THE ACTIONS OF D 600, ASPAMINOL AND PAPAVERINE ON CALCIUM-, POTASSIUM- AND HISTAMINE-INDUCED CONTRACTIONS OF ISOLATED RABBIT BASILAR ARTERY, AORTA, TAENIA COLI AND TRACHEAL SMOOTH MUSCLE

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Abstract—The effects of D 600, Aspaminol (1,1-diphenyl-3-piperidinobutanol hydrochloride) and papaverine on CaCl_2 -, KCl- and histamine-induced contractile responses of isolated rabbit basilar artery, aorta, taenia coli and tracheal smooth muscle were compared with each other. D 600 reduced CaCl_2 -induced maximal responses in basilar artery and aorta, and parallelly shifted the concentration-response curves for CaCl_2 to the right in taenia coli and trachea. D 600 also reduced histamine-induced maximal contractions in basilar artery and taenia coli. These reductions were not reversed by increasing CaCl_2 concentration in bathing fluids. High concentration of D 600 parallelly shifted the concentration-response curve for histamine to the right in aorta. Like D 600, Aspaminol, a nonspecific smooth muscle relaxant, parallelly shifted the concentration-response curve for histamine in aorta. Papaverine parallelly shifted the concentration-response curve for CaCl_2 in taenia coli and reduced the maximal responses in other tissues. Papaverine also reduced histamine-induced maximal responses in basilar artery, aorta and taenia coli. Influences of these smooth muscle relaxants on histamine-induced contraction in Ca-free buffer solution and on KCl-induced contraction in normal and high Ca (Ca: 12.5 mM) buffer solution were also studied. From the results obtained in this study, the effects of smooth muscle relaxants are considered to vary with the type of smooth muscle or the condition eliciting contraction, and the possible mechanisms of the contractions were discussed.

It is well known that activation of the contractile mechanism in smooth muscle is triggered by an increase in free Ca ions in the cytoplasm (1, 2). This contractile mechanism is considered to be achieved either by release of intracellularly sequestered (e.g., sarcoplasmic reticulum, mitochondria, plasma membrane) Ca or by the increase in influx of extracellular Ca (3–5). It is believed that all of the smooth muscle relaxants might elicit their actions by decreasing the level of activator Ca in the cytoplasm. Several

compounds which specifically block slow inward current at the cardiac plasma membrane had been designated as “Ca antagonists” by Fleckenstein et al. (6). In smooth muscles, these compounds specifically block the influx of Ca ions through a voltage sensitive Ca channel which is activated by the depolarization of plasma membrane (7, 8), and some of these compounds are used clinically in the therapy of various diseases such as hypertension, cardiac ischemia, arrhythmias, dysfunction of cerebral circulation and others (9–11). In smooth muscles, the sources of intracellular activator

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Ca or the influx pathway of extracellular Ca vary with the tissues, contracting agents or conditions. For example, K ion stimulates the influx of Ca by activating voltage sensitive Ca channel in most of the smooth muscles (7, 8, 12). Histamine causes the contraction of rabbit taenia coli mainly by increasing influx of extracellular Ca when compared with acetylcholine (13, 14). Acetylcholine, especially in high concentration, and carbachol release intracellularly sequestered Ca in guinea pig taenia coli and dog tracheal smooth muscle (15–17). In most of the vascular tissues, norepinephrine causes the contraction mainly by releasing Ca from an intracellular Ca store (12, 18, 19). Thus, it is also considered that the actions of smooth muscle relaxants vary with the differences of contractile mechanisms among smooth muscle tissues.

In this study, the actions of D 600, a Ca antagonist, on rabbit basilar artery, aorta, taenia coli and tracheal smooth muscle were investigated and were compared with the actions of nonspecific smooth muscle relaxants such as papaverine and Aspaminol.

Materials and Methods

Helically cut strips of basilar artery and aorta, strip of taenia coli and tracheal chain were prepared from male albino rabbits weighing 2–3 kg. These preparations were suspended in an organ bath containing 20 ml of Krebs buffer solution with following composition (mM): NaCl, 118; KCl, 4.5; CaCl_2 , 2.5; MgSO_4 , 1.0; KH_2PO_4 , 1.0; NaHCO_3 , 25.0; glucose, 6.0. Buffer solution was kept at $32 \pm 0.5^\circ\text{C}$ and bubbled with a mixture of 95% O_2 and 5% CO_2 in basilar artery and aorta and with air in taenia coli and trachea. Mechanical responses were measured isometrically under 0.25 g of resting tension in basilar artery and isotonically under 0.5 g load in aorta, taenia coli and trachea. All of the preparations were

allowed to equilibrate for at least 1 hr. CaCl_2 -induced contractile response was measured in high-K-Ca-free buffer solution of the following composition (mM): KCl, 122.5; MgSO_4 , 1.0; KH_2PO_4 , 1.0; NaHCO_3 , 25.0; glucose, 6.0. KCl-induced contraction was measured using solutions in which various concentrations of NaCl were replaced with KCl isosmotically. In some of the experiments, high Ca (Ca: 12.5 mM) buffer solution was used. Smooth muscle relaxants were added 5 min before the addition of contracting agents. In experiments to determine the actions of smooth muscle relaxants on contractions which are considered to occur by Ca released from an intracellular Ca store, tissues were incubated in buffer solution containing 1 mM Ca for 10 min in basilar artery, 45 min in aorta and 20 min in taenia coli, after the tissue Ca was depleted by repeated applications of histamine in Ca-free buffer solution. Thereafter, tissues were washed for 5 min with Ca-free buffer solution in the absence (control) or presence of smooth muscle relaxants following by the addition of histamine (10^{-4} M).

Drugs used: D 600 (methoxyverapamil; Knoll), Aspaminol (1,1-diphenyl-3-piperidinobutanol hydrochloride, Kowa), papaverine hydrochloride (Tokyo Kasei), histamine dihydrochloride (Wako Junyaku). Each value was presented as a mean with standard error, and statistical significance was evaluated by Student's *t*-test.

Results

Influences of D 600, Aspaminol and papaverine on CaCl_2 -induced contractile responses in isolated rabbit basilar artery, aorta, taenia coli and trachea (Fig. 1, Table 1): D 600 inhibited CaCl_2 -induced contractile responses of basilar artery and aorta in a noncompetitive manner, whereas it parallelly shifted the concentration-response curves for CaCl_2 to the right in taenia coli and

trachea. Aspaminol inhibited CaCl_2 -induced contractile response of basilar artery in a noncompetitive manner, whereas it parallelly shifted the concentration-response curves for

CaCl_2 to the right in other tissues. The inhibitory action of Aspaminol was most potent in trachea. Papaverine inhibited CaCl_2 -induced contractile responses of basilar

D 600

Aspaminol

Papaverine

Fig. 1. Concentration-response curves for CaCl_2 on isolated rabbit basilar artery, aorta, taenia coli and tracheal smooth muscle in the absence and presence of D 600 (top), Aspaminol (middle) and papaverine (bottom). The concentration of smooth muscle relaxants used are indicated by the following symbols. $\circ$: control, D 600 ($\bullet$: 10^{-8} M, $\blacktriangle$: 10^{-7} M, $\blacksquare$: 10^{-6} M), Aspaminol ($\blacktriangledown$: 3×10^{-6} M, $\bullet$: 10^{-5} M, $\blacktriangle$: 3×10^{-5} M, $\blacksquare$: 10^{-4} M), papaverine ($\blacktriangledown$: 3×10^{-6} M, $\bullet$: 10^{-5} M, $\blacktriangle$: 3×10^{-5} M, $\blacksquare$: 10^{-4} M). Each point and vertical bar represent the mean value and standard error, respectively. N: Number of experiments.

Table 1. Comparison of the antagonistic actions of D 600, Aspaminol and papaverine on CaCl_2 -induced contractions of isolated rabbit basilar artery, aorta taenia coli and tracheal smooth muscle

Drugs		Basilar artery	Aorta	Taenia coli	Trachea
D 600	(pA_2)			8.0 ± 0.1 (8)	8.3 ± 0.1 (8)
	(pD'_2)	7.4 ± 0.1 (6)	6.9 ± 0.1 (8)		
Aspaminol	(pA_2)		5.7 ± 0.1 (10)	5.1 ± 0.3 (8)	6.4 ± 0.1 (8)
	(pD'_2)	4.4 ± 0.1 (9)			
Papaverine	(pA_2)			5.4 ± 0.1 (8)	
	(pD'_2)	4.8 ± 0.1 (6)	4.1 ± 0.1 (8)		4.5 ± 0.1 (8)

Each value represents the mean $\pm$ S.E. of the number of experiments in the parenthesis.

artery, aorta and trachea in a noncompetitive manner, whereas it parallelly shifted the concentration-response curve for CaCl_2 to the right in taenia coli. Potencies of the relaxants are presented in Table 1 as pD'_2 values in the cases in which the maximal responses were reduced or pA_2 values in the cases in which the concentration-response curves were parallelly shifted to the right for convenience sake.

Influences of D 600, Aspaminol and papaverine on KCl-induced contractions in isolated rabbit basilar artery, aorta, taenia coli and trachea (Fig. 2, Table 2): D 600 reduced

maximal responses elicited by KCl in normal buffer solution in all of the tissues. The reduction was most remarkable in basilar artery. The reductions in basilar artery and aorta were not influenced by increasing concentration of CaCl_2 in bathing fluids, whereas the reductions in taenia coli and trachea were significantly reversed by increasing the concentration of CaCl_2 in bathing fluids. Aspaminol reduced maximal responses elicited by KCl in the same magnitude in all of the tissues. The reduction in basilar artery was not influenced by increasing the concentration of CaCl_2 in

D 600

Aspaminol

Papaverine

Fig. 2. Influence of increasing concentration of external Ca on the inhibitory actions of D 600 (top), Aspaminol (middle) and papaverine (bottom) to isosmotic KCl-induced contractions of isolated rabbit basilar artery, aorta, taenia coli and tracheal smooth muscle. The concentration of smooth muscle relaxant is shown in each figure. $\circ$: control, $\bullet$: treated. Solid lines indicate the results in normal buffer solution (Ca: 2.5 mM), and dotted lines indicate the results in high Ca buffer solution (Ca: 12.5 mM). Each point and vertical bar represent the mean value and standard error, respectively. N: Number of experiments.

Table 2. Influence of increasing external Ca concentration on the noncompetitive antagonistic activities of D 600, Aspaminol and papaverine to isosmotic KCl-induced contractions of isolated rabbit basilar artery, aorta, taenia coli and tracheal smooth muscle

Drugs		Basilar artery	Aorta	Taenia coli	Trachea
D 600	(Normal)	7.4±0.2 (5)	6.1±0.1 (8)	6.4±0.1 (5)	6.3±0.1 (8)
	(High Ca)	7.5±0.1 (5)	5.8±0.1 (8)	5.8±0.1** (5)	5.6±0.1** (8)
Aspaminol	(Normal)	4.9±0.1 (6)	5.0±0.02 (8)	4.9±0.1 (8)	5.0±0.02 (5)
	(High Ca)	4.8±0.1 (6)	4.0±0.2** (8)	4.4±0.1* (8)	4.0±0.1** (5)
Papaverine	(Normal)	4.9±0.1 (4)	3.9±0.1 (5)	4.5±0.1 (6)	4.7±0.2 (5)
	(High Ca)	4.8±0.1 (4)	4.1±0.1 (5)	3.7±0.2** (6)	4.5±0.2 (5)

Antagonistic activities are represented by pD'_2 values. Each value represents the mean±S.E. of the number of experiments in the parenthesis. *, **: Significantly different from the value in normal buffer solution ($P<0.05$, $P<0.01$, respectively).

bathing fluid, whereas the reductions in aorta, taenia coli and trachea were significantly reversed by increasing the concentration of $CaCl_2$ in bathing fluids. Papaverine also reduced maximal responses elicited by KCl in all of the tissues. The reductions in basilar artery, aorta and taenia coli were not influenced by increasing the concentration of $CaCl_2$ in bathing fluids, whereas the reduction in taenia coli was significantly reversed by increasing the concentration of $CaCl_2$ in bathing fluid.

Influences of D 600, Aspaminol and papaverine on histamine-induced contractions in isolated rabbit basilar artery, aorta and taenia coli (Figs. 3 and 4, Table 3): D 600 inhibited histamine-induced maximal responses of basilar artery, aorta and taenia coli in normal buffer solution. The order of potency of the inhibition was in taenia coli > basilar artery > aorta. In aorta, D 600 (10^{-5} – 10^{-4} M) shifted the concentration-response curve for histamine to the right with slight reduction of maximal response. The reductions of maximal responses in basilar artery and taenia coli were not reversed, but that in aorta was only slightly reversed by increasing concentration of $CaCl_2$ in bathing fluids. Aspaminol reduced histamine-induced maximal contractions in basilar artery and taenia coli. In aorta, Aspaminol shifted the concentration-response curve for histamine

to the right without reduction of maximal response. The reduction of the maximal response to histamine by Aspaminol was significantly reversed by increasing the concentration of $CaCl_2$ in bathing fluid in taenia coli, but not in basilar artery. Papaverine reduced histamine-induced maximal responses of basilar artery, aorta and taenia coli in normal buffer solution. These reductions were not influenced by increasing the concentration of $CaCl_2$ in bathing fluids. Figure 4 shows the influences of D 600, Aspaminol and papaverine on histamine-induced contractions of basilar artery, aorta and taenia coli in Ca-free buffer solution. Inhibitory activities of all the relaxants on histamine-induced contraction in Ca-free and normal buffer solution were almost the same as in basilar artery and aorta, but were not the same in taenia coli.

Tracheal smooth muscle did not contract in response histamine in Ca-free as well as normal buffer solution.

Discussion

As mentioned above, the actions of smooth muscle relaxants are considered to vary with the differences of tissues, conditions during the development of contraction and other factors (20, 21). In this study, the effects of D 600, Aspaminol and papaverine on contractile responses elicited

D 600

Aspaminol

Papaverine

Fig. 3. Influence of increasing concentration of external Ca on the inhibitory actions of D 600 (top), Aspaminol (middle) and papaverine (bottom) to histamine-induced contractions of isolated rabbit basilar artery, aorta and taenia coli. The concentration of smooth muscle relaxant used is shown in each figure. ○: control, ●: treated. Solid lines indicate the results in normal buffer solution (Ca: 2.5 mM), and dotted lines indicate the results in high Ca buffer solution (Ca: 12.5 mM). Each point and vertical bar represent the mean value and standard error, respectively. N: Number of experiments.

Fig. 4. Influences of D 600 (D), Aspaminol (A) and papaverine (P) on histamine (10⁻⁴ M)-induced contractions of isolated rabbit basilar artery, aorta and taenia coli after the external Ca was removed. See "Methods" for details. Control responses (C) are considered as 100%. Each value in parenthesis represents the number of experiments. Vertical bar represents the standard error.

Table 3. Influence of increasing external Ca concentration on the antagonistic activities of D 600, Aspaminol and papaverine to histamine-induced contractions of isolated rabbit basilar artery, aorta and taenia coli

Drugs		Basilar artery	Aorta	Taenia coli
D 600	(Normal)	4.5±0.2 (5)	3.4±0.1 (6)	6.1±0.2 (8)
	(High Ca)	4.2±0.1 (5)	<3.0§ (6)	5.8±0.2 (8)
Aspaminol	(Normal)	4.1±0.2 (4)	<2.5§ (4)	4.6±0.04 (5)
	(High Ca)	3.9±0.1 (4)	<2.5§ (4)	3.6±0.3** (5)
Papaverine	(Normal)	4.6±0.2 (5)	4.4±0.1 (8)	4.3±0.3 (5)
	(High Ca)	4.7±0.1 (5)	4.1±0.1 (8)	4.0±0.2 (5)

Antagonistic activities are represented by pD'₂ values. Each value represents the mean±S.E. of the number of experiments in the parenthesis. **: Significantly different from the value in normal buffer solution ($P<0.01$). §: The concentration-response curves for histamine shifted to the right by D 600 and Aspaminol.

by CaCl_2 in high-K depolarizing solution. KCl and histamine in both normal and high Ca buffer solution were compared with each other.

D 600, a Ca antagonist, inhibited CaCl_2 -induced contractile responses of the tissues used in this study. These results were confirmed by the experiments using KCl as an agonist in which D 600 reduced KCl-induced maximal responses in all of the tissues, and the reductions in taenia coli and trachea were significantly reversed by increasing external CaCl_2 concentration, but those in basilar artery and aorta were not reversed. These results suggest that D 600 might block the influx of extracellular Ca competitively in taenia coli and trachea and noncompetitively in basilar artery and aorta at the voltage sensitive Ca channel which is activated by KCl-induced membrane depolarization (7, 8). Schümann et al. (22) and van Breemen et al. (23) have reported similar findings that Ca antagonists such as nifedipine, diltiazem and others show non-competitive antagonism against CaCl_2 -induced contractile responses in vascular tissues. Van Breemen et al. (23) have further described that diltiazem inhibits Ca influx by interacting with the Ca pathway involved in excitation rather than competing with Ca for the entry in rabbit aorta. D 600 inhibited

histamine-induced contractile responses non-competitively in basilar artery and taenia coli. These results also suggest that D 600 might block influx of extracellular Ca noncompetitively in these tissues at the Ca channel activated by histamine. The fact that D 600 parallelly shifted the concentration-response curve for histamine in aorta may have something in common with the observations described by Fairhurst et al. (24) and Karliner et al. (25) that D 600 and verapamil inhibit specific ligand binding to membrane receptors; this result may have to be explained by other experimental steps such as a binding assay for histamine receptors. Tracheal smooth muscle did not contract by histamine as reported by Fleisch et al. (26). Aspaminol inhibited CaCl_2 -induced contractions in a competitive manner in aorta, taenia coli and trachea and in a noncompetitive manner in basilar artery. The result in taenia coli was consistent to that reported by Takayanagi et al. (27). These manners of inhibition were confirmed by the experiments using KCl in both normal and high Ca buffer solutions. From these results, it was suggested that Aspaminol might block influx of extracellular Ca competitively in aorta, taenia coli and trachea and noncompetitively in basilar artery at the voltage sensitive Ca channel. Furthermore, the results of the experiments using

histamine in both normal and high Ca buffer solution suggest that Aspaminol blocks influx of extracellular Ca competitively in taenia coli and noncompetitively in basilar artery. Aspaminol at a higher concentration (3×10^{-4} M) shifted the concentration-response curve for histamine slightly to the right without reduction of the maximal response. This result also may have to be explained by other experimental steps. Papaverine, a nonspecific smooth muscle relaxant, inhibited CaCl_2 -induced contractile responses in a competitive manner in taenia coli and in a noncompetitive manner in other tissues. Although the result in taenia coli is interesting and consistent with that reported by some investigators (28, 29), it is considered that further studies are necessary to clarify the reasons why papaverine showed competitive (or apparently competitive) antagonism only in taenia coli among the tissues used in this study. These results with papaverine were confirmed by the experiments using KCl in both normal and high Ca buffer solutions. The results described above indicate that there is a variety of properties in the Ca channel activated by KCl-induced membrane depolarization (so called voltage sensitive Ca channel) as well as that activated by histamine (possibly the so called receptor operated Ca channel) among different types of smooth muscle tissues. Moreover, it was indicated that the properties of the Ca channel activated by KCl-induced membrane depolarization might differ from that activated by histamine in each tissue type.

In aorta, potencies of the inhibitory effect of smooth muscle relaxants used in this study on histamine-induced contractions in normal buffer solution were similar to those in Ca-free buffer solution in which the contractions might be due to the release of Ca ions from an intracellular pool; while in taenia coli, there was no similarity, and inhibitory effects of relaxants in Ca-free buffer solution were

considerably smaller than those in normal buffer solution. Histamine is considered to cause not only membrane depolarization (12, 30–32), but also release of intracellularly sequestered Ca (12, 33) in vascular smooth muscle, whereas it causes the contraction mainly due to the influx of extracellular Ca in taenia coli (13, 14, 27). We have little information concerning agents which inhibit the release of intracellularly sequestered Ca in smooth muscles; however, the results in Ca-free buffer solution suggest that the inhibitions of histamine-induced release of intracellularly sequestered Ca by D 600, Aspaminol and papaverine in basilar artery and those by D 600 and papaverine in aorta might more or less contribute to the inhibitory effects of these relaxants in normal buffer solution, whereas they might be mainly due to the inhibition of histamine-induced influx of extracellular Ca in taenia coli. In addition, it seems that there is also variety in the properties of the histamine-sensitive Ca store among the smooth muscle tissue types because the inhibitory effects of relaxants on the contractions in Ca-free buffer solution varied with smooth muscle tissue types.

In conclusion, as well as the difference in dependence upon either an influx of extracellular Ca or a release of intracellularly sequestered Ca, the properties of Ca influx pathways (Ca channels) and cellular Ca stores seem to vary in a complex manner with the difference in tissue types and/or contracting agents. These might be attributable to the various actions of smooth muscle relaxants in various tissue types; and furthermore, it may become necessary to have further subclassifications of Ca channels and Ca stores.

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