nature. A similar endothelium-mediated mechanism of the vasodilator effect has also been described for endogenous biologically active substances: acetylcholine, serotonin, bradykinin, and histamine [9]. In addition, it has been shown that preliminary incubation with NG markedly reduces the sensitivity of vessels to acetylcholine [12].

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Ammonium Succinate Is an Effective Corrector of Cerebral Circulatory Hypoxia

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UDC 616.831-008.922.1-008.64-085.23:546.39

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 9, pp. 276-278, September, 1994
Original article submitted December 29, 1993

It is demonstrated that ammonium succinate is capable of increasing the survival of rats with acute brain ischemia. In transient brain ischemia therapeutic injection of ammonium succinate prevents the development of postischemic hypoperfusion and hypooxygenation of the brain. The antiischemic effect of ammonium succinate is due to a decrease of the oxygen affinity of hemoglobin and to limitation of the accumulation of malonic dialdehyde, a secondary product of lipid peroxidation, in the brain. No vasotropic activity of ammonium succinate is revealed.

Key Words: *brain ischemia; brain circulation; oxygen metabolism in the brain; ammonium succinate*

Antihypoxants are widely used as correctors of disturbances of the cerebral circulation due to the leading role of hypoxia in the pathogenesis of cerebrovascular disorders. Antihypoxants, which optimize the metabolic processes without exerting any of the possible, frequently unpredictable, adverse vasodilator effects [3], make up a promising group of antiischemic agents. The succinic acid (SA) derivatives exhibit a pronounced antihypoxic activity [4,8]. In view of the foregoing, we studied the antiischemic

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0007-4888/94/0009-0975$12.50

TABLE 1. Effect of AS, SA, and Cavinton on Survival of Rats (in %) 24 h after Occlusion of the Carotid Arteries

Experimental conditions	Control	Cavinton, 5 mg/kg	Doses, mg/kg							
			SA				AS			
			5	20	50	100	5	20	50	100
Preventive administration	20	55	20	30	20	–	20	25	60	–
Therapeutic administration	25	13	–	–	–	40	–	–	40	50

effects of ammonium succinate (AS) in comparison with those of SA and cavinton (vinpocetine) under conditions of acute transient brain ischemia and explored the possible mechanisms of the cerebroprotective effect of AS in ischemia.

MATERIALS AND METHODS

Antiischemic activity was assessed on a model of acute cerebral circulatory hypoxia on 112 male Wistar rats weighing 180-200 g. Ischemia was caused by occluding the common carotid arteries under light ether anesthesia. The test preparations were injected *per os*: in sessions of preventive administration every day during 7 days (the last injection being performed 1 h before the occlusion) and for therapeutic use once, 30 min after the onset of ischemia. The survival of animals after a 24-h ligation of the arteries *vs.* the control group served as the criterion of the efficacy of the preparations.

The effect of AS on the general cerebral circulation (GCC), on pO_2 in the parietal cortex, and on the systemic arterial pressure (SAP) was studied on the model of transient ischemia. The experiments were carried out on 14 urethane-anesthetized (1.3 g/kg, i.p.) Wistar rats weighing 200-250 g. Brain ischemia was caused by a 120-min occlusion of the two carotid arteries followed by recirculation. The intensity of GCC was assessed as the blood efflux from the sagittal sinus by the method of hydrogen clearance, pO_2 in the parietal cortex was determined by the polarographic method, and SAP was measured with a transducer from a Salyut physiological kit. AS was injected intraperitoneally in a dose of 50 mg/kg at the 30th min of ischemia. The vasotropic activity of the preparation was studied after Plotnikov [7] on 8 isolated segments of the rat thoracic aorta. The effect of the preparation on the oxygen affinity of hemoglobin was assessed by calculating p_{50} [2] in 17 urethane-anesthetized Wistar rats by a previously described method [6]. The antioxidant activity of AS was studied *in vitro* judging from the accumulation of malonic dialdehyde (MDA), a secondary product of lipid peroxidation (LPO), in the rat brain [5].

RESULTS

In the majority of rats of the control group with acute brain circulatory hypoxia generalized seizures developed by the 4th hour of ischemia; on day 1 the mortality of animals constituted 75-80% (Table 1). AS in a dose of 50 mg/kg used in the sessions of preventive treatment raised the survival threefold as compared to the control. A similar effect was obtained for injection of 5 mg/kg cavinton. Preventive administration of 5, 20, and 50 mg/kg SA had virtually no protective effect. The best result for the therapeutic use of the test compounds was shown by AS in a dose of 100 mg/kg: by the end of day 1 of ischemia the sur-

TABLE 2. Effect of AS (50 mg/kg) on pO_2 in the Parietal Cortex (%), GCC (ml×100 g^{-1}3min^{-1}), and SAP (mm Hg) in Rats during Brain Ischemia and Recirculation ($M \pm m$, $n=7$)

Parameter	Experimental conditions	Initial values	Ischemia, min				Recirculation, min		
			1	30	60	120	1	30	60
pO_2	control	100±0	38±5	43±4	40±5	45±10	67±5	70±3	73±3
	AS	100±0	42±12	52±9	60±8	60±12	101±8**	108±5**	115±6**
GCC	control	129±2	57±5*	62±8*	70±7*	73±8*	107±10	99±12*	95±13*
	AS	128±5	62±14*	70±8*	79±11*	94±19	111±6	145±18*,**	140±16*,**
SAP	control	92±5	78±3*	78±3*	85±2	96±5	76±6*	92±3	86±9
	AS	85±3	70±5*	68±6*	86±6	88±8	93±7	99±4*	96±5

Note. One and two asterisks denote reliable ($p<0.05$) differences *vs.* the baseline and control values, respectively.

vival of rats reached 50% *vs.* the control group. The protective effect of SA in the same dose was less pronounced and corresponded to the effect of AS in a dose of 50 mg/kg (Table 1). The intensity and duration of convulsive fits in rats administered SA and AS (preventive or therapeutic injections) decreased as compared with the same parameters in the control group and in the group given cavinton, which may be due to a lower degree of suppression of the succinate-dependent mitochondrial respiration and to the disconnection of oxidative phosphorylation [9].

The high efficacy of AS, which surpassed the efficacy of SA and cavinton for therapeutic administration, prompted further investigation of this preparation.

In the control group of rats a 2-h occlusion of the common carotid arteries was attended by a reduction of pO_2 and GCC, which by the end of the ischemic period reached 45 and 57% of the initial level, respectively. Disturbances of the cerebral hemodynamics in animals were aggravated by the hypotensive reaction to ischemia during the first 30-60 min. After recirculation postischemic cerebral hypoperfusion and hypooxygenation developed (Table 2). Injection of AS at the 30th min of ischemia did not result in a reliable change of the studied parameters during occlusion; however, during the postischemic period we observed a good prevention of hypooxygenation and hypoperfusion of the brain by the preparation, the values of GCC and pO_2 in the parietal cortex exceeding the initial level at the end of the follow-up; a hypotensive reaction was absent (Table 2).

The mechanism by which AS prevents postischemic disturbances was explored by studying the vasotropic effects of the preparation and its effect on LPO in the brain tissue and on the oxygen-transporting function of the blood.

In the experiments on isolated aorta segments the addition of AS in final concentrations of 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M to Krebs-Henseleit solution did not cause marked changes in the initial vascular tone, while the subsequent addition of KCl (100 mM) caused contractions which attained 760±95, 789±99, 775±116, and 796±110 mg, respectively, not differing reliably from the control (772±90 mg). Addition of AS in the same concentrations under conditions of depolarization-induced (100 mM KCl) contraction also led to insignificant shifts in vascular hypertone. The absence of a direct vasotropic effect of the preparation suggests the presence of other mechanisms of weakening of postischemic hypoperfusion. This effect may be mediated by a decrease in the accumulation of LPO products, which have a powerful vasoconstrictive effect [1]. Sixty minutes after injection of the preparation in final concentrations of 10^{-6}, 10^{-5}, and 10^{-3} M the MDA accumulation in rat brain homogenates for spontaneous LPO was noted to be moderately restricted (0.081±0.006, 0.077±0.006, and 0.067±0.003 μmol/mg protein, respectively, *vs.* 0.085±0.003 μmol/mg protein in the control, $p<0.05$).

The abrogation of brain tissue hypooxygenation by AS during the postischemic period is probably a complex effect, including the ability of the preparation to restrict hypoperfusion and to weaken the oxygen affinity of hemoglobin. One hundred twenty minutes after intraperitoneal injection of AS the blood p_{50} [4,7] in the control group of rats statistically reliably increased from 28.9±1.6 to 32.1±1.6 mm Hg, attesting to a pronounced weakening of the hemoglobin-oxygen binding, whereas in the control group the opposite effect - a decrease of p_{50} [4,7] from 29.7±1.3 to 27.7±1.3 mm Hg ($0.05<p<0.1$) - was observed. Such an effect of the preparation on the oxygen affinity of hemoglobin may effectively promote the correction of hypoxic states, since it has been established that a 2 mm Hg rise of p_{50} is equivalent to a 30% increase in the oxygen transport to the tissues [10].

Thus, AS markedly increased the survival of rats with acute brain ischemia, acting as an effective corrector of postischemic cerebral hypoperfusion and hypooxygenation. The antiischemic effect of the preparation is due to the decrease in the oxygen affinity of hemoglobin, as well as to the decrease in the accumulation of LPO products. The cerebroprotective influence of AS may result from its influence on the energy metabolism in brain cells.

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