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On the pharmacology of the glycine receptors on the cuneo-thalamic relay cells in the cat

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Summary

1. In cats either decerebrated or anaesthetized with sodium pentobarbitone, strychnine released by iontophoresis from electrodes containing a 5 mM solution in 165 mM of NaCl, abolished the action of glycine and β-alanine on cuneo-thalamic relay cells without disturbing their response to equally effective applications of γ-aminobutyric acid (GABA) and β-guanidino-propionic acid.

2. The loss of glycine sensitivity appeared to increase as long as the strychnine release continued until even the largest currents tolerated by the electrode were unable to eject effective amounts of glycine. Parallel shifts of the glycine log-current response curves, equivalent to an equipotent dose-ratio of about 2·0 only occurred when the duration of the strychnine applications by current in excess of 28 nA was restricted to a few minutes.

3. Without modifying either the frequency of the spike discharge or the amplitude or shape of the action potential, currents larger than 28 nA occasionally caused a loss of GABA sensitivity.

4. Strychnine administered either intravenously (0·2 mg/kg) or applied topically to the surface of the cuneate (0·5 mM) blocked the response to glycine without any obvious effect on the response to GABA.

5. It was concluded that the discovery of a strychnine-sensitive component of the inhibitory potentials recorded from the cuneate nucleus will reveal the physiological role of the glycine receptors on cuneo-thalamic relay cells.

Introduction

In the preceding paper (Kelly & Renaud, 1973a) γ-aminobutyric acid (GABA) and glycine were shown to be almost equally effective depressants of the activity of cuneate neurones excited either postsynaptically by glutamate or transynaptically by peripheral stimulation. The action of GABA but not glycine, was effectively blocked by iontophoretic release of bicuculline and picrotoxin. Inhibition of cuneate relay cells, therefore, can be initiated by two distinct populations of receptors. The objective of this study was to define the conditions under which strychnine can discriminate between GABA and glycine receptors by blocking selectively the response of cuneate cells to glycine.

Recently, Curtis, Duggan & Johnston (1971) concluded after a series of experiments on spinal interneurones and from a detailed review of the literature that relatively low concentrations of strychnine specifically block glycine receptors,

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perhaps competitively. Only at higher concentrations did strychnine block the responses to other depressants presumably as a result of a change in membrane permeability.

Earlier Galindo (1969) showed that strychnine released from a 20 mM aqueous solution with currents of 60–150 nA, had no effect on the response to GABA but restored synaptic transmission through the cuneate blocked by glycine. Only on rare occasions, however, could the interaction between glycine and strychnine be demonstrated on cells excited postsynaptically by iontophoretic glutamate.

In view of the experience of Curtis *et al.* (1971) the action of strychnine was re-examined under conditions (currents of 14–28 nA applied through electrodes containing strychnine 5 mM dissolved in 165 mM NaCl) where the amount released from the micropipette was as little as 0·03 to 0·01 of that tested by Galindo in an attempt to show that a specific interaction occurs with glycine receptors located postsynaptically on cells excited by glutamate.

Methods

The results to be presented in this paper were obtained from the same series of experiments on cats anaesthetized with sodium pentobarbitone or decerebrated under initial halothane/nitrous oxide anaesthesia, as those described in two companion papers (Kelly & Renaud, 1973a & b). Action potentials from cells identified as cuneo-thalamic relay cells, were recorded extracellularly through one channel of a multibarrelled glass pipette (tip outer diameter >5 μm) filled with 2·7 M NaCl while the other channels were used for iontophoresis.

Iontophoretic solutions

The outer barrels of the pipettes contained: γ-aminobutyric acid (GABA): 1 M, pH 4; glycine: 1 M, pH 3·5; Na L-glutamate: 1 M, pH 6–7; bicuculline hydrochloride: (a gift to Professor K. Krnjević from Dr. Manske, University of Waterloo, Canada) 5 mM in 165 mM saline, pH 3·5; picrotoxin (BDH): 5 mM in 165 mM saline, pH 7·5 (cf. Davidoff & Aprison, 1969); strychnine sulphate (BDH): 5 mM in 165 mM saline, pH 5·5; β-alanine: 1 M, pH 3·5; β-guanidino-propionic acid; 1 M, pH 4·0; δ-aminovaleric acid: 1 M, pH 4·0.

Log-current response curves

The construction of log-current response curves and the calculation of the equipotent ratio is described in the preceding paper (Kelly & Renaud, 1973a).

Results

In the preceding paper (Kelly & Renaud, 1973a), the response to glycine was shown to be almost unaffected by applications of bicuculline and picrotoxin which caused a nearly two-fold reduction in the GABA sensitivity of the postsynaptic cell. The glycine sensitivity of cuneate neurones can, however, be blocked very effectively by relatively brief applications of strychnine with currents 4 or 5 times smaller than those used to release GABA antagonists. In Fig. 1 photographic records of spike discharges show depressions of a glutamate-excited hair cell,

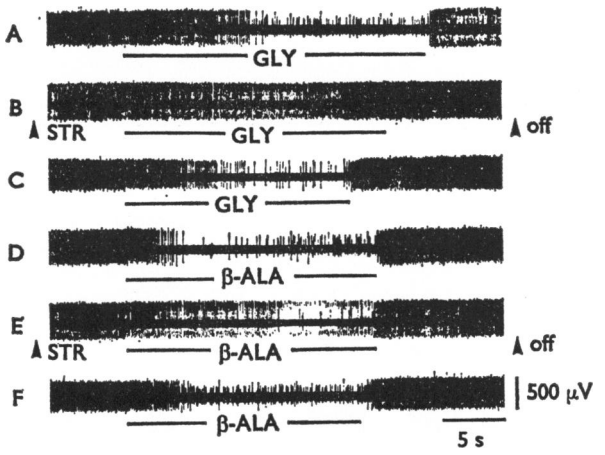

FIG. 1. The influence of iontophoretic strychnine on the depressant action of glycine (GLY) and β-alanine (β-ALA). Equipotent applications of glycine and β-alanine were applied alternately to a hair cell excited by a continuous 14 nA release of glutamate before (A) and (D), near the end of a 1·9 min, 14 nA application of strychnine (STR) (B) and (E) and 4·5 min after the strychnine application was terminated. Glycine 13 nA and β-alanine 23 nA reduced the overall frequency of the spike discharge to 50% during the 20 s application. Ratemeter records from this cell are shown in Figure 2.

evoked by equipotent doses of glycine and β-alanine, to be reversed extremely rapidly by a relatively short application of strychnine by a current of 14 nA. Unlike bicuculline, the potency of strychnine can only rarely be expressed in terms of the equipotent dose-ratio calculated from log-current response curves since the degree of block appeared to increase as long as the strychnine release continued, until even the largest currents tolerated by the electrode were unable to release effective amounts of glycine. Only by restricting the injection of strychnine either by using very low currents or extremely short application times were we able to compare the effect of strychnine on glycine with its effect on other amino acids. At higher concentrations, strychnine totally abolished the response to glycine and we could no longer quantify the comparisons.

The ratemeter records in Fig. 2 belong to the same cell whose spike discharge is illustrated in Fig. 1, and they show the sensitivity of the cell to glycine and β-alanine, at various intervals after the termination of a 6 min application of strychnine (28 nA). Before strychnine, log-current response curves showed 50% depression to be produced by glycine 13 nA and β-alanine 23 nA. The slope of the β-alanine curve was only one third as steep as that for glycine and therefore resembles that of GABA curves described in the preceding paper (Kelly & Renaud, 1973a). Three minutes after the onset of the 6 min strychnine application the slope of the glycine and β-alanine log-current response curves was unaltered, however, they were equally displaced to the right by a distance equivalent to an equipotent dose-ratio of 2. By the end of the application the responses to maximal doses of both amino acids were negligible. The records in Fig 2 which began 3·3 min after the strychnine application was terminated, show that the sensitivity to glycine (closed horizontal bars) and β-alanine (open horizontal bars) returned extremely slowly. Only after 17·5 min, were the depressions produced by 14 nA glycine and 25 nA β-alanine as great as those recorded during the pre-strychnine control period.

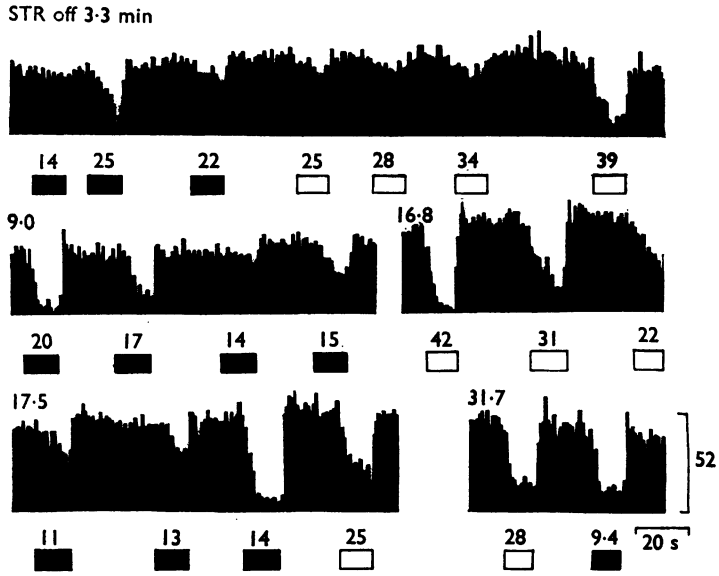

FIG. 2. The recovery of glycine and β-alanine sensitivity following a prolonged application of strychnine. Selected ratemeter records from the glutamate-excited hair cell whose spike discharge is shown in Fig. 1, which begin 3·3, 9·0, 16·8, 17·6 and 31·7 min after a 6 min application of strychnine (STR) 28 nA was terminated. Before the end of the strychnine application glycine and β-alanine currents of 84 nA no longer depressed the cell's discharge. Full recovery of the cell's response to doses of depressants which caused 50% depression during the pre-strychnine period was not complete for at least 18 minutes. The closed and open horizontal bars in this and the next figure identify glycine and β-alanine currents respectively and indicate the duration and the intensity of the current in nA. The vertical calibration shows spikes/minute.

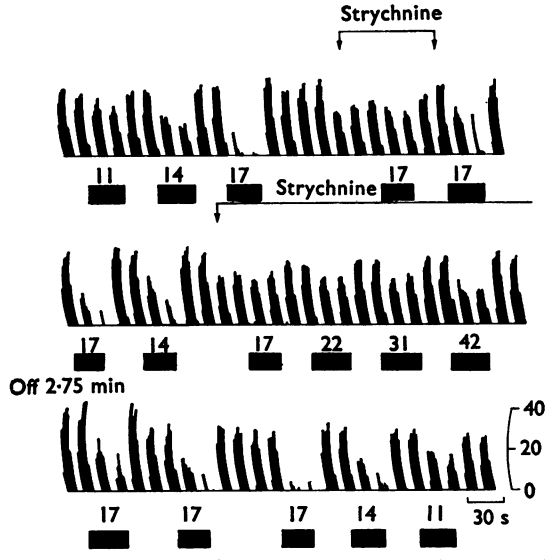

FIG. 3. The influence of strychnine applications of long and short duration on a cell excited intermittently by pulses of glutamate. Ratemeter records from a touch cell identified by antidromic excitation from the medial lemniscus. Recovery from a strychnine application of 28 nA for 1·3 min required 2·1 min and 5·5 min when the application lasted 8·8 minutes. During this longer application of strychnine, the glycine log-current response curve remained parallel to control curves and its displacement to the right was equivalent to an equipotent dose-ratio of 3·2. Inhibitory responses of this cell to transynaptic stimulation are shown in Fig. 1 of a companion paper (Kelly & Renaud, 1973b).

In another experiment, the firing rate of a touch cell, identified as a relay cell by antidromic spikes evoked from the medial lemniscus, was accelerated intermittently by short pulses of glutamate, as shown in Figure 3. A short application of strychnine 28 nA, only 1·3 min in duration, was quite sufficient to block the responses to glycine 17 nA, shown earlier in the absence of strychnine to evoke a maximal depression. Full glycine sensitivity returned approximately 2 min after the strychnine release was ended. After a longer application lasting 8·8 min, strychnine 28 nA blocked the response to glycine 34 nA and recovery was delayed for at least 5·5 minutes. A reversible parallel shift of the glycine log-current response curve to the right, equivalent to an equipotent dose-ratio of 3·16, occurred during a short 1·5 min application of twice this amount of strychnine (not illustrated).

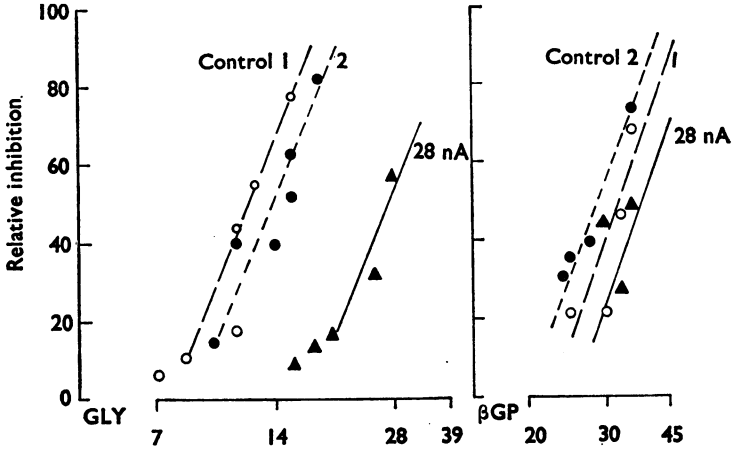

FIG. 4. The influence of strychnine on glycine and β-guanidinopropionic acid (βGP) log-current response curves. The percentage inhibition of the glutamate-evoked discharge of a touch cell by alternate 20 s applications of glycine and βGP are plotted as a function of their releasing currents on a logarithmic scale before (○), near the end of a 10·5 min, 28 nA strychnine application (▲) and after full recovery of the glycine sensitivity at 10·3 min (●). Note the difference in potencies; 50% inhibition is caused by 12·6 and 34·7 nA of glycine and βGP respectively.

TABLE 1. *Parameters calculated from γ-aminobutyric acid (GABA), glycine (GLY), β-guanidinopropionic acid (βGP) and β-alanine (βALA) log-current response curves in the presence and absence of strychnine*

Type	Strychnine Current nA	Strychnine Duration in min	Current required to evoke 50% depression GABA	GLY	βGP	βALA	Equipotent dose-ratio GABA	GLY	βGP	βALA
Hair	56	1·5		10·7				2·0		
Touch	56	1·5		12·9				3·2		
Hair Glut	56	2·0		14·0				2·2		
Hair	28	3·0		19·5				1·7		
Joint	56	8·6		12·9				3·2		
Hair	56	5·2	18·4				1·0			
Hair Glut	28	5·0	15·9				1·0			
Touch	14	10·0	17·2				1·9			
Hair	11	4·4		11·4	18·8			3·9	1·0	
*	14	5·0		11·4	18·8			6·0	1·3	
Touch Glut	28	10·5		12·6	34·7			2·1	1·0	
Hair	28	6·0		25·7	35·5			2·3	1·0	
Hair	14	7·8		16·4		23·7		2·3		3·3
Hair Glut	28	6·0		12·6		22·9		2·0		1·9
Hair Glut	56	3·8		14·8		39·8		2·0		2·1

Only occasionally did the glycine log-current response curves remain parallel before, during, and after longer applications of strychnine believed to create a steady concentration of antagonist near the post-synaptic membrane. In Fig. 4, the glycine and β-guanidino-propionic (βGP) acid log-current response curves from a touch cell excited by glutamate, show the changes which occurred towards the end of a 10·5 min application of strychnine 28 nA. The equipotent dose-ratio for glycine was calculated to be 2·14. The points plotted from the corresponding responses to βGP lie on three almost indistinguishable lines. Table 1 contains the results from a number of experiments in which the points obtained before, during, and after strychnine, fell on parallel log-current response curves. Strychnine applications with currents of 56 nA evoked glycine equipotent dose-ratios greater than 2·0 and the log-current response curves remained parallel provided the application lasted less than 2 minutes. Only when the strychnine current was relatively low (28 nA or less) did the log-current response curves seem parallel throughout a relatively long application. Similar doses of strychnine only rarely caused detectable changes of GABA and βGP curves.

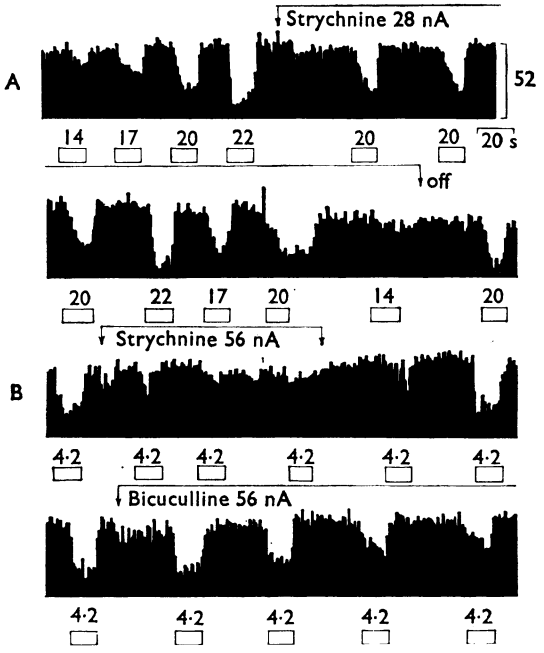

FIG. 5. The influence of larger applications of strychnine on the γ-aminobutryic acid sensitivity (A). Continuous ratemeter records show the GABA sensitivity of a hair cell excited by peripheral stimulation, to be unaffected by strychnine 28 nA. (B), Ratemeter records from a touch cell excited by glutamate 14 nA which compare the influence of strychnine and bicuculline 56 nA on repeated trials of the same GABA current. Neither the frequency nor the shape of the glutamate-evoked action potentials were altered by the glycine and GABA antagonists. Strychnine 14 nA applied to either of these cells was sufficient to cause a complete block of the response to equipotent glycine currents. The open horizontal bars below each record identify the GABA currents and indicate the duration and intensity of the releasing current.

The GABA-evoked depressions of the background discharge of the majority of hair cells excited by a turbulent air jet directed at the forepaw or by glutamate were, as shown in Fig. 5A, totally resistant to strychnine applications of 28 nA which often lasted at least 6 minutes. In this experiment a further application of

56 nA (not illustrated) was also ineffective. Very occasionally strychnine release with currents of 56 nA, caused a loss of GABA sensitivity. Ratemeter records in Fig. 5B and prints of continuous film in Fig. 6 show the spike discharges from two cells in which strychnine 14 nA caused a complete block of the response to glycine and interfered very little with the GABA sensitivity. However, a short but larger application of strychnine 56 nA, caused a marked reduction in (Fig. 6) or a complete block (Fig. 5B) of GABA-evoked depressions of the spike discharges. In both these experiments strychnine influenced the response to GABA without modifying either the frequency of the spike discharge or the amplitude or shape of the action potential. Indeed strychnine applied with currents as high as 112 nA to 165 mM NaCl solutions containing strychnine 5 mM, never caused the characteristic excitations and depressions which are a feature of experiments on spinal motoneurones (Curtis, 1962 ; Curtis, Hösli, Johnston & Johnston, 1968b ; Curtis, Hösli & Johnston, 1968a). A consistent finding in all 4 experiments where strychnine blocked GABA, was the rapidity with which the response to GABA disappeared. This rapid reduction in GABA sensitivity which immediately followed the onset of the strychnine release, is compared in Fig. 5B, with the more typical slow progressive block of GABA by a similar dose of bicuculline.

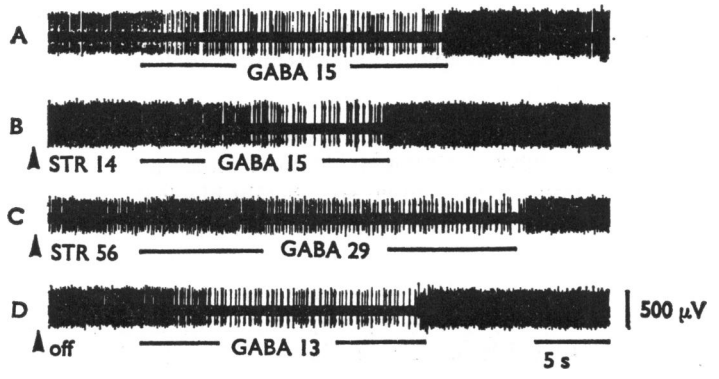

FIG. 6. The influence of strychnine on the γ-aminobutyric acid (GABA) sensitivity. Continuous film records from a touch cell excited by the constant release of glutamate 15 nA. Before (A) and after (B), a 10 min application of strychnine (STR) 14 nA. The response to glycine 14 nA was completely blocked near the origin of the strychnine application and the slowing of the onset of the GABA response shown in (B) only became apparent after 8 or 9 minutes. When after 10 min the strychnine current was raised to 56 nA for 1·7 min, the same degree of inhibition by GABA could only be achieved by doubling the release current (C). Record (D) shows the GABA sensitivity to have recovered after only 0·3 min whereas the recovery of glycine sensitivity was delayed several minutes.

β-Alanine applied to cuneate relay cells behaved exactly as it does in the spinal cord (Curtis *et al.*, 1968a) and was therefore classified as a glycine-like amino acid. Table 1 shows β-alanine to be a rather less potent depressant of cuneate relay cells than glycine, however, the reaction between β-alanine and the membrane receptors appears to be equally sensitive to strychnine.

Topical and intravenous strychnine

No systematic study was made of the action of strychnine administered either intravenously or topically to the surface of the cuneate. Earlier Galindo (1968, 1969) had shown strychnine administered by either of these two methods to be an extremely potent antagonist of glycine on cuneate neurones. We were content to

use the speed with which strychnine (0·2 mg/kg) intravenously and (0·5 mM) topically blocked the action of glycine as a control for our studies of the action of bicuculline administered by the same methods (cf. Kelly & Renaud, 1973b). The action of strychnine was always extremely powerful and abolished the response to glycine within 1–2 minutes.

Discussion

Strychnine was shown to abolish the inhibitory action of glycine and the structurally related amino acid β-alanine on cuneate neurones without disturbing the response to equally effective quantities of GABA and βGP. The influence of a limited application of strychnine was quantified from the roughly parallel displacement of the glycine log-current response curves. The equipotent glycine dose-ratio (Table 1) from cells excited transynaptically by peripheral stimulation did not differ from those of cells excited postsynaptically by iontophoretic glutamate. The glycine receptors of the cuneate nucleus may well resemble those of the spinal cord since Curtis *et al.* (1971) reported similar values for the influence of strychnine administered either iontophoretically or intravenously on the glycine equipotent dose ratio of spinal neurones. A comparison of the values reported by Kelly & Renaud (1973a) and Curtis *et al.* (1971) for the glycine current required for 50% depression of the spike discharge in the absence of strychnine, suggests that the two populations of receptors also resemble each other in terms of their respective sensitivities to glycine.

In spinal neurones the ionic currents that are responsible for the inhibition evoked by GABA and glycine appear to remain identical under a wide variety of conditions. Low concentrations of strychnine specifically block the glycine-evoked inhibitory currents without disturbing the ionic gradients and leave the GABA response unaltered (Curtis *et al.*, 1968a, b; Larson, 1969). Higher concentrations of strychnine can however limit the increase in membrane permeability (Araki, 1965) responsible for the inhibitory postsynaptic potential or alter the ionic gradients concerned (Pollen & Lux 1966). These changes should affect both the GABA current and the remaining glycine current. Our results are in accord with this idea since a loss of GABA sensitivity only occurred when strychnine had all but abolished the response to glycine. Even more dramatic was the rapidity with which the sensitivity to GABA returned at the end of the strychnine application. Characteristically the return of glycine sensitivity is extremely slow. Since the presence of glycine receptors on spinal cord motoneurones has been shown to be correlated with the action of the inhibitory transmitter, the demonstration of a similar group of receptors in the cuneate made the search for a strychnine-sensitive inhibitory input to cuneate relay cells an essential part of the study reported in the next paper (Kelly & Renaud, 1973b).

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