Sites and Mechanisms of Action of Lidocaine upon the Isolated Spinal Cord of the Frog

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The sites of action of lidocaine on the responses evoked by stimulation of lateral column (LC) and dorsal root (DR) were studied in the isolated, intra-arterially perfused spinal cord of the bullfrog. When the ventral root volley produced by stimulation was abolished by lidocaine, the presynaptic focal potential was almost unchanged. Intracellular recordings from motoneurons clearly demonstrated a marked reduction in amplitude of the EPSPs before the block of conduction of presynaptic fibers and the block of invasion of the neuron soma by antidromic spike potential. At low concentrations of lidocaine, the EPSPs elicited by LC stimulation produced shortening in time to peak, slowing in the decay time, decrease in amplitude and smaller changes in the later EPSPs of a train than the earlier ones. From the observations, it was concluded that the low concentrations of lidocaine affected primarily synaptic transmission in the spinal cord. The possible mechanisms of action of lidocaine were discussed.

INTRODUCTION

Local anesthetics block conduction of impulses of nerve fibers. Local anesthetics in low concentrations also affect neuromuscular transmission without impairing conduction in the presynaptic fibers by a direct action on the conductance increase of the end-plate membrane produced by acetylcholine (ACh), which reduces the sensitivity of the postsynaptic membrane to ACh. Local anesthetics may also decrease the transmitter output. Recently, the effects on alteration of ACh channels and end-plate currents have been extensively studied and the postsynaptic blocking action was attributed to a shortening of the elementary electrical events produced by ACh.

In the spinal cord, local anesthetics, when intravenously applied, depress monosynaptic and polysynaptic reflexes in the cat; some facilitation of the monosynaptic reflex may be present, probably due to release from inhibitory control. Curtis and Phillis also demonstrated that spike responses, whether elicited synaptically, antidromically, or directly, are suppressed by iontophoretic application of procaine, and proposed that the excitable membrane of the postsynaptic neuron soma may be more susceptible to procaine than that of the afferent fibers upon the cells. However, other investigators have suggested that lidocaine may affect spinal reflexes by selectively impairing conduction in the terminal portions of small diameter afferent axons, or by depressing interneurons of the polyneural reflex arc.

In the present study, wide ranges of lidocaine concentrations were applied intravascularly to the isolated spinal cord perfused from the ventral spinal artery of the frog. An attempt was made to identify which sites and mechanisms of action of lidocaine are primarily responsible for the changes in activities of motoneurons in response to lateral column (LC) and dorsal root (DR) activation.

MATERIALS AND METHODS

More than 50 bullfrogs (Rana catesbeiana) weighing 150–300 g were used. The techniques for preparing the isolated, intra-arterially perfused spinal cord preparation were similar to those described by Matsuura et al. The spinal cord with the 9th and 10th ventral and dorsal roots attached was excised...
from an anesthetized (sodium pentobarbitone; 30 mg/kg of weight) frog. The isolated spinal cord was perfused with oxygenated Ringer solution through a polyethylene cannula inserted into the ventral spinal artery. The composition of the Ringer solution was NaCl, 111 mM; KCl, 2 mM; NaHCO₃, 1.5 mM; CaCl₂, 1.8 mM; glucose, 0.1%. The pH was 7.2–7.4. Lidocaine dissolved in the Ringer solution was intra-arterially applied from the perfusing cannula. In some experiments, a solution with high Mg²⁺ and low Ca²⁺ was used without changing Na⁺ concentration. Experiments were carried out at room temperature (17–24 °C).

Bipolar Ag/AgCl electrodes were used for stimulation of ventral and dorsal roots. Bipolar needle tungsten electrodes insulated to the tips (tip diameter: 5–50 μm) were inserted into the lateral funiculus at the 3rd spinal segment for stimulation. The stimulus frequency was about 0.1–1.0/s.

Ventral root (VR) volleys were recorded from the 9th or 10th VR. The pre- and postsynaptic focal potentials to VR stimulation as well as to LC and DR stimulation were recorded by a glass microelectrode within the motoneuron pool of the ventral horn, while more dorsal sites were preferred for the recording of focal potentials of motoneurons to DR stimulation⁸,²⁴. Intracellular recordings of spinal motoneurons were made with glass microelectrodes filled with 4 M potassium acetate solution.

Microelectrodes were connected to pre-amplifiers with cathode follower input (Nihonkohden MZ-3B) and then displayed on an oscilloscope (Tecnix 502A). Recording electrodes for VR responses were also connected to DC and AC amplifiers and the responses were displayed on the oscilloscope, and photographed with a camera. The responses were also fed into a 4-channel analog data recorder (TEAC 400) through DC amplifiers for later analyses. Intracellular membrane potentials were always monitored with a pen recorder (Yokogawa 3047). In some cases, a number of serial responses were summed and averaged by an averaging computer (Nihonkohden, ATAC 150). Care was taken to eliminate troublesome interference from large stimulus artifact in the measurement of the amplitude of pre- and postsynaptic responses.

RESULTS

Presynaptic and postsynaptic component of the focal potentials and VR responses

The records of the focal potentials made within

![Figure 1](image_url)

**Fig. 1. Effects of lidocaine on the focal potential and the VR volley.** Each recording shows an average of 5 responses. Responses of the 10th VR (A–E) and focal potentials (F–J) due to stimulation of LC were recorded simultaneously. A and F, B and G, C and H, and D and I were obtained before, 47 s, 84 s, 148 s after application of 0.4 ml of 1 mM lidocaine, respectively. E and J, after application of 2 mM lidocaine (0.4 ml). Focal responses (K–P) by DR stimulation were obtained before (K), and after successive application of each 0.4 ml of 0.5 (L), 1 (M), 2 (N) and 5 (O) mM lidocaine solution. Each of the applications was conducted with a time interval of about 15 min. P shows recovery.
the motoneuron pool, elicited by LC stimulation showed two distinct components8. There was an initial fast positive-negative component which represents the presynaptic terminal potential and a subsequent slow negative potential produced by motoneuron activity (Fig. 1F). In all preparations examined after addition of lidocaine enough to abolish the VR responses elicited by LC stimulation, the depression of the presynaptic component of the focal potential always occurred after the decrease of the postsynaptic response or depression of the VR response. An example is shown in Fig. 1. When 0.4 ml of 1 mM lidocaine was applied, the amplitude of the postsynaptic focal response decreased in parallel with the depression of the VR responses (Fig. 1B–D and G–I). At the peak of lidocaine action the VR response was almost totally abolished (Fig. 1D), while some of the postsynaptic focal potential remained (Fig. 1I). On the other hand, the presynaptic focal potential was not affected (Fig. 1I), indicating that impulse conduction in the presynaptic terminal fibers was preserved at a level of lidocaine which caused almost total abolition of the VR volley produced by LC stimulation. A much larger amount of lidocaine first blocked the postsynaptic focal response completely and then later also blocked the presynaptic focal response (Fig. 1J).

The relationship between the amplitude of the presynaptic focal potential and the concentration of lidocaine was studied in the preparations where the perfusion fluids containing various concentrations of lidocaine were switched successively, as well as the relationships for the postsynaptic component and for the VR response (Fig. 2). The concentration required to decrease the postsynaptic focal potential and the VR volley was less than 0.01 mM. The VR response was totally abolished at a concentration less than 0.1 mM, while the amplitude of the presynaptic focal response was almost unchanged during perfusion with 0.2 mM lidocaine-Ringer solution. The changes in the responses were reversible.

The focal potential was also recorded from the region where the afferent terminals of the DR fibers make synaptic connection with the dorsal dendritic extensions of motoneurons. The greater sensitivity to lidocaine of the postsynaptic component evoked by DR stimulation was usually observed (Fig. 1K–P). In 5 preparations, the VR responses due to

![Fig. 2. Relationship between concentrations of lidocaine and the amplitudes of the responses on LC stimulation. A: presynaptic focal response. B: postsynaptic focal response. C: VR volley. Ordinate: percent of mean amplitude of the responses before drug applications. Abscissa: concentrations of lidocaine. Filled circles connected by line were obtained from one and the same preparation through successive perfusions with different concentrations of lidocaine solutions.](image)

the spinal reflex were recorded simultaneously with the focal responses, and it was found that in the presence of lidocaine VR activity disappeared before the postsynaptic focal response did.

*Intracellularly recorded motoneuron activities on response to lidocaine*

Intracellular recording from the motoneuron was confirmed by the short latency of the antidromic spike in response to a stimulus to the VR of the corresponding spinal segment. Most of the neurons produced a spike potential on LC stimulation when the stimulus intensity was adjusted to produce a just maximum monosynaptic response of the VR. DR stimulation usually produced spike potentials during the early phase of polysynaptic EPSPs, but not from the monosynaptic EPSPs. If a spike potential was not produced by both the LC and DR stimulation or if it was blocked at the initial segment or at the first Ranvier node upon VR stimulation during about 15 min of control recordings before application of lidocaine, the data were discarded. Successful, steady intracellular recordings were obtained from more than 40 neurons in the 20 spinal cords examined.
Fig. 3. Intracellular recordings of the responses due to antidromic and orthodromic stimulation. Responses of the motoneuron were produced by VR (top records), LC (middle) and DR (bottom) stimulation, and a few successive traces were superimposed in each record. A: control records before application of lidocaine. B and C were recorded in the presence of 1 mM lidocaine. B: potentials recorded just before and after the disappearance of the response to the EPSP evoked by a LC volley. C: responses, recorded just before and after the block of the invasion of the neuron soma by the antidromic spike potential.

The motoneuron whose responses are shown in Fig. 3A was excited orthodromically by each stimulus to the LC (middle record) and the DR (bottom) before application of lidocaine. The supra-threshold EPSPs resulted in spike generation, though the spike potentials are faint and truncated because of the high amplification. The threshold amplitude of the EPSP for spike generation was usually a little higher for DR stimulation than for LC stimulation as has been shown by others. The difference between the threshold amplitudes was greater in the neuron shown in Fig. 4.

The application of lidocaine caused a progressive decrease in amplitude of the EPSPs to both LC and DR volleys, which resulted in the failure of orthodromic spike generation. When the EPSP produced by LC stimulation just failed to generate a spike potential, the amplitude of the EPSP was a little larger than 80% of that before application of lidocaine (Fig. 3B, middle). At that time, however, the amplitude of the EPSP in response to a DR volley was less than one-fifth of its initial value (Fig. 3B, bottom).

The invasion of the cell body by antidromic impulse was present long after spike generation by orthodromic EPSPs was affected by lidocaine. The width of the antidromic spike potential increased with time during the action of lidocaine, and the inflection upon its rising phase due to the delay in conduction between the initial segment (IS) and the soma-dendritic (SD) region became apparent (Fig. 3C, top). These changes were eventually followed by block of the antidromic invasion of the SD regions. In some of the neurons examined, a block of antidromic SD-spike generation was found before abolition of spike initiation by orthodromic activation. Such early failure of the antidromic invasion was presumed to result from a slight deterioration of the cell impaled by microelectrode, since during perfusion with normal Ringer solution such neurons could not respond to repetitive antidromic stimuli at frequencies which caused no decrease in the size of the extracellular focal potentials.

The resting membrane potential was not changed appreciably by lidocaine even in the high concentrations required to abolish all spinal responses to electrical stimuli.

In some preparations, the intracellular records showed both the EPSP and the presynaptic activities in the terminal boutons ending on that neuron (Fig. 4). After addition of lidocaine to the perfusate, the spike potential initiated from the EPSP due to LC stimulation soon disappeared and the amplitude of the EPSP progressively decreased. However, the amplitude of the terminal potential remained almost unchanged just after abolition of the spike potential from the EPSPs (Fig. 4F). With time, the duration of the terminal potential increased and the onset of the EPSP was delayed.

Fig. 4. Effects of lidocaine on the responses of spinal motoneuron. Responses were recorded intracellularly. A-D: stimulus to DR (early responses) and LC (later responses). E-H: stimulus to LC, but recorded at magnified sweep speed. The stimulus intensity was kept constant in A-D and in E-H. A and E: control responses. B-D and F-H: responses after application of 1 mM lidocaine.
The effects of lidocaine on subthreshold EPSPs of the motoneuron are shown in Fig. 4A–D. Whether the EPSPs were elicited by DR or LC stimulation, the amplitude was decreased by lidocaine. In time, the monosynaptic and polysynaptic EPSPs disappeared (Fig. 4D).

When a frog spinal cord was perfused with lidocaine solution which did not cause conduction block of the presynaptic fiber, the time to peak amplitude was shortened, the time course of decay

Fig. 5. Time course of lidocaine EPSPs. A and C: the EPSPs in response to a stimulus to LC in normal, and in high Mg\(^{2+}\) (4 mM) and low Ca\(^{2+}\) (0.18 mM) Ringer solutions, respectively. B and D: the effects of 0.04 mM and 0.2 mM lidocaine on the EPSPs of A and C, respectively. E and F show the EPSPs by DR stimulation in 4 mM Mg\(^{2+}\) and 0.18 mM Ca\(^{2+}\) solution (E), and those in the solution containing 0.2 mM lidocaine (F). All responses are shown in DC recordings except for focal potentials which were recorded just outside the motoneuron and shown in the upper traces of A and B. Arrows in E and F indicate the electrotonic EPSPs.

Time course of lidocaine EPSP in normal and high Mg\(^{2+}\) solution

Fig. 6. Effects of lidocaine (0.04 mM) on repetitive EPSPs. Trains of 1–5 electrical stimuli were applied to LC; each 8 responses to the trains of different numbers of stimuli were averaged and the averaged responses were superimposed in A and B. In C and D, preceding EPSPs were subtracted with a data analyzer to show the last EPSPs of the responses to each train of 1–5 stimuli. A and C: in normal Ringer solution. B and D: in the lidocaine solution. All responses are shown in DC recordings.
was prolonged and the peak amplitude of the monosynaptic EPSPs was reduced (Fig. 5). Similar effects have been reported for procaine and lidocaine end-plate potentials\(^9,14,17,28\). The slowing of the decay by lidocaine was observed in both normal (Figs. 3, 4, 5B and 6D) and high Mg\(^{2+}\) Ringer solutions (Fig. 5D), but the magnitude of the effect was quite variable among motoneurons. At higher concentrations of lidocaine, the prolongation in decay was more pronounced. The polysynaptic EPSPs evoked by DR stimulation also showed a decrease in their peak amplitude and a prolonged time course during perfusion with the solution containing lidocaine, while the first component, i.e. the electrotonic EPSP\(^1,25,26\) (indicated by an arrow), remained stable in the presence of 0.2 mM lidocaine (Fig. 5E and F).

When EPSPs were elicited by a train of electrical pulses applied to LC, the successive EPSPs showed summation (Fig. 6A and B). In the presence of 0.04 mM lidocaine solution, the first EPSP was reduced to about 45% of the control (from 7.7 mV to 3.5 mV) and the 5th EPSP to about 69% of the control (from 6.4 mV to 4.4 mV) (Fig. 6B). The greater decrease in the earlier EPSPs can be easily observed in Fig. 6C and D, where the records were made by subtraction of the preceding EPSPs performed with the help of a data analyzer.

**DISCUSSION**

Lidocaine, at high concentration, impaired the conduction of orthodromic impulses along the primary afferent and descending LC fibers, and blocked the invasion to the motoneuron cell bodies of antidromic impulses of the axons, as has been already demonstrated by Curtis and Phillis\(^4\) for procaine. However, the absence of effects by low concentration of lidocaine on either the presynaptic terminal potentials elicited by the two types of orthodromic stimulation or the electrotonic EPSPs of the motoneurons provide evidence that the suppression of spike initiation from the EPSPs is not caused primarily by conduction block in the presynaptic nerve fibers. This has been shown to be true for the actions of general anesthetics in the central nervous system as well as for local anesthetics at the neuromuscular junction\(^5,9,17,24\).

The soma-dendritic spike potential produced by an antidromic volley remained in most of the intracellular recordings, long after the spike generation from the EPSP was blocked. The evidence suggests that the depression of the synaptically induced discharges by lidocaine cannot be ascribed to an effect of the electrically excitable membrane but to a decrease in amplitude of the EPSP. The decrease of EPSP amplitude before the increase of the firing threshold does not coincide with the observation of procaine action on the motoneuron of the cat\(^4\). The difference may result from a topical application by iontophoresis of the drug or some deterioration of the neuron. In some neurons, an increase in threshold of the excitable membrane may have contributed to the suppression of firing.

The present study showed the decrease in peak amplitude, the shortening in time to peak and the slowing in the decay time of EPSP in the presence of lidocaine, in agreement with previous findings on the end-plate\(^9,17,28\). The smaller change in the lateral EPSPs of the successive responses induced by a train of stimulation might reflect summation of the tail of the postsynaptic currents by lidocaine. At the end-plate, it has been reported that the reduction of EPSPs by local anesthetics is more severe for short application of ACh than for prolonged application of ACh\(^14,28\).

The postsynaptic blocking action of procaine at the end-plate has been supposed to originate from a greatly decreased amplitude of the elementary potential change\(^14\). It has been also proposed that lidocaine derivatives block open end-plate channels, changing the square pulses of single channel currents into burst of much shorter pulses\(^28\). From the same line of the explanations made on the end-plate, it may be proposed that low concentrations of lidocaine have a direct action on the postsynaptic membrane of the motoneuron. Further studies are necessary to elucidate the mechanisms of action of lidocaine on the central synapses in the spinal cord.

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