PHARMACOLOGY

Changes in Systemic Hemodynamics After Acute Administration of Diazepam Binding Inhibitor (DBI) and After Autoimmunization Against DBI

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Recently functional disturbance of the GABA-ergic system has come to be considered a factor in the development of arterial hypertension [4]. GABA-system dysfunction has been shown to produce an activation of the synaptic centers in the brain stem and spinal cord and a rise of the arterial pressure (AP) and heart rate (HR) [8,14]. The GABA receptor is known to be a complex containing binding sites to a variety of endogenous and exogenous agents, including benzodiazepines, which allosterically regulate GABA functions [12]. In 1983 a peptide was isolated from rat brain tissue which inhibited the binding of ligands (in particular, diazepam) with benzodiazepine receptors of the cell membrane (a diazepam binding inhibitor, or DBI). This endopeptide causes convulsions in rats, exhibits proconflict and stressogenic properties, and blocks GABA-ergic transduction [6]. In this connection, a study of the effect of DBI on the cardiovascular system and, in particular, on the cardiac output (CO), AP, and HR is of undoubted interest, especially since such data are not available in the literature.

In the first part of the present study we investigated the hemodynamic effects of a DBI active fragment - octadecaneuropeptide (ODN) - administrated intravenously in different doses. The fragment was synthesized at the Ferment Plant (Novosibirsk) according to Samukov's method [1]. In the second part of the experiment the opposite influence on the hemodynamics were studied, namely, a chronic depletion of the organism with endogenous ODN, which was achieved by inverse immunization, i.e, active autoimmunization against the peptide [2]. The results could be of value in the search for new means of achieving long-lasting activation of the GABA-ergic system as well as for the development of prolonged antistressor and antihypertensive drugs.

MATERIALS AND METHODS

Wistar rats weighing 250-280 g were used for the experiments. For measurement of the hemodynamic characteristics the rats were catheterized under nembutal narcosis in the femoral artery and jugular vein. A temperature-sensitive element was inserted in the arch of the aorta [3]. AP and HR in the femoral artery as well as CO by the method of thermodilution were measured in alert rats after 24 hours. The main experiment was carried out 24 hours after redetermination of the initial hemodynamics characteristics. In series I ODN in a concentration of 40,

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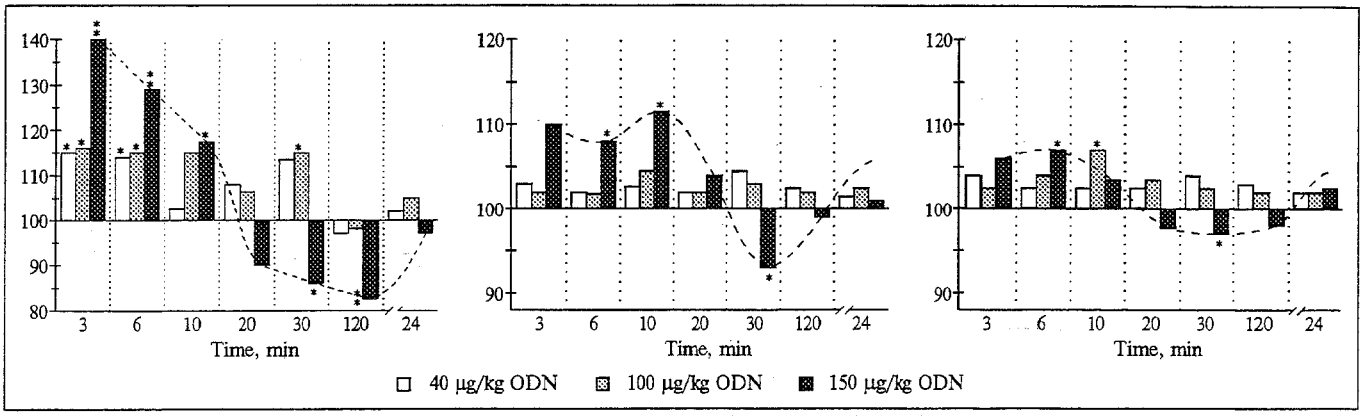

Fig. 1. Changes of CI, AP, and HR in response to i.v. injection of ODN (in % of initial level); Dotted line represents a biphasic response to the 150 μg/kg dose. Asterisks: the differences from the initial value are statistically reliable: * — $p<0.05$, ** — $p<0.01$.

100 or 150 mg/kg in a volume of 0.2 ml was injected intravenously in a bolus. Each animal received only one injection. Series II of the experiments was carried out on 3 groups of rats. The rats of the first group were immunized with a ODN-BSA (bovine serum albumin) conjugate. The immunization was performed by subcutaneous injections at four points of the back for a total of 5 times during 3 months. The antibody titer was 1:2560 at the beginning of the experiment [1]. The second (control) group received BSA injections and the third (control) group physiological saline injections according to the same scheme. In this last series, in addition to the mentioned parameters, the reaction to two types of stress (a sharp ring lasting 5 sec and tail clamping) was studied.

It should be pointed out that the rats were alert and free during the experiments. The data were processed statistically with a nonparametric test and Student's *t* test.

RESULTS

Series I. The initial hemodynamic parameters on average were the following: AP=103.0±4.6 mm Hg, cardiac index (CI) (CO/100 g body weight) = 33.6± ±3.1 ml/min/100g, HR=415±17.8 beats/min. Figure 1 illustrates the changes of these parameters during the first minutes, hours, and 24 hours after intravenous injection of ODN. ODN was injected through the catheter in the jugular vein in 3 different doses. A dose-dependent increase of CI (by 10-70% on average) was observed in practically all animals during the first 3-10 min after ODN injection. AP and HR were almost unchanged with the CI increase even during the first seconds after the injection, except in the case of the dose of 150 μg/kg, when a slight but significant rise of AP and HR occurred. Within an interval from 30 min to 2 hours after administration of 40 and 100 μg/kg ODN CI dropped to the basal level. The opposite result was observed with 150 μg/kg ODN: a biphasic response (rebound effect) was detected, i.e, the pronounced increase of CI during the first few minutes gave way to a drop to a level far lower then the initial CI value. Moreover, a significant drop of the blood temperature by 0.5-1°C was also noted.

After 24 hours the hemodynamic characteristics normalized in almost all rats. The behavior of the animals remained practically unchanged following ODN injection.

Series II . The hemodynamic parameters of the experimental groups are presented in Table 1. A drop of AP and a reduction of the peripheral resistance (PR) by 16% on average were detected in the animals immunized against ODN. These changes, including a certain increase of CI, which is apparently compensatory in nature, are typical for chronic mild hypertension. The immunized animals also proved to be less sensitive to stress, judging from the increase of AP and HR in response to the sharp ring or pain (Fig. 2, *a, b*).

TABLE 1. Hemodynamic Indexes in Resting Rats after Immunization

Hemodynamic Indexes	Animals		
	Control 1 (physiol. saline)	Control 2 (BSA)	Experiment (ODN-BSA)
AP, mm Hg	117.2±3.4	112.3±4.0	104.0±2.4*
HR, b/min	408.9±6.7	433.3±13.4	410.0±13.0
CI, ml/min/100g	34.7±2.6	32.4±1.5	36.6±0.5**
PR, rel. units	3.4±0.2	3.5±0.1	2.8±0.1*,**

Note: asterisks: significant differences $p<0.05$: * — as compared to control 1, ** — to control 2.

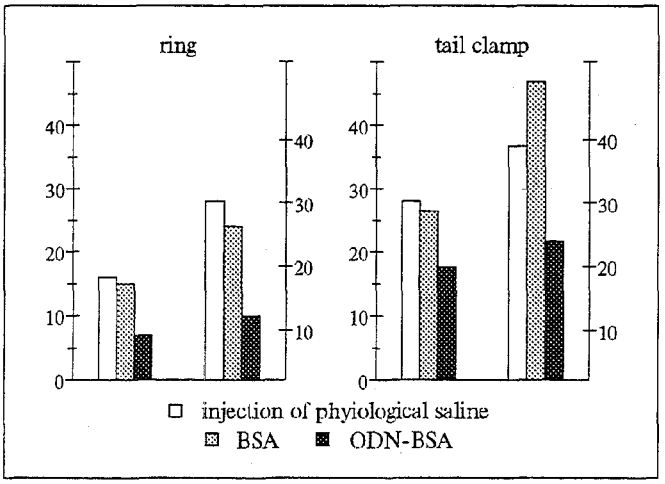

Fig. 2. AP (mm Hg) and HR (beats/min) changes in response to stress: *a*) ring, *b*) tail clamp.

In discussing these results, it should be remembered that DBI was found not only in the brain stem, spinal cord, and the central emotiogenic structures of the brain (cortex, hypothalamus, etc.) but also in a variety of peripheral organs: the adrenal glands, spleen, kidneys, blood vessels, heart, and blood plasma [5, 13]. The functions of DBI in the heart are still unknown, but "peripheral" benzodiazepine receptors are localized in the ventricles, and are probably involved in the regulation of the spike frequency of the papillary muscle [10]. Since AP was unchanged, the increase of CI, namely the stroke volume (Fig. 1), in the first few minutes after ODN injection is most likely connected with its direct cardiostimulatory influence, PR being decreased apparently to compensate for the CI increase. Besides, it evidently takes time to penetrate the blood-brain barrier. The injection of a high dose of ODN (150 μg/kg) led to a slight increase of AP and HR (Fig. 1), suggesting a GABA-mediated stimulatory influence on the CNS structures, which regulates the activity of the sympathetic nervous system. An increase of plasma catecholamine concentration was shown in rats [14] to which the GABA-receptor antagonist bicuculline was injected in the posterior hypothalamus.

The cardiodepressive effect observed 2 hours after the injection of 150 μg/kg ODN and the drop of the blood temperature probably resulted from the ODN-induced stimulation of GABA synthesis in the central structures and from the enhancement of inhibitory influences on the sympathetic nervous system [8,14]

Our data on the cardiostimulatory effect of ODN are indirectly confirmed by results reported previously [11], where diazepam (a DBI competitor for the GABA-receptor complex), potentiating the GABA effects, on the contrary, caused a reduction of CI and a pronounced rise of PR, and did not affect AP or HR.

As expected, the immunization against DBI exerted a certain sedative effect on the animals, which became less sensitive to the stress (Fig. 2, *a, b*). It is known that the heart component of the baroreceptor reflex is inhibited in stress: HR does not decrease with the AP elevation - on the contrary, both these values are raised. This is related to an increase of the sympathetic and a decrease of the vagus influences from the central vasomotor structures on the heart. It is possible that the lesser HR rise under stress in the rats immunized against ODN is due to a reduced excitability of the hypothalamic defence centers and that the stimulation of the GABA-ergic system function induced by the immunization against its inhibitor ODN plays a certain role in this process. Ppreviously we found changes in the sensitivity of the baroreceptor reflex in animals immunized against ODN. Apparently, ODN may be considered to be a factor in hemodynamics regulation which possesses a hyperkinetic effect. Given the fact that the early stages of spontaneous or stress-induced hypertension are characterized by a CO increase, the assumption can be made that acting upon the peptides which regulate the GABA-ergic system may be one of the new approaches in the search for antihypertensive and antistressor agents with an extremely long-lasting effect.

REFERENCES

1. I. P. Ashmarin, and O. A. Gomazkov, *Izv. Akad. Nauk SSSR*, Ser. Biol., No 1, 11-18 (1989).
2. I. P. Ashmarin, O. P. Vakulina, V. V. Rozhanets, *et al.*, *Byull. Eksp. Biol.*, **113**, № 3, 270-272 (1992).
3. T. P. Vakulina, V. B. Koshelev, B. G. Pinelis, *et al.*, *Ibid.*, **99**, № 1, 10-12 (1985).
4. G. V. Kovalev, and I. N. Tyurenkov, *Farmakol. Toksikol.*, № 1, 5-11 (1989).
5. J. A. Ball, P. W. J. Burnet, and B. A. Fountain, *Neurosci. Lett.*, **72**, 183-188 (1986).
6. J. Borman, *Trends Neurosci.*, **11**, № 3, 112-116 (1988).
7. P. R. Gehlert, H. J. Yamamura, and J. K. Wamsley, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **328**, № 4, 454-460 (1985).
8. R. A. Gillis, K. A. Yamada, and J. A. Di Micco, *Fed. Proc.*, **43**, No. 1, 32-38 (1984).
9. A. Guidotti, C. M. Forchitti, M. J. Corda, *et al.*, *Proc. Nat. Acad. Sci. USA*, **80**, 3531-3535 (1983).
10. M. Mestre, T. Carriot, C. Belin, *et al.*, *Life Sci.*, **35**, № 9, 953-962 (1984).
11. M. Sakemoto, H. Ohsumi, T. Yamaraki, and P. Okumura, *Acta Physiol. Scand.*, **139**, № 2, 281-287 (1990).
12. P. K. Schofield, M. G. Darlison, N. Fujita, *et al.*, *Nature*, **38**, № 6127, 221-227 (1987).
13. M. C. Tonon, L. Desy, P. Nicolas, *et al.*, *Neuropeptides.*, **15**, 17-24 (1990).
14. J. H. Wible, J. A. Di Micco, and F. C. Leift, *Hypertension*, **14**, № 6, 623-628 (1989).