Ca\(^{2+}\) Sensitization and Potentiation of the Maximum Level of Myofibrillar ATPase Activity Caused by Mutations of Troponin T Found in Familial Hypertrophic Cardiomyopathy*

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Human wild-type cardiac troponin T, I, C and five troponin T mutants (I79N, R92Q, F110I, E244D, and R278C) causing familial hypertrophic cardiomyopathy were expressed in *Escherichia coli*, and then were purified and incorporated into rabbit cardiac myofibrils using a troponin exchange technique. The Ca\(^{2+}\)-sensitive ATPase activity of these myofibrillar preparations was measured in order to examine the functional consequences of these troponin mutations. An I79N troponin T mutation was found to cause a definite increase in Ca\(^{2+}\)-sensitivity of the myofibrillar ATPase activity without inducing any significant change in the maximum level of ATPase activity. A detailed analysis indicated the inhibitory action of troponin I to be impaired by the I79N troponin T mutation. Two more troponin T mutations (R92Q and R278C) were also found to have a Ca\(^{2+}\)-sensitizing effect without inducing any change in maximum ATPase activity. Two other troponin T mutations (F110I and E244D) had no Ca\(^{2+}\)-sensitizing effects on the ATPase activity, but remarkably potentiated the maximum level of ATPase activity. These findings indicate that hypertrophic cardiomyopathy-linked troponin T mutations have at least two different effects on the Ca\(^{2+}\)-sensitive ATPase activity, Ca\(^{2+}\)-sensitization and potentiation of the maximum level of the ATPase activity.

EXPERIMENTAL PROCEDURES

Cloning and Mutagenesis of Human Cardiac Tn cDNAs—Human cardiac Tn (TnC, TnI, and TnT) cDNAs were amplified by reverse transcriptase-polymerase chain reaction of human heart mRNA purchased from CLONTECH (Palo Alto, CA). The polymerase chain reaction products were subcloned into the pUC 119 vector for screening by restriction analysis and DNA sequencing. The obtained wild-type Tn cDNAs were then constructed into a pET-3d vector for expression. To obtain mutant TnTs, mutations were generated by site-directed mutagenesis using the reported TnT treatment procedure (15–17). The Ca\(^{2+}\)-sensitive ATPase activity of these reconstituted myofibrils was examined. We thus found the HCM-linked TnT mutants to have at least two different effects on ATPase activity, which are Ca\(^{2+}\)-sensitization (I79N, R92Q, and R278C) and the potentiation of the maximum level (F110I and E244D). The Ca\(^{2+}\)-sensitizing effect of I79N and R92Q TnT mutants is consistent with our previous study using skinned fibers (18). These findings suggest that an enhanced contractility of the cardiac muscle, which occurs as a result of mutations in both groups, may be a common mechanism for the pathogenesis of HCM associated with these TnT mutations.

Expression and Purification of Recombinant Tns—The wild-type Tn and mutant TnTs were expressed in *Escherichia coli* BL21(DE3) and purified as described previously (18) with slight modifications. The induction of protein expression by isopropyl-\(\beta\)-thiogalactopyranoside was continued for 5.0 h, and then the cells were harvested and lysed. The lysate was then centrifuged at 15,000 rpm for 25 min at 4 °C. For the purification of TnT and TnC, 10 mM Tris/HCl (pH 8.0), 6 mM urea, 5 mM CDTA, and 15 mM 2-ME were added to the supernatant and the samples were applied to RESORCE Q column (Pharmacia Biotech Inc.) using a fast protein liquid chromatography system. For the purification of TnI, the pellet was resuspended with the same solution described above and the sample was applied to a Poros HS/M column (PerSeptive Biosystem).

Preparation of Myofibrils—To prepare myofibrils, fresh rabbit car-
ATPase assay consisted of 90 mM KCl, 5 mM MgCl₂, 20 mM MOPS (pH 7.0), and 0.5% 2-ME for 6 h at 4 °C. The solution was then changed to a mixture of 50% glycerol, 100 mM KCl, 20 mM MOPS (pH 7.0), and 0.5% 2-ME and the slices were kept in it overnight at 4 °C. The following morning, these slices were transferred to the new solution and stored at −20 °C. Myofibrils were prepared from the kept samples according to the method of Solaro et al. (20).

Tn Exchange in Myofibrils—Tn exchange was carried out according to our previously reported method (15–17) with slight modifications. Myofibrils (300 μg/ml) were incubated in a solution containing 20 mM MOPS/KOH (pH 6.2), 265 mM KCl, 2 mM EDTA, 2 mM MgCl₂, 0.5 mM 2-ME and 37.5 mM recombinant human cardiac TnT at 25 °C for 1 h while shaking. These myofibrils were washed with a solution containing 0.5% Brij-58, 20 mM MOPS (pH 7.0), 265 mM KCl, 2 mM MgCl₂, 0.5 mM 2-ME, and the myofibrils were resuspended in a solution (300 μg/ml) containing 60 mM KCl and 1 mM sodium bicarbonate after washing with the same solution. The myofibrils were then reconstituted with recombinant Tn (15.2 μg/ml) and TnC (11.6 μg/ml) on ice for 1 h.

ATPase Activity Measurement—The reaction mixture (200 μl) for the ATPase assay consisted of 90 mM KCl, 5 mM MgCl₂, 20 mM MOPS (pH 7.0), 1 mM CaCl₂-EGTA, 4 mM ATP, 40 μg of myofibrils. The reaction was started by adding ATP at 25 °C and was terminated by adding 1.6 ml of a mixture of 50% acetonitrile, 2.5 mM (NH₄)₆Mo₇O₂₄·4H₂O and 1.25 mM H₂SO₄. The contents were mixed carefully and 160 μl of 1N citric acid were added to tubes and the yellow color was measured at OD₅₅₀ (21). Ca²⁺ concentrations in the reaction mixtures were calculated as described previously (22).

Electrophoresis—SDS-PAGE was performed according to the procedure of Laemmli (23), with an acrylamide concentration of 12%. Alkaline gel electrophoresis was carried out according to the procedure described previously (24). Alkaline urea gel electrophoresis was done by the procedure of Pan and Solaro (25), with an acrylamide concentration of 13.5% at pH 8.5. The gel was stained with Coomassie Brilliant Blue R-250 and an optical densitometric scan was performed using Phoretix gel analysis software (Phoretix International Ltd.).

RESULTS

Comparison of Recombinant Human Cardiac Tn Subunit and Native Rabbit Cardiac Tn Subunit—Three subunits of human cardiac Tn (TnT, TnI, and TnC) were cloned by the reverse transcriptase-polymerase chain reaction technique and expressed using a pET-3d vector. As shown in Fig. 1A, recombinant human cardiac TnT and TnC had exactly the same electrophoretic mobility as rabbit cardiac TnT and TnC on 12% SDS-PAGE gel. Recombinant human cardiac TnI, however, had a little faster mobility than rabbit cardiac TnI. An alkaline gel electrophoresis analysis showed the recombinant human cardiac Tn subunit combined with the native rabbit cardiac Tn subunit to form binary or ternary complexes under nondenaturing conditions (Fig. 1B). As a result, the recombinant human cardiac Tn subunits had the ability to form a Tn complex in the same way as the native rabbit cardiac Tn subunits.

Effect of Wild-type TnT and I79N TnT Treatment on the ATPase Activity of Rabbit Cardiac Myofibrils—We previously reported the endogenous Tn complexes in myofibrils were replaced with exogenous TnT after treatment with an excessive amount of purified TnT in acidic solution and the ATPase activity of the treated myofibrils becomes insensitive to Ca²⁺ (15–17). Fig. 2 shows the ATPase activity of rabbit cardiac myofibrils treated with different amounts of wild-type (○, ◆) or I79N mutant (□, ■) TnT and the ATPase activity of these myofibrils were measured in the presence (pCa 5.18, open symbols) or absence of Ca²⁺ (pCa 7.05, closed symbols). The results are the mean ± S.E. of four experiments performed in triplicate.

ATPase Activity of Rabbit Cardiac Myofibrils—We previously reported the endogenous Tn complexes in myofibrils were replaced with exogenous TnT after treatment with an excessive amount of purified TnT in acidic solution and the ATPase activity of the treated myofibrils becomes insensitive to Ca²⁺ (15–17). Fig. 2 shows the ATPase activity of rabbit cardiac myofibrils treated with different amounts of recombinant TnT in the presence (pCa 5.18) and absence (pCa 7.05) of Ca²⁺. The ATPase activity in the presence of Ca²⁺ did not change after the treatment with TnT at any concentrations, whereas the ATPase activity in the absence of Ca²⁺ increased in a concentration-dependent manner and reached almost a maximum level after treatment with 20 μg/ml TnT (Fig. 2). The maximum ATPase activities of wild-type TnT-treated myofibrils were 22 ± 1.6 nmol-P/mg/min in the absence of Ca²⁺ and 32 ± 0.5 nmol-P/mg/min in the presence of Ca²⁺ (mean ± S.E., n = 4). The maximum ATPase activities of I79N TnT-treated myofibrils were 22 ± 1.5 nmol-P/mg/min in the absence of Ca²⁺ and 33 ± 3.0 nmol-P/mg/min in the presence of Ca²⁺ (mean ± S.E., n = 4). As a result, the maximum ATPase activity in the absence of Ca²⁺ increased up to approximately 65% of the maximum activity in the presence of Ca²⁺ by the treatments with either wild-type or I79N TnT at 37.5 μg/ml. In the following experiments, myofibrils were treated with this concentration of TnT.
**FIG. 3.** The gel electrophoresis pattern of TnT-treated and TnI-C-reconstituted myofibrils. A, SDS-PAGE pattern. The myofibrils were treated with 37.5 μg/ml wild-type or I79N mutant TnT and reconstituted with recombinant human TnI (15.2 μg/ml) and TnC (11.6 μg/ml). The samples were lysed in Laemmli’s sample buffer and separated on 12% SDS-PAGE. Lane 1, recombinant human cardiac TnT, I, C-mixture; lane 2, purified rabbit cardiac TnT, I, C-mixture; lane 3, untreated myofibrils; lane 4, wild-type TnT-treated myofibrils; lane 5, reconstituted myofibrils after treatment with wild-type TnT; lane 6, I79N TnT-treated myofibrils; lane 7, reconstituted myofibrils after treatment with I79N TnT. The data are representative of four other experiments. B, the alkaline urea gel electrophoresis pattern of TnT-treated myofibrils. The recombinant TnT-treated myofibrils were separated on alkaline gel (pH 8.5) containing 6 M urea and 13.5% acrylamide. Lane 1, recombinant human wild-type TnT; lane 2, native rabbit TnT; lane 3, untreated myofibrils; lane 4, wild-type TnT-treated myofibrils; lane 5, I79N TnT-treated myofibrils. All data are representative of four other experiments. C, the densitometry tracings for the alkaline urea gel electrophoresis pattern of the myofibrils. The gels stained with Coomassie Brilliant Blue R-250 were analyzed by an optical densitometric scan using Phoretix gel analysis software. (i) untreated myofibrils; (ii) wild-type TnT-treated myofibrils; (iii) I79N TnT-treated myofibrils. The tracing is typical of four other experiments.
myofibrils were measured in the absence of Ca$^{2+}$ (pCa 7.05). The results are expressed as a percentage of the ATPase activity of TnT-treated myofibrils without adding TnI and are the mean ± S.E. of three experiments performed in triplicate.

The effect of TnC on the restoration of the Ca$^{2+}$ sensitivity of TnT-treated and TnI-reconstituted myofibrils. The myofibrils treated with wild-type or I79N TnT were reconstituted with 15.2 µg/ml TnI and various amounts of TnC, and then ATPase activity of the reconstituted myofibrils were measured in the presence of Ca$^{2+}$ (pCa 5.18). The results are expressed as a percentage of the maximum ATPase activity of the reconstituted myofibrils and are the mean ± S.E. of three experiments performed in triplicate.

**TABLE I**

| pH  | Wild-type TnT | I79N TnT |
|-----|---------------|----------|
| 6.5 | 5.15 ± 0.080 (n = 3) | 5.55 ± 0.083 (n = 4*) |
| 7.0 | 6.01 ± 0.035 (n = 3) | 6.16 ± 0.080 (n = 4*) |
| 7.5 | 6.28 ± 0.036 (n = 3) | 6.24 ± 0.009 (n = 3) |

* p < 0.05 compared with wild-type TnT-treated myofibrils.

The native TnI was removed by wild-type TnT treatment and I79N TnT treatment, respectively (Fig. 3A). Although native rabbit cardiac TnT and recombinant human cardiac TnT had exactly the same electrophoretic mobility on 12% SDS-PAGE gel (Fig. 3A, lanes 1 and 2), the recombinant human cardiac TnT had a slightly faster mobility than rabbit cardiac TnT on alkaline urea gel (Fig. 3B, lanes 1 and 2). A densitometry scan on alkaline urea gel was performed to estimate the amount of TnT exchanged into myofibrils. On the typical scanning patterns of TnT-treated and untreated myofibrils (Fig. 3C), the density of both native rabbit TnT and TnC bands decreased by treatment with wild-type TnT or I79N TnT of which mobility were the same as actin. Although a quantitative analysis of TnTs could not be carried out because of the high background density, the increase in the density of the actin peak after TnT treatment indicated the incorporation of human recombinant TnTs into the myofibrils. The densitometry of the TnC bands showed the percentage of native rabbit TnC removed from myofibrils to be 65.0 ± 4.0% (mean ± S.E., n = 5) by wild-type TnT treatment and 63.9 ± 2.1% (mean ± S.E., n = 5) by I79N TnT treatment. These gel scan analyses, taken together with the ATPase assays, provided strong evidence that the extent of the recombinant human cardiac TnT incorporated into the myofibrils was measured in the absence of Ca$^{2+}$ (pCa 7.05). The results are expressed as a percentage of the ATPase activity of TnT-treated myofibrils without adding TnI and are the mean ± S.E. of three experiments performed in triplicate.

**FIG. 4.** The effects of recombinant human cardiac TnI and TnC on the myofibrils treated with wild-type or I79N TnT. A, inhibitory effect of TnI on ATPase activity of TnT-treated myofibrils. The myofibrils (300 µg/ml) were treated with 37.5 µg/ml wild-type (○) or I79N mutant (●) TnT for 1 h at 25 °C with a shake and then were reconstituted with various amounts of TnI at 4 °C for 1 h. The ATPase activity of the treated myofibrils was measured in the absence of Ca$^{2+}$ (pCa 7.05). The results are expressed as a percentage of the ATPase activity of TnT-treated myofibrils without adding TnI and are the mean ± S.E. of three experiments performed in triplicate. B, the effect of TnC on the restoration of the Ca$^{2+}$ sensitivity of TnT-treated and TnI-reconstituted myofibrils. The myofibrils treated with wild-type or I79N TnT were reconstituted with 15.2 µg/ml TnI and various amounts of TnC, and then ATPase activity of the reconstituted myofibrils were measured in the presence of Ca$^{2+}$ (pCa 5.18). The results are expressed as a percentage of the maximum ATPase activity of the reconstituted myofibrils and are the mean ± S.E. of three experiments performed in triplicate.

**FIG. 5.** The Ca$^{2+}$-activated ATPase activity of myofibrils reconstituted with human cardiac TnI and TnC after treatment of recombinant TnT. Myofibrils were treated with wild-type (○) or I79N mutant (●) TnT and then reconstituted with TnI and TnC. ATPase activity of the reconstituted myofibrils were measured at pH 7.5(—), pH 7.0(---), and pH 6.5 (- - -). The maximum ATPase activities of wild-type TnT-treated myofibrils were 15.8 ± 1.2, 32.3 ± 3.6, and 37.7 ± 0.67 (nanomole of P/mg/min, mean ± S.E., n = 3) for pH 6.5, 7.0, and 7.5, respectively. The maximum ATPase activities of I79N TnT-treated myofibrils were 16.2 ± 0.4, 31.0 ± 0.8, and 36.3 ± 1.5 (nanomole of P/mg/min, mean ± S.E., n = 3) for pH 6.5, 7.0, and 7.5, respectively. The results are expressed as a percentage of the maximum ATPase activity stimulated by Ca$^{2+}$ and are the mean ± S.E. of three experiments performed in triplicate.

**Determination of the Exchange Rate of Tn Complex in Myofibrils Treated with Wild-type TnT and I79N TnT.—** To determine the extent of the exchange of Tn complex in the myofibrils, both SDS-PAGE and alkaline urea gel electrophoresis were carried out. The densitometry of TnI bands in the SDS-PAGE pattern of the myofibrils showed that 65.3 ± 3.2% (mean ± S.E., n = 5) and 63.8 ± 4.0% (mean ± S.E., n = 5) of
myofibrils were approximately 65% and that the wild-type and I79N mutant TnTs were also equally effective in replacing the endogenous Tn complex in myofibrils.

**Effect of Recombinant Human Cardiac TnI and TnC on the Myofibrils Treated with Wild-type and I79N TnTs**—As shown in Fig. 4, the addition of the recombinant human cardiac TnI resulted in a decrease in the Ca$^{2+}$-insensitive ATPase activity of the myofibrils treated with wild-type or I79N TnT in a dose-dependent manner and were completely inhibited at 15.2 μg/ml (Fig. 4A). The amounts of TnI required for a 50% inhibition (IC$_{50}$) were 0.80 ± 0.05 μg/ml and 1.74 ± 0.21 μg/ml (mean ± S.E., n = 3) for wild-type TnT-treated and I79N TnT-treated myofibrils, respectively. This indicated that the inhibitory effect of TnI on actin-tropomyosin was significantly smaller in the I79N mutant TnT-treated myofibrils than the wild-type TnT-treated myofibrils. In the presence of Ca$^{2+}$-recombinant human cardiac TnC activated the suppressed ATPase activity of the TnI-reconstituted myofibrils in a dose-dependent manner and fully neutralized the ATPase activity at the concentration of 11.6 μg/ml (Fig. 4B). No significant difference in the neutralizing effect of TnC was detected between the myofibrils containing wild-type TnT and I79N TnT.

**pCa-ATPase Activity Relationships of the Myofibrils Treated with Wild-type TnT and I79N TnT, and Reconstituted with Recombinant Human Cardiac TnI and TnC at Different pH Values (pH 6.5, 7.0, and 7.5)—**Although no significant difference was seen in the pCa-ATPase activity relationships at pH 7.5, the Ca$^{2+}$ sensitivity (pCa$_{50}$, pCa at half-maximum activation) of the myofibrils reconstituted with I79N mutant TnT was significantly higher than the Ca$^{2+}$ sensitivity of myofibrils reconstituted with wild-type TnT at pH 7.0 and 6.5 (Fig. 5). The difference in the Ca$^{2+}$ sensitivity of the reconstituted myofibrils was thus larger at pH 6.5 than at pH 7.0 (Table I). However, no significant difference was observed in the maximum ATPase activity and the Hill coefficient (n$_H$, an index of cooperativity) between the myofibrils reconstituted with wild-type TnT and reconstituted with I79N mutant TnT at any pH.

**An SDS-PAGE Analysis of TnT Mutants, R92Q, F110I, E244D, and R278C—**Four other missense mutations, R92Q, F110I, E244D, and R278C mutant TnTs, were generated by the overlap extension method. An SDS-PAGE analysis showed all of these mutants to have the same electrophoretic mobility on 12% SDS-PAGE gel (Fig. 6A). The densitometric scan of the SDS-PAGE gel was performed to determine the amount of TnI removed from myofibrils by the treatment with TnTs. Data are the mean ± S.E. of five or four independent experiments. pCa values at half-maximum ATPase activity (pCa$_{50}$) and Hill coefficient (n$_H$) were calculated from the data shown in Fig. 7. The data are the mean ± S.E. of three or four experiments done in triplicate.

**TABLE II**

| TnT type | % of removed native TnI from myofibrils by treatment with TnTs | Myofibrillar ATPase activity |
|----------|---------------------------------------------------------------|----------------------------|
|          | pCa$_{50}$ | Maximum ATPase activity (μmol/mg/min) | After TnT treatment | After TnT treatment + TnC reconstitution | n$_H$ |
| Wild-type | 65.0 ± 4.0 (n = 5) | 6.01 ± 0.035 (n = 4) | 29.8 ± 2.9 (n = 4) | 32.3 ± 3.6 (n = 4) | 2.2 ± 0.6 (n = 4) |
| I79N     | 63.9 ± 2.1 (n = 5) | 6.16 ± 0.028 (n = 4) | 29.3 ± 3.0 (n = 4) | 31.0 ± 0.8 (n = 4) | 2.4 ± 0.2 (n = 4) |
| R92Q     | 61.2 ± 2.9 (n = 4) | 6.17 ± 0.003 (n = 3) | 30.3 ± 1.2 (n = 3) | 29.7 ± 0.2 (n = 3) | 2.1 ± 0.2 (n = 3) |
| F110I    | 60.8 ± 4.8 (n = 4) | 5.98 ± 0.063 (n = 3) | 27.4 ± 1.6 (n = 3) | 45.4 ± 1.8 (n = 3) | 1.6 ± 0.2 (n = 3) |
| E244D    | 70.5 ± 4.2 (n = 4) | 6.00 ± 0.036 (n = 3) | 27.8 ± 3.0 (n = 3) | 45.8 ± 4.2 (n = 3) | 1.6 ± 0.1 (n = 3) |
| R278C    | 72.1 ± 4.2 (n = 4) | 6.24 ± 0.064 (n = 4) | 31.6 ± 2.1 (n = 4) | 31.9 ± 2.6 (n = 4) | 1.2 ± 0.2 (n = 4) |

*p < 0.05 compared with wild-type TnT-treated myofibrils (t test).
the maximum ATPase activity. On the other hand, an increase in the maximum ATPase activity was observed with F110I and E244D TnT mutants, without any change in the Ca$^{2+}$ sensitivity of the ATPase activity (Fig. 8). No significant change in the cooperativity was observed between the wild-type TnT-treated myofibrils and mutant TnT-treated myofibrils (Table II). The summary in Table II clearly indicates these HCM-related TnT mutants to have the same ability as wild-type TnT to exchange native TnT in the myofibrils and are thus classified into at least two groups according to their effects on the Ca$^{2+}$-sensitive ATPase activity.

**DISCUSSION**

This is the first report to evaluate the properties of recombinant human cardiac TnT/C complex in myofibrils under physiological conditions. The results of the present study indicated the recombinant human cardiac Tn subunits and native rabbit cardiac Tn subunits to be almost identical. These findings led us to analyze the effects of HCM-linked TnT mutants on the Ca$^{2+}$-sensitive ATPase activity of rabbit cardiac myofibrils. We already reported that I79N and R92Q TnT mutants in HCM have a Ca$^{2+}$-sensitizing effect on the tension development of skinned fibers prepared from the rabbit ventricular muscle (18). In the present study, the Ca$^{2+}$-sensitizing effects of I79N and R92Q mutant TnTs on the myofibrillar ATPase activity were thus confirmed.

Regarding the molecular mechanism of action of these TnT mutants, the inhibitory effect of TnI on the Ca$^{2+}$-insensitive ATPase activity of myofibrils with I79N mutant TnT was found to be smaller than that with wild-type TnT, while the neutralizing effect of wild-type TnC was not affected by this mutation (Fig. 3, A and B). These findings suggest that the I79N mutant TnT reduced the affinity of TnI to actin-tropomyosin. The same mechanisms on the action of R92Q TnT mutant was also suggested in our previous study using skinned fibers (18). In this report, we showed that a mutation at Arg278 in TnT, in addition to the mutations at Ile 79 and Arg 92 in the TnT mentioned above, thus caused an increased Ca$^{2+}$ sensitivity of the ATPase activity without any change in the maximum level. It was also recently reported that the HCM-linked $\alpha$-tropomyosin mutant, D175N, is associated with an increase in the Ca$^{2+}$ sensitivity (26). It is therefore highly conceivable that an increase in the Ca$^{2+}$ sensitivity is one of the characteristic features of the HCM caused by the regulatory proteins associated with the thin filament.

The results of both the present and previous studies showing the Ca$^{2+}$-sensitizing effects of R92Q mutant on the ATPase activity...
activity of myofibrils and the tension development of skinned fibers apparently conflict with the results of a recent study by Marian et al. (27). They reported that the expression of R92Q mutant human cardiac TnT in cultured adult feline cardiac myocytes impaired the contractile performance as indicated by both fractional cell shortening and the peak velocity of shortening of the myocytes (27). Although the reason for this discrepancy in the results is not known, it might be due to the difference of the design of the experiments. No significant difference has been reported between the effects of I91N mutation in rat embryonic cardiac TnT, thus corresponding to I79N mutation in human, and wild-type TnT on Ca^{2+} sensitivity of the thin filaments examined by S-1 Mg-ATPase activity at pH 7.5 (28). While we found this mutant to behave in a manner similar to a wild-type at pH 7.5, the mutant had a Ca^{2+} sensitivity of the thin filaments examined by S-1 Mg-ATPase activity at pH 7.0 and 6.5, which was more significant at an acidic pH 6.5, which is also consistent with the previous observations on skinned fibers (18). This finding suggests that the I79N mutant may have a strong effect on the contractile performance during myocardial ischemia, although the effects of acidic pH on other TnT mutants have not yet been examined.

The most significant finding of the present study was that the maximum ATPase activity of the myofilaments was potentiated by the mutations at Phe^{110} and Glu^{244} in TnT (Fig. 8 and Table II). The potentiation effects of F110I and E244D TnT mutants were not observed after the TnT treatment but were observed only after subsequent reconstitution with TnC and TnI (Table II). This strongly suggests that the potentiation of the maximum ATPase activity is caused through a change in the interaction of TnT and TnI.

In the present study, we found that the effects of HCM-linked TnT mutations can be classified into at least two different types. The I79N, R92Q, and R278C TnT mutants enhanced Ca^{2+} sensitivity of myofilibrillar ATPase activity without affecting the maximum level of ATPase activity, whereas the F110I and E244D TnT mutants enhanced a maximum level of ATPase activity without affecting the Ca^{2+} sensitivity. These two groups of missense mutations in TnT might cause HCM in different manners. However, it is noteworthy that the mutations which cause an increase in the maximum ATPase activity alone have an apparent Ca^{2+} sensitizing effect at a submaximal concentration of Ca^{2+} due to a scale-up of the overall ATPase activity. Since the intact cardiac muscle has been reported to never be activated beyond the half-maximal level (29), an enhanced myofilament response to the submaximal Ca^{2+} may be a common phenomenon for the pathogenesis of HCM associated with these missense mutations in TnT. In several reports HCM-linked β-mysin heavy chain mutations and TnT mutations have been shown to impair cardiac performance and cause compensatory cardiac hypertrophy (30–32). However, our findings suggest that certain mutations might determine a hypercontractile state and thus appear to induce hypertrophy directly, instead of causing it indirectly via the mechanism of compensatory hypertrophy.

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