MORPHINE and some opioid peptides can cause centrally mediated hypotension and bradycardia reversible by naloxone (Laubie et al., 1977; Bolme et al., 1978). Antihypertensive drugs acting on \( \alpha_2 \)-adrenergic receptors in the central nervous system cause similar cardiovascular effects that can be antagonized by the \( \alpha_2 \)-receptor-blocking agent, yohimbine (Schmitt and Laubie, 1983). Recent findings indicate an interaction between the central adrenergic and opiate receptor systems. Naloxone and naltrexone were found to inhibit the antihypertensive effects of clonidine and \( \alpha \)-methyldopa in spontaneously hypertensive rats (SHR), whereas yohimbine was ineffective against morphine-induced hypotension and bradycardia (Farsang et al., 1980). Furthermore, superfusion of brain stem slices from SHR with clonidine or \( \alpha \)-methylnorepinephrine increased the release of \( \beta \)-endorphin immunoreactivity, suggesting the involvement of an endorphin-like substance in the antihypertensive action of central \( \alpha_2 \)-receptor agonists (Kunos et al., 1981). However, these physiological and biochemical interactions between the two types of drugs were absent in Wistar-Kyoto rats (WKY), the genetically matched normotensive counterpart of SHR (Farsang et al., 1980; Kunos et al., 1981). Therefore, the question arises whether the interaction observed in SHR is a genetically determined trait limited to that strain of rats, or whether it is related to the hypertensive process itself. In the present study, we attempted to answer this question by testing the in vivo interaction between \( \alpha_2 \)-receptor agonists and opiate antagonists in two additional models of experimental hypertension. Also, it has not yet been established whether the in vitro release of \( \beta \)-endorphin by \( \alpha_2 \)-receptor agonists is the mechanism underlying the in vivo adrenergic-opiate interaction. To test this possibility, we examined the effects of centrally administered naltrexone and \( \beta \)-endorphin antiserum on the cardiovascular responses to clonidine in rats with various forms of hypertension. The results suggest that both the release and the subsequent action of the released opioid occurs within the anatomical region of the brainstem.

**Methods**

Spontaneously hypertensive male rats of the Okamoto-Aoki strain and male Sprague-Dawley rats were obtained.
from Charles River of Canada Ltd. The animals were housed in an animal room with a light-dark cycle of 12:12 and were fed rat chow and tap water ad libitum. Rats were 3–6 months old when used, and weighed between 300 and 400 g.

Deoxycorticosterone-salt hypertension was induced in Sprague-Dawley rats by weekly injections of 10 mg of deoxycorticosterone pivalate (DOCP) subcutaneously and by providing 1% NaCl as the drinking fluid. This treatment was maintained for 6–8 weeks, by which time the animals had become hypertensive. The control group received vehicle only (corn oil) and drank tap water.

Renal hypertension was induced in Sprague-Dawley rats by unilateral nephrectomy and contralateral partial constriction of the renal artery with a silver clip narrowed to 0.2 mm. The operation was done under chloral hydrate anesthesia (100 mg/kg, ip). This procedure resulted in rapid development of hypertension, and the animals were used within 2–3 weeks after surgery. Controls were unilaterally nephrectomized only.

Systolic blood pressure and heart rate of unanesthetized animals were measured by the tail cuff technique, as described earlier (Kunos et al., 1978). All animals were accustomed to the procedure by regular measurements being taken twice weekly for several weeks before the experiments. Values for any given time point are means of four to six individual measurements. The same animals were used for each time point reported, and the means of individual measurements were averaged.

For measurements under anesthesia, animals received sodium pentobarbital, 45 mg/kg, ip, followed by a maintenance dose of 5 mg/kg at 60- to 90-minute intervals, as required. A polyethylene cannula was placed in the saphenous vein, the rats were heparinized (500 IU/kg, iv), and blood pressure was measured directly through a cannula placed into the femoral artery and connected to a Statham P23Db pressure transducer and a Grass model 7 polygraph. Heart rate was monitored continuously by a tachograph preamplifier (Grass, 7P44) driven by the R wave of the ECG signal.

For chronic cannulation of the 4th cerebral ventricle, rats were anesthetized with chloral hydrate (100 mg/kg, ip), and the head was fixed in a stereotaxic apparatus (David Kopf). A 13-mm-long stainless steel cannula (gauge 23) was inserted through a hole drilled in the skull so that its tip was located 1 mm above the aqueduct of Sylvius, by using the following coordinates (Konig and Klippel, 1967): anteroposterior 0.48 mm, lateral 0.0, horizontal 0.0, at an angle of 20°. The cannula was fixed to the skull with dental cement and remained in place for several weeks. Intracerebroventricular (icv) injections were done by briefly restraining the unanesthetized rat and using a Hamilton syringe with a plastic guide on the needle. Adequate placement of the cannula was verified by gross morphological inspection of the brain, when animals were killed 1 hour after the icv injection of 1% bromphenol blue. The dye was localized predominantly in the 4th ventricle, with smaller amounts in the 3rd ventricle and no appreciable amounts in other brain regions.

In experiments when drugs were given intracereventrally (ic), the rats were anesthetized with sodium pentobarbital, 45 mg/kg, ip, and the cisterna magna was exposed by a midline incision through the trapezoidal and rhomboid muscles, keeping the head flexed at 45°. The tip of a 10-cm-long polyethylene cannula (PE10) was inserted through the leptomeninges. This cannula was kept in place only for the duration of the experiment in the anesthetized animals. The correct position of the cannula was verified by efflux of clear CSF (cerebrospinal fluid).

For ic or icv injections, drugs were dissolved in sterile water to final concentrations of 5–50 µg/µl. The drug solution was diluted by mixing with 5 volumes of CSF, and the mixture (5–10 µl) was then slowly injected. Similar injections of water alone had no significant effects on blood pressure or heart rate in either conscious or anesthetized animals.

The β-endorphin antiserum used in these experiments was raised in rabbits against human β-endorphin. The antiserum, as well as bovine β-endorphin, was kindly provided by Dr. M. Chretien. The antibody showed no cross-reactivity with ACTH, α- or γ-endorphin, α- or β-MSH, met-enkephalin, but cross-reacted with β-lipotropin (Gianoulakis et al., 1981). It was injected as undiluted serum (titer: 1:20,000), one µl of which was sufficient to bind 1 µg of β-endorphin under in vitro conditions. Control rabbit serum was obtained from rabbits injected with Freund's adjuvant only.

The following drugs were used: sodium pentobarbital (Nembutal, Abbott Laboratories), chloral hydrate (Fisher Scientific Co.), deoxycorticosterone pivalate (Percorten, gift from Ciba-Geigy Co.), naltrindone HCl and naloxone HCl (gifts from Endo Laboratories), clonidine-HCl (gift from Boehringer-Ingelheim Co.), and a-methyldopa (Sigma Chemical Co.).

For statistical analyses, Student's paired or unpaired t-tests were used, as appropriate. Differences with a P value <0.05 were taken as significant.

**Results**

Awake SHR received 100 mg/kg a-methyldopa, intraperitoneally, which gradually reduced systolic blood pressure to a lower plateau within 60–90 minutes (Fig. 1). Naloxone, 2 mg/kg, given during the plateau phase, caused an acute rise in blood pressure that lasted approximately 30 minutes. The maximal increase in blood pressure caused by naloxone was 32 ± 5 mm Hg, whereas injection of the same volume of saline, tested on a different occasion
in the same rats, had no effect on blood pressure. In normotensive WKY rats, α-methyl dopa caused a much smaller reduction of blood pressure than in SHR, and naloxone given subsequently was without effect.

In DOCP-salt hypertensive rats, α-methyl dopa produced a hypotensive effect similar to that in SHR, the maximal reduction at plateau being 59 ± 6 mm Hg (Fig. 2). Naloxone, 2 mg/kg, given ip during the plateau phase, caused a rise in blood pressure similar to that seen in SHR, the maximal increase being 43 ± 2 mm Hg. In the corresponding normotensive Sprague-Dawley rats, α-methyl dopa caused only a transient hypotensive effect within the first 10 minutes of its injection. Naloxone had no effect on blood pressure in these normotensive animals.

Figure 3 illustrates that, in the renal hypertensive rats, α-methyl dopa had a hypotensive effect similar to that seen in SHR and in DOCP-salt hypertensive rats, causing a maximal reduction by 51 ± 5 mm Hg. On a different day, α-methyl dopa was retested 30 minutes after the ip injection of 2 mg/kg naltrexone, a long-acting opiate receptor antagonist (Misra et al., 1976). Naltrexone, rather than naloxone, was used in these experiments because (due to its short half-life) the blocking effect of naloxone is gone by the time the peak response to α-methyl dopa develops. After naltrexone, the maximal reduction of blood pressure by α-methyl dopa was only by 14 ± 4 mmHg (p < 0.001). In the normotensive controls, α-methyl dopa was ineffective in lowering blood pressure. Naloxone or naltrexone alone had no effect on blood pressure, either in the hypertensive or the normotensive animals (data not shown). α-Methyl dopa rather than clonidine was used in these experiments, because iv doses of clonidine that lower blood pressure in SHR are ineffective or have pressor effects in the other two hypertensive groups, probably as a result of supersensitivity of peripheral α-adrenoceptors, as demonstrated by Dadkar et al. (1979). This complication can be eliminated when clonidine is administered centrally.

In seven SHR implanted with a chronic cannula for icv injections, we tested the effects of centrally administered clonidine and β-endorphin on blood pressure and heart rate of the awake animals (Fig. 4). Clonidine, 10 μg, icv, produced prolonged hypotension and bradycardia (upper panels), and β-endorphin, 1 μg, icv, caused similar but smaller effects (lower panels). On a molar basis, the dose of β-endorphin was approximately 100 times lower than that of clonidine, which was necessary to avoid the respiratory depression occasionally seen with higher doses. In agreement with published results (Sitsen et al., 1982) the dose of β-endorphin used here had no effect on respiration in the conscious animals. The results shown in Figure 4 confirm the presence of both α2-adrenergic and opiate receptors mediating hypotension and bradycardia in structures in the vicinity of the 4th cerebral ventricle.

Figure 5 depicts the effects of the icv administration of 100 μg of naltrexone on the cardiovascular responses to clonidine, 10 μg/kg, iv, in five SHR. Naltrexone significantly attenuated the hypotensive response to clonidine, whereas the small reduction in the bradycardiac response was not significant.

To test further the possible release of an endorphin-like opioid by clonidine, we examined the effect of intracisternally administered β-endorphin antiserum on the cardiovascular response to iv clonidine in four anesthetized SHR. Figure 6 illustrates...
the effects of 5 μg/kg (iv) clonidine on mean blood pressure and heart rate 1 hour after the ic injection of either 2 μl of β-endorphin antiserum or the same volume of control rabbit serum. The effects of clonidine were significantly reduced by the β-endorphin antiserum, compared with the responses observed after control rabbit serum. The columns in the bottom panels indicate the peak changes in blood pressure and heart rate in response to clonidine. Whereas the hypotensive response was significantly reduced when tested either at 1 or 2 hours after the antiserum, the decrease in the bradycardiac effect of clonidine only became significant after 2 hours.

In these SHR, the anesthesia required for the maintenance of the intracisternal cannula resulted in a marked decrease in blood pressure to normotensive levels. To eliminate this complicating factor and to test whether the interaction observed is present in other forms of hypertension, we tested the effects of control rabbit serum and β-endorphin antiserum on the cardiovascular responses to clonidine in unanesthetized SHR, steroid-salt, and renal hypertensive rats. In these animals, the sera were injected icv through a chronic cannula implanted into the aqueduct of Sylvius. Nine and minutes after the injection of 2 μl of control serum or 2 μl of β-endorphin antiserum, the animals received clonidine and systolic blood pressure and heart rate were monitored at 5-minute intervals. In the SHR, clonidine (10 μg/kg) was injected intravenously, whereas in the other two groups, 5 μg of clonidine was injected icv to avoid its peripheral vasoconstrictor effect, which is enhanced in these animals (see above). The data in Table 1 indicate that—in all three groups—the hypotensive and bradycardiac effects of clonidine were significantly reduced by β-endorphin antiserum. Separate experiments in SHR indicated that the use of normal rabbit serum for controls is justified: clonidine, 10 μg/kg, iv, reduced
BP by 43 ± 11 mm Hg before and 42 ± 11 mm Hg after control rabbit serum, whereas the respective reductions in heart rate were 51 ± 6 and 62 ± 8 beats/min.

As the effects of clonidine are similar to the effects of baroreflex activation (Haeusler, 1974), we tested whether the reflex bradycardia in response to pressure rise is also inhibited by naloxone. Graded doses of phenylephrine (1–8 μg/kg) were injected intravenously as a bolus in five WKY and five SHR anesthetized with sodium pentobarbital. Blood pressure and heart rate were monitored directly through an intraarteral cannula connected to a pressure transducer and polygraph. Baroreceptor function curves were constructed by plotting the peak increases in mean blood pressure against corresponding peak decreases in heart rate, expressed as increases in pulse period, both before and after the ip injection of 2 mg/kg naltrexone. The slope of the regression relationship was used as an index of baroreflex sensitivity. The data in Figure 7 illustrate that naltrexone caused a slight upward shift, but no change in the slope of the regression line in five WKY, whereas, in the five SHR, there was a marked increase in slope, indicating increased baroreflex sensitivity. Thus, in contrast to the effects of clonidine in SHR, which were inhibited, baroreflex bradycardia was potentiated by an opiate receptor antagonist.

**Discussion**

Evidence is mounting in support of a role of endogenous opioid peptides in cardiovascular regulation. Enkephalins and β-endorphin produce cardiovascular effects when administered systemically (Lemaire et al., 1978) or centrally (Schaz et al., 1980), and naloxone reverses the hypotension associated with various pathophysiological conditions (Hodgson and Faden, 1981). Previous work from this laboratory has pointed to a possible role of endogenous opioids in the antihypertensive effect of drugs acting on central α2-adrenergic receptors; naloxone and naltrexone antagonized the antihypertensive action of clonidine and α-methyldopa in SHR (Farsang and Kunos, 1979; Farsang et al., 1980). As the two types of compounds do not interact with each other’s receptor sites in the brain (Farsang and Kunos, 1979), release of an endogenous opioid was postulated as the underlying mechanism. The absence of a similar interaction in normotensive WKY rats (Farsang et al., 1980) made it important to determine whether or not the interaction in SHR is also present in other models of hypertension. The present experiments demonstrate that opiate antagonists can inhibit or reverse the hypotensive action of α-methyldopa, not only in SHR, but also in steroid-salt and in renal hypertensive rats. Furthermore, a similar interaction between clonidine and naloxone has recently been found in a subgroup of patients with essential hypertension (Farsang et al., 1982). The adrenergic-opiate interaction suggested by these observations thus appears to be related to the hypertensive process itself. It also raises the intriguing possibility that endogenous opioids may be involved in the pathomechanism of hypertension.
The absence of an adrenergic-opiate interaction in normotensive animals has been a consistent and striking feature of the present, as well as of our previous studies (Farsang et al., 1980; Kunos et al., 1981). A similar lack of interaction between clonidine and naloxone has been found in normotensive Wistar rats (Elghozzi et al., 1982) and in normotensive human volunteers (Watkins et al., 1980), although failure of these authors to consider differences between normotensive and hypertensive conditions led them to incorrect generalizations. Nevertheless, in some recent studies on normotensive Sprague-Dawley rats (Bennett et al., 1982; Hamilton and Longman, 1982), or normotensive human opiate addicts (Resnick et al., 1980) naloxone or naltrexone did reverse clonidine-induced hypotension. Although such discrepancies may be due to differences in resting sympathetic tone resulting in variable responses to clonidine, more importantly, they suggest that hypertension potentiates an existing regulatory mechanism, rather than triggers its de novo development. That hypertension can activate regulatory mechanisms involving endogenous opioids is not without precedent: a naloxone-reversible reduction in pain sensitivity in various forms of hypertension has been reported (Zamir and Segal, 1979; Saavedra, 1981), and, together with the present observations, indicate a close relationship between mechanisms involved in the control of pain sensation and cardiovascular functions.

In slices of a brainstem preparation which included the hypothalamus, clonidine and a-methyl-norepinephrine increased the release of $\beta$-endorphin immunoreactivity in preparations obtained from SHR but not from WKY (Kunos et al., 1981). This finding strongly suggested that release of $\beta$-endorphin from the brain and subsequent stimulation of central opiate receptors inhibitory to sympathetic tone is the basis for the in vivo interaction between central $\alpha_2$-receptor agonists and opiate antagonists. However, a possible peripheral site of interaction has not been excluded. Clonidine was shown to increase circulating levels of $\beta$-endorphin in the peripheral blood of rats (Pettibone and Mueller, 1981a), due to a direct action of the drug on the anterior pituitary (Pettibone and Mueller, 1981b), and a similar effect has been found in hypertensive but not in normotensive people (Farsang et al., 1983). Also, $\beta$-endorphin is present in various peripheral tissues (Vuolteenaho et al., 1980; Imura and Nakat, 1981), from where it may be released by appropriate stimuli. Whatever the source of the released opioid, it may cause cardiovascular effects by interacting with peripheral opiate receptors that may either influence vascular tone or cardiac functions directly, or that may elicit a reflex decrease in central vasomotor activity (Willette et al., 1982). However, the present results favor a central site for the opioid action. The hypotension caused by centrally administered clonidine and $\beta$-endorphin confirms the presence of both $\alpha_2$-adrenergic and opiate receptors mediating such effects in the vicinity of the 4th ventricle. Inhibition of the hypotensive effect of iv clonidine by icv-administered naltrexone in SHR indicates that the opiate receptors involved in the action of clonidine have a similar localization, whereas the lack of simultaneous inhibition of the bradycardic effect suggests that the receptors involved in the control of heart rate are located farther away from the ventricular cavity. This possibility is also supported by the finding that inhibition of the bradycardic effect of clonidine after icv injection of $\beta$-endorphin antisem developed slower than the inhibition of the hypotensive response (Fig. 6). This dissociation is compatible with evidence in the literature indicating that different central sites are involved in the autonomic control of blood pressure and heart rate (Schmitt and Laubie, 1983).

Although some of the intraventricularly administered naltraxone may have leaked out into the peripheral circulation, the plasma concentrations thus achieved would be much lower than concentrations following systemic administration of the higher dose required to inhibit the actions of clonidine (Farsang et al., 1980). However, the most compelling evidence for a central action of a $\beta$-endorphin-like opioid comes from the experiments with $\beta$-endorphin antisem; inhibition of the effects of clonidine in rats with three different forms of hypertension is strong evidence that release of immunoreactive $\beta$-endorphin acting on opiate receptors in the brainstem is involved in the antihypertensive action of central $\alpha_2$-receptor agonists. Although substances injected into the 4th cerebral ventricle may reach distant brain regions by diffusion, the lower brainstem is the most likely site of action of the released $\beta$-endorphin, for several reasons. The cardiovascular effects of the icv-administered $\beta$-endorphin developed rapidly (Fig. 4B), and experiments with bromophenol blue have indicated that most of the dye remains in the 4th ventricle for up to 1 hour after icv injection (see Methods). Furthermore, $\beta$-endorphin injected into the 3rd ventricle causes pressor rather than depressor effects (Feldberg and Wei, 1978). Finally, microinjection of $\beta$-endorphin into the nucleus of the solitary tract (NST) of rats causes hypotension and bradycardia, whereas similarly injected met-enkephalin causes a pressor response and tachycardia (Petty and de Jong, 1982a). The lack of cross-reactivity of the antisem used in the present experiments with met-enkephalin (Gianoulakis et al., 1981) also discounts involvement of enkephalins in the depressor response to clonidine. However, peptides that contain the C-terminal segment of $\beta$-endorphin, against which the antibody is directed, will cross-react with the antibody and could conceivably mediate the effects of clonidine in the hypertensive animals.

In contrast to the inhibition of the effects of clonidine by opiate antagonists, the bradycardic response to baroreflex activation due to pressure rise was potentiated by naltrexone in SHR (Fig. 7).
observation leads to two conclusions. The first is that an endogenous opioid exerts tonic inhibition on the baroreflex in SHR but not in WKY. This opioid may be an enkephalin: attenuation of the vagal component of the baroreflex was observed after central injection of different enkephalin analogs in cats (Schaet al., 1980) and rabbits (Pettty and Reid, 1982), and the pressor effect of enkephalins is potentiated in spontaneously hypertensive as compared to normotensive rats (Schaet al., 1980). The second conclusion is that the pressor and depressor opioid systems are under different regulatory influences. Central \( \alpha_2 \)-receptor activation appears to selectively activate the depressor, endorphinergic system; conversely, the pathways activated by baroreceptor stimulation do not include the site of endorphin release or, if they do, the depressor effect of the released endorphins is masked by the stronger, pressor effects of other released opioids, possibly enkephalins, which are equally or more sensitive to block by naltrexone. This latter possibility is favored by unpublished observations in our laboratory that the bradycardic response of SHR to bilateral electric stimulation of the NST is potentiated by naltrexone but inhibited by local administration of \( \beta \)-endorphin antisemur. The above observations support the existence of opposing, pressor and depressor opioidergic systems, and indicate that the activity of both is potentiated in hypertension.

Outside the pituitary, the only brain region in which \( \beta \)-endorphin has been found in nerve cell bodies is the arcuate nucleus in the mediobasal hypothalamus (Rossier et al., 1977) from which endorphin-containing fibers reach various brain areas, including the NST (Palkovits, 1981). \( \beta \)-Endorphin released from such terminals may be involved in central cardiovascular regulation: in a recent report published while this manuscript was in preparation, the hypertensive and bradycardic response to micro-injections of \( \alpha \)-methylNoradrenaline into the NST of renal hypertensive rats was inhibited by similar injections of a \( \beta \)-endorphin antisemur (Pettty and de Jong, 1982b).

Although local release of a \( \beta \)-endorphin-like substance in the NST may explain the findings presented in this study, there are alternative possibilities. \( \beta \)-Endorphin in the hypothalamus can be released by depolarizing stimuli (Osborne et al., 1979), and the most likely source of the immunoreactive \( \beta \)-endorphin released by clonidine from the brainsstem of SHR (Kunos et al., 1981) is also the hypothalamus. Clonidine can also release \( \beta \)-endorphin from the anterior pituitary (Petttibone and Mueller, 1981b), and the peptide released from either side may reach brain stem sites important in cardiovascular control. Experiments to distinguish among these possibilities are in progress.

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