EFFECT OF NALOXONE IN DIFFERENT DOSES ON THE COURSE OF HEMORRHAGIC SHOCK IN RATS

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A single dose of naloxone and also its permanent administration (1-10 mg/kg) have been shown to improve the condition of animals (rats, cats, dogs) with shock due to acute blood loss [2, 4, 5]. It has been suggested that the effect of naloxone is linked with blocking of specific opiate receptors. Naloxone is known to have different levels of affinity for different types of opiate receptors [1], which differ in their physiological role. For instance, selective blocking of high-affinity receptors, with which naloxone mostly binds, preventing the development of analgesia in response to injection of morphine, has no effect on mortality of animals due to morphine overdosage [6].

The object of the present investigation was accordingly to compare the effects of naloxone, injected immediately after bleeding in a dose of 1 mg/kg, which is sufficient to displace practically completely, from its binding sites in the brain, labeled naloxone injected previously in saturating concentrations [8], and also in a dose of 0.4 mg/kg.

EXPERIMENTAL METHOD

Experiments were carried out on 52 male Wistar rats weighing 290-360 g. Under pentobarbital anesthesia (60 mg/kg intraperitoneally) 24 h before the experiment began polyethylene catheters filled with heparin solution were introduced into the caudal artery and internal jugular vein. Experiments were carried out on waking animals kept in a cage restraining their movements. Blood pressure (BP) was recorded by the direct method through the catheter in the caudal artery and the heart rate (HR) was obtained from pulse waves of BP. The respiration rate (RR) was recorded by means of a pneumatic cuff wrapped around the animal's chest. The animal was bled 30 min after being placed in the cage, when BP, HR, and RR had returned to normal, by taking blood into a heparinized syringe from the jugular vein. Blood was taken continuously until BP had fallen to its lowest possible level (40-50 mm Hg), at which it was maintained for 20 min so that the total blood loss was 40% of the circulating blood volume. Either physiological saline or naloxone solution was then injected in a volume corresponding in milliliters to the animal's body weight in grams, divided by 1000. The animals were then kept under observation for 2 h, and then for 24 h in an ordinary cage.

EXPERIMENTAL RESULTS

Data showing the time course of BP, HR, and RR are given in Table 1 and Fig. 1. HR of animals of all three groups showed no specific changes, and for that reason this parameter was subsequently disregarded. The animals of group 1 (n = 18) were given an injection of physiological saline after the end of bleeding (control). In the rats of group 1 the blood loss amounted to 39.8% and it caused BP to fall by 57.1%. RR remained virtually unchanged. After injection of physiological saline BP rose slowly to reach a maximum 40 min after the end of bleeding; the increase was 23.8% compared with the posthemorrhagic level. BP then fell and at the end of the first hour after injection of physiological saline BP was again indistinguishable from that in the posthemorrhagic period. The change in RR was very small, but by the 50th-60th minute it was 11.9-15.5% lower ($P < 0.05$, Student's t test). After

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TABLE 1. Time Course of BP and HR in Rats with Hemorrhagic Shock Treated with Different Doses of Naloxone (M ± m)

Period of investigation	BP, mm Hg			RR, cycles/min		
	injection of physiological saline	injection of naloxone		injection of physiological saline	injection of naloxone	
		0.4 mg/kg	1.0 mg/kg		0.4 mg/kg	1.0 mg/kg
Before bleeding	116,6±11,4	115,5±12,3	115,7±7,3	115,9±26,5	134,3±35,7	121,6±23,0
After bleeding	50,0±13,9	51,1±12,2	52,0±10,3	111,6±30,3	100,0±40,1	114,7±33,8
After injection of substance						
5 min	54,2±21,2	57,0±20,5	62,7±11,9***	96,8±37,8	95,9±52,7	129,1±39,1*
10 »	55,8±21,2	57,4±20,6*	63,2±12,3***	101,9±38,4	99,2±51,1	127,1±41,5*
20 »	63,0±18,5	63,2±13,3*	66,7±13,0***	117,3±25,4	117,5±23,0	127,2±34,5
30 »	61,4±12,9**	59,2±11,7	68,2±12,7***	114,9±29,3	109,4±22,0*	117,9±33,3*
40 »	63,5±10,3**	58,9±15,8	66,5±7,2**	114,8±27,6	101,1±26,4*	110,2±33,3*
50 »	61,1±12,3**	57,4±15,2	62,6±9,4*	107,1±25,9*	95,7±21,2*	108,8±31,9*
60 »	58,1±14,4	57,9±14,6	57,6±12,9	103,1±27,1*	88,1±22,8*	98,1±24,3*

Legend. Levels of significance of difference from posthemorrhagic level: *P > 95%, **P > 99%, ***P > 99.9%.

bleeding the pulse pressure also fell, from 11.5 ± 4.7 to 5.0 ± 3.1 mm Hg ($P < 0.001$), and 5 and 10 min after injection of physiological saline it was indistinguishable from its value before injection (4.4 ± 2.9 and 5.6 ± 3.1 mm Hg, respectively).

Of the 18 animals of this group five rats (27.8%) survived more than 24 h. The mean length of survival of animals that died was 43 min.

In the animals of group 2 (n = 20) the fall of BP after removal of 39.9% of the blood volume and near the time of injection of naloxone in a dose of 0.4 mg/kg was 55.8%. RR showed no significant change at this time. After injection of naloxone solution BP rose by 10.6-18.6% after 10-20 min ($P < 0.05$) and later it gradually decreased. HR decreased after the injection until the 30th minute after bleeding by 12.5%, and by the end of the first hour it was 24.3% below its posthemorrhagic value ($P < 0.01$). Just as in the rats of group 1 the pulse pressure was reduced from 17.9 ± 11.4 to 5.4 ± 4.1 mm Hg ($P < 0.05$); after injection of naloxone it showed no significant change.

Of the 20 animals of group 2 four rats (20%) survived more than 24 h. The mean duration of survival of animals that died was 49 min.

Naloxone was injected into the animals of group 3 (n = 14) in a dose of 1.0 mg/kg. These animals lost 41.1% of their blood volume and this led to a fall of 55.1% in BP. Immediately after injection of naloxone BP increased ($P < 0.001$) by 20.6% and was significnatly higher than in rats of both previous groups ($P < 0.05$, Fisher's test, dispersion analysis). Meanwhile RR increased by 11.2%, but by the 20th minute it no longer differed from its posthemorrhagic value. BP subsequently continued to rise, and the greatest increase (29.2%) occurred 30 min after injection; at that time it was significnatly higher ($P < 0.05$, Fisher's test), moreover, than in the rats of group 2. Next BP fell gradually, and toward the end of the first hour it no longer differed from its posthemorrhagic values. RR fell by 10.3% by the 40th minute, and by 20.1% toward the end of the first hour ($P < 0.05$). In the animals of group 3 the pulse pressure fell after bleeding from 14.9 ± 6.7 to 6.9 ± 2.9 mm Hg ($P < 0.05$). In response to injection of naloxone it rose to 12.0 ± 6.9 mm Hg ($P < 0.05$).

Of the 14 animals of this group five rats (35.7%) survived longer than 24 h. The mean duration of survival of the animals that died was 10 min and mortality during the first 2 h after bleeding in this group was significantly lower than in the previous groups ($P < 0.05$, Fisher's exact probability test).

The data given above show that a single injection of naloxone in a dose of 1.0 mg/kg after bleeding causes elevation of BP, which remains significantly higher for 50 min after the injection, an increase in RR during the first 10 min after injection, and an increase in the length of survival after bleeding and a decrease in the number of animals that die. These findings indicate an improvement in the animals' condition after receiving naloxone in a dose of 1.0 mg/kg after acute, massive blood loss compared with the condition of animals receiving an injection of physiological saline.

Meanwhile injection of naloxone in a dose of 0.4 mg/kg causes qualitatively different changes in BP and RR after blood loss compared with these parameters in animals receiving

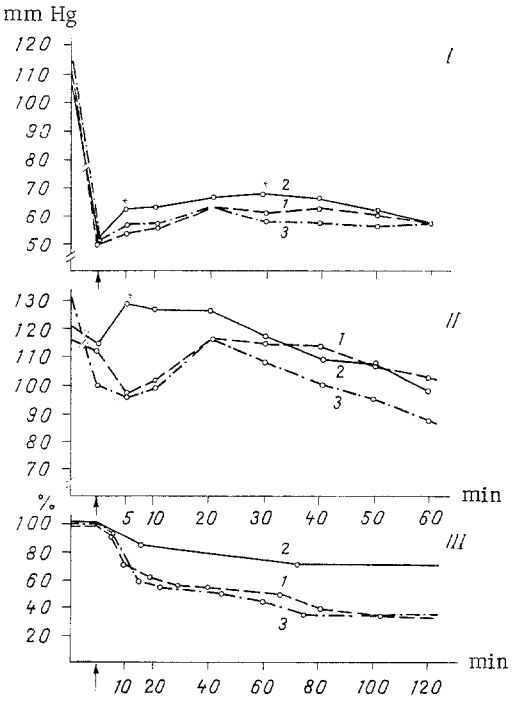

Fig. 1. Changes in BP (I), HR (II), and number of surviving animals (III) after injection of physiological saline (1) and naloxone in doses of 1.0 mg/kg (2) and 0.4 mg/kg (3) during development of hemorrhagic shock. Arrow indicates time of injection of drug. Asterisk indicates significance of differences between groups at $P < 0.05$ level.

physiological saline or naloxone in a dose of 1.0 mg/kg, and also an increase in the number of animals that died. This dynamics of the changes in BP and RR, moreover, is evidence of definite worsening of the state of the animals after injection of naloxone in the above-mentioned dose.

The experiments thus showed that a single injection of naloxone in a dose of 1.0 mg/kg significantly improves the state of the animals after acute blood loss, whereas in a dose of 0.4 mg/kg, on the other hand, naloxone causes worsening of the animals' state.

Qualitative differences observed in the effects of naloxone, in the doses used, can evidently be attributed to the fact that in small doses (0.4 mg/kg) the drug interacts chiefly with what are called high-affinity opiate receptors [1], and to a lesser degree it blocks the physiological effects of endogenous opioids, mediated through low-affinity receptors. We know that high- and low-affinity receptors differ both in their anatomical distribution [3] and in their physiological significance. It has been shown that it is low-affinity receptors which can mediate the inhibition of respiration caused by opiates in newborn rats [7]. The lethal properties of large doses of opiates in adult animals also are known [6]. On the basis of these findings and the facts described above it can be tentatively suggested that the depression of BP and RR observed during the development of hemorrhagic shock in rats is probably mediated through low-affinity opiate receptors, and the improving effect can be obtained only when these receptors are blocked. Meanwhile the role of high-affinity receptors in the development of hemorrhagic shock is still not clear.

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EFFECT OF NALOXONE IN HYPOTENSION INDUCED BY ACUTE BLOOD LOSS IN BABOONS (*Papio hamadryas*)

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Injection of naloxone is known to improve the condition of animals with pain-induced, exotoxic, hemorrhagic, endotoxic, and spinal shock [1, 4-6, 8]. This suggests the possibility of clinical use of antagonists of endogenous opioid peptides, which probably play an important role in the genesis of shock states [7], in the treatment of shock.

The object of the present invesgitation was to study the effect of naloxone on the time course of blood pressure (BP), heart rate (HR), and respiration rate (RR) in posthemorrhagic hypotension arising in one of the lower primates that is closest in its medico-biological characteristics to man.

EXPERIMENTAL METHOD

Experiments were carried out on 11 waking male baboons (*Papio hamadryas*) weighing 7-8 kg. The animals were lightly secured to an experimental platform lying on their back. Under local anesthesia a catheter was introduced into the right superficial lateral subcutaneous vein of the arm. The systolic BP was recorded by Korotkov's method in the left forelimb, HR was determined from the electrocardiogram, RR was recorded by means of a cuff fixed along the costal margin. Bleeding was carried out by removing 40% of the total circulating blood volume at the rate of 10 ml/min (total volume 180-210 ml) through a catheter introduced into the vein of the arm, into preserving solution. Either naloxone solution or physiological saline in a volume of 2 ml was injected through the same catheter 5 min after the end of bleeding, after which the catheter was rinsed with 2 ml of physiological saline. During the first 20 min after injection of naloxone BP was recorded every 30 sec, and during the next 2 h it was recorded three times every 5 min. The animal was reinfused with autologous blood at the end of 2 h.

The results were subjected to statistical analysis by dispersion analysis and Student's t test for small paired samples.

EXPERIMENTAL RESULTS

Data showing the time course of BP and RR are given in Fig. 1 and Table 1 . HR showed no specific changes in animals of all the groups, and accordingly this parameter was subsequently disregarded. Animals of group 1 (n = 3) were given an injection of physiological saline when BP was reduced by 31.7% as a result of bleeding (control). After injection of physiological saline BP was virtually unchanged, but starting from the 60th-70th minute it began to rise slowly, and by the 120th minute it was already significantly above the posthemorrhagic level, which it exceeded by 24.4%. There was little change in RR, but by the end of 2 h it showed a distinct tendency to fall.

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