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Research report

Behavioral effects of alpha-alkylated amino acid analogs in the C57BL/6J mouse

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HIGHLIGHTS

- AAAA are capable of neuromodulating effects.
- AIB and AEPG exhibit anxiolytic-like effects at lower dosage.
- Pharmacological properties of novel amino acid analogs are presented.

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ABSTRACT

Although a series of amino acid analogs have been shown to modulate brain function, information on the pharmacology of alpha-alkylated amino acids (AAAA) is limited. In particular there is no information on the effect of these amino acid analogs (AAA) on the elevated plus maze, the tail suspension test and the forced swim test. It was therefore the aim of the study to test a series of AAAA in these paradigms in order to explore behavioral activities of this compound class.

10 male mice per group aged between 10 and 14 weeks were used. Vehicle-treated controls were used in addition to intraperitoneal injections of 1, 10 and 100 mg/kg body weight of each, alpha-amino-isobutyic acid (AIB), isovaline (IVA), alpha-propyl-alanine (APA), alpha-butyl-alanine (ABA), alpha-pentyl-alanine (APnA), alpha-ethylphenylglycine (AEPG) and alpha-methyl-valine (AMV). The elevated plus maze (EPM), the tail suspension test (TST) and forced swim test (FST) were used for behavioral testing.

There were dose-dependent results: all compounds increased time and pathlength in the open arm of the EPM at least at one dose administered. In the TST and in the FST only the 100 mg dose was showing an effect.

The results show pharmacological activity modifying the EPM in low doses suggesting the use in treatment of behavioral traits and symptoms represented by or linked to the EPM including anxiety-related behavior including depression. Compounds acting at higher doses may be used to induce behavioral changes and thus serve as neurobiological-neuropharmacological tools.

1. Introduction

Amino acid analogs (AAA) are valuable tools for synthetic chemistry modifying pharmacological and chemical properties of peptides and proteins [1–4] and also serve as probes in neuropharmacology [5]. A series of AAA were shown to have biological and pharmacological properties and have been reported to cross the blood brain barrier [6], taken up by brain cells [7] and modify brain functions. Modulation of brain functions include changes of analgesic properties [8–10]; antagonism of allodynia [11] and AAA are changing feeding behavior [12–14], improve cognitive functions [15] or show convulsive [16], anticonvulsive [17] or hypnotic properties [18], mediate brain damage [19] or are neuroprotective [20].

Moreover, AAA were reported to bind to brain receptors including metabotropic [11,20–22], GABA [9,23], and NMDA receptors [12,24] or bind and modulate transporters of neurotransmitters [25].

Abbreviations: AAAA, alpha alkylated amino acids; AAA, amino acid analogs; AIB, alpha-amino-isobutyic acid; IVA, isovaline; APA, alpha-propyl-alanine; ABA, alpha-butyl-alanine; APnA, alpha-pentyl-alanine; AEPG, alpha-ethylphenylglycine (AEPG); AMV, alpha-methyl-valine (AMV); EPM, elevated plus maze; TST, tail suspension test; FST, forced swim test.

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Synthetizing and testing alpha-alkylated amino acid analogs (AAAA) for biological and pharmacological properties has been carried out in our laboratories before [26] and herein we tested alpha-alkylated amino acid analogs (AAAA) alpha-amino-isobutyric acid (AIB), isovaline (IVA; syn.: α-ethylalanine, 2-ethylalanine, (*R,S*)-amino-2-methylbutanoic acid), alpha-propyl-alanine (APA), alpha-butyl-alanine (ABA), alpha-pentyl-alanine(APnA), alpha-ethylphenylglycine (AEPG) and alpha-methyl-valine (AMV) for neuropharmacological effects in order to modulate behavior in three paradigms related to anxiety-related behavior, despair and depression [27]. For this purpose we selected the use of the elevated plus maze (EPM), the tail suspension test (TST) and the forced swim test (FST) that are well-documented to be used in the mouse system. Three different doses were applied intraperitoneally (IP) and indeed, these pharmacological treatments modified mouse behavior in a dose-dependent manner.

2. Materials and methods

2.1. Chemicals

AIB (syn.: α-aminoisobutyric acid, 2-methylalanine; 2-amino-2-methylpropanoic acid) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

Synthesis and purification of the other analogs was carried out in our laboratory according to the STRECKER procedure from the respective ketones and sodium cyanide and acidic hydrolysis of the resulting nitriles[28].

Purity of α,α-dialkyl α-amino acids was tested by various chromatographic techniques including thin-layer chromatography, high-performance liquid chromatography, gas-chromatography and ion-exchange chromatography [7,29].

2.2. Animals

Male C57BL/6J mice were used for the studies. Mice used were aged between 10 and 14 weeks because at this age development is complete and the aging processes are far from being started. Ten mice per group were used for this study. All mice were purchased from Charles River Laboratories (Germany) and maintained in cages made of makrolon and filled with autoclaved woodchips in the Core Unit of Biomedical Research, Division of Laboratory Animal Science and Genetics, Medical University of Vienna. An autoclaved standard rodent diet (Altromin®, Germany) and water in bottles was available ad libitum. The room was illuminated with artificial light at an intensity of about 200 lx in 2 m from 5 am to 7 pm. behavioral tests were performed between 8:00 h and 13:00 h.

All procedures were carried out according to the guidelines of the Ethics committee, Medical University of Vienna, and U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Federal Ministry of Education, Science and Culture, Austria (BMWF-66.009/0267-II/3b/2012). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.3. Behavioral studies

2.3.1. Elevated plus maze (EPM)

Mice were observed for anxiety-like behavior. The maze consisted of 4 arms (each 30 cm long and 5 cm wide) fixed to the height of 54 cm, and the arms were interconnected by a 5 cm × 5 cm wide central area. Two arms had 15 cm high side and end walls. Mice were observed for 5 min with video camcorder coupled to a computational tracking system in an arena. The mice were placed in the central area, head pointing away from the box. Following parameters were recorded: (a) the time spent in open arm and closed arm, (b) pathlength in open and closed arm [30].

2.3.2. Tail suspension test (TST)

TST was performed as described by [27]. TST is used to assess depression like behavior. The experiment was carried out by hanging the mouse by the tail at 70 cm above the surface. The duration of immobility (defined as the absence of all movement except for those required for respiration) was scored during 6 min. All test sessions were recorded by video camera positioned besides.

2.3.3. Forced swim test (FST)

FST was performed as described by Castagne et al. The experiment consisted of placing mice individually into a glass cylinder (25 cm tall × 10 cm in internal diameter) filled with water (23–25 °C) to a depth of 10 cm for 6 min. All test sessions were recorded by video camera positioned beside the cylinder. A mouse was judged to be immobile when making only those movements necessary to keep its head above water. The duration of immobility was scored during 6 min.

2.4. Chemical preparation and injection

All compounds were dissolved in saline and freshly prepared every day. Each animal was injected as per respective dosage group intraperitoneally (IP). The control group was injected with vehicle, saline. Animals received single IP injections 30 min prior to the test each day.

The compounds used were AIB, ISV, APA, ABA, APnA, AEPG and AMV at three individual single doses of 1 mg/kg, 10 mg/kg and 100 mg/kg intraperitoneally.

2.5. Statistical analysis

Group-wise comparisons were made by ANOVA, followed by unpaired Student's *t*-test. A probability level of $P < 0.05$ was considered as statistically significant. All calculations were performed using Graph Pad prism (http://www.graphpad.com/scientific-software/prism/).

3. Results and discussion

The major outcome of the study is the finding that dose-dependent anxiolytic-like effects by the intraperitoneal administration of low dose AAAA (1 mg/kg body weight) were observed in the EPM that has not been reported before.

3.1. Results of EPM

Anxiolytic-like effects were shown for all AAAA at various doses. Analysis of variance for results of pathlengths in the open arm of the EPM are shown in Fig. 1. All compounds showed an increase of the pathlength in the open arm at least at one dose. This effect was increasing with the administered dose in AIB and APnA treatments, while different doses exerted different effects so that in AEP only the l mg/kg body weight dose was increasing the pathlength in the open arm; higher doses were comparable to vehicle treatment. Statistical evaluation is provided in Table 1.

As shown in Fig. 2 and Table 1, the AIB and ISV-injected group spent more time in open arm as compared to the vehicle-treated group in a dose dependent manner. There was significant difference in time spent in the open arm between ISV injected animals and vehicle-injected animals. All treatments showed at all dosages that animals spent more time in the open arm but this was not related to the dose; at some doses in some treatments all doses had a comparable effect or there was no linear dose–response at all (Fig. 2).

3.2. Results of TST

As shown in Fig. 3 and Table 2 there was no significant effect of AAAA on immobility time at the low dose of 1 mg/kg. Only high AAAA doses of 100 mg/kg body weight showed reduction immobility time.

3.3. Results of FST

As in the TST only the high dose administration of all AAAA decreased immobility times in the FST as shown in Fig. 4 and Table 2.

It is intriguing that low dose AAAA given intraperitoneally showed pronounced behavioral effects in the EPM and this indeed may propose the use of this class of compounds as modifiers of mouse behavior in conditions with anxiety-like behavior. And indeed, AIB has been shown to exert effects on anxiety-related behavior at this low dose of 1 mg/kg body weight in the current study. The fact that this AAAA is not metabolizable and not a neurotransmitter [31] in the mammalian system may point to low or even absent toxicity of this compound making it an interesting candidate for further behavioral studies. The transport of AIB into the brain has been described and includes a sodium-dependent transporter system for amino acids uptake [32,33]. Our findings are, however, challenging the use of AIB as a marker for the measurement