PANCREATIC EPITHELIAL FLUID AND BICARBONATE SECRETION IS SIGNIFICANTLY ELEVATED IN THE ABSENCE OF PHERIPHERAL SEROTONIN

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Dear Editor,

We read the manuscript by Sonda et al.[1] recently published in Gut with great interest. The authors elegantly demonstrated that lack of peripheral serotonin (5-HT) in tryptophan hydroxylase 1 knockout ($TPH1^{-/-}$) mice remarkably limited pancreatic damage and leukocyte infiltration during the early phase of cerulein-induced acute pancreatitis (AP) and identified 5-HT as an important regulator of zymogen secretion in acinar cells. Although the study was very comprehensive, the ductal function of $TPH1^{-/-}$ mice was not investigated, which might be another key player in this protection. Notably, 5-HT was shown to inhibit fluid and HCO_3^- secretion of pancreatic ductal epithelial cells (PDEC)[2] that play pivotal role in pancreatic physiology and can influence the severity of AP[3]. Thus we investigated the possible alterations of pancreatic ductal secretion in $TPH1^{-/-}$ mice.

To achieve our aims intra/interlobular pancreatic ducts were isolated from the pancreas of wild type ($TPH1^{+/+}$) and $TPH1^{-/-}$ mice. HCO_3^- secretion was measured by three different, but complementary methods using microfluorometry[4], whereas fluid secretion was measured by the swelling technique using videomicroscopy[3]. *In vivo* basal pancreatic fluid secretion was determined in anesthetized mice[3].

The alkali-load technique (basolateral administration of 20mM NH_4Cl) revealed that the apical bicarbonate secretion was markedly increased in $TPH1^{-/-}$ mice compared to $TPH1^{+/+}$ (Fig.1.A,D). Notably, *in vitro* administration of 0.1µM 5-HT impaired the recovery in both cases (not shown). The inhibitory-stop method (basolateral administration of 0.2mM dihydro-4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (H_2DIDS) and 0.2mM amiloride) confirmed the marked increase of bicarbonate efflux in $TPH1^{-/-}$ mice (Fig.1.B,E).

After that we provided direct evidence that the Cl^-/HCO_3^- exchanger is crucial in the elevated ductal secretion, since the rate of pH_i recovery was significantly elevated in $TPH1^{-/-}$ mice after Cl^- withdrawal from the lumen of microperfused pancreatic ducts (Fig.1.C,F).

These three independent, but complementary methods clearly demonstrated an increased pancreatic bicarbonate secretion in $TPH1^{-/-}$ mice.

In the next step we compared the *in vitro* and *in vivo* pancreatic fluid secretion in $TPH1^{+/+}$ and $TPH1^{-/-}$ mice. Using sealed pancreatic ducts we showed that administration of HCO_3^- resulted in an immediate increase of the relative luminal volume, which was further increased by the administration of 5µM forskolin and 100µM 3-isobutyl-1-methylxanthine (IBMX). These experiments showed that both bicarbonate and secretagogue-induced *in vitro* fluid secretion is significantly elevated in $TPH1^{-/-}$ mice (Fig.2.A). *In vivo* pancreatic ductal fluid secretion was

analyzed in anesthetized mice, which was 0.33±0.05ml/hour/bwkg in $TPH1^{-/-}$ mice, almost twice as much as the $TPH1^{+/+}$ (0.18±0.017ml/hour/bwkg) (Fig.2.B).

These findings indicate that the fluid and HCO_3^- secretion is markedly increased in the absence of peripheral 5-HT, which may also contribute to the decreased severity of AP in $TPH1^{-/-}$ mice, besides the altered cytoskeleton dynamics of pancreatic acinar cells.

Figure 1. Pancreatic ductal bicarbonate secretion is markedly increased in $TPH1^{-/-}$ mice. **(A-C) pH_i traces** of PDEC in standard HCO_3^-/CO_2 solution. **(A)** Recovery from alkalosis during NH_4Cl administration, **(B)** the rate of acidosis caused by H_2DIDS and amiloride, **(C)** the rate of recovery after luminal Cl^- removal. **Bar charts** show summary data for the **(D)** base fluxes [-J(B^-/min)] (calculated from the dpH/dt as described earlier[4]) afterNH_4Cl, and **(E)** transporter inhibitor administration, or **(F)** luminal Cl^- removal. Data are shown as means ± SEM from 35-50 ROIs in 8-10 ducts. a: $p<0.01$ vs. $TPH1^{+/+}$.

Figure 2. Pancreatic fluid secretion is significantly increased in $TPH1^{-/-}$ mice. (A) Changes in the relative luminal volume of pancreatic ducts from $TPH1^{+/+}$ (red line, n = 8 from three animals) and $TPH1^{-/-}$ (blue line, n = 8 from three animals) mice. **(B)** The volume of pancreatic juice collected *in vivo* from anesthetized $TPH1^{+/+}$ and $TPH1^{-/-}$ mice. Data are shown as means ± SEM, n:5/group; a: $p<0.01$ vs. $TPH1^{+/+}$.

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