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Effects of Potassium Ion and Caffeine on Contraction and Cytosolic Free Ca^{2+} Levels in Vascular Smooth Muscle

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ABSTRACT

Effects of high concentration of KCl and caffeine on cytosolic Ca^{2+} level ($[Ca^{2+}]_{cyt}$), measured simultaneously with muscle tension using a fluorescent intracellular Ca^{2+} indicator fura 2, were examined in isolated smooth muscle of rat aorta. High K^+ (72.7 mM) solution induced sustained increase in both $[Ca^{2+}]_{cyt}$ and tension. In contrast to this, caffeine (20 mM) induced a rapid increase in $[Ca^{2+}]_{cyt}$ followed by a decrease to a level which was higher than the resting level. However, muscle tension showed only a transient increase followed by a decrease below the resting level. In a Ca^{2+}-free solution, high K^+ increased neither $[Ca^{2+}]_{cyt}$ nor tension, whereas caffeine induced a transient increase in both $[Ca^{2+}]_{cyt}$ and muscle tension.

These results suggest that high K^+-induced contraction in vascular smooth muscle of rat aorta is due to Ca^{2+} influx whereas caffeine-induced contraction is due to Ca^{2+} release from cellular store. Further, caffeine seems to have an additional effect to decrease the sensitivity of the contractile elements to Ca^{2+}.

Key Words: Caffeine, Fura 2, Cytosolic free Ca^{2+}, Rat aorta

INTRODUCTION

It is generally accepted that Ca^{2+} plays a central role in the regulation of smooth muscle contraction. Critical evaluation of the role of Ca^{2+} as an intracellular messenger in smooth muscle requires quantitative measurement of cytosolic free Ca^{2+} concentrations and comparison with contractile response. Recently, fluorescent dyes, such as quin 2 and fura 2, have become available to measure the change in cytosolic Ca^{2+} concentrations in various biological preparations (Tsien *et al.*, 1982; Grynkiewicz *et al.*, 1985). This technique has been successfully applied to the smooth muscle strips for the simultaneous measurement of contractile tension and cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]_{cyt}$) (Ozaki *et al.*, 1987). In this study, we used this technique to investigate the effect of two vasoactive agents, a high concentration of KCl and caffeine, on tension development and $[Ca^{2+}]_{cyt}$ in the smooth muscle strip of rat aorta.

MATERIALS AND METHODS

The thoracic aorta was isolated from male Wistar rat (250-300 g), cut into spiral strip (2 mm width and 7 mm length) and placed in a normal physiological salt solution (PSS). Endothelium was removed by gently rubbing the intimal surface with a finger moistened with PSS as described by Furchgott and Zawadzki (1980). The PSS contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) 20, ethylenediaminetetraacetic acid (EDTA) 0.01 and glucose 5.5. This solution was saturated with 100% O_2 gas at 37℃ at pH 7.4. High K^+ solution was made by substituting with equimolar KCl. Ca^{2+}-free solution was made by removing $CaCl_2$ from PSS and adding 0.5 mM ethyleneglycol bis (beta-aminoethylether)-N,N,N',

N'-tetraacetic acid (EGTA). Since endogenous heavy metal ions quench the fura 2-Ca^{2+} fluorescence, a chelator, 10 μM N,N,N',N'-tetrakis(2-pyridylmethyl) ethylenediamine (TPEN), was added to all the PSS used (Alsan *et al.*, 1985).

In order to measure $[Ca^{2+}]_{cyt}$, muscle strips were treated with 5μM acetoxymethyl ester of fura 2 (fura 2/AM) for 3-4 h at room temperature, as described previously (Ozaki *et al.*, 1987). The non-cytotoxic detergent, cremophor EL (0.5%), was added to increase the solubility of fura 2/AM. Experiments were performed with a fluorimeter designed to measure the fluorescence of living tissues (CAF-100, Japan Spectroscopic, Tokyo, Japan). Fura 2-loaded muscle strip was held horizontally in a temperature controlled, 7 ml volume organ bath. One end of the muscle strip was connected to a strain gauge transducer (Toyo Boldwin) to monitor the mechanical activity. A part of the muscle strip was illuminated alternatively with two excitation lights (340 nm and 380 nm). The fluorescence emitted from the muscle strip was collected to photomultiplier through 500 nm filter. The amount of the 500 nm fluorescence induced by 340 nm excitation and that induced by 380 nm excitation was measured and the ratio of these two fluorescence was automatically calculated by a computer equipped in the fluorimeter. It has been

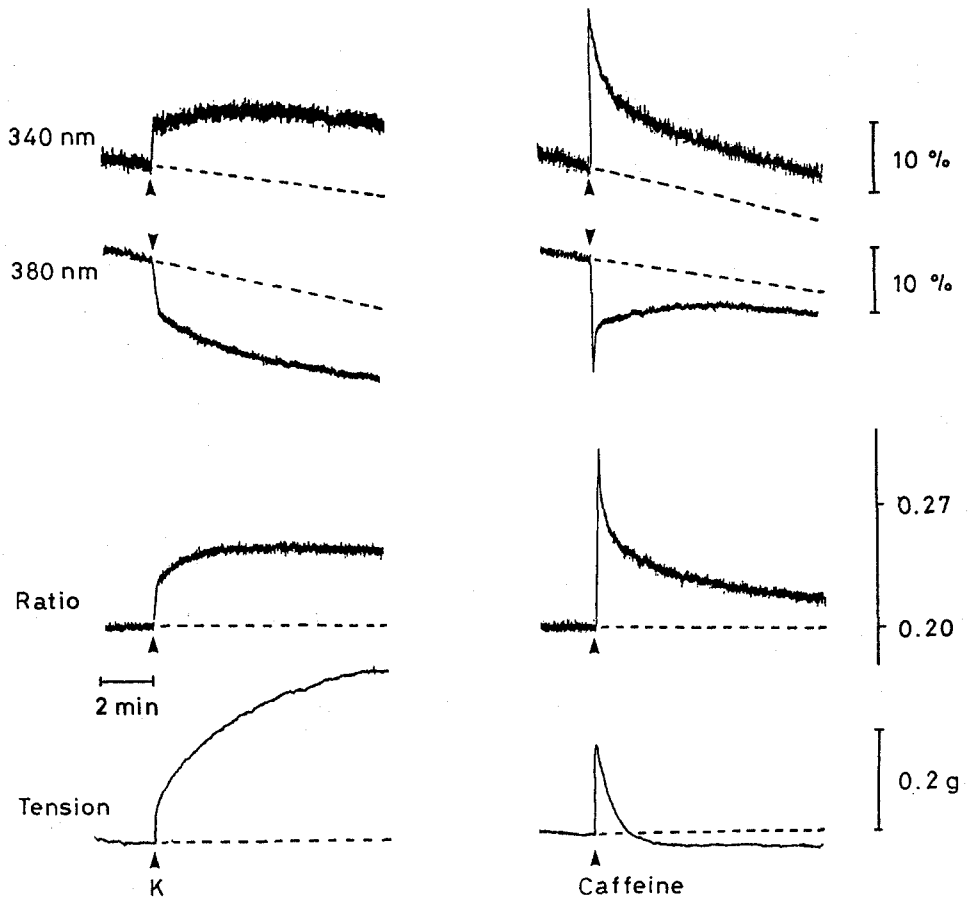

Fig. 1. Typical recordings of the changes in fura 2-Ca^{2+} fluorescence stimulated by 72.7 mM K^+ (K) and 20 mM caffeine in the presence of 1.5 mM Ca^{2+} simultaneously measured with muscle tension in a strip of rat aorta. Muscle was excited at 340 nm (first line) or 380 nm (second line) and emission at 500nm was monitored. The ratio of fluorescence at each excitation (third line) indicates the relative $[Ca^{2+}]$ cyt. Changes in muscle tension (fourth line) are also shown. Changes in the fluorescence induced by 340 nm excitation and that induced by 380 nm excitation are shown by relative value taking resting fluorescence level as 100%.

reported that this ratio represents relative $[Ca^{2+}]_{cyt}$ in the smooth muscle (Grynkiewicz *et al.*, 1985; Ozaki *et al.*, 1987).

Drugs used were noradrenaline bitartrate, caffeine (Wako Pure Chemicals), cremophor EL (Nakarai Chemicals), HEPES, EGTA, EDTA, TPEN and fura 2/AM (Dojin Chemicals).

RESULTS

In the PSS containing 1.5 mM Ca^{2+}, 72.7 mM K^+-solution induced sustained increase in the 500 nm fluorescence induced by the 340 nm exciation, sustained decrease in the 500 nm fluorescence induced by the 380 nm excitation, and sustained increase in the relative $[Ca^{2+}]_{cyt}$ indicated by the ratio of these two fluorescence, as shown in Fig. 1 (left). After a short latency period (less than 1 sec), muscle tension began to increase. The $[Ca^{2+}]_{cyt}$ stayed at a high level whereas muscle tension gradually increased during the 8 min observation period. Washing the muscle with normal PSS, the $[Ca^{2+}]_{cyt}$ rapidly decreased to the original level which was followed by a slow decrease in muscle tension (data not shown).

As shown in Fig. 1 (right), 20 mM caffeine induced a rapid increase in $[Ca^{2+}]_{cyt}$ followed by a decrease to a level which was higher than the resting level. However, muscle tension showed only a transient increase followed by a decrease below the resting level.

After the muscle strip of rat aorta was treated with 72.7 mM K^+ for 5 min, external solution was replaced with a high K^+ and Ca^{2+}-free PSS containing 0.5 mM EGTA. The Ca^{2+} removal rapidly decreased $[Ca^{2+}]_{cyt}$ which was accompanied by a slow decrease in muscle tension, as shown in Fig. 2. Addition of 20 mM caffeine after the Ca^{2+} removal induced rapid increments in both $[Ca^{2+}]_{cyt}$ and muscle tension followed by a decrease below the resting levels.

DISCUSSION

In the present experiments, we have demonstrated that high K^+ induced sustained increase in $[Ca^{2+}]_{cyt}$ which was accompained by a sustained contraction in vascular smooth muscle of rat aorta.

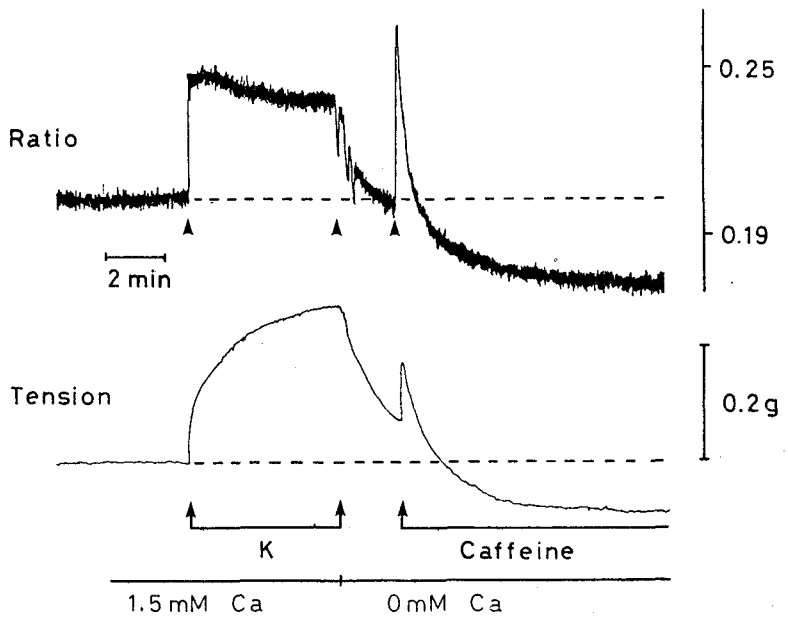

Fig. 2. Effect of caffeine on fluorescence ratio (relative $[Ca^{2+}]$ cyt ; upper line) and tension (lower line) in the absence of external Ca^{2+}. After treatment of the muscle for 5 min with 72.7 mM K^+ (K) solution, external solution was replaced with a Ca^{2+}-free solution containing 0.5 mM EGTA. After the fluorescence ratio decreased to the resting level, 20 mM caffeine (CAF) was applied.

The high K^+-induced increments in both $[Ca^{2+}]_{cyt}$ and tension were decreased by the removal of extracellular Ca^{2+}. Changes in $[Ca^{2+}]_{cyt}$ always preceded the changes in muscle tension. These results confirm the previous suggestion that the high K^+-induced contraction in smooth muscle is attributable to the increase in $[Ca^{2+}]_{cyt}$ resulted from an increase in Ca^{2+} influx (Karaki and Weiss, 1984).

In the presence of extracellular Ca^{2+}, caffeine induced a transient increase followed by a plateau phase in $[Ca^{2+}]_{cyt}$ which was higher than the resting level. In contrast to this phenomena, caffeine induced only a transient increase followed by a decrease below the resting level in $[Ca^{2+}]_{cyt}$ in the absence of extracellular Ca^{2+}. These results support the suggesting that caffeine releases Ca^{2+} from cellular store which results in a transient increase in $[Ca^{2+}]_{cyt}$ (Deth and Casteels, 1977; Karaki *et al.*, 1987; Karaki and Weiss, 1988). Further, caffeine induced a sustained increase in $[Ca^{2+}]_{cyt}$ only in the presence of external Ca^{2+}, suggesting that caffeine stimulates Ca^{2+} influx. Since caffeine has been shown to depolarize smooth muscle membrane (Sunano and Miyazaki, 1973), the caffeine-induced increase in Ca^{2+} influx may be mediated by the opening of voltage-dependent Ca^{2+} channels. However, caffeine induced only a transient increase in muscle tension which was followed by a decrease below the resting level either in the presence or absence of extracellular Ca^{2+}. These results indicate that caffeine does not induce smooth muscle contraction even in the presence of elevated $[Ca^{2+}]_{cyt}$, suggesting that caffeine decreases sensitivity of contractile elements to Ca^{2+}. As for the mechanism of inhibitory effect of caffeine, Ahn *et al* (1987) have shown that caffeine inhibits smooth muscle contraction by inhibition both Ca^{2+} influx and Ca^{2+} release. Present results indicate that caffeine has an additional mechanism of action to directly inhibit contractile elements. It is concluded that high K^+-induced contraction in vascular smooth muscle of rat aorta is due to Ca^{2+} influx whereas caffeine-induced contraction is due to the Ca^{2+} release from cellular store. Further, caffeine seems to have an additional effect to decrease the sensitivity of contractile elements to Ca^{2+}

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= 국문초록 =

혈관평활근 세포에서의 칼륨이온과 카페인의 영향 : 수축과 세포내 칼슘이온 농도에 대하여

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본 연구에서는 세포내의 칼슘이온과 결합하여 형광을 발하는 형광색소 fura 2를 이용하여 세포내의 칼슘이온 농도를 측정함과 동시에 장력을 측정하였으며 이에 대한 카페인과 고농도의 칼륨의 영향을 검토하였다.

72.7 mM의 칼륨 이온은 장력과 세포내 칼슘이온농도에서 각각 지속적인 증가를 보여주었으며, 20 mM의 카페인은 일과성의 빠른 세포내의 칼슘이온농도의 증가에 이어 감소를 보여주었으나 기본치 보다는 높았다. 그러나, 장력에 있어서는 카페인은 일과성의 증가에 이어 기본 장력보다 낮은 감소를 보여주었다.

한편, 칼슘이온 제거 용액에서 칼륨이온은 세포내의 칼슘이온 농도도 장력도 증가 시키지 못하였으나 카페인은 일과성의 세포내의 칼슘이온 농도와 장력의 증가를 보여주었다.

이상과 결과로부터 rat 대동맥에서의 고농도의 칼륨이온에 의한 수축은 세포 밖으로 부터의 칼슘이온 유입에 의한 것이며 반면에 카페인에 의한 수축은 세포내의 칼슘이온저장부위로 부터의 칼슘이온 유리에 의한 것임을 시사한다. 또한, 카페인은 세포내의 수축 단백의 칼슘 이온에 대한 감수성을 저하시키는 듯하다.