Correction of hypovolemic hypotension by centrally administered naloxone in conscious rabbits

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METHODS

For the main experiments, four female New Zealand White rabbits, weighing 1.96–3.06 kg (mean 2.58) and obtained from the colony at the Walter and Eliza Hall Institute for Medical Research, were used. For the preliminary experiments, four other female rabbits from the same source, weighing 2.15–2.73 kg (mean 2.38), were used. The experiments were approved by the Animal Ethics Committee of the Royal Melbourne Hospital.

Major surgical procedures. These have previously been described in detail (12). They were done under halothane anesthesia after induction with thiopental sodium (25 mg/kg iv) and endotracheal intubation. In all rabbits, an inflatable cuff was placed around the thoracic inferior vena cava (caval cuff). Two to three weeks later, an ultrasonic transit-time flow probe (type 65, Transonic Systems, Ithaca, NY) was placed extrapericardially around the ascending aorta. The tubes leading to the cuff and V4 catheter and the connecting plug of the flow probe were buried subcutaneously on the rabbit’s back. In all rabbits, the first study was done 2–3 wk after the last major surgical procedure, when the rabbits were well and gaining weight.

Van Leeuwen, Andrew F., Duncan W. Blake, and John Ludbrook. Correction of hypovolemic hypotension by centrally administered naloxone in conscious rabbits. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1371–H1377, 1998.—Our goal was to test directly whether the vasoconstrictor action of naloxone during hypovolemic hypotension is centrally mediated. In eight chronically instrumented rabbits, progressive central hypovolemia and fall in cardiac output (CO) were produced by gradually inflating a cuff on the thoracic vena cava. Central hypovolemia was then sustained for 8 min by holding CO constant. In the main experiment (n = 4), each rabbit was studied eight times over 4 experimental days. Saline or naloxone treatment commenced 10 min after the onset of sustained hypovolemia (early treatment) or 2 min after the onset of sustained hypovolemia (late treatment), given by intravenous infusion or into the fourth ventricle (V4). With saline treatment, there was spontaneous recovery of systemic vasoconstriction and arterial pressure during sustained hypovolemia. Late treatment with naloxone (4 mg/kg iv; 4–37 µg/kg V4) accelerated and exaggerated these changes. Thus, under conditions of constant CO and central blood volume, the vasodilatation of the compensatory phase of acute hypovolemia is not sustained, and intravenous naloxone’s vasoconstrictor action is via a brain stem mechanism.

Correction of hypovolemic hypotension by centrally administered naloxone is mediated by the brain stem and in particular at the δ-opioid receptors, which are prime candidates for the trigger to phase II of acute hypovolemia in rabbits (12, 18). Moreover, the weak affinity of n-methyl-naloxone for opioid receptors cannot be overcome by using very large doses, since these cause ganglionic blockade (2).

We have previously shown that intravenous naloxone given prophylactically prevents the occurrence of the hypotensive phase II of acute central hypovolemia by an action within the brain stem (5). We now address the question of where it acts to reverse phase II. We have compared the hemodynamic effects of injecting naloxone intravenously with those of injecting it into the fourth ventricle during phase II of acute, central hypovolemia.

IN CONSCIOUS MAMMALS, the hemodynamic response to acute, progressive reduction in central blood volume is biphasic (18). At first, there is progressive, baroreflex-mediated, systemic vasoconstriction that maintains blood pressure at near-normal levels (phase I). Then, if central blood volume and cardiac output (CO) reach critically low levels, sympathetically mediated vasoconstriction suddenly fails and blood pressure plummets (phase II). It has been repeatedly demonstrated in conscious rats and rabbits that naloxone, a nonspecific opioid-receptor antagonist, restores blood pressure if administered intravenously in large doses during phase II (18).

In conscious rabbits, this pressor action is associated with restoration of renal sympathetic postganglionic activity (7, 15, 21). This suggests, but does not prove, that the site of this action of intravenous naloxone is the central nervous system (CNS). It has been reported that n-methyl-naloxone (naloxone methobromide) failed to restore blood pressure (8) or renal sympathetic nerve activity (21) when given intravenously to conscious rabbits during phase II of hemorrhage in doses equivalent to effective doses of naloxone. Both sets of authors argued that since n-methyl-naloxone does not cross the blood-brain barrier, the sympathoexcitatory and pressor action of naloxone must be exerted within the CNS (8, 21).

However, although the blood-brain barrier is resistant to the passage of quaternary compounds in general and those of naloxone in particular, there are distinct limitations to using the quaternary n-methyl-naloxone to distinguish central from peripheral actions of the tertiary compound naloxone. In rat brain in vitro, n-methyl-naloxone has only 4–8% of the affinity of naloxone for opioid receptors (2). It should therefore not be assumed that naloxone and n-methyl-naloxone are equipotent as antagonists at opioid receptors in the brain stem and in particular at the δ-opioid receptors, which are prime candidates for the trigger to phase II of acute hypovolemia in rabbits (12, 18). Moreover, the weak affinity of n-methyl-naloxone for opioid receptors cannot be overcome by using very large doses, since these cause ganglionic blockade (2).

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Minor procedures in preparation for experiments. These were done under local anesthesia with 0.5% lidocaine hydrochlo-ride. The connecting plug for the flow probe and the ends of the tubes were retrieved. A catheter was advanced through a central ear artery to the root of the ear to measure arterial pressure and for blood sampling. Another catheter was inserted into a marginal ear vein through which to infuse drugs. The rabbit was placed in a 15 x 40 x 18 cm box fitted with a wire-mesh lid 90 min before the beginning of the experiment.

Hemodynamic variables. Arterial pressure was measured by connecting a transducer (model P23 XL, Viggo-Spectramed), placed at heart level 50 mm above the floor of the rabbit's box, to the ear artery catheter. The flow probe was connected to a flowmeter (model T206, Transonic Systems) to measure ascending aortic flow (CO). Heart rate (HR) was measured by a tachometer that was actuated by the flow pulse. Signals were amplified and recorded on a Grass polygraph (model 7) and sent to an IBM-PC for analog-to-digital conversion. This provided 10-s mean values for the primary variables mean arterial pressure (MAP, mmHg), CO (ml/min), and HR (beats/min) and for the derived variables cardiac index (CI = CO/kg body wt) and systemic vascular conductance index (SVCI = CI/MAP x 100, expressed as units).

Acute central hypovolemia. The caval cuff was gradually inflated with saline delivered by a micrometer-driven syringe, so that CI fell at a constant percentage of its baseline level per minute (progressive hypovolemia) (10). Across all rabbits, this rate was 8.6 ± 0.2%/min. When CI had fallen to 31% of its baseline level or MAP to 40 mmHg, whichever occurred first, CI was then maintained constant for a further 8 min (sus-
tained hypovolemia). The caval cuff was then deflated.

Arterial blood gases. In the main experiment, these were measured to control the confounding effects of hypoxia (1). Arterial PO2 (Pao2) and PC02 (Paco2) were measured on 0.6-ml arterial blood samples (Radiometer ABL4 acid-base analyzer, Copenhagen, Denmark), taken 2 min after the caval cuff was deflated.

Drugs. I-Naloxone hydrochloride (Sigma) was dissolved in sterile saline (154 mM NaCl). It was further diluted in saline to loading volumes of 2 ml for intravenous administration and 15 µl for V4 administration. Intravenous naloxone and saline were given as a 2-ml bolus over 1 min and then infused at 0.1 ml/min. V4 naloxone and saline were given as a 15-µl bolus over 1 min and then infused at 0.75 µl/min. On the basis of a preliminary experiment (see below), an intravenous bolus dose of naloxone of 4 mg/kg was used in the main experiment (see below), an intravenous bolus dose of 4 mg/kg was used in the main experiment.

The order of drug administration (saline or naloxone) and the timing of drug administration (early or late treatment). To achieve this, each rabbit was studied eight times. Two studies were done on each of 4 experimental days at an interval of 3 h. The interval between experiments was 3–5 days.

Protocol for preliminary experiment (n = 4). The goal was to establish that the changes in SVC1 and MAP during 8 min of sustained hypovolemia were reproducible and to determine the minimal intravenous dose of naloxone that would correct the low MAP and high SVC1 during sustained hypovolemia. Four studies were done on the same day in each rabbit at intervals of 90 min. Sustained hypovolemia was estab-
lished as described above. After 2 min, intravenous saline or naloxone was given in a loading volume of 2 ml, followed by infusion at 0.1 ml/min. The loading doses in the four studies were given in ascending order. They were 0 (saline), 40 µg/kg, 400 µg/kg, and 4 mg/kg.

Analysis of results. Phases I and II of the hemodynamic response to progressive hypovolemia were distinguished by the point at which SVC1 ceased falling and rose abruptly. The rates of change of MAP and SVC1 per minute during phase I were estimated by ordinary least-squares linear regression analysis, and the effects of treatments and routes were evaluated by conventional two-way analysis of variance (ANOVA).

The levels of the hemodynamic variables were recorded as 60-s averages at four points in each study (Fig. 1) and are given as between-rabbit means ± SE: 1) baseline, immediately preceding the commencement of saline or naloxone treatment, 10 min before progressive hypovolemia; 2) immediately preceding caval cuff inflation (onset of progressive hypovolemia); 3) after 2 min of sustained hypovolemia, immediately before treatment with saline or naloxone; and 4) after 8 min of sustained hypovolemia. The effects of treatments and routes on these levels were evaluated by two-way ANOVA.

The profiles of MAP, SVC1, and CI across successive 60-s time intervals during sustained hypovolemia were compared...
by the treatment × time and route × time interactions in repeated-measures ANOVA with the Greenhouse-Geisser correction for serial autocorrelation (9). These interactions tested the null hypothesis of parallelism over time with respect to treatment or route. Trends of MAP and SVCI over time during sustained hypovolemia with early or late saline treatment were also tested by repeated-measures ANOVA. Trends were tested by the main effect of time and differential effects of intravenous or V₄ route by the route × time interaction.

The statistical analyses were performed using the statistical package Systat 5.0 (SPSS, Chicago, IL).

RESULTS

Preliminary experiment. CI was held constant at 51 × 2% of its baseline level for 8 min. After 2 min, following injection of saline or naloxone (40–400 μg/kg iv), there was a steady fall in SVCI and rise in MAP (Fig. 2). In every rabbit, naloxone (4 mg/kg iv) caused an abrupt and exaggerated fall in SVCI and rise in MAP.

Early-treatment regimen. The treatments began 10 min before the commencement of caval cuff inflation (progressive central hypovolemia) and continued for the remainder of the study. Pretreatment with saline (iv or V₄) or naloxone (4 mg/kg iv) had no effect on the hemodynamic variables (P ≥ 0.15 always). However, V₄ naloxone (4–37 μg/kg) caused CI and SVCI to fall by 17 × 2 and 33 × 4%, respectively (P ≤ 0.02 always), and there was an inconsistent rise in MAP by 25 ± 7% (P = 0.06). During progressive hypovolemia and saline infusion (iv or V₄), the hemodynamic response was biphasic (Fig. 3). In phase I, SVCI fell progressively, MAP fell by only 12 ± 1 mmHg, and HR rose (not shown). When CI had fallen to 65 ± 2% of its baseline level, phase II commenced. SVCI rose suddenly, and MAP fell steeply to reach 35 ± 1 mmHg. Treatment with V₄ naloxone always prevented the occurrence of phase II during the 8-min period of progressive hypovolemia. During treatment with intravenous naloxone, in two rabbits, phase II had not occurred within 8 min, although CI had fallen to 33 ± 3% of baseline, and in the other two its onset was delayed until CI had fallen to 50 ± 5% of baseline. During phase I, the fall of SVCI and MAP over time was linear (Fig. 3). There was no consistent effect of treatment (saline or naloxone) or route (iv or V₄) on the rates of change of MAP or SVCI (P ≥ 0.23 always). Two minutes into the eight-minute period of sustained hypovolemia and during saline treatment (iv or V₄, respectively), CI was 57 ± 3 and 52 ± 5%, SVCI 109 ± 13 and 115 ± 16%, and MAP 55 ± 6 and 53 ± 4% of their baseline levels. During saline treatment, over the ensuing 6 min during which CI was held constant, SVCI fell progressively to a level similar to that at the end of phase I, and MAP progressively rose (P = 0.02 always), regardless of the route of saline infusion (P ≥ 0.64 always; Figs. 3 and 4). After early treatment with naloxone (iv or V₄), 2 min into the period of sustained hypovolemia, CI was much lower than after the early saline treatments because of the delay or abolition of phase II. CI was, respectively, 39 ± 3 and 38 ± 2%, SVCI 44 ± 6 and 47 ± 4%, and MAP 89 ± 5 and 81 ± 7% of their baseline levels. Over the ensuing 6 min during which CI was held constant and regardless of the route of naloxone administration, SVCI remained lower than after saline treatment and MAP higher (Figs. 3 and 4).
Late-treatment regimen. During progressive hypovolemia, the hemodynamic changes resembled closely those in the regimen of early treatment with saline (iv or V₄; Fig. 5). Specifically, phase II commenced when CI had fallen to 65 ± 2% of its baseline level. With saline treatment, during the 8-min period of sustained hypovolemia when CI was held constant, there was a progressive fall in SVCI and rise in MAP (P ≤ 0.01 always), regardless of the route of saline infusion (P ≥ 0.40 always; Figs. 5 and 6), in a pattern similar to that in the early-treatment regimen (Figs. 3 and 4) and preliminary experiment (Fig. 2). After naloxone treatment (iv or V₄), there was an abrupt fall in SVCI to a level below that reached during phase I and a rise in MAP to greater than the baseline level (Figs. 5 and 6). The effect of naloxone treatment was independent of route of administration, but the dose of V₄ naloxone was 108- to 1,000-fold less than that of intravenous naloxone.

DISCUSSION

We have made two main findings in these experiments. First, when cardiac output fell to a level that induced the hypotensive, vasodilator phase II and was then maintained constant at this low level for 8 min, there was spontaneous recovery of systemic vasoconstriction and arterial pressure in saline-treated rabbits (Figs. 2, 4, and 6). Second, this recovery was accelerated and exaggerated by administering naloxone, whether intravenously or into the fourth cerebral ventricle, confirming that this action of naloxone occurs within the brain stem (Figs. 2 and 6).

Using simulated rather than true hemorrhage enabled us to study each rabbit's response up to twice a day and eight times over 2 wk to complete a balanced,
within-rabbit experimental design. The caval cuff technique for simulating hemorrhage evokes hemodynamic and neurohumoral responses that closely resemble those of actual hemorrhage (10). It has the advantage that it can be repeated with reproducible hemodynamic effects several times in one day and over several days (10, 12). The caval cuff technique also allowed us to maintain cardiac output at a constant, low level during a period of sustained hypotension (Figs. 4 and 6), thus controlling the effects on cardiac output of the fluid transfer from the extravascular to intravascular compartments that occur after acute hemorrhage. There was no evidence that arterial hypoxia was a potential confounding variable (1).

In both the preliminary (Fig. 2) and main (Figs. 4 and 6) experiments, during the 8-min period of sustained hypovolemia and with saline treatment, there was a progressive recovery of systemic vasoconstriction and a progressive rise of blood pressure. It has been a consistent finding in conscious rabbits that, over a 5-min period after hypotensive hemorrhage, there is a steady rise in arterial pressure (7, 16, 17, 19, 21). However, in all except one of these studies, the possibility could not be excluded that a progressive increase in cardiac output, due to fluid shift from the extravascular to intravascular compartments, contributed to the rise in arterial pressure. The exception is the report by Schadt and colleagues (19), in which the modest recovery of arterial pressure could be attributed to a rise in systemic and regional vascular resistances. In our experiment, cardiac output was held constant; therefore the rise in arterial pressure was due entirely to

![Graphs showing hemodynamic changes](image-url)
systemic vasoconstriction (Figs. 4 and 6). It seems unlikely that this recovery was due to return of sympathetic vasoconstrictor drive, since two different groups of investigators have shown that, during the first 5 min after hypotensive hemorrhage, there was minimal, if any, increase in renal sympathetic nerve activity (7, 21). If only because of this, it seems unlikely that the signal to the brain which abolishes the arterial baroreflex and triggers phase II, whatever its origins (13), becomes attenuated or less effective over a matter of minutes. In a variety of mammals, including humans, vasoconstrictor hormones such as angiotensin II and especially arginine vasopressin are known to be released in the hypotensive phase of hemorrhage or acute central hypovolemia (18). There is direct evidence that both hormones contribute importantly to the recovery of arterial pressure after hypotensive hemorrhage in conscious rabbits (16, 17). For obvious reasons, it has not been possible to observe human volunteers whose central blood volume has been reduced by venesection, foot-down tilting, or lower body negative pressure for >1–2 min after they have entered the hypotensive phase II. Yet the hypotension of patients in “shock” from acute blood loss is usually attended by tachycardia and evidence of intense systemic vasoconstriction. Because these patients are usually observed >5–8 min after the onset of hypotension, the relative importance of recovery of sympathetic vasoconstrictor drive and the release of adrenal epinephrine, arginine vasopressin, and angiotensin II remains a matter for speculation.

In the early-treatment regimens of our experiment, the action of naloxone in preventing or delaying the onset of phase II merely confirm previous findings (5). Also the 108- to 1,000-fold smaller effective dose by the fourth ventricular route compared with intravenous route is very similar to the 90- to 900-fold difference previously reported (5).

In the late-treatment regimens of our experiment, when intravenous naloxone was given during the period of sustained hypovolemia, it rapidly restored blood pressure to prehypotensive levels by restoring systemic vasoconstriction (Figs. 2, 4, and 6). This is similar to the pressor and sympathoexcitatory effects of naloxone that have been demonstrated in conscious rabbits after true hemorrhage (7, 11, 16, 17, 19, 21). Our new finding is that fourth ventricular naloxone, in a dose that given intravenously is without effect (Fig. 2), is as effective in restoring systemic vasoconstriction and arterial pressure as intravenous naloxone in a dose 108–1,000 times greater (Fig. 6). We have previously shown that the prophylactic effect of naloxone referred to above is probably due to an action at δ1-opioid receptors in the brain stem (6, 12). When dye is infused into the fourth ventricle according to the naloxone infusion regimen employed in this experiment, there is staining of the pons, medulla, cerebellar vermis, and first cervical segment of the spinal cord (4). However, the precise site or sites of action of fourth ventricular naloxone have not been established. δ-Receptors have been identified in the nucleus tractus solitarii (NTS) (14), which is close to the tip of our fourth ventricular catheter. However, the rostral ventrolateral medulla (RVLM), in which the principal cardiovascular sympathetic premotor neurons are located (2, 20), is an equally likely candidate. Immuno-reactive enkephalin terminals with synaptic contacts have been described in the RVLM, some at least belonging to neurons located in the NTS, and microinjection of enkephalin analogs into the RVLM results in hypotension and bradycardia that are prevented or reversed by naloxone (2, 20). However, it remains to be demonstrated that the effects of microinjection of opioid agonists into the RVLM are prevented by δ-opioid-receptor antagonists.

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REFERENCES


