

INHIBITORY POSTSYNAPTIC ACTIONS OF TAURINE, GABA AND OTHER AMINO ACIDS ON MOTONEURONS OF THE ISOLATED FROG SPINAL CORD

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SUMMARY

The actions of glycine, GABA, α -alanine, β -alanine and taurine were studied by intracellular recordings from lumbar motoneurons of the isolated spinal cord of the frog. All amino acids tested produced a reduction in the amplitude of postsynaptic potentials, a blockade of the antidromic action potential and an increase of membrane conductance. Furthermore, membrane polarizations occurred, which were always in the same direction as the IPSP. All these effects indicate a postsynaptic inhibitory action of these amino acids. When the relative strength of different amino acids was compared, taurine had the strongest inhibitory potency, followed by β -alanine, α -alanine, GABA and glycine.

Topically applied strychnine and picrotoxin induced different changes of postsynaptic potentials, indicating that distinct inhibitory systems might be influenced by these two convulsants. Interactions with amino acids showed that picrotoxin selectively diminished the postsynaptic actions of GABA, while strychnine reduced the effects of taurine, glycine, α - and β -alanine. But differences in the susceptibility of these amino acid actions to strychnine could be detected: the action of taurine was more sensitively blocked by strychnine compared with glycine, α - and β -alanine.

With regard to these results the importance of taurine and GABA as transmitters of postsynaptic inhibition on motoneurons in the spinal cord of the frog is discussed.

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INTRODUCTION

Recordings from ventral and dorsal roots of the isolated spinal cord of the frog have been used in several laboratories to analyze pre- and postsynaptic actions of transmitter candidates^{3,16-18,39,42,45,46}. With such measurements the inhibitory actions of some amino acids and their interactions with presumed amino acid blockers, like picrotoxin, bicuculline and strychnine^{3,17,18,46} have been studied by means of a direct drug application to the bathing solution. The conclusive demonstration of postsynaptic actions of transmitter candidates, such as conductance increase of the membrane, and identity of reversal potentials⁴⁸ depends on intracellular recordings. Intracellular measurements in the spinal cord and the brain stem of the cat have provided good evidence for postsynaptic actions of GABA, glycine, β -alanine and taurine, when these substances were applied electrophoretically^{1,7,9,14,31,32,40,47}.

With electrophoretic application techniques, test substances can be released within the immediate vicinity of single neurons. However, this technique is coupled with many artefacts and gives little knowledge about the concentration of test substances. On the other hand test substances can be most easily applied to an isolated preparation, in controlled concentrations, by bathing the whole organ. Therefore it was considered valuable for pharmacological investigations to develop a technique which allows a quick exchange of the bathing solution without displacement of the intracellular recording electrode. With this technique in particular the actions of GABA, glycine, α - and β -alanine and taurine were investigated, because pronounced activities of these substances can be seen in extracellular recordings from spinal roots of the frog and characteristic actions can be seen in mammals (for review see ref. 15).

A comparison of the effects of amino acids either released iontophoretically or applied by bathing the cord was an additional interesting possibility offered by the isolated spinal cord preparation.

Furthermore, intracellular records from spinal motoneurons of the frog were used to study the effects of the convulsants strychnine and picrotoxin on both amino acid actions and postsynaptic potentials. In the spinal cord and in the brain stem of mammals strychnine can block the actions of glycine and glycine-like¹³ amino acids, *e.g.* α - and β -alanine and taurine^{5,11-14,20,28,29,38}, while picrotoxin can influence the actions of GABA^{8,11,19,25}. The possibility of adding amino acids to the bath in defined concentrations was used to investigate quantitative differences in the strychnine sensitivity of α -alanine, β -alanine, glycine and taurine.

A preliminary report has been already published⁴⁴.

METHODS

Experiments were performed with *Rana esculenta* (80–120 g body wt.). After decapitation a ventral laminectomy was performed in cooled Ringer solution. The spinal cord, including dorsal and ventral roots of the lumbar segments 8–10 (see ref. 24), was removed and placed in a recording chamber. The chamber consisted of a perspex block with a small groove (volume 1.5 ml), which was continuously super-

fused with Ringer solution by means of a roller pump (2–3 ml/min). The temperature of the perfusion fluid was adjusted to 16 °C by a Peltier element and monitored by a thermistor placed in the chamber. Ventral and dorsal roots of the lumbar segments of one side were placed on silver wire electrodes for stimulation, and covered with a mixture of paraffin and vaseline. Test solutions were stored in vessels which could be connected to the chamber by switching the tap of a distributor system. Solutions were gassed with a mixture of O₂ and CO₂ and were pH-stabilized at 7.4 with Tris-(hydroxymethyl)-methyl-2-aminoethane-sulfonic acid. pH was continuously measured with pH-electrodes. For insertion of microelectrodes, both the pia and the dura mater between ventral and dorsal roots were carefully removed. Recording electrodes (tip size below one μm) were filled either with a mixture of potassium citrate (3 M, 90%) and KCl (3 M, 10%) or only with potassium citrate (3 M). (Resistances were measured in Ringer solution 30–60 M Ω .) Stable intracellular records from motoneurons could commonly be achieved for 4–8 h.

Two types of applications of test substances were used. During intracellular recording from a motoneuron the cord was either superfused with Ringer solution containing amino acids, or the amino acids were applied iontophoretically. For iontophoresis a concentric 6-barreled iontophoresis electrode, surrounding a central recording electrode, was used⁴³. Tip distances between electrophoresis and recording orifices varied between 30 and 50 μm . These electrodes were connected to a 6-channel constant current generator, producing electrophoresis currents variable from 10 to 500 nA. For iontophoretic application, 0.5 M solutions of GABA, glycine and β -alanine were adjusted to a pH of 3.5–4 by addition of HCl. Signals were displayed on oscilloscopes and photographed with a camera. In many cases postsynaptic potentials were averaged with a laboratory computer. The membrane potential was monitored continuously by a compensation writer (maximum frequency 1.5 Hz, linearity \pm 0.5%).

RESULTS

(1) *Synaptic potentials*

Stimulation of dorsal roots with low strength only evoked depolarizing postsynaptic potentials, which are most likely EPSPs, since they could not be inverted by current injection. The latency for the onset of the depolarizing deflection (EPSP)²⁶ measured from the beginning of the shock artefact ranged between 2.5 and 3 msec (temp. 16 °C).

Hyperpolarizing deflections did not appear unless the stimulus strength was increased significantly above threshold. Fig. 1A illustrates the appearance and increase in amplitude of an IPSP^{34,36} with graded stimulus strength, cutting the rising phase of the EPSP.

This hyperpolarizing component of the PSP, however, in most cases reversed spontaneously within the first minutes after penetration of the cell, even when potassium citrate electrodes were used (Fig. 1B). Only in some cells hyperpolarizing

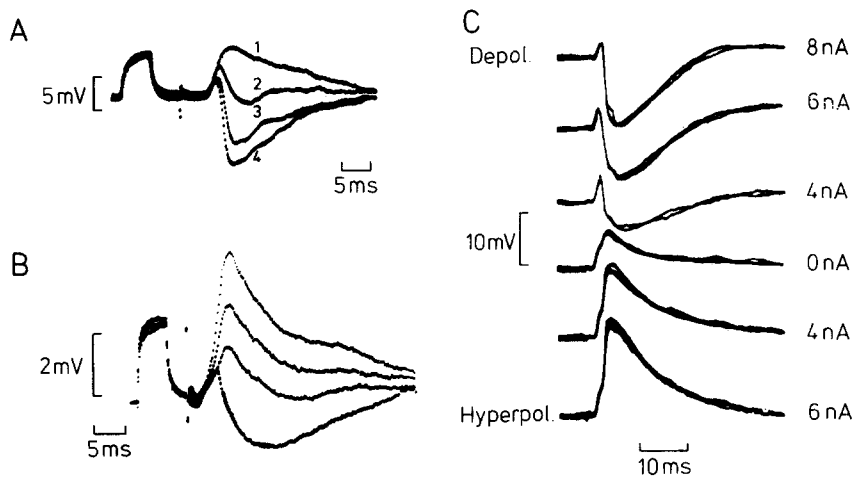


Fig. 1. Examples of postsynaptic potentials (PSPs) evoked by dorsal root (DR) stimulation in lumbar motoneurons of the isolated spinal cord of the frog. A: PSPs obtained with increasing strength of stimulation applied to DR: signal 1 at a low strength (0.8 V, T:0.5 V) could not be inverted by current injection. With increasing stimulus strength (1.0; 1.2; 1.5 V) additional hyperpolarizing deflections appeared (signals 2-4). Each signal in A, B was averaged from 4 single traces; and in C each specimen consists of 4 superimposed traces. (Duration of the test pulses 5 msec.) B: spontaneous inversion of the hyperpolarizing IPSP: 4 superimposed averaged signals recorded successively during the first 2 min after penetration. Lower trace was recorded first, the hyperpolarizing deflection reversed gradually. C: changes in direction and amplitude of PSPs during current injection: without current (0 nA) a depolarizing potential was recordable. Hyperpolarizing current (4-6 nA) increased the amplitude of PSPs (observe notch in the rising phase). As a result of depolarizing current (4-8 nA) a hyperpolarizing PSP-component appeared.

IPSPs remained stable for longer periods (10 min-1 h, see Fig. 2A). But in the majority of cells immediately after penetration only depolarizing PSPs were observed. Injections of depolarizing currents revealed a mixed pattern consisting of an early depolarization (EPSP), followed by a hyperpolarization (IPSP) (see Fig. 1C). Katz and Miledi³⁴ suggest that an extrusion of chloride ions from the electrode could be responsible for the inversion of the IPSP. Since the hyperpolarizing signal inverted even when potassium citrate pipettes were used, they assumed that this might be explained either by a leakage of NaCl into the cell around the point of impalement, or that there might be less discrimination between citrate and chloride in the frog than in mammalian neurons.

(2) Postsynaptic effects of amino acids

During intracellular recording the spinal cord was superfused for a few minutes with Ringer solution containing amino acids. Actions of amino acids were seen on membrane potential, postsynaptic potentials, action potentials, and membrane conductance. The effects were quantitatively dependent upon the amino acid concentration in the test solution. For comparison of the inhibitory strength of different amino acids, concentrations of $8 \times 10^{-3} M$ were used, at which clear effects of all substances

could be observed. An application time of 3 min proved to be long enough to reach a steady state of action, which could not be increased by extended applications. The delay in onset of the action was mainly due to the transport time in the tube system (1.5 min). The time between the entrance of the test solution into the recording chamber and the first detectable effect ranged between 20 and 25 sec.

(a) *Actions on membrane potential and PSPs*

Application of amino acids led to alterations in membrane potential, the direction of which corresponded always to the direction of the IPSPs recorded at the same time. Sometimes recordings of hyperpolarizing IPSPs could be obtained for about 1 h, in which case amino acid produced a membrane hyperpolarization (Fig. 2A). The DC recording of the membrane potential of Fig. 2 is superimposed with synaptic

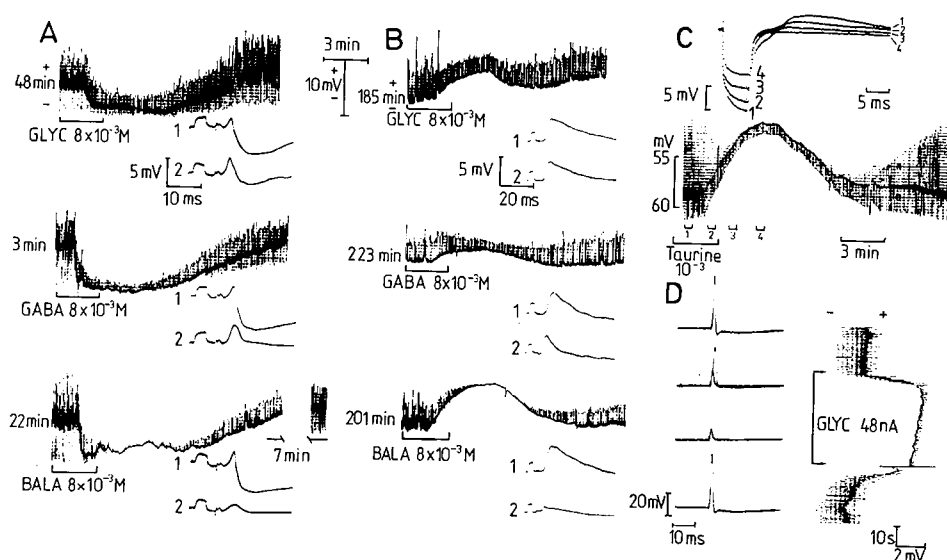


Fig. 2. Postsynaptic actions of amino acids. A: records from a cell with a stable hyperpolarizing IPSP. During amino acid application a hyperpolarization of the membrane potential develops (DC-recording of membrane potential is superimposed by synaptic potentials evoked by DR-stimulation with 0.2/sec). Changes of individual PSPs corresponding to each DC-recording are shown below (control 1, maximal effect 2). B: amino acid actions on the same cell after the hyperpolarizing IPSP had inversed spontaneously. Membrane polarizations due to amino acids are now depolarizing. Time of amino acid applications marked by bars (BALA = β -alanine). To the left of each record the time after penetration of the cell is noted. C: upper trace, electrotonic potential followed by a PSP after DR-stimulation; lower trace, DC-recording of membrane potential superimposed by DR-PSPs and a hyperpolarizing electrotonic potential (2 nA). Control: signal 1; signals 2-4 are taken during amino acid action. Each signal of the upper plot has been averaged during the time intervals marked by bars (1-4). The electrotonic potential which is much faster in rise time than the PSP in the DC-recording appears with a smaller amplitude because of the low frequency response of the compensation writer. Taurine reduces the membrane resistance synchronously with the amplitudes of the PSPs. D: during electrophoretic application of 48 nA glycine the SD-component of the antidromic AP is blocked and the IS-component is decreased. Membrane potential (registration from top downwards to the right) shows a depolarization during the glycine action (resting membrane potential 45 mV; iontophoresis current 48 nA).

potentials following rhythmic dorsal root (DR) stimulation (0.2/sec). The synaptic potential, shown on a fast time base in the insets of Fig. 2A, consists of an EPSP followed by a hyperpolarizing IPSP. In addition to the amino acid-induced hyperpolarization (referring to the thick line lying in between the inversion points of the deflections), there is a reduction of the amplitude of the IPSP (compare the averaged traces 1 with 2) evoked by all 3 amino acids tested in this experiment and an additional reduction of the EPSP during the strong action of β -alanine. More than 2 h later, the IPSPs had reversed into depolarizing transients (Fig. 2B, averaged traces). During this state of the cell, glycine, GABA, and β -alanine depolarized the cell membrane. The reduction in amplitude of the compound PSP occurring during amino acid application (averaged traces 2) appears to be of a magnitude similar to that seen in A. The parallel behavior of the amino acid polarization and the compound IPSP illustrated in Fig. 2A and B may indicate that identical equilibrium potentials, *i.e.* identical ionic processes, are involved in both events. Since IPSPs commonly reversed shortly after penetration of the cell, in the majority of neurons examined, amino acid applications caused a depolarization of the membrane potential.

(b) Actions on membrane resistance and PSPs

The membrane resistance, as indicated by the amplitude of an electrotonic potential produced by constant current pulses applied through the intracellular electrode, decreased during the application of GABA, glycine, α - and β -alanine and taurine. In Fig. 2C this is exemplified by the action of taurine. At a concentration of 10^{-3} M, taurine decreases the membrane resistance about 50%. Changes of membrane resistance were always correlated to a synchronous decrease of the amplitude of PSPs of the same relative strength. Therefore the changes of PSP amplitudes during amino acids application were used for a quantification of the inhibitory strength of different amino acids (see section 3).

(c) Actions on antidromic action potentials

The antidromic spike invasion of the SD membrane was blocked by all amino acids tested. This is exemplified by the action of glycine applied iontophoretically (Fig. 2D). Two sec after the injection of 48 nA glycine the soma dendritic component is blocked, while a depolarization of the membrane potential is developing. The remaining initial segment spike is decreased in amplitude during the action of glycine; probably due to the accompanying increase in membrane conductance⁴⁷. After switching off the iontophoresis current, the control situation is regained within about 10 sec.

(3) A quantitative comparison of the actions of amino acids

(a) GABA, glycine, β -alanine and taurine

The same order in the relative strength of action was obtained, when GABA, glycine and β -alanine were applied to the bath or by iontophoresis (numbers of iontophoretic measurements in parenthesis). In 52 experiments stable records for a comparison of amino acid actions were obtained in 118 (31) cells. In 68 (19) of 72 (20) cells

GABA reduced the amplitude of PSPs more than did glycine; in 4 (1) cells the actions of both amino acids were equal. In 40 (12) cells β -alanine was much more potent in diminishing PSP amplitudes than GABA. β -Alanine always produced a stronger polarization of membrane potential than GABA, and its action endured much longer. In 26 (10) cells β -alanine and glycine effects were compared. β -Alanine always showed much stronger and longer lasting actions.

Taurine was the most potent inhibitory amino acid observed in 36 cells from 18 spinal cord preparations. At a concentration of 10^{-3} M its action was comparable to that of β -alanine at a concentration of 8×10^{-3} M (see Fig. 4A). That the action of taurine based on the relationship of molar concentrations is 8 times as strong as the action of β -alanine is in contrast to observations of Curtis *et al.*¹⁶, who found the inhibitory effects of taurine on ventral root potentials (DR-VRP) of a similar strength to those of β -alanine.

(b) Other amino acids

The actions of 5-aminovaleric acid (AVA) and 6-aminocaproic acid (ACA), and α -alanine were also tested on membrane potential and PSPs^{16,27}. AVA produced membrane polarizations and slightly reduced the amplitude of PSP only at high concentrations (1.5×10^{-2} M). ACA had no detectable effect even at a concentration of 2×10^{-2} M. Actions of α -alanine (observed on 12 cells from 5 spinal cord preparations) were weaker than the action of β -alanine, but stronger than GABA-effects.

(4) Effects of strychnine and its interactions with amino acids

(a) Strychnine effects

Strychnine applied in a concentration of 10^{-6} – 5×10^{-4} M produced irreversible effects, confirming the observations of Kuno³⁷. After 6 min of superfusion with strychnine (5×10^{-4} M) spontaneous depolarizations appeared. These spontaneous depolarizations initially were so frequent that by summation a drop in membrane potential of 5–10 mV resulted (Fig. 3A). Postsynaptic potentials (PSPs) following DR stimulation were changed in a very characteristic way. The initial rising phase of the compound PSP remained unchanged but its decay time increased (Fig. 3B). With a latency of about 40 msec after the rising point of the PSP a strong depolarizing deflection developed although the stimulation strength was not increased. The average amplitude of this potential was 25 mV, and it gave rise to 5–10 action potentials. The whole duration of the postsynaptic potential was prolonged by strychnine from about 30 msec to more than 200 msec, indicating that strychnine probably allowed the activation of previously silent interneuronal pathways or the release of such pathways from an accompanying inhibition. This change in PSPs was observed even at very low doses of strychnine (10^{-6} M). At a concentration of 10^{-4} M spontaneous irregular depolarizations appeared, which after 5–10 min terminated in a rather regular frequency (Fig. 3C). In Fig. 3D two of these spontaneous depolarizations are shown, consisting of a slow deflection of 300–500 msec and a maximal amplitude of 25–30 mV, carrying 16–25 action potentials on top.

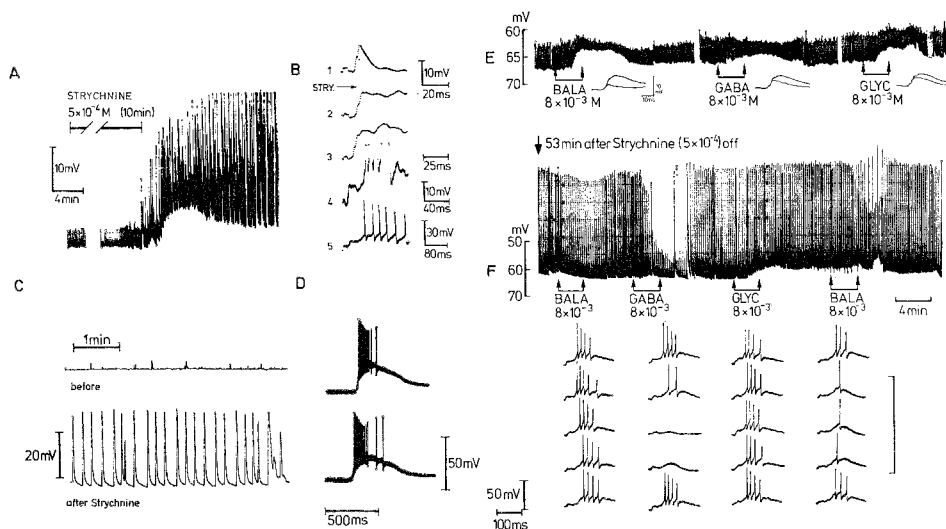


Fig. 3. Effects of strychnine and interactions with amino acids. A: registration of the membrane potential which is superimposed by postsynaptic potentials evoked by DR-stimulation (stimulation frequency 0.2/sec). Six min after strychnine ($5 \times 10^{-4} M$) PSPs increase in amplitude and spontaneous depolarizations appear. Signal amplitudes are partially cut off by the borderline of the writer. B: changes of DR-PSPs after strychnine superfusion, 1: control; 2–5: DR-PSPs after strychnine ($5 \times 10^{-4} M/10$ min) with different sweep speed and amplification. C: registration of membrane potential without DR-stimulation before and after strychnine. D: oscilloscope records of two of the spontaneous depolarizations illustrated in C. E: membrane potential with superimposed DR-PSPs; control applications of β -alanine (— BALA), GABA and glycine. F: application of amino acids at the same cell 53 min after superfusion with strychnine ($5 \times 10^{-4} M/10$ min). Below each bar corresponding to the amino acid application the effect on individual PSPs is illustrated.

(b) Strychnine interactions with amino acids

In Fig. 3E actions of β -alanine, GABA and glycine are demonstrated. At the same cell, 53 min after superfusion of the cord with strychnine ($5 \times 10^{-4} M$) for 10 min, amino acids were applied again (Fig. 3F). At the first application of β -alanine it appeared that the amplitudes of DR-PSPs were slightly diminished, while there was no detectable inhibitory effect of glycine. However, GABA showed a strong inhibitory action, reducing the amplitudes of PSPs by about 80%. A second application of β -alanine about 70 min after strychnine was switched off indicated that β -alanine could partially regain its inhibitory potency. (For strychnine interaction with taurine see c, below.)

If amino acids were applied during strychnine-induced spontaneous depolarizations, which correspond to a higher level of strychnine intoxication, no action of glycine, β -alanine or taurine was detectable, GABA, however, was able to reduce frequency and amplitude of the potentials for a period of about 10 min (not illustrated).

(c) Quantitative aspects of amino acid actions under strychnine

The effect of strychnine and its interaction with amino acids were studied in 18 preparations. In 14 different preparations the actions of amino acids were investigated

after the application of picrotoxin. Short applications of strychnine, even in low concentrations (10^{-6} M/10 min), reduced the actions of glycine, α - and β -alanine. The most remarkable effect, however, was the complete and irreversible suppression of the actions of taurine.

This observation studied in 18 preparations is exemplified in Fig. 4, where the actions of glycine, β -alanine and taurine are demonstrated before strychnine intoxication. When the amino acids were applied 20 min after the termination of a superfusion of the cord with strychnine in a concentration of 10^{-6} M for 10 min, typical changes of the PSPs were observed (compare Fig. 4A and B1 and 2). At this low concentration of strychnine, the action of taurine was blocked completely, but there was still a strychnine-resistant action of glycine and β -alanine observable.

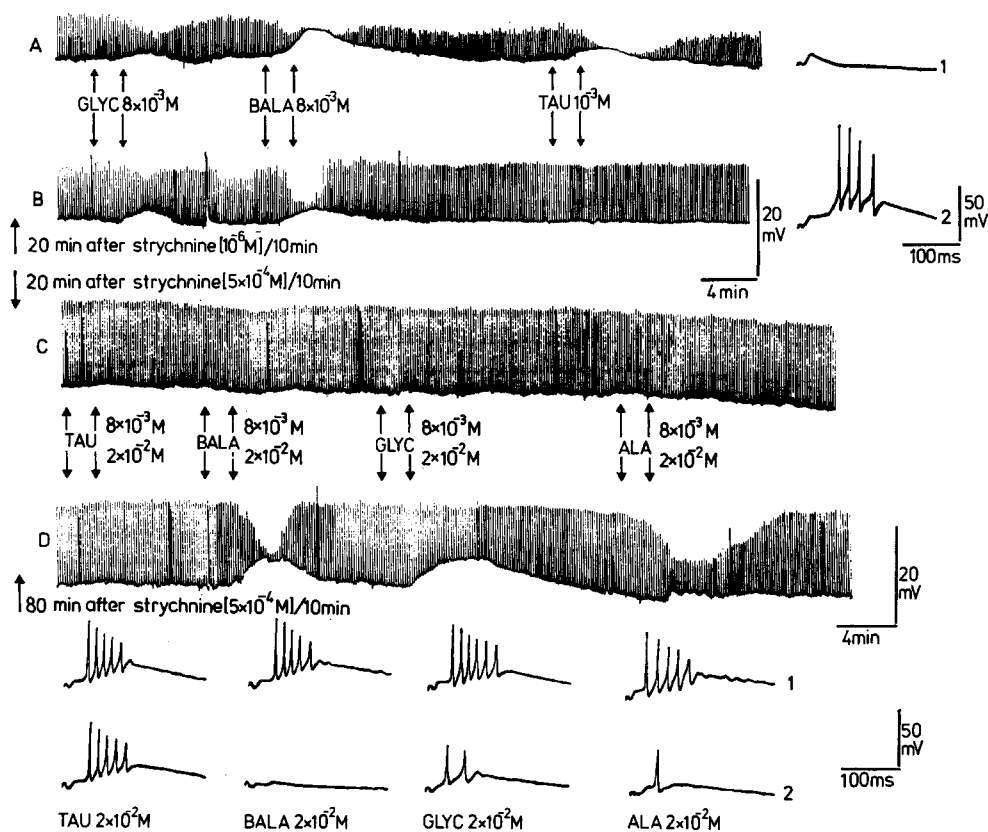


Fig. 4. Concentration dependent interactions of amino acids with strychnine. A: control application of glycine, β -alanine (BALA) and taurine. B: amino acid actions at the same cell 20 min after superfusion with strychnine (10^{-6} M/10 min). On the right side, corresponding to the DC recordings of membrane potential DR-PSPs before (1) and after (2) strychnine are illustrated. C: complete suppression of the actions of taurine, α - and β -alanine and glycine after application of strychnine in a concentration of 5×10^{-4} M for 10 min. (Control effect of α -alanine is not demonstrated.) D: effects of amino acids at the same cell with high concentrations (2×10^{-2} M) 80 min after the application of strychnine for 10 min. Below each action on the DC-recording of membrane potential control (1) and maximal effect (2) on DR-PSPs is illustrated.

Superfusion with strychnine in a concentration of $5 \times 10^{-4} M$ for 10 min suppressed all amino acid ($8 \times 10^{-3} M$) actions (Fig. 4C). If the concentration of these amino acids was increased to $2 \times 10^{-2} M$, α -alanine, β -alanine and glycine showed depolarizations of the membrane potential and reduced the amplitude of DR-PSPs as well as the number of action potentials elicited (Fig. 4D_{1,2}). Taurine, however, did not produce any inhibitory effects. The order of the relative strength of action of α -alanine, β -alanine, and glycine applied in high concentrations after strychnine was the same as in low concentrations without strychnine. In contrast, taurine which had the strongest inhibitory potency under normal conditions was completely ineffective in the presence of strychnine even when applied in this high concentration. On the same cell as illustrated in Fig. 4, taurine when applied at a concentration of $5 \times 10^{-2} M$ produced a weak reduction of PSP amplitudes (not illustrated).

(5) *Effects of picrotoxin and interactions with amino acids*

(a) *Picrotoxin effects*

When the spinal cord was superfused with picrotoxin ($5 \times 10^{-4} M$), the amplitudes of PSPs increased, and spontaneous long-lasting depolarizations appeared after about 5–10 min. Spontaneous depolarizations induced by picrotoxin appeared rather irregularly compared to those observed under strychnine (Fig. 5A). Two of these depolarizations photographed from the oscilloscope are shown in Fig. 5B. On their rising phase 8–10 action potentials were commonly generated and the whole duration of the signal varied between 20 and 30 sec being thus much longer than under strychnine. In Fig. 5C the compound DR-PSP before and after picrotoxin application ($5 \times 10^{-3} M$) is illustrated. The rising phase as well as the duration were increased with picrotoxin. Stimulation at the same strength now produced several action potentials. In contrast to strychnine, where the rising phase of the PSP remained unchanged and action potentials were elicited only during a late depolarizing deflection, under picrotoxin the rising phase of the PSP increased remarkably.

The spontaneous inversion of the hyperpolarizing IPSP in motoneurons of the frog³⁴ introduced difficulties when the influences of strychnine and picrotoxin upon the IPSP were to be determined. However, the pronounced differences in the change of the compound DR-PSP (E + IPSP inverted) after strychnine or after picrotoxin indicate that different inhibitory systems are blocked by these two convulsants.

(b) *Picrotoxin and its interaction with amino acids*

Picrotoxin diminished the effect of GABA selectively, since no interaction with β -alanine, glycine or taurine was observed. After control applications of glycine, GABA and β -alanine (Fig. 5D) the spinal cord was superfused with picrotoxin ($5 \times 10^{-4} M$) for 20 min. Although spontaneous picrotoxin induced depolarizations occurred during GABA and β -alanine actions, it seems clear that glycine and β -alanine still caused a reduction of the amplitude of PSPs and a membrane depolarization, while the action of GABA was completely blocked (Fig. 5E).

In all cases studied, picrotoxin (5×10^{-4} to $5 \times 10^{-3} M$) selectively influenced

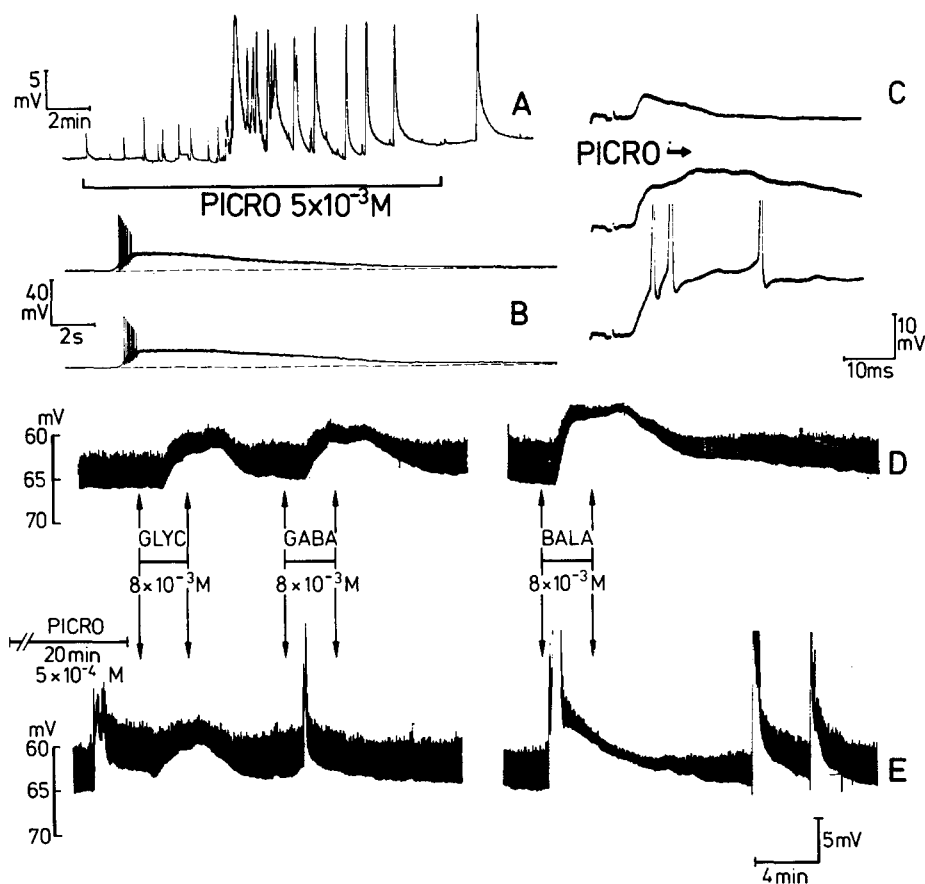


Fig. 5. Effects of picrotoxin on membrane potential, postsynaptic potentials and interactions with amino acids. A: registration of membrane potential. During the application of picrotoxin typical spontaneous depolarizations develop. B: oscilloscope records of two of these long-lasting spontaneous depolarizations. C: changes of DR-PSPs after picrotoxin ($5 \times 10^{-3} M/15$ min; from another experiment). D: membrane potential with superimposed DR-PSPs; control applications of glycine, GABA and β -alanine (BALA). E: amino acid applications at the same cell after 20 min superfusing the cord with picrotoxin ($5 \times 10^{-4} M$).

the GABA action ($8 \times 10^{-3} M$). In some experiments, however, and in contrast to Fig. 5 the action of GABA was only diminished. Taurine was also applied during picrotoxin intoxication, but in no case was the taurine action found to be influenced (not illustrated).

DISCUSSION

Electrical stimulation of ventral and dorsal roots or descending pathways of the frog spinal cord can evoke complex responses which are recordable in adjacent ventral or dorsal roots and which have been assumed to correspond to pre- and postsynaptic events^{4,6,22,23}. Many investigators have studied the action of inhibitory amino acids

on these signals^{3,16-18,39,42,45,46}. GABA, glycine, and β -alanine produce polarizations of the ventral roots, which can be taken as an indication of postsynaptic inhibition^{3,16,46}. These polarizations, however, were either hyper- or depolarizing and showed a great variability^{3,46}. Therefore analysis of the underlying processes on the motoneuron's membrane was rather difficult. For taurine Curtis *et al.*¹⁶ described a depression of ventral root reflexes, indicating that this amino acid may have a postsynaptic action. Our results show that GABA, glycine, α -alanine, β -alanine and taurine act postsynaptically on the motoneuron's membrane. Additional actions of amino acids on interneurons or presynaptic sites cannot be excluded with the technique of intracellular recording. Therefore nothing can be said about the role of GABA^{3,17,18,39,46}, taurine or β -alanine³⁹ mediating presynaptic inhibition. The weak inhibitory action of glycine compared with taurine, β -alanine and GABA is in correspondence with extracellular measurements in the spinal cord of amphibians of Curtis *et al.*¹⁶ and Fukuya²⁷. In contrast, in the spinal cord of the cat, glycine shows much stronger actions than GABA and β -alanine^{7,13,14}, while taurine produces only weak depression¹³. Chemical analysis has shown that glycine is present in the lumbar enlargement of the spinal cord of the bullfrog². Collins¹⁰ has recently compared the concentrations of free amino acids in the whole spinal cord. He found almost twice as much GABA as glycine and more than twice as much taurine than glycine. The relatively low concentration of glycine, coupled with its comparatively weak electrophysiological effects may indicate that glycine has a less important role as a transmitter in the spinal cord of the frog than GABA and taurine. However, further evidence for GABA and taurine as transmitters depend on an analysis of the distribution of these amino acids in different areas of the spinal cord. The amount of α -alanine in the spinal cord of the frog is about one-fifth that of glycine¹⁰, and for β -alanine concerning the frog no data are described. However, in the CNS of other vertebrates its concentration is near or below the level of detection^{16,33}. The strong postsynaptic inhibitory effects of these amino acids cannot be considered as a proof of their importance as inhibitory transmitters in the spinal cord of the frog, since their low concentration in the tissue is contradictory to any physiological meaning.

Certain types of postsynaptic inhibition in the spinal cord of the cat can be blocked by strychnine (for review see ref. 15), while other postsynaptic inhibitory processes are not sensitive to strychnine but can be suppressed by picrotoxin³⁵. The defined changes of the PSP under picrotoxin indicate an interference with inhibitory systems distinct from those which are affected by strychnine. As on spinal neurons of mammals^{8,11,19,25}, picrotoxin blocks the postsynaptic actions of GABA on spinal motoneurons of the frog. The actions of taurine, α - and β -alanine and glycine were never influenced. However, in many cases even under high concentrations of picrotoxin the action of GABA could only be diminished. Therefore picrotoxin must be considered as a specific, but weak blocker of the postsynaptic actions of GABA³⁰. In summary, the postsynaptic inhibitory action of GABA on spinal motoneurons and its high concentration in the tissue¹⁰ can be regarded as an indication that this amino acid is of importance as a transmitter in a picrotoxin-sensitive postsynaptic inhibition.

As described in spinal neurons of the cat^{5,11-14,20,28,29,38} strychnine blocked the

actions of taurine, α - and β -alanine and glycine, while GABA actions were not affected. Taurine, which before strychnine produced the strongest inhibitory effects of all amino acids tested, was the most sensitive. This observation supports the idea that the increase of excitability observed under strychnine may derive mainly from a blockade of postsynaptic receptors on which taurine may act as a natural transmitter. The relatively high concentration of this amino acid in the spinal cord of the frog¹⁰ also supports this hypothesis. The blockade of 'glycine receptors', as is postulated for spinal motoneurons of the cat¹², can hardly account for the powerful, irreversible change of excitation, considering the relatively weak inhibitory potency of glycine, its relatively low concentration in the tissue¹⁰ and its lower susceptibility to strychnine compared with taurine. Also in the Mauthner cell of the goldfish a strychnine sensitive inhibition is described, on which glycine cannot be the natural transmitter^{21,41}. Therefore the possibility cannot be ruled out that on spinal motoneurons of the frog glycine has not such an important role as a transmitter as in the cat spinal cord. Our results indicate that in the spinal cord of the frog taurine may be a more important transmitter of the strychnine-sensitive postsynaptic inhibition than glycine.

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