The direct inhibitory action of phentolamine on the contraction of rabbit basilar artery

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Summary

The effects of phentolamine on rabbit basilar artery were studied and compared with those on other smooth muscles.

The drug showed a dose-dependent depressing effect on high-potassium-induced contracture of rabbit basilar artery. The depressing effect was stronger on tonic than on phasic contraction. When phentolamine was applied during high-potassium-induced tonic contraction, dose-dependent relaxation was observed. The degree of phentolamine-induced-relaxation of high-potassium-induced tonic contraction was not affected by surgical denervation of the basilar artery, although catecholamine content was almost completely lost, as indicated by chemical and histochemical experiments. The relaxed tension caused by phentolamine was reversed by addition of Ca in a dose-dependent manner.

Verapamil also showed a dose-dependent relaxing effect on high-potassium-induced contracture, with this relaxed tension being reversed by the addition of Ca in a dose-dependent manner.

The relaxing effect of phentolamine on rabbit basilar artery was stronger than on rabbit aorta or guinea-pig taenia coli.

Phentolamine depressed serotonin- or histamine-induced contraction at a concentration more than 10 times higher than the concentration for depression of noradrenaline-induced contraction.

The depression of high-potassium-induced contracture and reversal by additional Ca, especially in denervated preparations, indicates that phentolamine acts directly on smooth muscle in a similar manner to that of Ca antagonists.

Introduction

Much work on cerebral angiospasm has been done. Among such work, one of the attempts to control experimental or clinical spasms has been the use of α -adrenergic blocking agents (Takamatsu *et al*, 1972; Flamm, Yasargil and Ransohoff, 1972; Smigiel and Sundt,

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1974; Walter, Neil-Dwyer and Parsons, 1980). In the cerebral vessels, adrenergic and cholinergic innervation has been demonstrated (Peerless and Yasargil, 1971; Peerless and Kendall, 1972; Edvinsson and Mackenzie, 1977). It has also been proven that patient with subarachnoid hemorrhage shows an increase in plasma catecholamine content (Peerless and Griffiths, 1972). These findings indicate the possibility that spasms occurring after subarachnoid hemorrhage may be related to elevated sympathetic activity or to increased vascular sensitivity to a sympathetic drive in association with other vaso-active agents (Walter, Neil-Dwyer and Parsons, 1980; Sohma, Saito and Sunano, 1981).

Phentolamine, a well known α -adrenergic blocking agent, possesses a releasing effect on cerebral blood vessels from vasospasm (Flamm, Yasargil and Ransohoff, 1972). It however, seems difficult to explain this effect by its α -adrenergic blocking action alone, since it has not been blocked by treatment with phenoxybenzamine (Flamm, Yasargil and Ransohoff; 1972). Phentolamine has also been shown to have an inhibitory action on high-potassium-induced contracture of rabbit basilar artery (Takayama, Sohma and Sunano; 1980). This result suggests that the action of phentolamine is independent of its α -adrenergic blocking action. The direct action of phentolamine on smooth muscle has also been reported in other vasculature (Nickerson and Hollenberg, 1967; Hoshi and Fujino, 1980). Hoshi and Fujino (1980), for example, reported that phentolamine showed inhibitory action on the Ca-contracture of depolarized aorta; contracture which can be explained by the inhibition of Ca influx.

The main aim of the present study is to investigate whether phentolamine acts directly on basilar artery to counteract extracellular Ca. To achieve this aim, the experiments were performed mainly in high-potassium-contracted preparations, since such contracture is well known to be induced mainly by influxed Ca without relation to the released transmitter. Denervated preparations were also used to confirm phentolamine's direct actions.

Methods

1 *Observation of the changes in tension*

Most experiments were performed using rabbit basilar artery. Animals were anesthetized with an intravenous injection of sodium pentobarbital and were sacrificed by bleeding from the femoral arteries. The basilar artery was removed with the brain stem by the transclival approach and was then removed from the brain stem under a stereoscopic microscope. A preparation about 0.2 mm in diameter and 10 mm in length was made by a spiral cut of the artery. A spiral cut of rabbit aorta and a straight cut of guinea-pig taenia coli were also used.

The preparations were mounted in a glass organ bath of 10 ml with 200 mg of resting tension, and then were incubated in a modified Tyrode solution of the following composition: NaCl 137 mM; KCl 2.7 mM; CaCl₂ 2.0 mM; MgCl₂ 1.0 mM; NaHCO₃ 11.9 mM; NaH₂PO₄ 0.4 mM; glucose 5.6 mM; equilibrated with a gas mixture of 95% O₂ and 5% CO₂. The high-K solution used in the present experiments was made by replacing all NaCl in the modified Tyrode solution with KCl.

Developed tensions were recorded isometrically by a mechano-electronic transducer (Sanei Sokki, Tokyo) and by a pen-writing oscillograph (Sanei Sokki, Tokyo).

2 *Surgical denervation*

The rabbits were anesthetized with an intraperitoneal injection of sodium pentobarbital, and were fixed in a supine position on the operating table. Bilateral superior cervical sympathectomy was carried out under an operating microscope. The animals were used for the experiments seven or fourteen days after surgery.

3 *Localization of catecholamines by fluorescence microscopy*

Specimens of rabbit basilar artery were immersed in 2% glyoxylic acid with 100 mM of phosphate buffer (pH 7.0) immediately after dissection. After immersion in this solution for about 30 minutes, they were spread on a glass microscope slide, dried, heated at 100°C for five minutes and then covered by a cover glass with entellan. A photograph was taken with a fluorescence microscope (Nikon Fluophot) (Axelsson *et al*, 1973).

4 *Measurement of catecholamines*

The entire rabbit basilar artery was dissected from the brain stem and blood was carefully removed. After weighing, the tissue was homogenized in 0.1 N perchloric acid containing 0.2% EDTA and 0.2% sodium metabisulfite at 0°C. Extracts were filtered (MILLEX, 0.22 μ m filter unit), and filtrates were purified by high speed liquid chromatography (HLC condition: precolumn 4.0 $\phi \times 10$ mm; main column 4.6 $\phi \times 250$ mm; resin NUCLEOSIL $_5C_{18}$ 5 μ m; fluent 0.2 M sodium phosphate pH 3.0; flow rate 0.8 ml/min). Noradrenaline and adrenaline were measured by the trihydroxyindole post labeled method (Sato and Suzuki, 1979).

Results

1 *Effects of phentolamine on high-K-induced contracture of rabbit basilar artery*

Fig. 1 shows high-K-induced contracture of basilar artery in the absence and presence of phentolamine. As shown (Fig. 1A control), the development of tension by high-K consisted of transient phasic and sustained tonic components. The tonic component attained its peak tension 40–60 minutes after the administration of high-K solution and thereafter was sustained without obvious decrease in tension. Phentolamine of 10^{-4} M caused depression of both phasic and tonic contractions; the tonic contraction being more strongly suppressed (Fig. 1A phentolamine). This concentration of the drug was chosen, since it was nearly ED 50 for depression of the phasic contraction. The height of the phasic contraction observed in the presence of phentolamine was $48.0 \pm 4.7\%$ ($M \pm SE$, $n=9$), while that of the tonic contraction measured when it achieved its peak was $20.5 \pm 4.2\%$ ($M \pm SE$, $n=9$) of the control observed in the absence of the drug (Fig. 1B).

When phentolamine was applied in the course of the tonic contraction, relaxation of the preparation was observed in a dose dependent manner (Fig. 2A). The threshold concentration for the relaxation was 5×10^{-7} M and maximal relaxation was observed by the application of 10^{-3} M (Fig. 2B). The tension in the presence of 10^{-3} M of phentolamine was $3.0 \pm 1.6\%$ ($M \pm SE$, $n=10$) of that observed in the absence of phentolamine (control). Thus, about 97.0% relaxation was observed by 10^{-3} M of phentolamine. By washing out phentolamine with K-Tyrode solution, recovery of the tonic tension was observed. The recovery was, however,

Fig. 1 Effect of phentolamine on high-K-induced contracture.

A: High-K-induced contracture in the absence (control) and presence (phentolamine) of phentolamine (10^{-4} M).

B: Changes in the height of phasic and tonic contractions. Each height in the absence of phentolamine was taken as 100% (white column) and those in the presence of phentolamine were expressed as percentages of the control (hatched column). Vertical lines in each column indicate $\pm$ SE ($n=9$).

Fig. 2 Relaxing effect of phentolamine on the tonic contraction of high-K-induced contracture of rabbit basilar artery (A) and its dose-response curve (B). The height of the tonic contraction in the absence of phentolamine was taken as 100% in B. Vertical lines indicate $\pm$ SE ($n=10$).

very slow and complete recovery was not observed until 30 minutes after washing out of the drug.

2 Surgical denervation

Since the possibility of involvement of intrinsic noradrenaline in high-potassium-induced contratture has been reported in vascular smooth muscle (Golenhofen, Hermstein and Lammel, 1933; Mikkelsen, Andersson and Bengtsson, 1978; Bengtsson, 1978), the influence of denervation on the action of phentolamine was studied. As shown in Fig. 3, a fluorescent micrograph showed a decrease or disappearance of catecholamine from nerve fibers and nerve terminals seven to fourteen days after surgical denervation (in all 20 animals examined). These changes were more prominent on the distal side of the basilar artery than on either the proximal side of the basilar artery or vertebral artery.

Noradrenaline content measured by the thrihydroxyindole post labeled method was also decreased markedly by surgical denervation (Fig. 4). The noradrenaline content of non

Fig. 3 Catecholamine fluorescence micrograph of rabbit basilar artery ($250\times$).
Upper: innervated basilar artery (control)
Lower: fourteen days after bilateral superior cervical sympathectomy

Fig. 4 Change in noradrenaline (NA) content of rabbit basilar artery 1 week (1 W) and 2 weeks (2 W) after surgical denervation. Each column indicates mean values of six preparations with $\pm$ SE.

-treated basilar artery was 2000.7 ± 183.2 ng/g wet tissue ($M \pm SE$, $n=11$), while the adrenaline content was 22.0 ± 6.1 ng/g wet tissue ($M \pm SE$, $n=11$). Seven days after bilateral cervical sympathectomy the noradrenaline content had fallen to 140.5 ± 71.9 ng/g wet tissue ($M \pm SE$, $n=6$, $P < 0.001$) and, fourteen days after, to 137.8 ± 49.8 ng/g wet tissue ($M \pm SE$, $n=6$, $p < 0.001$). The difference in the noradrenaline content after seven days and fourteen days was not statistically significant.

On the other hand, no significant difference in the adrenaline content was observed by surgical denervation. It was less than 20.0 ± 4.3 ng/g wet tissue ($M \pm SE$, $n=6$) seven days after denervation, and less than 19.5 ± 3.3 ng/g wet tissue ($M \pm SE$, $n=6$) fourteen days after.

In spite of the marked reduction in noradrenaline content, the relaxing effect of phentolamine was not affected by surgical denervation. As shown in Fig. 5, the degree of relaxation of the potassium-induced tonic contraction of the denervated preparation caused by 10^{-5} M and 5×10^{-5} M of phentolamine was almost the same as that of the innervated

Fig. 5 Relaxing effect of phentolamine in innervated (A), and denervated basilar artery (B).

Fig. 6 Changes in the height of the high-K-induced tonic contractions of rabbit basilar artery, aorta, and guinea pig taenia coli by treatment with 10^{-4} M phentolamine. The heights of the tonic contractions of each muscle were taken as 100% (white column) and those in the presence of phentolamine were expressed as percentages of the control (hatched column). $M \pm SE$, $n=10$, 10 and 7, respectively.

preparation.

3 Comparison of the relaxing effect of phentolamine with other smooth muscles

To investigate whether the relaxing action of phentolamine is related to α -adrenergic receptors, the effect of phentolamine on rabbit basilar artery was compared with the effect on aorta and taenia coli, which have been known to be stimulated respectively by α -adrenergic agents and cholinergic agents.

Fig. 6 shows the change in the tonic contraction of basilar artery, aorta of the rabbit and taenia coli of the guinea-pig by 10^{-4} M of phentolamine. As shown in this figure, phentolamine showed a strong depressing effect on the basilar artery, while the rabbit aorta and guinea-pig taenia coli were relatively insensitive to the drug. The heights of the tonic contractions of the

Fig. 7A Reversal of phentolamine-induced relaxation by increasing extracellular Ca concentrations. Dots indicate the increases in Ca concentration (from left to right, 3, 4, 5, 7, 9, 11, 13 mM of Ca, as final concentrations).

7B Dose-response curve for the reversal by Ca. Vertical lines at each point indicate $\pm$ SE ($n=6$).

basilar artery, aorta and taenia coli by the addition of 10^{-4} M of phentolamine were $15.5 \pm 3.1\%$ ($M \pm SE$, $n=10$), $43.7 \pm 3.5\%$ ($M \pm SE$, $n=10$), $58.5 \pm 3.1\%$ ($M \pm SE$, $n=7$) of those of the control.

4 Reversal of the relaxed tension by Ca

To study if the relaxing action was due to Ca-antagonistic action, the effects of changing extracellular Ca in the phentolamine-induced relaxation were studied.

Fig. 7A shows the effects of the increase in Ca concentration on the reduced tension in the presence of phentolamine. In this experiment, phentolamine of 10^{-4} M was applied when the tonic contraction attained its maximum and Ca was cumulatively added after the maximum relaxation had been achieved. As shown in this figure, the addition of Ca caused the reversal of the relaxed tension which increased with the increase in the added Ca concentration. Thus, a recovery up to $74.0 \pm 5.0\%$ ($M \pm SE$, $n=6$) of the control tension was observed with the addition of 9 mM of Ca (Fig. 7B). However, no further increase in tension was observed with further the increases in Ca concentration. Thus, complete recovery was not obtained by the addition of Ca in the presence of 10^{-4} M of phentolamine. When the relaxation induced a lower concentration of phentolamine, complete recovery was observed with a lower concentration of Ca.

5 Relaxation of high-K-induced tonic contraction by verapamil and reversal by Ca

Since Ca-antagonistic phentolamine action was suggested in the experiments described above, the effects were compared with those of verapamil.

A similar relaxing effect on the high-K-induced tonic contraction was observed by the

Fig. 8 Dose-response curve for verapamil-induced relaxation in high-K-induced tonic contractions of rabbit basilar artery. Each point indicates the mean value of 9 experiments with $\pm SE$.

Fig. 9 Dose-response curve for the effect of phentolamine on histamine-induced contraction. Histamine (5×10^{-5} M)-induced contraction in the absence of phentolamine was taken as 100% and those in the presence of various concentrations of phentolamine were expressed as percentages of the control. Each point indicates the mean value of 8 preparations with $\pm SE$.

application of verapamil. As shown in Fig. 8, the threshold concentration for the relaxation was 5×10^{-10} M, and maximum relaxation was achieved by 5×10^{-5} M of the drug, it being $8.2 \pm 2.9\%$ ($M \pm SE$, $n=9$) of the control tonic contraction height.

The relaxation was also reversed by increasing the Ca concentration; the relaxation by 5×10^{-7} M of verapamil was reversed to $87.6 \pm 5.1\%$ ($M \pm SE$, $n=6$) by the addition of 9 mM of Ca.

6 *Effects of phentolamine on drug-induced contractions*

The effects of phentolamine on drug-induced contractions were also studied.

Phentolamine of high concentration depressed the contractions by serotonin, histamine and, of course, by noradrenaline. Fig. 9 shows the dose-response curve for phentolamine on histamine-induced contraction. As shown in this figure, the threshold concentration for the depression was 10^{-6} M and maximal depression was achieved at 5×10^{-4} M. In a similar manner, contractions induced by 5×10^{-6} M of serotonin and by 5×10^{-5} M of noradrenaline were blocked completely by 5×10^{-4} M, and 10^{-5} M of phentolamine, respectively.

Discussion

Phentolamine showed an inhibitory action on high-K-induced contracture of the basilar artery. Since noradrenaline release by a high concentration of potassium has been reported (Lorenz and Vanhoutte, 1975; Vanhoutte and Verbeuren, 1976; Borda, Schuleib and Henry, 1977; Vanhoutte, Verbeuren and Webb, 1981), it was felt that the possibility that depression of the contracture by phentolamine might be brought about through its α -adrenergic blocking action should be taken into consideration. The possibility that phentolamine depresses high-K-induced contracture through its α -adrenergic blocking action has also been reported in other types of vascular smooth muscle (Vanhoutte and Verbeuren, 1976; Vanhoutte, Verbeuren and Webb, 1981; Sunano, 1981). In addition, it has been reported that a high concentration of phentolamine could block excitatory junctional potentials of the basilar artery (Karashima and Kuriyama, 1981). For these reasons, the possibility that phentolamine of the concentration used in the present experiments could block the action of released noradrenaline and cause relaxation of high-K-induced contracture of basilar artery was taken into consideration.

This possibility, however, was excluded by our experiment with denervated preparations. Catecholamine fluorescence could not be detected clearly seven to fourteen days after the denervation, and noradrenaline content had decreased to about 7% of the control. Such treatment has also been known to block the contractions of rabbit basilar artery induced by stimulation of intrinsic nerves, while not affecting contractions due to direct stimulation of smooth muscle (Lee, Su and Bevan, 1976). Nevertheless, phentolamine showed the same inhibiting or relaxing effect on the potassium-induced tonic contraction of the denervated preparations. These results indicated that the relaxation by phentolamine was not brought about through the α -adrenergic blocking action of the drug. The depressing effect shown by phentolamine on taenia coli indicates relaxation without any relation to transmitter release, since, in this muscle, noradrenaline has been shown to be an inhibitory transmitter and blockade of this transmission should cause the potentiation of the contraction (Bolton, 1979). The fact

that aortic smooth muscles were relatively more insensitive than basilar artery to phentolamine also indicates the relaxing action of the drug independent of released noradrenaline, since basilar artery has been shown to be relatively more insensitive than aorta to noradrenaline (Toda and Fujita, 1973; Allen *et al*, 1974; Edvinsson and Owman, 1974; Edvinsson, Owman and Sjöberg, 1976; Toda, Hayashi and Hattori, 1978).

In the present experiment, it was shown that the basilar artery was the most sensitive to phentolamine of all the smooth muscles tested, although no evidence to explain these differences was obtained. The relaxation of basilar artery therefore seems to be caused by the direct action of the drug in excitation-contraction coupling of the smooth muscle, as has been reported to be true of some other vascular smooth muscles (Nickerson and Hollenberg, 1967; Hoshi and Fujino, 1980).

High-potassium-induced contracture of smooth muscle has been shown to be no longer or not always dependent on extracellular Ca (Van Breemen *et al*, 1973; Johnishi and Sunano, 1978). Increases in Ca influx during potassium contracture have also been reported (Sperelakis, 1962; Chujiyo and Holland, 1963; Urakawa, and Holland, 1964). Potassium-induced contracture of the basilar artery is also highly sensitive to extracellular Ca and is abolished by the removal of Ca or by treatment with Ca antagonists (Briggs, 1962; Takayama, Sohma and Sunano, 1980). It was also demonstrated in the present experiments that the potassium contracture of the basilar artery of the rabbit was blocked almost completely by verapamil. The most probable explanation for this action of phentolamine is, therefore, that phentolamine blocked Ca influx and caused the relaxation.

In cat aorta, Hoshi and Fujino (1981) reported that Ca-induced contracture of depolarized smooth muscle was depressed by phentolamine and postulated that the drug did not act through α -adrenergic blocking action but through inhibiting action on Ca utilization. In the present experiments, the relaxation of the tonic contraction by phentolamine was counteracted by increases in Ca concentration. The relaxation by verapamil was also reversed by the addition of Ca. These results seem to support the possibility that phentolamine causes a blockade of Ca influx and, thus, induces relaxation.

As has previously been reported, the tonic contraction of potassium contracture may be sustained under a condition in which the influx and efflux of Ca are equilibrated at a certain elevated level of intracellular Ca (Sunano, 1976; Johnishi and Sunano, 1978; Shimodan and Sunano, 1981). Therefore, there may also be a possibility that the drug enhances Ca efflux and thus causes the reduction in intracellular free Ca. However, the reversal of the relaxation by Ca suggests the former possibility, since it has been reported that extracellular Ca does not inhibit, but enhances Ca efflux (Lüllmann and Siegfriedt, 1968; Raeymaekers, Wuytack and Casteels, 1974; Aaronson and Van Breemen, 1981).

Phentolamine also showed inhibiting actions on contractions induced by various agents. The basilar artery of the rabbit has been shown to be relatively insensitive to noradrenaline, and a high concentration of the drug is required to initiate contraction (Duckles and Bevan, 1976). Nevertheless, a much lower concentration of phentolamine could block contraction induced by a high concentration of noradrenaline. These results may also indicate less possibility of the involvement of released noradrenaline. In addition, it should also be noted

that phentolamine of a very high concentration has been required to block excitatory junctional potential of guinea-pig basilar artery (Karashima and Kuriyama, 1981).

Histamine has been reported to cause contraction of the cerebral vessels (Edvinsson and Mackenzie, 1977; Bolton, 1979). Phentoalmine can inhibit this contraction by histamine by acting on the same receptor which may be relatively nonselective, as has been reported to be the case in other smooth muscles (Macleod *et al.*, 1980). It is also possible that histamine causes the contraction mediated by released noradrenaline, and that phentoalmine blocks the contraction induced by released noradrenaline (Lobato *et al.*, 1981).

However, in the present experiments histamine caused a much larger contraction than that induced by noradrenaline and very high concentration of phentolamine was required to block the histamine-induced contracture. These results indicate that the inhibiting action of phentolamine was not mediated by its α -adrenergic blocking action. The inhibition of serotonin-induced contraction can also be explained in a similar manner, although it is more probable that the drug acts on the same receptor as that for noradrenaline.

These inhibiting actions of phentoalmine on drug-induced contractions may also be explained by the inhibition of Ca influx, since these contractions of basilar artery have been reported to be dependent on extracellular Ca and to be blocked by Ca-antagonists (Bolton, 1979; Harder, 1981). Thus, it was shown that phentolamine had a relaxing or inhibiting action on rabbit basilar artery by acting directly on smooth muscle. These effects may be brought about by phentoalmine's Ca-blocking action.

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