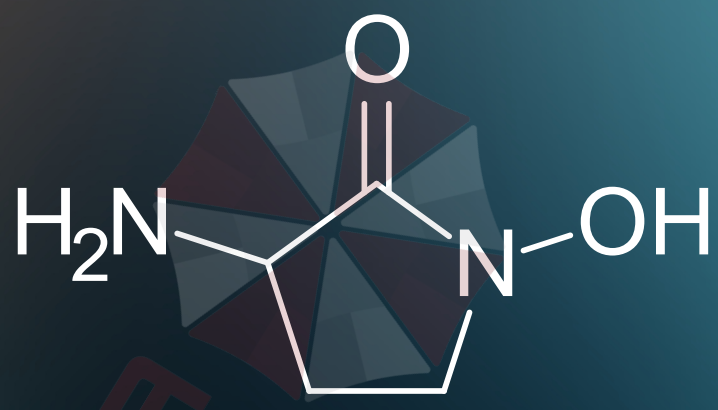


HA-966 and Its Enantiomers: Divergent Mechanisms and Behavioral Effects in Preclinical Models

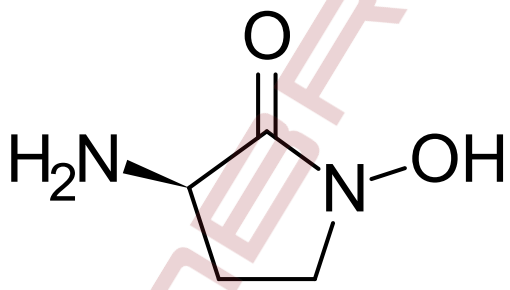
History

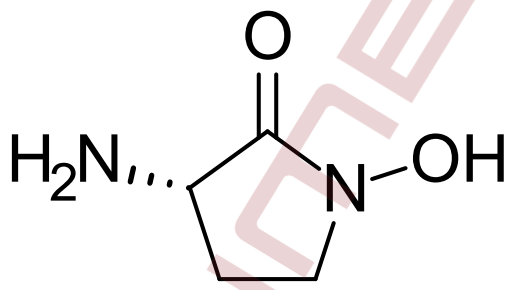
HA-966 (3-amino-1-hydroxy-pyrrolidin-2-one) is a mixture of its 2 pharmacologically distinctingly acting enantiomers. The (R)-enantiomer has a dual activity as a glycine receptor and NMDA receptor antagonist. The (S)-enantiomer has a different set of properties and is mostly known for its ataxic (movement inhibiting) effect. It was found that the R-HA-966 was primarily responsible for the desirable properties in most applications. However, the (S)-enantiomer also possesses some pharmacologically interesting properties.

The first synthesis of the compound dates back to 1959, most reports on the properties of HA-966 and its isomers date back to the 1960`s and 70`s. In the 1990`s there was a second wave of research on the distinct enantiomers of the compound in which a lot of animal experiments were conducted.[1]

Chemically, the HA-966 enantiomers are similar to the cyclic form of -amino-butyric acid (a endogenous neurotransmitter) and the chemical GBL. Both are known for their sedative properties.

From HA-966, a similar compound with the developmental name L-687,414 (CAS: 132695-96-6) was derived.





R-Enantiomer (left) and S-Enantiomer (right) of HA-966

Cell Culture Experiments

Racemic HA-966 is known to cause a rapid and selective increase in dopamine (DA) levels in certain brain areas, an effect that has been attributed to the compound's ability to block the spontaneous electrical activity in specific neurons. Systemic administration of racemic HA-966 produced a dose- dependent inhibition in the firing rate of dopaminergic neurons. The highest dose tested (40 mg/kg i.v.) completely inhibited the spontaneous activity of all cells tested. Pretreatment with naloxone (5 or 10 mg/kg i.v.) reduced the initial rate of decline in firing rate and the duration of inhibition but did not prevent a single dose of 40 mg/kg of racemic

Cyclic form of GABA (left) and GBL (right)

HA-966 from totally inhibiting dopamine cell impulse flow. Systemic administration of the (S)-enantiomer of HA-966 (30 mg/kg i.v.) inhibited neuronal activity in a manner analogous to a single injection of 40 mg/kg of racemic HA-966. Comparable doses of the (R)-enantiomer failed to affect neuronal activity in a similar manner but still affected it in a different way compared to the (S)-enantiomer.[2]

Preclinical Trials

The main action of HA-966 was found to be an antagonist at the glycine modulatory site of N-methyl- D-aspartate (NMDA) receptors. HA-966 mimics the effects of the endogenous neurotransmitter glycine and prevents these NMDA receptors (a subtype of glutamate receptors) from transmitting signals. However, glycine has a stronger binding affinity at this site.[3] This action was mainly attributed to the (R)-enantiomer. In contrast, the (S)-enantiomer was only weakly active as an NMDA-receptor antagonist, but nevertheless it possessed a marked sedative and muscle relaxant action in vivo.[4]

In addition, (R)-HA-966 also has been found to affect the dopamine metabolism of the brain in rats heavily. The serotonin system on the other hand seems to be unaffected by this enantiomer. It does not induce sedation, interfere with the rats habituation into a new environment or alter basal serotonin or cortisol levels.5 In contrast, the (S)-enantiomer was only weakly active as an NMDA-receptor antagonist, but nevertheless it possessed a marked sedative and muscle relaxant action in vivo.[5]

Social studies in rats found that racemic HA-966 had a significant pro-social and anti-anxiety effect in several tests. The results were similar to those produced by other classes of anxiolytic chemicals.[6]

In rats, pretreatment with PCP (5 and 10 mg/kg) or MK-801 (0.25 and 0.5 m/kg) dose-dependently stimulated dopamine turnover in several brain regions, but not in all. In contrast, pretreatment with (R)-HA-966 (10 and 30 mg/kg) did not affect dopamine turnover in any brain region. Pretreatment with (R)-HA-966 (10 and 30 mg/kg) significantly antagonized the stimulation of dopamine turnover induced by both PCP (10 mg/kg) and MK-801 (0.5 mg/kg). Pretreatment with PCP (3-30 mg/kg) or MK-801 (0.1-1.6 mg/kg) significantly increased locomotor activity in mice. In contrast, subcutaneous injection of (R)-HA-966 (10-100 m/kg) failed to stimulate activity. Pretreatment with (R)-HA-966 (10 and 30 mg/kg) dose-dependently antagonized both PCP (10 mg/kg) and MK-801 (0.4 mg/kg) induced hyperactivity in mice.[7]

Neuroprotective effects were also found for HA-966 under certain conditions. These were determined in rats which received NMDA, which can be neurotoxic in high concentrations. The animals received NMDA injections (15 nmol) and then were administered either racemic HA-966 (50:50 mixture of both enantiomers), or the purified (R) or (S) enantiomer 15 mins later. The (R)- enantiomer dose-dependently attenuated NMDA-induced brain injury, whereas the (S)-enantiomer was ineffective. When given intravenously to mice, racemic HA-966 and the (S)-enantiomer prevented seizures from low-intensity electroshock. Anticonvulsant effects of the (R)-enantiomer were much less potent (ED50 = 105.9 mg/kg). Induced ataxia measured was 17 times more potent with the (S)-enantiomer than with the (R)-enantiomer.[8,9] Another study confirmed to low anticonvulsant properties of the (R)-HA-966.[10]

(R)-HA-966 inhibits NMDA receptors with an IC50 of 12.5 µM, whereas (S)-HA-966 had an IC50 value of 339 µM. In agreement with findings with racemic HA-966, even high concentrations of (R)-HA-966 did not completely inhibit NMDA responses, suggesting that (R)-HA-966 is a low-efficacy partial agonist in the glycine binding pocket of the NMDA receptor. The sedative/ataxic effect of racemic

HA-966 was mainly attributable to the (S)-enantiomer, which was >25-fold more potent than the (R)-enantiomer. It is suggested that, as in the case of the sedative y-butyrolactone, disruption of dopaminergic mechanisms may be responsible for this action.[4]

Anxiolytic agents disinhibit suppressed behaviors in rodents in preclinical models of anxiety. (R)-HA- 966 significantly disinhibits both non-conditioned and conditioned behavior similar to the benzodiazepine diazepam (a common sedative and anxiolytic chemical), while the S enantiomer was devoid of anxiolytic activity and only produced behavioral sedation. Furthermore, (R)-HA-966 lacked side-effects in rodents commonly associated with the administration of benzodiazepines such as motor coordination and ataxia, significant interactions with ethanol, and amnesia. These data suggested that R-HA-966 site, was anxioselective and lacked some of the side-effects associated with benzodiazepine anxiolytics and hence made it interesting for potential clinical applications.[11] The anxiolytic effect of (R)-HA-966 was investigated in further animal models.[12]

A different study concocted in rats focused on the properties of the (S)-enantiomer. In these studies, (S)-HA-966 suppressed fear-induced behaviors: immobility and defecation. In other studies, (S)-HA- 966 (5 mg/kg i.p.) blocked acute cocaine-induced locomotion and resulted in sedation of the animals. In addition, the highest dose of (S)-HA-966 tested suppressed weight gain in control rats, unlike its enantiomer, (R)-HA-966. Because (S)-HA-966 has been proposed to act at the y-aminobutyric acid (GABA)B receptor, this study also investigated the effect of (S)-HA-966 in this receptor, but found no activation. This suggests (S)-HA-966 exerts its sedative effect via different mechanisms.[13]

In rhesus monkeys the administration of the racemic HA-966 (0.1–10 mg/kg, i.m.) 30 min before testing impaired visual test performance and recognition dose-dependently, starting at doses of 3.2 mg/kg; the memory deficit following the highest dose (10 mg/kg) was associated with longer response times.[14]

In experiments with hamsters, it was found as well that (R)-HA-966 had the potential to reduce the symptom burden of dystonia, which is a movement disorder often found in humans. R-HA-966, 30– 60 mg/kg i.p., potently reduced the severity of dystonic attacks in the hamster without inducing any behavioral adverse effects.[15]

Toxicity

Various animals received high doses of racemic HA-966 (50-100mg/kg). In most cases, this dose lead to a paralysis like state for several hours, but the animals did recover fully after several more hours. Lethal doses start at approximately 500 mg/kg in most animals.[1]

Clinical Trials

Pilot clinical trials showed that HA-966 appeared to benefit patients with tremors. Subsequent investigations at various clinical centers, however, produced some contradictory results. No more data on humans is available.[1]

Conclusion

HA-966 consists of 2 different pharmacological compounds, which are its enantiomers, (R)-HA-966 and (S)-HA-966. Their effects in animal and cell culture experiments vary wildly, meaning that one of them cannot simply be seen as a weaker form of the other, they rather two separate chemicals.

(R)-HA-966 shows the following effects in animal experiments:

- Anxiolytic
- Antagonized chemical induced hypermobility
- Suppressed weight gain

(S)-HA-966 shows the following effects in animal experiments:

- Anticonvulsant
- Sedative

It is also reported that HA-966 reduced pain reception in animals. These experiments were carried out with the racemic substance and it is not clear which enantiomer was responsible for this effect.[16]

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