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Icilin Activates the δ-Subunit of the Human Epithelial Na^+ Channel

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ABSTRACT

The amiloride-sensitive epithelial Na^+ channel (ENaC) regulates Na^+ homeostasis in cells and across epithelia. Four homologous ENaC subunits (α, β, γ, and δ) have been isolated in mammals. The chemical activators acting on ENaC, however, are largely unknown. More recently, we have found that capsazepine activates human ENaCδ (hENaCδ), which is mainly expressed in the brain. In addition, here we show that icilin, which is a tetrahydropyrimidine-2-one derivative unrelated structurally to capsazepine, markedly enhanced the activity of hENaCδβγ heteromultimer expressed in *Xenopus laevis* oocytes. The inward currents at a holding potential of −60 mV in hENaCδβγ-expressing oocytes were increased by the application of icilin in a concentration-dependent manner with an EC_{50} value of 33 μM. The icilin-elicited current was mostly abolished by the addition of 100 μM amiloride or by the removal of external Na^+. Homomeric hENaCδ was also significantly activated by icilin, whereas hENaCα activity was not affected by icilin, and icilin caused a slight inhibition of the hENaCαβγ current. Furthermore, icilin acted together with protons or capsazepine on hENaCδβγ. These findings identify icilin as a novel chemical activator of ENaCδ, providing us with a lead compound for drug development in the degenerin/ENaC superfamily.

The degenerin/epithelial Na^+ channel superfamily has striking functional diversity, including Na^+ homeostasis, acid sensing, peptide-gating, acidosis-evoked nociception, and mechanotransduction (Ugawa et al., 1998, 2002; Alvarez de la Rosa et al., 2000; Kellenberger and Schild, 2002; Welsh et al., 2002). The amiloride-sensitive epithelial Na^+ channel (ENaC) is an essential control element for Na^+ transport into cells and across epithelia. Four homologous ENaC subunits (α, β, γ, and δ) have been cloned in mammals (Canessa et al., 1993, 1994; McDonald et al., 1994, 1995; Waldmann et al., 1995). The δ-subunit was originally described to be expressed mainly in the human brain (Waldmann et al., 1995), and we have demonstrated that protons activate ENaCδ (Yamamura et al., 2004b). To explore physiological functions of ENaCδ in the human brain, it is necessary to identify the chemical modulator for ENaCδ. In pharmacological profiles of ENaCδ, it is well-published that the potassium-sparing diuretics, amiloride and benzamil, inhibit the activities of ENaCδ homomer and the heteromeric complexes with β and γ subunits (Waldmann et al., 1995; Ji et al., 2004). More recently, we have shown that capsazepine, which was originally developed as a competitive antagonist for transient receptor potential vanilloid subfamily 1 (Bevan et al., 1992; Szallasi et al., 1993; Caterina et al., 1997; Szallasi and Blumberg, 1999), is the first known chemical activator of ENaCδ (Yamamura et al., 2004a).

In this investigation, the effects of icilin, which is a tetrahydropyrimidine-2-one derivative (Wei and Seid, 1983) and is also an activator for transient receptor potential melastatin subfamily 8 and ankyrin-like subfamily 1 (McKemy et al., 2002; Story et al., 2003; Andersson et al., 2004; Chuang et al., 2004), were examined on the human ENaC (hENaC) current using electrophysiological analyses in the *Xenopus laevis* oocyte expression system. Here, we show that icilin activates hENaCδβγ in a concentration-dependent manner, and the icilin-evoked current was influenced by amiloride or external Na^+ removal. Icilin activated the hENaCδ homomer as well as hENaCδβγ, whereas the hENaCαβγ current was slightly reduced by icilin. These results indicate that icilin is a novel agonist for ENaCδ.

Materials and Methods

Molecular Biology. All experiments were approved by the Ethics Committee of the Nagoya City University Graduate School of Medical Sciences and were conducted in accordance with the Declaration

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ABBREVIATIONS: ENaC, epithelial Na^+ channel; h, human; NMDG, *N*-methyl-D-glucamine.

of Helsinki. The full-length hENaCα (GenBank accession number X76180), hENaCβ (X87159), hENaCγ (U48937), and hENaCδ (U38254) were isolated from human skin (for α, β, and γ subunits) or brain (for δ subunit) cDNA, as described previously (Yamamura et al., 2004a).

***X. laevis* Oocyte Electrophysiology.** Electrophysiological studies in *X. laevis* oocytes, using a two-electrode voltage-clamp technique, were performed as described previously (Yamamura et al., 2004a,b). In brief, cRNA transcript(s) (1 ng for homomeric channel or each 0.01 ng for coexpression) was injected into *X. laevis* oocytes, whereas the control oocytes were injected with an equal volume of diethyl dicarbonate-treated water, described as "native" throughout. After injection, oocytes were incubated at 20°C in a recording solution supplemented with 20 units/ml penicillin G, 20 μg/ml streptomycin, and either 10 (for hENaCα and αβγ) or 100 μM (for hENaCδ and δβγ) amiloride for 24 to 48 h before electrophysiological recordings. The recording solution had an ionic composition of 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES. The pH of the solution was adjusted to 7.5 with NaOH. The external Na^+-free solution was prepared by the replacement of 96 mM Na^+ with the equivalent *N*-methyl-D-glucamine (NMDG). All electrophysiological recordings were performed at a holding potential of −60 mV. The current-voltage relationships were measured using a ramp protocol from −100 to 50 mV for 30 s. The recording chamber was continuously perfused with solution at a flow rate of 5 ml/min. All electrophysiological experiments were carried out at room temperature (25 ± 1°C).

Drugs. Pharmacological reagents were obtained from Sigma Chemical (St. Louis, MO). Icilin was dissolved in dimethyl sulfoxide at the concentration of 100 mM as a stock solution. It was confirmed that up to 1% of dimethyl sulfoxide did not affect the oocyte currents.

Statistics. Pooled data are shown as the mean ± S.E. Statistical significance between two groups and among groups was determined by Student's *t* test and Scheffé's test after one-way analysis of variance, respectively. Significant difference is expressed in the figures (⋆⋆ or ##, $p < 0.01$). The data of the relationship between icilin concentrations and current responses were fitted using the following equation after normalization by the current amplitude in the absence of icilin (Figs. 4B and 5): $I_{Icilin}/I_{Control} = (I_{max}/I_{Contol})/\{1 + (K_d/[Icilin])^n\}$, where I_{max} is the maximum amplitude of the icilin-evoked current, K_d is the apparent dissociation constant of icilin, [Icilin] is the concentration of icilin, and n is the Hill coefficient.

Results

Activation of hENaCδβγ Current by Icilin. The effects of icilin, a class of compounds known as tetrahydropyrimidine-2-one derivatives (Wei and Seid, 1983), on the hENaCδβγ current were examined using a two-electrode voltage-clamp technique in the *X. laevis* oocyte expression system. When the hENaCδβγ heteromultimer was expressed in *X. laevis* oocytes, an inward current was induced at a holding potential of −60 mV, and the current was mostly blocked by 100 μM amiloride (Fig. 1A). The mean amplitude of the amiloride-sensitive current in hENaCδβγ-expressing oocytes was 575 ± 27 nA ($n = 29$, $p < 0.01$ versus native of 3 ± 1 nA, $n = 6$). In hENaCδβγ-injected oocytes, the application of 100 μM icilin was markedly increased in the inward current (1.79 ± 0.04-fold, $n = 23$, $p < 0.01$; Fig. 1C). The current increase by icilin was recovered to the resting level by the removal of icilin in all hENaCδβγ-expressing oocytes tested ($n = 15$). After washout for a few minutes, readministration of icilin caused current activation with a similar amplitude and kinetics to the first challenge ($n = 6$). The current-voltage relationship showed that the application of 100 μM icilin potentiated channel activity at all voltages examined in hENaCδβγ-expressed oocytes ($n = 5$; Fig. 1B). On the other hand, in native oocytes, the application of 100 μM icilin did not induce any current (1.01 ± 0.01-fold, $n = 6$, $p > 0.05$).

It was examined whether the activation of the inward current by icilin was mediated though either ENaCδ alone or the accessory β or γ subunit. In homomeric hENaCδ-expressing oocytes, the application of 100 μM icilin significantly elicited an inward current (1.22 ± 0.03-fold, $n = 5$, $p < 0.01$; Fig. 1C), as well as in hENaCδβγ-injected oocytes. Furthermore, we tested whether icilin was effective on another ENaC core unit, the α-subunit, in *X. laevis* oocytes. In contrast to hENaCδ- and δβγ-injected oocytes, the application of 100 μM icilin had no effect on the inward currents in hENaCα-expressing oocytes (0.99 ± 0.01-fold, $n = 8$, $p >$

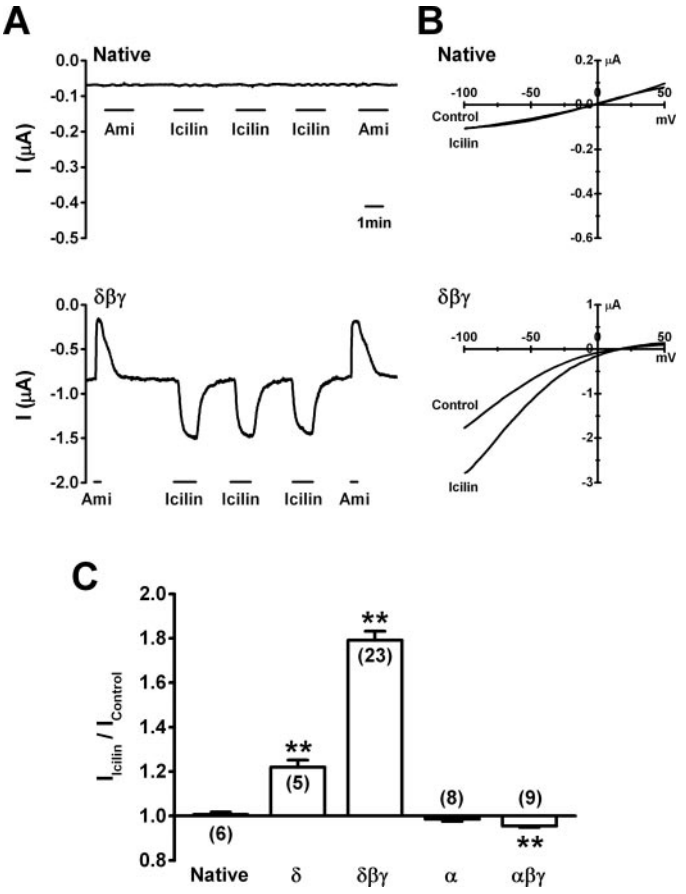

Fig. 1. Activation of hENaCδβγ current by icilin. Whole-cell currents were recorded at a holding potential of −60 mV in the *X. laevis* oocyte expression system using a two-electrode voltage-clamp technique. A, an hENaCδβγ-expressed oocyte possessed a larger inward current than a native oocyte. The larger current in an hENaCδβγ-expressing oocyte was mostly inhibited by 100 μM amiloride (Ami). In an hENaCδβγ oocyte, the application of 100 μM icilin enhanced the inward current, and this current increase was recovered by the removal of icilin. After washout for a few minutes, a readministration of icilin caused similar current activation. Note that neither the application of icilin nor amiloride induced any current in native oocytes. B, the current-voltage relationships in the absence and presence of 100 μM icilin in native and hENaCδβγ-injected oocytes are shown. Icilin stimulus enhanced hENaCδβγ activity at all voltages examined. C, the effects of 100 μM icilin on homomeric and heteromeric hENaCs expressed in *X. laevis* oocytes are summarized. The icilin-induced current was normalized by the current amplitude in the absence of icilin. The number of oocytes used is in parentheses. The statistical significance of the difference is expressed as ⋆⋆, $p < 0.01$ versus control.

0.05), and the hENaC$\alpha\beta\gamma$ current was significantly inhibited by 100 μM icilin (0.96 $\pm$ 0.01-fold, $n = 9$, $p < 0.01$).

Effects of Amiloride on Icilin-Induced Current. To confirm whether the icilin-induced current originated from the ENaC$\delta\beta\gamma$ expression, the effects of amiloride, an inhibitor of ENaC, on the inward current in the presence of icilin were examined. The 100 μM icilin-induced current in hENaC$\delta\beta\gamma$-injected oocytes (1421 $\pm$ 76 nA, $n = 6$) was dramatically inhibited by the addition of 100 μM amiloride (85 $\pm$ 3% decrease, $n = 6$, $p < 0.01$) and, moreover, significantly reduced the current to 209 $\pm$ 23 nA ($n = 6$, $p < 0.01$ versus the initial resting current of 785 $\pm$ 55 nA; Fig. 2). On the other hand, in native oocytes, the current amplitudes after the application of 100 μM icilin in the absence and presence of 100 μM amiloride (72 $\pm$ 1 and 69 $\pm$ 1 nA, respectively, $n = 6$) were not significant to the initial resting current (70 $\pm$ 1 nA, $n = 6$, $p > 0.05$).

Effects of External Na^+ Removal on Icilin-Evoked Current. In turn, the effects of external Na^+ removal were analyzed in hENaC$\delta\beta\gamma$-expressing oocytes. The Na^+-free solution was prepared by the substitution of 96 mM Na^+ in recording solution with the equivalent NMDG. In the absence of external Na^+, the amiloride-sensitive current was significantly small (14 $\pm$ 4 nA, $n = 5$, $p < 0.01$ versus before the removal of external Na^+) (Fig. 3). The current increase by the application of 100 μM icilin was significantly reduced by the removal of external Na^+ (39 $\pm$ 14 nA, $n = 5$, $p < 0.01$ versus 96 mM $[Na^+]_o$ of 599 $\pm$ 23 nA, $n = 5$).

Dose-Dependence of Current Activation by Icilin. The concentration-dependence of the icilin-induced current was examined in hENaC$\delta\beta\gamma$-expressed oocytes. In hENaC$\delta\beta\gamma$-expressed oocytes, changing the concentration of icilin in the range of 1 to 1000 μM showed that the inward current was significantly increased by icilin at a concentration of 10 μM and more ($n = 5$, $p < 0.01$ versus control of 743 $\pm$ 66 nA), and the enhancement was in a concentration-dependent manner (1547 $\pm$ 143 nA at 1000 μM, $n = 5$; Fig. 4). The EC_{50} value of icilin on the inward currents was 33 $\pm$ 1 μM, and the Hill coefficient was 1.3 $\pm$ 0.1 ($n = 5$).

Sensitization by Protons and Capsazepine on Icilin-Activated hENaC$\delta\beta\gamma$ Current. We reported previously that ENaC$\delta\beta\gamma$ activity was enhanced by external protons or capsazepine in the *X. laevis* oocyte expression system (Yamamura et al., 2004a,b). Therefore, the effects of icilin on

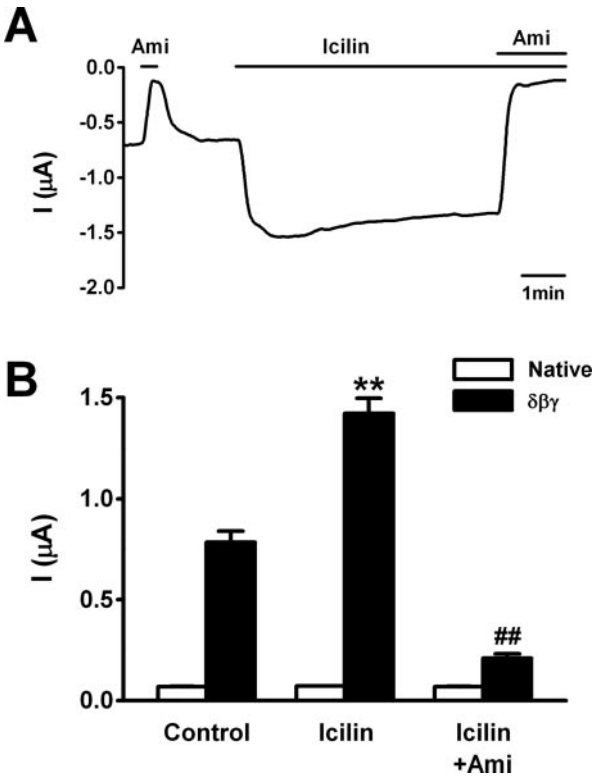

Fig. 2. Effects of amiloride on icilin-induced current. The effects of amiloride on the inward current evoked by icilin were examined in *X. laevis* oocytes. A, a typical current trace in an hENaC$\delta\beta\gamma$-expressing oocyte in response to icilin and amiloride (Ami) is represented. The current increase by 100 μM icilin was dramatically influenced by the addition of 100 μM amiloride. B, the effects of 100 μM icilin in native (□) or hENaC$\delta\beta\gamma$-expressed (■) oocytes in the absence and presence of 100 μM amiloride are summarized. The application of neither icilin nor amiloride induced any current in native oocytes. Experimental data were obtained from six oocytes for each. The statistical significance of the difference is expressed as ⋆⋆, $p < 0.01$ versus control, and ##, $p < 0.01$ versus icilin alone.

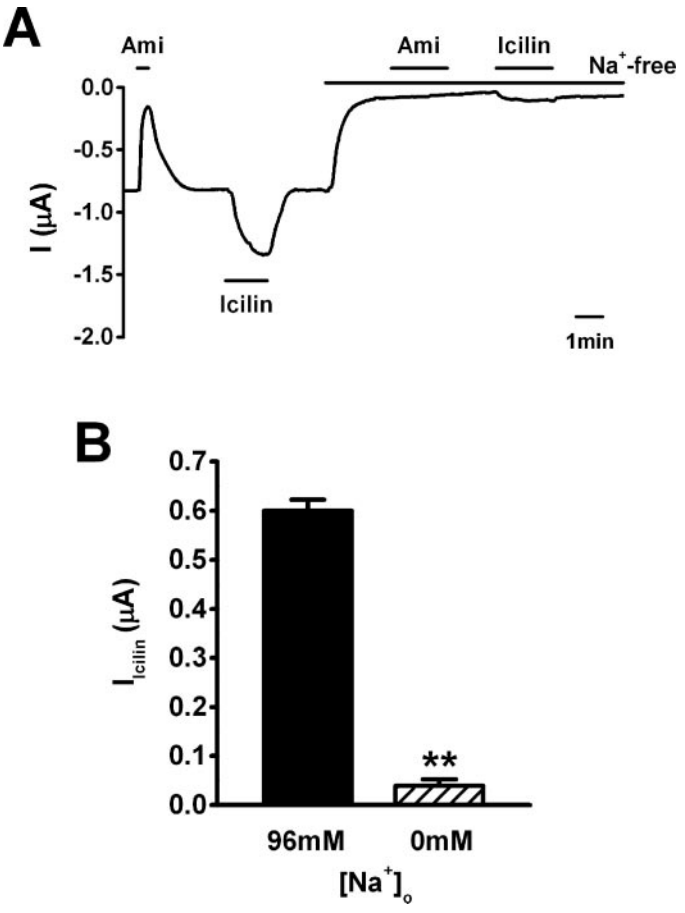

Fig. 3. Effects of external Na^+ removal on icilin-evoked current. The effects of external Na^+ removal on the inward current elicited by icilin were examined in *X. laevis* oocytes. A, a typical current trace in an hENaC$\delta\beta\gamma$-injected oocyte in response to icilin and amiloride (Ami) in the absence and presence of external Na^+ is shown. The external Na^+-free was prepared by the substitution of 96 mM Na^+ in the recording solution with the equivalent NMDG. In the absence of external Na^+, the current amplitude evoked by 100 μM icilin was smaller than that in the presence of external Na^+. B, the effects of 100 μM icilin in hENaC$\delta\beta\gamma$-expressed oocytes in the absence (▨) and presence (■) of external Na^+ are summarized. Experimental data were obtained from five oocytes for each. The statistical significance of the difference is expressed as ⋆⋆, $p < 0.01$ versus 96 mM $[Na^+]_o$.

the weak acidification of pH 7.0 medium, which was the subthreshold concentration of protons on hENaCδβγ current (by 23 ± 2 nA, $n = 5$), were examined in hENaCδβγ-expressed oocytes (Fig. 5A). The decrease in external pH from 7.5 to 7.0 caused a leftward shift of the dose-response curve for icilin on the inward current to the EC_{50} value of 16 ± 2 μM ($n = 5$, $p < 0.01$, versus pH 7.5) and the Hill coefficient of 1.2 ± 0.1. The maximum response of the icilin-induced current during the exposure to pH 7.0 (by 871 ± 144 nA, $n = 5$) was not statistically significant compared with that in pH 7.5 medium (by 804 ± 110 nA, $n = 5$, $p > 0.05$). On the other hand, the effects of icilin in the presence of 1 μM capsazepine, which by itself had a very small effect on hENaCδβγ current (by 120 ± 6 nA, $n = 5$), were examined in hENaCδβγ-injected oocytes (Fig. 5B). The dose-response curve for icilin on the inward currents was shifted to the left with an EC_{50} value of 12 ± 1 μM ($n = 5$, $p < 0.01$) and a Hill coefficient of 1.2 ± 0.1. The maximum amplitude of the icilin-evoked current in the presence of 1 μM capsazepine (by 873 ± 44 nA, $n = 5$) was not statistically significant compared with that in the absence of capsazepine ($p > 0.05$).

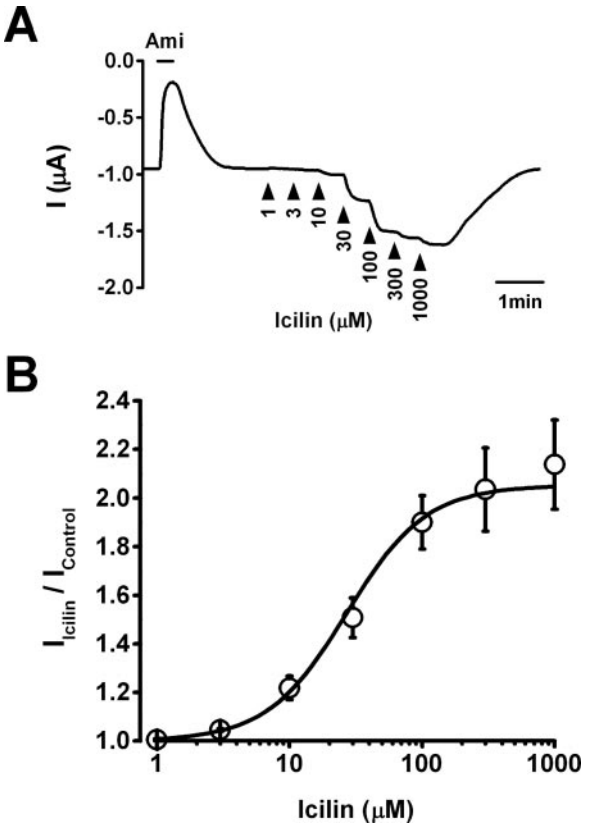

Fig. 4. Dose-dependence of current activation by icilin. The dependence on icilin concentration of the hENaCδβγ current was analyzed in *X. laevis* oocytes. A, a typical current trace of icilin responsiveness from 1 to 1000 μM in an hENaCδβγ-injected oocyte is represented. The activation of an inward current by icilin was observed in a concentration-dependent manner. B, icilin sensitivity in hENaCδβγ currents is plotted. The icilin-induced current was normalized by the current amplitude in the absence of icilin. The inward current was significantly potentiated by icilin at a concentration of 10 μM and more in hENaCδβγ-expressed oocytes ($p < 0.01$). The EC_{50} value for icilin on the inward currents was 33 ± 1 μM, and the Hill coefficient was 1.3 ± 0.1. Experimental data were obtained from five oocytes.

Discussion

Amiloride-sensitive ENaCs, members of the degenerin/ENaC superfamily, regulate essential control elements for Na^+ homeostasis in cells and across epithelia. Because the ENaCαβγ complex is expressed mainly in epithelia such as the kidney, lung, and colon to play a pathophysiological role, the physiological and pharmacological characterization has been well-documented (Alvarez de la Rosa et al., 2000; Kellenberger and Schild, 2002). On the other hand, the physiological function of the δ-subunit has not yet been identified. More recently, we have shown that ENaCδ is widely distributed throughout the brain and is activated by protons, indicating that it may act as a pH sensor in the human brain (Yamamura et al., 2004b). In addition, we have demonstrated that capsazepine is the first chemical agonist for ENaCδ (Yamamura et al., 2004a). In this investigation, we have found that the application of icilin, which is a tetrahydropyrimidine-2-one derivative unrelated structurally to capsaz-

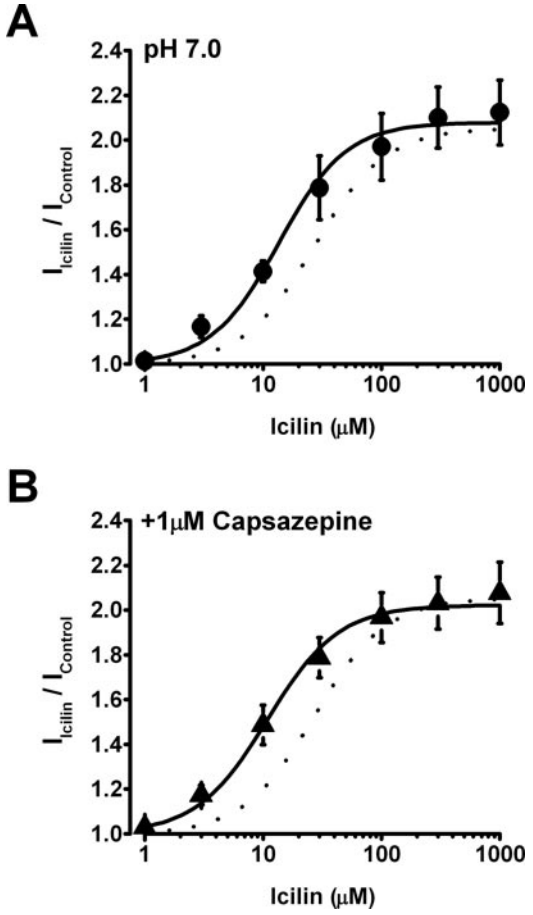

Fig. 5. Sensitization by protons and capsazepine on icilin-activated hENaCδβγ current. The effects of protons and capsazepine on the icilin-activated hENaCδβγ current in *X. laevis* oocytes were examined. A, in hENaCδβγ-injected oocytes, the decrease in pH from 7.5 to 7.0, which is the subthreshold concentration of protons on the hENaCδβγ current, caused a leftward shift of the dose-response curve for icilin on the inward current to an EC_{50} value of 16 ± 2 μM and a Hill coefficient of 1.2 ± 0.1. B, in the presence of 1 μM capsazepine, which by itself had a very small effect on hENaCδβγ current, the dose-response curve for icilin on inward currents was shifted to the left with an EC_{50} value of 12 ± 1 μM and a Hill coefficient of 1.2 ± 0.1. The icilin-induced current was normalized by the current amplitude in the absence of icilin. The broken line indicates a dose-response curve of icilin alone on the inward current obtained from Fig. 4B. Experimental data were obtained from five oocytes for each.

epine, activates hENaCδβγ in a concentration-dependent manner, and the enhancement is sensitive to amiloride.

When the heteromultimeric hENaCδβγ complex was expressed in *X. laevis* oocytes, the application of icilin at a concentration of 10 μM and more was markedly increased by an inward current. Because the icilin-induced current was significantly abolished by the addition of 100 μM amiloride, an inhibitor of ENaC, or by the removal of external Na^+ in hENaCδβγ-expressing oocytes, and icilin-elicited currents were not observed in native oocytes, the icilin-evoked currents originated from the ENaCδβγ expression. The icilin-induced current was maintained at a steady level during icilin exposure in hENaCδβγ-expressed oocytes (~5 min; Fig. 2A). After washout for a few minutes, the sequential challenge of icilin causes current activation to the same extent as the first trial in hENaCδβγ-expressing oocytes. These results indicate that icilin did not induce sensitization and desensitization to the activity of hENaCδβγ under these conditions, similarly to protons and capsazepine, which are also activators of ENaCδ (Ji and Benos, 2004; Yamamura et al., 2004a,b). In addition to the enhancement actions by icilin on transient receptor potential melastatin subfamily 8 and ankyrin-like subfamily 1 at micromolar concentrations (McKemy et al., 2002; Story et al., 2003; Andersson et al., 2004; Chuang et al., 2004), in this investigation, we clarified that icilin activates hENaCδβγ with an EC_{50} of 33 μM.

The sensitivity to icilin was increased in a weak acidic medium of pH 7.0. Protons activated the hENaCδ current, but pH 7.0 is the subthreshold concentration of protons in the hENaCδβγ current (Yamamura et al., 2004a,b; Ji and Benos, 2004). At this proton concentration, the dose-response for icilin on the inward currents was enhanced, indicating that the effect of icilin was sensitized by the addition of protons. Moreover, the concentration-dependence of hENaCδβγ for icilin was shifted to the left by the lower concentration of capsazepine (1 μM), which by itself had a very small effect on hENaCδβγ current (Yamamura et al., 2004a). Similar shifts in sensitivity by the mixture of two different ligands were observed: protons/capsaicin, capsaicin/2-aminoethoxydiphenyl borate, and protons/2-aminoethoxydiphenyl borate in transient receptor potential vanilloid subfamily 1 (Caterina et al., 1997; Hu et al., 2004) as well as protons/capsazepine in ENaCδβγ (Yamamura et al., 2004a). These results indicate that icilin acts synergistically with protons or capsazepine, activating factors of ENaCδ. Because the structural similarity between icilin (1-(2-hydroxyphenyl)-4-(3-nitrophenyl)-1,2,3,6-tetrahydropyrimidin-2-one) and capsazepine (*N*-[2-(4-chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2*H*-2-benzazepine-2-carbothioamide) seemed to be low, it is still unknown whether the mechanism of channel activation by icilin is similar to that by protons or capsazepine. It is interesting that icilin, capsazepine, and protons all activate ENaCδ but all partially inhibit ENaCα (Chalfant et al., 1999; Ji and Benos, 2004; Yamamura et al., 2004a,b), which may be a key point for elucidating the mechanism underlying channel modulation. These findings provide useful information for drug development in the degenerin/ENaC superfamily.

Acid-sensing ion channel 1a in the central nervous system has been implicated in long-term potentiation, suggesting that minute fluxes in synaptic pH may activate a proton-sensitive channel to enhance synaptic plasticity, learning, and memory (Bianchi and Driscoll, 2002; Wemmie et al., 2002). This raises the possibility that ENaCδ could also play a role in learning and memory in the human brain (Yamamura et al., 2004b). The expressed sequence tag and genome project databases show that an ENaCδ gene has been found only in humans and chimpanzees (GenBank accession numbers U38254 and O46547, respectively), and there is no evidence of the orthologs in rats and mice. The corresponding genomic assignments of ENaCδ were identified on human chromosome 1p36.3-p36.2 (Waldmann et al., 1996). Because the chemical agonists strongly influencing ENaCδ have been poorly investigated, icilin and capsazepine are potentially powerful tools for the electrophysiological analysis of ENaCδ and the elucidation of the clinical ENaCδ function in humans.

In conclusion, we found that icilin acts on ENaCδ and causes the activation of ENaCδ, indicating that icilin is a novel activator of ENaCδ. In addition to the physiological function of ENaCδ as a pH sensor in the human brain (Yamamura et al., 2004b), this finding provides a starting point for a number of exciting follow-up investigations into the physiological and pathological roles of ENaCδ in vitro and in vivo in humans.

Acknowledgments

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References

Alvarez de la Rosa D, Canessa CM, Fyfe GK, and Zhang P (2000) Structure and regulation of amiloride-sensitive sodium channels. *Annu Rev Physiol* **62:**573–594.
Andersson DA, Chase HW, and Bevan S (2004) TRPM8 activation by menthol, icilin and cold is differentially modulated by intracellular pH. *J Neurosci* **24:**5364–5369.
Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, Walpole CS, and Yeats JC (1992) Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. *Br J Pharmacol* **107:**544–552.
Bianchi L and Driscoll M (2002) Protons at the gate: DEG/ENaC ion channels help us feel and remember. *Neuron* **34:**337–340.
Canessa CM, Horisberger JD, and Rossier BC (1993) Epithelial sodium channel related to proteins involved in neurodegeneration. *Nature (Lond)* **361:**467–470.
Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, and Rossier BC (1994) Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. *Nature (Lond)* **367:**463–467.
Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, and Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature (Lond)* **389:**816–824.
Chalfant ML, Denton JS, Berdiev BK, Ismailov II, Benos DJ, and Stanton BA (1999) Intracellular H^+ regulates the α-subunit of ENaC, the epithelial Na^+ channel. *Am J Physiol* **276:**C477–C486.
Chuang HH, Neuhausser WM, and Julius D (2004) The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. *Neuron* **43:**859–869.
Hu HZ, Gu Q, Wang C, Colton CK, Tang J, Kinoshita-Kawada M, Lee LY, Wood JD, and Zhu MX (2004) 2-Aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2 and TRPV3. *J Biol Chem* **279:**35741–35748.
Ji HL and Benos DJ (2004) Degenerin sites mediate proton activation of δβγ-epithelial sodium channel. *J Biol Chem* **279:**26939–26947.
Ji HL, Bishop LR, Anderson SJ, Fuller CM, and Benos DJ (2004) The role of Pre-H2 domains of α- and δ-epithelial Na^+ channels in ion permeation, conductance and amiloride sensitivity. *J Biol Chem* **279:**8428–8440.
Kellenberger S and Schild L (2002) Epithelial sodium channel/degenerin family of ion channels: a variety of functions for a shared structure. *Physiol Rev* **82:**735–767.
McDonald FJ, Price MP, Snyder PM, and Welsh MJ (1995) Cloning and expression of the β- and γ-subunits of the human epithelial sodium channel. *Am J Physiol* **268:**C1157–C1163.
McDonald FJ, Snyder PM, McCray PB Jr, and Welsh MJ (1994) Cloning, expression and tissue distribution of a human amiloride-sensitive Na^+ channel. *Am J Physiol* **266:**L728–L734.
McKemy DD, Neuhausser WM, and Julius D (2002) Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature (Lond)* **416:**52–58.
Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA, Hwang SW, et al. (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* **112:**819–829.
Szallasi A and Blumberg PM (1999) Vanilloid (capsaicin) receptors and mechanisms. *Pharmacol Rev* **51:**159–212.
Szallasi A, Goso C, Blumberg PM, and Manzini S (1993) Competitive inhibition by

capsazepine of [^{3}H]resiniferatoxin binding to central (spinal cord and dorsal root ganglia) and peripheral (urinary bladder and airways) vanilloid (capsaicin) receptors in the rat. *J Pharmacol Exp Ther* **267:**728–733.
Ugawa S, Minami Y, Guo W, Saishin Y, Takatsuji K, Yamamoto T, Tohyama M, and Shimada S (1998) Receptor that leaves a sour taste in the mouth. *Nature (Lond)* **395:**555–556.
Ugawa S, Ueda T, Ishida Y, Nishigaki M, Shibata Y, and Shimada S (2002) Amiloride-blockable acid-sensing ion channels are leading acid sensors expressed in human nociceptors. *J Clin Investig* **110:**1185–1190.
Waldmann R, Bassilana F, Voilley N, Lazdunski M, and Mattei M (1996) Assignment of the human amiloride-sensitive Na^+ channel δ isoform to chromosome 1p36.3-p36.2. *Genomics* **34:**262–263.
Waldmann R, Champigny G, Bassilana F, Voilley N, and Lazdunski M (1995) Molecular cloning and functional expression of a novel amiloride-sensitive Na^+ channel. *J Biol Chem* **270:**27411–27414.
Wei ET and Seid DA (1983) AG-3–5: a chemical producing sensations of cold. *J Pharm Pharmacol* **35:**110–112.
Welsh MJ, Price MP, and Xie J (2002) Biochemical basis of touch perception: mechanosensory function of degenerin/epithelial Na^+ channels. *J Biol Chem* **277:** 2369–2372.
Wemmie JA, Chen J, Askwith CC, Hruska-Hageman AM, Price MP, Nolan BC, Yoder PG, Lamani E, Hoshi T, Freeman JH Jr, et al. (2002) The acid-activated ion channel ASIC contributes to synaptic plasticity, learning and memory. *Neuron* **34:**463–477.
Yamamura H, Ugawa S, Ueda T, Nagao M, and Shimada S (2004a) Capsazepine is a novel activator of the δ subunit of the human epithelial Na^+ channel. *J Biol Chem* **279:**44483–44489.
Yamamura H, Ugawa S, Ueda T, Nagao M, and Shimada S (2004b) Protons activate the δ-subunit of the epithelial Na^+ channel in humans. *J Biol Chem* **279:**12529–12534.

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