RECURRENT FACILITATION OF SPINAL REFLEXES

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ABSTRACT

Antidromic volleys in muscle nerves may facilitate monosynaptic reflexes originating from neighboring motoneurons. This facilitation has been studied in spinal cats. It is at its peak with a conditioning-test interval of 20 to 30 msec., and can last 50 to 100 msec. The threshold of facilitation is about the same as that of recurrent inhibition. Both phenomena appear to be activated by stimulation of the large motor axons. The latency of facilitation seems to be longer than that of recurrent inhibition by approximately 1 msec., suggesting the presence of at least one more synaptic delay. Facilitation often follows an inhibition of variable depth and duration. Frequently, however, the facilitation is not preceded by inhibition, and therefore it cannot be a rebound effect. The pharmacological properties of facilitation resemble those of recurrent inhibition. Dihydro-beta-erythroidine[1] partially blocks facilitation; the peak is decreased and occurs earlier, and the duration is shortened. Eserine increases the duration of facilitation and inhibition and sometimes enhances their magnitude. It is concluded that recurrent facilitation is mediated by the cholinergic axon collaterals, and that at least two interneurons are located between collateral and motoneuron. Possible mechanisms of facilitation are discussed.

INTRODUCTION

Antidromic stimulation of motor axons results in the conditioning of motoneurons lying in the same general region of the spinal cord as those neurons whose axons have been stimulated; the conditioning can take the form of inhibition or facilitation (Renshaw, 1941). Antidromic, or recurrent, inhibition has been studied by Renshaw (1941), Lloyd (1951), Eccles, Fatt, and Koketsu (1954), and others. It is now believed that the inhibitory action is mediated by the recurrent axon collaterals. Liberation of acetylcholine at their terminations results in the activation of interneurons, originally described by Renshaw (1946), now designated as Renshaw cells (Eccles, Fatt, and Koketsu, 1954). Renshaw cells inhibit the discharge of motoneurons on which they terminate. The physiology and pharmacology of this pathway have been studied in detail by Eccles, Fatt, and Koketsu (1954) and Eccles,

[1] The dihydro-beta-erythroidine hydrobromide used in these experiments was kindly provided by the Merck Sharp and Dohme Research Laboratory, Rahway, New Jersey.

Eccles, and Fatt (1956). Recently it has been demonstrated that the recurrent inhibitory curve is partially blocked by an agent which depresses cholinergic transmission, dihydro-beta-erythroidine (Brooks and Wilson, 1958), as would be expected from the observed action of dihydro-beta-erythroidine on Renshaw cell discharge.

While recurrent inhibition has been thoroughly investigated, the facilitation described by Renshaw (1941) has been largely ignored. However, evidence of facilitatory action following antidromic stimulation has been described. In some cases, antidromic inhibition may reverse, at a conditioning-test interval of approximately 40 msec., to a period of facilitation

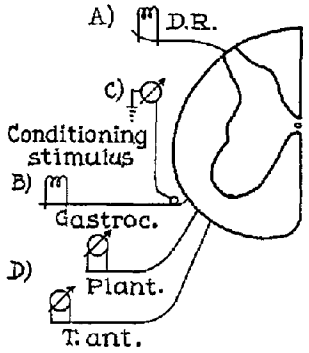

FIG. 1. Diagram of the usual experimental arrangement. For further explanation see text.

which then lasts another 75 msec. (Lloyd, 1951). When antidromic inhibitory action is tested by its effect on tonic discharge in single ventral root filaments, in excitable preparations, inhibition is often followed by double firing of the motoneuron (Granit, Pascoe, and Steg, 1957). This phenomenon has been attributed to rebound (Granit, Pascoe, and Steg, 1957).

The present experiments represent a study of some physiological and pharmacological properties of the facilitation produced by antidromic volleys. A preliminary report of these results has already been presented (Wilson, 1958 *a*).

Methods

Cats, with the spinal cord transected in the upper cervical region, were used in all experiments. The initial stages of the operation were carried out under ether; anaesthesia was discontinued 3 to 4 hours before the experiment was begun.

Laminectomy was performed in the usual manner. The dorsal roots on the side to be studied were cut from L4 down to at least S3. In experiments where eserine was to be injected the dorsal roots were severed bilaterally. The spinal cord was then covered by a pool of warm mineral oil, whose temperature was kept steady throughout the experiment. Leg nerves were dissected for stimulation and recording.

A maximal reflex was obtained by stimulation of the appropriate dorsal roots or rootlets, and recorded in muscle nerves. The antidromic conditioning volley was applied to selected muscle nerves. In a number of experiments it was desirable to condition the response of two different motoneuron pools with the same antidromic volley. Such an experimental arrangement is shown diagrammatically in Fig. 1. By appropriate selection of roots, it was usually possible to evoke a response both in plantaris and in one or more of the branches of the deep peroneal nerve with the same dorsal root test shock (Fig. 1 A). The monosynaptic reflexes in plantaris and in, for example, tibialis anterior, were then recorded in the peripherally cut muscle nerves (Fig. 1 D). An antidromic volley in one or both gastrocnemius nerves (Fig. 1 B) conditioned both of these test reflexes: the usual finding was that the response in plantaris was inhibited, that in tibialis anterior facilitated. In some experiments it was necessary to measure the size of the antidromic volley. This was done by placing an electrode on the ventral root near the point of its exit from the cord (Fig. 1 C). The height of the spike was determined by measuring the peak to peak amplitude of the triphasic potential.

At the end of many experiments the approximate longitudinal distribution of various nuclei within the spinal cord was determined. This was done by severing the ventral roots and stimulating their peripheral end. By recording in the peripheral nerves the potentials evoked in this manner, it was possible to obtain an approximate measure of the segmental distribution of the motoneuron pools utilized during the experiment.

All preparations were kept paralyzed by intravenous injections of flaxedil. Other drugs, such as dihydro-beta-erythroidine and eserine sulfate, were also administered intravenously.

RESULTS

Facilitation of the reflex discharge of various motoneuron pools by antidromic stimulation of muscle nerves has been studied in a number of cats. The test system consisted of the whole deep peroneal nerve or of its branches; in almost all cases various branches of the tibial nerve were used for conditioning. As pointed out by Renshaw (1941), the facilitation is at its peak when the interval between the conditioning and test shocks is of the order of 20 to 30 msecs. The duration of the effect varies. In some cases it may be as short as 50 msec., while at other times the facilitation may last as much as 90 to 100 msec. The mean duration of 31 cases was approximately 70 msec. The amplitude of the facilitation is variable, but is often 20 per cent or more; cases in which the facilitation exceeded 45 to 50 per cent have been obtained. A typical facilitation curve is shown in Fig. 2. While antidromic facilitation is seen frequently and may be quite strong, it is less powerful and is observed less regularly than is recurrent inhibition.

Renshaw (1941) found that facilitation usually followed a brief period of inhibition. Such effects have often been observed. There seems to be no relation between the depth of inhibition and the presence or absence of a subsequent facilitatory component. Facilitation that is not preceded by inhibition can also be obtained, as shown in Figs. 2 and 4.

Relation between the Size of the Antidromic Volley and the Degree of Inhibition and Facilitation:

The present series of experiments has confirmed the previous finding that recurrent inhibition has a very low threshold, and has shown that the same is true in the case of antidromic facilitation.

Several experiments have been performed in which antidromic stimulation of a given peripheral nerve resulted in inhibition of one motor nucleus and facilitation of another. In some of these the antidromic spike was measured by an electrode placed on the appropriate ventral root, near the point of its exit from the cord. The interval between the conditioning and test shocks

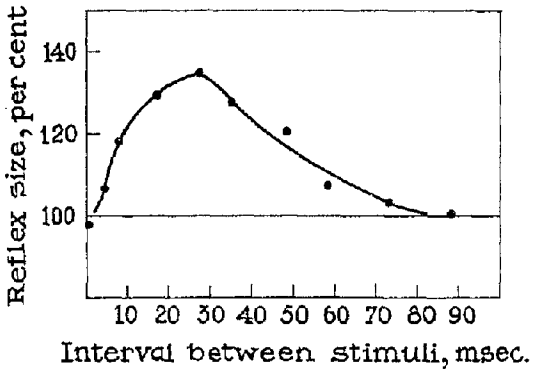

FIG. 2. Facilitation of reflex in deep peroneal nerve by an antidromic volley in the nerves to triceps surae. The test reflex was evoked by a stimulus to DR L6 and DR L7. Stimulus frequency, 0.3/sec. Oil pool temperature 35.5°C.

was kept constant, and the strength of the antidromic stimulus was gradually increased. The result of such an experiment is shown in Fig. 3. In this particular experiment definite inhibition and facilitation were present with stimulus strengths which evoked an antidromic spike less than 10 per cent maximal. Both inhibition and facilitation increased in degree as the strength of the conditioning stimulus was increased.

The shape of the curves which relate size of antidromic spike with degree of inhibition and facilitation has varied in different experiments; inhibition and facilitation do not necessarily grow at the same rate and may reach their maximum with different levels of antidromic spikes. In one experiment both inhibition and facilitation reached their peak when the antidromic spike was only 30 per cent of maximal. All the experiments, however, are consistent in two respects: (1) Inhibition and facilitation both have low thresholds, and the thresholds of the two phenomena are approximately the same. (2) Inhibition and facilitation both increase in intensity as the size of the antidromic volley is increased.

Latency of Recurrent Inhibition and Facilitation:

The latencies of recurrent inhibition and facilitation have been measured in a number of experiments. Renshaw (1941) has shown that inhibition of reflexes in the crural nerve by antidromic volleys in other branches to quadriceps is present when the test shock follows the conditioning volley by as little as 0.6 to 0.7 msec. In the present experiments, where inhibition was

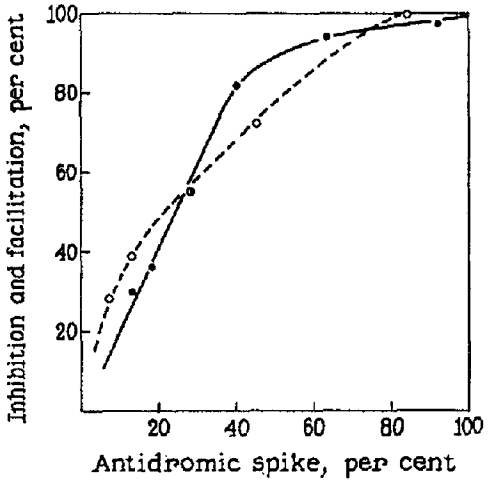

FIG. 3. Relation between size of antidromic spike and degree of facilitation and inhibition. An antidromic volley in the medial gastrocnemius nerve facilitated the reflex in tibialis anterior (○), inhibited the reflex in lateral gastrocnemius (●). The maximal conditioning that could be obtained was considered as 100 per cent. The conditioning-test interval for facilitation was set at 22 msec., and maximum facilitation reached was 18 per cent. The shock interval for inhibition was 6.5 msec.; maximum inhibition was 33 per cent. Stimulus frequency 0.4/sec.; temperature 36.5°C.

most often evoked by antidromic stimulation of the nerve to one or both heads of gastrocnemius, inhibition was usually present when the stimulus interval was of the order of 1.5 msec.

The latency of recurrent facilitation is more difficult to measure, as the facilitation is often preceded by an inhibition of variable duration and magnitude. In some experiments, however, facilitation has been obtained without any preceding inhibition. A result typical of several cases is illustrated in Fig. 4. The same test shock evoked monosynaptic reflexes in the nerves to plantaris and to extensor longus. Antidromic stimulation of the nerves to triceps surae inhibited the former and facilitated the latter. Inhibition started at a stimulus interval of about 1.6 msec. Facilitation did not begin till an interval of 2.8 to 3.0 msec. was reached. Stimulation of the cut ventral roots

at the end of the experiment showed that the extensor longus nucleus was located 82 per cent in L7, 18 per cent in L6. The plantaris nucleus was 94 per cent in L7, 3 per cent in each S1 and L6. In cross-section, these two nuclei lie at approximately the same distance from the gastrocnemius nucleus (Romanes, 1951). The distance of the conditioning pathway (to the spinal cord) was the same in the case of inhibition and facilitation; the distances inside the cord also seem to have been approximately the same. It appears that the latency of facilitation was, in this experiment, approximately 1.2 msec. longer than was the latency of inhibition.

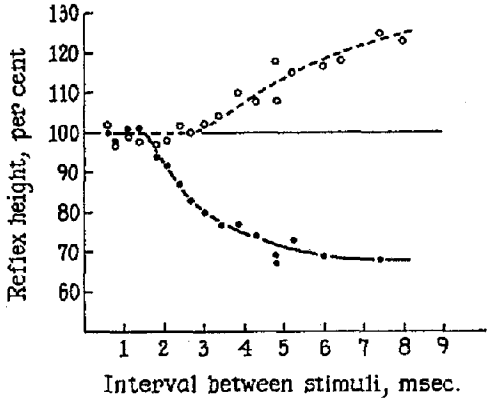

FIG. 4. Latency of recurrent inhibition and of facilitation. An antidromic volley in the nerves to triceps surae facilitated the reflex in extensor longus (○), inhibited the reflex in plantaris (●). Both test reflexes evoked by stimulation of rootlets of DR L7 and DR L6. Stimulus frequency 0.4/sec.; temperature 36.5°C.

Effect of Dihydro-beta-erythroidine on Antidromic Facilitation:

Dihydro-beta-erythroidine cuts short the duration of the repetitive discharge of Renshaw cells (Eccles, Fatt, and Koketsu, 1954). The hyperpolarization of motoneurons produced by antidromic stimulation is similarly affected: the initial part of the hyperpolarization is largely unchanged, the summit is depressed and reached earlier, and the late part of the potential is sharply reduced (Eccles, Fatt, and Koketsu, 1954). It has recently been shown that the inhibitory curve obtained by conditioning a monosynaptic test reflex with an antidromic volley in the appropriate ventral root or muscle nerve is modified in the same manner by dihydro-beta-erythroidine (Brooks and Wilson, 1958). Dihydro-beta-erythroidine exerts a similar action on the facilitation produced by antidromic volleys, as illustrated in Fig. 5. A monosynaptic reflex in one branch of the deep peroneal nerve was facilitated by antidromic stimulation of the nerves to triceps surae. Facilitation reached a

peak of 30 per cent at an interval of about 25 msec., and the reflex was back at the control level at about 80 msec. Intravenous injection of 1 mg./kg. dihydro-beta-erythroidine did not change the early course of the facilitation. However, just as is the case with recurrent inhibition, dihydro-beta-erythroidine sharply reduced the peak of facilitation; this peak occurred at 10 msec. instead of 30, and much of the later facilitation was removed. Qualitatively similar results have been obtained in several experiments. Injection of dihydro-beta-erythroidine always resulted in partial block of the facilitation; the amount of facilitation remaining varied from one experiment to another.

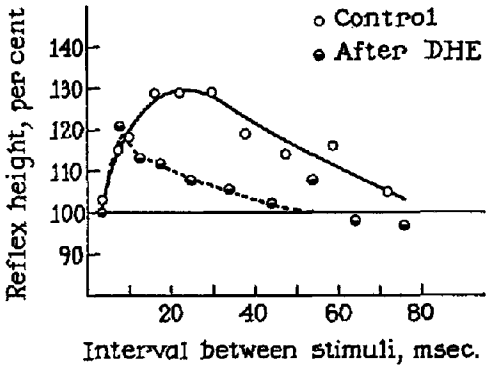

FIG. 5. Effect of dihydro-beta-erythroidine on facilitation. The monosynaptic reflex in a branch of the deep peroneal nerve was conditioned by an antidromic volley in the nerves to triceps surae (○). The second curve (◒) shows the effect of 1 mg./kg. dihydro-beta-erythroidine. Stimulus frequency 0.3/sec.; temperature 35.5°C.

Effect of Eserine on Antidromic Facilitation and Inhibition:

Following intravenous injection of eserine the duration of Renshaw cell discharge is greatly prolonged, while the early part of the discharge is not significantly altered (Eccles, Fatt, and Koketsu, 1954). The effect of eserine on antidromic inhibition and facilitation has been investigated in several experiments. These experiments are complicated by the fact that even very small doses may modify the size of reflex discharge. Despite this effect of the drug, some consistent results on the conditioning power of antidromic volleys have been observed.

Eserine increases the duration of the recurrent inhibitory curve, as shown in Fig. 6. Intravenous injection of 0.05 mg./kg. eserine increased the size of the test reflex, in this case the monosynaptic reflex in the nerve to biceps anterior. The same absolute depth of inhibition was reached at the peak of the curve. Over a considerable part of the decaying portion of the curve, the

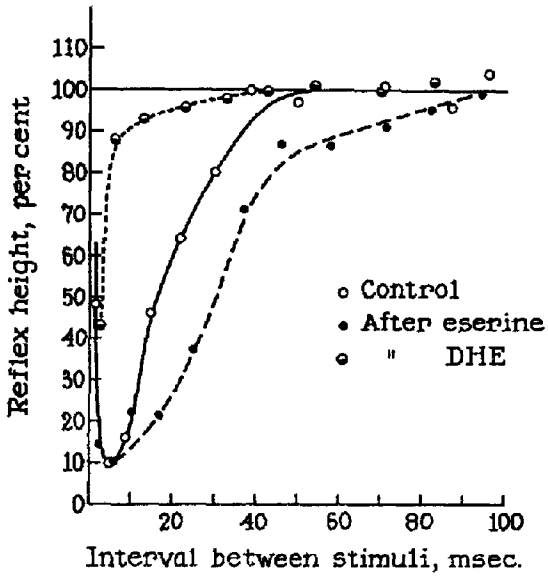

FIG. 6. Effect of eserine on recurrent inhibition. The monosynaptic reflex in the nerve to biceps anterior was inhibited by antidromic stimulation of biceps-semitendinosus. The graphs show control inhibition (○), inhibition after 0.05 mg./kg. eserine, intravenously (●), and inhibition after 0.5 mg./kg. dihydro-beta-erythroidine (◒). A second injection of 0.05 mg./kg. eserine had been given before the erythroidine was administered. Stimulus frequency 0.4/sec.; temperature 35.5°C.

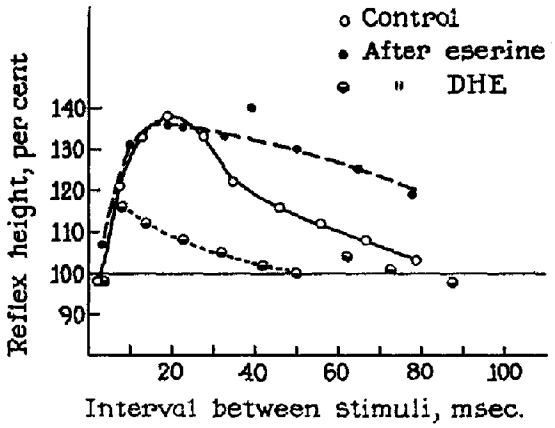

FIG. 7. Effect of eserine on facilitation. The monosynaptic reflex in the deep peroneal nerve was conditioned by an antidromic volley in triceps surae. The first curve (○) shows control facilitation. The second (●) shows the effect of the last of four doses of eserine. The first three doses were 0.05 mg./kg., the last dose 0.1 mg./kg. The third curve (◒) demonstrates the effect of 0.7 mg./kg. dihydro-beta-erythroidine. Stimulus frequency 0.4/sec.; temperature 35.5°C.

inhibited test reflex was smaller than it had been before eserine, despite the increase in the size of the uninhibited test reflex.

Similar results have been obtained with facilitation. It has been possible to inject a dose of eserine that did not modify the size of the test reflex, and yet was effective in changing antidromic facilitation. In this experiment successive injections of 0.05 mg./kg. eserine had no effect on the peak of the facilitation, but did slow its rate of decay and increase its duration. The final effect of 4 doses, the last one 0.1 mg./kg., is demonstrated in Fig. 7. A subsequent injection of 0.7 mg./kg. dihydro-beta-erythroidine had the usual action on the facilitation.

DISCUSSION

Antidromic stimulation of motor nerves clearly can bring about pronounced enhancement of the discharge of neighboring nuclei. It is of interest to compare the properties of this antidromic facilitation with those of recurrent inhibition.

With increasing antidromic volleys, interneurons, of the type since described as Renshaw cells, fire with decreasing latency, for a longer duration, and at a higher frequency (Renshaw, 1946). Renshaw cells are activated by stimulation of nerve fibers whose thresholds correspond to those of the large motor fibers, and no added effect is obtained with stimuli strong enough to excite the small motor axons (Eccles, Fatt, and Koketsu, 1954). Recurrent inhibition has also been described as appearing as the threshold for A fibers is reached; it may continue to increase in intensity until the stimulus reaches a value maximal for A fibers (Renshaw, 1941). Results similar to these have been obtained in the present experiments. In addition, it is clear that antidromic facilitation has approximately the same threshold as does recurrent inhibition. Both phenomena, therefore, are evidently activated by stimulation of the same group of axons, namely the large motor fibers.

A further similarity between the pathways subserving recurrent inhibition and facilitation is revealed by pharmacological studies. As previously described, dihydro-beta-erythroidine partially blocks both Renshaw cell discharge (Eccles, Fatt, and Koketsu, 1954) and the recurrent inhibitory curve (Brooks and Wilson, 1958). This drug has a similar action on the antidromic facilitation curve. Furthermore, eserine, which increases the duration of both Renshaw cell discharge and the antidromically evoked IPSP (Eccles, Fatt and Koketsu, 1954) increases the duration of both inhibition and facilitation. The close resemblance between the effects of eserine and dihydro-beta-erythroidine on inhibition and facilitation is shown in Figs. 6 and 7.

The pharmacological data, together with the evidence from the relation between size of antidromic volley and degree of facilitation and inhibition, suggest that the first link in the antidromic facilitation pathway consists of the cholinergic collaterals of the large motor fibers. For this reason the term

recurrent facilitation is suggested to describe the reflex enhancement brought about by antidromic conditioning.

A difference between the inhibitory and facilitatory pathways is revealed by measurements of the latencies of the two effects. As described in the second part of Results, several experiments show that the latency of recurrent facilitation is longer than that of recurrent inhibition by approximately 1, and sometimes more, msec. This suggests that the pathway for facilitation contains at least 1 more interneuron than does the inhibitory path.

Two main explanations appear to exist for the mechanism of recurrent facilitation, and the available data do not make it possible to distinguish between them. Firstly, it is possible that axon collaterals terminate on cells which excite other interneurons, which in turn facilitate motoneurons. Secondly, it may be that instead of true facilitation the reflex enhancement is actually due to a removal of inhibition.

If a background of inhibitory activity, depressing the excitability of a motoneuron pool, is lessened, the response of this pool to a test shock will be enhanced. This kind of effect has been postulated as responsible for the increase in monosynaptic reflex size often obtained with small doses of mephenesin (Taverner, 1952, Brooks and Koizumi, 1953) and meprobamate (Wilson, 1958 *b*). Renshaw cells evidently end not only on motoneurons but also on interneurons, since the spontaneous activity of many interneurons, scattered within the grey matter, can be inhibited for as long as several hundred milliseconds by an antidromic shock (Frank and Fuortes, 1956). Frank and Fuortes have suggested that this inhibition may be caused by a subsequent link in a pathway starting with axon collaterals. A simple hypothesis, consistent with the available data, is that axon collaterals activate Renshaw cells which, in turn, may terminate directly on motoneurons, inhibiting them; Renshaw cells may also terminate on interneurons, inhibiting them and thus disinhibiting motoneurons previously kept depressed by interneuron discharge. It is of interest that there is in the *Limulus* eye an inhibitory interaction between adjacent receptor units (Hartline, Wagner, and Ratliff, 1956) which has been compared to the inhibition between adjacent motoneurons in the spinal cord (Brooks and Wilson, 1958). Disinhibition, simulating facilitation, has been found in this *Limulus* preparation (Hartline and Ratliff, 1957). The hypothesis that antidromic facilitation may actually be a disinhibition due to Renshaw cell discharge is not necessarily in conflict with the observation that facilitation usually has a longer time course than does the inhibition, which is also due to Renshaw cell discharge. While recurrent inhibition usually has a duration of 40 to 50 msec., Renshaw cell discharge may, on occasion, last longer than 100 msec. (Eccles, Fatt, and Koketsu, 1954). Possibly the longer time course of facilitation may be due to properties of the second or third synapse in the facilitation pathway.

The facilitation studied in the present experiments clearly cannot be due to rebound since in a number of cases it has been seen without any previous inhibition.

The distribution of recurrent facilitation within the spinal cord, and the

occurrence of mixed inhibitory and facilitatory actions, are still under investigation. The function of facilitation is not yet clear. However, it must be considered together with the other influences which act to modify the excitability of motoneurons.

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