Effects of Central Endothelin-1 in Normotensive and Spontaneously Hypertensive Rats

T. I. Ponomareva, O. N. Khokhlova, E. A. Tukhovskaya, and A. M. Murashov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 3, pp. 275-278, March, 2003
Original article submitted August 30, 2002

Changes in blood pressure and sympathetic nerve activity induced by local injection of endothelin-1 into the rostroventrolateral medulla were studied in narcotized stroke-prone spontaneously hypertensive rats (SHRSP) and normotensive WKY rats. Endothelin-1 produced similar biphasic response in both rat strains: a transient increase in blood pressure and sympathetic nerve activity followed by progressive hypotension and bradycardia. Pretreatment with ET_B-receptor antagonist BQ788 inhibited sympathetic activation induced by endothelin-1, while pretreatment with the ET_A-receptor antagonist N-acetyl-[D-Trp^{16}]-endothelin-1 abolished the subsequent hypotension. The antihypotensive effect of ET_A-receptor blockade was most effective in normotensive rats. Our findings suggest that cardiovascular disorders in SHRSP rats can be related to peculiarities in the rostroventrolateral medullar endothelin system.

Key Words: *endothelin-1*; *rostroventrolateral medulla oblongata*; *sympathetic activity*; *ET_B-receptors*; *ET_A-receptors*; *spontaneously hypertensive rats*

Vasoactive peptide endothelin (ET) can act as a neurotransmitter and/or neuromodulator within the CNS. ET-receptors, ET, and ET-converting enzyme were detected in various brain structures including those involved in cardiovascular regulation [14]. Centrally administered ET-1 modulates activity of vasomotor neurons [1] and functions of the cardiovascular and respiratory system [7,8,10]. Abnormalities in the central endothelin system lead to the development of cardiovascular disorders. Changes in the binding of ET-1 were revealed in the rostroventrolateral medulla (RVLM) of spontaneously hypertensive rats (SHR) [4] and in the brain of SHRSP rats during severe hypertension and stroke development [11].

Here we studied the role of ET_A- and ET_B-receptors in the effects of exogenous ET-1 injected into RVLM, the major neuronal network involved in the sympathetic control. Another objective was to find out whether functional changes in RVLM endothelin system are characteristic of stroke-prone spontaneously hypertensive SHRSP rats.

MATERIALS AND METHODS

Experiments were carried out on WKY and SHRSP male rats aged 6-7 month narcotized with urethane (1.25 g/kg, intraperitoneally).

Blood pressure (BP), heart rate (HR), and renal nerve activity (RNA) after local unilateral injection of ET-1 (2 pmol, Sigma) into RVLM were recorded. ET-1 was injected 5 min after injection of specific ET_B-receptor antagonist BQ788 (20 pmol, Sigma), or specific ET_A-receptor antagonist ATET-1 (N-Acetyl-[D-Trp^{16}]-endothelin-1, 20 pmol, Sigma), or physiological saline (control). In special experiments, the rats were injected with the blockers without subsequent administration of ET-1. Each experimental group consisted of 6-9 rats.

Laboratory of Biological Tests, M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry (Pushchino Branch), Russian Academy of Sciences, Pushchino. ***Address for correspondence:*** khohlova@fibkh.serpukhov.su. Khokhlova O. N.

0007-4888/03/1353-0237$25.00 © 2003 Plenum Publishing Corporation

A polyethylene catheter was introduced into the femoral artery, and BP was recorded with an electric manometer. The left renal nerve was exposed via lateral peritoneal incision, placed on bipolar hooked silver electrodes and isolated from the surrounding tissue with paraffin grease. Electrical signals were amplified and filtered using a WPInstruments DAM-5A differential AC amplifier. BP and RNA signal processing was performed with a ADInstruments MacLab computer system for analysis of physiological signals.

Microinjections into RVLM were performed in stereotaxis. The animal was fixed in the position with maximum unbending of the atlanto-occipital joint, from which the medulla can be approached for the injection. A glass capillary with a pulled-out tip was stereotactically inserted into the RVLM according to the rat brain atlas coordinates [9]. The volume of injections was 0.2 μl. The site of electrode insertion was verified by histological investigation [6] and using a standard physiological test with injection of L-glutamate (2 nmol) evoking a hypertensive response [12].

The data were statistically processed using ANOVA-2 and Duncan tests for intergroup comparisons. The difference between the means were significant at $p<0.05$.

RESULTS

The mean baseline BP (BP_{mean}) and HR were 89±4 mm Hg and 266±13 bpm in narcotized WKY and 151±8 mm Hg and 281±13 bpm in SHRSP rats (n=36), respectively.

Local injection of ET-1 into RVLM induced a similar biphasic hemodynamic response with an initial BP increase followed by hypotension and bradycardia in both groups of rats (Fig. 1). Sympathetic activity

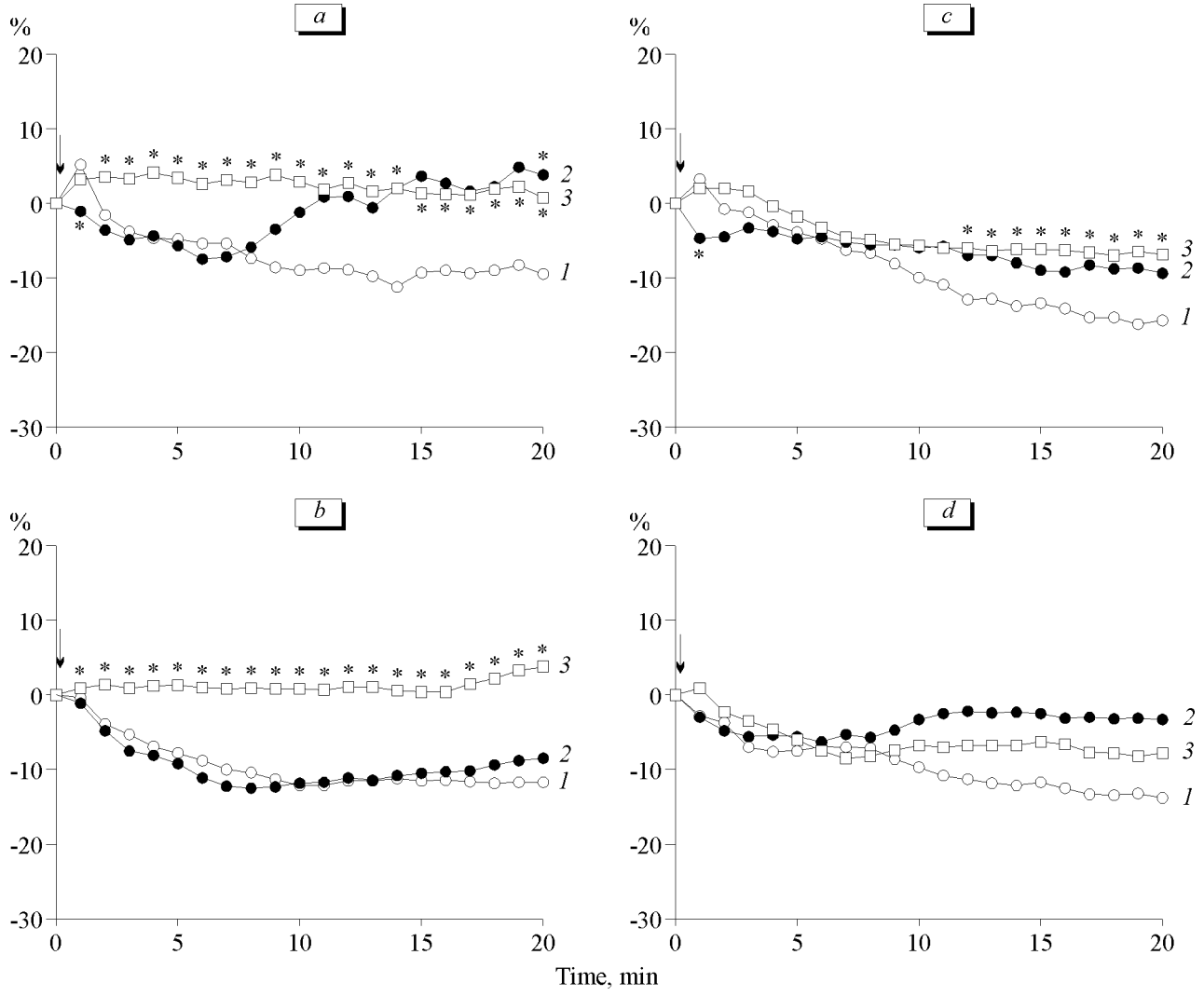

Fig. 1. Changes in mean arterial blood pressure (*a*, *c*) and heart rate (*b*, *d*) in WKY (*a*, *b*) and SHRSP rats (*c*, *d*) after injection of endothelin-1 (arrow) into the rostroventrolateral medulla in control (*1*) and after the pretreatment with BQ788 (*2*) or ATET-1 (*3*). Here and in Fig. 2: **p*<0.05 compared to the control (*1*).

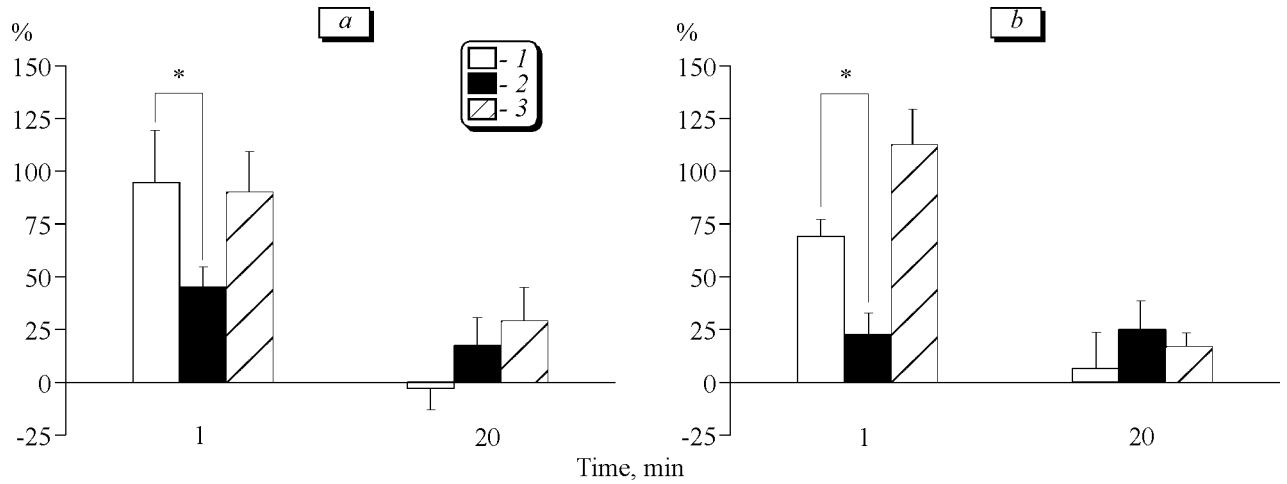

Fig. 2. Changes in renal sympathetic nerve activity in WKY (*a*) and SHRSP rats (*b*) after injection of endothelin-1 into the rostroventrolateral medulla in control (*1*) and after pretreatment with BQ788 (*2*) or ATET-1 (*3*).

was enhanced for 1 min postinjection (Fig. 2), which could play a critical role in initiation of the pressor response. It is important that SHRSP rats often died from apnea during hypotension, despite the hypotensive effect of ET-1 in these rats was similar to that in the normotensive rats.

To answer the question about participation of ET in tonic regulation of the sympathetic cardiovascular influences mediated by RVLM neurons and to find out which type of ET-receptors mediates the central effects of ET, the specific ET-receptor antagonists BQ788 and ATET-1 were injected into RVLM. Neither ET_A- nor ET_B-receptor antagonist in a dose of 20 pmol significantly changed the studied parameters (Table 1).

BQ788, but not ATET-1, prevented the ET-1-induced increase in BP (Fig. 1, *a, c*) and RNA (Fig. 2). By contrast, ATET-1 abolished the hypotensive effect of ET-1 in both rat strains, the effect being most pronounced in WKY rats. In normotensive rats, ET_B-receptor antagonist also alleviated ET-induced hypotension at the 20th minute (Fig. 1, *a*), while ATET-1 completely inhibited bradycardia (Fig. 1, *b*). In SHRSP rats, no significant decrease in HR was observed after injection of ATET-1 or BQ788 (Fig. 1, *c*).

Thus, ET-1 undoubtedly plays an important role in the central regulation of the cardiovascular function mediated by RVLM neurons. However, the data on the tonic effects of central ET are ambiguous. There is evidence that intracerebroventricular injection of ET_A-receptor antagonist BQ123 decreased BP and sympathetic activity in SHRSP rats [8]. By contrast, in other study injection of BQ123 into RVLM of normotensive Sprague-Dawley rats slightly increased BP [7]. Our findings suggest that ET produces no tonic effects on RVLM neurons in WKY and SHRSP rats.

In our experiments, local injection of ET-1 into RVLM enhanced sympathetic nerve activity in both normotensive and hypertensive rats. Recently, similar effects of ET-1 were observed in rats after intracerebroventricular injection of the peptide [8,10], but the responses to local administration of ET into RVLM were more short-lasting and followed by progressive decrease in BP and HR. A biphasic hemodynamic effect was observed in normotensive rats receiving in-

TABLE 1. Changes in Mean Blood Pressure (BP_{mean}), HR, and RNA in WKY and SHRSP Rats after Injection of BQ788 and ATET-1 into RVLM (*M±m*)

Parameter		BQ788		ATET-1	
		1 min	20 min	1 min	20 min
ΔBP_{mean}, %	WKY	2.4±1.0	9.2±3.8	4.1±3.1	2.9±6.2
	SHRSP	-0.2±1.1	4.9±4.8	-0.9±0.3	-4.0±1.7
ΔHR, %	WKY	1.1±2.2	-2.6±6.3	-1.4±0.9	-4.1±2.2
	SHRSP	1.0±0.9	6.2±2.1	-0.4±0.6	-4.6±3.8
ΔRNA, %	WKY	24.3±18.3	-16.7±11	-1.6±11.13	-10.7±10.3
	SHRSP	23.7±17.8	-7.5±14.4	-13.1±9.7	-11.2±15.8

jections of ET-1 into RVLM [7]. The severity of ET-induced hypotension and bradycardia in SHRSP and WKY rats was similar. However, high mortality of SHRSP rats under these conditions attests to specific changes in the ET system. It was established that the hypotensive response to intracerebroventricular injection of ET-1 in Sprague-Dawley rats was due to reduction of blood flow to the brain and peripheral organs, which in turn can result from cerebral ischemia [3,5]. Pretreatment with BQ123, a specific antagonist of ET_A-receptors, abolished the regional circulatory effects of ET-1 [5]. This agrees with our findings that ET_A-receptor antagonist inhibits hypotension induced by ET-1. The lesser degree of inhibition of the hypotensive response in SHRSP rats can be due to higher susceptibility of these rats to cerebrovascular injuries.

The stimulating effect of ET-1 on sympathetic activity is mediated predominantly via ET_B-receptors. There are contradictory data on the evolvement of these receptors into the central sympathetic control. On the one hand, BQ123 abolished hypotension and decrease in blood flow induced by ET-1 [5]. On the other hand, BQ123 inhibited the pressor response to intracerebroventricular ET-1 in rats [10]. Predominant localization of ET_B-receptors in the brain tissue and ET_A-receptors in cerebral vessels confirms the involvement of ET_B-receptors in mediation of ET neurotransmitter functions [2,13]. Moreover, activation of ET_B-receptors (but not ET_A-receptor) induces dopamine release in the striatum [14].

Thus, injection of exogenous ET-1 into RVLM induces a short-term sympathetic activation in both normotensive and hypertensive rats, primarily via stimulation of ET_B-receptors. The development of a subsequent hypotension mediated by ET_A-receptors often leads to death in SHRSP rats. Low efficiency of inhibition of hypotension by ET-receptor antagonists in SHRSP rats, compared to normotensive WKY rats, attests to impairment of ET system in SHRSP model of congenital cardiovascular pathology.

The work was supported by Russian Foundation of Fundamental Researches (grant No. 01-04-48963).

REFERENCES

1. W. H. Cao, T. Kuwaki, M. Unekawa, *et al*., *J. Cardiovasc. Pharmacol*., **2**, Suppl. 8, S196-S198 (1993).
2. A. P. Davenport, R. E. Kuc, M. J. Ashby, *et al*., *Br. J. Pharmacol*., **123**, 223-230 (1998).
3. A. Gulati, A. Kumar, and B. T. Shahani, *Life Sci.*, **58**, 437-445 (1996).
4. A. Gulati and S. Rebello, *Ibid*., **48**, 1207-1215 (1991).
5. A. Gulati, S. Rebello, and A. Kumar, *Am. J. Physiol*., **273**, H1177-H1186 (1997).
6. O. N. Khokhlova, A. N. Murashev, and O. S. Medvedev., *Byull. Eksp. Biol. Med.,* **131**, No. 4, 336-339 (2001).
7. R. Mosqueda-Garcia, K. Yates, J. O'Leary, and T. Inagami, *Hypertension*, **26**, 263-271 (1995).
8. K. Nakamura, S. Sasaki, J. Moriguchi, *et al*., *J. Cardiovasc. Pharmacol*., **33**, 876-882 (1999).
9. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, San Diego (1997).
10. N. F. Rossi, D. S. O'Leary, D. Woodbury, and H. Chen, *Am. J. Physiol.*, **279**, E18-E24 (2000).
11. P. Savage and A. Y. Jeng. *Can. J. Physiol. Pharmacol*., **80**, No. 5, 470-474 (2002).
12. S. Sesoko, H. Muratani, M. Yamazato, *et al*., *Am. J. Physiol*., **274**, R1119-R1124 (1998).
13. S. Shibata, M. Niwa, and A. Himeno, *Cell. Mol. Neurobiol*., **17**, 151-156 (1997).
14. M. Van den Buuse and K. M. Webber, *Prog. Neurobiol*., **60**, 385-405 (2000).