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κ-Opioid receptor agonist protects against ischemic reduction of 2-deoxyglucose uptake in morphine-tolerant rats

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Abstract

We examined the effects of μ-opioid receptor agonist and antagonists, and κ-opioid receptor agonist on the hypoxia/hypoglycemia-induced reduction in 2-deoxyglucose uptake of rat hippocampal slices. Naloxone, a μ-opioid receptor antagonist and (5,7,8)-(+)-3,4-dichloro-*N*-methyl-*N*-(7,8,1-pyrrolidinyl)-1-oxaspirol{4,5}dec-8-yl)-benzeneacetamide methanesulfonate, U-62,066E, a κ-opioid receptor agonist, showed neuroprotective actions against the hypoxia/hypoglycemia-induced deficit in glucose uptake. In contrast, morphine exhibited an exacerbating action. These results suggest that blockade of μ-opioid receptor- and stimulation of κ-opioid receptor-mediated functions has a protective role against the hypoxia/hypoglycemia-induced decreases in glucose metabolism in hippocampal slices. Chronic administration of morphine (10 mg/kg) for 9 days affected neither the basal nor the hypoxia/hypoglycemia-induced reduction in 2-deoxyglucose uptake. Rats treated with morphine chronically exhibited not only tolerance to the analgesic effect but also tolerance to the exacerbating action. However, chronic morphine did not modify U-62,066E-induced neuroprotection. These findings indicate that the receptor mechanisms of neuroprotection produced by the activation of κ-opioid receptors may not be involved in μ-opioid receptor function.

Keywords: Morphine; Ischemia; κ-Opioid receptor; 2-Deoxyglucose; Hippocampal slice; Tolerance

1. Introduction

Agonists of the μ-opioid receptor subtype such as morphine exacerbate ischemic neurological deficits, whereas antagonists of these receptors such as naloxone attenuate ischemic brain damage in animal models of ischemia (Hosobuchi et al., 1982; Baskin et al., 1984) and human patients (Baskin and Hosobuchi, 1981).

Dynorphine A-(1–13), an endogenous κ-opioid receptor agonist, and selective non-peptide κ-opioid receptor agonists such as U-50,488H, U-62,066E, GR89696 and CI-977 can produce neuroprotective effects in certain animal models of cerebral ischemia. For example, U-62,066E, U-50,488H and their analogues reduce mortality and hippocampal CA1 neuronal necrosis following transient bilateral carotid occlusion in gerbils and in rats (Tang, 1985; Silvia et al., 1987; Hall and Pasara, 1988; Contreras et al., 1991). Similarly, GR89696 and CI-977 have recently been shown to reduce cerebrocortical infarct volume in a permanent, unilateral middle cerebral artery occlusion model in mice, rats and cats (Mackay et al., 1993; Birch et al., 1991; Kusumoto et al., 1992). In addition, there have been several reports of the attenuating effects of κ-opioid receptor agonists on ischemia-induced memory dysfunction in several learning performance paradigms (Ohno et al., 1991; Itoh et al., 1993a,b).

Because glucose is a major energy substrate in nervous tissue, 2-deoxyglucose uptake is an index of regional energy consumption (Kadekaro et al., 1987). Recently, we demonstrated that 2-deoxyglucose uptake under hypoxic/hypoglycemic (ischemic) conditions was reduced in hippocampal slices (Shibata et al., 1992; Shibata and Watanabe, 1993). This decrease in 2-deoxyglucose uptake was prevented by pretreatment with neuroprotective drugs (Shibata et al., 1992). Therefore, we examined whether morphine, naloxone, and U-62,066E affect the hypoxia/hypoglycemia-induced reduction of 2-deoxyglucose uptake in hippocampal slices.

Chronic administration of morphine has been reported to be associated with down-regulation of μ-

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opioid receptors in certain areas of the brain and spinal cord but not with down-regulation of κ- and δ-opioid receptors (Bhargava et al., 1989, Bhargava et al., 1991a,b). Although tolerance occurs to the analgesia induced by both κ- and μ-opioid receptor agonists, there is no cross-tolerance between the two (Von Voigtlander et al., 1983; Dykstra et al., 1987; Dykstra and Massie, 1988; Nakazawa et al., 1990). These lines of evidence have suggested that the neuroprotective effect of κ-opiate receptor agonists may be unaffected by chronic morphine treatment. To assess this possibility, we examined the effects of morphine, naloxone, and U-62,066E on the hypoxia/hypoglycemia-induced reduction of 2-deoxyglucose uptake in hippocampal slices in morphine-tolerant rats.

2. Materials and methods

2.1. Animals

Young (7-week-old) male Wistar rats were purchased from Kyudo Animal (Fukuoka, Japan) and maintained in temperature-controlled animal quarters, with food and water ad libitum. The animals were housed under a 12 h light/dark cycle.

2.2. Analgesic assay

Analgesic activity was determined by the hot-plate test. Briefly, the rats were placed on a metal surface maintained thermostatically at 55 ± 0.5° C (Muromachi Kikai, MK-350, Japan). The hot-plate latency was taken as the time between the placing of the rat on the surface and the rat licking its hind-paw or attempting to escape by jumping. A cut-off time of 60 s was used in the absence of any response; the rats that showed no responses within 60 s were removed from the hot-plate and assigned a response latency of 60 s. The analgesic assay was held just prior to and 30 min after drug injection on day 1 and day 9. Analgesic tolerance was determined in rats treated with 10 mg/kg of morphine once each day for 9 consecutive days. On day 10, these rats were used for in vitro experiments.

2.3. Slice preparations

The rats were stunned and decapitated under light ether anesthesia, and their brains were rapidly removed to ice-cold Krebs-Ringer solution. Hippocampi were then sliced parasagitally using a tissue chopper (set at a thickness of 0.45 mm). The hippocampal slices (2.8 ± 0.3 mg wet weight, 1.9 ± 0.21 μg protein) contained the dorsal part of the hippocampal formation including the dentate gyrus and subiculum.

The composition of the control Krebs-Ringer solution, equilibrated with 95% O_2-5% CO_2, was (in mM): NaCl 129, $MgSO_4$ 1.3, $NaHCO_3$ 22.4, KH_2PO_4 1.2, KCl 4.2, glucose 10.0, $CaCl_2$ 1.5. The buffer was maintained at a pH of 7.3–7.4.

2.4. 2-Deoxyglucose uptake

Preparations were preincubated in normal Krebs-Ringer solution for 1 h in a recirculation chamber (100 ml volume). Slices were exposed to normal Krebs-Ringer solution containing the drug for 20 min, and were then placed in hypoxic/hypoglycemic solution containing the drug for a further 20 min. After this procedure the slices were removed and placed in normal Krebs-Ringer solution for 6 h. For induction of hypoglycemia the glucose in the incubation medium was replaced by 10 mM sucrose and for induction of hypoxia the solution was pre-equilibrated with 95% N_2-5% CO_2 gas mixture for at least 1 h.

After a 6-h washout, the slices were removed and then incubated in normal Krebs-Ringer solution containing 0.1 μCi/ml of 2-deoxy-D-[^{14}C]glucose (specific activity, 50 mCi/mmol; Amersham) for 45 min at 37 ± 0.2° C. The incubation chamber was arranged to recirculate 15 ml of buffer at 4.4 ml/min with continuous bubbling of humidified 95% O_2-5% CO_2 through the buffer as it entered the chamber. The procedure for monitoring 2-deoxyglucose uptake has been reported previously (Shibata et al., 1992; Shibata and Watanabe, 1993). Briefly, the slices were removed from the preincubation chamber, drained and then placed in the incubation chamber for 45 min. Incubations were terminated by removing the slices from the incubation chamber, rinsing them with 20 ml of warm preincubated buffer, and placing them in a chamber identical to the preincubation chamber for 30 min. At the end of the wash-out period, the slices were placed on dry ice to stop metabolism. The slices were then homogenized in 1 ml of phosphate buffer containing 0.5% perchloric acid, and 450 μl of the homogenate was used to determine total protein using a Bio-Rad protein assay kit (Bio-Rad, Richmond, USA). The radioactivity in another 450 μl of the homogenate was measured in a liquid scintillation counter after being solubilized with 6 N NaOH at 60° C and neutralized with 6 N HCl. Background dpm (30–50 dpm) was < 5% of total dpm. Background dpm was subtracted from total dpm.

2.5. Drugs and data analysis

Morphine hydrochloride was purchased from Takeda (Osaka, Japan), and naloxone were from Sigma (USA). U-62,066E: (5,7,8)-(+)-3,4-dichloro-*N*-methyl-*N*-(7,8,1-pyrrolidinyl)-1-oxaspirol{4,5}dec-8-yl)-benzeneacetamide methanesulfonate and MR-2266: [(−)2-(3-furylmethyl)-5,9-diethyl-2′-hydroxy-6,7-benzomorphan

were gifts from Upjohn and Boehringer Ingelheim, respectively. MR-2266 was dissolved in 0.1 N HCl and the pH was adjusted to approximately 6 with NaOH. All the other drugs were dissolved in distilled water. Data are expressed as means ± S.E. The significance of differences between groups was determined using analysis of variance (ANOVA) followed by Duncan's test or Student's *t*-test.

3. Results

3.1. Effects of opiate drugs against hypoxia/hypoglycemia-induced reduction in 2-deoxyglucose uptake

In previous experiments, we observed the time course of the recovery of 2-deoxyglucose uptake in hippocampal slices following 10-, 15- and 20-min hypoxia/hypoglycemia, and the decrease in 2-deoxyglucose uptake by slices was dependent upon the duration of ischemia (Shibata et al., 1992). 2-Deoxyglucose uptake in control hippocampal slices was 260 ± 3.6 dpm/μg protein per 45 min (n = 14) and that in slices exposed to ischemia for 20 min was 127 ± 3.1 dpm/μg protein per 45 min (n = 14) after a 6-h wash-out.

The hypoxia/hypoglycemia-induced decrease in 2-deoxyglucose uptake by hippocampal slices was significantly prevented by treatment with naloxone ($F(4,57) = 7.1$, $P < 0.01$) in a dose-dependent manner (Fig. 1). In contrast to naloxone, morphine significantly potentiated the decline of 2-deoxyglucose uptake in a concentration-dependent manner ($F(3,48) = 5.0$, $P < 0.01$) (Fig. 1). When morphine (1 μM) was perfused in the presence of naloxone (1 μM), both the exacerbating effect of morphine and the protective effect of naloxone against the reduced 2-deoxyglucose uptake by hypoxia/hypoglycemia disappeared (Fig. 1).

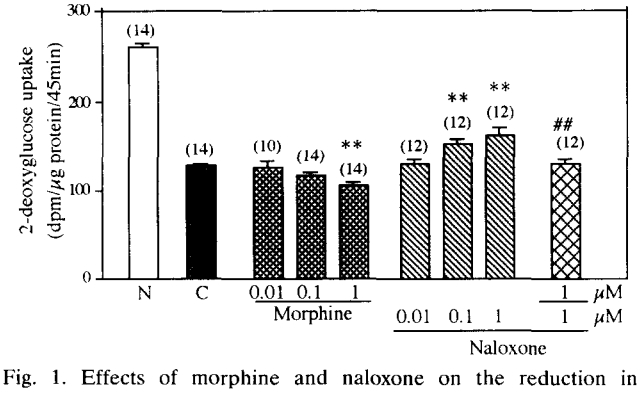

Fig. 1. Effects of morphine and naloxone on the reduction in 2-deoxyglucose uptake in hippocampal slices induced by 20 min of hypoxia/hypoglycemia. 2-Deoxyglucose uptake was measured after a 6-h wash-out following 20-min hypoxia/hypoglycemia. Values shown are means ± S.E.M., and numbers in parentheses are the number of slices. Drugs were perfused 20 min before and throughout the hypoxia/hypoglycemia period (20 min). $^{**}P < 0.01$ vs. vehicle-treated control slices (C) (Duncan's test) and $^{\#\#}P < 0.01$ vs. 100 nM morphine or naloxone administration alone (Duncan's test).

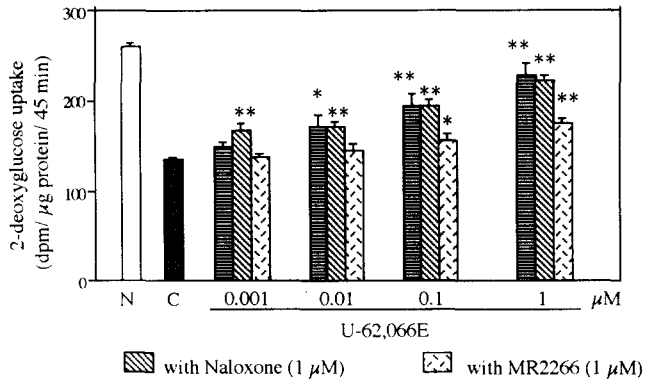

Fig. 2. Effects of MR2266 or naloxone on U-62,066E-induced protection against decreased 2-deoxyglucose uptake induced by 20 min of hypoxia/hypoglycemia. 2-Deoxyglucose uptake was measured after a 6-h wash-out following 20-min hypoxia/hypoglycemia. Values shown are means ± S.E.M. for 6 animals. Drugs were perfused 20 min before and throughout the hypoxia/hypoglycemia periods (20 min). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle-treated control slices (C) (Duncan's test) and $^{\#\#}P < 0.01$ vs. U-62,066E administration alone (Duncan's test).

The hypoxia/hypoglycemia-induced decrease in 2-deoxyglucose uptake of hippocampal slices was significantly prevented by treatment with U-62,066E ($F(4,55) = 15.1$, $P < 0.01$, one-way ANOVA) (Fig. 2). The neuroprotective effect of U-62,066E was significantly attenuated by MR2266 (1 μM), a κ-receptor antagonist ($F(4,110) = 3.4$, $P < 0.01$, two-way ANOVA), but not by naloxone 1 μM ($F(4,110) = 0.76$, $P > 0.05$, two-way ANOVA) (Fig. 2). The hypoxia/hypoglycemia-induced decrease in 2-deoxyglucose uptake was unaffected by MR2266 per se ($F(3,44) = 0.5$, $P > 0.05$) (data not shown). Treatment of hippocampal slices with morphine (1 μM), naloxone (1 μM) or U-62,066E (1 μM) for 40 min in normal non-ischemic solution did not affect 2-deoxyglucose uptake after a 6-h wash-out, and 2-deoxyglucose uptake was 266 ± 14 (n = 5) dpm/μg protein per 45 min for control, 266 ± 11 (n = 5) for morphine, 261 ± 16 (n = 5) for naloxone and 266 ± 12 (n = 5) for U-62,066E, respectively.

3.2. Effects of chronic morphine on analgesia

Morphine caused a dose-dependent analgesic effect in the hot-plate test. Tolerance to morphine analgesia developed when 10 mg/kg of drug was administered once daily for 9 consecutive days (Fig. 3). 24 h after the last injection of the drug, all animals were processed for the in vitro experiment.

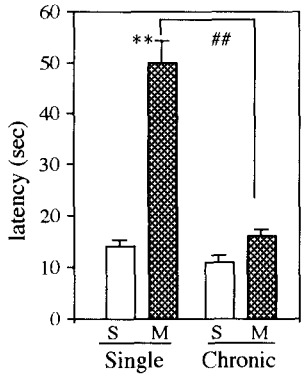

Fig. 3. Tolerance to morphine-induced analgesia in rats. Rats were given the hot-plate test 30 min after the first injection of 10 mg/kg morphine. Then, rats were treated with 10 mg/kg morphine once a day for 8 consecutive days. On day 9, they were again given the hot-plate test 30 min after the injection. Each column represents the mean ± S.E.M. of hot-plate latencies for 6 animals. The significance of differences from the saline-treated rats ($^{**}P < 0.01$) and from single administered rats ($^{\#\#}P < 0.01$) was determined by Student's *t*-test.

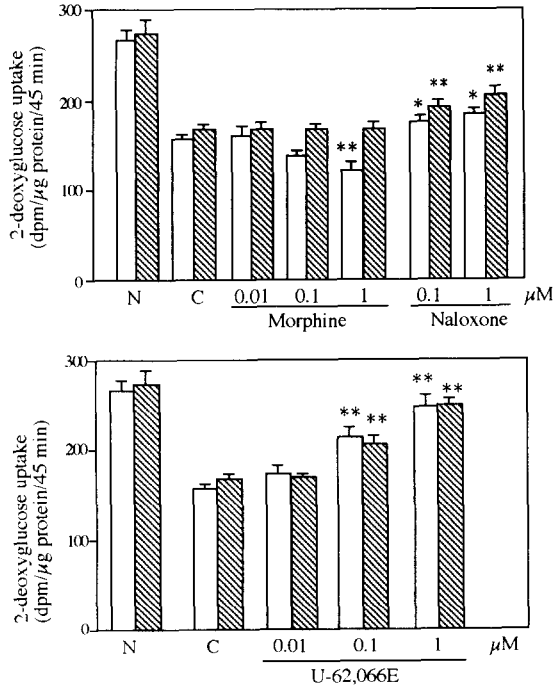

Fig. 4. Effects of morphine, naloxone and U-62,066E on the hypoxia/hypoglycemia-induced reduction in 2-deoxyglucose uptake of hippocampal slices obtained from morphine-tolerant rats. Open columns: saline-treated rats; hatched columns: morphine-tolerant rats. 2-Deoxyglucose uptake was measured after a 6-h wash-out following 20-min hypoxia/hypoglycemia. Values shown are means ± S.E.M. for 6 animals. Drugs were perfused 20 min before and throughout the hypoxia/hypoglycemia period (20 min). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle-treated control slices (C) (Duncan's test) and $^{\#\#}P < 0.01$ vs. single administration (Duncan's test).

3.3. Effects of drugs against the hypoxia / hypoglycemia-induced reduction of 2-deoxyglucose uptake in morphine-tolerant rats

2-Deoxyglucose uptake was 266 ± 11 ($n = 12$) in hippocampal slices from chronic saline-treated rats, and 274 ± 15 ($n = 12$) in those from chronic morphine-treated animals. Thus, there were no significant differences in 2-deoxyglucose uptake between these two groups. Hypoxia/hypoglycemia for 20 min reduced the 2-deoxyglucose uptake of hippocampal slices from morphine-naive rats to 59 ± 2.2% ($n = 12$) and from morphine-tolerant animals to 61 ± 2.1% ($n = 12$). The responses to hypoxia/hypoglycemia were similar in both groups.

The exacerbating effect of morphine against the decline in 2-deoxyglucose uptake induced by hypoxia/hypoglycemia disappeared in chronic morphine-treated rats, whereas the protective effect of naloxone was slightly potentiated by chronic morphine administration (Fig. 4). Chronic treatment with morphine did not attenuate the protective effect of U-62,006E against the hypoxia/hypoglycemia-induced decrease in 2-deoxyglucose uptake (Fig. 4).

Treatment of hippocampal slices with morphine (1 μM), naloxone (100 nM) or U-62,066E (1 μM) for 40 min in normal non-ischemic solution did not affect 2-deoxyglucose uptake of hippocampi obtained from chronic morphine-treated rats, and 2-deoxyglucose uptake was 285 ± 20 ($n = 5$) dpm/μg protein per 45 min for control, 285 ± 13 ($n = 5$) for morphine, 288 ± 13 ($n = 5$) for naloxone and 282 ± 15 ($n = 5$) for U-62,066E, respectively.

4. Discussion

The present results demonstrated that the hypoxia/hypoglycemia-induced decrease in 2-deoxyglucose uptake in hippocampal slices was attenuated by naloxone, a μ-opioid receptor antagonist, but potentiated by morphine. When morphine was perfused in the presence of naloxone, both the exacerbating effect of morphine and protective effect of naloxone disappeared. This indicates that the morphine-induced exacerbation is due to activation of μ-opioid receptors and not to side effects of this agent.

Naloxone has been found to reduce physiological, histological and behavioral changes after focal or global ischemia in many experimental models and species (Hosobuchi et al., 1982; Baskin et al., 1986; Capdeville et al., 1986). In contrast to naloxone, morphine exacerbates neurologic deficits associated with focal and global ischemia (Hosobuchi et al., 1982; Baskin et al., 1984).

In pyramidal cells of the hippocampal CA1 region in rats, μ-opioid receptor activation results in inhibition

of inhibitory postsynaptic potentials followed by enhancement of excitatory postsynaptic potentials (Henderson, 1983; Swearengen and Chavkin, 1987; Neumaier et al., 1988). These excitatory effects are predominantly mediated through μ- and δ-opioid receptors rather than through κ-opioid receptor (Chavkin et al., 1985; Dunwiddie et al., 1987). The CA1 region of the hippocampus has been reported to be particularly vulnerable to ischemia in vitro (Shibata et al., 1992). Thus, the excitatory effect of morphine on CA1 hippocampal neurons may be due to its exacerbating action against the ischemia-induced deficit in 2-deoxyglucose uptake, as an excitatory transmitter such as vasopressin potentiated the ischemia-induced decrease in the CA1 field potentials (Tanaka et al., 1994).

The early release of excitatory amino acids has been shown by in vivo microdialysis experiments following cerebral ischemia. The release of excitatory amino acids has been implicated in cell damage after cerebral ischemia (Korf et al., 1988; Globes et al., 1988). These increases in extracellular excitatory amino acids are significantly attenuated by opiate receptor antagonists (Graham et al., 1993).

The results of the present study demonstrated that a κ-opioid receptor agonist U-62,066E attenuated the reduction of 2-deoxyglucose uptake induced by ischemia. The data, therefore, substantiate and extend previous evidence that κ-opioid receptor agonists reduce mortality and hippocampal CA1 neuronal necrosis following transient bilateral carotid occlusion in gerbils and rats (Hall and Pasara, 1988; Silvia et al., 1987; Tang, 1985). Similarly, the κ-opioid receptor agonists GR89696 and CL-977 have recently been shown to reduce ischemic brain damage in rat, mouse and cat models of focal cerebral ischemia (Birch et al., 1991; Kusumoto et al., 1992; Mackay et al., 1993). In the present experiment, the neuroprotection provided by U-62,066E was blocked by the reputed κ-opioid receptor antagonist MR2266 (Romer et al., 1980), but not by naloxone. This finding suggests that the neuroprotective effect of U-62,066E is predominantly mediated via κ-opioid receptors. Although there is growing evidence indicating the neuroprotective efficacy of κ-opioid receptor agonists, the precise pharmacological mechanism underlying the neuroprotective effects of such agents is not known.

U-62,066E may modulate the neurotoxic action of glutamate via presynaptic inhibition of its release. In vitro, κ-opioid receptor agonists inhibit the excitatory transmission in the hippocampus (Pinnock, 1992; Wagner et al., 1992) and the evoked release of glutamate and aspartate from rat cortical slices (Bradford et al., 1986; Lambert et al., 1991) and guinea pig hippocampal mossy fiber synaptosomes (Gannon and Terrian, 1991). In addition, U-50,488H is reported to decrease the entry of ^{45}Ca into rat cortical synaptosomes (Xiang et al., 1990). We demonstrated that adenosine A1 receptor agonists had neuroprotective effects against the hypoxia/hypoglycemia-induced deficit in 2-deoxyglucose uptake through presynaptic inhibition of glutamate release (Tominaga et al., 1992).

In the present experiment, chronic treatment with morphine exhibited tolerance in both analgesia and exacerbation. This result suggests that both effects of morphine appear to be mediated mainly by activation of μ-opioid receptors. Previous studies demonstrated that chronic administration of morphine was associated with down-regulation of μ-opioid receptors in certain areas of the brain and spinal cord but not with down-regulation of κ- and δ-opioid receptors (Bhargava et al., 1991a,b, 1989; Wimpey et al., 1989). Previous behavioral studies in rats have found no evidence of the existence of cross-tolerance between μ- and κ-opioid receptor agonists (Von Voigtlander et al., 1983; Dykstra et al., 1987; Dykstra and Massie, 1988; Nakazawa et al., 1990). Therefore, the observed failure of the exacerbating effect by morphine in morphine-tolerant rats may be related to the down-regulation of μ-opioid receptors as occurs in morphine-tolerant animals. However, chronic administration of morphine did not modify U-62,066E-induced protection against the deficit in 2-deoxyglucose uptake.

In summary, the present results showed that κ-opioid receptor agonists have neuroprotective effects on the hypoxia/hypoglycemia-induced decrease in 2-deoxyglucose uptake in morphine-naive and also morphine-tolerant rats.

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