A DECREASE IN THE RATE OF Na^+/Ca^{2+} EXCHANGE LEADS TO INCREASED CYTOSOLIC Ca^{2+} ACTIVITY $[Ca^{2+}]_i$ AFTER cAMP STIMULATION IN THE RECTAL GLAND OF *SQUALUS ACANTHIAS*

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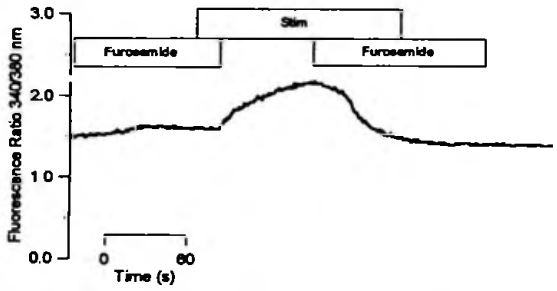

Last year we have reported that $[Ca^{2+}]_i$ is increased in rectal gland tubule (RGT) cells by cAMP. This increase in $[Ca^{2+}]_i$ is important for stimulation-secretion-coupling inas-much as it enhances the K^+ con-ductance of the basolateral mem-brane and thus the driving force for Cl^- secretion across the luminal membrane (Warth, R. et al.: Pfluegers Arch Eur J Physiol 436:133-140, 1998). The finding in RGT was surprising because we had found very recently that $[Ca^{2+}]_i$ falls in colonic crypt cells when secretion of Cl^- is stimulated by cAMP (Fischer K.G. et al.: Pfluegers Arch Eur J Physiol 432:735-740, 1996). We have also shown that the cAMP-mediated fall in $[Ca^{2+}]_i$ in colonic crypts is due to the depolarization and argued that the same should occur in RGT. In the present study we have examined further the effect of cAMP stimulation on RGT $[Ca^{2+}]_i$.

RGT were perfused *in vitro* and fura-2 fluorescence was measured as reported recently (Greger, R. et al. Bull Mt Desert Isl Biol Lab 23:8-10, 1983, Warth, R. et al. Pfluegers Arch Eur J Physiol 436:133-140, 1998). First we confirmed our previous finding (Warth et al.: Pfluegers Arch Eur J Physiol 436:133-140, 1998) that addition of a stimulation „cocktail" (Stim) consisting of 0.5 mmol/l cAMP, 0.5 mmol/l adenosine and 10 µmol/l forskolin caused an increase of the fura-2 fluorescence ratio 340/380 nm (FFR corresponding to $[Ca^{2+}]_i$) from 1.25 ± 0.03 to 1.99 ± 0.07 (n = 27). This, according to pertinent calibrations, corresponds to a very substantial increase in $[Ca^{2+}]_i$ by one order of magnitude.

Next we examined whether the known increase in cytosolic Na^+ activity (Greger, R. et al. Pfluegers Arch Eur J Physiol 402:376-384, 1984) may be causally involved in this $[Ca^{2+}]_i$ increase. This would be expected if Na^+/Ca^{2+} exchange was of relevance for the regulation of $[Ca^{2+}]_i$. Thus, the stimulation solution was added in the presence of furosemide ($3x10^{-4}$ mol/l). As evident from Fig. 1 furosemide inhibited the increase in $[Ca^{2+}]_i$. Removal of furosemide led to the expected increase of $[Ca^{2+}]_i$, and re-addition of furosemide reduced $[Ca^{2+}]_i$. In a larger series of experiments FFR was 1.46 ± 0.07 (n = 9) in the presence of furosemide and 1.42 ± 0.07 (n = 18) in the presence of furosemide and Stim. These data suggest that the inhibition of Cl^- secretion by furosemide and hence low cytosolic Na^+ activities prevent the cAMP induced increases in $[Ca^{2+}]_i$.

If Na^+/Ca^{2+} exchange was of importance for the regulation of $[Ca^{2+}]_i$ in RGT one would predict that this export of Ca^{2+} would oppose Ca^{2+} influx via store controlled Ca^{2+} influx channels. Such channels are of importance for Ca^{2+} transduction in RGT with cholinergic stimulation and after cAMP stimulation (Warth, R. et al. Pfluegers Arch Eur J Physiol 436:133-140, 1998).

Therefore we have examined the question of whether the increased $[Ca^{2+}]_i$ plateau observed with cholinergic stimulation (carbachol (CCH) 10^{-4} mol/l) in RGT was dependent on extracellular Na^+. CCH led to the previously described spike and plateau phase. The FFR in the plateau phase was 1.45 ± 0.1 ($n = 6$). In these experiments FFR was increased markedly when bath Na^+ was reduced to 5 mmol/l to 2.28 ± 024 and it fell to 1.27 ± 0.07 when CCH was removed and Na^+ re-added to the bath. These data confirm the hypothesis.

The present results underline the importance of Na^+/Ca^{2+} exchange for $[Ca^{2+}]_i$ homeostasis in RGT. This exchange is probably one of the major Ca^{2+} extrusion systems in this gland. The rate of this exchanger is determined by the electrochemical driving forces for Ca^{2+} and Na^+: a reduction of bath Na^+ as well as an increase in cytosolic Na^+ activity reduce its rate. The former will never occur physiologically, the latter is of high physiological importance. We have shown that stimulation enhances cytosolic Na^+ activity very substantially. This increase in cytosolic Na^+ activity slows Ca^{2+} export and hence leads to an increase in $[Ca^{2+}]_i$. Furthermore, with cholinergic stimulation of the gland the $[Ca^{2+}]_i$ plateau will be higher whenever the gland is also stimulated by cAMP and cGMP mobilizing agonists (eg. VIP, CNP) because the increase in cAMP or cGMP will increase cytosolic Na^+ activity and reduce Na^+ dependent Ca^{2+} extrusion.

Ca^{2+} appears to be an important second messenger for Cl^- secretion even in this gland. Its role in other primarily Ca^{2+} controlled glands has been beyond any discussion for many years (Petersen, O.H. J Physiol (Lon) 448:1-51, 1992). In the rectal gland this has probably not been realized because the two pathways: cAMP /cGMP and Ca^{2+} appear to interact in co-operative fashion. Whilst eg. in the colonic crypt cell cAMP alone reduces $[Ca^{2+}]_i$, full co-operation of both pathways requires the presence of cAMP producing and Ca^{2+} releasing agonists such as acetylcholine or ATP. The situation in the rectal gland is quite different. Here the mobilization of cAMP alone secures an increase in $[Ca^{2+}]_i$ and fully supports upregulation in both, the cAMP-mediated Cl^- conductance and the Ca^{2+}-controlled K^+ conductance. This may also explain why the Ca^{2+} transduction in this gland has not been noticed for so many years.

Supported by Deutsche Forschungsgemeinschaft: grant Gr 480/13 to RFG.