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The influence of intracerebroventricular administration of (±) propranolol and (±) verapamil on experimental myocardial ischemia and necrosis in rats

M. **Blidaru***, B. **Cuparencu****, L. **Pleşca-Manea***

* Department of Pathophysiology, University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj-Napoca, Romania,
** Department of Pharmacology, Faculty of Medicine and Pharmacy, University Oradea, Romania

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In albino rats, infarctoid myocardial lesions were produced by intraperitoneal (i.p.) administration of isoproterenol (75 mg/kg, during 3 days). In other groups, the descending anterior left coronary artery was ligated. In both experimental settings, the intracerebroventricular (i.c.v.) administration of (±) propranolol (100–200–300 µg/animal/day, during 7 days) or (±) verapamil (40–80–160 µg/animal/day, during 7 days) afforded a significant protection (with the exception of the lowest dose) on the investigated parameters: arrhythmias, ischemic zone (in coronary ligated rats), lactate dehydrogenase and aspartate aminotransferase activity of the serum, focal necrosis (in isoproterenol treated rats). This protective activity is lower than that afforded by i.p. administered (±) propranolol (5 mg/kg, during seven days) or (±) verapamil (5 mg/kg, during seven days).

From these data it may be concluded that (±) propranolol and (±) verapamil have a protective action on the experimental myocardial ischemia and necrosis in rats, not only when the drugs come in direct contact with the heart, but also acting upon the central nervous system.

Keywords: rats, myocardial ischemia, intracerebroventricular, (±) propranolol, (±) verapamil, protection

Correspondence should be addressed to
Dr. Mihai Blidaru
Str. Bizusa nr. 4, ap. 1.
3400 Cluj-Napoca, Romania
E-mail: mblidaru@mailcity.com

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In the clinical literature there are numerous data that suggest the participation of central nervous system (CNS) in the pathogenesis of myocardial ischemia and myocardial infarction (for a review see [1]).

In the experimental medicine, the available data are rather contradictory. The ambiguity of results can be explained by the diversity of experimental methods used, by the various aspects investigated and mainly by state of being under general anesthesia or state of consciousness of the animals [2].

In the experiments here presented, in order to investigate the participation of CNS in the pathogenesis of myocardial ischemia and myocardial infarction, we used the intracerebroventricular administration of two well-known protective agents (±) propranolol and (±) verapamil in two experimental models: a) the infarctoid damage of the heart induced by high doses of isoproterenol (ISO), b) the ligation of the descending anterior coronary artery. We hypothesized that if the administration of low doses of these compounds affords a significant protection, it is likely that the neural actions of these drugs would play a certain role in their protective activity. Implicitly, the participation of CNS in the pathogenesis of the above-mentioned disorders is more than a simple speculation.

Materials and methods

The experiments were carried out in albino rats (Wistar strain) of either sex, weighing 180 to 250 g. They were fed a standard equilibrated diet and received water *ad libitum*.

I.a. The experimental group, in which ISO was used, was processed as follows: during 7 days the animals were injected intraperitoneally (i.p.) with either saline (control group), or (±) propranolol (5 mg/kg) or (±) verapamil (5 mg/kg) (experimental groups). Beginning with the 5th day, an i.p. administration of ISO (75 mg/kg/day) was carried in the 5th, 6th, and 7th days. Prior to ISO administration and at the end of experiments, the electrocardiogram (ECG) was recorded by means of CARDIOR device in D_1 lead with a speed of 25 mm/second. One day after the last ISO administration, the blood was sampled by punction of the retro-orbital sinus. The lactate dehydrogenase (LDH) and aspartate aminotransferase (ASAT) activities were determined with an automatic Beckman analyzer, using enzymatic kits. The results were expressed as international activity units (I.U.). Then, the animals were decapitated, the heart was removed and processed for histological examinations. The thin sections were stained with hematoxylin-eosin. They were examined with a Zeiss microscope at various magnifications.

I.b. In another variant, (±) propranolol (100 μg–200 μg–300 μg/day) or (±) verapamil (40 μg–80 μg–160 μg/day) were administered in a lateral cerebral ventricle during 7 days. The microinjections were performed in volumes of 10 μl. The lateral cerebral ventricles were located according to the stereotaxic atlas of de Groot [3]. The trephination of the skull and the implantation of permanent metallic cannulas were performed two days prior to the start of the experiment in ether anesthesia.

II.a. The experiments in which the descending anterior left coronary artery was ligated were carried out in the following way: during 7 days, the rats received an i.p. injection of saline (1 ml/animal). The ECG was recorded. Next day, in ether anesthesia the chest was opened. The ligature of the artery was performed according to the technique of Leprán et al. [4], slightly modified by us. After 10 minutes, the ECG recordings were repeated. Then, the animals were decapitated, and the heart was removed. The size of ischemia was visually delimited by using an "Edding 3000 permanent marker". The ischemic area was then cut off and weighed on an analytical balance.

II.b. This experimental model was used in two circumstances: 1. For the i.p. administration of (±) propranolol and (±) verapamil which were performed in the same experimental conditions as already mentioned; 2. For the i.c.v. microinjection of the drugs (see I.b.)

Criteria for the evaluation of antiarrhythmogenic activity

Two criteria were taken into account:

1. The arrhythmogenic index (AI) which was expressed as percent of the ratio: number of ectopic beats/total number of heart beats recorded within 10 minutes.
2. The arrhythmogenic score (AS) initially used by Lown and Wolf [5], then adapted for animals by Johnston et al. [2]. It was used also by Pleşca et al. [6]. In the present paper, we used the score of Pleşca et al. [6], in a slight modification. The arrhythmogenic score has the following aspect:

ARRHYTHMOGENIC SCORE		
Degrees of severity /10 minutes	Ventricular changes	Atrial changes
0	Without any change	Without any change
1	Up to 5 ventricular extrasystoles (VE)	Up to 5 atrial arrhythmic episodes
2	Up to 10 VE	Up to 10 atrial arrhythmic episodes
3	Up to 15 VE	Up to 15 atrial arrhythmic episodes
4	Up to 20 VE	Up to 20 atrial arrhythmic episodes
5	More than 20 VE	More than 20 atrial arrhythmic episodes
6	Ventricular fibrillation	–

The lethality, expressed as percent was recorded in all groups.

Statistical evaluation. The significance of results was appreciated by the non-paired "**t**" test [7]. The comparison of results was made between the control groups and the experimental ones. For the arrhythmogenic score, the significance was calculated taking into account the initial number of animals (first day), and separately the number of survivors (10 minutes after ISO administration or after coronary ligation).

Drugs

1. Isoproterenol sulfate. The administered solution was prepared (for stabilization) by dissolving the drug in a 1‰ ascorbic acid solution.
2. (±) Propranolol hydrochloride
3. (±) Verapamil hydrochloride

(±) Propranolol and (±) verapamil were dissolved in saline.
The doses are expressed as salts.

Results

1. The i.p. administration of high doses of ISO induced the well-known diffuse necrosis of the myocardium [8]. The measurements of serum LDH and ASAT activity have revealed very significant increases. These elevations were interpreted by the authors who dealed with the cardiac effects of high doses of ISO as a consequence of cytolysis of cardiomyocites [8]. ISO administration induced severe cardiac arrhythmias as it was demonstrated by the increase of arrhythmogenic index (AI) and of the arrhythmogenic score (AS) (Table I, Fig. 1). The lethality reached 40% in an interval of 7 days. The i.p. injection of (±) propranolol (5 mg/kg) during the last three days of ISO administration induced the following changes, when the results were compared to those obtained in control ISO-treated rats: serum LDH and ASAT activities decreased very significantly (Table I); AI was drastically diminished as well as the AS (Table I, Fig. 2); the lethality was reduced to half (20%) (Table I). (±) Verapamil administration induced approximately the same changes as (±) propranolol. The lethality was decreased to 10% (Table I). The morphopathological picture was greatly improved.

2. When (±) propranolol and (±) verapamil were microinjected i.c.v. in the experimental model of myocardial diffuse necrosis induced by i.p. ISO injection the following results were obtained:

(±) Propranolol in a dose of 100 µg induced a significant decrease of LDH activity, but not that of ASAT. The AI and AS were very significantly reduced.

The lethality was practically not influenced (Table II). The double of this dose (200 µg) induced very significant diminutions of all parameters (Table II, Fig. 3). The same was valid for (±) propranolol given in a dose of 300 µg (Table II). Between the results obtained with 200 µg and those given with300 µg, there were no significant differences.

Table I

Effects of i.p. administration of (±) *propranolol and* (±) *verapamil on the infarctoid lesions induced by i.p. injection of isoproterenol (ISO) (means ± standard error)*

Group	Number of rats/group	LDH I.U./L	ASAT I.U./L	Arrhythmogenic index (AI)	Arrhythmogenic score (AS)	Lethality %
1. Control - saline -	10	496.2 ± 36.57	17 ± 0.93	0	0	0
2. Control - ISO - p (versus 1)	10* 6**	2377.5 ± 96.92 <0.001	143.16 ± 5.91 <0.001	0.46 ± 0.04 <0.001	4.8 ± 0.38*** 4 ± 0.36**** <0.001*** <0.001****	40
3. ISO + (±) propranolol (5 mg/kg) p (versus 2)	10* 8**	1491.62 ± 32.02 <0.001	86.87 ± 2.97 <0.001	0.09 ± 0.009 <0.001	2.1 ± 0.65*** 1.12 ± 0.12**** <0.001*** <0.001****	20
4. ISO + (±) verapamil (5 mg/kg) p (versus 2)	10* 9**	1998.2 ± 38.67 =0.001	115.33 ± 2.80 <0.001	0.17 ± 0.03 <0.001 <0.001****	2.2± 0.48*** 1.77 ± 0.27**** = 0.001***	10

* = initial numbers of rats/group; ** = number of animals remained alive during the experiment

*** = calculated taking into account the initial number of animal

**** = calculated taking into account the number of survivors

(±) Verapamil microinjected in a dose of 40 µg failed affect any of the recorded parameters. When the dose was elevated up to 80 µg, all the values were very significantly diminished. The same was valid for the highest dose tested (Table II). Between the last two doses, no significant differences were found. The morphopathological aspects of the myocardium were improved. However, this improvement was less expressed than in rats administered i.p. the same agents.

3. The left coronary artery ligation induced a marked ischemia of the myocardium. The ischemic area weight was 31.96±1.20 percent of the heart weight (Table III). At the same time, AI and AS were very significantly increased, when compared to saline treated rats (Table III, Fig. 4). It is noteworthy that in this

experimental model, AI, AS and lethality were significantly higher than those seen in myocardial diffuse necrosis induced by ISO.

The i.p. administration of both (±) propranolol and (±) verapamil elicited very significant reduction of all the recorded parameters (Table III, Fig. 5). No significant differences between the effects of these drugs were seen.

Table II

Effects of i.c.v. administration of (±) propranolol and (±) verapamil on the infarctoid lesions induced by i.p. administration of ISO (means ± standard error)

Group	Number of rats /group	LDH I.U./L	ASAT I.U./L	- AI -	- AS -	Lethality %
1. Control - ISO -	15* 10**	1375.50 ± 17.50	98 ± 2.88	0.39 ± 0.03	4.26 ± 0.39*** 3.4 ± 0.33****	33.3
2. ISO + (±) propranolol (100 µg) p (versus 1)	10* 7**	1326.28 ± 13.80 <0.05	94.28 ± 3.30 >0.1	0.24 ± 0.03 <0.01	3.4 ± 0.58*** 2.28 ± 0.18**** = 0.1*** = 0.01****	30
3. ISO + (±) propranolol (200 µg) p (versus 1)	15* 12**	1290 ± 27.12 <0.01	85 ± 2.83 <0.01	0.15 ± 0.02 <0.001	2.20 ± 0.51*** 1.25 ± 0.13**** <0.01*** <0.001****	20
4. ISO + (±) propranolol (300 µg) p (versus 1)	10* 8**	1297.5 ± 26.27 = 0.01	82.37 ± 4.43 <0.01	0.11 ± 0.02 <0.001	2.30 ± 0.63*** 1.37 ± 0.18**** <0.01*** <0.001****	20
5. ISO + (±) verapamil (40 µg) p (versus 1)	10* 7**	1380 ± 36.12 >0.1	96.42 ± 8.28 >0.1	0.42 ± 0.05 >0.1	4.30 ± 0.44*** 3.57 ± 0.36**** >0.1*** >0.1****	30
6. ISO + (±) verapamil (80 µg) p (versus 1)	15* 12**	1229.16 ± 14.39 <0.001	81.66 ± 1.60 <0.001	0.19 ± 0.02 <0.001	2.46 ± 0.48*** 1.58 ± 0.14**** <0.01*** <0.001****	20
7. ISO + (±) verapamil (160 µg) p (versus 1)	10* 8**	1233.75 ± 15.91 <0.001	79.37 ± 6.43 <0.01	0.17 ± 0.03 <0.001	2.6 ± 0.60*** 1.75 ± 0.25**** = 0.01*** <0.001****	20

* = initial number of rats/group
** = number of animals which did not die within the experiment
*** = calculated taking into account the initial number of animals
**** = calculated taking into account the number of survivors

Table III

Effects of i.p. administration of (±) propranolol and (±) verapamil on descending anterior left coronary artery ligation – induced ischemia (means ± standard error)

Group	Number of rats /group	Ischemic area		- AI -	- AS -	Lethality %
		Weight (mg)	% of the heart weight			
1. Control	10*	285	31.96	0.68	5.3 ± 0.26***	50
- left coronary artery ligation -	5**	± 6.25	± 1.20	± 0.07	4.6 ± 0.24****	
2. Left coronary artery lig.	10*	214	24.31	0.20	2.7 ± 0.57***	20
+ (±) propranolol (5 mg/kg)	8**	± 8.80	± 0.38	± 0.02	1.87 ± 0.22****	
p (versus 1)			<0.001	<0.001	= 0.001***	
					<0.001****	
3. Left coronary artery lig.	10*	192.12	22.11	0.26	2.9 ± 0.54***	20
+ (±) verapamil (5 mg/kg)	8**	± 12.22	± 0.97	± 0.02	2.12 ± 0.22****	
p (versus 1)			<0.001	<0.001	<0.01***	
					<0.001****	

* = initial number of rats/group
** = number of animals which did not die within the experiment
*** = calculated taking into account the initial number of animals
**** = calculated taking into account the number of survivors

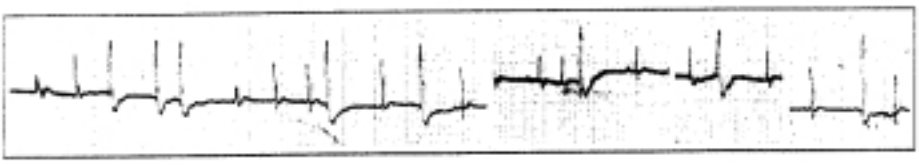

Fig. 1. Recording of an ECG in an ISO administered rat

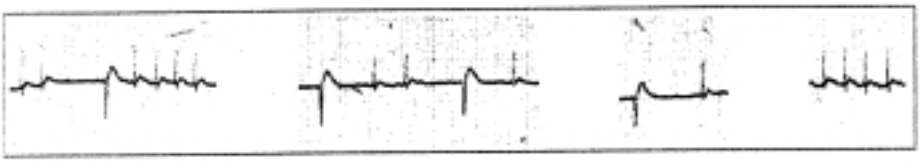

Fig. 2. ECG tracing recorded in rat which received ISO and (±) propranolol i.p.

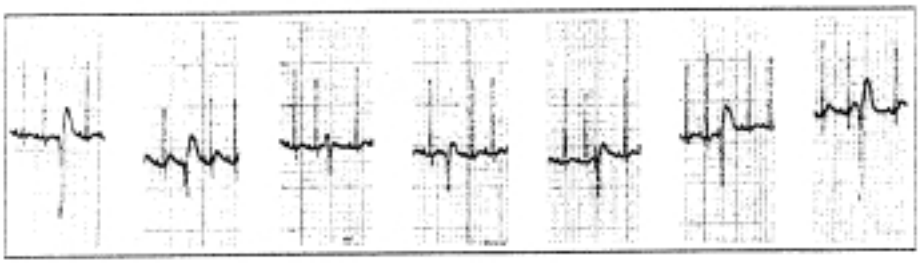

Fig. 3. ECG tracing recorded in a rat administered ISO and (±) propranolol i.c.v.

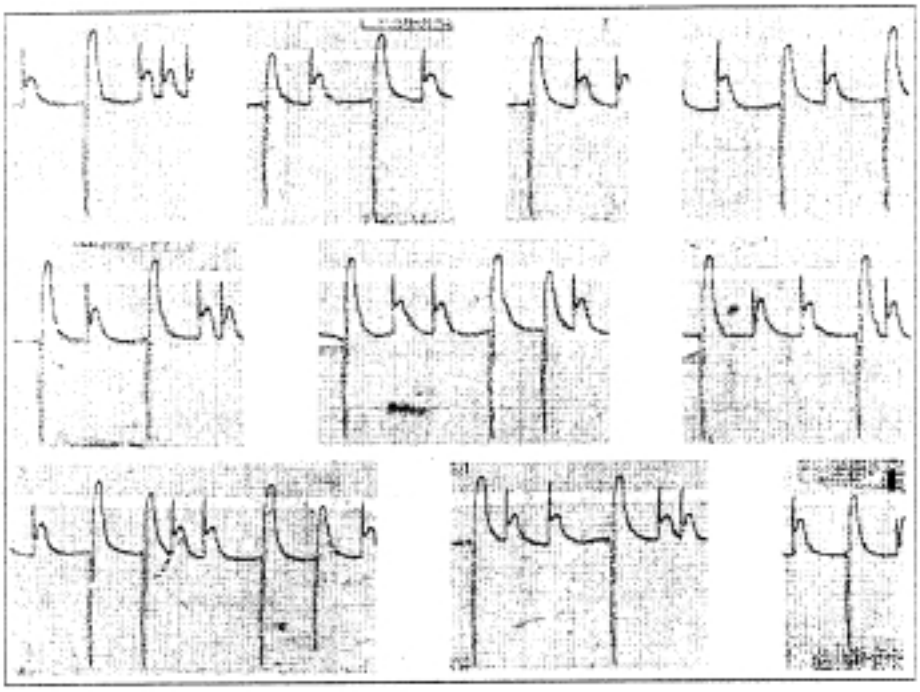

Fig. 4. Recording of an ECG in rat with descending anterior left coronary ligation

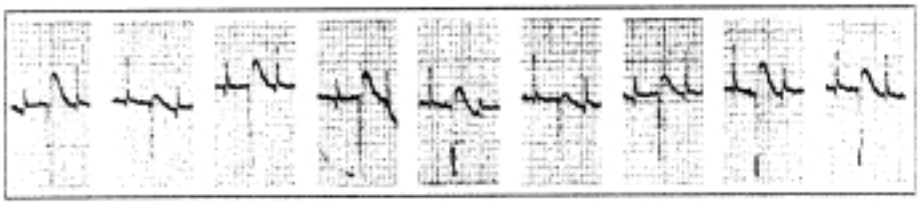

Fig. 5. As in Figure 4. The rat was received (±) verapamil i.p.

Table IV

Effects of i.c.v. administration of (±) propranolol and (±) verapamil on descending anterior left coronary artery ligation – induced ischemia (means ± standard error)

Group	Number of rats /group	Ischemic area		- AI -	- AS -	Lethality %
		Weight (mg)	% of the heart weight			
1. Control - left coronary artery lig. -	20* 7**	306.42 ± 8.94	32.88 ± 0.98	0.84 ± 0.11	5.55 ± 0.15*** 4.71 ± 0.18****	65
2. Left coronary artery lig. + (±) propranolol (100 µg) p (versus 1)	10* 5**	295.2 ± 25.40	33.29 ± 0.91 >0.1	0.69 ± 0.11 >0.1	5 ± 0.39*** 4 ± 0.44**** >0.05*** >0.05****	50
3. Left coronary artery lig. + (±) propranolol (200 µg) p (versus 1)	20* 11**	262.72 ± 6.71	28.90 ± 0.54 <0.001	0.43 ± 0.03 <0.001	4.55 ± 0.32*** 3.36 ± 0.24**** <0.01*** <0.001****	45
4. Left coronary artery lig. + (±)propranolol (300 µg) p (versus 1)	10* 7**	241.42 ± 12.95	28.06 ± 0.98 <0.01	0.38 ± 0.07 = 0.01	3.9 ± 0.54*** 3 ± 0.43**** <0.001*** = 0.001****	30
5. Left coronary artery lig. + (±) verapamil (40 µg) p (versus 1)	10* 4**	279 ± 19.89	31.42 ± 1.22 >0.1	0.73 ± 0.13 >0.1	5.4 ± 0.30*** 4.5 ± 0.5**** >0.1*** >0.1***	60
6. Left coronary artery lig. + (±) verapamil (80 µg) p (versus 1)	20* 12**	237.25 ± 3.72	27.55 ± 0.76 <0.001	0.51 ± 0.07 <0.01	4.65 ± 0.30*** 3.75 ± 0.27**** = 0.01*** = 0.01***	40
7. Left coronary artery lig. + (±) verapamil (160 µg) p (versus 1)	10* 6**	246 ± 18.06	27.85 ± 1.48 <0.01	0.46 ± 0.10 = 0.01	4.3 ± 0.57*** 3.16 ± 0.60**** <0.01*** = 0.01****	40

* = initial number of rats/group

** = number of animals which did not die within the experiment

*** = calculated taking into account the initial number of animals

**** = calculated taking into account the number of survivors

4. When (±) propranolol was microinjected i.c.v. in a dose of 100 μg, no significant effects were observed (Table IV). The dose of 200 μg, induced very significant diminutions of the ischemic area of AI, AS and of the lethality (Table IV). The dose of 300 μg, induced practically the same effects as did the previous dose, with the exception of AS which was more diminished (Table IV).

(±) Verapamil administered in a dose of 40 μg, failed to affect any of the phenomena observed (Table IV). The double of this dose (80 μg) induced very significant decreases of all investigated parameters (Table IV, Fig. 6). The highest tested dose (160 μg) induced practically the same changes, as did the dose of 80 μg (Table IV). The lethality was reduced by (±) propranolol at the dose of 200 μg and particularly by 300 μg. (±) Verapamil diminished lethality at the doses of 80 and 160 μg (Table IV).

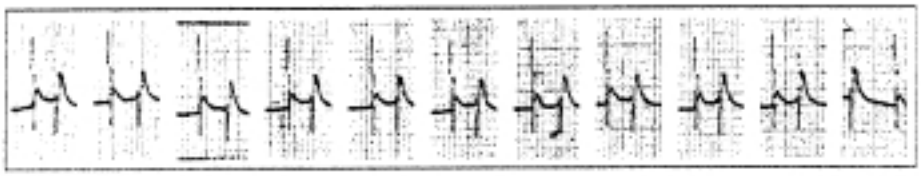

Fig. 6. As in Fig. 4. but the rat received (±) verapamil i.c. v.

Discussion

1. The way to induce diffuse myocardial necrosis induced by high doses of ISO is an easy method to elicit similar lesions to those seen in myocardial infarction. However, it has the drawback that the lesions are multiple and not limited as is seen in myocardial infarction.

The pathogenesis of the lesions produced by ISO is clarified in large extent. The excessive stimulation of cardiac beta-adrenoceptors, induce a massive influx of calcium through the calcium channels. Calcium evokes the cytolytic phenomena. Thus, the blockade of the beta-adrenoceptors of heart as well as the calcium channels closure would afford a notable protection [9]. This was achieved in our experiments by the use of (±) propranolol and (±) verapamil, respectively.

As a consequence of the myocardiocytolysis, the serum level of lactate dehydrogenase (LDH) and aspartate aminotransferase (ASAT) is very significantly increased. At the same time, severe cardiac arrhythmias were seen. Death occurred in 33–40% of the animal.

All these phenomena were dramatically reduced by i.p. administration of (±) propranolol and (±) verapamil. It noteworthy that despite the fact that the improvement of the investigated parameters were less expressed in the case of verapamil, the lethality was more reduced than that seen in (±) propranolol-treated rats.

In conclusion, our experiments have confirmed the well-known protective effects of i.p. administered (±) propranolol and (±) verapamil in the experimental model of diffuse myocardial necrosis induced by high doses of ISO.

2. When (±) propranolol and (±) verapamil were microinjected i.c.v. in increasing doses, in the ISO experimental model, the decrease of LDH and ASAT activities were less expressed than in the case of i.p. Exceptions are ASAT activities, which are not influenced by the lowest dose (100 μg) of (±) propranolol and both LDH and ASAT activities, which are not affected, by the lowest dose (40 μg) of (±) verapamil. With regard to AI and AS, although the prevention afforded by i.c.v. administration of (±) propranolol and (±) verapamil is very significant, the lowest doses of both agents afforded only a marginal or no protection. This is particularly valid for verapamil, which at a dose of 40 μg failed to afford a significant protection.

The histopathological picture was improved by i.c.v. administration of both (±) propranolol and (±) verapamil, although in a less extent than if these agents were given i.p.

Summarizing, the i.c.v. administration of (±) propranolol and (±) verapamil afforded a significant protection in ISO–induced myocardial damage. However, this protection is less expressed than that afforded by i.p. injection. The difference can be explained alternatively in two ways: 1.) The effects of these drugs are exclusively due to their action upon the CNS structures involved in cardiovascular control; 2.) The drugs leaked totally into the periphery. This leakage ensured the protection of the heart against the damage induced by ISO. This question is not well understand. However, we think that the effects of the agents when microinjected in the ventriculocerebral cavities are due mainly to a central nervous action. In addition, it is obvious that a part of the compounds would leak into the periphery. For this assumption, the lack of difference of lethality in i.p. and i.c.v. administered drugs in various doses seems to be probatory.

An important statement was the partial disagreement observed between LDH and ASAT modifications and the arrhythmogenic activity, which was apparent under the influence of the investigated drugs when they were given i.p. So, (±) verapamil (5 mg/kg, i.p.) induced a less reduction of LDH and ASAT activities when compared to (±) propranolol. Concomitantly, the arrhythmogenic activity was not different. The lethality was diminished to half in comparison with propranolol. Interestingly, this lack of correlation was not observed when the drugs were administered i.c.v. The lethality was influenced in the same degree as the arrhythmogenicity.

Based on the above data it can be concluded that between the enzymatic and ECG changes there is no obligatory relationships and there is a certain parallelism between the arrhythmogenicity and lethality.

3. The model of ligation of the descending anterior coronary artery was used in order to study the influence of drugs upon the earliest phase of myocardial infarction – the ischemia. Indeed, the interval of ten minutes, which elapsed between ligation and the sacrifice of the animal, was insufficient to produce necrotic damage. Therefore, the cytolytic enzymes activity was not determinate.

The i.p. administration of (±) propranolol and (±) verapamil afforded a very significant protection, diminishing the ischemic area and the AI and AS. The protection afforded by both agents was practically equal.

4. The i.c.v. microinjections of (±) propranolol and (±) verapamil also afforded a significant protection in coronary ligated rats, albeit less expressed than the i.p. injection. The protective activity was absent at the lowest doses. Between the medium and highest dose there were no significant differences. Among the weight of the ischemic area, the arrhythmogenicity and the lethality, there was a good correspondence.

The statement that both AI and AS are larger in ligatured rats than in ISO-administered ones, may be interpreted in the sense that ischemia is a more arrhythmogenic factor than necrosis, at least in these experimental conditions.

Thus, the i.c.v. administration of (±) propranolol and (±) verapamil afforded a significant protection in both ISO – induced myocardial necrosis and in ischemia produced by coronary artery ligation.

The mechanism(s) of action of i.c.v. administered (±) propranolol and (±) verapamil is(are) a matter of debate. In our opinion, these drugs acting upon the brain centers that control cardiovascular parameters (heart rate, rhythm, blood pressure, cardiac output) exert a positive influence on the disorders brought about by myocardial ischemia and necrosis. In our previous studies it was shown that verapamil and propranolol possess a central antiarrhythmogenic activity [10, 11]. Other investigations revealed that i.c.v. propranolol administration reduces the cardiac output and prevent the increase of the peripheral resistance during sleep apnea [12]. Moreover, it is not excluded that these drugs acting centrally, would have a “trophic” effect, at least partly preserving the function and morphological integrity of the heart. It seems reasonable to admit that the pathological changes of the heart are perceived by central nervous system. They modify the normal function of the cardiovascular centers. (±) Propranolol and (±) verapamil would have a “normalizing” activity. This is reflected by positive changes of heart functions and structure.

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