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The effects of neuroleptic and tricyclic compounds on BK_{Ca} channel activity in rat isolated cortical neurones

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1 The actions of several neuroleptic and tricyclic compounds were examined on the large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel present in neurones isolated from the rat motor cortex.

2 Classical neuroleptic compounds including chlorpromazine and haloperidol applied to the intracellular surface of inside-out patches produced a concentration-dependent reduction in BK_{Ca} channel activity. Similar effects were observed when these compounds were applied to the extracellular surface of outside-out patches.

3 In contrast, the atypical neuroleptic compounds clozapine and sulpiride did not affect BK_{Ca} channel activity (100 nM – 1 mM) in either inside-out or outside-out patches, while 10 μM pimozide produced 73% of the inhibition produced by 10 μM chlorpromazine.

4 BK_{Ca} channel activity was also unaffected by application of structurally related tricyclic compounds including the anti-cholinesterase tacrine and the anti-epileptic carbamazepine. The tricyclic antidepressant drug amitriptyline was found to inhibit BK_{Ca} channel activity but was much less effective than the classical neuroleptic compounds.

5 It is concluded that compounds belonging to the classical neuroleptic group of drugs inhibit BK_{Ca} channel activity in the rat motor cortex in a structurally-specific manner. This observation may be of clinical significance as it may contribute to some of the side effects associated with classical neuroleptic drug therapy.

Keywords: Neuroleptics; BK_{Ca} channel; tricyclic compounds; cerebral cortex

Introduction

The class of drugs known as the neuroleptics are a chemically diverse group of compounds which are used therapeutically in order to treat various psychotic disorders including schizophrenia. Although these compounds are believed to achieve their antipsychotic effects via the antagonism of dopamine receptors (Creese *et al*., 1976), they are known to exert a wide range of other pharmacological actions including the ability to inhibit several voltage and ligand gated ion channels (e.g. Ogata & Narahashi, 1989; Ogata *et al*., 1990; Revah *et al*., 1990) including K^+ channels (Müller *et al*., 1991; Ogata & Tatebayashi, 1993).

At the single channel level, various phenothiazine and butyrophenone neuroleptics have been shown to inhibit the activity of the large conductance, calcium-activated K^+ (BK_{Ca}) channel present in canine airway smooth muscle in a voltage-dependent manner, but only when applied to the cytoplasmic surface of excised patches (McCann & Welsh, 1987). In light of this previous study, the principal aim of the present one was to investigate the actions of neuroleptic compounds upon BK_{Ca} channel activity in the rat motor cortex with a view to comparing BK_{Ca} channel pharmacology in the two tissues.

Furthermore, Wooltorton & Mathie (1993) have demonstrated that the delayed rectifier K^+ current present in isolated sympathetic neurones is inhibited by neuroleptic compounds such as chlorpromazine in a structurally-specific manner. Therefore the present study was extended to investigate the effects of other tricyclic compounds upon BK_{Ca} channel activity in order to determine whether similar structural constraints pertain to the action of neuroleptic compounds upon cortical BK_{Ca} channel activity. Some of these results have been communicated to the Physiological Society (Lee *et al*., 1995a).

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Isolation of cortical neurones

Coronal slices (350 μm – 400 μm thick) were cut from brains of male Sprague-Dawley rats (100 – 300 g weight) with a vibratome. Portions of the motor cortex were dissected from the coronal sections and were incubated in pronase E (1.5 mg ml^{-1}) in ACSF gassed with 95% O_2 and 5% CO_2 at 30°C for 10 min and then triturated, by use of flame-polished Pasteur pipettes of decreasing internal diameter. The dispersed cells were plated onto tissue culture dishes and left for 30 min – 1 h before use, by which time the cells had adhered to the plates.

Electrophysiological recording and data analysis

All experiments were performed with either the inside-out or the outside-out configuration of the patch-clamp technique. Recording electrodes were pulled from borosilicate glass capillaries and when filled with electrolyte had resistances of 8 – 12 MΩ. Single channel events were detected by a List EPC-7 amplifier and were stored on digital audio tape (DAT; DTC-1000ES) for subsequent replay into a Gould 2200 chart recorder. The potential across the membrane is described following the usual sign convention for membrane potential (i.e. inside negative). Single channel current analysis was accomplished off-line by use of the analysis programme PAT 6.2. as implemented on an Elonex 386 SX 160 microcomputer (Dempster, 1993). Data segments between 30 and 90 s duration were replayed at the recorded speed, filtered at 1.0 kHz with an 8-pole Bessel filter and digitized at 5.0 kHz with a Data Translation 2801A interface. The average channel activity (N_fP_o) where N_f is the number of functional channels in the patch and P_o is the open state probability, was determined by construction of an all-points current amplitude distribution. To calculate N_fP_o, the total time spent at each current level was expressed as a proportion of the total time recorded by fitting gaussian distributions to the peaks obtained (Lee *et al*., 1994). Changes in N_fP_o as a result of drug effects are expressed as a percentage of control. The effects of increasing concentrations

of inhibitor ([I]) upon channel activity were fitted by non linear regression to the following equation:

$$\% \text{ inhibition of control activity} = 100/(1 + (IC_{50}/[I])^{n_H})$$

where IC_{50} is the half maximal inhibitory concentration and n_H is the Hill coefficient. In order to determine statistically whether the neuroleptics and related compounds affected BK_{Ca} channel activity in a voltage-dependent manner, the percentage inhibition produced by a given concentration of drug was assessed for significant differences at different holding potentials by use of one way analysis of variance (ANOVA). All data in the text, tables and figures are presented as mean values ± s.e.mean unless otherwise stated. Concentration-inhibition curves were assessed for statistical differences by fitting the curves simultaneously (by use of the NAG library routine E04FDF as implemented on the Cambridge IBM 3081/3084) and assessing the residual sum of squares when one of the parameters was constrained to be the same for both curves by calculating the *F*-statistic (Rodbard, 1974);

$$F = [(SS_2 - SS_1)/(df_2 - df_1)]/(SS_1/(df_1)$$

where SS_2 is the sum of squares when a parameter was constrained to be the same for both curves (df_2 difference in degrees of freedom, i.e. $df_2 = 1$ when only a single parameter) and SS_1 is the sum of squares when the parameters are left to float freely with their respective curves (df_1 degrees of freedom).

Methods

Drugs and solutions

The artificial cerebrospinal fluid (ACSF) for rat cortical slices contained (in mM): NaCl 128.0, KCl 5.0, NaH_2PO_4 1.2, $CaCl_2$ 2.4, $MgCl_2$ 1.3, $NaHCO_3$ 26.0, D-glucose 10.0 (pH 7.4). Before single channel recording, the cells were washed thoroughly with normal external saline which consisted of (in mM): NaCl 135.0, KCl 5.0, $CaCl_2$ 1.0, $MgCl_2$ 1.0, HEPES 10.0 (solution A). For inside-out patch studies the pipette contained (in mM): KCl 140.0, $CaCl_2$ 1.0, $MgCl_2$ 1.0, HEPES 10.0 (solution B) whilst the bath contained either (in mM): KC1 140.0, $CaCl_2$ 0.8, $MgCl_2$ 1.0, EGTA 1.0, HEPES 10.0 (solution C) which resulted in free Ca^{2+} and Mg^{2+} concentrations of 1.0 μM and 1 mM, respectively, or (in mM): KCl 140.0, $CaCl_2$ 1.0, $MgCl_2$ 1.0, EDTA 1.0, HEPES 10.0 (solution D) which resulted in free Ca^{2+} and Mg^{2+} concentrations of 10.0 μM and 1 mM, respectively. For outside-out patch recordings, the pipette contained solution D whilst the bath contained solution A. Solutions A–D and the ACSF were adjusted to pH 7.2 and 7.4, respectively, with either NaOH or KOH. The concentrations of free divalent ions present in each solution were calculated by use of the binding constants as described by Fabiato & Fabiato (1979).

All drugs were obtained from Sigma (Poole, Dorset, U.K.) with the exception of quinpirole which was a gift from Dr P.R. Boden (Parke Davis Neuroscience Research Centre, Cambridge, U.K.). Amitriptyline, dopamine, fluphenazine and tacrine (all HCl salts) were made up as 100 mM stock solutions, in distilled H_2O, whilst chlorpromazine, haloperidol, carbamazepine, clozapine, pimozide, quinpirole and sulpiride were made up as 0.1–1.0 M stock solutions in dimethylsulphoxide (DMSO). All stock solutions were made freshly on the day of the experiment. DMSO (1%) was without effect upon BK_{Ca} channel activity ($n = 4$). Experiments were performed at room temperature (20–24°C).

Results

In single channel recordings made from excised membrane patches isolated from acutely dispersed cortical neurones at least one BK_{Ca} channel with mean unitary conductance of 245 ± 6pS ($n = 6$, measured in symmetrical 140 mM KCl) was found in all patches investigated ($n = 61$). BK_{Ca} channel activity increased when the intracellular Ca^{2+} concentration was raised from 0.1 μM to 1–10 μM, by depolarization or by the addition of 1–5 mM ATP to the intracellular surface (Lee *et al*., 1995b).

Effects of classical neuroleptic compounds

In order to assess the effects of so called 'classical neuroleptic compounds' upon BK_{Ca} channel activity, the concentration of free Ca^{2+} bathing the channels intracellular surface was raised to 10 μM in order to induce BK_{Ca} channel P_o values between 0.5 and 0.8 over the voltage range tested (±40 mV). Under these conditions, application of chlorpromazine to the intra-

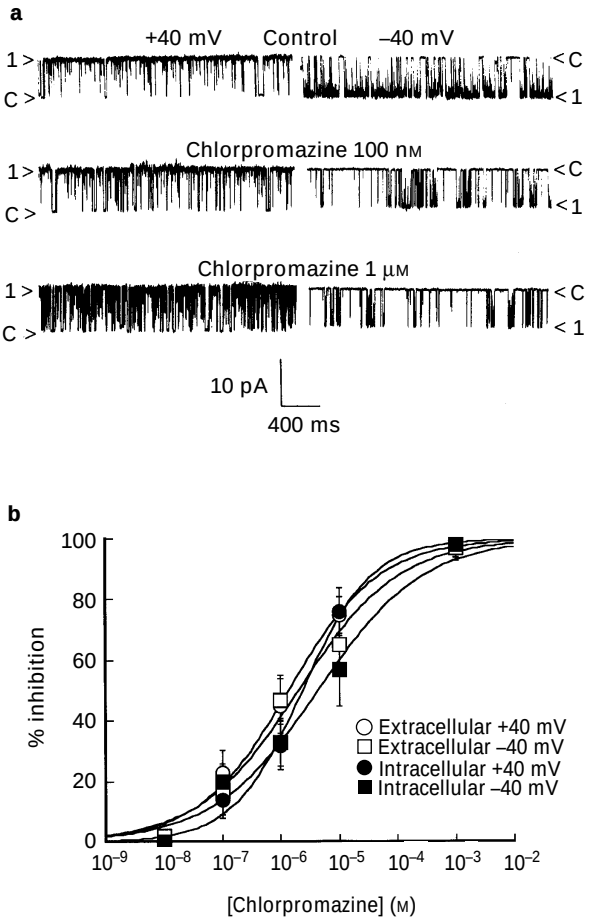

Figure 1 The effect of the neuroleptic, chlorpromazine upon BK_{Ca} channel activity. (a) Single BK_{Ca} channel current recordings (inside-out configuration) illustrating the effect of 100 nM and 1 μM chlorpromazine upon channel P_o at +40mV and −40 mV. Recordings were made in the presence of symmetrical 140 mM KCl (i.e. solutions B and D). The P_o values were as follows. Control +40 mV, 0.77; −40 mV 0.52; 100 nM chlorpromazine +40 mV, 0.62; −40 mV, 0.39; 1 μM chlorpromazine +40 mV, 0.29; −40 mV, 0.21. The symbol 'C>' refers to the closed state of the channel in this and subsequent figures. (b) The effect of increasing concentrations of chlorpromazine upon BK_{Ca} channel activity. The data were obtained at membrane holding potentials of +40 mV and −40 mV with application of the drug at the intracellular or extracellular surface of excised patches. Channel activity in the presence of chlorpromazine is expressed as a fraction of the N_fP_o measured before the introduction of the drug. Each point (10^{-8} to 10^{-3} M) is the mean of between 3 and 6 separate determinations with the s.e.mean indicated by the vertical lines. Where no vertical lines are visible, the error was within the size of the symbol. The values for the IC_{50} (half-maximal inhibitory concentration) were obtained by fitting the data by non-linear regression to a modified Hill equation with KaleidaGraph (Abelbeck Software, U.S.A.).

cellular surface of inside-out patches reduced channel activity in a concentration-dependent-manner (Figure 1a). Chlorpromazine inhibited BK_{Ca} channel activity with IC_{50} values of 1.3 ± 0.5 μM and 2.0 ± 1.1 μM at $+40$ mV and -40 mV, respectively (Figure 1b) with corresponding Hill coefficients of 0.82 and 0.75, respectively. These IC_{50} values are not significantly different and therefore suggest that under the present experimental conditions, chlorpromazine inhibits BK_{Ca} channel activity in a voltage-independent manner. Concentrations of chlorpromazine above 10 μM were associated with a voltage-independent and reversible reduction in BK_{Ca} channel current amplitude, characterized by the flickery nature of

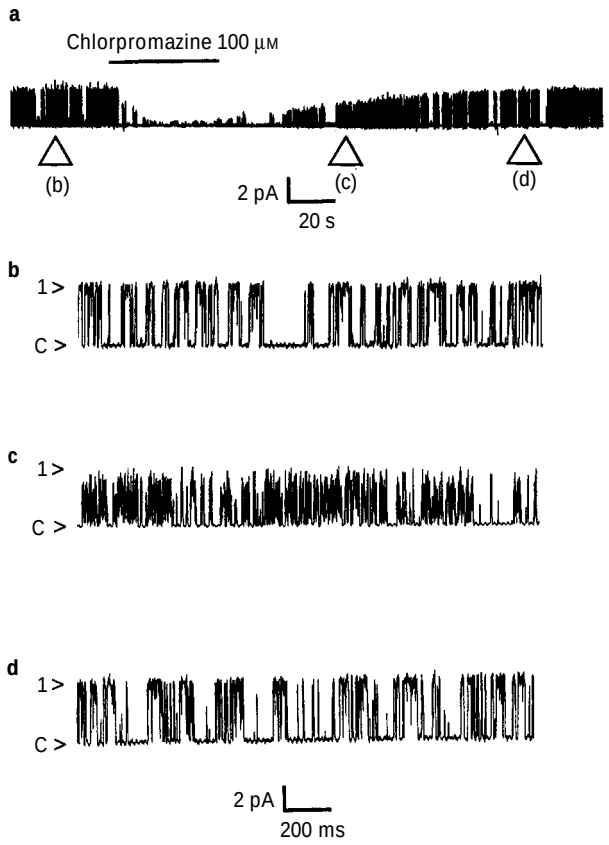

Figure 2 (a) Continuous recording showing the effect of 100 μM chlorpromazine upon BK_{Ca} channel activity in an inside-out patch at $+20$ mV in the presence of symmetrical 140 mM KCl. Application of chlorpromazine caused a reduction in BK_{Ca} channel activity in addition to a decrease in channel unitary amplitude. Both effects were reversible on washout. The letters beneath this trace indicate the time periods shown on an expanded timescale in parts (b) to (d). The P_o values were as follows: (b) 0.45; (c) 0.34 and (d) 0.52.

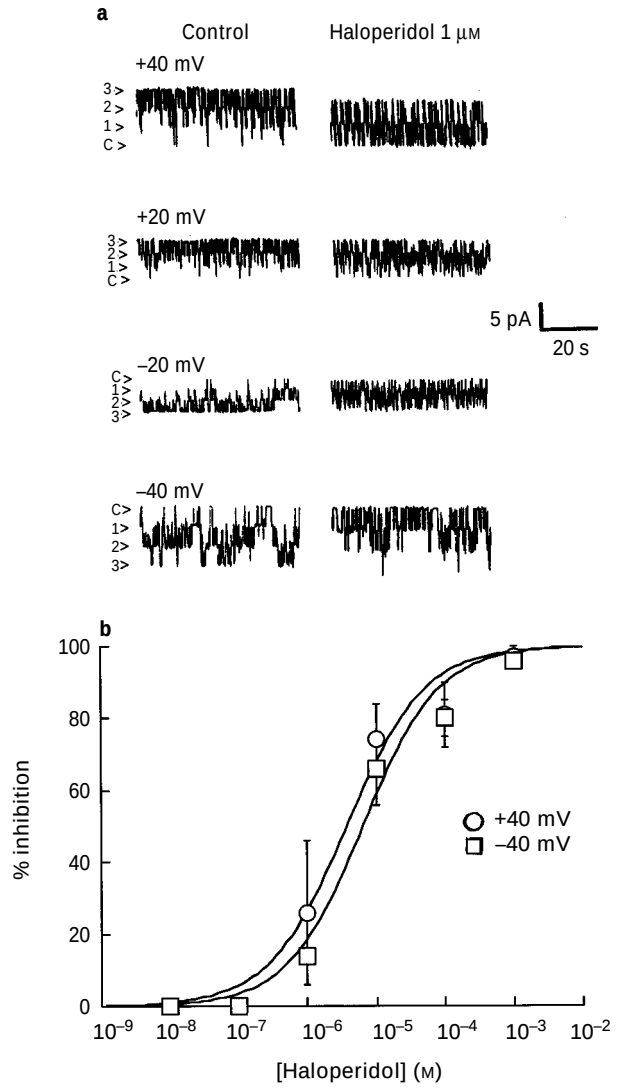

Figure 3 The effect of haloperidol upon BK_{Ca} channel activity (a) BK_{Ca} channel current recordings illustrating the effect of 1 μM haloperidol upon channel activity at various membrane holding potentials (inside-out configuration). Recordings were made in the presence of symmetrical 140 mM KCl. The N_fP_o values were as follows: control $+40$ mV, 2.11; $+20$ mV 2.02; -20 mV 1.75; -40 mV 1.32; 1 μM haloperidol $+40$ mV, 1.45; $+20$ mV, 1.21; -20 mV, 1.12; -40 mV 0.95. (b) The effect of increasing concentrations of haloperidol upon BK_{Ca} channel activity at membrane holding potentials of $+40$ mV and -40 mV. Each point (10^{-8} to 10^{-3} M) is the mean of between 3 and 6 separate determinations with the s.e.mean indicated by vertical lines. Where no vertical lines are visible, the error was within the size of the symbol. Data were fitted as described in Figure 1b.

Table 1 Summary of the effects of the neuroleptics and structurally related compounds upon the activity of the BK_{Ca} channel present in neurones acutely dissociated from the rat motor cortex

Compound class	*Name*	*% inhibition* $+20$ *mV*	-20 mV
Phenothiazine neuroleptic	Chlorpromazine 10 μM	75.3 ± 4.3 ($n=5$)	73.2 ± 3.5 ($n=4$)
	Fluphenazine 10 μM	69.2 ± 3.2 ($n=3$)	67.2 ± 2.2 ($n=3$)
	Thioridazine 10 μM	72.3 ± 3.2 ($n=3$)	71.2 ± 2.1 ($n=3$)
Butyrophenone neuroleptic	Haloperidol 10 μM	72.2 ± 3.2 ($n=4$)	73.2 ± 2.2 ($n=4$)
Dibenzazepine neuroleptic	Clozapine 10 μM	N/E ($n=3$)	N/E ($n=3$)
Diphenylbutyl-piperazine neuroleptic	Pimozide 10 μM	55.3 ± 3.2 ($n=3$)	57.2 ± 3.2 ($n=3$)
Benzamide neuroleptic	(±)-Sulpiride 1.0 μM	N/E ($n=3$)	N/E ($n=3$)
Tricyclic antidepressant	Amitriptyline 10 μM	22.3 ± 1.4 ($n=3$)	21.3 (19.7, 22.8; $n=2$)
Dibenzazepine antiepileptic	Carbamazepine 100 μM	N/E ($n=4$)	N/E ($n=4$)
Tricyclic anticholinesterase	Tacrine 100 μM	N/E ($n=3$)	N/E ($n=3$)

Data were obtained from excised inside-out patches in the presence of symmetrical 140 mM KCl and 10 μM free Ca^{2+}. Values presented are the mean ± s.e.mean N/E, no effect.

channel openings which were unresolvable due to the limited frequency response of the patch clamp amplifier (Figure 2). In addition to chlorpromazine, two related phenothiazine compounds, fluphenazine and thioridazine, were tested on the cortical BK_{Ca} channel. As summarized in Table 1, both compounds inhibited channel activity with a potency similar to that of chlorpromazine and in a manner which was also independent of membrane voltage.

Another class of compounds which exhibit classical neuroleptic activity but are structurally different from the phenothiazines are the butyrophenones. Therefore the archetypal butyrophenone neuroleptic drug haloperidol was tested. As found with the phenothiazines, a concentration-dependent reduction in BK_{Ca} channel activity was observed when haloperidol was applied to the intracellular surface of excised inside-out patches in symmetrical 140 mM KCl (10 μM free Ca^{2+}, Figure 3). The inhibition of channel activity was independent of membrane holding potential with IC_{50} values of 2.5 ± 0.6 μM and 3.5 ± 0.5 μM at +40 mV and −40 mV and associated Hill coefficients of 0.76 and 0.79, respectively. As observed with chlorpromazine, at concentrations above 10 μM, haloperidol was associated with a reversible reduction in BK_{Ca} channel current amplitude due to the flickery nature of the channel openings (not shown).

The effects of chlorpromazine and haloperidol on the BK_{Ca} channels were also examined on outside-out patches in symmetrical 140 mM KCl to determine whether the compounds were similarly potent when applied to the extracellular surface of the membrane. Both chlorpromazine (Figure 4a) and haloperidol produced concentration-dependent reductions of the BK_{Ca} channel activity under these conditions. The inhibition was independent of voltage with IC_{50} values for chlorpromazine of 2.3 ± 0.6 μM and 8.1 ± 5.4 μM at +40 mV and −40 mV with Hill slopes of 0.72 and 0.58 (Figure 1b). Similar results were seen with haloperidol with IC_{50} values of 1.1 ± 0.9 μM and 3.6 ± 0.9 μM and Hill slopes of 0.81 and 0.95 at +40 mV and −40 mV, respectively (data not shown).

In a series of further experiments, chlorpromazine and haloperidol were applied to the extracellular surface of outside-out patches in the presence of a quasi-physiological ionic gradient (extracellular; solution A, intracellular; solution D). Under these conditions, both compounds were found to inhibit BK_{Ca} channel activity with the same potency to that observed when they were applied to the intracellular surface of inside-out patches in symmetrical 140 mM KCl. For example, 1 μM chlorpromazine reduced BK_{Ca} channel activity by $38 \pm 3.2\%$ ($n = 4$) when applied to the intracellular surface of an inside-out patch held in symmetrical KCl at +20 mV, whilst the same concentration of chlorpromazine reduced BK_{Ca} channel activity by $36 \pm 3.2\%$ ($n = 3$, Figure 4b) when applied to the extracellular surface of an outside-out patch under a quasi-physiological gradient. Similarly, 1 μM haloperidol reduced BK_{Ca} channel activity by $36 \pm 2.5\%$ ($n = 4$) when applied to the intracellular surface of an inside-out patch held in symmetrical KCl at +20 mV, compared with $33 \pm 1.8\%$ ($n = 3$) inhibition when applied to the extracellular surface of an outside-out patch under a quasi-physiological gradient.

In contrast to the inhibitory effects of these neuroleptic dopamine receptor antagonists, both dopamine (100 μM) and the D_2 receptor agonist quinpirole (100 μM) failed to affect BK_{Ca} channel activity when applied to the intracellular surface of excised inside-out patches or to the extracellular surface on outside-out patches ($n = 3$ for each, data not shown).

Effects of atypical neuroleptic compounds

The actions of certain 'atypical neuroleptic compounds' were also tested upon BK_{Ca} channel activity. These are a group of structurally unrelated compounds which resemble the classical neuroleptics in their antipsychotic activity but which produce minimal extrapyramidal side effects (Kerwin, 1994). In contrast to the effects of the phenothiazine and butyrophenones, these compounds were found to be much less effective inhibitors of BK_{Ca} channel activity. For example the benzamide compound (±)-sulpiride was unable to affect BK_{Ca} channel activity when applied to either inside-out or outside-out patches at concentrations of up to 1 mM ($n = 3$ for each; Table 1). Similarly, the dibenzyldiazepine clozapine (100 nM – 10 μM) failed to affect BK_{Ca} channel activity when applied to either inside-out ($n = 3$; Table 1) or outside-out patches (Figure 5a) at ±40 mV. In contrast, the diphenylbutylpiperazine, pimozide (10 μM), did inhibit BK_{Ca} channel activity ($n = 3$) but was significantly less effective than the classical neuroleptic compounds ($P < 0.05$, Table 1).

Effects of related tricyclic compounds

In an attempt to investigate the structural specificity of BK_{Ca} channel inhibition in more detail the effects of a number of related tricyclic compounds were also examined. Amitriptyline

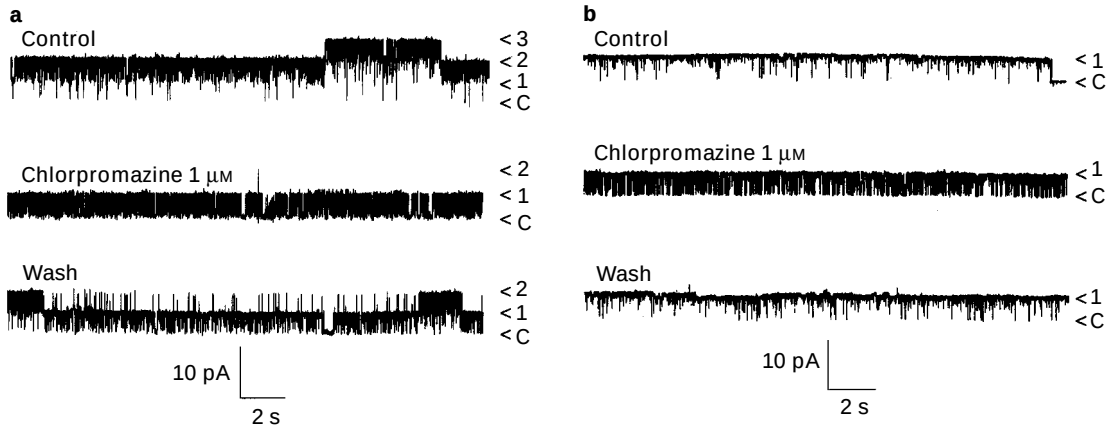

Figure 4 Single channel current showing that 1 μM chlorpromazine inhibits BK_{Ca} channel activity when applied to the external surface of an excised outside-out patch. (a) The recording was made in symmetrical 140 mM KCl at +20 mV. The pipette and bath contained solution D (10 μM free Ca^{2+}). Initially three BK_{Ca} channels were active with a N_fP_o of 1.92. Application of 1 μM chlorpromazine resulted in a decrease in activity (reduction in the number of active channels from 3 to 2) and N_fP_o fell to 0.78. The inhibition was partially reversible as N_fP_o recovered (but the number of active channels remained at 2) to 1.39 upon washout of chlorpromazine. (b) In this recording, the patch was held at +20 mV in the presence of a quasi-physiological ionic gradient. The pipette contained solution D (10 μM free Ca^2) while the bath contained normal saline, solution A. At the start of the recording, there was one BK_{Ca} channel active in the patch with a P_o of 0.67. Application of 1 μM chlorpromazine reduced the channel P_o to 0.34 and this was reversible on wash (P_o 0.62). A much smaller conductance channel was also observed in this recording but was not examined in the present study.

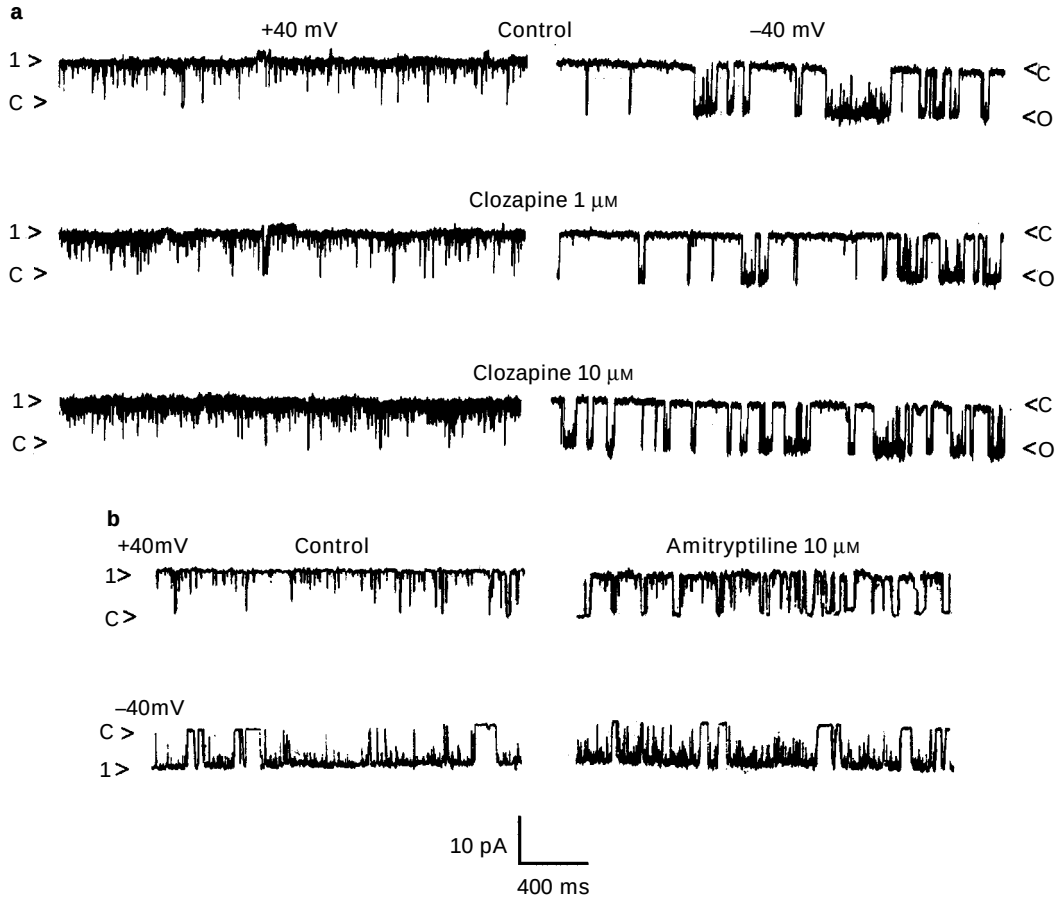

Figure 5 The effect of the tricyclic antidepressant, amitriptyline and the atypical neuroleptic, clozapine on BK_{Ca} channel activity. (a) Single channel currents were recorded in the outside-out patch configuration at +40 mV and −40 mV in the presence of symmetrical 140 μM KCl (10 μM free Ca^{2+}). The P_o values were as follows for +40 mV and −40 mV, respectively; control 0.96 and 0.34; 1 μM clozapine 0.92 and 0.29; 10 μM clozapine 0.86 and 0.43. (b) Single channel from an excised inside-out patch held at +40 mV in the presence of 140 mM KCl (10 μM free Ca^{2+}). The P_o values were as follows for +40 and −40 mV, respectively,: control 0.96 and 0.34; 1 μM clozapine 0.92 and 0.29; 10 μM clozapine 0.86 and 0.43.

is structurally related to chlorpromazine but exhibits little neuroleptic activity and is used clinically for its antidepressant properties. This compound was a weak inhibitor of the BK_{Ca} channel showing only 20% inhibition of BK_{Ca} channel activity at 10 μM (Figure 5b, Table 1). The anticholinesterase tacrine was also tested since it contains a tricyclic ring and has been shown to inhibit potassium channel currents (Robbins & Sim, 1990). However, it was an even less effective inhibitor of the BK_{Ca} channel than amitriptyline having no effect on channel activity at concentrations up to 100 μM ($n=3$, Table 1). The dibenzazepine carbamazepine which is structurally related to clozapine and has previously been shown to activate potassium currents present in cultured cortical neurones (Zona *et al.*, 1990) was also tested. However this compound was unable to stimulate or inhibit BK_{Ca} channel activity when applied to either the intracellular surface (Table 1) or the extracellular surface of excised membrane patches at a concentration of 100 μM ($n=3$ for each configuration) in the presence of either 1 μM or 10 μM intracellular free Ca^{2+}.

Discussion

The principle findings of the present study are that classical, but not atypical, neuroleptic compounds, inhibit rat motor cortex BK_{Ca} channel activity. Within the classical category of neuroleptics two structurally different groups are known, the phenothiazines and the butyrophenones and drugs from both groups (e.g. chlorpromazine and haloperidol respectively) were shown to be equally effective inhibitors of BK_{Ca} channel activity. Furthermore, non-neuroleptic compounds, structurally related to the classical neuroleptics (e.g. amitriptyline, tacrine and carbamazepine), were relatively ineffective inhibitors of rat motor cortex BK_{Ca} channel activity. Thus it is at present unclear which structural aspects of the classical neuroleptic compounds are responsible for the inhibition of this channel.

The inhibition of BK_{Ca} channel activity occurs independent of membrane voltage (between $\pm$40 mV) and is unaffected by the side of the membrane to which the drugs are applied. These latter observations are not in agreement with a previous study, which showed that phenothiazine and butyrophenone neuroleptics inhibit BK_{Ca} channel activity in canine airway epithelial cells in a voltage-dependent manner only when applied to the intracellular aspect of the patch (McCann & Welsh, 1987). The reasons for these differences are not obvious, although it is possible that there may be tissue-related differences in the molecular architecture of the two BK_{Ca} channel types. However, since these compounds are highly lipophilic, they would be expected to pass readily through lipid bilayers even at pH 7.2 (pH used in both studies), where they would exist mainly in an ionized form (pK_a values of 9.3 and 8.7 for chlorpromazine and haloperidol, respectively). Therefore it is difficult to explain why these compounds were found to act only from the intracellular surface in this former study. In an attempt to distinguish betweeen an extracellular or intracellular site of action, it would prove useful to reduce the lipophilicity of these compounds by modifying the proportion of uncharged forms thereby restricting their mode of action to the side of their application. One way would be to alter the intra/extracellular pH or by utilizing permanently charged analogues.

Previously, it has been suggested that these agents can affect neuronal processes via a non-specific perturbation of the membrane lipid due to their high lipid solubility (Seeman, 1997). However, since the atypical neuroleptics together with the other tricyclic compounds used in the present study are also extremely lipid soluble, but have much weaker effects on BK_{Ca} channel activity, it is unlikely that simple membrane perturbation can account for the effects observed. These results are thus similar to those obtained by Wooltorton & Mathie (1993) on the delayed rectifier current ($I_{K(V)}$) present in isolated sympathetic neurones. Such findings led these authors to suggest that the tricyclic ring structure possessed by these compounds was not as important as the central chain in determining potency. They concluded that the ring may provide an important anchorage on or near to the channel with the central carbon chain then determining the inhibitory potency of the drug. Furthermore, McCann & Welsh (1987) suggested that the neuroleptics mediate their inhibitory effects upon the BK_{Ca} channel via their ability to interact with the open conformation of the channel. In relation to this, it is known that chlorpromazine blocks the nicotinic acetylcholine receptor/channel by interaction with a specific binding site situated in the M2 (pore forming) region of the receptor. This binding site is made up of seven amino acids which lie at three distinct positions on the five subunits which form the functional nicotinic receptor/channel (Changeux *et al*., 1987; Revah *et al*., 1990). Recently, the amino acid sequence of the BK_{Ca} channel has been published and its putative pore forming region (H5) shown to possess considerable homology to that of other voltage-dependent K^+ channels (Adelman *et al*., 1992). However, this H5 region does not appear to possess any sequence homology to the chlorpromazine binding site present in the nicotinic receptor (Noda *et al*., 1983). Thus it is possible that the neuroleptics may bind to a novel sequence of amino acids present in the pore forming region of both Ca^{2+}- and voltage-gated K^+ channels.

It is believed that the neuroleptics achieve their antipsychotic effects due to their ability to antagonize dopamine D_2 receptors (Seeman, 1980). In the present study, the ability of the neuroleptics to inhibit BK_{Ca} channel activity does not appear to be related to their ability to antagonize dopamine receptors since the atypical neuroleptics, which are also highly potent dopamine antagonists (Seeman & Van Tol, 1994), were much less effective inhibitors of the BK_{Ca} channel. Furthermore, both dopamine and the D_2 receptor agonist quinpirole were without effect upon BK_{Ca} channel activity recorded in the outside-out patch configuration. The present findings taken together with those obtained previously (Ogata & Narahashi, 1989; Ogata *et al*., 1990; Nakazawa *et al*., 1995) suggest that the traditional neuroleptics can inhibit a wide range of neuronal voltage-activated K^+, Ca^{2+} and Na^+ currents at very low (submicromolar in the present study) concentrations. It is difficult to equate the concentrations employed in these studies with those used clinically since these drugs are highly bound to plasma proteins (Bowman & Rand, 1984). However, concentrations of chlorpromazine in the range 9–60 μM (Huang & Ruskin, 1964) have been found in schizophrenic patients. Thus, these results suggest that the traditional neuroleptics are capable of interfering with a variety of ion channels at therapeutic concentrations and may thus produce complex effects upon neuronal excitability. Therefore at normal therapeutic concentrations classical neuroleptic compounds such as chlorpromazine may inhibit BK_{Ca} channel activity and in so doing affect neuronal excitability directly and/or modulate the effects of neurotransmitters upon neuronal excitability. In contrast, it is unlikely that the atypical neuroleptics used in this study would affect BK_{Ca} channel activity at their therapeutic concentrations (e.g. approximately 200 nM for sulpiride; Alfredsson *et al*., 1979). At present, the reason why atypical neuroleptics produce fewer extrapyramidal side effects than their classical counterparts remains poorly understood. Previously, it has been suggested that the atypical neuroleptics modulate the activity of dopaminergic neurones present in the cortico-limbic system more selectively than agents which cause extrapyramidal side effects (White & Wang, 1983). Alternatively it has been suggested that the lower incidence of extrapyramidal side effects associated with the atypical neuroleptics is due to their different receptor antagonist profiles (see Seeman & Van Tol, 1994). However, on the basis of the present findings, it is also possible that the deleterious effects produced by the classical antipsychotics may arise via their ability to inhibit BK_{Ca} channel activity, in areas of the brain where this channel plays an important role in the regulation of neuronal electrical excitability. Thus there is an apparent correlation between the ability of the neuroleptics to inhibit BK_{Ca} channel activity and their tendency to produce extrapyramidal side effects (Reynolds, 1992).

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