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Glutamate inhibitory action of matrine at the crayfish neuromuscular junction

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1 The effect of some alkaloids from *Sophora flavescens* (matrine, oxymatrine and N-methylcytisine) on glutamate-induced responses was investigated using electrophysiological techniques at the crayfish neuromuscular junction.

2 At concentrations greater than 0.1 mM, matrine depressed both glutamate-induced responses and neurally evoked excitatory junctional potentials. Oxymatrine was less powerful than matrine, and N-methylcytisine was not effective. Matrine also depressed quisqualate-induced responses at this site, but did not have an effect on γ-aminobutyric acid (GABA)-induced responses. Matrine had no influence upon either the resting membrane potential or the input resistance of the crayfish opener muscle.

3 The inhibition of the glutamate-induced response by matrine was not affected by the membrane potential of the muscle fibre.

4 Matrine reduced the size of extracellularly recorded excitatory junctional potentials without affecting their quantal content.

5 Matrine did not affect the decay of extracellular excitatory junctional currents at the resting membrane potential.

6 The results presented clearly demonstrate that matrine has an inhibitory action on glutamate-induced responses.

Introduction

The root of the plant, *Sophora flavescens* Aiton, contains several alkaloids such as matrine, oxymatrine and N-methylcytisine (Figure 1). It is known that the crude extract of the plant kills maggots and harmful insects in oxen and horses. In herb medicine, it has been used as a stomachic, diuretic, antipyretic and anodyne. Recently, the effect of these alkaloids was tested on the motor activity of helminth worms

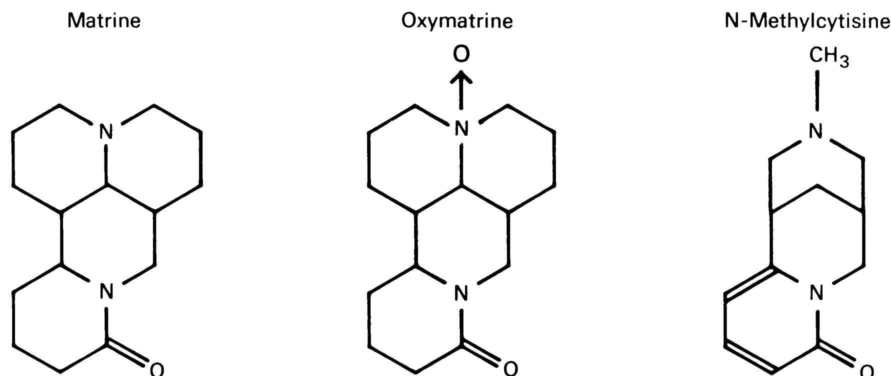

Figure 1 Chemical structures of alkaloids from the root of the plant, *Sophora flavescens* Aiton.

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(Anantaphruti *et al.*, 1982; Terada *et al.*, 1982). The results of those experiments prompted us to investigate the possible action of the alkaloids on neuromuscular transmission in the crayfish. The crayfish neuromuscular junction provides us with insight into chemical transmission processes at many synapses (Katz, 1966; Shinozaki, 1980), and is a useful model for studying the mechanism of action of drugs in the mammalian central nervous system. Since the crayfish neuromuscular junction is sensitive to glutamate which is a candidate for the excitatory transmitter, this junction is useful for the examination of the effect of drugs on the glutamatergic synapses. In the present study, electrophysiological techniques were used to elucidate pharmacological properties of these alkaloids at the crayfish neuromuscular junction and to obtain information on whether these alkaloids may be useful as a pharmacological tool in the field of neuroscience.

Methods

The methods used were similar to those described previously (Ishida & Shinozaki, 1980; Shinozaki & Ishida, 1983a). The opener muscle of the dactyl in the first leg of the crayfish (*Cambarus clarkii*) was used. Potential changes produced by nerve stimulation and by application of drugs were recorded either intracellularly from the muscle fibre with a 3 M KCl filled microelectrode or extracellularly from the neuromuscular junction with a 2 M NaCl filled microelectrode. The intracellular microelectrode was in-

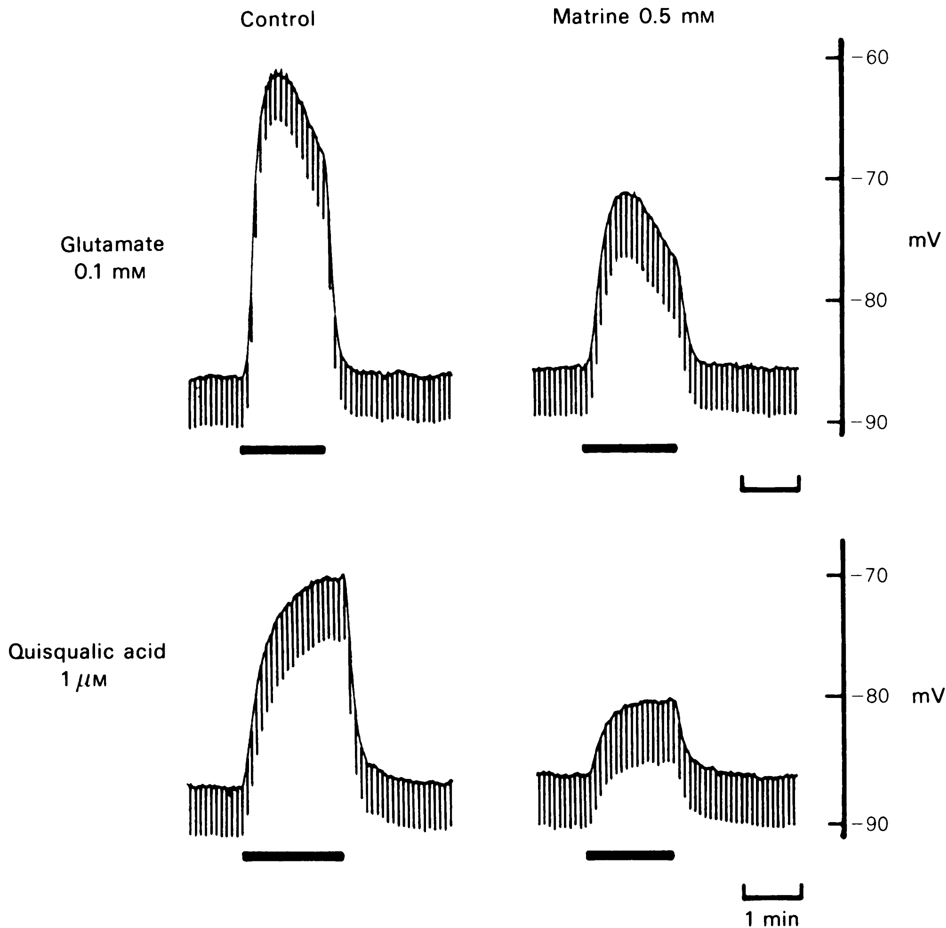


Figure 2 Matrine depressed the glutamate- and quisqualate-induced responses in the crayfish opener muscle. Glutamate or quisqualic acid was added to the bathing solution at a concentration of 0.1 mM or 1 μM, respectively, for the period indicated in the absence and presence of 0.5 mM matrine. The vertical lines represent the electrotonic potentials produced by hyperpolarizing current pulses delivered from a constant current device. All data were obtained from the same muscle fibre.

serted into the middle portion of a muscle fibre, and an extracellular microelectrode was placed on the surface of the muscle at a spot showing the largest extracellular excitatory junctional potentials (e.j.ps). In some experiments, the membrane potential of the muscle fibre was clamped with two intracellular microelectrodes and the currents induced by iontophoretic application of L-glutamate to the excitatory junction were measured. In order to measure the input resistance of the muscle fibre, two microelectrodes were inserted separately into the middle portion of a muscle fibre less than 50 μm apart, one for recording voltage changes and the other for passing hyperpolarizing current pulses (duration 200 ms). The decay time constant of extracellular e.j.ps was determined by fitting straight lines to the semilogarithmic plots of their decay phases by the method of least-squares (Shinozaki & Ishida, 1983c). Regressions were calculated between 80% and 20% of the peak amplitude, and the plots were seen to fall close to a straight line.

Drugs

Sodium L-glutamate, mono (Wako) and γ-amino-n-butyric acid (GABA; Tokyo Kasei) were used. Quisqualic acid was isolated from the plant, *Quisqualis indica* L. Matrine, oxymatrine and N-methylcytisine were generous gifts from Dr T. Noro (Shizuoka College of Pharmacy, Shizuoka, Japan) and Dr M. Terada (Department of Parasitology, Hamamatsu University School of Medicine, Shizuoka, Japan).

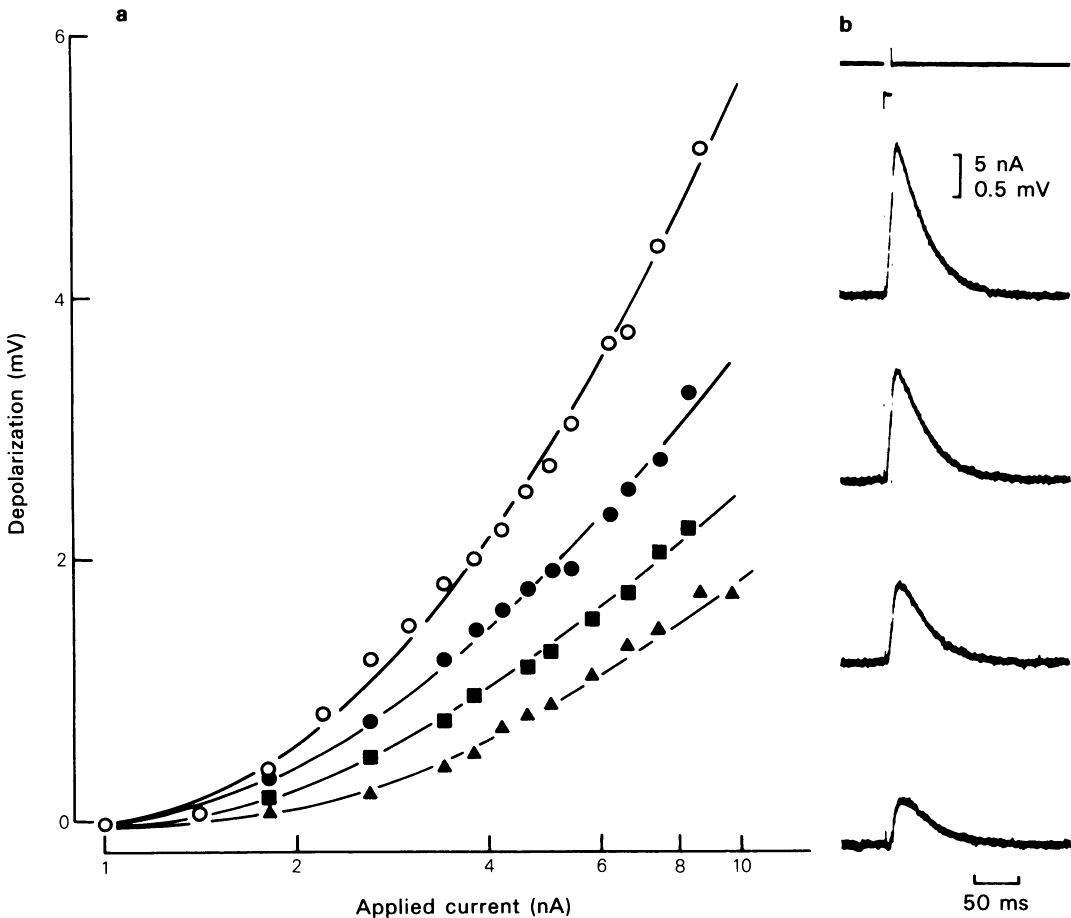


Figure 3 Logarithmic dose-response curves for glutamate-induced responses obtained in the absence and presence of various doses of matrine. Ordinate scale, amplitude of glutamate-induced potentials (mV). Abscissa scale, logarithmic current intensity (nA) of glutamate pulses (10 ms duration). (○) Control, (●) matrine 0.2 mM, (■) 0.5 mM, (▲) 1 mM. Records on the right side (b) represent the glutamate-induced potentials in the absence and presence of 0.2, 0.5 and 1 mM from the second of the records, respectively. Above the potential records is the monitored injection current which is common to all potential records.

Results

Effects of matrine on the glutamate response

Glutamate (0.1 mM) was applied in the solution which superfused the preparation at a constant flow rate (4ml min^{-1}). Matrine, at a concentration of 0.5 mM, reduced the intracellularly recorded depolarization to 50 to 60% of the control without affecting the time-to-peak of the depolarization. There was no apparent difference between the control and matrine-treated preparations in the rate of the decline of the depolarization during application of glutamate. Matrine, even at higher concentrations, did not affect either the resting membrane potential or the input resistance of the cell. Removal of matrine gradually but completely restored the glutamate-induced response to normal. Oxymatrine also has an inhibitory action on glutamate-induced responses, but it was less powerful than matrine. N-Methylcytisine at a concentration of 1 mM did not depress the response of the crayfish muscle to glutamate. The electrotonic potential induced by the hyperpolarizing constant current pulse was reduced significantly in the presence of 0.1 mM GABA. Matrine at a concentration of 0.5 mM did not affect the GABA-induced response. Trains of e.j.ps and inhibitory junctional potentials (i.j.ps) induced by trains of pulses at 83 Hz for 96 ms or 125 Hz for 80 ms, respectively, were alternately evoked at intervals of 5 s. When the responses were found to be sufficiently stable, matrine was added to the bathing

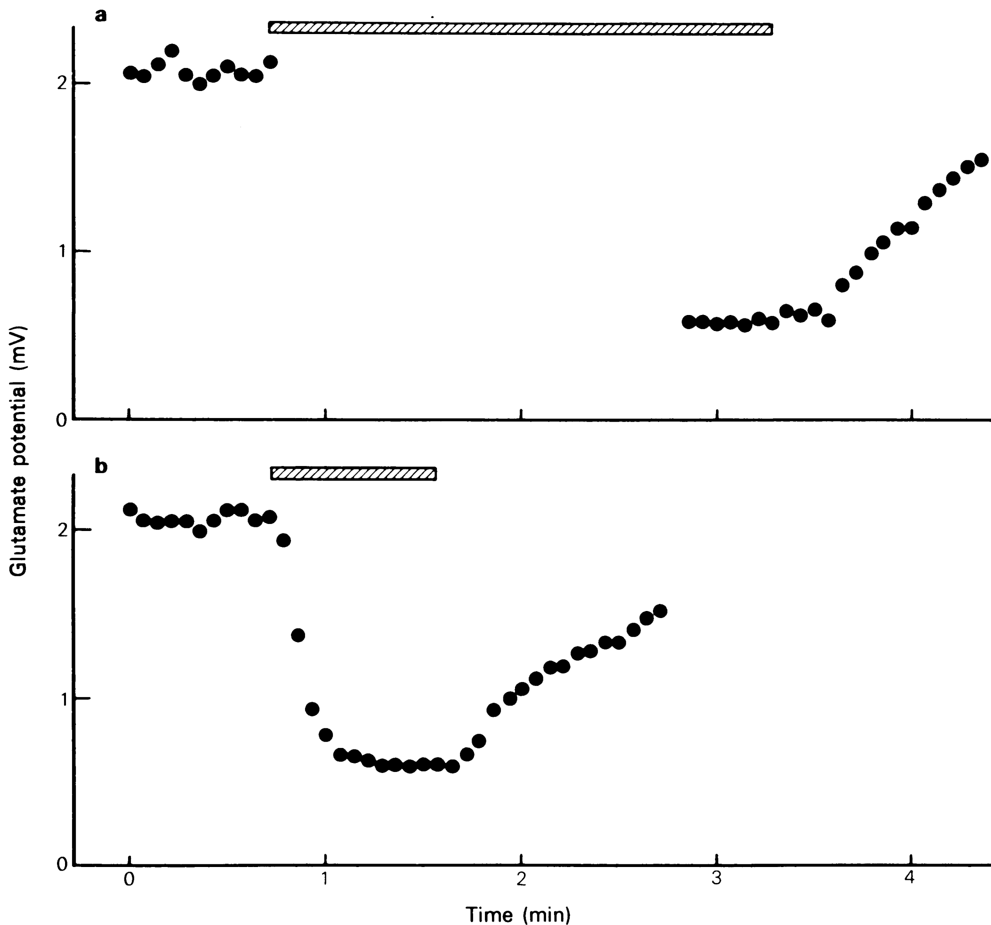


Figure 4 Matrine depressed the amplitude of glutamate-induced pokccive of the interpulse interval of glutamate pulses. The potentials induced by iontophoretic application of glutamate were evoked at an interval of 4 s. Matrine was applied in the bathing solution at a concentration of 1 mM for the period indicated. Ordinate scale, amplitude of glutamate-induced potentials (mV). (a) Matrine was applied but iontophoretic application of glutamate was discontinued for about 2 min. The amplitude of the first response at the end of the 2 min period was reduced to a similar level to that of glutamate-induced responses which were induced repetitively (b).

solution at a concentration of 0.5 mM. The amplitude of e.j.ps was reduced but that of i.j.ps was not affected by matrine. The effect of matrine on e.j.ps will be described in detail later. At a concentration of 0.5 mM, matrine depressed the depolarization caused by bath application of 1 μM quisqualate to less than a half of the control (Figure 2). The potentials which were induced by iontophoretic application of glutamate or quisqualate were alternately evoked at an interpulse interval of 10 s using a double-barrel micropipette in the presence of matrine 0.5 mM. The amplitude of the quisqualate-induced potential was affected by matrine to a similar degree as that of the glutamate-induced potential.

The amplitudes of glutamate-induced potentials were restricted to levels less than 5 mV, since a large depolarization produces muscle contraction which may disturb the position of the micropipette. Matrine reduced the amplitude of glutamate-induced potentials at concentrations greater than 0.1 mM. When the amplitudes of glutamate-induced potentials were plotted against the intensity of applied currents of glutamate pulses in the absence and presence of various amounts of matrine, a parallel shift of the logarithmic dose-effect curve for glutamate was not observed in the presence of matrine, but the curve declined with increasing matrine concentrations (Figure 3).

Postjunctional inhibitory effects can occur by block of either receptors or ionic channels or by changes in the rates of opening or closing ion-channels. Several compounds have been demonstrated to possess an inhibitory action on the glutamate-induced response at the crayfish neuromuscular junction (Shinozaki, 1980) and some are presumed to be open-channel blockers (Shinozaki & Ishida, 1983a, b; Ishida & Shinozaki, 1983). Is it possible to explain the matrine action by the assumption that matrine is an open-channel blocker at the crayfish neuromuscular junction? When the open-channel blocker is given alone without application of the agonist, a decrease in the number of available receptors is not expected. Immediately before bath application of 1 mM matrine glutamate pulses were discontinued, and about 2 min after the application of matrine, potentials were induced by glutamate again in the presence of the drug. The amplitude of the first glutamate-induced response after resuming the iontophoretic pulses was decreased to a similar level to that of responses induced by the repetitive application of glutamate at very short intervals (Figure 4). If matrine is an open-channel blocker which slowly dissociates from the channel, the results shown in Figure 4 would not be obtained. When membrane current responses were induced by a train of glutamate pulses, their amplitudes gradually diminished with successive pulses. The shorter the interpulse interval, the greater was the decrease in response amplitude per pulse. In the presence of matrine the degree of this gradual decrease of response amplitude with repetitive applications of glutamate was similar to that in the control even when the interpulse interval was very short, while every glutamate-induced potential was decreased in size. In these cases effects of the channel block and desensitization of the receptor are mixed, but similar data were obtained in the concanavalin A treated muscle (Mathers & Usherwood, 1976; Shinozaki & Ishida, 1978) in which the time-dependent decrease in size could be completely blocked (Figure 5). If the dissociation of an open-channel blocker from the channel is significantly slow, amplitudes of glutamate-induced potentials which were repetitively induced at a short interval should be decreased and the degree of the decrease would be dependent on the equilibrium constant of

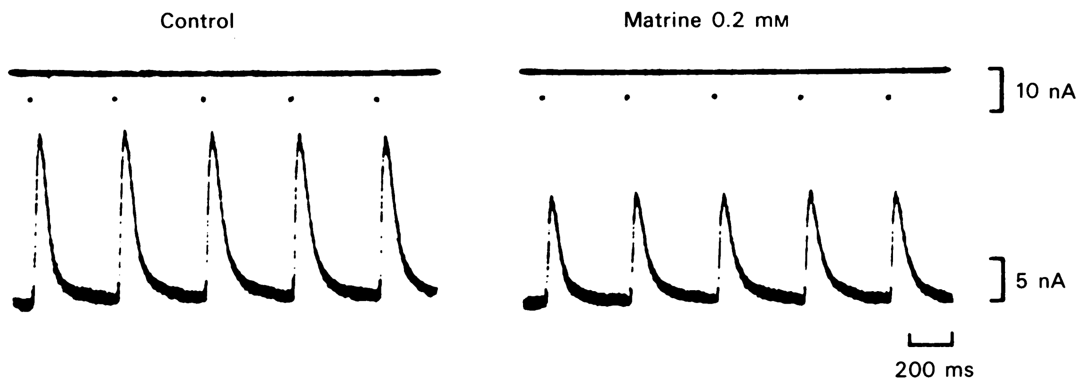


Figure 5 Current responses induced by a train of glutamate pulses (duration 10 ms) in the concanavalin A treated muscle. The amplitude of glutamate-induced currents was decreased in the presence of 0.2 mM matrine but a gradual decline of their amplitudes with successive pulses was not observed. Upper traces: monitored injection currents. Lower traces: glutamate-induced currents.

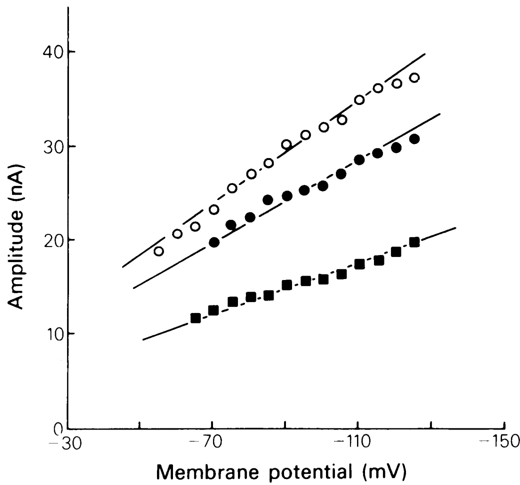
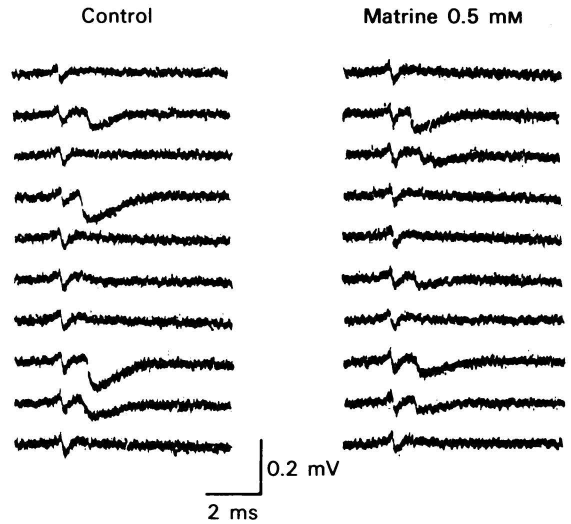


Figure 6 The relationship between the peak amplitudes of glutamate-induced currents and membrane potential in the presence of various concentrations of matrine. Membrane current responses were induced by glutamate pulses (duration 10 ms) at an interval of 15 s. Ordinate scale, amplitude of glutamate-induced currents. Abscissa scale, membrane potential of the muscle fibre (mV). (○) Control; (●) 0.2 mM matrine; (■) 1 mM matrine. The voltage-dependent inhibition of glutamate-induced currents was not observed in the range of the membrane potential tested.



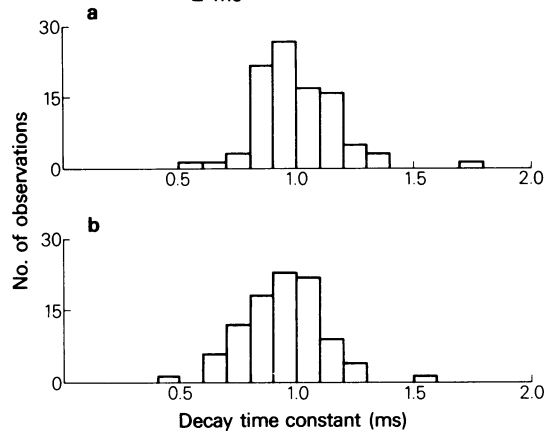


Figure 7 Records of extracellularly recorded e.j.ps and histograms of their decay time constants. Potential records: e.j.p.s were elicited by trains of stimuli applied to the nerve at a frequency of $12\,s^{-1}$. The quantal content was relatively small and consequently nerve impulses often failed to induce extracellular e.j.ps. Histograms: (a) control, (b) during application of matrine 0.5 mM. The average decay time constant was 1.00 ± 0.02 (mean $\pm$ s.e., $n = 96$) ms in (a) and 0.94 ± 0.02 ms in (b).

the channel block reaction (Adams, 1976; Katz & Miledi, 1978; Dreyer *et al.*, 1978). Therefore, our results suggest either that the dissociation of matrine from the channel is not slow or that matrine is not an open-channel blocker.

If matrine blocks the receptor, little change is expected in the voltage dependence of the amplitude of the glutamate-induced current responses. In the experiment shown in Figure 6, the peak amplitude of glutamate-induced currents at various membrane potentials was plotted in the absence and presence of matrine. Effects of some channel blockers have been shown to be dependent on the membrane potential (Adams, 1976; Feltz *et al.*, 1977; Albuquerque & Gage, 1978). Figure 6 suggests that the inhibition of glutamate-induced currents is not voltage-dependent in the presence of matrine. If the depression of glutamate-induced currents was due to channel block in which the blocker dissociated from the channel very rapidly, it is expected that the time course of the glutamate-induced current may apparently be changed as shown in the previous study (Shinozaki & Ishida, 1983b). However, there was no apparent change in the time course of the glutamate-induced current at any of the membrane potentials tested.

Effect of matrine on extracellular e.j.ps

The decay rate of nerve evoked synaptic currents is thought to reflect the average channel open-time (Magleby & Stevens, 1972a, b; Anderson & Stevens, 1973). If matrine is an open-channel blocker with a large forward rate constant, the decay rate of evoked synaptic currents would be hastened by matrine. The values of the decay time constant of extracellular synaptic potentials were hardly affected by matrine at a concentration of 0.5 mM in spite of the fact that the

amplitude of extracellular e.j.ps was significantly reduced (Figure 7). The channel block theory predicts that transformation from the active open-channel to the blocked open-channel is a function of the blocker concentration (Adams, 1976; Peper *et al.*, 1982; Rang, 1982). Even at matrine concentrations greater than 1 mM, the decay rate of extracellular e.j.ps was not changed but their amplitudes were markedly decreased.

If the reduction in peak amplitudes of extracellular e.j.ps results from asynchronous opening of channels during the rising phase, so that some become blocked before others have opened, then the early part of the rising phase should be unaffected by matrine. If, on the other hand, a different mechanism is operating to reduce the effectiveness of the excitatory transmitter in opening channels, the block should be evident throughout the rising phase. The ratios of the peak amplitude of extracellular e.j.ps to the time-to-peak were 0.86 ± 0.07 $V s^{-1}$ (mean ± s.d., $n = 33$) and 0.61 ± 0.07 $V s^{-1}$ ($n = 38$) in the absence and presence of matrine 0.5 mM respectively, at the resting membrane potential. From these results it can be seen that the block appears to be present during the earliest part of the rising phase, suggesting that the open-channel block cannot fully account for the entire action of matrine on the excitatory transmitter at the crayfish neuromuscular junction.

When the muscle fibre was treated with matrine, a dose-dependent decrease in the peak amplitude of successive e.j.ps was observed. The effective concentrations of matrine to decrease the e.j.p. amplitude were greater than 0.1 mM. Oxymatrine at a concentration of 1 mM reduced the amplitude of intracellular successive e.j.ps to a similar level to that seen with 0.2 mM matrine, and N-methylcytisine was not effective (Figure 8). To know whether matrine decreased the unit size of extracellular e.j.ps, a quantal analysis was performed. The excitatory axon was stimulated at various frequencies of $10-20 s^{-1}$, and the number of quanta released per impulse was estimated from the number of failures of extracellular e.j.ps. The average unit size was decreased by 0.5 mM matrine to $73 \pm 2\%$ ($n = 9$) of the control. On the other hand, the quantal content was hardly affected by matrine, the ratio of the control quantal content to the test being 0.96 ± 0.05 ($n = 9$).

Discussion

Matrine depressed glutamate-induced responses at the crayfish neuromuscular junction at concentrations greater than 0.1 mM which were almost the same as those of the established glutamate inhibitors. Matrine also depressed the e.j.p. amplitude without

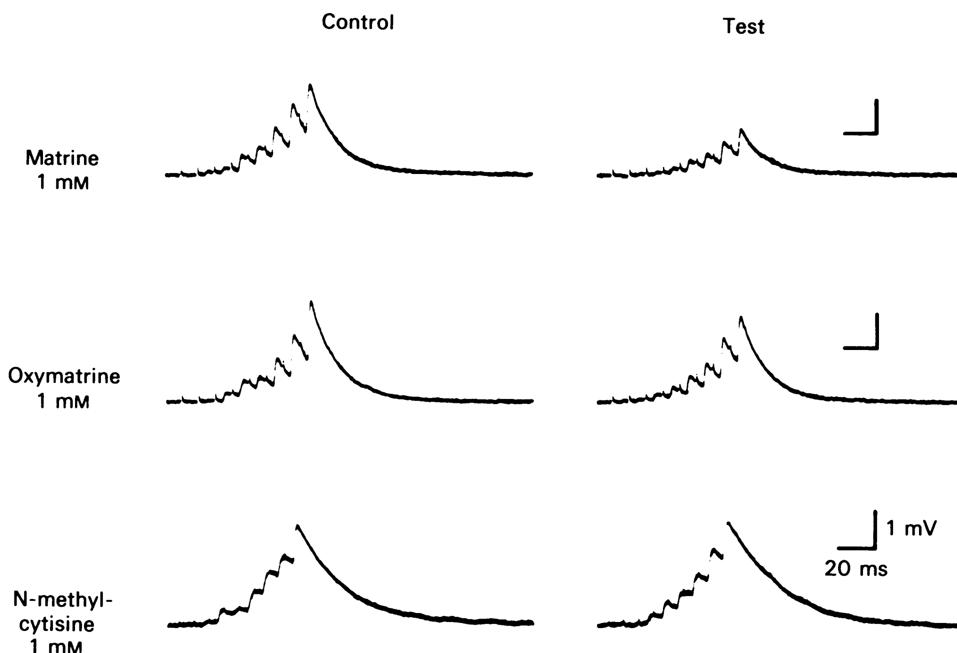


Figure 8 Records of intracellularly recorded e.j.ps in the absence and presence of alkaloids. E.j.ps were induced by trains of stimuli at 100 Hz for 80 ms. Each e.j.p. shown in the records has an amplitude almost equivalent to the average of 30 e.j.ps recorded before or after the application of the drugs.

affecting the quantal content. Matrine did not have any influence on GABA-induced responses at the crayfish neuromuscular junction. The results presented here clearly demonstrate that matrine has a postsynaptic action at the crayfish neuromuscular junction. The amplitudes of the ACh-induced potentials and the endplate potential (e.p.p.) of frog sartorius muscle were decreased by matrine to a similar extent to that of spontaneous miniatrure e.p.ps, but matrine required concentrations two orders of magnitude higher than well-known competitive neuromuscular blockers such as (+)-tubocurarine and pancuronium (unpublished observations). Therefore, we suggest that matrine may have an inhibitory action on excitatory synapses irrespective of species or agonists. It has been reported that streptomycin depresses both ACh-induced responses at vertebrate endplates (Jindal & Desphande, 1960; Farley *et al.*, 1982) and glutamate-induced responses at invertebrate neuromuscular junctions (Onodera & Takeuchi, 1977; Usherwood, 1981) in a similar concentration range (above 10^{-4} M) to matrine. Streptomycin is not a nicotinic competitive inhibitor at the vertebrate endplate. It has been reported that streptomycin reduces the amplitude of excitatory junction currents (e.j.c.s) at the locust neuromuscular junction and increases their decay rate at a concentration of 0.25 mM (Usherwood, 1981), suggesting that streptomycin is an open-channel blocker at the locust neuromuscular junction. At this junction the neuromuscular block caused by streptomycin was voltage dependent. Other cholinergic antagonists are known to depress the glutamate-induced responses in the crab (Lingle *et al.*, 1981), the crayfish (Shinozaki et al., 1982; Shinozaki & Ishida, 1983a, b) and insects (Yamamoto & Washio, 1979; 1983; Cull-Candy & Miledi, 1983). In these cases they act as open-channel blockers. Unlike these compounds, matrine did not satisfy all necessary criteria for open-channel blockers, and open-channel block did not fully account for the entire action of matrine at the crayfish neuromuscular junction. From the results obtained in the present study, a possibility is suggested that matrine is a non-competitive inhibitor which might act at a receptor site distinct from the recognition site for agonists.

Terada *et al.* (1982) showed an excitatory action of matrine on the motility of *Angiostrongylus cantonensis*, isolated frog rectus and mouse ileum, in which a distributed supply of nerve endings is scattered all along the fibre surface, and concluded that these effects could be related to the release of transmitters caused by matrine. However, in crayfish opener muscle which also has a similar multijunctional neuromuscular system, matrine has no influence upon presynaptic events. At the present time, there is no further information about pharmacological properties of matrine at the other synapses because of the limited availability of matrine. Clearly it is of interest to know more about the pharmacological properties of this drug and to compare its action at many excitatory synapses.

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