Br. J. Pharmac. (1984), **83**, 813–820

Effects of caroverine and diltiazem on synaptic responses, L-glutamate-induced depolarization and potassium efflux in the frog spinal cord

Yoshihisa Kudo & Shoji Shibata*

Mitsubishi-Kasei Institute of Life Sciences, Minamiooya 11, Machida, Tokyo 194, Japan and University of Hawaii*, Honolulu, Hawaii, U.S.A.

1 The frog spinal cord was used to determine the characteristics of the actions of caroverine and diltiazem, two organic Ca^{2+}-antagonists, on synaptic responses and L-glutamate-induced depolarization.

2 Caroverine and diltiazem (10^{-4} M) depressed the dorsal root potential (DR-DRP) induced by electrical stimulation of an adjacent dorsal root. Diltiazem also depressed the ventral root potential (DR-VRP), whereas caroverine augmented both the polysynaptic component in the ventral root reflex and the size of the DR-VRP.

3 The root potentials induced by high frequency stimulation (20 Hz, for 1 s) were markedly depressed by these Ca^{2+}-antagonists at a concentration of 10^{-4} M.

4 When the preparation was perfused with normal medium, the compounds depressed L-glutamate-induced depolarizations in ventral and dorsal roots.

5 In preparations treated with tetrodotoxin (TTX) (2×10^{-7} M), the antagonizing actions of the drugs against L-glutamate-induced depolarizations in the ventral root were markedly reduced or abolished, while significant antagonizing actions on the depolarization in the dorsal root were still observed.

6 The increase in extracellular K^+ activity induced by L-glutamate in the TTX-treated preparation was significantly reduced by the compounds.

7 Caroverine and diltiazem had no effect on the presynaptic nerve spike and on the focal synaptic potential induced by a single stimulation of a dorsal root; however, the focal synaptic potential induced by high frequency stimulation (20 Hz, 1 s) was attenuated.

8 Motoneuronal action potentials were abolished by the drugs, while the excitatory postsynaptic potential remained unaffected.

9 The present results suggest that caroverine and diltiazem are not specific L-glutamate antagonists in the frog spinal cord, but that they block the initiation of an action potential without affecting presynaptic nerve conduction, transmitter release or transmitter-receptor interactions. The inhibitory effects of these compounds on L-glutamate-induced K^+-efflux are discussed with reference to their Ca^{2+}-antagonizing actions.

Introduction

Recent studies on the crayfish neuromuscular junction have shown that certain organic Ca^{2+}-antagonists, diltiazem and caroverine, block the potential induced by L-glutamate but have no inhibitory effects upon the excitatory junctional potentials (Ishida & Shinozaki, 1980; 1983). Such differential effects of the substances upon L-glutamate-induced and synaptically evoked potentials calls into question the widely-held view that L-glutamate is the excitatory synaptic transmitter at the crayfish neuromuscular junction (Takeuchi & Onodera, 1973; Atwood, 1976). Since L-glutamate is also regarded as a likely candidate for the excitatory neurotransmitter of the vertebrate central nervous system (cf. Nistri & Constanti, 1979), the question raised by the experiments in crayfish should also be examined in this system. Since L-glutamate-induced depolarizations are accompanied by an influx of Ca^{2+} as well as of Na^+

(Ramsey & McIlwain, 1970; Onodera & Takeuchi, 1976; Sonnhof & Bühle, 1981; Kudo & Oka, 1982), the differential actions of Ca^{2+}-antagonists on L-glutamate-induced and synaptically evoked potentials may be related to the Ca^{2+}-dependent actions of L-glutamate.

In the present study we used the frog spinal cord in order to clarify the mode of action of caroverine and diltiazem on vertebrate central neurones. The effects of these compounds were tested on L-glutamate-induced depolarization and on synaptic activity, and it was found that the Ca^{2+}-antagonists had inhibitory actions on membrane excitability and the L-glutamate-induced efflux of K^+, but seemed to have no direct action in antagonizing L-glutamate.

Methods

Isolated, intra-arterially perfused spinal cord of the bullfrog

The technique for preparing the isolated, intra-arterially perfused spinal cord of bullfrogs (*Rana catesbiana*) was similar to that described by Kudo *et al.* (1975). The spinal cord was isolated and arranged in a chamber kept at room temperature. A glass cannula having a tip diameter of about 200 μm was inserted into the anterior spinal artery for perfusing the spinal cord with amphibian Ringer solution of the following composition (mM): NaCl 115, KCl 2.7, $CaCl_2$ 1.8 and glucose 5.5. The pH was adjusted to 7.6 by the addition of $NaHCO_3$. The perfusion rate was about 0.3 ml min^{-1}.

Recording of the root potentials and root reflexes by the sucrose gap method

The sucrose gap method used in the present study has been described in detail previously by Kudo *et al.* (1975). Briefly, the 9th or 10th ventral and dorsal roots were arranged in two separate pools separated by a stream of sucrose, thus isolating the spinal cord and the peripheral stumps of the ventral or dorsal roots by a sucrose gap. Potential differences between the spinal cord and the peripheral root stumps were detected by a pair of Ag-AgCl electrodes and recorded by a two pen d.c. recorder (Technicorder, Type 3047, Yokokawa). An adjacent dorsal root was stimulated by a pair of Ag-AgCl electrodes (0.1 Hz, duration 0.5 ms, 3–8 V, Nihonkohden SEN 3101).

Microelectrode study

Field potential The focal synaptic potential and presynaptic nerve spike were recorded according to the method used in rat spinal cord (Ono *et al.*, 1979). A dorsal root (9th) was stimulated submaximally (0.2 Hz, duration 0.05 ms) and the focal synaptic potential and presynaptic nerve spike were recorded by a glass microelectrode filled with 1 M NaCl, inserted through the ventral surface of the spinal cord to a depth of approx. 1000 μm.

Intracellular recording The resting membrane potential and action potentials of motoneurones evoked by stimulation of a dorsal root (orthodromic) or a ventral root (antidromic stimulation) were detected by glass microelectrodes filled with 1 M potassium citrate (20–30 MΩ resistance).

Potassium-sensitive microelectrodes were prepared according to the technique described by Walker (1971) and Vyskocil & Kriz (1972), and modified by Kudo (1978). The potassium-sensitive microelectrode was filled with 0.5 M KCl, with a 200–500 μm long column of Corning ion exchanger resin (477317) in its tip. The reference electrode was filled with solution of 0.15 M NaCl. The tips of these electrodes were assembled as close as possible together under microscopic observation and were fixed in position with wax. Extracellular potassium activity of the spinal cord was measured at depths of between 1000 and 1250 μm from the ventral surface of the spinal cord.

Drugs and application system

Drugs used were: caroverine fumarate, 1-(2-diethylaminoethyl)-3-(p-methoxybenzyl)-1,2-dihydro-2-quinoxalinone fumarate (Mitsubishi Chemical Industries), diltiazem (Tanabe), monosodium L-glutamate (Wako Pure Chem.), γ-aminobutyric acid (Wako Pure Chem.) and tetrodotoxin (Sankyo Co.).

Test drugs were dissolved in a Ringer solution and applied by exchanging the normal solution with one containing drugs using an electric valve system (Anger-Branswick, Model 373) connected to a timer device. Amino acids were dissolved in normal Ringer solution and were applied once every 5 min through fine polyethylene cannulae inserted into an arterial cannula using a microtubepump (LKB Multiperpex pump 2115) and timer-controlled microelectric valves (Anger-Branswick Model 336).

Results

Effects on evoked root potentials, reflexes and L-glutamate-induced depolarization

As shown in Figure 1 and Table 1, caroverine (10^{-4} M) significantly reduced the amplitude of the

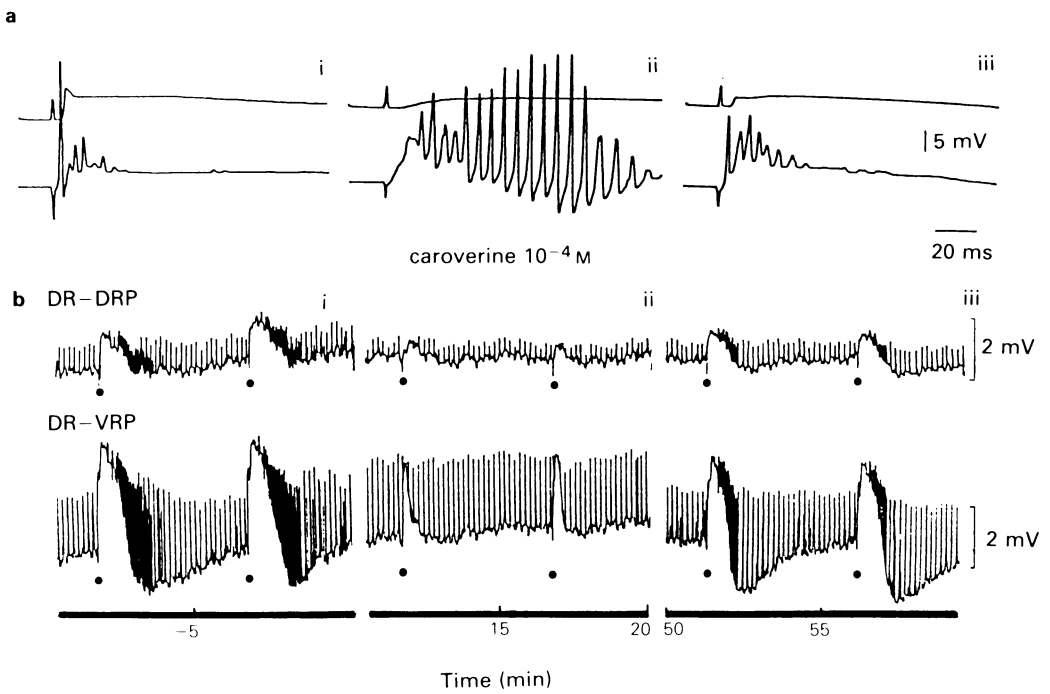

Figure 1 Effects of caroverine on ventral and dorsal root potentials and reflexes and on L-glutamate-induced depolarizations. (a) Effect of caroverine (10^{-4} M) on ventral and dorsal root potentials and reflexes induced by stimulation of the dorsal root. Upper traces: dorsal root potentials and reflexes. Lower traces: ventral root potentials and reflexes. These potentials were averaged (five traces) by a signal averager, the output of which was recorded by an X-Y recorder. (i) Before the application of caroverine (10^{-4} M); (ii) 20 min after application; (iii) 30 min after washing. (b) Effects of caroverine on root potentials and L-glutamate-induced depolarizations. Upper trace: dorsal root potentials. Lower trace: ventral root potentials. At the dot, L-glutamate (5×10^{-3} M, 0.1 ml; 5×10^{-7} mol) was injected (once every 5 min).

Table 1 Effects of caroverine and diltiazem on ventral and dorsal root potentials evoked by electrical stimulation and by L-glutamate in normal medium

			Root potentials (mV)			
			Electrically evoked		*L-Glutamate-induced*	
Compounds	*Dose* (M)	n	VRP	DRP	VRP	DRP
Control		5	2.3±0.7	2.1±0.6	3.0±0.4	2.0±0.3
Caroverine	10^{-4}		2.5±0.5	1.2±0.4*	1.8±0.4	0.8±0.2**
Control		5	2.1±0.4	2.3±0.3	3.2±0.4	2.3±0.5
Diltiazem	10^{-4}		0.8±0.3*	1.1±0.5*	2.3±0.6	1.5±0.2*

Means of amplitudes of root potentials observed just before the application (control) and following 20 min of application of test compounds are indicated with s.e. * and **: Statistically significant difference from control: $P < 0.05$ and $P < 0.01$, respectively (paired t test).

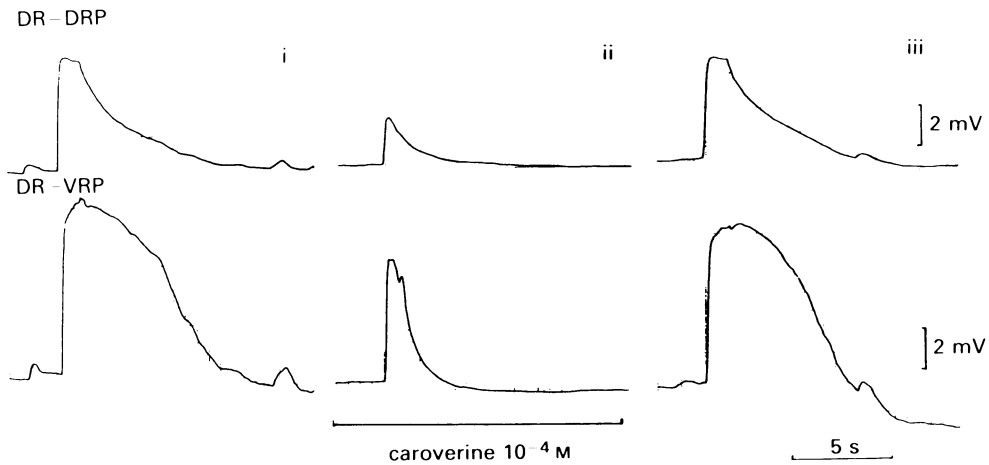

Figure 2 Effects of caroverine on ventral and dorsal root potentials evoked by high frequency stimulation. Stimulation (20 Hz, for 1 s) of 9th dorsal root. Upper and lower traces indicate dorsal and ventral root potentials, respectively. (i) Before the application of caroverine (10^{-4} M); (ii) 10 min after application; (iii) 30 min after washing.

dorsal root potential (DR-DRP) and reflex (DR-DRR) induced by the stimulation of an adjacent dorsal root. Although the first spike potential of the ventral root reflex (DR-VRR) was also reduced markedly within 20 min of the drug application, the amplitude of the ventral root potential (DR-VRP) and the late polysynaptic component of DR-VRR were markedly augmented. However, the root potentials induced by high frequency stimulation (20 Hz, for 1 s) were markedly reduced by caroverine (Figure 2 and Table 2). The minimum effective dose of the drug was 10^{-5} M. When a dose of 3×10^{-5} M was applied, it caused an augmentation of the DR-VRP (166.7 ± 31.2% of the predrug, control response). However, at a concentration of 3×10^{-4} M, caroverine abolished the DR-VRP within 20 min. On the other hand, diltiazem (10^{-4} M) significantly reduced both DR-DRP and DR-VRP as well as the root potential induced by high frequency stimulation (Table 2). Unlike caroverine, this compound had no facilitatory effects on the DR-VRP at doses ranging from 10^{-5} to 3×10^{-4} M. The effects of caroverine and diltiazem were partially reversed by washing for 30 min with drug-free Ringer solution.

As shown in Table 1, caroverine and diltiazem (10^{-4} M) reduced the amplitude of L-glutamate-induced depolarizations in the dorsal root (primary afferent terminal) and in the ventral root (motoneurone). The effect on the maximum amplitude of the depolarization in the ventral root was not statistically significant. However, both these substances abolished the L-glutamate-induced afterdischarges in the motoneurone and shortened the time course of the depolarization (Figure 1b). Thus, if the percentage inhibition was calculated based upon the 'area under the curve' of the L-glutamate-induced depolarization, marked inhibitory effects of these drugs were recognized in the ventral root as well as in the dorsal root. Caroverine and diltiazem (10^{-4} M) had a negligible effect on the response to GABA of the ventral and dorsal roots.

As shown in Figure 3b,c and Table 3, caroverine

Table 2 Inhibitory effects of caroverine and diltiazem on root potentials evoked by high frequency stimulation of the dorsal root

Compounds	*Dose* (M)	n	*% Inhibition of potentials* VRP	DRP
Caroverine	10^{-4}	4	60.1 ± 16.1	77.2 ± 8.9
Diltiazem	10^{-4}	4	66.4 ± 8.5	68.6 ± 7.9

Percentage inhibition by the compounds of root potentials evoked by high frequency stimulation (20 Hz, for 1 s) was calculated based upon the amplitudes of root potentials observed after a 10 min application of test agents; responses before the application were taken as control.

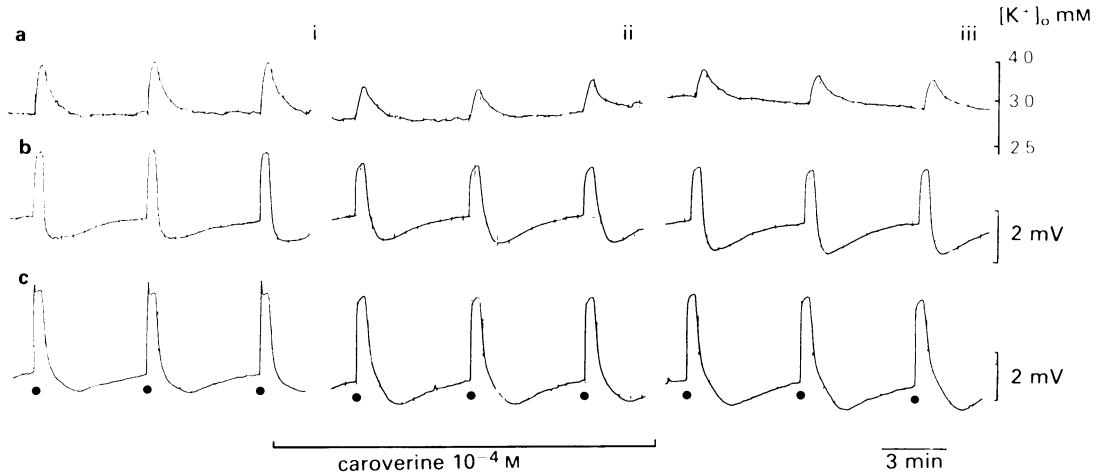

Figure 3 Effects of caroverine on L-glutamate-induced K^+-efflux and root potentials in the tetrodotoxin-treated preparation. At the dot, L-glutamate (5×10^{-3} M, 0.1 ml; 5×10^{-7} mol) was injected once every 5 min: (i) Before the application of caroverine (10^{-4} M); (ii) 20–30 min after application; (iii) 20–30 min after washing.

and diltiazem (10^{-4} M) significantly reduced L-glutamate-induced depolarization in the dorsal root while having no significant effect on L-glutamate-induced depolarization in the ventral root, in the preparation treated with tetrodotoxin (TTX) (2×10^{-7} M).

Effects on the L-glutamate-induced increase in extracellular K^+ activity

As shown by Kudo & Fukuda (1976), L-glutamate caused a marked increase in extracellular K^+ activity. In the TTX-treated preparation, a one shot injection of L-glutamate (5×10^{-3} M, 0.1 ml; 5×10^{-7} mol) caused more than 1 mM equivalent increase in extracellular K^+ activity (Table 3 and Figure 3a). Such an increase in K^+ activity was significantly reduced by the application of caroverine and diltiazem (10^{-4} M). This effect was accompanied by a significant decrease in the size of the dorsal root depolarization.

Effects on presynaptic nerve spike and focal synaptic potential

Extracellular microelectrode recording was used in four spinal cords to test the effects of caroverine and diltiazem on presynaptic nerve conduction and the focal synaptic potential. Caroverine and diltiazem (10^{-4} M) had little effect on the presynaptic nerve spike and the focal synaptic potential induced by a single stimulation (Figure 4a). By contrast, the focal synaptic potential induced by high frequency stimulation (20 Hz, 20 s^{-1}) was markedly reduced while the presynaptic nerve spike remained unaffected (Figure 4b).

Table 3 Effects of caroverine and diltiazem on L-glutamate-induced potentials and alteration of extracellular K^+-activity in the tetrodotoxin (TTX)-treated preparations

Compounds	*Dose* (M)	n	*Root potentials* (mV) VRP	DRP	$[K^+]_o$ (mM)
Control	10^{-4}	5	2.3±0.5	2.0±0.2	1.1±0.2
Caroverine			2.4±0.5	1.5±0.1*	0.6±0.2*
Control	10^{-4}	4	2.8±0.6	2.4±0.4	1.6±0.3
Diltiazem			2.9±0.7	1.7±0.2*	0.5±0.1**

Means of amplitudes of root potentials and increase in K^+-activity observed just before the application (control) and following 20 min of application of test compounds are indicated with s.e. * and **: Statistically significant difference from control, $P < 0.05$ and $P < 0.01$, respectively (paired t test).

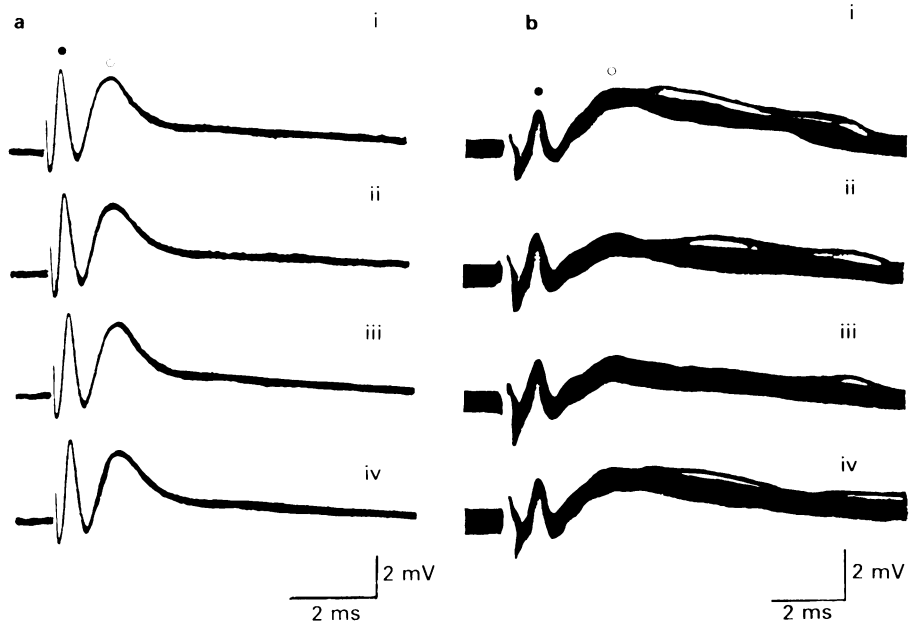

Figure 4 Effects of caroverine on the field potential induced by a single, or by high frequency stimulation of the dorsal root. (a) The field potential evoked by a single stimulation of the dorsal root (five traces superimposed). (b) The field potential evoked by high frequency stimulation (20 Hz, for 1 s) (20 traces superimposed). Filled circle indicates presynaptic nerve spike. Open circle indicates focal synaptic potential. (i) Before the application of caroverine (10^{-4} M); (ii) and (iii) 10 min and 20 min after application, respectively; (iv) 30 min after washing.

Effect on the motoneurone

The action potential generated in a motoneurone, by stimulation of the dorsal root or by antidromic stimulation of the ventral root was inhibited by caroverine (10^{-4} M) in five motoneurones of five separate spinal cords tested (Figure 5). Within 10 min of the application of caroverine, the overshoot of the action potential was reduced and the notch of the initial segment spike potential became visible in its rising phase, while the falling phase was slightly prolonged. Within 20 min the action potential was nearly abolished; however the excitatory postsynaptic potential remained unaffected (Figure 5a). The action potential elicited by antidromic stimulation was also abolished within 20 min of the drug's application (Figure 5b). These effects of caroverine were reversed completely within 30 min following washing. Similar inhibitory actions on the action potentials were observed in preparations treated with diltiazem (10^{-4} M) (two neurones tested).

The membrane resistance measured by passing constant current pulses (5×10^{-9} A) through one barrel of a double barrel microelectrode was slightly increased within 20 min of the application of caroverine (10^{-4} M) (9.5 ± 2.1% increase, $n = 3$).

Discussion

Caroverine and diltiazem inhibited L-glutamate-induced depolarizations in normal medium. Furthermore, these compounds inhibited the DR-DRR, the DR-DRP and the first spike potential in the DR-VRR. However, the effects of these substances upon the L-glutamate-induced potential in ventral roots were abolished after treatment with TTX. The results are indicative that the apparent antagonistic actions of these organic Ca^{2+} antagonists against L-glutamate in normal media are not attributable to the specific action on L-glutamate at its receptor.

The presynaptic nerve spike and the focal synaptic potentials were not affected by caroverine and diltiazem at concentrations sufficient to block the evoked root potentials and L-glutamate-induced depolarization. Excitatory postsynaptic potentials were not affected by these drugs, but the initiation of an action potential was suppressed. The action potential evoked by antidromic stimulation of the ventral root was also blocked by the compounds. These results lead us to speculate that caroverine and diltiazem interfere with the initiation of action potentials in spinal neurones by blocking Na^+-channels in a manner similar to that described for other organic Ca^{2+}-

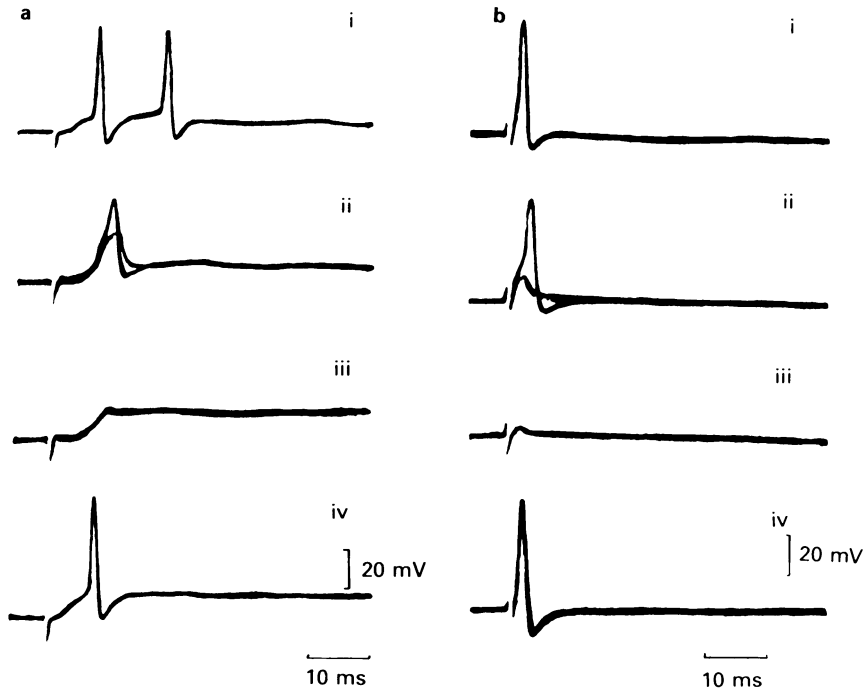

Figure 5 Effects of caroverine on action potentials recorded intracellularly. (a) Action potentials evoked by orthodromic stimulation of the dorsal root. (b) Action potentials evoked by antidromic stimulation of the ventral root. (i) Before the application of caroverine (10^{-4} M); (ii) and (iii) 10 min and 20 min after application, respectively; (iv) 30 min after washing.

antagonists (Nachshen & Blaustein, 1979). The effective depressant action of caroverine and diltiazem on focal synaptic potentials and root potentials induced by high frequency stimulation seemed to parallel the effects of these substances on the afterfiring induced by L-glutamate. The fact that highly activated neurones are more sensitive to the drugs than are less active ones, suggests the increased susceptibility of active (open) channels to these agents, as suggested previously by Ishida & Shinozaki (1983). Alternatively, such differences in sensitivity may be attributed to their Ca^{2+} antagonizing action; Ca^{2+} influx into the rapidly responding presynaptic nerve terminal exhibiting a high demand for Ca^{2+} influx compared with the case of single stimulation, was reduced.

The antagonistic actions of caroverine and diltiazem upon the effects of L-glutamate in normal media may be explained as a result of the inhibition of interneurones which are excited by the acidic amino acid, causing recruitment. However, in TTX-treated preparations, these compounds inhibited dorsal root depolarizations induced by L-glutamate by approx. 30%. The extracellular K^+ activity measured simultaneously in the dorsal horn was reduced by more than 50%. If the L-glutamate-induced depolarization in dorsal roots was the result of elevated K^+ activity produced by the excitation of postsynaptic receptors, as suggested by Evans (1980), the inhibitory effect of caroverine and diltiazem on dorsal root depolarization can be ascribed to reduced extracellular K^+ activity. The potassium permeability at the neuronal membrane has been shown to be increased by an injection of Ca^{2+} (Meech, 1974) and Parod & Putney (1978a,b) have described the receptor-mediated Ca^{2+} influx which controls membrane permeability to K^+. The inhibitory effect of caroverine and diltiazem on extracellular K^+ activity may be explained by their inhibitory actions on Ca^{2+} influx induced by L-glutamate.

The effects of caroverine were qualitatively similar to those of diltiazem except for the augmentation of late polysynaptic components in the ventral root potential. Such an augmentation was never observed in the dorsal root. Although the membrane resistance gradually increased by about 10% after 20 min of caroverine application, this effect was too small and too slow to account for such a drastic and fast de-

veloping augmentation of ventral root potentials. Since motoneurones receive inhibitory as well as excitatory input from interneurones, the evoked responses in the normal condition consisted of the summation of excitatory and inhibitory actions. Thus, apparent excitatory effects may be induced by a preferentially suppressant action of caroverine on inhibitory interneurones. As primary afferent terminals receive input from interneurones having excitatory actions, the effect of caroverine on the dorsal root may appear to be solely inhibitory. However, we have no evidence to support this speculation.

In conclusion, caroverine and diltiazem were not specific L-glutamate antagonists in the frog spinal cord, but they blocked the initiation of the action potential without affecting presynaptic nerve conduction, neurotransmitter release and receptor-transmitter interactions. Inhibitory actions of these compounds on K^+-efflux and dorsal root depolarization induced by L-glutamate may be attributed to their Ca^{2+}-antagonizing action. Thus, at least in the frog spinal cord, such organic Ca^{2+}-antagonists cannot be provided as a tool for elucidating the possible involvement of L-glutamate as an excitatory synaptic transmitter.

We are grateful to Dr T. Philip Hicks (University of Calgary) for his helpful criticism and corrections of the manuscript and to Ms Misayo Nakai for typing the manuscript.

References

ATWOOD, H.T. (1976). Organization and synaptic physiology of crustacean neuromuscular systems. *Prog. Neurobiol.*, **7**, 291–391.

EVANS, R.H. (1980). Evidence supporting the indirect depolarization of primary afferent terminals in the frog by excitatory amino acids. *J. Physiol.*, **298**, 25–35.

ISHIDA, M. & SHINOZAKI, H. (1980). Differential effects of diltiazem on glutamate potentials and excitatory junctional potentials at the crayfish neuromuscular junction. *J. Physiol.*, **298**, 301–319.

ISHIDA, M. & SHINOZAKI, H. (1983). Reduction of glutamate responses by caroverine at the crayfish neuromuscular junction. *Brain Res.*, **266**, 174–177.

KUDO, Y. (1978). The pharmacology of the amphibian spinal cord. *Prog. Neurobiol.*, **11**, 1–76.

KUDO, Y. & FUKUDA, H. (1976). Alteration of extracellular K^+-activity induced by amino acids in the frog spinal cord. *Jap. J. Pharmac.*, **26**, 385–387.

KUDO, Y. & OKA, J. (1982). The role of calcium ion in the L-glutamate-induced depolarization in the frog spinal cord. *Comp. Biochem. Physiol.*, **72C**, 231–236.

KUDO, Y., ABE, N., GOTO, S. & FUKUDA, H. (1975). The chloride dependent depression by GABA in the frog spinal cord. *Eur. J. Pharmac.*, **32**, 251–259.

MEECH, R.W. (1974). The sensitivity of Helix aspersa neurones to injected calcium ions. *J. Physiol.*, **237**, 259–277.

NACHSHEN, D.A. & BLAUSTEIN, M.P. (1979). The effect of some organic "calcium antagonists" on calcium influx in presynaptic nerve terminals. *Molec. Pharmac.*, **16**, 579–586.

NISTRI, A. & CONSTANTI, A. (1979). Pharmacological characterization of different types of GABA and glutamate receptors in vertebrates and invertebrates. *Prog. Neurobiol.*, **13**, 117–235.

ONO, H., FUKUDA, H. & KUDO, Y. (1979). Mechanisms of depressant action of baclofen on the spinal reflex in the rat. *Neuropharmac.*, **18**, 647–653.

ONODERA, K. & TAKEUCHI, A. (1979). Permeability changes produced by L-glutamate at the excitatory postsynaptic membrane of the crayfish muscle. *J. Physiol.*, **255**, 669–685.

PAROD, R.J. & PUTNEY JR., J.W. (1978a). An alpha-adrenergic receptor mechanism controlling potassium permeability in the rat lacrimal gland acinar cell. *J. Physiol.*, **281**, 359–369.

PAROD, R.J. & PUTNEY JR., J.W. (1978b). The role of calcium in the receptor mediated control of potassium permeability in the lacrimal gland. *J. Physiol.*, **281**, 371–381.

RAMSEY, R.L. & McILWAIN, H. (1970). Calcium content and exchange in neocortical tissues during the cation movements induced by glutamate. *J. Neurochem.*, **17**, 781–787.

SONNHOF, U. & BÜHRLE, CH. (1981). Analysis of glutamate-induced ion fluxes across the membrane of spinal motoneuron of the frog. In *Glutamate as a Neurotransmitter.* ed. Di Chiara, G. & Gessa, G.L. pp. 195–204. New York: Raven Press.

TAKEUCHI, A. & ONODERA, K. (1973). Reversal potentials of the excitatory transmitter and L-glutamate at the crayfish neuromuscular junction. *Nature, New Biol.*, **242**, 124–126.

VYSKOCIL, F. & KRIZ, N. (1972). Modifications of single and double-barrel potassium specific microelectrodes for physiological experiments. *Pflügers Arch. ges. Physiol.*, **337**, 265–276.

WALKER JR., J.L. (1971). Ion specific liquid ion echanger microelectrodes. *Analyt. Chem.*, **43**, 39A–93A.

(*Received May 21, 1984.*)