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Effectiveness of Systemic Delivery of Hypothalamic Neuropeptides, OrexinA and OrexinB, on Sleep-Wakefulness Cycle Ultradian Structure and Food Motivation in Rats

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ABSTRACT. Present study investigated possible differences between the effects of intravenous injection of OrexinA and OrexinB on the ultradian structure of sleep-wakefulness cycle and food motivation in rats. Two doses of these neuropeptides (5μg/ml and/or 10μg/ml) were injected in the tail vein. Continuous EEG registration of baseline sleep-wakefulness cycle during 5 h period daily (11.00 a.m. – 16.00 a.m.) was started after post-surgery recovery period. Three baseline ultradian structures were registered on each animal so each animal served as a control for itself. After establishment of the stable baseline SWC ultradian structure OrexinA and/or OrexinB (from PHOENIX PHARMACEUTICALS) were injected in the tail vein at 10.55 a.m., than EEG registration of sleep-wakefulness cycle ultradian structure was started at 11.00 a.m., as in baseline recordings. Significant data were obtained about the whole effectiveness of i.v. OrexinA and ineffectiveness of i.v. OrexinB on sleep-wakefulness cycle ultradian structure. In contrast to i.v. OrexinB i.v. OrexinA produced significant increase of active wakefulness incidence, total time and percentage, whole suppression of REM sleep, and substantial changes in non-REM sleep stages - reduction of their incidence, total time and percentage. Because OrexinA has similar affinity to both Orexin receptor-1 and Orexin receptor-2 while OrexinB reveals a 10-fold higher affinity to Orexin receptor-2 it is possible to speak about the significance of Orexin receptor-1 for the sleep-wakefulness cycle disorders described in the present study. Therefore we can suggest that systemic administration of antagonists for Orexin receptor-1 can be effective for the aim of clinical therapy of insomnia.

Key Words: OrexinA; OrexinB; Systemic administration, sleep-wakefulness disorders

Hypothalamic neuropeptides named as Orexin [1] and/or Hypocretin [2] were discovered independently by two scientific groups in the lateral, posterior and perifornical hypothalamus. Two sub-groups of this neuropeptides – OrexinA (ORXA and/or Hypocretin1) and OrexinB (ORXB and/or Hypocretin2) and their respective Orexin-1 (ORXR1) and Orexin-2 (ORXR2) receptors were soon identified [1]. Starting from the

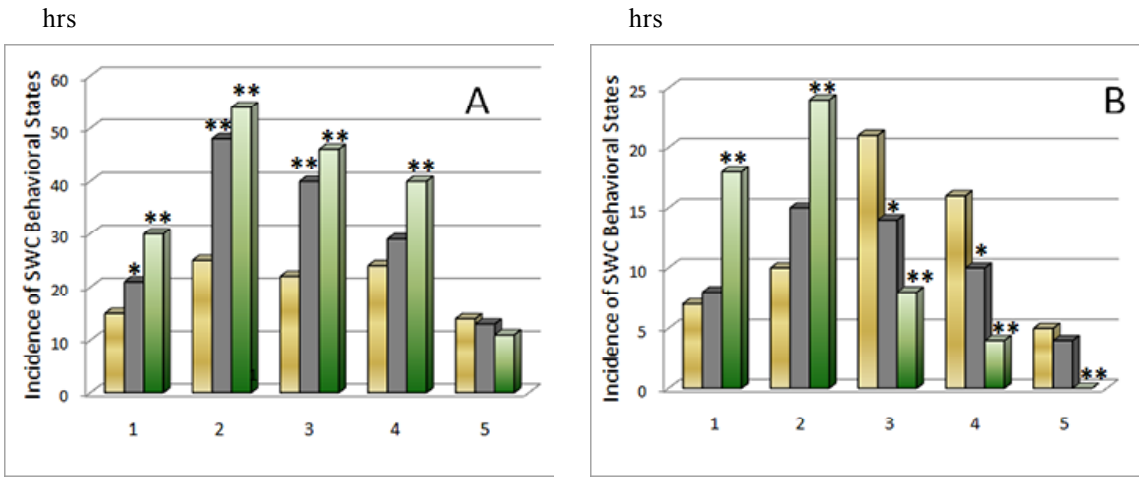

Fig. 1. Changes in the incidence of various behavioral states of SWC under the impact of ORXA On A – Changes in the 5 hour post-injection EEG registration period, on B - Changes in the first 2 hour post-injection EEG registration period. On the abscissa: 1 – active wakefulness incidence; 2- passive wakefulness incidence; 3 - incidence of light non-REM sleep; 4 - incidence of deep non-REM sleep; 5 - incidence of REM sleep. Yellow columns – date from control rats, gray columns – date from experimental group I with i.v. injection of 5μg/ml OrexinA, green columns - date from experimental group II with i.v. injection of 10μg/ml OrexinA. * = $p < 0.05$, ** = $p < 0.01$.

discovery of Orexin-containing neurons much information has been gathered concerning their participation in the regulation of feeding and energy homeostasis [1], modulation of neuro-endocrine and cardiovascular functions [3], learning and memory [4], regulation of the sleep-wake cycle (SWC) [5,6] and narcolepsy [7,8] but despite all of this their precise function is not known so far.

Recently, we have found for the first time that serial electrical activation of hypothalamic Orexin-containing neuronal areas promotes regulation of sleep homeostasis and recovery of wakefulness, and in general, recovery of SWC behavioral states from experimental comatose state and barbiturate-induced sleep [9]. Overall, Orexin neurons are thought to sustain wakefulness and suppress REM sleep [5, 6]. However, we think that increment in wakefulness time and suppression of REM sleep can't be happen without serious disturbances in the time parameters and quality of non-REM sleep stages. Investigations carried out until today indicate that ICV injection of Orexin in mice and rats increase wakefulness potently and suppress non-REM sleep [6,10,11].

In the present study we were interested whether there is the difference between the effects of intravenous (i.v.) injection of ORXA and ORXB. The possibility of some differences in the effects of ORXA and ORXB is indicated indirectly by some facts, namely: ORXA has similar affinity to both ORXR1 and ORXR2 while ORXB reveals a 10-fold higher affinity to ORXR2 than to ORXR1 [12]; ORXA contains two sets of disulfide bonds playing a key role in receptor activation and prolonged half-life, compared to ORXB [13].

Therefore the aim of the present study was to find out whether i.v. ORXA and ORXB can equally be effective in producing of disturbances in the SWC ultradian structure, quality of various behavioral states and food motivation and if so whether their effects are similar.

Materials and Methods

Subjects. Investigation was carried out on 20 wild white rats (weight 200-250 g) that were divided into four experimental groups (n=5 in each group).

Surgery. Surgery for implantation of recording electrodes in: neocortical areas; hippocampus and neck muscles was made under general anesthesia (50 mg/kg Ketamine+10 mg/kg Xylazine, intraperitoneally).

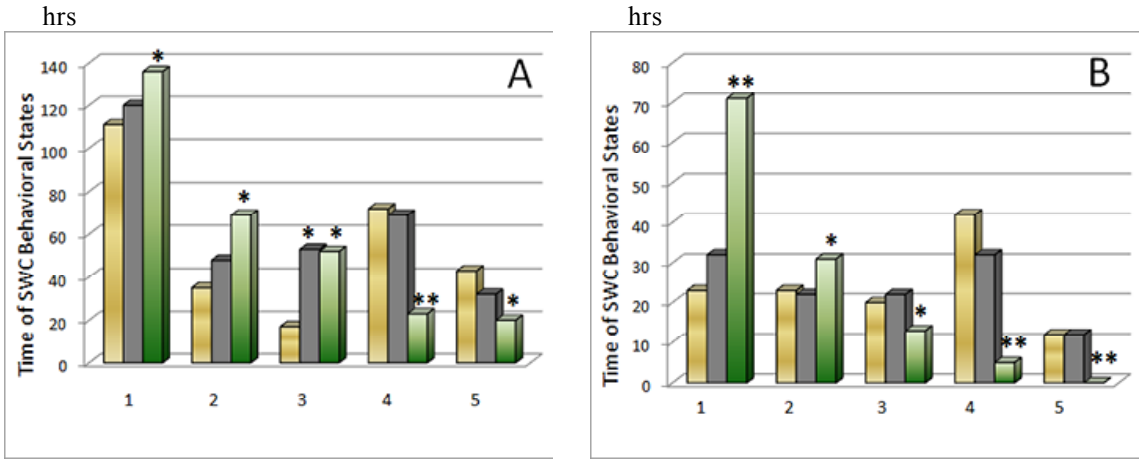

Fig. 2. Changes in the total time taken by various behavioral states of SWC under the impact of ORXA On A – Changes in the 5 hour post-injection EEG registration period, on B - Changes in the first 2 hour post-injection EEG registration period. On the abscissa: 1 – active wakefulness incidence; 2- passive wakefulness incidence; 3 - incidence of light non-REM sleep; 4 - incidence of deep non-REM sleep; 5 - incidence of REM sleep. yellow columns – date from control rats, gray columns – date from experimental group I with i.v. injection of 5μg/ml OrexinA, green columns - date from experimental group II with i.v. injection of 10μg/ml OrexinA. * = $p < 0.05$, ** = $p < 0.01$.

Stainless steel screws served as recording electrodes. Animals were under special care during 7-10 recovery days after surgery.

Experiment I. Continuous EEG registration of baseline SWC during 5 h period daily (11.00 a.m. – 16.00 a.m.) was started after post-surgery recovery period. Three baseline SWC ultradian structures were registered on each animal so each animal served as a control for itself. After establishment of the stable baseline SWC ultradian structure ORXA (from PHOENIX PHARMACEUTICALS) was injected in the tail vein at 10.55 a.m., than EEG registration of SWC ultradian structure was started at 11.00 a.m., as in baseline recordings. Animals from group I received i.v. 5 μg/ml of ORXA, from group II - 10 μg/ml of ORXA.

Experiment II. Procedure for experiment II was the same as for experiment I, with the difference that this time i.v. ORXB (from PHOENIX PHARMACEUTICALS) was injected in the tail vein. Animals from group III received i.v. 5 μg/ml of ORXB, from group IV - 10 μg/ml of ORXB.

EEG and EMG registration of SWC was made by SAGURA EEG/PSG system.

Statistical treatment. Statistical treatment of obtained results was made by Student's t test, * = $p < 0.05$, ** = $p < 0.01$ were taken as the levels of significance.

Results and Discussion

It appeared that none of the doses of ORXA and ORXB applied i.v. have produced statistically significant changes in the amount of daily food consumption. This fact contradicts the literary data about the involvement of Orexin neuropeptides in food intake. One of the reasons for this may be the difference in the effects of i.v. ORXA and ORXB from the effects of their intra-cerebral microinjection.

Changes in SWC ultradian structure were assessed by sleep latency, incidence of various behavioral states, total time taken by SWC behavioral states, percent ratio of various behavioral states of SWC, REM sleep latency. Data were analyzed for the whole 5 h post- injection as well as for the first 2 h post-injection periods. We have found that i.v. ORXA significantly altered ultradian structure of SWC. It was manifested in a sharp increase of sleep latency. Incidence of SWC behavioral states in the whole 5 h EEG registration period was changed significantly (Fig.1A). The number of active and passive wakefulness episodes increased dose-dependently with more than 3-fold growth in response to10 μg/ml of ORXA (Fig.1A1 and 1A2).

Incidence of light non-REM and deep non-REM sleep were changed in a different manner.

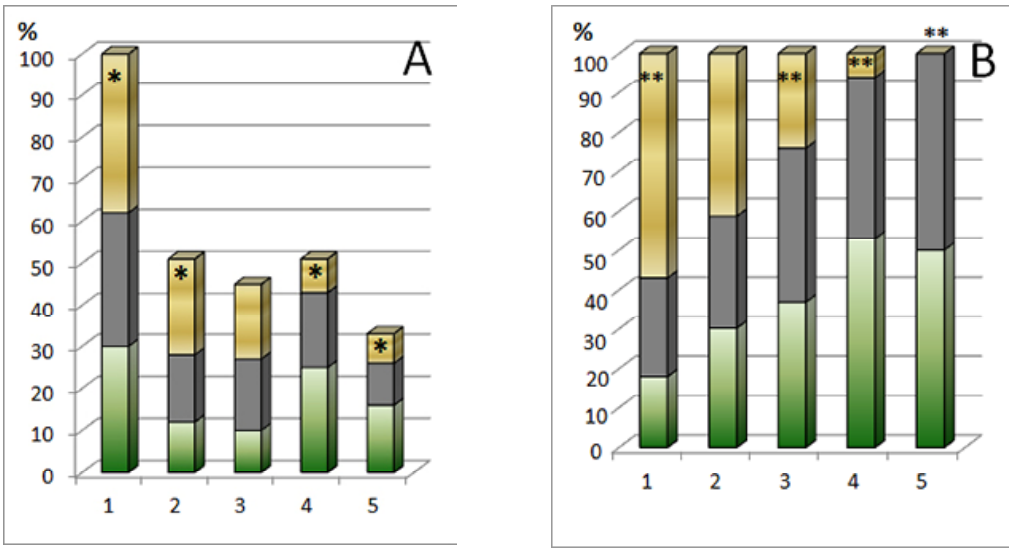

Fig. 3. Changes in the percentage of various behavioral states of SWC under the impact of ORXA – On A – Changes in the 5 hour of EEG registration, on B - Changes in the first 2 hour of EEG registration. On the abscissa: 1- percentage of active wakefulness; 2 – percentage of passive wakefulness; 3 – percentage of light non-REM sleep; 4 – percentage of deep non-REM sleep; 5 – percentage of REM sleep. Green columns – baseline date, gray columns – date from experimental group I with i.v. injection of 5μg/ml ORXA, yellow columns - date from experimental group II with i.v. injection of 10μg/ml ORXA, on the ordinate – percentage for SWC behavioral states.* = $p < 0.05$, ** = $p < 0.01$.

The rate of light non-REM sleep episodes was enhanced dose-dependently (Fig.1A3) while incidence of deep non-REM sleep was significantly decreased in response to both doses of ORXA. As for REM sleep incidence it showed significant diminution under the impact of both doses of ORXA.

The analysis of the data separately for the first 2 h post-injection hours showed that the effects of i.v. ORXA are especially pronounced during this period. Incidence of active wakefulness rises very strongly and dose-dependently (Fig.1B1), but incidence for the passive wakefulness, light non-REM and deep non-REM sleep significantly decreases, with the strongest effect exerted by i.v. injection of 10 μg/ml of ORXA (Fig.1B2, 1B3, 1B4). REM sleep incidence significantly declined in response to 5 μg/ml of ORXA, moreover under the impact of i.v. 10 μg/ml of ORXA its episodes did not appear during the 2 h EEG registration period at all (Fig.1B5).

Significant changes in total time taken in 5 hour post-injection EEG registration period by various behavioral states of SWC have been found under the impact of i.v. OrexinA (Fig.2A). It was manifested in the dose-dependent increase of total time of active and passive wakefulness (Fig.2A1, 2A2, black columns), 10 μg/ml of ORXA resulted also in a significant decrease of deep non-REM (Fig.2A4) and REM sleep (Fig.2A5) total times. Total time taken by various behavioral states of SWC was changed intensively in the first 2 h post-injection period of EEG registration (Fig.2B). 10 μg/ml of ORXA was especially effective in this period. It produced, along with significant increase in active wakefulness time, substantial diminution of deep non-REM sleep total time (Fig.2A4). REM sleep was totally disappeared during the 2 h post-injection period and REM time equaled to zero (Fig.2A5). Only 10 μg/ml of i.v. ORXA produced significant changes in the percentage of SWC behavioral states. These were expressed in substantial rising of percentage share of active and passive wakefulness and light non-REM sleep while percentage for deep non-REM and REM sleep decreased significantly (Fig.3A4 and 3A5). Effects of 10μg/ml of i.v. ORXA were especially intensive in the first 2 h post-injection period of EEG registration (Fig.3B). There was a 3-fold increase in the percentage of active wakefulness. Considerable rising was noted in the percentage of passive wakefulness. Another important result for this period was substantial

diminution of deep non-REM sleep percentage and whole delete of REM sleep during the first 2 h post-injection period of EEG registration (Fig.3B5).

None of the doses of ORXB (5 μg/ml and/or 10μg/ml) injected i.v. did not produce statistically significant behavioral and SWC changes.

Thus, for producing of SWC changes i.v. ORXA was wholly effective in the dose of 10μg/ml in the first 2h post-injection period. The main results of its action are: 1. rising in active wakefulness incidence, time and percentage; 2.decrease in deep non-REM sleep incidence, time and percentage and whole diminution of REM sleep. In sum, present study represents data about the whole effectiveness of i.v. ORXA at the dose of 10μg/ml and supports results of earlier authors showing also effectiveness of i.v. ORXA.

It was concluded that doses of i.v. ORXA, up to 10μg/kg, did not lead to an increase in content of this neuropeptide in cerebrospinal fluid whereas 1.0μg/kg OrexinA administered directly to the nasal mucosa produce significant increase 10 min after application [14]. Despite these it was shown that systemic administration of ORXA in femoral vein inhibits neurogenic dural vasodilatation, while ORXB had no significant effect even at the highest dose. Our results support the data about effectiveness of i.v. 10 μg/ml ORXA in comparison with ineffectiveness of i.v. ORXB in the same dose. In addition, we interested whether there is the difference between the effects of i.v. ORXA and ORXB on the SWC ultradian structure that is whether their effects on SWC are similar. In response to this question we have shown that there is a significant difference between the effects of i.v. injection of ORXA and ORXB. Moreover i.v. ORXB, in contrast to i.v. ORXA, is ineffective in producing of SWC changes.

In support of this it was shown that systemic administration of ORXA in femoral vein inhibits neurogenic dural vasodilatation, while ORXB had no significant effect even at the highest dose. Available results indicate to the significance of hypothalamic Orexin-containing neurons for normal functioning of wakefulness system [5,6]. In this line we demonstrated that i.v. injection of ORXA at the dose of 10μg/ml is wholly effective for the enhancement of active wakefulness incidence, total time and percentage. Results obtained in this work support the data of previous studies showing significant decrease of REM sleep in result of intra-cerebro-ventricular delivery of ORXA [6]. Overall, Orexin neurons are thought to sustain wakefulness and suppress REM sleep [5,6] however, we think that increasing in wakefulness time and suppression of REM sleep can not happen without disturbances in the time parameters and quality of non-REM sleep stages. There are some indications in the literature that ICV injection of orexin in mice and rats increase wakefulness potently and suppress both non-REM and REM sleep [6,10,11]. But it was noted early that systemic administration of Hypocretin-1 produces an increase in activity level, longer waking periods and decrease in REM sleep, without changes in non-REM sleep. Therefore, in present study special attention was devoted to the changes of non-REM sleep stages. We have shown that i.v. ORXA significantly suppress non-REM sleep evoking substantial decrease in the incidence; total time and percentage of deep non-REM sleep. Interestingly, non-REM sleep changes were retained even after recovery of cyclic alternation between SWC behavioral states that is during 5 hour post-injection period of EEG registration of SWC. This result indicates to the involvement of hypothalamic Orexin neurons in the mechanisms modulating non-REM sleep too. This fact can be explained, at least partly, by acting of orexinergic outputs on the monoaminergic and cholinergic neurons that are the main parts of complex mechanisms triggering sleep behavioral states.

As a result, present study represents significant data about the whole effectiveness of i.v. ORXA and ineffectiveness of i.v. ORXB on SWC ultradian structure, namely in contrast to i.v. ORXB i.v. ORXA produced significant increase of active wakefulness incidence, total time and percentage, whole suppression of REM sleep and significant changes in non-REM sleep stages expressed in the reduction of its incidence, total time and percentage. One possible

explanation for such differences in the effects of these neuropeptides may the difference in the affinity between ORXA and ORXB to the ORXR1 and ORXR2 [1,12]. Because ORXA has similar affinity to both ORXR1 and ORXR2 while ORXB reveals a 10-fold higher affinity to ORXR2 [12] it is possible to speak about the significance of ORXR1 for the SWC changes described in the present study. Therefore we can conclude that systemic administration of antagonists for the ORXR1 that bind ORXA can be effective for the aim of clinical therapy of insomnia.

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ადამიანისა და ცხოველთა ფიზიოლოგია

ჰიპოთალამური ნეიროპეპტიდების, ორექსინA-ს და ორექსინB-ს, შიდავენური შეყვანის ეფექტურობა ვირთაგვების ძილ-ღვიძილის ციკლის ულტრადიანულ სტრუქტურასა და კვებით მოტივაციაზე

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(წარმოდგენილია აკადემიის წევრის თ. ნანეიშვილის მიერ)

შეისწავლებოდა ორექსინA და ორექსინB-ს (შეძენილია PHOENIX PHARMACEUTICALS-დან) შიდავენური შეყვანის ეფექტები ძილ-ღვიძილის ციკლის ულტრადიანულ სტრუქტურაზე და კვებით მოტივაციაზე ვირთაგვებში. ორექსინA-ს და ორექსინB-ს სხვადასხვა დოზები (5 მკგ/მლ და 10 მკგ/მლ) შეიყვანებოდა კუდის ვენაში. შრომაში მიღებულია მნიშვნელოვანი მონაცემები ორექსინA-ს შიდავენური შეყვანის მაღალეფექტურობის და ორექსინB-ს არაეფექტურობის შესახებ. ორექსინA-ს შიდავენური შეყვანა, ორექსინB-სგან განსხვავებით, მნიშვნელოვნად ზრდიდა აქტიური ღვიძილის სიხშირეს, ჯამურ დროს და პროცენტულობას, სრულად თრგუნავდა REM ძილს და non-REM ძილის სტადიების სიხშირის, ჯამური დროისა და პროცენტულობის რედუქციას ახდენდა. ვინაიდან ორექსინA-ს ერთნაირი აფინურობა აქვს ორექსინ-1 და ორექსინ-2 რეცეპტორების მიმართ, ხოლო ორექსინB 10-ჯერ მეტ აფინურობას ამჟღავნებს ორექსინ-2 რეცეპტორების მიმართ, შესაძლოა აქცენტის გაკეთება ორექსინ-1 რეცეპტორების როლზე აღწერილ ძილის დარღვევებში. მიგვაჩნია, რომ ორექსინ-1 რეცეპტორების ანტაგონისტების გამოყენება შეიძლება ეფექტური იყოს უძილობის კლინიკური თერაპიისთვის.

REFERENCES

1. *Sakurai T., Amemiya A., Ishii M., Matsuzaki I., Chemelli R. M., Tanaka H., Williams S. C.,* et. al. (1998) Cell. 92: 573–585.
2. *De Lecea L., Kilduff T.S., Peyron C., Gao X., Foye P. E., Danielson P. E.,* Fukuhara et. al. (1998) Proc. Nat. Acad. Sci. USA 95: 322–327.
3. *Samson W. K., Bagley S.L., Ferguson A. V., White M.M.* (2007) Amer. J. Physiol. 292: R382- R87.
4. *Raoof R., E., Esmaeili-Mahani S., Abbasnejad M., Raoof M., Sheibani V., Kooshki R., Amirkhosravi L., Rafie F.* (2015) Neuropeptides, 50: 9-26
5. *Bonnavion P., De Lecea L.* (2010) Current Neur. and Neurosci. Reports, 10: 174-179.
6. *Mieda M., Sakurai T.* (2015) In: Orexin and Sleep, molecular, functional and clinical aspects (Eds. Sakurai T., Pandi-Perumal S.R., Monti J.M.), 53-65.
7. *Nishino S., Ripley B., Overeem S., Lammers G.J.,* (2000) Lancet. 355: 39–40.
8. *Burlet S., Tyler C. J., Leonard C. S.* (2002) Neurosci., 22: 2862–2872.
9. *Nachkebia N., Mchedlidze O., Chkhartishvili E., Chijavadze E.,* et. al., (2013), Proc. Georgian Natl. Acad. Sci. Biomed. Series, 39: 125-133.
10. *Piper D. C., Upton N., Smith M.I., Hunter A.J.* (2000) Eur. J. Neurosci. 12: 726–730.
11. *Bourgin P., Huitron-Resendiz S., Spier A.D., Fabre V., Morte B., Criado J.R.,* Sutcliffe et. al., (2000) Neurosci. 20: 7760–7765.
12. *Smart D., Jerman J. C., Brough S. J., Neville W. A., Jewitt F., Porter R. A.* (2000) British J. Pharmacol. 129: 1289–1291.
13. *Selbach O.* Orexins. (2008) In: Encyclopedia of Molecular Pharmacology (Eds. Offermans S., Rosenthal W.) 2nd edition, Springer, 1.
14. *Hanson L. R., Martinez P. M., Taheri S., Kamsheh L., Mignot E., Frey W.H.* (2004) Drug Delivery Technol. 4: 65–71.

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