Flavonoids belong to a large group of plant polyphenols that are consumed daily in large amounts. Our previous findings have shown that baicalein, a major flavonoid derived from the medicinal herb Scutellariae radix, induces Cl⁻ secretion across rat colonic mucosa. The current study examines the effect of baicalein on Cl⁻ secretion in human colonic epithelial (T84) cells and its interaction with Ca²⁺ and cAMP-dependent secretagogues. We have employed a technique that allows concurrent monitoring of short-circuit current (I_SC) and [Ca²⁺], in polarized epithelium. Basolateral application of baicalein induced a concentration-dependent increase in I_SC. The increase in I_SC was because of Cl⁻ secretion and was not accompanied by any discernible increase in [Ca²⁺]. Baicalein acted synergistically with Ca²⁺ but not cAMP-dependent secretagogues. In the presence of baicalein, the carbachol and histamine induced increases in I_SC that were markedly potentiated while increases in [Ca²⁺] were not significantly enhanced. Baicalein treatment uncoupled Cl⁻ secretion from inhibitory effects normally generated by muscarinic activation. Baicalein treatment also resulted in increased cAMP content and activated PKA activity. Nystatin permeabilization studies revealed that baicalein stimulated an apical Cl⁻ current but did not activate any basolateral K⁺ current. These data suggest that baicalein potentiates Ca²⁺-mediated Cl⁻ secretion through a signaling pathway involving cAMP and protein kinase A, most likely through the cystic fibrosis transmembrane conductance regulator in the apical membrane.

Flavonoids belong to a large group of naturally occurring plant polyphenols that are consumed daily in large amounts (1). They are present in many medicinal plants, and traditional folk remedies have employed herbal medicines containing flavonoids for centuries (2). Baicalein is a major bioactive flavone constituent of the medicinal herb Scutellariae radix, which is the dried root of Scutellaria baicalensis Georgi (3). It has been extensively reported to possess a wide range of biological activities, including anti-inflammatory (4), anti-viral (5), anti-oxidant (6–8), and anti-cancer activities (9, 10).

In the intestinal tract, flavonoids have been demonstrated to affect gastrointestinal motility both in vivo and in vitro (2). Some flavonoids have been shown to possess anti-diarrheal effects and to inhibit intestinal motility or protect animals against castor oil-induced diarrhea (e.g. apigenin from Petroselinum sativum). Other flavonoids have been shown to stimulate colonic epithelial ion transport processes. The best known examples are quercetin (a flavonol) and genistein (an isoflavone). Recent studies have shown that quercetin stimulates Cl⁻ secretion across rat colonic mucosa (11, 12). This secretory activity does not depend on cAMP but depends on Ca²⁺, possibly via a Ca²⁺/calmodulin-dependent pathway. The cAMP pathway and inhibition of phosphodiesterase appear not to be responsible for the secretory activity of the flavonol (13). On the other hand, genistein can activate both wild type and mutant cystic fibrosis transmembrane conductance regulator (CFTR), probably through direct binding of the channel (14). In the rat colon, genistein has been found to stimulate Cl⁻ secretion and to inhibit Na⁺ and Cl⁻ absorption. Moreover, pre-stimulation of the cAMP pathway is a prerequisite for the secretory action of genistein (15, 16). Currently, there are no reports describing any effect of baicalein on colonic secretion in human intestinal cells.

Our previous finding provided the first evidence that baicalein induces Cl⁻ secretion across rat colonic mucosa, possibly via a cAMP-dependent pathway (17). Therefore, the underlying pro-secretory mechanism(s) of baicalein differs from that of quercetin or genistein. Consequently, we examined the effect of baicalein on Cl⁻ secretion in human colonic epithelial (T84) cells and its interaction with Ca²⁺-dependent secretagogues. In the present study, we employed a technique that allows concurrent monitoring of agonist-induced short-circuit current (I_SC) and [Ca²⁺], in a polarized epithelium (18). Here, we show that baicalein stimulates Cl⁻ secretion and potentiates Ca²⁺-mediated Cl⁻ secretion via a cAMP- and PKA-dependent pathway in human intestinal cells.

EXPERIMENTAL PROCEDURES

Culture of Cells—Experiments were performed using the human intestinal T84 cell line obtained from American Type Culture Collection. T84 cells were maintained in Dulbecco's modified Eagle's medium/ F-12 supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) non-essential amino acids, 100 IU/ml penicillin, and 100 μg/ml streptomycin. For simultaneous measurements of [Ca²⁺], and I_SC, cells were seeded onto Transwell-col membranes (Costar, Cambridge, MA) with 0.4-μm pore diameter (culture area 0.1 cm²) as previously described (18). Cells reached confluence after 9 to 10 days, with a resistance greater than 300 Ωcm².

Received for publication, June 17, 2004, and in revised form, July 2, 2004
Published, JBC Papers in Press, July 3, 2004, DOI 10.1074/jbc.M406787200

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* This work was supported by Research Grants Council of the Hong Kong Special Administrative Region Project number CUHK4171/02M and a direct grant for research from The Chinese University of Hong Kong (Ref. No. 2003.1.070) to W.-H. K. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; CCH, carbachol; DPC, diphenylamine-2-carboxylic acid; IBMX, 3-isobutyl-1-methylxanthine; PKA, protein kinase A; [Ca²⁺], intracellular calcium concentration; I_SC, short-circuit current; Fura-2-AM, Fura-2 acetoxymethyl ester; ΔI_SC, change in short-circuit current; I_kbaso, apical membrane Cl⁻ currents; I_kbaso, basolateral membrane K⁺ currents.
Our previous study demonstrated that baicalein stimulates Cl− secretion in rat colon (17). In the present study, the effects of baicalein on [Ca2+]i (Fig. 1A) and Isc (Fig. 1B) were monitored simultaneously in T84 cells. The 340/380 nm fluorescence ratio was used to represent the changes in [Ca2+]i. Basolateral application of 100 µM baicalein elicited an increase in Isc (n = 10, Fig. 1B). Meanwhile, baicalein did not induce any discernible increase in fluorescence ratio (Fig. 1A). Apical application of baicalein (10–300 µM) did not induce any significant concentration-dependent increases in Isc or [Ca2+]i (data not shown). When cells were exposed to 2-min pulses of increasing concentrations (10–300 µM) of baicalein, a concentration-dependent
increase in $I_{SC}$ occurred (Fig. 1C), with an EC$_{50}$ value of 28.2 ± 5.5 μM. In Cl⁻-free solution, basolateral application of baicalein (100 μM) did not induce any increase in $I_{SC}$ ($n = 5$). The data confirm that the baicalein-induced increase in $I_{SC}$ in T84 cells is because of Cl⁻ secretion.

**Baicalein Potentiates Cl⁻ Secretion Stimulated by Ca²⁺-dependent Agonists**—Although baicalein did not stimulate a substantial increase in Cl⁻ secretion as seen in rat colon (17), it may interact with other known secretagogues acting via cAMP or Ca²⁺. Experiments were performed to determine whether baicalein acted synergistically with Ca²⁺-dependent agonists, such as the muscarinic receptor agonist CCh. As seen in Fig. 2, CCh (10 μM, basolateral) induced a transient increase in $I_{SC}$ ($ΔI_{SC} = 6.9 ± 0.5 μA/cm^2$, $n = 21$) in the T84 monolayers. CCh produced a rapid increase in $I_{SC}$ that peaked at 1–2 min after drug application and returned to baseline values within 15 min (Fig. 2B). At the same time, CCh stimulated an increase in Fura-2 fluorescence ratio ($Δ$Ratio = 0.28 ± 0.02, $n = 21$), with maximum ratio reached within 10–20 s (Fig. 2A). The fluorescence ratio then declined gradually and reached a plateau, but it did not return to baseline until CCh was washed off. The EC$_{50}$ values for the CCh-induced increase in $I_{SC}$ and Fura-2 fluorescence ratio were 31.1 ± 9.2 and 5.1 ± 3.1 μM, respectively.

To test whether baicalein could potentiate the CCh-induced secretory effect, cells were first exposed to baicalein on the apical or basolateral side. After 2 min, CCh (10 μM, basolateral) was added to the cells in the presence of baicalein. Fig. 2D shows that baicalein (basolateral) markedly enhanced the $I_{SC}$ response to CCh ($ΔI_{SC} = 14.1 ± 3.4 μA/cm^2$, $n = 23$). The peak increase in $I_{SC}$ differs significantly from that of control ($ΔI_{SC} = 6.9 ± 0.5 μA/cm^2$, $n = 21$, $p < 0.05$). Similar results were obtained when the monolayers were stimulated with CCh in the presence of 100 μM baicalein applied to the apical side ($ΔI_{SC} = 12.43 ± 3.12 μA/cm^2$, $n = 23$, $p < 0.05$). Meanwhile, the response of [Ca$^{2+}$], to CCh in the absence (Fig. 2A) or presence (Fig. 2C) of baicalein was qualitatively the same (CCh alone: $Δ$Ratio = 0.28 ± 0.02, $n = 21$; baicalein + CCh: $Δ$Ratio = 0.29 ± 0.02, $n = 23$, $p > 0.05$). Therefore, the secretory response to CCh was significantly potentiated by baicalein added to either side of the epithelia. However, baicalein treatment did not alter the response to [Ca$^{2+}$]. In the presence of baicalein (apical or basolateral), the CCh-induced $I_{SC}$ increase was abolished in Cl⁻-free solution while the CCh-induced [Ca$^{2+}$], increase remained unchanged ($n = 5$, data not shown), further confirming that baicalein potentiated the CCh-stimulated Cl⁻ secretion in T84 cells. The threshold concentration of baicalein for the augmentation of CCh-elicited $I_{SC}$ was repeatedly observed at 10 μM ($n = 10$, data not shown).

To determine whether the ability of baicalein to potentiate Ca²⁺-dependent secretory responses was specific for CCh, the H$_2$-receptor agonist histamine was used. Addition of histamine (100 μM) to the basolateral bathing solution of T84 cell monolayers increased both [Ca$^{2+}$], and $I_{SC}$ ($Δ$Ratio = 0.26 ± 0.03, $ΔI_{SC} = 3.9 ± 0.5 μA/cm^2$, $n = 6$), as shown in Fig. 3A and B. Prior additions of apical (Fig. 3C, A and D) or basolateral (data not shown) baicalein (100 μM) increased potentiation of $I_{SC}$ responses to 7.6 ± 1.1 μA/cm² (Fig. 3D, $n = 6$, $p < 0.05$) and 8.0 ± 2.0 μA/cm² ($n = 5$, $p < 0.05$), respectively. However, there was no significant difference in [Ca$^{2+}$], responses elicited by histamine in the absence (Fig. 3A, $Δ$Ratio = 0.26 ± 0.03, $n = 6$) or presence of baicalein in the apical (Fig. 3C, $Δ$Ratio = 0.28 ± 0.02, $n = 7$, $p > 0.05$) or basolateral side ($Δ$Ratio = 0.30 ± 0.02, $n = 7$, $p > 0.05$). Therefore, the potentiation effect of baicalein on histamine-induced $I_{SC}$ was similar to that of CCh.

**Baicalein Uncouples the Inhibitory Effects of Carbachol on Subsequent Cl⁻ Secretory Response**—A previous study has shown that carbachol exhibits stimulatory and inhibitory effects on Cl⁻ secretion (26). The long-term effect of this muscarinic agonist on Cl⁻ secretion is inhibitory (27–29). We tested whether baicalein affected muscarinic receptor-mediated inhibition of Ca²⁺-dependent Cl⁻ secretion. As shown in Fig. 4. A and B, T84 cell monolayers stimulated with CCh (10 μM, basolateral) exhibited increases in both [Ca$^{2+}$], and $I_{SC}$. However, such a brief exposure to CCh was capable of inhibiting the subsequent $I_{SC}$ response of the cells to a second challenge of CCh (1st peak: $ΔI_{SC} = 5.9 ± 0.7 μA/cm^2$; 2nd peak: $ΔI_{SC} = 2.4 ± 0.9 μA/cm^2$, $n = 4$, $p < 0.05$). In contrast, if baicalein (30 μM, basolateral) was added after withdrawal of CCh activation as shown in Fig. 4D, a markedly potentiated CCh-induced $I_{SC}$ occurred (1st CCh challenge: $ΔI_{SC} = 5.4 ± 0.6 μA/cm^2$; 2nd CCh challenge: $ΔI_{SC} = 15.0 ± 4.4 μA/cm^2$, $n = 7$, $p < 0.05$). Under this condition, the first CCh challenge no longer inhibited the
secretion of baicalein elicited an increase in the monolayers were challenged by CCh, the same concentration of CCh after 10 min. Fura-2 ratio (C/11006) and concentration of CCh from T84 epithelia receiving two carbachol (C/11005) stimulations. Monolayers were first exposed to 10 μM CCh on the basolateral (bl) side. CCh was washed off, and the monolayer was challenged with the same concentration of CCh after 10 min. Fura-2 ratio (C/11006) were measured in the monolayer using a protocol similar to that described in A and B with 100 μM baicalein added to the basolateral side of the monolayer before the second challenge of CCh. Essentially identical responses were obtained in eight experiments.

second CCh-induced Isc response, despite the change in fluorescence ratio (Fig. 4C) being significantly decreased (1st CCh challenge: Δratio = 0.30 ± 0.02; 2nd CCh challenge: Δratio = 0.25 ± 0.03, n = 7, p < 0.05). Therefore, baicalein reversed the subsequent refractoriness of the cells to CCh-induced Cl secretion. On the other hand, as shown in Fig. 4D, the baicalein-induced Isc response was also significantly potentiated when the monolayers were pre-stimulated with CCh (10 μM, bl). In control experiments, the Isc responses to baicalein (30 μM, basolateral) were only 0.3 ± 0.1 μA/cm² (n = 9). However, after the monolayers were challenged by CCh, the same concentration of baicalein elicited an increase in Isc (4.6 ± 1.5 μA/cm², n = 7, p < 0.05).

Interaction of Baicalein with Forskolin and the Effect on Cl− Secretion—We next examined whether baicalein would interact with a cAMP-dependent secretagogue of Cl− secretion. Therefore, experiments were performed to test the effects of baicalein on Cl− secretion in the presence of the adenylate cyclase activator, forskolin. Applications of baicalein and forskolin had no discernible effect on [Ca²⁺]i, data not shown. In the presence of low concentrations of forskolin (0.1 and 1 μM), application of baicalein elicited an increase in isc (Fig. 5, A and B, n = 4). In contrast, when the monolayers were maximally stimulated by 10 μM forskolin (Fig. 5D), subsequent addition of baicalein (100 μM, apical) inhibited the Isc from 96.9 ± 13.5 to 86.3 ± 12.5 μA/cm² (n = 4). The inhibitory effect of baicalein on forskolin-induced Isc was similar when baicalein was applied to the basolateral side (n = 4, data not shown). Moreover, washing out of baicalein was associated with overshooting of Isc (Fig. 5, A–D). These data suggest that forskolin and baicalein may share a common intracellular signaling pathway (e.g., cAMP) for stimulating Cl− secretion. Thus, upon maximal stimulation of cAMP-dependent Cl− secretion, baicalein may exert an inhibitory effect.

Baicalein Stimulates cAMP Accumulation and PKA Activity—Our previous data have shown that baicalein does not induce increased [Ca²⁺]i, but potentiates the isc response to Ca²⁺-dependent agonists such as CCh and histamine. Furthermore, baicalein does not further increase the isc after a maximal forskolin-induced isc response. Therefore, it is likely that baicalein exerts its effect via a cAMP-dependent pathway in T84 cells. Preliminary study has shown that maximal cAMP stimulation was detected when the cells were exposed to baicalein (100 μM) for 2 min (compared with those measured at 5 and 10 min). As shown in Fig. 6A, the intracellular cAMP content under basal conditions was 2.53 ± 0.26 pmol/mg protein (n = 7). After incubation with baicalein (100 μM), cAMP levels increased significantly to 4.62 ± 0.41 pmol/mg protein (n = 7, p < 0.05). To test whether decreased cAMP degradation contributed to increased cAMP levels, the broad, specific phosphodiesterase inhibitor IBMX was added to T84 cells, and the resulting effects on baicalein-responsive cAMP levels were evaluated. As shown in Fig. 6B, IBMX (100 μM) alone cause an increase in cAMP levels to 3.57 ± 1.20 pmol/mg protein, which is not significantly different from baicalein (n = 5, p > 0.05). However, after incubation with baicalein (100 μM) in the presence of IBMX (100 μM), there was a significant increase in cAMP levels (6.32 ± 1.01 pmol/mg protein, n = 6, p > 0.05). In the presence of forskolin (1 or 10 μM) and IBMX (100 μM), baicalein (100 μM) could not increase cAMP content (n = 5, p > 0.05, data not shown). The data suggest that degradation of intracellular cAMP by phosphodiesterase(s) may alter the process of baicalein-induced Cl− secretion.

PKA activity was also measured in T84 cells using a commercial based assay (Promega) that measures the phosphorylation of Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly), a synthetic substrate specific for PKA. As shown in Fig. 7A, phosphorylated peptide migrated toward the anode. Addition of baicalein (100 μM) for 5 and 15 min significantly increased the PKA activity by about 20 and 25% (n = 7), respectively, when compared with cells treated with vehicle control alone (Fig. 7B).

Baicalein Stimulates Transepithelial Ic(cep) but Not Ikd(b) in Nystatin-permeabilized Monolayers—To further determine whether baicalein was activating an apical Cl− conductance or basolateral K+ conductance in the T84 monolayer, the pore-forming antibiotic nystatin was used to selectively permeabilize either the apical or basolateral membranes. The appropriate transepithelial ion gradients were also established to measure the apical Ic(cep) and basolateral Ik as described under “Experimental Procedures.” Under these asymmetrical conditions, the Cl− gradient was reversed; thus, activation of the apical membrane Cl− conductance would cause a rapid down-
Baicalein-induced Cl⁻ Secretion in T84 Cells

Fig. 6. Effect of baicalein on intracellular cAMP level in T84 cells. A, cells were treated with either vehicle (control) or baicalein (100 μM). Each column represents the mean ± S.E. Significant elevations of cAMP content occurred in baicalein-treated cells compared with untreated control cells (*, p < 0.05, Student’s t test). B, cAMP production was compared following addition of baicalein (100 μM), IBMX (100 μM), and baicalein (100 μM) with IBMX (100 μM). Each column represents the mean ± S.E. Significant elevations in cAMP production were compared within these three groups (*, p < 0.05, analysis of variance with Scheffe’s test).

Fig. 7. Effect of baicalein on PKA activity. A, confluent T84 cells in culture dishes were treated with either vehicle alone (control) or 100 μM baicalein for 5 and 15 min. The positive and negative controls provided by the assay kit are shown in lanes 4 and 5. PKA activity was measured as a function of fluorescence intensity. This photo is representative of seven to eight experiments. B, summarized data showing the relative fluorescence level as compared with control level. Each column represents the mean ± S.E. (*, p < 0.05, Student’s t test).

Fig. 8. Effect of baicalein on apical Cl⁻ currents and basolateral K⁺ currents in nystatin-permeabilized T84 monolayers. A, after establishment of an apical-to-basolateral Cl⁻ gradient and permeabilization of basolateral membrane with nystatin (360 μg/ml), addition of baicalein (100 μM, apical) elicited an inward current, consistent with an absorptive Cl⁻ flow. Glibenclamide (300 μM, apical) blocked this absorptive Cl⁻ current response. B, after establishment of an apical to basolateral K⁺ gradient and permeabilization of apical membrane with nystatin (360 μg/ml, ap), addition of baicalein (100 μM, bl) failed to increase I(Cl)bl whereas subsequent addition of 1,1-ethyl-2-benzimidazolone (300 μM, bl) elicited an increase in basolateral membrane currents. The dashed line indicates the zero current level. C, summarized data showing average changes in I(Cl)bl induced by baicalein (100 μM, ap) in the presence of DPC (300 μM, ap), bumetanide (100 μM, bl), or glibenclamide (300 μM, ap). Each column represents the mean ± S.E. (*, p < 0.05, Student’s t test compared with control).

DISCUSSION

Baicalein is a major flavonoid derived from the medicinal herb S. radix, the dried root of the plant S. baicalensis. Baicalein has been shown to possess a wide range of pharmacological effects including anti-inflammatory (4), anti-tumor (9, 10), anti-oxidative (6–8), and anti-human immunodeficiency virus activities (5). Recently, our laboratory has shown that baicalein...
stimulates Cl⁻ secretion in isolated rat colonic mucosa. The mechanism appears to act via the activation of cAMP-dependent apical Cl⁻ channels and basolateral K⁺ channels. Baicalein also stimulates the accumulation of cAMP in intact rat colonic mucosa (17). Because S. radix is often used in traditional remedies to treat gastrointestinal disorders, the present study focused on the effect of baicalein on human colonic secretory functions using T84 monolayer as a cellular model.

With simultaneous measurement techniques, the concurrent effects of agonists on anion secretion and intracellular calcium increase could be examined in detail. The present study demonstrates that baicalein induced a Cl⁻–dependent secretory response in human colonic T84 cells. This was supported by the effect of Cl⁻ ion replacement in the bathing solution, which completely abolished the I_{SC} response. However, baicalein alone could produce only a modest change in I_{SC} response, which was much smaller than that induced in rat colon (17). Nonetheless, baicalein could markedly potentiate the Ca²⁺–dependent Cl⁻ secretion. The CCh-induced I_{SC} increased by 81 and 105% in the presence of apical and basolateral baicalein, respectively. The results also indicate that apical and basolateral baicalein potentiated the histamine-induced I_{SC} responses by 95 and 104%, respectively. Thus, baicalein can act from both membranes and synergistically enhance the I_{SC} response to Ca²⁺–dependent secretory agonists, suggesting that the effect is not mediated by cellular surface components such as membrane receptors. The marked synergistic effect further suggests that this flavonoid may have substantial in vivo stimulatory effects on colonic Cl⁻ secretion when combined with endogenous Ca²⁺–dependent secretagogues, such as muscarinic agonists or histamine released from mast cells.

Interestingly, the synergism between CCh and baicalein was also apparent when the two compounds were applied in the reverse order, i.e. CCh following by baicalein (Fig. 4D). When the cells were briefly exposed to CCh, subsequent application of baicalein could induce a substantial increase in I_{SC} even though the CCh had been washed off. The observed synergism between CCh and baicalein suggests that they do not share the same effector pathway mediated by intracellular Ca²⁺. Moreover, the results also indicate that baicalein can reverse the inhibitory effect of the first CCh challenge on subsequent CCh-induced Cl⁻ secretion. As in other flavonoids, one possible mechanism for modulating Ca²⁺–dependent Cl⁻ secretion involves the ability to inhibit tyrosine kinase activity (35, 36). Consequently, generation of inositol 3,4,5,6-tetrakisphosphate by a tyrosine kinase-dependent pathway could negatively regulate Ca²⁺–dependent Cl⁻ secretion (37). Baicalein has been shown to inhibit tyrosine kinase activity in human T-lymphoid leukemia cells (38). However, further experiments are required to investigate the effect of baicalein on the generation of these regulatory signals because baicalein may functionally uncouple CCh-induced Cl⁻ secretion from these signals. Consequently, the interaction between CCh and baicalein suggests that intestinal Cl⁻ secretion may be regulated by positive feedback. That is, CCh enhances the baicalein I_{SC} response, which in turn potentiates the CCh effect on Cl⁻ secretion.

Regarding the potentiation by baicalein of CCh- or histamine-induced Cl⁻ secretion in T84 cells, one possible mechanism involves increasing and/or prolonging the cytosolic Ca²⁺ signaling, as done by aspartyl protease inhibitors (39). These agents potentiate muscarinic activation of Cl⁻ secretion by increasing the magnitude and duration of CCh-induced increases via activation of a long-lived, store-operated Ca²⁺ entry (39). However, data from simultaneous measurements of [Ca²⁺], and I_{SC} (Figs. 1, A and B, and 4, C and D) suggest that baicalein itself does not stimulate any increase in [Ca²⁺]. Also, the CCh- and histamine-induced increases in [Ca²⁺], were not altered by baicalein (Figs. 2C and 3C). Therefore, the cellular mechanism does not involve modulation of Ca²⁺ signaling pathways. In T84 cells, Cl⁻ ions exit through the calcium-activated Cl⁻ channels (CaCC) or cAMP-dependent CFTR Cl⁻ channels (37). Our data suggest that the CaCC do not mediate the Cl⁻ secretory response. Thus Cl⁻ movement across the apical membrane occurs via another class of Cl⁻ channels, namely the CFTR Cl⁻ channels. A less likely possibility is that baicalein activates a distinct Cl⁻ channel that requires regulation by CFTR. Using enzyme-linked immunosorbent assay, baicalein was shown to increase cAMP content in T84 cells (Fig. 6). The cAMP content was increased by baicalein in the presence of the phosphodiesterase inhibitor IBMX, excluding the possibility that baicalein might have inhibited phosphodiesterase. PKA, which is downstream of cAMP in the signaling pathway, was also activated by baicalein (Fig. 7). Elevation of cAMP and activation of PKA, as shown in this study, are key regulatory components of CFTR activity (37). Additionally, the CFTR blocker glibenclamide (30, 31) inhibited I_{SC} in the presence of baicalein (Fig. 8C), indicating that Cl⁻ secretion was dependent on apical CFTR activity. It is therefore most likely that the I_{SC} response to baicalein was mediated by increases in cellular cAMP levels, which then activate PKA and subsequently lead to the opening of apical CFTR Cl⁻ channels. The augmentation of the Ca²⁺–mediated I_{SC} can also be explained by this cAMP-dependent pathway. Cl⁻ secretion in colonic epithelia is regulated in a cooperative fashion by cAMP and [Ca²⁺] (40). A number of studies have described that an agonist of cAMP could synergistically enhance the secretory response to Ca²⁺–mobilizing agonists in T84 cells, despite the varied degree of synergism observed (41–43). Chloride secretion induced by CCh could be augmented in the presence of agents that increase cAMP levels. Histamine can promote chloride secretion, which resembles that of CCh in that it is associated with a rise in [Ca²⁺], and is potentiated by cAMP-mediated secretagogues (44). These agents increase basolateral K⁺ permeability and CFTR Cl⁻ conductance on the apical membrane, accounting for a potentiated I_{SC} response (45). Several known flavonoids, such as flavopiridol (46) and citrus flavonoids (47), have been shown to potentiate Ca²⁺–dependent secretions in T84 cells in a similar fashion.

The stimulation of transepithelial Cl⁻ secretion requires the activation of apical membrane Cl⁻ channels and/or basolateral K⁺ channels. Our data show that baicalein could activate an apical membrane Cl⁻ conductance in nystatin-permeabilized T84 monolayers (Fig. 8A). Although baicalein has no effect on basolateral K⁺ conductance (Fig. 8B), its ability to significantly increase I_{SC} produces more apical Cl⁻ exit pathways than are present in the basal state. The results indicate that the basolateral membrane was rate-limiting to Cl⁻ secretion across T84 cells. Our finding also shows that baicalein-induced Cl⁻ secretion is sensitive to glibenclamide, which blocks apical CFTR. Taken together, the present study provides evidence that cAMP-PKA signaling is crucial to the I_{SC} response to baicalein, most likely by targeting apical CFTR Cl⁻ channels.

Although baicalein appears to exert its effect via a cAMP-PKA-dependent pathway, the increase in I_{SC} induced by the adenylate cyclase activator forskolin was diminished by baicalein (Fig. 5D). This result is in contrast to the findings obtained in rat colon, in which baicalein further increased the I_{SC} after a maximal stimulation of cAMP-dependent secretion by forskolin (17). In T84 cells, baicalein induced a further increase in I_{SC} only after the cells were stimulated by forskolin at low concentrations (0.1 and 1 μM), at which CFTR channels may not be maximally activated. However, baicalein did not pro-
mote further $I_{SC}$ response or cAMP production after maximal stimulation of the cells by forskolin (10 μM). Again, this lack of an additive response is consistent with both baicalein and forskolin activating CFTR. In contrast, the forskolin-stimulated $I_{SC}$ responses were inhibited by baicalein by an average of 8%. Moreover, when baicalein and forskolin were removed from the bathing solution, overshooting of the $I_{SC}$ occurred (Fig. 5, A–D). Interestingly, a similar inhibitory effect by genistein on forskolin-induced $I_{SC}$ has been observed in T84 cells (48). Illek et al. (48) have shown that genistein inhibited forskolin-stimulated $I_{SC}$ secretion by 11% in T84 monolayers. They suggest that the inhibitory effect of genistein is because of a blockage of K+ conductance in the basolateral membrane. When cAMP-dependent channels were maximally activated by forskolin, basolateral K+ conductance inhibition was induced by genistein and the forskolin-induced $I_{SC}$ increase was inhibited. Therefore, similar to genistein, the overall secretory response to baicalein may be composed of both stimulatory and inhibitory components. The dominance of inhibitory and stimulatory interactions induced by baicalein may explain why baicalein induces only a modest secretory response in $I_{SC}$ changes. Moreover, baicalein did not induce a significant increase in basolateral K+ conductance, thus hyperpolarizing the membrane and increasing the electrochemical driving force for Cl− exit across the apical membrane, which is a rate-limiting step during cAMP-mediated Cl− secretion.

In summary, this study provides the first evidence of the cellular mechanism by which baicalein modulates Ca2+-dependent secretion in human intestinal cells. Cl− secretion across the T84 monolayers can be stimulated by the flavonoid baicalein. Mechanisms involve cAMP-, PKA-, and luminal cAMP-dependent Cl− channels, most likely CFTR. Baicalein also markedly potentiates Ca2+-dependent secretagogues, such as CCh and histamine. Therefore, baicalein may act directly on the intestinal mucosa to enhance the activity of muscarinic and other Ca2+-dependent secretagogues in potentiating an otherwise normal secretory response or in readying the cells for a secretory response to physiologic agonists. In addition, the inhibitory effect of CCh was reversed in the presence of baicalein, and the baicalein-induced Cl− secretion was also potentiated by CCh. Such interactions and mechanisms of action may therefore have important impacts on intestinal Cl− secretion.

Acknowledgments—We thank Wallace C. Y. Yip for excellent technical support. The miniature Ussing chamber was obtained from Dr. E. H. Larsen (Zoophysiological Laboratory A, August Krogh Institute, University of Copenhagen, Denmark).

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Cellular Mechanism for Potentiation of Ca\(^{2+}\)-mediated Cl\(^{-}\) Secretion by the Flavonoid Baicalein in Intestinal Epithelia
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doi: 10.1074/jbc.M406787200 originally published online July 3, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M406787200

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