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Effects of [D-Ala^2, D-Leu^5]Enkephalin and [D-Pen^2, L-Pen^5]Enkephalin on Apomorphine-Induced Motor Activity in the Mouse

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UKAI, M., T. TOYOSHI AND T. KAMEYAMA. *Effects of [D-Ala^2, D-Leu^5]enkephalin and [D-Pen^2, L-Pen^5]enkephalin on apomorphine-induced motor activity in the mouse.* PHARMACOL BIOCHEM BEHAV **41**(1) 171–176, 1992.—The effects of intracerebroventricular injections of opioid peptides such as DADL ([D-Ala^2, D-Leu^5]enkephalin) and DPLPE ([D-Pen^2, L-Pen^5]enkephalin) with different degrees of selectivity for delta- over mu-receptor on apomorphine (0.1, 0.3, 1.0 and/or 3.0 mg/kg)-induced motor activity were investigated in the mouse using multi-dimensional behavioral analyses. Lower doses (0.1 and 0.3 mg/kg) of apomorphine failed to affect significantly motor activity, whilst higher doses (1.0 and 3.0 mg/kg) of the drug produced a marked increase in linear locomotion, circling, rearing, and/or grooming behaviors. DADL (0.03, 0.1 or 0.3 μg) by itself did not influence behaviors, while the peptide (0.1 and 0.3 μg) produced a marked inhibition on apomorphine (1.0 but not 3.0 mg/kg)-induced increase in rearing behaviors. Furthermore, the inhibitory effect of DADL (0.3 μg) on the apomorphine (1.0 mg/kg)-induced increase in rearing was reversed by treatment with the alkylating agent β-FNA (β-funaltrexamine) (5.0 μg). In contrast to the effects of DADL, the much more delta-selective opioid agonist DPLPE (0.3, 1.0 or 1.75 μg) had no marked effects on apomorphine (1.0 mg/kg)-induced behaviors. These results suggest that delta opioid receptors do not play a principal role in the apomorphine-induced increase in circling, rearing or grooming behaviors.

DADL ([D-Ala^2, D-Leu^5]enkephalin) DPLPE ([D-Pen^2, L-Pen^5]enkephalin) Apomorphine Locomotor activity Mouse

IT has been reported that opioids affect locomotor activity in rodents (7,10). We have analysed the behavioral effects of opioids by using multi-dimensional behavioral analyses of locomotor activity based upon a capacitance system (8). For instance, γ-endorphin produces a marked increase in linear locomotion without affecting other behaviors (8), whilst α-neo-endorphin, β-endorphin and dynorphin A(1–17) produce a marked decrease in linear locomotion, circling, rearing and/or grooming behaviors (8, 20, 27). More recent findings show that higher doses of DADL and DPLPE produce a marked increase in circling behaviors (26). It is further documented that opioids interact with dopamine systems in vivo (11, 22, 23) as well as in vitro (18,28). For instance, kappa opioid agonists such as bremazocine, dynorphin A(1–13) as well as U-50,488H and the mu opioid peptide DAGO [D-Ala^2, NMePhe^4, Gly(ol)]enkephalin, unlike delta opioid peptides, diminish the K^+- or electrically evoked release of dopamine in rat brain slices (3, 6, 13, 18, 28). Dynorphin A(1–13) and DAGO inhibit the marked increase in rearing behaviors induced by higher doses (0.56 and 1.0 mg/kg) of apomorphine (25). However, there seems to be no direct behavioral evidence as regards the relationship between delta opioid receptors and dopamine neurons.

In the present study, the effects of the delta-selective ligands DADL and DPLPE with different degrees of selectivity for delta- over mu-receptor on the behaviors induced by a variety of different doses of apomorphine were examined by using multi-dimensional behavioral analyses. In addition, the effects of DADL were characterized with the alkylating agent β-FNA (15).

METHOD

Subjects

Male ddY mice (Japan SLC, Inc.) weighing between 20–30 g were employed in the experiments. The animals were randomly assigned to groups consisting of 8 to 10 mice per group. Before the experiments, the mice were given free access to food and water, and individual mice were housed in a cage in a constantly illuminated room at a temperature of 23 ± 1°C and a relative humidity of 55 ± 2.5%. The mice were used only once and were unfamiliar with the test box. The experiments were conducted between 10:00 a.m. and 6:00 p.m. in a sound-attenuating room.

Multi-Dimensional Analysis

Immediately before multi-dimensional behavioral analyses, mice were selected according to the number of revolutions (range from 125 to 150 per 10 min for criterion) using wheel

TABLE 1

SPONTANEOUS MOVEMENTS IN MICE AFTER DADL, APOMORPHINE (APO) AND THEIR COMBINATIONS

Treatments	Behaviors[a]			
	Linear Locomotion	Circling	Rearing	Grooming
SAL (SC)[b]				
+ DADL 0.03 μg (ICV)	0.7 ± 0.3	1.7 ± 0.6	1.2 ± 0.3	1.0 ± 0.2
+ DADL 0.1 μg (ICV)	0.9 ± 0.2	1.5 ± 0.2	1.6 ± 0.3	1.4 ± 0.2
+ DADL 0.3 μg (ICV)	0.7 ± 0.3	1.8 ± 0.6	1.2 ± 0.3	1.0 ± 0.2
APO 0.1 mg/kg (SC)				
+ SAL (ICV)	0.7 ± 0.2	1.2 ± 0.3	1.3 ± 0.1	1.1 ± 0.2
+ DADL 0.03 μg (ICV)	1.3 ± 0.3	1.2 ± 0.2	1.4 ± 0.2	1.1 ± 0.2
+ DADL 0.1 μg (ICV)	1.1 ± 0.4	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.2
+ DADL 0.3 μg (ICV)	0.5 ± 0.3	1.3 ± 0.4	1.3 ± 0.3	0.8 ± 0.2
APO 0.3 mg/kg (SC)				
+ SAL (ICV)	0.7 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.1
+ DADL 0.03 μg (ICV)	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
+ DADL 0.1 μg (ICV)	0.6 ± 0.4	1.0 ± 0.3	0.8 ± 0.1	0.7 ± 0.2
+ DADL 0.3 μg (ICV)	0.6 ± 0.3	0.5 ± 0.1	1.0 ± 0.4	0.6 ± 0.2
APO 1.0 mg/kg (SC)				
+ SAL (ICV)	2.6 ± 0.7	2.9 ± 0.4	3.6 ± 0.4*	2.3 ± 0.5*
+ DADL 0.03 μg (ICV)	2.3 ± 0.6	2.3 ± 0.4	3.0 ± 0.5*	1.9 ± 0.6
+ DADL 0.1 μg (ICV)	2.9 ± 0.6	3.4 ± 1.3	2.0 ± 0.2†	1.8 ± 0.2
+ DADL 0.3 μg (ICV)	1.7 ± 0.7	3.9 ± 0.7*	2.2 ± 0.2†	1.7 ± 0.2
APO 3.0 mg/kg (SC)				
+ SAL (ICV)	3.7 ± 1.1	4.9 ± 0.6*	3.0 ± 0.2*	2.0 ± 0.2
+ DADL 0.03 μg (ICV)	5.0 ± 2.6*	3.9 ± 0.7*	3.4 ± 0.5*	1.8 ± 0.2
+ DADL 0.1 μg (ICV)	4.3 ± 1.3	4.2 ± 1.0*	3.4 ± 0.4*	2.4 ± 0.3*
+ DADL 0.3 μg (ICV)	3.1 ± 0.6	3.4 ± 0.7	3.6 ± 0.4*	2.2 ± 0.2*

[a]Each value depicts the mean ± S.E. for 8 mice.
[b]SAL: 0.9% saline.
*Denotes significant difference from saline control, $p<0.05$.
†Denotes significant difference from apomorphine 1.0 mg/kg, $p<0.05$.

cages to exclude individual differences of animals in locomotor activity as much as possible. About 30% of the mice were discarded for failing the criterion in the first measurement. The mice discarded were repeatedly put into wheel cages for selection on different days. Finally, we could use almost all of the mice purchased in the study, although not all of the mice were given the same number of screening in wheel cages. Behavior was observed over a period of 30 min, cumulating counts at 15-min intervals, but the results in the latter period (15–30 min) were not figured or tabulated because apomorphine did not produce any marked effects on behaviors in the second (15–30 min) period. The Animex II, equipped with an electronic microcomputer, was used for measuring the behavior (8, 21, 27). The sensor consisted of three pairs of electrodes and formed a capacitor bridge. Once a mouse was placed in the space (150 × 210 × 140 mm) between the electrodes connected to field detectors, the value of the capacitor then depended upon the location of the mouse within that space. When converting the analog signal received by the detectors to a digital form, the D.C. voltage movement spectrum analyser classified the movement into 9 degrees (1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 and 1/256). The surface length of the cage in which mice showed behavioral responses (linear locomotion, circling, rearing and grooming) was 490 mm in distance. The 490 mm distance consisted of the length of the cage bottom (210 mm) and the two cage walls (140 × 2 mm). Thus the counters corresponded to the following sizes of movements: 1/1 (×490.0 mm) = 490.0 mm, 1/2 (×490.0 mm) = 245.0 mm, 1/4 (×490.0 mm) = 122.5 mm, 1/8 (×490.0 mm) = 61.3 mm, 1/16 (×490.0 mm) = 30.6 mm, 1/32 (×490.0 mm) = 15.3 mm, 1/64 (×490.0 mm) = 7.7 mm, 1/128 (×490.0 mm) = 3.8 mm, and 1/256 (×490.0 mm) = 1.9 mm. The movement of greatest magnitude was principally registered on the 1/1 counter and the movement of the smallest magnitude, such as tremor, on the 1/256 counter. Specific patterns of behavior, induced by a drug, were registered on the counters as follows, linear locomotion on 1/1, circling on 1/4, rearing on 1/16 and grooming on 1/64 (8). The sensitivity (%) of the device was adjusted according to the body weight (g) as follows, 20–21 g = 27%, 22–23 g = 26%, 24–25 g = 25%, 26–28 g = 24% and 29–30 g = 23%. Each value in the tables was labeled ratio mean ± S.E. after calculating ratios for each of the animals. The ratios for each of the animals = (each of the actual values of drug-treated animals)/(mean actual value of controls). Additionally, a continuous recording of the animal behaviors (behavioral traces) was made with X-Y recorders (Watanabe Inc., Nagoya, Japan) connected to the field detectors of the Animex II.

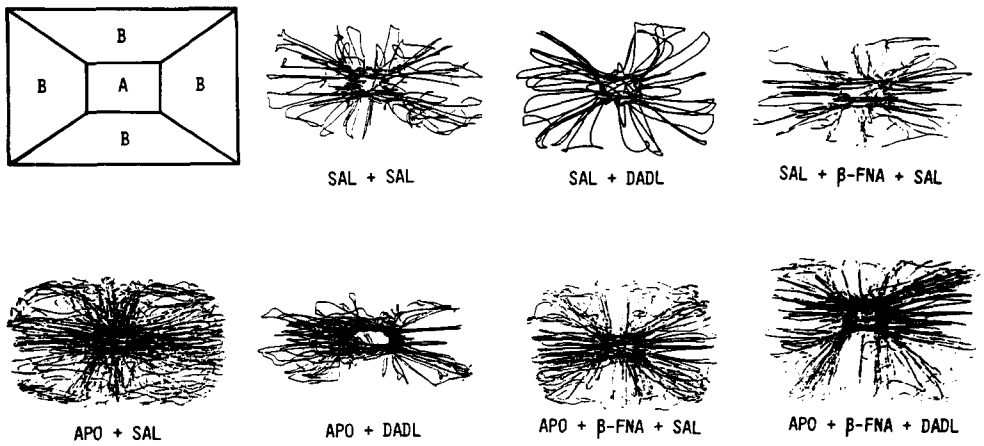

FIG. 1. Behavioral traces in each of the mice treated with DADL, apomorphine (APO), β-FNA and their combinations. Top left-hand part of the figure shows; (A) cage bottom; (B) cage walls. SAL: 0.9% saline, SC and ICV, DADL: 0.3 μg/mouse, ICV, β-FNA: 5.0 μg/mouse, ICV and APO: 1.0 mg/kg, SC.

Drugs and Treatments

Apomorphine hydrochloride (Sigma, St. Louis, MO), DADL (Peptide Institute Inc., Minoh, Osaka, Japan), DPLPE (Peninsula Laboratories, Inc., Belmont, CA) and β-FNA hydrochloride (Research Biochemicals, Inc., Natick, MA) were employed throughout. Apomorphine (SC) and β-FNA (ICV) were administered 25 min and 22–24 h, respectively, before the start of behavioral measurements. The peptides dissolved in sterile isotonic saline in polypropylene containers were injected intracerebroventricularly 10 min before the start. The unilateral injection site was 2 mm from either side of the midline on a line drawn through the anterior roots of the ears (5). The injection was made with a 4 mm long needle attached to a 50 μl Hamilton microsyringe. The needle was inserted perpendicularly through the skull and into the brain of the unanaesthetized mouse. Solutions were injected in a volume of 10 μl per mouse over a period of 20 s as previously described (8). The site was checked by injecting a 1:10 dilution of India ink in isotonic saline (0.9% NaCl, pH 7.5). Histological examinations revealed particles of the ink in the lateral and 3rd ventricles but not in the others. As previously described (8), neither insertion of the needle nor injection of 10 μl of isotonic saline solution had a significant influence on behaviors during observation periods, although slight

TABLE 2

SPONTANEOUS MOVEMENTS IN MICE AFTER DPLPE, APOMORPHINE (APO) AND THEIR COMBINATIONS

	Behaviors[a]			
Treatments	Linear Locomotion	Circling	Rearing	Grooming
SAL (SC)[b]				
+ DPLPE 0.3 μg (ICV)	2.3 ± 0.7	1.6 ± 0.3	1.6 ± 0.2	1.2 ± 0.2
+ DPLPE 1.0 μg (ICV)	1.3 ± 0.6	1.5 ± 0.5	1.6 ± 0.3	1.2 ± 0.2
+ DPLPE 1.75 μg (ICV)	0.1 ± 0.1	2.0 ± 0.3	1.2 ± 0.3	1.1 ± 0.3
APO 0.1 mg/kg (SC)				
+ SAL (ICV)	0.7 ± 0.2	1.2 ± 0.3	1.3 ± 0.1	1.1 ± 0.2
+ DPLPE 0.3 μg (ICV)	0.5 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.1
+ DPLPE 1.0 μg (ICV)	1.1 ± 0.4	1.0 ± 0.2	0.9 ± 0.3	0.8 ± 0.1
+ DPLPE 1.75 μg (ICV)	0.2 ± 0.2	1.6 ± 0.3	1.6 ± 0.3	1.1 ± 0.1
APO 1.0 mg/kg (SC)				
+ SAL (ICV)	2.6 ± 0.7	2.9 ± 0.4*	3.6 ± 0.4*	2.3 ± 0.5*
+ DPLPE 0.3 μg (ICV)	3.3 ± 1.3	3.3 ± 1.3*	3.9 ± 0.2*	2.7 ± 0.1*
+ DPLPE 1.0 μg (ICV)	3.2 ± 1.0	3.0 ± 0.5*	3.4 ± 0.3*	1.9 ± 0.3
+ DPLPE 1.75 μg (ICV)	2.8 ± 0.8	3.9 ± 0.4*	3.5 ± 0.4*	1.9 ± 0.2

[a]Each value depicts the mean ± S.E. for 8 mice.
[b]SAL: 0.9% saline.
*Denotes significant difference from saline control, $p<0.05$.

TABLE 3

SPONTANEOUS MOVEMENTS IN MICE AFTER DADL, APOMORPHINE (APO), β-FNA AND THEIR COMBINATIONS

Treatments	Behaviors[a]			
	Linear Locomotion	Circling	Rearing	Grooming
SAL (SC)[b]				
+ β-FNA 5.0 μg (ICV) + SAL (ICV)	0.7 ± 0.2	1.0 ± 0.3	1.1 ± 0.2	0.7 ± 0.2
+ SAL (ICV) + DADL 0.3 μg (ICV)	0.3 ± 0.1	2.2 ± 0.6	1.3 ± 0.3	1.0 ± 0.2
+ β-FNA 5.0 μg (ICV) + DADL 0.3 μg (ICV)	0.8 ± 0.1	0.9 ± 0.2	1.3 ± 0.2	0.9 ± 0.1
APO 1.0 mg/kg (SC)				
+ SAL (ICV) + SAL (ICV)	2.2 ± 0.5	3.6 ± 0.7*	3.3 ± 0.3*	1.7 ± 0.2*
+ β-FNA 5.0 μg (ICV) + SAL (ICV)	2.9 ± 0.6*	4.0 ± 0.4*	3.6 ± 0.5*	1.8 ± 0.2*
+ SAL (ICV) + DADL 0.3 μg (ICV)	1.3 ± 0.5	3.7 ± 0.8*	2.3 ± 0.2*†	1.6 ± 0.2*
+ β-FNA 5.0 μg (ICV) + DADL 0.3 μg (ICV)	2.6 ± 0.6*	4.3 ± 0.5*	3.3 ± 0.3*‡	1.9 ± 0.2*

[a]Each value depicts the mean ± S.E. for 10 mice.
[b]SAL: 0.9% saline.
*Denotes significant difference from saline control, $p<0.05$.
†Denotes significant difference from apomorphine 1.0 mg/kg, $p<0.05$.
‡Denotes significant difference from apomorphine 1.0 mg/kg + DADL 0.3 μg/mouse, $p<0.05$.

sedation was seen only within a few minutes after ICV injections. The three tables provided illustrate the observations which form three separate study periods. There were vehicle control groups in the three studies (Tables 1 and 2: saline, SC + saline, ICV, Table 3: saline, SC + saline, ICV + saline, ICV), and the activity scores in control groups were reproducible from study to study.

Data Analysis

Data for actual values were analysed statistically by means of a one-factor analysis of variance (ANOVA). Post hoc analysis for between-group differences was carried out by the Newman-Keuls method for multiple comparisons (31). Effects were considered statistically significant if $p<0.05$. Data in tables indicate ratios derived from actual values for the clearer presentation of results.

RESULTS

Effects of Various Drugs on Behavioral Traces

A 1.0 mg/kg dose of apomorphine produced a marked increase in linear locomotion, circling and rearing behaviors, while DADL (0.3 μg) decreased rearing behaviors induced by apomorphine (1.0 mg/kg) (Fig. 1). The effects of DADL were antagonized by a 5.0 μg dose of β-FNA (Fig. 1).

Effects of DADL on Apomorphine-Induced Behaviors

The following three doses of DADL were chosen, because they alone have been shown not to produce behavioral abnormality such as continuous circling behaviors (26). ANOVA revealed a significant relation ($p<0.01$) in the following sizes: $F(19,140)=3.02$ in linear locomotion, $F(19,140)=6.29$ in circling, $F(19,140)=11.78$ in rearing, $F(19,140)=5.76$ in grooming (Table 1). DADL (0.03, 0.1 or 0.3 μg) alone had no marked effects on behaviors similar to the results reported previously (26) (Table 1). Apomorphine (0.1 or 0.3 mg/kg) alone or in combination with DADL (0.03, 0.1 or 0.3 μg) had no significant effects on different behaviors (Table 1). A 1.0 mg/kg dose of apomorphine produced a significant increase in rearing and grooming behaviors. The apomorphine (1.0 mg/kg)-induced increase in rearing behaviors was significantly inhibited by treatment with DADL (0.1 and 0.3 μg) (Table 1). A higher dose (3.0 mg/kg) of apomorphine markedly increased circling, rearing and grooming behaviors, but DADL (0.03, 0.1 or 0.3 μ g) did not significantly affect the behaviors induced by a 3.0 mg/kg dose of apomorphine (Table 1).

Effects of DPLPE on Apomorphine-Induced Behaviors

Since the higher doses (3.0 and 10.0 μg) of DPLPE alone have been reported to affect different behaviors such as continuous linear locomotion and circling (26), the following three doses (0.3, 1.0 and 1.75 μg) of DPLPE were chosen not to evoke behavioral abnormality. Thus a 1.75 μg dose of DPLPE was only below the range of behaviorally active doses. ANOVA showed a significant relation ($p<0.01$) in the following behaviors: $F(11,84)=9.83$ in circling, $F(11,84)=17.17$ in rearing and $F(11,84)=7.87$ in grooming (Table 2). Apomorphine (0.1 mg/kg) alone or in combination with DPLPE (0.3, 1.0 or 1.75 μg) did not influence motor activity (Table 2). DPLPE (0.3, 1.0 or 1.75 μg) did not affect the behavioral changes induced by a 1.0 mg/kg dose of apomorphine (Table 2).

Effects of β-FNA on the Effects of DADL

ANOVA indicated a significant relation ($p<0.01$) in the following sizes: $F(7,56)=5.43$ in linear locomotion, $F(7,56)=8.6$ in circling, $F(7,56)=15.38$ in rearing and $F(7,56)=5.67$ in grooming (Table 3). β-FNA (5.0 μg) alone or in combination with DADL (0.3 μg) did not affect motor activity (Table 3). In addition, β-FNA (5.0 μg) failed to influence the apomorphine (1.0 mg/kg)-induced alterations of behaviors (Table 3). DADL (0.3 μg) again produced an inhibitory effect on the apomorphine (1.0 mg/kg)-induced increase in rearing (Table 3). A 5.0 μg dose of β-FNA antagonized the inhibitory effect of DADL (0.3 μg) on the apomorphine (1.0 mg/kg)-induced increase in rearing behaviors (Table 3).

DISCUSSION

In multi-dimensional analyses of behavior, neither a 0.1 nor a 0.3 mg/kg dose of apomorphine influenced behaviors, but 1.0

and 3.0 mg/kg doses produced a marked increase in behaviors, particularly circling, rearing and/or grooming. The delta-selective opioid agonist DADL (1.0 and 3.0 μg) has been shown to produce a marked increase in circling behaviors (26), whereas the peptide at lower doses (0.03, 0.1 or 0.3 μg) does not markedly affect behaviors (26) as in this study. DADL (0.1 and 0.3 μg) produced an antagonistic effect on the apomorphine (1.0 mg/kg)-induced increase in rearing behaviors. The results suggest that, since a 1.0 mg/kg dose of apomorphine interacts exclusively with postsynaptic dopamine receptors (16), DADL plays an inhibitory role on postsynaptic dopamine neurons. Likewise, other enkephalin analogues have been shown to antagonize the behavioral effects of apomorphine (1,14). Furthermore, Stoof and Kebabian (17) have shown that dopamine receptors are divided into at least two categories such as D_1 and D_2. The behavioral effects of apomorphine (1.0 mg/kg) seem to be attributable to D_2 rather than D_1 dopamine receptors (29,30), although the drug may also have considerable effects on D_1 receptors. DADL would interact with dopamine neurons through D_2 dopamine receptors in the brain.

In contrast, DPLPE (0.3 and 1.0 μg) did not affect apomorphine (1.0 mg/kg)-induced behaviors. Since DPLPE has been reported to possess roughly 300-fold selectivity for delta over mu opioid receptors, while DADL is only about 10-fold selective (2). It appears that delta opioid receptors themselves do not play an important role in the dopamine-elicited behaviors in rodents, although delta opioid receptors may have only minimal effects on apomorphine-induced changes in behavior. Therefore, the inhibitory effects of DADL on apomorphine-induced increase in rearing were presumed at least in part to be mu-mediated, because the present results indicate that the relatively mu-selective opioid antagonist β-FNA (5.0 μg) reversed the effects of DADL (0.3 μg). Similar results were obtained by Locke and Holtzman (10). It has also been demonstrated that DADL has relatively high affinity for mu opioid receptors in binding assays (4, 9, 12) and that DADL but not DPLPE produces discriminative stimulus effects in common with morphine (19). In fact, our previous study has demonstrated that the mu-selective peptide DAGO specifically inhibits the apomorphine-induced increase in rearing and grooming behaviors as in the present study (24).

It is probable that opioid receptors have potent effects on nigrostriatal-mediated locomotion. These locomotor effects appear to be opioid in nature and mediated by the nigrostriatal dopamine system (11). The effects of DADL on apomorphine-induced behaviors may be mediated through nigrostriatal pathways in the brain.

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