REVISTA ESPAÑOLA DE FISIOLOGIA, 33, 221-226. 1977

Influence of the Ionic Environment on the Responses to Adrenaline and Potassium of the Isolated Rat Tail Artery *

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(Received on January 27, 1977)

J. H. POLIDORO, A. F. HENRY, A. C. TAQUINI, Jr., and E. A. SAVINO. *Influence of the Ionic Environment on the Responses to Adrenaline and Potassium in the Isolated Rat Tail Artery.* Rev. esp. Fisiol., 33, 221-226. 1977.

Isolated non-stripped segments of the rat tail artery, after stimulation by catecholamines or high-K, developed an isometric contraction strong enough so as, to allow easy recording of complete dose response curves. Lowering external Na to 17 % of its standard concentration decreased the responses to the higher range of K concentrations whereas it enhanced those to the lower range of K concentrations. Raising external Na by 38 % or decreasing it 50 % did not alter responses to adrenaline. But, lowering Na enhanced those responses when arteries were rendered hypodynamic by prior immersion in a low-Ca medium. The curves depicting the relationship between Ca concentrations and tension induced by adrenaline or high-K were also studied. Curves of the arteries exposed to adrenaline were placed to the left with respect to those of K.

The present data provide evidences that Na is involved in the excitation-contraction coupling processes of arterial smooth muscle. However, its role is affected when Na concentration is far below the physiological one. The effects of changing Na concentrations vary according to the K and Ca concentrations. It also reinforced the belief that adrenaline may act by increasing the Ca inward movement beyond that elicited by mere depolarization.

The need of a small artery *in-vitro* preparation was remarked some years ago by FRIEDMAN and FRIEDMAN (7). This want was met by using strips of arterioles or small arteries (2) and by perfusing segments of the tail artery of the rat (10). However, contractions obtained by the former procedure were rather weak not allowing easy recording of dose-response curves, whereas employing the latter technique, the contractile responses could be only indirectly measured by counting drops

* This Research was partially supported by a grant of the Secretaría de Ciencia y Técnica.

of the effluent perfusate or recording the vessel diameter.

On the other hand, several studies concerning the influence of the ionic environment on the contractile activity of arterial smooth muscle reported controversial results (1, 3, 5, 6, 10, 17, 21). Then, it was considered interesting to study the effects of changing the external concentrations of Na ([Na]) and Ca ([Ca]) on the contractile responses to K and catecholamines of an almost intact preparation of the ventral tail artery of the rat, recording isometric tension directly. The ultimate aim was to provide further knowledge about excitation-contraction coupling processes of the vascular smooth muscle.

Materials and Methods

Male albino rats 180-220 g body weight were killed by decapitation and the ventral tail artery was immediately removed. Then, using a low-power microscope it was freed of adventitial tissue and when indicated also longitudinally opened. One or two segments of 2 cm length were excised from the middle portion of the artery and then each one suspended in 20 ml organ baths, attached from one end to a glass holder and from the other to the transducer lever arm under a resting tension of 1,500 mg. This tension was applied because it was in the plateau range of the length/tension relationship of the preparation (9). Isometric tension was recorded through a force-displacement strain-gauge connected to a direct writing oscilograph.

The composition of the media employed are shown in Table I. To perform the K dose-response curves, media of different K concentration were made by appropriately mixing the depolarizing solution and the standard or the 17 % low-Na Tris compensated media. All the solutions contained dextrose 11 mM, the pH was 7.4, 95 % O_2-5 % CO_2 was continuously bubbled and the temperature was maintained at 37° C. Drugs were added from concentrated stock solutions.

Table I. *Composition of the incubation media.*

1: Standard medium. 2: 17 %, low-Na Tris-compensated medium. 3: 17 % low-Na Li-compensated medium. 4: 50 % low-Na Tris-compensated medium. 5: 38 % high-Na medium. 6: Depolarizing medium.

Ions	Media					
	1	2	3	4	5	6
Na	145.0	25.0	25.0	72.5	200.0	25.0
K	6.0	6.0	6.0	6.0	6.0	126.0
Ca	2.5	2.5	2.5	2.5	2.5	2.5
Mg	1.3	1.3	1.3	1.3	1.3	1.3
Tris	0.0	120.0	0.0	77.5	0.0	0.0
Li	0.0	0.0	120.0	0.0	0.0	0.0
Cl	127.4	104.5	127.4	118.0	182.3	128.6
HCO_3	25.0	25.0	25.0	25.0	25.0	25.0
PO_4	1.2	0.0	1.2	0.0	1.2	1.2
SO_4	1.2	1.2	1.2	1.2	1.2	1.2

All the preparations were allowed to recover in the standard medium while being stimulated every 25-30 min. Experiments started when stable performance was attained, usually after 2-hours. Drugs used were adrenaline hydrochloride (Parke-Davis), noradrenaline bitartrate (Winthrop) and val-5-angiotensin II amide (Hypertensin, Ciba). All drug concentrations refer to the free base final M concentration in the organ-bath. Results were statistically compared by means of Student's «t» test.

Results

The responses of the tail artery to maximal additions of adrenaline, noradrenaline and K were of about equal strength whereas angiotensin elicited a feeble and transient slow contraction (Fg. 1.). Despite thorough washing, the vessel remained unresponsive to further additions of the peptide, at least over the ensuing 90 min. Cumulative exposure to the catecholamines showed that adrenaline dose-response curve is placed to the left with

respect to that of noradrenaline. It was also observed that noradrenaline curve was shifted leftward when longitudinally opened arteries were used (Fig. 2).

Potassium dose-response curves were made by adding every 25 min, media prepared by mixing the depolarizing and the standard solutions. Maximum contraction was attained at 130 mM K and the vessel was unresponsive at 45 mM K (Fig. 3). To ascertain about the effects of changing [Na] on the K dose-response

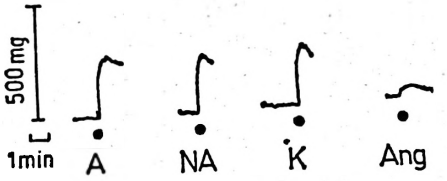

Fig. 1. *Isometric responses to stimulating agents.*
A: Adrenaline 3×10^{-6}. NA: Noradrenaline 1×10^{-4}. K: Depolarizing medium. Ang: Angiotensin 10^{-6}.

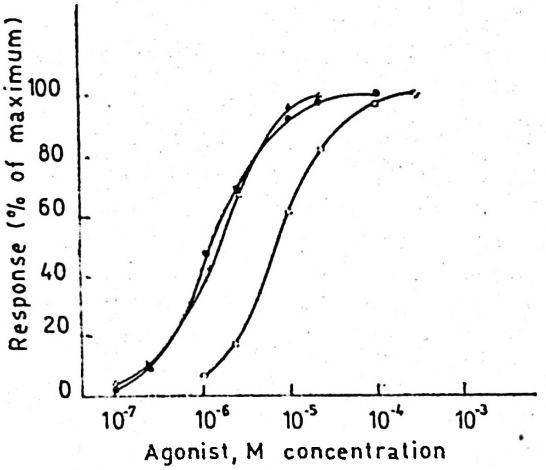

Fig. 2. *Average cumulative dose-response curves.*
O—O Noradrenaline, ED_{50} $7.7 \pm 0.2 \times 10^{-6}$ (n: 6). ●—● Noradrenaline on longitudinally opened arteries, ED_{50} $1.3 \pm 0.3 \times 10^{-6}$ (n: 8), $p > 0.001$ vs. intact arteries. △—△ Adrenaline, ED_{50} $1.0 \pm 0.2 \times 10^{-6}$ (n: 6). Bars represent standard error of the mean.

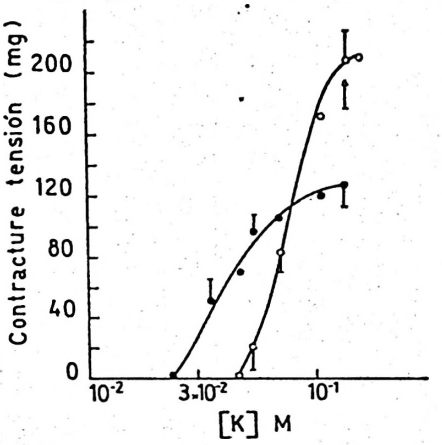

Fig. 3. *Average dose-response curves to K.* O—O Arteries incubated in standard medium (n: 6). ●—● Arteries incubated in the 17 % low-Na Tris-compensated medium (n: 6). △ Control response before starting low-Na incubation.

curves, these were also performed with arteries incubated in the 17 % low-Na Tris compensated medium. Depolarizing solutions were prepared by mixing with this medium, instead of the standard one, to maintain [Na] at 25 mM throughout the experiment. Average curves (Fig. 3) indicate that incubation in the low-Na medium elicited a significant decrease of the maximum contraction whereas the responses to the lower range of K concentrations were enhanced.

To know about the effects of changing [Na] on the responses to stepwise increasing addition of adrenaline, the arteries were stimulated after being immersed for 30 and 60 min in the experimental ionic environment. Raising [Na] to 200 mM without any concomitant osmotic adjustment or using a 50 % low-Na Tris-compensated medium did not alter the strength of contractile responses nor did they shift dose-response curves. Incubation in the 17 % low-Na Tris- or Li-compensated media reduced the maximum response to adrenaline (Fig. 4) without any concomitant shift of the dose-response curves. When experiments were performed with

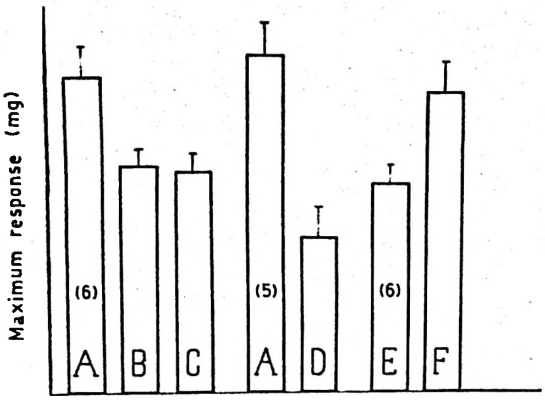

Fig. 4. *Effects of lowering Na on the maximum responses to adrenaline.*
A: Control in standard medium. B and C: After 30 and 60 min respectively, in 17 % low-Na, Tris-compensated medium. D: After 30 min in 17 % low-Na, Li-compensated medium. E: Control in 0.17 mM Ca medium. F: After 30 min in low-Ca, Tris-Compensated low-Na medium. Number of arteries in parenthesis. Bars represent standard error of the mean. B, C, D and F compared to its control, $p > 0.02$.

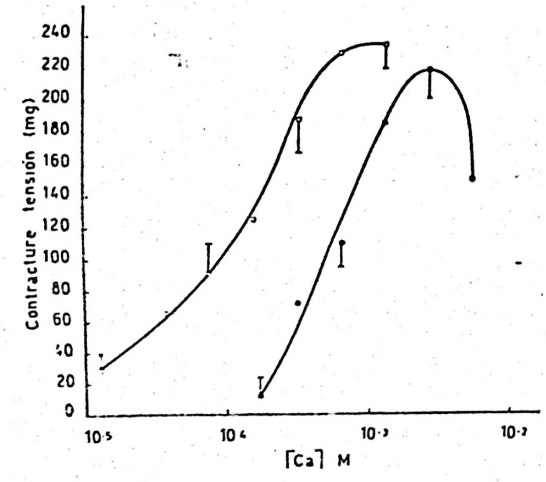

Fig. 5. *Relationship between Ca concentration and contractile responses to adrenaline and K.*
O—O Depolarizing medium, ED_{50} $4.6 \pm 0.4 \times 10^{-4}$ (n: 10). ●—● Adrenaline 3×10^{-5}, ED_{50} $1.3 \pm 0.8 \times 10^{-4}$ (n: 6), $p > 0.001$ vs. arteries in depolarizing medium. Bars represent standard error of the mean.

medium containing 0.17 mM Ca, exposing the arteries to the 17 % low-Na medium elicited an increase of the contraction induced by the adrenaline addition (Fig. 4).

In order to investigate the Ca requirements for the contractile responses to adrenaline and high-K, bathing solutions with different Ca concentrations were changed 30 min before each stimulation. Depolarizing media had the same Ca concentration used in the preceding period. Fig. 5 indicate that the average curve relating [Ca] and contractile force of the adrenaline stimulated vessels is placed leftward with respect to that obtained with the depolarizing medium. Maximum responses were attained at 0.62 and 2.5 mM Ca, and ED_{50} at 0.13 and 0.46 mM Ca with arteries stimulated by adrenaline and high-K respectively.

Discussion

The present experiments showed that the non-stripped segment of the rat tail artery developed, after different stimulations, an isometric contraction strong enough, so as to allow easy recording of complete dose-response curves. As the artery is devoid of longitudinal smooth muscle (8, 9) its responses must reflect, in all probability, the activity of the circular-spiral muscle fibres. Thus, the recorded contractions may be considered as an indirect measurement of the vasoconstrictor potentiality wherein geometric influences such as wall/lumen ratio are avoided.

In agreement with prior observations, using strips of resistance vessels (2) and the perfused tail artery (10), the preparation was more sensitive to adrenaline than to noradrenaline. It was also observed that sensitivity to noradrenaline was enhanced when the arteries were longitudinally opened. Since the agonist could diffuse through the intima, this finding very probably results from the prevention of noradrenaline uptake by the sympathetic

nerves (4) and agrees with prior observations using the perfused tail artery (11).

HINKE *et al.* (11) reported that at 51 mM K the perfusate flow rate of the tail artery was reduced by 85 %. In the present study, maximum contraction was attained at 130 mM K and responses to 51 mM K were near threshold. These data suggest that the preparations differ somewhat. Perfusion at the rather low 50 mm Hg pressure may not be appropriate to record complete dose-response curves of vasoconstrictor ability, probably mainly because when the vessel lumen is almost closed the contractile elements may still be far from being fully activated.

Immersing the arteries in low-Na medium evoked an enhancement of the responses to the lower range of [K] in accordance with prior results (10), whereas it decreased those to the higher [K]; thus suggesting that [Na] effects would vary according to the membrane electric potential. Reactions to low-Na media also suggest that an inward Na current or a Na-Ca exchange mechanism may be involved in electro-mechanical coupling processes.

A 38 % increase or a 50 % decrease of [Na] did not change responses to adrenaline. But, when Na was lowered to 17 % of its standard concentration, the responses declined significantly. These findings do not agree with BOHR (1, 16) and HINKE and WILSON'S (10) observations, but in general coincide with the findings of several other investigators (5, 6, 12, 20, 21). These data indicate that the role of Na gets impaired only when its concentration is far below the physiological one, showing that it is unlikely that [Na] would be involved in the regulation of the vascular tone. On the other hand, upon the hypodynamic arteries by immersion in low-Ca medium, the 17 % low-Na medium increased the responses to adrenaline. In this respect, it may be hypothesized that the effects of low-Na were surmounted by the increase of the ratio [Ca]/[Na], based on LÜTTGAU and NIEDERGERKE'S report (13). When [Ca] is far in excess to that needed for full activation of contraction (>0.62 mM, Fig. 5) this ratio may not be operative, in accordance with the present data with standard [Ca].

The curve depicting the relationship between [Ca] and tension of adrenaline-induced concentrations was placed to the left with respect to that of high-K (Fig. 5). This correlates well with previous reports using the perfused tail artery (11), strips of the rabbit aorta (19) and the portal vein (14, 15), and provides further support to the belief that catecholamines act by increasing Ca inward movement beyond that produced by mere depolarization (17, 18). The present curves are placed to the left when compared to those of HINKE *et al.* (11), probably because of the lower concentration of agonists they used.

Acknowledgement

We thank Miss Norma Infante for her technical assistance.

Resumen

Después de ser estimulados por catecolaminas o altas concentraciones de K^+, los segmentos aislados no estriados de la arteria de la cola de rata desarrollaron una contracción isométrica lo suficientemente fuerte como para permitir el fácil diseño de las curvas correspondientes a las diferentes dosis. El descenso de Na^+ externo a un 17 % de su concentración normal disminuyó las respuestas de K^+ en sus niveles más altos, mejorando las de los niveles más bajos. Elevar el Na^+ externo un 38 % o disminuirlo un 50 % no alteró las respuestas a la adrenalina. Sin embargo, cuando las arterias se hacían hipodinámicas por inmersión previa en un medio bajo de Ca, el descenso de Na mejoraba esas respuestas. También se estudiaron las curvas que muestran la relación entre las concentraciones de Ca y la tensión inducida por la adrenalina o la alta concentración de K.

Los presentes datos evidencian que el Na está involucrado en los procesos acoplantes de excitación-contracción del músculo arterial liso.

Su papel se ve afectado, sin embargo, cuando la concentración de Na es muy inferior a la fisiológica. Los efectos producidos al cambiar las concentraciones de Na varían según las concentraciones de K y Ca. Se confirma que la adrenalina actúa al incrementar el movimiento de Ca hacia el interior por encima del que puede producir la mera despolarización.

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