Attenuation of Mechanical Hypersensitivity by an Antagonist of the TRPA1 Ion Channel in Diabetic Animals

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Background: The TRPA1 ion channel modulates excitability of nociceptors, and it may be activated by compounds resulting from oxidative insults. Diabetes mellitus produces oxidative stress and sensory neuropathy. The authors tested the hypothesis that diabetes-induced endogenous compounds acting on the TRPA1 ion channel contribute to development and maintenance of mechanical hypersensitivity.

Methods: Diabetes mellitus was induced by streptozotocin. Mechanical hypersensitivity was assessed by the monofilament and paw pressure tests. Chembridge-5861528 (CHEM; a TRPA1 channel antagonist, a derivative of HC-030603) or vehicle was administered acutely or twice daily for 10 days in diabetic animals. For comparison, effects of CHEM were assessed in a group of healthy control animals.

Results: Acute administration of CHEM attenuated mechanically induced withdrawal responses in diabetic and control groups. The maximal effect (over 50% elevation of the paw pressure threshold) by acute administration of CHEM was obtained in 30 min. The lowest dose producing a significant attenuation was 10 mg/kg in the diabetic group and 30 mg/kg in the healthy controls. Chronic administration of CHEM (30 mg/kg twice daily) for a week in the diabetic group attenuated development of mechanical hypersensitivity.

Conclusions: Reduction of pain-related behavior by a lower dose of the TRPA1 channel antagonist in the diabetic than in the control group suggests that endogenous compounds resulting from diabetes mellitus and acting on the TRPA1 channel contribute to diabetic hypersensitivity. Prolonged antihypersensitivity effect after chronic treatment suggests that daily administration of a TRPA1 channel antagonist may prevent development of diabetic hypersensitivity.

Materials and Methods

Experimental Animals

The experiments were performed with male Hanover-Wistar rats (180–250 g; Harlan, Horst, Netherlands). All experiments were approved by the ethical committee for experimental animals studies of the State Provincial Office of Southern Finland (Hämeenlinna, Finland), and the experiments were performed according to the guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available. The animals were housed in polycarbonate cages with a deep layer of saw dust, one to three animals in each cage, in a thermostatically controlled room at 24.0 ± 0.5°C. The room was artificially illuminated from 8:30 AM to 8:30 PM. The animals received commercial pelleted rat feed (CRM-P pellets; Special Diets Services, Witham, Essex, England) and tap water ad libitum.

DM was induced by tail vein injection of streptozotocin (60 mg/kg; Sigma-Aldrich, St. Louis, MO) in citrate buffer (pH 4.5). Streptozotocin-induced DM is known to cause a marked hypersensitivity to various types of stimuli. The development of DM was confirmed 3 and 10 days later by measurements of blood glucose concentra-
tion (One Touch Ultra; Life Scan Inc, Milpitas, CA). Weight of the animals was assessed every other day. For comparison, one experimental group consisted of healthy control rats.

Assessment of Pain-related Behavior in Diabetic Animals

The rats were habituated to the experimental conditions by allowing them to spend 1–2 h daily in the laboratory during 2 to 3 days preceding any testing. For assessment of mechanical hyperalgesia, the hind limb withdrawal threshold to noxious mechanical stimulation (the paw pressure test) was determined with a Basile Analgesy-meter (Ugo Basile, Varese, Italy). With this device, a mechanical force was applied at a rate of 32 g/s to the hind paw until limb withdrawal or the cutoff force of 250 g. At each time point, two threshold determinations for the hind paw were performed at 1-min intervals. The mean of the two threshold values for each hind paw at each time point was used in further calculations.

To assess tactile allodynia-like behavior elicited by mechanical stimulation of the skin in diabetic animals, the frequency of withdrawal responses to the application of monofilaments (von Frey hairs) to the hind paw was examined. Nine hairs with forces varying from 1.0 g to 60 g (North Coast Medical, Inc., Morgan Hill, CA) were applied five times at a frequency of approximately of 0.5 Hz. Hairs were tested in ascending order of force. A visible lifting of the stimulated hind limb was considered a withdrawal response.

Influence of Chembridge-5861528 (CHEM) on thermal nociception in diabetic animals was assessed in a separate session by determining radiant heat-induced limb withdrawal latency. For this purpose, Hargreaves’ method was employed by using radiant heat equipment (Plantar test model 7370; Ugo Basile). For this, the rat was placed on a glass plate, and radiant heat was applied to the plantar surface of the hind paw from beneath, until spontaneous withdrawal of the hind paw was achieved. An electronic counter in the equipment registered the time in seconds from heat application to limb withdrawal, and this was considered as the latency of limb withdrawal. The cutoff point was arbitrarily set at 15 s, after which time heat application was discontinued to prevent possible tissue damage.

Assessment of Pain-related Behavior in Healthy Control Animals

Paw pressure threshold in the hind paw was assessed in the control group as in the diabetic group (see above the section on assessment of pain-related behavior in diabetic animals). The heat-induced tail-flick response was determined using a radiant heat device (Socrel DS-20, Ugo Basile) that automatically records the latency to removal. To avoid tissue damage, the three consecutive measurements at each time point were made at 1-min intervals, and the beam was applied to three different spots in the tail. The mean latency at each time point was used in further calculations. The same sites were used in pretreatment and posttreatment conditions. The stimulus intensity was adjusted so that the mean baseline latency was 2.2–2.5 s and the cutoff latency was 9 s.

Assessment of Acute Drug Treatment on Motor Performance

Rotarod test was performed to assess the effect of acute drug treatment on motor behavior. In the Rotarod test, the animals were placed on a revolving drum (a constant speed of 26 rounds/min). The latency until the animal dropped from the drum was determined with a stop watch. Before any drug testing, the rats were habituated to the Rotarod test during two previous days. The maximum observation period was 1 min, after which the animal that was still on the drum was removed. The Rotarod test was repeated three times at 1-min intervals, and the longest latency for each rat in each condition was used in further calculations.

Course of the Behavioral Study

When assessing modulation of pain-related behavior by acute drug treatments, vehicle, or CHEM (a TRPA1 channel antagonist, a derivative of HC-030031) at a dose of 3, 10, or 30 mg/kg was administered intraperitoneally in diabetic rats 10–20 days after induction of DM. Healthy animals served as a control group. In the diabetic group, also the effect of a comparison drug (gabapentin at a dose of 100 mg/kg) that is known to be effective against diabetic hypersensitivity was assessed 10–20 days after induction of diabetes. Each animal participated in 4–5 drug-testing sessions at 2-day intervals. The order of testing various drug treatment conditions was counterbalanced within groups. In each drug-testing session with the diabetic group, the paw pressure and monofilament tests were performed before acute drug/vehicle treatment and at three time points after the treatment (15, 30, and 60 min after drug administration).

After noting that the antihypersensitivity effect of CHEM was not completely over in the 1-h session used in the first testing sessions, we performed a separate study assessing time course of the antihypersensitivity effect induced by acute administration of 30 mg of CHEM or vehicle control for a longer period (up to 3 h) in a group of diabetic animals. Moreover, to assess whether CHEM influences heat nociception in diabetic animals, radiant heat-evoked hind limb withdrawal latency was determined in a separate group of diabetic animals treated with 30 mg of CHEM or vehicle. A change in skin temperature is a significant confounding factor when assessing radiant heat-induced response latencies in diabetic animals; therefore, skin temperature in the hind paw was measured with BAT-12 Microprobe Thermometer (Physitemp Instruments, Clifton, NJ) 1 min before
application of each heat stimulation to the paw. To assess whether diabetic animals were hypersensitive to heat before drug treatments, heat-evoked hind limb withdrawal threshold and skin temperature in the hind limb were measured also in a group of healthy control animals.

In healthy controls, the paw pressure test was performed as in the diabetic group. The tail flick test used for assessing heat nociception in healthy animals was performed before drug treatment and at four time points after it (8, 18, 33, and 63 min after treatment). In the diabetic group, blood glucose was determined before induction of diabetes and continuing until 10 days after induction of diabetes. Body weight and paw pressure threshold were determined at 2-day intervals starting 20 days after induction. Blood glucose was assessed also in a group of healthy control animals.

When assessing the effect of chronic drug treatment in the diabetic group, vehicle or 30 mg/kg CHEM was administered intraperitoneally twice daily from the third to the tenth day after induction of diabetes. Blood glucose was assessed before induction of diabetes and 3 and 20 days after induction. Body weight and paw pressure threshold were determined at 2-day intervals starting before induction of diabetes and continuing until 10 days after induction of diabetes. In the chronic treatment condition, paw pressure threshold was determined in the afternoon, 6 h after the morning dose of CHEM or vehicle (before administration of the evening dose). Potential influence of CHEM on the paw pressure threshold measured at this time point was considered to represent a nonacute effect, as indicated by our present results with acute drug treatments. Drug testing was performed in a blinded fashion; i.e., the experimenter assessing pain-related behavior did not know which animals were treated with vehicle and which ones with the studied drug. After completion of the study, the animals were anesthetized and sacrificed under deep anesthesia.

Prolonged effect of CHEM at a twice daily dose of 30 mg/g was assessed also in a group of healthy control animals. CHEM treatment lasted 7 days, and the paw pressure threshold was determined before the start of the daily treatment and 6 h after the morning dose of CHEM every other day during the treatment period.

Motor effects by acute treatment with vehicle or CHEM at a dose of 30 mg/kg were assessed in eight healthy control animals and in eight diabetic animals. Drop latency in the Rotarod test was assessed 30 min after administration of CHEM or vehicle control. Half of the animals were first tested with vehicle and 2 days later with CHEM, and the rest of the animals were tested in the opposite order. In one additional control group, the effect by gabapentin at a dose of 100 mg/kg on Rotarod performance was assessed in eight healthy animals 30 min after administration of gabapentin. Moreover, to assess the sensitivity of the Rotarod assay, one group of healthy animals was tested 15 min after intraperitoneal administration of pentobarbitone at a subanesthetic dose of 20 mg/kg.

In Vitro Studies for Characterization of the TRPA1 Channel Antagonist Properties of CHEM
Since the properties of CHEM as a TRPA1 channel antagonist have not been published earlier, we determined its properties as a TRPA1 channel antagonist using in vitro methods, as described below.

Cell Culture
Human TRPA1-inducible HEK-293 cells (HEK-Lacl-trpA1 clone B22) were cultured in Dulbecco’s modified Eagle’s medium supplemented with newborn-calf serum (10%), 25 mM HEPES, 1 mM Na-pyruvate, 0.3 mg · ml⁻¹ genetin, 100 U · ml⁻¹ streptomycin, and 20 μg · ml⁻¹ hygromycin. The cells were split twice a week at ratios of 1:4 and 1:6.

Determination of TRPA1 and TRPV1 Antagonism by CHEM and HC-030031
Intracellular calcium elevations evoked by allyl isothiocyanate (5 μM) and 4-HNE (30 μM) in TRPA1-expressing cells by capsaicin (1 μM) in TRPV1 transfected cells were measured using the fluorometric imaging plate readers FlexStation or FLIPR TETRA (Molecular Devices, Union City, CA). The day before experiments, cells were plated onto 96-well poly-D-lysine-coated, clear-bottom black walled plates (BIOTAC®, Bedford, United Kingdom) at a density of 40,000 cells/well in medium supplemented with 1 mM IPTG. After removing the growth medium, cells were loaded with Calcium 3 or 4 Assay reagent (Molecular Devices) diluted in Probenecid Ringer’s, and incubated for 45–60 min in the dark. Probenecid Ringer’s was composed of (in mM): NaCl 150, KCl 3, MgCl₂ 1.2, glucose 5, HEPES 20, and probenecid 2.5, pH 7.4. The osmolarity was adjusted to 322 mOsm. To determine antagonism, cells were preincubated with HC-030031 and CHEM-diluted in Probenecid Ringer’s for 1 h in the dark. Fluorescence measurements were subsequently conducted with FLEXstation or FLIPR, with the agonists added by the internal pipettor during the recording period. 4-HNE was used within 4 h of hydrolysis from HNE-DMA. All experiments were performed at 37°C. The fluorescence value, maximum minus minimum, was calculated for each well with SOFTmax PRO 3.2 and Screenworks 2.0 softwares (Molecular Devices). Fitting of the agonist dose-response results were performed with the 3-parameter Hill equation and antagonists with the free Hill equation in Sigma Plot 8.0 (Systat Software Inc., San Jose, CA).

Drugs
CHEM (a TRPA1 channel antagonist, a derivative of HC-030031; fig. 1) was synthesized by ChemBridge Corporation (San Diego, CA). In behavioral studies, it was dissolved in 0.5% methylcellulose that was used as vehicle control. In all in vivo experiments, CHEM was administered intraperitoneally. Streptozotocin was used...
for induction of DM. Gabapentin that was used as a comparison drug was obtained from Sigma-Aldrich. Sodium pentobarbitone used in a Rotarod comparison group was obtained from OrionPharma Inc. (Espoo, Finland). Other chemical compounds used in in vitro experiments were purchased from Sigma-Aldrich, except for HC-030031 (a prototype TRPA1 antagonist), which was purchased from ChemBridge.

Statistics
Paw pressure thresholds, response rates in the monofilament test, tail flick latencies, body weight, and blood glucose levels were assessed using one- or two-way analysis of variance (1-w- or 2-w-ANOVA) followed by t test with a Bonferroni correction for multiple comparisons (comparisons of three or more groups), or with a t test (comparison of two groups). Repeated measures ANOVA was used when assessing within-subject factors (e.g., a change of weight and paw pressure threshold during prolonged drug treatment) and nonrepeated measures ANOVA when assessing between-subject factors. A nonparametric Kruskal-Wallis (KW) test followed by Dunn’s test was used in the analysis of Rotarod data. P < 0.05 was considered to represent significant difference. Statistical analyses were performed with Prism 4 for Windows software (GraphPad Inc., San Diego, CA).

Results
Acute Treatment of Diabetic Animals with a TRPA1 Channel Antagonist
All streptozotocin-treated animals developed DM, as shown by the increase of the mean blood glucose from the pretreatment value of 6.6 mmol/l (range, 5.6–7.7 mmol/l) to 26.1 mmol/l (range, 20.2–32.2 mmol/l) in 10 days and to 27.2 mmol/l (range, 25.1–29.7 mmol/l) in 20 days. Diabetes induced in 10 days a significant hypersensitivity to mechanical stimulation when compared with responses of nondiabetic control animals in the paw pressure (P < 0.005, t test; fig. 2A) and monofilament tests (F₈,₃₀₅ = 35.9, P < 0.0001, 2-way-ANOVA; fig. 2B).

Acute treatment of diabetic animals with CHEM (a TRPA1 channel antagonist) was studied 10–20 days after

Fig. 1. Chemical structures of two TRPA1 channel antagonists: HC-030031 and Chembridge-5861528.

Fig. 2. Influence by acute treatment with Chembridge-5861528 (CHEM, a TRPA1 channel antagonist) on pain-related behavior in diabetic animals. (A) Paw pressure test thresholds of diabetic and nondiabetic control animals before drug treatments. (B) Withdrawal response rates evoked by monofilaments in diabetic and nondiabetic control animals before drug treatments. (C) Paw pressure test thresholds 30 min after administration of CHEM. (D) Withdrawal responses rates evoked by monofilaments 30 min after administration of CHEM. (E) Time course of the paw pressure threshold elevation induced by 30 mg/kg CHEM. (F) Withdrawal response rates evoked by monofilaments after administration of 10 mg/kg CHEM versus 100 mg/kg gabapentin. All results in graphs C–F were from diabetic animals. Increase in the paw pressure threshold (left column) and decrease in the monofilament-evoked response rate (right column) are considered to represent antihypersensitivity effect. In graphs D–F, drug doses represent mg/kg; 0 mg/kg represents vehicle-treatment group. Error bars represent SEM. In A and B, n₃ₐ₍d₀₃ₔ = 11, n₃₈₈₉₉ = 8. In C and D, n₉₅₈₉₉ = 8, n₃₅₉₉₉ = 9. In E, n₉₅₈₉₉₆₀₆₉₆₉ = 17, n₉₅₈₉₉₆₀₆₉₆₉ = 8, n₅₃₅₉₉₆₀₆₉₆₉ = 15, n₉₅₈₉₉₉₉₉₉₉ = 7. In F, n₉₅₈₉₉₉₉₉₉₉ = 8, n₉₅₈₉₉₉₉₉₉₉ = 8, *P < 0.05, **P < 0.01, ***P < 0.005 (in A, t test; in B–E, t test with a Bonferroni correction for multiple comparisons; in B, the reference was the corresponding value in the nondiabetic group; in C, the reference was the 0 mg/kg group; in D and E, the reference was the corresponding value in the 0 mg/kg group).
induction of diabetes. Intraperitoneal administration of CHEM produced a dose-related antihypersensitivity effect in the paw pressure (F(3,34) = 14.5, P < 0.0001, 1-w-ANOVA; fig. 2C) and monofilament tests (F(3,27) = 65.6, P < 0.0001, 2-w-ANOVA; fig. 2D). The lowest dose of CHEM producing a significant antihypersensitivity effect in diabetic animals, independent of the test, was 10 mg/kg. A further increase of the CHEM dose from 10 to 30 mg/kg did not increase the antihypersensitivity effect (fig. 2, C and D). The onset of the CHEM-induced antihypersensitivity effect was 15 min, the maximal effect was obtained in 30 min, and the antihypersensitivity effect induced by CHEM was completely over 120 min after its acute administration (fig. 2E). At a dose of 10 mg/kg, CHEM produced an antihypersensitive effect that was not different from that induced by gabapentin at a dose of 100 mg/kg, as indicated by thresholds in the paw withdrawal latency before drug administrations in diabetic animals (7.0 ± 0.3 s, n = 8; t test). The negligible changes of the heat-evoked limb withdrawal latencies of diabetic animals after administration of 30 mg of CHEM (increase by 0.07 ± 0.8 s, n = 8) or vehicle (increase by 0.28 ± 0.38 s, n = 7) were not significant (t test). Before drug treatments, the mean skin temperature in the hind paw of diabetic animals was 27.3 ± 0.9°C, which was slightly but not significantly lower than the paw skin temperature in healthy control animals (28.8 ± 0.9°C, n = 8; t test). Skin temperature of diabetic animals was not significantly changed 30 min after administration of 30 mg/kg of CHEM or vehicle (t test; not shown).

**Prolonged Treatment of Diabetic Animals with a TRPA1 Channel Antagonist**

To assess the antihypersensitivity effect by prolonged treatment of diabetic animals with a TRPA1 channel antagonist, CHEM was administered intraperitoneally twice daily for a week at a dose of 30 mg/kg. Treatment with CHEM started on the third day after the induction of diabetes. After the start of twice daily CHEM treatment or vehicle treatment, mechanical hypersensitivity was tested every second day in the afternoon, 6 h after the administration of the morning dose (before administration of the evening dose). When compared with prolonged treatment of diabetic animals with vehicle, prolonged treatment with CHEM significantly attenuated development of mechanical hypersensitivity as revealed by the paw pressure test (F(1,80) = 31.4, P < 0.0001; fig. 3A). After prolonged treatment, blood glucose levels were not different in the CHEM-treated group (mean, 29.9 mmol/l; range, 22.1–32.8 mmol/l) when compared with the vehicle-treated group (mean, 30.5 mmol/l; range, 27.9–32.8 mmol/l). Although diabetes produced a decrease of body weight with elapsed time from its induction (F(4,80) = 3.59, P < 0.01) independent of the experimental group (F(4,80) = 0.32), prolonged treatment with CHEM failed to influence weight development when compared with vehicle-treated diabetic animals (F(4,80) = 3.74; fig. 3B). Except for reduced hypersensitivity in the CHEM-treated group, no other differences in the behavior of CHEM-treated versus vehicle-treated diabetic animals were noticed in the experiments.

*Acute Treatment of Healthy Control Animals with a Trpa1 Channel Antagonist*

In healthy control animals, acute treatment with CHEM produced a dose-related elevation of the paw pressure threshold (F(3,23) = 14.48, P < 0.0001; fig. 4A). The lowest dose of CHEM producing a significant paw pressure threshold elevation in healthy controls was 30 mg/

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**Fig. 3. Influence by prolonged treatment with Chembridge-5861528 (CHEM, a TRPA1 channel antagonist) on pain-related behavior in diabetic animals. (A) Paw pressure threshold of diabetic animals treated for a week either with CHEM or vehicle (Veh). (B) Body weight in diabetic animals during prolonged treatment with CHEM or vehicle. D3–D10 indicate numbers of days from the induction of diabetes mellitus (DM), and pre refers to time point before its induction. Prolonged treatment with CHEM (30 mg/kg twice daily) or vehicle started on D3 and ended on D10. Paw pressure threshold was determined in each day 6 h after administration of the morning treatment with CHEM or vehicle; i.e., paw pressure threshold was determined at a time point when the acute treatment effect by CHEM was over. Error bars represent SEM (n = 9 in each group). ** P < 0.01, *** P < 0.005 (t test with a Bonferroni correction for multiple comparisons; reference: the corresponding value in the Veh-group).
kg. Latency of the heat-induced tail-flick response was increased in a dose-related fashion by acute treatment with CHEM (F3,23 = 6.13, P < 0.005; fig. 4B). The lowest dose of CHEM producing a significant heat antinociception in healthy controls was 30 mg/kg. The heat-evoked withdrawal response had returned to the predrug level by 63 min after acute treatment with CHEM (fig. 4C).

Prolonged Treatment of Healthy Control Animals with a TRPA1 Channel Antagonist

According to one-way ANOVA, daily treatment with CHEM at a twice daily intraperitoneal dose of 30 mg/kg for 1 week failed to produce a significant change in baseline paw pressure threshold of healthy control animals, as measured 6 h after administration of each morning dose of CHEM (F4,44 = 1.08); 1 week after the chronic treatment with CHEM, the paw pressure threshold was in the same range as before start of the treatment (93 ± 2 g vs. 91 ± 2 g, respectively; mean ± SEM, n = 9).

Effect by Acute Treatment with CHEM on Motor Behavior

Effect of CHEM on motor behavior was assessed with the Rotarod test in healthy and diabetic animals. CHEM failed to impair motor performance. This is indicated by the finding that all healthy and diabetic animals tested were able to perform the Rotarod test until the maximum of 60 s after administration of vehicle as well as CHEM at a dose of 30 mg/kg (fig. 5). Gabapentin at the dose of 100 mg/kg produced a slight decrease of the drop latency from the maximum of 60 s to 50–55 s in two animals, whereas six of the gabapentin-treated animals stayed on the Rotarod for the maximum duration of 60 s. Whereas motor performance in the Rotarod test varied between the experimental groups (KW = 20.38, P < 0.0001), the effect of gabapentin on motor performance was not significant (Dunn’s test; reference of the vehicle-treated group; fig. 5A). To assess sensitivity of the currently used Rotarod test, one group of eight healthy animals was tested 15 min after intraperitoneal administration of pentobarbitone at a low dose. Pentobarbitone at a dose of 20 mg/kg produced a marked impairment of motor performance in the Rotarod test.
Only three of the pentobarbitone-treated animals were able to stay on the Rotarod for periods varying from 55 to 60 s, whereas five of the pentobarbitone-treated animals were not able to stay on the Rotarod at all; consequently, their drop latency was 0 s. Over all animals, pentobarbitone induced a highly significant suppression of motor performance \((P < 0.001, \text{ Dunn's test; reference of the vehicle-treated group; fig. 5A)}\).

In Vitro Study: Properties of CHEM as a TRPA1 Channel Antagonist

HC-030031 (a prototype TRPA1 channel antagonist) dose-dependently antagonized TRPA1 responses with IC50 values of 41.8 \(\pm\) 3.5 \(\mu\)M \((n = 4)\) and 48.4 \(\pm\) 3.4 \(\mu\)M \((n = 5)\), when allyl isothiocyanate and 4-HNE were agonists, respectively. CHEM antagonized similarly allyl isothiocyanate- and 4-HNE-evoked TRPA1 responses, with IC50 values of 14.3 \(\pm\) 0.7 \(\mu\)M \((n = 6)\) and 18.7 \(\pm\) 0.3 \(\mu\)M \((n = 6)\), respectively. Neither HC-030031 nor CHEM showed any TRPA1 agonism up to the highest tested dose of 100 \(\mu\)M \((n = 4)\). In TRPV1-transfected cells, HC-030031 and CHEM were not able to attenuate agonist-induced responses and were not agonists themselves up to the highest tested dose of 100 \(\mu\)M \((n = 2)\).

Discussion

The TRPA1 Channel Contributes to Maintenance of Diabetic Hypersensitivity

The current results indicate that hypersensitivity induced by experimental DM is reduced by acute treatment with a TRPA1 channel antagonist. This finding suggests that endogenous compounds potentially resulting from oxidative stress of diabetes contribute to maintenance of hyperexcitability. This is in line with earlier findings showing that oxidative stress caused by diabetes increases release of reactive compounds, such as 4-HNE, that are known to activate nociceptive primary afferent neurons through the TRPA1 channel. Although the duration of diabetes at the time of drug testing was short (5–20 days), earlier studies indicate that the streptozotocin-induced experimental animal model of diabetes used in the current study induces significant oxidative stress in various organs already within this time period. The antihypersensitivity effect induced by the TRPA1 channel antagonist in diabetic animals may have been submodality selective; pain-related responses evoked by heat were not significantly influenced by CHEM at a dose that produced a significant attenuation of mechanically evoked withdrawal responses. Alternatively, diabetic animals at the time of testing were hypersensitive to mechanical stimulation but not to heat; therefore, hypersensitivity may have been a more important factor in determining suppression of pain-related behavior by a TRPA1 receptor antagonist than submodality of test stimulation. Interestingly, acute treatment with a TRPA1 channel antagonist attenuated pain-related behavior also in healthy control animals. It is, however, important to note that the lowest dose of the TRPA1 channel antagonist producing a significant suppression was an order of magnitude lower in the diabetic group than the healthy control group. This finding indicates that the TRPA1 channel plays a more important role in tonic regulation of pain-related behavior in diabetic animals than in healthy controls.

Prolonged Treatment with a TRPA1 Channel Antagonist Prevents Development of Diabetic Hypersensitivity

Chronic treatment of diabetic animals with a TRPA1 channel antagonist administered twice daily for 1 week prevented development of baseline hypersensitivity. This is indicated by the finding that hypersensitivity, as revealed by the paw pressure test, developed only in the diabetic group treated with vehicle but not in diabetic animals receiving the TRPA1 antagonist twice daily for a week. During chronic treatment with the TRPA1 channel antagonist, the paw pressure threshold was determined at time points (6 h after the morning dose) when the acute treatment effect by the drug was over. Therefore, it may be proposed that the TRPA1 channel plays a role not only in maintenance but also in development of diabetic hypersensitivity. This preemptive effect by a TRPA1 channel antagonist was selective for diabetes; chronic treatment with a TRPA1 channel antagonist failed to produce a change in the baseline paw pressure threshold in healthy control animals.

Although the TRPA1 channel was originally identified as a cold-activated channel, later studies have suggested that its role in sensing cold is less clear than that in sensing chemical irritants and reactive compounds. In addition, there is evidence indicating that the TRPA1 channel can be activated also by mechanical stimulation and heat, which is in line with the current findings. Recent studies indicate that the TRPA1 channel plays a role in mechanical hyperalgesia. Interestingly, inflammatory hyperalgesia was associated with an increased proportion of the TRPA1 protein in the dorsal root ganglion neurons, suggesting that an increased expression of the TRPA1 channel may contribute to inflammatory hyperalgesia. It remains to be studied if prolonged exposure to diabetes and an accompanying oxidative stress induced an increased expression of TRPA1 channels and if the prolonged administration of the TRPA1 channel antagonist prevented it, providing a mechanism for the prevention of diabetic hypersensitivity.

Lack of Marked Side Effects by the TRPA1 Channel Antagonist

No marked side effects by acute or prolonged treatment with a TRPA1 receptor antagonist were observed.
in this study. This is indicated by the finding that, when compared with vehicle-treated diabetic animals, treatment of diabetic animals with a TRPA1 channel antagonist failed to produce a change in body weight or any obvious change in behavior such as sedation or motor impairment. TRPA1-like immunoreactivity has been found on motoneurons,\(^3,3^3\) suggesting that the TRPA1 receptor might influence some aspect of motor behavior. The failure to influence motor performance by the currently used dose of the TRPA1 antagonist, however, supports the interpretation that the present suppression of pain-related behavior by the TRPA1 antagonist was rather due to sensory than motor action.

Conclusions

Present results indicate that the TRPA1 channel contributes to maintenance and development of diabetic hypersensitivity. Daily treatment with a TRPA1 channel antagonist for a week failed to produce marked side effects. Together, these results suggest that a TRPA1 channel antagonist might prove useful in prevention as well as in acute suppression of diabetic hypersensitivity.

References


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