The Effect of Strychnine on Membrane Properties of Spinal Motoneurons in the Cat

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ABSTRACT

Strychnine (Stry.) has been used, as an instrument for studies of experimental epilepsy, though its precise mode of action has remained obscure. One mechanism of action was partially clarified in 1954 by the demonstration that subconvulsive doses of Stry. reduce the amplitude of inhibitory postsynaptic potentials (IPSPs) in the cat’s spinal motoneurons (MN). Because of the rapid onset of its action and the absence of effects upon monosynaptic excitatory postsynaptic potentials (EPSPs), it was proposed that Stry. competed with some unidentified transmitter for inhibitory receptor sites on the postsynaptic membrane. Electrophoresis of Stry. is known to block the inhibitory effects of glycine, a likely candidate as an inhibitory transmitter on MN in the cat spinal cord. A Stry. resistant inhibition seems to exist not only in the higher portion of the CNS, but also for the spinal MN. Gamma amino butyric acid (GABA) is a candidate for this synaptic transmitter.

In Nembutal anesthetized cat, intracellular recording of spinal MN was performed during Stry.-induced seizure.

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To conclude, it can be said that there were no consistent changes in the MN action potential which would reflect an action of Stry. upon MN’s membrane properties important to seizure generation. It is still to be resolved whether the increase in polysynaptic EPSP amplitude is due to a Stry. effect upon the membrane properties of excitatory interneurons or to an effect only upon the inhibitory as well as the EPSPs.

INTRODUCTION

The convulsant properties of strychnine have attracted the attention of neuropharmacologists for over 150 years (Wall et al., 1956). It has been used as the instrument for analysing electrical activities of the central nervous system (Brookes and Fuortes, 1952) and for studies of experimental epilepsy (Dusser De Barenne, 1953; Bremer, 1953; Chang, 1951; Wall and Horwitz, 1951; Purpura and Grundfest, 1957; Goldring and O’Leary, 1960; Pollen and Sie, 1964), though its precise mode of action has remained obscure. Many attempts have been made to explain the mechanism by which strychnine influences neuronal activity.

The mechanism of its action was partially clarified in 1954 by the demonstration that subconvulsive doses of strychnine reduce the
amplitude of inhibitory postsynaptic potentials (IPSPs) in cat spinal motoneurons (Eccles et al., 1954). Because of the rapid onset of action and the absence of effects upon monosynaptic excitatory postsynaptic potentials (EPSPs), it was proposed that strychnine competed with some unidentified transmitter for inhibitory receptor sites on the postsynaptic membrane (Eccles, 1957). This hypothesis was supported by the observation that local electrophoresis of strychnine onto motoneurons also reduced IPSPs without effecting EPSPs or the field potentials generated by presynaptic inhibitory fibers (Curtis, 1962). Furthermore, electrophoresis of strychnine has been reported to block the inhibitory effects of glycine, a candidate for being the inhibitory transmitter on motoneurons in the cat spinal cord (Werman et al., 1968; Curtis et al., 1967, 1968 a and b).

Since it is probable that strychnine blocks some spinal postsynaptic inhibition by preventing the access of the transmitter substance to the appropriate sub-synaptic receptor sites (Curtis, 1963), it may be proposed that this transmitter, presumably the same for all strychnine-sensitive inhibition, differs from the transmitter responsible for the strychnine-resistant postsynaptic inhibitions of cerebral, cerebellar, olfactory (Green et al., 1962), thalamic and hippocampal neurons (Andersen et al., 1963). In more recent reports of experiments on the cat, evidence has been presented that gamma-amino butyric acid (GABA) is an inhibitory transmitter and its receptor is insensitive to strychnine but blocked by bicuculline (Curtis et al., 1968, 1971).

The factors causing epileptic discharges can be very different and may involve different parts of the neuronal membrane. Therefore, according to the classification proposed by Grundfest (1961), there are three different types of ionic channels in the cell membrane can be affected by an epileptogenic drug:

1) The resting or leakage channels, the permeability of which to potassium, sodium and chloride ions determine the resting membrane potential (RMP). In addition the active sodium-potassium transport system is important for the generation of the RMP.

2) The subsynaptic channels for sodium, chloride and potassium ions, which are activated by the chemical transmitters and generate the EPSP and IPSP.

3) The potential-dependent and electrical excitable sodium and potassium channels which are generating the action potential.

In experimental epilepsy increased excitation or excitability of the neurons has to exist in order to elicit seizure discharges. This can be due to a reduced RMP, to an increase in the synaptic excitation/or reduced inhibition, or finally, in changes in the excitability of the cell membrane due to changes in the characteristics of the electrically excitable ionic channels.

As has been described above, strychnine is known to affect the chemically excitable membrane, i.e. being a competitive antagonist to glycine. The aim of the experiments to be described was the following:

1) According to the literature strychnine generates seizures even in structures in which it does not block the IPSPs (cerebral cortex, hippocampus and cerebellum). The usual method to induce strychnine seizure in the structures cited above is the topical application of strychnine on their surface. The effect of doses of topical application does not allow a comparison of the effects on the spinal level following intravenous injection. For a better
comparison, the “threshold” for the generation of spontaneous seizures in the isolated cerebral cortex and the spinal cord of cats following intravenous injection was determined in a series of experiments.

2) In the spinal cord the IPSPs are blocked already in subconvulsive doses. Therefore it is possible, that even in the spinal cord the interaction of strychnine with the glycine receptors might not be the main source of the general epileptogenic action. It seemed necessary to re-check with more refined methods the report of Fuortes and Nelson (1963) that strychnine does not change the amplitude and time course of the action potential of the motoneuron. As had been described also by Larson (1969) there should be no change of the RMP with small doses of strychnine. Therefore an attempt was made to find out, whether higher doses of strychnine (up to 0.85 mg/kg) could influence those membrane properties.

3) The constant effect of strychnine on the excitatory input of the motoneuron is a rapid and strong increase in the amplitude of the polysynaptic EPSP. An attempt was made to decide which factors were responsible for this phenomenon.

4) Bicuculline, a known GABA-antagonist, was injected for blockade of strychnine-resistant inhibitory components in polysynaptic PSPs.

5) Mephenesin is known as a very effective antagonist of strychnine seizure (Smith, 1965). To test, whether substances of its class, i.e. central muscle-relaxants like MY 301, act only by depressing the transmission in polysynaptic pathways (or also changes membrane properties of motoneurons), doses of up to 175 mg/kg were given.

6) GABA derivative, Lioresal, was used to compare its effect with the effect of MY 301 in blocking seizures induced by strychnine or by bicuculline.

MATERIALS AND METHODS

I. Animal preparation

A total of 35 adult cats of both sexes, weighing between 1.5 to 3.5 kg were used. They were anesthetized by an intraperitoneal injection of sodium pentobarbital (Nembutal, Abbott, 30 mg/kg). In some cases supplemental doses (5 to 10 mg/kg) were given 3 to 4 hours after the initial injection.

The trachea was intubated. The lumbar nerve segments of the spinal cord were exposed by laminectomy. Following dural incision, the dura mater ligaments of the ipsilateral (recording) side were cut. Nerves of the biceps/semittendinosus (BST), quadriceps, gastrocnemius and tibialis muscles were dissected in the left leg. The skin flaps were arranged to form a pool, so that the spinal cord and the hind limbs nerves could be bathed in paraffin. Under a dissecting microscope, the ventral roots L6-S1 were transected at their dorsal exits and were used for antidromic stimulation of the motoneurons. In some cases, only the left dorsal roots L7 and S1 were used for orthodromic activation. The muscle nerves were hooked around bipolar stimulating electrodes for orthodromic stimulation. The dorsal roots of L5 and L6 were cut and the proximal stumps deflected to the other side. The cord was rotated to the contralateral side and supported by a piece of cotton. Care was taken to protect the radicular arteries during the micro-dissection. Movements of the spinal cord were minimized by rigid clamping of the spinal process, L1, vertebral column at L4 and the pelvis. Following the procedures, the animals were paralyzed.
with intravenously administered gallamine triethiodide (Flaxedil, Boehringer & Sohn, 2 mg/kg) and artificially respired (Animal Respirator Pump, Phipps & Bird, Richmond). A bilateral pneumothorax, minimized movements associated with respiration.

The rectal temperature was maintained at 37±1°C by a heating pad and the paraffin-filled pools maintained at 38°C by tubings immersed in the paraffin through which water was circulated by a thermostat.

Strychnine sulfate was dissolved in Ringer saline and administered intravenously in consecutive doses of either 0.10 or 0.15 mg/kg. In some experiments, interaction between strychnine and GABA-derivative β-(4-chlorophenyl)-gamma-aminobutyric acid (Lioresal, Ciba-Geigy, Basel, 2 mg/kg), guaiacol glyceryl ether (MY 301, Dr. Christian Brunnengraber, Chemische Fabrik & Co., Lübeck, 50 to 100 mg/kg) or bicuculline (0.25 to 0.50 mg/kg), were studied.

In 4 cats, with an isolated spinal cord (C1-2 section) preparation, the difference in sensitivity between the cortex and the spinal cord for the generation of spontaneous seizure due to convulsive doses of strychnine were investigated. In these experiments, in contrast to the following section, only mass recording techniques were used: electrocorticogram (ECG) recorded by a small disk placed on the motor cortex and the cord dorsum potential recorded from a silver ball electrode on the dorsal surface of the spinal cord at the level of L7. In each case evoked potentials were measured: those of the motor cortex following electrical stimulation of the ventralis oralis posterior (Vop) nucleus of the thalamus and those from the ventral root or dorsal surface of the spinal cord after stimulation of the dorsal root L7, quadriceps or BST muscle nerves. For these studies consecutive doses of strychnine, 0.10 to 0.50 mg/kg up to total of 3.6 mg/kg were administered intravenously.

II. Recording techniques

Intracellular recording was performed with single glass micropipettes filled with 3 M potassium chloride and having tip diameters ranging from 0.5 to 3 μ. The resistance of the microelectrodes in the spinal cord varied from 3 to 30 MOhm. A bridge circuit was used to balance the potentials developed across the electrodes while passing current into the cell by the recording electrode. Only those electrodes which displayed negligible rectification while passing up to 20 nA (nA or 10⁻⁹ A) of current were used. The connection between the microelectrode electrolyte solution and the preamplifier input (Mento, N-950 intracellular probe system, Mineapolis, Minnesota) was made with Ag-AgCl junction. The reference electrode was an Ag-AgCl junction to a Ringer-soaked cotton pad which made a low resistance contact with the animal.

The electrode was inserted into a motoneuron from the dorsolateral surface at the level of the L7 or S1 roots by a micro-manipulator (L.P.C., Paris). The preamplifier output was subsequently amplified by Tektronix type 3A9 and 2A63 differential amplifiers for display on a Tektronix 7704 or 565 oscilloscope and a pen-writer (Rikadenki, Tokyo). Action potentials were electronically differentiated using a Tektronix 3A8 operational amplifier with an external circuit (time constant: 10 µsec). The oscilloscope traces were photographed on moving films (Fa. Tönnies, Germany or Nihon Kohden, Gokyo, Japan).

For measurement of postsynaptic potentials, 15 consecutive sweeps of 125 msec duration were averaged with a computer of Average
Traneient (CAT-400, North Haven, Connecticut, U.S.A.) and printed out by a VARI- 
ANT X-Y-Plotter.

Audiomonitoring of the signal was provided by an AM-3 (Grass instruments, Quincy, 
Mass.). Stimulation was provided by a Grass instruments S-8 which received timing signals 
from a Digitimer type 3290(Devices, London). An additional stimulator channel was provided 
by a Devices isolated stimulator, type 2533A.

III. Data selection

Motoneurons were identified by antidromic activation via the ventral roots. The criteria 
used for the selection of data from the recorded neurons were i) a membrane potential 
greater than 50 mV, ii) spike amplitude greater than 60 mV and iii) a stable resting 
potential over a period of 20 to 30 min. About 70 neurons were impaled which gave at least 
10 min recording but only 29 neurons fulfilled all the requirements noted above. In electrically 
differentiated recording (Fig. 3, 1B), the rates of rise and fall of the action potential 
were expressed by the amplitude of the component E1 to E4. E2 represents presumably 
the sodium activation of the action potential, while E4 represents potassium activation and 
E2/E4 is therefore the interval between sodium and potassium activation (Ito & 
Oshima, 1964, Klee et al., 1974 c).

RESULTS

I. Doses of strychnine inducing spontaneous seizure activity in the spinal cord and the cerebral cortex

Recording from the cord dorsum or the ventral root L7 of the isolated spinal cord 
elicited a negative cord dorsum potential, following stimulation of the dorsal root L7 
or quadriiceps or BST muscle nerves. Intra-
venous administration of strychnine, (0.30 to 
0.45 mg/kg (0.35±0.07 mg/kg) (Table 1) 
resulted in the appearance of accentuated sharply positive spikes following the negative 
wave in 4 experiments, i.e., spontaneous seizures of the spinal cord were induced 
represented by continuous discharges of sharply positive waves intermingled with the negative 
waves.

Surface recording fron the motor cortex, 
following electrical stimulation of Vop-nucleus 
of the thalamus showed positive-negative surface potentials. There was no change in 
the surface recording with doses of (0.3-0.4 
mg/kg) strychnine. In contrast to the spinal cord, 2.0-3.7 mg/kg (2.87±0.97 mg/kg) 
of strychnine were required to elicit the accentuation of the negative potentials(increase 
in both amplitude and duration), and the spontaneous discharges, so-called strychnine 
spikes.

Therefore, the convulsive doses of strychnine is about eight times higher for the motor cortex compared to the spinal cord in spinalized animals (see Table 1).

II. The effect of strychnine on membrane characteristics of the motoneuron

a. The effect of strychnine on the membrane potential

1. The effect of subconvulsive doses

The intracellular resting membrane potential (RMP) was recorded continuously by a pen- 
writer and measured every minutes for the first 5 min and later every 2 min following 
each injection of strychnine. Table 2 shows maximum changes in the RMP within 15 
min following the first injection (0.10-0.15 
mg/kg) in 15 motoneurons. As will also be described later, the effect of strychnine on 
the RMP was inconsistent: with subconvulsive
Table 1. Doses of Strychnine Necessary to Induce Spontaneous Seizure Activity in the Spinal Cord and the Cerebral Cortex.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Convulsive doses of strychnine (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spinal cord</td>
</tr>
<tr>
<td>1</td>
<td>0.35</td>
</tr>
<tr>
<td>2</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>0.30</td>
</tr>
<tr>
<td>4</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>0.35±0.07*</td>
</tr>
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</table>

* Standard error of mean.

doses of strychnine 10 neurons were depolarized by 0.5 to 10 mV (3.6±1.04 mV) and 5 other neurons were hyperpolarized by 1 to 5 mV (2.4±1.5 mV).

As is shown in Fig. 1A, the membrane potential of one neuron was hyperpolarized by up to 5 mV following the injection of 0.10 mg/kg strychnine. The hyperpolarized state persisted during the 15 min of recording. In another neuron shown in Fig. 1B the RMP was almost unchanged during 40 mNt of recording following the injection 0.15 mg/kg strychnine. But more frequently the membrane was slightly depolarized following the first injection and recovered, e.g. the neuron shown in Fig. 1C. In this cell, the membrane was depolarized by a maximum of 18 mV immediately after the first injection (0.15 mg/kg), thereafter the resting potential increased (hyperpolarized), exceeding the control level.

2. The effect of doses inducing convulsions

In cases of multiple injection of strychnine up to convulsive doses (more than 0.20 mg/kg), because of convulsive movement it was extremely difficult to have a stable recording; cell damage due to the instability in the recording apparatus often appeared. But in 6 neurons recordings were obtained success-

Table 2. Maximum Changes in the Resting Membrane Potential (RMP) within 15 Min. following Each Injection of Strychnine

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Initial RMP (mV)</th>
<th>First injection</th>
<th>Second injection</th>
<th>Final concentration of stry. (mg/kg)</th>
<th>Recording after inject stry. (min.)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Dosage (mg/kg)</td>
<td>Max. changes in RMP (mV)</td>
<td>Dosage (mg/kg)</td>
<td>Max. changes in RMP (mV)</td>
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</tr>
<tr>
<td>1</td>
<td>75</td>
<td>0.15</td>
<td>-1.5</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>0.15</td>
<td>+5.0</td>
<td>0.15</td>
<td>+4.5</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>0.15</td>
<td>-10.0</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>63</td>
<td>0.15</td>
<td>+1.0</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>0.15</td>
<td>-0.5</td>
<td>0.10</td>
<td>+1</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>0.15</td>
<td>-1.0</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>0.10</td>
<td>+2.0</td>
<td>0.10</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>0.10</td>
<td>+2.0</td>
<td>0.10</td>
<td>-8</td>
</tr>
<tr>
<td>9</td>
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<tr>
<td>11</td>
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<td>-9.0</td>
<td>0.10</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
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<td>0.10</td>
<td>-9.0</td>
<td>0.10</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>55</td>
<td>0.10</td>
<td>-2.0</td>
<td>0.10</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
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<td>0.10</td>
<td>+2.0</td>
<td>0.10</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>0.10</td>
<td>-5.0</td>
<td>0.10</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>62</td>
<td>0.10</td>
<td>-4.0</td>
<td>0.10</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>50</td>
<td>0.10</td>
<td>-2.0</td>
<td>0.10</td>
<td>0</td>
</tr>
</tbody>
</table>

(+) Depolarization, (-): Hyperpolarization.
Right two columns: Final concentration of strychnine and duration of recording after injection of strychnine.
Effect of Strychnine on Spinal Motoneurons

Fig. 1. Changes in resting potential following successive injections of strychnine.

A: Effect of strychnine during 35 min on the RMP of a motoneuron. Initial hyperpolarization following the first injection (○; 0.10 mg/kg), depolarized after the second (convulsive) injection (□; 0.20 mg/kg).

B: Effect of strychnine during 70 min on the RMP of a motoneuron, showing unchanged membrane potential although convulsions occurred; ○; first injection, □; second injection, ; each 0.15 mg/kg.

C: Effect of successive injections of strychnine, total 0.70 mg/kg, on the RMP for 80 min. Immediately after the first injection the membrane depolarized, later the potential increased gradually exceeding the control level; ○; 0.15 mg/kg, □; 0.10 mg/kg.

Fully over a time, up to 125 min.

In different cells convulsive doses (0.20 to 0.45 mg/kg) of strychnine resulted in different effects on the RMP. As is shown in Fig. 1A, initial hyperpolarization after the first injection was followed by a depolarization up to 23 mV after the subsequent convulsive injection (total 0.30 mg/kg). The cell later returned partially to the initial potential but remained depolarized by about 5 mV.

The cell of Fig. 1B showed almost unchanged RMP during the 30 min of recording following a convulsive injection (total of 0.30 mg/kg. Fig. 1C shows a long term (80 min) effect of high doses of strychnine on the membrane potential, a total 0.70 mg/kg. In this cell a gradual hyperpolarization followed the second injection (convulsive doses) exceeding the control value by 16 mV. There was a sharp drop in RMP with the last injection followed by a return to the control value.

It could be observed that the magnitude of RMP changes during strychnine was dependent on the initial resting potential, i.e., the changes in RMP were greater with lower RMP and vice versa. Fig. 2 shows graphically this dependency for 18 cells. The changes in

Fig. 2. The dependence of the maximum changes of the membrane potential on the control membrane potential.

Strychnine sensitivity of the RMP (mV/mg) of 18 motoneurons plotted against the RMP at the beginning of each injection (abscissa). Filled symbols: strychnine, 0.15 mg/kg; open symbols: 0.10 mg/kg of strychnine. The changes in RMP with strychnine were greater in those cells having initial RMP smaller than approximately −60 to −65 mV and small in those cells having RMP greater than −65 mV.
RMP with strychnine were greater in those cells having a RMP smaller than approximately -60 to -65 mV and relatively small in those cells having RMP greater, i.e., more negative. Moreover, for those cells with a RMP less -65 mV, strychnine induced depolarization in all but three cases.

b. The changes of action potential following strychnine application

The recording of the antidromic action potential is usually displayed in the following manner: A fast and delayed sweep representing the DC recording of the action potential (Fig. 6, A1b) and its electrically differentiated AC recording (a), together with a slower sweep speed DC recording of the antidromic action potential with similar gain as in the fast delayed sweep recording (c). High gain AC recording (same speed as in a) shows a truncated antidromic action potential and the postsynaptic potentials (d).

Among 25 neurons 19 neurons could be activated by antidromic stimulation during the entire course of the experiment. In the other cases the antidromic spike could only be elicited during the synchronous generation of an EPSP. As already described for the changes in RMP, strychnine had also no consistent effect on the spike potential. Nine neurons showed reduction of the amplitude by 7-20% with the subconvulsive doses of strychnine and in 6 cases there were only minimal changes (less than ±5%). In contrast, in 4 neurons the amplitude of the spike was increased by 3-8%.

The cell illustrated in Fig. 5, A2 and Fig. 9, B1 are the examples of the antidromic action potential which showed no changes in its amplitude with the subconvulsive doses of strychnine (0.10-0.20 mg/kg). As is shown in Fig. 3, 2A, Fig. 4, A2-B2 and Fig. 5, A3

Fig. 3. Changes in a mixed monophasic EPSP and the antidromic action potential following a single injection of strychnine.
1A: Antidromic action potential with calibration signal and zero reference line. 5 sweeps were superimposed.
1B: Electrical differentiated recording of the same signal as in 1A.
2A: 10 min after 0.10 mg/kg strychnine injection, amplitude of the action potential reduced by 2%, E2 reduced by 3%, E3 & E4 also reduced by 10%, tE2/E4 not changed.
2B: 12 min after the injection.
1C: Polysynaptic EPSP evoked by suprathreshold stimulation of the dorsal root L7, 5 sweeps were superimposed. The early peak of the fast depolarizing potential is the peak amplitude of the monosynaptic EPSP and is followed by the polysynaptic EPSP.
1D: 3 min after 0.10 mg/kg strychnine injection, amplitude of polysynaptic components of the PSP was increased by 200% and also its duration prolonged by 250%, while the monosynaptic component remained the same as the control.
2C: Slower sweep speed recording (see calibration signal) after 4 min
2D: 14 min after the injection, marked increase in amplitude and duration of the polysynaptic EPSP, amplitude of the monosynaptic EPSP is still not changed.

the amplitude of the action potential of these cells were reduced by 2-5% following the injection of 0.10-0.20 mg/kg strychnine. In contrast the cells demonstrated in Figs. 6,
Effect of Strychnine on Spinal Motoneurons

Fig. 4. Changes in a antidromic action potential and its electrical differentiation following a single injection of strychnine.
A1: Antidromic action potential of a L7 motoneuron evoked by stimulation of the ventral root, L7 (upper trace) and its electrically differentiated recording (lower trace).
A2: 4 min after 0.10 mg/kg strychnine injection,
B1: 23 min after the injection,
B2: 30 min after the injection.
Throughout the recording time the amplitude of the antidromic action potential is slightly increased (about 2%) while E2 & E3 increased by 5% (A2 and B1), E4 is not changed.

A2-B2 and 7, the upper part of the diagram, show increases in the amplitude after the injection of 0.15 mg/kg strychnine.

With the doses of strychnine being able to trigger convulsions (0.30-0.40 mg/kg), the amplitude of the action potential was unchanged (Fig. 9, C1) or slightly reduced (Figs. 6, B3 and 8, A3). Even with doses as high as 0.70-0.85 mg/kg, the effect of strychnine on the spike amplitude could be very different: the action potential demonstrated in Fig. 8, B4 increased by 11%, 87 min after the injection of a total of 0.70 mg/kg strychnine. The amplitude of the action potential, shown in Fig. 5, B3, was reduced by 13% with total doses of 0.85 mg/kg strychnine.

Fig. 5. Maximal change of an antidromic action potential after a total injection of 0.85 mg/kg strychnine.
A1: Antidromically evoked action potential (upper tracing) and its electrically differentiated recording (lower tracing) of a motoneuron in the L7 segment.
A2: 15 min after injection of 0.10 mg/kg strychnine,
A3: 5 min after the second injection of strychnine, (total: 0.20 mg/kg). Amplitude of action potential decreased by 5%, E2 decreased by 7%, E3, E4 and tE2/4 unchanged.
B1: 22 min after the fourth injection of strychnine (total: 0.40 mg/kg). Amplitude of action potential reduced by 20%, E2 reduced by 15%, B2: 20 min after the sixth injection of strychnine (total 0.70 mg/kg). E2 reduced by 25%, E4 reduced by 23%.
B3: 15 min after the seventh injection of strychnine (total: 0.85 mg/kg). Amplitude of action potential reduced by 13%, E2 and E4 reduced by 17%, E3 reduced by 26%, tE2/4 reduced by 7%.

The relationship between the changes of the amplitude of antidromically activated action potential and the changes of the RMP was following: Among 19 neurons studied, 13 neurons showed reduction of the spike amplitude associated with depolarization of membrane potential and in 4 neurons the
mic discharges occurred. Comparing (a) and (b) in A2 and A3 with A1 shows that despite the effects of strychnine on the EPSP the antidromic action potential has become faster (a) and increased in the total amplitude (b).

B1: 48 min after injection. Spontaneous orthodromic discharges still occur, (c) and (d).

B2: 86 min after the injection the amplitude of the polysynaptic EPSP reduced but still was larger than in control. Action potential is still larger and faster compared to A1.

B3: 3 min after second injection of 0.15 mg/kg strychnine. Again the amplitude of polysynaptic EPSP increased markedly with multiple discharges, while the antidromic action potential is now reduced.

amplitude increased parallel with an increase of the RMP. In 2 neurons the amplitude even increased despite a depolarization of the RMP.

In the electrically differentiated recording the rates of rise and fall of the action potential are expressed by the amplitude of the components E1 to E4 (see inset in Fig. 3, 1B). It can be assumed that E2 represents the activation of the sodium channels, E4 the delayed activation of the potassium channels while E3 might be related to sodium inactivation (Ito & Oshima, 1964; Klee et al., 1974c). Quite often the increase in amplitude of the action potential was associated with an increase in E2 (Figs. 4 and 7). Component E4 was often unchanged or decreased when the amplitude of action potential also decreased. Fig. 3, 2A and Fig. 5, B2 show reduced component E4 together with reduced amplitude of spike potential. In Fig. 5, A3 component E4 was unchanged although the spike amplitude is reduced. the t E2/E4, representing the interval between sodium and potassium activation, was unchanged with subconvulsive doses of strychnine (Fig. 3, 2A and Fig. 5, A3), while the
Effect of Strychnine on Spinal Motoneurons

Fig. 7. Changes of spike-and membrane parameters and the amplitude of a polysynaptic EPSP during 9 min of strychnine action.

**Upper part:** Percentage changes of parameters of action potential and membrane potential after single injection of 0.15 mg/kg strychnine; amplitude of action potential (▲) increased during the first 6 min, then returned to the control value. Duration of action potential (●) showed reciprocal relationship to its amplitude. Component E2 (●) and E3,4 (○) were also increased after the injection, RMP (○) shows slight depolarization throughout the recording period.

**Lower part:** Plot of the amplitude of polysynaptic EPSP (x) reveals immediate response, increased by 230% of control value within 30 sec of injection, then increased further up to 560% during the next 2 min.

interval was prolonged (7-10%) with convulsive doses (0.30 mg/kg) (Fig. 9, C1).

The hyperpolarizing after-potential was unchanged with subconvulsive doses of strychnine (Fig. 9, B1). It was reduced by approximately 30% with convulsive doses (0.40 mg/kg or more) of strychnine (Fig. 8, A4) if the RMP increased.

III. The effect of strychnine on the post-synaptic potentials (PSPs)

a. The monosynaptic excitatory postsynaptic potential (EPSP)

The monosynaptic EPSPs elicited by elect- 

Fig. 8. Increased amplitude of an antidromic action potential following strychnine injection up to 0.7 mg/kg.

A1: DC recording of antidromic action potential (b), its slow sweep speed recording (c) and electrically differentiated AC recording (a), (d); high gain recording of (c).

A2: (c) and (d); Response to the orthodromic stimulation, eliciting polysynaptic EPSP.

A3: 4 min after injection of 0.3 mg/kg strychnine, amplitude of action potential is reduced by 7% (b) and its duration increased.

A4: 5 min after injection of 0.40 mg/kg strychnine. Amplitude of action potential exceeds the control value and the action potential became faster, i.e. the rising and falling time is shorter (increased amplitude in (a)). Hyperpolarizing after-potential is reduced together with an increase in the RMP.

B1: 6 min after total of 0.55 mg/kg strychnine. The amplitude of the action potential increased further and its duration became shorter (compare to A4).

B2: 26 min after 0.55 mg/kg strychnine. Orthodromic shock resulted in amplitude of polysynaptic EPSP increased beyond the firing level eliciting orthodromic discharges.

B3 and B4: 9 and 21 min after 0.70 mg/kg
strychnine. Amplitude of action potential increased, amplitude of polysynaptic EPSP remained increased.

rival stimulation of the synergistic muscle nerves or the dorsal root, were not changed by subconvulsive doses nor by convulsive doses of strychnine. Only if the stimulus intensity was increased (superthreshold) a polysynaptic EPSP was superimposed on the decaying phase of the monosynaptic EPSP; its amplitude sometimes reached the firing level eliciting an action potential.

Fig. 3 column C and D shows changes in mixed mono-and poly-synaptic EPSP, evoked by stimulation of the dorsal root, following a single injection of strychnine. The early peak of the fast rising depolarizing potential is the peak amplitude of the monosynaptic EPSP which is followed by the polysynaptic EPSP (1C). Following injection of strychnine (0.10 mg/kg) the amplitude of the polysynaptic component was increased, while the peak amplitude of the monosynaptic EPSP remained the same as in control (1D vs. 1C). In the cell shown in Fig. 9, column 2, a monosynaptic EPSP was elicited by the dorsal root stimulation, suprathreshold stimulus strength (A2). Following the injection of strychnine 0.10 mg/kg, the monosynaptic component of the EPSP, the fast rising depolarizing potential up to the firing level, was unchanged, while the delayed polysynaptic component exceeded the firing level eliciting multiple orthodromic discharges (B2-D2).

Averaged amplitude of the monosynaptic EPSP evoked by stimulation of BST muscle nerves (Fig. 10A, second potential) were unchanged following injection of 0.15mg/kg strychnine, while started on falling phase another polarization (Fig. 10B). Following subsequent convulsive injection the fast rising component

Fig. 9. Comparison of the effects of strychnine on an antidromic action potential vs. a monosynaptic EPSP, elicited by superthreshold stimulation.
A1: Antidromic action potential and its electrically differentiated recording; slower sweep speed and high gain recording of action potential (lowest tracing), uppermost tracing is the reference zero line with time marks every msec for the slower sweep speed.
A2: Monosynaptic EPSP elicited by the dorsal root stimulation (suprathreshold stimulus strength).
B1: 5 min after 0.10mg/kg strychnine, unchanged action potential and hyperpolarizing after-potential.
B2: 3 min after the injection, monosynaptic component of the EPSP is not changed, while delayed polysynaptic component exceeds the firing level eliciting multiple orthodromic discharges.
C1: Injection of 0.30 mg/kg strychnine, amplitude of action potential is unchanged, E2 reduced by 11%, E3, 4 reduced by 20%, E2/4 increased by 10%.
C2: Increased number of orthodromic discharges following the test stimulus.
D1: Amplitude of action potential increased slightly, while the amplitude of polysynaptic EPSP increased strongly (D2).

of the monosynaptic EPSP was unchanged but the polysynaptic EPSP increased on its falling phase (Fig. 10D).

b. The polysynaptic EPSP

The most prominent and constant effects of strychnine observed in these experiments were those on the polysynaptic EPSPs. In contrast to the monosynaptic EPSPs, the amplitude of the polysynaptic EPSPs elicited by the stimulation of the nerves of synergistic muscle or the dorsal root, increased by 200–550% even with the subconvulsive doses of strychnine (Fig. 7, bottom) together with an increase in their duration by about 150 to 200%. The increase can be observed immediately (within 30 sec after the injection) and the amplitude of the polysynaptic EPSP increased further showing its maximum within 10 to 15 min. Often the depolarization reached firing level eliciting orthodromic discharges. In 20 to 25 min after the injection the amplitude can be reduced but is still 200–300% larger than the control value over more than an hour. As is shown in Fig. 6, the polysynaptic EPSP of this cell increased in amplitude (400%) and duration (260%), 6 min after the first injection (A2).

With convulsive doses of strychnine, the increased EPSPs gave rise to multiple bursting discharges. These depolarizing waves represent the epileptic events of the cellular level and occur synchronously with negative-positive waves in the cord dorsi potential recorded from the surface of the spinal cord. Fig. 10 shows the effect of a pair of shocks, one to

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Fig. 10. Different effects of strychnine on polysynaptic and monosynaptic EPSP. X-Y-Plot.

A: Averaged polysynaptic EPSP evoked by stimulation of the dorsal root followed by a monosynaptic EPSP evoked by stimulation of BST muscle nerve. 15 consecutive sweeps of 125 msec duration were averaged with a CAT 400 computer and printed out by X-Y-plotter. Calibration signal: 10 mV, 2 msec.

B: 10 min after injection of 0.15 mg/kg strychnine. Enormous increase in amplitude of the polysynaptic EPSP giving rise to multiple discharges, while the amplitude of the monosynaptic EPSP is not changed; the delayed falling phase is followed by another depolarization.

C: 30 min after the injection. Additional increase of the polysynaptic EPSPs.

D: 5 min after strychnine injection (total 0.30 mg/kg). Polysynaptic EPSP increased further, monosynaptic EPSP; fast rising component is not changed while its falling phase exhibits polysynaptic EPSP.
the dorsal root followed by another stimulation of the BST nerve. The response of dorsal root stimulation is a polysynaptic EPSP while the BST gave rise to a monosynaptic EPSP followed by a polysynaptic component. The later one has already been described in the foregoing paragraph. The figure shows the X-Y plot from an average computer (CAT-400). Ten min after injection of 0.15 mg/kg strychnine (B), the polysynaptic EPSP showed an increase of amplitude giving rise to multiple discharges. Five min after the second injection (total 0.3 mg/kg) it further increased (D).

It should be noted that compared to the minimal changes in the action potential (Fig. 3, 2A and B), the amplitude of the polysynaptic EPSPs were increased by at least 100% after the first injection of 0.1-0.15 mg/kg (Fig. 3, 2C and D).

c. Strychnine-resistant inhibition

As already described, the main points of interest for this study were the following:

1) Is strychnine in doses of 0.1-0.8 mg/kg induce changes in the membrane properties of the motoneurons, i.e. changes of the resting potential and the action potential? 2) How can we explain the increase in the amplitude of the polysynaptic EPSP?

For this later question a different mechanism could be responsible: a) The reduction or blockade of inhibitory components mixed within the polysynaptic PSP by strychnine could cause an increase of the depolarization, i.e. increase the amplitude of the polysynaptic EPSP. If this is correct, strychnine could reduce block those phenomena of inhibitory processes which seems to be due to a contamination of the EPSPs by IPSPs. If, on the other hand, these phenomena, e.g. 1) conductance changes, 2) changes of the EPSP amplitude during hyperpolarization and 3) increase in EPSP amplitude following chloride injection still persist after strychnine injection, one has to draw the conclusion, that a substantial part of the inhibition of the motoneuron is of a strychnine-resistant character. This then would lead to the conclusion that this hypothesis is not the main reason for the changes of the EPSP amplitude.

b) Strychnine might increase the excitability of the excitatory interneurons. In this case a given stimulus would elicit a greater number of spikes in these inter-neurons, which would then lead to a more frequent synaptic bombardment of the motoneuron.

c) The polysynaptic EPSP can differ from the monosynaptic EPSP in that the polysynaptic EPSP might increase in amplitude during hyperpolarization and have conductance changes expressed by the reduction of the antidromic action potential. Up to now it is impossible to decide whether this difference is due to a contamination of the polysynaptic EPSPs with IPSPs or express fundamental differences between the two types of synaptic potentials (see review: Klee, 1074a). This could even include different transmitter substances.

Considering the fact that strychnine should block some inhibitory post-synaptic potentials, a systematic investigation of the polysynaptic EPSP using three methods described below could be able to decide, to what extent IPSP components in the polysynaptic EPSP might be responsible for the different reaction of these EPSPs vs. the monosynaptic EPSP.

1. The mixture of IPSP in the polysynaptic PSP

Stimulation of the polysynaptic pathways can cause the additional activation of group II fibers giving rise to mixed EPSP/IPSP
sequences. Because of their different synaptic delay, the peak of the IPSP will coincide with the peak, the falling phase of the EPSP and/or speed up the decay of the EPSP as well. As is shown in Fig. 8, A2 c. and d, stimulation of dorsal root L7 elicited a mixed polysynaptic PSP. It is composed of a fast rising EPSP, which then decays rapidly, and is followed by an IPSP.

Because the existence of a strychnine-resistant inhibition is now well established, and because of the problems concerning the increase of the amplitude of poly-synaptic EPSP discussed above, it was interesting to know to what extent inhibition was still present in the poly-synaptic PSPs after the injection of strychnine. For this purpose different methods were used to demonstrate phenomena which might be due to inhibitory components in the PSP.

The methods used were the following:

i) Measurement of the conductance during the slope of the EPSP. As has been demonstrated (Klee, 1971, 1974a), polysynaptic EPSP shows, in contrast to monosynaptic EPSP, conductance changes during different phases of the polysynaptic EPSP. Whether this is due to fundamental differences between the two types of EPSP (also in respect to the localization of the synapses on the cell soma and/or dendrites) or only due to the mixture of polysynaptic EPSP with IPSP still remains unclear. This method is similar to the so-called short-circuit effect of the endplate potential on the action potential of the muscle fiber.

ii) The injection an inward current.

Similar to the above described differences between the two types of EPSPs in respect to conduction changes, polysynaptic EPSP differs from monosynaptic EPSP in that the amplitude of the polysynaptic EPSPs increased with increased membrane potential in contrast to the unchanged amplitude of monosynaptic EPSPs (Eccles, 1957, Klee and Wagner, 1967). Again, this effect could be explained by the contamination of the polysynaptic EPSP with IPSPs, but could also be due to differences in the localization of the synapses being involved (Klee, 1974a). Therefore this technique was used as an additional tool to look for inhibitory components.

iii) The injection of chloride ions into the neurons.

IPSPs are generated by a diffusion of chloride ions across the membrane. The amplitude and direction of the change of the membrane potential is given by the relationship between the resting potential and the chloride equilibrium potential ($E_{Cl}$) (the later being near $-70$ mV in cat motoneurons, Eccles, 1957). Increasing the internal chloride concentration shifts the $E_{Cl}$ to a less negative potential, i.e. an IPSP can be changed to a depolarizing potential (Eccles, 1957). Therefore, any inhibitory component mixed within a polysynaptic EPSP should be unmasked following a chloride injection. The IPSP components will be reversed to depolarizing potentials and being added to the depolarizing amplitude of the EPSP.

In simplifying the situation for an easier understanding in the following paragraphs those phenomena which could be due to IPSP components and also to different characteristics of the polysynaptic EPSPs will be expressed by the term: "inhibitory components".

2. The determination of inhibitory components

a. The measurement of conductance changes during the slope of the polysynaptic PSP

Antidromic action potentials were superim-
posed on the rising and falling phase of polysynaptic EPSPs, often resulting in a reduction of the amplitude of the action potential conditioned by an EPSP (compared to the antidromic spike potential without EPSP) due to the short circuiting action of the polysynaptic EPSP (Fig. 11, A1). Fig. 13 demonstrates the method of conductance measurement using the amplitude of the antidromic spike. Fig. 13, A1 and A3 show the maximum reduction of the amplitude (marked by arrows) of the antidromic action potential during the falling phase of a polysynaptic EPSP. Also in Fig. 13, B2 with faster sweep speed the amplitude of the antidromic action potential, which was conditioned by the polysynaptic EPSP, showed reduction by 4 mV compared to the superimposed unconditioned antidromic action potential. These findings could be taken as suggestion that conductance changes during the falling phase of the polysynaptic EPSP might be due to a contamination of the EPSP with IPSP.

Both before and after the administration of strychnine, often the antidromic spike was reduced during the time course of the polysynaptic potential. As is shown in Fig. 11 the amplitude of the conditioned antidromic action potential was reduced again by 4 mV compared to the unconditioned one (A1). Following injection of strychnine, 0.1 mg/kg, the conditioned antidromic action potential shows reduction of its amplitude by 7 mV (A2). After the subsequent injection of strychnine (total 0.4 mg/kg) (B2 and B3), the unconditioned antidromic activation of the cell was blocked, while the antidromic spike elicited together with the EPSP shows further reduction of its amplitude (B1 and B2 vs. A3).

In another experiment the antidromic action potential was superimposed on the polysynaptic EPSP, again varying the shock interval

![Diagram](image_url)

**Fig. 11.** Demonstration of a strychnine-resistant inhibition in the slope of a polysynaptic EPSP by measuring the amplitude of the antidromic action potential.

A1: Action potentials of two different amplitudes and their electrically differentiated recordings. Antidromic action potentials were superimposed on the falling phase of a polysynaptic EPSP, resulting in a reduction of the amplitude of the conditioned action potential by about 4 mV compared with the antidromic spike evoked without EPSP, due to the short circuiting action of the polysynaptic EPSP. Low gain and slow sweep speed recording in the lower line shows two antidromic action potentials; one is superimposed on the falling phase of the polysynaptic EPSP and the other is not conditioned by an EPSP.

A2: 3 min after 0.10 mg/kg strychnine injection. Slow sweep speed recording shows a orthodromic action potential followed by two antidromic action potentials; one is with conditioning by a polysynaptic EPSP and the other is without conditioning. Delayed fast sweep speed recording of the conditioned antidromic action potential shows 7 mV reduction in amplitude compared to the unconditioned one. Duration of the spike is prolonged and the membrane potential is slightly depolarized.

A3: 8 min after 0.30 mg/kg strychnine. During this doses of strychnine the conductance increase due to the EPSP still reduced the spike amplitude by 7 mV.
B1: 11 min after 0.30 mg/kg strychnine. The antidromic activation during the EPSP is evoked. The conditioned antidromic spike is reduced.

Fig. 12. Temporary reduction of a strychnine-resistant inhibition by injection of bicuculline.
A1: Control recording. Antidromic action potentials were superimposed on the polysynaptic EPSP, varying the shock interval between orthodromic and the antidromic stimulation. The amplitude of the conditioned action potentials were unchanged on the rising phase, while it was blocked on the peak and during the falling phase of the polysynaptic EPSP, probably due to the contamination by inhibitory components within the polysynaptic PSPs.
A2 & A3: Injection of 0.10 mg/kg strychnine does not take off the blockade which presumably is due to a strychnine-resistant inhibition.
B1 & B2: Administration of 0.25 mg/kg bicuculline resulted in the temporary reduction of a strychnine-resistant inhibition. Blockade of the action potential disappeared on the falling phase of the polysynaptic EPSP, but the blockade reappeared 4 min after the administration of bicuculline (B3).

between the orthodromic and the antidromic stimulus, the amplitude of the conditioned action potentials were unchanged on the rising phase (not shown in the figure), while the soma-dendritic spike (SD) was blocked on the peak and during the falling phase of the

Fig. 13. Demonstration of the method of conductance measurement using the amplitude of the antidromic spike and the effect of bicuculline on membrane potential and spike amplitude.
Recording method is same as Fig. 12.
A2: Unconditioned antidromic action potential.
A1, 3: Arrows indicate maximum reduction of the amplitude of the antidromic action potential during the falling phase of the polysynaptic EPSP.
B1: Expanded recording of unconditioned action potential and its electrically differentiated recording by a delayed sweep.
B2: Two different antidromic action potentials are shown; one is conditioned and the other is unconditioned by a polysynaptic EPSP. Conditioned one shows reduction of its amplitude by 4 mV.
B3: Conditioned antidromic action potential by a polysynaptic EPSP. In slow sweep recording an orthodromic spike is followed by a conditioned antidromic spike.
C1: Shows 7 mV reduction of conditioned action potential.
C2: 10 sec after 0.25 mg/kg bicuculline. Reduction of membrane potential. Reduction of amplitude of the conditioned spike potentials.
C3: Further reduction of RMP 30 sec after injection. Orthodromic activation is nearly blocked, antidromic potential strongly reduced.
polysynaptic EPSP (Fig. 12, A1). Injection of 0.1 mg/kg strychnine did not take off this blockade of the somatic spike due to a presumably strychnine-resistant inhibition (Fig. 12, A2 and A3).

According to recent investigations (Werman et al., 1968; Curtis et al., 1988b), GABA is the inhibitory transmitter for the strychnine-resistant inhibitory PSP. Administration of bicuculline (0.25 mg/kg), which is known to be a GABA antagonist (Curtis et al., 1971), resulted in the temporary reduction of the effect of the strychnine-resistant inhibition (B1 and B2). The blockade of the SD-action potential disappeared on the falling phase of the polysynaptic EPSP, but its blockade reappeared 4 min after the administration of bicuculline (B3).

After demonstrating the conductance changes during a polysynaptic EPSP by comparing the amplitude of the conditioned and unconditioned antidromic action potential (Fig. 13, A1 and A3), the effect of bicuculline alone was investigated i.e. bicuculline was injected without preliminary application of strychnine. This injection resulted in seizures causing reduction and instability of membrane potential and reduction of the conditioned spike potentials. But, as can be seen in Fig. 13, C2 vs. B3, the antidromic activation is not blocked any more after the application of bicuculline. Thirty sec later the membrane potential and spike amplitude was reduced and the orthodromic activation nearly blocked.

β. The injection of inward current

As already described, polysynaptic EPSP mostly show an increase in amplitude during hyperpolarization. If this is due to inhibitory components, being reversed in polarity if the membrane potential is made more negative than $E_{PSP}$, then a drug which blocks inhibitory PSP should also prevent the increase in

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**Fig. 14** Strychnine-resistant inhibition and the effect of the injection of an inward current on the amplitude of a polysynaptic EPSP.

A1: High gain recording of polysynaptic EPSP (upper tracing) and its low gain recording with same sweep speed (lower tracing) in control state.

A2: Antidromic action potentials and their differentiated recording. The faster rising action potential is conditioned by a polysynaptic EPSP.

B3: 5 min after 0.10 mg/kg strychnine. An antidromic action potential was superimposed on a polysynaptic EPSP.

B4: Delayed sweep shows the same situation as in B3. Differences of the amplitude between the conditioned and the unconditioned action potentials after strychnine injection (6% reduction) expressed the conductance changes due to the EPSP.

C5: An increase in amplitude (70%) of the polysynaptic EPSP during injection of the inward current. Upper trace; without current injection, polysynaptic EPSP, after injection of 0.40 mg/kg of strychnine, lower trace; by passing an inward current of approximately 10
nA, 50msec duration, the membrane was hyperpolarized and the amplitude of the polysynaptic EPSP increased.

**Fig. 15.** The effects of chloride injection and current injection on the amplitude of a polysynaptic EPSP. X-Y-plot.

Recording method is same as in Fig. 10.

A: Average trace of the polysynaptic EPSP being increased after injection of 0.20 mg/kg strychnine.

B: Unmasking of IPSP component in polysynaptic EPSP by chloride injection. Average polysynaptic EPSP, following 20sec of injection of Cl ions from a KCl-filled electrode by 20 nA current; the amplitude increased by roughly 15%.

C: A strong (30%) increase in amplitude of a polysynaptic EPSP during the injection of an inward current.

The amplitude of a polysynaptic EPSP during the injection of a hyperpolarizing, i.e. inward current.

In 3 cells inward current pulses of 10 nA and 50 msec duration were injected before and after the application of strychnine. In control the amplitude of polysynaptic EPSP was increased with hyperpolarizing current, and following injection of strychnine, the amplitude was increased in the same manner by the inward current.

Fig. 14 shows an example of this type of experiment. The differences of the amplitudes between the conditioned and the unconditioned action potentials after injection of strychnine (7% reduction) express the conductance increase during the polysynaptic EPSP (Fig. 14, B4). Injection of an inward current resulted in an increase in amplitude (70%) of the polysynaptic EPSP (Fig. 14, C5).

Another cell, demonstrated in Fig. 15, C, shows a 30% increase in amplitude of a polysynaptic EPSP during the injection of inward current following strychnine 0.2 mg/kg injection.

7. The injection of chloride ions

The injection of chloride ions can be considered to be the most direct method to demonstrate the existence of IPSP components in mixed PSP.

In 5 neurons Cl⁻ was delivered from the
recording glass microelectrode filled with KCl by a hyperpolarizing current passing through the electrode. This procedure increased the amplitude of polysynaptic EPSP by an average of 50% in the control state which is due to the displacement of the equilibrium potential for the IPSP (E_{IPSP}) toward a less negative potential, resulting in a reversal of the polarity of the IPSP components. This effect lasts for about 2–4 min.

As is shown in Fig. 15 A, averaged amplitude of the polysynaptic EPSP was increased after injection of 0.2 mg/kg strychnine. Following 20 sec of injection of Cl^- from a KCl-filled electrode by 20 nA current, the amplitude of polysynaptic EPSP increased by roughly another 15%, unmasking the IPSP component in polysynaptic EPSP by chloride injection (Fig. 15).

In conclusion, the foregoing described experiments showed a persistence of conductance increase during the time course of polysynaptic EPSP after strychnine injection, a persistent increase in EPSP amplitude of polysynaptic EPSP during hyperpolarization and the possibility of unmasking an IPSP component in polysynaptic EPSPs by chloride injections after strychnine application.

Unfortunately the number of experiments in which all three methods could be applied to a single neuron is too small to draw final conclusions about the possible differences between mono-and polysynaptic EPSP.

IV. The antagonism of MY 301 (guaiacol glyceryl ether) and Lioresal (β-(4-chlorophenyl)-gamma-amionobutyric acid) to the phenomena induced by strychnine injection

a. The effect of MY 301

As proposed by some authors (Wall et al., 1965; Fuortes and Nelson, 1963; Klee and Faber, 1974b), strychnine may, besides depressing the IPSPs, increase the excitability of presynaptic elements more than the excitability of the motoneuron itself.

MY 301 (guaiacol glyceryl ether) a substance chemically very similar to mephenesin, is known to have stronger depressing effect on polysynaptic pathways and interneurons than on the monosynaptic excitation (Smith, 1965).

In 4 strychnine treated neurons, after injection of 50–100 mg/kg of MY 301, a strong reduction of amplitude and duration of the polysynaptic EPSP was observed (Wagner and Klee, 1968). Administration of MY 301 resulted in the stopping of convulsive activities induced by strychnine (Table 3). The anticonvulsant effect was almost immediate. Also this drug blocked the orthodromic multiple bursting discharges and reduced the amplitude of polysynaptic EPSP which was enhanced by strychnine (50% reduction within 3 min of action of MY 301). In one experiment, MY 301 ceased the seizure activity induced by the summed action of bicuculline (0.5 mg/kg) and strychnine (0.1 mg/kg).

Table 2. Interaction of strychnine with Lioresal and MY 301

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Total dosage of strychnine (mg/kg)</th>
<th>Doses of Lioresal stopping seizure (mg/kg)</th>
<th>Doses of MY 301 stopping seizure (mg/kg)</th>
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<tr>
<td>21</td>
<td>0.5</td>
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<td>50</td>
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*: In this experiment (Exp. No. 20), Lioresal could not block the seizure activity induced by the summed action of bicuculline (0.5 mg/kg) and strychnine (0.1 mg/kg) but it was blocked by MY 301 (50 mg/kg).
The RMP and amplitude of antidromic action potential were unchanged with doses of MY 301 blocking seizure activity. But increasing the concentration of the drug, more than 125 mg/kg, the RMP was reduced (depolarized) by about 10 mV and the amplitude of the action potential was also reduced by about 15% together with an increase of its duration. Administration of 175 mg/kg of MY 301 blocked antidromic activation.

b. The effect of Lioresal

The high concentration of GABA in mammalian nervous tissue suggests that this amino acid may be a transmitter in the central nervous system (Astrup et al., 1951; Purpura et al., 1959; Roberts et al., 1960). Furthermore, in more recent reports of experiments on the cat, evidence has been presented that GABA is an inhibitory transmitter and its effects are insensitive to strychnine but blocked by bicineulline (Curtis et al., 1968b, 1971).

The GABA-derivative β-(4-chlorophenyl)-gamma-aminobutyric acid (Lioresal) was applied intravenously. Unlike GABA, Lioresal readily crosses the blood brain barrier(Pederson et al., 1970).

In 4 experiments, administration of Lioresal (1~4 mg/kg) resulted in stopping the convulsive activities induced by strychnine (Table 3). The anticonvulsant effect was also almost immediate. The enhanced polysynaptic EPSP showed reduction (about 50%) in amplitude 2 min following injection of Lioresal (2 mg/kg).

But as was expected this drug (1 mg/kg) could not stop the seizure induced by the summed action of bicineulline (0.5 mg/kg) and strychnine (0.1 mg/kg) in contrast to the effect of MY 301 (see forgoing paragraph). The RMP and the antidromic action potential were unchanged by anticonvulsant doses (up to 3 mg/kg) of Lioresal.

DISCUSSION

By means of the intracellular microelectrode technique in anterior horn cells of the spinal cord, Eccles et al. (1954) found that IPSPs were reduced by strychnine without changes in membrane potential and amplitude of excitatory processes. They postulated that strychnine blocks the so-called "direct" and the recurrent inhibition in the spinal cord by competing with the inhibitory transmitter on the postsynaptic receptor sites. It was proposed that this mechanism might be responsible for the convulsant action of strychnine also in other parts of the nervous system. These studies, and those of Kuno (1967) in spinal motoneurons of the frog, proved that the convulsant action of strychnine was not due to sustained depolarization of the cell membrane as had been proposed by Brooks and Fuortes (1952). Blocking of recurrent inhibition was shown by Curtis (1962) to occur even when strychnine was administered by electrophoretic microinjection in the vicinity of a single motoneuron suggesting that the inhibitory blockade is not due to blocking of the cholinergic synapse of the Renshaw cell as proposed by Alving (1961).

According to the studies in the cerebral cortex, cerebellar cortex and hippocampus of the cat (Andersen et al., 1963; Crawford et al., 1963; Klee, 1966) the induced and the spontaneous IPSPs in these cells not only remained unchanged but were even enhanced at an early stage of strychnization.

These data suggested, that a different chemical transmitter might be involved in the transmission of IPSP in the spinal cord vs. parts of the central nervous system. Even in spinal motoneuron a type of inhibition, being
strychnine-resistant was described (Kellerth, 1965; Klee et al., 1966). The strychnine sensitive IPSPs seem to be generated by a liberation of glycine from the terminals of those interneurons being involved in the pathways of the direct and recurrent inhibition (Werman et al., 1968; Curtiss, 1968).

The strychnine-resistant IPSP might be generated by a liberation of GABA from different terminals at the motoneuron membrane (Curtis et al., 1971).

The problem to which this investigation tried to add further data is the following: Is the blockade of one type of inhibition by strychnine the main factor for the generation of epileptic discharges/or does this factor only add to other mechanisms and decrease the "threshold" for the occurrence of epileptic discharges in the spinal cord? This idea is supported by the following facts: i) Strychnine blocks direct IPSPs with subconvulsive doses (0.05−0.10 mg/kg) (Eccles et al., 1954). ii) Strychnine induces seizures in those structures in which the IPSPs seem to be uninfluenced (cerebral cortex etc.). iii) In experiments in which IPSPs are partially blocked by ammonium salts (Lux et al., 1970) increased excitability either never occur or is only a very short lasting phenomena (1−2 min). From this facts the question arises, whether beside its action on the IPSP, strychnine could induce changes on other membrane properties, which might lead to epileptogenic seizures.

Recent investigations (Klee et al., 1973; Faber and Klee, 1974), have shown that strychnine has selective and unique effects on both electrical and chemical excitability, the chemical excitable membranes being more sensitive than the electrically excitable regions.

As had been already proposed by Fuortes and Nelson (1963) the generator for the strychnine seizure might be located in the pool of excitatory interneurons. Strychnine might change their excitability by interfering directly with their membrane properties. Eventually this effect might be comparable to the effect of strychnine on snail neurons.

As has been postulated by Ayala et al. (1973) in a recent review article about the possible generation of epileptogenic activity, seizure activity might be induced by an enhanced positive feedback via recurrent excitation. Their model could explain the generation of seizure activity in the cerebral and hippocampal structures, but not in the spinal cord: Motoneurons of the cat process only a recurrent inhibition via the Renshaw-cells, but no recurrent excitation. Therefore the increased excitation has to come directly from the excitatory interneurons being activated by peripheral inputs. This hypothesis is illustrated in Fig. 16 (Klee 1974b). Strychnine could increase the synaptic drive coming from the excitatory interneurons being labelled as E-Pool in the right side of the scheme. As has been calculated by Calvin (1972) only 2−8% of the neuronal input has to be of abnormal activity in order to drive a "normal" nervous structure into epileptic activity.

For methodical reasons (size, fragility) it is more or less impossible, to make intracellular recordings from interneurons over a necessary long period of several minutes. Therefore the experiments being described try to draw conclusion indirectly, because the recordings were done from the relatively big (40−60μ) motoneuron in the lumbosacral spinal cord.

1. The effect of strychnine on the action potential and the resting potential of motoneurons

If one considers that strychnine acts directly and preferentially on membrane properties of
some nerve cells, i.e. interneurons, one would guess that at least with higher doses similar effects should be observed from their follower cells, i.e. the motoneurons. As has been found, at least in the range up to 0.85 mg/kg, strychnine shows no effect on motoneuron RMP or action potential which would be considered to be a specific one, i.e. an effect that shows clearly changes in their excitability. Even with the fastest sweep speed of 200 μsec/div. the action potentials shown in Fig. 4 and Fig. 5 exhibit small changes in amplitude, rise and falling time and duration.

As has been described, the changes of the membrane potential and the action potential were not consistent and not unidirectional: As can be seen in Fig. 1, the membrane potential can be constant during 2 injections (B), or can be first decreased, then recover and finally be increased, i.e., hyperpolarized (C). As shown in Tab. 2, with one exception (Exp. 13) experiments which allowed a recording over 30 min, or more, showed little change of the RMP (2.5±1.4mV) which would be in the range of accuracy of the recording method and of fluctuations in the RMP which can occur over a period of recording. The minor changes in RMP are not only related to the duration of recording (which includes the amount of membrane sealing around the electrode), but also to the RMP before the strychnine injection (Fig. 2). The data clearly show that the epileptogenic action of strychnine is not due to a general depolarization of the cell membranes.

The changes in the amplitude of the action potential were not only related to the RMP changes. Reduction in action potential amplitudes of neurons associated with a reduced RMP can easily be explained on the basis of the dependency of the action potential on the cell's RMP. In contrast, reduction of the amplitude of the action potential in neurons, the RMP of which are unchanged or even increased, are of interest. As demonstrated in Fig. 5 action potential amplitude, as well as its rise and falling time, can be reduced by 15%, despite an increase in the RMP. Because of the equal reduction in both, E2 and E4, an equal reduction in both, inward and outward current underlying spike genesis, might be causing these changes.

In general, the possible influence of the anesthetic, of changes in pH or circulation, seems to be negligible in order to explain the somewhat different effect of strychnine on motoneurons.

Doses of strychnine which were able to increase polysynaptic EPSPs (0.1 mg/kg) did rarely cause changes in spike parameter if there was no change of the membrane potential. But as shown in Fig. 3, although the RMP is unchanged, the spike amplitude and the rise time of the spike (E2) of this cell was decreased.

This effect of strychnine becomes more pronounced in those experiments in which the total amount of the drug was increased to 0.7~0.85 mg/kg. As has been described for Fig. 5, although the RMP after 125 min is even higher than in the control, strychnine causes a gradual decrease in spike amplitude, rate of rise and rate of fall to 15~18% respectively. But none of the data show a TEA-like action of strychnine on the motoneuron membrane, i.e. a significant increase in the spike duration, especially a pronounced effect on E4; also E2/E4 kept being constant for the whole experiment in the cell of Fig. 5.

These results confirm former experiments described but not illustrated by Fuortes and Nelson (1963) in respect to a missing effect of strychnine on the antidromic action potential of the cat motoneuron following injections of
up to 0.3 mg/kg strychnine, and by Larson (1969), concerning the changes of the 
RMP. Unfortunately we were unable to record from 
cells after injection of higher doses of strychnine. Therefore the data allow two conclu-
sions: 1) Strychnine does not act directly on 
membrane properties of mammalian neurons or: 2) The effective doses for the motoneurons 
is higher than 0.8 mg/kg.

For the last conclusion one might take into 
account the results concerning the differences 
in the sensitivity of the spinal cord vs. the 
cerebral cortex following successive intrave-
nous injections of strychnine. As has been 
postulated by Curtis (1968) the topical appli-
cation of strychnine in concentration of 
6~25 mM, used in most experiments, might 
exert a non-specific effect of strychnine on 
different structures in the cerebral cortex. 
We tried to determine the ratio between the 
doses generating epileptic discharges in the 
isolated cat motor cortex following intrave-
nous injection of strychnine, and the doses 
being necessary to provoke seizure discharges 
in the spinal cord. This ratio was 8.2 i.e. 
an 8-times higher concentration of strychnine 

is necessary to induce epileptogenic discharges 
in cortical neurons than in spinal neurons. If 
the motoneurons share similar sensitivity with 
the cortical neurons, a dose of 0.8 mg/kg of 
strychnine is only 30% of the dose necessary 
to induce strychnine seizure in the cerebral 
cortex.

As has been calculated by Curtis, an 
intravenous injection of 0.1 mg/kg strychnine 
into a cat might give proposing an equal 
distribution of the drug in the aqueous com-
partment of the animal: a final concentration of 
10^{-8}M strychnine. The data concerning 
the sensitivity of the motor cortex neurons 
would show therefore, that a concentration of 
2.8 × 10^{-8}M strychnine (instead of 2.5 × 
10^{-8}M following topical application) is able to 
induce seizure activity in the cortex. This 
relative small difference between the two 
structures would not allow the speculation 
that the generation of strychnine seizures in 
cortical areas is due to an "uncontrolled", 
nonspecific effect of the drug (3.5 × 10^{-8} vs. 
2.8 × 10^{-8}M).

2. The increase of the amplitude of 
 polysynaptic EPSPs

The most prominent and constant effect of 
even small doses of strychnine is the imme-
diate increase in the amplitude of the polysyn-
aptic EPSPs. In this respect strychnine acts 
similarly on the spinal cord as does pentyle-
netrazol and penicillin (see review by Esplin 
and Esplin, 1969). If as proposed, strychnine 
is blocking direct IPSPs transmitted by gly-
cine, it seems highly probable that the increase 
of the EPSPs is due to a blockade of 
EPSPs in the polysynaptic potential.

If this hypothesis is true, different pheno-
mena-being described as an indication of IPSP 
mixed with polysynaptic EPSP-should be 
blocked after the injection of strychnine. As 
has been shown (Fig. 11,12,14 and 15) 
follow ing the injection of different amounts 
of strychnine, the phenomena of a possible 
inhibitory nature still exist as expressed by 
reduction of the antidromic action potential 
(Fig. 11,12 and 14), increased amplitude of 
the EPSP during injection of an inward 
current (Fig 14 and 15) and increased 
depolarization following chloride injection 
(Fig. 15).

These data would lead to two conclusions: 
1) Most of IPSP components mixed within 
the polysynaptic EPSPs are transmitted by 
GABA since they are strychnine resistant and 
can be blocked/or reduced following 
the injection of bicuculline (Fig. 12).
Effect of Strychnine on Spinal Motoneurones

2) Polysynaptic EPSP differs from monosynaptic EPSP in that at least the conductance change during the EPSP generation and the increase in amplitude during hyperpolarization is rather a characteristic of the polysynaptic EPSP than due to a contamination of the EPSP with IPSPs (see discussion in Klee, 1974).

As has been described by Kostyuk et al., (1971) and Kuno and Weakly (1972) the polysynaptic EPSP differ also from the monosynaptic EPSP by their pronounced tendency to summate following repetitive stimulation. Small changes in this unique characteristic might be responsible for the increase of these EPSP after strychnine injection.

3. The counteraction of MY 301 and Lioresal to strychnine seizure

a. MY 301

As has been known, mephenesin (Smith, 1965) and MY 301 (Klee and Wagner, 1967; Wagner and Klee, 1968) are very specific antagonists in blocking strychnine induced epileptic phenomena. In doses of 50 mg/kg seizure discharges can be stopped. In a recent paper Klee and Faber (1974) described the effect of mephenesin on snail neurons. In this preparation the inward current of the cells is completely blocked by concentrations of 10−25 mM. In order to check the hypothesis that mephenesin/or MY 301 is affecting membrane properties rather than polysynaptic transmission, higher doses were injected. In two experiments we could find a complete blockade of the antidromic activation using concentration of up to 175 mg/kg. This effect was accompanied by a depolarization of the membrane and an increase in spike duration. There seems to be no contradiction between these results and the hypothesis given by Klee and Faber (1974).

b. Lioresal

Lioresal, a GABA derivative which easily crosses the blood-brain-barrier, was also tested as an anticonvulsant to strychnine. Doses between 1 and 4mg/kg of the substance were able to block the strychnine seizure. This would imply that roughly this substance is about 10 times more potent in depressing the polysynaptic EPSP amplitude than MY 301.

But if the GABA mediated inhibition is strychnine resistant and persists in being mixed with the polysynaptic EPSPs (see forgoing chapter) one wonders how small doses of a GABA derivative can be such a powerful anticonvulsant. On the other hand, in one experiment, it could be shown that 2 mg/kg Lioresal were unable to block seizure activity which was induced by a combined injection of bicuculline and strychnine. As bicuculline is supposed to occupy the receptor site for GABA, Lioresal could exert no effect. An additional injection of MY 301 stopped this epileptic seizure immediately.

CONCLUSION

1. Using successive injections of strychnine, 0.1 mg/kg increments the amounts of this drug necessary to elicit spontaneous discharges in the isolated motor cortex and the spinal cord of the cat were determined.

2. The dose of strychnine necessary to elicit seizure discharges in the isolated motor cortex of the cat was 8 times higher than the dose that elicited seizure in the spinal cord (2.87 vs. 0.35 mg/kg).

3. With subconvulsive as well as convulsive doses of strychnine (0.1−0.85 mg/kg), the resting membrane potential (RMP) was variously unchanged, slightly hyperpolarized or slightly depolarized.
4. The antidromic action potential showed relatively little change even in doses as high as 0.85 mg/kg given over a period of two hours. Generally a gradual reduction of the spike amplitude and its rates of rising and falling could be observed, despite an unchanged RMP. The duration of the spike was rather constant.

5. The most consistent and prominent changes observed were the immediate increase in amplitude and duration of the polysynaptic excitatory postsynaptic potential (EPSP), which began with doses as low as 0.1 mg/kg.

6. In contrast, the amplitude of the monosynaptic EPSP was always unchanged.

7. To determine whether the increase of the amplitude of polysynaptic EPSP was due to a reduction or blockade of IPSP components mixed with the EPSPs, the EPSPs were checked for phenomena which could signal the possible existence of IPSP components.

8. A reduction of the antidromic action potential during the time course of the polysynaptic EPSP, an increase in the polysynaptic EPSP during hyperpolarization due to current injection and an increase of EPSP amplitude following chloride injection could be demonstrated before and after injection of strychnine, up to 0.4 mg/kg.

9. Therefore one has to conclude that either the IPSP components contaminating the EPSPs are strychnine-resistant or that polysynaptic EPSPs have a quite different characteristic than monosynaptic EPSP, i.e. the phenomena do not signal inhibitory processes.

10. MY 310, a drug similar to mephensin and Lioresal, a GABA derivative, were both able to block the strychnine seizure. Lioresal was roughly 10 to 20 times more potent than MY 301.

11. The data allow no conclusion as to whether strychnine affects the membrane properties of excitatory interneurons or interferes only with excitatory as well as inhibitory PSP. In the dose range of 0.8 mg/kg the motoneuron membrane showed no effects which would clearly reflect an action of strychnine important for epileptic phenomena.

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