

Effects of *m*-nisoldipine on delayed afterdepolarization of canine Purkinje fibers

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ABSTRACT Effects of *m*-nisoldipine (*m*-Nis) on delayed afterdepolarization (DAD) and triggered activity (TA) were studied using standard microelectrode technique. The development of DAD and TA were markedly inhibited by pre-treatment with *m*-Nis ($1-4 \mu\text{mol} \cdot \text{L}^{-1}$) as well as *m*-Nis treatment after DAD and TA had been elicited. The amplitude of DAD was reduced from 15.3 ± 2.7 to 2.3 ± 2.0 mV, and the duration from 980 ± 45 to 130 ± 27 ms. The occurrence of TA was also reduced or prevented by *m*-Nis. These effects of *m*-Nis might be attributed to its blocking effects on voltage-dependent calcium channel and the resultant alleviation of intracellular calcium overload.

KEY WORDS *m*-nisoldipine; electrophysiology; Purkinje fibers; microelectrodes

Triggered activity (TA) induced by delayed afterdepolarization (DAD) has been implicated as a possible cellular mechanism for arrhythmias in some experimental models and in human heart diseases^(1,2). DAD is transient or oscillatory depolarization occurring after full repolarization. The ionic mechanism underlying DAD may be related to the increase in myoplasmic Ca^{2+} concentration⁽³⁾. In this paper, the effects of *m*-nisoldipine (*m*-Nis), a new dihydropyridine calcium channel blocker, on DAD and TA induced by ouabain and high Ca^{2+} were investigated using standard microelectrode and on-line microcomputer analyzing system.

MATERIALS AND METHODS

Tissue preparation Hearts were rapidly removed from dogs of either sex anesthetized with sodium pentobarbital ($30-35 \text{ mg} \cdot \text{kg}^{-1}$,

iv) and immersed in oxygenated Tyrode's solution. Several subendocardial Purkinje fibers (0.3-0.5 mm in diameter, 5-10 mm long) were excised. The isolated fibers were pinned to the silicon rubber on the floor of the tissue chamber and perfused with Tyrode's solution at a flow rate of $5 \text{ ml} \cdot \text{min}^{-1}$. The perfusate was equilibrated with O_2 . The temperature of perfusate was maintained at $35 \pm 1^\circ\text{C}$ and was monitored with a thermistor. The preparation was driven by the rectangular pulse (1 Hz) from the stimulator (SEN-3201) controlled by a microcomputer (APPLE-II), through a bipolar electrode. Transmembrane potentials were led to the microelectrode amplifier (MEZ-8201) by a standard intracellular glass microelectrode. The amplified signal was fed to the microcomputer and monitored with a storage oscilloscope (VC-11). Microcomputer collected the transmembrane potential signals at a rate of 1000 byte / s and analyzed the parameters of DAD and action potentials.

Induction of DAD Ouabain and high Ca^{2+} were used in the experiment to induce DAD and TA. The concentration of ouabain was carefully chosen to induce a stable and reproducible DAD and TA in the pilot experiment. After equilibration in the oxygenated Tyrode's solution for 1 h, the preparation was superfused with Tyrode's solution containing ouabain $0.8 \mu\text{mol} \cdot \text{L}^{-1}$ and CaCl_2 $5.4 \text{ mmol} \cdot \text{L}^{-1}$. The DAD was elicited and its amplitude gradually increased within 30-40 min while the external stimulation to the preparation continued. Once the amplitude of DAD reached the threshold, the TA would be evoked after the full repolarization from the previous activation (Fig 1). In most

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preparations, DAD remained stable for at least 1 h.

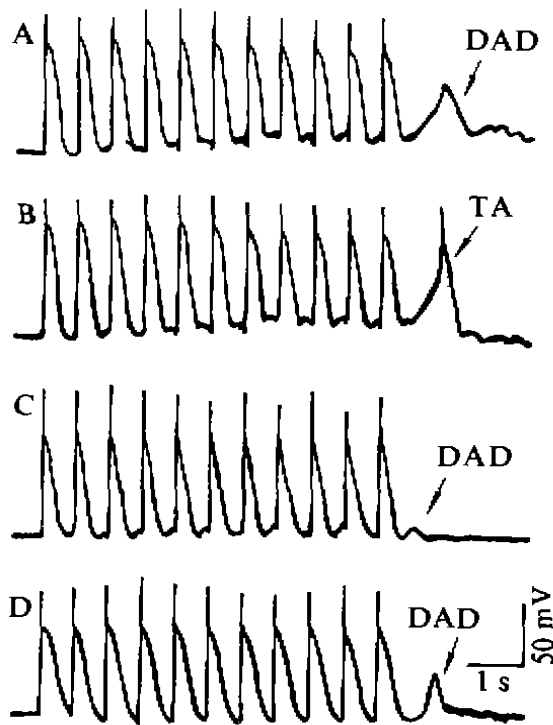


Fig 1. Effects of *m*-Nis $2 \mu\text{mol} \cdot \text{L}^{-1}$ on DAD and triggered activity (TA) induced by ouabain, high Ca^{2+} and overdriving (BCL=600 ms). A) control, DAD evoked; B) control, DAD, and TA were evoked; C) *m*-Nis treatment for 30 min; amplitude and duration of DAD were reduced and TA disappeared. D) washout for 30 min.

Experimental protocol In the first part of the experiment, the effects of ouabain and high Ca^{2+} on the transmembrane potentials were examined. The characteristics of DAD induced by the ouabain and high Ca^{2+} were also studied.

The second part was undertaken to assess the prophylactic effects of *m*-Nis on the development of DAD and TA. After equilibrium in the oxygenated Tyrode's solution for 1 h, the Purkinje fiber exposed to ouabain and high Ca^{2+} was treated with *m*-Nis or its solvent. The preparations in this part of the ex-

periment were divided into 3 groups at random: 1) control group: perfused with Tyrode's solution containing ouabain $0.8 \mu\text{mol} \cdot \text{L}^{-1}$ and Ca^{2+} $5.4 \text{mmol} \cdot \text{L}^{-1}$; 2) solvent control group: perfused with Tyrode's solution containing ouabain $0.8 \mu\text{mol} \cdot \text{L}^{-1}$, Ca^{2+} $5.4 \text{mmol} \cdot \text{L}^{-1}$ and solvent; 3) *m*-Nis treated groups: perfused with Tyrode's solution containing ouabain $0.8 \mu\text{mol} \cdot \text{L}^{-1}$, Ca^{2+} $5.4 \text{mmol} \cdot \text{L}^{-1}$, and *m*-Nis 0.5 – $4 \mu\text{mol} \cdot \text{L}^{-1}$. This group was to observe whether *m*-Nis might prevent the occurrence of DAD and TA while the preparation was exposed to ouabain and high Ca^{2+} .

The last part was designed to evaluate the inhibitory effects of *m*-Nis on DAD and TA, induced by ouabain and high Ca^{2+} . Purkinje fibers were first exposed to ouabain and high Ca^{2+} . After stable DAD and TA had been induced, *m*-Nis was then applied to the perfusate.

Parameters of DAD were measured from the first DAD after the cessation of a train of driven activity at a specific basic cycle length (BCL). Using a program designed by our department, the following parameters of DAD were defined automatically by a on-line microcomputer analyzing system: 1) amplitude of DAD, 2) duration of DAD, 3) coupling interval (CI): the time from the upstroke of the last action potential to the peak of DAD, 4) reduction in maximal diastolic potential (ΔMDP) during overdriving. Parameters of transmembrane action potential were also analyzed by the microcomputer: amplitude of action potential (APA), maximal diastolic potential (MDP), maximal rate of depolarization (V_{max}), duration of 50% and 90% repolarization (APD_{50} , APD_{90}) and plateau phase duration (PPD).

Solutions and drugs Modified Tyrode's solution was prepared just before the experiment by mixing the stock solutions (NaCl 147, CaCl_2 1.8, MgCl_2 1.05, KCl 5.4, Tris

10 mmol · L⁻¹, glucose 1.5 g · L⁻¹). The composition of solvent and resources of *m*-Nis were described elsewhere⁽⁴⁾. *m*-Nis solution was prepared before each experiment from stock solution and kept away from intense light.

RESULTS

Effects of ouabain and high Ca²⁺ on the transmembrane potentials of canine Purkinje fiber Exposure to ouabain and high Ca²⁺ for 5, 10, and 15 min caused a prolongation in APD₅₀, APD₉₀, and PPD; MDP, APA, and V_{max} showed no significant change in the first 15 min, and greatly reduced at 20, 30, and 40 min (Tab 1). The durations of action potential measured at 30 and 40 min were shortened, in contrary to the prolongation in the first 15 min of exposure to ouabain and high Ca²⁺. Following reperfusion with oxygenated Tyrode's solution for 60 min, a partial recovery of the transmembrane potential was seen.

Stability and characteristics of DAD model In the pilot experiment, ouabain 0.4, 0.8, and 1.5 μmol · L⁻¹ were used to induce DAD. Ouabain was unable to induce DAD at 0.4 μmol · L⁻¹ within 2 h, whereas DAD induced by ouabain (1.5 μmol · L⁻¹) was likely to develop into sustained TA associated with an irreversible reduction in MDP and loss

of excitability. An intermediate concentration of ouabain (0.8 μmol · L⁻¹) was chosen to develop stable DAD and TA. The amplitude of DAD reached its peak magnitude within 30–40 min, and lasted at least 1 h. Elevation of Ca²⁺ concentration increased the amplitude of DAD, making the model more suitable to assess the effect of the drugs.

The steady amplitude of DAD was 10.3 ± 1.2 mV. The magnitude of DAD and the occurrence of TA were directly related to the frequency and the number of the driven activity. The amplitude of DAD was correlated negatively with the driving BCL, and positively with the number of driven activity (Fig 2). The amplitude of DAD was increased from 6.2 to 11.7 mV as the BCL decreased from 1000 to 300 ms (the number of driven

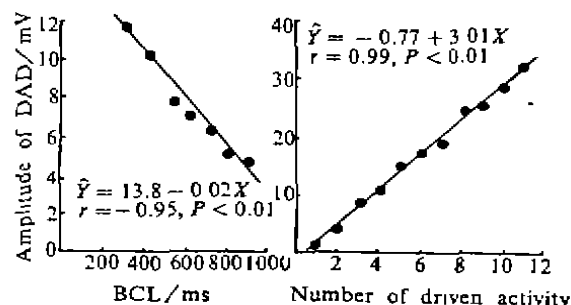


Fig 2. Effects of BCL and number of driven activity and on the amplitude of DAD induced by ouabain (0.8 μmol · L⁻¹) and high Ca²⁺ (5.4 mmol · L⁻¹), n = 5.

Tab 1. Effects of ouabain (0.8 μmol · L⁻¹) and high Ca²⁺ (5.4 mmol · L⁻¹) on transmembrane potentials of canine Purkinje fibers. n = 6, $\bar{x} \pm s$, *P > 0.05, **P < 0.05, ***P < 0.01 vs control.

| | MDP / mV | APA / mV | V _{max} / V · s ⁻¹ | APD ₅₀ / ms | PPD / ms | APD ₉₀ / ms |
|-------------------------------|----------------|------------|--|------------------------|------------|------------------------|
| Control | -78.2 ± 2.4 | 119 ± 3. | 472 ± 18 | 281 ± 9 | 273 ± 5 | 399 ± 7 |
| Ouabain+high Ca ²⁺ | | | | | | |
| 5 min | -80.4 ± 3.2* | 124 ± 2* | 467 ± 8* | 309 ± 8* | 301 ± 4*** | 449 ± 4** |
| 10 min | -78.5 ± 2.1* | 123 ± 3* | 475 ± 4* | 331 ± 3*** | 319 ± 5*** | 465 ± 7*** |
| 15 min | -76.9 ± 2.5* | 121 ± 2* | 467 ± 8* | 315 ± 6** | 311 ± 2*** | 445 ± 2** |
| 20 min | -73.0 ± 3.2* | 117 ± 3* | 390 ± 12*** | 319 ± 2* | 309 ± 4*** | 439 ± 4** |
| 30 min | -66.9 ± 4.8*** | 110 ± 3*** | 362 ± 6** | 241 ± 8** | 250 ± 9*** | 346 ± 8*** |
| 40 min | -62.3 ± 7.4*** | 102 ± 6** | 298 ± 6** | 155 ± 4** | 201 ± 9** | 233 ± 9** |
| Washout | -70.3 ± 10.4** | 108 ± 8*** | 367 ± 18*** | 243 ± 10** | 287 ± 12* | 285 ± 17** |

Tab 2. Effects of pre-treatment with *m*-Nis and *m*-Nis after DAD had been elicited on DAD and triggered activity induced by ouabain and Ca^{2+} , $5.4 \text{ mmol} \cdot \text{L}^{-1}$ in canine Purkinje fibers. $\bar{x} \pm s$. * $P > 0.05$, ** $P < 0.05$, * $P < 0.01$ vs control.**

| Group / $\mu\text{mol} \cdot \text{L}^{-1}$ | <i>n</i> | DAD | | Couple interval / s | Reduction in MDP / mV | Prevalence of triggered activity / % |
|--|----------|----------------------|--------------------|---------------------------|-----------------------------|--|
| | | Amplitude / mV | Duration / ms | | | |
| Control | 24 | 15.3 ± 2.7 | 980 ± 45 | 1.1 ± 0.11 | 8.1 ± 1.8 | 19 (79%) |
| <i>m</i> -Nis treatment before ouabain | | | | | | |
| 0.5 | 6 | $16.4 \pm 2.4^*$ | $987 \pm 44^*$ | $1.1 \pm 0.12^*$ | $8.0 \pm 1.6^*$ | 5 (83%)* |
| 1.0 | 6 | $10.1 \pm 2.1^{***}$ | $906 \pm 61^{**}$ | $1.0 \pm 0.11^{**}$ | $1.9 \pm 1.4^{***}$ | 0*** |
| 2.0 | 6 | $3.1 \pm 2.6^{***}$ | $341 \pm 52^{***}$ | $1.1 \pm 0.14^*$ | $1.0 \pm 1.2^{***}$ | 0*** |
| 4.0 | 6 | $2.3 \pm 2.0^{***}$ | $130 \pm 27^{***}$ | $1.0 \pm 0.13^*$ | $1.0 \pm 1.8^{***}$ | 0*** |
| <i>m</i> -Nis treatment after ouabain | | | | | | |
| 1.0 | 8 | $4.0 \pm 1.4^{**}$ | $468 \pm 57^{**}$ | $1.1 \pm 0.12^*$ | $7.0 \pm 2.1^*$ | 1 (12.5%)*** |
| 2.0 | 8 | $2.6 \pm 1.8^{***}$ | $338 \pm 62^{***}$ | $1.1 \pm 0.12^*$ | $6.3 \pm 2.1^{**}$ | 0*** |
| 4.0 | 8 | $2.7 \pm 2.7^{***}$ | $322 \pm 77^{***}$ | $1.0 \pm 0.11^*$ | $1.4 \pm 1.8^{***}$ | 0*** |

activity was 10). For DAD occurring at a specific pace rate (BCL = 600 ms), the amplitude of DAD was increased from 1.5 to 32.9 mV as the number of driven activity was increased from 1 to 11.

Prophylactic effects of *m*-Nis on DAD and TA Exposure to ouabain and high Ca^{2+} induced DADs in all 24 preparations, out of which 19 (79%) were accompanied by TA (Tab 2). The amplitude of DAD was 15.3 ± 2.7 mV with a duration of 980 ± 45 ms. Gradual reduction of MDP (Δ MDP) was also noted during the course of overdriving (Fig 1A, B). The solvent had no effects on DAD and TA. In the *m*-Nis treated group, the amplitude of DAD was greatly decreased, and the TA failed to occur. The amplitude and duration of DAD were significantly decreased in a concentration-dependent manner as compared with those of the control group. The decrease in MDP was negligible under the action of *m*-Nis. Overdriving failed to evoke TA in *m*-Nis concentrations from 1–4 $\mu\text{mol} \cdot \text{L}^{-1}$, mainly as a result of decrease in the amplitude of DAD.

Inhibitory effects of *m*-Nis on induced DAD and TA DAD induced by 30 min ex-

posure to ouabain and high Ca^{2+} remained stable for at least 1 h in the control group. After recording several control DAD, *m*-Nis was added to the perfusate to reach a concentration of 1, 2 or 4 $\mu\text{mol} \cdot \text{L}^{-1}$. The inhibitory effects of *m*-Nis on DAD reached its peak level in about 30 min. The amplitude and duration of DAD as well as Δ MDP were decreased to 17.6%, 32.8%, and 17.3% of those before medication, respectively. Owing to the decrease in amplitude of DAD, the TA failed to appear. The results were summarized in Tab 2.

DISCUSSION

DAD is oscillatory potential related to abnormal rise in intracellular Ca^{2+} concentration during various interventions. In this study, ouabain combined with high Ca^{2+} and overdriving was used successfully to produce a stable and reproducible DAD model. Cardiac glycosides are known to inhibit $\text{Na}^+ - \text{K}^+$ pump, causing an increase in intracellular Na^+ level. This could result in a rise of intracellular Ca^{2+} concentration via $\text{Na}^+ - \text{Ca}^{2+}$ exchange mechanism. Elevation of Ca^{2+} in the perfusate and overdriving might potentiate

the effect of ouabain.

Our results showed that the amplitude and duration of DAD as well as the occurrence of TA were evidently dependent on the BCL and the number of driven activity. Increasing the number of driven activity or shortening the BCL might enhance the action of ouabain and high Ca^{2+} , resulting in an increased amplitude and duration of DAD, reduced MDP, and enhanced susceptibility of TA. Either increasing the number of driving or shortening the BCL was likely to favor the accumulation of intracellular Ca^{2+} , since the sequestration of Ca^{2+} by the sarcoplasmic reticulum had lagged behind the Ca^{2+} influx during repeated activations. The results were consistent with the hypothesis that intracellular calcium overload plays an important role in the development of DAD and TA⁽⁵⁾.

m-Nis ($1-4 \mu\text{mol} \cdot \text{L}^{-1}$) markedly prevented or reduced the development of DAD and TA induced by ouabain, high Ca^{2+} , and overdriving. The amplitude and duration of DAD, as well as ΔMDP were all greatly reduced by pretreatment with *m*-Nis as well as *m*-Nis treatment after DAD and TA had been elicited. TA was completely prevented by *m*-Nis ($1-4 \mu\text{mol} \cdot \text{L}^{-1}$).

Intracellular calcium overload had been postulated as a responsible mechanism in several DAD models⁽⁵⁾. Calcium overload induced by several interventions would lead to a damped oscillatory release of calcium from intracellular stores (mainly the sarcoplasmic reticulum) and an underlying transient inward depolarising current (I_{u}). The initiation of I_{u} (nonselective cation channel activated by Ca^{2+} ⁽⁶⁾ and electrogenic $\text{Na}^+-\text{Ca}^{2+}$ exchange mechanism⁽⁷⁾) is dependent on a process sensitive to intracellular Ca^{2+} concentration, though the charge-carrier mechanism of I_{u} has remained controversial⁽⁸⁾. Factors that favor the intracellular calcium accumula-

tion, such as adrenoceptor stimulation, high Ca^{2+} perfusion, digitalis intoxication and overdriving might elicit or enhance DAD and TA. On the contrary, high K^+ , acetylcholine, Mn^{2+} as well as calcium channel blockers, which decrease the intracellular calcium concentration, could depress or prevent DAD and TA.

Calcium channel blockers (nifedipine, nisoldipine, verapamil) have been reported to reduce the Ca^{2+} influx by blocking the voltage-dependent calcium channel⁽⁹⁾. Accordingly, it is reasonable to propose that *m*-Nis could possess the same effect, thereby resulting in a reduction of calcium influx during activation and an alleviation of intracellular calcium overload. Although the marked prophylactic and inhibitory actions of *m*-Nis on DAD and TA were demonstrated in our experiment, more persuasive evidence for the mechanisms underlying the action of *m*-Nis will require further studies using voltage clamp technique and intracellular Ca^{2+} concentration measurement.

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REFERENCES

- 1 Cranefield PF, Aronson RS. Initiation of sustained rhythmic activity by single propagated action potentials in canine cardiac Purkinje fibers exposed to sodium-free solution or to ouabain. *Circ Res* 1974; 34 : 477-81.
- 2 Gough WB, Zeiler RH, El-Sherif N. Effects of diltiazem on triggered activity in canine 1 day old infarction. *Cardiovasc Res* 1984; 18 : 339-43.
- 3 January CT, Fozzard HA. Delayed afterdepolarizations in heart muscle: Mechanisms and relevance. *Pharmacol Rev* 1988; 40 : 219-27.
- 4 Fu SX, Li YS, Jin CJ, Ren LM. Effects of *m*-nisoldipine and nisoldipine on hemodynamics on anesthetized dogs. *Acta Pharmacol Sin* 1988; 9 : 43-8.
- 5 Levy MN. Role of calcium in arrhythmogenesis.

Circulation 1989; 80 : 23-30.

- 6 Kass RS, Tsien RW. Fluctuation in membrane current driven by intracellular calcium in cardiac Purkinje fibers. *Biophys J* 1982; 38 : 259-69.
- 7 Kass RS, Tsien RW, Weingart R. Ionic basis of transient inward current induced by strophantidin in cardiac Purkinje fibers. *J Physiol (Lond)* 1978; 281 : 209-26
- 8 Cranefield PF. Action potentials, afterpotentials, and arrhythmias. *Circ Res* 1977; 41 : 415-23.
- 9 Struyker-Boudier HAJ, Smits JFM, De Mey JGR. The pharmacology of calcium antagonists: a review. *J Cardiovasc Pharmacol* 1990; 15 Suppl 4 : 1-10.

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间尼索地平对犬浦肯野氏纤维迟发性后除极化的影响

R 963
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提要 利用细胞内微电极技术, 观察了间尼索地平 (*m-Nis*) 预防给药或诱发 DAD 后再给予 *m-Nis* ($0.5-4 \mu\text{mol} \cdot \text{L}^{-1}$), 对 DAD 和 TA 均有明显的抑制作用, DAD 的幅度由 15.3 ± 2.7 减小到 2.3 ± 2.0 mV, DAD 时程由 980 ± 45 减小到 130 ± 27 ms, TA 的发生也明显减少或完全被抑制, *m-Nis* 这些效应可能与其阻断钙通道、减轻细胞内钙超载有关。

关键词 间尼索地平; 电生理; 浦肯野纤维; 微电极

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Antithrombotic activity of verapamil

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ABSTRACT The antithrombotic activity of verapamil (Ver) was assessed in an arterial thrombosis model and an *ex vivo* thrombosis model. Ver 2 and 4 $\text{mg} \cdot \text{kg}^{-1}$ ip markedly prolonged the time of thrombotic occlusion in rat carotid artery induced by electric stimulation. The weight of thrombus formed *ex vivo* was reduced by Ver 0.2 $\text{mg} \cdot \text{kg}^{-1}$ iv. However, Ver showed no effects on blood viscosity.

KEY WORDS verapamil; thrombosis; blood viscosity

The incidence of reocclusion following successful reperfusion in acute myocardial infarction (AMI) by thrombolytic therapy was reported of 15-30% or more⁽¹⁾. A subsequent adjunctive treatment to intervene thrombosis and to prevent the reocclusion remains a requisite⁽²⁾. Thromboxane A₂ (TXA₂) and/or

epoprostenol (Epo) play an important role in thrombosis, and calcium channel blockers show antiplatelet effects⁽³⁾ and beneficial actions on TXA₂ and/or Epo formation⁽⁴⁾. But their action on reocclusion after AMI is not yet identified, nor have the data of the agents on thrombosis been found. Here we report the effects of verapamil (Ver), a calcium channel blocker on thrombosis and the influence of Ver on blood viscosity.

MATERIALS AND METHODS

Arterial thrombosis Lewis rats (♀ and ♂, wt $271 \pm s$ 24 g) were anesthetized by sodium pentobarbital 1 h after saline or Ver ip. The right carotid artery was isolated up to 15 mm long. Thrombosis was induced by electric stimulation of 1.6 mA, which lasted 7 min⁽⁵⁾. The thrombosis was indicated by occlusion

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