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PHARMACOLOGY

EFFECT OF ALLOFERON 1 ON CENTRAL NERVOUS SYSTEM IN RATS

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Abstract: Alloferon 1 is an insect-derived peptide with potent antimicrobial and antitumor activity. It was isolated from blood of an experimentally infected insect, the blow fly *Callifora vicina*. Synthetic alloferon 1 reveals a capacity to stimulate activity of NK cells and synthesis IFN in animal and human models. Moreover, it was demonstrated antiviral and antitumor activity of alloferon 1 in mice. There are no data on influence of alloferon 1 on central nervous system. The aim of present study was to determine an effect of alloferon 1 on rats' central nervous system by some behavioral tests: open field test, hole test, score of rats irritability, and determination of memory consolidation in the water maze test. Moreover, a probable antinociceptive effect of alloferon 1 in rats was determined by a tail immersion test and hot plate test. Experiments were performed on female Wistar rats. Seven days before experiments, rats were anesthetized with ketamine and xylazine and polyethylene cannulas were implanted into the right lateral brain ventricle (*i.c.v.*). On the day of experiment, alloferon 1 dissolved in a volume of 5 μL of saline was injected directly *i.c.v.* through implanted cannulas at doses of 5–100 nmol. It was found that alloferon 1 had slight effect on locomotor and exploratory activity, induced some decrease of rat irritability and a weak impairment of rats memory (only at the low dose of 5 nmol). On the other hand, the higher dose of this peptide exerts significant antinociceptive effect. Obtained results indicate that alloferon 1 do not exert any evidently toxic effect on central nervous system in rats. Therefore, alloferon 1 may be good new drug with antitumor and antinociceptive activity.

Tridecapeptide alloferon 1 (HGVSGHGQHG VGH) (Al 1) has been isolated from blood of experimentally infected larvae of the blow fly *Callifora vicina* (1). It does not possess any similarity of aminoacid sequence with other known immunomodulatory peptides. On the other hand, this peptide shows some functional similarity with interferon and therefore was named alloferon as a nonvertebrate-derived regulator of cytotoxic lymphocytes (1). Synthetic alloferon 1 reveals a capacity to stimulate activity of NK cells, and synthesis of interferon (IFN) in animal *in vivo* and in human models *in vitro* (1). Moreover, it was demonstrated antiviral and antitumor effect of alloferon 1 in mice (1, 2). It was shown that several peptides of different length and aminoacid sequence of peptide chain act on central nervous system (CNS) (3–6). However, there are no data on influence of alloferon 1 on CNS. Present study was undertaken in order to define any effect of synthetic alloferon 1 on CNS in rats, determined by some behavioral tests.

METHODS

Animals

Experiments were performed on female Wistar rats weighting 230–280 g obtained from the animal farm of the Medical University of Silesia in

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Katowice. The animals were kept under 12 h light/12 h dark cycle (light from 6 a.m. to 6 p.m.) in a constant temperature with free access to the water and a standard food (Labofeed B. Kcynia, Poland).

Intracerebroventricular (*i.c.v.*) cannulation

One week before the beginning of the experiments, rats were implanted with polyethylene cannulas (length 35 mm, internal diameter 0.4 mm and external diameter 0.7 mm) into the right lateral brain ventricle (*i.c.v.*) using the same technique as in our previous investigations (3, 4). After intraperitoneal (*i.p.*) injection of xylazine hydrochloride (Xylavet 2% sol., ScanVet, Poland, 10 mg/kg *i.p.*) and of ketamine hydrochloride (Bioketan, Biovet, Poland, 100 mg/kg *i.p.*) heads of animals were fixed in the stereotaxic frame. Polyethylene cannulas (Tomel, Tomaszów Mazowiecki, Poland) were introduced into the right lateral brain ventricle (*i.c.v.*) according to the following coordinates: a depth 4 mm from the surface of the skull, 2 mm to the right from sagittal suture and 2 mm caudal from the coronal suture and fixed to the skull with dental cement (Duracryl, Spofa Dental, Prague, Czech Republic).

Drugs

Alloferon 1 was synthetized in the Faculty of Chemistry, University of Wrocław, Poland (7). It was injected directly *i.c.v.* through implanted cannulas in four doses of 5, 25, 50 and 100 nmol dissolved in 5 μL of 0.9% NaCl to unanesthetized rats using a Hamilton microsyringe. Control animals were treated *i.c.v.*with 5 μL of 0.9% NaCl.

Behavioral tests

Locomotor and exploratory activity was determined 10 min and 24 h after *i.c.v.* injection by means of an open field according to Janssen et al. (8), and 16 min and 24 h after injection in the hole test according to File et al. (9, 10). The open field test was performed in the dark room where was placed round black table with eight white lines. The punctual light was switching on the center of the table where all white lines cut through. Rats were placed on the table and for 3 min were count: ambulation, rearing, peeping, grooming and number of defecation. In the hole test during 3 min number of head dips were counted in rats placed on the wooden box with 16 circular holes of 7 cm diameter.

Irritability was measured 15 min and 24 h after *i.c.v.* injection of Al 1 using the score of Nakamura and Thoenen (11). In this test, rats reaction on blowing is measured, using 0–3 point score: blowing, touching with the glass rod of whiskers and back and holding by hand.

Antinociceptive effect was determined by two tests: the tail immersion (12) and a hot plate test (13) using apparatus HP – 41 (COTM, Białystok, Poland). The latencies for the tail immersion test were recorded before experiment and 10 min, 60 min and 24 h after injection, while the latencies for a hot plate test were recorded also before, and next 11 min, 61 min and 24 h after Al 1 injection.

Latencies determined in the tail immersion test were converted to percent of analgesia according to the formula:

$$\begin{matrix}\text{\% of analgesia}\\ \text{(\% of maximal}\\ \text{antinociceptive effect)}\end{matrix} = \frac{T_x - T_o}{10 - T_o} \times 100$$

The determined latency time of each animal in the hot-plate test was converted to the coefficient: percent of analgesia according to the formula:

$$\begin{matrix}\text{\% of analgesia}\\ \text{(\% of maximal}\\ \text{antinociceptive effect)}\end{matrix} = \frac{T_x - T_o}{20 - T_o} \times 100$$

where T_x – individual latency time determined at time intervals after Al 1 administration; T_o – individual latency time determined before Al 1 administration; 10 – maximal latency time in the tail immersion test (in s); 20 – maximal latency time in the hot-plate test (in s).

Space memory was determined by means of a water maze test according to Plech et al. (14). The time between placing the animal into a central part of the maze and entering onto platform was measured. A mean latency time for three probe trials of each animal was calculated. Latency time was measured one day before beginning of the experiment and 65 min and 24 h after *i.c.v.* injection of Al 1.

At the end of experiments, rats were sacrificed by xylazine and ketamine injection and *post mortem* the placements in the brain of cannulas' tips was checked by injecting *i.c.v.* 10 μL of 2% methylene blue dye solution and investigations of frontal brain slices cut with freezing microtome.

Data were subjected to ANOVA and *post-hoc* Dunnett test (15) (significance $p < 0.05$).

All these experiments were performed in accordance with guidelines for investigations of experimental pain in conscious animals (16).

The protocol of this study was approved by the local ethical committee of the Medical University of Silesia (KNW-0022/LKE-1-77/09).

RESULTS

Alloferon 1 decreases number of rearing (at doses: 5 and 25 nmol) and number of peeping (at

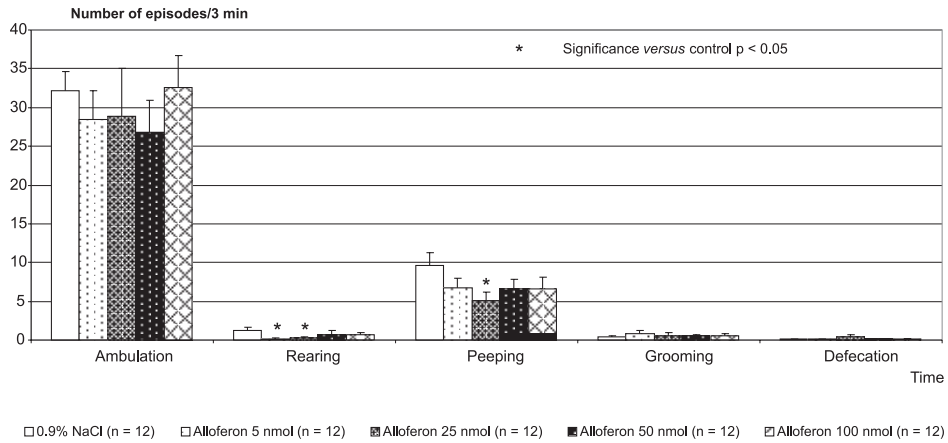

Figure 1. Affect of alloferon 1 on rat's behavior measured with an open field test 10 min after *i.c.v.* injection

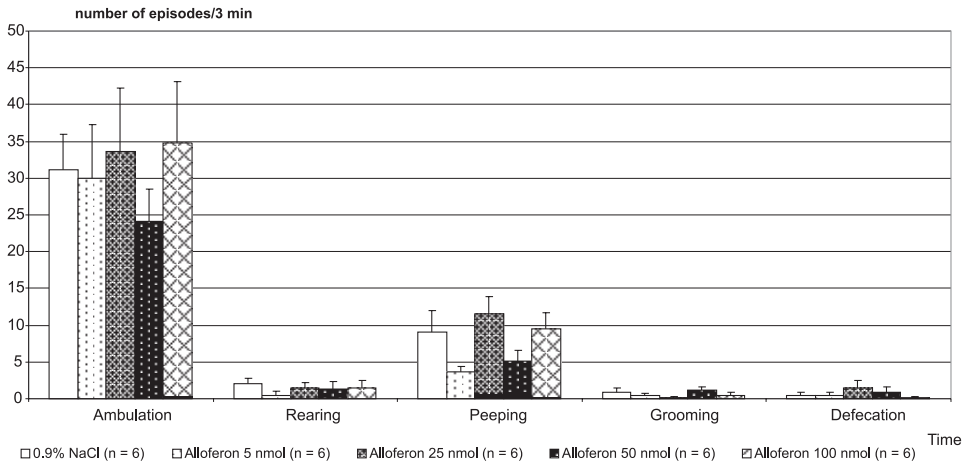

Figure 2. Effect of alloferon 1 on rat's behavior measured with an open field test 23 h after *i.c.v.* injection

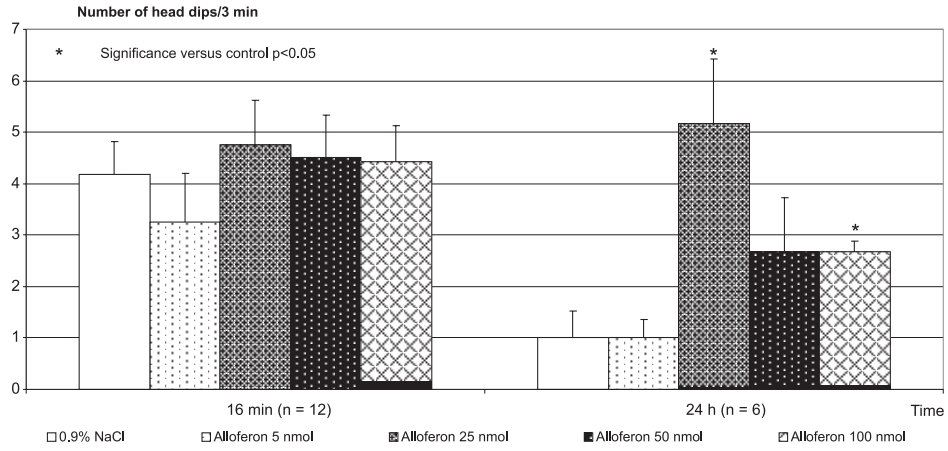

Figure 3. Effect of alloferon 1 on rat's exploratory activity measured by a hole test after *i.c.v.* injection

a dose 25 nmol) in rats 10 min after *i.c.v.* injection (Fig. 1). It does not change number of ambulation, grooming and defecation (Fig. 1) and it does not influence on rats behavior observed in the open field test after 24 h after *i.c.v.* injection (Fig. 2). Alloferon slightly increases rats' exploratory activity 24 h after *i.c.v.* administration, specially at a dose of 25 nmol (Fig. 3). The same dose 25 nmol of Al 1 exerts significant activity lowering rats' irritability (Fig. 4). Investigations of antinociceptive effect of Al 1 show slight antinociceptive effect at both used tests: tail immersion and hot plate test (Figs. 5 and 6). However, Al 1 at a dose 25 nmol *i.c.v.* 61 min after injection exerted some hyperalgesia determined by a hot plate test (Fig. 6). Alloferon exerts also a weak impairment on rats' memory measured by a water maze test (Fig. 7).

DISCUSSION

The method used in this study of direct, intracerebral administration – into the lateral brain ventricle (*i.c.v.*) of tridecapeptide Al 1 made it possible to overcome the blood-brain barrier and to determine the effect of this peptide on the function of rat brain. It is commonly known, since several years that different peptides poorly penetrated the blood-brain barrier (17). The method of *i.c.v.* administration of different peptides and of other drugs was used in several our earlier studies (18–20). *I.c.v.* administration of peptides or drugs resulted in their broad penetration to different brain areas of the rat (21, 22). Synthetic Al 1 was given *i.c.v.* in four increasing doses: 5, 25, 50 and 100 nmols. Such range of dosing was chosen in order to find their effect on rats behavior. Several of different synthetic peptides

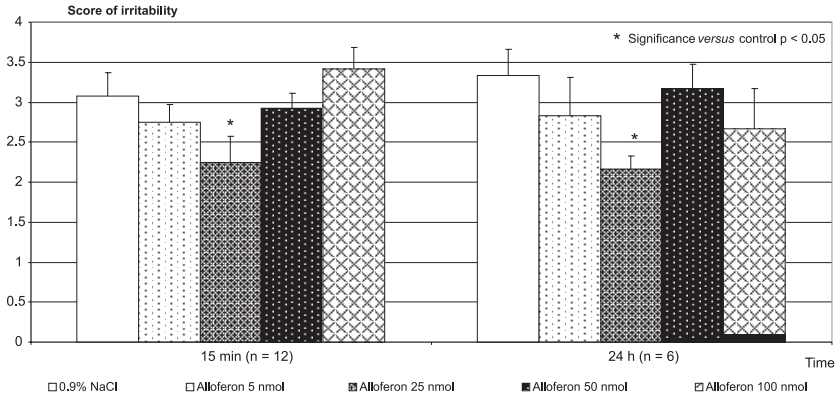

Figure 4. Effect of alloferon 1 on rat's irritability measured ny Nakamura-Thoenen score after *i.c.v.* injection

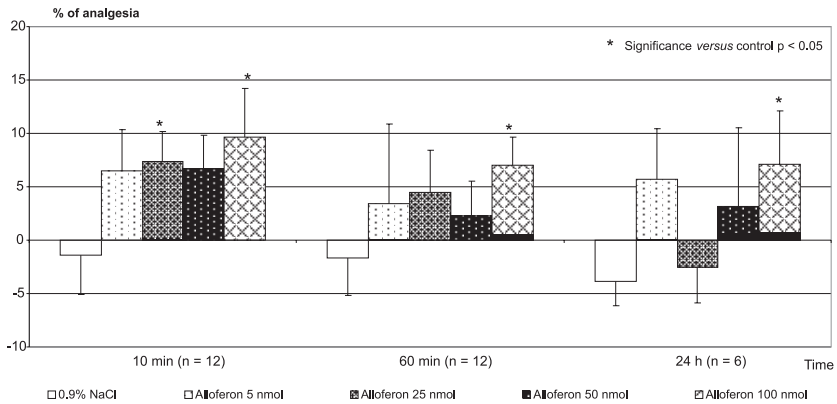

Figure 5. Antinociceptive effect of alloferon 1 determined by the tail immersion test after *i.c.v.* injection

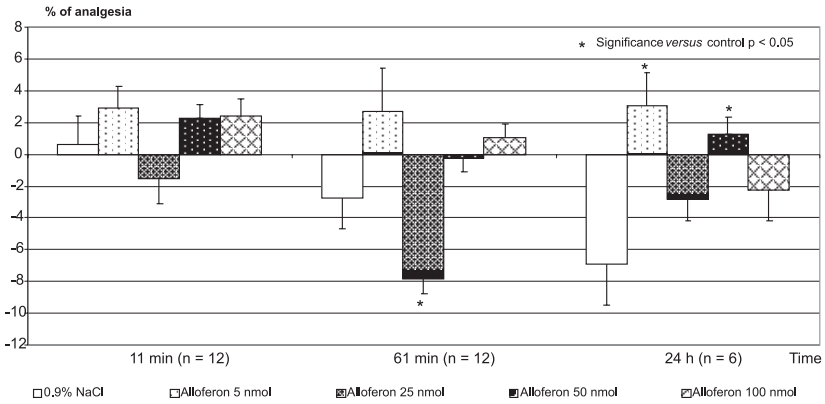

Figure 6. Antinociceptive effect of alloferon 1 determined by a hot plate test after *i.c.v.* injection

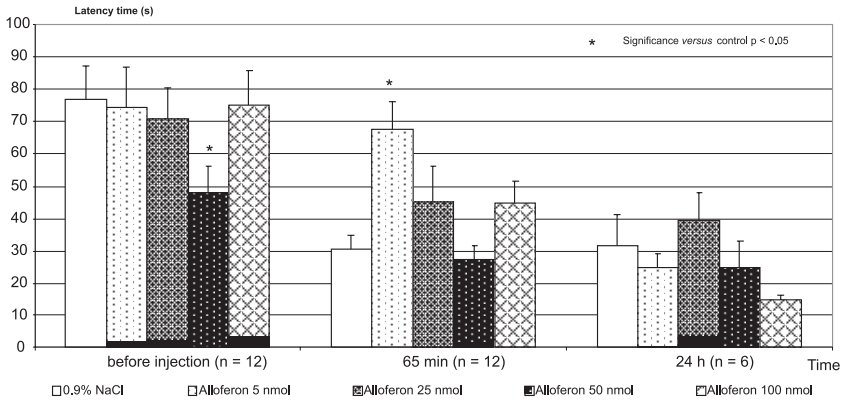

Figure 7. Memory effect of *i.c.v.* administration of alloferon 1 determined by water maze test

applied *i.c.v.* and in similar range of doses cause evident behavioral effects in rats (20, 23). Two used behavioral tests: of the hole and of an open field evaluated rats locomotor and exploratory activity. These both forms of rats behavior expressed an activity of the CNS. Obtained results proved that Al 1 slightly affected rats exploratory activity, as it was observed a prompt effect (in 10 min after it *i.c.v.* administration) of a decrease of the number of rats rearings in an open field test. However, this effect was not confirmed in the hole test. It was not found any prompt decrease on the number of head dips determined in a hole test. Moreover, *i.c.v.* administration of Al 1 elicited in rats a late (after 24 h after it *i.c.v.* administration) increase of rats exploratory activity, expressed as an increase of the number of rats head dips counted in a hole test. Thus, different, not distinct changes of rats locomotor and exploratory activity indicated that Al 1 not disturbed the function of rats CNS. The results of the study of two other forms of rats behavior i.e., the determination of the intensity of rats irritability and the investigation of memory consolidation also did not show any neurotoxic effect of Al 1. All applied doses of Al 1 neither increase rats irritability nor elongated the latency time of entering rats onto platform in a water maze test in comparison to untreated control animals. All behavioral evaluations were performed on female rats. It is known that different behavioral effects in rats, including locomotor and exploratory activity, are modulated by gender, estrus cycle as well as by progesterone (24). Estradiol seems to be a main ovarian steroid modulating also an acquisition of conditioned avoidance response (25). On the other hand, it was found in earlier investigations that synthetic pentapeptide proctolin, the first discovered

insect neuropeptide, applied directly into the lateral brain ventricle to uncastrated female rats did not change rats exploratory activity in every of four phases of estrus cycle (26). The same, equimolar dose of proctolin, of 100 nmols applied *i.c.v.* in castrated female rats, also did not change their exploratory activity in comparison to control group (26). The results of our present investigations were obtained on intact, uncastrated female rats. These animals either of experimental or control groups were in different phases of estrus cycle, therefore, it was possible to avoid a distinct effect of estrus phase on rats behavior. Moreover, there were used different behavioral tests. Thus, we expect that probable effect of estrus cycle had not prominent influence on results of performed investigations of Al 1 activity.

Antinociceptive effect of the same, standard, equimolar doses of Al 1: 5–100 nmol was investigated in rats by two classic methods: the tail immersion and the hot plate. It was found a slight antinociceptive effect of Al 1. It was presented in several reports that opioid and non-opioid peptides displayed analgesic effect in rats and mice (19, 23, 27, 28). At present, the mechanism of analgesic effect of Al 1 is unknown. We hope that further study of this peptide may reveal the mechanism responsible for its analgesic effect. To our knowledge this report is a first on neurotoxic effect of Al 1.

In summary, all presented here behavioral studies allow to conclude that Al 1 is not a distinct neurotoxic agent for rats.

CONCLUSION

The results of present study did not prove any neurotoxic effect of Al 1 in rats, expressed as an impairment of rats behavior.

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