The Analog of Arginine-Vasopressin (6-9) Fragment, Ac-D-SPRG, Exhibits Antidepressant Action in Rats in Case of Intranasal Injection

ARTICLE *in* NEUROCHEMICAL JOURNAL · JULY 2015

Impact Factor: 0.3 · DOI: 10.1134/S1819712415030034

5 AUTHORS, INCLUDING:

Andrey Sinjushin

Lomonosov Moscow State University

27 PUBLICATIONS **57** CITATIONS

ISSN 1819-7124, Neurochemical Journal, 2015, Vol. 9, No. 3, pp. 201–205. © Pleiades Publishing, Ltd., 2015.

EXPERIMENTAL ARTICLES

The Analog of Arginine-Vasopressin (6-9) Fragment, Ac-D-SPRG, Exhibits Antidepressant Action in Rats in Case of Intranasal Injection[1]

A. S. Belyakova[a, 2], A. A. Sinjushin[b], O. G. Voskresenskaya[a], A. A. Kamensky[a], and V. P. Golubovich[c]

[a] *Department of Human and Animal Physiology, M. V. Lomonosov Moscow State University, Moscow, Russian Federation*
[b] *Department of Genetics, M.V. Lomonosov Moscow State University, Moscow, Russian Federation*
[c] *Institute of Bioorganic Chemistry, National Academy of Sciences, Minsk, Republic of Belarus*

Received April 7, 2015

Abstract—The antidepressant properties of newly synthesized analog of arginine-vasopressin fragment analog, Ac-D-SPRG, were tested using Porsolt's swimming test on white rats. It was demonstrated that this substance when injected intranasally decreases the depression in comparison with the control group.

Keywords: depression, forced swimming test, arginine-vasopressin, neuropeptide, intranasal injection
DOI: 10.1134/S1819712415030034

INTRODUCTION

The arginine-vasopressin (AVP) is the first biologically active peptide artificially synthesized [1, 2]. While its peripheral functions and effects have been comparatively finely studied to date, the influence of AVP, its analogs and fragments on higher nervous activity and behavior of animals and humans are still to be investigated.

The influence of AVP on animal behavior, anxiety and depression together with training with positive and negative reinforcement was for the first time demonstrated in the works of De Wied with coauthors in 1960s [3–6]. It was demonstrated that the given effects were not mediated by the hormonal action of peptide.

It was found out that the products of vasopressin proteolysis might exhibit high behavioral activity [1]. It was hypothesized that such action is inherent not only for a vasopressin itself but also for the fragments resulting from the degradation of a hormone caused by the proteolytic action of blood enzymes. The vasopressin molecule was initially proposed to split into ring structure, pressinamide, and a C-terminal part [5], It was later found out that peptidases cleave the initial vasopressin into few linear fragments from 4th to 9th amino acid residues [4, 5]. Then the following possible sites of hydrolysis of peptide bonds were postulated in a vasopressin molecule. (1) The dipeptidyl aminopeptidase cleaves the N-terminus between Tyr and Phe causing a formation of AVP(1-2) and AVP(3-9) fragments. (2) Either prolyl endopeptidase or peptidyl peptidase A can cleave a bond between Pro and Arg thus producing AVP(1-7) and AVP(8-9). (3) The action of thrombin or plasmin on C-terminus results in a cleavage of bond between Arg and Gly and a formation of AVP(1-8) and AVP(9).

The available literature sources evidence for the functional significance of C-terminal part of vasopressin molecule in generation of the behavioral effects. Theoretical studies on possibilities of metabolic vasopressin fragments enabled to find out eight cysteine-containing C-terminal fragments: AVP(3-9), (4-9), (5-9), (6-9), (3-8), (4-8), (5-8), (6-8). The fragments AVP(3-9), (4-9), (5-9), and (6-9) are produced in blood with higher probability than the others.

The table represents the statistical data which indicate that AVP(3-9) fragment is produced with the highest likelihood. However, this oligopeptide arises on the first stage of degradation and is itself degraded by nine peptidases. The lifetime of this fragment is too short so it can be excluded from the analysis. The

The probability of generation of different AVP fragments after vasopressin degradation in blood, after [7]

Fragment	Probability of generation
AVP(3-9)	0.07
AVP(4-9)	0.06
AVP(5-9)	0.05
AVP(6-9)	0.05

[1] The article is published in the original.

[2] Corresponding author; address: Leninskie gory, 1, str. 12, Moscow, 119234, Russia; phone: +7 (495) 939-43-09; fax: +7 (495) 939-54-90; e-mail: alixletter@yandex.ru.

probability that AVP(3-9) reaches the goal is almost zero [7]. Oppositely, the formation of AVP(6-9) occurs due to activity of a single enzyme, dipeptidyl aminopeptidase I, which cleaves the N-terminal part of AVP(4-9) fragment. On the one hand, the likelihood of AVP(6-9) formation in blood is high enough. On the other hand, it is degraded with five peptidases only, so the efficient reaching the goal is possible enough [7].

The metabolic fragments of vasopressin are known to possess the neurotropic activity which is comparable (or often exceeds) the one of an intact molecule. These oligopeptides stimulate behavioral characteristics, improve training efficiency and memory [8, 9]. It was earlier demonstrated that the most expressed neurotropic effect characterizes the fragment AVP(6-9): Cys^6-Pro^7-Arg^8-Gly^9-NH_2 [10]. To obtain the new analog with an expressed nootropic action in small doses, it is desirable to synthesize a peptide with Cys^6 substituted with another amino acid. Such modification can enhance the resistance of a molecule against blood peptidases. Search for biologically active was carried out with a special reference to link between structure and activity, involving elements of effector-receptor interactions. Combination of two methods (isolation of biologically active conformation and active fragments of peptide regulators) enables to obtain the substances with a predictable profile of action. As a result of a conducted analysis, the tetrapeptide with Cys^6 replaced with D-Met (Ac-D-MPRG) was synthesized. Studies on its action demonstrated that its intranasal injection in small doses in animals leads to the increased exploratory behavior, decreased anxiety and depression together with accelerated training with both negative and positive reinforcement [11, 12]. Replacement of C-terminal Cys with D-Met in AVP led to production of new peptide with an expressed nootropic action, which exhibits its effect in case of intranasal injections in minute doses.

During the presented stage of research, the C-terminal cysteine in AVP fragment was replaced with D-serine. We studied the influence of the obtained analog, Ac-D-SPRG, on depression characteristics of animals in case of a single intranasal injection.

METHODS

Animals. The work was carried out using the pubescent males of non-linear white rats with weight of 220–250 g. Every group included averagely 18 animals. All animals were housed in groups of ten individuals in plastic containers with iron lattice, on a 12 : 12-h light-dark schedule in a temperature-controlled (22°C) chamber. All rats were freely supplied with water and food.

Forced swimming test. The degree of depression was studied in the "forced swimming" test [13, 14] with some modifications. The animals were not subjected to a pre-test before the test itself. Every animal was placed in a center of a plastic cylindrical container (height 60 cm, diameter 40 cm) with volume of 60 L, being filled with water (28°C) on two thirds of its volume. Such experimental set up disabled any support for animals, even with tails. The following parameters were scored during 10 min: joint time of active and passive swimming, joint time of immobilization; number of periods of active and passive swimming, number of immobilization periods; the latent period and duration of the first immobilization and first active swimming; average duration of period of active and passive swimming; average duration of the immobilization period.

The following behavioral patterns were recognized: (1) immobilization—a rat was floating in water without active movements except for rare singular strokes which enable rat's head to be kept above water level; (2) active swimming—an animal was producing active swimming movements with all legs and tail, thus rapidly moving within a basin; (3) passive swimming—a rat made regular swimming movements usually without broaching with its forepaws from the water.

After a swimming, the animals were removed from the basin, dubbed with towel and placed into a heated cage for 20–25 min.

Locomotor activity. The locomotor activity and physical endurance were scored in the experimental apparatus comprising the vertical nylon bed with cells of 1.5 × 1.5 mm and total area of 30 × 60 cm. This bed is boarded with wooden panels from above and on all perimeters. Every animal was placed on the bed, and the time between this placement and drop was scored. This time was measured three times. A total duration for three repeats was estimated as a measure of endurance.

Drug treatment. The Ac-D-SPRG was injected intranasally [15, 16] in doses of 0.01, 0.1, 1.0, and 10.0 μg/kg in a volume of 1 μL per 10 g of body mass 5, 15, and 30 min before tests. The control animals were treated with an equivalent volume of solvent (distilled water). According to available data on dynamics of AVP accumulation in blood after intranasal injection, the highest concentration of AVP in serum is achieved in 20–30 min after administration [16]. After 5 min, the concentration of AVP in serum comprises about half-maximum, so we have chosen these intervals as informatory.

The intranasal way of injection was chosen for more rapid delivery of the peptide into a brain avoiding hematoencephalic barrier [15]. Except this, the given mode of injection is the least stressful which is important for studies on the behavioral reactions in animals.

Statistical analysis. For statistical treatment, the standard methods of analysis were applied. The nonparametrical Mann-Whitney test was used. The results were treated with Statistica 6.0 software package and MS Excel.

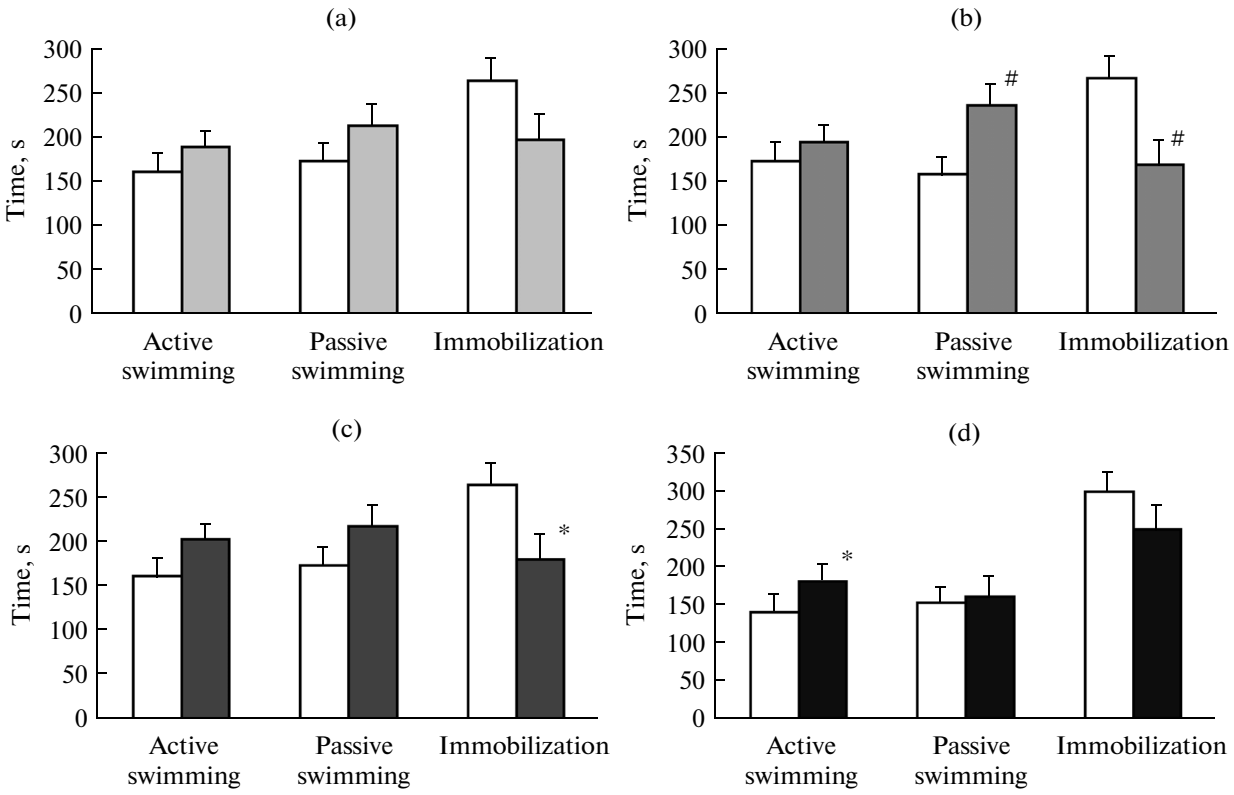

Fig. 1. Effects of Ac-D-SPRG injection 5 min before tests, in doses of 0.01 μg/kg (a), 0.1 μg/kg (b), 1.0 μg/kg (c), and 10.0 μg/kg (d). Average duration of phases and standard error are presented here and on other graphs. White bars = control, * = difference from the control significant at $P < 0.05$, # at $P < 0.01$.

RESULTS

Locomotor Activity

For all studied doses of Ac-D-SPRG, the locomotor activity revealed in the test with vertical bed was on equal level both in control and experimental groups (data not shown). Regardless of time interval between drug administration and testing, the differences between two groups were insignificant (Mann–Whitney test).

The Forced Swimming Test

Administration of Ac-D-SPRG 5 min before the testing. In case of injection of Ac-D-SPRG in all doses 5 min before the "forced swimming" test, many of registered characteristics significantly differed from the control.

When injecting the analog in dose of 0.01 μg/kg, the significant increase of the number of periods of active swimming in comparison with a control group was recorded. The total duration of active swimming was also increased, although not statistically significantly (Fig. 1a). The average duration of immobilization period was significantly decreased.

The injection of Ac-D-SPRG in a dose of 0.1 μg/kg caused the significant increase of the first period of active swimming compared with the control animals. The total duration of active swimming was insignificantly increased, while the total time of passive swimming and number of passive swimming periods increased reliably (Fig. 1b). Except this, the total time of immobilization shortened significantly (Fig. 1b), while the first immobilization episode occurred significantly later than in control.

The injection of Ac-D-SPRG in a dose of 1 μg/kg led to the significantly higher number of active swimming periods than in a control group. The total duration of active swimming was insignificantly increased, while number of immobilization periods and the total duration of immobilization were decreased reliably (Fig. 1c). The first immobilization episode also occurred significantly later in an experimental group.

The significant increase of the duration of active swimming was recorded after injection of Ac-D-SPRG in a dose of 10 μg/kg (Fig. 1d). Except this, the tendency towards the increase of the duration of the first period of active swimming was recorded: only a single control animal exhibited this period exceeding 90 s compared with five individuals in an experimental group ($p = 0.058$, Mann–Whitney test).

One may conclude that the injection of studied tetrapeptide in all doses leads to the decrease of depression in animals. The obtained results indicated that

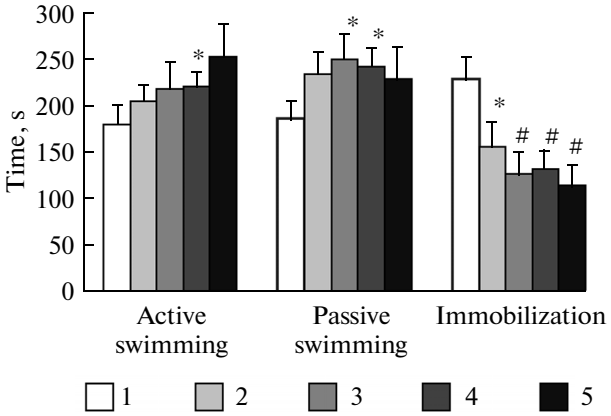

Fig. 2. Effects of Ac-D-SPRG injection 15 min before tests. 1 = control, 2 = 0.01 μg/kg, 3 = 0.1 μg/kg, 4 = 1.0 μg/kg, 5 = 10.0 μg/kg. See Fig. 1 for further designations.

such action is the most expressed for doses of 0.1 and 1.0 μg/kg.

Administration of Ac-D-SPRG 15 min before the testing. The injection of the analog 15 min before the "forced swimming" test also had the significant influences on most recorded features in all doses (Fig. 2).

When injecting Ac-D-SPRG in a dose of 0.01 μg/kg, the reliably later occurrence of the first immobilization period was observed, while the total duration of immobilization was also shortened. A total number of immobilization episodes decreased reliably.

The treatment of animals with 0.1 μg/kg of Ac-D-SPRG caused the reliable increase of the total duration of passive swimming together with the decrease of duration and number of periods of immobilization. The later occurrence of the first immobilization period was also revealed.

A dose of 1 μg/kg caused the significantly longer duration of the first active swimming period in comparison with a control group. The total duration of active and passive swimming was also reliably longer, and the total duration of immobilization (together with the number of immobilization episodes) shorter. We also registered the significantly later occurrence of the first immobilization period.

The injection of Ac-D-SPRG in a dose of 10 μg/kg caused the reliably later occurrence of the first immobilization period. The number of immobilization periods and their joint duration were also significantly decreased in comparison with a control.

Administration of Ac-D-SPRG 30 min before the testing. The intranasal injection of Ac-D-SPRG 30 min before the test also had certain effects in all studied doses. Applying a dose of 0.01 μg/kg caused the reliably prolonged total duration of passive swimming combined with the shortened total immobilization time. The first immobilization episode also occurred reliably later (Fig. 3).

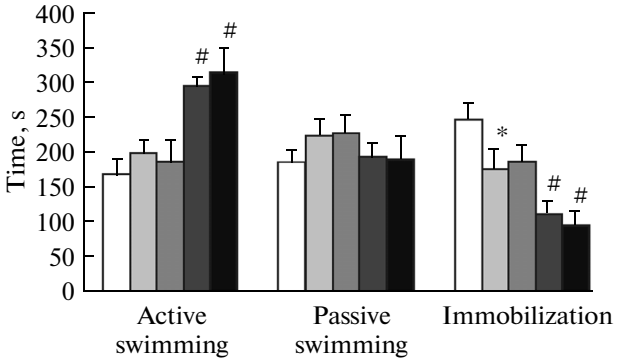

Fig. 3. Effects of Ac-D-SPRG injection 30 min before tests. See Figs. 1 and 2 for further designations.

A dose of 0.1 μg/kg caused the decrease of the total immobilization duration and later occurrence of the first immobilization period. The significantly longer first active swimming period was recorded after the injection of 1 μg/kg of Ac-D-SPRG. This effect was accompanied by the prolonged total time of active swimming, the decreased joint duration of immobilization and fewer number of immobilization episodes. The first episode of immobilization occurred significantly later than in a control group of animals.

The influence of Ac-D-SPRG in a dose of 10 μg/kg was significant and similar to one of a dose of 1 μg/kg.

DISCUSSION

We have demonstrated that Ac-D-SPRG, the synthetic analog of AVP(6-9) fragment, causes decrease of depression in white rats in a modified forced swimming test in a range of doses from 0.01 to 10 μg/kg. These data finely accord with evidences for AVP participation in stress-inhibiting reactions of organism. For example, endogenous AVP synthesis in lateral septum causes decrease of immobilization duration in forced swim test. The application of antagonist of V1 receptors with usage of reverse microdialysis causes the opposite results [17].

The question on pathogenesis of depression still remains under discussion. It is however proposed that different forms of depression are connected with malfunctioning of the HPA axis. Rats of HAB line exhibit the increased reactivity of the HPA axis in response to emotional stressor of moderate intensity [18]. Injection of antagonist of V1a receptors leads to decrease of anxiety and significant switch in choice of stressor avoidance: active reaction replaces passive one. This evidences for antidepressant action of antagonist. Hence, AVP modulates behavioral and neuroendocrinal characters dealing with regulation of depression in animals [19]. It was also demonstrated that activity of the HPA axis decreases in case of knockout of gene of V1b receptors [20]. Some data exist which confirm

that usage of selective antagonists of V1b receptors has an antidepressant effect [21–24].

The intranasal injection of 10 μg/kg of AVP led to decrease of immobilization period, and the first immobilization episode occurred later in treated animals. The tetrapeptide Ac-D-MPRG caused prolonged duration of active swimming (and hence decreased immobilization time) in doses of 0.01 and 10 μg/kg [25].

Both Ac-D-SPRG and Ac-D-MPRG have antidepressant action in case of intranasal injection. The mechanism of such action of tetrapeptide analog is of certain interest but it is precocious to classify the studied peptide as either agonists or antagonist of any AVP receptors. It is also connected with the fact that role of different receptors in genesis of anxiety and depression is still poorly studied (see above). To date, the regulation of depressive states is proposed to be mediated by AVP receptors of V_{1b} type [21, 22, 26]. So, one may propose the existence of interaction of newly synthesized peptide with this type of AVP receptors which is however needs further investigation.

The antidepressant action of Ac-D-SPRG is doubtless. The results of test for locomotor activity indicate that physical fitness in both control and experimental animals is on similar level. This indicates that Ac-D-SPRG does not modulate locomotion activity and all differences in the forced swimming test can be explained by induced changes in depression state. Immobilization occurs earlier and lasts longer in control animals which evidences for generation of "behavioral despair" state in them. This state can be estimated as an experimental analog of despair and pessimism in clinical symptoms of depression.

CONCLUSIONS

During an experimental study of effect of newly synthesized analog of arginine-vasopressin, we have observed the decrease of immobilization period, longer periods of active and passive swimming in treated animals. This phenomenon evidences for antidepressant action of studied tetrapeptide. These results correspond to observations of application of AVP itself and another tetrapeptide analog, Ac-D-MPRG. The original analog Ac-D-SPRG has the potential as a basis for antidepressant drug and surely comprises significant interest for studies on pathogenesis of depressive states.

REFERENCES

1. Du Vigneaud, V., Ressler, C., Swan, J.M., Roberts, C.W., and Katsoyannis, P.G., *J. Am. Chem. Soc.*, 1954, vol. 76, pp. 3115–3121.
2. Du Vigneaud, V., The isolation and proof of structure of the vasopressins and the synthesis of octapeptide amines with pressor-antidiuretic activity, *Proceedings of the Third International Congress of Biochemistry*, New York: Academic Press, Inc., 1956, pp. 49–54.
3. De Wied, D., *Int. J. Neuropharmacol.*, 1965, vol. 4, pp. 157–167.
4. De Wied, D., *Nature,* 1971, vol. 232, pp. 58–60.
5. De Wied, D., *Life Sci.*, 1976, vol. 19, pp. 685–690.
6. De Wied, D. and Gispen, W.N., Behavioral effects of peptides, in *Peptides in Neurobiology*, US: Springer, 1977, pp. 397–425.
7. Tonkovich, I.N., Mathematical modeling and prediction of degradation of peptide compound, *PhD Thesis*, Minsk, 2000.
8. Burbach, J.P., Kovacs, G.L., de Wied, D., van Nispen, J.W., and Greven, H.M., *Science*, 1983, vol. 221, pp. 1310–1312.
9. Lin, C., Lin, R., and Du, Y., *Hippocampus*, 1993, vol. 3, pp. 193–204.
10. Golubovich, V.P., Martinovich, V.P., Usenko, A.P., Slobodchikova, L.K., Titov, S.A., and Ashmarin, I.P., *Pharm. Chem. J.*, 1988, vol. 22, pp. 440–444.
11. Ponomareva, N.S., Voskresenskaya, O.G., Kamensky, A.A., Golubovich, V.P., and Ashmarin, I.P., *Zhurn. Vyssh. Nerv. Deiat. im. I.P. Pavlova*, 1998a, vol. 48, pp. 471–477.
12. Ponomareva, N.S., Voskresenskaya, O.G., Kamensky, A.A., Golubovich, V.P., and Ashmarin, I.P., *Russ. J. Physiol.*, 1998b, vol. 84, pp. 1363–1369.
13. Porsolt, R.D., Anton, G., Blavet, N., and Jaltre, M., *Europ. J. Pharmacol.,* 1978, vol. 47, pp. 379–386.
14. Detke, M.J., Rickels, M., and Lucki, I., *Psychopharmacology*, 1995, vol. 121, pp. 66–72.
15. Pietrowsky, R., Struben, C., Molle, M., Fehm, H.L., and Born, J., *Biol. Psychiatry,* 1996, vol. 39, pp. 332–340.
16. Born, J., Lange, T., Kern, W., McGregor, G.P., Bickel, U., and Fehm, H.L., *Nature Neurosci.*, 2002, vol. 5, pp. 514–516.
17. Koolhaas, J.M., Everts, H., de Ruiter, A.J., de Boer, S.F., and Bohus, B., *Progr. Brain. Res.*, 1998, vol. 119, pp. 437–448.
18. Landgraf, R., Wigger, A., Holsboer, F., and Neumann, I.D., *J. Neuroendocrinol.*, 1999, vol. 11, pp. 405–407.
19. Wigger, A., Sanchez, M.M., Mathys, K.C., Ebner, K., Frank, E., Liu, D., et al., *Neuropsychopharmacology*, 2004, vol. 29, pp. 1–14.
20. Tanoue, A., Ito, S., Honda, K., Oshikawa, S., Kitagawa, Y., Koshimizu, T.A., et al., *J. Clin. Invest.*, 2004, vol. 113, pp. 302–309.
21. Griebel, G., Simiand, F., Gal, C.S.-L., Wagnon, J., Pascal, M., Scatton, B., et al., *Proc. Natl. Acad. Sci. USA*, 2002, vol. 99, pp. 6370–6375.
22. Griebel, G., Simiand, J., Stemmelin, J., Serradeil-Le, G.C., and Steinberg, R., The vasopressin V1b receptor as a therapeutic target in stress-related disorders, in *Current Drug Targets-CNS and Neurological Disorders*, Princeton (NJ): Bentham Science Publishers, 2003, vol. 2, pp. 191–200.
23. Serradeil-Le, G.C., Wagnon, J., Simiand, J., Griebel, G., Lacour, C., Guillon, G., et al., *J. Pharmacol. Exp. Ther.*, 2002, vol. 300, pp. 1122–1130.
24. Stemmelin, J., Lukovic, L., Salome, N., and Griebel, G., *Neuropsychopharmacology*, 2005, vol. 30, pp. 35–42.
25. Voskresenskaya, O., Kamensky, A., and Golubovich, V., *Europ. Neuropsychopharmacol.*, 2007, vol. 17, pp. S249–S250.
26. Iijima, M., Yoshimizu, T., Shimazaki, T., Tokugawa, K., Fukumoto, K., Kurosu, S., Kuwada, T., Sekiguchi, T., and Chaki, S., *Brit. J. Pharmacol.*, 2014, vol. 171, pp. 3511–3525.