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Effect of naloxone on renal cortical microcirculation in haemorrhagic shock

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Abstract. In order to assess the effect of opioid receptor antagonists, naloxone and noradrenaline, on renal cortical microcirculation, India ink infusion was made through the renal artery, one hour after treatment with each drug, in dogs subjected to haemorrhagic shock. Naloxone (1 mg/kg) treatment showed a dual beneficial effect of significant improvement ($P < 0{\cdot}001$) in the mean arterial pressure without increasing the renal resistance as indicated by the presence of ink particles in about 75% of the cortical glomeruli. However, in the case of noradrenaline (2 μg/kg/min)-treated animals, although mean arterial pressure increased significantly ($P < 0{\cdot}001$) only very few glomeruli (25%) in the cortical region showed ink particles, demonstrating severe vasoconstriction. In the control group infused only with saline, although most of the glomeruli (92%) were filled with ink particles, there was a significant decline in the mean arterial pressure ($P < 0{\cdot}001$).

Keywords. Naloxone; renal microcirculation; haemorrhagic shock.

1. Introduction

Many laboratories have reported that haemorrhagic shock is associated with a decrease in the renal cortical blood flow (Carrier *et al* 1966; Logan *et al* 1971; Rector *et al* 1972). This decrease in outer cortical blood flow has been attributed to the enhanced sympathetic activity, increased release of catecholamines and diminished perfusion pressure. More recently a characteristic biphasic response in the renal nerve activity with an initial transient sympathetic activation followed by a more pronounced sympathetic inhibition during prolonged haemorrhagic shock has been reported by Skoog *et al* (1985) and Koyama *et al* (1988). The decrease in renal nerve activity during the later stages of hypovolemic shock appears to be mediated through opioid receptors (Burke and Dorward 1988). Endogenous opioid peptides released during haemorrhagic shock, play an important role in the cardiovascular suppression seen in haemorrhagic shock and the opioid receptor antagonist naloxone reverses these effects on the cardiovascular system (Holaday 1983). It is also reported from this laboratory (Reghunandanan *et al* 1988) that renal clearances are improved after administration of naloxone in haemorrhaged dogs. Schadt *et al* (1984) reported no significant change in the renal resistance after administration of naloxone in rabbits subjected to hypovolemic shock.

We have performed angiohistopathological studies on the renal cortex of dogs subjected to haemorrhagic shock, to determine whether the opioid antagonist

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Abbreviation used: MAP, Mean arterial pressure.

naloxone has any effect on renal cortical glomerular blood flow. An attempt has also been made to compare this effect of naloxone with that of noradrenaline.

2. Materials and methods

Twenty one Mongrel dogs of either sex were used for the present study. They were anaesthetized with Nembutal (sodium pento barbitone), 30 mg/kg intravenously (i.v.). Soft endotracheal tubes were inserted in the trachea after tracheostomy to make them breathe atmospheric air spontaneously. Femoral vessels of both sides were exposed and cannulated. The femoral artery of one side was connected to a polygraph for recording blood pressure while that of the other side was used for producing haemorrhage. The femoral vein of one side was used for infusion of drugs and saline. Renal vessels were exposed by a flank incision. After 30 min of surgery the animals were administered heparin (250 units/kg body weight) and bled over a period of 15 min until a mean arterial pressure (MAP) of 45 mm Hg was achieved (t_0). The MAP was maintained at this pressure for 1 h ($t_0 - t_{60}$) by adjusting the height of the bottle in which the shed blood was collected. The average loss of blood in each animal during this period was 43·5 ± 1 ml/kg body weight. After 1 h, *i.e.* at t_{60}, the reservoir was clamped and the drug treatment started for the different animal groups. Either naloxone as i.v. bolus in a dose of 1 mg/kg in 2 ml of saline or noradrenaline (2 μg/kg/min) as i.v. infusion in 0·5 ml of saline per min was administered. The control group of animals received only saline at 0·5 ml/min i.v.

After 1 h of treatment with drugs at t_{120} min India ink was infused through the renal artery under arterial pressure till it started coming out through the renal vein. Immediately the vessels were ligated and the kidneys removed before sacrificing the animals with overdosage of Nembutal. Since the saline-treated animals died before t_{120} in our previous experiment (Reghunandanan *et al* 1988), in the present case, the kidneys were removed from this group of animals just before death i.e. at t_{90}. Each of the kidneys was fixed in 10% formalin solution. Blocks from the cortical region of the kidney were made. Sections were cut and stained with haematoxilin and eosin after routine processing. Kidneys of three dogs without haemorrhage were also perfused with India ink and processed in the same way. Three hundred glomeruli were counted under high power objective (40 ×) from each kidney taken from various regions of the cortex. Statistical analysis was performed by using Student's paired t test and χ^2 test.

3. Results

MAP increased significantly ($P < 0{\cdot}001$) in both noradrenaline- and naloxone-treated groups of animals subjected to haemorrhagic shock (table 1). In the control group of animals infused only with saline the blood pressure deteriorated further ($P < 0{\cdot}001$). The section of the normal dog's kidney showed that almost all the glomeruli in the cortical region were completely filled with India ink particles (table 2). Only about 25% of the glomerular capillaries were partially filled with India ink in the renal cortex of the dogs subjected to haemorrhagic shock and treated with noradrenaline whereas about 75% of the glomeruli were partially filled with ink particles in the case of the naloxone group of animals (table 2). Statistical analysis

Table 1. Effect of naloxone, noradrenaline and saline on MAP in dogs subjected to haemorrhagic shock.

	A (Basal)	B (t_{60})	C (t_{120})	*P* value *B* vs *C*
Naloxone	118 ± 4·0	45 ± 2·2	81 ± 3·4	< 0·001
Noradrenaline	116 ± 6·8	45·5 ± 1·6	80 ± 6·0	< 0·001
Saline	112 ± 3·1	45·0 ± 0·5	30 ± 3·0	< 0·001

Values are mean ± SE. For saline group values at column C are taken at t_{90}. The number of animals in each group is 6.

Table 2. Status of the renal cortical glomerular perfusion after infusing India ink through renal artery in various groups of dogs.

Experimental group	Glomeruli counted	Glomeruli perfused	Perfusion (%)
Normal (no haemorrhage)	900	880	98
Naloxone	900	672	75
Noradrenaline	900	140	25
Saline	900	825	92

The number of animals in each group is 3.

of the data (χ^2 test) revealed that the difference between the two groups was highly significant ($P < 0{\cdot}01$). In the control group of dogs, the kidneys taken just before death (t_{90}) showed ink particles in a majority of the glomeruli (92%).

4. Discussion

In the present study MAP deteriorated significantly ($P < 0{\cdot}001$) from 45 ± 0·5 (t_{60}) to 30 ± 3 (t_{90}) in the control group of dogs subjected to haemorrhagic shock. At the same time it is interesting to note that there was a reduction in the renal resistance as indicated by the presence of ink particles in the majority of the glomeruli (92%) in the cortical region of the kidney perfused with India ink and removed just before the death of the animal at t_{90} min. These observations demonstrate a decrease in total peripheral and renal resistance. This might be due to sympathetic inhibition, as reported by Skoog *et al* (1985) and Koyama *et al* (1988), that increased renal nerve activity at the beginning of haemorrhage decreased significantly when the hypotension is prolonged. Endogenous opioid peptides released during shock may also be responsible for this effect (Burke and Dorward 1988).

On the other hand in noradrenaline (2 μg/kg/min)-treated dogs there was a severe vasoconstriction in the cortical region of the kidney as indicated by the observation that only very few glomeruli (25%) and its surrounding area of the cortex showed ink particles (table 2) at t_{120} min. This is in spite of the significant increase in the MAP from 45 mm Hg at t_{60} to 80 mm Hg at t_{120} (table 1). The increased renal vasoconstriction seen in this case is supported by reports that sympathetic fibers are found mainly in the renal cortex, and intrarenal infusion of noradrenaline produces

a pattern of progressive cortical ischaemia and consequent renal necrosis leading to death (Mitchel 1951; Frank *et al* 1956; Carrier 1969).

In the group of animals treated with the opioid receptor antagonist naloxone, after haemorrhage, a majority (75%) of the glomerular capillaries in the cortical region were partially filled with ink particles (table 2). This histological picture is indicative of the partial patency of the majority of the vasculature in the cortical region even at the low flow state. There was also improvement in MAP in this case (table 1). A supporting and correlating observation for this change in the vasculature of the kidney with increased MAP is provided by the improvement in the renal clearances observed in the previous study (Reghunandanan *et al* 1988). Naloxone treatment while improving MAP in haemorrhagic shock also produces the dual benefit of not increasing total peripheral resistance (Lechner *et al* 1985), renal resistance (Schadt *et al* 1984) and preventing its decline in the later stages of shock. Endogenous opioid peptides released during haemorrhagic shock may limit the sympathetic activity including that of renal nerve activity (Burke and Dorward 1988; Koyama *et al* 1988). This may be responsible for the decreased norepinephrine release seen during haemorrhagic shock (Schadt and Gaddis 1984). Naloxone, by antagonizing this peptide-mediated effect, removes the limit and allows the sympathetic activity to continue. Alpha adrenergic receptors appear to mediate this effect as it is attenuated by phenoxy benzamine pretreatment (Bond *et al* 1985; Lechner *et al* 1985).

To conclude, the outcome of this study suggests that any drug which can improve both cardiovascular status and renal function without increasing renal resistance, can be of immense help in combating the circulatory derangements associated with haemorrhagic shock. In this context, naloxone seems to meet the requirements.

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