Analysis of Ouabain-Induced Contractions in Isolated Coronary Arteries

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Abstract—Contractile responses to ouabain in helical strips of dog and monkey coronary arteries were investigated. Ouabain (5×10⁻⁸ to 5×10⁻⁶ M) caused a dose-related contraction in dog and monkey arteries; the response of monkey coronary arteries was significantly greater. In dog coronary arteries, contractile responses to high concentrations of ouabain were potentiated by treatment with propranolol. In the arteries contracted with ouabain, the addition of phentolamine caused a relaxation. Contractile responses of dog coronary arteries to ouabain were markedly suppressed by exposure to Ca²⁺-free media or by treatment with verapamil. Reduction of external concentration of K⁺ or lowering the temperature of bathing media did not selectively influence the ouabain-induced contraction. These results suggest that ouabain-induced contractions of dog coronary arteries are associated mainly with an increase in the Ca²⁺-influx, which does not result from an inhibition of the Na⁺, K⁺-activated ATPase nor from an activation of α adrenoceptors by noradrenaline released from adrenergic nerves. Ouabain in high concentrations seems to liberate noradrenaline from adrenergic nerves, which preferentially activates β adrenoceptors in dog coronary artery.

It has been demonstrated that ouabain-induced contractions of monkey, dog and rabbit vascular smooth muscle are caused by an increase in the influx of Ca²⁺ across cell membranes, which results from an inhibition of the Na⁺ pump at low concentrations of the glycoside (dog cerebral arteries (1)) and from a release of noradrenaline from adrenergic nerves in high concentrations (dog mesenteric, renal and femoral arteries (1) and dog portal and saphenous veins (2–4)). In coronary arteries, it has been reported that cardiac glycoside-induced contractions of porcine and bovine coronary arteries can be easily prevented by Ca²⁺-antagonistic inhibitors (5, 6). However, the mechanism of action of glycosides in coronary arteries has not been determined. The present study was thus undertaken to clarify the response of isolated dog and monkey coronary arteries to ouabain and to determine the mechanism of action of ouabain in the dog arteries.

Materials and Methods

Mongrel dogs of either sex, weighing 9–13 kg, and Japanese monkeys (Macaca fuscata) of either sex, weighing 7–12 kg, were anesthetized with intravenous injections of pentobarbital sodium (30 mg/kg) and killed by bleeding from common carotid arteries. The heart was rapidly removed. The distal portion of the ventral interventricular and circumflex branch of left coronary arteries (0.6–0.9 mm OD) was isolated from the heart. The arteries were cleaned and helically cut into strips approximately 20 mm long. The specimens were vertically fixed between hooks in the muscle bath (20 ml capacity) containing the nutrient solution that was gassed with a mixture of 95% O₂–5% CO₂ and was maintained at 37±0.5°C. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihon-Kohden...
Kogyo, Tokyo, Japan). The resting tension was adjusted to 1.5 g for dog arteries, which was demonstrated to be optimal for producing the maximum contraction (7), and to 1.0 g for monkey arteries. Constituents of the solution were as follows (mM): NaCl, 120; KCl, 5.4; NaHCO₃, 25.0; CaCl₂, 2.2; MgCl₂, 1.0; and dextrose, 5.6. The pH of the solution was 7.35–7.41. Before the start of experiments, all preparations were allowed to equilibrate in the bathing media for 40–90 min during which time the fluids were replaced every 10–15 min.

Isometric contractions and relaxations were recorded on an ink-writing oscillograph (Sanei Sokki, Tokyo, Japan). The contractile response to 30 mM K⁺ was first obtained, and then preparations were washed 3 times with control fluids and equilibrated for 40–50 min. Ouabain was added directly to the bathing media in cumulative concentrations unless otherwise mentioned. Responses to high concentrations of ouabain could not be reproduced in the same preparations; therefore, a one dose-response relationship was obtained from each strip. Contractile responses to 30 mM K⁺ were taken as 100%, and contractions induced by ouabain relative to those induced by K⁺ were presented. In some experiments, arterial strips were treated for 20 min with propranolol or verapamil before the response to ouabain was obtained. Some preparations were exposed for 30 min to the Ca²⁺-free solution or K⁺-deficient solution. In some preparations, the temperature of the bathing medium was lowered to 27°C. Before the experiments were commenced, preparations were equilibrated for 90 min at 27°C. Results shown in the text and figures are expressed as the mean±S.E.M. Statistical analyses were made using Student’s t-test. Drugs used were ouabain octahydrate (Sigma), phentolamine mesylate, verapamil hydrochloride (Eisai), (±)-propranolol hydrochloride, prostaglandin (PG) F₂α (Ono Co.), and papaverine hydrochloride.

Results

Effect of ouabain on coronary arteries isolated from dogs and monkeys: In helically cut strips of coronary arteries isolated from dogs, the addition of ouabain in concentrations ranging from 5×10⁻⁸ to 5×10⁻⁶ M caused a dose-related contraction (Fig. 1). Further increase in the concentration of ouabain caused a relaxation from the contracted level. When the ouabain (2×10⁻⁵ M)-induced relaxation stabilized, the addition of propranolol (10⁻⁶ M) caused a marked contraction. Treatment with 10⁻⁶ M propranolol potentiated ouabain-induced contractions of dog coronary arteries (Fig. 2). In arteries pretreated with propranolol (10⁻⁶ M) and contracted with ouabain (2×10⁻⁵ M), the addition of phentolamine (10⁻⁶ M) caused a relaxation; the mean absolute relaxation was 320±102 mg (n=7) from an increased level of tension by ouabain (480±129 mg).

Coronary arteries isolated from monkeys responded with a dose-dependent contraction to ouabain in concentrations ranging from 5×10⁻⁸ to 10⁻⁴ M (Fig. 1). Contractile

![Fig. 1. Concentration-response curves for ouabain in strips of dog (○) and monkey (□) coronary arteries. Constrictions induced by 30 mM K⁺ were taken as 100%; mean absolute values were 2986±202 mg (n=22) in the dog arteries and 1325±222 mg (n=10) in the monkey arteries. Vertical lines represent the S.E.M. Numbers in parentheses indicate the number of preparations used. Significantly different from values in dog arteries, *P<0.001.](image-url)
responses of monkey coronary arteries to ouabain (10\(^{-6}\) to 2 \times 10^{-5} M) relative to those induced by 30 mM K\(^+\) in control and Ca\(^{2+}\)-free media were 7.8±1.3\% (n=9) and 1.1±0.3\% (n=9), respectively: the difference was statistically significant (P<0.001) (Fig. 3, left). The addition of 2.2 mM Ca\(^{2+}\) contracted the arteries to a level similar to that attained by ouabain in control media. Treatment for 20 min with 2 \times 10^{-7} M verapamil also suppressed the contractile response to ouabain (5\times10^{-8} to 5\times10^{-6} M) (Fig. 3, right). The suppression was partially reversed by the addition of Ca\(^{2+}\) (2.2 mM).

Reduction of [K\(^+\)]\(_o\) (external concentration of K\(^+\)) to 1.2 mM (1/4 normal [K\(^+\)]\(_o\)) did not significantly alter the coronary arterial tension. In the coronary arteries exposed to normal media (5.4 mM [K\(^+\)]\(_o\)) and partially precontracted with prostaglandin F\(_2\alpha\), the addition of 5 mM K\(^+\) caused a contraction, whereas the same concentration of K\(^+\) relaxed the arteries exposed to reduced [K\(^+\)]\(_o\), (0.4, 1.4 and 2.7 mM), the magnitude of relaxations being related inversely to [K\(^+\)]\(_o\) (Fig. 4, lower panel). The K\(^+\) (5 mM)-induced relaxation was abolished by treatment with ouabain (10\(^{-6}\) and 5\times10^{-6} M). Contractile responses of coronary arteries to 5\times10^{-6} M ouabain was suppressed by a reduction of [K\(^+\)]\(_o\) to 1.4 mM (Fig. 4, upper panel). The mean reduction of the response to ouabain at reduced [K\(^+\)]\(_o\) was 71.9±12.9\% (n=7, significantly different from the response at normal [K\(^+\)]\(_o\), P<0.001). Similar suppression by a reduction of [K\(^+\)]\(_o\) was also obtained in contractions induced by PGF\(_2\alpha\) (62.5±6.9\% inhibition, n=7, P<0.001) in a concentration (5\times10^{-7} M) sufficient to produce similar magnitudes of contractions to those induced by 5\times10^{-6} M ouabain (Fig. 4, upper panel).

When the temperature of the bathing medium was lowered from 37\(^\circ\) to 27\(^\circ\)C, the arterial tension was not significantly altered. Relaxations induced by the addition of 5 mM K\(^+\) in the arteries exposed to 1/4 [K\(^+\)]\(_o\) medium were significantly suppressed by lowering the temperature (Fig. 5, right). However, ouabain (5\times10^{-8} to 5\times10^{-6} M)-induced contractions were unaffected by lowering the temperature of the bathing
medium to 27°C (Fig. 5, left). The relaxation induced by a high concentration of ouabain (2×10^-6 M) was prevented at 27°C (Fig. 6). Similar results were obtained in additional 3 preparations. The addition of propranolol (10^-6 M) caused a greater contraction from the stabilized level at 37°C than at 27°C. Contractions induced by K^+ in a concentration of 10 mM sufficient to produce a similar magnitude of contractions to that with 5×10^-6 M ouabain were suppressed by lowering the temperature to 27°C. Mean values of the K^+ (10 mM)-induced contractions at 37°C and 27°C were 676±88 mg and 333±94 mg (n=7), respectively; the difference was statistically significant (P<0.05).

Fig. 3. Modification of the ouabain-induced contraction by removal of external Ca^{2+} (left) and treatment with 2×10^-7 M verapamil (right). Left: Ouabain (5×10^-6 M)-induced contraction in control media (left column) and in Ca^{2+}-free (with 0.1 mM EGTA) media (middle column), addition of 2.2 mM Ca^{2+} to Ca^{2+}-free media containing ouabain (right column). Contractions induced by 50 mM K^+ in control media were taken as 100%; mean absolute values in experiments in normal media and in Ca^{2+}-free media were 3456±400 mg (n=9) and 3457±240 mg (n=9), respectively. Each column represents the mean contraction, and vertical lines show the S.E.M. Numbers in the columns indicate % controls. Numbers of the preparations used were 9 in control media and 9 in Ca^{2+}-free media, obtained from the same dogs. Significantly different from the control, aP<0.001. Right: Contractile responses to ouabain in control (○) and verapamil-treated strips (●). Numbers in parentheses show the number of preparations used. Contractions induced by 30 mM K^+ in control media were taken as 100%; mean absolute values in experiments in control and verapamil treated strips were 2883±365 mg (n=6) and 2374±335 mg (n=6), respectively. Significantly different from the controls, aP<0.01, bP<0.05, cP<0.001. Vertical lines represent the S.E.M.

Discussion

The present study revealed that contractile responses to ouabain of coronary arteries isolated from dogs and monkeys appreciably differed, the responses of monkey coronary arteries being appreciably greater. Contractile responses to ouabain were markedly suppressed by exposure to Ca^{2+}-free media or by treatment with verapamil, a Ca^{2+} antagonist, the inhibition by verapamil being partially reversed by raising [Ca^{2+}]_o. These results suggest that the ouabain-induced contraction is mainly associated with the influx of Ca^{2+} across cell membranes. Suppression of glycoside-induced contractions in porcine and bovine coronary arteries by Ca^{2+}-antagonistic inhibitors has been reported (5,
It has been suggested that the ouabain-induced contraction of dog and monkey arteries, except for coronary arteries, is associated with an inhibition of the electrogenic Na+ pump (or Na+, K+-activated membrane ATPase) by low concentrations of K+.

**Fig. 4.** Modification by reduced [K+]o of the response of dog coronary arteries to 5 × 10⁻⁷ M prostaglandin F₂α (●), 5 × 10⁻⁶ M ouabain (○) and 5 mM K⁺ (△). Contractile responses to 30 mM K⁺ were taken as 100%; mean absolute values in experiments with PGF₂α and ouabain in normal [K⁺]o and 1/4 [K⁺]o were 2024 ± 299 mg (n=7), 2854 ± 412 mg (n=7) and 2640 ± 260 mg (n=7), respectively. Before the addition of 5 mM K⁺ in the lower figure, the strips were partially precontracted with PGF₂α. Contractions at normal [K⁺]o (5.4 mM) were expressed as % of contractions induced by 30 mM K⁺ (2897 ± 235 mg, n=6); relaxations at reduced [K+]o were expressed as % of relaxations induced by 10⁻⁴ M papaverine. Mean absolute values of papaverine-induced relaxations were 285 ± 38 mg in 1/2 [K⁺]o, 273 ± 48 mg in 1/4 [K⁺]o, and 135 ± 24 mg (n=6) in media without K⁺. Numbers in parentheses indicate the number of preparations used. Vertical lines represent the S.E.M. Significantly different from the control, "P<0.001, "P<0.01, "P<0.02, ’P<0.05.

**Fig. 5.** Left: Concentration-response curves for ouabain at 37°C (○) and 27°C (●). Contractions induced by 30 mM K⁺ at 37°C were taken as 100%; mean absolute values in experiments at 37°C and 27°C were 3040 ± 296 mg (n=8) and 3275 ± 378 mg (n=8), respectively. Numbers in parentheses indicate the number of preparations used. Right: Relaxations induced by 5 mM K⁺ in 1/4 [K⁺]o at 37°C (left column) and 27°C (right column). Relaxations induced by 10⁻⁴ M papaverine were taken as 100%; mean absolute values were 391 ± 51 mg (n=8) at 37°C and 331 ± 62 mg (n=8) at 27°C. Numbers in the columns indicate % controls. Each column represents the mean relaxation and vertical lines show the S.E.M. Significantly different from the control,  "P<0.01.
of ouabain (cerebral arteries) and with noradrenaline released from adrenergic nerves by high concentrations of the glycoside (mesenteric, renal, and femoral arteries) (1). Therefore, whether or not the ouabain-induced contraction in dog coronary arteries is caused by the membrane ATPase inhibition was investigated.

Both reduction of $[K^+]_o$ and lowering the temperature of the bathing medium are known to inhibit Na$^+$, K$^+$-ATPase activity (8–10). In the present study, the K$^+$ (5 mM)-induced relaxation was related inversely to $[K^+]_o$ and was abolished by ouabain, suggesting an involvement of membrane ATPase activation in the relaxation. Lowering the temperature of the bathing medium also attenuated the K$^+$-induced relaxation. Coronary arterial tension was not affected by $[I^+]_o$ deprivation and lowering the temperature, but significantly increased by ouabain. Treatment of the arteries with reduced $[K^+]_o$ inhibited the ouabain-induced contraction, as did the contraction induced by PGF$_2\alpha$. Such a result may indicate that in the bathing medium deprived of K$^+$, where the Na$^+$, K$^+$-ATPase activity is expected to be inhibited, contractions induced by ouabain are not selectively reduced. Lowering the temperature of the bathing medium did not influence contractions induced by ouabain, but suppressed contractions induced by K$^+$ (10 mM).

These results suggest that the ouabain-induced contraction of dog coronary arteries is not associated with the inhibition of the Na$^+$, K$^+$-ATPase activity and that ouabain and K$^+$ do not share the same mechanism underlying arterial contractions.

High concentrations of ouabain caused relaxations, which were reversed by the addition of propranolol in dog coronary arteries. Pretreatment with propranolol enhanced contractions induced by high concentrations of ouabain (5×10$^{-6}$ and 2×10$^{-5}$ M). These results may indicate that the activation of $\beta$ adrenoceptors by noradrenaline released from adrenergic nerves innervating the arterial wall counteracts the contraction induced by high concentrations of ouabain. The noradrenaline-releasing effect of ouabain has been reported in other vessels (1–4). Noradrenaline released by an electrical stimulation of adrenergic nerves and applied exogenously predominantly activates $\beta$ adrenoceptors in dog coronary arteries, producing a relaxation (11). The relaxations induced by high concentrations of ouabain were attenuated by lowering the temperature of bathing media from 37°C to 27°C (Fig. 6), whereas isoproterenol-induced relaxations are not influenced (N. Toda, unpublished data). The vascular contraction (12, 13) and the release of noradrenaline (14, 15) induced by adrenergic

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**Fig. 6.** Typical recordings of the response of dog coronary arteries to ouabain at 37°C (upper trace) and 27°C (lower trace). The strips were obtained from the same dog. Horizontal lines just left of the tracings represent the level prior to the addition of ouabain in concentrations lower than 10$^{-6}$ M. Prop. =10$^{-6}$ M propranolol, Phentol. =10$^{-6}$ M phentolamine.
nerve stimulation are reportedly attenuated at low temperature. Therefore, lowering the temperature appears to interfere with the release of noradrenaline from adrenergic nerves by ouabain as well as the release induced by nerve action potentials. In contrast to the dog arteries, ouabain does not seem to release noradrenaline in concentrations sufficient to significantly alter the monkey coronary arterial tone from adrenergic nerves, since the arteries contracted with high concentrations of ouabain were not influenced by treatment with phentolamine nor by propranolol.

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