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Failure of Naloxone to Influence Surgical Reversal of Two-Kidney, One-Clip Hypertension in the Rat (42453)

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Abstract. The rapid fall in blood pressure after removal of the constricting clip in two-kidney one-clip (2K-1C) hypertension in the rat is not fully explained by inhibition of the renin–angiotensin system or change in sodium balance. It has been postulated that compounds released in the renal venous effluent following unclipping of 2K-1C rats have a central opiate-like action and endogenous opioids are recognized to have profound hypotensive properties. To investigate this, we removed the clip from, or performed a sham operation in, early phase (<6 weeks) 2K-1C hypertensive rats during an infusion of naloxone, an opioid antagonist, or vehicle alone. The infusion of naloxone did not affect the pattern of blood pressure fall in either unclipped or sham-operated rats. Both naloxone-treated and control groups were similarly normotensive at 24 hr postoperation, the MAP being significantly lower than in the sham-operated groups, which regained previously hypertensive levels. Heart rate was unchanged 24 hr postoperatively in all groups. Morphine-induced bradycardia and hypotension were significantly reduced by naloxone infusion. Thus, naloxone infusion had no effect on blood pressure or heart rate in either the sham-operated or the unclipped groups, indicating that endogenous opioids do not have a major role in the reversal of renovascular hypertension under these circumstances.

The rapid fall in blood pressure after removal of the constricting clip in two-kidney one-clip hypertension in rats has been well demonstrated (1–3), although the precise mechanism is still not fully understood. Studies on the hemodynamic effects of unclipping suggest that this fall is due to the lowering of total peripheral resistance (4, 5) that occurs while hypertensive structural changes are still present (6), thus implying that vessel tone has become subnormal. Surgical reversal during pharmacological inhibition of the renin–angiotensin system indicated that this pressor mechanism was not fully responsible for the maintenance, or its suppression for the reversal of this form of hypertension (3). Sodium retention does not appear to be responsible for the elevated blood pressure (1, 2). The role of changes in the activity of the sympathetic nervous system in the maintenance of the elevated blood pressure and in its surgical reversal is more controversial (7, 8). Attention therefore has been directed to possible vasodepressor mechanisms. It is therefore of interest that a recent study has postulated that compounds released in the renal venous effluent after unclipping suppressed tonic efferent sympathetic nervous system activity and also had an effect on behavior, consistent with a central opiate-like action (9). In other studies, induction of hypertension in the two-kidney one-clip model was associated with altered activity of the endogenous opioid system (10). Endogenous opioids have been shown to have profound cardiovascular effects in other pathophysiological states (11–13). In addition to hemodynamic changes invoked by unclipping, cardiovascular effects of any anesthetic used during this procedure will contribute to the initial rapid fall in blood pressure, and therefore obfuscate the true time course of the reversal of hypertension. Furthermore, circulating endorphins are increased with ether anesthetic (14); hence it is possible that release of endogenous opioids may contribute to the rapid fall in blood pressure seen after unclipping either directly or indirectly via the autonomic nervous system or via the effects of the anesthetic agent.

To investigate further the potential role of opioids in the reversal of two-kidney, one-clip hypertension, rats were unclipped during an infusion of an opioid antagonist, naloxone. In order to control for the effects of the anes-

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thesia, a sham unclipped group was included and additional groups of unclipped and sham-operated rats were infused with vehicle alone.

Materials and Methods. Female Wistar rats (170–190 g wt) were used throughout and all surgical procedures were performed under ether anesthesia. Two-kidney one-clip hypertension was produced by placing a silver clip (internal diam 0.2 mm) on the left renal artery through a loin incision. The right kidney was not disturbed. Indirect blood pressures were measured by a photoelectric method under light ether anesthesia (15) and animals with systolic blood pressures greater than 150 mm Hg between 3 and 6 weeks after clipping were used. Blood samples for measurement of plasma renin concentration (PRC) were taken under a light ether anesthetic at least 24 hr before the insertion of catheters (2).

Operative procedure. Polythene catheters were placed in the left carotid artery (P50 internal diameter (i.d.) 0.58 mm, external diam (e.d.) 0.96 mm) and right jugular vein (P30 i.d. 0.50 mm, e.d. 1.0 mm) via a neck incision and then exteriorized between the scapulae and protected by a light flexible metal coil, attached to the animals by a linen jacket. The coil was attached to an overhead lightly counterbalanced arm to ensure minimal tension as previously described (3). On recovery from the anesthesia, the rats were placed in a plastic container measuring 30 × 30 × 38 cm with free access to food and water. The venous line was kept patent by an infusion of dextrose (50 g/liter) at a rate of 0.56 ml/hr overnight. The arterial line was flushed with 0.5 ml of heparinized dextrose (50 g/liter: 10 units heparin/ml) and was connected to a Statham P23 i.d. transducer and blood pressure recorded on a Grass polygraph.

The rats were allocated to four groups, each containing eight rats:

(1) Dextrose (50 g/liter)
(2) Naloxone (5 mg/kg)
] unclipping

(3) Dextrose (50 g/liter)
(4) Naloxone (5 mg/kg)
] sham unclipping

The following morning, the blood pressure was recorded for 30 min and then the rat was exposed to ether anesthetic. A 0.625-ml bolus intravenous injection was given of either dextrose or naloxone hydrochloride (2.5 mg/kg) followed by an infusion of dextrose or 5 mg/kg naloxone (average dose 1 mg/hr) dissolved in dextrose respectively at the rate of 0.56 ml/hr. The rats were then subjected to a further operation through the original loin incision, which consisted either of unclipping or sham unclipping. Sham unclipping consisted of exposure of the clip and cleaning but not removing it. This operation was completed within 10 min and the rats then were allowed to recover from the anesthetic. Blood pressure was recorded and infusions continued throughout the operation and for a further 24 hr. Heart rate was recorded before and 24 hr postoperation. Mean arterial blood pressure (MAP) was calculated from diastolic plus one-third of the pulse pressure.

Morphine challenge. Twenty-four hours postoperation, resting heart rate was recorded and in some rats (three unclip, seven sham receiving naloxone infusion; three unclip, seven sham receiving dextrose infusion) an intraarterial bolus of 4 mg/kg morphine sulphate was given. The change in pulse rate was recorded and expressed as a percentage of the resting pulse rate (16) and the maximum fall in MAP measured.

Statistics. All results are expressed as mean values ± SEM. Paired t tests were used to compare values within each group, one-way analysis of variance was used to compare values in different groups and two-way analysis of variance was used to compare the pattern of blood pressure response over the 24-hr postoperative period between groups. The PRC was transformed into logarithms before such comparisons were made since PRC is logarithmically and not normally distributed, but results in the text are expressed as the arithmetic means ± SEM.

Results. The weight (overall mean; 208 ± 2.4 g) and number of days post clipping at the time of the second operation (32.5 ± 0.8) was similar in all four groups ($P > 0.05$). There was no significant difference in plasma renin concentration (PRC) between the groups ($F = 0.45$, $P > 0.05$, Table I). Similarly, there was no significant difference in initial mean arterial blood pressure or heart rate between the four groups studied ($F = 2.51$ and 0.26,

TABLE I. PLASMA RENIN CONCENTRATION (PRC), DIRECT MEAN ARTERIAL BLOOD PRESSURE, AND HEART RATE PREOPERATION AND 24 hr POSTOPERATION IN RATS WITH EARLY TWO-KIDNEY, ONE-CLIP HYPERTENSION (MEANS ± SEM)

Group ($n = 8$)	PRC (pmol AI/ml/hr)	Direct mean arterial pressure (mm Hg)		Heart rate (beats/min)	
		Pre-op	24 hr Post-op	Pre-op	24 hr Post-op
Unclip; dextrose	1163 ± 384	163 ± 5	126 ± 11*†	401 ± 18 (n = 7)	394 ± 23 (n = 7)
Unclip; naloxone	618 ± 124	178 ± 10	120 ± 4*†	402 ± 13	429 ± 15
Sham; dextrose	731 ± 192	188 ± 6	174 ± 7	402 ± 14	429 ± 15
Sham; naloxone	557 ± 129	182 ± 6	175 ± 7	417 ± 16	414 ± 23

* $P < 0.01$ Comparison of pre-op and 24 hr post-op values between groups.
† $P < 0.01$ Comparison of pre-op and 24 hr post-op values within each group.

respectively, $P > 0.05$; Table I) and no significant difference in the fall in blood pressure with the anesthetic before removal of the clip (overall mean 57.7 ± 4.1 mm Hg, $F = 0.13$, $P > 0.05$; Fig. 1).

Unclipped groups (Table I). The MAP in both groups showed a small rise postoperatively to a maximum within 4 hr which was similar in both groups ($P > 0.05$). Both groups were normotensive at 6 hr and remained so, such that at 24 hr blood pressure was significantly lower than the preoperative value ($P < 0.01$). There was no significant difference between the two groups in the pattern of blood pressure change over the 24-hr period ($F = 0.46$, $P > 0.05$) or in the MAP at 24 hr ($P > 0.05$). MAP at 24 hr was significantly lower in both the unclipped groups compared to the sham-operated groups ($P < 0.01$).

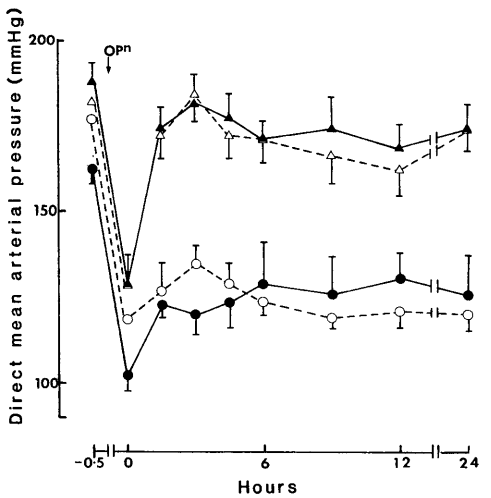

FIG. 1. Direct mean arterial pressure (mm Hg) pre- and postoperation in rats unclipped (○) or sham unclipped (△) during naloxone infusion and in rats unclipped (●) or sham unclipped (▲) during dextrose (50 g/liter) infusion. Data expressed as means ± SEM.

There was no significant difference between pre- and 24-hr postoperative heart rate in either group, and no significant difference between the groups ($P > 0.05$).

Sham-operated rats. Following the sham operation, the MAP in both groups rapidly returned to hypertensive levels (within 2–4 hr). There was no difference between the two groups in the pattern of blood pressure change ($F = 2.76$, $P > 0.05$) or in the MAP and heart rate at 24 hr ($P > 0.05$) and, within each group, there was no significant difference between the preoperative and 24-hr postoperative MAP and heart rate ($P > 0.05$).

Effect of morphine sulphate. The intraarterial injection of 4 mg/kg morphine sulphate caused a fall in heart rate of 61 ± 4% (256 ± 18 b/min) and a fall in MAP of 63 ± 9 mm Hg in the dextrose-treated group. The naloxone-treated group showed a fall in heart rate of only 3 ± 1% (12 ± 3 b/min) and in MAP of 12 ± 4 mm Hg ($P < 0.01$ compared to vehicle only). There was no apparent difference between the sham and unclipped rats in either the naloxone or the dextrose group, but statistical analysis was not performed in view of the small numbers.

Discussion. The rapid fall in blood pressure following surgical removal of the clip in early (<6 weeks) two-kidney, one-clip hypertension in the rat has been shown to be mediated by a reduction in peripheral resistance (4, 5) which occurs before reversal of structural hypertensive changes in the vessels has taken place (6). Although prolonged blockade of the renin–angiotension system by oral administration of captopril over a 7-day period in the two-kidney, one-clip model results in a fall in blood pressure to normotensive levels (17), administration of saralasin or captopril as an infusion over 12- to 15-hr period (comparable to this study) did not normalize blood pressure. Moreover, this did not alter the pattern of response of blood pressure to unclipping (3, 18). Furthermore, cumulative sodium balance becomes positive following reversal of hypertension in this model (2), suggesting that a diuresis and natriuresis is not responsible for the fall in blood pressure. Attention has therefore focused on two well-characterized intrarenal vasodepressor systems: prostaglandins and kinins. However, there was no alteration in the pattern of response of blood pressure to unclipping when indomethacin or trasylol was infused continuously in two-kidney, one-clip hypertensive rats in doses known to produce substantial inhibition of the prostaglandin or kinin system, respectively (19).

Endogenous opioid systems, which are distributed widely throughout the central and autonomic nervous system, are an alternative candidate for this vasodepressor role. Studies utilizing opioid antagonists have indicated that opioids are important in the central regulation of cardiovascular function (11–13, 20). Naloxone, a relatively pure opiate antagonist (21), has been widely used, and while it has a greater affinity for μ-opiate receptors, it is also effective at δ-receptors at higher concentrations. It has a rapid onset of action in animals, readily crossing the blood–brain barrier (22), and whether by peripheral or intracerebroventricular administration reverses endotoxin-induced hypotension (23). This pressor effect is stereospecific and is dependent on an intact sympathomedullary system. It has therefore been postulated that endogenous opioids act to decrease sympathetic efferent activity centrally (11, 24), and that naloxone blocks this inhibitory effect.

It has been shown that unclipping rats with two-kidney one-clip hypertension leads to a suppression of tonic efferent sympathetic activity (25), possibly through central mechanisms, consistent with the activation of the endogeneous opioid system. In the present experiment, infusion of naloxone had no effect on the pattern of blood pressure response following unclipping. It is difficult to be certain that all central and peripheral opioid receptors were blocked by naloxone, but the regimen used was comparable to that used in previous work (11) and should have been sufficient to antagonize the actions of endogenous opioids at both μ- and δ-central receptors. Opiate receptor blockade was confirmed in this experiment by the absence of a bradycardia following the systemic administration of morphine sulphate (26) in naloxone-infused rats and the significant reduction of the morphine-induced vasodepressor response. In view of its rapid onset of action, the bolus of naloxone given at the time of operation is likely to have blocked opioid receptors by the time the clip was removed. Therefore if endogenous opioid release had been responsible for sustaining the fall in blood pressure after operation, one might have expected to see a higher blood pressure in the naloxone-treated group 2-6 hr postoperatively. Endogenous opioids have been implicated in some of the hemodynamic effects of anesthetic agents (27). However, in the present experiment, infusion of naloxone in the sham-operated group had no effect on blood pressure compared with that of the control group, suggesting that any opioid stimulation following induction of anesthesia had a minimal effect on blood pressure response postoperatively.

Intravenous naloxone increases the sensitivity of the baroreceptors in both the conscious and anesthetized rabbit, implying a tonic reduction in sensitivity by endogenous opiates (28). Hemodynamic studies in reversal of renovascular hypertension show that despite a marked fall in blood pressure there is no corresponding tachycardia at either 2 or 24 hr (4), implying decreased baroreceptor sensitivity or rapid resetting of baroreceptor threshold.

Sensitivity of baroreceptors is decreased in renovascular hypertension (29), but rapidly returns toward normal after unclipping (30, 31). In our experiment, there was no change in heart rate at 24 hr compared to the heart rate before unclipping in either the naloxone-treated or control group and hence naloxone did not increase the sensitivity of the baroreceptors over this period or prevent the resetting induced by unclipping.

In conclusion, the present experiment suggests that release of endogenous opioids does not play a major part in the reversal of renovascular hypertension either directly or indirectly through the sympathetic nervous system. Other vasodepressor systems such as the putative renomedullary lipid complex suggested by Muirhead and co-workers (32) which may have a direct effect on resistance vessels remain to be evaluated.

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