METHODS

Effects of Impaza on Sexual Behavior in Different Experimental Models

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Experiments on different models for sexual behavior (seasonal and age-related inhibition of sexual function, animals with initially reduced sexual function) showed that ultra-low doses of anti-NO-synthase antibodies (Impaza) stimulate sexual motivation and copulative behavior in rats. The effects of the drug on different aspects of sexual behavior depend on the chosen model.

Key Words: *sexual behavior; sexual motivation; copulative behavior; ultra-low doses of anti-NO-synthase antibodies*

Investigations of reproductive system functioning and ways for its regulation are not only of fundamental, but also great practical importance, since reproductive health is important factor determining human quality of life. Despite a variety of available preclinical models for sexual behavior investigation, the creation of an adequate model is still an unsolved problem because of difficulties in extrapolation of the obtained data to human [3,4]. Sexual behavior in rodents is presented by a sequence or "cascade" of behavioral events and can be divided into motivational (appetitive) and terminal (consummatory) phases. The boundaries of these phases are not clear; some components can be present in both phases. Unlike terminal phase, stereotypical for each species, motivational behavior is more variable, since survival frequently depends on animal ability to learn various strategies for achievement of the aims [6]. Human sexual behavior is more complex, includes more phases, and comprises socially conditioned elements.

In spite the fact that European and USA regulatory authorities (FDA, EMEA) so far have no official recommendations concerning investigations in this area, plenty of papers were published during last years concerning animal sexual behavior and discussing both reflex response (erection mechanisms) and more complex forms of behavior (sexual desire and its manifestations).

The objective of this work was the analysis of differences in the effects of Impaza in several models of erectile dysfunction.

Impaza is a preparation containing ultra-low doses of antibodies against endothelial NO-synthase. Impaza increases activity of endothelial NO-synthase, restores NO production by endothelium during sexual stimulation, increases cyclic guanosine monophosphate content in smooth muscles and promotes their relaxation, what results in increased blood filling of cavernous bodies and intracavernous pressure gain (increase in penis length in different phases of the copulative cycle) and provides satisfactory strength and duration of erection [2].

The product increases libido and satisfaction from coitus and positively affects spermatogenesis. Regular administration of Impaza promotes rise of testoster-

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one serum level (when baseline level is moderately reduced). Impaza possesses endothelium-protective effects and can be used in complex therapy of diseases associated with endothelial dysfunction [2].

MATERIALS AND METHODS

Copulative behavior of male rats was studied in three different models: seasonal inhibition of reproductive function (winter period: January-February) [1], age-related inhibition of reproductive function in open field test using method [5] (experiments were performed in Institute of Pharmacology North Division of Russian Academy of Medical Sciences in Laboratory of Embryotoxicity and Teratogenicity) and using method [4] in rats with age-related inhibition of reproductive function [7] and in sexually mature rats with initially reduced sexual function (experiments were carried out in Faculty of Psychology of Tromsø University, Norway) [8]. It has to be noted that some authors disclaim the existence of seasonal inhibition of reproductive function in laboratory animals, noted in autumn-winter period in Institute of Pharmacology North Division of Russian Academy of Medical Sciences.

For studying the effects of Impaza on paired sexual behavior in open field using method [5] on model of seasonal and age-related inhibition of reproductive function (Table 1), experimental animals were divided into 2 groups: control and experimental (Impaza). Water Impaza solution or distilled water was administered for 5 days in a dose 5 ml/kg. On day 5 (after the last Impaza dose), paired sexual behavior was investigated. The total mount number (TMN) and coupling number (CN) were registered for 15 min.

To investigate the effects Impaza on copulative behavior using method [3], selection of the rats with impaired erectile ability was performed on the basis of copulative behavior assessment made in 3 screening tests. Sexually mature male Fisher 344 and Wistar rats (n=60) participated in the screening test. Erectile ability was assessed by the intromission coefficient (IC; intromission number/(mount number+intromission number). The animals passed the screening had erectile ability below average. Impaza was administered orally for 28 days to old male rats in dose 6.6 ml/kg and to sexually mature rats in two doses (3 or 9 ml/kg). Rat copulative behavior was studied before Impaza administration and on days 7, 14 and 28 of treatment. Experiments were recorded on video. The effects of the preparation were estimated using IC value. In addition, sometimes video data was used to assess the length of erected penis, looking out the preputium during mount and/or intromission or ejaculation. Video records of mounts (with subsequent intromission/ejaculation or without) were analyzed using overlapping of images. Image of penis with maximal erection was used for measurement.

The effects of Impaza on sexual motivation were investigated using method [4]. Like copulative behavior, sexual motivation in rats was investigated before drug administration and on days 7, 14 and 28 of treatment. To this end, a male rat was placed in the experimental chamber connected with two cages, where receptive female and mature male were placed as drive stimuli. Animal movements within the chamber were recorded for 10 min, and then the time spent near the female (T1) and near the control male (T2), number of visits into the corresponding ones, and motor activity were analyzed. Preference coefficient (PC) in favor of females in comparison with male (ratioT1/T1+T2) and increase in T1 time were used as criterion of the influence on sexual motivation.

RESULTS

Despite of the use of several approaches for sexual behavior investigation, all experiments revealed unidirectional effects of Impaza. The drug in the studied doses affected the main aspects of sexual behavior, motivational (appetitive) and terminal (consummatory) phases of sexual behavior.

Thus, in experiment employing method [5], daily administration of Impaza (5 ml/kg for 5 days) to animals from the group with seasonal or age-related inhibition of reproductive function resulted in activation of their sexual behavior, which was seen from significant increase in TMN and IC compared to the control (Table). In experiments on animals with age-related impairment, an increase in sexual activity in comparison with control was noted in 55.5% animals (p<0.05).

On day 28, PC increased by 24.1% in comparison with baseline in the experiment performed using method [4] with administration of 6.6 ml/kg Impaza daily for 28 days to male Fisher 344 rats with physiological inhibition of sexual function. Moreover, this parameter decreased by 4.8% in the control group. In old animals of both control and experimental groups copulations were absent.

Administration of 9 ml/kg Impaza to sexually mature Fisher 344 rats 2.6-fold increased IC in comparison with the control (p<0.05). Beneficial effects of Impaza were also observed in terms of penis length during ejaculation: Impaza (9 ml/kg) increased penis length by 8.6%, which is quite important, since erection strength at the moment preceding intromission is the factor determining intromission occurrence or nonoccurrence in the future.

The effects of Impaza on sexually mature Wistar rats differed from that on Fisher 344 rats. Impaza (3 ml/kg) had a stimulation effect on sexual motiva-

TABLE 1. Main Parameters of Experimental Investigation of Impaza

Model	Investigation site	Animals (age, weight)	Dose, treatment period	Results
Analysis of copulative behavior				
Seasonal sexual function inhibition (open field according to method [5])	Institute of Pharmacology, North Division of the Russian Academy of Medical Sciences	Mongrel albino male rats (*n*=17, 4 months, 400-450 g)	5 ml/kg, 5 days	TMN increased 2.6-fold CN was increased 4-fold*
Age-related sexual dysfunction (open field using method [5])	Institute of Pharmacology, North Division of the Russian Academy of Medical Sciences	Mongrel white male rats (*n*=17, 16 months, 600-700 g)	5 ml/kg, 5 days	TMN increased 1.5-fold CN was increased 1.8-fold*
Age-related sexual dysfunction [3]	Tromsø University, Norway	Male Fischer 344 rats (*n*=30, 20 months, 420-490 g)	6.6 ml/kg, 28 days	No copulation
Sexually mature rats with impaired erectile function (IC below average) [3]	Tromsø University, Norway	Male Fischer 344 (*n*=30, 5 months, 310-330 g) and Wistar (*n*=30, 5 months, 330-360 g) rats	3 ml/kg, 28 days 9 ml/kg, 28 days	Fisher 344 rats: Impaza 9 ml/kg increased IC 2.6-fold* Wistar rats: Impaza 3 ml/kg increased certain parameters of copulative behavior in rats
Analysis of sexual motivation				
Age-related sexual function impairment [4]	Tromsø University, Norway	Male Fischer 344 rats (*n*=30, 20 month, 420-490 g)	6.6 ml/kg, 28 days	PC was increased 1.8-fold (in comparison with control group)
Sexually mature rats with impaired erectile function (IC below average) [3]	Tromsø University, Norway	Male Fischer 344 (*n*=30, 5 month, 310-330 g) and Wistar (*n*=30, 5 months, 330-360 g) rats	3 ml/kg, 28 days 9 ml/kg, 28 days	Fisher 344 rats: PC was unaffected Wistar rats: Impaza 3 ml/kg had an effect on PC

Note. *$p<0.05$ compared to the control group.

tion on Wistar rats, increasing PC by 17.2% on day 28 of treatment in comparison with baseline level ($p<0.05$). Copulative behavior was not significantly affected, although after treatment with Impaza in a dose of 9 ml/kg we observed a trend toward increase in intromission number and percentage of animals with ejaculation.

There are no data confirming the differences in sexual behavior of Fisher 344 and Wistar rats. Fisher 344 rats are known to be more anxious and susceptible to stress than Wistar rats [12]. Some authors revealed interstrain differences in the level and affinity of certain neurotransmitters, morphine sensitivity [13], and cytochrome P450 system [11]. Our findings show that despite Fisher 344 rats are more fearful and susceptible to stress, they demonstrate higher level of sexual motivation than Wistar rats. According to recent data, cytochrome P450-dependent arachidonic acid metabolite, 11,12-epoxyeicosantrienoic acid (EET), produces consistent cavernous body relaxation through NO-cGMP-signaling and ATP-sensitive K^+-channels [14]. EET injection into rat penis blocks the rise of intracavernous pressure in response to electric stimulation of the main pelvic ganglion [9]. Moreover, cytochrome P450 can possess its own NOS-activity [10]. Under physiological condition, NO produced by P450, eNOS, or nNOS may cause erection after interaction with EET. From the fact that P450 system is more active in Fisher 344 rats than in other strains [11], one may assume their higher sensitivity to Impaza, which

explains the increase in erection was observed after long-term Impaza administration in Fisher 344 rats, whereas this effect was absent in Wistar rats.

Thus, experiments performed on several models of impaired erectile function revealed unidirectional stimulating effects of Impaza on various aspects of sexual behavior; the degree of Impaza effect depended on the model employed.

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