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TRIS(HYDROXYMETHYL)AMINOMETHANE INHIBITS CALCIUM UPTAKE IN VASCULAR SMOOTH MUSCLE

PRASAD D.M.V. TURLAPATY, BELLA T. ALTURA and BURTON M. ALTURA

Department of Physiology, State University of New York, Downstate Medical Center, Brooklyn, NY 11203 (U.S.A.)

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Summary

This report demonstrates that the commonly used buffering agent Tris-(hydroxymethyl)aminomethane (Tris) in concentrations of 5 and 30 mM inhibits calcium (Ca^{2+}) uptake in both rat aortic and portal venous smooth muscle. The data indicates that total exchangeable Ca^{2+} in portal vein is reduced by about 35% in 5 or 30 mM Tris, while the intracellular exchangeable Ca^{2+} is not significantly altered. On the other hand, in aortic smooth muscle, while 30 mM Tris reduces total exchangeable Ca^{2+} by about 20%, intracellular Ca^{2+} is reduced by 44% in 5 mM Tris and by 55% in 30 mM Tris. The present studies, thus, reveal that Tris exerts significant inhibitory effects on exchangeability and transmembrane movement of Ca^{2+} in at least two different types of smooth muscle.

Tris(hydroxymethyl)amino methane, $H_2NC(CH_2OH)_3$, termed Tris or Tham, is commonly used as a buffering agent in physiological media for studies of the role of Ca^{2+} in excitation-contraction coupling events in muscle. It has also been widely used for the study of calcium fluxes, calcium binding and in the calculation of distribution of this ion in various tissues. This and other artificial buffers have been substituted for phosphate and HCO_3^- in order to prevent precipitation of various di- and trivalent cations, which are helpful in delineating the transmembrane fluxes of calcium and its binding sites.

In the interim, several reports have appeared in the literature describing adverse effects of Tris in various cellular systems. For example, Tris has been shown to: (i) exert inhibitory effects on neuromuscular transmission in smooth and cardiac muscle [1]; (ii) decrease the magnitude of contraction to adrenergic nerve stimulation in vascular tissue [2]; (iii) decrease spontaneous

tone in small arterioles [3]; (iv) attenuate acetylcholine-induced responses in aplysia neurons [4]; (v) interact with substrates and enzymes, thereby modifying cellular metabolism [5]; and (vi) inhibit (Ca^{2+}+Mg^{2+})-ATPase of red blood cells [6]. We have recently reported that Tris buffer inhibits Ca^{2+}- and drug-induced contractile responses in smooth muscle of rat aorta and portal vein and have tentatively concluded that Tris might interfere with the movement of Ca^{2+} [7].

In view of these reports which describe inhibitory effects of Tris on responses in excitable membranes, and the common usage of this buffer in the study of Ca^{2+} movements, there was a need to evaluate, precisely, the effects of Tris on Ca^{2+}, if any. We now report that 5 and 30 mM Tris buffer markedly inhibit exchangeability and uptake of radiocalcium in two types of vascular smooth muscle when compared to that obtained in a phosphate- and bicarbonate-buffered medium.

Aortic strips and portal veins, obtained from male Wistar rats (300—400 g), were set up isometrically in vitro as described previously [8] and equilibrated for 2 h in either normal Krebs-Ringer bicarbonate solution [8] or Tris-Ringer solution [7]. The tissues were aerated with a 95% O_2/5% CO_2 mixture in the case of Krebs-Ringer bicarbonate solution and with 100% O_2 in the Tris-Ringer solution and kept at 37°C at a pH of 7.4. Both Krebs-Ringer bicarbonate and Tris-Ringer solutions were made iso-osmolar [7]. The tissues were then incubated for 30 min in 4-ml muscle chambers, using identical solutions as above and containing 0.016 μCi ^{45}Ca. After this time, each tissue was either rinsed in cold Krebs-Ringer bicarbonate or Tris-Ringer solution for 10 s (conventional method) or for 2, 5 or 10 min in 50 mM La^{3+}, Ca^{2+}-free, 5 mM Tris-Ringer solution (lanthanum method, as modified by Godfraind [9]). The strips were then blotted, weighed, and the ^{45}Ca uptake counted in a Searle Mark III liquid scintillation counter after solubilization with 1 ml NCS solubilizer at 50°C for 6 h. The "conventional method" reveals the total exchangeable Ca^{2+} of the tissues (as verified by uptake experiments for different time periods) [10]. With the "lanthanum method", the ^{45}Ca content is proposed to represent exchangeable intracellular calcium, as the concentration of lanthanum used has been shown to (i) replace Ca^{2+} at superficial binding sites and (ii) block influx and markedly retard efflux of Ca^{2+} within the 5-min contact time [9,11]; the latter has also been observed in the present study.

Table I summarizes the results obtained in Krebs-Ringer bicarbonate solution and Tris-Ringer solutions with the conventional and lanthanum methods. The data indicates that total exchangeable calcium in portal vein has been reduced by about 35% in 5 or 30 mM Tris-Ringer solution, while the intracellular exchangeable calcium was not significantly altered. The inhibition by Tris of spontaneous mechanical activity and drug-induced contractions in this tissue [7] could be explained solely by a reduction in the availability of extracellularly bound Ca^{2+}. The 2 min lanthanum wash experiments indicate that the calcium decrease observed in Tris buffer is, indeed, an extracellular bound fraction (Table I), since in 2 min, extracellular spaces should be depleted equally in Krebs-Ringer bicarbonate and Tris-Ringer-bathed tissue.

In aortic smooth muscle (Table II), the data indicate that while 30 mM Tris-Ringer solution reduces total exchangeable Ca^{2+} by about 20%, intracel-

TABLE I

EFFECTS OF Tris BUFFER ON TOTAL EXCHANGEABLE AND INTRACELLULAR CALCIUM CONTENT OF RAT PORTAL VEIN

Values are given as mmol/kg wet wt. (mean ± S.E.). Numbers in parentheses represent number of different tissues used for each experiment.
The composition of Krebs-Ringer bicarbonate is: 118.0 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 10.0 mM glucose. For Tris-Ringer solutions, phosphate and bicarbonate were eliminated and Tris(hydroxymethyl)aminomethane in 5 or 30 mM concentration was used.

Medium	Total exchangeable calcium	2-min La^{3+} wash (calcium fraction)	5-min La^{3+} wash (cellular calcium)
Bicarbonate-Ringer	3.15 ± 0.25 (18)	2.18 ± 0.12 (6)	1.29 ± 0.25 (8)
Tris-Ringer			
5 mM	2.11 ± 0.17* (6)	1.44 ± 0.09* (6)	1.00 ± 0.11 (8)
30 mM	2.08 ± 0.09* (6)	1.25 ± 0.05* (6)	0.90 ± 0.07 (5)

*Significantly different from paired bicarbonate-Ringer values ($P < 0.001$).

TABLE II

EFFECTS OF Tris BUFFER ON TOTAL EXCHANGEABLE AND INTRACELLULAR CALCIUM CONTENT OF RAT AORTA

Values are given as mmol/kg wet wt. (mean ± S.E.). Numbers in parentheses represent number of different tissues used for each experiment.

Medium	Total exchangeable calcium	2-min La^{3+} wash (calcium fraction)	5-min La^{3+} wash (cellular calcium)
Bicarbonate-Ringer	4.09 ± 0.12 (18)	2.41 ± 0.11 (6)	0.83 ± 0.08 (8)
Tris-Ringer			
5 mM	3.79 ± 0.28 (6)	1.42 ± 0.14* (6)	0.47 ± 0.06* (6)
30 mM	3.29 ± 0.21* (6)	1.16 ± 0.08* (6)	0.37 ± 0.06* (6)

*Significantly different from paired bicarbonate-Ringer solution ($P < 0.005$).

lular Ca^{2+} is reduced by 44% in 5 mM Tris-Ringer and by 55% in 30 mM Tris-Ringer. Contractile inhibition seen with Tris in this issue [7] can also be explained by these results, since intracellular Ca^{2+} is thought to play an important role in agonist-induced contraction in aortic smooth muscle [7,12]. The present studies thus reveal that Tris exerts significant inhibitory effects on exchangeability and transmembrane movement of Ca^{2+} in at least two different types of smooth muscle.

Tris has been shown by others, indirectly, to have effects on this ion. For example, it was seen to: (i) antagonize the effect of calcium in acetylcholine induced-contractile responses of rat skeletal muscle [13]; (ii) decrease ionized Ca^{2+} in red blood cells [14]; and (iii) inhibit Ca^{2+}-induced contractions of potassium-depolarized rat aorta and portal vein [7]. It has also been shown to form stable complexes with divalent metal ions [15]. The molecular structure of Tris suggests that it could bind to the membrane with three negatively charged hydroxyl groups, leaving the positive amino group free to repel the movement of cations across the muscle membranes.

In view of the evidence obtained, in this study, that Tris inhibits Ca^{2+} exchange and movement across smooth muscle cell membranes, conclusions drawn from previous studies may need to be reevaluated. We suggest, therefore, that at the very least, quantitative conclusions as to Ca^{2+} distribution and movement obtained in physiological salt solutions employing Tris as the buffering agent, should be looked at with extreme caution. Alternatively, phosphate and HCO_3^- may be necessary in excitable cells for a normal Ca^{2+}

distribution. Lengthy studies using Tris as a buffer seem injudicious for visualization of Ca^{2+} turnover, binding, and its apportionment.

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