3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate Effect on Induced Contractions by Hydrogen Peroxide on Trachea Smooth Muscle

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Experimental researches noticed the relaxing effect of Nifedipine on the guinea pig tracheal smooth muscle constrictor agents comparatively with the prevention of contraction induced by these agents. The aim of this study was to evaluate these considerations in the hydrogen peroxide constrictor effect.

Keywords: nifedipine, hydrogen peroxide, beta-adrenergic receptors, cholinergic receptors

Nifedipine (3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) was developed by the German pharmaceutical company Bayer, most initial studies being performed in the early 1970s.

The use of nifedipine and related calcium channel antagonists was much reduced in response to 1995 trials that mortality was increased in patients with coronary artery disease who took nifedipine [16]. This study was a meta-analysis, and demonstrated harm mainly in short-acting forms of nifedipine (that could cause large fluctuations in blood pressure) and at high doses of 80 mg a day and more [1-5].

Nifedipine is a dihydropyridine calcium channel blocker. Its main uses are as an antianginal (especially in Prinzmetal’s angina) and antihypertensive, although a large number of other indications have recently been found for this agent, such as Raynaud’s phenomenon, premature labor, and painful spasms of the esophagus in cancer and tetanus patients. It is also commonly used for the small subset of pulmonary hypertension patients whose symptoms respond to calcium channel blockers (fig 1).

The approved uses for nifedipine are the long-term treatment of hypertension (high blood pressure) and angina pectoris. In hypertension, recent clinical guidelines generally favor diuretics and ACE inhibitors, although calcium channel antagonists, along with thiazide diuretics, are still favored as primary treatment for patients over 55’s [6-8].

Sublingual nifedipine has previously been used in hypertensive emergencies. This was found to be dangerous, and has been abandoned. Sublingual nifedipine causes blood-pressure lowering through peripheral vasodilatation. It can cause an uncontrollable decrease in blood pressure, reflex tachycardia, and a steal phenomenon in certain vascular beds. There have been multiple reports in the medical literature of serious adverse effects with sublingual nifedipine, including cerebral ischemia/infarction, myocardial infarction, complete heart block, and death. As a result of this, the FDA reviewed all data regarding the safety and efficacy of sublingual nifedipine for hypertensive emergencies in 1995, and concluded that the practice should be abandoned because it was neither safe nor efficacious [9-12].

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Experimental part

There were studied 12 spiral tracheas, obtained from male guinea pig (200g) sacrificed under narcosis. Preparations were put in an organ bath containing 50 mL Krebs-Henseleit solution at 37 Celsius degree, continuously gassed with a mixture of 95% O₂ and 5% CO₂ in order to maintain oxygen tension and a pH of 7.4.

A force transducer for recording isometric contraction, displayed on an xy inscriptor, has been used in order to follow up tracheal smooth muscle contractility.

After an equilibrium period of 60 min with 6 intermediate changes of solution, a dose-response curve was performed using hydrogen peroxide in concentration 10⁻³ M with two intermediate changes of buffer solution. Then the maximal contraction response of the tracheal smooth muscle was performed. Based on this maximal response the samples were incubated 10 minutes with Nifedipine M/40 (solved in ethyl alcohol 1%) 10⁻⁵, 5 . 10⁻⁵ and 10⁻⁴ M which reduced the contractile effect of hydrogen peroxide (considered maximal at 100%).

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In the second experiment the preparations were incubated ten minutes first with Nifedipine M/40 (solved in ethyllic alcohol 1%) 10^-5, 5 . 10^-5 and 10^-4 M followed by a single dose 10^-3 M hydrogen peroxide M/10. We observed the reducing hydrogen peroxide constrictor effect evaluated by the time of total relaxing of the sample which appeared after the initial contraction induced by the hydrogen peroxide addition in the organ bath.

For each study we have performed 10 experiments in duplicate.

Statistical analysis included calculation of mean values, standard deviation and Student`s test.

Results and discussions

Nifedipine effect on hydrogen peroxide contractile response

Incubation with 10^-3 M hydrogen peroxide M/10 produced the appearance of guinea pig tracheal smooth muscle contraction with the maximal amplitude in 2-3 min (plateau phase).

The addition of Nifedipine, 10 min produced the decrease of hydrogen contractile response to total relaxation depending on the Nifedipine dose used.

Results signalled out an relationship between Nifedipine dose and total relaxing time of the tracheal smooth muscle.

Preincubation with Nifedipine (10^-3 M) induced the total relaxation in 20 min, a dose of 5 . 10^-2 in 12 min and preincubation with 10^-4 M Nifedipine induced total relaxation in 8 min (fig 2).

We used the same protocol as we observed the decrease of the 10^-3 M hydrogen peroxide M/10 contractile response, administrated after ten minutes Nifedipine preincubation with different doses 10^-3 , 5 . 10^-4 and 10^-4 M, separated by three washing procedures with Krebs-Henseleit solution.

We evaluated the decrease of the hydrogen peroxide contractile response depending on the Nifedipine dose used.

A dose of 10^-3 M Nifedipine reduced the hydrogen peroxide contractile response with 50% (mean value), a dose of 5 . 10^-2 reduced this effect with 60% and a dose of 10^-4 M had a reducing effect with 80% (fig 3).

Conclusions

We observed that the lower doses were necessary in order to obtain the same maximal contractile response comparatively with the rat tracheal smooth muscle. These results pointed out a higher muscarinic receptors density or a higher receptor affinity.

Incubation with the hydrogen peroxide had the same effect like in rats experimental studies. These data confirmed the results of another experimental studies which noticed the similarity of hydrogen peroxide effect in tracheal smooth muscle on both species rats and guinea pig.

An explanation for the difference in sensitivity to hydrogen peroxide between these two responses might be found in the fact that the beta-adrenergic receptors are coupled to adenylate cyclase which contains an sulfhydryl group and is rapidly inactivated by hydrogen peroxide.

So, hydrogen peroxide determinate the imbalance between the sympathetic and parasympathetic receptor response, enhanced the cholinergic receptors response.

Experimental studies of Kramer and Leurs noticed the decrease of beta adrenoreceptors in vivo and in vitro after incubation with oxygen free radicals generating systems.

In vitro studies demonstrated that Nifedipine had a relaxing effect on tracheal smooth muscle in human, guinea pig, dog and rat. This effect is depending on dose, is slow and the total relaxation appeared after 20-30 minutes at high doses of Nifedipine.

Excitation-contraction coupling in the airway muscle cell is dependent on variations of the intracellular calcium concentration. Calcium may enter the cell through voltage/receptor operated channels or through the cell membrane. Voltage dependent channels are activated by depolarizing stimuli and are sensitive to calcium channels blockers such as Nifedipine and explained the relaxing effect of this agent on airways level.

Nifedipine decreased the contractile response of hydrogen peroxide in guinea pig tracheal smooth muscle.

Hydrogen peroxide acts on the beta-adrenergic and cholinergic receptors and provided a role in the constriction of airways at this level.

This study confirmed the literature data which established the Nifedipine role in reducing contraction (in this study of hydrogen peroxide) comparatively with the contraction prevention.

References

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