EFFECT OF A COMBINATION OF Ca^{2+} ANTAGONISTS ON INOTROPIC AND CHRONOTROPIC FUNCTION OF THE HEART AND CORONARY VASCULAR RESISTANCE

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Combined administration of antianginal agents belonging to different groups can yield a therapeutic effect in patients with ischemic heart disease (IHD) if its course is severe or refractory to monotherapy [5, 6]. In these cases a combination of several Ca^{2+} antagonists also may be used [4-6, 14]. Meanwhile the character and magnitude of the changes in resistance of the coronary vessels and the inotropic and chronotropic function of the heart during the separate and combined action of several Ca^{2+} antagonists remains completely unstudied. The investigation described below was carried out to shed light on this problem.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated hearts of 15 cats whose coronary vessels were perfused with blood from a donor cat by means of a constant delivery pump [8]. Changes in resistance to the blood flow in the coronary arteries were judged by changes in the perfusion pressure (PP) in them. The systolic isometric tension of the myocardium (SITM) and the heart rate (HR) were recorded with an isometric strain-gauge transducer, sutured to the anterior wall of the left ventricle. The arteriovenous difference in O_2 concentration in the coronary blood (A/V O_2) was determined and calculated by means of two oxyhemograph transducers (036M) and an analog computer (MN-7), and changes in the O_2 consumption of the myocardium were judged from its value. The parameters for study were recorded on an N-327/5 inkwriting instrument. Initially each Ca^{2+} antagonist was injected in one stage in increasing doses (corinfar 0.1 — 1 — 10 μg; verapamil 1—10—100 μg; senzit 10—100—1000 μg) into the afferent tube of the perfusion pump in 0.1 ml of physiological saline. In the case of combined administration the Ca^{2+} antagonists were used in doses giving a moderate but distinct effect on the parameters studied in a particular experiment. The preparations were injected simultaneously from two syringes in a volume of 0.2 ml. The sources of the solutions used in the experiments were: verapamil from "Lek," Yugoslavia; corinfar* from "Germed," Fast Germany, and senzit,** from "Chinoin," Hungary. The last two solutions were prepared from the dry substance, dissolved in 15% ethyl alcohol. The corinfar solution was made up in a dark room immediately before use. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

The Ca^{2+} antagonists studied caused a significant decrease in PP in the coronary arteries in all doses used, the magnitude of which depended on dose of the drug (Table 1). Meanwhile corinfar in a dose of 0.1 μg ($0.35 \cdot 10^{-9}$ mole)

Alternative names: *nifedipine; **fendiline.

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TABLE 1. Changes in Coronary Vascular Resistance, Cardiac Activity, and Myocardial Oxygen Consumption Following Intracoronary Injection of Corinfar, Verapamil, and Senzit (M ± m)

Parameter	0,1	Corinfar, μg		Verapamil, μg			Senzit, μg		
		1	10	1	10	100	10	100	1000
Initial PP, mm Hg	99,0±6,1	97,6±5,6	98,0±5,6	109,1±9,47	108,8±7,5	115,8±8,4	105,8±8,2	108,6±7,8	110,3±8,5
Δ%	−15,2±2,65*	−26,0±4,0*	−35,8±4,6*	−5,5±1,4*	−13,3±1,8*	−39,6±5,3*	−10,0±1,9*	−19,2±2,8*	−33,4±4,2*
	15(13)	15(15)	15(15)	13(9)	13(13)	13(13)	17(16)	18(17)	17(17)
		<0,05	>0,1		<0,001	<0,001		<0,001	<0,001
SITM, Δ%	−17,7±2,4*	−33,5±1,6*	−59,81±2,7*	−7,9±1,6**	15,0±2,2	−55,8±4,0*	−11,7±1,6*	−20,0±2,9	−40,7±4,3*
	8(8)	8(8)	8(8)	12(6)	12(12)	12(12)	12(12)	13(13)	12(12)
Initial HR,	127,6±3,1	126,5±3,6	131,7±3,9	138,5±3,0	134,9±3,2	133,5±3,0	131,5±4,5	128,7±4,2	127,0±4,9
beats/min, Δ %		−4,8±0,2*	−8,8±2,0**		−5,7±1,3**	−18,7±2,9*	−4,0±0,3**	−5,1±0,5**	−11,5±1,3*
	11(0)	11(5)	11(11)	11(0)	11(5)	11(11)	12(3)	13(6)	12(12)
A/V O_2, Δ %	−7,9±1,6*	−16,9±2,4*	−34,9±3,7*	−4,4±1,3*	−8,2±1,3*	−30,6±2,9*	−4,5±0,8*	−9,5±1,06*	−20,3±3,2*
	11(10)	11(10)	11(11)	10(5)	11(11)	11(11)	10(10)	17(13)	17(17)

Legend Δ%) Difference between maximal changes in parameter in per cent of initial; —) decrease in value of parameter. Numbers in front of parentheses indicate number of experiments carried out, numbers between parentheses indicate number of experiments in which a change in the parameter was noted. *$p < 0.001$, **$p < 0.01$ — significance of differences between changes in parameter compared with those in response to previous dose.

gave a stronger coronary dilator effect than verapamil in a dose of 1 μg ($2 \cdot 10^{-9}$ mole) or senzit in a dose of 10 μg ($28 \cdot 10^{-9}$ mole). This general rule also was maintained when the drugs were given in a larger dose (Table 1). Consequently, it can be concluded from the ratio of the molar concentrations and the intensity of the vasodilator effect that corinfar is about 6 times more active than verapamil and 80 times more active than senzit.

The data in Table 1 indicate that definite differences exist in the action of corinfar, verapamil, and senzit on the inotropic and chronotropic function of the heart. For instance, corinfar in the first two doses (0.1 and 1 μg) depressed SITM more often and by a greater degree than verapamil (1 and 10 μg) or senzit (10 and 100 μg). On the other hand, after injection of 0.1 μg corinfar and 10 μg verapamil, an almost equal decrease was observed in SITM and PP, but under these circumstances verapamil gave a negative chronotropic effect. When corinfar and verapamil were used in the largest dose, changes observed in SITM, PP, and the myocardial oxygen consumption were about equal, whereas verapamil led to more marked slowing of HR than corinfar (Table 1).

Consequently, though they belong to the same group, the Ca^{2+} antagonists studied possess heterogeneous properties, making their combined administration justified.

In the case of the combined use of corinfar (in two experiments 1 μg, in five experiments 10 μg) and verapamil (in three experiments 10 μg, in four — 100 μg) the reduction of PP was 32.5 ± 4.7% whereas if they were used separately in the same doses, the corresponding reduction was 26.8 ± 3.3 and 31.8 ± 7.5%.

Thus in all cases the chnanges in PP were virtually equal in value. The duration of coronary vasodilatation following the combined use of the drugs (279.4 ± 36.2 sec) likewise was virtually indistinguishable from that observed after the use of corinfar (267.0 ± 45.2 sec) and verapamil (202.0 ± 61.8 sec) separately. Meanwhile, after combined administration of these drugs, there was a greater decrease in strength of the cardiac contractions and in HR than when they were used separately in the same doses. Thus the decrease in SITM in response to intracoronary injection of corinfar was 17.5 ± 2.2% of verapamil 33.2 ± 7.8% but of a combination of both 56.0 ± 9.7%. The corresponding reduction of HR was 3.2 ± 1.2, 10.1 ± 3.7, and 26.3 ± 5.5%. According to these results, during combined administration of corinfar and verapamil, compared with their use separately, the intensity of the negative chronotropic effect of the drugs is increased by many times, whereas the negative inotropic action undergoes virtual summation. Whereas with the combined use of corinfar and verapamil the reduction of the myocardial oxygen consumption (—26.8 ± 3.2%) was significantly greater than when corinfar was used (—13.5 ± 3.4% $p < 0.05$), and rather greater than when verapamil was given (—16.8 ± 2.7%), the duration of this reaction with a combination of the drugs (501.1 ± 78.2 sec) was significantly greater than with the separate use both of corinfar (256.2 ± 27 sec, $p < 0.02$) and of verapamil (292.0 ± 48.6 sec, $p < 0.05$).

In response to combined administration of corinfar (in three experiments 1 μg, in four experiments 10 μg) and senzit (100 μg in all experiments) the reduction of PP in the vessels of the heart was more marked (—38.2 ± 3.8%) than when they were used separately (corinfar —28.1 ± 5.2, senzit —21.9 ± 3.2%, $p < 0.02$). The duration of the coronary dilator action of corinfar (385.3 ± 96.6 sec) was greater than that of senzit (158.0 ± 25.9 sec, $p < 0.05$), but the combined use of

the drugs gave the longest coronary dilator effect (474.7 ± 97.0 sec). Compared with the separate effects of corinfar and senzit on cardiac activity, their combination led to a many times greater reduction of HR (by —4.7 ± 1.6%, —2.0 ± 0.9%, and —25.4 ± 7.1%, respectively), a more marked reduction of SITM (by —18.6 ± 5.2, —16.6 ± 5.0, and —32.8 ± 4.3% respectively), and a much greater decrease in the oxygen consumption of the myocardium (by —14.3 ± 3.8, —11.6 ± 4.3, and —33.8 ± 7.7% respectively). The period of reduced myocardial oxygen consumption following a combination of corinfar and senzit lasted 435.7 ± 58.8 sec, significantly longer than that which followed the use of corinfar alone (252.6 ± 25.0 sec, $p < 0.05$) or senzit alone (185.0 ± 52.0 sec, $p < 0.02$).

The combined administration of verapamil (10 μg in three experiments, 100 μg in four) and senzit (100 μg in all experiments) did not lead to any significant strengthening of the coronary dilator effect compared with its value when the two drugs were used separately, although there was a tendency in this direction. For instance, PP decreased under the influence of verapamil by 25.2 ± 5.0%, under the influence of senzit by 22.5 ± 3.5%, but under the influence of both preparations by 34.4 ± 5.2%. The decrease in PP produced by a combination of these Ca^{2+} antagonists (289.5 ± 41.4 sec) lasted longer than after intracoronary injection of senzit (158.6 ± 21.7 sec, $p < 0.05$) but did not differ from the effect of verapamil (271.0 ± 28.0 sec).

The combined use of senzit and verapamil, like a combination of corinfar and verapamil, or corinfar and senzit, led to summation of the negative inotropic effect and to potentiation of the negative chronotropic effect by several times. For instance, the reduction of SITM and HR after verapamil was 30.1 ± 9.3 and 14.0 ± 4.5 respectively, after senzit 15.0 ± 6.6 and 3.6 ± 1.6% respectively, and after a combination of both it was 52.4 ± 9.7% and 30.3 ± 9.1% respectively. With a combination of verapamil and senzit, moreover, the myocardial oxygen consumption was reduced significantly more (—24.8 ± 4.4%) than with verapamil alone (—12.1 ± 2.8%, $p < 0.05$) or senzit alone (—5.5 ± 1.3%, $p < 0.01$). The duration of the period of reduction of the myocardial oxygen consumption after verapamil was 250.0 ± 60.0 sec, after senzit 139.6 ± 35.0 sec, and after a combination of both 275.8 ± 12.4 sec.

Thus the investigation revealed a hitherto unknown feature of the action of Ca^{2+} antagonists, namely strengthening by several times of the negative chronotropic effect of corinfar, verapamil, and senzit when combination of two of them is used, whereas the negative inotropic effect of the drugs only undergoes summation. During the combined use of senzit with corinfar or verapamil the duration of the coronary dilator action is longer than when these drugs are given separately.

The Ca^{2+} antagonists studied are different derivatives: corinfar is a dihydropyridine, verapamil a phenylalkylamine, and senzit a diphenylalkylamine [9, 12], and they differ from one another in the mechanism of their inhibitory action on transmembrane and intracellular Ca^{2+} ion currents [2-4, 11, 13]. Some of them prevent the entry of Ca^{2+} into the cell, others block Ca^{2+} release from the intracellular reserves, while a 3rd group, besides the mechanisms indicated above, promote Ca^{2+} outflow from the cell or its transport into intracellular depots.

During the combined administration of two Ca^{2+} antagonists interaction may perhaps take place between the mechanisms of their blocking effect on the intracellular Ca^{2+} flow. Moreover, since cells of the sinus node are more sensitive than myofibrils to changes in the transmembrane Ca^{2+} flow [12], the effect of a combination of mechanisms inhibiting the Ca^{2+} flow is to make the negative chronotropic action many times stronger.

It can thus be concluded from these results that the basic mechanism of potentiation of the antianginal effect of combined administration of several Ca^{2+} antagonists is a significantly greater reduction of the myocardial oxygen consumption combined with a longer period of dilatation of the coronary arteries than when the drugs are used separately.

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HYDRA PEPTIDE MORPHOGEN ACTIVATES Na/H EXCHANGE IN HUMAN ERYTHROCYTES

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Hydra peptide morphogen (HPM), which consists of 11 amino-acid residues, was first; isolated from Hydra and sea anemones (Coelenterata) [7]. It was later found in the plasma, intestine, and brain (hypothalamus and pons) of mammals and man [1, 6, 12]. HPM has now been placed in the neuropeptide class. Its functions are being studied from all aspects.

HPM in a dose of 20 μg/kg has been shown to activate ornithine decarboxylase in the liver of intact and partially hepatectomized rats [5], evidence of its involvement in the regulation of growth and regeneration. It has been suggested that the peptide is produced and utilized by tumor cells of nervous and endocrine tissue as a growth factor [13]. In previous investigations we found that HPM activates cell division of different kinds of albino rat epithelium over a wide range of doses. The modulating action of HPM on concentrations of cyclic nucleotides in regenerating liver and muscle has been noted [4]. However, the pattern of intracellular transmission as a whole under the influence of HPM has not been adequately studied.

Much information indicating involvement of the Na/H exchange system in the regulation of proliferative activity of many tissues had been obtained [2]. Accordingly, it was decided to study the effect of HPM on the velocity of Na/H antitransport.

EXPERIMENTAL METHOD

Fresh donor's blood containing heparin (50 U/ml) was used. Erythrocytes were sedimented (1000 g, 10 min, 2-4°C), the plasma and white blood cells were removed, and the erythrocyte suspension was washed with physiological saline and kept on ice. The velocity of Na/H exchange was determined as the amiloride-inhibited component of the rate of proton efflux under conditions of creation of an electrochemical proton gradient, with values of the intra- and extracellular pH of 6.45 and 8.00 respectively (H-induced Na/H exchange), and estimated in microequivalents H/min. The techniques used and the order of the calculation are given elsewhere [2, 9]. The amiloride was obtained from "Sigma" (USA) and the anion transport inhibitor (SITS) from "Serva" (West Germany). Heparin was obtained from "Gedeon Richer" (Hungary). HPM

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