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Effect of nicardipine and amlodipine on the brain free arachidonic acid level in hypoxic rats

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INTRODUCTION

The brain damage caused by hypoxia is dominantly due to the entering of extracellular calcium ions (Ca^{2+}) into the neurones, mostly through the L-type of voltage sensitive calcium channels *(2, 4, 8, 11, 12),* and partially due to their accumulation in mitochondria and the loss of ATP production *(15).* The increase in the intracellular Ca^{2+} concentration leads to the activation of phospholipases A_2 and C and the release of the brain free fatty acids (FFA) that have detrimental effects on mitochondria and plasma membrane functions *(16),* particularly free arachidonic acid (FAA) *(15).* Accumulation of their metabolites causes additional cell damage *(16).*

In order to prevent massive influx of Ca^{2+} into neurones and following damage of the brain cells, we investigated the effects of 1,4 dihydropyridines, nicardipine and amlodipine, on the brain FAA level in hypoxia-exposed rats.

MATERIAL AND METHODS

Animals

The study was carried out on female Hannover-Wistar rats, weighing 200-250 g. The animals were maintained on a 12hr light-dark cycle at a room temperature of 22-24°C and allowed free access to food and water. The animals were randomly divided into 11 groups. One group consisted of intact, drug naive rats with no past experimental history.

Hypoxia

All other animals were subjected to the controlled hypoxia conditions. They were placed in a hypoxia cage consisting of an airtight plexiglas box into which a mixture of pure nitrogen and oxygen was delivered. The oxygen concentration was gradually reduced by the animal breathing until the level of 3.5V% of oxygen was reached. It was then maintained constant by an automatically regulated release of pure oxygen from separate chamber. The percentage of oxygen was continuously measured and controlled by an oxygen measuring device. The animals were maintained in such conditions until losing the righting reflex. Then they were transferred to the normal atmosphere and were decapitated 15 minutes later. The brains were quickly removed and frozen in liquid nitrogen.

One group of animals was subjected only to the hypoxia procedure but was not treated with any drug. Other hypoxic rats received intraperitoneal (i.p.) injections of the vehicle solution containing ethanol and propylene glycol 400 (50:50, V/V) or various doses (0.03; 0.1, 0.3 or 1.0 mg/kg) of nicardipine or amlodipine. The drugs tested were dissolved in the vehicle solution, in a total volume of 1 ml/kg and given 30 minutes before hypoxia-exposure.

Biochemical analyses

The frozen brains were weighed and homogenized in about 25 ml of a chloroform/methanol (2:1, V/V) mixture for lipid extraction. Nonadecanoic acid (19:0) was added as an internal standard. The extract was dried, lipids were dissolved in 1 ml of the chloroform/methanol (2:1, V/V) mixture and aliquots were used for analyses. The FFA were separated by preparative thin-layer chromatography (developing solvents: petroleum ether/ether/acetic acid (97:3:1, by vol.) and petroleum ether/ether/acetic acid (80/20/1, by vol.)). Streaks on thin-layer plates were visualised by water spray, scraped off and eluted with chloroform/methanol (2:1, V/V).

FFA methyl esters were prepared by methanolysis with methanol/hydrochloric acid (8.3:1.7, V/V) for 20 minutes at 120°C, extracted with petroleum ether and quantified by gas chromatography using an internal standard.

Statistical analysis

Levels of the brain FAA are expressed in mg/g tissue. Statistical significance was calculated according to ANOVA, followed by Duncan's multiple range test. The criterion for significance was $p \leq 0.05$.

RESULTS

Table 1 shows the brain FAA levels in intact, drug naive rats and in hypoxic rats decapitated 15 minutes after losing the righting reflex. An overall of ANOVA on the brain FAA levels revealed the significant effect of the treatment [$F(2, 15) = 5.93$; $p = 0.013$]. Duncan's multiple range test indicated that the brain FAA contents in hypoxic drug naive, or hypoxic rats receiving the vehicle solution were significantly higher in relation to the intact animals. It is also evident that administration of the vehicle solution had no significant influence on the brain FAA level in the hypoxic rats [$p = 0.16$] (Table 1).

TABLE 1

The brain FAA level in the intact rats and in the hypoxic animals decapitated 15 minutes after losing the righting reflex.

TREATMENT	FAA (mg/g)
Intact *Drug naive*	0.0029 ± 0.0021
Hypoxia *Drug naive*	0.0361 ± 0.0091*
Hypoxia *Vehicle solution*	0.0217 ± 0.0073*

The results are expressed as means ± S.E.M.; n = 6 for each group; * $p \leq 0.05$; Significantly different from intact, drug naive rats.

The brain FAA content in the hypoxic animals was not statistically altered neither by the doses tested of nicardipine [$F(4,25) = 0.21$; $p = 0.93$] nor amlodipine [$F(4,25) = 0.69$; $p = 0.60$] (Table 2). Thus the brain FAA levels in the hypoxic animals treated with the vehicle solution did not differ significantly in relation to the levels in hypoxic animals receiving various doses of the calcium channel blockers tested.

TABLE 2

The brain FAA levels in hypoxic rats treated with vehicle solution or with various doses of nicardipine or amlodipine, decapitated 15 minutes after losing the righting reflex.

TREATMENT	DOSE mg/kg i.p.	FAA (mg/g)
Vehicle solution	–	0.0217 ± 0.0073
Nicardipine	0.03	0.0221 ± 0.0047
	0.1	0.0300 ± 0.0039
	0.3	0.0267 ± 0.0099
	1.0	0.0276 ± 0.0107
Amlodipine	0.03	0.0367 ± 0.0084
	0.1	0.0272 ± 0.0077
	0.3	0.0315 ± 0.0069
	1.0	0.0359 ± 0.0073

The results are expressed as means ± S.E.M.; n=6 for each group.

DISCUSSION

The results of our experiments demonstrate that an accumulation of the brain FAA occurs during the period of posthypoxic normoxic reoxygenation in hypoxia-exposed rats. This finding is in agreement with our earlier report *(21)* in which we determined the dynamics of the brain FAA pool in hypoxic animals decapitated at various time intervals after losing the righting reflex. Maximum level of FAA was detected 15 minutes after cerebral hypoxia had been obtained. According to this result all hypoxic animals were decapitated at the mentioned time interval after losing the righting reflex.

Nicardipine is a dihydropyridine calcium channel blocker with ability to cross the brain-blood barrier *(6)*. Mentioned calcium channel blocker is a very potent cerebro-arterial dilatator *(5)* with proven beneficial effects in stroke *(13)*, subarachnoid haemorrhage *(18)* and ischemia *(14)*. Nicardipine can improve the retention deficit in animals exposed to hypoxia *(19)*. This drug does not significantly inhibit the IP3-receptor binding *(9)*, it has limited effects on calcium-calmodulin binding, and does not protect against delayed neuronal death *(7)*. Some authors report it to be efficient only in high doses *(1)*.

Amlodipine is a potent regulator of the L-type of voltage-sensitive calcium channels on peripheral blood vessels with delayed hypotensive effect *(17)*. This drug may also significantly prolong the shortened retest step-through latency of the passive avoidance task in hypoxia-treated rats *(20)*.

In our experimental conditions neither nicardipine nor amlodipine influenced significantly the increase in the brain FAA level in hypoxia-exposed rats. Inactivity of nicardipine in our study is not in agreement with the results of Kidooka et al. *(10)* who found that mentioned drug in a dosage of 1 mg/kg effectively attenuated the liberation of FFA, particularly FAA, in the decapitation model of global cerebral ischemia. The differences in mentioned results could be caused by difference in used methodological procedures.

Both calcium antagonists tested in our study are active on the L-type of voltage-sensitive calcium channels. Therefore, we suggest that in our experimental conditions the inhibition of the Ca^{2+} influx via mentioned channels is not sufficient for the reduction of hypoxia-induced accumulation of the FAA. This result is also consistent with the observations that different modes of the elevation in cytosolic Ca^{2+} concentration (influx of extracellular Ca^{2+} through voltage-sensitive L and N channels, NMDA and non-NMDA receptor- sensitive channels, via membrane Na^+/Ca^{2+} exchanger or Ca^{2+} release from intracellular stores) are the critical events in the pathogenesis of hypoxic-ischemic brain injury *(3)*.

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ABSTRACT

Effect of nicardipine and amlodipine on the brain free arachidonic acid level in hypoxic rats

Background and purpose: A number of investigations support the hypothesis that a common denominator of hypoxic cellular damage in the brain is an increase in intracellular Ca^{2+} concentration that causes degradation of membrane phospholipids and liberation of cerebral free fatty acids, particularly free arachidonic acid (FAA). The aim of our study was to examine the effects of the calcium channel blockers, nicardipine and amlodipine, on the level of the brain FAA in rats exposed to hypoxia.

Material and methods: The study was carried out on Hannover-Wistar rats. The drugs tested had been injected 30 minutes prior to hypoxia exposure. Rats were decapitated 15 minutes after losing the righting reflex. The brains of all animals were taken out and were frozen in liquid nitrogen. FAA was quantified using gas chromatography method.

Results: The results of our experiments show that an accumulation of the brain FAA occurs during the period of posthypoxic normoxic reoxygenation in hypoxia-exposed rats. The tested doses of nicardipine and amlodipine did not influence the increase in the brain FAA level in hypoxic rats.

Conclusions: The inhibition of the Ca^{2+} influx via L-type of voltage-sensitive calcium channels is not sufficient for the attenuation of the release of the brain FAA in hypoxic rats.

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