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REPETITIVE TRANSIENTS IN INTRACELLULAR Ca^{2+} IN CULTURED HUMAN VASCULAR SMOOTH MUSCLE CELLS

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SUMMARY

Human uterine vascular smooth muscle cells have been isolated and maintained in culture. When these cells are exposed to bathing solutions with nominally zero sodium, using potassium, *N*-methyl-D-glucamine or Tris as substitutes, repetitive transient increases in intracellular calcium are observed. These transients are abolished when the calcium concentration of the bathing solution is reduced to nominally zero suggesting a role for extracellular calcium in the activation or maintenance of the transients. The hypothesis is proposed that the underlying mechanism involves a calcium influx through the reversed operation of a sodium–calcium exchange mechanism and the cyclical activation of calcium-induced calcium release from the sarcoplasmic reticulum. Noradrenaline (10^{-6} M) and caffeine (20–30 mM) reversibly inhibited the transients. The inhibitory action of these agents could not be mimicked by dibutyryl cAMP suggesting that cAMP does not mediate the inhibition. Caffeine alone had no effect on resting calcium. Thimerosal (1–100 μM), an agent thought to activate a second type of calcium-induced calcium release mechanism activated repetitive transient increases in intracellular calcium which behave in a similar manner to those activated by sodium removal. These data are consistent with the presence of a thimerosal-activated calcium-induced calcium release mechanism in these cultured human cells. It is proposed that this mechanism is different from the calcium-induced calcium release mechanism, described in other cell types, which is activated by caffeine.

INTRODUCTION

The contractile state of vascular smooth muscle is regulated by the concentration of intracellular free calcium ($[Ca^{2+}]_i$). The mechanisms by which Ca^{2+} is elevated can involve either the entry of extracellular Ca^{2+} through voltage- or ligand-gated ion channels or by the release of Ca^{2+} from intracellular stores. It is now evident that intracellular Ca^{2+} is stored in at least two different releasable pools, defined by the primary mechanism which brings about its release. In some cell types it has been demonstrated that there is an inositol trisphosphate (IP_3)-sensitive pool while in other cells there may be a second pool, activated by a Ca^{2+}-induced Ca^{2+} release (CICR) mechanism (Berridge, Cobbold & Cuthbertson, 1988). The functional significance of these different pools in vascular smooth muscle is not clear.

In many cell types in which a CICR mechanism has been identified, it has been shown to be sensitive to methylxanthines. For example in cardiac and skeletal muscle, caffeine is known to elevate $[Ca^{2+}]_i$ by increasing the permeability of the sarcoplasmic reticulum to

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