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AUGMENTATION BY CHLORDIAZEPOXIDE OF THE INHIBITORY EFFECTS OF TAURINE, β-ALANINE AND γ-AMINOBUTYRIC ACID ON SPIKE DISCHARGES IN GUINEA-PIG CEREBELLAR SLICES

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1 Chlordiazepoxide (Cdp, 1 to 100 μM) enhanced the inhibitory action of externally applied γ-aminobutyric acid (GABA) upon spontaneous spike discharges in guinea-pig cerebellar slices; the actions of externally applied β-alanine and taurine, but not externally applied glycine, were also enhanced by Cdp.

2 It was suggested that Cdp might exert its action by enhancing the increase of membrane permeability to K^+ induced by the amino acid, but not to Cl^-.

3 Cdp (5 to 100 μM) reversed the antagonism of picrotoxin to the inhibitory action of externally applied GABA and also the antagonism of strychnine to the actions of externally applied β-alanine and taurine.

4 The inhibition of the spontaneous spike discharges of Purkinje cells, evoked by electrical stimulation of the slice, was also enhanced by Cdp (10 to 100 μM).

5 The blocking action of picrotoxin (10 or 20 μM) on the stimulus-evoked inhibition of spike discharges was reversed by Cdp (10 μM).

6 In a similar manner, strychnine (10 or 20 μM) was also found to block the stimulus-evoked inhibition of spike discharges. It is suggested that in the cerebellum strychnine-sensitive amino acid(s) may be involved in synaptic transmission. Strychnine blockade was also reversed by Cdp (10 μM).

Introduction

Recent investigations suggest that augmentation of pre- and postsynaptic responses of (γ-aminobutyric acid) GABA-ergic neurones is the most consistent action of the benzodiazepines (Polc, Mohler & Haefely, 1974; Haefely, Kulcsar, Mohler, Pieri, Polc & Schaffner, 1975; Costa, Guidotti, Mao & Suria, 1975; Dray & Straughan, 1976; Kozhechkin & Ostrovskaya, 1977; Choi, Farb & Fischbach, 1977; Macdonald & Barker, 1978). A possible effect of the benzodiazepines on postsynaptic glycine receptors (Young, Zukin & Snyder, 1974) has not been supported by physiological studies (Dray & Straughan, 1976; Curtis, Game & Lodge, 1976; Choi *et al.*, 1977; Macdonald & Barker, 1978). It has also been demonstrated that the benzodiazepines may bind with specific receptors on synaptosomal membrane prepared from the brain of the rat (Squires & Braestrup, 1977), the mouse (Chang & Snyder, 1978) and human (Braestrup, Albrechtsen & Squires, 1977). Specific interaction between GABA and benzodiazepines binding sites on the membrane prepared from rat cerebral cortex has also been reported (Tallman, Thomas & Gallager, 1978).

Spontaneous spike discharge frequency in guinea-pig cerebellar slices can be suppressed in a dose-dependent manner by several putative neurotransmitter amino acids such as GABA, glycine, β-alanine and taurine (Okamoto & Quastel, 1973; 1976; Okamoto, Quastel & Quastel, 1976). We have studied the effects of chlordiazepoxide (Cdp) on the actions of these amino acids upon spike discharge frequency in the cerebellar slices. The effects of Cdp on the actions of amino acids other than GABA and glycine were of particular interest in this study. An attempt was made to investigate the ionic mechanisms which mediate the action of Cdp. The effect of Cdp on the antagonistic effects of picrotoxin and strychnine were also investigated. Finally in order to demonstrate that the effect of Cdp on the action of bath-applied amino acids might be concerned with synaptic events, the effects of Cdp on electrically-evoked inhibition of spontaneous firing were also investigated.

Methods

Preparation of cerebellar slices

The guinea-pig was killed by stunning, and a slice (20 to 25 mg, 0.3 mm thick) was prepared, with a Stadie-Riggs tissue slicer, from the isolated cerebellum. Sections cut perpendicular to the pial surface

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were used for experiments in which the spontaneous discharge was recorded. Sections cut parallel to the pial surface were used for experiments in which the inhibition of the spontaneous discharge was evoked by electrical stimulation. The slice was placed on a nylon mesh in the superfusion chamber which was kept at 37°C by a water jacket, and superfused with control medium at 37°C for at least 15 min before the first penetration with an electrode. The level of superfusing medium was maintained just deep enough to cover the upper surface of the slice.

Solutions and their application to the slice

All solutions were applied to the slice by superfusion through a glass capillary placed just above the surface of the slice and as close as possible (approx. 0.5 mm) to a recording electrode. The flow of superfusion medium was controlled by the tip size of the capillary so as to give a constant rate of about 1 ml/min. Details of the superfusion system have been described previously (Cooke & Quastel, 1973; Okamoto & Quastel, 1973; 1976).

The control medium consisted of (mM): NaCl 125, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 1, $NaHCO_3$ 24 and glucose 11, pH. 7.4.

As solutions at pH 7.4 which had the same composition as the control medium and contained 10 to 100 μM Cdp were found to become turbid on standing in reservoirs at 37°C for 2 to 3 h, the pH of the solutions used was adjusted to 4.0 by addition of HCl. Although no obvious deterioration of electrical activity of the slice occurred for at least 2 to 3 h during continuous exposure to solutions at pH 4.0, the slice was routinely superfused with control medium (pH 7.4) in between applications of solutions at pH 4.0.

The low[Cl^-] (4 mM) medium (pH 4.0) was prepared by replacement of NaCl by Na_2SO_4, KCl by K_2SO_4 and $MgCl_2$ by $MgSO_4$, the $CaCl_2$ (2 mM) was not replaced; the pH was adjusted by H_2SO_4 instead of HCl. The potassium-free medium (pH 4.0) was prepared by replacement of KCl by NaCl. All solutions to be tested were kept at 37°C and continuously bubbled with 95% O_2 and 5% CO_2 in reservoirs surrounded by a water jacket.

Recording of spontaneous spike discharge frequency

Spontaneous spikes were obtained from the slice, cut perpendicular to the cerebellar pial surface, with an extracellular glass microelectrode (2 to 3 MΩ, filled with 2.5 M NaCl) placed in the molecular layer, 300 to 350 μM from the pial surface of the slice.

Only cells that displayed steady continuous discharges (20 to 100 spikes/s) of relatively large spikes (≥1 mV) were used. All cells consistently responded to all amino acids tested. The cells were considered to be Purkinje cells on the basis described previously (Okamoto & Quastel, 1976; Okamoto *et al.*, 1976).

Discharge frequencies of spikes (spikes/s) were counted by a pulse counter, and the instantaneous spike frequency was recorded simultaneously on an ink-writer oscillograph (Model RJG-3002, Nihon Kohden, Tokyo) and a digital printer. The values obtained during the period of greatest inhibition were used for calculation of the percentage inhibition of the spike discharge (see Okamoto & Quastel, 1976).

Recording of peristimulus histograms of spike discharges

The slice cut parallel to the pial surface was mounted in the superfusion chamber with its pial surface facing upward, and was superfused with solutions containing substances to be tested in a manner similar to that described above. Electrical stimuli (5 to 7 V square pulses, 0.1 ms duration, 2/s) were applied to the slice through bipolar silver-wire electrodes placed off-beam to the recording electrode on the pial surface of the slice (Eccles, Ito & Szentágothai, 1967). A data processor (Model ATAC-350, Nihon Kohden, Tokyo) was used to obtain peristimulus histograms. The number of spikes which occurred in each 200 μs bin during a period of 50 to 80 ms was cumulated for 100 sweeps. The resultant histogram was displayed on the oscilloscope and later reproduced on an X-Y recorder. Stimulus artifacts were made as small as possible by the use of a reference electrode placed in the solution close to the recording electrode, and eliminated from the final record by means of a window discriminator.

Dose-response curves

The dose-response curves which gave the best fit to observed data were obtained in a manner similar to that described in detail in previous papers (Okamoto & Quastel, 1976; 1977). In short, the theoretical dose-response curve was derived from the following equation (1).

$$p = p_{max}/\{1 + (K/[A])^n\} \quad (1)$$

where p is the percentage inhibition of spike discharge frequency induced by the amino acid, K is the concentration of the amino acid, [A], giving 50% of the maximum inhibition (p_{max}) which is 100, and n is the estimate of the number of molecules of the amino acid thought to combine with a single receptor site.

Materials

All amino acids and drugs used were purchased from Yako Pure Chemical Industries Ltd., Tokyo. Chlordiazepoxide was kindly donated by Dr T. Kamioka of The Biology Department of The Central Research Laboratories of Sankyo Co. Ltd., Tokyo.

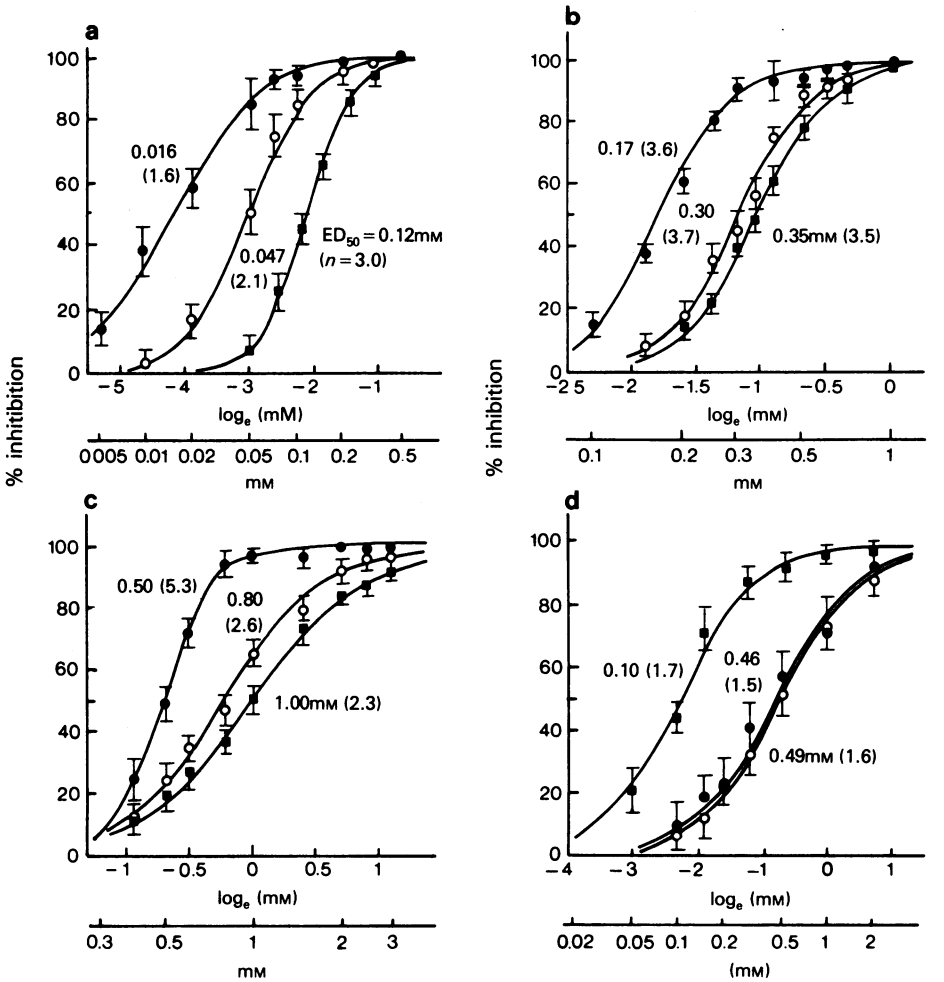

Figure 1 Log dose-response curves for the inhibitory action of amino acids on spontaneous spike discharge frequency in the presence and absence of chlordiazepoxide (Cdp). (a) γ-Aminobutyric acid; (b) β-alanine; (c) taurine and (d) glycine. Ordinate scales: percentage inhibition of discharge frequency induced by the amino acid. Abscissa scales: amino acid concentration in mM($-\log_e$ scale). (■) Values obtained in pH 7.4 medium in the absence of Cdp; (○) values obtained in pH 4.0 medium in the absence of Cdp; (●) values obtained in pH 4.0 medium in the presence of 10 μM Cdp. The mean values were obtained from 7 to 12 cells in several slices. Vertical bars show the s.e. mean. All lines are theoretical curves calculated using equation (1) and gave the best fit to the observed values. Concentrations of amino acids that gave 50% inhibition (ED_{50}) are given beside each curve. The number given in parentheses is n (equation 1); the number of amino acid molecules thought to combine with a single receptor site.

Results

Effects of chlordiazepoxide upon the inhibitory action of GABA, β-alanine, taurine and glycine on spontaneous spike discharges

When the pH of the superfusing medium was changed from 7.4 to 4.0, there usually occurred a slow rise in spike discharge frequency, which started approximately 30 s after the change and reached a plateau within 1 min. The final frequency of the discharge was 30 to 40% higher than the original discharge frequency in the medium at pH 7.4. At pH 4.0, however, spike size and discharge pattern were similar to those at pH 7.4 once a steady frequency had been attained.

When the medium at pH 4.0 containing 10 μM Cdp (no amino acid present) was applied to the slice after equilibrating with the medium at pH 4.0 for at least

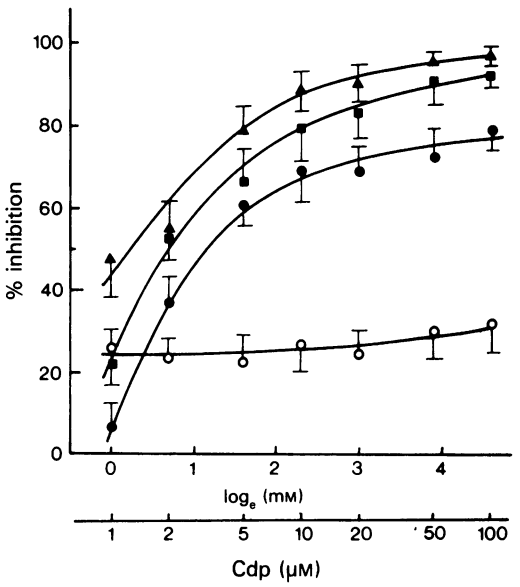

Figure 2 Effect of chlordiazepoxide (Cdp, 1 to 100 μM) on the inhibitory action of γ-aminobutyric acid (GABA), β-alanine, taurine and glycine (fixed doses). Ordinate scale: percentage inhibition of discharge frequency induced by the amino acid. Abscissa scale: concentration of Cdp in μM ($-\log_e$ scale). Effects of 0.01 mM GABA (●), of 0.15 mM β-alanine (■), of 0.4 mM taurine (▲) and of 0.2 mM glycine (○), in the presence of 1 to 100 μM Cdp. Values plotted are the means from 7 to 9 cells obtained in different slices. Vertical bars show the s.e. of the mean. Experiments were carried out in the medium at pH 4.0.

3 min, no noticeable change occured in the frequency and the pattern of spike discharges.

However, the inhibitory actions of the amino acids on the spontaneous spike discharge frequency was found to be affected by the change in the pH of the medium to 4.0. Figure 1 shows how the log dose-response curve for GABA was shifted to the left along the abscissa with slight flattening (Figure 1a); the curve for β-alanine was slightly shifted to the left (Figure 1b); that for taurine was also shifted to the left with a slight increase of the slope (Figure 1c), and that for glycine was shifted to the right without a change in the slope (Figure 1d). Although the potency of the amino acids was affected by the change in pH, the slopes of the dose-response curves were virtually unaltered (Figure 1).

The log dose-response curves for the inhibitory actions of GABA, β-alanine and taurine obtained at pH 4.0 were shifted to the left by the addition of 10 μM Cdp to the medium as shown in Figure 1a, b and c respectively, whereas no displacement was observed of that for glycine (Figure 1d). K for GABA was decreased from 47 μM to 16 μM, indicating an enhancement of the inhibitory action of GABA. Only a slight change in n from 2.1 to 1.6 occurred (Figure 1a). A similar shift was observed with β-alanine, where K decreased from 0.30 mM to 0.17 mM and n was unchanged (Figure 1b). In the case of taurine, a shift of the curve to the left (K decreased from 0.80 mM to 0.50 mM) was accompanied by the steepening of the slope (n increased from 2.6 to 5.3) (Figure 1c).

As shown in Figure 2 the effect of Cdp at higher concentrations (1 to 100 μM) on the inhibitory actions of GABA, β-alanine and taurine was invariably an enhancement. No antagonistic actions were observed at the concentrations of Cdp tested (Gähwiler, 1976; Steiner & Felix, 1976; Nistri & Constanti, 1978; Macdonald & Barker, 1978).

Effects of chlordiazepoxide on actions of other substances

The excitatory actions of L- and D-glutamates, L-aspartate, acetylcholine and high (10 to 20 mM) external K^+ on the spontaneous spike discharges (Okamoto & Quastel, 1973) were unaffected by Cdp (10 to 50 μM).

The inhibitory action of L-glutamate (0.05 to 10 mM) on the spike discharges of unidentified cells which may be located in the granule layer of the cerebellar slice (Yamamoto, Yamashita & Chujo, 1976) was also unaffected by the presence of Cdp (10 to 50 μM).

Ionic dependence of the augmenting action of chlordiazepoxide on the inhibition evoked by GABA

The ionic mechanism which may underly the augmentation by Cdp of the inhibitory effect of GABA was studied in a low[Cl^-] and K^+-free media. As described in the previous paper (Okamoto *et al.*, 1976), the inhibitory actions of GABA, glycine, β-alanine and taurine might be exerted via an increase of neuronal membrane permeability to Cl^- (P_{Cl-}) and probably to K^+ (P_{K+}). It was thus conceivable that Cdp could act by enhancing the increase of either one or both of these changes in ionic permeability.

Superfusion of the slice (after equilibration with the medium at pH 4.0) with low[Cl^-] (4 mM), and high[SO_4^{-2}] (66 mM) medium considerably lowered the plateau level of spontaneous spike discharge frequency after an initial 3 min period of complete suppression of the discharge. GABA applied to the slice under these conditions consistently evoked an extremely rapid increase in the spike discharges as shown in Figure 3a and c. This excitatory action of GABA was reproducible for at least 30 min and was sensitive to picrotoxin, in a manner similar to that

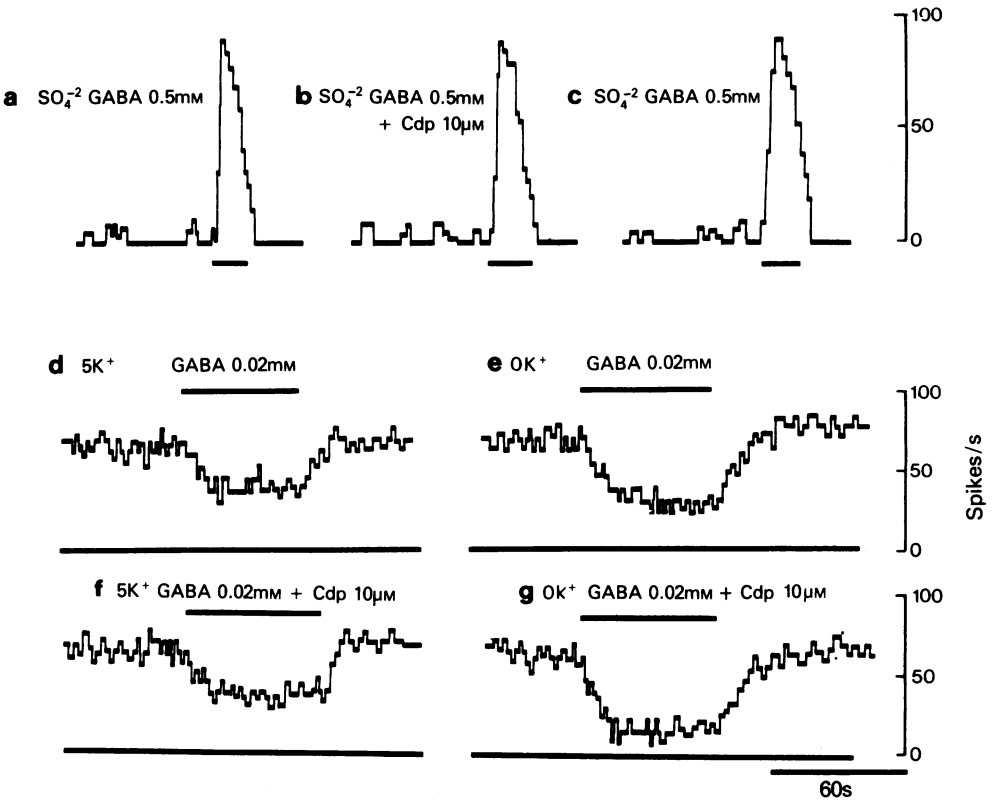

Figure 3 The effect of chlordiazepoxide (Cdp) on the excitatory action of γ-aminobutyric acid (GABA) in the low[Cl^-], high[SO_4^{-2}] medium and on the inhibitory action of GABA in the control and K^+-free media. Vertical scale: the number of spikes per s. Horizontal scale: time, 60 s. Horizontal bars: the periods of application of GABA and Cdp. (a to c) The effects of 0.5 mM GABA in the low[Cl^-] (4 mM), high[SO_4^{-2}] (66 mM) medium in the absence of Cdp (a and c) and in the presence of 10 μM Cdp (b); the record (c) was obtained about 2 min after (b). Records (a to c) were obtained from a single cell after equilibrating the slice with the low[Cl^-] medium for 3 min. (d to g) Show the K^+-dependency of the action of Cdp and GABA: (d) the effects of 0.02 mM GABA in the control ($5K^+$) medium; (e) in the K^+-free ($0K^+$) medium, and (f) the effects of 0.02 mM GABA and 10 μM Cdp in the $5K^+$ medium; (g) in the $0K^+$ medium. Records (d to g) were obtained from a single cell, and records (e and g) were obtained after equilibrating the slice with the $0K^+$ medium for 3 min. Experiments were carried out in media at pH 4.0.

observed in solutions at pH 7.4 (Okamoto *et al.*, 1976). The excitatory action of GABA is most probably due to a reversal of the direction of Cl^- flow which leads to the efflux of Cl^- and a depolarization of the neuronal membrane (see Okamoto *et al.*, 1976). However, the presence of Cdp (10 μM) had no effect on the excitation evoked by GABA. A typical example from observations made on 10 different cells is shown in Figure 3b. We therefore tentatively suggest that the enhancement by Cdp of GABA-evoked responses is not due to an enhancement of the increase in P_{Cl^-} by the amino acid.

Earlier it was found (Okamoto *et al.*, 1976) that the removal of K^+ from the superfusion medium enhanced the inhibitory actions of GABA, glycine, β-alanine and taurine at pH 7.4, and also diminished the excitatory actions of these amino acids in a low[Cl^-] medium (pH 7.4). These results indicate that the amino acids also increase membrane permeability to K^+ (P_{K^+}).

Figure 3d and e shows that the inhibitory action of GABA is also enhanced by the absence of K^+ in the medium at pH 4.0; in 8 cells the enhancement was 172 ± 15% (mean ± s.e. mean) (Figure 3d and e). In the K^+-free medium the addition of 10 μM Cdp caused a further and significant increase in the action of GABA 221 ± 15% ($n = 7$) (Figures 3f and g). This suggests that the enhancing action of Cdp on the

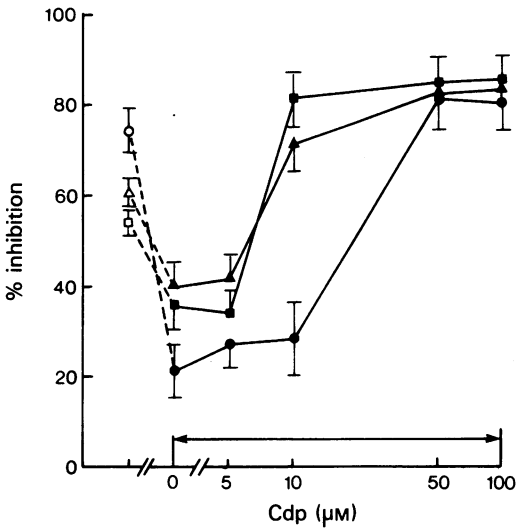

Figure 4 The action of chlordiazepoxide (Cdp) on picrotoxin antagonism to the inhibitory effect of γ-aminobutyric acid (GABA) and the strychnine antagonism of the effects of β-alanine and taurine. Ordinate scale: percentage inhibition of discharge frequency induced by the amino acid. Abscissa scale: concentration of Cdp in μM ($-\log_e$ scale). Circles: inhibitory effects of 0.1 mM GABA alone (○) and in the presence of 10 μM picrotoxin together with 0, 5, 10, 50 and 100 μM Cdp (●). Squares: inhibitory effects of 0.3 mM β-alanine alone (□) and in the presence of 10 μM strychnine together with 0, 5, 10, 50 and 100 μM Cdp (■). Triangles: inhibitory effects of 0.8 mM taurine alone (△) and in the presence of 10 μM strychnine together with 0, 5, 10, 50 and 100 μM Cdp (▲). Horizontal bar with arrows indicates the range over which the convulsants (10 μM) were added. Values plotted are the means from 6 to 10 cells. Vertical bars show the s.e. mean. Experiments were carried out in media at pH 4.0.

action of GABA might be mediated by an increase in P_{K^+}.

Reversal by chlordiazepoxide of the antagonistic actions of picrotoxin and strychnine

In view of the findings that the benzodiazepines reverse the antagonism by convulsants, picrotoxin and bicuculline, of the depolarizing action of GABA in the rat sympathetic superior cervical ganglia *in vitro* and in rat brain stem neurones *in vivo* (Bowery & Dray, 1976; 1978), the action of Cdp on picrotoxin- and strychnine-induced blockade of the inhibitory amino acids in the cerebellum *in vitro* was investigated. As shown in Figure 4, the antagonistic actions of both picrotoxin (10 μM) and strychnine (10 μM) were diminished, in a dose-dependent manner, by the presence of Cdp (5 to 100 μM). Although the reversal of strychnine blockade by Cdp has not been reported previously, no apparent difference was observed in the potency of Cdp as an antagonist of the actions of picrotoxin and strychnine.

Effects of chlordiazepoxide and convulsants on stimulus-evoked inhibition

In order to examine the effects of Cdp and convulsants on synaptically-evoked inhibition of spike discharges, off-beam electrical stimulation was applied to the pial surface of the slice (cut parallel to pial surface) (Eccles *et al.*, 1967).

Effect of chlordiazepoxide The inhibition of spike discharges evoked by the electrical stimulation was prolonged by the presence of 10 to 100 μM Cdp either in the medium at pH 4.0 (Figure 5i and k; 5ø and q) or in the medium at pH 7.4 (Figure 5c to f). Records (Figure 5b to f) were obtained in the control medium at pH 7.4 immediately after a 3 min period of superfusion with the media at pH 4.0 containing 0 to 100 μM Cdp.

Effect of picrotoxin Picrotoxin (10 μM) shortened the inhibition evoked by electrical stimulation either at pH 7.4 (Figure 5g and h) or pH 4.0 (Figure 5i and j), and almost complete blockade was observed in all 8 cells tested in the presence of 20 μM picrotoxin. The blockade was reversible upon washing for about 3 min with the control medium.

Effect of strychnine A similar blocking action was also demonstrated with strychnine (10 μM) either at pH 7.4 (Figure 5m and n) or pH 4.0 (Figure 5ø and p). Like picrotoxin, the blockade by 20 μM strychnine was almost complete and reversible.

Effect of chlordiazepoxide in the presence of convulsants Cdp (10 μM) applied together with picrotoxin (10 μM) or strychnine (10 μM), in the medium at pH 4.0, resulted in the partial reversal of the blockade evoked by these convulsants (Figure 5j and l; 5p and r). In the medium at pH 4.0 the stimulus-evoked inhibition in the absence of the convulsant was not noticeably prolonged by 10 μM Cdp. Higher concentrations (50 or 100 μM) of Cdp only exhibited slightly greater reversal of the convulsant-evoked blockade, and prolonged the stimulus-evoked inhibition in the absence of the convulsant as shown in Figure 5i and k, and Figure 5ø and q. Cdp at lower concentrations (2 to 5 μM) showed little effect either on the stimulus-evoked inhibition or the blockade by the convulsants, in the medium at pH 4.0.

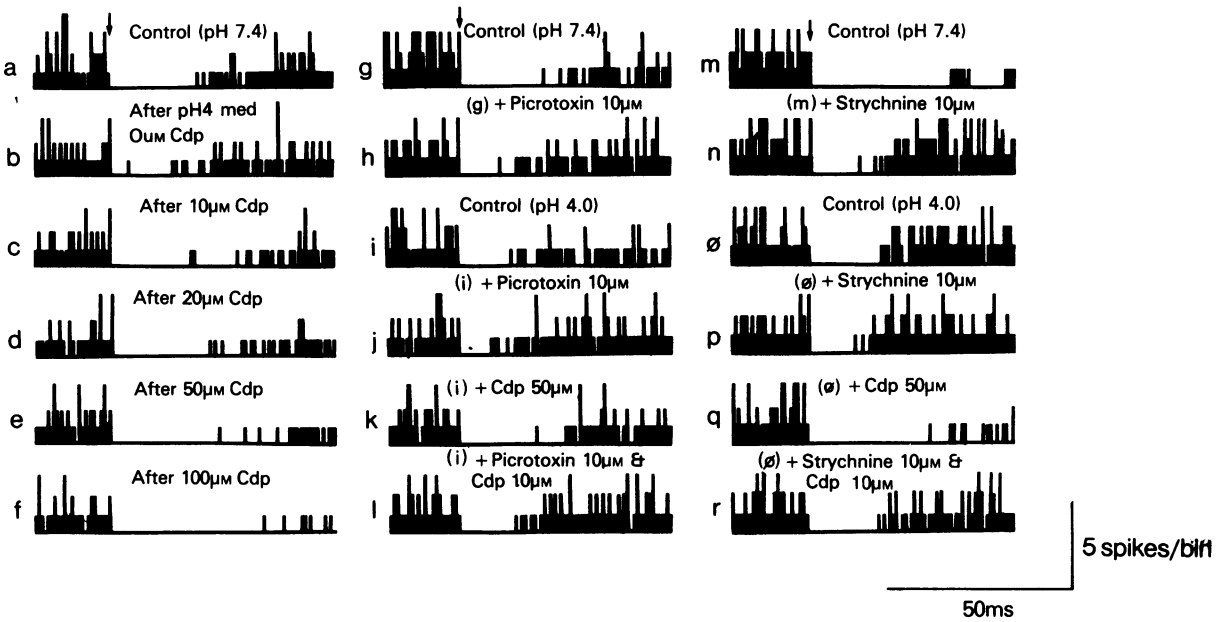

Figure 5 Peristimulus histograms showing the effects of chlordiazepoxide (Cdp) and convulsants on the stimulus-evoked inhibition of spike discharges. Electrical stimuli (5 to 7 V square pulses, 0.1 ms duration, 2/s) were applied at the time indicated by vertical arrows. The number of spikes appearing in each 200 μs bin were cumulated for 100 sweeps. Vertical scale: the number of spikes per bin, five spikes. Horizontal scale: time, 50 ms. (a) In medium at pH 7.4 (control); (b to f) in medium at pH 7.4 immediately after superfusion (for 3 min) with media at pH 4.0 containing Cdp, 0 μM(b), 10 μM(c), 20 μM(d), 50 μM(e) and 100 μM(f); (g to l) effects of picrotoxin and Cdp; (m to r) effects of strychnine and Cdp. The changes induced by Cdp and the convulsants were reversed upon washing. Records (a to f), (g to l) and (m to r) were obtained from three different cells; similar results were also obtained from seven other cells.

Discussion

Effects of chlordiazepoxide on the inhibitory action of GABA and glycine

The results obtained with guinea-pig cerebellar slices (Figure 1a) confirm previous findings of the synergistic action of the benzodiazepines and GABA in the CNS (Kozhechkin & Ostrovskaya, 1977; Macdonald & Barker, 1978; Choi *et al.*, 1978).

The antagonism of benzodiazepines to GABA, which has been demonstrated in cultured rat cerebellar neurones (Gähwiler, 1976), cultured mouse spinal neurones (Macdonald & Barker, 1978) and in Purkinje cells of the rat and cat *in vivo* (Steiner & Felix, 1976), was not observed in guinea-pig cerebellar slices (Figure 2).

The lack of the action of Cdp on the inhibitory effect of glycine (Figure 1d) agrees with previous findings (Dray & Straughan, 1976; Curtis *et al.*, 1976; Macdonald & Barker, 1978; Choi *et al.*, 1978).

Effects of chlordiazepoxide on the inhibitory action of taurine and β-alanine

It was demonstrated, for the first time, that the inhibitory effects of taurine and β-alanine on spontaneous spike discharges in guinea-pig cerebellar slices are also sensitive to the augmenting action of Cdp (2 to 100 μM) (Figure 1b and c).

Since Cdp (1 to 100 μM) does not increase spontaneous spike discharge frequency and since the dose-response curve of glycine (Figure 1d) is unaffected by Cdp, it seems unlikely that the augmentation produced by Cdp is solely mediated by the mobilization of endogenous GABA from presynaptic storage sites as proposed by Haefely *et al.* (1975). However, the possibility that Cdp might mobilize taurine or β-alanine from presynaptic storage sites cannot be ruled out.

Ionic mechanism of the augmenting action of chlordiazepoxide

The results (Figure 3) suggest that Cdp might enhance GABA-evoked increase in P_{K^+}. The enhancement of GABA-evoked increase in P_{Cl^-}, however, cannot be ruled out. Direct measurements of P_{Cl^-} would be helpful.

Nistri & Constanti (1978) have found that flurazepam antagonizes both glutamate- and GABA-evoked depolarizations of frog spinal dorsal root and suggested this is due to the blockade of receptor-activated Na^+ channels on the postsynaptic membranes. However, in the present study the excitatory action of L-glutamate on spike discharges, was unaffected by Cdp. As suggested in an earlier paper (Okamoto *et al.*, 1976), the excitatory actions of glycine, taurine and β-alanine, but not GABA, in a low[Cl^-] medium, might be partly mediated by the increase in P_{Na^+}. Thus, it seems possible that the enhancement of the inhibitory action of taurine and β-alanine by Cdp (Figure 1b and c) might result from the blockade of a depolarizing component mediated by an increase in P_{Na^+}. In any case, Cdp seems to affect only a restricted number of receptor-activated ionic channels and has no effect on those activated by glycine (Figure 1d) and L-glutamate.

Reversal by chlordiazepoxide of the antagonistic actions of picrotoxin and strychnine

Although it has been reported that barbiturates may reverse the bicuculline- and picrotoxin-antagonisms of GABA and the strychnine-antagonism of glycine (Bowery & Dray, 1976; 1978; Brown & Constanti, 1978), there appears to be no previous findings of the reversal by the benzodiazepines of the blockade by strychnine of the action of amino acids. Our results (Figure 4) indicate apparent reversal by Cdp of the strychnine blockade of the inhibitory action of bath-applied taurine and β-alanine, in addition to the reversal of the picrotoxin antagonism to GABA. The reversal by Cdp of the strychnine blockade of the stimulus-evoked inhibition (Figure 5) also suggests the possibility that Cdp may reverse the action of strychnine-sensitive transmitter(s), in the cerebellum, released by the electrical stimulation.

Prolongation of stimulus-evoked inhibition by chlordiazepoxide

The prolongation of stimulus-evoked inhibition by the benzodiazepines has also been described by Curtis, Lodge, Johnston & Brand (1976) in the cat cerebellum *in vivo* and by Tsuchiya & Fukushima (1978) in the cat hippocampus *in vivo*. Thus, the results given in Figure 5a to f, 5i and k, and 5ø and q are in agreement with these previous findings *in vivo*.

As the synaptic transmission between cerebellar Purkinje cell and interneurones is thought to be mediated by amino acid(s), the resemblance between the actions of Cdp on bath-applied amino acids (Figure 1) and on the stimulus-evoked inhibition (Figure 5a to f; 5i and k; 5ø and q) might suggest that the enhancing action of Cdp on the bath-applied amino acids is directly concerned with synaptic events.

Blockade by strychnine of the stimulus-evoked inhibition

The blockade by strychnine of the stimulus-evoked inhibition of spike discharges (Figure 5m to p) was rather unexpected in view of the previous observations of the picrotoxin- or bicuculline-sensitive, but strychnine-resistant, nature of the inhibitory synaptic processes in the cerebellum of the frog (Woodward, Hoffer, Siggins & Oliver, 1971) and of the cat (Anderson, Eccles, Løyning & Voorhoeve, 1963; Curtis & Felix, 1971). However, there appear to be no experimental findings in previous publications on the effects of strychnine on stimulus-evoked inhibition in guinea-pig cerebellum.

The possibility that taurine might function as a neurotransmitter in the cerebellum, particularly in the stellate cell, of the rat has been suggested by Nadi, McBride & Aprison (1977) and Frederickson, Neuss, Morzorati & McBride (1978). Chemical observations such as the presence of a high-affinity uptake process and the calcium-dependent release, of labelled taurine, were also demonstrated in guinea-pig cerebellar slices (Okamoto & Namima, 1978). Therefore, the result shown in Figure 5m to p could suggest the involvement of strychnine-sensitive amino acid(s), possibly taurine, in synaptic transmission, at least in the guinea-pig cerebellum.

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