BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **245,** 226–230 (1998)
ARTICLE NO. RC988411

Thyroid Hormone Regulates Expression of Shaker-Related Potassium Channel mRNA in Rat Heart

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Received February 27, 1998

Effects of thyroid hormones on cardiac function or rhythm have been known; however, the mechanism is still unclear. In the present study examined were effects of triiodethyronine (T3) on voltage-gated potassium channel gene expression in rat heart since the potassium channels were presumed to modulate cardiac functions. The mRNA expression of five voltage-gated potassium channel gene α subunits (Kv1.2, Kv1.4, Kv1.5, Kv2.1, and Kv4.2) in heart was examined by ribonuclease protection assay in rats which were treated with T3 or propylthyouracil (PTU). All these genes except Kv1.4 mRNA were apparently expressed in the normal rat heart ventricle. Kv1.2 mRNA expression in ventricle was markedly suppressed by T3-treatment and enhanced by PTU-treatment. Interestingly, upregulation of Kv1.4 mRNA expression and downregulation of Kv1.5 mRNA expression were concomitantly induced in the ventricle by the PTU-treatment. In addition, the downregulation of the ventricular Kv1.5 mRNA expression induced by PTU was restored by T3 replacement. No changes of Kv2.1 and Kv4.2 mRNA expression were observed in the ventricles by the T3- or PTU-treatment. In heart atrium the same findings were observed. Kv1.4 mRNA expression, which was detectable in control rat atrium, also decreased significantly by T3-treatment. In contrast, no changes of Kv1.2, Kv1.4, and Kv1.5 mRNA expression in rat brains were induced by T3-treatment. These findings suggest that thyroid hormone specifically influences mRNA expression of Shaker-related potassium channel genes in rat hearts through a common T3 receptor-mediated regulation at a transcriptional level. © 1998 Academic Press

Key Words: **potassium channel; thyroid hormone; T3 receptor.**

Recently a large number of voltage-gated potassium channel genes have been identified in various animals and tissues. In rat hearts, mRNA expression of five types of voltage-gated potassium channels (Kv1.2, Kv1.4, Kv1.5, Kv2.1 and Kv4.2) has been demonstrated at relatively high levels (1). Several studies reported hormonal regulation of the potassium channels. Kv1.5 mRNA expression was shown to be upregulated by glucocorticoids and downregulated by thyrotropin-releasing factor (TRH) in cultured pituitary cells (2, 3). In cardiocytes, glucocorticoids stimulated cAMP generation, resulting in upregulation of the Kv1.5 mRNA expression at a transcription level through a cAMP response element (CRE) of Kv1.5 gene (4, 5).

Although thyroxine-treatment was shown to induce shortening of the effective refractory period in rabbit atrium (6), little is known about this mechanism. On the other hand, thyroid hormones have been noticed to change cardiac functions and cardiac action potential duration, which may be modulated by the voltage-gated potassium channels. Taken together, it may be hypothesised that thyroid hormones modulates voltage-gated potassium channel gene expression resulting in the shortening of the effective refractory period in the heart. To test this hypothesis, effects of triiodethyronine (T3) or propylthyouracil (PTU) on the mRNA expression of a subunits of five potassium channels (Kv1.2, Kv1.4, Kv1.5, Kv2.1 and Kv4.2) were examined in rat hears by ribonuclease protection assay.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats (Charles River Japan Inc., Atsugi, Japan), aged 8-wk (weight 170 to 180 g) were used in all experiments. In experiment I, three groups of rats (n = 5 each) received 4 mg of propylthyouracil (PTU), 25 mg of triiodethyronine (T3, liothyronine sodium; Takeda pharmaceutical Co., Tokyo, Japan) or 0.25 ml of distilled water as a control per 100 g body weight, respectively, through a gastric tube daily for 4-wk. Expression of voltage-gated potassium channel mRNA in the heart ventricle and atrium was examined by ribonuclease protection assay as described below. In experiment II, rats were given T3 (25 mg per 100 g body weight) for 1 and 3 days (n = 5) or PTU (4 mg per 100 g body weight) for 1, 2 and 3-wk (n = 5) daily by a gastric tube. To test whether the downregulation of Kv1.5 mRNA expression in the ventricle by the PTU treatment may be caused by suppression of T3 synthesis, 5 rats

0006-291X/98 $25.00

treated daily with PTU for 2-wk were given an administration of T3 (25 mg per 100 g body weight) 24 hours before removal of their hearts. In experiment III, effect of the T3 treatment on Kv1.2, Kv1.4, and Kv1.5 mRNA expression in the brain was examined 24 hours after T3 administration (25 mg per 100 g body weight, n = 5).

The ears were removed at sacrifice and weighed after rinsing in PBS to remove excess blood. Blood was collected by cardiac puncture under ether anesthesia for measurement of plasma T3 and T4 by a RIA method. The T3 effect on the heart was also evaluated by a ratio of heart to body weight.

Isolation of RNA. The hearts were divided into atrium (left and right atrial appendages) and left ventricle. Total cellular RNA was purified from the left ventricle, atrium and whole brain by the acid guanidinium-phenol-chloroform method.

Voltage-gated potassium channel cDNA cloning. Rats cDNAs for voltage-gated potassium channels were cloned by reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA isolated from normal rat ventricule was reverse transcribed to cDNA using oligo dT primer. The following unique primers were synthesized for cloning of rat voltage-gated potassium channel cDNA fragments: Kv1.2 cDNA encoding 1143 to 1401 [RBK2] (7); sense [5′-TTCCGGGATGAGAACGAGGA-3′] and anti-sense [5′-TTAGCTGAGAAGCCAGAGGA-3′], Kv1.4 cDNA encoding 673 to 905 [RHK1] (8); sense [5′-TTCCAGAAACTCTGTTGGGA-3′] and anti-sense [5′-TTTGTGAGAGAAGAGGAGGA-3′], Kv1.5 cDNA encoding 2394 to 2741 [Kv1] (9); sense [5′-TTCCCGCTGCCTGGTAACCA-3′] and anti-sense [5′-ATGCGAACCAGGGTGGACGG-3′], Kv2.1 cDNA encoding 1536 to 1953 [drk1] (10); sense [5′-GGGATCCCCCGAAAAGGCCA-3′] and anti-sense [5′-CTCTGGTTTCTTCGTGGAGA-3′], Kv4.2 cDNA encoding 1962 to 2359 [Rshall] (11); sense [5′-GGATCCAGCTTCGAGACACA-3′] and anti-sense [5′-CTCCAGTAACCACCCCAGAA-3′]. Using the cDNA as a template and the primers, cDNA was amplified by PCR and the cDNA was cloned in pBluescript II SK+ vector (Stratagene, San Diego, CA). The sequences of the cloned genes were examined by an automated DNA sequencer (ABI 373A; Perkin-Elmer Japan, Urayasu, Japan). As a rat housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (kindly provided by Dr. R. Wu, Cornell University, Ithaca, NY) of 114 bp length corresponding to 673 to 787 was subcloned in pGEM 3Z vector (Promega Co., Madison, WI). The plasmids were linearized with EcoR I (Kv1.2, Kv1.4 and Kv1.5) or BamH I (Kv2.1, Kv4.2 and GAPDH).

Ribonuclease protection assay. Total cellular RNA and tRNA (Promega Co.) of 10 mg were hybridized with ^{32}P-labeled Kv1.2, Kv1.4, Kv1.5, Kv2.1, Kv4.2 antisense cRNA probes (1×10^5 counts/min each) combining with GAPDH antisense probe, synthesized by in vitro transcription using the linearized templates, at 45°C overnight, and unhybridized probes were digested with ribonuclease A (0.3 mg/ml; Sigma, St. Louis, MO) and ribonuclease T1 (30 units/ml; GIBCO/BRL, Grand Island, NY) at 30°C for 1 h. The ribonucleases were inactivated with proteinase K-treatment (0.5 mg/ml, Promega Co.) at 37°C for 30 min. After phenol-chloroform extraction and ethanol precipitation, the hybridized probes protected from the ribonuclease digestion were denatured at 85°C for 3 min and electrophoresed on 6% polyacrylamide gels. The dried gels were exposed to X-ray films (Fujiphoto, Tokyo, Japan) at −70°C for 20 h to detect potassium channel mRNA expression followed by exposure to new X-ray films for 6 h to detect GAPDH mRNA expression. For quantitation of each mRNA expression, the autoradiography bands were analyzed by a computerized densitometry using NIH Image software (1.59, NIH Division of Computer Research and Technology, Bethesda, MD). Data are presented as a ratio of specific mRNA (exposed for 20 h) to GAPDH mRNA (exposed for 6 h) to equalized the quantity of RNA within each sample.

Staistical analysis. Results are presented as mean value ± SD. The Mann-Whitney U test was used to compare difference between unpaired samples and $p<0.05$ was considered significant.

RESULTS

Experiment I: Effect of T3- or PTU-Treatment on Plasma T3 and T4 Levels and on Body and Heart Weights

Plasma T3 and T4 levels were significantly decreased in the rats treated with PTU for 4-wk compared with control rats (50.0 ± 0.0 ng/dl vs. 92.0 ± 11.0 ng/dl, $p < 0.01$) and (1.0 ± 0.0 mg/dl vs. 4.6 ± 1.0 mg/dl, $p < 0.01$), respectively. By the T3-treatment for 4-wk plasma T3 and T4 levels were also significantly decreased (50.0 ± 10.0 ng/dl vs. 92.0 ± 11.0 ng/dl, $p < 0.01$) and (1.0 ± 0.0 mg/dl vs. 4.6 ± 1.0 mg /dl, $p < 0.01$), respectively. No significant difference in the rate of body weight increase during the period was observed among the groups (control rats: 40.9 ± 8.3%, PTU-treated rats: 31.7 ± 3.3%, T3-treated rats: 23.8 ± 9.5%). The ratio of heart weight to body weight was significantly smaller in the PTU-treated rats, comparing to control rats (0.33 ± 0.01% vs. 0.41 ± 0.03%, $p < 0.05$). Conversely, the ratio was significantly larger in the T3-treated rats, comparing to controls (0.57 ± 0.01% vs. 0.41 ± 0.03%, $p < 0.01$) or PTU-treated rats (0.57 ± 0.01% vs. 0.33 ± 0.01%, $p<0.05$).

Effect of T3- or PTU-Treatment on Voltage-Gated Potassium Channel mRNA Expression in Heart Ventricle

(1) Kv1 gene family. Kv1.2 mRNA expression in the heart ventricles increased approximately 2-fold by the PTU-treatment, compared with the control rats (11.1 ± 2.5% vs. 22.5 ± 3.5%) and strongly suppressed by the T3-treatment to a nearly undetectable level (Fig. 1A). Kv1.4 mRNA expression in the ventricle increased approximately 7-fold (14.8 ± 0.4%) in the PTU-treated rats, although it was faint in the control rats (2.0 ± 1.1%) and in the T3-treated rats (2.0 ± 0.1%) (Fig. 1B). On the other hand, ventricular Kv1.5 mRNA expression decreased approximately 1/5-fold by the PTU-treatment from 27.8 ± 6.6% to 5.5 ±0 .4%, but not by the T3-treatment (22.1 ± 5.7%) (Fig. 1C).

(2) Kv2 and Kv4 gene families. No apparent changes in ventricular Kv2.1 mRNA expression were observed in all three groups; control rats (23.4 ± 0.7%), PTU-treated rats (23.4 ± 2.3%) and T3-treated rats (22.2 ± 0.2%) (Fig. 1D). Kv4.2 mRNA expression in ventricle was also not affected by PTU- (20.5 ± 1.3% vs 27.8 ± 4.8%) or T3-treatment (28.2 ± 5.3% vs 27.8 ± 4.8%) (Fig. 1E).

Experiment II

(1) Time-course of ventricular Kv1.2 and Kv1.5 mRNA expression during T3-treatment. The ventricular Kv1.2 mRNA expression decreased approximately

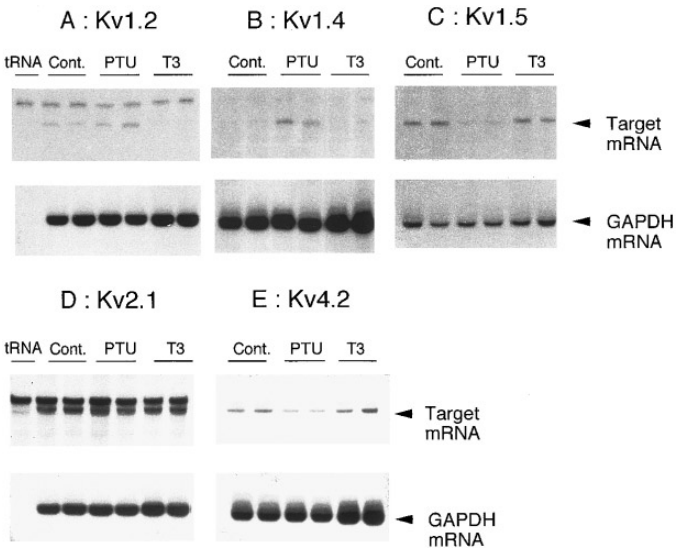

FIG. 1. Effects of T3- or PTU-treatment for 4 weeks on voltage-gated potassium channel mRNA expression in rat ventricles (Experiment I). The representative mRNA expression in two ventricle samples each is represented by ribonuclease protection assay. Kv1.2 mRNA expression is apparently suppressed by T3-treatment (A). In contrast, PTU-treatment enhances Kv1.2 mRNA expression slightly and Kv1.4 mRNA expression markedly (B), but suppresses Kv1.5 mRNA expression conspicuously (C). On the other hand, No changes of Kv2.1 (D) and Kv4.2 (E) mRNA expression are induced by the T3- or PTU-treatment although Kv4.2 mRNA expression is slightly attenuated by the PTU-treatment. Cont: control, PTU: propylthyouracil, T3: triiodethronine.

1/5 fold (34.4 ± 0.4% vs. 7.8 ± 0.4%) 24 h after the T3 administration, whereas Kv1.5 mRNA expression increased approximately 1.4 fold (14.8 ± 2.3% vs. 21.3 ± 4.7%). These changes became more prominent after T3 administration for 3 days (Kv1.2: 5.3 ± 0.5%, Kv1.5: 22.8 ± 2.1%) (Fig. 2).

Consecutive administration of PTU for 1-wk resulted in a decrease of plasma T3 (control: 92.0 ± 11.0 ng/dl, PTU 1-wk: 50.0 ± 0 ng/dl, PTU 2-wk: 60.0 ± 14.1 ng/dl, PTU 3-wk: 55.0 ± 7.1 ng/dl) and a decrease of Kv1.5 mRNA expression in the ventricles (control: 77.4 ± 8.1%, PTU 1-wk: 36.4 ± 7.6%, PTU 2-wk: 16.9 ± 2.0%, PTU 3-wk: 7.4 ± 1.8%) (Fig. 2).

(2) Effect of T3 replacement to the PTU-treated rats. The downregulation of Kv1.5 mRNA expression in the ventricle induced by the PTU-treatment for 2 wk was restored by a single injection with T3 (24.0 ± 15.2% from 2.8 ± 1.5 % comparing to 26.2 ± 14.6 %) (Fig. 2).

Experiment III: Expression of the Voltage-Gated Potassium Channel mRNA Expression in Rat Atrium and Brain

In the atrium, the change in expression of mRNA for Kv1.2 and Kv1.5 was similar to that in the ventricle after T3- or PTU- treatment. Kv1.2 mRNA expression in the atrium was also markedly suppressed by T3-treatment and enhanced by PTU- treatment as shown in the ventricle (data not shown). Kv1.4 mRNA expression was apparently detectable in the atriums of control rats (21.6 ± 10.3%) and also consistently decreased by T3-treatment (3.1 ± 2.0%) (Fig. 3). No changes of Kv2.1 and Kv4.2 mRNA expression in the atrium were observed by the T3- or PTU-treatment (data not shown).

The expression of Kv1.2 mRNA was intense and that of Kv1.4 and Kv1.5 mRNA was less in normal rat brain. These expression in brain was not influenced by the T3-treatment (Kv1.2: 420.9 ± 195.2% vs. 431.1 ± 138.2%, Kv1.4: 9.7 ± 2.5% vs. 11.8 ± 2.8%, Kv1.5: 19.0 ± 3.5% vs. 24.8 ± 12.2%) (Fig. 3).

DISCUSSION

Our results showed expression of mRNA for Shaker-related potassium channels (Kv1.2, Kv1.4 and Kv1.5) in rat hearts were regulated by T3- or PTU-treatment whereas no significant effects were observed on other voltage-gated potassium channel mRNA expression by the same treatment. Kv1.2 expression in the hearts

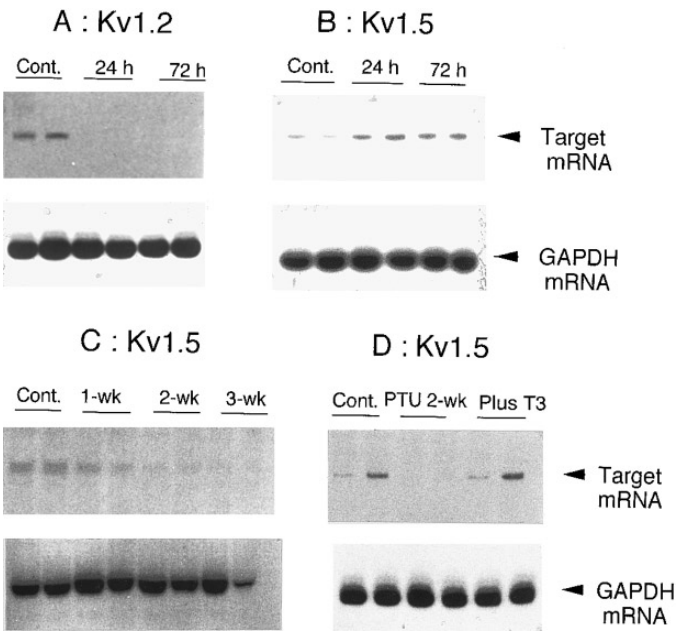

FIG. 2. Changes of Kv1.2 and Kv1.5 mRNA expression in the ventricles during T3 administration (Experiment II). Kv1.2 mRNA expression is negligible 24 h after administration of T3 (A) and Kv1.5 mRNA expression is intensified at 24 h (B). Kv1.5 mRNA expression is gradually suppressed by PTU-treatment (C) and the suppression of Kv1.5 mRNA expression is restored up to the control level 24 h after T3 replacement (D). 24 h: 24 hours, 72 h: 72 hours, PTU 2 W: PTU-treatment for 2 weeks, plus T3: PTU-treatment for 2 weeks followed by T3 replacement.

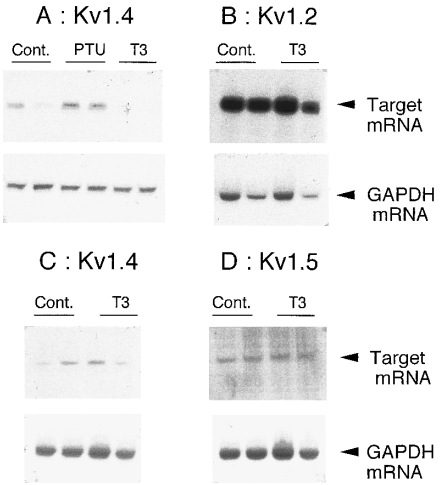

FIG. 3. Kv1.4 mRNA expression in rat atrium after T3- or PTU-treatment and Kv1.2, Kv1.4 and Kv1.5 mRNA expression in the brain after T3-treatment. Kv1.4 mRNA expression detectable in the control rat atrium is enhance by PTU-treatment and suppressed by T3-treatment (A). No changes of Kv1.2 (B), Kv1.4 (C) and Kv1.5 (D) mRNA expression are obvious in the brain after T3-treatment.

was downregulated and Kv1.5 mRNA expression was upregulated by T3 administration. Conversely, administration of PTU in rats for 4-wk enhanced Kv1.2 and Kv1.4 mRNA expression and suppressed Kv1.5 mRNA expression in rat hearts. Furthermore, the PTU-induced suppression of Kv1.5 mRNA expression was restored by T3 replacement. These observations suggest the Shaker-related potassium channel mRNA expression in hearts is regulated by T3.

The PTU-induced augmentation of the Kv1.2 and Kv1.4 mRNA expression in the hearts suggests that T3 acts to suppress expression of these potassium channels in a physiologic condition. In contrast, the suppression of Kv1.5 mRNA expression in the hearts by PTU-treatment may indicate that the Kv1.5 mRNA expression in the heart is dependent on a level of T3 in the circulation. This presumption was confirmed by the restoration of the PTU-induced Kv1.5 mRNA expression with the T3 replacement shown in the present study.

There may be discrepancy in the expression level of the voltage-gated potassium channels in hearts between the current results and previous studies. Kv1.4 mRNA expression was hardly detectable in normal rat ventricles in the present study, although it was apparently shown in the previous studies (1, 12). Since Kv1.4 mRNA expression in the ventricle was also demonstrated to decrease gradually during development , the different finding between the two studies would be explained by age-dependent gene expression (13). In addition, Kv1.4 immunoreactive protein has been hardly detectable in normal rat ventricles, which may support our current results (14).

Since T3-treatment modulated mRNA expression of Shaker-related potassium channels but not other voltage-gated potassium channels in hearts, Shaker-related potassium channel expression was suggested to be regulated by T3 through a common T3 receptor-mediated transcription. T3 binds to DNA-binding proteins termed T3 receptor (T3R) and then the T3/T3R complex binds to thyroid hormone response element (TRE) locating on upstream of T3-dependent genes and modulates their transcription. Two types of TRE, positive and negative TRE, have been identified (15).The positive TRE enhances transcription of genes when T3/T3R complex is bound, whereas the negative TRE can directly reduce transcription of genes. To be more complicated, T3R binds to positive TRE without complexing with T3 and the binding of T3R alone reduces gene transcription and T3R alone binding to negative TRE can stimulate gene transcription(16). The modulation of Kv1.2, Kv1.4 and Kv1.5 mRNA expression by T3- or PTU-treatment demonstrated in the present study suggests that the positive TRE is present in the 5′ flanking region of the rat Kv1.5 gene and negative TRE in the 5′ flanking region of the rat Kv1.2 and Kv1.4 genes. Since the T3-mediated modulation of Shaker-related potassium channel gene expression was not observed in brain, there should be other regulatory elements for their transcription in the heart. It has been shown that three types of T3R (c-erbAa1, c-erbAa2, c-erbAb1) are present in rat hearts whereas c-erbAb2 T3R has been identified in rat brain (17, 18, 19). The differential distribution of T3R subtypes may be related with tissue-specific regulation of Shaker-related potassium channel genes by thyroid hormone.

Clinically ventricular arrhythmias are rare in hyperthyroid state (20). This may be accounted by the suggested counter regulation of Kv1.5 gene and Kv1.2 or Kv1.4 gene in the ventricle, which may stabilize the action potential duration by their mutual compensation. Conversely, atrial fibrillation occurs in 9 - 22% of hyperthyroid patients (21). Since shortening of the action potential duration can induce atrial fibrillation, the preferential atrial arrhythmogenic effect of thyroid hormones could be due to quantitative imbalance between Kv1.5 gene upregulation and Kv1.2 or Kv1.4 gene downregulation in the atrium. In normal rat hearts, distribution of Kv1.2, Kv1.4 and Kv1.5 may be different in the ventricle and the atrium as shown previously (1). Kv1.5 may be much more abundant in the atrium than the ventricle in human hearts and may define the ultrarapidly activating component of the delayed rectifier current (iKur) (22, 23, 24). Therefore, the modulation of Shaker-related potassium channel gene expression by thyroid hormones might cause atrial arrhythmogenic effect but no ventricular effects. To confirm our speculation, studies should be done in combination with electrophysiological examination and

to clarify an implication of α subunits assembly and interaction of β subunits (25, 26, 27).

CONCLUSION

T3-regulated mRNA expression of Shaker-related potassium channel genes (Kv1.2, Kv1.4 and Kv1.5) in rat hearts with no effects on other voltage-gated potassium channel expression suggested a presence of regulatory elements common to Shaker-related potassium channel gene transcription. Furthermore, thyroid hormone may regulate heart function and rhythm through counter effect on the expression of individual gene for Shaker-related potassium channels.

ACKNOWLEDGMENT

We thank Kan Yoshida for expert technical assistance.

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