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RADOVI XCIV, knj. 34.

Konjhodžić, Faruk

2005

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AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE
АКАДЕМИЈА НАУКА И УМЈЕТНОСТИ БОСНЕ И ХЕРЦЕГОВИНЕ
ACADEMY OF SCIENCES AND ARTS OF BOSNIA AND HERZEGOVINA

WORKS

VOLUME XCIV

Department of Medical Sciences
Volume 34

Centre of Medical Research
Volume 4

Editorial Board
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SARAJEVO 2005

HEMODYNAMIC AND RESPIRATORY RESPONSES OF DOGS TO HEMORRHAGE AFTER TREATMENT WITH DIHYDROERGOTOXINE AND NALOXONE

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Abstract

The endogenous opiate receptor antagonist (naloxone, NAL) and alpha-adrenergic receptor antagonist (dihydroergotoxine, DHETX) were infused separately or simultaneously in dogs to determine their effects in hemorrhagic shock. Mean arterial pressure, heart rate, electrocardiogram, respiratory rate, hematocrit and plasma protein concentration were measured during sustained posthemorrhagic hypotension (180 min, 40 mmHg) in 22 dogs. Animals were divided into four groups: DHETX-treated (n=6), NAL-treated (n=5), DHETX+NAL-treated (n=5), and SAL (saline)-treated (control group, n=6). The treatment was performed before bleeding. After 3 hours of posthemorrhagic hypotension, all shed blood was returned to the dogs, and animals passed through a postretrasfusion period for 60 min. The animals which survived experimental procedure were observed in next 24 hours. Under the present experimental conditions, prophylactic administration of DHETX had better effects on preservation of parameters mesaured and survival of dogs than NAL, while DHETX+NAL treatment had the worst effects (no one dog survived).

Key words: hemorrhagic shock, dogs, opiate receptor blockade, alpha adrenergic receptor blockade

Introduction

Endogenous opiates might play a role in altering cardiovascular hemodynamics during hypovolemic shock (1), and modulate the hemodynamic instability, neuroendocrine, and cytokine responses to hemorrhagic shock (2). Furthermore, a role of the opioid peptides in cardiovascular responses to several shock and trauma paradigms has also been suggested based on the cardiotonic and improved hemodynamic responses to treatments with naloxone (1, 3, 4). Although several studies failed to demonstrate improved cardiovascular status in hemorrhaged animals treated with naloxone (5, 6), including failure to demonstrate improved survival, it is still commonly believed that activation of endogenous opioid system plays a depressor and hence a detrimental role in cardiorespiratory recovery after bleeding (7).

Previous experimental results show that naloxone improved the hemodynamic and biochemical state of cats in hemorrhagic shock (3). Vargish et al. (8) reported a significant positive inotropic effect in dogs after naloxone infusion during hemorrhagic shock. Gu et al. (9) showed that the opiate antagonist, naloxone, significantly potentiated the inotropic effect of infused epinephrine in the canine isolated heart/lung. We have previously reported that the alpha adrenergic antagonist phenoxybenzamine is benefitial in canine hemorrhagic shock (10, 11, 12). The present study was designed to investigate a possible link between effects of naloxone, an opiate receptor antagonist, and dihydroergotoxine, an alpha adrenergic receptor antagonist, given separately or simultaneously during posthemorrhagic hypotension in anesthetized dogs, more exactly, to investigate whether naloxone and dihydroergotoxine could improve cardiovascular and respiratry function in dogs exposed to prolonged poshemorrhagic hypotension.

Material and Methods

A total of 22 mongrel dogs (10-25 kg) of both sexes were anesthetized with chloralose (0.1 g/kg) intravenously and intubated with endotracheal tubes. Additional chloralose was given whenever necessary to maintain anesthesia. Femoral arteries and veins of either side were cannulated with polyethylene catheters filled with a 0.9% saline solution containing heparin. Right femoral vessels were used for bleeding (artery) and for a drugs and saline infusion (vein). Left blood vessels were used for mean arterial pressure (MAP) measurement (artery) with Statham P23 pressure transducer and for blood sampling (vein). The electrodes are attached to the legs for electrocardiogram (ECG) lead II registration. The pneumograph was placed around the chest and connected to a volumetric pressure transducer for respiratory rate (RR) measurement. All parameters (MAP, ECG, RR), including heart rate (HR) automaticaly obtained from ECG, were continuously recorded on a Grass 7D polygraph.

Following the surgical preparation, heparin (500 units/kg) was given, and animals were allowed to stabilize for approximately 30 min. Control values were recorded thereafter and their mean was taken as baseline value (100%); the blood samples were drawn for determination of hematocrit (microhematocrit technique) and plasma protein concentration (Biuret-method), too.

After procedure described above, experimental animals have been introduced in the state of hemorrhagic shock by the bleeding during 15 minutes until the fall of MAP to the level of 40 mm Hg. This level of posthemorrhagic hypotension was

maintained 180 minutes by giving back or removing blood as necessary (Fig 1., period from 0 to 180 min). At the end of hypotensive period all shed blood, which remained in reservoir, was retransfused. The animals were observed in the next 60 minutes of postretransfusion period, catheters were removed, the wounds were closed, and the animals were returned to their cages. Survival was determined at 24 hours.

Four groups of hemorrhaged dogs were studied as follows: DHETX (n=6) (dihydroergotoxine treated, bolus 0.1 mg/kg in 8 ml 0.9% saline + 0.1 mg/kg/h in 12 ml 0.9% saline during 30 min infusion); NAL (n=5) (naloxone treated, bolus 2 mg/kg in 8 ml saline + 2 mg/kg/h in 12 ml saline during 30 min infusion); DHETX+NAL (n=5) (DHETX and NAL treated, each in same dose and way as separately); and SAL (n=6) (control group, 0.9% NaCl treated, volume and way as in first 3 groups). DHETX, NAL (separately or simultaneously) and vehicle (0.9% NaCl) were given before hemorrhage.

Two-milliliter blood samples were collected at -15, 0, 30, 60, 120, 180, and 240 min for analysis of hematocrit and plasma protein concentration. Statistical analysis was performed by Student's t-test. The level of statistical significance was assessed at $P<0.05$.

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Results

Table 1. shows the values of bleedout volumes in the moment of decrease of MAP to 40 mm Hg in all experimental groups. Observing all volume of blood in ml or expressed in ml/kg of body weight, we can notice a significant differences between treated groups. The bleedout volume until hypotension of 40 mmHg is smallest in control group, but is the biggest in NAL-treated group.

Table 1.

BLEEDING VOLUME TO MAP=40mmHg				
Groups:	n	Average of body weight (kg)	Total blood volume (ml)	Blood loss per kg body weight (ml/kg)
SAL	6	17,0	174	10,27
DHETX	6	13,9	382	27,48
NALOXONE	5	12,5	536	42,88
DHETX+NAL	5	14,8	476	32,16

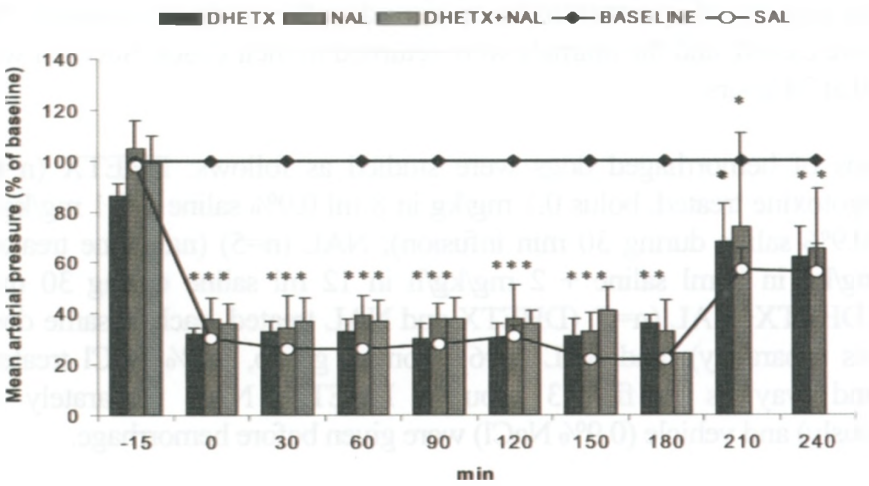


Fig. 1. Changes in mean arterial pressure (% of baseline) in hemorrhaged dogs treated with dihydroergotoxine (DHETX), naloxone (NAL), dihydroergotoxine+naloxone (DHETX+NAL) and saline (SAL). Values are mean ± SEM. Significant differences in each group: *P<0.05 compared with baseline value (100%). Significant differences between different groups at same time point (P<0.05): **a** = DHETX vs. SAL; **b** = NAL vs. SAL; **c** = DHETX+NAL vs. SAL; **d** = DHETX vs. NAL; **e** = DHETX vs. DHETX+NAL; **f** = NAL vs. DHETX+NAL.

Hemorrhage induced sharp decreases in MAP (Fig. 1.). Changes of MAP in terms of percentage regarding the values before bleeding (100%, baseline) in all groups are significant (P<0.001) during the hypotensive period. However, the differences between groups have not been significant during the hypotensive period, what is reasonable considering the used method. The rastauration of MAP after retransfusion is the best in NAL- treated group (74% of baseline), but in DHETX-treated group 68% and in control group 57% of baseline. In DHETX+NAL-treated group none animals survived the complete experimental procedure.

This improved blood pressure profile was not due to a difference in severity of the shock protocol between the treated and untreated shock groups. The bleedout volumes until MAP was reduced to 40 mmHg were 10.27 ml/kg for control dogs; 27.48 ml/kg for DHETX-treated dogs; 42.88 ml/kg for NAL-treated dogs; and 32.16 ml/kg for DHETX+NAL-teated dogs (Table 1). The increased bleedout volumes experienced by the treated shock dogs would be expected to

produce a more severe shock state resulting in a lower postretransfusion MAP, however, the opposite occurred (except for DHETX+NAL-treated dogs).

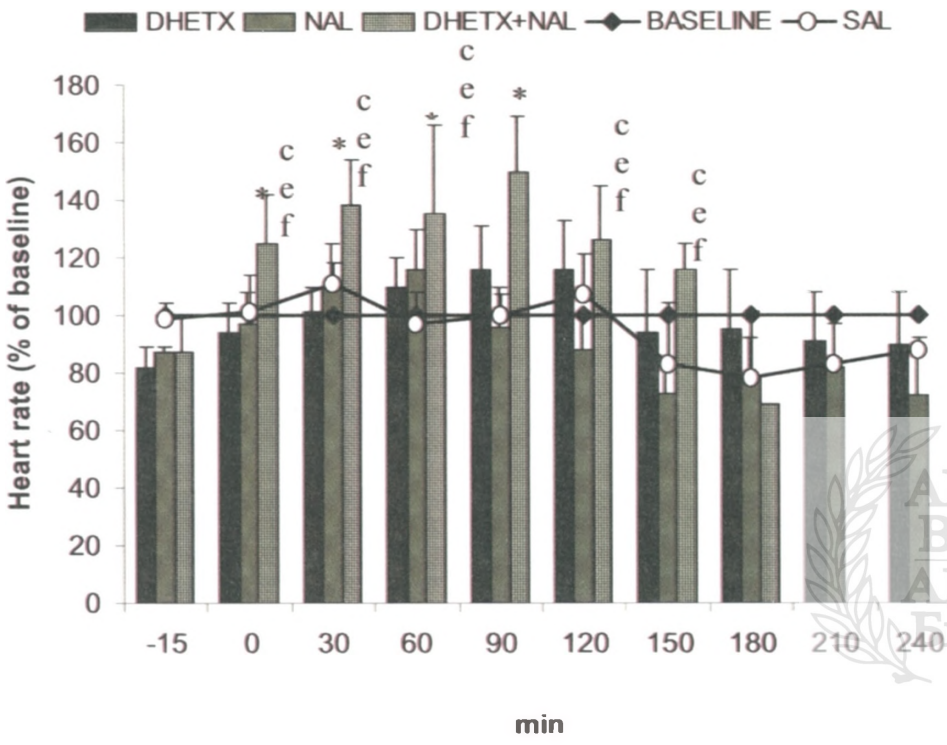


Fig. 2. Changes in heart rate (% of baseline) in hemorrhaged dogs. Values are mean ± SEM. Groups and symbols are the same as in Figure 1.

Figure 2. shows changes of HR during the experimental procedure. After the hemorrhage, HR increased in dogs of all groups regarding the baseline values. During the hypotensive period (until 90 minutes) the increase of HR is only in DHETX+NAL-treated group higher than prehemorrhagic value. After the initial increase we noticed a slow tendency of decrease of HR in dogs of control, DHETX-treated and NAL-treated groups which is more intensive from 120 to 240 min. During all hypotensive period (from 0 to 150 min) HR at DHETX+NAL-treated dogs was significantly higher than in other three groups.

Figure 3. illustrates the representative ECG during prolonged posthemorrhagic hypotension in four experimental groups. Beside the DHETX-treated group, in

all groups a significant changes in ECG appeared. In NAL-treated group and in DHETX+NAL-treated group those changes are more visible than in the control group. Tachycardia is present in all experimental groups, but the elevation of ST-segment is a characteristics only in NAL-treated and DHETX+NAL-treated groups, but the height of R-wave is decreased significantly. In all groups the significant shortening of PQ-interval was observed. In DHETX +NAL-treated group the characteristic pictures of ECG disappears gradually, and in terminal phase of experiment we can not notice PQRST-complexes. In NAL-treated group changes of ECG appear already during infusion of NAL, what means before the hemorrhage. In DHETX-treated dogs recorded electrocardiograms are extremly good, except the tachycardia mentioned above, we can not notice any other changes.

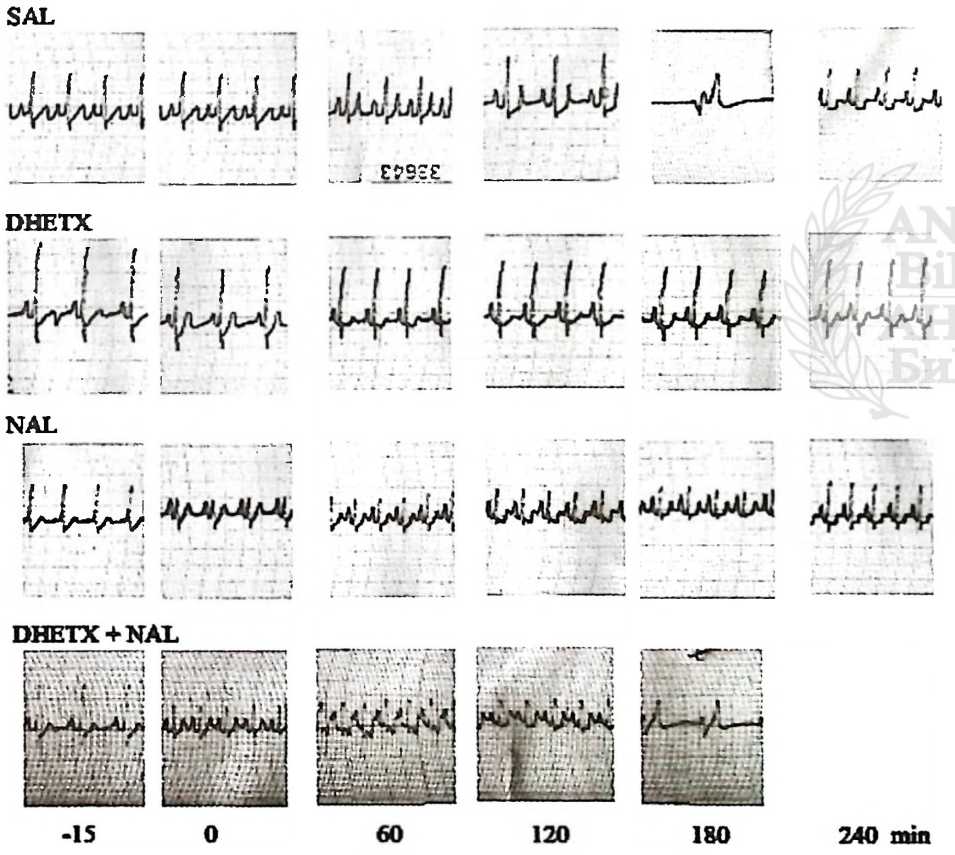


Fig. 3. This series of representative electrocardiograms shows the effects of naloxone and dihydroergotoxine given separately or simultaneously during posthemorrhagic hypotension in anesthetized dogs.

Changes in values of respiratory rate (RR) are presented in Fig 4. In NAL-treated group there are the biggest aberrations regarding the basal values. This could be absolutely disturbed breathing, where the normal phases of breathing is disappearing, i.e., instead of normal breathing it appear panting. Changes are significant comparing with other three groups. The most stable breathing is recorded in DHETX- treated group.

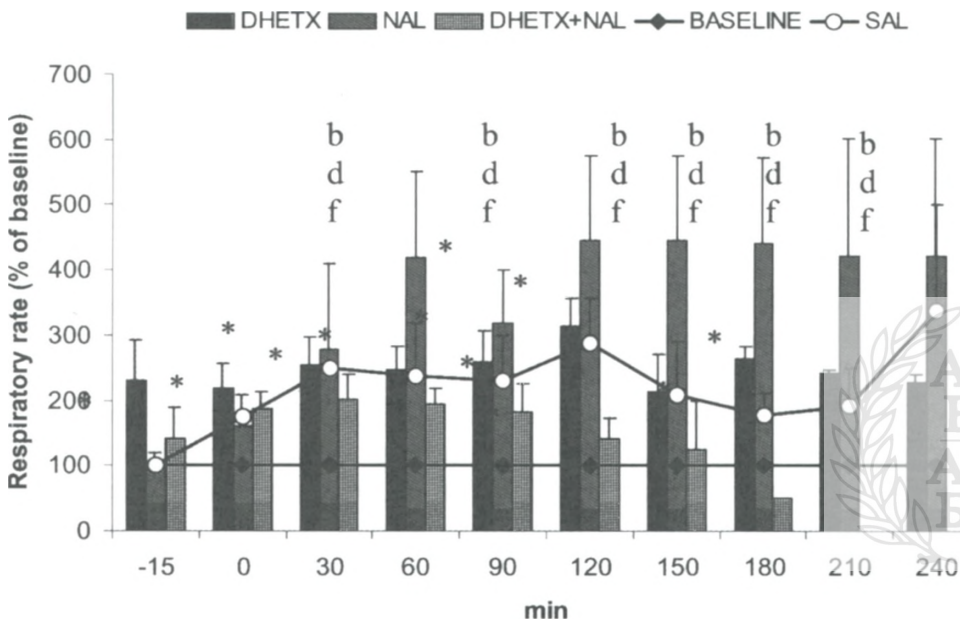


Fig. 4. Changes in respiratory rate (% of baseline) in hemorrhaged dogs. Values are mean ± SEM. Groups and symbols are the same as in Figure 1.

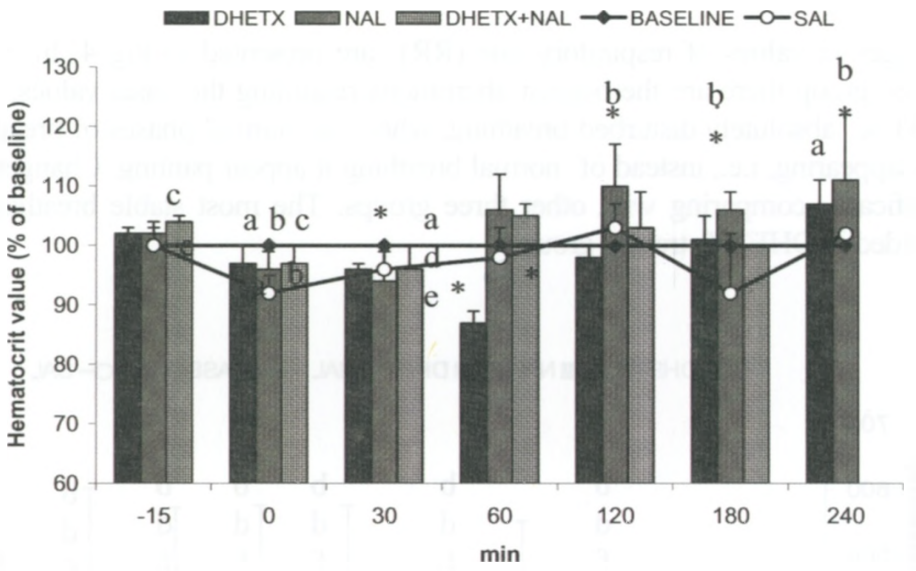


Fig. 5. Changes in hematocrit (% of baseline) in hemorrhaged dogs. Values are mean ± SEM. Groups and symbols are the same as in Figure 1.

Immediately after the hemorrhage and fall of MAP to 40 mmHg (0 min) and in 30 minutes, the hematocrit values (Ht) in dogs of all four groups are lower regarding the baseline values (Fig 5.). The fall of Ht in DHETX-treated dogs continue also in 60 minutes of hypotensive period, while in other groups, Ht shows a slow tendency of increase until the end of experimental procedure.

Figure 6. summarizes the decreases in plasma proteine concentrations during the course of the 240-min experiment for the four experimental groups of dogs. By testing of differencies between groups we established that only in NAL-treated group the values of proteinemia are significantly different as compared to other groups, especially in later phase of experimental procedure.

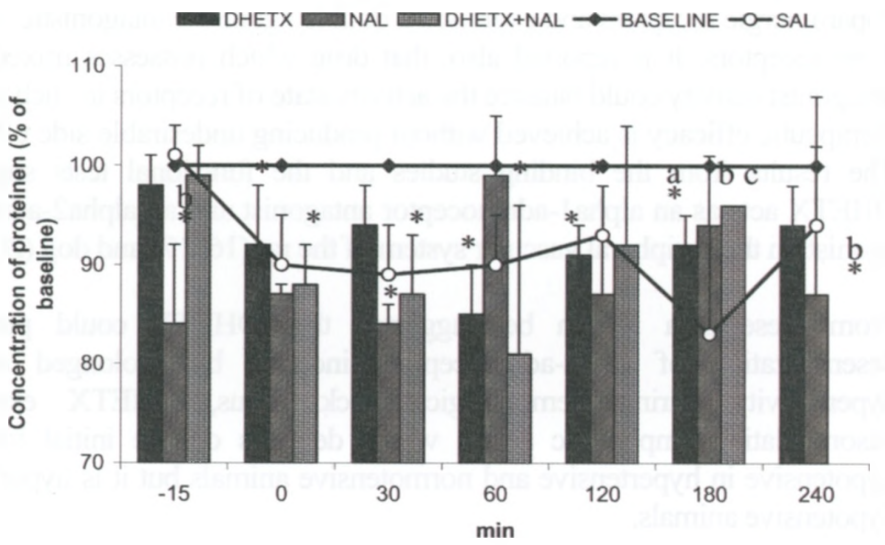


Fig. 6. Changes in plasma proteine concentration (% of baseline) in hemorrhaged dogs. Values are mean ± SEM. Groups and symbols are the same as in Figure 1.

Discussion

This study was carried out to investigate whether naloxone (NAL), an opiate receptor antagonist, and dihydroergotoxine (DHETX), an alpha adrenergic receptor antagonist, could improve cardiovascular and respiratory function in dogs exposed to prolonged posthemorrhagic hypotension.

It is well known that the bleedout provokes an strong activation of opioide (1-7) and adrenergic systems (10-12). Three hours long experimental posthemorrhagic hypotension of around 40 mm Hg is lethal for 70-90% of animals regardless transfusion or retransfusion (12, 13). Our previous data (11, 12) have showed that phenoxybenzamine (alpha adrenergic antagonist) in hemorrhagic shock in dogs delay the appearance of irreversible disturbances, improve cardiovascular functions, liver and pancreatic blood flow, and finally, increase the percentage of survival as compared to untreated animals. In available litterature we did not find data about what kind of effects DHETX has in hemorrhagic shock. Investigating hemodynamic effects of different ergot alcaloids, Sušič et all (14) are established marked effect of DHETX on cardiovascular system with clear antihypertensive effects. It was proved that DHETX possess a high afinity for alfa-2 adrenoceptors which it antagonize competitively (15, 16).

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Several studies have shown that DHETX can interfere with at least three types of receptor (15-19): alpha-adrenergic, 5-hydroxytryptaminergic and dopaminergic receptors and exercises a double agonistic/antagonistic activity on these receptors. It is reported also, that drug which possesses mixed agonist-antagonist activity could balance the activity state of receptors in such a way that therapeutic efficacy is achieved without producing undesirable side effects (19). The results from the binding studies and the functional tests suggest that DHETX acts as an alpha1-adrenoceptor antagonist and an alpha2-adrenoceptor agonist on the peripheral vascular system of the rat (16, 17) and dog (20).

From these data it can be suggested that DHETX could prevent the desensitization of alpha-adrenoceptors induced by prolonged adrenergic hyperactivity during hemorrhagic shock. Thus, DHETX exercises a vasoregulating amphoteric action which depends on the initial tonus: it is hypotensive in hypertensive and normotensive animals but it is hypertensive in hypotensive animals.

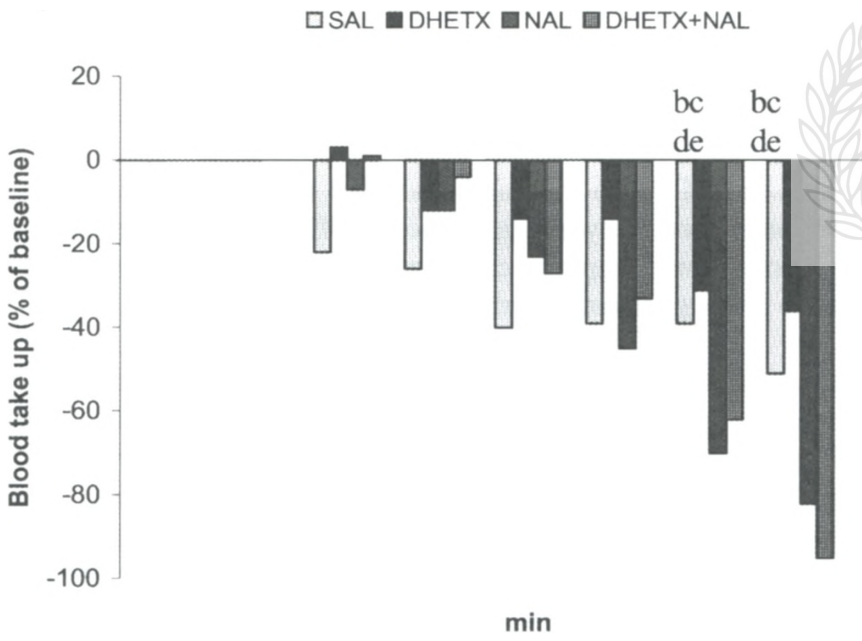


Fig. 7. Changes of volume spontaneously returned blood from reservoir into circulation (% of volume recorded after hemorrhage of dogs to 40 mmHg of MAP). Groups and symbols are the same as in Figure 1.

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The results of our work shows that DHETX given before the hemorrhage, has a favourable effects in sense of more stable reactions of cardiovascular and respiratory system during hemorrhagic shock procedure (Fig 1-4.). It is well known that the blockade of alpha adrenoceptors brings to relaxation of postcapillary sphincters and decrease of hydrostatic pressure in capillaries (10) what results in decrease of intravascular fluid loss. The dynamics of changes of Ht (Fig 5.) and proteinemia (Fig 6.) point at this. The dynamics of blood return in circulation from the reservoir (take-up) with aim to maintain the controled hypotension at level of 40 mmHg also confirm those facts (Fig 7.). DHETX-treated group is the most stable in balancing of spontaneous blood return from the reservoir in blood circulation; to the end of hypotensive period (180 min) it was spontaneously returned around 35% of blood which was recorded after the hemorrhage of dogs to 40 mmHg of MAP (Table 1.). In control and NAL-treated groups spontaneous blood return from reservoir in circulation starts immediately after the finish of bleedout and continue during the whole hypotensive period, and because of that only small quantity of blood remain for retransfusion. The least blood in reservoir at the end of hypotensive period remained in group of DHETX+NAL-treated dogs, only 5% from the initial hemorrhaged volume.

The number of survived animals is the biggest in group of DHETX-treated dogs (4/6, 66%), in NAL-treated group 3/5, 60%), in control group (2/6, 33%), but in DHETX+NAL-treated group no one animal survived (Table 2.).

Table 2.

SURVIVAL IN HEMORRHAGIC GROUPS								
Groups:	n	Hypotensive period			Postretransfusive period			% of survival
		60 min	120min	180min	60min	120min	48 hours	
SAL	6	5	5	3	2	2	2	33,33
DHETX	6	6	5	4	4	4	4	66,66
NALOXONE	5	4	4	4	3	3	3	60,00
DHETX+NAL	5	5	3	1	0	0	0	0,00

Results about the activity of naloxone in circulatory shock are contradictory. In the literature are more data about the favourable influence of NAL on the improvement of cardiovascular hemodynamics and survival of state of shock (1, 3, 4, 8, 13) than data which confirm opposite effects (5, 6). In our experiments three of five dogs survived what is much better than results in untreated group.

Respiratory rate in NAL-treated group increased from 60 min more than 4-fold regarding to the baseline value (Fig 4.). It is known that opioids inhibit the peripheral chemoreceptors by the μ opioid receptors, while activity of respiratory center is inhibited by μ and δ receptors (21). Since NAL has a high affinity to μ receptors, it blocks the central and peripheral opoid activity (22). In this way respiratory center is deblockated and acts »without brake» that leads to enormous increasing of RR in NAL-treated dogs, probably potentiated by effects of peripheral hypoxia presented in a shock.

According to effects of NAL on RR noted above, the low increase of RR in DHETX+NAL-treated group is unexpected. We suppose that favorable effects of DHETX on cardiovascular parameters dominate in this group, by which the following effects are achieved: better blood supplying of CNS, more adequately information receiving of central and peripheral chemoreceptors from periphery, as well as substantial deceleration of respiratory center activity.

The hemodynamic component to the protective effect of naloxone may involve a modest positive inotropic effect, and maybe, potentiated the affect of epinephrine at beta-adrenoceptor or beyond (9). The maintenance of blood pressure after retransfusion in NAL-treated dogs sustains a normal tissue perfusion pressure neccessary for normal blood flow. In these conditions it is showed that NAL-treated shock cats exhibited lower circulating amino nitrogen concentrations and plasma cathepsin D and myiocardial depressant factor (MDF) activities than shock cats receiving saline (3). The metabolic component appears to involve the stabilization of lysosomal membranes and the prevention of proteolysis, possibly by nonspecific effects preventing the formation of MDF as well as having direct value in the maintenance of normal circulatory function. These possibly nonspecific actions of naloxone may act in concert with its well-known specific opiate antagonism to protect shock animals by a dual mechanism.

DHETX+NAL treatment had the worst effects on hemodynamic and respiratory stability and survival of hemorrhaged dogs (no one dog survived). We assumed that DHETX excluded the favorable effect of NAL on heart function, while NAL excluded the protective effects of DHETX on peripheral blood vessels.

Conclusion

Under the present experimental conditions, prophylactic administration of DHETX had better effects on preservation of parameters mesaured and survival of dogs (66%) than NAL (60%), while DHETX+NAL treatment had the worst effects (no one dog survived).

Apstrakt

Nalokson (NAL), antagonista endogenih opijatnih receptora i dihidroergotoksin (DHETX), antagonista alfa-adrenergičnih receptora su aplicirani pojedinačno ili istovremeno psima u cilju određivanja njihovih efekata u hemoragijskom šoku. Srednji arterijski pritisak, frekvenca rada srca i respiracije, hematokritska vrijednost i koncentracija proteina u plazmi su mjereni tokom tročasovne posthemoragijske hipotenzije kod 22 psa. Životinje su bile podjeljene u četiri grupe: DHETX-tretirane (n=6), NAL-tretirane (n=5), DHETX+NAL-tretirane (n=5) i NaCl-tretirane (n=6, kontrolna grupa). Aplikacija supstancija je izvršena prije iskrvarenja. Nakon tročasovne posthemoragijske hipotenzije cjelokupna preostala količina iskrvarene krvi je vraćena psima, i životinje su držane još jedan sat u postretransfuzionom periodu. Preživljavanje pasa je praćeno još sljedećih 24 sata. Ustanovljeno je da u ovim eksperimentalnim uslovima profilaktično davanje DHETX-a pokazuje veći zaštitni efekat u očuvanju praćenih parametara i preživljavanja pasa u odnosu na NAL, dok je tretman sa DHETX+NAL imao najgore efekte (ni jedan pas nije preživio).

Ključne riječi: hemoragijski šok, psi, blokada opijatnih receptora, blokada alfa-adrenergičnih receptora

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