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World J Gastroenterol 2003;9(11):2505-2508
World Journal of Gastroenterology
 ISSN 1007-9327

• BASIC RESEARCH •

# Effect of tetramethylpyrazine on exocrine pancreatic and bile secretion

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**Received:** 2003-05-11 **Accepted:** 2003-08-19

## Abstract

**AIM:** To investigate the effect of tetramethylpyrazine (ligustrazine, TMP) on the secretion of exocrine pancreas (and biliary).

**METHODS:** In *in vivo* study, we investigated the effect of TMP on the secretion of pancreatic-bile juice (PBJ) in rats. Using human pancreatic duct cell line, CAPAN-1, combined with the short-circuit current ($I_{SC}$) technique we further studied the effect of TMP on the pancreatic anion secretion.

**RESULTS:** Administration of TMP (80 mg/kg, ip) significantly increased the secretion of PBJ ($P<0.05$), but the pH of PBJ and the secretion of pancreatic protein were not significantly affected. Basolateral addition of TMP produced a dose-dependent increase in $I_{SC}$ ($EC_{50}$=1.56 mmol/L), which contained a fast transient $I_{SC}$ response followed by a slow decay. Apical application of $Cl^-$ channel blockers, DPC (1 mmol/L), decreased the response by about 67.1 % ($P<0.001$), whereas amiloride (100 μmol/L), a epithelial sodium channel blockers, had no effect. Removal of extracellular $HCO_3^-$ abolished TMP-induced increase in $I_{SC}$ by about 74.4 % ($P<0.001$), but the removal of external $Cl^-$ did not. Pretreatment with phosphodiesterase inhibitor, IBMX(0.5 mmol/L), decreased the TMP-induced $I_{SC}$ by 91 % ($P<0.001$).

**CONCLUSION:** TMP could stimulate the secretion of PBJ, especially pancreatic ductal $HCO_3^-$ secretion via cAMP or cGMP-dependent pathway. It need further study to investigate the roles of cAMP or cGMP in the effect of TMP on the secretion of exocrine pancreas.

Zhao WC, Zhu JX, Tang N, Gou YL, Rowlands DK, Chung YW, Xing Y, Chan HC. Effect of tetramethylpyrazine on exocrine pancreatic and bile secretion. *World J Gastroenterol* 2003; 9 (11): 2505-2508
http://www.wjgnet.com/1007-9327/9/2505.asp

## INTRODUCTION

Tetramethylpyrazine (TMP, $C_8H_{12}N_2$, molecular mass 136.20 u, also known as ligustrazine) is an active alkaloid contained in the rhizome of *Chuanxiong*[1]. TMP has been widely used for the treatment of patients with cardiovascular and cerebrovascular diseases[1-5]. Its proposed pharmacological actions include antagonizing calcium mobilization[6], inhibiting platelet aggregation[7] and increasing intracellular cAMP level by inhibiting phosphodiesterase activity[8]. Recently it has been reported that TMP has antioxidant effect, reducing free radical generation[9,10] and decreasing nitric oxide production[11,12]. However, the effect of TMP on the pancreatic exocrine secretion are unknown. Since TMP is known to activate cAMP, it may act similarly to secretin, a physiological regulator of pancreatic secretion by elevating intracellular cAMP to act on pancreatic epithelial CFTR, which is a cAMP/cGMP-regulated $Cl^-$ channel[13,14] and involved in pancreatic $HCO_3^-$ secretion. In the present study, we investigated the effect of TMP on the secretion of pancreatic-bile juice (PBJ), which includes both pancreatic protein and $HCO_3^-$. We also undertook the present study using the short-circuit current ($I_{SC}$) technique to investigate TMP effect on $HCO_3^-$ secretion by human pancreatic duct cell line, CAPAN-1, which retains most of the properties of pancreatic duct cells[15,16].

## MATERIALS AND METHODS

### *Materials*

Hydrochloride tetramethylpyrazine was purchased from the First Chengdu pharmaceutical Factory (Chengdu, China). RPMI 1640 medium and fetal bovine serum, trypsin-EDTA were supplied by Gibco Laboratories (New York). Hanks' balanced salt solution (HBSS), diphenylamine-2, 2'-dicarboxylic acid (DPC), glucose, calcium gluconate, N-2-hydroxethypiperazine-N'-2-ethanessulfonic acid (HEPES), sodium bicarbonate, DMEG, penicillin-streptomycin (P/S) and Bradford reagent were supplied by Sigma Chemical (St.Louis, MO). Calcium chloride, magnesium sulfate, potassium chloride, sodium chloride, were obtained from Merck (Darmstadt, Germany). Potassium gluconate and sodium gluconate were from BDH Chemicals (Poole, England). Tris was from Amersham Biosciences (Stockholm, Sweden).

### *Methods*

**Animal preparation** Animal experimentation was conducted according to institutional guidelines. Adult male Sprague-Dawley rats, weighing 220-280 g, were housed under controlled temperature (23 ℃) and fasted for 12 h with free access to water before surgery. Midline abdominal incisions were made under xylazine and ketamine anesthesia (13 and 87 mg /kg body weight, respectively, im), followed by insertion of a polyethylene tube into the proximal duodenum for diversion of PBJ to the duodenum. A pancreatic duct cannula was made by inserting a polyethylene tube at the junction between the pancreatic duct and the duodenal wall for collection of PBJ[17-19].

**Collection and measurement of exocrine pancreatic-bile secretions** The animals were divided into two groups randomly: TMP group (TMP 80 mg/kg, ip. pH 2.3) and control

group (9 g/L NaCl solution, ip. pH 2.3). After a 30-minute stabilization period, pancreatic-bile secretions were collected every 15 minutes for 90 minutes. The volume was measured by a 1mL syringe and the pH value of PBJ was determined by a pH analyzer. 10 μL of PBJ was taken and diluted for pancreatic protein determination using spectra MAX 250 and Brandford reagent. The remaining undiluted PBJ was pumped into the duodenum via the duodenal cannula during the next collection period. Administrations were given after 2-time collections (30 minutes) and the second 15-minute-secretion was the basal PBJ secretion.

**Cell culture** Experiments were performed on the human pancreatic duct cell line, CAPAN-1 at a passage of 50-58. Cells were grown in RPMI 1640 medium with 200 mL/L fetal bovine serum and 10 g/L penicillin-streptomycin (P/S) as described previously[20,21]. Briefly, a volume of 0.2 mL of the cell suspension ($1.5\times10^9$/L) was plated onto each permeable support, which was made of a millipore filter and a silicon ring with a confined area of 0.45 $cm^2$, floating on culture medium and incubated at 37 ℃ with 50 mL/L $CO_2$ and 950 mL/L $O_2$ for 7-8 days. The cells were used for $I_{SC}$ once the monolayers reached confluence.

**Short-circuit current measurement** The basic principles of the short-circuit current experiments performed in the present study were the same as previously described[20,21]. Monolayers grown on permeable supports were clamped vertically between two halves of the Ussing chamber and bathed in Krebs-Henseleit (K-H) solutions with following compositions (mmol/L): NaCl, 117; KCl, 4.7; $MgSO_4$, 1.2; $KH_2PO_4$, 1.2; $NaHCO_3$, 24.8; $CaCl_2$, 2.56; Glucose, 11.1; with an osmalarity of 285 gassed with 950 mL/L $O_2$ and 50 mL/L $CO_2$. In some experiments, gluconate was used to replace $Cl^-$. For $HCO_3^-$-free solution, HEPES and Tris were used and the solution was gassed in air. All the electrodes were connected to the voltage-current clamp amplifier. The signal output from the amplifier was the $I_{SC}$ measured and recorded online by chart recorders. A 0.1 mV voltage pulse was applied intermittently across the epithelium and the transepithelial resistance was calculated from the corresponding current changes.

### *Statistical analysis*

The data were collected and analyzed by SPSS statistical package 10.0. The results were expressed as $\bar{x}\pm S_{\bar{x}}$. Comparisons between groups of data were carried out using Student' s paired or unpaired *t*-test. Comparisons in one group of data were carried out using One-way ANOVA. A *P*-value less than 0.05 was considered to be statistically significant. $EC_{50}$ values were determined by non-linear regression by GraphPad Prism software.

## RESULTS

### *Effect of TMP on secretion of PBJ*

Table 1 shows the volume of pancreatic-bile juice (PBJ) collected at different time points before and after intraperitoneal administration of TMP or 9 mL/L NaCl (control). Compared with the basal secretion, the PBJ secretion in TMP treated rats was increased by 7.0 % ($P<0.05$), 22.3 % ($P<0.001$), 14.3 % ($P<0.01$), 18.2 % ($P<0.01$) and 14.2 % ($P<0.01$) at 15, 30, 45, 60 and 75minutes, respectively ($n=13$) (Figure 1). However, the same treatment with of 9 mL/L NaCl solution did not produce significant effect ($n=12$) (Figure1). Protein content in PBJ and pH of PBJ were shown to have no differences between TMP-treated and control groups (data not shown).

### *Effect of TMP on CAPAN-1 cell line*

The CAPAN-1 monolayer clamped in Ussing chambers bathing with normal K-HS ($Cl^-/HCO_3^-$-containing) exhibited a potential difference of 0.52±0.04 mV, basal $I_{SC}$ of 5.70±0.53 μA/$cm^2$ and transmembrane resistance of 93.6±4.5 Ω$cm^2$ ($n=77$). In $Cl^-$-free ($n=9$) and $HCO_3^-$-free ($n=9$) K-H solution, the transepithelial potential difference was 0.62±0.15 mV and 0.86±0.28 mV and the basal $I_{SC}$ was 1.90±0.50 μA/$cm^2$ and 3.98±1.32 μA/$cm^2$, respectively, and the resistance of the monolayers were 208.6±33.6 Ω$cm^2$ and 161.8±25.6 Ω$cm^2$.

**Table 1** Volume of secretion of pancreatic bile juice ($\bar{x}\pm S_{\bar{x}}$, μL)

| | Groups | TMP $n=13$ | Control $n=12$ |
|---|---|---|---|
| | Basal | 372.3±22.2 | 342.5±15.8 |
| | 15 min | 398.5±23.3 | 326.7±1.74 |
| After | 30 min | 455.4±18. 8 | 344.2±14.5 |
| administration | 45 min | 425.4±19.8 | 331.7±17.5 |
| | 60 min | 440.0±25.8 | 357.5±18.8 |
| | 75 min | 425.0±25.2 | 342.5±18.2 |

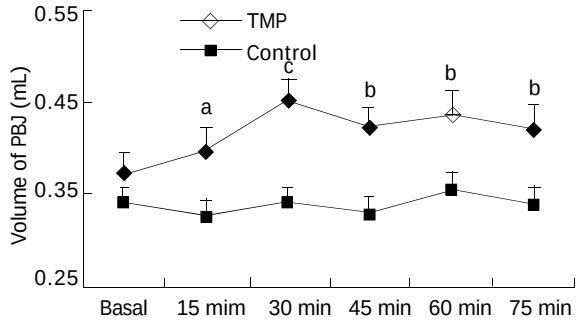


**Figure 1** Effect of TMP on Volume of pancreatic-bile juice secretion. The volume of pancreatic-bile juice secretion collected before (basal) and 15, 30, 45, 60 and 75 minutes after intraperitoneal administration of TMP (80 mg/kg) and 9 mL/L NaCl in rats. The results were expressed as $\bar{x}\pm S_{\bar{x}}$. $^aP<0.05$, $^bP<0.01$, $^cP<0.001$, as compared to basal values.

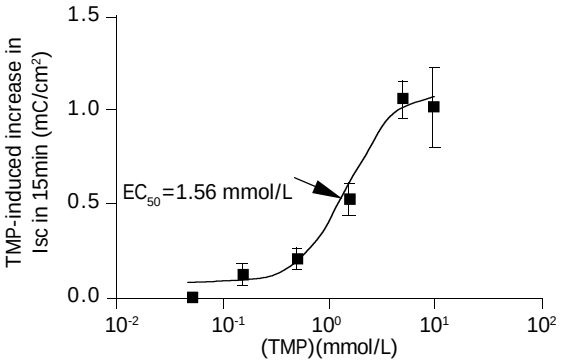


**Figure 2** Concentration-response curve for TMP-induced $I_{SC}$ in CAPAN-1 cells. Different concentrations of TMP were added to basalateral side. Each data point was obtained from at least 4 individual experiments. Arrow shows $EC_{50}$.

Basolateral addition of TMP (0.05, 0.15, 0.5, 1.5, 5 and 10 mmol/L) produced a dose-dependent increase in $I_{SC}$. $EC_{50}$ was about 1.56 mmol/L (Figure 2). TMP (5 mmol/L)-induced $I_{SC}$ increase was biphasic: a fast transient peak followed by a slow decay. The total transported charges for 15 minutes (the area under the curve of the TMP-induced $I_{SC}$ response) were about 1.06±0.99 mC/$cm^2$ ($n=27$, Figure 3.A and C). TMP-induced current increase was inhibited by 67.1 % after apical pretreatment of 1 mmol/L DPC, a non-specific $Cl^-$ channel blockers ($n=8$, $P<0.001$) (Figure 3.B and C). However apical application of epithelial sodium channel blockers, amiloride

(100 μmol/L) did not affect the TMP-produced response ($n$=3, Figure 3.C). After the removal of external $HCO_3^-$, TMP-induced $I_{SC}$ was blocked by 74.4 % ($n$=9, $P$<0.001) (Figure 3. D), but the removal of external $Cl^-$ did not reduce but increase the TMP-induced $I_{SC}$ ($n$=8, $P$<0.05) (Figure 3.D). Pretreatment of phosphodiesterase inhibitor, IBMX (0.5 mmol/L) for 15 minutes decreased the TMP-induced $I_{SC}$ by 91 % ($n$=4, $P$<0.001) (Figure 4.A and B).

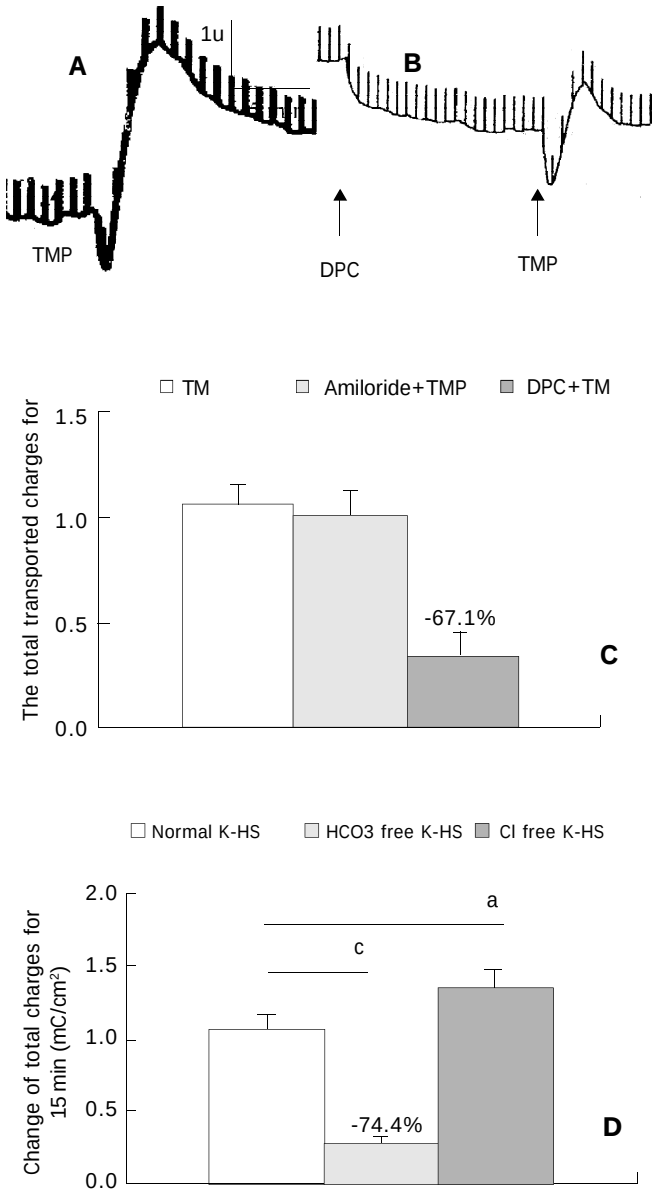


**Figure 3** TMP-induced $HCO_3^-$ secretion in CAPAN-1 cells. Comparison of TMP (5 mmoL/L)-induced $I_{SC}$ (mC/cm²) obtained in control (A, C), pretreatment with DPC (1 mmoL/L) (B, C), Amiloride 100 umoL/L (C), $HCO_3^-$-free solution (D), $Cl^-$-free solution (D), Values are $\bar{x}\pm S_{\bar{x}}$, $^aP$<0.05, $^cP$<0.001.

## DISCUSSION

While TMP has been widely used clinically for treating cardiovascular disorders and cerebrovascular diseases[1-5], little is known about its effect on pancreatic secretion. In the present study, intraperitoneal application of TMP increased secretion of pancreatic-bile juice (PBJ), but the protein content and pH value were not affected. It is known that PBJ consists of both bile and pancreatic juice, the volume of the basal secretion (or agonist induced) of pancreatic juice is normally very low, only about 9-18 μL in 15 minutes in rat[17,18], as compared to that of

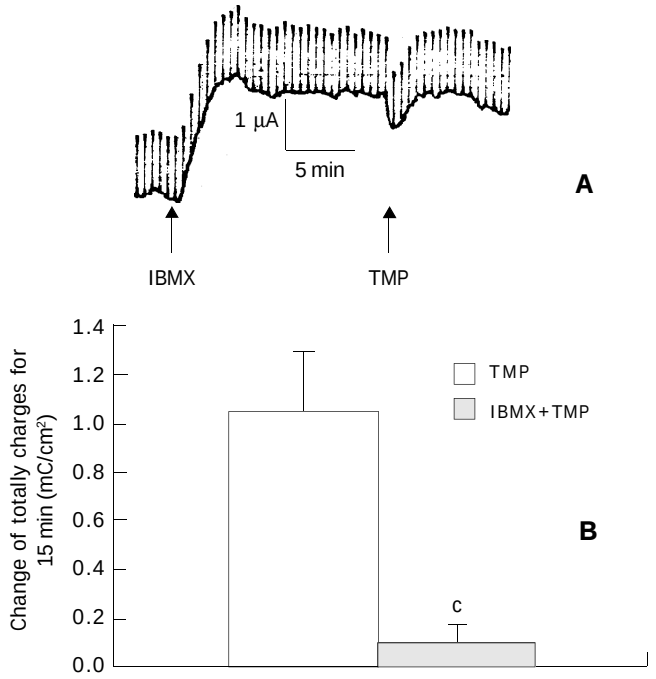


**Figure 4** Pretreatment with IBMX decreased TMP–induced $I_{SC}$ by 91 %. Values are $\bar{x}\pm s$, $^cP$<0.001.

the liver. Although TMP increased the secretion of PBJ by 26-83 μL in 15 minutes, it was difficult to assess the contribution to exocrine pancreatic secretion from our experiments *in vivo*. Therefore, CAPAN-1 cell line was used to further demonstrate the effect of TMP on the pancreatic secretion. Our data suggested that TMP could directly stimulate $HCO_3^-$ secretion in human pancreatic duct cells.

According to the $HCO_3^-$ secretory model[22], the intracellular $HCO_3^-$ was accumulated from the tissue fluid via the basolateral membrane[23-25] and mainly secreted by a $Cl^-/HCO_3^-$ exchanger[26,27] and/or directly via cAMP/cGMP-dependent $Cl^-$ channel (CFTR)[13,26,28]. Recently, it was reported that CFTR could also secrete $HCO_3^-$ as $Cl^-/HCO_3^-$ exchanger[29,30]. The TMP- increased $I_{SC}$ in CAPAN-1 could be blocked by $Cl^-$ channel blockers, as well as phosphodiesterase inhibitors, which were known to increase cAMP and cGMP, and abolished by removal of extracellular $HCO_3^-$, suggesting that TMP can stimulate pancreatic $HCO_3^-$ secretion via CFTR since it has been shown to be activated by both cAMP and cGMP. The TMP-induced increase in the volume of PBJ observed in the rats could be due to an increase in pancreatic $HCO_3^-$ secretion, as well as bile secretion, leading to enhanced water secretion. However, the inability of TMP to increase the pH of PBJ appeared to contradict to its observed effect on the increase in $HCO_3^-$ secretion. This could be explained by two possible reasons. One is that the mechanism of $HCO_3^-$ secretion in human pancreatic duct cells was different from that in rats since the $HCO_3^-$ concentration of pancreatic juice in rats was only 70 mmol/L while in human was 145 mmol/L[22]. The other is that the $HCO_3^-$ secreted by the pancreas might be diluted by bile juice and thus having less prominent effect on pH. It is interesting to note that TMP could stimulate $HCO_3^-$ secretion that could be inhibited by extracellular $Cl^-$ as evidenced by the increase in the TMP-induced $I_{SC}$ observed in the absence of extracellular $Cl^-$. However, the mechanism for the observed inhibition of $HCO_3^-$ secretion by extracellular $Cl^-$ remains to be elucidated.

In conclusion, the present *in vivo* and *in vitro* results suggest that TMP can promote the secretion of PBJ in rats and $HCO_3^-$ secretion in human pancreatic duct cells, which may be beneficial to improving digestive function.

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**Edited by** Wang XL