BAK FOONG PILLS STIMULATE ANION SECRETION ACROSS NORMAL AND CYSTIC FIBROSIS PANCREATIC DUCT EPITHELIA

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The present study examined the effect of Bak Foong Pills (BFP), an over-the-counter traditional Chinese medicine (China registration no. Z980035), on anion secretion and the underlying signaling pathways in normal and cystic fibrosis pancreatic duct cell lines, CAPAN-1 and CFPAC-1, respectively, using the short-circuit current technique. Apical addition of BFP ethanol extract (600 μg/ml) induced a fast transient \( I_{SC} \) peak that was followed by a slower but more sustained increase in \( I_{SC} \) in CAPAN-1 cells. However, the response to BFP in CFPAC-1 was predominantly the first transient peak. Apical addition of DIDS (200 μM) inhibited the first peak by more than 60% in both cell lines without significantly affecting the second \( I_{SC} \) rise. More than 85% of the BFP-induced first transient in both cell lines was inhibited when extra and intracellular Ca\(^{2+}\) was chelated or emptied by pretreatment with BAPTA (100 μM) and thapsigargin (10 μM), respectively. Acute addition of PMA (1 μM), a PKC activator, blocked more than 95% of the BFP-induced first peak in both cell lines, consistent with previously reported PKC modulation of Ca\(^{2+}\)-dependent pancreatic anion secretion. The BFP-induced second \( I_{SC} \) rise in CAPAN-1 could be inhibited by 73.6% and 71.13% by pretreatment of the cells with MDL-12330A (20 μM), an adenylate cyclase inhibitor and Rp-cAMP (200 μM), a cyclic AMP antagonist, respectively. However, less than 25% of the \( I_{SC} \) was inhibited by combined treatment with BAPTA and thapsigargin. The second rise was also completely blocked by DPC (2 mM) or Glibenclamide (1 mM). The results indicate that BFP ethanol extract stimulates pancreatic duct anion secretion in normal and CF cells via different signaling pathways involving both Ca\(^{2+}\) and cAMP.

INTRODUCTION

Bak Foong Pills (BFP, also known as Bai Feng Wan) (China registration no. Z980035) has long been used for treating gynecological disorders and improvement of overall body functions (Zheng et al., 2000; Wan et al., 1998). Using the short-circuit current technique, we have recently demonstrated a stimulatory effect of BFP on human colonic epithelia cell line T84 (Zhu et al., 2002). The results indicated that the effect of BFP was mediated predominantly by activating adenylate cyclase leading to activation of cAMP-dependent Cl\(^{-}\) channels. The same study also observed that a small fraction of the BFP-induced \( I_{SC} \) in T84 cells was sensitive to DIDS, a Cl\(^{-}\) channel blocker known to block Ca\(^{2+}\)-activated Cl\(^{-}\) channels, suggesting the possible involvement of Ca\(^{2+}\) signaling pathway.

The present study further investigated the signaling pathways mediating the effect of BFP by making use of the pancreatic duct cell lines,
CAPAN-1 and CFPAC-1, derived from normal and cystic fibrosis (CF) patients, respectively. CAPAN-1 has been shown to possess most of the properties of pancreatic ductal epithelial cells (Cotton, 1998; Madden and Sarras, 1988; Kyriazis et al., 1982), and contain apical cAMP-dependent and Ca\(^{2+}\)-dependent Cl\(^{-}\)/p1 channels, both of which are involved in HCO\(_3\)/p1 secretion (Levrat et al., 1988; Becq et al., 1992; Cheng et al., 1998). CFPAC-1, a human CF pancreatic duct cell line, exhibits defective cAMP-dependent but intact Ca\(^{2+}\)-dependent anion secretion (Zeng et al., 1997; Cheng et al., 1999a; Cheng et al., 1999b). Both of the pancreatic duct cell lines appear to be useful models for the study of separate signaling pathways involved in human pancreatic ductal secretory mechanisms. Therefore, we undertook the present study to further investigate the signaling mechanisms of BFP using these cell lines in conjunction with the short-circuit current technique.

MATERIALS AND METHODS

Materials

Hank’s balanced salt solution (HBSS) and fetal bovine serum was from Gibco Laboratories (New York, NY, U.S.A.). 4,4’-diisothiocyanostilbene-2,2’-disulfonic acid (DIDS), phorbol 12-myristate 13-acetate (PMA), Rp-cAMP and glibenclamide were from Sigma (St Louis, MO, U.S.A.). BFP was provided by Eu Yan Sang (Hong Kong) Limited. Diphenylamine-2,2’-dicarboxylic acid (DPC) was obtained from Riedel-de Haen Chemicals (Hannover, Germany). Calbiochem (San Diego, CA, U.S.A.) was the source for the adenylate cyclase inhibitor MDL-12330A, BAPTA and thapsigargin.

BFP ethanol extract

The procedure of making BFP ethanol extract has been described previously (Zhu et al., 2002). 1 kg of BFP powder in 70% ethanol at a ratio of 1 to 10 (g/ml) was put in round-bottom flask and boiled under reflux for 2 h. The mixture was filtered and the residue of BFP was subject to the same treatment for a second time. The filtrates from the two treatment procedures were collected and put in the vacuum rotary evaporator for concentration. The extract was collected and lyophilized by freeze dryer. BFP was resuspended to desired concentrations in Krebs–Henseit solution.

Solutions

Krebs–Henseit (K-H) solution had the following composition (mM): NaCl, 117; KCl, 4.5; CaCl\(_2\), 2.5; MgCl\(_2\), 1.2; NaHCO\(_3\), 24.8; KH\(_2\)PO\(_4\), 1.2; Glucose, 11.1. The solution was gassed with 95% O\(_2\) and 5% CO\(_2\), and the pH 7.4.

Cell culture

Human pancreatic duct cell lines, CAPAN-1 and CFPAC-1 were purchased from American Type Culture Collection (Maryland, U.S.A.). Culture procedures for CFPAC-1 cells, grown in Iscove’s modified Dulbecco’s medium with 10% FBS, and for CAPAN-1 cells, grown in RPMI 1640 medium with 15% FBS, have been described previously (Becq et al., 1992; Cheng et al., 1999a). When cells were disassembled from the culture flask, 0.25% trypsin-EDTA was added with extra care to avoid striking on cell layer directly. Quickly afterwards, less than 1 min, most of the trypsin was removed leaving about 0.5 ml in the flask which was then incubated for 2–3 min. Cells were then resuspended in serum-containing medium with gentle pipetting of the cells to break up the cell aggregations. The suspension was then transferred into a centrifuge tube for spinning at 800 g for 5 min to remove any trypsin left. Supernatant was discarded and the cells were resuspended with a desirable volume of medium to make up to a final cell concentration of 1.5 × 10\(^6\)/ml. A volume of 0.25 ml of the cell suspension was then plated onto each permeable support (area of 0.45 cm\(^2\)) floating on culture medium and incubated at 37\(^\circ\)C in 95% O\(_2\)–5% CO\(_2\) for 5–7 days till the monolayers reached confluence and were ready for I\(_{SC}\) measurement.

Short-circuit current measurement

The measurement of I\(_{SC}\) has been described previously (Becq et al., 1992; Nguyen et al., 2001). In most of the experiments, the change in I\(_{SC}\) was defined as the maximal rise in I\(_{SC}\) or the area under the I\(_{SC}\) response curve following agonist stimulation and they were normalized to current change per unit area of the epithelial monolayer (µA/cm\(^2\) or µCl/cm\(^2\)). In each experiment, a transepithelial potential difference of 0.1 mV was applied. The change in current in response to the applied potential could be used to monitor the transepithelial resistance of the monolayer.

Data analysis

Results were expressed as mean ± standard error mean (SEM). The number of experiments
represents independent measurements on separate monolayers. Comparisons between groups of data were made by Student’s unpaired t-test. A P value of less than 0.05 was considered statistically significant.

RESULTS

BFP-induced different I\textsubscript{SC} responses in normal and CF cells

When cultured monolayers were clamped in Ussing chambers bathing with normal K-H solution (Cl\textsuperscript{-} / HCO\textsubscript{3}\textsuperscript{-}-containing), a potential difference of 0.51 ± 0.08 mV (n=17) and 0.29 ± 0.024 mV (n=34) (P<0.01, Fig. 1A), basal I\textsubscript{SC} of 8.78 ± 0.89 μA (n=17) and 3.18 ± 0.36 μA (n=34) (P<0.001, Fig. 1B), and transepithelial resistance of 32.49 ± 5.58 Ω cm\textsuperscript{2} (n=17) and 42.73 ± 5.38 Ω cm\textsuperscript{2} (n=21) (P=0.2, Fig. 1C) were observed in CAPAN-1 and CFPAC-1 cell lines, respectively. Apical addition of BFP ethanol extract (600 μg/ml) induced a biphasic increase in I\textsubscript{SC} in CAPAN-1 with a fast transient peak followed by a slower but more sustained rise (Fig. 1D). However, the BFP-induced I\textsubscript{SC} response in CFPAC-1 was predominantly the first transient peak (Fig. 1E). The BFP-induced first transient peak, which was normally reached and declined within 1 minute, was 5.21 ± 0.47 μA/cm\textsuperscript{2} in CAPAN-1 (n=9) and 3.54 ± 0.40 μA/cm\textsuperscript{2} in CFPAC-1 (n=17). The BFP-induced second rise in I\textsubscript{SC} in CAPAN-1 cells usually lasted about 10 to 15 min, and therefore the total charges transported (the area under the curve of the I\textsubscript{SC} response) were used to describe the response, which averaged to be 1010.5 ± 87.83 μC/cm\textsuperscript{2}. Occasionally, a second rise in I\textsubscript{SC} in response to BFP was also notable in CFPAC-1 cells, but was insignificant as compared to that observed in CAPAN-1 cells. In both cell lines, treatment with BFP ethanol extracts produced significant increases in transepithelial resistance (Fig. 1C).

Involvement of Ca\textsuperscript{2+}-dependent pathway

Apical application of DIDS (200 μM), a Ca\textsuperscript{2+}-dependent Cl\textsuperscript{-} channel blocker, inhibited basal I\textsubscript{SC} by 9.6 ± 1.2% and 133.5 ± 16.6%, and increased transepithelial resistance from 32.67 ± 8.46 to 43 ± 9.09 Ω cm\textsuperscript{2} and from 29.1 ± 3.2 to 38.4 ± 3.83 Ω cm\textsuperscript{2} respectively in CAPAN-1 (n=6, P<0.05) and CFPAC-1 cells (n=9, P<0.001). The BFP-induced first peak was inhibited by more than 60% in both cell lines (from 3.54 ± 0.40 to 1.31 ± 0.13 μA/cm\textsuperscript{2} in CFPAC-1 cells, n=9, P<0.001; from 5.21 ± 0.47 to 0.44 ± 0.28 μA/cm\textsuperscript{2} in CAPAN-1 cells, n=6, P<0.001) without significant effect on the second rise in I\textsubscript{SC} (Fig. 2A, B, C), indicating that the BFP-induced first peak might involve Ca\textsuperscript{2+} mobilization. In order to confirm this, BAPTA (100 μM) was used to chelate extra-cellular Ca\textsuperscript{2+}, and thapsigargin (10 μM), a vesicular Ca\textsuperscript{2+} pump inhibitor, was used to empty intracellular Ca\textsuperscript{2+} store. The combined treatment resulted in more than 85% reduction (from 3.54 ± 0.40 to 0.48 ± 0.15 μA/cm\textsuperscript{2} in CFPAC-1 cells, from 5.21 ± 0.47 to 0.19 ± 0.09 μA/cm\textsuperscript{2} in CAPAN-1 cells, n=6, P<0.001) in the BFP-induced first peak in both cell lines (Fig. 2C), but less than 25% reduction in the second rise in I\textsubscript{SC} in CAPAN-1 cells.

Our previous studies on both CAPAN-1 and CFPAC-1 cells have demonstrated that activation of PKC suppressed Ca\textsuperscript{2+}-activated pancreatic anion secretion (Cheng et al., 1999a). In the present study, we also examined whether PKC had any modulatory effect on the BFP-induced I\textsubscript{SC}. Apical addition of 1 μM PMA, a PKC activator, blocked more than 95% of the fast peak in both cell lines, from 5.21 ± 0.47 to 0.18 ± 0.18 μA/cm\textsuperscript{2} in CAPAN-1 cells (n=5, P<0.001) and from 3.54 ± 0.40 to 0.11 ± 0.11 μA/cm\textsuperscript{2} in CFPAC-1 cells (n=4, P<0.001), but did not affect the second I\textsubscript{SC} rise in CAPAN-1 (Fig. 3A, B, C). Consistent with the previous observed inhibitory effect of PKC on Ca\textsuperscript{2+}-activated pancreatic anion secretion, the presently observed inhibitory effect of the PKC on BFP-induced first peak suggested the involvement of Ca\textsuperscript{2+}.

Involvement of cAMP-dependent pathway

In order to demonstrate whether cAMP was involved in mediating the BFP-induced effect, an adenylate cyclase inhibitor, MDL-12330A, or a cyclic AMP antagonist, Rp-cAMP, was added 10 minutes before addition of BFP extract. Pretreatment with MDL did not significantly affect the BFP-induced first peak in both cell lines, but inhibited the second rise by 73.7%, from 1010.48 ± 367.85 to 266.67 ± 96.15 μC/cm\textsuperscript{2} (n=4, P<0.01) (Fig. 4) in CAPAN-1 cell line. Rp-cAMP 200 μM also inhibited the second rise by 76.2%, from 1010.48 ± 87.83 to 240 ± 113.4 μC/cm\textsuperscript{2} (n=3, P<0.01). Both of the first and second rises by BFP could be remarkably inhibited by the combined pretreatment with PMA and MDL 12330A in CAPAN-1 (n=4, P<0.001) (Fig. 5). The second rise
could be completely blocked by Cl⁻ channel blockers DPC (n=4) or glybenclamide (n=3) but not DIDS (see above). Thus, it appeared that the BFP-induced second rise in I_{sc} was predominantly cAMP-dependent.

**DISCUSSION**

BFP has been used for treatment of gynecological disorders for several centuries in China with well-known effects for improving overall body functions...
However, its beneficial effects on the gastrointestinal tract have not been investigated until recently (Zhu et al., 2002). In the previous study, we have demonstrated that BFP ethanol extract exerted a stimulatory effect on gastrointestinal Cl⁻/p1 secretion by predominantly activating adenylate cyclase and apical CAMP-dependent Cl⁻/p1 channels, namely CFTR, in T84 human colonic cell line (Zhu et al., 2002). The study also indicated possible activation of Ca²⁺-dependent Cl⁻ channels.

The present study has further investigated the effect of BFP on pancreatic duct anion secretion and the underlying signaling pathways using normal and CF human pancreatic duct cell lines. While CAPAN-1 possesses both apical Ca²⁺-dependent and CAMP-dependent Cl⁻ channels (CFTR) (Cheng et al., 1998; Nguyen et al., 2001; Marino et al., 1991), CFPAC-1 has intact Ca²⁺-dependent Cl⁻ channels but defective CFTR (Cheng et al., 1999a; Zsembery et al., 2000; Chan et al., 1996). In the present study, BFP induced a

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**Fig. 2.** Involvement of Ca²⁺-dependent pathway in BFP-induced $I_{sc}$. The BFP-induced first rise was inhibited by pretreatment of 100 μM DIDS ($n=9$) in CAPAN-1 (A) and in CFPAC-1 (B) or 100 μM BAPTA and 10 μM thapsigargin (TSG, $n=6$) (C). Values are means ± SEM; ***P<0.001 (compared to control).
biphasic $I_{\text{SC}}$ response in CAPAN-1 but mostly a fast single peak in CFPAC-1, suggesting that different signaling pathways are involved in mediating the effect of BFP. Several lines of evidence suggest that the BFP-induced first transient $I_{\text{SC}}$ peak in both cell lines was mediated by Ca$^{2+}$, which include: (1) inhibition by DIDS, a blocker of the Ca$^{2+}$-dependent Cl$^{-}$ channels; (2) sensitivity to treatment with BAPTA and thapsigargin which prevents both extra and intracellular Ca$^{2+}$ mobilization; and (3) suppression by PMA, a PKC activator, which has previously been shown to inhibit Ca$^{2+}$-dependent anion secretion in these two cell lines (Cheng et al., 1999a). Furthermore, the BFP-induced first transient peak also exhibits kinetics similar to the Ca$^{2+}$-dependent ATP-induced $I_{\text{SC}}$ previously observed in the two cell lines (Cheng et al., 1999a; Chan et al., 1996).

Fig. 3. Effect of PKC activator on the BFP-induced $I_{\text{SC}}$ in normal and CF cell lines. (A) Representative $I_{\text{SC}}$ recording obtained from CAPAN-1 cells showing that pretreatment with PMA (1 μM) inhibited first peak, but not the second response. (B) Representative $I_{\text{SC}}$ recording obtained from CFPAC-1 cells. The first peak was inhibited by pretreatment with PMA (1 μM). (C) Comparison of the magnitude of the BFP-induced first rise in the presence and absence of PMA 1 μM in CAPAN-1 and CFPAC-1 cells ($n=5$). Values are means ± SEM; ***$P<0.001$ (compared to control).
The present study also suggests that the BFP-induced second rise in ISC in CAPAN-1 cells is predominantly mediated by cAMP. The evidence includes: (1) suppression by adenylate cyclase inhibitors, MDL-12330A and cAMP antagonist, Rp-cAMP, but less sensitive to treatment with BAPTA and thapsigargin; (2) inhibition by DPC and glibenclamide, but not DIDS; and (3) the presence of prominent second rise in CAPAN-1 but not CFPAC-1 cells where CFTR, a cAMP-activated Cl- channel itself, is defective (Cheng et al., 1999a; Cheng et al., 1999b; Zsembery et al., 2000; Chan et al., 1996). While the evidence suggests that the BFP-induced second rise in ISC is

![Fig. 4](image)

**Fig. 4.** Effect of adenylate cyclase inhibitor (MDL 12330A) on BFP-induced second peak in I_{sc} in CAPAN-1 cells. Comparison of the total charges transported in BFP-induced I_{sc} response in the absence and presence of MDL 12330A (n=4). Values are means ± SEM; **P<0.01 (compared to control).

![Fig. 5](image)

**Fig. 5.** Effect of the combined treatment of PKC activator (PMA) and adenylate cyclase inhibitor (MDL 12330A) on the CAPAN-1 cells. (A) Representative I_{sc} recording obtained from apical addition of PMA (1 μM) showing inhibition of first peak. (B) Representative I_{sc} recording with arrow showing BFP-induced first and second rises notably inhibited by apical addition of PMA (1 μM) and basolateral addition of MDL 12330A (20 μM).
predominantly cAMP-dependent, it should be noted that a small fraction of the current could be inhibited by treatment with BAPTA and thapsigargin, and a small second rise in \( I_{SC} \) was also observed occasionally in CFPAC-1 cells, indicating a small contribution of \( Ca^{2+} \) in mediating the activation of the second rise in \( I_{SC} \). Taken together, the present results are consistent with the previous findings in human colonic \( T_{84} \) cells (Zhu et al., 2002), suggesting activation of both \( Ca^{2+} \) and cAMP-dependent pathways in mediating the effect of BFP. However, it should be noted that the fast transient peak observed in the pancreatic duct cells was not observed in \( T_{84} \) cells, perhaps due to different kinetics in \( Ca^{2+} \) mobilization between the colonic cells and pancreatic duct cells.

The fact that BFP can stimulate both cAMP and \( Ca^{2+} \)-dependent secretory pathways in epithelial cells from the GI tract has implications for its clinical use for treating GI disorders since its activation of both \( Ca^{2+} \) and cAMP-dependent secretion would be beneficial when maximal GI secretion is desired. It remains to be elucidated whether the dual effect of BFP on signaling pathways is generated by different pharmacologically active components.

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REFERENCES


