Role of α_2-Adrenoceptors in Brain Resistance to Total Ischemia

V. I. Kulinskii and T. N. Medvedeva

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Neuroprotective effect of epinephrine is revealed against the background of prazosin, β-antagonists and their combinations protect against cerebral ischemia. α_2-Antagonists (but not α_1) block the neuroprotective effect of these combinations, methyldopa, guanabenz, clonidine, and oxymetazoline and decrease brain resistance to ischemia. α_2-Adrenoceptors participate in the endogenic mechanism of brain resistance to ischemia and in the neuroprotective effect.

Key Words: *adrenoceptors; neuroprotectors; cerebral ischemia*

Epinephrine does not change the resistance of brain to total cerebral ischemia (CI), although its effect is manifested against the background of propranolol [1]. Similar effects of other adrenoceptor antagonists were not studied. α_2-Agonists produce a marked neuroprotective effect (NPE), i.e., the cerebral protective effect, which was demonstrated in various models of CI [1,2,6-8]. It is generally accepted that to prove the role of certain receptors in any physiological effect means to use blockade analysis. NPE of clonidine [1] and dexmedetomidine [6] is blocked by α_2-antagonists. The respective data are still absent for agonists of dihydroxyphenylethanolamine and aminoguanidine groups. Moreover, idazoxan, an efficient blocker of α_2-receptors, protects the brain against CI [5,8]. The aim of the present study was to elucidate the role of α_2-adrenoceptors in resistance to total CI with the help of the blockade analysis.

MATERIALS AND METHODS

The study was conducted on 564 (CBA×C57Bl) F_1 and CBA mice weighing 19-27 g and on 27 randomly bred rats of both sexes weighing 150-200 g. Since α_2-agonists are powerful analgetics with sedative effect [4,9,10], no anesthesia was necessary. We used R⁻-epinephrine hydrotartrate, propranolol (Kharkov Plant of Endocrine Preparations), methyldopa, oxymetazoline, corynanthine, and idazoxan (Sigma), rauwolscine (ICN), clonidine and prazosin (Chemico-Pharmaceutical Institute, Moscow). Guanabenz acetate was synthesized by Dr. V. Yu. Kovtun, and alprenolol, nifenalol, and practolol by Dr. I. B. Simon. Methyldopa, guanabenz, prazosin, and practolol were suspended in 1% Tween-80 and administered intraperitoneally, while the other substances were injected subcutaneously as water solutions (injection volume 10 ml/kg). Epinephrine was administered 15 min before the agonists; the respective periods for other agents were: clonidine, 30 min; oxymetazoline, 1 h; guanabenz and methyldopa, 2 h; the antagonists, 15 min (with exception of prazosin, 30 min). Rauwolscine and idazoxan were given repeatedly 45 min after guanabenz and methyldopa. The decapitation model of total CI was used in the study, with estimation of duration of agonal respiration (gasping). The series were compared according to nonparametric *U* test [3].

RESULTS

Epinephrine was entirely inactive in hybrids, but in CBA mice there was a tendency to a small increase in the duration of gasping (Table 1). Not only pro-

Department of Biochemistry, State Medical University, Irkutsk

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pranolol [1], but also three other β-antagonists and the α_1-blocker prazosin exhibited protective activity (15-42%). Combined administration with epinephrine resulted in a clear-cut NPE, but for propranolol and prazosin it was significantly higher than for the antagonist itself. Upon combined administration of propranolol and prazosin, their NPE increased 2-fold ($p<0.02$). Epinephrine produced an additional NPE against this background. Idazoxan drastically reduced NPE of the prazosin+propranolol combination and epinephrine ($p<0.001$). Evidently, the high NPE of the last two combinations is realized not via β- or α_1-receptors, but via the α_2-adrenoceptors.

The high NPE of methyldopa, guanabenz, and clonidine [1] is supported by extensive experimental data. An analogous effect is found with oxymetazoline (Table 2), an antagonist of α_1- and α_2-adrenoceptors [9,10]. The activity of the combination prazosin+clonidine is higher than that of clonidine, and the NPE of methyldopa and guanabenz is potentiated by prazosin. Another α_1-antagonist, corynanthine, potentiates NPE of clonidine, but does not significantly affect the activity of oxymetazoline. NPE of clonidine is also potentiated by propranolol (by 2.4 times, $n=9$, $p<0.001$). Rauwolscine in doses of 11 and 28 μmol/kg and idazoxan (1.1, 2.75, and 5.5 μmol/kg) equally decrease (by 17-19%) natural resistance of hybrid mice to CI; in rats idazoxan produces a stronger effect (decrease by 32-35%, $p<0.001$). The total blockade of clonidine-evoked NPE was achieved at higher doses of rauwolscine (28 μmol/kg) or idazoxan (5 μmol/kg). Rauwolscine blocks NPE almost completely in the case of clonidine and oxymetazoline and almost completely in the case of methyldopa and guanabenz (Table 2). However, a small NPE, which is reduced by 2-6.5 times, can still be observed against the shortened duration of gasping caused by rauwolscine itself. Idazoxan almost completely blocks NPE of clonidine, guanabenz, and oxymetazoline (the respective data did not differ from the control ones) and virtually entirely blocks the methyldopa-produced NPE. As compared with the shortened gasping period under idazoxan itself, NPE of agonists are decreased by 3-10 times.

TABLE 1. Effect of Epinephrine and Its Combinations with Different Adrenoblockers on the Duration of Gasping in Mice (Sec)

Blockers, μmol/kg	Mouse line			
	[CBA×C57Bl] F_1		CBA	
	without epinephrine	epinephrine, 5.5 μmol/kg	without epinephrine	epinephrine, 5.5 μmol/kg
Without blockers (control)	16.3 (14—18) n=60	16.1 (14—18) n=17	16.0 (14—17) n=5	14.6 (13—17) n=11	16.1 (14—18) n=8
Alprenolol, 35	—	—		20.8*** (19—24) n=6	21.5***°°° (19—24) n=8
Nifenalol, 192	—	—		16.8* (15—20) n=6	17.2* (15—21) n=6
Practolol, 376	—	—	19.7 (16—24) n=6		22.2***°°° (20—24) n=6
Propranolol, 34	20.8*** (17—26) n=20	26.9******°°° (23—33) n=24		—	—
Prazosin, 2.4	18.8*** (16.5—23) n=21	20.8*****°°° (19—23) n=11		—	—
Prazosin, 2.4+ Propranolol, 34	32.9*** (27—38) n=16	38.9***°°° (32—45) n=16		—	—
Idazoxan, 5.5+ Prazosin, 2.4+ Propranolol, 34	22.2*** (17—29) n=6	29.5***°°° (26—33) n=6		—	—
Idazoxan, 5.5	13.5*** (11—15) n=21	13.8***°° (13—14) n=4		—	—

Note. Here and in Table 2: the variation range is given in parentheses. *$p<0.05$, **$p<0.01$, ***$p<0.001$ relative to baseline; +$p<0.05$, ++$p<0.01$, +++$p<0.001$ relative to the antagonist; °$p<0.05$, °°$p<0.01$, °°°$p<0.001$ relative to the agonist.

TABLE 2. Influence of Different α-Adrenoblockers on Neuroprotective Effect of α_2-Agonists

Agent, μmol/kg	Gasping duration, sec				
	without agonists	methyldopa	guanabenz	clonidine	oxymetazoline
		dose, μmol/kg			
		1900 by 2 h	20 by 2 h	3.75 by 30 min	6.74 by 1 h
Without blockers (control)	16.3 (14—18) *n*=60	28.4*** (23—35) *n*=35	39.3*** (32—46) *n*=27	26.6*** (22—31) *n*=33	28.7*** (24—34) *n*=9
Prazosin, 2.4	18.8*** (17—23) *n*=19	35.0***+++ooo (29—43) *n*=10	49.9***+++ooo (51—63) *n*=8	29.6***+++ooo (25—33) *n*=9	—
Corynanthine, 28	17.7 (15—25) *n*=7	—	— (27—37)	32.0***+++ooo (20—29) *n*=6	23.8*** *n*=6
Rauwolscine, 28	13.3*** (10—16) *n*=20	20.6***+++ooo (17—25) *n*=7	24.4***+++ooo (20—28) *n*=7	16.7+++ooo (15—19) *n*=10	15.2+ooo (13—17) *n*=6
Idazoxan, 5.5	13.5*** (11—15) *n*=21	17.6***ooo (16—20) *n*=7	16.1+++ooo (14—19) *n*=7	14.5ooo (12—16) *n*=12	16.5+++ooo (15—17) *n*=6

The decrease in the resistance to CI by two α_2-blockers and the existence of NPE in prazosin, four β-antagonists, and, in particular, in combination of prazosin with propranolol (in addition, NPE is drastically reduced by idazoxan) supports our hypothesis [1] on the dual effect of endogenous catecholamines on the resistance to CI: it is enhanced via α_2- and decreased via β- and α_1-adrenoceptors. The latter is probably mediated via β_1-subtype receptors, because we found NPE of practolol, a β_1-selective blocker [9]. Potentiation by α_1-antagonists of NPE evoked by α_2-agonists, as well as potentiation of clonidine-produced NPE by propranolol, is probably related to the blockade of this adverse effect of endogenous catecholamines. The blockade analysis revealed the dual effect of exogenous epinephrine as well. Presumably, the controversy over the influence of exo- and endogenous catecholamines on the resistance to CI [6-8] is a result of their opposite effects mediated via different adrenoceptors.

Retention (oxymetazoline) and enhancement (methyldopa, guanabenz, clonidine) of NPE of these agonists by prazosin and corynanthine preclude the participation of α_1-adrenoceptors in this effect. The major role of α_2-adrenoceptors is now proved not only by the fact that they are affected by all these agents (most selectively by guanabenz and α-methylnorepinephrine synthesized in the organism from methyldopa [4,9,10]), but also by complete or profound blockade of NPE, which is produced by all these agents, by rauwolscine or idazoxan, i.e., by two independent methods. Reliability of this inference is rather high, since α_2-agonists and epinephrine belong to three different chemical groups: dihydroxyphenylethanolamines (epinephrine and methylnorepinephrine), imidazolines (clonidine and oxymetazoline), and aminoguanidines (guanabenz). The α_2-antagonists are also different: rauwolscine is an alkaloid, while idazoxan is a derivative of benzodioxan [4,9]. As oxymetazoline has a high and prazosin minimal affinity for $\alpha_{2A,D}$-receptors [9]; our data show that these receptors participate in NPE produced by α_2-agonists. In total CI idazoxan has no NPE and lowers natural resistance to CI. These discrepancies may originate from different character of CI models, as well as from various modes of idazoxan administration: in this work it was given before, while in [5,8] after CI. However, α_2-antagonists have a strong protective effect both before [1,2,6] and after CI [7,8].

On the other hand, idazoxan is known to block not only α_2, but also imidazoline I-receptors [4,9]. Therefore, the fact that when administered in a smaller dose, idazoxan produces stronger inhibition of NPE evoked by α_2-agonists, than a selective α_2-antagonist rauwolscine, testifies to the participation in its action not only of α_2-, but also I-receptors. However, I-receptors are activated by imidazolines, but not by dihydroxyphenylethanolamines or guanabenz [4,9], and NPE of the latter in the optimal conditions is larger than that of imidazoline [1]. Moreover, rau-

wolscine produces stronger inhibition of NPE evoked by imidazolines, which contradicts the hypothesis on their additional protection mechanism. Direct experiments with more selective agonists and antagonists of I-receptors are necessary to shed light on the problem of involvement of these receptors in NPE.

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