Genetic AT\textsubscript{1A} receptor deficiency attenuates cold-induced hypertension

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Sun, Zhongjie, Xiuqing Wang, Charles E. Wood, and J. Robert Cade. Genetic AT\textsubscript{1A} receptor deficiency attenuates cold-induced hypertension. Am J Physiol Regul Integr Comp Physiol 288: R433–R439, 2005. First published October 21, 2004; doi:10.1152/ajpregu.00466.2004.—The aim of this study was to test our hypotheses that AT\textsubscript{1A} receptors play a role in the pathogenesis of cold-induced hypertension (CIH) and in the cold-induced increase in drinking responses to ANG II. Two groups of wild-type (WT) and two groups of AT\textsubscript{1A} receptor gene knockout (AT\textsubscript{1A}-KO) mice were used (6/group). Blood pressures (BP) of the four groups were similar during the control period at room temperature (25°C). After the control period, one group of WT and one group of AT\textsubscript{1A}-KO mice were exposed to cold (5°C), while the remaining groups were kept at 25°C. BP of the cold-exposed WT group elevated significantly within 1 wk of exposure to cold and increased gradually to a maximum level by week 5. However, there was only a slight increase in BP of the cold-exposed AT\textsubscript{1A}-KO group. The maximal cold-induced increase in BP (ΔBP) is significantly less in AT\textsubscript{1A}-KO group (11 ± 3 mmHg) than in WT group (49 ± 6 mmHg), indicating that AT\textsubscript{1A} receptor deficiency attenuates cold-induced elevation of BP. Interestingly, both WT and AT\textsubscript{1A}-KO mice developed cardiac and renal hypertrophy to the same extent. AT\textsubscript{1A}-KO caused a significant increase in urine and plasma levels of nitric oxide (NO), indicating that the renin-angiotensin system inhibits NO formation probably via AT\textsubscript{1A} receptors. Cold exposure inhibited endothelial NO synthase protein expressions and decreased urine and plasma levels of NO, which may be mediated partially by AT\textsubscript{1A} receptors. AT\textsubscript{1A}-KO completely abolished the cold-induced increase in drinking responses to ANG II. We conclude that 1) AT\textsubscript{1A} receptors play an essential role in the pathogenesis of CIH but not cardiac hypertrophy; 2) the role of AT\textsubscript{1A} receptors in CIH may be mediated partially by its inhibitory effect on the NO system; and 3) cold-induced increase in drinking response to ANG II is mediated by AT\textsubscript{1A} receptors.

blood pressure; cardiac hypertrophy; mice; nitric oxide; endothelial nitric oxide synthase

IT IS AN ESTABLISHED OBSERVATION that people who live and work in cold areas have a high incidence of hypertension and related cardiovascular diseases (8, 10, 19, 22, 30, 31, 41, 54). Cold temperatures (weather) make hypertension more severe in hypertensive patients (8, 19, 22, 30, 41). The high rate of cardiovascular diseases in the warm Southeast of the United States is due to selective migration, old age, and special dietary patterns or nutrient intakes (20, 25, 28). In the United States, the cold winter season has the highest incidence of cardiovascular diseases in a year (3, 39). It has been reported that normal human subjects have seasonal variations in blood pressure (BP) with higher pressure in winter (5, 26, 36, 55, 57). Exposure of the bare face to cold could induce a significant elevation of systolic and diastolic BP in normotensive subjects dressed in cold-protective clothing (17). An analysis of the relation between outdoor temperature and BP in men in central London from 1986 to 1992 indicated that cold exposure of normal life in winter is sufficient to induce significant and prolonged hypertension in the general population (10). Therefore, cold temperatures are a risk factor contributing to the high incidence of hypertension and the high cardiovascular mortality in cold regions or in winter. Thus it is important to study the effect of cold temperatures on cardiovascular functions, because it has major implications for humans.

EARLY STUDIES FROM THIS LABORATORY (14, 43–48, 50) have shown that chronic exposure of rats to mild cold (5°C or 41°F) is accompanied by a significant elevation of (systolic, diastolic, and mean) BP and cardiac hypertrophy. Thus rats develop hypertension naturally in cold, namely cold-induced hypertension (CIH). This is presently the only naturally occurring form of experimental hypertension that does not require surgery, administration of large doses of hormones or drugs, or genetic manipulation. Therefore, it is important to understand fully the mechanism contributing to this unique model of hypertension. Chronic cold exposure activates the sympathetic nervous system (34, 47, 48, 50) but inhibits \( \alpha_1 \)-adrenergic receptors (11–13). Both in vivo and in vitro vascular responsiveness to phenylephrine (\( \alpha_1 \)-adrenergic receptor agonist) is decreased in cold-exposed animals (11–13, 34), indicating that cold exposure downregulates \( \alpha_1 \)-adrenergic receptors. Previous studies from this laboratory (44, 45, 47, 48, 50) have suggested that the hyperactivity of the sympathetic nervous system initiates CIH via activation of the renin-angiotensin system (RAS).

The RAS has been shown to be involved in the initiation of CIH (16, 40, 43, 47–50). Plasma renin activity increases initially and returns to control level at 3 wk after exposure to cold (16). However, BP continues to increase after 3 wk of cold exposure and remains elevated throughout exposure to cold (43–45, 47, 48, 50, 51). It has been found that chronic cold exposure increases the ANG II-induced water intake (16, 50, 51), ANG II-induced vascular contractile response (40), ANG II-induced c-Fos expression (51), AT\textsubscript{1} receptor mRNA expression (43, 47), and AT\textsubscript{1} receptor binding (35). The AT\textsubscript{1} receptor binding was increased in aorta, heart, kidneys, brain hypothalamus, and brain stem of cold-exposed rats (35, 51). Therefore, AT\textsubscript{1} receptors are upregulated by chronic cold exposure. However, two subtypes of AT\textsubscript{1} receptors have been identified in mouse and rat (AT\textsubscript{1A} and AT\textsubscript{1B}) (37). It is well known that the AT\textsubscript{1A} receptor that is involved in BP regulation, fluid balance, and hormone secretion (aldosterone, AVP, etc.). It has been reported that ANG II increases cardiac protein synthesis in the
adult rat heart (33) and is involved in cardiac hypertrophy (56). Consistently, expression of AT1 receptors is associated with cardiac hypertrophy in humans (32). The AT1A receptor is a putative major effector of the growth response of the RAS. Thus we hypothesize that AT1A receptors play a role in the development of CIIH and cardiac hypertrophy. Because chronic cold exposure increases the drinking response to ANG II (16, 50, 51), we hypothesize that the cold-induced increase in the drinking response to ANG II is mediated by AT1A receptors. The objective of this experiment is to test these hypotheses by using AT1A receptor gene knockout (AT1A-KO) mice chronically exposed to cold. AT1A receptor KO mice are born in expected numbers, and the histomorphology of their kidneys, heart, and vasculature are normal (23).

**MATERIALS AND METHODS**

**Animals.** This study was carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The project, of which this study was a part, was approved by the Institutional Animal Care and Use Committee.

Two groups of wild-type (WT) and two groups of AT1A-KO mice (6 mice/group) (Jackson Laboratories, Bar Harbor, ME) were used. AT1-A-KO is produced by gene targeting leading to a null mutation of the AT1A gene and backcrossed to establish a congenic line with C57BL/6J-129 background. Two groups of wild-type (WT) and two groups of AT1A-KO mice were moved into a cold-climate-controlled walk-in chamber (5 ± 2°C). The remaining groups were kept in an identical warm chamber (room temperature, 25 ± 2°C) and served as controls. Relative humidity was controlled automatically at 45 ± 5% in both thermal environments. All mice were housed individually in wire mesh cages without bedding throughout the experiment. It has been confirmed that the temperature inside the cage is the same as that of the chamber. All the mice were provided with Teklad Rodent Diet (Harlan standard 8604, 24.4% protein, 4.4% fat, 3.6% fiber) and tap water. For all mice, lights were on from 0700 to 1900. The animals were handled frequently (3 times/day, 5 min/time) to minimize handling stress. BP, body weight, and colonic temperature were measured weekly in all mice during exposure to cold. Colonic temperature was measured as described previously (43). During the 1st, 3rd, and 5th wk of exposure to cold, a 24-h urine sample was collected for measurement of urinary output of nitrite and nitrate [NOx; index of nitric oxide (NO) production]. During the 3rd week of exposure to cold, drinking response to ANG II (100 μg/kg body wt sc) was tested in all mice. At the end of week 5 of exposure to cold, all mice were killed by decapitation, and blood was collected in EDTA for measurement of plasma concentrations of NOx. The heart and kidneys were removed and weighed. Heart and aorta were stored at −80°C for measurement of endothelial NO synthase (eNOS) by Western blot analysis. Brain hypothalamus, liver, part of the heart, and the left kidney were saved for measurement of AT1A receptor mRNA by quantitative real-time (QRT) RT-PCR.

**QRT-RT-PCR.** QRT-RT-PCR was performed utilizing the 5700 Sequence Detector (PE Biosystems) as described in our previous studies (49, 52). Briefly, specific quantitative assays for mouse AT1A receptor mRNA and 36B4 (27) were developed by using Primer Express software (PE Biosystems) following the recommended guidelines based on cDNA sequences from GenBank. Sense and antisense primers for mouse AT1A receptor gene were 5’-GAAGCCCTGGT-TCCACCCGATCCGACGTAC-3’ and 5’-GGATGACGCCCCAGCT-GAAATCAGCACATCC-3’, respectively. The quantity of AT1A receptor mRNA was normalized on the basis of 36B4 content in the same sample (AT1A receptor/36B4). 36B4 is a housekeeping gene.

**Western blot analysis.** Tissues were homogenized at 4°C in water (wt/vol, 1:9) containing 10 mM EDTA and 1 mM PMSE. The homogenate was centrifuged at 10,000 g for 30 min at 4°C and the supernatant was adjusted to pH 4.5 with acetic acid and incubated for 1 h at 4°C. The acidic supernatant was recenterfuged at 100 g for 20 min. The supernatant was then neutralized to pH 7.4 and assayed for protein content (Bio-Rad Protein Assay). Supernatant (25 μg protein/lane) was electrophoresed on a 10% polyacrylamide gel in SDS, and the gel subsequently was electroblotted onto a polyvinylidene difluoride membrane. The membrane was blocked with 5% (wt/vol) nonfat milk in Tris-buffered saline, pH 7.4, and 0.1% (vol/vol) Tween 20 for 1 h at room temperature. The membrane was then incubated overnight at 4°C with mouse anti-eNOS antibody (BD Transduction Laboratories, 1:2,500 dilution) followed by an hors eradish peroxidase-labeled anti-mouse IgG (BD Transduction Laboratories, 1:500 dilution) for 1 h at room temperature. After the membrane was thoroughly washed, the reaction was revealed by using enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ). The density of the bands was quantitated with a computerized imaging system (M4, Imaging Research, Brock University, St. Catherines, ON, Canada).

**Measurement of nitrite/nitrate.** Urine and plasma levels of NOx were measured by using an NO analyzing system (Antek) as described previously (6, 7, 49).

**Statistical analysis.** Statistical analysis of the data for BP was carried out by a repeated-measures one-way ANOVA. The cold-induced increase in BP (ΔBP) was analyzed between the WT and the AT1A-KO group by a one-way ANOVA. Data for body weight and urine concentrations of NOx were analyzed by a three-way ANOVA (main factors: time, temperature, and strain), followed by two- or one-way ANOVA. Data for organ weight, tissue eNOS protein expression level, tissue AT1A receptor mRNA expression level, plasma concentration of NOx, and the drinking response to ANG II were analyzed by a two-way ANOVA (main factors: temperature and strain), followed by a one-way ANOVA. The Newman-Keuls procedure was used to assess the significance of differences between means. Significance was set at the 95% confidence limit.

**RESULTS**

**BP, body and organ weights, and colonic temperature.** Basal systolic BP was significantly (P < 0.05) higher in WT mice than in AT1A-KO mice (98 ± 7 vs. 77 ± 5 mmHg) during the control period at room temperature (warm, 25°C) (Fig. 1A). When exposure to cold (5°C) was initiated, the BP of the WT group continued to increase thereafter and rose to 149 mmHg by week 5. However, BP of the AT1A-KO group increased slightly (P < 0.05) above its precold exposure measurements within 1 wk of exposure to cold. The BP of this group continued to increase thereafter and rose to 149 mmHg by week 5. However, BP of the AT1A-KO group increased slightly. The repeated-measures ANOVA revealed a significant (P < 0.001) strain × time interaction (F = 3.74). The maximal cold-induced increase in BP (ΔBP = 11 ± 3 mmHg, compared with precold exposure level) of the AT1A-KO group was significantly (P < 0.01) less than (ΔBP = 49 ± 6 mmHg) that of the WT group (Fig. 1A). Thus the cold-induced elevation of BP was significantly attenuated in AT1A-KO mice. BPs of both groups kept in warm (25°C) temperature remained unchanged during the period of observation.
Body weights of the four groups did not differ significantly from one another throughout the experiment (Fig. 1B). Kidney and heart weights (in milligrams per gram per body weight) of both cold-exposed groups were increased significantly above those of the WT group maintained at 25°C (Fig. 2). There was a significant ($P < 0.001$) strain × temperature interaction ($F = 3.49$). There was no significant difference of heart and kidney weight between the AT1A-KO and WT groups in the same temperature condition.

Colonic temperature was not significantly different among the four groups at either of the five time points of observation. AT1A-KO did not affect core temperature. Colonic temperature was 37.7 ± 0.1°C for the cold-exposed mice and 37.4 ± 0.1°C for the warm-adapted mice.

QRT-RT-PCR analysis of AT1A receptor mRNA expression. As shown in Table 1, AT1A receptor mRNA expression was increased significantly in brain hypothalamus, heart, liver, and kidneys of the cold-exposed WT mice compared with that of the WT mice kept in warm (25°C) temperature. AT1A receptor mRNA was not detectable in both AT1A-KO groups.

Western blot analysis of eNOS protein expression. There was a significant ($P < 0.001$) strain × temperature interaction ($F = 3.52$). Cardiac eNOS protein expression was decreased significantly in the cold-exposed WT group compared with that of the WT group kept in warm (25°C) temperature (Fig. 3). However, chronic cold exposure failed to decrease cardiac eNOS expression in the AT1A-KO group. There was no significant difference of cardiac eNOS expression between the cold-exposed AT1A-KO group and the warm-adapted AT1A-KO group.

Urinary output of NOx and plasma level of NOx. Urinary NOx output was significantly greater in the AT1A-KO group than in the WT group kept at 25°C during weeks 1, 3, and 5. Because similar changes were found at all of the three time points of observation, the data were combined in this analysis. There was a significant ($P < 0.001$) strain × temperature interaction ($F = 3.49$). There was no significant difference of cardiac eNOS expression between the cold-exposed AT1A-KO group and the warm-adapted AT1A-KO group.

Table 1. Quantitative real-time analysis of AT1A receptor mRNA expression in brain hypothalamus, heart, liver, and kidney of four groups of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Hypothalamus</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1A-KO-cold</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AT1A-KO-warm</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>WT-cold</td>
<td>58.4 ± 6.3†</td>
<td>32.7 ± 4.6*</td>
<td>29.5 ± 5.7†</td>
<td>47.4 ± 8.3†</td>
</tr>
<tr>
<td>WT-warm</td>
<td>27.3 ± 4.7</td>
<td>19.5 ± 3.1</td>
<td>14.4 ± 4.4</td>
<td>21.6 ± 5.3</td>
</tr>
</tbody>
</table>

Values are means ± SE measured when the animals ($n = 6$) were killed at 5 wk after exposure to cold. WT, wild-type mice; AT1A-KO, AT1A receptor gene knockout mice; ND, not detectable. *$P < 0.05$, †$P < 0.01$ compared with WT-warm group.
points, only data from \textit{week 1} are shown (Fig. 4A). Chronic exposure to cold significantly decreased urinary NOx output in the WT group. Urinary NOx output was also significantly decreased in the cold-exposed AT1A-KO group compared with its counterpart in warm temperature. However, urinary NOx output was significantly greater in the AT1A-KO than in the WT group in the cold environment. Thus cold exposure did not decrease the urinary NOx output of the AT1A-KO mice to the level of the cold-exposed WT mice.

Plasma concentration of NOx was significantly greater in the AT1A-KO group than in the WT group in the warm environment (25°C) (Fig. 4B). Chronic cold exposure decreased plasma level of NOx in both AT1A-KO and WT mice compared with their counterparts kept at 25°C. However, plasma concentration of NOx was significantly greater in the cold-exposed AT1A-KO group than in the cold-exposed WT group. Thus cold exposure failed to decrease plasma level of NOx of the AT1A-KO mice to that of the cold-exposed WT mice.

\textbf{Drinking response to ANG II.} During \textit{week 3} of exposure to cold, the drinking response to ANG II was tested in all animals (Fig. 5). There was a significant increase in ANG II-induced water intake in the cold-exposed WT group compared with that of the WT group kept in room temperature (warm, 25°C). However, water intake of the cold-exposed AT1A-KO group did not differ from that of the warm-adapted AT1A-KO group during the 2-h observation after subcutaneous administration of ANG II. The water intake of both AT1A-KO groups was significantly less than that of the WT group kept in warm temperature.

\textbf{DISCUSSION}

The major finding of this study is that targeted disruption of the AT1A receptor gene significantly attenuates cold-induced elevation of BP (Fig. 1A). The result provides for the first time that AT1A receptors are essential for the development of CIH. AT1A receptor mRNA was not detectable in brain hypothalamus, heart, liver, and kidneys of AT1A-KO mice, confirming complete deletion of the AT1A receptor gene (Table 1). BP is lower in the AT1A-KO mice than in the WT mice at room temperature (25°C), supporting the notion that AT1A receptors are involved in the maintenance of normal BP.

We have recently reported (49) that null mutation of angiotensinogen (\textit{Agt}) gene delays and attenuates cold-induced elevation of BP, which suggests that the RAS is involved in the
development of CIH. The present study reveals that the role of the RAS in CIH is mediated by AT1A subtype receptors with the RAS intact.

Chronic cold exposure increased ANG II-induced water intake in WT mice (Fig. 5), suggesting upregulation of ANG II receptors in cold-exposed mice. Our previous study (47) indicated blockade of central AT1 receptors by losartan abolished the cold-induced increase in the drinking responses to ANG II in rats, which suggests that AT1 receptors are responsible for this phenomenon. The finding of the present study that ANG II failed to increase drinking response in AT1A-KO mice (Fig. 5) further suggests that the cold-induced increase in drinking responses to ANG II is solely mediated by AT1A subtype receptors.

As indicated in Table 1, AT1A receptor mRNA expression level was increased in both central and peripheral organs of cold-exposed WT mice. These results suggest that AT1A receptors are upregulated by chronic cold exposure. It is interesting to note that the kidney and brain hypothalamus have high levels of AT1A receptor mRNA in WT mice (Table 1). It has been reported (29, 42) that the RAS and AT1A receptors in kidneys and brain are important in BP regulation. Indeed, the renal RAS and central AT1A receptors play a role in the development of CIH (47, 48, 50).

Both AT1A-KO and WT mice developed cardiac hypertrophy to the same extent (Fig. 2A). Thus AT1A-KO failed to prevent or abate cardiac hypertrophy, although it attenuates the cold-induced elevation of BP (Fig. 1A). This is in agreement with our previous studies (15, 45) that antihypertensive agents effectively reduced or prevented CIH but failed to prevent or attenuate cardiac hypertrophy. Thus cold-induced cardiac hypertrophy represents a unique model of experimental cardiac hypertrophy, because its occurrence is independent of pressure overload and tachycardia. It has been reported (18, 56) that the RAS is involved in cardiac hypertrophy. The AT1A receptor is a putative major effector of the growth response of the RAS. Expression of AT1 receptors is associated with human cardiac hypertrophy (32). However, the present finding indicates that AT1A receptors may not be involved in cold-induced cardiac hypertrophy. In addition, Agrt-KO does not affect the development of cardiac hypertrophy in mice exposed to cold (49). These data suggest that the RAS may not mediate cold-induced cardiac hypertrophy and that other systems are active in producing the hypertrophy in this model. It has been reported that AT1A-KO does not prevent pressure overload-induced cardiac hypertrophy (21). Substantial cardiac hypertrophy occurred in aorta-banded mice for both AT1A receptor-deficient and WT mice (21). There is evidence that pressure overload-induced cardiac hypertrophy is mediated by AT2 receptors (38). Cold-induced cardiac hypertrophy is a natural form of cardiac hypertrophy, which does not require surgical occlusion of aorta or genetic manipulation. Therefore, it will be both interesting and important to make a thorough investigation into the pathogenesis of this unique animal model of cardiac hypertrophy. A histological study is required to examine the quality of the change in the hearts of these two genotypes. Chronic cold exposure also increased kidney weight of both AT1A-KO and WT mice to a similar extent (Fig. 2), suggesting that the development of cold-induced renal hypertrophy is independent of AT1A receptors.

Another interesting finding of this experiment is that cold exposure inhibited eNOS expression in the heart (Fig. 3) and aorta of WT mice. A decrease in eNOS expression could contribute to the cold-induced elevation of BP. Interestingly, cold exposure failed to decrease eNOS expression in AT1A-KO mice. These results suggest that the inhibitory effect of cold exposure on eNOS expression may be mediated by AT1A receptors. It has been reported that ANG II could inhibit eNOS and decrease NO production (1, 58). This is apparently mediated by AT1 receptors, because activation of AT2 receptors stimulates the formation of NO (2, 4). As discussed above, chronic cold exposure upregulates AT1 receptors that may contribute to cold-induced inhibition of eNOS. Thus it is reasonable to hypothesize that the role of AT1A receptors in CIH may be mediated partially by its inhibition on eNOS. On the other hand, CIH and subsequent cardiovascular hypertrophy may impair the endothelial system and cause NO dysfunction, which can further amplify CIH and related cardiovascular disorders. AT1A receptor gene KO did not affect eNOS expression at room temperature (warm, 25°C).

Consistently, NO production is decreased by chronic cold exposure as evidenced by a significant reduction in urinary output of NO (Fig. 4A) and plasma level of NO (Fig. 4B) in cold-exposed WT mice. Cold exposure decreased NO production in AT1A-KO mice but failed to decrease it to the level of the cold-exposed WT mice. This suggests that cold-induced decrease in NO production may be mediated partially by AT1A receptors. In contrast, cold exposure did not decrease eNOS expression in AT1A-KO mice (Fig. 3). Thus mechanisms, in addition to inhibition of eNOS that may be involved in cold-induced decrease in NO production, need to be investigated. Possible involvement of inducible NOS and neuronal NOS in cold-induced reduction of NO formation needs to be investigated. Interestingly, AT1A-KO increased NO production in
warm-adapted mice that is apparently unrelated to eNOS. This indicates that AT1A receptors may have tonic inhibition on NO formation under physiological conditions.

The mechanism of the slight increase in BP of the AT1A-KO group (Fig. 1) is not known but is apparently unrelated to AT1A receptors. It has been reported that, in the absence of AT1A receptors, AT1B receptors may partially replace the function of AT1A receptors in BP regulation and ANG II-induced vasoconstriction (33, 59). However, AT1B receptors have been reported to be not involved in the development of two-kidney, one-clip Goldblatt hypertension in AT1A-KO mice (9). Therefore, the mechanism of the slight increase in BP of the cold-exposed AT1A-KO mice remains to be investigated.

Previous studies (13, 43) from this laboratory have shown that rats could maintain their core temperature and survive during chronic exposure to cold (5°C). The present data clearly indicate that AT1A-KO and WT mice could maintain their core temperature and grew at approximately the same rate throughout the experiment (Fig. 1B). Body weights of the two cold-exposed groups did not decrease, suggesting that both AT1A-KO and WT mice adapted well to chronic cold exposure.

In summary, the present finding reveals that AT1A receptors are essential to the development of CIH. The critical role of AT1A receptors in CIH may be mediated, in part, by its inhibition on eNOS and NO formation. The present results are significant, because they provide a potential target for preventive and therapeutic interventions that are particularly important for people who live and work in cold areas, especially those who work daily in cold environments (meat packers, butchers, custodians of freezer lockers, ice cream manufacturers, and so forth). The findings may help to control cold-induced elevation of BP and related cardiovascular diseases (myocardial infarction, stroke, and so forth) more appropriately and more effectively in hypertensive patients in winter. Although CIH has been studied extensively, little is known about the pathogenesis of cold-induced cardiac hypertrophy, which is independent of both pressure overload and tachycardia. Cardiac hypertrophy is an identified risk factor for myocardial infarction. Thus it will be interesting and important to investigate the mechanisms of cold-induced cardiac hypertrophy, a nonpharmacological, nonsurgical, and nongenetic model of cardiac hypertrophy.

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