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Behavioral Effects of Norepinephrine and Dibutyryl 3',5' AMP in Centrally Sympathectomized Rats

R. BRUS, Z. S. HERMAN AND F. KOSTMAN

Department of Pharmacology, Institute of Biology and Physiology, Silesian School of Medicine Marksa 38, 41-808 Zabrze, Poland

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BRUS, R., Z. S. HERMAN AND F. KOSTMAN. *Behavioral effects of norepinephrine and dibutyryl cyclic 3',5' AMP in centrally sympathectomized rats.* PHARMAC. BIOCHEM. BEHAV.2(6) 719–724,1974.– Male Wistar rats were injected intraventricularly with two doses of 250 μg of 6-hydroxydopamine (6-OHDA) at a 48 hr interval. Seven days after the second injection, 50 μg of norepinephrine (NE) or 100 μg of dibutyryl cyclic AMP (DCAMP) were injected intraventricularly. There were no differences in gross behavior but an increase in irritability was observed in rats treated with 6-OHDA compared with controls. NE increased locomotor activity and irritability of animals. Chemical sympathectomy intensified locomotor excitation and irritability caused by NE. DCAMP caused an increase of locomotor activity, irritability and convulsions 30 min after injection. These behavioral phenomena were intensified in animals treated with 6-OHDA. It is suggested that 6-OHDA sensitizes the central nervous system to action of NE and DCAMP.

6-Hydroxydopamine Norepinephrine Dibutyryl cyclic 3',5' AMP Behavior

6-HYDROXYDOPAMINE (6-OHDA) injected into the lateral ventricles of the brain [30,31] or into the cisterna magna [4] reduces whole brain norepinephrine (NE) and dopamine (DA) content in a dose-dependent manner by destruction of catecholamine nerve terminals. It was shown by us [11] that during the first hour after 6-OHDA injection into the lateral ventricle of rats the behavior of animals was similar to that observed after injection of reserpine or a benzoquinolizine derivative. Six hr after the drug injection signs of sedation occurred. But during this period of observation we have noted disturbances in the level of NE, DA and also 5-hydroxytryptamine in different areas of the brain [11]. However many authors have shown that 7–10 days after treatment with 6-OHDA the gross behavior of animals was largely normal in spite of low levels of catecholamines in the brain and an altered catecholamine metabolism [3, 5, 12, 31]. We have also observed [11] that a few days after 6-OHDA treatment, when the level of NE and DA was evidently decreased in different parts of the rat's brain, the behavior of these animals was undistinguishable from the behavior of normal rats. The dissociation between behavioral phenomena and catecholamine levels after 6-OHDA treatment was further indicated by the results of other investigations [21, 25, 26]. Héry *et al.* [12] have shown that 6-OHDA pretreatment did not affect the total locomotor activity of the animals but changed the pattern of activity between light and darkness since the motor activity was twice normal during the light period.

On the other hand it was shown that 6-OHDA caused increased irritability [15], a rage similar to that evoked by septal or ventromedial hypothalamic lesions [6] or increase in footshock-induced fighting [27].

It seemed to us that the model of central sympathectomy induced by 6-OHDA treatment may be used to examine the behavioral effects of substances acting on the central nervous system. Since it was shown in this laboratory that NE and dibutyryl cyclic 3',5' AMP (DCAMP) injected into the lateral ventricle of the rat brain caused evident dose-dependent behavioral changes [9,10], we decided to examine behavioral effects of these substances in rats with chemical central sympathectomy elicited by 6-OHDA.

METHOD

Experiments were carried out on 94 male rats of Wistar strain weighing 200–230 g, from Central Animal Farm of Silesian School of Medicine. Two hundred fifty μg of 6-OHDA (Kistner Lab. Göteborg) were injected into the left lateral ventricle of the rat brain in a volume of 10 μl. Injections were made under light ether anesthesia according to Herman [9]. 6-OHDA was dissolved in artificial cerebrospinal fluid (ACSF) [16] containing 0.1% ascorbic acid a few seconds before injection. Two days later the next dose of 250 μg of 6-OHDA was injected in the same way into the right lateral ventricle. Control animals were treated with

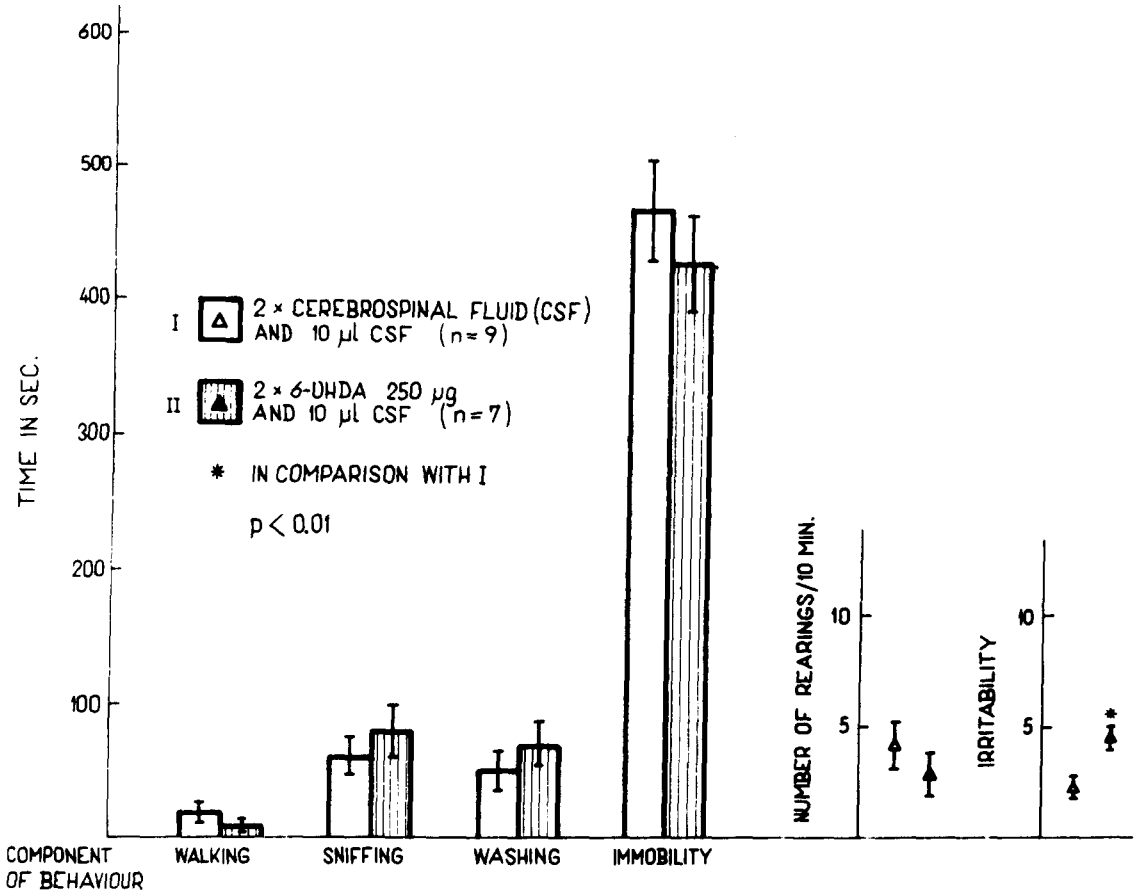

FIG. 1. The behavior of rats treated with 6-hydroxydopamine (6-OHDA).

ACSF in the same way. Seven days after the second injection of 6-OHDA or vehicle, 50 µg norepinephrine bitartrate (NE), (SIGMA) or 100 µg of N^6,O^2-Dibutyryl adenosine 3′,5′ cyclic phosphate, monosodium 5 1/2 H_2O (DCAMP), (CALBIOCHEM) or 10 µl of ACSF fluid were injected into the left lateral ventricle. Doses of applied substances are expressed as free salts. Immediately after the injection, rats of experimental and of control groups were placed in single glass cages with dimensions of 40 × 25 × 25 cm. After 1 min of adaptation the time of walking, washing, sniffing, convulsions and immobility was measured during the 10 min period in seconds to the nearest 5 sec using a stopwatch. The number of rearings was also counted. The irritability of animals was measured using the method of Nakamura and Thoenen [15] after completing the 10 min period of behavioral observation. Rats which received DCAMP were observed by the above described methods and again 30 min after DCAMP injection.

RESULTS

Seven days after the treatment with 6-OHDA the behavior of these rats was similar to untreated rats, except in irritability, which was significantly increased (Fig. 1). NE in rats pretreated with ACSF increased the time of sniffing, decreased the time of immobility and inceased the irritability of animals as compared with rats injected only with ACSF. In rats pretreated with 6-OHDA, NE increased significantly the time of walking and sniffing, decreased immobility, and increased the number of rearings and the irritability of animals as compared with rats pretreated with ACSF (Fig. 2).

Immediately after injection of DCAMP in rats pretreated with ACSF a decrease in the time of walking and an increase in the time of immobility and a very short lasting period of convulsions were observed compared with rats treated with ACSF. Immediately after application of DCAMP in rats pretreated with 6-OHDA a decrease in the time of walking, sniffing and washing was observed in comparison with control group. The time of immobility was significantly increased. The number of rearings was reduced and increased irritability of animals was seen. In these animals the time of convulsions was significantly increased compared with rats pretreated only with ACSF (Fig. 3).

Thirty min after placement into the observation cages animals pretreated and injected with ACSF showed complete immobility. In rats pretreated with ACSF and injected with DCAMP 30 min after DCAMP application walking, sniffing, washing, convulsions, rearings and increased irritability was observed. The evident decrease in immobility was shown.

In rats pretreated with 6-OHDA, DCAMP, 30 min after injection, obviously increased the time of washing and convulsions, and significantly increased irritability compared with animals pretreated with ACSF (Fig. 4).

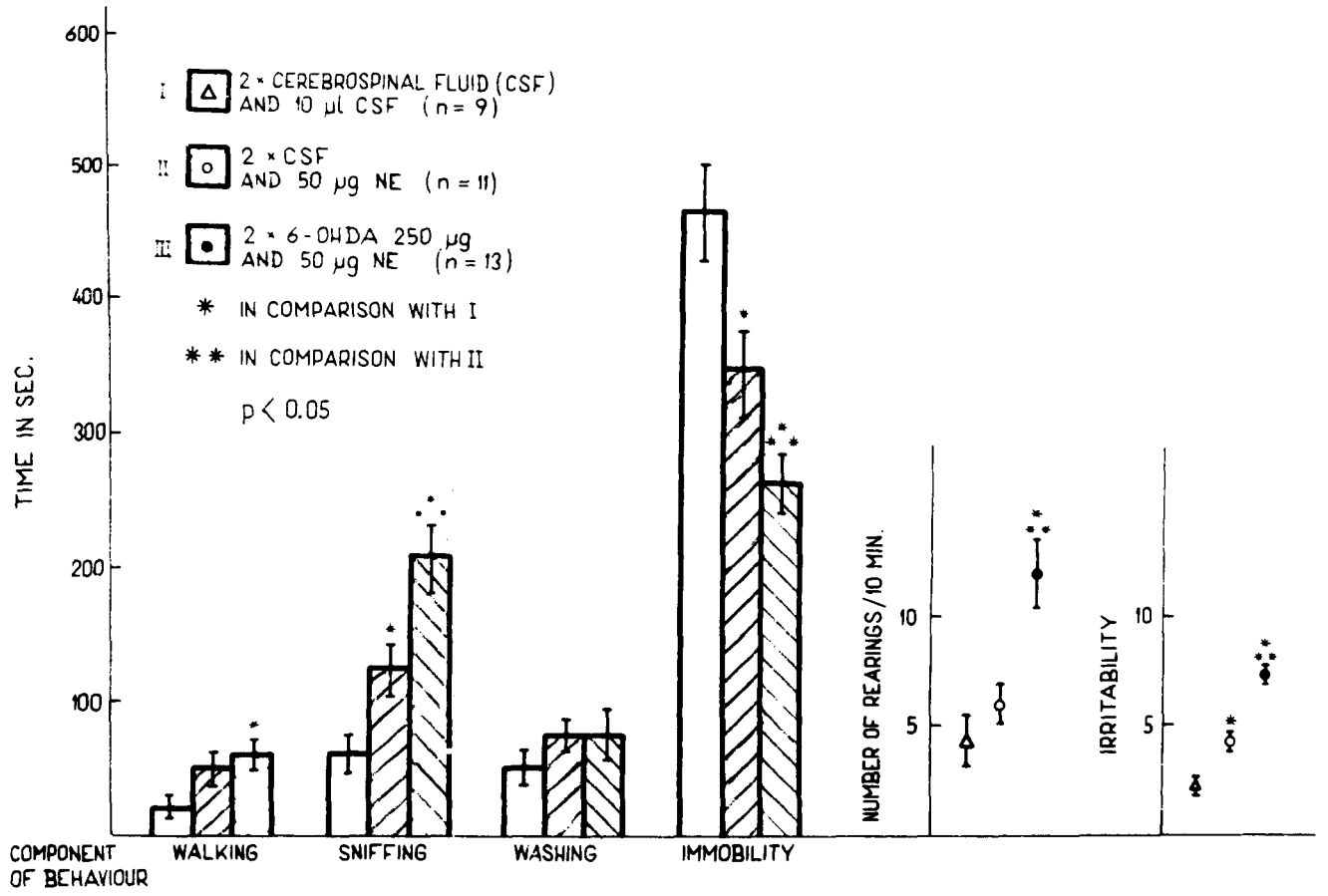

FIG. 2. Behavioral effects of norepinephrine (NE) in rats treated with 6-hydroxydopamine (6-OHDA).

DISCUSSION

Behavioral studies in our experiments were made 7 days after the second injection of 250 µg of 6-OHDA. We used a procedure very similar to Samanin and Bernasconi [19] who observed that 7–10 days after injection of two doses of 250 µg of 6-OHDA the content of NE was 12% of control and the content of DA was 41% of control. Therefore in our experimental condition the level of both amines was reduced. We have studied 3 types of behavior: locomotor activity, rearings which are considered as an indicator of exploratory activity [14] and irritability. In our experiments 6-OHDA enhanced excitatory effects elicited by NE or DCAMP.

Recently it was suggested that 6-OHDA and 6-hydroxy DOPA elicit the supersensitivity of central nervous system to putative neurotransmitters [18].

The phenomenon of supersensitivity to catecholamines after degeneration was observed in the peripheral sympathetic nervous system by several authors [7, 8, 23, 33]. Haeusler *et al.* [8] suggested that chemical and surgical sympathectomy induced the presynaptic as well as postsynaptic types of supersensitivity of isolated nictitating membrane of cat for NE, and obtained the evidence for the absence of the postsynaptic supersensitivity of the heart. Both types of supersensitivity for NE of isolated rabbit atria and aortic strips produced by 6-OHDA were observed [23].

Anden *et al.* [1] have observed that some animals given L-DOPA rotated in a direction which indicated that L-DOPA was more active on the denervated side than on the innervated. They suggested that this might be due to the denervation supersensitivity. The supersensitivity to the 5-HT after degeneration of the descending serotonin pathways was also described [22].

Ungerstedt studied the effect of L-DOPA and apomorphine in rats after unilateral degeneration of the nigrostriatal DA system by intracerebral injection of 6-hydroxydopamine, and suggested that 6-OHDA caused postsynaptic supersensitivity [29]. The author observed enhanced rotational response to both drugs. This effect coincided with the disappearance of DA from the corpus striatum.

Thoa *et al.* [28] have reported vigorous spontaneous fighting in rats first treated intracisternally with 6-OHDA and then intraperitoneally with L-DOPA and methyldopahydrazine. In explanation of this phenomenon they considered two possibilities: a denervation hypersensitivity for exogenous DOPA or dopamine, or destruction of inhibitory system which blocked the behavioral effect of DOPA.

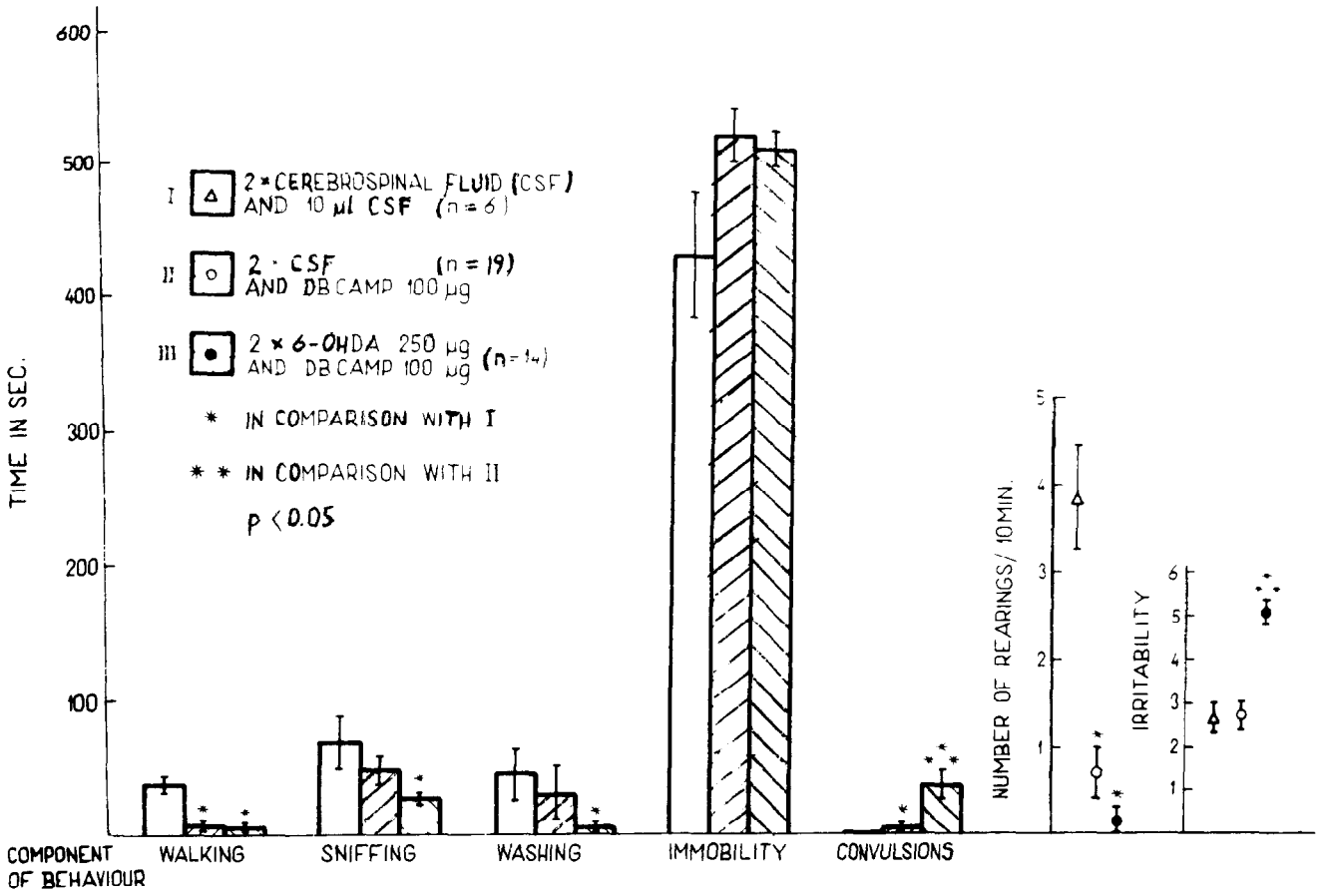

FIG. 3. Immediate behavioral effects of dibutyryl cyclic 3'5'AMP (DCAMP) in rats treated with 6-hydroxydopamine (6-OHDA).

Other authors suggested a supersensitivity to putative neurotransmitters by the exaggerated behavioral response to L-DOPA or to apomorphine in mice and rats treated with 6-OHDA [2, 13, 20, 32], or by the 6-OHDA elicited supersensitivity to NE in self-stimulation tests [24].

Palmer [17] has suggested that adrenergic denervation supersensitivity to catecholamines may be mediated by brain adenyl cyclase.

Our results in the light of above mentioned data support a hypothesis that 6-OHDA sensitizes the central nervous system to NE. We have also shown the supersensitivity to DCAMP of the brain, after chemical sympathectomy elicited by 6-OHDA. Our results support previously reported findings that excitatory type of behavior occurs not before 30 min after intraventricular injection of DCAMP [10].

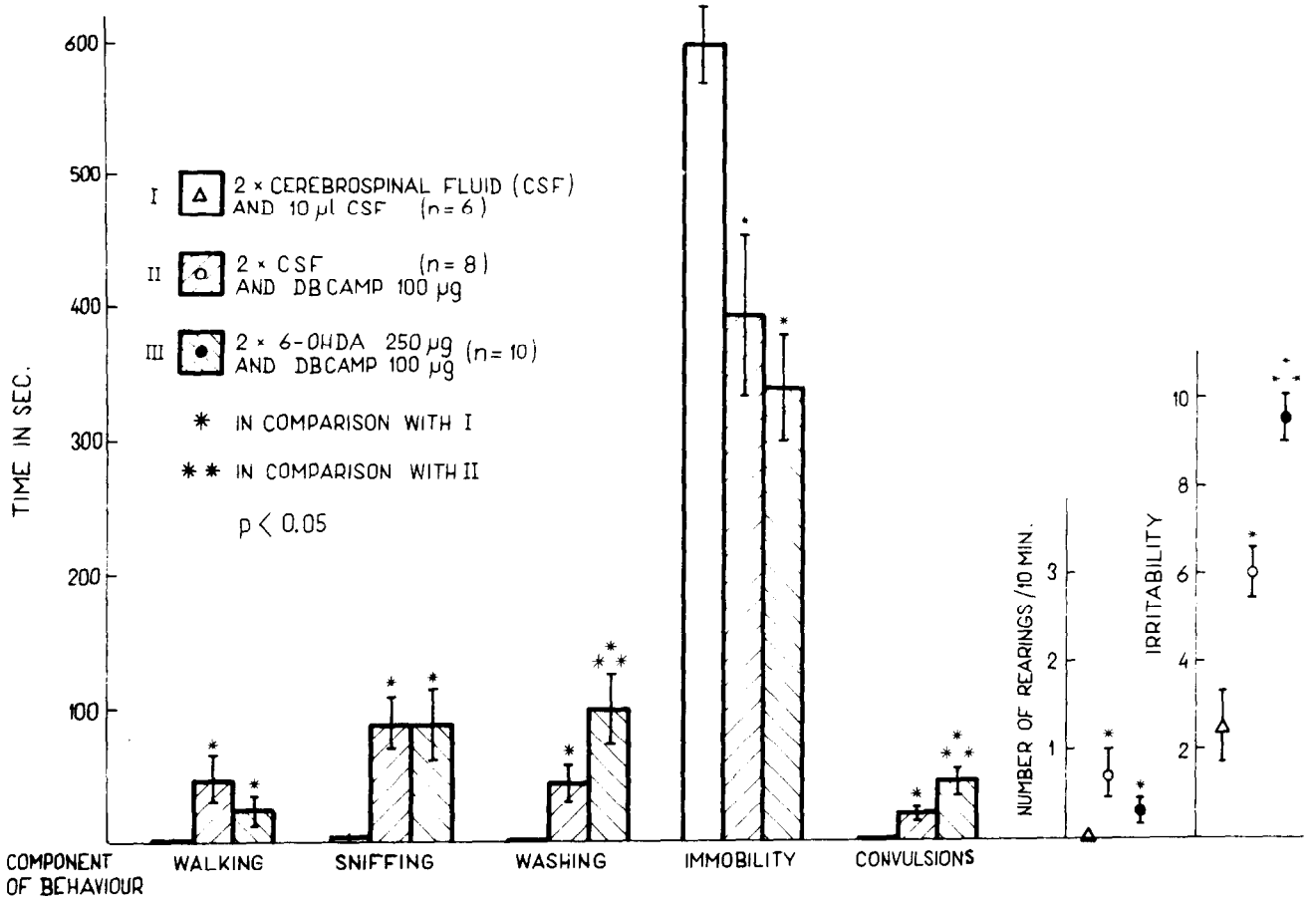

FIG. 4. Behavioral effects 30 min after injection of dibutyryl cyclic 3'5' AMP (DCAMP) in rats treated with 6-hydroxydopamine (6-OHDA).

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