Biochemical Pharmacology, 1965, Vol. 14, pp. 1679–1681. Pergamon Press Ltd., Printed in Great Britain.

SHORT COMMUNICATIONS

The interaction of α- and β -L-Aspartyl-L-Glutamic Acid and of their N-Acetly derivatives on central neurones and certain isolated peripheral tissues

(*Received* 25 *May* 1965; *accepted* 13 *July* 1965)

AUDITORE and Hendrickson[1] recently claimed to have isolated a substance from human brain other than acetylcholine which had activity similar to acetylcholine on the frog rectus abdominis and guinea pig ileum. Their evidence indicated that the active compound was an acyl derivative, probably N-acetyl-, of the dipeptide aspartylglutamic acid. No information about either the optical configuration or mode of peptide linkage was given. Subsequently the isolation of N-acetylaspartylglutamic acid both from rabbit and from horse brain was reported.[2]

We have now investigated the action of both the α- and β- isomers of L-aspartyl-L-glutamic acid and of their N-acetyl derivatives on the frog rectus abdominis, on the guinea pig ileum and on the

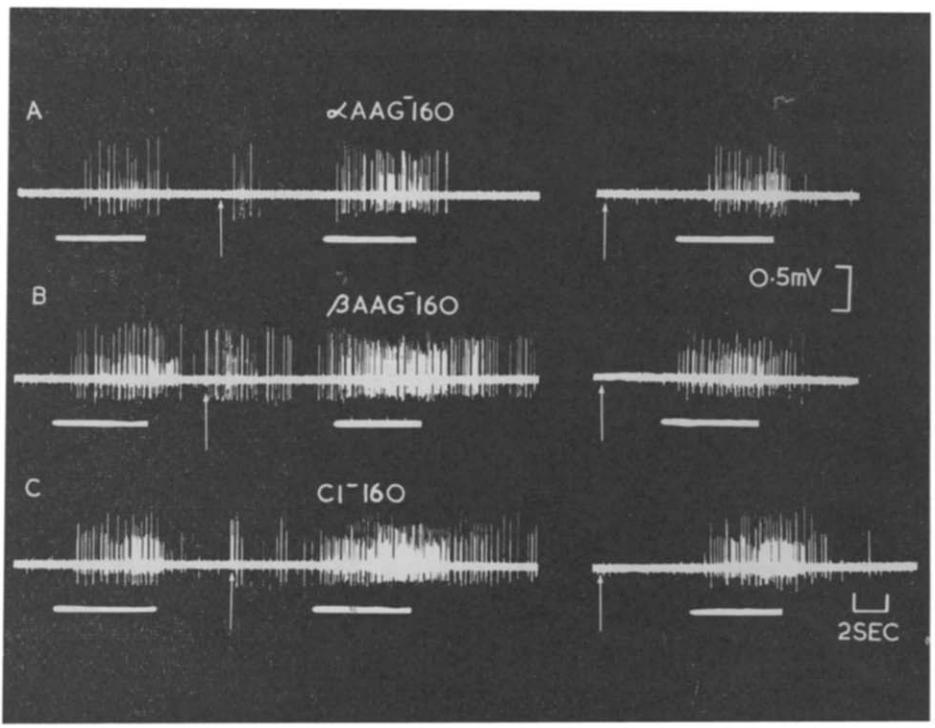

FIG. 1. Extracellular spike responses from two units at 0·8 mm in the piriform cortex. L-Glutamate (32 nA) was applied for 5 sec, horizontal line. Test applications were made for 30 sec periods between the arrows.

A. α-acetylaspartylglutamate, 160 nA (α AAG⁻ 160)
B. β-acetylaspartylglutamate, 160 nA (β AAG⁻ 160)
C. a tip negative current of 160 nA through the NaCl barrel (Cl⁻160)

The gap in each record is of 15 sec and the last response was taken 5 sec after the test application. The changes in excitability may be accounted for in terms of current effects.

nA = nanoampères.

neurons of the cat piriform cortex. The N-acetyl derivatives of the free peptides (kindly given to us by Dr. G. T. Young of Oxford University) were prepared using acetic anhydride at 0° and purified with Zeocarb 225 in the hydrogen form. The purity of both the free and acetylated peptides was confirmed by automatic titration and paper electrophoresis.

Our results indicate that neither the α- nor the β- isomers of the dipeptides nor their N-acetyl derivatives could have been responsible for the activity on the frog rectus and on the guinea pig ileum observed by Auditore and Hendrickson. Neither of the tissues responded to amounts of the dipeptide (neutralised to pH 7 before use) or of their acetylated derivatives ranging from 20 to 500 μg. They also did not appear to potentiate or depress the usual responses of these tissues to acetylcholine.

It seems unlikely, too, that either the free or the acetylated peptides have the requisite properties to be central transmitter substances. Tested on 22 cells of the cat's piriform cortex, the free peptides, applied by the iontophoretic technique[3, 4, 5] were without effect. On 22 out of 27 other cells the acetylated dipeptides were again without effect, as illustrated in Fig. 1. This particular cell was quiescent but could be excited by L-glutamate. The initial excitation and potentiation of L-glutamate-induced firing by the α- and β- acetylpeptides (Records A and B) was not significantly different from that of the current control (Record C). On 5 cells, however, a weak excitant action was seen as is illustrated in Fig. 2. These units were excited by L-glutamate but not by a negative (chloride ion) current (Record A). The record at B shows excitation by both the peptides but at a low level compared to that of L-glutamate. These results do not suggest any possibility that their action was comparable to that of a neurotransmitter.

We conclude that neither the acetylated derivatives nor the free peptides have any significant

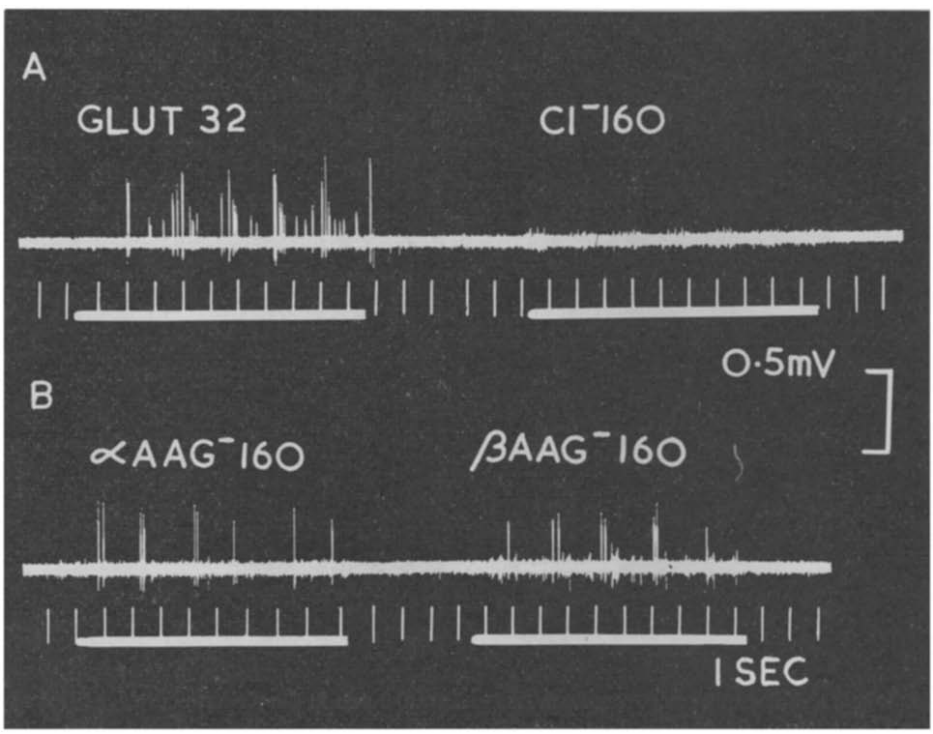

FIG. 2. Extracellular spike potentials from units at 0·4 mm in the piriform cortex. Drugs applied for 10 sec periods at the horizontal lines. At A, when L-glutamate (GLUT 32) was applied there was unit firing within 2 sec. A current of 160 nA tip negative through the NaCl barrel ($Cl^{-}160$) was without effect. In B, (record continued) α-acetylaspartylglutamate, 160 nA (α $AAG^{-}160$) caused excitation of the large unit after a short delay. Similarly when β- acetylaspartylglutamate 160 nA (β $AAG^{-}160$) was applied there was also excitation.

nA = nanoampères.

excitation action on either the neurones or on the muscle preparations we tested. One explanation of the results of Auditore and Hendrickson which occurs to us is that they may have tested their isolated peptide without neutralising it. The synthetic compounds were very acidic and, when tested on the rectus abdominus without first being neutralised, were found to cause contractions in the same manner as other acidic solutions will do. The neutralised peptides, however, has no activity.

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Biochemical Pharmacology, 1965, Vol. 14, pp. 1681–1683. Pergamon Press Ltd., Printed in Great Britain.

Reduction of the toxicity of radiomimetic alkylating agents in rats by thiol pretreatment—V. The effect of thiol pretreatment on the anti-tumour action of Merophan

(*Received* 4 *March* 1965; *accepted* 13 *July* 1965)

IT IS well established that many thiols can protect animals against the toxic effects of certain tumour inhibitory nitrogen mustards[1–4] Combined treatment of thiol and nitrogen mustard would only be of advantage in cancer chemotherapy over treatment with nitrogen mustard alone if the protection given to the animal by the thiol were greater than the protection given to the tumour. The selectivity of anti-tumour action of the nitrogen mustard as judged by therapeutic index would remain unaltered if host protection was paralleled by protection to the tumour. Peczenic[5] and Therkelsen,[6] and recently Rutman,[7] have shown that for certain tumours the therapeutic effectiveness of HN2 can be increased by pretreating the animals with either cysteamine or 3 amino mercaptobutane.

The effect of either A.E.T. or cysteine pretreatment on the therapeutic index of the aromatic nitrogen mustard Merophan (*o*-di-2-chloroethylamino-DL-phenylalanine) measured in rats bearing the Walker carcinoma and mice bearing the ADJ/PC5 plasma cell tumour has been investigated.

MATERIALS AND METHODS

The plasma cell tumour was implanted into BALB/C$^-$ mice as previously described.[8] Merophan was given as a single i.p. dose ten days after transplantation, when the tumours measured between 4 × 4 and 8 × 8 mm. Ten days after the injection the animals were killed and the tumours weighed. Treated tumour weights were expressed as a percentage of control tumour weights and these percentages plotted against log dose. The dose to inhibit tumour growth to 10 per cent of the control value (90 per cent inhibitory dose, $I.D._{90}$) was obtained by interpolation on the curve. The LD_{50} was similarly obtained by plotting the percentage survivors against log dose. The therapeutic index, which is a measure of the selectivity of the drug or drug combination, was the ratio LD_{50}/ID_{90}. The dose ratio was 1·5 for mice and 2 for rats. The dose range was sufficient to include the LD_{100} and ID_{90} in the one experiment.

The Walker tests in Chester Beatty male albino rats* were carried out in the same manner except that Merophan was injected the day after transplantation. Each point on the survival and tumour inhibition curves was obtained by treatment of groups of 6–10 animals. Thiols were given intraperitoneally 30 min before the nitrogen mustard except where otherwise stated. All compounds were given in aqueous solution adjusted to pH 7·0. For measurement of non-protein SH levels a sample

* Carried out under the supervision of F. J. C. Roe and B. C. Mitchley.