The Evoked Potential in the Slice of Guinea Pig Hippocampus, and the Effects of Zinc on It *

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INTRODUCTION

In 1951, Tokuoka[24] attempted the partial resection of pancreas as a cure for genuine epilepsy and reported that the operation was effectual in alleviating epileptic fits in most of the cases. It has been suggested in the following reports of his collaborators that the disturbance of zinc metabolism might have some corelation with the abnormal brain excitability; e. g. Fuchimoto et al.[7] reported in mice that intracerebral injection of zinc (-chloride) produced the convulsive seizures with a latency of about 50 seconds.

Since zinc does not readily penetrate the blood-brain barrier, systemic administration is inadequate to investigate its direct effect on the brain. Intracerebral injection has, also, some disadvantages. For instance, it is difficult to assess accurately the concentration of the chemicals around the nerve-cells. Recently, however, Yamamoto and Kawai[27] reported the experiments, in which electrophysiological responses were successfully recorded from the slice prepared from the mammalian cortex and incubated in the artificial medium. It was reported in their paper[30] that the train of discharges (seizure discharge) was evoked, in the chloride-free medium, by a single shock applied to the slice of guinea pig hippocampus. Therefore, this method seems to be adequate for investigating the effects of zinc on the excitability of nerve-cell in the brain.

In the present paper, a study carried out along these lines is reported.

MATERIALS AND METHODS

After stunning the guinea pig by a blow on the back of the neck, the animal was sacrificed by a second blow on the back of the chest, which stopped the heart beat[25]. The brain was taken out and divided sagittally along the midline. The surface of the hippocampus facing the thalamus was exposed by removing the brain stem. A slice, 0.3~0.5 mm thick, was prepared from the exposed portion of the hippocampus using a thin bladed scalpel and a slicing guide[25]. The slice

*A study, carried out in the Department of Physiology under the supervision of Prof. G. Kawabata.

was unfolded on the teflon mesh with its cut surface, about 5×5 mm^2 in area, upward. The bathing medium used was the Krebs-Ringer tris buffer solution[25], which is called normal medium in the present study, about 37°C in temperature. The slice was incubated in this medium saturated with 95 % oxygen and 5 % carbon dioxide in an apparatus similar to that reported by Gibson and McIlwain[10]. During the recording of electrical activity, the surface level of the incubating medium was lowered to that of the teflon mesh. A pair of stimulating Ag-AgCl electrodes was placed on the slice, and the electrical stimulus, 5～10 Volt square pulse with 0.1 msec duration, was given every 5 seconds. A ball-tipped silver wire was placed on the slice, a few millimeter away from the site of stimulation, as the recording electrode. A reference one was placed in the medium.

The electrical activity was recorded by means of biophysical amplifier (RB-2, NIHON KOHDEN) and ink-writing electromagnetic oscillograph. The time constant of the amplifier was adjusted to 0.1 second.

RESULTS

Figure 1 shows the recorded potentials observed in normal medium. A single shock generated slow negative (upward) wave, 200～400 msec in duration. (The positive deflection in the figure was not treated in this paper.) Yamamoto and Kawai[29] reported that a similar potential was recorded from the dentate gyrus, in vitro, and suggested that the slow negative wave would be the excitatory postsynaptic potential (EPSP) in the layer of granular cells. In the present experiment, there was much variation in the latency, and the duration was very long. It is plausible, however, that they were caused by nuclear delay and/or by synaptic interaction, since the hippocampus has, histologically, the polysynaptic neuronal networks.

Fig. 1.

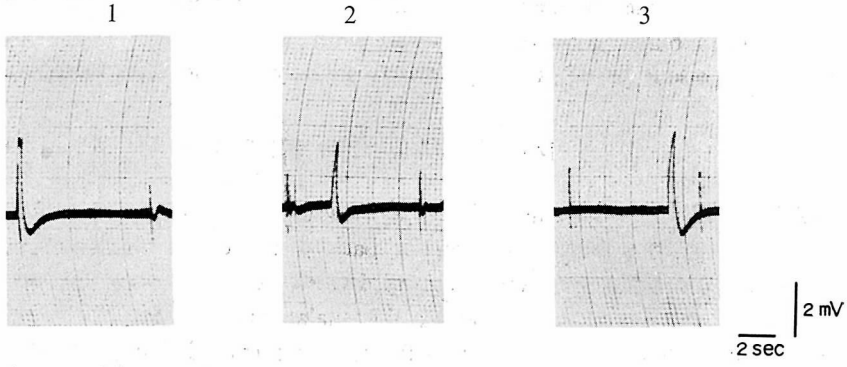

Fig. 1. The potentials evoked in the slice of guinea pig hippocampus by direct stimulation in the normal medium. Strength of the stimulus was kept constant throughout this experiment. Upward deflection indicated the negativity of the recording electrode. The fast diphasic deflections are the stimulus artifacts. (The same in the following figures.)

Fig. 2. 1 2 3

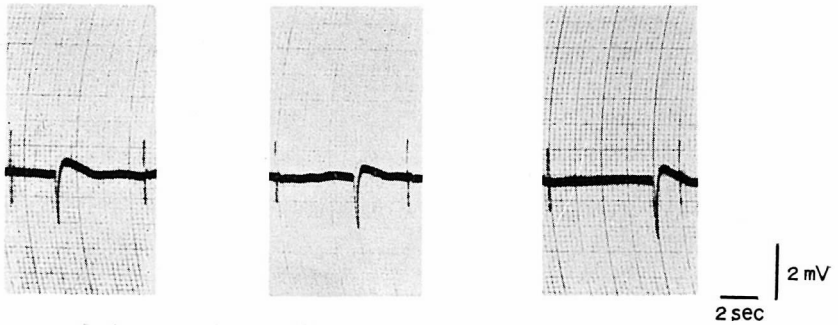

Fig. 2. The potentials when the slice was inverted and led from the surface of pia mater.

Figure 2 shows the records when the slice was inverted and the potential was led from the surface of pia mater. The potential was entirely reversed in electrical polarity. This phenomenon was also observed by the above authors[28], and would be explained by assuming that the adjacent field of the cut surface was depolarized by the transmitted impulses. In other words, the electrical stimulation strongly depolarized the cell soma and dendrites underlying the cut surface, to which (sink) the current flowed from the surface (source) of pia mater.

Figure 3 shows the effects of 1×10^{-6} M (molar) acetylcholine chloride (Ach). There was no obvious changes in the potential evoked by the stimulus.

Fig. 3. 1 2 3

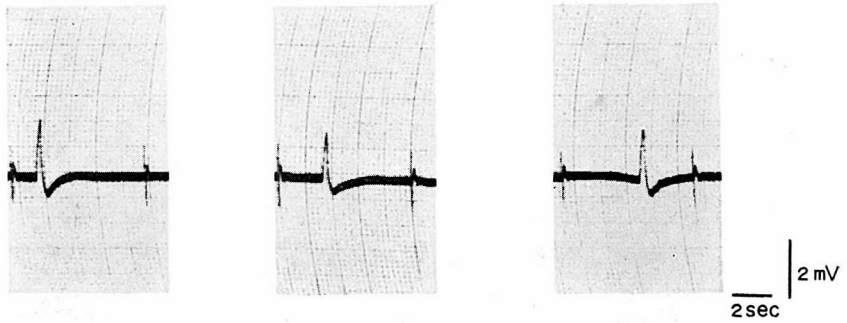

Fig. 3. Effects of Ach chloride. Record 1: Control response in the normal medium. Record 2: 2~3 min after 1×10^{-6} M Ach was added to the medium. Record 3: 2~3 min after washing the slice with normal medium.

Fig. 4.

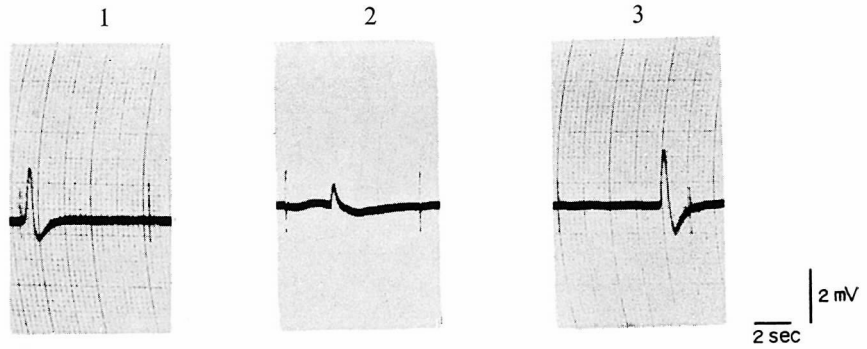

Fig. 4. Effects of Ach chloride together with physostigmine sulfate. Record 1: Control response in the normal medium. Record 2: 2~3 min after 1×10^{-6} M Ach chloride was added to the medium together with 1×10^{-6} M physostigmine sulfate. Record 3: 2~3 min after washing the slice with normal medium.

Figure 4 shows the effects of Ach when it was applied at a concentration of 1×10^{-6} M together with 1×10^{-6} M physostigmine sulfate. The potential was markedly depressed in amplitude, but it recovered in 10 minutes when the slice was washed with normal medium. The fact that Ach was effective in such a low concentration suggests that the drug may play some important physiological role in the hippocampus.

In order to recognize whether the potential represents a synaptic potential or not, chloride ion in the normal medium was completely replaced by propionate ion. In this chloride-free medium, the same stimulus provoked seizure discharges, and they were eliminated when the slice was washed with normal medium. These findings were illustrated in Fig. 5.

Fig. 5.

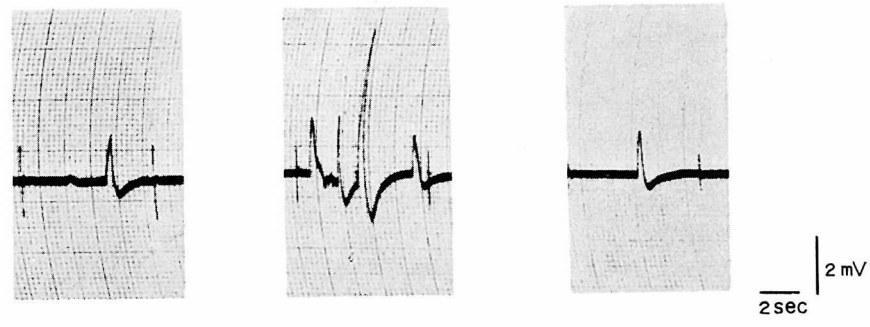

Fig. 5. Record 1: Control response in the normal medium. Record 2: Potentials in Cl-free medium, in which Cl ion was replaced completely with propionate ion. Record 3: 2~3 min after washing the slice with normal medium.

Eccles et al. reported that the inhibitory postsynaptic potential (IPSP) in the spinal motoneuron was brought about by an increase in permeability of postsynaptic

membrane to chloride ion, and that the same ionic mechanism would play a main role in generating IPSPs in the higher nervous center[1)3)13)].

In the experiment illustrated in Fig. 5, the seizure discharges would be caused by the decrease of chloride ion in the extracellular space. It is probable that, in the chloride deficient medium, the generation of IPSPs would be suppressed because of the high concentration gradient, or of the low permeability of that ion in the postsynaptic membrane. In any case, the generation of IPSPs has to be considered in the present experiment (see Discussion).

Figure 6 shows the effects of 1×10^{-4} M zinc chloride in the normal medium, the pH of which was about 7.0. The amplitude of potential was markedly depressed as compared with the control.

Fig. 6.

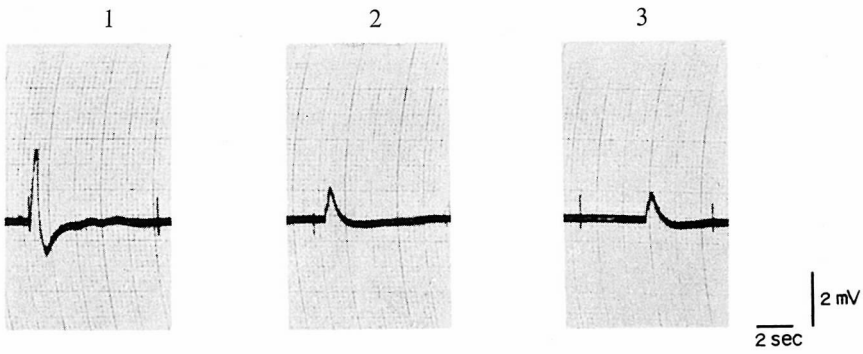

Fig. 6. Effects of zinc on the potential. Record 1: Control response in the normal medium. Record 2: 2~3 min after 1×10^{-4} M zinc chloride was added to the medium. Record 3: 2~3 min after washing the slice with normal medium.

Fig. 7.

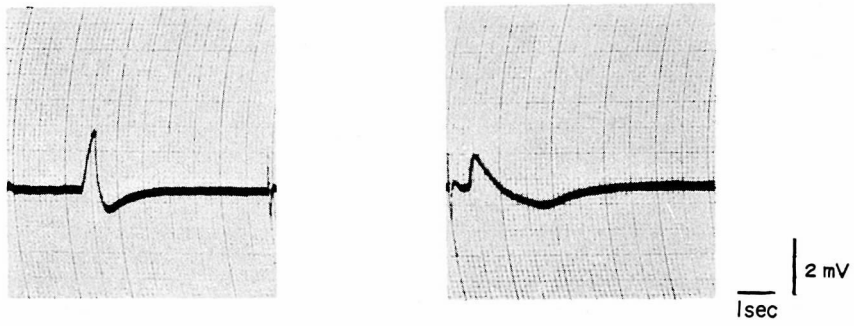

Fig. 7. Effects of zinc on the potential. Record 1: Control response in the normal medium. Record 2: 15 min after application of 1×10^{-4} M zinc chloride.

Another records are shown in Fig. 7, in which a prolongation of the potential-duration, especially the slowing of falling phase, can be seen. The potential, however, did not recover by 10 minutes, when the slice was washed with normal

medium. Figure 8 is a schematic illustration of the potential, which was obtained at 15 th minute after the application of 1×10^{-4} M zinc chloride to the tissue.

Fig. 8.

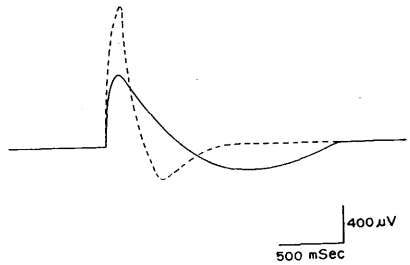

Fig. 8. Schematic drawing of the shape of the potential after application of 1×10^{-4} M zinc chloride. Each tracing was obtained from ten records with three preparations. Dotted curve was the control potential in the normal medium. Solid curve, the potential after 15 min in zinc medium.

The results represented in Figs. 6 and 7 suggest that zinc induced irreversible changes in the evoked potential of the hippocampus.

Fig. 9.

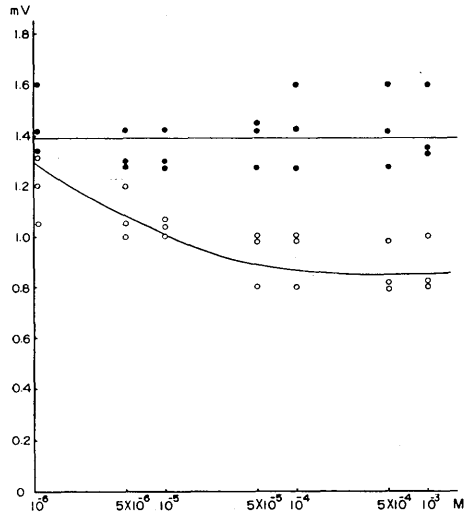

Fig. 9. The relationship between the amplitude of the potential and zinc concentrations. Abscissa is logarithmic scale of zinc concentration and ordinate, evoked potential in millivolt. Solid circles show an experiment with normal medium, and open ones, with zinc chloride medium. Each circle indicates the mean value of ten records from a preparation.

Fig. 10.

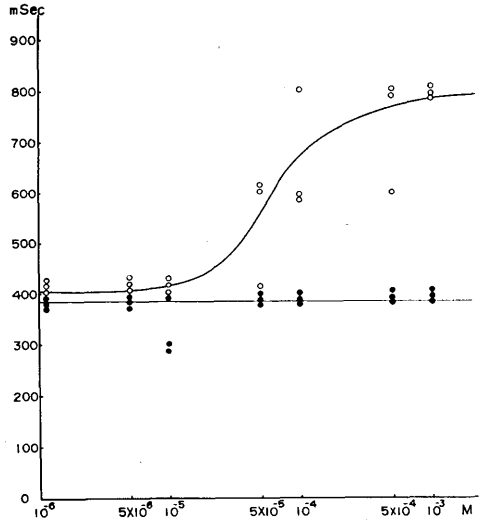

Fig. 10. The relationship between the potential-duration and zinc concentrations. Abscissa is logarithmic scale of zinc concentration and ordinate, evoked potential-duration in millisecond. Solid circles show an experiment with normal medium, and open ones, with zinc chloride medium. Each circle indicates the mean value of ten records from a preparation.

Figure 9 and Fig. 10 show the relation between the potential and zinc concentrations. In these figures, the abscissa is the logarithm of the zinc concentration. The ordinate is the amplitude of evoked potential in millivolt in Fig. 9, and potential-duration in millisecond in Fig. 10, respectively. Solid circles shows an experiment with normal medium, and open ones, with the medium containing zinc chloride. Each circle indicates the mean value of ten records from one preparation. The depression of the potential-amplitude and the prolongation of potential-duration are shown clearly in these figures.

DISCUSSION

A series of studies concerning the relation between zinc metabolism and brain excitability have been carried out in our laboratory[8)9)11)19)20)26)], and the author has an impression that the zinc must have some important role on the synaptic transmission in the hippocampus. The special histological geometry of the hippocampal cells and the laminar arrangement of synapses are the favourable conditions for the present study.

It was reported by Mathisen and Blackstad[18)] in the hippocampus of albino rat, that the high activity of acetylcholinesterase (AchE) was found histochemically in the layers receiving cingulum fibres or where axonal arborizations of basket cells form axo-somatic synapses. On the other hand, Otsuka et al.[21)] and Fujii[8)9)] reported in mice that systemically or intracerebrally injected ^{65}Zn was preferentially localized in h_3 (CA_3), h_4 (CA_4) and h_5 region of hippocampus. (Notation ; $h_1 \sim h_5$ is a subdivision after Rose[22)] and $CA_1 \sim CA_4$, after Lorente de Nó.[16)]) These regions are likely to be coincident with the layers above mentioned. Watanabe[26)] carried out further histochemical study and indicated that the intracerebral injection of zinc diminished the activity of AchE in 4th or 5th day, when the injected zinc was distinctly localized in these layers. Therefore, it is probable to assume that the inhibition of AchE activity caused by zinc would result in the abnormal accumulation of Ach.

In the present study, although the simple administration of Ach did not affect the evoked potential in the slice, it was effective when applied together with physostigmine, a well-known inhibitor of AchE. The potential was markedly depressed in amplitude, but recovered after washing the slice with the normal medium discontaining the drugs. The effective concentration of Ach was considerably low (10^{-6} M). These results are in good agreement with those reported by Yamamoto and Kawai[27)]. They interpreted the evoked potential (negative wave) as the EPSPs in the layer of granular cells and apical (proximal) dendrites. Thereby, the high concentration of Ach, resulted from the inhibition of AchE, might depress the synaptic activation of cell-soma and/or of dendrite. In this connection, Bullock et al.[2)] reported in the giant axon and the fin nerve of squid

that the nerve action potential was abolished when the AchE was inactivated by eserine. Katz and Thesleff[15], on the other hand, observed no depolarization in the endplate of frog sartorius when the high concentration of Ach was applied for a long period. The latters interpreted the results as a 'desensitization' produced by Ach, i. e. the Ach-receptor in the membrane was changed from the effective state to the refractory one.

The effects of zinc on the evoked potential, in the present study, were the depression of the potential in amplitude and the prolongation of its duration. These effects were irreversible, i. e. both the amplitude and the duration did not recover when the slice was washed with normal medium. The depression of the amplitude was the same phenomenon as that of Ach application together with the physostigmine. The inhibition of AchE activity by the intracerebrally injected zinc is described above. Therefore, it seems likely that the high concentration of Ach in the synaptic regions would be resulted, also in the case of zinc application. In this connection, Sandow et al.[23] reported in the frog sartorius muscle that the absence of muscle response to indirect stimulation was due to a specific blockade of transmission caused by zinc at the neuromuscular junction. They suggested that the blockade was mediated by a decrease in the endplate potential.

Mashima and Washio[17], however, reported in the frog semitendinosus muscle that the zinc caused the increase in the effective duration of action potential. They postulated that the main cause of this increase was the slowing of the falling phase of action potential, due to the delay in the increase of potassium conductance of active membrane. Edman and Grieve[4] reported the same experimental results in the frog sartorius muscle. According to Hodgkin-Huxley's ionic theory, the retardation of the increase of potassium conductance means the prolongation of refractory period. In fact, Issacson and Sandow[12] reported in the frog sartorius muscle that the administration of zinc chloride ($0.005 \sim 0.5$ mM) caused the remarkable increase in the absolute refractory period and that the ability to discharge repetitively was depressed in the zinc-treated muscle fibre. Although careful criteria are necessary to discuss the present study referring these results obtained with muscle fibre, it is probable that the zinc primarily affects the postsynaptic membrane and/or neuron-soma and changes its excitability. If this change were not uniform in each nerve cell, there would be the desynchronization in the generation of EPSPs described above. The desynchronization is reflected, in the present study, as the decrease of the evoked potential in amplitude and as the prolongation of its duration.

Lastly, the inhibitory postsynaptic potential (IPSP) has to be considered. Kandel et al.[14] reported that very large prolonged IPSPs were induced in the pyramidal cells of CA_2, CA_3, and CA_4 regions of the cat hippocampus by stimulation of the fimbria. Andersen et al.[1] kept up this work and found that virtually all of the pyramidal cells in CA_3 region responded to the stimulation of fimbria, septum,

commissura or local afferent fibres by the IPSP of the same character. The latter workers reported that there was, perhaps, an interneuron on the inhibitory pathway and that the IPSP was associated with a powerful inhibitory action on the generation of impulses, both those arising spontaneously and those evoked by appropriate stimulation. Although there is no pyramidal cell layer in the slice of guinea pig hippocampus, investigated in the present study, the generation of train of discharges in the chloride-free medium (fig. 5) suggests that the stimulation of local afferent fibres would bring about an inhibitory synaptic action on or close to the granular cell somata. It is possible that the zinc would have some effects on this inhibitory action. However, the author has few experimental result to consider this effect further.

In conclusion, there are three possible mechanisms concerning the effects of zinc on the generation of impulses in the hippocampus : i. e. (1st) The AchE in the synaptic region is depressed by zinc and the resulted high concentration of Ach blocks the impulse-transmission between the presynaptic fibres and nerve cells. (2nd) Zinc primarily affects the postsynaptic membrane and/or neuron-soma and changes the excitability of nerve cell. (3rd) The inhibitory synaptic action of afferent impulse is affected by the zinc. It is easy to suppose that abnormal impulse conduction and desynchronization in the activities of nerve cells are liable to be produced by these mechanisms, operated either simply or together. Therefore, the assumption that the increase of zinc in the hippocampus would induce generalized seizures is likely to be reasonable. However, further study would be required to decide the mechanism by which zinc brings about the functional changes in the hippocampus or in the whole brain.

SUMMARY

1. A thin slice was prepared from the hippocampus of guinea pig. It was activated by the direct electrical stimulus and the potential was recorded at the dissected surface nearby the stimulating point.

2. The recorded potential, called as evoked one in the present paper, was biphasic : i. e. a slow negative wave of 200 $\sim$ 400 msec in duration was followed by a positive one of about the same duration and small amplitude. The potential reversed in polarity, when the slice was inverted and it was recorded from the surface of pia mater.

3. Acetylcholine (1×10^{-6} M), in the presence of physostigmine, depressed the evoked potential in amplitude, but the potential recovered in 10 minutes when the slice was washed with the Krebs-Ringer's solution discontaining the drug (normal solution).

4. When chloride ion in the incubating medium was completely replaced by propionate ion, the same stimulus provoked a train of discharges, which could not

be observed after washing with the normal solution.

5. In the presence of zinc chloride, the decrease of amplitude and the prolongation of the duration of the potential, especially the slowing of falling phase, were observed. The threshold concentration was 5×10^{-6} molar. The changes of the potential did not recover after washing.

6. A block of impulse-transmission at the presynaptic terminal, a change in excitability of postsynaptic membrane and/or of neuron-soma and an alteration of inhibitory synaptic action of afferent impulse were discussed as the possible affective mechanisms of zinc.

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