Br. J. Pharmacol. (1993), **109**, 1239–1246

Differential inhibition of a transient K^+ current by chlorpromazine and 4-aminopyridine in neurones of the rat dorsal root ganglia

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1 K^+ currents were recorded from neurones of the newborn rat cultured dorsal root ganglia, by a whole cell variation of the patch-clamp technique.

2 Chlorpromazine (CPZ), a neuroleptic, reversibly reduced the amplitude of the transient K^+ current (referred to as 'I_T' hereafter) with a dissociation constant (K_d) of 4.5 μM. The inhibition of the delayed rectifier K^+ current (I_{DR}) was much less potent (K_d, 120 μM). CPZ (100 μM) had no effect on the inward rectifier K^+ current.

3 The blocking action of CPZ on I_T was about seven times more potent than that of 4-aminopyridine (4-AP) which had a K_d of 31 μM. The inhibition of I_T followed one-to-one binding stoichiometry with both drugs.

4 The decay time course of I_T was not affected by CPZ, whereas 4-AP markedly accelerated the decay phase of I_T.

5 The steady-state inactivation curve of I_T was shifted in the negative direction (about 5 mV) by CPZ, whereas the curve was shifted in the positive direction (about 13 mV) by 4-AP.

6 The recovery from inactivation as measured by a conventional double pulse protocol was described by two exponential components in the control solution. CPZ markedly reduced the first component and slowed down the recovery from inactivation. In contrast, in the presence of 4-AP, the peak amplitude of I_T was rather increased by a preceding I_T possibly through voltage-dependent unbinding of 4-AP molecules.

7 These results indicate that CPZ has a preferential blocking action on I_T and the mechanism underlying this block is markedly different from the mechanism underlying the blocking action of 4-AP.

Keywords: Chlorpromazine; psychotropic drugs; neuroleptic; potassium channel; dorsal root ganglion; transient potassium current; aminopyridine; channel block; cultured neurone

Introduction

The neuroleptic drugs are chemically diverse compounds which are widely prescribed for the treatment of psychiatric disorders such as schizophrenia. Their antischizophrenic actions are thought to involve blockade of dopamine receptors in the brain (Seeman, 1981). In addition, it is well known that neuroleptics have quinidine-like direct membrane effects in the heart which may be related to some of their cardiovascular side actions (Risch *et al.*, 1981). A potent blocking action of chlorpromazine (CPZ), a phenothiazine neuroleptic, on cardiac Na^+ (Ogata & Narahashi, 1989; Ogata *et al.*, 1989), neuronal Na^+ (Ogata & Tatebayashi, 1989; Ogata *et al.*, 1990) and neuronal Ca^{2+} channels (Ogata & Narahashi, 1990) may explain the quinidine-like direct membrane effects of the neuroleptic drugs.

With the exception of a few drugs such as tetrodotoxin (TTX) for Na^+ channels, many of the blockers of ion channels are not absolutely selective in their blocking action. A number of papers reported a direct action of CPZ on Ca^{2+}-activated K^+ channels (e.g., Dinan *et al.*, 1987; McCann & Welsh, 1987). However, a direct action of CPZ on other types of K^+ channels remains to be demonstrated. An activation of voltage-gated K^+ channels plays a pivotal role as the 'neuronal brake' in regulation of cellular excitability, since K^+ currents counteract inward currents through Na^+ or Ca^{2+} channels. Voltage-gated K^+ channels form the largest and most diversified class of ion channels. Therefore, it seems of particular interest to examine responses to CPZ of different types of K^+ channels, since each channel type has different roles in cellular functioning (Adams & Nonner, 1990). We, therefore, investigated the action of CPZ on different types of K^+ currents in cultured neurones from rat dorsal root ganglia (DRG).

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Methods

Culture procedures

Dissociated cultures of rat DRG neurones were prepared as described by Tatebayashi & Ogata (1992). Rats (1 to 3 days old) were killed by decapitation under ether anaesthesia. DRG were dissected out and incubated at 36°C for 30–40 min in Ca^{2+}- and Mg^{2+}-free saline, containing 0.25% trypsin (Type XI, Sigma). The ganglia were then mechanically dissociated with a fire-polished Pasteur pipette. The cells were plated on glass cover-slips coated with poly-L-lysine (Sigma) and maintained in a humidified incubator containing 5% CO_2 in air at 35°C in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum (GIBCO), penicillin (40 iu ml^{-1}), and streptomycin (40 ng ml^{-1}). After 1–2 days in culture, cytosine β-(+)-arabinofuranoside (Sigma) was added to cultures to suppress the growth of non-neuronal cells. Subsequent medium changes were done at 3–4 day intervals. Cells between 2–10 days in culture were used.

Electrical recording

The methods for electrical recording used were similar to those previously described (Ogata *et al.*, 1990). Membrane currents were recorded with the whole-cell patch-clamp tech-

nique (Hamill *et al.*, 1981). The d.c. resistance of suction electrodes was 1.5–3 MΩ. The pipette solution contained (in mM): K-glutamate 130, Mg-ATP 3, $MgCl_2$ 2.5, glucose 5, HEPES 5 and EGTA 5. The pH of the pipette solution was adjusted to 7.0 with KOH. The external solution contained (in mM): tetramethylammonium-Cl 120, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 and glucose 25. The pH of the external solution was adjusted to 7.4 with NaOH. TTX at a concentration of 0.2 μM was added to block inward TTX-sensitive Na^+ current. Cells having TTX-insensitive Na^+ current (Ogata & Tatebayashi, 1992) were not used for experiments. Since the external solution was Na^+-free, a possible involvement of Na^+-dependent K^+ current (Coraboeuf & Carmeliet, 1982; Escande *et al.*, 1987; Bader *et al.*, 1990) was eliminated. Ca^{2+} currents were eliminated by 50 μM Cd^{2+} which completely blocked Ca^{2+} currents in rat DRG (Tatebayashi & Ogata, 1992). Thus, Ca^{2+}-dependent components of K^+ currents could be eliminated. Membrane currents passing through the pipette were recorded by a current-to-voltage converter designed by M. Yoshii (Ogata *et al.*, 1990). Compensation for the series resistance was performed by adding a part of the output voltage of the current recording to the command pulse.

All the experiments were performed with an on-line system which has been developed by M. Yoshii and N. Ogata, using a personal computer (PC-286V, EPSON, Tokyo, Japan). Capacitative and leakage currents were subtracted digitally by the P-P/4 procedure (Ogata *et al.*, 1990). Exponential fits were determined by computer using a non-linear sum of the least squares fitting routine. The liquid junction potential between internal and external solutions was about 11 mV. The data shown here were compensated for this effect by adjusting the zero-current potential to the liquid junction potential. Only cells showing an adequate voltage and space clamp (Ogata *et al.*, 1989) were used. All experiments were done at room temperature (21–23°C). Results are expressed as mean ± s.e.mean, and n represents the number of cells.

Drugs

Drugs were applied through a rapid microsuperfusion system (Ogata & Tatebayashi, 1991). This system uses a fine polyethylene needle (20 μm tip internal diameter) placed near (about 50 μm) the cell. Electromagnetic valves were used to apply test solutions. This microsuperfusion system enabled us a rapid (latency, less than 0.5 s) and localized application of

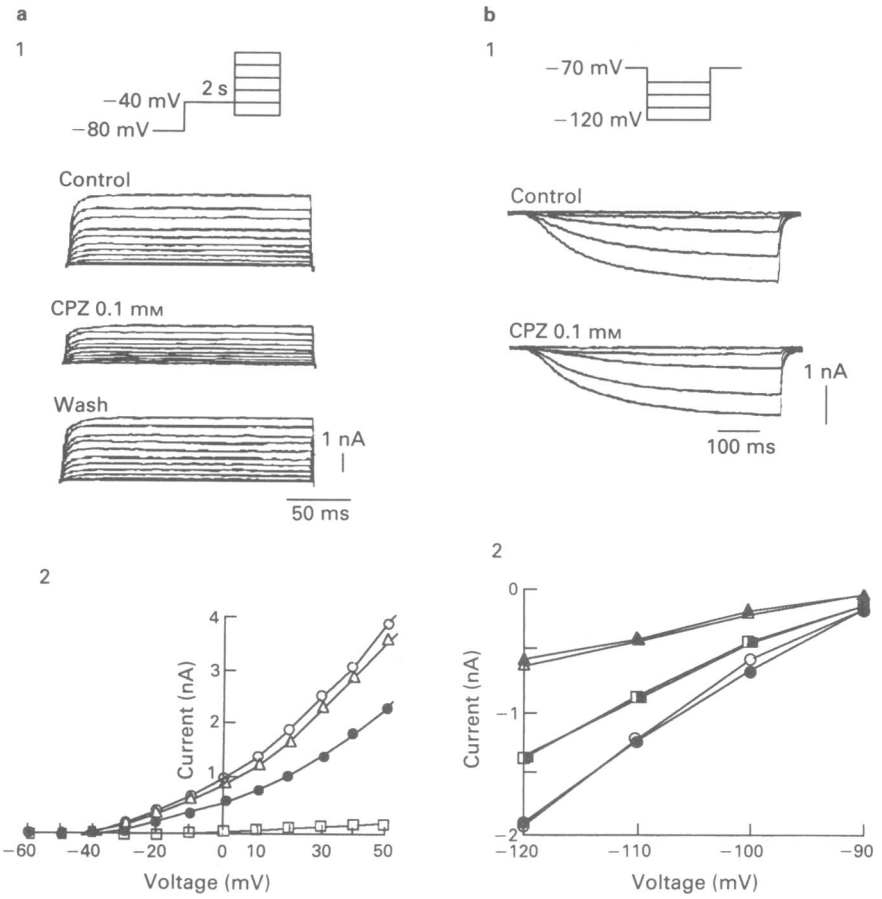

Figure 1 Effects of chlorpromazine (CPZ) on the delayed rectifier K^+ current, I_{DR}, (a) and on the inward (anomalous) rectifier K^+ current, I_{IR}, (b). Insets in a_1 and b_1 illustrate pulse protocols for selective activation of I_{DR} and I_{IR}, respectively. (a_1) From a holding potential (V_h) of − 80 mV, a prepulse to − 40 mV was given for 2 s to inactivate I_T, and immediately following the prepulse, a test pulse to potential levels positive to − 40 mV was given. Only a period of the test pulse is illustrated. (b_1) From V_h of − 70 mV, test pulses to potential levels negative to − 90 mV was given. To further eliminate a possible contamination by transient outward K^+ currents, 4-aminopyridine (4-AP) in a concentration of 1 mM was added to the medium in recordings of I_{DR}. I_{IR} in b_1 was recorded in 50 mM K^+ (by replacing NaCl with equimolar amounts of KCl). a_2 and b_2 plot current-voltage curves for I_{DR} and I_{IR}, respectively. In a_2, amplitudes of I_{IR} in the control solution (○), in the presence of 0.1 mM CPZ (●) or 25 mM TEA (□) and after washing (△) were plotted against test pulse potential. TEA was applied by replacing TMA-Cl with equimolar amounts of TEA-Cl. The current-voltage curves for I_{IR} were obtained from the same neurone in different external K^+ concentrations. (△, ▲), (□, ■) and (○, ●) were recorded in 5 mM, 25 mM and 50 mM K^+, respectively; open symbols, in the control solution; filled symbols, in the presence of 0.1 mM CPZ.

test solutions and their rapid washout. Solutions containing chlorpromazine-HCl (CPZ, Sigma) 4-aminopyridine (4-AP, Sigma) or α-dendrotoxin (α-DTX, Alomone Labs, Jerusalem, Israel) were prepared immediately before the experiment. The pH of the solution containing 4-AP was re-adjusted to 7.4 before application.

Results

K^+ currents in DRG neurones

In DRG, we identified four types of voltage-gated K^+ currents, namely, the delayed rectifier (I_{DR}), inward (anomalous) rectifier (I_{IR}), transient K^+ (refered to as 'I_T' hereafter), and Ca^{2+}-activated K^+ currents ($I_{K(Ca)}$). $I_{K(Ca)}$ was not studied, because direct interaction between $I_{K(Ca)}$ and CPZ could only be investigated with single channel recordings from excised patches of the membrane, and such an investigation has already demonstrated direct interaction between $I_{K(Ca)}$ and CPZ (McCann & Welsh, 1987).

The three types of K^+ currents were easily differentiated on the basis of their pharmacological and kinetic properties (see below). I_{DR} and I_{IR} were observed in most of the DRG cells examined. However, I_T was observed preferentially in a certain group of cells having relatively larger diameters, i.e. I_T was found in 68 cells out of 80 cells having diameters larger than 40 μm, whereas majority of the cells having smaller diameters (less than 20 μm) were devoid of any transient type of K^+ currents. Among the 40 smaller diameter cells examined, I_T was found in only 9 cells. Thus, we used cells having diameters larger than 40 μm.

Effects of CPZ on I_{DR} or I_{IR}

Figure 1 shows the effect of CPZ on I_{DR} or I_{IR}. CPZ at a concentration of 0.1 mM reversibly reduced the amplitude of I_{DR} to about 50% of the control (Figure $1a_{(1)}$). A large portion of I_{DR} was blocked by 25 mM TEA (Figure $1a_{(2)}$). The time course of I_{DR} and its voltage-dependence of activation as evaluated with current-voltage curves in Figure $1a_{(2)}$ were not affected by CPZ. Figure 1b shows the lack of effect of CPZ on I_{IR}. From a holding potential (V_h) of − 70 mV, test pulses to potential levels negative to − 90 mV induced a time-dependent inward current ($b_{(1)}$). This current, I_{IR}, was readily blocked by 1 mM Cs^+ (not illustrated), and its amplitude (consequently, conductance) was augmented by an increase in external concentration of K^+ ($b_{(2)}$). These features of I_{IR} were identical to those of the typical inward (anomalous) rectifier current in marine egg cells (Hagiwara & Yoshii, 1979). I_{IR} was not affected by a high concentration (0.1 mM) of CPZ.

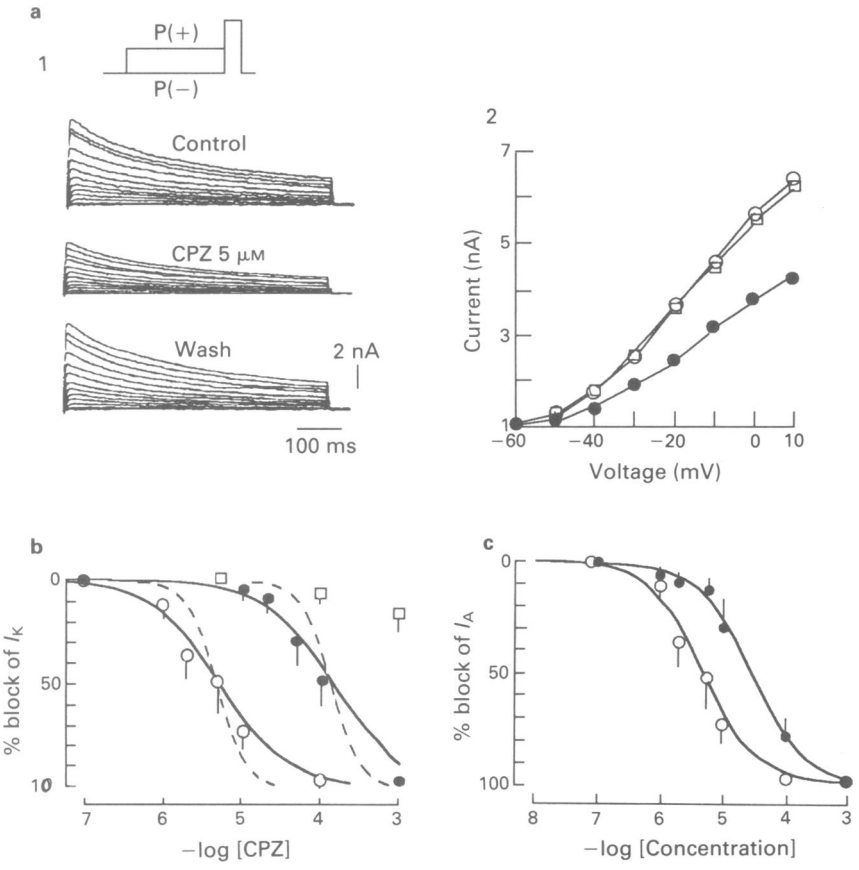

Figure 2 Effects of chlorpromazine (CPZ) on the slowly inactivating K^+ current, I_T. Inset in a_1 illustrates pulse protocol. K^+ currents were evoked by command pulses applied directly from V_h of − 80 mV ($P_{(-)}$) or applied subsequent to a prepulse to − 40 mV ($P_{(+)}$). I_T was obtained by subtracting the current produced by $P_{(+)}$ from the current produced by $P_{(-)}$. (a_2) Current-voltage curves. Amplitudes of I_T in the control solution (○), in the presence of CPZ (●) and after washing (□) were plotted against test pulse potential. (b) Concentration-response curves for CPZ block of different types of K^+ currents. Each point represents the mean ± s.e.mean from 5 experiments. Curves were drawn according to the following equation:

$$I_K = 1/\{1 + ([CPZ]/K_d)^n\}$$

where K_d is the dissociation constant of the binding reaction (4.5 μM for I_T and 120 μM for I_{DR}), [CPZ] is the concentration of CPZ, and n is 1 (solid line) or 2 (broken line). (○) I_T; (●) I_{IDR}; (□) I_{IR}. (c) Concentration-response curves for block of I_T by CPZ (○) and 4-aminopyridine (4-AP) (●). Lines were drawn according to the above equation (4.5 μM for CPZ and 31 μM for 4-AP).

Characterization of I_T

I_T (Figure 2a$_{(1)}$) could be evoked in the absence of I_{DR}. A holding potential of -40 mV inactivates I_T (see Figure 4) and appreciable amount of I_{DR} was not activated at this potential (see Figure 1). A hyperpolarizing prepulse to -120 mV from V_h of -40 mV removed the inactivation of I_T, and a step back to V_h evokes I_T in isolation. A conventional double pulse protocol showed that a hyperpolarizing prepulse longer than 2 s was required to obtain the maximal amplitude of I_T (data not illustrated). It was also possible to isolate I_T using subraction between currents obtained with or without depolarizing prepulse to -40 mV. To exclude further a possible contamination by I_{DR}, recordings of I_T were carried out in the presence of 5 mM TEA which had no detectable effect on I_T.

I_T activated rapidly at potentials positive to -60 mV but shows only slow (inactivation time constant, τ_{intact}, an order of seconds, see Figure 3) and incomplete inactivation, I_T was inhibited by low concentrations of 4-AP (2–100 μM, see Figure 2c) and α-DTX (44 ± 8% inhibition by 10 nM, $n = 5$). This current was noticeably different from the 'classical' transient K^+ current (collectively called 'I_A' or 'A-current') which rapidly inactivates ($\tau_{inact.}$ less than 100 ms) and is generally insensitive to 4-AP at concentrations below 100 μM (Rogawski, 1985). A similar slowly inactivating K^+ current has been reported in a subpopulation (A-cell) of rat nodose ganglion (visceral afferent) neurones (Stansfeld *et al.*, 1986). This nodose K^+ current was also inhibited by low concentrations of 4-AP (1–30 μM) and DTX (3–10 nM). Thus, I_T recorded in DRG may be analogous to the slowly inactivating K^+ current recorded in A-cells of nodose ganglia. However, there are differences in channel kinetics (e.g., an activation threshold of the nodose slowly inactivating K^+ current was about -70 mV) which may be due to differences in cell type (dorsal root vs nodose), preparation (cultured cell vs freshly-dissociated cell), or recording conditions (patch-clamp vs microelectrode voltage-clamp).

Effects of CPZ on I_T

As shown in Figure 2a$_{(1)}$, CPZ at a concentration of 5 μM reduced the amplitude of I_T to 60% of the control. The effect was completely reversible. The time course of the current was not affected by CPZ. Figure 2a$_{(2)}$ shows the current-voltage curves for I_T obtained in the presence or absence of CPZ. The curve was proportionally reduced by CPZ at each potential level. The effects of CPZ on three types of K^+ currents are summarized in Figure 2b. The concentration required for 50% block (dissociation constant, K_d) was 120 μM for I_{DR} and 4.5 μM for I_T, respectively. Figure 2c shows the concentration-response curves for the block of I_T by CPZ and 4-AP. K_d for 4-AP was 31 μM. In each case, the concentration-response curves were fitted by a 1:1 stoichiometry.

Effects of CPZ and 4-AP on the decay time-course of I_T

It is well-established that 4-AP has a preferential blocking action on the transient K^+ currents (Gustafsson *et al.*, 1982; Thompson, 1982), and thus this agent has been utilized as a pharmacological tool in studies of K^+ channels. Therefore, we compared the action of CPZ on I_T with the action of 4-AP. Figure 3a illustrates changes in current envelope during applications of CPZ and 4-AP. The currents in the presence of drugs (filled arrows) were normalized to the currents measured before application of drugs (open arrows). The current in the presence of CPZ completely overlapped with the control current, whereas the current in the presence of 4-AP had a faster decay phase as compared with the control current. The decay phase of I_T in the control solution was usually described by three exponential components (b). The three time constants of 70, 405 and 2067 ms in the control solution were reduced by 4-AP to 53, 240 and

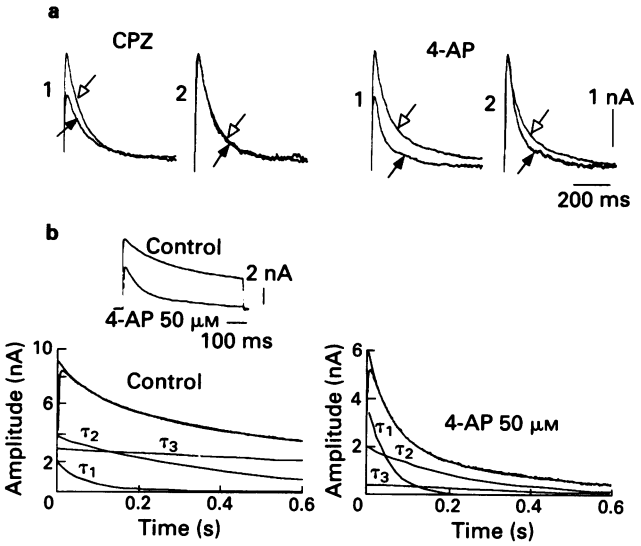

Figure 3 Effects of chlorpromazine (CPZ) and 4-aminopyridine (4-AP) on the decay phase of I_T. (a) I_T was recorded before and during applications of drugs. The currents in the presence of CPZ or 4-AP (filled arrows in traces labelled 1) were scaled to the currents measured before application of drugs (open arrows in traces labelled '1') and shown superimposed (traces labelled '2'). (b) The decay phase of I_T in the control solution or in the presence of 4-AP was fitted by exponential functions. τ_1, τ_2 and τ_3 in Control, 70, 405 and 2067 ms; τ_1, τ_2 and τ_3 in 4-AP 50 μM, 53, 240 and 450 ms. For further explanation, see text.

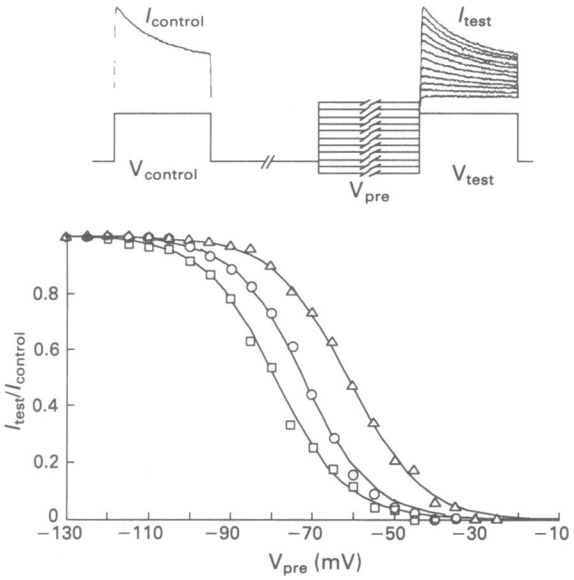

Figure 4 Steady-state inactivation curves for I_T measured in the control solution (○) and in the presence of 5 μM chlorpromazine (□) or 50 μM 4-aminopyridine (△). Inset illustrates pulse protocol. Two identical step depolarizations to -40 mV for 0.6 s were applied 20 s before ($V_{control}$) and immediately subsequent (V_{test}) to the prepulse (V_{pre}) from V_h of -120 mV. The potential level of V_{pre} was changed from -130 mV to -25 mV in 5 mV steps. The peak amplitude of I_{test} was normalized to that of $I_{control}$ and plotted as a function of V_{pre}. The smooth curves were drawn according to the equation:

$$I_{test}/I_{control} = 1/(1 + \exp[(V_{pre} - V_i)/\kappa])$$

where V_i is the prepulse potential when I_T is one-half maximal, and κ is the slope factor.

450 ms, respectively. Such an acceleration of the decay phase by 4-AP was observed in all of the 10 cells examined, and the slower phase of the decay was more sensitive to 4-AP.

Effects of CPZ and 4-AP on the steady-state inactivation of I_T

Figure 4 shows the steady-state inactivation curves for I_T measured in the control solution and in the presence of CPZ or 4-AP. The steady-state inactivation curve in the control solution (circles) had $V_{\frac{1}{2}}$ of -74 mV. CPZ and 4-AP had opposite effects on the steady-state inactivation characteristics. The steady-state inactivation curve was shifted 5 mV (5.5 ± 1.2 mV, $n = 4$) in the negative direction by CPZ (squares), whereas it was shifted by 13 mV (13.1 ± 2.1 mV, $n = 4$) in the positive direction by 4-AP (triangles). The slope factor was not affected by either drug. These observations strongly suggest different modulatory actions of these drugs on the inactivation process of I_T.

Effects of CPZ and 4-AP on the recovery process of I_T

I_T fully recovered from inactivation after a recovery period (ΔT) of 6400 ms in the control solution (Figure 5a, open circles). When the medium contained CPZ, the recovery process was considerably delayed at relatively shorter ΔT (squares). However, an overall period required for recovery of I_T was similar in either the control solution or in the presence of CPZ. The recovery process of I_T was fitted by exponential functions (Figure 5b). In the control solution, the recovery proceeded as the sum of two exponentials having time constants of 260 and 1400 ms. In the presence of CPZ, a third exponential function with a time constant of 800 ms was required to give a best fit in addition to the two time constants used for the control time course. Essentially similar results were obtained in two additional experiments.

In contrast to CPZ, 4-AP increased the peak amplitude of I_T evoked subsequently to the prepulse (Figure 5a, triangles). Such a paradoxical increase of I_T by 4-AP reached a maximum at ΔT of 160 ms and declined very slowly with a time constant of 7.2 ± 1.7 s ($n = 5$). The increase was no longer observed at ΔT of 20 s. These observations were reproducible in all of the 4 cells examined. These results indicate that in the presence of 4-AP, use-dependent unblocking of I_T is induced by a preceding activation of I_T.

Effects of CPZ and 4-AP on the frequency-dependent modulation of I_T

A striking difference between CPZ and 4-AP was noted in their actions on frequency modulation of I_T. Figure 6a shows typical examples of current traces recorded during ten repet-

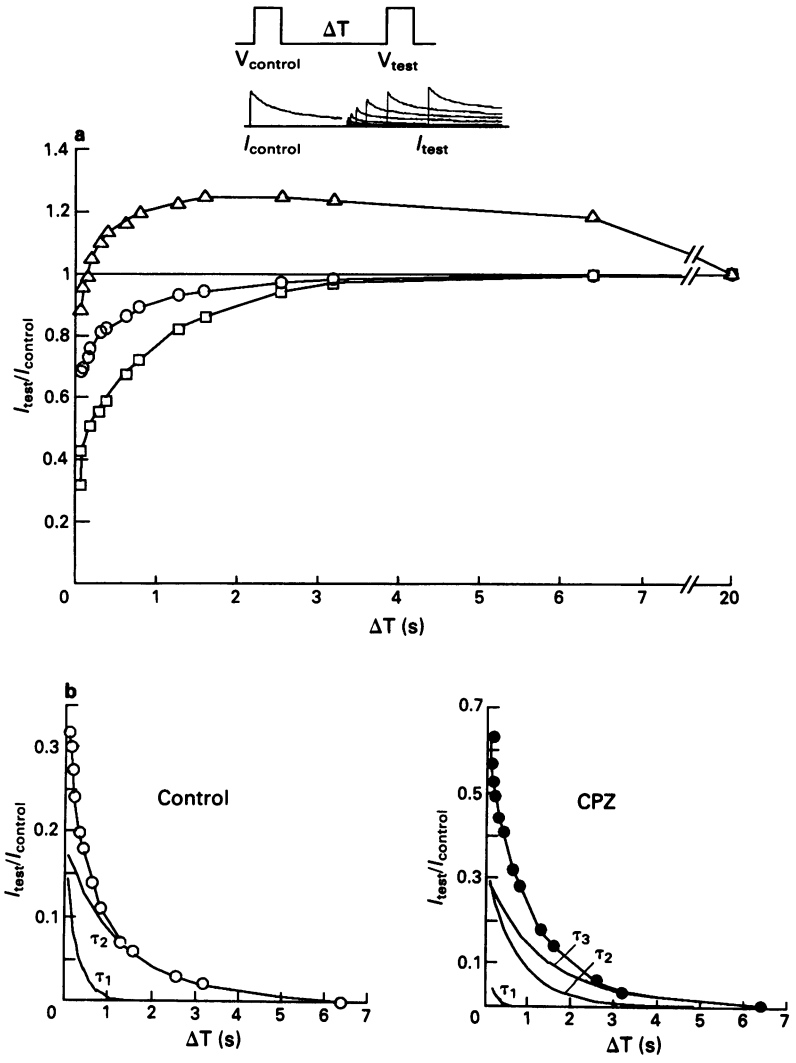

Figure 5 Effects of chlorpromazine (CPZ) and 4-aminopyridine (4-AP) on the recovery process of I_T from inactivation. (a) Two identical test pulses ($V_{control}$ and V_{test}) to -30 mV for 0.5 s were given with varying inter-pulse interval (ΔT) from V_h of -90 mV. The amplitude of I_{test} normalized to that of $I_{control}$ were plotted against ΔT in the control solution (○) and in the presence of 5 μM CPZ (□) or 50 μM 4-AP (△). (b) The value, $1\text{-}I_{test}/I_{control}$, was plotted against ΔT. The time course of $(1\text{-}I_{test}/I_{control})$ was fitted by exponentials. τ_1 and τ_2 in Control, 260 and 1400 ms; τ_1, τ_2 and τ_3 in CPZ, 260, 800 and 1400 ms. For further explanation, see text.

itive test pulses applied with inter-pulse interval (ΔT) of 100 ms, in the control solution and in the presence of CPZ or 4-AP. The peak current amplitude for each pulse in the train was normalized to that of the first pulse (I_T) and plotted against the pulse number (Figure 6b).

In the control solution, the peak amplitude of I_T was progressively decreased during ten repetitive test pulses. The decrease was smaller when ΔT became more prolonged from 80 ms to 3200 ms, and with ΔT of 6400 ms, there was no longer any detectable decrease in I_T amplitude throughout the ten test pulses. When the same pulse protocol was examined in the presence of CPZ, I_T was successively attenuated as in the case of the control solution. The degree of the attenuation of I_T induced by test pulses having comparable ΔT was always greater when compared with the decrease in the control solution.

In the presence of 4-AP, I_T was rather increased in most cases. The maximal increase was obtained following the third pulse. It should be noted that, in the presence of 4-AP, the I_T evoked subsequently to a 5 s pause after the ten repetitive pulses (I_{11} in Figure 6a) was much larger than I_T evoked during ten repetitive pulses. Thus, it is suggested that, during repetitive pulses in the presence of 4-AP, there was an interplay between the inactivation process resultant from the preceding activation and the increased channel availability due to unbinding of 4-AP molecules from the channel. At the time of I_{11} activation, the inactivation process could be recovered while the increased channel availability is still present (see Figure 5a). In contrast to the case of 4-AP, the peak amplitude of I_{11} in the control solution or in the presence of CPZ was identical to that of I_1.

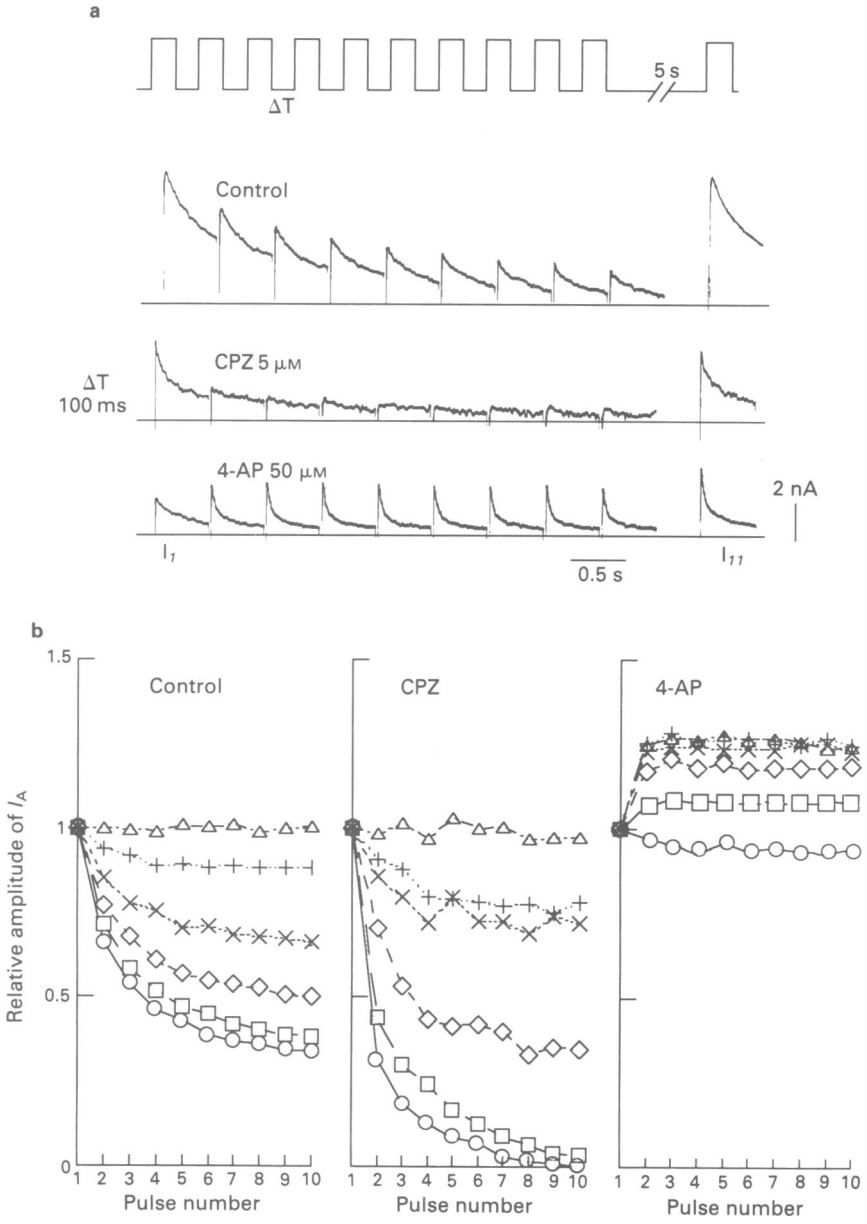

Figure 6 Effects of chlorpromazine (CPZ) and 4-aminopyridine (4-AP) on the frequency modulation of I_T. (a) Inset illustrates pulse protocol. From V_h of − 90 mV, ten consecutive test pulses to − 30 mV for 0.5 s were applied with varying inter-pulse interval (ΔT), and an additional test pulse (I_{11}) was given after 5 s pause at V_h. Typical examples of current traces in the control solution (upper) and in the presence of CPZ (middle) or 4-AP (lower) evoked with ΔT of 100 ms are illustrated. (b) The peak current amplitude for each pulse in the train was normalized to that of the first pulse (I_1) and plotted against the pulse number. Left, in the control solution: middle, in the presence of 5 μM CPZ: right, in the presence of 50 μM 4-AP: (○) 80 ms; (□) 160 ms; (◇) 640 ms; (×) 1600 ms; (+) 3200 ms and (△) 6400 ms.

Discussion

The present results demonstrate that CPZ blocks I_T (K_d, 4.5 μM) in preference to two other voltage-gated K^+ currents, I_{DR} and I_{IR}. Although I_{DR} was weakly sensitive (K_d, 120 μM) to CPZ, I_{IR} was practically insensitive to CPZ. It has been shown that $I_{K(Ca)}$ is depressed by 1–2 μM CPZ in rat hippocampal neurones (Dinan *et al.*, 1987) and in dog airway smooth muscle (McCann & Welsh, 1987). Thus, it appears that CPZ has a preferential depressant action on particular types of voltage-gated K^+ channels.

It is difficult to equate concentrations of CPZ in the experimental perfusion system with those in the serum or cerebrospinal fluid of humans, due to various factors that influence the tissue concentrations of CPZ such as the prolonged trapping of CPZ in a closed enterohepatic circuit or strong binding of CPZ to plasma or tissue proteins (Dingell *et al.*, 1961; Baldessarini, 1990). Nevertheless, concentrations used in this study appear to be well within the range of concentrations attained in schizophrenic patients treated with this drug (9–60 μM, Hugang & Ruskin, 1964; 3 μM, Surawicz & Lasseter, 1970).

Neuroleptics also inhibit calmodulin; however, an involvement of calmodulin inhibition appears to be unlikely, because N- (6-aminohexyl)-5-chloro-1-naphthalenesulphonamide (W-7), a calmodulin antagonist (Hidaka *et al.*, 1981), did not influence I_T. In addition, the blocking action of CPZ was observed when we used fluoride as an intracellular impermeant anion which disrupts cellular metabolism by chelating Ca^{2+} ions (data not shown). A similar conclusion has been drawn in CPZ-induced blocks of neuronal Na^+ (Ogata *et al.*, 1990) and Ca^{2+} (Ogata & Narahashi, 1990) channels or of $I_{K(Ca)}$ in airway smooth muscle (McCann & Welsh, 1987).

It is well known that neuroleptic drugs lower the seizure threshold and induce discharge patterns in the EEG that are associated with epileptic seizure disorders, and therefore, neuroleptic agents should be used with extreme caution in epileptic patients and in patients undergoing withdrawal from central depressants such as alcohol or barbiturates (Baldessarini, 1990). There has been no rational explanation for this side effect of the neuroleptic drugs.

$I_{K(Ca)}$ is considered to underlie the long-lasting after-hyperpolarization (AHP) observed in several types of neurones which acts as a powerful neuronal brake (Alger & Nicoll, 1980). I_T which is activated in the subthreshold range of cellular excitation is thought to reduce the excitatory effect of depolarizing membrane currents in a time-dependent manner. In addition, a recent investigation suggested that an inactivating type of K^+ current may also be involved in the generation of the AHP (Ruppersberg *et al.*, 1991). Thus, CPZ may affect cellular excitability through inhibition of $I_{K(Ca)}$ and I_T.

I_T had properties similar to those of the slowly inactivating K^+ current found in nodose A-cells (Stansfeld *et al.*, 1986), e.g., these two currents are highly sensitive to 4-AP and DTX whereas they are relatively insensitive to TEA. A comparable K^+ current has been shown also in rat hippocampal neurones (I_D, Storm, 1988). The slowly inactivating K^+ currents in nodose and hippocampal neurones have been shown to play an important role in spike adaptation, such that inhibition of these currents induces striking repetitive firing (Standfeld *et al.*, 1986; Storm, 1988). In addition, it has been shown that 4-AP induces a similar repetitive firing in rat dorsal root (but not ventral root) fibres (Baker *et al.*, 1985). Thus, there is a possibility that the slowly inactivating K^+ current is a major target underlying the proconvulsant side effect of CPZ.

The blocking action of CPZ on I_T was strikingly different from that of 4-AP which blocks inactivating K^+ currents in a variety of tissues (Gustafsson *et al.*, 1982; Thompson, 1982; Stansfeld *et al.*, 1986; Storm, 1988). The action of 4-AP on I_T was characterized by a prominent acceleration of the decay phase of I_T (see Figure 3), whereas such a kinetic effect was not observed in the case of CPZ. Two possible explanations might be considered for the acceleration of the I_T decay. One possibility is that 4-AP binds more strongly to the open (activated) channel state than to the closed state ('open-channel block'). Indeed, the open-channel block of the inactivating K^+ current by 4-AP has been reported in molluscan neurones (Thompson, 1982) or in neuroblastoma x glioma hybrid cells (Robbins & Sim, 1990). The other possibility is that 4-AP preferentially blocks the slower component of I_T. I_T may be composed of multiple components, since the decay phase of I_T was fitted best, by three exponential components (Figure 3b). Two types of transient K^+ currents have been reported in cardiac muscle cells (Coraboeuf & Carmeliet, 1982; Escande *et al.*, 1987). These possibilities could be distinguished by use of single channel recordings.

An additional striking difference between actions of CPZ and 4-AP was the contrasting effects of these drugs on the steady-state inactivation characteristics (Figure 4). The positive shift of the steady-state inactivation curve by 4-AP suggests a possible voltage-dependent unblocking action of this drug as has been suggested for the A-currents in molluscan neurones (Thompson, 1982) and in dog ventricular muscle (Simurda *et al.*, 1989). This notion is further strengthened by the use-dependent facilitation of I_T in the presence of 4-AP (Figures 5 and 6).

In contrast to 4-AP, CPZ shifted the steady-state inactivation curve in the negative direction, i.e., the block was increased by membrane depolarization (Figure 4). This effect of CPZ was observed also in blocks of Na^+ or Ca^{2+} channels and attributed to the greater affinity of CPZ for the inactivated state of channels than for the resting channels (Ogata *et al.*, 1990) consistent with the 'modulated receptor model' (Hille, 1977; Hondeghem & Katzung, 1977). The slowed recovery time course (Figure 5) and the use-dependent depression of I_T (Figure 6) in the presence of CPZ may be due to slow repriming of CPZ-bound channels from inactivation.

In conclusion, CPZ causes preferential block of I_T in neurones of the rat dorsal root ganglia. Although its channel selectivity was low as compared with the channel selectivity of 4-AP, the concentration of CPZ required to produce a comparable inhibition of I_T was much lower than the concentration of 4-AP. Since CPZ also depresses the neuronal Na^+ (Ogata *et al.*, 1990) and Ca^{2+} (Ogata & Narashi, 1990) channels, its overall effect on the neuronal activity may be diverse depending on the types of neurone. These direct membrane effects of CPZ appear to be related to some aspects of clinical efficacy of this drug.

We thank Professor Hirosi Kuriyama for pertinent support and advice. This study was supported by the Japanese Ministry of Education (Scientific Research 63570096).

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(Received February 16, 1993
Revised April 6, 1993
Accepted April 8, 1993)