Hemodynamic Changes in Alert Rats with Acute Streptozotocin-Induced Diabetes Administered Combined V2/V1 Vasopressin Antagonist

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The development of many complications of diabetes mellitus is caused by microcirculatory dysfunction. The diabetic "triopathy," that is, retinal involvement, renal disease, and neuropathy, is denoted by the general term "microangiopathy." In accordance with the hemodynamic hypothesis, an altered regional hemodynamics is the factor triggering the development of microcirculatory dysfunction in diabetes [9,14,17]. It is noteworthy that although maintenance of the optimal blood sugar level is currently the only way to prevent diabetic microangiopathy, there are more and more reports demonstrating that the recovery of the microvascular hemodynamics may inhibit the development of vascular complications as well, and therefore such a therapeutic approach holds good promise [13,15]. Vasodilatation and increased regional bloodflow observed at the early stages of diabetes mellitus seem to be related to raised blood sugar level. Hyperglycemia reduces vascular smooth muscle cell contractility [12] and is the principal factor responsible for plasma osmolarity increase in diabetes [5]. This plasma hyperosmolarity in turn may induce changes in the regional hemodynamics, reducing the irritability of vascular smooth muscle cells [3] and stimulating endogenous vasopressin production. The plasma vasopressin level is in fact increased in rats with short-term streptozotocin-induced diabetes [5].

The present research was aimed at elucidating the possible role of vasopressin in changes of the systemic and regional hemodynamic parameters in alert rats with acute streptozotocin-induced diabetes of 24 h duration [1].

MATERIALS AND METHODS

Endogenous vasopressin is known to induce hemodynamic changes both as a circulating hormone and as a neurotransmitter. Therefore, to elucidate the hemodynamic effect of vasopressin we studied the effect of its antagonist on the systemic and regional hemodynamic parameters in rats with acute experimental diabetes.

Since vasopressin affects the hemodynamics not only via V1 but via V2-like receptors as well [7], we used a combined V2/V1 vasopressin antagonist [Adamantanacetyl^1, o-Et-D-Tyr^2, Val^4, Aminobutyryl^6, Arg^{8,9}]-vasopressin. Experiments were carried out on male Wistar rats weighing 300-400 g. Cardiac output and blood flow in the organs were assessed by the labeled microspheres technique described in detain previously [2]. Ether-anesthetized animals were catheterized in the left heart ventricle (through the right carotid artery) for microsphere introduction, in the abdominal aorta (through the fight femoral ar-

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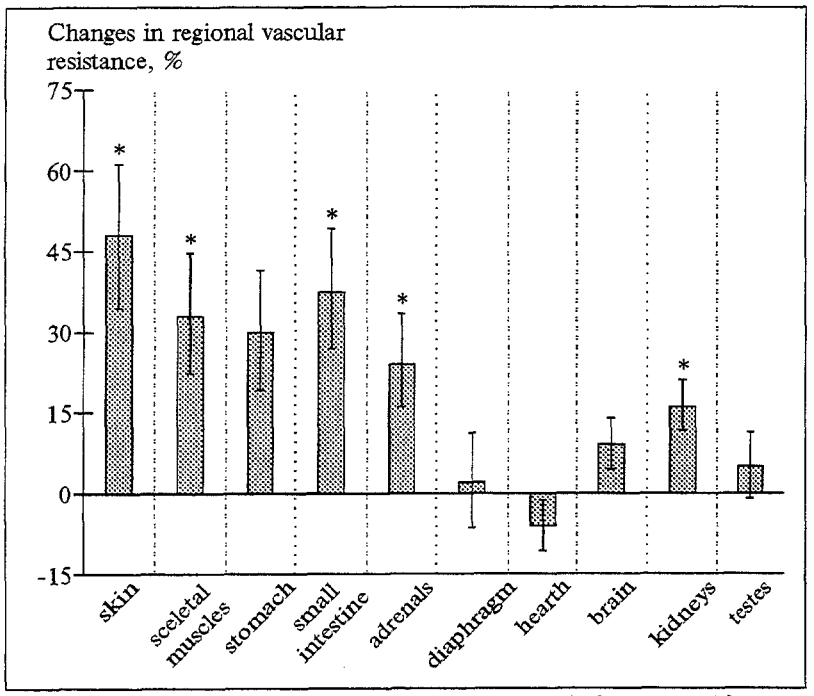

Fig. 1. Changes in regional vascular resistance of alert rats with acute 24-h-long streptozotocin-induced diabetes 10 min after combined V2/V1 vasopressin antagonist injection vs. baseline level. Asterisk: $p<0.05$ vs. the level before vasopressin antagonist injection (paired t test).

tery) for recording the mean arterial pressure and blood sampling, and in the right jugular vein for infusions of substances. After a 48 h recovery period the animals were weighed and streptozotocin (STZ) prediluted in 0.9% NaCl solution was injected in a dose of 60 mg/kg. One day after STZ injection the rats were tested for glucosuria with multistix reagent strips (Ames, Great Britain) and weighed, and the systemic and regional hemodynamic parameters were measured. Thirty minutes after the first measurement combined V2/V1 vasopressin antagonist was infused in a dose of 50 μg/kg, prediluted in 0.9% NaCl solution. Ten minutes after antagonist infusion, the hemodynamic parameters were measured for the second time. At the end of the experiment the blood sugar concentrations were measured with a glucometer (Miles, USA). For the control intact rats were used in which the systemic and regional hemodynamic parameters were measured twice, before and after vasopressin antagonist i.v. injection (50 μg/kg). After the experiment the animals were killed with a high nembutal dose, and the organs and tissues in which blood flow was assessed were weighed and placed in disposable test tubes. All the measurements of the number of microspheres were carried out using a Compugamma 1282 gamma scintillation counter (LKB-Wallac, Finland). The cardiac index (CI), total peripheral vascular resistance (TPVR), stroke volume (SV), regional blood flow (RBF), and regional vascular resistance (RVR) values were calculated according to standard formulas [2]. The data are presented as $M\pm m$. The Student paired t test was used for statistical processing. The differences were considered reliable at bilateral significance level $p<0.05$.

RESULTS

Glucosuria was observed in all the rats ($n=10$) 24 h after STZ injection. The blood sugar concentration was elevated 415±15 mg/100 ml.

The systemic and regional hemodynamic parameters in alert rats with acute streptozotocin-induced diabetes before and 10 min after vasopressin antagonist infusion are presented in Table 1.

Marked hemodynamic changes were observed in diabetic animals 10 min after vasopressin antagonist administration. These changes were a lowered CI (49.0±3.1 vs. 68.7±5.2 ml/min/100 g before antagonist injection, $p<0.05$) and SV 0.40±0.03 vs. 0.57±0.06 ml before antagonist infusion, $p<0.05$) and an increased TPVR (1.84±0.15 vs. 1.31±0.12 mm Hg/ml/min per 100 g before antagonist infusion, $p<0.05$). The mean arterial pressure (AP) and heart rate (HR) were unchanged. The systemic hemodynamic changes after vasopressin antagonist infusion were associated with a reliable ($p<0.05$) blood flow reduction in the skin, skeletal muscles, stomach, small intestine, and kidneys. The changes in RVR 10 min after vasopressin antagonist infusion in comparison with that before infusion are presented in Fig. 1.

Our data indicate that blocking of the vasopressin V1 and V2 receptors involves marked hemodynamic changes in alert rats with acute streptozotocin-induced diabetes of 24 h duration. Vasopressin antagonist injection to intact animals did not lead to significant changes in the hemodynamics (data not presented).

We previously demonstrated a hyperkinetic type of circulation to be characteristic of alert rats with acute streptozotocin-induced diabetes of 24 h duration; such a type of circulation manifests itself in an increased CI and reduced TPVR and in an increased blood flow in a number of vascular regions [1]. In accordance with the hemodynamic hypothesis of diabetic microangiopathy development [9,14,17], such changes in hemodynamics may lead to the development of capillary hypertension and microvessel sclerosis. The findings of the present research demonstrate that combined V2/V1 vasopressin antagonist significantly improves the hemodynamic situation in short-term experimental diabetes, this indicating a

TABLE 1. Hemodynamic Parameters in Alert Rats with Acute Streptozotocin–Induced Diabetes ($n=10$) before and 10 min after Injection of Combined V2/V1 Vasopressin Antagonist ($M\pm m$)

Hemodynamic parameters	Before antagonist injection	10 min after antagonist injection
AP	85.0±2.9	86.1±2.3
CI	68.7±5.2	49.0±3.1*
SV	0.57±0.06	0.40±0.03*
HR	364±12	363±12
TPVR	1.31±0.12	1.84±0.15*
Regional bloodflow in:		
skin	0.25±0.05	0.19±0.04*
skeletal muscle	0.16±0.02	0.13±0.01*
stomach	1.60±0.23	1.20±0.09*
small intestines	6.74±0.75	5.11±0.42*
adrenals	8.60±1.36	7.86±1.76
diaphragm	1.23±0.11	1.32±0.13
heart	10.22±0.74	11.24±0.93
brain	1.69±0.11	1.60±0.07
kidneys	7.86±0.43	6.93±0.41*
testes	0.44±0.06	0.43±0.05

Note. Asterisk: $p<0.05$ vs. value before antagonist injection (paired t test).

possible contribution of vasopressin to the hemodynamic shifts in diabetes.

Circulating vasopressin is known to cause vasoconstriction and to increase TPVR by acting on the vascular V1 receptors [8]. However, a depressive effect of vasopressin has been reported as well. For instance, infusion of exogenous vasopressin to rats after V1 receptor blocking resulted in a drop of TPVR [16]. Some authorities report that in dogs endogenous vasopressin produced in response to physiological stimuli may depress the activity of the sympathetic nervous system via the central V1 receptors accessible to vasopressin circulating in the blood [6]. In alert rats the pressor response to exogenous vasopressin infusion is selectively weakened by nonbaroreflex inhibition of the vascular sympathetic tone [8]. Moreover, a direct vasodilative effect of vasopressin V2 antagonist has been described in rats [7].

Hence, we may assume that the depressor effect of vasopressin may surpass its pressor effect in rats with acute streptozotocin-induced diabetes. Note that in the case of a chronically elevated plasma vasopressin level in rats it is not always possible to detect the pressor effect of the hormone [10]. Injection of vasopressin selective V1 antagonist to rats with heart failure also did not result in TPVR reduction, despite the chronically elevated plasma vasopressin level [11]. These data indicate that the chronic hemodynamic effect of vasopressin may differ considerably from its acute effect. The incapacity of vasopressin to exert a pressor effect for a prolonged increase of its plasma concentration may be the result of desensitization of the vascular V1 receptors. In diabetes a special role in weakening the vasopressin pressor effect may be played by nonspecific reduction of vascular smooth muscle cell contractility resulting from stepped-up intracellular metabolism of glucose to sorbitol [12], as well as from disturbed excitation of the vascular smooth muscle due to the increased plasma osmolarity [3].

We may thus conclude that the lowered TPVR observed in rats 24 h after STZ injection is, at least partially, vasopressin-dependent and may result from a reduced vascular sympathetic tone and/or from a direct vasodilative effect of vasopressin.

In spite of the significant increase of TPVR injection of combined vasopressin V2/V1 antagonist did not change AP, because it was associated with a reliable reduction of CI (Table 1), this possibly reflecting a relationship between vasopressin and the baroreflex [4].

Our data provide evidence of an important role of vasopressin in the changes occurring in the systemic and regional hemodynamic parameters in alert rats with acute streptozotocin-induced diabetes of 24 h duration.

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Causes of Death in Animals Poisoned with Hydrogen Peroxide

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Numerous cases of fatal and nonfatal poisoning with hydrogen peroxide (HP) have been described [14]. The cause of death has been variously attributed to gas embolism [5-7], cardiac weakness [4], or respiratory failure [8]. However, the reported information on how HP affects the cardiovascular and respiratory systems is based solely on clinical findings which are often contradictory and do not give a clear picture of the pathological changes occurring in the body. On the other hand, attempts have been made over many years to use HP clinically for oxygenating blood in cases of asphyxia or hypoxia [9-11]. The purpose of the present study was to determine the exact causes of death in HP-poisoned animals.

MATERIALS AND METHODS

Randomly bred rats aged 9-10 months were used (n = 285). As the purpose of this study was to elucidate the causes of death in acute poisoning with HP, the animals were poisoned with the peroxide at the LD_{100} level (500 mg/kg) - a dose producing typical changes that arise in cases of HP intoxication. HP was injected subcutaneously as a 10% aqueous solution.

A PDM-3 instrument was used for measuring parameters of external respiration. Arterial pressure was measured in an iliac artery and venous pressure in a femoral vein, with graphic recording on an electrokymograph. The ECG was recorded in six leads (I, II, III, aVR, aVL, and aVF) using an EK2T-02 electrocardiograph. (Because the question of how ECGs should be interpreted in laboratory animals has not been resolved, the ECGs recorded in animal experiments for clinical purposes are difficult to interpret [12].) Stroke output and minute volume were calculated using an integral rheogram [3]. The gaseous composition of the blood (pO_2, pCO_2), its pH, and the percentage of oxyhemoglobin in it were determined in a Micro-Astroup analyzer (Radiometer). Arterial blood was sampled from an iliac artery and venous blood from a femoral vein (from the left paw before the HP injection and from the right paw after it). A pharmacological analysis was also performed

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