THE ROLE OF CALCIUM IN SQUALUS ACANTHIAS RECTAL GLAND STIMULATION BY CYCLIC AMP

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Dibutyryl cyclic AMP (db-cAMP) stimulates chloride secretion by the shark rectal gland. We explored the possible role of calcium and calmodulin in the activation of transcellular chloride transport in isolated rectal gland cells stimulated by db-cAMP. We used inhibitors of cell membrane Ca^{++} transport (verapamil, nifedipine and diltiazem), inhibitors of calmodulin (W-13, calmidazolium) and 8-(N,N-Diethylamino) octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB-8), a compound reported to block the release of calcium from sarcoplasmic reticulum in skeletal and cardiac muscle. In addition, we used Quin-2/AM, a compound which fluoresces when it binds calcium, to determine whether large changes in intracellular calcium were responsible for the stimulation.

Isolated cells were prepared as previously described (MDIBL Bull. 20:38-39, 1980). The only modification was the addition of 70mM trimethylamine oxide (TMAO) to all solutions.

Oxygen consumption was measured as an indicator of transport activity in a volume of 2 ml (100 microliters of cells plus 1.90 ml shark Ringers) at 25° C using a polarographic oxygen electrode. The cells were incubated for thirty minutes to achieve a steady baseline, and then were stimulated with 1mM db-cAMP and 0.5mM theophylline. Stimulated oxygen consumption was determined 20 minutes after addition of the db-cAMP. Micromoles oxygen consumed per gram of cells per hour was determined using the slope of the oxygen consumption, the volume of solution in the chamber and the weight of cells. Compounds to be tested were added during the initial incubation period unless otherwise specified.

Quin-2/AM was loaded into the cells by incubating 300 microliters of the cell suspension for one hour in 2.7ml of shark Ringers with 30 micromolar Quin-2/AM at 25°. The cells were then washed two times and resuspended in shark Ringers. Aliquots were added to 2ml of Ringers and fluorescence measured at an excitation wavelength of 339 and emission wavelength of 490 using an Aminco-Bowman Spectrophotofluorometer.

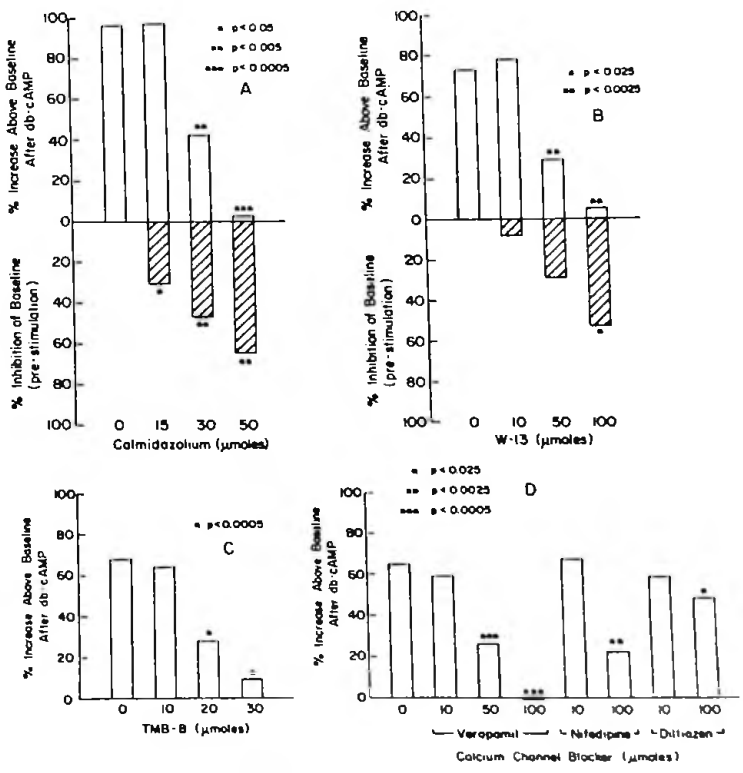
Baseline oxygen consumption, though highly variable from one group of cells to the next, was consistently inhibited by 10^{-4}M ouabain to 76±6% of the control value. db-cAMP produced a stimulation (reported as percent increase above baseline) of 70± 23%. This entire increase was ouabain-sensitive, with return of the oxygen consumption to 68±9% of the original baseline after the addition of 0.1mM ouabain. Calmidazolium inhibited db-cAMP

stimulation of oxygen consumption at concentrations equal to or greater than 3 x 10^{-5}M, with little or no effect at 1.5 x 10^{-5}M. However, the baseline oxygen consumption was also significantly decreased by concentrations of calmidazolium as low as 1.5 x 10^{-5}M (Figure 1-A).

Another calmodulin antagonist, N-(4-aminobutyl)-5-chloro-2-naphthalenesulfonamide hydrochloride (W-13), reportedly a more specific inhibitor, had no effect on stimulation of oxygen consumption at a concentration of 10^{-5}M but at 5 x 10^{-5}M and 10^{-4}M it inhibited stimulation significantly (Figure 1-B). W-13 decreased baseline oxygen consumption only at concentrations equal to or greater than 10^{-4}M. W-12, a W-13 analogue which (due to the deletion of a chloride ion) is a much weaker inhibitor of calmodulin, had no significant effect on either the baseline oxygen consumption or stimulated oxygen consumption at concentrations or 10^{-5}M, 5 x 10^{-5}M or 10^{-4}M.

TMB-8 was found to inhibit stimulation significantly at concentrations above 10^{-5}M (Figure 1-C). The baseline oxygen consumption was not affected by concentrations up to 10^{-4}M.

Verapamil inhibited db-cAMP stimulation of oxygen consumption by the rectal gland cells at concentrations of 5 x 10^{-5}M or 10^{-4}M. Nifedipine and diltiazem had less effect with only moderate inhibition at a concentration of 10^{-4}M (Figure 1-D). None of the Ca^{++} channel blockers tested had a significant effect on baseline oxygen consumption.



After loading with Quin-2/AM, the cells were found to have normal baseline oxygen consumption with a moderately decreased response to db-cAMP (40% increase with stimulation versus a normal increase of 70±23%). Baseline fluorescence increased by approximately 14% when the cells were lysed with triton in the presence of 2.5mM calcium, and decreased by approximately 30% when lysed in the presence of 10mM EGTA. The addition of 1mM db-cAMP and 0.5mM theophylline did not increase the level of Quin-2 fluorescence,

implying no detectable change in intracellular calcium concentrations when transcellular chloride transport is activated. TMB-8 did not cause any appreciable change in fluorescence either.

These data must be interpreted in light of the probability that calcium-binding proteins such as calcium channels, protein kinases and calmodulin have similarities in molecular structure. Pharmacological agents that inhibit one class of calcium-related proteins are therefore likely, at high concentrations, to inhibit others. Our results strengthen the likelihood that calmodulin (known to be present in rectal gland cells) and calcium are involved in stimulated secretion. Large changes in intracellular ionic calcium were not detected by the fluorescence technique we used.