CARDIOVASCULAR EFFECTS OF CENTRALLY ACTING OREXIN A IN HAEMORRHAGE-SHOCKED RATS

Orexin A influences the central cardiovascular regulation, since after intracerebroventricular (icv) administration it evokes short-lasting increases in mean arterial pressure (MAP) and heart rate (HR) in normotensive animals. The aim of the present study was to examine haemodynamic effects of orexin A in haemorrhage-shocked rats. Experiments were carried out in anaesthetized Wistar rats subjected for a critical irreversible haemorrhagic hypotension of 20-25 mmHg, which resulted in the death of all saline icv-treated control animals within 30 min. Orexin A (0.5-1.5 nmol; icv) administered at 5 min of critical hypotension evoked dose-dependent long-lasting increases in MAP, HR and renal, mesenteric and hindquarters blood flows, with a 100% survival of 2 h after treatment (1.5 nmol; icv). Changes in MAP and peripheral haemodynamics were inhibited by intravenous pretreatment with \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptor antagonists prazosin (0.5 mg/kg) and yohimbine (1.0 mg/kg), respectively. Moreover, both antagonists significantly decreased the survival rate to 16.6 and 33.3% (P<0.05 vs. orexin A [1.5 nmol]-treated group). In contrast, \( \beta \) adrenoceptor antagonist propranolol (1.0 mg/kg) completely blocked orexin A-induced HR changes, without influence on MAP, peripheral blood flows and the survival rate. Therefore, we conclude that centrally acting orexin A evokes the resuscitating effect in haemorrhage-shocked rats due to the activation of the sympathetic nervous system.

Key words: orexin A, haemorrhagic shock, sympathetic nervous system, rats
INTRODUCTION

Orexin A (hypocretin-1) and orexin B (hypocretin-2) are 33- and 28-amino acid hypothalamic peptides, respectively, proteolytically derived from a single precursor – 130-amino acid prepro-orexin (1). Although orexin-containing cell bodies are localized in the dorsal and lateral hypothalamus, orexin-containing fibers are widely distributed in the cerebral cortex, circumventricular organs, hypothalamus, thalamus, medulla oblongata and spinal cord (2). This suggests that orexinergic neurons may have connections with other neuronal systems. Indeed, orexins are able to influence many functions of the CNS, including feeding behavior (3), sleep-wake cycle (4), hormone secretion (5), locomotor activity (3) and respiratory regulation (6). Moreover, they affect the central cardiovascular regulation, since there are increases in mean arterial pressure (MAP) and heart rate (HR) after orexin A administration into the brain lateral ventricle (icv) (7), cisterna magna (8) or after microinjections to the nucleus of the solitary tract (NTS) (9) and to the rostral ventrolateral medulla (RVLM) (8) in normotensive animals. Orexins act as neuromediators in NTS and RVLM, influencing the excitability of cardiovascular center neurons, moreover, it is suggested that they are able to regulate global cardiovascular function. Indeed, studies by Machado et al. demonstrate that orexin A acting on RVLM neurons increases sympathetic outflow targeted to the heart and blood vessels (10).

Since the second phase of cardiovascular regulation in haemorrhagic shock is characterized by sympathoinhibition, the aim of the present study was to examine haemodynamic effects of orexin A in haemorrhage-shocked rats. The experimental haemorrhagic shock model by Guarini et al. (11) was chosen, as in the previous studies (12-13), to examine orexin A action at constant initial values of both critical MAP and the volume of circulating blood.

MATERIAL AND METHODS

Animals

Studies were carried out in 36 male Wistar rats weighing 260-310 g (4-5 months old). The animals were housed in individual cages in the animal colony, under controlled conditions of temperature (20-22°C), humidity (60-70%), lighting (12 h light/dark cycle) and provided with food and water ad libitum. Each studied group consisted of six animals. All experimental procedures were performed according to the EU directives and reviewed by the Ethics Committee of the Medical University of Silesia.

Surgical preparations

For icv treatment the rats were prepared 5-7 days before the experiment by implantation, under ketamine/xylazine (100 mg/kg/10 mg/kg; ip) anaesthesia, of polyethylene cannula into the right brain lateral ventricle (14). All icv injections were made in a volume of 10 µl over a period of 60 s, and the correctness of injections was verified as described previously (14).
On the day of the experiment, after induction of general anaesthesia with ethylurethane (1.25 g/kg; ip), the rats were implanted with catheters filled with heparinized saline (300 IU/ml) in the right carotid artery and the right jugular vein.

**Cardiovascular parameter measurements**

MAP and HR were measured using the pressure transducer RMN-201 (Temed, Zabrze, Poland) and the electrocardiograph Diascope 2 (Unitra Biazet, Białystok, Poland), respectively. Electromagnetic probes (Type 1RB2006, Hugo Sachs Elektronik, March-Hugstetten, Germany) were implanted around the right renal and the superior mesenteric arteries to monitor renal (RBF) and mesenteric (MBF) blood flow, and around the distal abdominal aorta to monitor perfusion of the hindquarters (HBF) using Transit Time Flowmeter Type 700 (Hugo Sachs Elektronik, March-Hugstetten, Germany). All measurements of blood flow were started after a 30 min adaptation period to avoid influences of probe implantation.

**Haemorrhagic shock protocol**

Irreversible haemorrhagic shock, according to the method of Guarini et al. (11), was produced by intermittent blood withdrawal from the catheter inserted into the right jugular vein over a period of 15-25 min, until MAP stabilised at 20-25 mmHg.

**Experimental protocol**

Orexin A (0.5, 1.0, 1.5 nmol; icv) was injected in three groups of haemorrhage-shocked rats 5 min after termination of bleeding. Since a dose of 1.5 nmol of orexin A produced a pressor effect with a 100% survival at 2 h, it was used for the further studies.

To examine the role of the sympathetic system in orexin A-induced cardiovascular effects, in separate groups, immediately after bleeding termination the animals were pre-treated with prazosin (0.5 mg/kg; iv), yohimbine (1 mg/kg; iv) or propranolol (1 mg/kg; iv), and 5 min later, orexin A (1.5 nmol; icv) was administered. To reduce the number of the animals used, we compared the results with those obtained in the control saline icv-treated groups published earlier (14, with kind permission of Springer Science and Business Media). The animals were continuously monitored for 2 h after treatment, or until death, if it occurred earlier. All the experiments were performed between 8 a.m. and 2 p.m.

**Drugs**

The following drugs were used: orexin A (synthesised by I. Kato and A. Kuwahara), prazosin hydrochloride, (±)-propranolol hydrochloride, xylazine hydrochloride, yohimbine hydrochloride (Research Biochemicals, Natick, MA, USA), ethylurethane (Riedel-de Haën, Seelze, Germany), ketamine (Gedeon Richter, Budapest, Hungary), heparin (Polfa, Warszawa, Poland). All adrenoceptor antagonists were administered iv in a fixed volume of 0.2 ml. All drug solutions were prepared freshly on the day of the experiment. The doses of drugs refer to the free base.

**Statistics**

All values are given as means ± standard deviation with P < 0.05 considered as the level of significance. Statistical evaluation was performed by paired t-test. The Fisher’s exact test was used to examine significant differences in survival rates.
Fig. 1. Influence of prazosin (0.5 mg/kg; ▲, △), yohimbine (1 mg/kg; ◆, ◇), propranolol (1 mg/kg; ■, □) and saline (●, ○) on MAP (A), HR (B), and changes in renal (RBF), hindquarters (HBF) and mesenteric blood flow (MBF) after injection of orexin A (1.5 nmol; icv; filled symbols) and saline (5 µl; icv; open symbols) in haemorrhage-shocked rats. Six animals per group. Adrenoceptor antagonists were administered at –5 min, and orexin A 5 min later. For MAP: since 10 min in all groups, except for propranolol-pretreated group, P<0.05 vs. saline-pretreated orexin A-treated group; for HR: since 15 min in all groups, except for prazosin- and yohimbine-treated groups P<0.05 vs. saline-pretreated orexin A-treated group; for peripheral blood flows: in all control groups since 15 min P<0.05 vs. saline-pretreated orexin A-treated group, and in orexin A-treated groups * - P<0.05 vs. saline-pretreated group. Initial RBF, HBF and MBF in the control saline-pretreated group were 6.23 ± 1.12, 8.82 ± 1.71 and 7.88 ± 1.84 ml/min, respectively. Haemodynamic parameters in the control groups were taken from (14) with kind permission of Springer Science and Business Media.
RESULTS

Initial pre-bleeding values of MAP, HR and peripheral blood flows in all groups did not reveal significant differences.

The total bleeding volume for the induction of critical hypotension in all animals was 2.36 ± 0.27 ml/100 g b.w. Bleeding from MAP 76.2 ± 5.2 mmHg to 20-25 mmHg was associated with a decrease in HR from 351 ± 14 beats/min to 237 ± 32 beats/min.

Cardiovascular effects of orexin A in critically hypotensive rats

In haemorrhage-shocked rats, orexin A (0.5-1.5 nmol; icv) evoked dose-dependent increases in MAP, HR and peripheral blood flows, with a 100% survival of 2 h after treatment (1.5 nmol; icv). Orexin A at a dose of 0.5 nmol produced only transient increases in MAP and HR, maximally up 7.1 ± 2.3 mmHg and 22 ± 6 beats/min (data not shown), respectively, without influence on the survival rate at 2 h in comparison to the control saline-treated group. In contrast, orexin A administered at doses of 1.0 and 1.5 nmol evoked long-lasting rises in MAP, maximally up to 14.2 ± 4.6 and 32.3 ± 7.4 mmHg, and HR, up to 44 ± 12 and 102 ± 28 beats/min, respectively (data not shown). The effects were accompanied by increases in the survival rates at 2 h to 50% and 100% (for the dose of 1.5 nmol of orexin A P < 0.05 vs. the control animals).

Influence of adrenoceptor antagonists on survival rates and regional haemodynamic effects of orexin A

Orexin A (1.5 nmol; icv)-induced changes in MAP and peripheral blood flows were inhibited by α1- and α2-adrenoceptor antagonists prazosin (0.5 mg/kg; iv) and yohimbine (1.0 mg/kg; iv), respectively (Fig. 1). Moreover, both antagonists significantly decreased the survival rate to 16.6 and 33.3% (P<0.05 vs. orexin A-treated group). In contrast, β-adrenoceptor antagonist propranolol (1.0 mg/kg; iv) completely blocked orexin A-induced HR changes, without influence on MAP, peripheral blood flows and the survival rate (Fig. 1).

Pre-treatment with adrenoceptor antagonists did not influence survival rates in the control saline icv-treated groups, and all the animals died within 30 min (Fig. 1).

DISCUSSION

The present data show for the first time that the orexinergic system, similarly to other non-opioid systems, such as the melanocortinergic, histaminergic, cholecystokininergic, thyreoliberinergic and cholinergic systems (15 - 17), evokes the resuscitating effect in haemorrhage-shocked rats. Moreover, the study
demonstrates the involvement of the sympathetic nervous system in orexin A-induced resuscitating action.

The integrated response to acute haemorrhage consists of two phases of regulation – an initial sympathoexcitatory phase, in which arterial pressure is maintained well due to an increase in the sympathetic system activity, and the second, sympathoinhibitory phase, which is associated with inhibition of cardiovascular centre neurones activity (18). The pressure-controlled model of haemorrhagic shock by Guarini et al. is a model of severe irreversible hypotension and is associated with an early initiation of the sympathoinhibitory phase of regulation (11). In the present study, we demonstrate that in these conditions, orexin A given icv evokes a long-lasting pressor effect with rises in renal, mesenteric and hindquarters blood flows. Although studies by Hirota et al. show that also in young-adult (12-14 weeks) normotensive anaesthetised rats orexin A administered icv elicits increases in MAP and HR (19), however, in contrast to normovolaemic rats, in haemorrhage-shocked animals the action is long-lasting and is present after central administration of lower doses of orexin A. We hypothesise that the differences can be explained by an activation of additional compensatory mechanisms in shock conditions, similarly as we have reported previously in central histamine-evoked resuscitating effect (20).

The mechanisms of orexin A-induced influence on the cardiovascular regulation include the central activation of the sympathetic system, since orexin A (0.3 – 3.0 nmol; icv) dose-dependently increases renal sympathetic nerve activity and plasma catecholamines in normotensive conscious rats (21). Moreover, there is an induction of c-fos mRNA in the hypothalamic paraventricular nucleus after icv administration of orexin A and B and, therefore, this site is probably involved in orexins action after icv injection (21).

To further study the role of the sympathetic system in the central orexin A-induced resuscitating effect, adrenoceptor antagonists have been administered before orexin A injection. As we previously reported, critical hypotension of 20-25 mmHg is associated with extremely low RBF, HBF and MBF and high regional vascular resistance (22, 23). Interestingly, orexin A produces an increase in blood flows and we suggest that the effect can be explained by compensatory mechanisms-mediated vasoconstriction of the venous part of the circulatory system and mobilisation of blood from its reservoirs. Similar changes we reported earlier in central histamine-induced resuscitating action which is also associated with the activation of the sympathetic nervous system (14). In addition, an increase in regional blood flows in orexin A-treated animals can be explained by hyporeactivity to vasopressor agents, since there is a peripherally-mediated loss of vascular reactivity in profound haemorrhagic shock (24).

The present results demonstrate that α1- and α2-adrenoceptor antagonists diminish MAP and peripheral blood flow changes evoked by orexin A. Similar effects we observed in central histamine-induced activation of the sympathetic system in haemorrhagic shock (14). We suggest that there are two different
mechanisms involved - prazosin, acting directly, is able to decrease vascular resistance; on the other hand, yohimbine inhibits hepatic blood volume responses to sympathetic nerve stimulation (11). Since the hepatic vascular bed is an important blood reservoir, α2-adrenoceptor blockage may diminish the mobilisation of blood in response to central orexin A-induced activation of the sympathetic system. Thus, our present results are in agreement with the hypothesis by Guarini et al. (11) that the peripheral resistance changes leading to mobilisation of blood from venous reservoirs are of essential importance for the spontaneous reversal of haemorrhagic shock.

On the other hand, propranolol blocks orexin A-induced HR increase, however, without influence on peripheral blood flows and survival rates. Studies by Antunes et al. demonstrate that also in anaesthetised rats, propranolol markedly diminishes increases in HR after orexin A intrathecal administration (25). Moreover, these results are consistent with our previous studies in which β-blockage prevented HR changes, with no influence on the pressor action elicited by centrally acting histamine in haemorrhage-shocked rats (14).

Results of the present study are similar to those obtained in our earlier experiments concerning endogenous histamine-induced resuscitating effect in haemorrhagic shock in rats. Since there are functional interactions between the orexinergic and histaminergic systems (26), further studies are needed to clarify the precise neuronal mechanisms responsible for the activation of compensatory mechanisms in haemorrhagic shock conditions.

In conclusion, centrally acting orexin A evokes the resuscitating effect in haemorrhage-shocked rats due to the activation of the sympathetic nervous system. The mechanisms of orexin A-induced resuscitating effect include changes in peripheral vascular resistance, but not in heart rate.

REFERENCES


Received: November 21, 2006  
Accepted: November 24, 2006

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