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Significance of Various Adrenoreceptors for Brain Resistance to Total Ischemia

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In contrast to the β-agonist isoprenaline and the α_1-agonist phenylephrine, the α_2-agonists clonidine, guanobenz, and methyldopa are highly effective against total brain ischemia. Epinephrine in itself is inactive but displays protective activity against the background of the β-antagonist propranolol. The α_2-antagonist rauwolscine, but not α_1-antagonists, abolish the protective effect of clonidine.

Key Words: *adrenoreceptors; brain protectors; brain ischemia*

Protectors with receptor activity are highly effective in various critical states, including brain ischemia (BI) [2]. The effects of the adenosine receptor agonists [4,9] in ischemia are well known, whereas those of the agonists of adrenoreceptors have not been studied in sufficient detail. In focal [8] and incomplete BI [6,7], the α_2-agonists clonidine [6] and dexmedetomidine [7,8] improve neurological status [6,7] and reduce histologically revealed damage to the brain cortex [8]. The data for the hippocampus and caudal nuclei are ambiguous [6-8] and evidence on the effects of naturally occurring catecholamines is controversial. It is thought that in incomplete BI catecholamines actually aggravate neurological status and have no effect on total ischemia [6]. The adrenoreceptor agonists have not been compared, and the effects of a_2-agonists have not been investigated. The present study was designed to address these topics.

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MATERIALS AND METHODS

Experiments were performed on 350 mice of both sexes weighing 16-25 g. The following preparations were used: R(-)-epinephrine hydrotartrate, RS-isoprenaline hydrochloride, anapriline (propranolol) (both from the Kharkov Plant of Endocrine Preparations), phenylephrine (Serva), α-methylhydroxyphenylalanine (methyldopa), corynanthine (both from Sigma), rauwolscine (ICN), clonidine and prazosin (both from the Chemico-

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TABLE 1. Effects of Agonists of Various Adrenoreceptors on the Duration of Gasping in Mice (CBA×C57Bl) F_1

Compound	*n*	Dose, μmol/kg	Time, min	Duration of gasping, sec	Effect, %
Control	55	-	5-420	16.8 (14-22)	-
Isoprenaline	9	11	5	15.7 (13-17)	-7
Phenylephrine	13	49	15	16.9 (13-22)	+1
Epinephrine	15	5.5	15	16.2 (14-18)	-4
Anapriline	19	34	15	20.8 (17-26)	+24*
Anapriline+epinephrine	18	34+5.5	15+15	27.8 (23-33)	+65*
Clonidine	23	3.75	45	24.8 (21-29)	+48*
Guanobenz	11	20	180	42.0 (29-64)	+150*
Methyldopa	14	1900	300-420	36.2 (30-52)	+115*

Note. Here and in Table 2: deviation limits are given in parentheses, *$p<0.001$ compared with the control.

Pharmaceutical Institute, Moscow), and guanobenzacetate synthesized by Dr. V. Yu. Kovtun (Farmzashchita, Moscow).

Prazosin, guanobenz and methyldopa were suspended in 1% Tween-80 and injected intraperitoneally; the other agents were injected subcutaneously as aqueous solutions. The volume of injection was 10 ml/kg. Dosage and times of administration were optimal for the manifestation of the pharmacological effect. The decapitation model of total BI with evaluation of the duration of agonal respiration (gasping) was used. The Wilcoxon-Mann-Whitney *U* test [4] was employed because of considerable deviations from the norm.

RESULTS

The β-agonist isoprenaline and the α_1-agonists phenylephrine and epinephrine were inactive, while all studied α_2-agonists (clonidine, guanobenz and methyldopa) markedly prolonged the duration of gasping (Table 1). This testifies to the significance of α_2-adrenoreceptors, since these compounds are chemically different: clonidine is a phenylaminoimidazoline derivative, guanobenz is a benzylydeneaminoguanidine derivative, and the active metabolite methyldopa (α-methylepinephrine) is a derivative of phenylethylamine [5,10]. However, it is unclear why epinephrine, which is known to activate α_2-adrenoreceptors, was inactive. It may be related to the fact that epinephrine is an agonist of all four types of adrenoreceptors and its opposite effects are mutually abolished, as was demonstrated for radioprotective [1], anticalorigenic, and antihypoxic effects of catecholamines [3]. In fact, against the background of the β-antagonist propranolol, which increases the brain's resistance to ischemia, the protective effect of epinephrine is pronounced. Since all three α_2-agonists displayed protective activity, it can be assumed that the protective effect of epinephrine is realized via the α_2-adrenoreceptors. In order to evaluate the role of the α_2-adrenoreceptors, we studied the influence of the α_1-antagonists prazosin and corynanthine and of the α_2-antagonist rauwolscine on the protective effect of clonidine (Table 2). Obviously, prazosin and corynanthine did not block but rather increased the activity of clonidine. By contrast, rauwolscine reduced in a dose-dependent manner and even prevented the protective effect of clonidine. This becomes particularly striking when the effect in all the

TABLE 2. Blockade of the Cerebrospinal Effect of Clonidine by Antagonists of Various Adrenoreceptors

Antagonist	Dose, μmol/kg	Time prior to clonidine injection, min	Duration of gasping, sec	
			without clonidine	clonidine, 3.75 μmol/kg for 45 min
(CBA×C57Bl) F_1				
Control	-	30	16.8 (14-22) *n*=55	24.8^{2} (21-29) *n*=23
Prazosin	2.4	30	18.5^{1} (16-21) *n*=17	$29.6^{1,4,7}$ (25-33) *n*=9
Outbred				
Control	-	15	16.4 (11-21) *n*=75	27.4^{2} (18-33) *n*=8
Corynanthine	28	15	18.0 (15-25) *n*=6	$32.0^{1,3,5}$ (27-37) *n*=6
Rauwolscine	11	15	13.4^{1} (12-15) *n*=8	$20.5^{1,4,6}$ (14-26) *n*=13
Rauwolscine	28	15	13.0^{1} (10-16) *n*=9	$16.7^{3,7}$ (15-19) *n*=10

Note. Significance of differences: $^{1}p<0.01$, $^{2}p<0.001$ (compared with the control); $^{3}p<0.01$, $^{4}p<0.001$ (compared with the antagonist); $^{5}p<0.05$, $^{6}p<0.01$, $^{7}p<0.001$ (compared with clonidine).

series is compared with the control. Compared with the effect of rauwolscine, which reduces the period of gasping, the effect of clonidine is more pronounced. However, even in this comparison the effect of clonidine is much weaker than that in the series without rauwolscine. It should be stressed that corynanthine and rauwolscine are chemically similar (they are diastereoisomers of yohimbine) but differ markedly pharmacologically: corynanthine is a selective α_1-antagonist and rauwolscine is a selective α_2-antagonist [5,10]. Therefore, the experiments with the blockers confirm the significance of the α_2-adrenoreceptors for realizing the protective effect of clonidine. This is consistent with the blockade of the protective effect of dexmedetomidine by the α_2-antagonist atipamezol [7]. The increase in the basal duration of gasping after the administration of propranolol and prazosin and a decrease after rauwolscine indicate that endogenous catecholamines have a dual effect on brain resistance to total ischemia: they reduce it via the β- and α_1-adrenoreceptors and potentiate it via the α_2-adrenoreceptors.

Thus, the protective effect of α_2-agonists is typical not only of focal and incomplete BI but also of total BI. It has been demonstrated for different chemical compounds and, consequently, is of a universal nature. The role of α_2-adrenoreceptors in the protective effect of clonidine has been confirmed by blockade with the selective α_2-antagonist rauwolscine.

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