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Research Article

Variations in Responses of Vascular Smooth Muscles to Na-K Pump Inhibition

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ABSTRACT

There is a paucity of information concerning the variability of Na^+-K^+-ATPase activity in various vascular preparations. In this study, we have investigated, comparatively, K^+-induced relaxation in different vascular tissues, to establish the heterogeneity of the activity of this enzyme. Isometric contractions of ring preparations of porcine tail artery and rat aorta as well as longitudinal strips of rat portal vein, mounted in 20ml organ baths were studied, under an initial load of 1g, at 37^{o}Cand pH of 7.4. The protocols examined were: Contractile responses to phenylephrine, 80mM K^+ and K^+-free exposure as well as relaxation responses to K^+ following exposure to K^+-free PSS. Phenylephrine, 80mM K^+ and K^+-free exposure elicited contractile responses in all tissue preparations. Following 30 minutes exposure to K^+-free PSS and pre-contraction induced by EC_{70} (M) concentration of phenylephrine, addition of 5mM K^+ elicited relaxation responses only in rat aortic rings and rat portal vein strips. Rings from Porcine tail artery failed to relax to K^+ in all experiments. The magnitude of K^+-induced relaxation was in the order: Rat Portal Vein > Rat Aorta. The times-to-peak of K^+-free-induced contractile responses were 2.0 ± 0.0, 3.5 ± 0.5 and 6.25 ± 3.13 minutes for rat portal vein (n=6), rat aorta (n=6) and porcine tail artery (n=6), respectively. The magnitudes of relaxation in response to 5mM K^+ for rat portal vein and rat aorta were 86.5 ± 16.75 and 60.00 ± 10.0%, respectively while the respective durations of K^+-induced relaxation were: 30 ± 0 and 180 ± 22 seconds (n=6). The results suggest considerable heterogeneity in the activity of Na^+-K^+-ATPase enzyme in vascular smooth muscles from the rat portal vein, rat aorta and porcine tail artery.

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INTRODUCTION

Na^+-K^+-ATPase enzyme plays a major role in the maintenance of membrane potential in excitable tissues (Skou et al, 1992). In vascular smooth muscle, the activity of this enzyme contributes to regulation of transmembrane potential, vascular tone and hence, blood flow (Blaustein, 1988). Inhibition of the electrogenic Na^+-K^+ pump by various agents e.g. ouabain and K^+-free exposure leads to increase in vascular reactivity.

It has been reported that K^+- induced relaxation following a period of K^+-free exposure serves as a functional indicator of Na^+-K^+-ATPase activity (Webb and Bohr, 1978, Ebeigbe 1981). There is a paucity of information in the literature concerning the variability of Na^+-K^+-ATPase activity in various vascular preparations. In this study, we have investigated, comparatively, the effect of K^+- free induce contraction and potassium-induced relaxation in different vascular tissues, to establish the heterogeneity of the activity of this enzyme, in different parts of the vasculature.

MATERIALS AND METHODS

Tissue Preparation:

Segments of aorta and portal vein were obtained from wistarrats which had been freshly sacrificed by

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stunning. Porcine tails were obtained from a local slaughter house, placed in physiological salt solution (PSS) and the arteries dissected out. All arterial tissues were cleaned free of adhering connective tissues and cut into 2-3mm rings. Portal veins were slit open longitudinally. The tissues were placed between L-shaped wire loops in 20ml organ baths containing PSS (Portal veins were attached to the wire loops by means of a thread). The upper loop was attached to a Grass Model FT03 force transducer connected to a Grass Model 7P polygraph (Grass Instruments Co., Quincy, MA, USA). The composition of the PSS was (mM): NaCl 119, KCl 4.7, $NaHCO_3$ 24.9, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.6, glucose 11.5. The PSS was bubbled throughout with 95% O_2 - 5% CO_2 gas mixture with the pH and temperature maintained at 7.4 and 37ºC respectively. K^+-free PSS was prepared by equimolar replacement of K^+ with Na^+. The vessels were given a resting tension of 1g. An equilibration period of 90 minutes was allowed. After equilibration, rings were stimulated twice at 15 minutes intervals with a depolarising solution (80mM K^+); the average of these contractions represented the reference (100%) for comparing subsequent contractile responses (Ebeigbe and Cabanie, 1992).

The protocol for studying K^+-induced relaxation was as follows: vascular preparations were exposed to K^+-free PSS for 30 minutes (this resulted in slowly-developing contractile responses in rat aortic and porcine tail artery rings). Thereafter, K^+ was reintroduced to the bath to induce relaxation. In portal vein strips, pre-contraction was induced by EC_{70} concentration of phenylephrine, five minutes to the end of the K^+-free exposure.

Data are presented as means ± SEM. Graphs and statistical analyses were by means of OriginPro 9.1 software and Student's *t*-test. A *p* value less than 0.05 was considered significant, while *n* values denote number of animals from which vessels were obtained. Tests were carried out on at least six vessel preparations. EC_{70} (concentration producing 70% contraction) values were derived graphically. The time-to-peak of K^+-free contraction was estimated as the interval between application of K^+-free PSS and attainment of peak contraction.

RESULTS

Contractile Responses To Phenylephrine, High K^+ and K^+- free Exposure

Phenylephrine, 80mM K^+ and K^+-free exposure elicited contractile responses in all tissue preparations. The respective EC_{50} values for phenylephrine-induced contractions in rat portal vein (n =8), rat aorta (n = 8) and porcine tail artery (n = 10) are: 0.5 (±0.1) x 10^{-7}, 1.0 (±0.06) x 10^{-7} and 1.3 (±0.2) x10^{-7} M.

K^+-free PSS exposure resulted in contractile responses in rat aortic and porcine tail artery rings. The times-to-peak of K^+-free-induced contractile responses were 2.0 ± 0.0, 3.5 ± 0.5 and 6.25 ± 3.13 minutes for rat portal vein (n=6), rat aorta (n=6) and porcine tail artery (n=6). Fig. 1 shows the time course K^+-free-induced contractile responses in rat aortic and porcine tail artery rings.

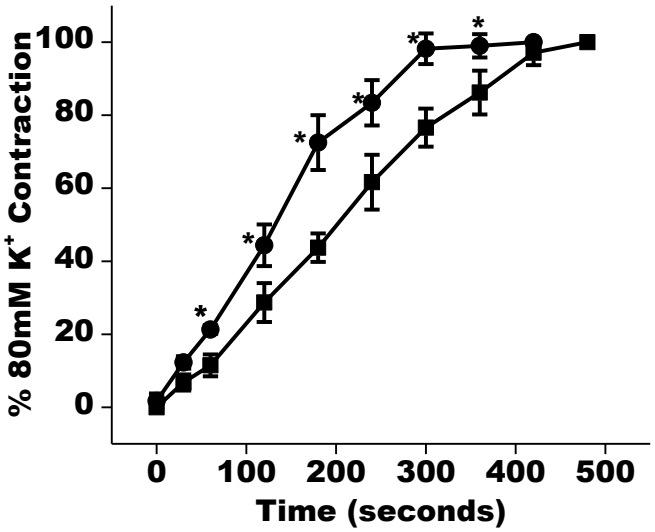

Fig. 1.
Time course of contractions induced by K^+-free exposure in rings from rat aorta (circle, n = 8) and porcine tail arteries (square, n = 6). Values are Means ± SEM; *p<0.05

In rat portal vein strips, K^+-free exposure resulted in increases in the magnitude and frequency of spontaneous contractions (Table 1).

Table 1.
Spontaneous contractions of rat portal vein

Condition (n)	Amplitude (mg)	Frequency (spikes/min)
Normal PSS (8)	380 ± 11.56	24.0 ± 2.6
K^+–free exposure (8)	393 ± 0.68*	42.7 ± 4.1*

Enhanced amplitude and frequency of spontaneous contractions of rat portal veins following exposure to K^+-free PSS. Values are means ± SEM; *p<0.05

K^+-induced relaxation

In tissues exposed to K^+-free PSS, K^+-induced relaxation was examined following pre-contraction induced by EC_{70} concentration of Phenylephrine (rat portal vein and rat aorta) or K^+-free-induced contraction (porcine tail artery). Addition of 5mM K^+ elicited relaxation responses only in rat aortic rings and

rat portal vein strips (Fig. 2). Rings from porcine tail artery failed to relax to K^+ (within the duration observed) in all experiments.

In both the rat portal vein and rat aorta, K^+-induced relaxation was transient, followed by a rebound contraction. The magnitude of 5mM K^+-induced relaxation was in the order: Rat Portal Vein > Rat Aorta (Fig. 3): 86.5 ± 16.75 and 60.00 ± 10.0% respectively for rat portal vein and rat aorta while the respective durations of K^+-induced relaxation were: 30.0 ± 0 and 180.0 ± 12 seconds ($n \geq 6$).

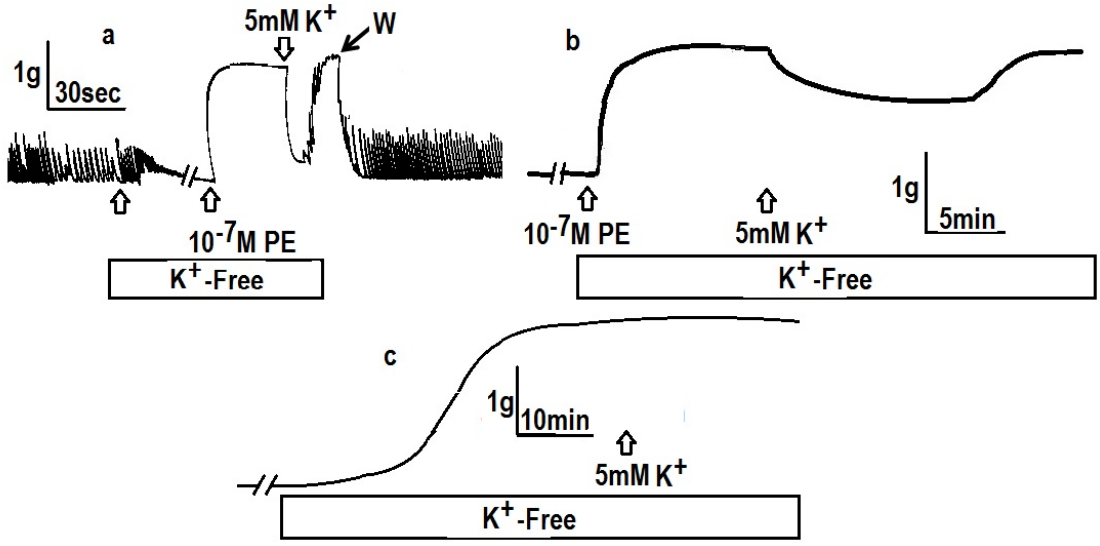

Fig. 2.
Representative tracings showing the relaxation responses induced by addition of 5mM K^+ in rat portal vein strip (a) and rat aortic ring (b) pre-contracted with 10^{-7}M phenylephrine during exposure to K^+-free PSS. Note the rapid and transient relaxation in rat portal vein and the lack of relaxation response in porcine tail artery (c).

DISCUSSION

Inhibition of Na-K pump by K^+-free exposure results in transmembrane ion changes which increase intracellular Ca^{2+} levels and induce vascular smooth muscle contraction (Webb and Bohr, 1978; Ebeigbe, 1981; Lamb et al, 1988). The contractile response induced by K^+-free exposure has been attributed to membrane depolarization and enhanced Ca^{2+} influx via potential sensitive channels, depolarization-induced release of noradrenaline from adrenergic nerve endings as well as raised intracellular Na^+ and Ca^{2+} via Na^+-Ca^{2+} exchanger (Blaustein, 1988; Vanhoutte& Lorenz, 1984; Webb and Bohr, 1978).

This study reports that there is considerable variability in the responses of vascular smooth muscles from the rat portal vein, rat aorta and porcine tail arteries to Na-K pump inhibition.

In the rat portal vein, K^+-free exposure resulted in enhancement of both magnitude and frequency of the spontaneous contractions (Table 1) whereas contractile responses were observed in rat aortic and porcine tail artery rings. It has been reported that the portal vein serves as a model for the study of resistance vessels and that the active rhythmic vasomotion of this vessel is comparable to the rhythmic vasomotion of small resistance and precapillary sphincter vessels (Pegram, 1980). Thus the Na-K ATPase enzyme activity of the rat portal vein is likely to be similar to what obtains in resistance vessels. The faster time-to-peak of K^+-free contractions in rat aortic rings (Fig. 1) compared to porcine tail arteries suggests a greater sensitivity of rat aortic smooth muscle to Na-K pump inhibition. It is possible that there are differences in the membranes of rat aortic smooth muscle and those of porcine tail arteries which permit greater permeability to Ca^{2+} and Na^+ in aortic rings, comparable to reported observations on mesenteric arteries of SHR and WKY rats (Dominiczak and Bohr, 1990).

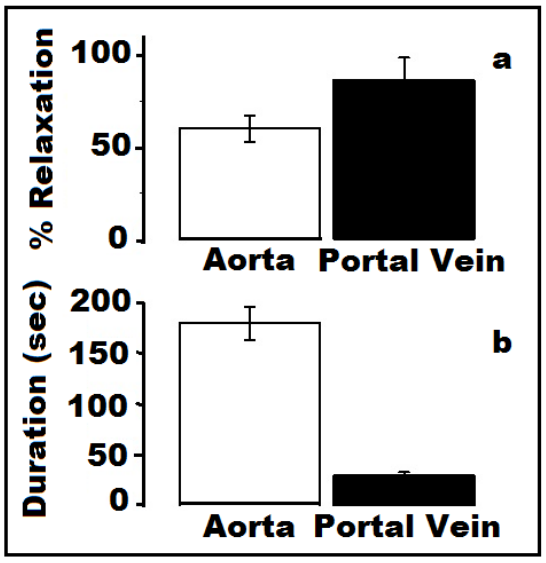

Fig. 3. Magnitude of 5mM K^+-induced relaxation, expressed as percentage of 10^{-7}M phenylephrine pre-contraction (a) and duration of K^+-induced relaxation (b). Values are means ± SEM; n=6), *P<0.05

The rapidity, magnitude and transiency of K^+-induced relaxation in the rat portal vein, which is often employed as a model for resistance arteries (Pegram, 1980), suggest a greater contribution of Na-K ATPase activity to the maintenance of membrane potential in this vessel compared to the rat aorta and the porcine tail artery. The longer duration of K^+-induced relaxation in rat aortic rings (Fig. 3) suggests that the K^+-induced membrane hyperpolarization (and activation of the Na-K pump) occurs at a slower rate. It has also been suggested that the rate of K^+-induced relaxation also reflects contractile protein dephosphorylation and re-establishment of resting intracellular free Ca^{2+} concentration in smooth muscle (Arvola et al, 1992). As shown in Fig. 2, K^+-induced relaxation of rat aortic rings occurs slowly in contrast with the rapid relaxation in rat portal vein. The contraction/relaxation cycle of the rat portal vein depends more on extracellular Ca^{2+} whereas rat aortic smooth muscle contraction is dependent on both influx and intracellularly-mobilized Ca^{2+} (Bolton, 1979; Ebeigbe, 1982a; 1982b); this is perhaps responsible for the differential K^+-induced relaxation between rat portal vein and rat aorta observed in this study. The reason for the observed lack of K^+-induced relaxation response in porcine tail artery is not clear but may be attributed to a passive role of Na-K ATPase enzyme in membrane potential changes in this vascular smooth muscle.

In conclusion, the present study shows that considerable heterogeneity exists in the activity of Na^+-K^+-ATPase enzyme in vascular smooth muscles from the rat portal vein, rat aorta and porcine tail artery

Acknowledgements

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REFERENCES

Arvola P, Porsti I, Vuorinen P, Pekki A, Vapaatalo A (1992). Contractions induced by potassium-free solution and potassium relaxation in vascular smooth muscle of hypertensive and normotensive rats *Br J Pharmacol*. (1992). **106:** 157-165

Blaustein MP (1988). Sodium-calcium exchange and the control of contractility in cardiac muscle and vascular smooth muscle. *J. Cardiovasc. Pharmacol.* 12 (Suppl): S56-S68.

Bolton TB (1979).Mechanisms of action of transmitters and other substances on smooth muscle.Physiol Rev **59**: 606-718

Dominiczak AF, Bohr DF (1990). Cell membrane abnormalities and the regulation of intracellular calcium concentration in hypertension.*Clin. Sci.***79**: 415-423.

Ebeigbe AB (1981). Evidence against an electrogenic mechanism in hypoxic relaxation of vascular smooth muscle.*IRCS Med Sci.* **9:** 497.

Ebeigbe (1982a) Influence of calcium-depletion on ultrastructure and contractility of the rat portal vein.*W Afr J Med***1**: 29-33.

Ebeigbe AB (1982b). Calcium pools for noradrenaline and potassium-induced contractions of rat portal vein. *Canad J PhysiolPharmacol***60**: 1225-1227

Ebeigbe AB, Cabanie M (1992). Responses of isolated human epigastric arteries to histamine.*J AutonNerv Syst.* 39: 201-210.

Lamb FS, Moreland RS, Webb RC (1988). Calcium and contractile responses to ouabain and potassium-free solution in aortae from spontaneously hypertensive rats.*J Hypertens.* **6** (10): 821-8.

Pegram BL (1980). The portal vein as a model for resistance vessels. In: Vascular Neuroeffector mechanisms. Ed by JA Bevan et al. Raven Press, NY.

SkouJC, Esmann M. (1992). The Na^+-K^+-ATPase. *J Bioenerg Biomembr***24**: 249-261.

Vanhoutte PM, Lorenz RR (1984). Na^+, K^+-ATPase inhibitors and the adrenergic neuroeffector interaction in the blood vessel wall.*J CardiovascPharmacol***6** (suppl. 1): S88-S94.

Webb RC and Bohr DF (1978). Potassium-induced relaxation as an indicator of sodium-potassium ATPase activity in vascular smooth muscle.*Blood Vessels***15**: 198-207.