ELSEVIER

European Journal of Pharmacology 399 (2000) 29–34

ejp

www.elsevier.nl/locate/ejphar

Effects of clomethiazole on spreading depression in the rat hippocampal slice

Trevor W. Stone *

Institute of Biomedical Life Sciences, Division of Biomed. and Life Sciences, West Medical Building, University of Glasgow, Glasgow G12 8QQ, Scotland, UK

Received 2 February 2000; received in revised form 10 April 2000; accepted 26 April 2000

Abstract

Clomethiazole is neuroprotective in a variety of animal models of ischaemic stroke, but the mechanism is unclear. This study examined whether clomethiazole is able to modify spreading depression elicited in rat hippocampal slices. When spreading depression was induced by superfusion with high K^+ medium (50 mM), clomethiazole at 100 μM reduced its duration. Both the amplitude and duration of spreading depression were reduced at 200 μM. Clomethiazole at 200 μM tended to reduce the amplitude of the K^+-induced shift in direct current (DC) potential but this was not statistically significant. When a pair of K^+ pulses were presented, 30 min apart, the second produced a smaller DC potential than the first. Clomethiazole at 200 μM increased the size of the ratio of these responses. Superfusion with a hypoxic solution induced spreading depression observed as a shift in the DC field potential. The amplitude of this was decreased significantly by clomethiazole at 200 μM. With intracellular recordings, the effects of clomethiazole were quantified by measuring the time from the peak K^+-induced depolarisation to the recovery of membrane potential following the period of hyperpolarisation. Clomethiazole did not reduce this period significantly. It is concluded that clomethiazole can reduce some forms of spreading depression, but only at the higher concentrations tested. It is unlikely that this effect contributes to its neuroprotective properties.

Keywords: Clomethiazole; Chlormethiazole; Spreading depression; Hippocampus; Hypoxia; Neuroprotection

1. Introduction

Clomethiazole has recently been shown to be neuroprotective in a variety of animal models of acute ischaemic stroke (see Green, 1998). It protects against neuronal damage in global models (Cross et al., 1991, 1995; Baldwin et al., 1993) and in focal models involving both transient (Sydserff et al., 1995b) and permanent (Sydserff et al., 1995a) middle cerebral artery occlusion and photochemically induced infarction (Snape et al., 1993; Baldwin et al., 1994). Its neuroprotective activity appears to be exerted at doses lower than those, which produce sedative or anticonvulsant effect (Cross et al., 1995). Protection has also been recently observed clinically in a large, multicentre placebo-controlled clinical trial (Wahlgren et al., 1999).

The mechanism by which clomethiazole produces neuroprotection remains unknown although it may be related to the γ-amino-butyric acid-(GABA)-mimetic properties of the drug (Green, 1998). Leão (1944) described the phenomenon of spreading depression in the central nervous system (CNS), a propagating wave of neuronal inactivation characterised by a profound increase of membrane conductance. Spreading depression can be induced in vivo by a range of insults including mechanical distortion or a period of cerebral ischaemia, and in brain slices by hypoxia or a high K^+ challenge. The increase of membrane conductance is accompanied by a large rise of extracellular K^+ concentration coupled with a fall of extracellular calcium as it moves into the intracellular compartment. Interest in spreading depression stems partly from the fact that these ionic shifts and calcium influx can induce an irreversible loss of neurotransmission and subsequent neuronal damage. Agents, which shorten the duration of spreading depression, such as chlorpromazine and gangliosides, appear to be neuroprotective (Somjen et al., 1990; Balestrino and Somjen, 1986). There are other parallels with in vivo studies of neuroprotection, as reduced slice temperature

* Tel.: +44-141-330-4481; fax: +44-141-330-4100.
E-mail address: t.w.stone@bio.gla.ac.uk (T.W. Stone).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00331-9

reduces or prevents the occurrence of spreading depression and the irreversible loss of synaptic function (Taylor and Weber, 1993). The present study was designed to determine whether clomethiazole had any influence on spreading depression in hippocampal slices.

2. Methods

Male Wistar rats (150–250 g) were anaesthetised with urethane (1.5 g kg^{-1} i.p.) and cooled on ice whilst breathing oxygen enriched air until rectal temperature reached 30°C. This procedure was recommended by Newman et al. (1992) to enhance the viability of slices. The animals were then killed by cervical dislocation, decapitated, and the brain rapidly removed to ice-cold artificial cerebrospinal fluid (aCSF) of composition (mmol l^{-1}) KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5 $MgSO_4$ 1.2, glucose 10 saturated with 95% O_2 and 5% CO_2. The hippocampi were dissected free of surrounding tissue and were cut transversely into slices 450 μm thick using a McIlwain tissue chopper. Slices were maintained in an incubation chamber saturated with 95% O_2/5% CO_2 for at least 1 h before being transferred to a submersion recording chamber and superfused with pre-gassed aCSF (34–35°C) at a constant flow rate of 2–3 ml/min. Drugs were added to the superfusion fluid.

Test pulses (100 μs, 200–450 μA) were delivered at 10 or 20 s intervals via a concentric bipolar electrode placed in the stratum radiatum of the CA1 area. Recordings of extracellular population spikes were made from the stratum pyramidale using glass microelectrodes of tip diameter approximately 2 μm and filled with 0.9% NaCl (resistances ~5 MΩ). Responses were amplified, displayed on oscilloscopes and recorded both digitally onto magnetic tape and directly onto a fast response thermal chart recorder (DASH IV, AstroMed). The potentials were quantified as the amplitude of the population spike, (measured as the difference between the peak negativity and the averaged values of the two peak positivities of the population spike).

Direct current (DC) field responses were measured using glass microelectrodes of tip diameter approximately 5 μm and filled with 200 mM NaCl. The tips were positioned in the stratum pyramidale. Recordings were amplified using a Neurolog NL 102 DC amplifier.

2.1. Intracellular recording

Intracellular recordings were made using sharp microelectrodes fabricated on a Narashige vertical puller and filled with 1 M KCl or potassium acetate (90–120 MΩ). Potentials were amplified using a Neurolog NL 102 amplifier or Axoclamp-2A amplifier operated in bridge balance mode. Neurones were used if they displayed a stable resting potential greater than 60 mV and a spike of at least 70 mV amplitude.

2.2. Spreading depression

Spreading depression can be studied either by observing the changes in extracellular DC field potential, which accompany the fall of membrane potential and elevation of extracellular K^+, or by observing the loss of neuronal activity, which results from these changes. In the present study, spreading depression was normally induced by perfusing slices briefly with a solution containing high K^+. The solution contained 50 mM K^+ chloride substituted for sodium chloride to preserve osmolarity. This was perfused for 15 s.

2.3. Hypoxia

Some slices were examined using a hypoxic medium, which was made of the same ionic composition in distilled water as the normal CSF, but which was not gassed. The pH of this medium was adjusted to within the range 7.0–7.4 by the addition of 1 M HCl, before the addition of calcium. Superfusion of slices with this medium elicited a spreading depression DC potential shift, which commenced within 7.8 ± 2.4 min of the start of the hypoxic perfusion. As soon as the DC shift was apparent, the normal medium was restored immediately so as to minimise damage to the slice.

For the recordings of DC potentials, the duration of K^+ perfusion was increased to 45 s in order to induce a sufficiently large and measurable response.

2.4. Statistics

Data were usually subjected to analysis of variance followed by Dunnett's test for a comparison of several data sets against a control set, or the Student–Newman–Keuls test for a comparison of selected data sets.

3. Results

3.1. Superfusion with high potassium

The pattern of responses obtained during K^+ superfusion of slices in which population spikes were evoked by orthodromic stimulation is illustrated in Fig. 1. The response to K^+ consisted of a multiphasic change of neuronal potentials. An initial increase of potential size was produced by the immediate depolarisation produced by potassium, and this was followed by a loss of neuronal responses due to over-depolarisation. This in turn was followed by a partial recovery, the magnitude of which was variable between slices, after which there was a secondary loss of responses to orthodromic stimuli, reflecting the occurrence of spreading depression, followed by a final period of recovery. This sequence is similar to that described by Jing et al. (1991), and occurred in approxi-

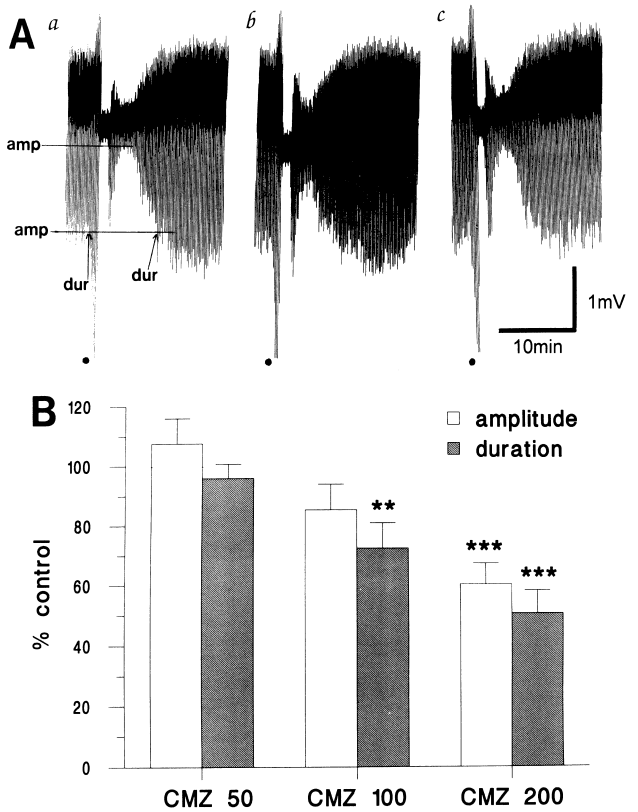

Fig. 1. (A) Each sweep of this record represents the population spike recorded extracellularly in the CA1 region of a hippocampal slice. Stimuli are delivered at 0.1 Hz. High (50 mM) potassium containing medium was superfused for 15 s indicated by the dots below the records. There follows a transient period of increased potential size followed by a period of overdepolarisation, partial recovery on washout and a secondary loss of potential caused by spreading depression. Record (a) was taken under control conditions in the absence of drugs. Superfusion with clomethiazole at 200 μM produced a decrease in the amplitude of the spreading depression response (measured between the two lines marked 'amp' in record a) (b and B) with subsequent washout (c). There was no change in the duration of the spreading depression (measured between the two arrowheads marked 'dur' in record a, in this example. (B) Histogram to show the effect of different concentrations of clomethiazole on the amplitude and duration of the spreading depression-associated loss of evoked potentials. Clomethiazole was used at 50 μM ($n = 16$), 100 μM ($n = 24$) and 200 μM ($n = 21$). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ relative to control slices.

mately 40% of the slices tested. Clomethiazole had no significant effect on the initial potassium-induced increase of spike size, nor the subsequent loss of potential due to over-depolarisation.

In order to quantify the amount of spreading depression, the amplitude and duration of the secondary loss of neuronal responsiveness was measured. The amplitude of depression was measured relative to the control potential amplitude before the addition of potassium. The duration was measured by constructing a line through the mean of ten evoked potentials before potassium addition, and extrapolating to the first potential to cross that line during recovery (i.e. as the period from the initial change of potential size to the time of recovery of the control size). In some slices, this was followed by a period of increased potential size (Fig. 1). The effect of clomethiazole was to reduce these parameters when tested at the higher concentrations (Fig. 1B). These parameters were not changed by superfusion with clomethiazole at 50 μM, although the duration was reduced significantly at 100 μM and both amplitude and duration were affected at 200 μM (Fig. 1B). Clomethiazole at 200 μM tended to produce a gradual decline in the size of the evoked potentials, though this usually reached a plateau after about 15 min at approximately 75% of the control size.

When tested on normal synaptic transmission in these slices, no concentration of clomethiazole in the range 10–200 μM produced any change in the amplitude of the population spikes, as reported previously (Stone, 1988; Addae and Stone, 1988).

3.2. DC potentials

As an alternative approach to that of monitoring evoked potentials, the potassium-induced shift in DC potential was recorded (Fig. 2A). Clomethiazole at 100 or 200 μM reduced the amplitude of these potentials (Fig. 2B).

When a pair of potassium pulses were presented, 30 min apart, the second produced a smaller DC potential than the first. A series of slices was therefore studied in which the ratio between the second and first pulses (SD2/SD1) was examined in control slices and slices treated with clomethiazole at 50, 100 or 200 μM. The action of clomethiazole was to increase the size of this ratio, at the highest dose of 200 μM (Fig. 2C).

3.3. Hypoxia

Superfusion with a hypoxic solution has been reported to induce spreading depression in hippocampal slices, observed as a shift in the DC field potential. In the present study, a voltage shift occurred after 7.8 ± 2.4 min of perfusion with hypoxic medium and the amplitude and duration of this was measured. Clomethiazole did not change the duration of the response ($F[3,21] = 1.367$) but did decrease the amplitude significantly at 200 μM from a control amplitude of 4.2 ± 0.22 mV to a level of 2.9 ± 0.18 mV ($F[3,21] = 6.573$; $P = 0.0026$).

3.4. Intracellular recordings

A similar pattern of events was observed using intracellular recordings to that seen with field potential recording. As shown in Fig. 3A, the immediate effect of 50 mM K^+ was to produce a marked depolarisation of membrane potential, with a corresponding increase of neuronal firing. This was followed by a hyperpolarisation and loss of spiking activity as the neurone repolarised, a change, which would correspond with the secondary loss of re-

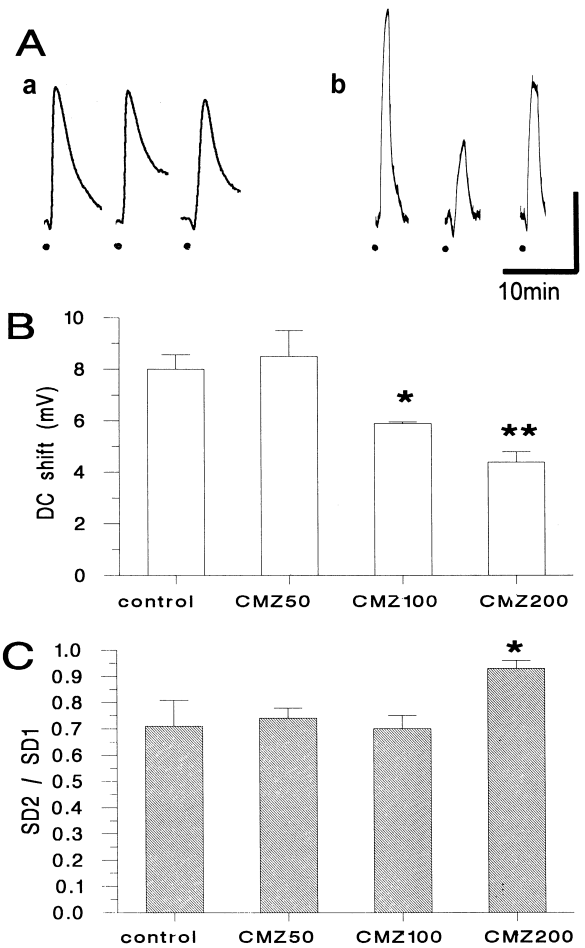

Fig. 2. (A) Records of DC potentials produced by superfusion with medium containing potassium, 50 mM, perfused at the time indicated by the dots below the records. The first three responses are taken from a single slice 30 min apart in the absence of any drug treatment. The first of the three responses to the right of A indicates a control response to potassium in a different slice. The effect of clomethiazole at 200 μM (middle record on the right) is to reduce the size of the potential in this example, while the third response illustrates partial recovery on washout of the drug. Responses were evoked 30 min apart, but only partial recovery was seen on most slices after the application of clomethiazole. The pooled data ($n = 6$ at each concentration) are summarised in B. (C) The effect of clomethiazole on the SD2:SD1 ratio of DC responses to 50 mM potassium. The ratio was increased only at the highest concentration of clomethiazole. Calibrations in (A) 4 mV for series (a); 2 mV for series (b); 10 min. $^{*}P < 0.05$ relative to control slices ($n = 8$ at each concentration).

sponsiveness seen extracellularly. In Fig. 3B, a sequence is illustrated in which a cell was recorded intracellularly in parallel with an extracellular field potential record. The initial rapid depolarisation seen intracellularly (Fig. 3Ba) occurs in parallel with the extracellular early loss of the field potential (Fig. 3Bb). The intracellular repolarisation is accompanied both by a loss of action potentials and a profound reduction of membrane input resistance, which parallels the secondary loss of extracellular potentials and reflects the occurrence of spreading depression.

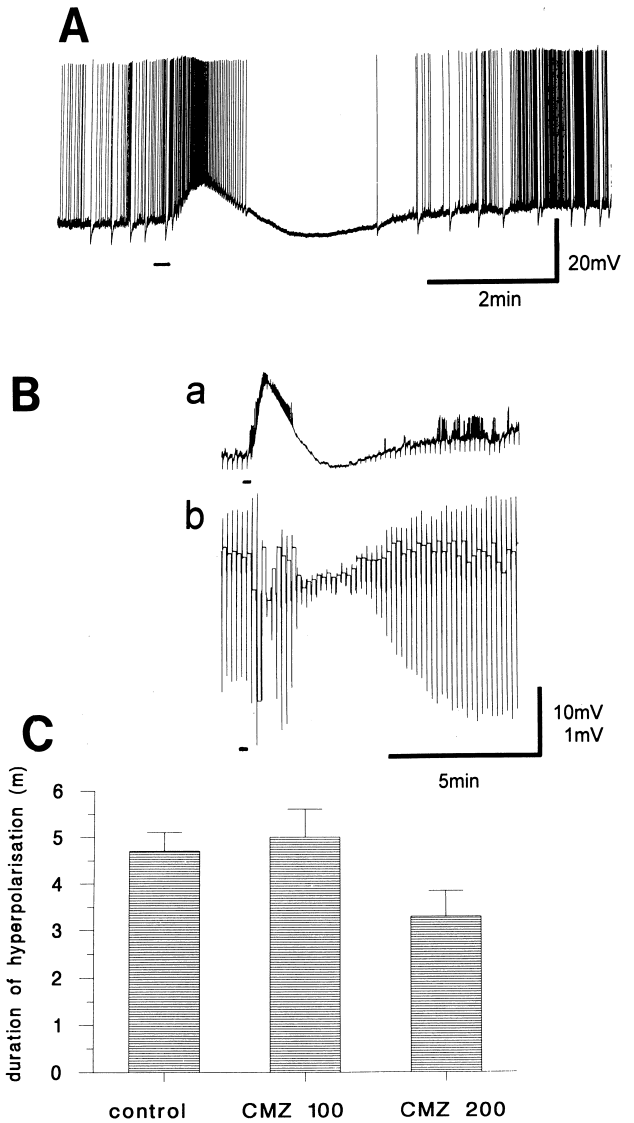

Fig. 3. (A) Intracellular recording of a CA1 neurone following superfusion of medium containing 50 mM potassium during the period indicated by the bar below the record. The cell is firing spontaneously at approximately 1 Hz and the rate of firing increases with the potassium-induced depolarisation. Repolarisation is accompanied by an increased action potential threshold leading into a period of hyperpolarisation and subsequent depolarisation. (B.a) An intracellular record is illustrated in which high potassium medium (applied during the bar below the records) induced depolarisation accompanied by a series of action potentials. (Action potentials in this record are truncated due to the low frequency response of this recorder). These cease as the threshold for initiation increased during the repolarisation phase. The regular vertical pulses indicate the delivery of hyperpolarising current pulses (300 ms, 0.2 nA) to monitor membrane input resistance and reveal a profound loss of membrane resistance during the depolarisation and repolarisation phases. (b) Illustrates evoked population potentials recorded in parallel with (a). The period of intracellular repolarisation and loss of membrane resistance corresponds to the secondary loss of population potentials associated with spreading depression. (C) Histogram to show the effect of different concentrations of clomethiazole on the duration of the high potassium-induced hyperpolarisation. Clomethiazole was used at 100 μM ($n = 4$) and 200 μM ($n = 6$). The duration of the hyperpolarisation was not changed significantly by clomethiazole.

Recordings have been made of eight cells in different slices, which were held long enough to perform tests with at least one concentration of clomethiazole. The effects of clomethiazole were quantified by measuring the time from the peak K^+-induced depolarisation to the recovery of membrane potential following the hyperpolarisation. Clomethiazole did not reduce this period significantly (Fig. 3C).

4. Discussion

Many different triggering stimuli have been used including ouabain (Basarsky et al., 1998) oxygen-free or low oxygen/low glucose media, (Jing et al., 1993, 1994; Taylor and Weber, 1993; Huang et al., 1996; Hershkowitz et al., 1993) hypotonic media, (Chebabo et al., 1995) or high K^+ media (Jing et al., 1993, 1994, 1997; Footitt and Newberry 1998; Taylor et al., 1997; Obrenovitch et al., 1996; Basarsky et al., 1998). In view of this variability, two different methods have been used here to evoke spreading depression. In all cases, clomethiazole had no effect at the lowest concentration tested, but tended to reduce spreading depression at the higher concentrations. In most cases, a significant reduction of spreading depression was obtained at 200 μM clomethiazole.

The therapeutic implications of a decrease of spreading depression are uncertain. Plasma levels of around 10 μM clomethiazole are associated with neuroprotection (Cross et al., 1995), so that it seems unlikely that the effects on spreading depression contribute substantially to this. Nevertheless, the neuroprotective activity seems to be reflected in the effect of clomethiazole on the DC potentials. The less-than-unity ratio of SD2:SD1 size would suggest that a degree of neuronal damage had occurred in response to the first period of activation. The ability of clomethiazole to raise the DC potential ratio towards unity would then be consistent with a neuroprotective action against the longer period of potassium stimulation.

Spreading depression may be due to the efflux of glutamate (Van Harreveld, 1959) or of potassium (Grafstein, 1956) from neurones or glial cells. One possibility, then, is that clomethiazole could interfere with the actions of glutamate. However, the role of glutamate in spreading depression is unclear. Jing et al. (1993) have shown that glutamate antagonists can delay and reduce the amplitude of spreading depression, but do not prevent it. Similarly, Young and Somjen (1992) found that glutamate antagonists did not modify the change of calcium levels during spreading depression and other groups have obtained results, which do not support the involvement of glutamate (Obrenovitch and Zilkha, 1995; Obrenovitch et al,. 1996). Indeed, the pharmacology of spreading depression appears to depend on the manner of its induction. Electrically induced (Mody et al., 1987) or K^+-induced spreading depression (Obrenovitch and Zilkha, 1996; Footitt and Newberry, 1998) was blocked by NMDA receptor antagonists. On the other hand, spreading depression induced by 4-aminopyridine was not prevented by glutamate antagonists in some studies (Avoli et al., 1996) while hypoxia-induced spreading depression can be prevented by kynurenic acid (Hershkowitz et al., 1993) but not by NMDA receptor antagonists (Aitken et al., 1988).

The interactions between clomethiazole and amino acid receptors are themselves unclear. In animals, clomethiazole is able to prevent the neuronal damage produced by kainic acid (MacGregor et al., 1998) but not by NMDA (Green et al., 1998). However, clomethiazole does not displace NMDA or other glutamate receptor ligands in binding studies (Green et al., 1998) and does not reduce their effects electrophysiologically (Addae and Stone, 1988). Clomethiazole does not alter neuronal sensitivity to adenosine (Stone, 1988).

The most likely mechanism of action of clomethiazole is that of GABA potentiation. Clomethiazole is able to potentiate the electrophysiological actions of GABA (Simmonds and Turner, 1987; Zhong and Simmonds, 1997) and open chloride channels on central neurones (Hedlund and Ogren, 1987). These effects are produced at concentrations of around 200 μM, comparable with those effective in reducing spreading depression here. There is also evidence that clomethiazole can open such channels even in the absence of GABA (see Green 1998). It is possible, therefore, that clomethiazole reduces the excitability of hippocampal neurones sufficiently by this mechanism that it is able to combat or reduce the ionic shifts associated with spreading depression.

Acknowledgements

Clomethiazole was a gift from Astra Arcus Pharmaceuticals.

References

Addae, J.I., Stone, T.W., 1988. Effects of anticonvulsants on responses to excitatory amino acids applied topically to rat cerebral cortex. Gen. Pharmacol. 19, 455–462.

Aitken, P.G., Balestrino, M., Somjen, G.G., 1988. NMDA antagonists: lack of protective effect against hypoxic damage in CA1 region of hippocampal slices. Neurosci. Lett. 89, 187–192.

Avoli, M., Nagao, T., Kohling, R., Lucke, A., Mattia, D., 1996. Synchronization of rat hippocampal neurones in the absence of excitatory amino acid-mediated transmission. Brain Res. 735, 188–196.

Baldwin, H.A., Jones, J.A., Cross, A.J., Green, A.R., 1993. Histological, biochemical and behavioural evidence for the neuroprotective action of chlormethiazole following prolonged carotid artery occlusion. Neurodegeneration 2, 139–146.

Baldwin, H.A., Williams, J.L., Snares, M., Ferreira, T., Cross, A.J., Green, A.R., 1994. Attenuation by chlormethiazole administration of the rise in extracellular amino acids following focal ischaemia in the cerebral cortex of the rat. Br. J. Pharmacol. 112, 188–194.

Balestrino, M., Somjen, G.G., 1986. Chlorpromazine protects brain tissue

in hypoxia by delaying spreading depression-mediated calcium influx. Brain Res. 385, 219–226.

Basarsky, T.A., Duffy, S.N., Andrew, R.D., MacVicar, B.A., 1998. Imaging spreading depression and associated intracellular calcium waves in brain slices. J. Neurosci. 18, 7189–7199.

Chebabo, S.R., Hester, M.A., Aitken, P.G., Somjen, G.G., 1995. Hypotonic exposure enhances synaptic transmission and triggers spreading depression in rat hippocampal tissue slices. Brain Res. 695, 203–216.

Cross, A.J., Jones, J.A., Baldwin, H.A., Green, A.R., 1991. Neuroprotective activity of chlormethiazole following transient forebrain ischaemia in the gerbil. Br. J. Pharmacol. 104, 406–411.

Cross, A.J., Jones, J.A., Snares, M., Jostell, K.-J., Bredberg, U., Green, A.R., 1995. The protective action of chlormethiazole against ischaemia-induced neurodegeneration in gerbils when infused at doses having little sedative or anticonvulsant activity. Br. J. Pharmacol. 114, 1625–1630.

Footitt, D.R., Newberry, N.R., 1998. Cortical spreading depression induces an LTP-like effect in rat neocortex in vitro. Brain Res. 781, 339–342.

Grafstein, B., 1956. Mechanism of cortical spreading depression. J. Neurophysiol. 19, 154–171.

Green, R., 1998. Clomethiazole (Zendra) in acute ischemic stroke: basic pharmacology and biochemistry and clinical efficacy. Pharmacol. Ther. 80, 123–147.

Green, A.R., Misra, A., Hewitt, K.E., Snape, M.F., Cross, A.J., 1998. An investigation of the possible interaction of clomethiazole with glutamate and ion channel sites as an explanation of its neuroprotective activity. Pharmacol. Toxicol. 83, 90–94.

Hedlund, B., Ogren, S.-S., 1987. Chlormethiazole acts on chloride channels in cultured spinal cord neurones. Neurosci. Lett. 78, 217–221.

Hershkowitz, N., Katchman, A.N., Veregge, S., 1993. Site of synaptic depression during hypoxia: a patch-clamp analysis. J. Neurophysiol. 69, 432–441.

Huang, R., Aitken, P.G., Somjen, G.G., 1996. Hypertonic environment prevents depolarization and improves functional recovery from hypoxia in hippocampal slices. J. Cereb. Blood Flow Metab. 16, 462–467.

Jing, J., Aitken, P.G., Somjen, G.G., 1991. Lasting neuron depression induced by high potassium and its prevention by low calcium and NMDA receptor blockade. Brain Res. 557, 177–183.

Jing, J., Aitken, P.G., Somjen, G.G., 1993. Role of calcium channels in spreading depression in rat hippocampal slices. Brain Res. 604, 251–259.

Leão, A.A.P., 1944. Spreading depression of activity in the cerebral cortex. J. Neurophysiol. 7, 359–390.

MacGregor, D.G., Graham, D.I., Stone, T.W., 1998. The attenutation of kainate-induced excitotoxicity by clomethiazole and its enhancement by dizocilpine, muscimol and adenosine receptor agonists. Exp. Neurol. 148, 110–123.

Mody, I., Lambert, J.D.C., Heinemann, U., 1987. Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. J. Neurophysiol. 57, 869–888.

Newman, G.C., Qi, H., Hospod, F.E., Grundmann, K., 1992. Preservation of hippocampal brain slices with in vivo or in vitro hypothermia. Brain Res. 575, 159–163.

Obrenovitch, T.P., Zilkha, E., 1995. High extracellular potassium, and not extracellular glutamate, is required for the propagation of spreading depression. J. Neurophysiol. 73, 2107–2114.

Obrenovitch, T.P., Zilkha, E., 1996. Inhibition of cortical spreading depression by L701,324 a novel antagonist at the glycine site of the NMDA receptor complex. Br. J. Pharmacol. 117, 931–937.

Obrenovitch, T.P., Zilkha, E., Urenjak, J., 1996. Evidence against high extracellular glutamate promoting the elicitation of spreading depression by potassium. J. Cereb. Blood Flow Metab. 16, 923–931.

Simmonds, M.A., Turner, J.P., 1987. Potentiators of responses to activation of GABA receptors. Neuropharmacology 26, 923–930.

Snape, M.F., Baldwin, H.A., Cross, A.J., Green, A.R., 1993. The effects of chlormethiazole and nimodipine on cortical infarct area after focal cerebral ischaemia in the rat. Neuroscience 53, 837–844.

Somjen, G.G., Aitker, P.G., Balestrino, M., Herreras, O., Kawasaki, K., 1990. Spreading depression-like depolarisation and selective vulnerability of neurons: a brief review. Stroke 21, 179–183.

Stone, T.W., 1988. Interactions of carbamazepine, clomethiazole and pentobarbitone with adenosine on hippocampal slices. Gen. Pharmacol. 19, 67–72.

Sydserff, S.G., Cross, A.J., Green, A.R., 1995a. The neuroprotective effect of chlormethiazole on ischaemic neuronal damage following permanent middle cerebral artery ischaemia in the rat. Neurodegeneration 4, 323–328.

Sydserff, S.G., Cross, A.J., West, K.J., Green, A.R., 1995b. The effect of chlormethiazole on neuronal damage in a model of transient focal ischaemia. Br. J. Pharmacol. 114, 1631–1635.

Taylor, C.P., Weber, M.L., 1993. Effect of temperature on synaptic function after reduced oxygen and glucose in hippocampal slices. Neuroscience 52, 555–562.

Taylor, D.L., Urenjak, J., Zilkha, E., Obrenovitch, T.P., 1997. Effects of probenecid on the elicitation of spreading depression in the rat striatum. Brain Res. 764, 117–125.

Van Harreveld, A., 1959. Compounds in brain extracts causing spreading depression of cerebral cortical activity and contraction of crustacean muscle. J. Neurochem. 3, 300–315.

Wahlgren, N.G., Ranasinha, K.W., Rosolacci, T., Franke, C.L., Erven, P.M.M., Ashwood, T., Claesson, L., For the CLASS study group, 1999. Clomethiazole acute stroke study (CLASS). Results of a randomised controlled trial of clomethiazole versus placebo in 1360 acute stroke patients. Stroke 30, 21–28.

Young, J.N., Somjen, G.G., 1992. Suppression of presynaptic calcium currents by hypoxia in hippocampal tissue slices. Brain Res. 573, 70–76.

Zhong, Y., Simmonds, M.A., 1997. Interactions between loreclezole, chlormethiazole and pentobarbitone at GABA(A) receptors: functional and binding studies. Br. J. Pharmacol. 121, 1392–1396.