CONTRACTILE RESPONSES OF ISOLATED CANINE BLOOD VESSELS TO ATP AND ADP

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Abstract—Effects of ATP and ADP on helically cut strips of canine different blood vessels were studied, in comparison with those of K^+ and norepinephrine (N.E.). ATP and ADP were equally effective in causing transient contractions in contrast to sustained contractions induced by K^+ and N.E.. The mesenteric, renal arteries and portal veins were sensitive to both nucleotides. The response to the nucleotides and K^+ was more dependent on Ca^{++} and Mg^{++} concentrations in bathing media than that to N.E.. Treatment of the strips with EGTA in Ca^{++} -free media quickly abolished the contractile responses to ATP, ADP and K^+ . Application of 0.1 mM Cd^{++} attenuated the contractile responses to K^+ more markedly than those to N.E., but did not affect ATP action. Verapamil at 0.5 μ M inhibited the K^+ -induced contraction alone, and 2 mM procaine inhibited the response to N.E. alone, but rather intensified the ATP-induced contraction. It may be concluded that ATP and ADP produce a transient contraction of isolated canine blood vessels by a mechanism relating to an increased mobilization of loosely bound Ca^{++} in cell membranes.

ATP and related compounds have been shown to produce a vasodilation in most vascular beds *in situ*, but to cause either contraction or relaxation in a variety of isolated blood vessels and at different vascular tones. ATP and ADP produced relaxing responses in strips of rabbit portal vein (1) and dog coronary and renal arteries (2), in contrast, contractile responses of strips of rabbit aorta (3, 4), rat portal vein (5), and isolated perfused rat renal artery (6) were observed. It has been reported that the contractile responses were modified by Ca^{++} and Mg^{++} concentrations in bathing media; an increase of Ca^{++} : Mg^{++} ratio enhanced contractile responses and a decrease of ratio *vice versa* (5, 6). However, the mechanism of contractile effects of ATP and ADP has not been elucidated.

In the present experiment, effects of ATP and ADP were studied in isolated different blood vessels of the dog and the mechanisms of their contractile effects were studied in comparison with those of KCl and norepinephrine, since the contractile mechanisms of both agents have been fairly well clarified (7-10).

MATERIALS AND METHODS

Mongrel dogs of both sexes, weighing 10-15 kg, were anesthetized with sodium pentobarbital and sacrificed by bleeding from the carotid arteries. The superior mesenteric, renal, coronary and femoral arteries and the mesenteric, portal and renal veins were isolated. Helical strips of approximately 15 $\times$ 3 mm were cut from these arteries and veins, and each strip was fixed vertically between hooks under a resting tension of 1-2 g in a 20 ml organ bath containing Krebs solution. Constituents of the solution were as follows (mM): Na^+ ,

143.5; K^+ , 5.9; Ca^{++} , 2.5; Cl^- , 125.8; $H_2PO_4^-$, 2.5; HCO_3^- , 25.0; dextrose, 5.0. The nutrient solution was maintained at $37^\circ C$ and aerated with a mixture of 95% O_2 and 5% CO_2 . The pH of the solution was 7.2–7.4. Ca^{++} and Mg^{++} concentrations of Krebs solution were changed as required. The strips were allowed to equilibrate for 90–120 min before experiments were commenced. In experiments using qualified Krebs solution, the strips were exposed to these solutions for 60 min, during the exposure period, the solution was replaced every 20 min. EGTA (ethyleneglycol-bis-(β -aminoethylether) N,N'-tetraacetic acid) was dissolved in 1 N NaOH and added to Ca^{++} -free solution at 1 mM. Preparations were exposed to EGTA-added, Ca^{++} -free solution for 5 or 40 min and then to Ca^{++} -free solution for 5 or 20 min, respectively. The hook anchoring the upper end of strips was connected to the lever arm of a force-displacement transducer (Nihonkoden Kogyo Co., SB-1TH). The contractile responses of strips to stimulatory agents were recorded on an ink-writing oscillograph (Nihonkoden Kogyo Co., PMP-3004). Response of each strip to each agent in normal nutrient solution was taken as 100% and the response to the same concentration of the same agent after various treatments was expressed as % of controls. In most experiments, 30 mM K^+ , 10^{-6} – 2×10^{-6} M norepinephrine and 10^{-3} – 3×10^{-3} M ATP were used. Results shown in tables and figures are expressed as mean values $\pm$ standard errors of the means. Statistical analyses were made using the Student's *t*-test.

The agents used were potassium chloride (K^+ , Wako Pure Chem.), dl-norepinephrine hydrochloride (N.E., Sankyo), adenosine triphosphate disodium (ATP, Kowa), adenosine diphosphate disodium (ADP, Kyowa Hakko), atropine sulfate (Tanabe Seiyaku), phentolamine hydrochloride (Ciba), phenoxybenzamine hydrochloride (Nikken Kagaku), diphenhydramine hydrochloride (Kowa), cadmium chloride (Cd^{++} , Wako Pure Chem.), procaine hydrochloride (proc., Daiichi Seiyaku) and verapamil hydrochloride (ver., Eisai).

RESULTS

Effects of ATP and ADP on the strips of isolated different blood vessels

Application of 3×10^{-3} – 10^{-2} M ATP and ADP to strips of the superior mesenteric and renal arteries of the dog produced dose-dependent contractions. Contractions which developed immediately after application of ATP and ADP reached a peak within 1 to 2 minutes, and were followed by relaxation to the original tension within 2 to 3 minutes. In contrast, the sustained contraction, consisting of the initial phasic and successive tonic phase, was induced by 30–50 mM K^+ and 10^{-6} – 10^{-5} M N.E.. ATP and ADP were about equally effective in causing contraction of each blood vessel (Fig. 1). Repeated applications of 10^{-3} – 3×10^{-3} M ATP and ADP at 10 min intervals, during which time the prepara-

FIG. 1. Contractile responses of the superior mesenteric arteries to ATP, ADP, KCl and norepinephrine.

tions were washed with aerated normal media, produced similar contractile effects. In the superior mesenteric and renal arteries and portal veins, the maximum contraction induced by 10^{-2} M ATP and ADP was 75–85% of the contraction caused by 50 mM K^+ and 10^{-5} M N.E.. The minimum effective concentration of ATP and ADP was 3×10^{-4} M for coronary arteries and mesenteric veins, and 10^{-3} M for the femoral arteries and renal veins, respectively. The maximum contraction induced by 10^{-2} M ATP in the former two vessels was only one half the contraction seen in the mesenteric arteries, and that in the latter two vessels was one-third to one-fourth. The next experiments were carried out on strips of superior mesenteric arteries.

Effects of adrenergic and cholinergic blockers and antihistaminics on ATP and ADP contraction

The contractile effects of 10^{-3} – 3×10^{-3} M ATP and ADP on the vascular strips were not significantly inhibited by treatment with phentolamine (10^{-6} – 10^{-5} M), phenoxybenzamine (5×10^{-5} M), atropine (10^{-6} M) and diphenhydramine (10^{-6} M). Each of these blockers at the above concentrations suppressed the contraction induced by the corresponding agonist, N.E. (2×10^{-6} M), acetylcholine (10^{-5} M) and histamine (10^{-5} M).

Effects of Ca^{++} and Mg^{++} on ATP-, ADP-, K^+ - and N.E.-contraction

Decrease in external Ca^{++} from 2.5 to 0.5 mM attenuated the maximal contraction induced by 10^{-3} – 3×10^{-3} M ATP and ADP to $60.5 \pm 3.2\%$ ($N=5$) the control contraction, and the contraction induced by 30 mM K^+ to $51.6 \pm 7.7\%$ ($N=8$), while such decrease in Ca^{++} failed to significantly reduce the contractile effects of N.E. (Fig. 2). Removal of Ca^{++} from the bathing media produced a further reduction in the contractile responses to

FIG. 2. Effects of lowering Ca^{++} concentrations on the contractile responses of superior mesenteric arteries to norepinephrine, K^+ and ATP. ●—● normal Ca^{++} media (2.5 mM), ○—○ low Ca^{++} media (0.5 mM), ▲—▲ Ca^{++} -free media, △—△ EGTA-added, Ca^{++} -free media. The tension developed at 3×10^{-5} M norepinephrine, 5×10^{-2} M K^+ and 10^{-2} M ATP in normal media was taken as 100% for each agent. *: Significant difference from values of the developed tension in normal media, $p < 0.01$, **: $p < 0.005$, ***: $p < 0.001$. Figures in parentheses indicate number of preparations used.

FIG. 3. Effects of high Ca^{++} and Mg^{++} -free on the contractile responses of the superior mesenteric arteries to norepinephrine, K^+ and ATP. $\bullet$ — $\bullet$ normal media, $\circ$ — $\circ$ high Ca^{++} media (Ca^{++} 5.0 mM), $\triangle$ — $\triangle$ Mg^{++} -free media. The tension developed at 3×10^{-5} M norepinephrine, 5×10^{-2} M K^+ and 10^{-2} M ATP in normal media was taken as 100% for each agent. *: Significant difference from values of the developed tension in normal media, $p < 0.01$, **: $p < 0.005$, ***: $p < 0.001$. Figures in parentheses indicate number of preparations used.

high concentrations of ATP and ADP as well as K^+ , shifting their dose-response curves to the right and downward, and a moderate reduction ($30.9 \pm 9.4\%$, $N=4$) in the response to N.E.. Treatment with 1 mM EGTA for 5 min in Ca^{++} -free media abolished the contractile responses of the strips to 10^{-8} – 3×10^{-3} M ATP and ADP also to 30 mM K^+ , but decreased to a lesser extent the response to 2×10^{-6} M N.E. ($80.8 \pm 7.7\%$, $N=6$). Further inhibition in the contractile responses was induced by treatment with EGTA for 40 min. Increase of external Ca^{++} to 5 mM significantly enhanced the maximum contraction induced by 3×10^{-8} – 10^{-2} M ATP and ADP, and shifted their dose-response curves to the left and upward (Fig. 3). Similar potentiation of the effect of K^+ was also demonstrated, while the dose-response curve of N.E. was not significantly altered.

Doubling the concentration of external Mg^{++} had no significant influence on the effects of these four agonists. Further increase in Mg^{++} to 4-fold the normal inhibited the contractile responses to both nucleotides ($31.5 \pm 6.9\%$, $N=5$) and K^+ ($55.3 \pm 10.6\%$, $N=5$), but did not significantly alter the N.E.-induced contraction. Removal of Mg^{++} from normal media shifted dose-response curves for ATP, ADP and K^+ to the left and upward, but failed to alter the dose-response curve of N.E.. Removal of both Ca^{++} and Mg^{++} from the media reduced the contractile responses to these agonists to a lesser extent than those observed in Ca^{++} -free solution.

Effects of ATP, ADP, K^+ and N.E. after addition of Ca^{++} to EGTA-added, Ca^{++} -free media

Although the application of Ca^{++} (2.5 mM) to the strips, which had been exposed to EGTA-added, Ca^{++} -free media for 40 min, caused no contraction, the simultaneous ap-

TABLE 1. Effects of ATP, K⁺ and norepinephrine (N.E.) after addition of Ca⁺⁺ to EGTA-added, Ca⁺⁺-free media

Agonists	ATP 3×10^{-3} M			K ⁺ 3×10^{-2} M			N.E. 2×10^{-6} M		
	Tension (g)	%	n	Tension (g)	%	n	Tension (g)	%	n
Control	1.67±0.09	100	31	2.03±0.09	100	4	2.38±0.28	100	11
0 min	1.28±0.19*	76.6	14	2.18±0.23	107.4	4	2.55±0.38	107.1	4
1.0	1.49±0.20	89.2	7	---	---	---	---	---	---
2.0	1.52±0.12	91.0	6	---	---	---	2.59±0.36	108.8	6
3.0	1.83±0.28	109.6	3	---	---	---	---	---	---

Contractile responses of the superior mesenteric arteries to ATP, K⁺ and norepinephrine (N.E.) after Ca⁺⁺ (2.5 mM) application with lapse of time between addition of each agent and Ca⁺⁺. Tension developed in normal media was taken as 100% for each agent. n; Number of preparations used. t; Time interval between applications of Ca⁺⁺ and each agent. *: Significant difference from value of the developed tension in normal media, $p < 0.05$.

plication of 3×10^{-3} M ATP with Ca⁺⁺ produced a transient contraction, the magnitude being significantly less than that induced by ATP in normal media. ATP when added 3 min after the application of Ca⁺⁺ caused a transient contraction which was almost the same as that seen in control media. Similar responses were observed after application of ADP. On the other hand, contractile responses to K⁺ and N.E. were not influenced when Ca⁺⁺ (2.5 mM) was added after long exposure of the strips to EGTA-added, Ca⁺⁺-free media (Table 1).

Effects of Cd⁺⁺, ver. and proc. on ATP-, K⁺- and N.E.-contraction

Treatment of mesenteric arterial strips with 0.02 mM Cd⁺⁺ for 20 min slightly inhibited the contractile response to 30 mM K⁺, and did not alter the response to 10^{-6} M N.E. and 3×10^{-3} M ATP. The addition of 0.1 mM Cd⁺⁺ markedly decreased the response to K⁺, but only slightly reduced the response to N.E.. At this concentration of Cd⁺⁺, the response to ATP was not influenced (Table 2). When preparations were exposed for 20 min to ver. in a concentration of 5×10^{-7} M, contractions produced by 30 mM K⁺ decreased to $47.0 \pm 5.6\%$ (N=8) of control, whereas the contractions caused by 10^{-6} M N.E. and 10^{-3} M ATP were not significantly altered. Treatment for 5 min with 2 mM proc. suppressed the contractile response to 10^{-6} M N.E., but did not change the contraction induced by 30 mM K⁺. ATP-induced contraction was rather enhanced by proc. (Table 2).

DISCUSSION

The present study showed that ATP and ADP were equally effective in causing con-

TABLE 2. Effects of Cd^{++} , verapamil (ver.) and procaine (proc.) on the contractile responses of the superior mesenteric arteries to K^+ , norepinephrine (N.E.) and ATP

Agonists	$\text{K}^+ 3 \times 10^{-2} \text{ M}$			$\text{N.E. } 10^{-6} \text{ M}$			$\text{ATP } 3 \times 10^{-3} \text{ M}$			$\text{ATP } 10^{-3} \text{ M}$		
Antagonists	Tension (g)	%	n	Tension (g)	%	n	Tension (g)	%	n	Tension (g)	%	n
Control	2.32 ± 0.19		26	1.84 ± 0.13		39	2.08 ± 0.22		15	1.24 ± 0.12		20
10^{-4} M	$0.49 \pm 0.07^{***}$	-78.9	5	$1.06 \pm 0.15^{**}$	42.4	11	2.03 ± 0.17	-2.4	6	—		
Cd^{++}												
$5 \times 10^{-4} \text{ M}$	$0.47 \pm 0.06^{***}$	-79.7	5	$0.38 \pm 0.07^{***}$	-79.3	6	1.81 ± 0.28	-13.0	9	—		
ver. $5 \times 10^{-7} \text{ M}$	$1.09 \pm 0.19^{**}$	-53.0	8	1.68 ± 0.08	-8.9	9				1.18 ± 0.18	-4.8	9
proc. $2 \times 10^{-3} \text{ M}$	2.19 ± 0.13	-5.6	6	$0.48 \pm 0.07^{***}$	-73.9	9				$2.15 \pm 0.26^{**}$	-73.4	12

The strips were exposed to Cd^{++} and verapamil (ver.) for 20 min or procaine (proc.) for 5 min prior to application of these three agents. %; Percent changes in the developed tension in experimental conditions relative to that in control media. n; Number of preparations used. **; Significant difference from values of the developed tension in control media, $p < 0.005$. ***; $p < 0.001$.

traction of isolated different blood vessels of the dog. Among the blood vessels tested, the superior mesenteric and renal artery and portal vein showed relatively high responses to both nucleotides. However, the maximum contraction induced by both nucleotides in these blood vessels was smaller than that induced by 50 mM K^+ and 10^{-5} M N.E.. The contractions induced by ATP and ADP were quite different from those caused by K^+ and N.E.: both nucleotides produced a transient contraction, while K^+ and N.E. produced a sustained contraction. On the basis of the phasic pattern of contraction, it is suggested that the nucleotide-induced contraction is due to release of membrane bound and intracellularly stored Ca^{++} (11).

The contractile responses of the mesenteric arterial strips to ATP and ADP were not influenced by adrenergic, cholinergic blockers and antihistaminics as reported in the isolated perfused rat renal artery (6) and the rat portal vein (5). These data indicate that the effects of ATP and ADP are not related to the transmitter release but rather to their direct action on the smooth muscle of blood vessels.

The contractile responses of isolated mesenteric arterial strips to ATP and ADP were closely related to Ca^{++} and Mg^{++} concentrations in bathing media; high Ca^{++} : Mg^{++} ratio favoring contraction, and low ratio opposing contraction as evidenced in the rat portal vein (5) and the isolated rat renal artery (6). Especially, the response to the nucleotides and K^+ was more susceptible to removal of Ca^{++} than that with N.E.. Such different susceptibility in the response to K^+ and N.E. has been reported by other workers (12-15). Thus Ca^{++} available for the nucleotide-induced contraction may be supplied from external fluids across cell membranes and also from intracellular stored sites as shown in K^+ -induced contraction (12, 13). However, as described above it has been postulated that the transient contraction is associated with a release of membrane bound and intracellularly stored Ca^{++} (11), whereas the tonic contraction is associated with a sustained increase in the Ca^{++} -influx (7, 9). In the present experiment, it was confirmed that the simultaneous application of Ca^{++} with ATP to the strips, which had been exposed to EGTA-added, Ca^{++} -free media, produced a contraction smaller than that induced by ATP in normal media, but the same maneuver with K^+ or N.E. elicited a contraction as large as that which occurred in normal media. These findings suggest that the Ca^{++} -influx is not mainly related to ATP- and ADP-induced contraction. Recently, Toda (15) demonstrated that Cd^{++} suppresses vascular contractions in response to stimulatory agents, including K^+ , N.E., histamine etc., by interference with Ca^{++} -influx. The present experiments showed that Cd^{++} attenuated the contractile responses to vascular strips to K^+ more markedly than that to N.E., and this divalent cation failed to inhibit the ATP-induced contraction. Verapamil was also effective in inhibiting the K^+ -induced contraction, but ineffective in attenuating the contractions induced by N.E. (17) and ATP. These results support the idea that the contractile response to ATP is not ascribable to an increased Ca^{++} -influx but to a release of stored Ca^{++} .

Proc. did not inhibit ATP-induced contraction but rather intensified it. While the contractile response to N.E. was suppressed by proc., the response to K^+ was not modified, the findings being in agreement with Hudgins and Weiss' report (13). These authors demon-

strated a specific antagonism of proc. to N.E.-induced contraction by preventing Ca^{++} -release from firmly bound stores. These findings suggest that ATP-induced transient contraction is not associated with firmly bound Ca^{++} -stores. On the other hand, it has been reported that part of Ca^{++} available for K^+ -induced contraction must be loosely-bound Ca^{++} with cell membrane, since it is rapidly depleted by EDTA or removal of external Ca^{++} (12, 13). This may be true with both nucleotides since EGTA rapidly abolished the contractions.

From these studies, it may be concluded that ATP and ADP caused a transient contraction of isolated canine blood vessels by a mechanism related to an increased mobilization of loosely bound Ca^{++} in cell membranes.

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