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Conductance States Activated by Glycine and GABA in Rat Cultured Spinal Neurones

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Summary. The conductance properties of single Cl^- channels activated by glycine and gamma-aminobutyric acid (GABA) were examined in rat spinal cord neurones grown in cell culture. The majority (85%) of spinal neurones were sensitive to both glycine and GABA as were most (83%) outside-out patches tested. Glycine and GABA activated multiple conductance state Cl^- channels with linear current-voltage properties when the chloride activities of the solutions bathing both sides of the membrane were similar. Glycine activated six distinct conductance states with conductances of 14, 20, 30, 43, 64 and 93 pS, whereas GABA activated five states with conductances of 13, 20, 29, 39 and 71 pS. The 30 and 43 pS states and the 20 and 29 pS states were observed most frequently with glycine and GABA, respectively. As the values of the glycine- and GABA-activated conductance states form a geometric progression when arranged in ascending order, we concluded that the channels do not consist of a cluster of identical pores. Additional conductance states (50 and 100 pS) were activated by glycine occasionally. The similarity between the conductances of the states activated by the two transmitters is consistent with the proposal that they both activate the same type of Cl^- channel.

Key Words glycine · GABA · chloride channels · spinal cord neurones and tissue culture

Introduction

Glycine and gamma-aminobutyric acid (GABA) play important roles as neurotransmitters at many inhibitory synapses in the mammalian central nervous system (for review *see* Krnjevic, 1974). Despite their pharmacologically distinct actions, glycine and GABA both activate Cl^- channels when applied to spinal neurones (Curtis et al. 1968*b*; Bormann, Hamill & Sakmann, 1987). Furthermore, the loss of sensitivity to glycine or GABA after desensitizing applications of the other transmitter, led Barker and McBurney (1979) to propose that both receptors were coupled to a common conductance mechanism in mouse cultured spinal neurones. Using single-channel recording techniques, Hamill, Bormann and Sakmann (1983) demonstrated that both transmitters activated multistate Cl^- channels in the same preparation. Glycine activated conductance states of 21, 31 and 45 pS and GABA activated conductances of 19 and 30 pS. Because of the similarity of the GABA-activated conductances to the two lowest states activated by glycine, and the similar anion selective properties of the responses to both transmitters, Hamill et al. (1981) proposed that both receptors were coupled to the same type of Cl^- channel. In the same preparation, however, other workers have reported that GABA-activated channels can display only one conductance state (Jackson et al., 1982; Mathers, 1985), while Bormann et al. (1987) have updated the number of states they observe to four, for GABA and glycine. This discrepancy and our interest in the relationship between the actions of glycine and GABA, prompted this re-examination of the conductance states activated by these transmitters. We report here that GABA activates five distinct conductance states and glycine activates at least six states in rat cultured spinal cord neurones. The similarity between the values of the lower five glycine-activated conductance states and the GABA-activated conductance states supports and extends the proposal that both receptors are coupled to the same type of channel. In contrast with work on mouse neurones, the receptor type is not the lone determinant of the most frequently occurring conductance state. Some of these results have been published in abstract form (McBurney, Smith & Zorec, 1985).

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Materials and Methods

Tissue Culture

Cultures of spinal cord neurones were prepared as described previously (Hughes et al., 1987; Smith, 1987). In brief, the spinal cords were removed from 13–15 day old embryonic rats, dissociated and plated onto a near-confluent bed of cortical astrocytes. Culture medium consisted of a mixture of Dulbecco's minimum essential medium (MEM), Ham's F12 and α-MEM in the ratio 3 : 6 : 1, that was augmented with rat serum (4%), chick embryo extract (1%) and L-glutamine (1%). All components except the rat serum were obtained from GIBCO. The neurones were incubated in medium at 37°C in a 5% CO_2 and 95% air atmosphere for 8–42 days before use.

Recording Techniques

Recordings were made in the whole-cell or outside-out patch configurations (Hamill et al., 1981) from spinal neurones with cell bodies of 10–25 μm in diameter. Coverslips containing the cells were placed in a recording chamber mounted on the stage of an inverted phase contrast microscope (Invertoscope, Zeiss) and viewed at 400× magnification. Recording electrodes were prepared with outer diameters of about 1–2 μm, coated with Sylgard (Dow Corning) and heat polished. All recordings were made with an EPC-5 or EPC-7 amplifier (List Electronics) that was modified to permit the offsetting of liquid junction potentials of up to ±100 mV. Recordings were made at room temperature, which was fairly constant (within ±1°C) during any single experiment, but varied between 15 and 27°C overall. The current and voltage signals were stored on an FM tape recorder (4DS, Racal) operated at 3.75 in sec^{-1} (dc – 2.5 kHz; −3 dB) or following pulse code modulation (modified 701ES, Sony; Lamb, 1985) on a video recorder (NV-830, Panasonic). The frequency response of the pulse code modulation-video recorder system was limited to dc −7.5 kHz by substituting the usual filters for six-pole Bessel filters (Barr and Stroud). This modification prevented the response to step changes in the current or voltage traces from "ringing."

Solutions

The composition of bathing medium was (in mM): 140 NaCl, 3.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 HEPES. Electrodes were filled with either ES1 (in mM: 140 CsCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, 10 HEPES) or ES2 (same as ES1, but 20 mM CsCl replaced with 20 mM TEACl). All solutions were titrated to pH 7.2 with NaOH. On occasion tetrodotoxin (TTX, Sigma) and cadmium ions (Cd^{2+}) were added to the bathing medium and test solution. Such modifications are described in the relevant text and figure legends.

The chloride activities (aCl) of all solutions were estimated by interpolation from standard tables (Robinson & Stokes, 1959).

Test solutions were applied to whole cells or isolated membrane patches by pressure ejection (<0.05 nm^{-2}) from micropipettes (2–10 μm outer tip diameter) or from a modified U-tube tool (Hughes et al., 1987). Membrane potentials were corrected for the liquid junction potentials that resulted from the different ionic composition of electrode and bathing solutions (Kaneko & Tachibana, 1986).

Data Analysis

Two methods of obtaining mean current amplitudes were used. The majority of recordings were replayed from tape (either 3.75 or 0.9375 in sec^{-1} from the FM recorder), effectively low-pass filtered at 1 or 3 kHz (−3 dB) and captured on the screen of an oscilloscope (Gould 4020). Measurements were then made directly from the screen, or from records, subsequent to the traces being dumped at low speed onto a chart recorder (Gould 2400S). Mean current amplitudes were usually obtained by measuring 10–20 openings to each current level during an application. Mean current levels were also estimated by measuring a larger number of events and constructing a histogram (*see below*). Where estimates of the mean current levels obtained from histograms were compared with means obtained by measurement of only some of the events, the difference between the two values was on average 2.5 ± 1.0% (n = 6) of the histogram derived value. Single-channel recordings used to produce amplitude histograms were filtered at 1 kHz (−3 dB; two-pole Bessel), digitized at 10 times this frequency by a laboratory computer (Digital Equipment, PDP 11/23+ equipped with a Data Translation analog-to-digital data acquisition system) and viewed on a graphics terminal screen. The time taken for the output of this system of acquisition to make an almost full transition in response to a step change at the input, was measured as 0.6 msec. The digitized current traces were displayed on a graphics terminal screen and the apparent amplitude and duration of each event derived from placements of a cursor. To limit error in the measurement of channel amplitudes, events were only included in the histogram when the rise or decay time was between 0.5 and 0.7 msec. Events with rise times shorter that this were unresolved and therefore ignored. Longer rise times were presumed to result from two openings occurring close together in time. In the absence of a continuous cursor (Colquhoun & Sigworth, 1983), the time course of the transition was measured directly from the digitized record on the terminal screen. Measurements are described in the format arithmetic mean ± SD of the mean.

Results

Actions of Glycine and GABA on Membrane Patches

The majority of interneurones and motoneurones in the spinal cord of the anaesthetised cat (Curtis, Hosli & Johnston, 1968*a*; Curtis et al., 1968*b*) are sensitive to both glycine and GABA. Likewise, the majority of rat spinal neurones in cell culture respond to the application of both transmitters. Out of the 75 cells tested, 64 (85%) responded to both agents applied at concentrations of 50 μM, 7 (9%) were sensitive to GABA only, 2 (3%) to glycine only and 2 (3%) were sensitive to neither.

When the actions of glycine and GABA were compared on outside-out patches, a similar result was obtained. Of the 48 patches tested with both glycine and GABA (50 μM), 40 (83%) responded to both transmitters. Five of the patches (10%) were sensitive to GABA only while 3 (6%) responded to

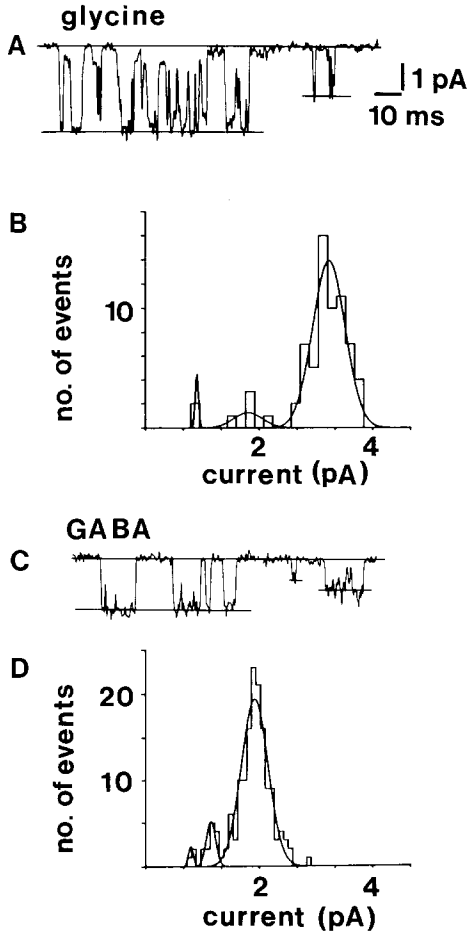

Fig. 1. Glycine- and GABA-activated channels open to discrete conductance levels. (*A*) Examples of the glycine-activated (10 μM) channels in this outside-out patch, which was held at −70 mV. Room temperature was 27°C. (*B*) The distribution of the sizes of current passing through 69 resolved glycine-activated channel openings in this patch. The bin size was 0.16 pA. (*C*) Examples of the channels activated by 10 μM GABA in another outside-out patch. Room temperature was 22°C. (*D*) The distribution of the sizes of currents passing through 158 GABA-activated channel openings. The bin size was 0.082 pA. The solid lines in (*A*) and (*C*) mark the zero current level and the means of the Gaussian curves

glycine only. In all these recordings, it was clear that the channels activated had a variety of conductance levels.

Glycine and GABA Activate Multiple Conductance State Channels

By measuring the amplitudes of the elementary current events activated by glycine and GABA, it was possible to produce amplitude histograms like those in Fig. 1. The openings in these two patches fell into discrete groups that could be reasonably fitted by normal distributions. Decisions on boundaries between conductance states were made following inspection of the histogram, and then curves drawn using the means and SD derived from the data within each grouping. Examples of glycine-activated channels and a histogram are shown in Fig. 1*A* and *B*. From the histogram, it was concluded that in this recording glycine-activated channels exhibited three current levels of 0.96 ± 0.03, 1.87 ± 0.26 and 3.32 ± 0.29 pA. In another outside-out patch, GABA-activated currents recorded at the same voltage (−70 mV) had mean amplitudes of 0.81 ± 0.04, 1.17 ± 0.08 and 1.96 ± 0.24 pA (Fig. 1*C* and *D*). A single event with a greater amplitude (2.9 pA) was also seen.

Figure 2*A* and *C* shows traces of single-channel currents activated by glycine and GABA (both 50 μM) in an outside-out patch held at −69 mV. Openings to three discrete levels were observed when glycine was applied, whereas GABA produced openings to only two levels. The broken lines denote the mean sizes of the currents. The current-voltage relationships for the two most common conductance states were linear over the range −130 to 50 mV (Fig. 2*B* and *D*) for both transmitters. Linear regression revealed conductances of 30 and 42 pS and 20 and 29 pS for these glycine and GABA-activated conductance states, respectively. The reversal potentials of the fitted lines were between −4 and −8 mV in this patch, close to the value expected for Cl^- specific conductances. Glycine was applied seven times to the patch described in Fig. 2. During four of these applications, the 42-pS state was the most frequently occurring (68–88% of the 19–84 measured events). However, during two other applications, the 30-pS state was seen a greater number of times (67–74% of the 25–75 measured events), while the 20- and 42-pS states were seen with equal frequency on the remaining occasion (49% of the 35 events measured). In the five applications of GABA to the same patch, the 29-pS state occurred most frequently (74–92% of the 18–129 events measured) on all occasions, except one, when the 20-pS state was the most frequent (all of 15 events measured). Additional openings to a greater level with a slope conductance of 85 pS, were observed during the first two applications of glycine (Fig. 2*A* and *B*).

The Conductances of the Different States

In general, the conductances of the channel openings activated by both agents in outside-out patches were similar to those seen in Fig. 2. However, the

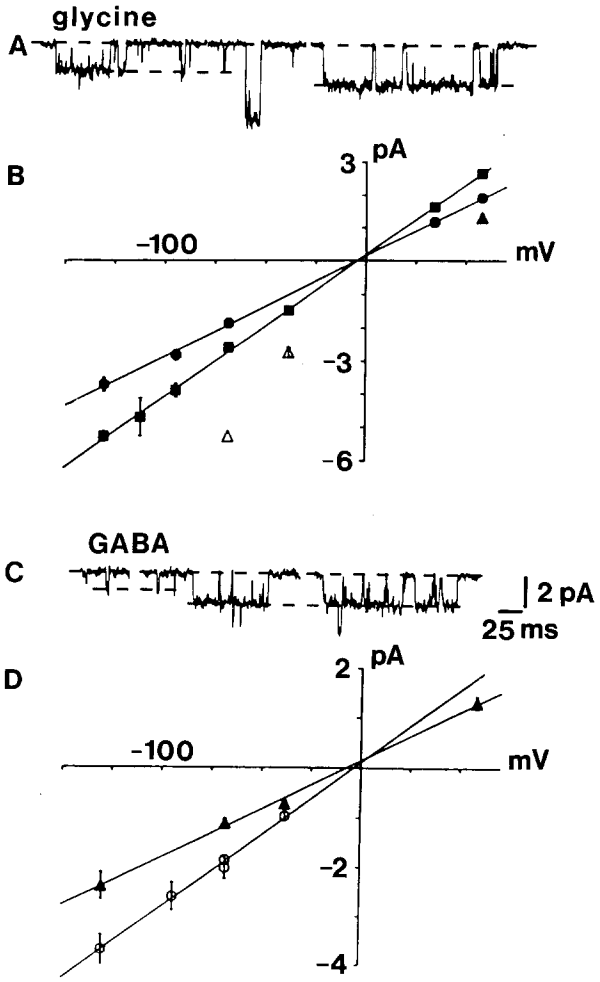

Fig. 2. The current-voltage relationship for glycine- and GABA-activated channels in one outside-out patch. (*A*) Examples of the glycine-activated (50 μM) channels with the patch held at −69 mV. (*B*) The current-voltage plot for glycine-activated conductance states. (*C*) Examples of the channels activated by 50 μM GABA in the same outside-out patch at −69 mV. (*D*) The current-voltage relationship for GABA-activated conductance states. Room temperature was 17°C

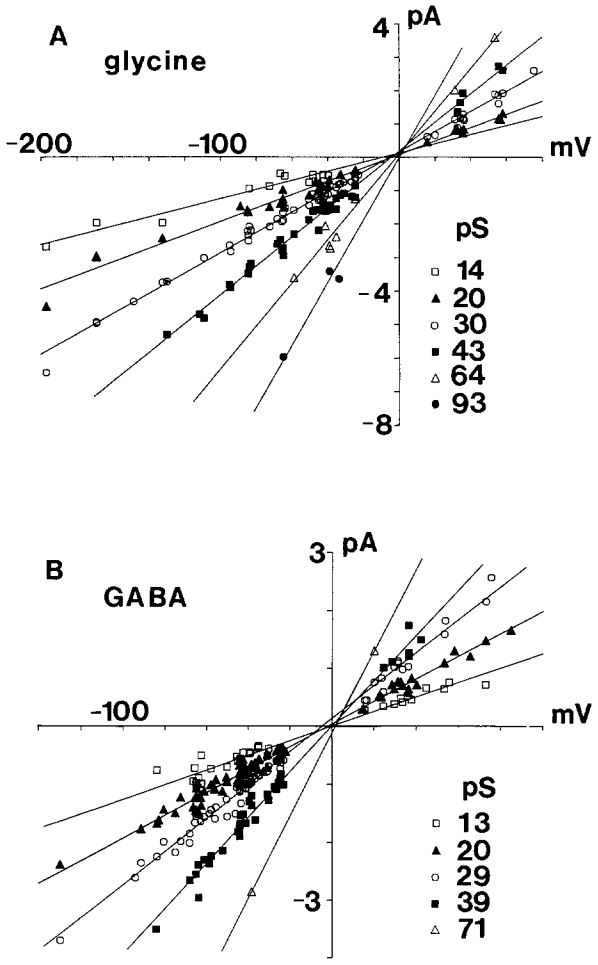

Fig. 3. The conductances of the states activated by glycine and GABA. All recordings were made in BM1 with ES1 as the electrode solution (E_{Cl} = −2 mV) at temperatures between 17 and 19°C. Composite current voltage plots of the glycine- (*A*) and GABA-activated (*B*) conductance states. The data for these plots was derived from 69 applications of glycine to a total of 21 patches and 109 applications of GABA to 35 patches. The grand mean conductances are expressed on the respective plots

variation in the number, size and frequency of occurrence of conductance states between different patches were factors that complicated the grouping of the data necessary to obtain the mean values.

A composite current-voltage plot for the different conductance states was produced by grouping the data according to the conductances derived from the individual current-voltage plots, for a total of 21 and 35 patches for glycine and GABA, respectively (Fig. 3*A* and *B*). Only data obtained from experiments performed at 17–19°C was included in the composite plots. The grand mean conductances derived from the slopes of the lines were 14, 20, 30, 43, 64 and 93 pS for the glycine-activated conductance states and 13, 20, 29, 39 and 71 pS for the channels activated by GABA. On occasion, channel amplitudes were observed to switch from one conductance level to another. While this occurred infrequently, the reversible nature of such excursions suggested that they were not simply due to the simultaneous opening and closing of channels of different sizes. Movements between most of the different states were observed for both GABA and glycine. However, excursions from one state to all others was not seen in any single patch. Therefore, it can be concluded that at least some of the channels activated by glycine and GABA can adopt more than one conductance level.

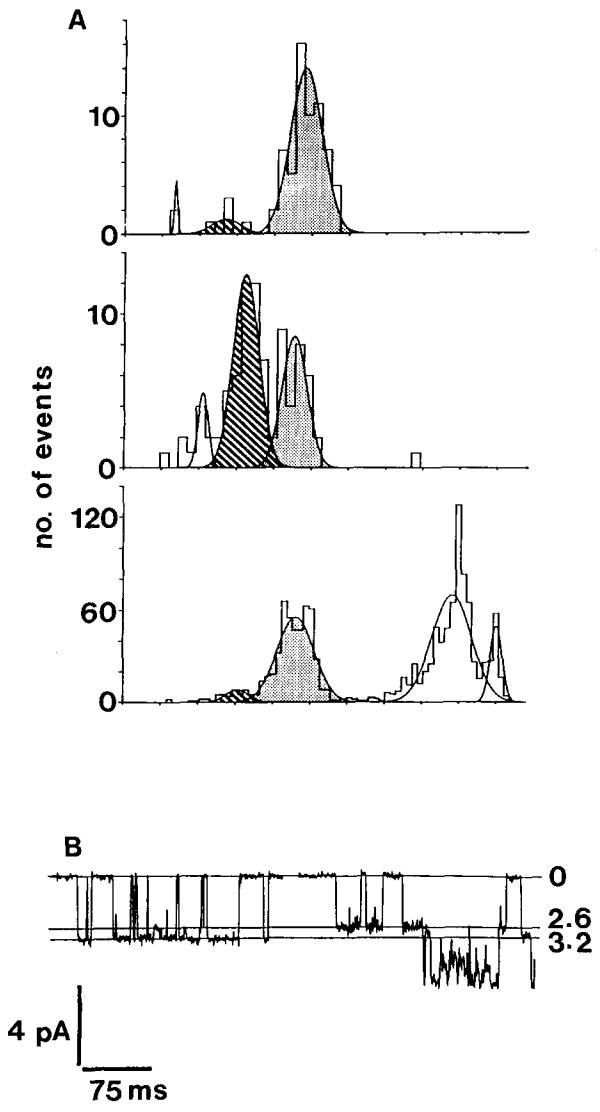

Fig. 4. The distributions of the conductance states activated by glycine in three outside-out patches. (*A*) Histograms normalized by dividing current by driving voltage. In the upper histogram, events fell into three distributions with means of 14, 28 ± 4 and 49 ± 4 pS, the number of occurrences of each state (*n*) being 2, 5 and 62, respectively. The middle histogram exhibits three normal distributions with means of 21 ± 1, 32 ± 3 and 45 ± 3 pS ($n = 7$, 45 and 30), and in the lowest histogram the conductance levels are fitted with five normal distributions with means of 31 ± 3, 46 ± 5, 67 ± 1, 88 ± 5 and 100 ± 1.6 pS ($n = 43, 473, 7, 641$ and 137, respectively). The bin sizes for the three histograms are 2.4, 2.4 and 1.46 pS, respectively. In the experiments described in the upper two histograms, the electrode solution was ES2, room temperature 27°C and the patch was held at −70 mV. In the other, ES1 was used, room temperature was 17°C and the patch was held at −65 mV. The patches were all bathed in BM1. (*B*) Examples of single-channel events activated by glycine in the patch described in the lowest histogram in (*A*). Two sizes of opening are observed in this part of the current trace. The two lines drawn through the open channel noise seen here correspond to 2.6 and 3.2 pA

Frequency of Occurrence of the Conductance States

While analyzing our experimental results, it became clear that no single glycine- or GABA-activated conductance state occurred most frequently in all patches, or even during different applications to the same patch (*see* p. 47). The conductance histograms of glycine-activated states (Fig. 4*A*) illustrate this variability vividly. Together these three patches demonstrate all the different conductance states described in Fig. 3*A*. Shading distinguishes the occurrence of the same conductance state in different patches. Openings to a level around 46 pS accounted for between 35–90% of all events in the three patches and was the main or most frequent state in only one of these. The 30-pS level was also most frequent in one patch (53% of all events), but made up only 3% of the events in the lowest histogram. In this patch, the 88-pS level, which was not identified in the other two patches, accounted for 49% of all the openings and was the main state. However, comparison of the lower histogram with the other two is complicated by a difference in temperature of 10°C. The effect of temperature on the conductance of this channel is unknown, but estimates for other channels predict a Q_{10} of 1.1–1.2 (Hille, 1984). Such values suggest the mean conductances obtained at 27°C for the upper and middle histograms are equivalent to conductances of 12–13, 23–25 and 41–45 ps and 18–19, 27–29 and 38–41 ps, respectively at 17°C. All these values are close to those for the mean conductance states described in Fig. 3, with the exception of the middle state in the upper histogram. Therefore, even accounting for the 10°C temperature difference it is obvious that the most frequent conductance level can vary between different patches.

During 61 of the 68 applications of glycine (50 μM) and 101 of the 109 applications of GABA (50 μM) included in Fig. 4, it was possible to estimate which state occurred most frequently. The proportion of these applications in which a state occurred most frequently and the proportion of applications in which a state was observed at least twice are given in the Table. The 30-pS state was the main state most often when GABA or glycine were applied. However, in a significant proportion of the patches, the 43-pS (28%) and the 20-pS (44%) states were the main states for glycine- and GABA-activated channels, respectively (Table, column *A*). The 20-pS state occurred in a greater proportion of the patches tested with GABA than any other, whereas the 30-pS state was observed the most often in the patches tested with glycine (Table,

column *B*). Overall, the pattern was for glycine to activate the 30- and 43-pS states most frequently, whereas, in the presence of GABA the 20- and 29-pS states were most favored. However, it is clear from Fig. 4 that significant deviations from this general pattern do occur, contradicting the opinion that the agonist is the lone determinant of channel conductance (Hamill et al., 1983).

Additional Conductance States

Part of the variability within the conductance states described in Fig. 3 may be explained by the presence of additional conductance levels. Occasionally, openings were observed that appeared to lie between two of the states that have been described (*see* Fig. 4*B*). The left-hand part of the trace shows openings to a level around 3.2 pA, denoted by the lowest continuous line. Three transient partial closures to the next lowest line (2.6 pA) are apparent, two of which are followed by a return to the 3.2-pA level and the other by a closure to the current baseline. Only 3 sec later (right-hand trace), the channel was seen to open to the 2.6-pA level more frequently. The 2.6- and 3.2-pA levels correspond to conductances of 41 and 51 pS. Inspection of the histogram obtained for this application (lowest histogram, Fig. 4*A*) shows there are two peaks within the normal distribution that had a mean conductance of 46 pS. The modal values of these peaks (43 and 50 pS) are close to the conductance levels illustrated in Fig. 4*B* and we, therefore, propose that there are other conductance levels besides those obtained from the grand mean values in Fig. 3.

The Relationship Between Adjacent Conductance States

Reports on the properties of three other types of multistate Cl^- channels have indicated that all conductance states are multiples of an elementary conductance (Miller, 1982; Geletyuk & Kazachenko, 1985; Krouse, Schneider & Gage, 1986). Because of this elegant relationship, and the presence of rapid transitions between fully open and closed states, it was suggested that the Cl^- channels consisted of a cluster of identical pores that were coupled in some way. Interestingly, it was proposed that the glycine- and GABA-activated channels also operated in this fashion (Geletyuk & Kazachenko, 1985). Examination of the relationship between the sizes of the conductance states activated by glycine (14, 20, 30, 43, 64 and 93 pS) and GABA (13, 20, 29, 39 and 71 pS) in rat spinal neurones provides some clarification of this matter. Instead of forming an arithmetic progression, like other multistate Cl^- channels, consecutive conductance levels for glycine and GABA appear to be part of a geometric progression. Specifically, each conductance state is about 50% bigger than the next smallest. However, it could be argued that the real relationship is an arithmetic progression and that the additional states seen in Fig. 4 were wrongly omitted. Supposing this to be true, the difference of 7 pS between the modal values of 43 and 50 pS in Fig. 4*B* (lowest histogram), could account for the grand mean conductance values quite well. However, for the Cl^- channels made up of clusters of pores, the probability of occurrence of the different states follows a binomial distribution (Miller, 1982; Krouse et al., 1986). Were this the case for the glycine-activated channel, openings to states between the 30- and 43-pS states should have been observed in Fig. 3*A*. Given the high frequency of occurrence of 50- and 88-pS states, but the paucity of events between these values, the data in Fig. 4*A* (lowest histogram) is also difficult to reconcile with a binomial distribution.

Table.

Glycine			GABA		
State (pS)	A	B	State (pS)	A	B
14	0	18	13	3	36
20	15	51	20	44	77
30	56	94	29	47	65
43	28	60	39	7	36
64	0	13	71	0	2
93	2	5			

A: the proportion (%) of the applications of glycine or GABA (50 μM) in which a given conductance level was the main state. *B:* the proportion (%) of applications of glycine or GABA (50 μM) in which a state was observed at least twice.

Discussion

Chloride-Channel Receptor Coupling

The similarity, reported here, between some of the conductance states activated by glycine (14, 20, 30, 43 and 64 pS) and GABA (13, 20, 29, 39 and 71 pS) supports and extends the proposal by Hamill et al. (1983) that GABA and glycine activate a similar type of channel. Given the distinct nature of the receptors to glycine and GABA on spinal neurones (Curtis et al., 1971*a*; Curtis, Duggan & Johnston, 1971*b*; Smith, 1987) and assuming that the channels

are identical, two simple models of receptor-channel coupling are consistent with this proposal. In the first model, every Cl^- channel is coupled to either a GABA or a glycine receptor and consequently there should be no interaction between the two agents. However, in the other model, every channel is coupled to both types of receptor. In support of the latter hypothesis, it has been reported that the sustained application of either GABA or glycine reduces the sensitivity of spinal neurones to the other transmitter (Barker & McBurney, 1979). However, this report has remained unsubstantiated, and has been questioned by other workers (Gold & Martin, 1984).

Indeed, our demonstration that a minority of spinal neurones and outside-out patches are sensitive to only glycine or GABA is inconsistent with the second model. In addition, the poor correlation between the size of currents activated by glycine (15 μM) and GABA (10 μM) on voltage-clamped spinal neurones is also unexpected if every channel is coupled to both types of receptor (Smith, 1987). While these results support the idea that each channel is only associated with one type of receptor, they do not permit us to rule out the possibility that some are coupled to both. Interestingly, glycine receptors have recently been reported to exist in apposition to GABA-containing boutons in the rat spinal cord (Triller, Cluzeaud & Korn, 1987).

Although there are very close similarities between the conductances of the three lowest glycine- and GABA-activated states, the matches are not as good for states four and five (Fig. 3). This may reflect a structural difference between the channels activated by the two transmitters or, alternatively, the difference in frequency with which each transmitter activates distinct conductance states. The observation that the openings to the 46-pS level in Fig. 4*B* are composed of two separate sizes of event gives some support to this last idea. For if openings to both of these levels were not usually being resolved and glycine were to activate the higher of these two states more often than the lower, and GABA the converse, this would explain the small difference between the size of the conductance states. The large variability around the mean in the values of the conductance states activated by glycine and GABA (*see* Fig. 3) may also result from our grouping unresolved, distinct conductance states together.

Frequency of Occurrence of the Conductance States

In contrast to glycine, GABA was not observed to activate a state higher than 30 pS by Hamill et al. (1983). However, a GABA-activated state comparable to the 43-pS state of glycine seen here, has been reported in other preparations (Bormann & Clapham, 1985; Cottrell, Lambert & Peters, 1985). Recently, in addition to the conductance states initially reported, Bormann et al. (1987) have confirmed that GABA activates a 45-pS state in spinal neurones, and that both transmitters activate an additional state of 12 pS. Despite this update in the number of states observed in mouse neurones to 4 (glycine: 12, 20, 30 and 46 pS; and GABA: 12, 19, 30 and 44 pS), it still remains that a greater number of states are seen in rat neurones. This may not reflect a real difference to the number of conductance states that can be adopted, but rather may stem from differences in the frequency with which a state is occupied. Some support for this suggestion is provided by Hamill et al. (1983), who reported that, "certain patches did display glycine-activated currents with amplitudes larger than the main current level. . . ." This suggestion may also explain why other workers see only one GABA-activated conductance level in spinal neurones (21 pS, Jackson et al., 1982; 29 pS, Mathers, 1985) and in pituitary cells (14 pS, Inenaga, Mason & Tibbs, 1987; 20 pS, Kehl, *personal communication*). In agreement with Bormann et al. (1987), differences in the frequency of occurrence of the states were apparently determined, at least in part, by the type of agonist. However, whereas in mouse spinal neurones glycine activated the 45-pS state most frequently in 85% of the patches, in rat neurones the main state for glycine was 43 pS in only 28% of all applications; the 30-pS state being the main state most commonly overall (56%). GABA activated the 29-pS state as the main state most often in mouse (94%) and rat (47%) spinal neurones, but in rat neurones the 20-pS state was the main state occurring with almost equal frequency (44%). So, although on average glycine tended to activate higher conductance states more frequently than GABA in rat spinal neurones, the differences were not as marked as observed in mouse neurones (Hamill et al., 1983; Bormann et al., 1987).

The Relationship Between Different Conductance States

The conductance states most commonly activated by glycine and GABA form a geometric progression. Consequently, it seems unlikely that the glycine- and GABA-activated channels consist of a cluster of coupled, identical ion-conducting pores as has been proposed for the other multistate Cl^- channels (Miller, 1982; Geletyuk & Kazachenko,

1985; Krouse et al., 1986). The observation of additional conductance states in Fig. 4 severely limits any interpretation of the geometric progression exhibited by conductances of adjacent states. Consequently, it has not been possible to relate this relationship to the mechanism of permeation of the channel by anions.

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