STUDII CLINICO-ŞTIINŢIFICE

The Effect of Hypotensive Drugs on Carbonic Anhydrase I Activity. *In vitro* Studies

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Efectul hipotensivelor asupra activității anhidrazei carbonice I. Studii *in vitro*

Anhidraza carbonică (CA) se conține în tot corpul uman. Izoenzima I se evidențiază mai des în endoteliul vascular. Scopul studiului – a demonstra, *in vitro*, acțiunea de inhibare a hipotensivelor asupra CA I prin intermediul receptorilor adrenergici sau prin acțiunea directă a vasodilatatoarelor. S-a stabilit viteza reacției prin care CO_2 este hidratat în absența enzimelor catalitice, în prezența CA I și cu participarea CA I în asociere cu hipotensive. La expunerea rezultatelor s-a folosit ecuația Michaelis Menten conform metodei lui Eadie Hoffstee. A fost folosită soluție în concentrațiile de la 10^{-8} M până la 10^{-4} M. S-a stabilit că clonidina în concentrație de 10^{-4} M reduce activitatea CA I cu 50%, prazosina – cu 42%, reserpina – cu 30%. Epinephrina în asociere cu clonidina de concentrație 10^{-4} M, crește activitatea CA I numai cu 31%, *vs* 68% – când sunt testate fiecare în parte. Yohimbina, în prezența clonidinei manifestă același efect asupra CA I indiferent de concentrația soluției. Norepinephrina în prezența prazosinei are o activitate redusă asupra CA I – 25%, *vs* 61% – în testare separată. Concluzie**:** Clonidina, prazosina și reserpina reduc activitatea CA I. Studiile *in vitro* demonstrează că clonidina inversează efectul activității catecolaminelor. S-a stabilit o inhibiție competitivă între Yohimbină și clonidină. Prazosina are un efect opus celui de activare al norepinephrinei în prezența CA I.

Cuvinte-cheie: remedii hipotensive, anhidraza carbonică, antiadrenergice.

Действие гипотензивных средств на активность углекислой ангидразы I. Исследование *in vitro*

Углекислая ангидраза (УА) содержится во всех тканях организма человека. Изофермент I чаще обнаруживается в сосудистом эндотелии. Цель исследования – установить *in vitro* ингибирующее действие гипотензивных средств на УА I с участием адренергических рецепторов или непосредственным действием гипотензивных препаратов. Была установлена скорость реакции гидратации CO_2 в отсутствии каталитических ферментов, в присутствии УА I и при участии УА I в сочетании с гипотензивными средствами. При изложении результатов было использовано уравнение Michaelis Menten в соответствии с методикой Eadie Hoffstee. Был использован раствор в концентрациях от 10^{-8} М до 10^{-4} М. Установлено, что клонидин в концентрации 10^{-4} М снижает активность УА I на 50%, прозосин – на 42%, резерпин – на 30%. Епинефрин в сочетании с клонидином в концентрации 10^{-4} М увеличивает активность УА I лишь на 31%, а при их отдельном тестировании – на 68%. Иохимбин в присуствии клонидина имеет тот же эфект на УА I независимо от концентрации раствора. Норепинефрин в присуствии празосина снижает свое действие на УА I на 25%, в то же время как при их отдельном тестировании оно составило 61%. Вывод: Клонидин, празосин и резерпин снижают активность УА I. Исследования *in vitro* показали, что клонидин приводит к обратному действию катехоламинов. Установлено ингибирующее конкурентное действие иохимбина и клонидина. Празосин в присуствии УА I проявляет ингибирующий эффект, а норепинефрин, наоборот – активирующий.

Ключевые слова: гипотензивные средства, углекислая ангидраза, антиадренергетический эффект.

Introduction

Carbonic anhydrase was discovered in 1932 by Meldrum and Roughton (1). This enzyme catalyzes the simplest reaction in the human body: the reaction between CO_2 and water (2). Over the last few years, the role of this enzyme has been reconsidered as more important because of pH variation determined by this reaction in the human body and its physiological and physiopathological implications (3). We will abbreviate carbonic anhydrase as CA in this study.

The aim of this study is to demonstrate that, in vitro, CA I is inhibited by hypotensive drugs which antagonize adrenergic receptors or direct vasodilators. This study is a fragment of a larger study which tries to demonstrate the implication of CA I in hypertension physiopathology. Clonidine is an antihypertensive drug, agonist of alfa 2 adrenergic receptors. It is also the central action of the sympathetic nervous system (4). Prazosin is an antagonist of alfa 1 receptors and its action leads to arterial and venous vasodilatation (5). Reserpine is a Rauwolfia alkaloid which causes vasodilatation and antihypertensive effect by depleting the norepinephrine stores (6).

Material and Methods

We have studied the effect of clonidine, prazosin and reserpine on CA I and II by in vitro studies. We also studied the effect of epinephrine or yohimbine association with clonidine and norepinephrine association at prazosin. All our associations were made with echimolar concentrations between 10^{-8} M and 10^{-4} M (3).

Experimental method: Speed of reaction without catalyzer: determination of time needed for reaction in the absence of the enzyme. The time is noted as T_0 (8). Speed of reaction in the presence of catalyzer: time needed for the

reaction in the presence of the enzyme. Time is noted as T. The volumetric activity of CA is determined by the formula: $(T_0 - T)/T$ (U.E/ml) (8). 1 U.E. is the quantity of enzyme used to double the speed of reaction (9). Speed of the reaction may be modified by activators or inhibitors association. Effect of it may be monitored by application of activators or inhibitors in reaction medium and by measuring the speed of the reaction. These determinations are made by the relative doses/responses at concentrations between 10^{-8} and 10^{-6} M (8). The initial speed of reaction of CO_2 hydration has been measured by the spectophotometry pH indicator method. This involved the use of the Hi-Tech stopped-flow spectrophotometer SF 51 MX (Hi-Tech, England), at 400nm, using the Hepes 20mM - p-nitrophenol system. The initial pH was 7.5 and the temperature was a constant 25 °C (9). For initial speed measurement, we varied the concentration of substrate between 5 mM and 30 mM CO_2. The concentrations of CO_2 we used were measured by spectophotometry at 340 nm using the automatic analyzer IMPACT 400. We have integrated the results using linearization of the Eadie-Hoffstee method with the Michaelis Menten equation. To calculate the median and standard deviation we used the Student test. All values we have used were a median of five different measurements, at every probe.

Results

Clonidine decreased CA I and CA II activity by a dose/response correlation, starting from concentrations of 10^{-8} M, and at 10^{-4} M inhibition, the results were 50% for CA I and 15% for CA II. Prazosin decreased CA I and CA II activity by a dose/response correlation, starting from concentrations of 10^{-8} M, and at 10^{-4} M inhibition, the results were 42% for CA I and 20% for CA II. Reseprine decreased CA I and CA II activity by a dose/response correlation, starting from concentrations of 10^{-8} M, and at 10^{-4} M inhibition, the results were 30% for CA I. Contrary to clonidine and prazosin, reseprine is a strong activator of CA II. At 10^{-4} M the cativator effect is 178%. Results in detail are given in tables 1, 2 and 3, and in figures 1 and 2.

Table1

Effects of clonidine, prazosin and reseprine on CA I and CA II

Concentration (M)	Clonidine		Prazosin		Reserpine	
	CA I	CA II	CA I	CA II	CA I	CA II
10-8 M	-11%	0	-9%	0	-9%	35%
10-7 M	-19%	0	-16%	-5%	-14%	67%
10-6 M	-32%	-6%	-28%	-9%	-20%	92%
10-5 M	-41%	-11%	-35%	-14%	-27%	136%
10-4 M	-50%	-15%	-42%	-20%	-30%	178%

Table 2

Effects of ephedrine and yohimbine association with clonidine on CA I activity

Concentration (M)	Ephedrine	Ephedrine + Clonidine	Yohimbine	Yohimbine + Clonidine
10-8 M	12%	6%	-17%	-17%
10-7 M	29%	14%	-23%	-23%
10-6 M	52%	21%	-35%	-35%
10-5 M	63%	28%	-41%	-41%
10-4 M	68%	31%	-52%	-52%

Table 3

Effects of prazosin and norepinephrine association on CA I activity

Concentration (M)	Norepinephrine	Norpeinephrine + Prazosin
10-8 M	14%	5%
10-7 M	26%	10%
10-6 M	40%	16%
10-5 M	53%	20%
10-4 M	61%	25%

Discussions

Our results show that clonidine and prazosin restrict CA I activity much more than CA II activity. In vitro, clonidine antagonizes the activator activity of catecholamines on CA I. This may be proof of direct action of clonidine on active situs of CA I.

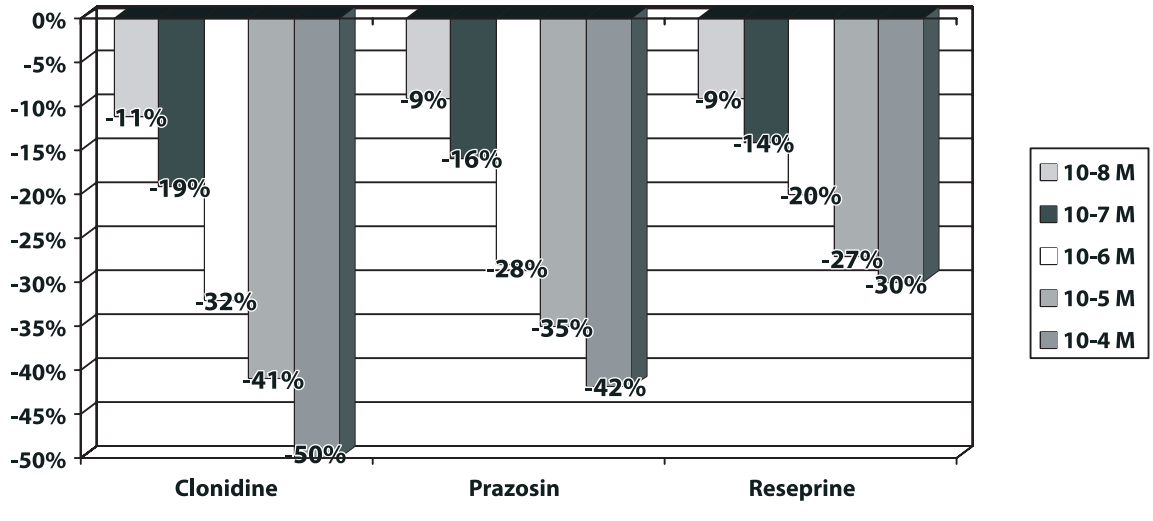

Fig. 1. Effects of Clonidine, Prazosin and Reserpine on CA I activity.

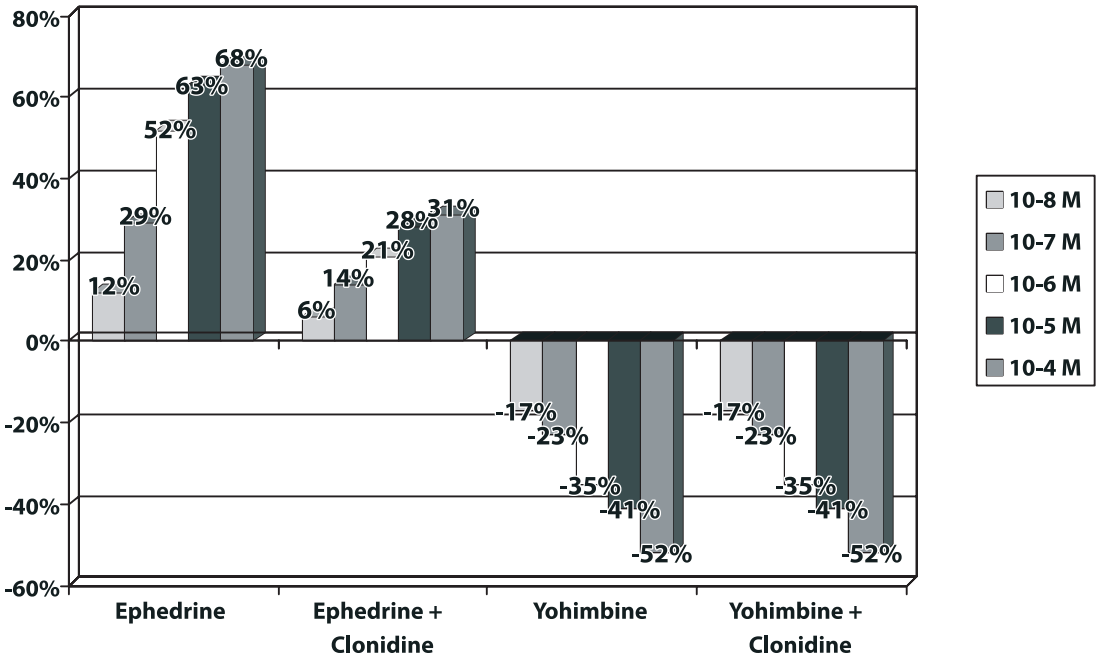

Fig. 2. Effects of ephedrine and yohimbine association with clonidine on CA I

We suggest that the proof of Langer et al. (9) - who have demonstrated that clonidine is blocked by yohimbine - is real but incomplete. There is a competitive inhibition between these two substances, and the yohimbine affinity for CA I is so important that to completely remove clonidine from active situs of CA I, so that there is no effect of clonidine on CA I.

To completely understand the anomaly of inhibition and stimulation of alpha 2 adrenergic receptors, we have to recognise that these receptors, with regards to their activity on cardiovascular system, are presinapticaly, and regulate the release of adrenergic mediators. Alfa2 agonist (clonidine) leads to inhibition of adrenergic mediators release, vasodilatation and hypotension. Alfa2 antagonists stimulate the adrenergic mediators release, vasoconstriction and hypertension. These processes are partially explained by the action of these mediators on CA I activity. The inhibition of CA I by clonidine explains its hypotensive activity despite its adrenergic agonism, but doesn't explain why yohimbine, one of the most powerful inhibitors of CA I has the opposite effect on the central nervous system. With regards to peripheric circulation, yohimbine has a vasodilator and hypotensive effect probably through alfa1 antagonsim. In the case of alfa2 adrenoreceptors, the pH theory is not able to explain why substances with the same effect on CA I activity have different effects on alfa2 receptors (10). Prazosin is a very selective antagonist of alpha 1 adrenergic receptors. It has a 1000 times greater affinity for alpha 1 compared with alfa2. In our in vitro studies, prazosin antagonized the effect of norepinephrine on CA I activity. Our studies emphasize that clonidine and prazosin, which both are ligands of alpha adrenergic receptors, have also a direct inhibitory effect on CA I activity. Reserpine lowers the CA I activity but is a strong activator for CA II. In the future we intend to monitor the correlation between the decreasing of CA I activity and the hypotensive effect of adrenergic antagonist.

Conclusions

1. Clonidine and prazosin predominately decrease the activity of CA I and in a lesser manner CA II activity.
2. In vitro, clonidine antagonizes activator effects of cathecolamines on CA I. This may be proof of the direct action of clonidine on active situs of CA I.
3. There is a competitive inhibition between clonidine and yohimbine and the affinity of yohimbine for CA I is so important that is completely remove clonidine form the active situs of CA I, this completely remove the effect of clonidine on CA I.
4. Prazosin antagonizes the effect of norepinephrine on CA I.
5. Prazosin and clonidine are direct inhibitors of CA I.
6. Reserpine, according to our in vitro studies, decrease the CA I activity, while it is a strong activator of CA II.

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Клинико-функциональные изменения сердечно-сосудистой системы у больных хронической обструктивной болезнью легких

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Clinico-Functional Changes in the Cardio-Vascular System in Patients with Chronic Obstructive Pulmonary Disease

The study included 55 patients, 25 (mean age 54,2±1,5 years) of whom were diagnosed with exertional angina pectoris of II-III degree and COPD of medium severity, 15 (mean age 57,5±1,3 years) with exertional angina pectoris of II-III degree, and 15 (mean age 56,0±1,5 years) with COPD of medium severity. The survey included Doppler-echocardiography, daily ECG monitoring, assessment of respiratory function, the study of lipid metabolism and blood gases. The study found that 76% of the patients with exertional angina II-III in combination with COPD complained of dyspnoea and palpitations. Daily ECG monitoring showed they more often recorded various rhythmic irregularities and painless ischemic heart disease, as well as more pronounced systole-diastolic left ventricular dysfunction.

Key words: chronic obstructive pulmonary disease, stable angina, painless ischemia.

Dereglări clinico-funcţionale ale sistemului cardiovascular la pacienţii cu bronhopneumopatie obstructivă cronică

Au fost studiaţi 55 de bolnavi, dintre care 25 (cu vârsta medie 54,2±1,5 ani) au fost diagnosticaţi cu angină pectorală de efort CF II-III şi BPOC de gravitate medie; 15 (cu vârsta medie 57,5±1,3 ani) – cu angină pectorală de efort CF II-III şi 15 bolnavi (cu vârsta medie 57,5±1,3 ani) cu BPOC de gravitate medie. Examinarea bolnavilor a inclus doppler-ecocardiografia, monitorizarea nictemerală a ECG (metoda Holter), evaluarea respiraţiei externe, studierea metabolismului lipidic şi a gazelor sangvine. În rezultatul cercetărilor a fost stabilit că pacienţii cu angină pectorală de efort CF II-III şi BPOC prezentau dispnee şi palpitaţii. În timpul monitorizării nictemerale a ECG, la aceşti pacienţi se înregistrau mai des diverse dereglări de ritm şi forme asimptomatice ale BIM, cât şi dereglarea mai manifestă a funcţiei sistolo-diastolice a ventriculului stâng.

Cuvinte-cheie: bronhopneumopatie obstructivă cronică, angină pectorală stabilă, ischemie asimptomatică.

Актуальность проблемы

В современном обществе хроническая обструктивная болезнь легких (ХОБЛ), наряду с артериальной гипертонией, ишемической болезнью сердца (ИБС) и сахарным диабетом, входит в группу ведущих хронических заболеваний; на их долю приходится более 30% среди всех других форм патологии человека. Всемирная организация здравоохранения относит ХОБЛ к заболеваниям с высоким уровнем социального бремени, она широко распространена как в развитых, так и в развивающихся странах. По прогнозу на период до 2020 г., составленному экспертами ВОЗ, ХОБЛ станет не только одной из самых распространенных болезней человека, но войдет в число лидирующих причин смертельных исходов [13, 14]. Необходимо подчеркнуть, что лечебные и профилактические программы при ХОБЛ в значительной степени зависят от сопутствующих заболеваний, на фоне которых она протекает. Наиболее частыми сопутствующими заболеваниями ХОБЛ являются: кахексия, гипотрофия и атрофия скелетных мышц, артериальная гипертония, ИБС, сердечная недостаточность, васкулопатии малого круга кровообращения. Прогноз наиболее неблагоприятен при сочетании ХОБЛ с группой сердечно-сосудистых заболеваний. Больные, страдающие тяжелыми формами ХОБЛ, относятся к группе высокого риска внезапной смерти. Одной из причин, ведущей к развитию внезапной смерти, являются различные нарушения ритма сердца. Данная клиническая проблема нуждается в более глубоком