Caesium ions: a glycine-activated channel agonist in rat spinal cord neurones grown in cell culture

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1 The chloride (Cl^-) currents activated by caesium ions (Cs^+), glycine and γ -aminobutyric acid (GABA) were compared following their application to rat neurones that had been grown in cell culture. Recordings were made using the whole-cell patch clamp technique under voltage clamp conditions.

2 In spinal cord neurones, bicuculline methiodide antagonized GABA-activated currents more effectively than Cs^+ - or glycine-activated currents. However, strychnine was more effective at blocking the currents activated by Cs^+ or glycine than those activated by GABA.

3 Of the 3 agonists, only GABA activated currents in cells from the intermediate lobe of the rat pituitary.

4 In spinal neurones the size of the currents activated by 70 mM Cs^+ was correlated to the size of the currents activated by 15 μM glycine ($P < 0.005$; $n = 10$, Spearman's rank correlation), but there was no significant correlation between the size of the currents activated by these agents and 10 μM GABA.

5 The joint application of glycine and Cs^+ activated currents that were approximately twice as big as the sums of the currents activated by separate applications of the same doses. This synergism was consistent with Cs^+ acting at the same receptor as glycine (7 μM glycine being equivalent to 31 ± 7 mM Cs^+).

6 It was concluded that Cs^+ activates the same Cl^- channel as the inhibitory neurotransmitter glycine.

Introduction

Curiously, the monovalent cation caesium (Cs^+) has recently been shown to activate a chloride (Cl^-) channel in rat spinal neurones (McBurney *et al.*, 1985; Hughes *et al.*, 1987). The physiologically important alkali metal ions, sodium and potassium, were dismissed as candidates for the role of the endogenous ligand of this channel as they did not mimic the effect of Cs^+ . However, the observation that the Cs^+ -activated channel exhibited the same conductance states (Hughes *et al.*, 1987) as the Cl^- channels activated by glycine and γ -aminobutyric acid (GABA) (Smith *et al.*, 1988), prompted us to compare some other properties of these agonists. We present the results of these studies here and conclude that Cs^+ is a glycine channel agonist. Some of these

results have been presented previously in abstract form (Smith, 1987a; Smith & McBurney, 1987).

Methods

Tissue culture

Cultures of spinal cord neurones were prepared as described previously (Smith, 1987b). Briefly, spinal cords were dissected 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%) and chick embryo extract (1%). Glucose (28 mM), L-glutamine (2 mM), sodium bicarbonate (10.5 mM) and 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid

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Figure 1 The action of 5 μM bicuculline methiodide (Bic) on the currents activated by 3 Cl^- channel agonists. (a) GABA (10 μM), (b) Cs^+ (70 mM), (c) glycine (15 μM). All applications were made with a U-tube tool for 2 s, with at least 70 s between applications. Cs^+ test solutions were produced by replacing 70 mM of the Na^+ in the bathing medium and the patch electrode contained ES4 ($V_H = -70$ mV, $E_{\text{Cl}} = -5$ mV).

(HEPES, 4.2 mM) were also added to the culture medium. All components except the rat serum were obtained from GIBCO. The neurones were incubated at 37°C in 5% CO_2 and 95% air for 11–35 days before use. Pars intermedia cells from the rat pituitary were prepared as described by Kehl *et al.* (1987).

Recording techniques

Coverslips supporting the cells were placed in a recording chamber mounted on the stage of an inverted phase contrast microscope (Invertoscope D. Zeiss) and viewed at $\times 400$ magnification. Recording electrodes were prepared with outer diameters of about 1–2 μm and heat polished. Whole-cell recordings (Hamill *et al.*, 1981) were made from spinal neurones with cell bodies of 10–25 μm in diameter.

All recordings were made at room temperature (19–22°C) with an EPC-7 amplifier (List Electronics) that was modified to permit the offsetting of junction potentials of up to ± 100 mV. The current and voltage signals were stored following pulse code

modulation (modified Sony 701ES; Lamb, 1985) on a video recorder (NV-830, Panasonic).

Solutions

During recordings, the cells were continuously superfused with a medium containing (in mM): NaCl 140, KCl 3.5, CaCl_2 2, MgCl_2 1, HEPES 10 and glucose 5. The electrode filling solutions were either ES3 (Na isethionate 114, NaCl 6, CaCl_2 1, MgCl_2 2, EGTA 11 and HEPES 10) or ES4 (CsCl 125, CaCl_2 1, MgCl_2 2, EGTA 11 and HEPES 10). All solutions were titrated to pH 7.2 with NaOH, except for ES3 where TEOH was used. The Cl^- activities of all solutions were estimated by interpolation from standard tables (Robinson & Stokes, 1959) and used to calculate the Cl^- equilibrium potential (E_{Cl}). Test solutions were made up in the bathing medium and applied to whole cells from a modified U-tube tool (Hughes *et al.*, 1987). Cs^+ -containing solutions were made by replacing Na^+ on a stoichiometric basis. Membrane potentials were corrected to account for

the liquid junction potentials that resulted from the different ionic composition of electrode and bathing solutions (Kaneko & Tachibana, 1986).

Data analysis

The majority of recordings were replayed from tape, filtered at 1 or 3 kHz (-3 dB) and captured on the screen of an oscilloscope (Gould 4020). Measurements were then made directly from chart records, subsequent to the traces being dumped at low speed onto a chart recorder (2400S, Gould). Measurements are described in the format arithmetic mean $\pm$ s.d.

Results

The actions of bicuculline and strychnine on GABA-, Cs^+ - and glycine-activated currents

The receptors for glycine and GABA have been distinguished by their different sensitivity to the antagonists strychnine and bicuculline (Curtis *et al.*, 1971a,b). In an attempt to determine the site of action for Cs^+ we therefore compared the effects of these antagonists on the currents activated by GABA, Cs^+ and glycine.

Figure 1 illustrates the effect of bicuculline methiodide on similar sized currents activated by GABA, Cs^+ and glycine in a spinal neurone (holding voltage (V_H) = 70 mV). Solutions containing antagonists also contained the control level of agonist. The GABA ($10 \mu\text{M}$)-activated current was reversibly reduced by 81% of its control value by $5 \mu\text{M}$ bicuculline methiodide (Figure 1a), whilst the same concentration of bicuculline methiodide reduced the Cs^+ - and glycine (70 mM and $15 \mu\text{M}$ respectively; Figure 1b,c)-activated currents by 11 and 8%, respectively. In 6 similar recordings $5 \mu\text{M}$ bicuculline methiodide reduced the GABA-activated currents by $82 \pm 5\%$ but had no significant effect on the Cs^+ (50 or 70 mM) - or glycine (10 or $15 \mu\text{M}$)-activated currents (reductions of 3 ± 15 and $5 \pm 7\%$, respectively). However, in 2 of these neurones, $10 \mu\text{M}$ bicuculline methiodide reduced the Cs^+ (70 mM)-activated currents by 20% and the glycine ($15 \mu\text{M}$)-activated currents by 19 and 22%.

In recordings from 6 other spinal neurones, using the same recording protocol, strychnine ($5 \mu\text{M}$) reduced the peak currents activated by GABA ($10 \mu\text{M}$), Cs^+ (70 or 100 mM) and glycine ($15 \mu\text{M}$) by 48 ± 9 , 80 ± 11 and $94 \pm 6\%$. One such recording is illustrated in Figure 2. Unlike the GABA-activated currents, the responses to Cs^+ and glycine were truncated in the presence of strychnine; peaking sooner and falling at greater rates than in the control applications (see Figure 2). In 3 of these cells the strychnine concentration was increased to $10 \mu\text{M}$, and

Figure 2 The action of $5 \mu\text{M}$ strychnine on the currents activated by 3 Cl^- channel agonists. (a) GABA ($10 \mu\text{M}$), (b) Cs^+ (70 mM), (c) glycine ($15 \mu\text{M}$). Solutions were as described in Figure 1 and were applied from a U-tube tool ($V_H = -70 \text{ mV}$).

this inhibited the GABA-activated currents by $73 \pm 4\%$.

The different sensitivity of the responses to bicuculline methiodide and strychnine, was inconsistent with Cs⁺ and GABA activating the same receptor. However, the degree of inhibition of the peak currents by $5 \mu\text{M}$ strychnine was also different for Cs⁺ and glycine ($P < 0.05$, paired t test). This difference may have reflected different mechanisms of action for Cs⁺ and glycine, or problems arising from making measurements under non-equilibrium conditions. In an attempt to differentiate between these 2 possibilities the effect of a lower concentration of strychnine ($2 \mu\text{M}$) was examined in greater detail.

As before, control solutions containing GABA ($10 \mu\text{M}$), Cs⁺ (70 mM) and glycine ($15 \mu\text{M}$) were applied with a U-tube tool to spinal neurones ($V_H = -70 \text{ mV}$). Test solutions contained in addition $2 \mu\text{M}$ strychnine, and reduced the peak responses to Cs⁺ and glycine by 62 ± 11 and $81 \pm 9\%$ on average, in the 5 neurones tested, GABA ($10 \mu\text{M}$) was applied to 4 of these neurones, and the resulting currents were reduced by $21 \pm 6\%$ by $2 \mu\text{M}$ strychnine.

The effects of $2 \mu\text{M}$ strychnine on glycine- and Cs⁺-activated currents are compared in Figure 3 for a typical cell. Even when test and control currents were both measured at the time at which the control currents peaked (denoted by double arrow heads in Figure 3a), the glycine-activated current was antagonized by a greater amount than the Cs⁺-activated current (93 ± 6 and $78 \pm 9\%$, respectively; $P < 0.05$, paired t test). However, this difference was at least partly explained by the observation that the current activated by glycine took longer to peak than the Cs⁺-activated current. The glycine-activated currents peaked, on average, $960 \pm 210 \text{ ms}$ after the switching of the U-tube valve, compared with $650 \pm 90 \text{ ms}$ for the Cs⁺-activated currents ($P < 0.05$, paired t test, $n = 5$).

The currents activated by both Cs⁺ (70 mM) and glycine ($15 \mu\text{M}$) usually desensitized during the course of an application (see Figures 1, 2 and 3). This factor complicated our attempts to obtain a meaningful measure of the level of antagonism produced by strychnine at equilibrium. However, by measuring the test current: control current ratio (r_0) at 100 ms intervals throughout the application period (Figure 3b) the maximum level of antagonism that occurred during an application was estimated. This r_0 value will reflect the antagonism by strychnine if the rates of desensitization are unchanged in the presence and absence of the antagonists. In this cell the r_0 values fell during the application period to 0.02 and 0.08 for glycine and Cs⁺ respectively. On average the levels of antagonism increased during the applications to give r_0 values of 0.01 ± 0.01 and 0.09 ± 0.08 for glycine- and Cs⁺-activated currents respectively

Figure 3 Antagonism of glycine- and Cs⁺-activated currents by $2 \mu\text{M}$ strychnine. (a) Responses aligned relative to the beginning of the 1.5 s application period, which is denoted by the arrowheads above the highest traces. The 2 smaller arrowheads on either side of the current traces indicate the time at which the control responses to glycine and Cs⁺ peaked. (b) Plots of r_0 against time for glycine- and Cs⁺-activated currents, where r_0 is the ratio of the test current: control current. Bath and electrode solutions were as described in Figure 1.

($P = 0.08$, paired t test). It is pertinent to note that whilst the degree of antagonism of the responses to glycine achieved an apparently steady level by the end of the application period, this was not the case for the responses to Cs⁺.

The actions of GABA, glycine and Cs⁺ on cells of the rat pituitary

From the comparisons of the actions of bicuculline methiodide and strychnine on GABA-, Cs⁺- and glycine-activated currents it seemed more probable that Cs⁺ was an agonist of glycine-activated channels than of GABA-activated channels. To determine whether Cs⁺ had any action on GABA-activated channels, we compared the effects of GABA, glycine and Cs⁺ on cells from the pars intermedia of the rat pituitary, as these cells contain GABA-activated Cl⁻ channels (Kehl *et al.*, 1987) but are insensitive to glycine (data not shown).

The cells were voltage clamped at 0 mV ($E_{\text{Cl}} = -64 \text{ mV}$) and the test solutions applied with a

U-tube tool for 500 ms at intervals of 80–120 s. GABA ($100\ \mu\text{M}$) activated outward currents of $294\text{--}1314\ \text{pA}$ (mean = $699\ \text{pA}$) but glycine ($100\ \mu\text{M}$) and Cs^+ ($140\ \text{mM}$) produced no detectable effect in the same 4 cells.

A comparison of the sizes of the currents activated by GABA, glycine and Cs^+

Unlike the pars intermedia cells, spinal neurones were always found to respond to GABA, glycine and Cs^+ . However, using fixed concentrations of agonists the ratios of the sizes of the currents were not always constant. In 10 spinal neurones GABA, glycine and Cs^+ were applied at concentrations of $10\ \mu\text{M}$, $15\ \mu\text{M}$ and $70\ \text{mM}$ to give mean currents of 421 ± 345 , 509 ± 379 and $358 \pm 240\ \text{pA}$, respectively. The ratios of the currents elicited for $\text{Cs}^+:\text{GABA}$, $\text{Cs}^+:\text{glycine}$ and $\text{glycine}:\text{GABA}$ were 1.38 ± 1.93 , 0.78 ± 0.19 and 1.77 ± 2.4 . It can be seen that the standard deviation of the $\text{Cs}^+:\text{glycine}$ ratio is over an order of magnitude smaller than the values for the other 2 ratios.

The variation in the relative magnitude of the currents can be seen more clearly in Figure 4. Here the sizes of the current activated by each agonist are plotted against the size of the currents activated by the other 2 agents. There is a significant correlation between the sizes of the currents activated by glycine and Cs^+ ($P < 0.005$, Spearman's rank correlation test) but none between GABA- and Cs^+ - or GABA- and glycine-activated currents at the $P = 0.05$ level.

Does Cs^+ activate the same channel as glycine?

The above results are consistent with Cs^+ activating the same channel as glycine but not GABA. However, they do not exclude the possibility that Cs^+ and glycine activate similar but distinct types of channel. We considered 3 different receptor-channel configurations to explain the activation of Cl^- channels by Cs^+ , all of which were based on the assumption that Cs^+ acts through a single type of receptor. It was proposed that Cs^+ and glycine act at distinct receptors that are both coupled to similar, but independent channels, or that they act at distinct receptors that are both coupled to the same Cl^- channel, or that both agonists act at the same receptor. To differentiate between these hypotheses we compared the effects of applying Cs^+ and glycine separately and jointly to the same neurone; for if either of the first two hypotheses are correct the sums of currents activated by the separate application of both agonists, should be equal to or greater than the currents elicited by the simultaneous application of glycine

Figure 4 Correlations between the size of the currents activated by GABA ($10\ \mu\text{M}$), glycine ($15\ \mu\text{M}$) and Cs^+ ($70\ \text{mM}$) in 10 spinal neurones. All cells were voltage clamped at $-70\ \text{mV}$ and superfused with BM1 (Cs^+ test solutions produced by replacing an equivalent concentration of Na^+). The electrode solution was ES4 ($E_{\text{Cl}} = -5\ \text{mV}$). (a) Glycine- against Cs^+ -activated currents. Spearman's correlation coefficient (r_s) = 0.94. (b) GABA- against Cs^+ -activated currents; r_s = 0.47. (c) GABA- against glycine-activated currents; r_s = 0.60. Lines represent linear regression to the data points.

and Cs^+ . However, it is known that double logarithmic transformations of the Cs^+ and glycine dose-response plots are fitted by lines with mean slopes of 1.9 and 2.0, respectively (Smith, 1987b). Therefore, if Cs^+ and glycine compete for the same receptor, their joint application should activate larger currents than the sum of the currents elicited by separate applications.

In Figure 5a a spinal neurone was voltage clamped at $-70\ \text{mV}$ ($E_{\text{Cl}} = -5\ \text{mV}$) and test solutions applied from a U-tube tool. The sums of the

Figure 5 Glycine and Cs⁺ act synergistically on spinal neurones. (a) Currents activated by glycine, Cs⁺ or both agonists together. Only 2 of the 5 applications of 7 μ M glycine are illustrated. Agonist concentrations are given next to each trace. (b) The plot of the mean normalized currents against Cs⁺ concentration with ($\bullet$) and without (Δ) 7 μ M glycine for 3 spinal neurones. Currents were normalized by being divided by the mean response to 7 μ M glycine for each neurone. Straight lines were drawn between the points representing the sums of the responses to separate applications of glycine and Cs⁺. Solutions were as described in Figure 4.

currents activated by glycine (7 μ M) and Cs⁺ (14, 24, 34 and 42 mM) were less than the peak currents elicited by the simultaneous application of glycine and Cs⁺ (Figure 5a, note the reduced gain in the right-hand panel). The mean data from 3 such experiments are illustrated in (Figure 5b). Each current was normalized by being divided by the mean current ($n = 5$) activated by 7 μ M glycine in that particular cell. The means ($n = 3$ cells) of these normalised currents were plotted against the concentration of Cs⁺ (Figure 5b). Straight lines were drawn between the points representing the sums of the responses to separate applications of glycine and Cs⁺. These lines clearly lie below the currents measured during the simulta-

neous applications of both agents ($\bullet$), and so the first and second hypotheses are untenable.

In an attempt to account for the apparent synergism between Cs⁺ and glycine quantitatively the data from the 3 cells were replotted (Figure 6). Double logarithmic plots were constructed of the Cs⁺-activated currents against Cs⁺ concentration (Δ), and the points fitted to a line by linear regression. The 3 lines have slopes of 1.9 (a), 1.7 (b) and 1.9 (c). The concentration of Cs⁺ required to produce a current equal to that activated by 7 μ M glycine ($[Cs^+]_{eqv}$) was interpolated from each line (26, 27 and 39 mM in a, b and c respectively). Using these values the data derived from the simultaneous appli-

Figure 6 The synergism between Cs⁺ and glycine can be explained by assuming they act at the same receptor. The Cs⁺-activated currents (Δ) from the 3 cells described in Figure 5 were replotted on double logarithmic axes and fitted to a straight line by linear regression. Values for $[Cs^+]_{eqv}$ were obtained by interpolation. (a) Slope = 1.9; $[Cs^+]_{eqv}$ = 26 mM. (b) Slope = 1.7; $[Cs^+]_{eqv}$ = 27 mM. (c) Slope = 1.9; $[Cs^+]_{eqv}$ = 39 mM. The responses to joint applications of Cs⁺ and glycine were replotted using the actual $[Cs^+]$ plus the $[Cs^+]_{eqv}$ ($\bullet$) as the values on the abscissa scale.

cation of both agents were replotted ($\bullet$) so that the $[Cs^+]$ equalled the actual value plus $[Cs^+]_{eqv}$. These additional points lie close to the lines derived from the responses to Cs⁺ alone, which would be expected

if both agonists bound to the same receptor and their concentration-effect relationships had the same form over the range of concentrations used.

Discussion

Cs⁺ is not a GABA channel agonist

Cs⁺ has been shown to activate Cl⁻ channels with conductance properties similar to those activated by glycine and GABA, in spinal neurones (Hughes *et al.*, 1987). The inability of bicuculline methiodide (5 μ M) to reduce Cs⁺-activated currents, while antagonizing similar-sized responses to GABA, argues against Cs⁺ acting at the same receptor as GABA. The different effects of strychnine on Cs⁺- and GABA-activated currents is also inconsistent with them binding to a common receptor. If 2 agonists activate the same channel, then under conditions of voltage clamp it is expected that the ratio of the elicited currents will be constant for fixed concentrations of agonist. The poor correlation between the size of currents activated by fixed concentrations of Cs⁺ and GABA, indicated that these 2 agents activated different populations of Cl⁻ channels. However, such data did not exclude the possibility that Cs⁺ acted in some part, by causing the opening of channels sensitive to GABA. Pars intermedia cells possess GABA-activated Cl⁻ channels which are modulated by benzodiazepines and antagonized by bicuculline (Kehl *et al.*, 1987), but they do not respond to Cs⁺ (140 mM). If the same level of Cs⁺ insensitivity is exhibited by the GABA-activated channel in cells from the pars intermedia and spinal neurones, then the observed effects of Cs⁺ on spinal neurones would not have involved the activation of the GABA sensitive channel at all. Therefore, the major effect of Cs⁺ in spinal neurones probably involves activation of a GABA-insensitive Cl⁻ channel.

Cs⁺ is a glycine channel agonist

Glycine- and Cs⁺-activated currents were similarly sensitive to bicuculline methiodide. A comparison of the effects of strychnine on the similarly sized currents activated by Cs⁺ and glycine, showed that the latter were apparently more sensitive to strychnine. Maximum blockade by strychnine occurred more slowly than the maximum activation of channels by either glycine or Cs⁺ and during control applications, Cs⁺-activated currents peaked earlier than those activated by glycine. These factors may explain

why the level of antagonism by strychnine was greater for glycine- than for Cs⁺-activated currents, when it was assessed by measuring test: control peak current ratios (Figure 2).

From the τ_0 values for the duration of application, the amount of antagonism was observed to increase with time as the relative effects of strychnine and agonist approached 'equilibrium' (Figure 3). Strychnine was also slightly more effective against glycine than Cs⁺ when judged by this method. However, this small, insignificant difference ($P = 0.08$) may be accounted for by the following explanations. The level of antagonism of the Cs⁺-activated current was still increasing at the end of the application (Figure 3b), whereas the effect of strychnine on glycine had apparently achieved a steady state. This could be responsible for some of the observed difference and presumably stems from the tendency for responses to Cs⁺ to persist longer than the comparable currents activated by glycine (see Figures 1, 2 and 5). One other possibility is that the currents activated by Cs⁺ (70 mM) may have involved the occupation of a greater proportion of the total number of receptors than when glycine (15 μ M) was applied. This may seem unlikely because the ratio of the sizes of currents for Cs⁺:glycine was 0.78. However, it was evident that Cs⁺-activated currents showed more desensitization than the glycine-activated currents, and so the relative size of the currents activated by the 2 agonists is an imperfect estimate of the relative degrees of receptor occupation. Taking into consideration these explanations, the effects of strychnine are consistent with Cs⁺ and glycine activating the same type of receptor.

The absence of a response to Cs⁺ or glycine in pars intermedia cells, and the close correlation between the sizes of current activated by fixed concentrations of Cs⁺ and glycine are 2 factors consistent with Cs⁺ activating the same channel as glycine. Comparisons of the frequency of occurrence of the different conductance states of the relevant Cl⁻ channels (see Hughes *et al.*, 1987; Smith, 1987b), are also consistent with the proposal that Cs⁺ activates the same channel as glycine but not GABA.

Of the three models (see Results section) relating the receptor sites for glycine and Cs⁺ the first two can be dismissed on account of the synergism observed when the agonists were applied jointly. Moreover, the data were consistent qualitatively with Cs⁺ and glycine acting at the same receptor.

By using the peak currents as approximations of the currents at equilibrium, it was possible to estimate $[Cs^+]_{eqv}$ (Figure 6). The assumption was then made that 7 μ M glycine would have the same effect as $[Cs^+]_{eqv}$. The validity of this assumption depends on the kinetic scheme for the interaction between the agonists and its receptor. For instance with even a

relatively simple scheme such as that used to describe the activation of other channels with 2 binding sites (Colquhoun *et al.*, 1987), and supposing $k_1 = k_2$ and $k_{-1} = k_{-2}$:

the assumption only holds if the ratio β/α is the same for Cs⁺ and glycine, or if the open probability for the channels is suitably low (e.g. for β/α ratios of 3 and 30 the error < 10% if the open probability < 0.025). However, although these conditions are quite plausible a satisfactory kinetic scheme has not yet been derived for glycine (see Smith, 1987b) and so the original assumption may be invalid. In favour of the assumption, it has been shown that the double logarithmic dose-response relationships for Cs⁺ and glycine are both described by straight lines with similar slopes for the range of concentrations used in Figure 6 (Smith, 1987b), which is consistent with the assumption that 7 μ M glycine will behave like $[Cs^+]_{eqv}$. The currents elicited with the joint applications of Cs⁺ and glycine were therefore replotted using the actual $[Cs^+]$ plus $[Cs^+]_{eqv}$ and it is evident that these data lie close to the line extrapolated from the Cs⁺ dose-response plot (Figure 6). This result is consistent with the third model, which predicts that both agents compete for the same receptor. This approach is not as rigorous as would be preferred; however, it does show that the synergism can be accounted for in terms of only one type of receptor. Hence, the data do not necessitate the postulation of an additional modulatory site, equivalent to those activated by barbiturates and steroids and involved in the enhancement of GABA-activated channels (Barker & Ransom, 1978; Cottrell *et al.*, 1987).

On the sites of action of Cs⁺

In conclusion there is good evidence that Cs⁺ activates the same channel as glycine, probably via the same receptor, in rat spinal cord neurones. Nevertheless it is difficult to envisage how a simple monovalent alkali metal cation can interact with a receptor that is normally activated by glycine; an amino acid which exists almost completely as a zwitterion at pH 7.2 (White *et al.*, 1973).

The large number of shells of electrons around the nucleus of Cs⁺ makes it difficult to estimate the shape of the hydrated ion. Thus we have been unable to compare the hydrated structures of Cs⁺ and glycine.

Interestingly, other similarities between the actions of Cs⁺ and glycine have been described recently.

Biscoe *et al.* (1987), using mouse spinal neurones, showed that glycine blocks a potassium channel that is also blocked by Cs^+ . So whilst there is no obvious structural similarity between Cs^+ and glycine they have similar effects on at least 2 types of channel. However, Cs^+ does not enhance N-methyl-D-aspartate-activated currents (Smith & McBurney, unpublished observation) in the way described for glycine (Johnson & Ascher, 1987).

In addition, to its well documented action of blocking K^+ channels (Hille, 1984), and its ability to activate Cl^- channels, Cs^+ also blocks the activation of acetylcholine-activated channels in chromaffin and sympathetic ganglion cells (Hirano *et al.*, 1987; A. Mathie, personal communication). Surprisingly,

Cs^+ antagonizes acetylcholine-activated currents without causing a change in the noise spectrum, and it is therefore possible that Cs^+ is acting as a competitive antagonist at this receptor.

It is clear that Cs^+ can no longer be considered solely as a blocker of K^+ channels, and in future, its other actions should be considered whenever it is used.

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