Capsazepine Is a Novel Activator of the \( \delta \) Subunit of the Human Epithelial \( Na^+ \) Channel*

Received for publication, August 4, 2004, and in revised form, August 9, 2004
Published, JBC Papers in Press, August 11, 2004, DOI 10.1074/jbc.M408929200

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The amiloride-sensitive epithelial \( Na^+ \) channel (ENaC) regulates \( Na^+ \) homeostasis into cells and across epithelia. So far, four homologous subunits of mammalian ENaC have been isolated and are denoted as \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \). The chemical agents acting on ENaC are, however, largely unknown, except for amiloride and benzamil as ENaC inhibitors. In particular, there are no agonists currently known that are selective for ENaC\( \delta \), which is mainly expressed in the brain. Here we demonstrate that capsazepine, a competitive antagonist for transient receptor potential vanilloid subfamily 1, potentiates the activity of human ENaC\( \delta\gamma \) (hENaC\( \delta\gamma \)) heteromultimer expressed in Xenopus oocytes. The inward currents at a holding potential of \(-60 \text{ mV}\) in hENaC\( \delta\gamma \)-expressing oocytes were markedly enhanced by the application of capsazepine (\( \geq 1 \mu \text{M} \)), and the capsazepine-induced current was mostly abolished by the addition of 100 \( \mu \text{M} \) amiloride. The stimulatory effects of capsazepine on the inward current were concentration-dependent with an \( EC_{50} \) value of 8 \( \mu \text{M} \). Neither the application of various other vanilloid compounds (capsaicin, resiniferatoxin, and olvanil) nor a structurally related compound (dopamine) modulated the inward current. Although hENaC\( \delta \) homomer was also significantly activated by capsazepine, unexpectedly, capsazepine had no effect on hENaCo and caused a slight decrease on the hENaCo\( \beta\gamma \) current. In conclusion, capsazepine acts on ENaC\( \delta \) and acts together with protons. Other vanilloids tested do not have any effect. These findings identify capsazepine as the first known chemical activator of ENaC\( \delta \).

The degenerin/epithelial \( Na^+ \) channel superfamily has striking functional diversity including \( Na^+ \) homeostasis, acid sensing, peptide-gating, acidosis-evoked nociception, and mechano-transduction (1–5). The amiloride-sensitive epithelial \( Na^+ \) channel (ENaC)\( ^{1} \) is an essential control element for \( Na^+ \) transport into cells and across epithelia. Four homologous ENaC subunits (\( \alpha \), \( \beta \), \( \gamma \), and \( \delta \)) have been cloned in mammals (6–10). There is an overall \(-37\%\) amino acid identity between the \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \) subunits. ENaCa is expressed mainly in epithelia, such as the kidney, lung, and colon, and binds with these \( \beta \) and \( \gamma \) subunits to play a pathophysiological role (2, 4), whereas the \( \delta \) subunit is widely distributed throughout the brain and is also expressed in the heart, kidney, and pancreas (10, 11). More recently, we have demonstrated that protons activate ENaC\( \delta \), indicating that it may contribute to pH sensation and/or pH regulation in the human brain (11). Most interestingly, the expressed sequence tag and genome project databases show that an ENaC\( \delta \) gene has been found only in humans and chimpanzees (GenBankTM accession numbers U38254 and O46547, respectively), and there is no evidence of the orthologues in rats and mice. The corresponding genomic assignments of ENaC\( \delta \) were identified on human chromosome 1p36.3-p36.2 (12).

The functional expression and activity of ENaC are regulated by various endogenous and exogenous compounds. It has been described that aldosterone increases the transcription of serine/threonine kinase, and serum- and glucocorticoid-regulated kinase to reduce Nedd4-2-mediated degradation of ENaCa\( \beta\gamma \), leading to the increased expression of ENaCa\( \beta\gamma \) at the cell surface (13, 14). Arachidonic acid reduces the surface expression of ENaCa\( \beta\gamma \) while altering the rates of delivery and internalization of functional channels (15). In addition, syntaxin 1A interacts with cell surface ENaCa\( \beta\gamma \) to rapidly decrease the open probability (16). On the other hand, ENaC\( \delta\beta\gamma \) can bind with a copper transporter, Murr1, by interacting at the C-terminal domain of ENaC\( \delta \) to decrease the activity of the channel (17). In pharmacological profiles of ENaC, it is well known that the potassium-sparing diuretics, amiloride and benzamil, inhibit the activities of ENaCa, ENaC\( \delta \), and the complexes with \( \beta \) and \( \gamma \) subunits (6–11). Extracellular protons reduce the activity of ENaCa (18), whereas ENaC\( \delta \) is activated by protons (11, 19). In addition, ENaCa\( \beta\gamma \) is activated by cAMP and blocked by Ni\( ^{2+} \) (20). In contrast to the relatively well documented ENaCa\( \beta\gamma \) in pharmacological studies, it has been poorly investigated for ENaC\( \delta \). In particular, chemical activators for ENaC\( \delta \) have not yet been reported.

In this investigation, the effects of capsazepine on human ENaC (hENaC) current were examined by using electrophysiological analyses in the Xenopus oocytes expression system. Capsazepine, which possesses a vanilloid moiety, has been developed as a synthetic competitive antagonist for the transient receptor potential vanilloid subfamily 1 (TRPV1) (21–24). Here we show that capsazepine activates hENaC\( \delta\beta\gamma \) in a concentration-dependent manner, although other vanilloids (capsaicin, resiniferatoxin, and olvanil) were not affected on the hENaC\( \delta\gamma \) current. Most interestingly, capsazepine causes potentiation of the hENaC\( \delta \) current but not hENaCa activity. To our knowledge, capsazepine is the first agonist to activate ENaC\( \delta \).

* This work was supported by a grant-in-aid for scientific research from the Japan Society for the Promotion of Sciences (to H. Y. and S. S.). 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.

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The abbreviations used are: ENaC, epithelial \( Na^+ \) channel; hENaC, human epithelial \( Na^+ \) channel; PCR, polymerase chain reaction; RT, reverse transcription; TRPV1, transient receptor potential vanilloid subfamily 1; MES, \( 2-(\text{N-morpholino})\text{ethanesulfonic acid} \);
**EXPERIMENTAL PROCEDURES**

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 of Helsinki. Human samples free from neurological disorders were taken within 24 h after death with permission from the families of the deceased. Ethical principles and considerations were observed regarding forensic and related research using human organs and fluids obtained from autopsies. Human tissues were rapidly frozen on dry ice immediately after their removal at autopsy and were kept at −80 °C until use. The total RNA was extracted from homogenates of human tissues using ISOGEN (Nippon Gene, Tokyo, Japan), following digestion with RNase-free DNase (Promega, Madison, WI). The reverse transcription (RT) was performed as follows. We heated the reaction mixture of total RNA (3 μg) and 500 ng of an oligo(dT)12–18 primer (Invitrogen) in 8 μl of diethyl dicarbonate-treated water at 70 °C for 10 min, chilled it for 1 min, added 11 μl of the reaction buffer (as a final concentration, 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl2, 10 mM dithiothreitol, and 0.5 mM individual dNTPs), and incubated it at 50 °C for 2 min. The mixture was incubated at 50 °C for 90 min after the addition of 200 units of SuperScript II reverse transcriptase (Invitrogen), and then heated at 70 °C for 15 min. After this RT procedure, we added 80 μl of distilled water to the reaction mixture and used it for PCR. To isolate the full-length ENaCs from human skin (for hENaCβγ,5) and brain (for δ subunit) cDNA, oligonucleotide primers were designed as follows: for hENaCα (GenBank™ accession number X76180), +, 5′-gaa ttc gcc gcc ace ATG GAG GGG CCC ACC GAA CAG CAG GAC T-3′, and −, 5′-tct aga TCA GGG CCC CCC CAG ACA AAG GGA GGA ACT GGC CCC T-3′; for hENaCβ (X87159), +, 5′-gat ttc gcc gcc ace ATG CAC GTG AAG TAC CTG CTC GAG GGC CT-3′, and −, 5′-tct aga TTA GAT GCC ATC ACC CTC ACT GTC AGA CAG CTG GAC GCC CA-3′; for hENaCγ (U48937), +, 5′-gaa ttc gcc gcc ace ATG GGC CCC GGG GAG AAC ATC AAA GCC A-3′, and −, 5′-tct aga TCA GAG CTC ATC CAG CAT CTG GGT ATC TGT GAG CT-3′; for hENaCδ (U38254), +, 5′-gaa ttc gcc gcc ace ATG GCT GAG CAC CGA AGC ATG GAC GGG AGA-3′, and −, 5′-tct aga TCA GGT CCT AGT CAG CTC AAG GGG CTG GGG CCC AGC CCA GCT-3′. The sequences indicated by lowercase letters are EcoRI (gaat ttc), EcoRV (gat ttc), XbaI (tct aga) recognition sites, and the Kozak sequence (gcc gcc ace), which were added to the insert PCR products into vector DNA in the proper orientation and to promote effective translation, respectively. The RT reaction product (2 μl) was amplified using GeneAmp PCR System 2700 (Applied Biosystems) in a total volume of 25 μl of a solution containing 15 mM Tris-HCl, pH 8.0, 50 mM KCl, 0.2 mM individual dNTPs, 1.5 mM MgCl2, 15 pmol of sense and antisense primers for each, 10% dimethyl sulfoxide, and 2.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems). The thermal cycler program used for PCR amplification included a 0.5-min denaturation step at 94 °C, a 0.5-min annealing step at 58 °C, and a 2-min primer extension step at 72 °C after a preheating step at 94 °C for 9 min. The amplification was performed for 45 cycles. Thereafter, the reaction mixture was heated at 72 °C for 15 min. The PCR products were separated on a 1% agarose gel in Tris acetate/EDTA buffer, recovered from the gel fragments, cloned, and sequenced.

Xenopus Oocyte Electrophysiology—Electrophysiological studies in Xenopus oocytes, using a two-electrode voltage clamp technique, were performed as described previously (11). In brief, cRNA(s) (2 ng for homeric channel or each 0.02 ng for co-expression) was injected into Xenopus 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 αβδγ) or 100 μM (for hENaCδ and βγ) amiloride for 24–48 h before electrophysiological recordings. The recording solution had an ionic composition of 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM HEPES. The pH of the solution was adjusted to 7.5 with NaOH. The recording solution for pH experiments was prepared with the equivalent MES (pKa = 6.15) instead of HEPES (pKa = 7.55) and adjusted to a suitable pH with either NaOH or HCl. All electrophysiological recordings were performed at a holding potential of −60 mV. The current-voltage relationships were measured by using a ramp protocol from −100 to 50 mV for

**FIG. 1.** Activation of hENaCβγ current by capsazepine. Whole-cell currents were recorded at a holding potential of −60 mV in the *Xenopus* oocyte expression system using a two-electrode voltage clamp technique. A, hENaCβγ-expressed oocyte possessed a larger inward current than a native oocyte. The larger current in an hENaCβγ-expressing oocyte was mostly inhibited by 100 μM amiloride (Ami). In an hENaCβγ oocyte, the application of 10 μM capsazepine (CZ) enhanced the inward current, and this current increase was recovered by the removal of capsazepine. After washout for a few minutes, the re-addition of capsazepine caused similar current activation. B, the current-voltage relationships in the absence and presence of 10 μM capsazepine in native and hENaCβγ-injected oocytes are shown. The capsazepine stimulus enhanced the hENaCβγ activity at all voltages examined. Note that neither the application of capsazepine nor amiloride induced any current in a native oocyte.
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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).

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 Scheffe’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 either capsazepine or proton concentrations and current responses were fitted by using the following equation after normalization by the maximum current amplitude (see Figs. 3B and 6): $I/I_{\text{max}}(\%) = 100/[1 + (K_d/[A])^n]$, where $I_{\text{max}}$ is the maximum amplitude of the capsazepine- or proton-evoked current; $K_d$ is the apparent dissociation constant of capsazepine or proton; [A] is the concentration of capsazepine or proton, and n is the Hill coefficient.

RESULTS

Activation of hENaCβγ Current by Capsazepine—The effects of capsazepine, a well known competitive antagonist for TRPV1 (21–24), on the hENaCβγ current were examined using a two-electrode voltage clamp technique in the Xenopus oocyte expression system. When hENaCβγ heteromultimer was expressed in Xenopus oocytes, an inward current was induced at a holding potential of −60 mV, and the current was mostly inhibited by 100 μM amiloride (Fig. 1A). The mean amplitude of the amiloride-sensitive inward current in hENaCβγ-expressing oocytes was $615 ± 24$ nA (n = 54, p < 0.01 versus native of 1 ± 1 nA, n = 17). In hENaCβγ-injected oocytes, the application of 10 μM capsazepine was markedly increased in the inward current by $785 ± 42$ nA (n = 26, p < 0.01). The current increase by capsazepine was recovered to the resting level by the removal of capsazepine in all hENaCβγ-expressing oocytes tested (n = 26). After washout for a few minutes, the re-addition of capsazepine caused current activation with similar amplitude and kinetics to first challenge (n = 6). The current-voltage relationship showed that the application of 10 μM capsazepine enhanced the channel activity at all voltages examined in hENaCβγ-expressing oocytes (n = 4; Fig. 1B). On the other hands, in native oocytes, the application of 10 μM capsazepine did not induce any current (n = 14), because the capsazepine-induced currents were mediated by the activation of ENaCβγ.

Effects of Amiloride on Capsazepine-induced Current—To confirm whether the capsazepine-induced current originated from ENaCβγ expression, the effects of amiloride, an inhibitor of ENaCs, on the inward current in the presence of capsazepine were examined. The 10 μM capsazepine-induced current in hENaCβγ-injected oocytes (1570 ± 53 nA, n = 26) was dramatically blocked by the addition of 100 μM amiloride (88 ± 1% decrease, n = 26, p < 0.01 versus capsazepine) and, moreover, significantly inhibited the current to 194 ± 20 nA (n = 26, p < 0.01 versus the initial resting current of 785 ± 33 nA; Fig. 2). In native oocytes, the current amplitudes after the application of...
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**Fig. 4. Effects of capsazepine-related compounds on hENaCδβγ currents.** The effects of capsazepine-related compounds on the inward current were measured in hENaCδβγ-expressing oocytes. The changes in current amplitude after exposure to these compounds at a concentration of 10–100 μM for 5 min in hENaCδβγ-injected oocytes are summarized. Vanilloid compounds were 30 μM capsazepine, 10 μM resiniferatoxin, and 30 μM 8-olvanil; the antagonist for nonselective cation channel containing TRPV1 was 30 μM dopamine. The inward current was markedly enhanced by 30 μM capsazepine, although the effects of other compounds tested were not statistically significant (p > 0.05). Experimental data were obtained from six oocytes for each. The statistical significance of the difference is expressed as p < 0.01 (** versus control).

10 μM capsazepine in the absence and presence of 100 μM amiloride (65 ± 4 nA and 66 ± 4 nA, respectively, n = 14) were not significant to the initial resting current (64 ± 4 nA, n = 14, p > 0.05).

**Dose Dependence of Current Activation by Capsazepine—The concentration dependence of capsazepine-induced current was examined in hENaCδβγ-expressing oocytes. In hENaCδβγ-expressing oocytes, changing the concentration of capsazepine ranged from 0.01 to 100 μM, which showed that the inward current was significantly increased by capsazepine at a concentration of 1 μM and more (n = 7, p < 0.05 versus control of 703 ± 79 nA), and the enhancement was in a concentration-dependent manner (2346 ± 153 nA at 100 μM, n = 7; Fig. 3). The EC_{50} value of capsazepine on the inward currents was 7.8 μM, and the Hill coefficient was 1.3.

**Effects of Capsazepine-related Compounds on hENaCδβγ Currents—Capsazepine possesses a vanilloid moiety, which is a key factor for the binding for TRPV1 (24–26). To examine whether the opening action for ENaCδβγ was shared by structurally related compounds of capsazepine, the effects of capsazepine-related compounds on the inward current were measured in hENaCδβγ-expressing oocytes. The change in current amplitude after exposure to the following compounds at a concentration of 10–100 μM for 5 min in hENaCδβγ-expressing oocytes is shown in Fig. 4: vanilloid compounds were 30 μM capsazepine, 10 μM resiniferatoxin, and 30 μM 8-olvanil; the antagonist of a nonselective cation channel containing TRPV1 was 30 μM ruthenium red; and the molecule structurally similar to capsazepine was 100 μM dopamine. Regardless of this, the inward current was markedly enhanced by 30 μM capsazepine as shown in Fig. 3 (n = 6, p < 0.01), and the effects of other compounds were not statistically significant (n = 6 for each, p > 0.05).

**Selective Activation of hENaCδ Not hENaCa by Capsazepine—**It was examined whether the activation of the inward current by capsazepine was mediated through either ENaCδ alone or the accessory β or γ subunits. When the hENaCδ homomer was expressed in Xenopus oocytes, a 100 μM amiloride-sensitive inward current was induced at −60 mV (69 ± 6 nA, n = 5; Fig. 5A), as described previously (11). The application of 30 μM capsazepine significantly evoked an inward current in hENaCδ-expressing oocytes (by 67 ± 3 nA, n = 5, p < 0.01), as well as in hENaCδβγ-injected oocytes (by 1133 ± 80 nA, n = 21, p < 0.01; Fig. 5). Furthermore, we tested whether capsazepine was effective on another ENaC core unit, the α subunit, in Xenopus oocytes. When the hENaCa homomer or αβγ heteromultimer was expressed in Xenopus oocytes, these inward currents induced at −60 mV were mostly blocked by lower amiloride at 10 μM (Fig. 5A). The mean amplitude of the amiloride-sensitive current in hENaCδ- and αβγ-expressed oocytes at −60 mV was 34 ± 9 nA (n = 6) and 822 ± 54 nA (n = 11), respectively. Unexpectedly, in contrast to hENaCδ and δβγ, the application of 30 μM capsazepine had no effect on the inward currents in hENaCδαγ oocytes (by 5 ± 5 nA decrease, n = 6, p > 0.05), and moreover, the hENaCδβγ current was slightly decreased by 30 μM capsazepine (by 71 ± 5 nA, n = 11, p < 0.01).

**Sensitization by Capsazepine on Proton-activated hENaCδγ Current—**We reported previously (11) that ENaCα activity was enhanced by external protons in the Xenopus oocyte expression system. Therefore, the effects of capsazepine on proton-activated hENaCδβγ current in Xenopus oocytes were examined. In hENaCδβγ-expressed oocytes, the gradual decrease in pH from 7.5 to 4.0 resulted in significantly increased currents in a proton concentration-dependent manner (2279 ± 92 nA, pH 4.0, n = 10, p < 0.01 versus control of 821 ± 69 nA; Fig. 6A). The half-maximal pH for activation of the hENaCδβγ current was 5.9 ± 0.1, and the Hill coefficient was 0.35 ± 0.02 (n = 10). In the presence of 1 μM capsazepine, which by itself had a very small effect on hENaCδβγ current (by 144 ± 20 nA, n = 10; see Figs. 3 and 6B), the dose-response curve for protons on hENaCδβγ currents was shifted to the left (half-maximal pH of 6.3 ± 0.1 and Hill coefficient of 0.42 ± 0.02, n = 10). The maximum amplitude of the acid-evoked current in the presence of 1 μM capsazepine (by 1502 ± 102 nA, n = 10) was not statistically significant with that in the absence of capsazepine (by 1458 ± 117 nA, n = 10, p > 0.05). Conversely, the dose response to capsazepine was strongly potentiated by weak acidification to pH 7.0 (Fig. 6B), which was a subthreshold concentration of protons on hENaCδβγ current (by 25 ± 4 nA, n = 10; see Fig. 5A). The decrease in pH from 7.5 to 7.0 caused a left shift of the dose-response curve for capsazepine from the EC_{50} value of 8.3 ± 0.6 μM (Hill coefficient of 1.4 ± 0.1, n = 10) to 2.4 ± 0.2 μM (Hill coefficient of 1.0 ± 0.1, n = 10). The maximum response of the capsazepine-induced current during the exposure to pH 7.0 (by 1574 ± 122 nA, n = 10) was not statistically significant to that in pH 7.5 medium (by 1566 ± 130 nA, n = 10, p > 0.05).

**DISCUSSION**

Amiloride-sensitive ENaCs, members of the degenerin/ENaC superfamily, regulate essential control elements for Na⁺ homeostasis into cells and across epithelia. The heteromultimeric ENaCδβγ complex is expressed mainly in epithelia such as the kidney, lung, and colon to play a pathophysiological role (2, 4). More recently, we have shown that homomeric 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 (11). In pharmacological aspects, the chemical agents for ENaCα are well documented, although those for ENaCδ are largely unknown except for amiloride and benzamil as ENaC inhibitors. In particular, no chemical activators have been reported for ENaCδ to date. In this investigation, the effects of capsazepine, a well-known competitive TRPV1 antagonist (21–24), on hENaCδβγ were examined by electrophysiological studies in the Xenopus oocytes expression system. We have found...
that the application of capsazepine activates hENaCδβγ in a concentration-dependent manner, and the enhancement is sensitive to amiloride. The most interesting finding in this investigation is that capsazepine activated the hENaCδ current but not hENaCγ activity.

When the heteromultimeric hENaCδβγ complex was expressed in *Xenopus* oocytes, the application of capsazepine at a concentration of 1 μM and more was markedly increased by an inward current. Because the capsazepine-induced current was significantly abolished by the addition of 100 μM amiloride, an inhibitor of ENaCs, in hENaCδβγ-expressing oocytes, and capsazepine-evoked currents were not observed in native oocytes, the capsazepine-induced currents originated from the ENaCδβγ expression. The capsazepine-induced current was maintained at a steady level during capsazepine application in hENaCδβγ-expressed oocytes (see Fig. 2A). It is exciting that the chemical agonists for the ion channel induce sensitization or desensitization of its activity, such as capsaicin for TRPV1 (23) and 2-aminoethoxydiphenyl borate for TRPV1 and TRPV3 (27, 28). After washout for a few minutes, the sequential challenge of capsazepine causes current activation to the same extent as the first trial in hENaCδβγ-expressing oocytes (see Fig. 1A). These results indicate that capsazepine did not induce sensitization and desensitization to the activity of hENaCδβγ under these conditions.

The sensitivity to capsazepine was increased in a weak acidic medium of pH 7.0. Protons are activated the hENaCδ current (11, 19), but pH 7.0 is the subthreshold concentration of protons in the hENaCδβγ current. At this proton concentration, the dose response for capsazepine on the inward currents was enhanced, indicating that the effect of capsazepine was sensitized by the addition of protons. Inversely, the pH dependence...
Capsazepine protects against neuronal injury caused by oxygen glucose deprivation by inhibiting hyperpolarization-activated nonspecific cation channel current (32) and inhibits the transient receptor potential melastatin subfamily 8 (33). In addition to its competitive antagonism of TRPV1 and its inhibitory actions on these channels, in this investigation we have clarified that capsazepine activates hENaCβγ with an EC<sub>50</sub> of 8 μM, providing us with a novel target for drug development and screening in the degenerin/ENaC superfamily.

A vanilloid structure containing capsazepine is a key factor for acting on TRPV1 (24–26). Therefore, it was examined whether the vanilloid structure contributed to the mechanism underlying the activation of hENaCβγ. No activation was observed in response to vanilloid compounds (capsaicin, resiniferatoxin, and olvanil), an antagonist for nonselective cation channel (ruthenium red), and a molecule structurally similar to capsazepine (dopamine) at the concentration of 10–100 μM in hENaCβγ-injected oocytes. In contrast, the application of capsazepine significantly activated the hENaCβγ current. These results suggest that hENaCβγ activation by capsazepine may be independent of the vanilloid structure.

The most interesting finding in this investigation was that capsazepine activated hENaCβγ but not hENaCα. The homoergic hENaCβγ, as well as hENaCαβγ, was activated by capsazepine, indicating that capsazepine acts directly on hENaCβγ itself. The extent to which the activation in hENaC alone (1.6 ± 0.1-fold by 30 μM capsazepine, n = 5) was significantly smaller than that in hENaCβγ (2.6 ± 0.2-fold, n = 21, p < 0.05), suggesting that the accessory β and/or γ subunit(s) may modulate the channel current activated by capsazepine. Another ENaC core unit, human α subunit with 37% amino acid identity to δ subunit, failed to increase the current amplitude by the application of capsazepine, and unexpectedly, capsazepine caused a slight reduction of the hENaCαβγ current. It has been described that there are differences in Na<sup>+</sup> permeability (α:δ = 2.6:1 as I<sub>Lp</sub>/I<sub>Na</sub>), and the amiloride sensitivity (α:δ = 0.1:2.6 μM as I<sub>CAMP</sub> between α and δ subunits (4, 6, 7, 10). Because the chemical agents influencing strongly either the α or δ subunit have been poorly investigated, capsazepine is a potentially powerful tool for the electrophysiological analysis of ENaCδ and the elucidation of the clinical ENaCδ function in humans.

In conclusion, we found that capsazepine acts selectively on hENaCβγ rather than hENaCα and causes the activation of hENaCβγ, showing that capsazepine is the first known activator for hENaCβγ. In addition to the function of ENaCα as a pH sensor in human brain as described previously (11), this finding in our study 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—We thank Katsuyuki Tanaka and Kenji Kajita for technical assistance.

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J. Biol. Chem. 2004, 279:44483-44489.
doi: 10.1074/jbc.M408929200 originally published online August 11, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M408929200

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