CONTRACTILE RESPONSES OF SPIRAL STRIPS OF LARGE BLOOD VESSELS FROM RABBITS TO TRANSMURAL STIMULATION AND TYRAMINE

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Transmural electrical stimulation with repetitive pulses of short duration causes contraction of isolated vascular smooth muscles, which is suggested to correlate with excitation of adrenergic nerve terminals innervating the vascular wall (1,2). This is supported by findings which indicated that field stimulation causes contraction and an increased efflux of $^3$H-noradrenaline from rabbit main pulmonary arteries (3) and that a biologically active (gut-relaxing) substance is released from stimulated rabbit ear arteries (4). Tyramine releases noradrenaline from the stored site that is not accessible to nerve impulses (5). Excitatory responses of the vascular smooth muscle to electrical and chemical stimulation are thought to relate directly to functional noradrenaline involved in the different pools of nerve terminals.

The aim of the present study was to examine contractile responses of ascending and thoracic aortae and the main pulmonary and superior mesenteric arteries to transmural stimulation, tyramine and dopamine, in comparison with noradrenaline. Since arterial strips have a capacity to accumulate and retain noradrenaline when applied exogenously at high concentrations (6,7), the contractile response to the electrical and chemical stimulation was also investigated in arterial strips after exposure to noradrenaline or dopamine.

METHODS

Albino rabbits of both sexes weighing 1.8 to 2.2kg were sacrificed by bleeding from common carotid arteries. The main pulmonary artery, ascending aorta and a part of the thoracic aorta (middle part between the aortic arch and the diaphragm) and the superior mesenteric artery were isolated and adherent tissues removed. These vessels were spirally cut into strips of approx. $5 \times 25$ mm in size (for the former three vessels) and $3 \times 25$ mm (for the mesenteric artery). Specimens were suspended in a muscle bath of 100 ml capacity containing the nutrient solution and adjusted to a resting tension of 2 g. Isometric contractions of the vascular smooth muscle were recorded on a two-channel penwriter (Sanei Sokki Co.) by means of a force-displacement transducer (Nihonkoden Kogyo Co.). Temperature of the bath was maintained at $37 \pm 0.5^\circ$C and the solution bathing the vascular strips was continuously aerated with a mixture of 95% oxygen and 5% carbon dioxide. Before measurements were begun, preparations were allowed to equilibrate for longer than 2 hr in the nutrient solution of the following composition (mM): $Na^+$, 162.1; $K^+$, 5.4; $Ca^{++}$,
2.2; Cl\textsuperscript{−}, 157.0; HCO\textsubscript{3}\textsuperscript{−}, 14.9; dextrose, 5.6. During the equilibration period the nutrient solution was twice replaced with fresh solutions.

The strips were placed between a pair of stimulating electrodes of the platinum plate, 5 × 15 mm in size and 2 mm apart. Gaps between the electrodes and the strip were wide enough to allow undisturbed vascular contraction and yet sufficiently narrow to stimulate intramural nerve terminals effectively. For transmural stimulation of the preparation a train of square pulses delivered by an electronic stimulator (Type MSE-3R, Nihonkoden Kogyo Co.) was passed through the electrodes.

Noradrenaline, tyramine and dopamine were applied directly to the nutrient solution of the muscle bath in cumulative concentrations. Maximum tension developed by various concentrations of the amines and by transmural electrical stimulation was measured. Absolute values of the tension increment were compared. Mean values of the contraction relative to that obtained when preparations were stimulated by $5 \times 10^{-4}$ M noradrenaline and to transmural stimulation of 0.3 msec duration with 60 V (for the pulmonary artery) or 80 V intensity (for the other three vessels) at a frequency of 20/sec for 10 sec were also compared. Contractile responses to transmural stimulation were obtained after 15 to 20 min exposure to adrenergic and cholinergic antagonists. The results shown in the text, figures and tables are expressed as mean values ± standard errors of the means.

From 4 rabbits sacrificed by exsanguination the ascending and thoracic aortae and the main pulmonary and superior mesenteric arteries were removed, frozen in liquid nitrogen-cooled isopentane, freeze-dried and exposed to formaldehyde vapour at 80°C for 1 hr following the fluorescence technique introduced by Hillarp and Falck. After the tissue was embedded in paraffin wax, sections were transversely cut at 8 to 10 μm and examined with the Leitz fluorescence microscope with a BG 12 3 mm filter in the exciting light pathway, a Leitz 51 secondary filter and a high-power dark field condenser. Ten sections were obtained every 200 to 300 μm intervals from all tissue blocks. The fluorescence intensity of one transversely-cut section was estimated semiquantitatively.

Drugs used include dl-noradrenaline hydrochloride, tyramine hydrochloride, dopamine hydrochloride, atropine sulfate, phenoxymethylamine hydrochloride, tolazoline hydrochloride, dl-propranolol hydrochloride, bretylium tosylate (Wellcome Foundation Ltd.) and tetrodotoxin (Sankyo Pharmaceutical Co.).

RESULTS

Effects of changes in individual parameters of transmural stimulation on contractile responses

Spirally-cut strips of the pulmonary artery, ascending aorta and thoracic aorta were stimulated electrically through bipolar platinum electrode. Mean values of the contractile tension developed by the transmural electrical stimulation under different stimulus conditions are illustrated in Fig. 1. Threshold intensity of the stimulation was approx. 10 V in these arterial strips. Tension was increased with increasing intensity until a plateau was attained at 40 V in the pulmonary artery and at 60 V in both aortae. Shortening of the pulse duration to 0.1 msec markedly reduced the tension (upper right in Fig. 1). Tension increment of the
Fig. 1. Tension developed by transmural stimulation applied under different stimulus conditions. The tension increment induced by stimulation of 0.3 msec duration, with 60 V (for the pulmonary artery) or 80 V intensity (for both aortae) at a frequency of 20/sec for 10 sec was taken as 100%. Each point represents the mean value obtained from 7 to 17 preparations. Vertical bars represent standard errors of the means.

Fig. 2. Contractions induced by transmural stimulation. Upper recording: parameters measured of contraction of the pulmonary artery. Lower: contractions of the pulmonary artery (P), ascending aorta (AA) and thoracic aorta (TA). The horizontal bar just under the tracings represents the application of stimulation.
arterial strips was directly related to stimulation frequency and stimulation period (lower right and left in Fig. 1). No remarkable difference in the contraction of the three kinds of strips was observed under variations in the pulse duration, stimulation frequency and stimulation period.

Analysis of components of contractile responses to transmural stimulation

Contractile responses of the pulmonary arterial strips to electrical stimulation and variables of the response measured are illustrated in Fig. 2 (upper part). The duration of the contraction at the level of one-half the maximum tension was termed "duration". The "time to peak" represents the interval between the onset of stimulation and the top of the contraction. Differences in actual contractile response of three vessels are presented in Fig. 2 (lower part). Mean values of the variables of the contraction induced by stimulation of 0.3 msec duration with 60 V (for pulmonary artery) or 80 V intensity (for both aortae)
at 20/sec for a period of 10 sec are illustrated in Fig. 3. The tension developed was significantly greater in strips of the pulmonary artery and the ascending aorta than in those of the thoracic aorta (P<0.05), whereas the “duration” and the “time to peak” were in the order, thoracic aorta > ascending aorta > pulmonary artery.

In the arterial and aortic strips transmural stimulation applied at 5/sec caused a slow but steady rise in the tension until the stimulation was terminated, whereas stimulation at 100/sec caused a rapid rise, the maximum tension being attained 1 to 3 sec after termination of the stimulation. The latency from the onset of stimulation to the initiation of contraction varied inversely with stimulation frequency. The contractile response to stimulation with the same number of pulses (200 pulses) at different frequencies was compared (Fig. 4). In pulmonary arterial strips the duration of the contraction varied inversely with stimulation frequency. The tension developed at 5/sec was significantly less than that at 20 and 100/sec (P<0.05). In strips of the ascending aorta the duration and the tension developed depended entirely on stimulation frequency, although the tension increment induced at 20 and 100/sec did not appreciably differ (the tension developed at 5/sec and that at 20 and 100/sec significantly differ, P<0.01). In strips of the thoracic aorta no appreciable difference in the tension and the duration was observed at the three frequencies, although in some preparations the tension developed at 5/sec was always less than that at 20 and 100/sec.

Effects of drugs on responses to transmural stimulation

Following treatment with 10⁻⁴ M phenoxybenzamine, 10⁻⁴ M tolazoline, 2×10⁻⁵ M bretylium and 10⁻⁶ M tetrodotoxin the contractile response to electrical stimulation of 0.1 and 0.3 msec duration was negligible. On the other hand, a prolongation of pulse duration to 1.0 msec potentiated considerably the contraction, which was not totally abolished even by the higher concentrations of phenoxybenzamine (5×10⁻⁶ M), bretylium (10⁻⁴ M) and tetrodotoxin (3×10⁻⁶ M).

Tension developed by the transmural stimulation was not influenced by atropine in concentrations up to 5×10⁻⁶ M. Tests were attempted to determine whether or not the nerve stimulation could release noradrenaline in concentrations sufficient to stimulate beta-adrenoceptive receptors in the wall of the pulmonary artery and the aortae. The tension of the vascular strips was increased 1.5 to 2.2 g following an elevation of K⁺ to 20 to 35 mM or the application of Ba²⁺ at 1 to 3 mM, and phenoxybenzamine at 10⁻⁶ M sufficient to block the excitatory effect of the neural stimulation was applied. Under these conditions the electrical stimulation caused neither contraction nor relaxation, whereas noradrenaline in concentrations higher than 10⁻⁴ M could induce slight relaxation (100 to 300 mg) which was antagonized by 10⁻⁶ M propranolol.

Responses of the mesenteric artery to transmural stimulation

In 7 of 14 strips of the superior mesenteric artery, electrical stimulation of the pulse duration less than 0.3 msec did not produce contraction but in the remaining 7 produced slight contraction (69 ± 15 mg, N= 7). In 6 of the 7 strips which did not respond to trans-
Fig. 5. Modification by noradrenaline of the effect of transmural stimulation applied to the mesenteric arterial strips. Horizontal bars just under the tracing represent stimulation application.

Fig. 6. Dose-response curves of noradrenaline, tyramine and dopamine in strips of the pulmonary and mesenteric arteries and the ascending and thoracic aortae. Tension developed by $5 \times 10^{-5}$ M noradrenaline was taken as 100%. Figures in parentheses indicate the number of preparations. Vertical bars represent standard errors of the means. Number of preparations for the value at $3 \times 10^{-3}$ and $1.2 \times 10^{-3}$ M tyramine: 5 pulmonary arteries, 7 ascending aortae, 8 thoracic aortae and 3 mesenteric arteries.
mural stimulation a marked tension increment was produced by stimulation following 5 to 20 min exposure to noradrenaline in concentrations of $5 \times 10^{-7}$ to $2.5 \times 10^{-6}$ M. Repeated applications of the stimulation caused a decline in the contractile response. An occurrence of the contraction by electrical stimulation after treatment with noradrenaline is shown in Fig. 5. Treatment with noradrenaline significantly increased the contractile response to transmural stimulation in 5 of 7 strips in which slight contraction was elicited by the stimulation. The mean values of the contraction prior to and following noradrenaline were $68 \pm 21$ mg (N=5) and $354 \pm 140$ mg (N=5), respectively.

*Effects of noradrenaline, tyramine and dopamine*

Noradrenaline in concentrations ranging from $2.5 \times 10^{-8}$ to $5 \times 10^{-4}$ M caused a dose-dependent increase in the tension of strips of the pulmonary and mesenteric arteries and the ascending and thoracic aortae (Fig. 6). In 7 of 18 strips of the mesenteric artery noradrenaline at $2.5 \times 10^{-6}$ to $2.5 \times 10^{-4}$ M caused an increase in the tension in association with spontaneity (Fig. 5). Similar rhythmical spontaneous contractions are seen in spiral strips of the rat aorta in response to noradrenaline (8).

A dose-dependent increase in the tension was also produced by tyramine in concentrations from $6 \times 10^{-6}$ to $6 \times 10^{-4}$ M (Fig. 6). Further increase in the concentration to $3 \times 10^{-3}$ M elicited no additional increase or a slight increase in the tension in 4 of 5 pulmonary arteries and 6 of 8 thoracic aortae but a decrease in the remaining 1 and 2, respectively. In strips of the ascending aorta and the mesenteric artery tyramine at $3 \times 10^{-3}$ M caused additional tension increment but at $1.2 \times 10^{-2}$ M caused an inhibition. The dose-response curve of dopamine was approx. parallel with that of noradrenaline (Fig. 6), dopamine being about 1/50 as potent as noradrenaline.

Absolute values of the tension increment induced by $5 \times 10^{-7}$ M noradrenaline, $5.3 \times 10^{-4}$ M dopamine and $6 \times 10^{-4}$ M tyramine were compared in strips of the arteries and aortae (Fig. 7). Noradrenaline produced the most marked contraction in the ascending aorta. Dopamine action was similar in the three vessels used. The effects of tyramine in these vessels did not significantly differ. In these strips, the tyramine action was re-evaluated after 10 to 15 min exposure to noradrenaline ($10^{-7}$ or $5 \times 10^{-7}$ M) or dopamine ($5.3 \times 10^{-5}$ M). Tyramine was applied after the tension developed by noradrenaline or dopamine had been stabilized. The results are summarized in Fig. 8. Treatment with the amines caused a significant potentiation of the tyramine action. On the other hand, tyramine action was not potentiated following treatment with noradrenaline of strips of the thoracic aorta and the pulmonary artery.

Concentrations of noradrenaline required to produce contraction equivalent to that in response to tyramine and transmural stimulation are shown in Table 1. The potency of tyramine relative to noradrenaline was in the following order, pulmonary artery > thoracic aorta = mesenteric artery > ascending aorta, whereas that of transmural stimulation was in the order of pulmonary artery > ascending aorta > thoracic aorta > mesenteric artery.
FIG. 7. Tension developed by noradrenaline, tyramine and dopamine in different vascular strips. Number of preparations used: same as shown in Fig. 6. The value of the ascending aorta in response to noradrenaline is significantly different from those of the pulmonary artery and the thoracic aorta (P<0.01).

FIG. 8. Modification by noradrenaline and dopamine of the excitatory effect of tyramine in strips of the mesenteric artery. Tyramine was applied after 10 to 15 min exposure to noradrenaline. Tension developed by 6×10^-4 M tyramine in control solutions was taken as 100%. Figures in parentheses indicate the number of preparations.

TABLE 1. Contractile responses of different vascular strips to transmural stimulation and tyramine demonstrated as concentrations of noradrenaline equipotent to the electrical and chemical stimulation.

|                  | Pulmonary artery | Ascending aorta | Thoracic aorta | Mesenteric artery |
|------------------|------------------|-----------------|----------------|--------------------|
| **Transmural**   |                  |                 |                |                    |
| stimulation      | 5/sec, 40 sec*   | 2.8±0.8(7)      | —              | —                  |
|                  | 20/sec, 10 sec   | 7.0±1.8(8)      | 4.2±1.0(5)     | 3.4±1.7(6)         |
|                  | 100/sec, 2 sec   | 6.2±1.9(8)      | 5.0±1.9(5)     | 3.4±1.5(6)         |
| **Tyramine**     |                  |                 |                |                    |
|                  | 3.0×10^-3 M      | 6.4±1.7(6)      | 6.6±1.9(10)    | 2.4±1.1(4)         |
|                  | 1.2×10^-4 M      | 37±11(6)        | 3.0±0.5(10)    | 11±2.6(10)         |
|                  | 6.0×10^-4 M      | 90±24(6)        | 21±2.5(10)     | 18±4.4(10)         |

A train of pulses of 0.3 msec duration with 60 V (for the pulmonary artery) or 80 V intensity (for both aortae) were used for transmural stimulation. * Frequency of stimulation and stimulation period. Figures in parentheses represent the number of preparations.
Histochemical studies

Ascending aortae were characterized by linear fluorescence along elastic fibers of the outer layer of the media and in the adventitia. In pulmonary arteries, dispersed fluorescence spots were observed in the outer layer of the media and in the adventitia. Fluorescence networks were observed mainly in the adventitia of mesenteric arteries.

Fluorescence intensity of the ascending and thoracic aortae and the main pulmonary and superior mesenteric arteries was compared. The intensity was arbitrarily expressed as (-), (±), (+), (+ +), (+ + +), (+ + + +) and (+ + + + +), depending on the number of groups of fluorescence spots and lines or networks. Mean values of the intensity expressed as means of the number of ‘plus’; (±) was evaluated as 1/2 (+). Fluorescence intensity was in the following order, ascending aorta > pulmonary artery > mesenteric artery > thoracic aorta (Table 2).

| Rabbit No. | Ascending aorta | Pulmonary artery | Mesenteric artery | Thoracic aorta |
|------------|-----------------|------------------|-------------------|----------------|
| 1          | 2.0             | 0.6              | 1.6               | 0              |
| 2          | 1.8             | 2.5              | 0.6               | 0.5            |
| 3          | 6.0             | 5.0              | 2.5               | 0.6            |
| 4          | 1.7             | 0.6              | 3.3               | 0              |
| Mean       | 2.6±0.79        | 2.2±1.00         | 2.0±0.58          | 0.3±0.16       |

Figures in the table represent the number of (+). Each figure represents the mean value of the scores obtained from ten sections cut transversely.

DISCUSSION

Results obtained in rabbit aortae and pulmonary arteries were consistent with findings seen in aortic strips, car vessels and portal veins from rabbits (2,4,8,9) in that the contractile response to electrical stimulation applied with short pulse duration was eliminated by phenoxycyanazine, tolazoline, bretylium and tetrodotoxin. The response appears to have some relation to the adrenergic excitatory mechanism.

The excitatory effect of transmural stimulation was not altered by atropine in concentrations sufficient to block cardiac slowing induced by transmural cholinergic stimulation at the S-A node. Following treatment with phenoxycyanazine or tolazoline the contractile response never reversed to a relaxation as would be expected from stimulation of adrenergic inhibitory or cholinergic receptors in the presence of pharmacologically-induced suppression of the adrenergic excitatory mechanism. It appears that transmural stimulation does not cause a mobilization of vascular amines in an amount sufficient to excite beta-adrenoceptive and choloineceptive receptors in strips of these large vessels placed under the experimental conditions.

Susceptibility to transmural stimulation of strips of different large vessels used in the
present study differed. The main pulmonary artery, ascending aorta, thoracic aorta and superior mesenteric artery were in the descending order of the susceptibility. As far as the large vessels utilized here are concerned, the further the distance from the heart, the less was the contractile response to transmural stimulation. This may reflect geographical differences in the amount of functional (or easily-released) noradrenaline included in the vascular wall. Histochemical study proved that a stronger fluorescence of catecholamines was detected in the ascending aorta and pulmonary artery than in the thoracic aorta. However, fluorescence comparable to that in the pulmonary artery could be seen in the superior mesenteric artery in which only slight contractions or none at all could be elicited following excitation of intramural nerve terminals. Differences between the data obtained from physiological and histochemical studies on mesenteric arteries may be due to differences in localization of the amine fluorescence; in the mesenteric artery most of the fluorescence locates in the adventitia, whereas in the other three the fluorescence locates in the media as well as in the adventitia. Further, histochemically-detected catecholamines do not represent the amount of the amines functioning when the nerve was excited electrically. Exposure of mesenteric arterial strips to noradrenaline markedly potentiated the contractile response to transmural stimulation. This declined with repeated application of the stimulation. Similar potentiation of the response to tyramine was induced in preparations exposed to noradrenaline or dopamine. The ability of arterial smooth muscle to accumulate and retain noradrenaline has been demonstrated (7). Thus, it could be postulated that a noradrenaline which had accumulated in the preparations was easily mobilized to excite adrenoceptive receptors, whereas catecholamines detectable as fluorescence in the wall of the mesenteric artery placed under the experimental conditions was not easily released by the electrical and chemical stimulation. On the other hand, strips of the pulmonary artery and the thoracic aorta responded well to transmural stimulation and tyramine, but the response was not potentiated by treatment with noradrenaline.

Results comparing the action of tyramine relative to that of noradrenaline in different vascular strips (Table 1) indicated that the order of susceptibility of the strips to tyramine differed from that of transmural stimulation. The difference of the susceptibility could be associated with different noradrenaline pools, one containing the amine mobilized by tyramine and the other accessible to electrical excitation (5).

SUMMARY

1. Contractile response of spiral strips of the ascending and thoracic aortae and the main pulmonary artery to transmural stimulation of the pulse duration shorter than 0.3 msec (60 or 80 V intensity, frequencies up to 100/sec) was almost totally abolished by phenoxybenzamine, tolazoline, bretylium or tetrodotoxin, whereas in stimulation of a longer duration abolishment was not complete.

2. Maximum tension increment induced by transmural neural stimulation was in the order, pulmonary artery=ascending aorta>thoracic aorta, whereas the duration of contraction at the level of half maximum tension and the time to the maximum tension were
in the order, thoracic aorta>ascending aorta>pulmonary artery. In strips of the superior mesenteric artery transmural stimulation caused no or only slight contractions. After treatment with noradrenaline the excitatory effect of the stimulation was potentiated.

3. Vascular strips were always less susceptible to tyramine than noradrenaline. In mesenteric arterial strips the effect of tyramine was potentiated after treatment with noradrenaline or dopamine. Dose-response curves of dopamine and noradrenaline were nearly parallel, although potency of dopamine was about 1/50 that of noradrenaline.

4. Intensity of catecholamine fluorescence was in the order of ascending aorta>pulmonary artery>mesenteric artery>thoracic aorta.

5. It appears that there are geographical differences in the amount of functional noradrenaline involved in the vascular wall; the further the distance from the heart, the less is functional noradrenaline in large vessels.

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