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ORIGINAL ARTICLE

Effect of somatostatin analog-octreotide on the adjuvant arthritis in rat

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Abstract

OBJECTIVES: The aim of present study was the estimation of the anti-inflammatory effects of the somatostatin analog-octreotide in the adjuvant arthritis in rats.

MATERIAL AND METHODS: The arthritis was induced by intradermal injection of Freund's adjuvant at the foot's pulvinar of the posterior paw in August's strain rats. The animals received: 0.9% NaCl, octreotide (oct), dexamethasone (dx) and oct together with dx. The clinical signs, body weight, weight of spleens and thymuses were estimated. The cellularity of the thymus was evaluated by computer assisted morphometry and levels of IL-1 beta in serum were measured by the ELISA method.

RESULTS: In the rats treated with oct in comparison to the untreated group the reduction of the clinical signs, a decrease of the body weight fall, inhibition of the thymus weight fall, the lower spleen's weight, an increase of the thymus cellularity and an increase of the IL-1 beta level in serum were found. The rats treated with dx showed greater to oct-treatment reduction of the clinical signs and greater loss of the thymus weight. The spleen's weight was similar to the healthy group. A significant reduction of the thymus cellularity and a decrease of the IL-1 beta level were also found. The rats treated with dx and oct jointly showed in comparison with dx alone a weaker reduction of the clinical signs, lower thymus weight fall, an increase of the IL-1 beta level in serum, without the significant influence on the other parameters.

CONCLUSION: The octreotide shows the anti-inflammatory effect in adjuvant arthritis, but it is weaker in comparison to dexamethasone. Treatment with dx and oct together does not result in the more pronounced anti-inflammatory action of glucocorticoid alone.

Introduction

Somatostatin (Sst), besides well known inhibiting effect on endocrine and exocrine secretions, exhibits many other properties including the less recognized immunomodulatory and anti-inflammatory activities [1, 2, 3]. Sst is synthesized by endocrine and neural cells in many parts of the body, including the dorsal root ganglia. Then it is transported to the peripheral sensory nerve terminals, and from this site it is released under the influence of the local injurious factors [1]. The expression of Sst has been found in the inflammatory foci [4], and this neurohormone is considered to be a local anti-inflammatory factor. Sst may restrict the inflammatory processes in several ways. It inhibits the migration of circulatory leukocytes to the inflammatory site [5], suppresses the blood vessels permeability and induces vasoconstriction and inhibits angiogenesis [6]. Sst inhibits the release of substance P, the main mediator of pain which stimulates release of such inflammation mediators as leukotrienes and prostaglandins [7, 8]. Sst receptors (SS-R) were identified on mononuclear leukocytes [9] and on T lymphocytes [10], the cells which play the important roles in the inflammatory and autoimmune diseases. The high affinity Sst receptors were also found in the immune organs, such as the spleen and thymus [11]. The anti-inflammatory properties of Sst are shared by Sst analogs, including the octapeptide analog octreotide [12, 8]. Because of the much longer duration of action, these analogs are more suitable for therapeutical application than the native hormone. At present, they are mainly used for the treatment of acromegaly and neuroendocrine tumors of the digestive tract. The trials of their usage for the antiphlogistic properties are still limited and concern mainly the Graves ophthalmopathy [12, 13, 14]. One of the conditions which may be considered as a candidate for the therapy with Sst analogs is rheumatoid arthritis (RA). The presence of SS-R on synovial membranes in patients with RA was demonstrated in vivo by using receptor scintigraphy [15]. The degree of clinical symptoms in RA is positively correlated with Sst receptor density in the joints [16]. The presence of SS-R in blood vessels of synovium of the patients suffering from RA was also detected by the in vivo autoradiography [6].

The trials of intraarticular injection of somatostatin analogs in patients with rheumatoid arthritis are reported [16, 17, 18, 19]. The treatment resulted in a significant improvement: the reduced pain at rest and on movement, joint tenderness, morning stiffness and spontaneous pain. The thickness of the synovial membrane measured with echography in patients with RA decreased at the treated joint [18]. The commonly used animal model of RA is adjuvant arthritis (AA) in rats [20]. The aim of this study was to estimate the anti-inflammatory effect of the best known Sst analog octreotide in AA and to compare the octreotide effect with that of the glucocorticoid dexamethasone, which the well-known and very potent suppressor of the inflammatory reaction [21, 22, 23]. We tried also to see whether octreotide and dexamethasone exert a synergistic or additive action.

Material and Methods

The study was carried out on 83 three-month-old male August rats with the body weight of 260–300 g. The animals were housed at 23–25°C, in 12:12 light-dark schedule (lights on 7.00 am–7.00 pm), with food and water freely available. During the experiment animals were housed in standard plastic cages, 5–8 per cage.

Induction of adjuvant arthritis

The adjuvant arthritis (AA) was induced by intradermal injection of 0.05 ml Freund's adjuvant at the foot's pulvinar of the posterior paw. The Freund's adjuvant was prepared as follows: Mycobacterium tuberculosis H 37 RA No. 3114 (Difco) was added to a liquid paraffin oil and ground together in a glass tissue homogenizer, 1 ml of the solution contained 5 mg Mycobacterium.

Treatment protocols

Two independent experiments were performed. In the first experiment, 24 rats were used. The animals were divided into three groups. The intact rats were the control group. Between 10 and 21 days after the administration of the Freund's adjuvant, the rats with AA received subcutaneus injections of either octreotide (Sandostatin-Novartis), 100 μg/kg or 0.25 ml of 0.9% NaCl twice a day. The animals were killed 21 days after the adjuvant administration and 12 hours after the last injection of octreotide or saline.

The second experiment was carried out on 59 rats. The AA was induced in the same way. The adjuvant-treated rats were randomly assigned to one of five groups receiving the following treatments during 7 days: 1) 0.25 ml 0.9% NaCl twice a day, 2) 100 μg/kg octreotide twice a day; 3) dexamethasone 1mg/kg once a day, 4) dx 0.5 mg/kg once a day; 5) octreotide 100 μg/kg twice a day plus dx 0.5 mg/kg once a day. The treatments started one day before the induction of AA. The additional group of 6 healthy rats was

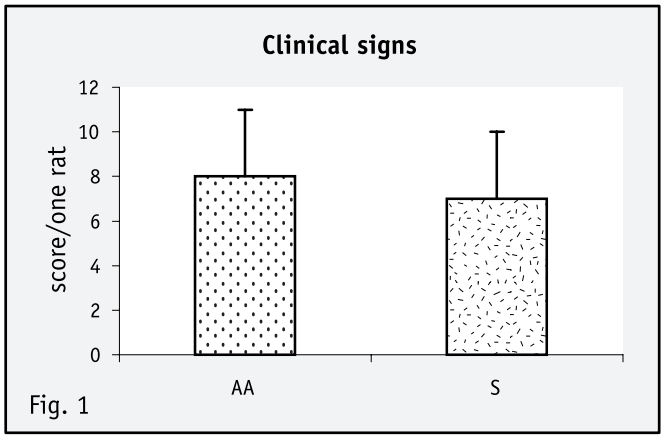
treated with 0.9% NaCl. The experiment was finished on 15th day after the induction of AA and 8 days after the last injection of the drugs.

In both the experiments the evaluation of the clinical signs and body weightings were performed on the last day of experiment, by the person not knowing which treatment was given in the evaluated groups. Arthritis was scored on a 4-point scale for each paw and the combined scores for the four paws of each animal were used for the analysis, following Cerani et al. [24]. A score of 0 was assigned if the paw appeared normal, 1-was assigned if redness was present, 2-indicated that the paw was red and slightly swollen, 3-was assigned if the swelling was greater and movement of the joint was restricted, and 4-swelling was marked and/or the joint was immobilized.

The animals in both collected experiments were sacrificed by decapitation. After sacrificing the spleens, thymus and blood samples were collected. The tissues were weighed, fixed in Bouin's fluid and than embedded in paraffin wax. Histological morphometry of the thymus preparations was performed by means of an image analysis system consisting of an IBM-compatible computer equipped with an optical mouse, A Ver 2000 card (frame grabber, true-color, real-time), produced by ADDA Technologies (Taiwan), and color TV camera Panasonic (Japan) linked to a Carl Zeiss Jenaval microscope (Germany). The system was programmed (program MultiScan, produced by CSS-Poland) to calculate the number of objects (automatic function with manual correction). The randomly selected high power fields (0.013 mm^2 each) were analyzed. The levels of IL-1 beta in serum were measured by the ELISA method (Endogen). The numerical data were evaluated statistically by means of the Mann-Whitney U test. The value of $p \leq 0.05$ was considered as significant.

Results

The results obtained from both experiments were very similar and are described here jointly. In the rats treated with oct in comparison to the untreated groups with AA, the slight reduction of clinical signs score (Figs. 1, 2) was found. This reduction was more pronounced in the dx-treated rats (Fig. 2), the administration of dx jointly with oct did not cause further reduction of the parameter (Fig. 2). The body weight fall in the rats treated with oct in comparison to other groups with AA was slightly attenuated (Figs. 3, 4). The spleen's weight was the highest in the untreated rats with AA. The spleen's weight was significantly lower in the rats treated either with oct or with dx in comparison to the untreated AA rats (Fig. 5). This parameter in the rats treated with dx and oct jointly was very similar to the control group without inflammation (Fig. 5). A significant reduction of thymus weight and cellularity vs. heathy controls was observed in the rats with untreated AA (Figs. 6, 7, 9). In the rats treated with a higher dose of dx the further reduction of both parameters was found (Fig. 9). In the oct-treated group, both parameters were higher than those in the group with untreated AA (Figs. 6,



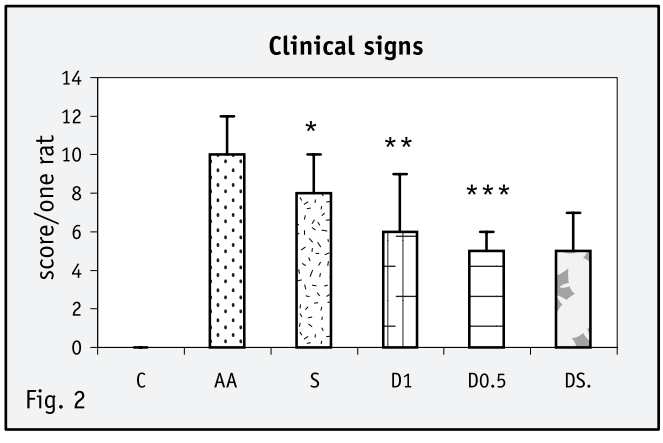


Fig. 1. Effect of octreotide 100 µg/kg twice a day (=**S**) on the clinical signs in rats with adjuvant arthritis. **AA**=untreated rats with adjuvant arthritis.
Data are presented as as means+/-S.E.M.

Fig. 2. Effects of octreotide 100 µg/kg twice a day (=**S**); dexamethasone 1 mg/kg once a day (**D1**); dexamethasone 0.5 mg/kg once a day (=**D 0.5**); oct 100 µg/kg twice a day plus dexamethasone 0.5 mg/kg once a day (=**SD**) on the clinical score in rats with adjuvant arthritis. **AA**=untreated rats with adjuvant arthritis.
Levels of significance: *p<0.05 vs. AA, **p<0.01 vs. AA, ***p<0.001 vs. AA.
Data are presented as as means+/-S.E.M.

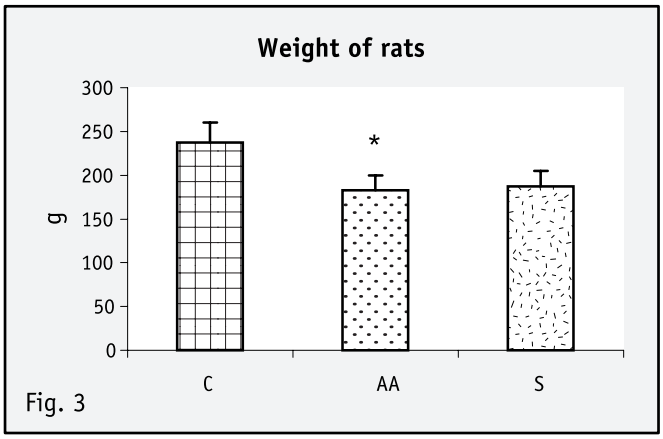


Fig. 3

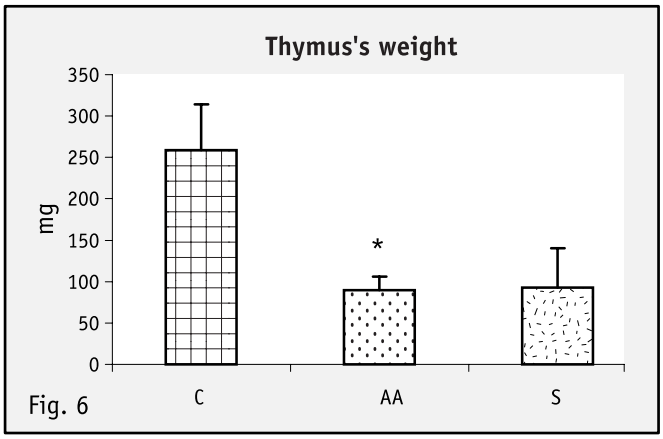
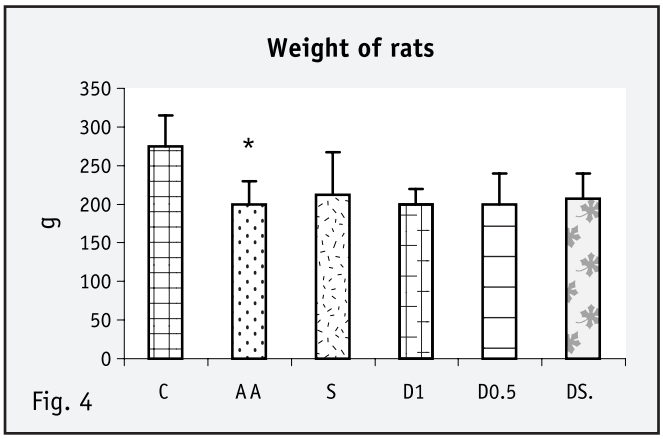


Fig. 6



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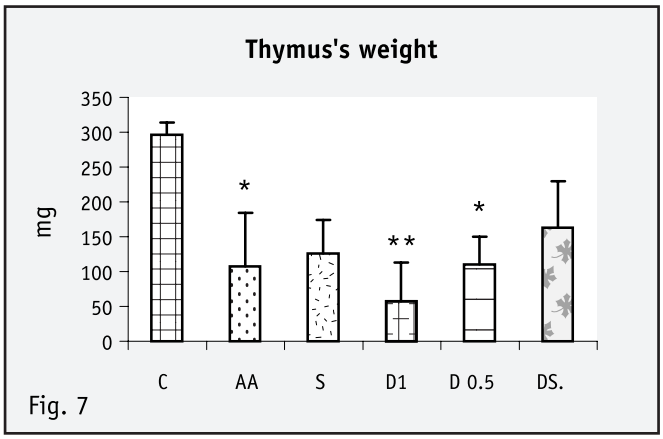

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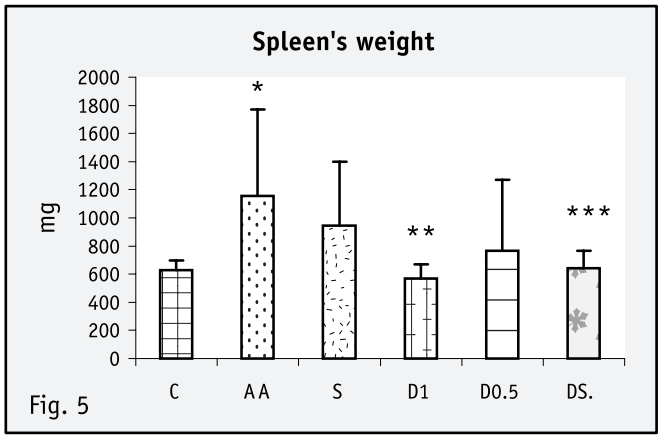


Fig. 5

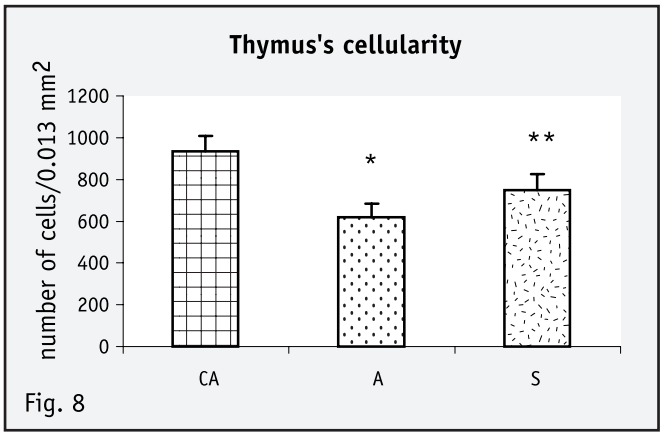


Fig. 8

Fig. 3. Effects of octreotide 100 μg/kg twice a day (**S**) on the body weight of rats. **C**=control group without inflammation; **AA**=untreated rats with adjuvant arthritis. Levels of significance:*p<0.001 vs. C. Data are presented as as means+/-S.E.M.

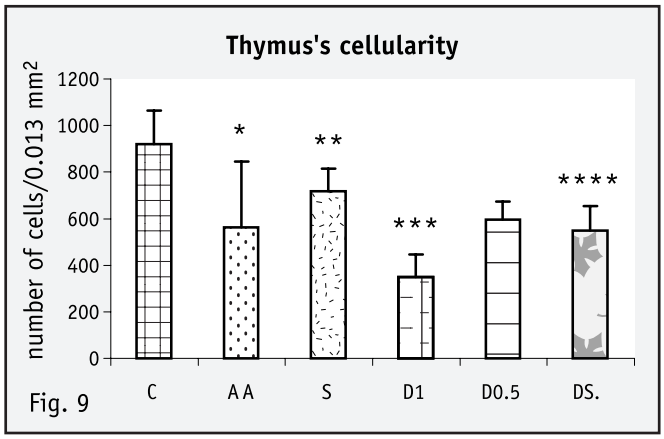
Fig. 4. Effects of octreotide 100 μg/kg twice a day (**S**); dexamethasone 1 mg/kg once a day (=**D1**); dexamethasone 0.5 mg/kg once a day (=**D 0.5**); oct 100 μg/kg twice a day plus dexamethasone 0.5 mg/kg once a day (=**SD**) on the body weight of rats with AA. **C**=the control group without inflammation; **AA**=untreated rats with adjuvant arthritis. Levels of significance:*p<0.001 vs. C. Data are presented as as means+/-S.E.M.

Fig. 5. Effect of octreotide 100 μg/kg twice a day (=**S**); dexamethasone 1 mg/kg once a day (=**D1**); dexamethasone 0.5 mg/kg once a day (=**D 0.5**); oct 100 μg/kg twice a day plus dexamethasone 0.5 mg/kg once a day (=**SD**) on the spleen's weight in rats with AA. **C**=control group without inflammation; **AA**=untreated rats with adjuvant arthritis. Levels of significance: *p<0.01 vs C; **p<0.001 vs. S; ***p<0.01 vs. S. Data are presented as as means+/-S.E.M.

Fig. 6. Effect of octreotide 100 μg/kg twice a day (=**S**) on the thymus's weight in rats with AA. **C**=control group without inflammation; **AA**=untreated rats with adjuvant arthritis. Levels of significance:*p<0.001 vs. C.
Data are presented as as means+/-S.E.M.

Fig. 7. Effect of octreotide 100 μg/kg twice a day (=**S**); dexamethasone 1 mg/kg once a day (=**D1**); dexamethasone 0.5 mg/kg once a day (=**D 0.5**); oct 100 μg/kg twice a day plus dexamethasone 0.5 mg/kg once a day (=**SD**) on the thymus's weight in rats with AA. **C**=control group without inflammation; **AA**=untreated rats with adjuvant arthritis. Levels of significance: *p<0.001 vs. C; **p<0.05 vs. AA. Data are presented as as means+/-S.E.M.

Fig. 8. Effect of octreotide 100 μg/kg twice a day (=**S**) on the thymus's cellularity in rats with AA. **C**=control group without inflammation; **AA**=untreated rats with adjuvant arthritis. Levels of significance: *p<0.001 vs. C; **p<0.05 vs. AA.
Data are presented as as means+/-S.E.M.



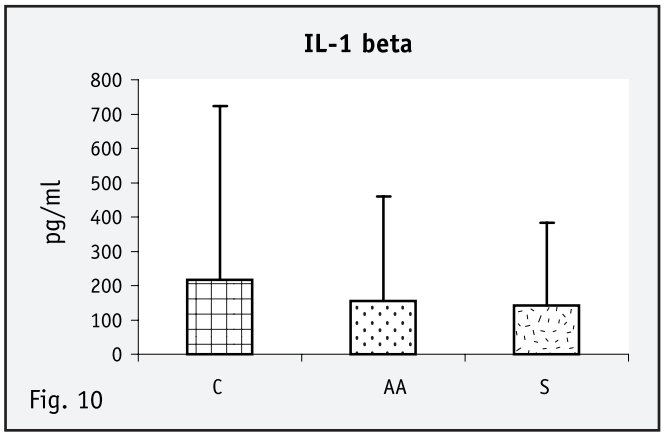


Fig. 9. Effect of octreotide 100 µg/kg twice a day (=**S**); dexamethasone 1 mg/kg once a day (=**D1**); dexamethasone 0.5 mg/kg once a day (=**D 0.5**); oct 100 µg/kg twice a day plus dexamethasone 0.5 mg/kg once a day (=**SD**) on the thymus's cellularity in rats with AA. **C**=control group without inflammation; **AA**=untreated rats with adjuvant arthritis. Levels of significance: *p<0.001 vs. C; **p<0.05 vs AA; ***p<0.01 vs S; ****p<0.01 vs. D1.
Data are presented as as means+/-S.E.M.

Fig. 10. Effect of octreotide 100 µg/kg twice a day (=**S**) on the IL-1 beta levels in serum in rats with AA. **C**=control group without inflammation; **AA**=untreated rats with adjuvant arthritis.
Data are presented as as means+/-S.E.M.

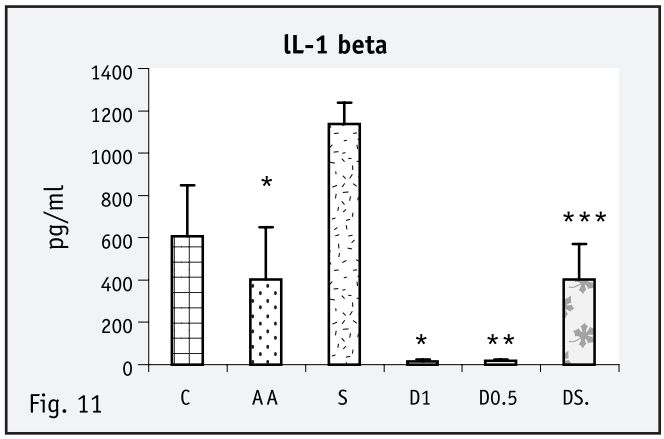


Fig. 11. Effect of octreotide 100 µg/kg twice a day (=**S**); dexamethasone 1 mg/kg once a day (=**D1**); dexamethasone 0.5 mg/kg once a day (=**D 0.5**); oct 100 µg/kg twice a day plus dexamethasone 0.5 mg/kg once a day (=**SD**) on the IL-1 level in serum in rats with AA. **C**=control group without inflammation; **AA**=untreated rats with adjuvant arthritis. Levels of significance: *p<0.05 vs. AA; **p<0.01 vs AA; ***p<0.01 vs. D0.5.
Data are presented as as means+/-S.E.M.

7, 8, 9). The administration of oct concomitantly with dx resulted in a braking of decreasing thymus weight and cellularity (Fig. 7, 9). The serum levels of IL-1 beta were lower in untreated AA in comparison to the rats without inflammation, and the treatment with oct caused an increase of this level above the control values (Figs. 10, 11). The lowest values of IL-1 beta were observed in dx-treated groups, whereas in the animals treated with dx and oct jointly IL-1 beta level did not differ from this noted in the untreated AA rats (Fig. 11).

Discussion

The data presented above show that the somatostatin analog-octreotide exerts an anti-inflammatory effect in the rat adjuvant arthritis model. This finding corroborates with the observations of other authors concerning the other aseptic inflammation models [5, 22, 25, 26]. The anti-inflammatory effect of oct is rather moderate and is clearly weaker than that observed after the treatment with dexamethasone. On the other hand, the dose of oct, calculated as the molar mass, was lower than those of dx. The combined treatment with a low dose of dx and oct did not enhance the anti-inflammatory effect of the former. The lack of additive or synergistic effects of dx and oct in the AA experimental model speaks against the joint use of these drugs in the antiphlogistic therapy. However, in the rats treated jointly with oct and dx a smaller reduction of thymus weight was observed than that found in rats treated with dx alone. In numerous papers a local increase of IL-1 beta level in the synovial fluid of the rats with AA was reported [27, 28, 29]. Unexpected, we have found that IL-1 beta levels in the systemic blood serum are lower in AA rats as compared to healthy controls. This disturbance may depend on the increased secretion of the endogenous corticosterone which is a well documentated phenomen in AA [30]. This explanation is supported by the finding that both doses of dx dramatically suppressed IL-1 beta levels in our AA rats. Interestingly, oct,

when given together with dx, reversed the suppressive effect of the further on IL-1 beta level.

Summing up, the data presented above indicate that octreotide possesses some, although moderate, antiphlogistic action in the rat adjuvant arthritis model. The search for other Sst analogs exhibiting a higher antiphlogistic potency is needed.

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