Effects of \( \omega \)-Amino Acids and Related Compounds on Staphylococcal Infections in Mice: a Combined Prophylactic-Therapeutic Procedure

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Received for publication 21 January 1970

By a short-term combined prophylactic-therapeutic procedure, the following compounds were found to be active against staphylococcal infections in Swiss mice: \( \gamma \)-aminobutyric acid, \( \gamma \)-amino-\( \beta \)-hydroxybutyric acid (GABOB), \( \delta \)-amino-valeric acid (DAVA), \( \epsilon \)-aminocaproic acid (EACA), trans-4-aminomethylcyclohexanecarboxylic acid (trans-AMCHA), taurine, and cysteic acid. Many of these compounds had displayed limited or no activity by a previously used prophylactic procedure. Although DAVA and GABOB were the most potent of the straight-chain \( \omega \)-amino acids, trans-AMCHA displayed the greatest antistaphylococcic activity of the \( \omega \)-amino acids thus far investigated. Homocarnosine (\( \gamma \)-aminobutyrl histidine, which also was active by the prophylactic procedure) equalled trans-AMCHA in activity. Taurine was similar in potency to DAVA, and the activity of cysteic acid approximated that of EACA.

Although many antibiotics have been employed for the treatment of staphylococcal infections, such diseases continue to be a problem. The development of multiple antibiotic-resistant strains of the organism suggests the desirability of investigating other means of combating the infection.

Nutini and Lynch (7, 8) reported that a crude extract of bovine brain protected mice against infection by \textit{Staphylococcus aureus}. In a series of experiments designed to isolate the active compounds, ion-exchange chromatography indicated the presence of three groups of active substances which were distributed among the acidic, neutral, and basic fractions of the amphoteric compounds in the brain extract (2). Analytical studies of the extract by the automatic amino acid analyzer, paper chromatography, thin-layer chromatography, high-voltage electrophoresis, microbiological assay, etc., revealed the presence of 18 \( \alpha \)-amino acids as well as other ninhydrin-positive compounds, including cysteic acid, taurine, \( \beta \)-alanine, \( \gamma \)-aminobutyric acid (GABA), and some amino acid derivatives and peptides (K. Tanaka et al., Abstr., 17th Annual Meeting, Chem. Soc. Japan, p. 283, 1964). Further studies with the amino acid analyzer and an Amberlite CG-120, 2,6-lutidine column followed by a diazo-coupling reaction confirmed the presence of carnosine and homocarnosine in the bovine extract (11, 12).

In the earlier experiments, the test chemicals were given to mice subcutaneously for a period of 5 days before the infection, with an interval of 6 to 24 hr between the last drug injection and the organism challenge. This method is called "long-term" treatment. The bovine brain extract and homocarnosine, a dipeptide in the basic fractions of the extract, were both shown to have potent antistaphylococcic activity by this method, but GABA, which was the major component in the neutral fractions, was ineffective under these conditions in Swiss mice (6, 11). This might have been due to the rapid excretion of GABA, since Takada et al. (10) and Mori (5) had reported that most of the \( \omega \)-amino acids disappeared from the blood stream within 6 hr after their subcutaneous administration. Later this was confirmed in our laboratory by giving the chemicals subcutaneously to rabbits. Consequently, we have now employed a "short-term" technique in which the test chemicals were given to mice shortly before and after infection. By this method, GABA displayed
effectiveness. The present paper also gives the results of a series of ω-amino acids and related compounds, among which taurine and cysteic acid were found to be active antistaphylococcal factors in the acidic fractions of bovine brain extract.

MATERIALS AND METHODS

Organism. The strain of S. aureus used in the present investigations and termed "original" strain was isolated in our laboratory from an infected tonsil and has been maintained in the lyophilized state. It is penicillin-resistant, is highly chromogenic, ferments a number of sugars, including mannitol, mannose, maltose, lactose, galactose, glucose, and fructose, and produces coagulase, catalase, gelatinase, deoxyribonuclease, phosphatase, urease, and ω-toxin.

Culture. Culture conditions were standardized, and the third subculture from the lyophilized mother culture was used. The subcultures were grown at 37° C for 24 hr in Staphylococcus Medium 110 (DiCo). The organisms from the third subculture were twice washed and suspended in TC Tyrode Solution (DiCo), and the concentration was adjusted turbidimetrically, with a nephelometer, for injection into animals. The transmission levels on the scale of the instrument were taken as a reference of the density of the suspensions and were correlated with viable bacterial counts. Animals were inoculated subcutaneously with 0.5 ml of a suspension having 70% transmission or 2 × 10^8 organisms by count. This dosage was approximately 1.5 times the LD₅₀.

Animals. Swiss albino female mice maintained on the Rockland diet, ranging in age from 10 to 14 weeks old and in weight from 18 to 23 g, were used in all experiments. All mice were randomized for individual experiments. These mice were propagated in our laboratory from stock originally obtained from Texas Inbred Mouse Co., Houston, Tex.

Treatment. The chemicals under investigation, most of which were procured from Nutritional Biochemical Corp., were administered by two methods. The long-term method used in the present work was a prophylactic procedure employed by many of the early investigators in our laboratory. It consisted of the daily subcutaneous inoculation of the test material over a period of 5 days into the experimental animals, followed 6 hr after the last treatment with a subcutaneous inoculation of organisms. In some of the more recent work, the drug was administered over 3 days with a 24-hr interval before challenge (6, 11). The short-term method of the present paper was a combined prophylactic and therapeutic procedure in which half of the total dose of test material was given subcutaneously 2 hr before and the second half 4 hr after the subcutaneous challenge with organisms. The mortality of animals was recorded for a period of 4 days postchallenge.

RESULTS

Comparative studies of short- and long-term procedures. Table 1 shows the results of antistaphylococcal activity of homocarnosine, GABA, and related compounds. In this table, long- and short-term effectiveness are compared in terms of per cent protection [(mortality control)-(mortality treated)/(mortality control) × 100] on the 4th day after infection with S. aureus. All of the chemicals tested were found to give greater antistaphylococcal protection and more constant results by the short-term procedure than by the long-term one. The greater short-term activity was particularly noticeable for trans-4-Aminomethylcyclohexanecarboxylic acid (trans-AMCHA) but was significant for the other compounds.

Activity of GABA. The antistaphylococcal activity of 5 mg of GABA was lower than that of the same amount of homocarnosine by both the long- and short-term techniques. However, the concentration of GABA in brain extract was found to be 12.8 μg per mg (dry weight) of extract (automatic amino acid analyzer), about 46 times the homocarnosine content of 0.28 μg per mg [by modified McManus (3) method]. Thus, GABA may play a part in the activity of brain extract. The better protection afforded by higher levels of GABA is illustrated in Table 2.

Activity of ω-amino acids and related compounds. Table 3 shows the results of further investigations of the active compounds identified in bovine brain extract, as well as the synthetic compounds, with the short-term procedure. ω-Aminovaleric acid (DAVA) was the most potent compound in a series of the single straight-chain ω-amino acids, and GABA had essentially the
same activity as \(\epsilon\)-aminocaprylic acid (EACA), which was less active than DAVA. Neither \(\beta\)alanine nor glycine had any significant activity.

The synthetic amino acid, AMCHA, has been introduced as a potent antifibrinolytic agent by Mangyo (4), Okamoto and Okamoto (9) and Dubber et al. (1). They found that trans-AMCHA was a more potent antifibrinolytic agent than either EACA or a mixture of trans and cis forms of AMCHA. In our laboratory, trans-AMCHA also proved to be the most active antistaphylococcal agent tested by the short-term procedure (Table 4). It is to be noted that the histidine peptides of the \(\omega\)-amino acids were markedly more active than the corresponding \(\omega\)-amino acids: glycylhistidine > glycine, \(\beta\)alanylhistidine > \(\beta\)-alanine, and \(\gamma\)-aminobutyrylhistidine > GABA (Table 3; A. Fujii, Y. Tsuchiya, and K. Tanaka, Abstr., 158th National Meeting, American Chemical Society, New York, N.Y., September 1969, no. 50 MEDI).

**Activity of taurine.** The results in the preceding section demonstrated the dependence of antistaphylococcal activity on chemical structure. They suggested that taurine and cysteic acid in the acidic fractions of brain extract might have some activity since these compounds possess an \(\omega\)-amino residue with a sulfonic residue in place of a carboxyl. Table 5 shows the results of experiments using taurine and cysteic acid in the short-term procedure. The levels of protection obtained by 5 mg of taurine and cysteic acid per animal were 53 and 37\%, respectively. Thus, both taurine and cysteic acid were also demonstrated to be active antistaphylococcal factors in the acidic fraction of the brain extract.
### Table 4. Antistaphylococcal activity in mice of e-aminocaproic acid (EACA) and aminomethylcyclohexanecarboxylic acid (AMCHA) (short-term procedure)

| Compound | No. of mice | Per cent protection on 4th day | Per cent mortality on 4th day<sup>b</sup> |
|----------|-------------|--------------------------------|------------------------------------------|
|          |             | Mean | sd | Mean | Upper limit | Lower limit | Mean | Upper limit | Lower limit |
| EACA     | 48          | 33   | 7  | 90   | 95          | 77         | 60   | 71          | 48         |
| Mixture of cis- and trans-AMCHA | 20 | 50 | 7 | 90 | 95 | 63 | 45 | 64 | 31 |
| trans-AMCHA | 79 | 71 | 5 | 89 | 94 | 81 | 26 | 34 | 21 |

<sup>a</sup> Dose, 5 mg per mouse.  
<sup>b</sup> F (0.05).

### Table 5. Antistaphylococcal activity in mice of taurine and cysteic acid (short-term procedure)

| Compound | No. of mice | Per cent protection on 4th day | Per cent mortality on 4th day<sup>b</sup> |
|----------|-------------|--------------------------------|------------------------------------------|
|          |             | Mean | sd | Mean | Upper limit | Lower limit | Mean | Upper limit | Lower limit |
| Taurine  | 60          | 53   | 3  | 83   | 90          | 73         | 35   | 47          | 32         |
| Cysteic acid | 59 | 37 | 5 | 83 | 90 | 73 | 51 | 62 | 42 |

<sup>a</sup> Dose, 5 mg per mouse.  
<sup>b</sup> F (0.05).

### DISCUSSION

A short-term combined prophylactic-therapeutic procedure, employed on the basis of physiological effects and activities related to the rate of absorption, distribution, and excretion, demonstrated the effectiveness of GABA, DAVA, EACA, and especially trans-AMCHA as antistaphylococcal agents, although in the earlier reports (6, 11) none of these chemicals showed significant activity by a strictly prophylactic procedure. The activity of these materials in the short-term procedure would seem to depend upon their presence in the host system. Treatment in the early stages of subcutaneous staphylococcal infection is essential because the majority of deaths occur within 24 hr, with a few on the 2nd day, after which the increment of mortality drops appreciably (6, 11).

Although homocarnosine was very effective in the short-term experiments, it also protected in the long-term ones as well, as previously reported (6, 11, 12). Bovine brain extract, which contains carnosine and homocarnosine together with β-alanine and GABA (11, 12), was equally effective in both short- and long-term procedures. Thus, the relatively rapidly excreted ω-amino acids display short-term anti-infectious activity, whereas their histidine peptides have both short- and long-term activity. If the simple ω-amino acids are the active agents in vivo, the longer-lasting activity of the peptides (11, 12; unpublished data) might be due to the liberation of the ω-amino acids by the slow hydrolysis of the peptides in the body. In unpublished experiments, oral administration to mice of 10 mg of homocarnosine in divided doses (6 hr before and 6 hr after challenge with S. aureus) resulted in 100% protection, whereas oral administration of a similar quantity of trans-AMCHA failed to provide protection. Further pharmacological studies will be necessary to resolve the question of short-term versus long-term activity.

All of the active compounds thus far investigated possessed (i) a carboxylic or sulfonic residue and (ii) an ω-amino group. The length of the carbon chain or cyclohexane ring between basic and acidic groups seemed to determine the degree of activity. DAVA was the most active compound among the straight-chain ω-amino acids, being superior to GABA and EACA. trans-AMCHA, with similar molecular length, was the most potent of the simple ω-amino acids. The problem of chemical structure has been dealt with elsewhere (A. Fujii, Y. Tsuchiya, and K. Tanaka, Abstr.,...
158th National Meeting, American Chemical Society, New York, N.Y., September 1969, no. 50 MEDI).

Application of the short-term treatment and the general idea of the chemical structure of active compounds led to the present finding that taurine and cysteic acid were among the antistaphylococcal factors in the acidic fraction of the brain extract. The α-amino acids tested had little or no activity. There is some evidence to suggest that there may be as yet unidentified active factors in the brain extract. Our present information allows us to suggest that taurine in the acidic fraction, GABA in the neutral fraction, and homocarnosine in the basic fraction are among the main active antistaphylococcal principles in brain extract.

The antistaphylococcal activity of these chemicals may be connected with host-defense mechanisms in some way because none of the chemicals showed significant bactericidal and bacteriostatic activity in vitro.

ACKNOWLEDGMENTS

We thank Daiichi Seiyaku Co., Ltd., for gifts of AMCHA and trans-AMCHA. We are also grateful to Sperti Drug Corp. and the Lt. David Van Alstyne III Foundation for grants in aid.

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