Beneficial action of naloxone in splanchnic artery occlusion shock[1]

M.T. Curtis and A.M. Lefer[2]

Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia (PA 19107, USA), 22 May 1980

Summary. The effects of naloxone on mean arterial blood pressure, myocardial depressant factor (MDF) activity and survival, after splanchnic artery occlusion were studied in cats. Naloxone significantly improved survival in splanchnic artery occlusion shock and prevented the accumulation of MDF in the plasma.

The opiate antagonist naloxone is beneficial in endotoxic, hypovolemic and spinal shock[3-7]. The protective action of naloxone may be mediated by a direct antagonism of the actions of the pituitary peptide β-endorphin or it may be due to other actions. Plasma β-endorphin levels have been shown to increase under stressful conditions in rats[8] and may increase under conditions leading to shock. Systemic infusion of β-endorphin has been shown to produce hypotension[9], and increases in plasma β-endorphin concentrations may contribute to the circulatory collapse seen in shock states. In the experiments described in this study, we investigated the effect of naloxone during splanchnic artery occlusion (SAO) shock. SAO shock is a lethal form of circulatory shock which is directly attributable to prolonged ischemia of the splanchnic region[10]. This type of shock is characterized by a fall in systemic blood pressure upon release of the occlusive clamps as well as an accumulation of myocardial depressant factor in the circulation[11-14]. We report here that naloxone significantly diminishes hypotension, reduces the accumulation of the cardiotoxic peptide, MDF, and improves survival after reestablishment of splanchnic flow in SAO shock.

Methods. Adult male cats ranging in weight from 2.4 to 4.5 kg were anesthetized with i.v. injected sodium pentobarbital (30 mg/kg) and for supplemental anesthesia as needed. Catheters were placed in the right carotid artery and the left jugular vein to record mean arterial blood pressure (MABP) and central venous pressure (CVP) respectively with Statham P23 Db pressure transducers. The right femoral vein was cannulated to serve as a route for drug infusion.

3 groups of cats were studied: 1. Sham shock cats given naloxone (8 mg $\cdot$ kg^{-1} injection followed by a 2 h infusion of 8 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}), 2. SAO shock cats given naloxone vehicle (0.9% NaCl), and 3. SAO shock cats given naloxone (8 mg $\cdot$ kg^{-1} injection followed by a 2 h infusion of 8 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}). SAO shock was induced by complete ligation of the celiac, superior mesenteric and inferior mesenteric arteries. The clamps were removed 2.25 h later and the animals were observed for an additional 1.5 h.

Sham shock cats (Sham SAO) underwent all surgical procedures experienced by the SAO shock animals, except that the splanchnic arteries were not occluded. Cats in all 3 groups were given an injection of 8 mg $\cdot$ kg^{-1} naloxone or 2.0 ml of 0.9% NaCl 5 min before time zero. This was followed by an infusion of 8 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} of naloxone or 2.0 ml of 0.9% NaCl starting 1 h prior to the release of the clamped vessels and continuing until 1 h after release of the clamped vessels.

At the termination of the experiment, a 25–35-ml sample of blood was drawn from the arterial catheter for bioassay of MDF activity. Following deproteinization[15], protein free plasma was eluted on a Bio-gel P-2 gel filtration column[14] and the eluates were assayed on isolated papillary muscles as described previously[13]. MDF activities were expressed as MDF units/10 ml of plasma. 1 MDF unit is equal to a 1% decrease in developed tension at a frequency of 1 Hz and a temperature of 37 °C[16].

Statistical comparisons were made using χ^2 for survival, and unpaired t-test for all other values.

Results. The figure shows the mean arterial blood pressures of 3 experimental groups during the course of the experiment. Cats in all groups exhibited comparable initial mean arterial blood pressures.

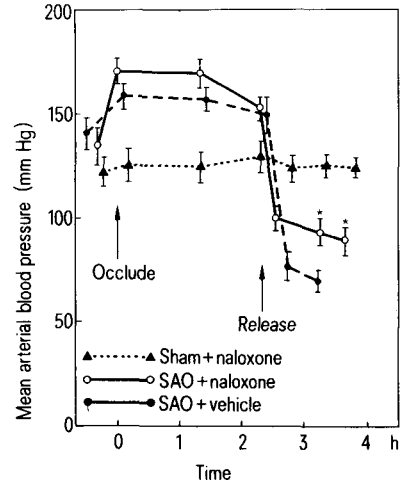
The sham shock cats exhibited stable arterial blood pressures over the entire experimental time course. Upon occlusion of the splanchnic vessels, the 2 shock groups exhibited a moderate increase in blood pressure. Over the final hour of the occlusion period, the 2 SAO shock groups exhibited a moderate decline in mean arterial blood pressure. Immediately after release of the clamps, mean arterial blood pressure declined to 77 $\pm$ 8 mm Hg in shock cats given vehicle but only to 100 $\pm$ 5 mm Hg in shock cats treated with naloxone ($p < 0.02$).

SAO shock cats given only the vehicle demonstrated rapid deterioration of cardiovascular function. SAO shock cats given naloxone also demonstrated a decline in blood pressure after release of the clamps. However, blood pressure of the shock group given naloxone was significantly higher

Survival rates in cats subjected to SAO shock or to a sham shock procedure

Experimental group	No. surviving animals/total No. animals	Percent survival
Sham SAO + naloxone	9/9	100%
SAO shock + vehicle	1/7	14%*
SAO shock + naloxone	6/7	86%

* $p < 0.01$ from the other groups.



Mean arterial blood pressures expressed in mm Hg over the course of the experimental period in 9 sham shock cats given naloxone (sham + naloxone), in 7 splanchnic artery occlusion shock cats given naloxone (SAO + naloxone) and in 7 splanchnic artery occlusion shock cats given vehicle (SAO + vehicle). All values are means $\pm$ SEM. Asterisks indicate statistical significance between SAO groups, $p < 0.02$.

than the blood pressure of the shocked cats given only vehicle at all times after release of the clamps.

The table summarizes survival data in the 3 groups of cats. Presented in the table is the ratio of animals surviving in each group to total number of animals in the group and the percent survival at the end of the experiments. In the sham shock group, all animals survived. The SAO shock group given vehicle had only one survivor out of 7 at the end of the experiment (14% survival). In contrast to this, 6 of 7 animals survived in the SAO shock group treated with naloxone, yielding a survival rate of 86%.

No significant MDF activities were observed in sham shock cats given naloxone. Plasma MDF activities averaged between 8 and 19 units/10 ml in the 8 cats studied. However, in 5 untreated SAO shock cats, final plasma MDF activity was 64 ± 4 units (mean $\pm$ SEM) indicating a significantly elevated MDF formation and accumulation. In contrast, 5 naloxone treated SAO shock cats exhibited plasma MDF activities of 28 ± 3 units, a value significantly lower than untreated shock cats ($p < 0.01$). These data show that naloxone significantly prevents the formation of the cardiotoxic peptide, MDF, during SAO shock in cats.

Discussion. The therapeutic action of naloxone in various types of circulatory shock has been proposed as evidence for the involvement of the endogenous opiates, specifically the pituitary peptide β-endorphin[3-7]. In this paper, we present data showing that naloxone is beneficial in splanchnic arterial occlusion shock in cats. Therefore, the endogenous opioid β-endorphin may be involved in the pathophysiology of SAO shock. Other factors to be considered in the pathogenesis of SAO shock are 1. the production of the cardiotoxic peptide MDF by the ischemic splanchnic region, 2. depression of myocardial function, and 3. massive release of lysosomal hydrolases[11,13,17-20].

We have shown that plasma accumulation of the cardiotoxic peptide MDF was prevented by naloxone in SAO shock. In addition to maintenance of circulatory function that may be due to the antagonism of endogenous opioid peptides, naloxone may contribute to improved survival through stabilization of lysosomal membranes and a reduction of plasma proteolysis. We have presented data showing that naloxone stabilizes lysosomal membranes and reduces proteolysis in vitro in cats during hemorrhagic shock[21]. These actions of naloxone would contribute to the lower circulating levels of the cardiotoxic peptide MDF which we observed in the present experiments and would improve survival[22], since both lysosomal hydrolases and MDF contribute significantly to mortality during circulatory shock[12].

1 Supported in part by a research grant from the W.W. Smith Foundation.
2 Acknowledgments. We gratefully acknowledge the expert technical assistance of Maureen Messenger.
3 A.I. Faden and J.W. Holaday, Science *205*, 317 (1979).
4 J.W. Holaday and A.I. Faden, Nature *275*, 450 (1978).
5 J.W. Holaday and A.I. Faden, Physiologist *22*, 57 (1979).
6 J.W. Holaday and A.I. Faden, Fedn Proc. *39*, 606 (1980).
7 D.G. Reynolds, R.B. Lechner, N.J. Gurll and T. Vargish, Physiologist *22*, 106 (1979).
8 J. Rossier, E.D. French, C. Rivier, N. Ling, R. Guillemin and F.E. Bloom, Nature *270*, 618 (1977).
9 I. Lemaire, R. Tseng and S. Lemaire, Proc. natl Acad. Sci. USA *75*, 6240 (1978).
10 J. Milliken, A. Nahor and J. Fine, Br. J. Surg. *52*, 699 (1965)
11 T.M. Glenn and A.M. Lefer, Circulation Res. *27*, 783 (1970).
12 A.M. Lefer and J. Martin, Circulation Res. *26*, 59 (1970).
13 A.M. Lefer and J. Martin, Am. J. Physiol. *218*, 1423 (1970).
14 J.N. Leffler, V. Litvin, Y. Barenholz and A.M. Lefer, Am. J. Physiol. *224*, 824 (1973).
15 A.M. Lefer and Y. Barenholz, Am. J. Physiol. *223*, 1103 (1972).
16 A.M. Lefer, Fedn Proc. *37*, 2734 (1978).
17 G. Bounous and A.H. McArdle, J. Surg. Res. *9*, 339 (1969).
18 A. Janoff, G. Weissmann, B.W. Zweifach and L. Thomas, J. exp. Med. *116*, 451 (1962).
19 E.E. Kobold and A.P. Thal, Surg. Gynec. Obstet. *117*, 315 (1963).
20 L.F. Williams, Jr, A.H. Goldberg, B.J. Polansky and J.J. Byrne, Surgery *66*, 138 (1969).
21 M.T. Curtis and A.M. Lefer, Am. J. Physiol. *239*, 416 (1980).
22 A.M. Lefer, Mod. Concepts cardiovasc. Dis. *42*, 59 (1973).

Occurrence of DDT and BHC residues in human milk in India[1]

R.L. Kalra and R.P. Chawla

Department of Entomology, Punjab Agricultural University, Ludhiana (India), 22 January 1980

Summary. Samples of human milk from Punjab, India have shown the presence of DDT and BHC residues in amounts greater than those reported from most of the other countries.

During recent years increasing concern has been voiced about the presence of pesticide residues in human milk and their effects on breast-fed infants[2-4]. Residues of DDT and BHC have been reported earlier in adipose tissues[5-7] and blood[8] in the general population in India. This communication forms the first report on DDT and BHC residues found in human milk in India.

Materials and methods. During 1979, samples of milk were collected from 75 lactating women residing in the Punjab State (India), within a week after delivery. Pesticide residues were extracted by blending 3-5 ml of the subsamples with 2 vol. of n-hexane-acetone (1:1, v/v). The homogenate was allowed to stand till clear separation into 2 layers occurred. After the removal of the upper organic phase, the lower phase was re-extracted twice with 10 ml portions of n-hexane. The combined n-hexane extract, after concentration to 20 ml, was transferred to a separating funnel, to which 5 ml of concentrated sulphuric acid (specific gravity, 1.84) was added dropwise.

The contents of the separating funnel were shaken gently and allowed to stand. The lower sulphuric acid layer

DDT and BHC residues in human milk samples in India

Pesticide	Residues in whole milk (PPM)	
	Mean	Range
p,p'-DDE	0.25	0.02 -0.80
p,p'-DDT	0.26	0.02 -1.62
Total DDT	0.51	0.04 -2.35
alpha-BHC	0.031	0.002-0.160
beta-BHC	0.158	0.012-0.720
gamma-BHC	0.007	ND* -0.020
Total BHC	0.195	0.014-0.820

* Not detected.