Chlormethiazole inhibits epileptiform activity by potentiating GABA$_A$ receptor function

Ruth M. Empson, Veronica J. Gee, Malcolm J. Sheardown, Nigel R. Newberry

Vernalis Research Ltd, Oudene Court, 613 Reading Road, Winnersh, Wokingham RG41 5UA, UK

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Abstract

Chlormethiazole has sedative, hypnotic, anticonvulsant and neuroprotective properties. Using in vitro grease-gap recordings, we show that it inhibits epileptiform activity in neocortical slices superfused with Mg$^{2+}$-free medium (IC$_{50}$~200 μM). At an antiepileptic concentration (300 μM), chlormethiazole potentiated the action of exogenously applied GABA (1 mM) but did not affect responses to the glutamate receptor agonists N-methyl-D-aspartate (10 μM) or L-quisqualic acid (3 μM). The GABA$_A$ receptor antagonist N-methyl-bicuculline (50 μM) reduced chlormethiazole’s potency to inhibit the epileptiform activity. These results indicate that chlormethiazole’s anticonvulsant action is likely mediated by potentiating GABA$_A$ergic inhibition rather than by antagonising glutamatergic excitation. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chlormethiazole (clomethiazole, Heminevrin, Zendra$^\text{®}$) was first patented in 1938 (Swiss patent 200,248 to Hoffman La Roche). It has sedative, hypnotic and anticonvulsant properties and it is neuroprotective in animal models of stroke [5]. Its main clinical use has been as an anticonvulsant, particularly in the treatment of ethanol withdrawal syndrome [11,18] and status epilepticus [12]. Despite its age, the mechanism responsible for its anticonvulsant action remains unclear. In a key study using ionophoretic drug applications onto single neurones in the rat brain in vivo, Gent and Wacey [4], showed that chlormethiazole preferentially potentiated the inhibitory action of GABA — higher ejection currents were needed to depress glutamate-induced excitation. That work has been confirmed and extended [1–3,6,8,9,13,15,17,19]. It is now generally agreed that chlormethiazole acts to potentiate GABAergic function rather than inhibiting glutamatergic function. However, its anticonvulsant potency has rarely been directly compared to its potency to interact with amino acid receptor function. Here we succeed in relating its anticonvulsant effects to its actions on both GABAergic and glutamatergic receptor function by using a robust in vitro electrophysiological preparation: the cortical “wedge” of Harrison and Simmonds [10]. We demonstrate that chlormethiazole inhibits epileptiform activity in a concentration-dependent manner. Because this preparation responds to quisqualic acid, NMDA and GABA, we were able to relate its antiepileptic potency to its actions on three amino acid receptor-mediated responses. We conclude that at antiepileptic concentrations chlormethiazole does not directly inhibit glutamatergic receptor function and most likely acts by potentiating GABA$_A$ receptor function, as previously suggested.

2. Materials and methods

Unanaesthetised male Sprague–Dawley rats (80–120 g, Harlan–Olac) were killed by rapid decapitation (Home
Office-approved Schedule 1 method). Coronal sections of forelimb/frontal neocortex (500-μm-thick) were cut in ice-cold artificial cerebrospinal fluid (aCSF) using an Oxford Vibratome, trimmed to ≈1–1.5-mm-wide pieces of tissue and placed in a two-compartment chamber with the ventral part of the cortex protruding through a greased-gap in the Perspex partition dividing the two chambers. The chamber containing the dorsal part of the neocortex (=0.5 ml) was continuously perfused (2–2.5 ml/min) with aCSF while the aCSF in the chamber containing the corpus callosum was static. The experiments were performed at room temperature. The superfusing aqueous aCSF initially contained, in mM, NaCl 135, KCl 3, NaH2PO4 1.25, MgCl2 1, CaCl2 2, glucose 5 and NaHCO3 26 and was pre-equilibrated with 95% O2/5% CO2. Twenty minutes after setting up the slices the superfusate was switched to one without MgCl2 which led to the appearance of spontaneous, synaptically-mediated, depolarising, epileptiform discharges — sometimes called paroxysmal discharges. The frequency of these events was counted over 10 min periods. In some experiments, 0.5 μM tetrodotoxin was included in the MgCl2-free medium to prevent the synaptically driven spontaneous events. Depolarising responses were induced by superfusing the glutamate agonists, l-quisqualic acid (3 μM) and N-methyl-D-aspartate (NMDA, 10 μM), and γ-aminobutyric acid (GABA, 1 mM) for 1 min periods every 20 min. Previous research has shown that these responses are mediated by AMPA, NMDA and GABA receptors, respectively [10,14,16]. When studied together, the agonists were applied alternately (see Fig. 1). Chlormethiazole was superfused for ≥30 min and the agonists reapplied in its presence. The drug sources were as follows: chlormethiazole (Tocris), NMDA (Sigma), l-quisqualate (Tocris), GABA (Sigma), N-methyl-bicuculline (Sigma), tetrodotoxin (TTX, Calbiochem), (±)-APV (Tocris and Sigma). The salts were Analar grade. Data are expressed as mean±S.E.M. Statistical comparisons were made with the unpaired Student’s t-test.

3. Results

In the “Mg2+-free” superfusate, the spontaneously occurring epileptiform events occurred at a rate of 1.7±0.17 times per minute (n=33). These events were mediated in part by NMDA receptors as they were antagonized by (±)-APV with an IC50 of 12 μM (not shown, cf. [9]). Chlormethiazole reduced the frequency of these events in a concentration-dependent manner (IC50≈200 μM, Fig. 1B). At 300 μM, it had no significant effect on the depolarisations induced by either quisqualate or NMDA (Fig. 1A). In contrast, at this concentration it significantly increased responses to superfused GABA by +60±14% over control values (n=7, e.g. Fig. 1A). We

![Figure 1](image-url)

Fig. 1. The mechanism of action of chlormethiazole (CMZ, 300 μM) is exemplified in A by its inhibition of epileptiform activity, its potentiation of the GABA response (1 mM, G) and its lack of action on NMDA (10 μM, N) or quisqualic acid (3 μM, Q)-induced responses. The epileptiform events, that slow in the presence of chlormethiazole, are the frequent brief upward deflections of the baseline. In B, the concentration–response curve to chlormethiazole is shown together with the effect of N-methyl-bicuculline (BIC, 50 μM) on it (n=5–6). In C, the pooled data show the effect of 1 mM chlormethiazole on quisqualate (3 μM, Q) and NMDA (10 μM, N)-induced depolarisations. Chlormethiazole does not inhibit responses in the presence of tetrodotoxin (TTX, 0.5 μM) and N-methyl-bicuculline (BIC, 50 μM) (n=4–7) (*P<0.05).
therefore considered that chlormethiazole may be inhibiting synaptic activity in this slice by potentiating GABAergic inhibition. The superfusion of the GABA receptor antagonist N-methyl-bicuculline (50 μM) increased the frequency of epileptiform events by on average 40% suggesting that there was indeed a GABAergic inhibitory tone in the slice. Furthermore, chlormethiazole’s inhibition of spontaneous epileptiform activity was antagonised by this compound with the IC50 for chlormethiazole being shifted to >1 mM (Fig. 1B).

An action of chlormethiazole on glutamate receptor function was demonstrable at the highest concentration tested (1 mM) when chlormethiazole did reduce the effects of quisqualate (although not significantly) and particularly, NMDA (Fig. 1C). This effect was probably mediated indirectly since it was not seen if synaptic inhibition was prevented by the combined application of the sodium channel blocker tetrodotoxin (0.5 μM) and the GABA receptor antagonist N-methyl-bicuculline (50 μM) (Fig. 1C).

4. Discussion

The principal finding of this study is that chlormethiazole inhibits epileptiform activity in neocortical slices in vitro. At concentrations of the drug that inhibit epileptiform activity, it potentiates the action of GABA but has no significant effect on the actions of two glutamate agonists. Additionally, a GABA receptor antagonist inhibits its “anti-epileptic” action. This is the first demonstration of all these actions in an in vitro model of epilepsy. The value of using an in vitro rather than an in vivo preparation has allowed the direct comparison of three amino acid neurotransmitter functions in the same preparation and removed any confounding interactive effects of the anaesthetic on the actions of chlormethiazole. Moreover, the actions of chlormethiazole to potentiate GABA responses and inhibit the epileptiform activity occurred at concentrations similar to those observed to be neuroprotective in a gerbil model of ischaemia, ≈200 μM [7]. This suggests that the GABA potentiation mechanism may also be relevant to the therapeutic, neuroprotective actions of chlormethiazole.

The action of chlormethiazole on neocortical GABA receptor function was consistent with previous studies showing that it binds to a site on this receptor that is distinct from the barbiturate and benzodiazepine sites [2,8,9,13,19]. We confirmed the weak antagonist action on quisqualate-induced responses on this preparation [17] and the depression of NMDA–induced responses as seen in vivo [3,6,15]. However, both of the effects we observed were likely to be indirect as blocking GABAergic synaptic transmission prevented them. It has been suggested from radioligand binding experiments that chlormethiazole reduces NMDA-induced responses indirectly [3,6], but the possibility that chlormethiazole acted at a novel site on the NMDA receptor could not be excluded. In our experiments, we clearly demonstrated that chlormethiazole reduces glutamate receptor function indirectly by potentiating GABAergic neurotransmission.

In conclusion, our results support the hypothesis that chlormethiazole exerts its anticonvulsant actions predominantly via a potentiation of GABAergic inhibition rather than by its weak antagonism of glutamate receptor activation.

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