CYTOSOLIC Ca^{2+}, A SECOND MESSENGER IN THE RECTAL GLAND TUBULE OF *SQUALUS ACANTHIAS*

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Isolated tubules of rectal glands where perfused in vitro (Greger, R., et al., *Pfluegers Arch. Eur. J. Physiol.* 402:376-384, 1984). Transepithelial voltage (V_{te}), transepitelial resistance (R_{te}), and equivalent short circuit current (I_{SC}), basolateral membrane voltage (V_{bl}), whole-cell conductance (G_m), and membrane capacitance (C_m) were measured by established techniques (Greger, R. and Schlatter E., *Pfluegers Arch. Eur. J. Physiol.* 396:315-324, 1983; Greger, R. and Schlatter E., *Pfluegers Arch. Eur. J. Physiol.* 402:63-75, 1984; Hug, M. et al., *Pfluegers Arch. Eur. J. Physiol.* 434:779-784, 1997). In addition, cytosolic Ca^{2+} in these in vitro perfused tubules was monitored by Fura-2 fluorescence using a filter-wheel photon counting system (Nitschke, R., et al., *Pfluegers Arch. Eur. J. Physiol.* 417:622-632, 1991). Data are expressed as mean ± 1 SEM. Paired students t-test was used to examine for statistical differences. A P-value of < 0.05 was chosen to indicate statistical significance.

Carbachol increased cytosolic Ca^{2+} activity in a concentration dependent manner with a half maximal effect at 10 µmol/l (n=5). The Ca^{2+} transients were characterized by a rapid spike and a plateau phase. The spike corresponded to store release and the plateau to influx of Ca^{2+} from the extracellular space. This influx was voltage-dependent inasmuch as a depolarization of the cells by an increase in bath K^+ concentration to 60 mmol reduced the plateau significantly (n=5). Also, a reduction in the bath Ca^{2+} concentration to 10 µmol/l abolished the plateau (n=8). Carbachol increased I_{sc} significantly from -208 ± 43 to -399 ± 81 µA/cm^2 (n= 34). It had no further effect in RGTs previously stimulated by cAMP: -951 ± 144 to -996 ± 136 µA/cm^2 (n=16).

In paired experiments carbachol reduced transepithelial resistance from 14.2 ± 1.0 to 13.5 ± 0.92 Ωcm^2 (n=34) and hyperpolarized V_{bl} significantly from -87 ± 1 mV to -90 ± 1.5 mV (n=18). It had a similar effect on membrane voltage in whole-cell patches (-87 ± 1.9 versus -91 ± 2.1 mV (n=15)) and increased membrane conductance significantly from 14 ± 1.8 to 16 ± 1.7 nS (n=11). C_m was unaltered with 4.83 ± 0.29 pF versus 4.95 ± 0.30 pF (n=12). These data are compatible with the conclusion that carbachol, by an increase in cytosolic Ca^{2+} activity, increases a basolateral K^+ conductance in these cells.

Next, we examined why carbachol loses its effect when the rectal gland tubule is stimulated by cAMP producing agonists such as VIP or adenosine (cf. above). Fura-2 fluorescence measurements indicated that adenosine increases cytosolic Ca^{2+} activity with a half maximal effect at 20 µmol/l (n=4-7). This increase in cytosolic Ca^{2+} activity was much slower than that caused by carbachol. It was dependent on the presence of extracellular Ca^{2+} suggesting that it was due to increased Ca^{2+} influx. A similar effect on cytosolic Ca^{2+} was exerted by VIP (1 µmol/l, n=8) by 8-(4-chlorophenylthio)-adenosine 3':5'-cyclic monophosphate (= 8CPT-cAMP, 500 µmol/l, n=5) and by a combination of 8CPT-cAMP and forskolin (500 µmol/l and 10 µmol/l, respectively, n=10).

These data indicate that Ca^{2+} transduction is important for secretion in rectal gland tubule cells in two ways: 1. Agonists, like carbachol, and ATP (data not shown) activate secretion by

releasing Ca^{2+} from stores and increasing Ca^{2+} influx. These agonists only increase the basolateral K^+ conductance. 2. The agonists acting via cytosolic elevation of cAMP have a dual effect: they enhance the luminal Cl^- conductance and by increasing cytosolic Ca^{2+} also increase a basolateral K^+ conductance. These data explain why the agonist carbachol can only be effective if the gland is not maximally stimulated by agonists acting via cAMP. This study has been supported by DFG Gr 480/12.