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Neurochemical actions of the desglycinyl metabolite of remacemide hydrochloride (ARL 12495AA) in mouse brain

[1]John Paul Leach, Graeme J. Sills, Elaine Butler, Gerard Forrest, George G. Thompson & Martin J. Brodie

Epilepsy Unit, University Department of Medicine and Therapeutics, Western Infirmary, Glasgow G11 6NT, Scotland

1 Remacemide hydrochloride, a recently developed antiepileptic drug, is believed to exert its effects, at least in part, via its desglycinyl metabolite, ARL 12495AA.

2 We have investigated the effects of ARL 12495AA on several neurochemical parameters in mouse brain. Adult male ICR mice were randomized into two groups and administered ARL 12495AA (0–75 mg kg^{-1}) intraperitoneally, either as a single dose or once daily for 5 days.

3 Six hours after the final dose, animals were killed and their brains removed. Brain tissues were analysed for concentrations of γ-aminobutyric acid (GABA), glutamine and glutamate and for the activities of GABA-transaminase (GABA-T) and glutamic acid decarboxylase (GAD).

4 Single dose ARL 12495AA was without effect on any of the parameters investigated.

5 Repeated ARL 12495AA treatment did not alter brain concentrations of GABA and glutamine, but at a high dose there was a trend toward reduced brain glutamate concentrations ($P=0.10$).

6 Repeated administration of ARL 12495AA at a high dose significantly increased GABA-T activity ($P<0.05$) and decreased that of GAD ($P<0.05$).

7 These findings may have relevance to the clinical use of remacemide hydrochloride in human epilepsy.

Keywords: Remacemide; active metabolite; γ-aminobutyric acid (GABA); glutamate; glutamine; γ-aminobutyric acid-transaminase (GABA-T); glutamic acid decarboxylase (GAD); antiepileptic drugs; epilepsy

Introduction

Remacemide hydrochloride (RMD) is a new antiepileptic drug (AED) which emerged from a drug discovery programme aimed at developing a novel compound with a three-dimensional structure similar to that of phenytoin (Rogawski & Porter, 1990). Although experimentally and clinically active, RMD undergoes significant metabolism to the desglycinyl derivative ARL 12495AA (Heyn *et al*., 1994). This metabolite has a longer elimination half-life than RMD (Stables *et al*., 1995) and has a more potent pharmacological profile than the parent compound (Muir & Palmer, 1991).

Both RMD and its active metabolite are effective against a wide range of experimental seizures, including those induced by maximal electroshock, hippocampal kindling, N-methyl-D-aspartate (NMDA), kainate and 4-aminopyridine (Stagnitto *et al*., 1990; Garske *et al*., 1991; Palmer *et al*., 1992; Cramer *et al*., 1994). The drug is also active in the audiogenic-seizure sensitive DBA/2 mouse (Rogawski & Porter, 1990). RMD is currently undergoing clinical trial for the treatment of complex partial seizures with or without secondary generalization (Palmer *et al*., 1993). Initial studies have shown that the drug is well tolerated (Stables *et al*., 1995), although it may interfere with the hepatic metabolism of carbamazepine (Leach *et al*., 1996) and phenytoin (Leach *et al*., 1997a).

The mechanism of action of RMD remains to be determined. Both RMD and ARL 12495AA prevent sustained repetitive firing of cultured spinal cord neurons via a blockade of voltage-sensitive sodium channels (Wamil *et al*., 1996). There is also evidence to suggest that the drug may exert its effects, at least in part, by an action at the NMDA subtype of glutamate receptor (Hu & Davies, 1995; Subramaniam *et al*., 1996). Although RMD itself is only weakly active at this site, ARL 12495AA displaces [^{3}H]-dizocilpine binding to the NMDA channel at clinically relevant concentrations (Subramaniam *et al*., 1996). Further evidence has also suggested that ARL 12495AA inhibits the veratridine-induced release of glutamate from mouse isolated neocortical slices (Srinivasan *et al*., 1995).

It is clear that much, if not all, of the experimental data gathered on RMD to date has addressed the effects of the drug on neuronal excitation. There would appear to be no studies, positive or otherwise, on any potential actions of RMD on inhibitory mechanisms in the brain. As a result, we have investigated the effects of single and repeated treatment with ARL 12495AA, the desglycinyl metabolite of RMD, on several γ-aminobutryric acid (GABA) - related neurochemical parameters in mouse brain.

Methods

Male ICR mice (25–30 g) were obtained from Harlan Olac (Bicester, UK) and were housed in a controlled temperature and humidity environment with day/night cycle conditions and access to food and water *ad libitum*. All experimental work was governed by the Animals (Scientific Procedures) Act, 1986 (U.K.).

Determination of protein concentrations, amino acid (GABA, glutamate, glutamine) concentrations and the activites of the enzymes GABA-transaminase (GABA-T; EC 2.6.1.19) and glutamic acid decarboxylase (GAD; EC 4.1.1.15) was performed in accordance with the methods described by Sills and colleagues (1997).

Single dose studies

Mice were randomized into four treatment groups ($n=12$/group) and ARL 12495AA was administered (i.p.) in doses of 10, 25, 50 and 75 mg kg^{-1}. A fifth group (control) received vehicle (0.9% saline) alone. At 6 h post-administration, the animals were killed and their brains removed. Brains were divided into two hemispheres by a saggital incision and stored at -70°C until required.

[1] Author for correspondence.

Multiple dose studies

Mice were randomized into four treatment groups ($n=12$/group) and ARL 12495AA was administered (i.p.) in doses of 10, 25, 50 and 75 mg kg^{-1}. A fifth group (control) received vehicle (0.9% saline) alone. Treatment was continued once daily for 5 days. At 6 h after the final dose, the animals were killed and their brains removed. Brains were divided into two hemispheres by a saggital incision and stored at $-70°C$ until required.

Neurochemical assays

Six left hemispheres from each group (control and drug treatments) in each phase (single and multiple dose) were assayed for GABA concentrations. Six right hemispheres from each group in each phase were employed for the simultaneous determination of glutamate and glutamine concentrations. The remaining six left hemispheres were assayed for GABA-T activity and the remaining six right hemispheres were employed for determination of GAD activity.

Drugs

ARL 12495AA ((±)-1-methyl-1,2-diphenylethylamine) was obtained from Astra Charnwood (Loughborough, U.K.) and was prepared daily for intraperitoneal (i.p.) injection in 0.9% saline to varying concentrations for uniformity of injection volume.

Radiolabelled GABA (γ-[^{14}C(U)]-aminobutyric acid) was obtained from NEN Research Products (Stevenage, U.K.). All chemicals (reagent grade) were obtained from Sigma Chemical Company (Poole, U.K.) and solvents (h.p.l.c. grade) were purchased from Rathburn Chemicals Ltd. (Walkerburn, U.K.).

Statistical methods

Statistical analysis was performed by use of MINITAB for Windows statistical package (Version 10.1) on a Viglen 4DX266 microcomputer. All results were calculated as the percentage of mean control values. Group results were then expressed as mean percentages ± s.e.mean. Statistical differences from control were determined by one way analysis of variance with Dunnett's correction for multiple comparisons.

Results

Amino acid concentrations

Single dose and repeated treatments with ARL 12495AA were without effect on the concentrations of both GABA (Figure 1) and glutamine (Figure 2) in mouse brain at 6 h post-administration. Single dose treatment with ARL 12495AA was similarly without effect on mouse brain glutamate concentrations (Figure 3). However, following repeated treatment with higher doses (50 and 75 mg kg^{-1}), there was a trend ($P=0.10$) toward reduced glutamate levels (Figure 3).

Enzyme activities

Single doses of ARL 12495AA were without effect on the activities of both GABA-T (Figure 4) and GAD (Figure 5) at 6 h post-administration. Repeated drug treatments significantly ($P<0.05$) increased the activity of GABA-T (Figure 4) and significantly ($P<0.05$) reduced the activity of GAD (Figure 5) at 6 h after the final dose.

Discussion

RMD is a promising new anticonvulsant drug (Leach & Brodie, 1995) with a wide range of activity in animal seizure models (Stables *et al*., 1995) and has provided encouraging evidence of efficacy in man (Palmer *et al*., 1993). Current opinion suggests that the drug acts, at least in part, through an active desglycinyl metabolite, ARL 12495AA (Heyn *et al*.,

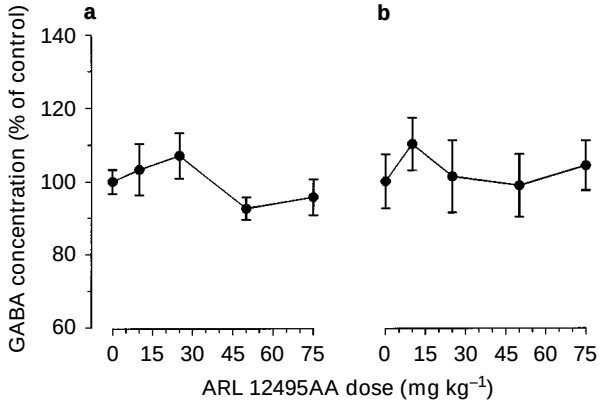

Figure 1 Effect of ARL 12495AA (0–75 mg kg^{-1}) on mouse brain GABA concentration at 6 h after acute (single dose; a) and chronic (once daily for 5 days; b) administration. Results ($n=6$) are expressed as the percentage of individual control values and vertical lines denote s.e.mean.

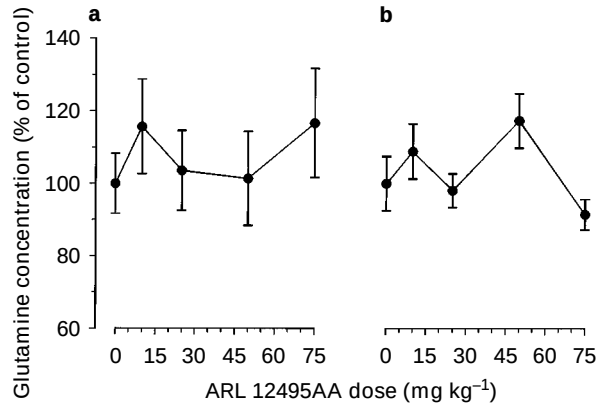

Figure 2 Effect of ARL 12495AA (0–75 mg kg^{-1}) on mouse brain glutamine concentration at 6 h after acute (single dose; a) and chronic (once daily for 5 days; b) administration. Results ($n=6$) are expressed as the percentage of individual control values and vertical lines denote s.e.mean.

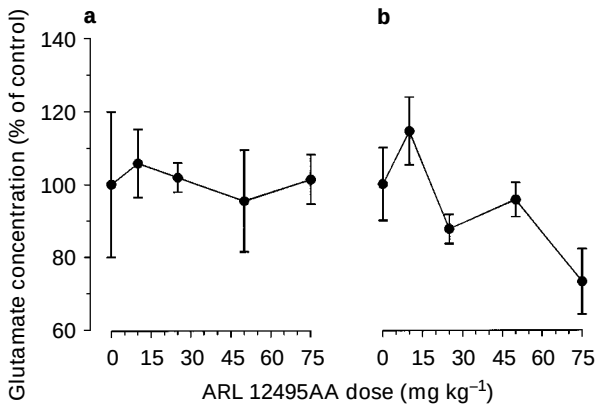

Figure 3 Effect of ARL 12495AA (0–75 mg kg^{-1}) on mouse brain glutamate concentration at 6 h after acute (single dose; a) and chronic (once daily for 5 days; b) administration. Results ($n=6$) are expressed as the percentage of individual control values and vertical lines denote s.e.mean.

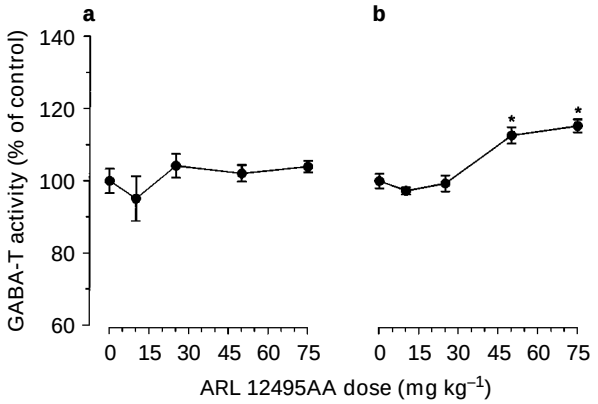

Figure 4 Effect of ARL 12495AA (0–75 mg kg^{-1}) on mouse brain GABA-T activity at 6 h after acute (single dose; a) and chronic (once daily for 5 days; b) administration. Results ($n=6$) are expressed as the percentage of individual control values and vertical lines denote s.e.mean. Statistical significance ($^{*}P<0.05$) was determined by ANOVA with Dunnett's correction.

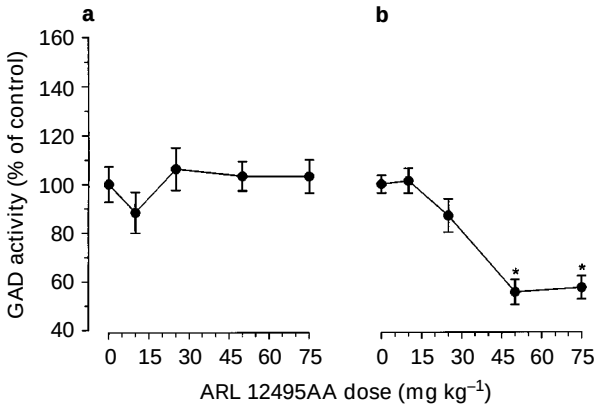

Figure 5 Effect of ARL 12495AA (0–75 mg kg^{-1}) on mouse brain GAD activity at 6 h after acute (single dose; a) and chronic (once daily for 5 days; b) administration. Results ($n=6$) are expressed as the percentage of individual control values and vertical lines denote s.e.mean. Statistical significance ($^{*}P<0.05$) was determined by ANOVA with Dunnett's correction.

1994), and exerts its antiepileptic action via an antagonism of both the voltage-sensitive sodium channel (Wamil *et al*., 1996) and the NMDA subtype of glutamate receptor (Hu & Davies, 1995). We have investigated the effects of single and repeated treatment with the desglycinyl derivative of RMD on several GABA-related neurochemical parameters in mouse brain. This approach, arguably applicable to the investigation of the mechanism of action of all AEDs, has been employed in our laboratory to highlight previously unreported neurochemical effects of gabapentin (Leach *et al*., 1997b), vigabatrin and tiagabine (Leach *et al*., 1997c).

Single and repeated treatments with ARL 12495AA were without effect on the concentrations of GABA and glutamine in mouse brain. However, a trend towards reduced mouse brain glutamate levels was observed following repeated administration of the drug. The contribution of this effect, which just failed to reach statistical significance at higher drug doses, to the mechanism of RMD action is difficult to ascertain. It is possible that subtle changes in glutamate concentration were masked by the tissue volume employed. The use of techniques which afford greater regional resolution, such as microdissection and microdialysis, may help to determine the relevance of this effect more clearly. To date, RMD and ARL 12495AA have demonstrated effects on glutamate receptors (Subramaniam *et al*., 1996) and glutamate release (Srinivasan *et al*., 1995), respectively. These, together with a potential to reduce glutamate levels in the brain, may further advocate the use of the drug not only in epilepsy, but in many other conditions, such as cerebral ischaemia, Huntington's disease and amyotrophic lateral sclerosis, in which glutamate excitotoxicity has been implicated.

Repeated treatment with ARL 12495AA significantly increased brain GABA-T activity and reduced the activity of GAD. The effect on GABA-T activity, with an increase to 115% of control, was notably more modest than the inhibition of GAD, which was reduced to 55% of control values. One might postulate that ARL 12495AA has a direct inhibitory action on GAD. RMD has been implicated as an inhibitor of cytochrome P450 subfamily 3A4 (Leach *et al*., 1996; 1997a). The smaller increase in GABA-T activity remains unexplained. However, it is conceivable, that ARL 12495AA, like gapapentin (Goldlust *et al*., 1995), has specific effects on other enzymes of the GABAergic system

One perhaps surprising observation was the failure to demonstrate a significant reduction in brain GABA levels, despite the potentiation of GABA-T and inhibition of GAD. Further studies in isolated enzyme preparations and cell cultures are required to clarify this apparent anomaly. Until such time, these results could be interpreted to suggest that RMD compromises GABAergic neurotransmission and, as such, may be the basis for the proconvulsant effect observed at high drug doses in other preclinical studies (Palmer *et al*., 1991; 1993). Interestingly, a clinical proconvulsant effect is well recognised with high dose phenytoin (Osorio *et al*., 1989; Reynolds 1989; Murphy *et al*., 1991), the drug from which RMD was developed and with which it shares a common mechanism of action and a similar experimental anticonvulsant profile.

The clinical relevance of these previously described actions of ARL 12495AA is difficult to ascertain. They were manifested following repeated treatment with the pure desglycinyl metabolite at doses higher than those employed clinically with RMD itself. In addition, it is impossible to say whether such a compromise of GABAergic neurotransmission would, in fact, be proconvulsant. Gale (1989, 1992) demonstrated clearly that potentiation of GABAergic transmission with localized injections of vigabatrin into the brain could result in either seizure protection or seizure propagation depending upon the site of injection. Finally, it must also be noted that most, if not all, AEDs would appear to have multiple neurochemical effects (Leach *et al*., 1997b, c). Although these are often manifested at different drug concentrations, it is difficult to claim which effect is the most relevant to the clinical action of any particular compound.

In conclusion, repeated treatment with high doses of the desglycinyl derivative of RMD appears to reduce mouse brain glutamate concentration but also potentiates GABA-T activity and inhibits GAD. These results would suggest that, at a high dose, RMD might act as a proconvulsant. It is clear that further, more detailed studies of the mechanism of RMD action are required to disseminate the individual components of the neurochemical response to this drug and to determine which of these components is relevant to its clinical activity.

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