_2$ potentiates the response to adrenergic nerve stimulation by increasing the contractions mediated by α-adrenoceptors, which are activated by noradrenaline released from adrenergic nerve terminals. Similar results have been reported in experiments with $cTXA_2$, another TXA_2 analogue (Nakajima & Toda, 1986). In guinea-pig mesenteric arteries, however, transmission at the adrenergic neuromuscular junction is reportedly inhibited by $cTXA_2$ (Makita, 1983).

Contractions of dog mesenteric, cerebral and coronary arterial strips induced by $cTXA_2$, $PGF_{2\alpha}$, PGE_2 and PGD_2 are suppressed by treatment with DPP (Toda, 1982a,b) and ONO3708 (Toda *et al.*, 1986). These antagonists are expected to antagonize selectively the action of prostaglandins and TXA_2 analogues, since the response to other vasoconstrictors, such as 5-hydroxytryptamine, K^+ and noradrenaline, were not attenuated with the concentrations used in the present study. The potentiation by $PGF_{2\alpha}$ of the contraction and the ^{3}H-overflow evoked by adrenergic nerve stimulation was not suppressed by DPP (Nakajima & Toda, 1986). Thus, the phloretin compound appears to antagonize the postjunctional action of prostaglandins and TXA_2 analogues. In the present study, the potentiating effect of $sTXA_2$ on contractions in response to adrenergic nerve stimulation and noradrenaline was completely inhibited by DPP. Further, treatment with ONO3708 (10^{-7} M) also suppressed the potentiation by $sTXA_2$ of contractions in response to nerve stimulation. Taken together with the previous result obtained with $cTXA_2$, TXA_2 would be considered to increase arterial tone by acting directly on smooth muscle and indirectly via sensitization of α-adrenoceptors to noradrenaline released from adrenergic nerves.

TXB_2 is an endogeneous and stable metabolite of TXA_2. The vasoconstrictor potency of TXB_2 is much less than that of TXA_2 analogue; approximately 1/10,000 as a molar ratio. TXB_2, in concentrations (10^{-7} to 10^{-6} M) insufficient to alter the basal tone, potentiated the contractile response to electrical stimulation of adrenergic nerves but did not alter the response to noradrenaline. DPP failed to inhibit the potentiating effect of TXB_2 (10^{-7} and 3×10^{-7} M) on the response to electrical stimulation, but partially

inhibited the potentiation produced by TBX_2 10^{-6} M. Similar results were also obtained with the other prostaglandin antagonist ONO3708. On the other hand, TXB_2 at 3×10^{-6} and 10^{-5} M, potentiated the contractile response to noradrenaline, which was completely suppressed by DPP. Therefore, TXB_2 potentiates the contractile response to adrenergic nerve stimulation, possibly due to actions on both prejunctional and postjunctional sites; in low concentrations (3×10^{-7} M or lower), the prejunctional mechanism is responsible for the potentiation via the increased noradrenaline release from nerves, and in the higher concentrations, the postjunctional mechanism is also involved in the potentiation through an enhancement of arterial responses to noradrenaline. Such a postjunctional mechanism of potentiation by the thromboxane analogue was postulated above. Whether or not TXB_2 and TXA_2 share the same postjunctional receptors remains to be determined.

Interestingly, TXB_2 has an ability to potentiate the response to nerve stimulation through a prejunctional mechanism, which could not be observed with TXA_2 analogues. Therefore, TXB_2, but not TXA_2, appears to have a specific site on nerve terminals. The actions of TXB_2 on this site were not inhibited by DPP. Inability of this antagonist to block prejunctional actions of PGD_2 and $PGF_{2\alpha}$ has been demonstrated in previous papers (Nakajima & Toda, 1984; 1986). The lack of DPP antagonism may not be due to problems of its access to prejunctional sites, because ONO3708, a TXA_2 analogue, also failed to inhibit the prejunctional actions of TXB_2. These data indicate that the prejunctional receptor sites for prostaglandins and TXB_2 may be functionally different from the postjunctional sites.

The vascular action of TXA_2 locally produced is expected to be quite short, whereas the action of its stable metabolite TXB_2 is persistent. Based on the findings obtained in the present study, the vascular action of TXB_2, although it has been considered to be inert (Kolata, 1975), would not be negligible if the stable metabolite were to accumulate in the vicinity of vascular neuroeffector regions. The ability of blood vessels to synthesize TXA_2 locally has been demonstrated (Hagen *et al.*, 1979; Saltzman *et al.*, 1980), although no information is available concerning extracellularly accumulated concentrations of its metabolite.

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